WRAC Project Termination Report
Part I: Summary

PROJECT TITLE: **The Ecological Role and Potential Impacts of Molluscan Shellfish Culture in the Estuarine Environment**

PROJECT WORK PERIOD: 4/1/99 - 3/31/03; no cost extension approved through 3/31/2005

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REASON for TERMINATION: Objectives completed

PROJECT OBJECTIVES:

1. Assess the immediate and longer-term response of eelgrass to aquaculture practices, including bottom-culture (planting, harvest dredging, hand harvesting) and long-line operations (planting and harvesting).
2. Compare benthic infauna and epifauna species diversity, density, and biomass across habitat types (eelgrass, mud, bottom-culture oysters, long-line oysters); manipulate habitat and assess response of infauna and epifauna.
3. Compare densities and behavior of fish and invertebrates, especially juvenile salmonids and Dungeness crabs, across habitat types.

PRINCIPAL ACCOMPLISHMENTS:

Objective 1: Assess the immediate and longer-term response of eelgrass to aquaculture practices, including bottom-culture (planting, harvest dredging, hand harvesting) and long-line operations (planting and harvesting).

We approached the examination of the effects of oyster aquaculture on eelgrass from two directions 1) A survey of existing oyster aquaculture areas in Willapa Bay, Washington where grow-out and harvesting practices are fairly diverse and 2) an experimental approach in both Willapa Bay and Humboldt Bay to examine individual practices and their effect on eelgrass growth and survival over time. A new WRAC project (Ruesink et al. 2005) takes this experimental approach one step further to distinguish individual mechanisms and processes like the ability of oysters to influence eelgrass seedling production and survival, nutrients via feces and pseudofeces production and light via their ability to filter water column particulates.

We found that there is substantial spatial and temporal variability in oyster growing practices and eelgrass density, growth, and survival. This variability must be taken into account when evaluating and comparing the effects of shellfish aquaculture. Nonetheless, we were able to show a consistent trend in the effects of harvest practices with reduced eelgrass density in all areas where oysters were cultivated and approximately two thirds lower density observed in areas where a harvest dredge implement had been used versus that found in nearby eelgrass meadows. Beds where oysters were picked by hand and where long-line culture was used had intermediate densities and cover. Individual plant growth rates were also reduced in oyster culture areas, probably reflecting smaller plant size.

Experimental studies in both Willapa Bay and Humboldt Bay suggest that this impact is relatively short term if an area is left to recover and can be mitigated if necessary. Harvest dredge experiments in Willapa Bay resulted in significant eelgrass loss, particularly in muddy sediments, but eelgrass density recovered to a level equivalent to nearby control plots within a short < 1 year time-frame on sandy substrate and within 2 years on muddy substrate. Long-line culture experiments in Humboldt Bay revealed that increased spacing between lines could reduce impacts (most significant impacts occurred at spacings < 2.5 feet) and our experimental results were directly comparable with observations in existing commercial scale plots at 2.5 and 5 foot spacing. Simply planting oysters directly on the substrate surface had an apparent effect on eelgrass density after they were allowed to grow, but only at high planting density (300 seed bags/acre) and only on muddy substrate. No effects were observed on eelgrass growth.

Objective 2: Compare benthic infauna and epifauna species diversity, density, and biomass across habitat types (eelgrass, mud, bottom-culture oysters, long-line oysters); manipulate habitat and assess response of infauna and epifauna.

We compared the composition and diversity of benthic infauna and epibenthic meiofauna assemblages in various habitats, not only to evaluate the direct effects of oyster aquaculture, but also to evaluate whether these effects might be important for consumers, primarily juvenile salmon and Dungeness crab (see Objective 3 below). We sampled meiofauna and infauna in three habitats in Willapa Bay (oyster ground culture, eelgrass, and open sand/mud) using an epibenthic pump and a 10.5 cm diameter core respectively. We found that habitat structure clearly affected density and species composition with almost an order of magnitude higher density and the most diverse assemblages occurring in eelgrass and oyster habitats. This was particularly true for epibenthic meiofauna and for harpacticoid copepods, which have been previously shown to be important components in the diet of juvenile salmon. Benthic infauna were also sampled in experimental oyster long-line culture plots in Humboldt Bay where negligible differences were seen between plots with different line spacing, but three loosely coherent groups could be defined for eelgrass reference sites, experimental oyster-longlines, and ground-culture and adjacent eelgrass sites. Seasonal signals were also evident with highest densities and biomass occurring in August and November in Humboldt Bay and density increasing over the spring and early summer months sampled in Willapa Bay.

Objective 3: Compare densities and behavior of fish and invertebrates, especially juvenile salmonids and Dungeness crabs, across habitat types.

Our approach to this objective was three-fold: 1) to conduct field surveys and capture fish and invertebrates over various habitats in the field, 2) to tag fish and crab and observe their behavior in the field and/or large enclosures, and 3) to examine fish behavior in experimental mesocosms. We focused our field efforts in Willapa Bay because we presumed that juvenile salmon would potentially be more abundant (at least 3 species – chum, coho and chinook), sampling them less of an issue because no ESA listings were yet in place for these stocks, and an assortment of representative oyster aquaculture activities take place in this estuary. Oyster aquaculture beds present a formidable sampling problem which thwart traditional use of beach seines, trawls and other nets used to capture fish and invertebrates in these estuarine systems. We initially tested and used small minnow traps and breeder traps in Humboldt Bay and Willapa Bay respectively, but found that they do not efficiently capture enough organisms to conduct statistical comparisons. Larger 0.9m depth free standing fyke/hoop nets with 15 m wings fished over 24 hour tidal cycles were a much better passive sampling device and we used these to describe seasonal fluctuations in the estuarine bottom-oriented fish/invertebrate (hereafter referred to as nekton) community and to examine differences between species richness and density in three habitats (oyster bottom-culture, eelgrass, and open mud). Multivariate canonical correlation analysis indicated that nekton community composition was significantly related to location in the estuary, but not habitat. While some species were positively associated with individual habitats (eg. tubesnouts with eelgrass), the majority including Dungeness crab, staghorn sculpin, shiner perch, and other typical estuarine residents were not. We documented a significant increase in abundance for most of these species over the summer season which has been shown previously using subtidal trawls, but our data indicates that shallow estuarine tideflats including oyster aquaculture beds are also extensively utilized.

Juvenile salmon were captured using the passive fyke/hoop net gear, but it was clearly not the most effective technique for these often surface oriented fish, so we developed an experimental 6.1m wide two-boat tow-net with a squashed design that enabled deployment over a tideflat in shallow water at high tide. We successfully fished this net over ground-cultured oyster beds, eelgrass beds, open mudflat habitats, and open channel in an extensive array of locations in Willapa Bay. Juvenile salmon were counted and measured and diet samples were collected via gastric levage on a subsample of these fish in order to compare with data collected on available food items in the epibenthos/benthos under objective 2 above. A neuston net tow was also made at each of these sampling locations and sites to characterize pelagic food items present. We used the temporal data collected on juvenile salmon presence with fyke nets to design the tow net survey. Juvenile chinook salmon (*Oncorhyncus tshawytscha*) were the most abundant species over time in the area of the estuary we fished where oyster aquaculture activities typically take place and were present with a declining trend throughout the period we sampled. Juvenile chum salmon (*Oncorhyncus keta*) were equally abundant, but only present for a short period in spring and coho (*Oncorhnycus kisutch*) were less abundant and present only during spring. Significant differences in tow net catches of chinook and chum salmon were present by location within estuary, but not habitat and neither location nor habitat was significant for coho. Chinook and chum catches were highest near the mouth of the estuary at Stackpole Harbor in Willapa Bay. Although some temporal and spatial trends were noted, no significant habitat effect was found in analyses of diet content or stomach fullness for any of these three salmon species.

The lack of a significant influence of benthic habitat on juvenile salmonid distribution and diet composition is supported by a finding that most prey items we observed in their guts were derived from pelagic rather than benthic sources. Floating insects were the most important prey taxa for juvenile chinook salmon and along with larvaceans were also important in chum salmon diets. Coho fed mostly on crab megalopae and fish. The only common prey taxa associated with benthic habitat were harpacticoid copepods which were important for chum salmon. Harpacticoid copepods were found in the neuston tows apparently associated with eelgrass blades that contacted the net or rafting eelgrass and other habitat. This suggests that juvenile salmon that utilize these low intertidal areas where oyster aquaculture is present may either be foraging pelagically or moving to other nearby habitats (e.g. salt marsh) with greater resources.

Habitat associated behavior of juvenile salmon may be due to use as potential cover from predators as well as for feeding, so we evaluated salmon behavior in laboratory and field mesocosms under controlled scenarios. Acoustically tagged chinook salmon were released into a large enclosure in Willapa Bay where they were individually tracked and analysis suggests they associated primarily with eelgrass as three dimensional structure (after correcting for time spent close to the enclosure edge). Wild chinook salmon caught in the Salmon River estuary in Oregon were placed in much smaller laboratory mesocosms where the proportion of time individual fish spent occupying each of three habitats (oyster, eelgrass, open mud) was recorded with video cameras. A mock heron predator was introduced to evaluate the potential change in habitat preference due to perceived predation risk. Large chinook salmon smolts (x = 95.6 mm fork length) captured in August were clearly influenced by and preferred eelgrass habitat when exposed to a predator while smaller smolts (x = 51.4 mm fork length) displayed no such effect.

The nursery role of complex intertidal habitat including oyster aquaculture areas by juvenile 0+ Dungeness crab (*Cancer magister*) is well documented (Feldman et al. 2000), however use of these areas by 1+ and older animals is less well studied. We captured significant numbers of these larger crab in the passive fyke nets, but could not detect significant differences between habitats. Additional studies using baited traps, acoustic telemetry and tagged crabs, and finally fyke shaped funnels and underwater video clearly demonstrated that these crab migrate from subtidal channels to intertidal areas during night time high tides and prefer open sand/mud habitat over eelgrass, oyster, and *Spartina* dominated areas. In contrast, red rock crab (*Cancer productus*) appear to reside in littoral areas and prefer on-bottom oyster culture areas.

IMPACTS

Shellfish farming practices have come under increased regulatory scrutiny in the last decade due primarily to their enhanced visibility in the public eye. People are moving to and becoming more interested in activities that take place along the coast and the cumulative effect of human activities threatens not only terrestrial, but also freshwater and marine species like salmonids on the West coast (Kareiva et al. 2000). Although many farming practices have changed very little over the last 100 years, few studies have investigated the influence of these practices on other fauna in west coast estuaries. Therefore the “best available science” with which agencies choose to address the regulatory challenges (such as ESA) is very weak or simply doesn’t exist, leading these agencies to be very cautious about any perceived impacts to habitats and/or organisms that have been studied in more detail (e.g. eelgrass and salmon). This cautious approach has already begun to influence existing aquaculture practices in California and Oregon where growers have been forced to abandon culture areas or switch to off-bottom culture, particularly in areas where eelgrass is present (Chew 2001). Recent listings of additional salmon stocks under ESA in Washington promise that similar actions will be considered there in the very near future, again with little scientific understanding of the consequences.

Our results to date indicate complicated interactions between different shellfish farming techniques and the response of other commercially and ecologically important species. Some harvest techniques associated with oyster aquaculture can reduce eelgrass density, and recovery rates may vary spatially due to substrate type with effects being greater and recovery slower (up to 2 years) in soft muddy sediment. Surveys and manipulative dredging and longline studies suggest the following gradient of impact on eelgrass: harvest dredging > narrow-spaced longlines > widely-spaced longlines > hand-picked ground culture. Simply planting oysters at dense concentrations displaces some eelgrass and changes both cover and density values, again with site specific results. Eelgrass growth however, does not seem to be affected by oyster presence at least in Willapa Bay where nutrients are abundant, but work on this aspect of interactions continues (see new WRAC project report, Ruesink et al. 2005). Among mobile species, some species show habitat associations that could lead to changes in their distribution due to the presence of aquaculture operations, while others appear to show no such trend. Juvenile salmon were caught across various habitats with no significant habitat association, while other fish such as tubesnouts are clearly associated with eelgrass and others such as rock crab are clearly associated with oyster habitat. Analyses of epibenthic and benthic prey from various habitats in both estuaries suggests that both oyster and eelgrass provide habitat for greater densities of these organisms than open mudflat. Juvenile salmon gut contents reveal that most prey items were derived from pelagic rather than benthic sources and thus are not likely to be influenced by aquaculture. Behavioral observations in laboratory mesocosms and field enclosures however indicate that juvenile salmon do use eelgrass habitat preferentially for cover in response to the threat of predation. Our research suggests that Dungeness crab also use intertidal areas in a more complex manner than previously thought, preferring oyster habitat as small 0+ crab in their first year of life, but choosing open intertidal mudflat for extensive migrations as older animals and these areas may subsidize large estuarine crab populations.

The shellfish growers have taken a pro-active role in this issue. In addition to working towards their own environmental management system, they are contributing substantially to this research effort to obtain the science necessary to make informed decisions. We are strategically positioned to make the results of our studies immediately available to both the regulatory agencies and the growers as part of their efforts. Per discussion by the WRAC board, work to prepare guidelines to assist shellfish farmers in avoiding and reducing adverse impacts on estuarine habitats and maximizing beneficial impacts, particularly enhancing habitat for juvenile salmonids will be conducted by Dan Cheney at PSI with outreach funds or some other outside source. The shellfish industry membership organization, the Pacific Coast Shellfish Growers Association (PCSGA), and individual growers will continue to be involved through all phases of this task through collaborative workshops and meetings. PCSGA recently completed a broad set of environmental codes of practice (ECOP) for the west coast shellfish industry. The results of this study will be incorporated into the ECOP when additional funds are received.

We have begun to communicate results of this WRAC-supported research at a variety of regional and national meetings and publish the results in peer reviewed literature (see list below). Our primary goal is not to communicate “impacts” of aquaculture, but to explain options for estuarine management. It is clear from our study that oyster aquaculture influences the system. Some of the changes that occur are “pulses” after which the ecosystem returns to a previous state. These pulse perturbations are exemplified by declines and subsequent recoveries in eelgrass cover after the dredge harvest experiment. Other environmental changes are “presses”, for instance, long-term changes in habitat distribution (e.g. new oysterbeds in areas where eelgrass once was abundant). Such changes probably benefit some species and may negatively affect others. An important step that remains to be completed is to examine the importance of these changes and interactions on broader temporal and spatial scales in west coast estuaries. While managers and regulators are constrained by a “no net loss” policy for estuarine wetlands and therefore seek to protect existing eelgrass beds as habitat, our results suggest that oyster aquaculture beds and even open mud habitats have value. It may also be that some eelgrass beds are more important than others on a landscape scale (e.g. as protective cover near channel edges for juvenile salmon) and that the configuration of oyster habitat is also important because it provides food and cover (e.g. for juvenile 0+ crab and numerous other typical estuarine species). With respect to both pulse and press perturbations, a range of options exist for growers to carry out economically-viable and environmentally sustainable practices. The information we provide should help them choose options that reduce the magnitude and duration of undesired impacts.

RECOMMENDED FOLLOW-UP ACTIVITIES:

We recommend several follow-up activities , some of which are already underway or at least partially funded and expected to occur. The first is continued funding for and completion of the project on eelgrass-oyster/geoduck interactions. The second, as noted above and discussed previously by the WRAC board, is using the results from this study to prepare guidelines to assist shellfish farmers in avoiding and reducing adverse impacts on estuarine habitats and maximizing beneficial impacts (particularly enhancing habitat for juvenile salmonids) and incorporating this in their environmental codes of practice. This could be funded via outreach funds or some other outside source, but is a necessary progression. Finally, we see great value in examining the importance of changes and interactions between oyster aquaculture and other estuarine habitats on broader temporal and spatial scales in west coast estuaries. This topic is the subject of one objective in the USDA- ARS funded project “Integrated Pest Management Practices for Pacific Shellfish Production” for which Brett Dumbauld now serves as lead scientist. Initial steps are underway to map intertidal habitats in Willapa Bay. The primary focus of this research project is however initially to examine the interaction and influence of burrowing shrimp on oyster aquaculture and eelgrass as habitat. Further research on juvenile salmonid behavior relative to landscape features such as eelgrass meadows, eelgrass corridors and shellfish aquaculture beds would be useful once the habitat maps are created.

SUPPORT

Support for this project included an annual industry contribution of $48,820 which represented a $15,000 cash contribution from Coast Oyster Company and $33,820 in-kind match from Taylor Shellfish Company, Coast Oyster Company, and Nisbet Oyster Company as outlined in the original proposal. These contributions allowed us to work in Willapa Bay, but the cash and in-kind contributions from Coast Oyster are the only reason we were able to complete work in Humboldt Bay, California which has become a test case for the industry along the entire coast.

Year	WRAC-USDA	University	Industry	Other Federal	Other*	Total	Total Support
1999-2000	100,000		48,820				$148,820
2000-2001	100,000		48,820				$148,820
2001-2002	100,000		48,820				$148,820
2002-2003	100,000		48,820				$148,820
2003-2004	continued						
2004-2005	continued						

SUBMITTED BY:____
Work Group Chair Date

APPROVED:____
Technical Advisor Date

WRAC Project Termination Report
Part II: Detail

PROJECT TITLE: **The Ecological Role and Potential Impacts of Molluscan Shellfish Culture in the Estuarine Environment**

PROJECT WORK PERIOD: 4/1/99 - 3/31/03; no cost extension approved through 3/31/2005

AUTHOR: Brett Dumbauld

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PROJECT OBJECTIVES:

1. Assess the immediate and longer-term response of eelgrass to aquaculture practices, including bottom-culture (planting, harvest dredging, hand harvesting) and long-line operations (planting and harvesting).
2. Compare benthic infauna and epifauna species diversity, density, and biomass across habitat types (eelgrass, mud, bottom-culture oysters, long-line oysters); manipulate habitat and assess response of infauna and epifauna.
3. Compare densities and behavior of fish and invertebrates, especially juvenile salmonids and Dungeness crabs, across habitat types.

TECHNICAL SUMMARY AND ANALYSIS

Extensive areas of estuarine tidelands in Washington, Oregon, and California are currently used for commercial cultivation of Pacific oysters (*Crassostrea gigas*). Aquaculture practices have recently come under greater environmental and public scrutiny as natural resource agencies and government officials direct more attention towards protecting ecosystems for their biological productivity, complex habitats, and diverse assemblages of species. Resource agencies, particularly those involved with water quality, have traditionally viewed the commercial shellfish industry as a key indicator of a healthy estuarine environment. Concern has arisen recently because aquaculture can influence the distribution of habitat types and abundance of species. In order to balance preservation of aquatic species and shellfish cultivation, managers must understand how shellfish growing activities affect the dynamics of estuarine systems. On the east coast of North America, native oysters provide well-documented benefits in improving water quality and fish habitat (Newell 2004; Jackson et al. 2001), yet little research has been conducted to quantify ecological effects of shellfish culture on the Pacific coast. Furthermore, little is known of the cascading effects on higher trophic level species, either directly through habitat utilization or indirectly through shifts in the prey base provided by these habitats.

Eelgrass (*Zostera marina*) meadows provide critical habitat for several species and support rich biological communities (Simenstad et al. DATE). Eelgrass is protected in Washington State under a “no net loss” policy (Fresh 1994), and in Oregon and California policies are even more stringent. Previous studies in Oregon estuaries have demonstrated that commercial aquaculture on racks and stakes can have a significant impact on eelgrass and its associated community (Everett et al. 1995). However, little work has been done with respect to on-bottom ground culture and long-line culture, which account for the vast majority of oyster culture operations. Despite this lack of information, aquaculture activities are already being restricted on state leases in all three states due to the potential for negative impacts, and the U.S. Army Corps of Engineers is currently deciding on requirements for Section 4 permits.

The listing of West Coast salmon stocks under the Endangered Species Act (ESA) represents the most immediate challenge for aquaculture. Aquaculture activities could be restricted if they negatively influence juvenile salmon habitat and prey resources in west coast estuaries. Under the “taking” provision in the Endangered Species Act (ESA), shellfish harvest and other culture activities could be restricted during peak periods of estuarine use by threatened or endangered salmonids.

The study we present here was designed to provide the best available science on both positive and negative effects of aquaculture activities in Pacific Northwest estuaries. Two locations were chosen for the studies; Willapa Bay in Washington State because this estuary alone produces up to 9% of the US product and because juvenile salmon and other large fish and invertebrates were easier to study there (several species abundant and no species yet on ESA lists), and Humboldt Bay in northern California because the oyster aquaculture industry was already under regulatory pressure to change practices in this estuary due to potential impacts. The goal was to examine a variety of culture practices and document the magnitude of initial effects if they occurred, as well as recovery period for habitat, particularly eelgrass, prey base, and important larger fish and invertebrate species. This study is now complemented by a second project which seeks to better define specific interactions between shellfish and eelgrass (Ruesink et al. 2005). Results will continue to be available to incorporate into the aquaculture industry’s comprehensive environmental management system, codes of practice, and a programmatic biological assessment should that be the avenue chosen to satisfy ESA requirements, and to aid estuarine managers and regulators in conservation activities. Results for studies in Humboldt Bay are reported separately (Rumrill and Poulton 2004, Appendix 1), but are referred to here when appropriate, and more importantly discussion is incorporated in the accomplishment and impact sections of Part 1 of this report. Results for Willapa Bay studies are outlined by objective below.

Objective 1: Assess the immediate and longer-term response of eelgrass to aquaculture practices, including bottom-culture (planting, eelgrass mowing, harrowing, harvest dredging, hand harvesting) and long-line operations (planting and harvesting).

Large-scale comparisons of eelgrass. We conducted a survey in intertidal areas of Willapa Bay to describe the distribution and growth of eelgrass within and adjacent to currently cultured oyster beds. There is substantial variability in oyster planting and harvest schedules and harvest frequency that influence results and therefore, with assistance from growers, we selected beds of several culture/harvest types (harvest dredged, handpicked, and longlines) that had similar planting and harvest histories. We selected nearby eelgrass meadows as control sites. All measures were made in May at the time of eelgrass peak growth and included growth (measured by marking individual eelgrass blades with needles and harvesting to re-measure after several days) and density (percent cover and stem count). Eelgrass density showed variability between sites (Nemah and Long Island) and culture type, but all cultured areas had lower density and cover than uncultured meadow areas (Figure 1). Harvest dredged sites had the lowest eelgrass density with approximately one third of the eelgrass cover seen in uncultured areas. Individual plant growth rates (g/plant/day) and growth rates per area were lower in all cultured areas than in eelgrass meadows (Figure 2). Eelgrass in long line beds showed the slowest growth, but when density and growth rates are combined to give a measurement of aerial productivity, harvest dredged beds had the lowest overall eelgrass production. Size-corrected growth rates (not shown) were not consistently related to either culture type or oyster density.

Experimental dredge harvest of eelgrass. In addition to the above observational data, we conducted experimental harvest dredge operations to examine the effects of oyster harvest implements on eelgrass. The sites were located on the Washington State Oyster Reserves. The experimental unit consisted of three treatments: eelgrass + oysters (dredge harvested), eelgrass (dredge harvested), and eelgrass (control). We marked off three one acre treatment plots at both the Nemah and Long Island oyster reserves in August 2000. Prior to the manipulations, we recorded eelgrass measurements (stem count, above ground and below ground biomass) on each bed to insure that there were no pre-treatment differences. Eelgrass was present at an average density of 51 stems m^{-2} on all 6 of the plots and there was no significant difference between sites or treatments (Figure 3, ANOVA, $p = 0.94$ and $p = 0.17$ respectively). WDFW contracted with an oyster grower who conducted experimental harvest operations from March 13 - 17, 2001. Because it had been some time since pre-harvest measurements were taken, a second set of eelgrass measurements was made on March 5- 7, just prior to the harvest operation. A significant treatment difference (ANOVA, $p < 0.001$) was detected in pre-harvest stem density with the eelgrass + oyster treatment already exhibiting a lower stem count than the other two treatments. This difference persisted after the experimental harvest operation when we sampled on April 11, however eelgrass counts were equally low on the eelgrass + dredge treatment (Figure 3) indicating that the harvest operation had a significant effect at both sites. We revisited the sites in May 2001 and took percent cover and longest blade measurements to see whether the harvest implement may have acted like a mower and also reduced remaining blade length. Blade length was significantly lower on the treated plots ($p < 0.01$). Sites were revisited on July 7, 2001 near the peak of the eelgrass growth period one year after the initial measurements were made and again each year for four years thereafter in August 2002, July 2003, April 2004 and May 2005. Some recovery had already taken place in July 2001, particularly at the Nemah site where there was no significant difference between the eelgrass+ harvest treatment and the control plot. Eelgrass density has fluctuated since that time with density generally highest on the un-harvested plot but no statistically significant difference between means. Recovery was much slower however, at the Long Island (Jensen Spit) location where a significant difference in eelgrass density remained through 2002 (Figure 3). Recovery was complete at this site for the harvest treatment in 2002 and trends for this treatment and the control were similar thereafter, but little recovery took place in the eelgrass + oysters+ harvest treatment over time. Eelgrass density declined over the five year observation period at both sites independent of the harvest operation. The apparent difference in eelgrass response between the two locations is intriguing. Although initial harvest effects were observable, density in all three treatments was somewhat variable at the Nemah site and therefore harvest effects less obvious and recovery quicker. In contrast, eelgrass density increased gradually on the control plot at Jensen Spit making initial harvest effects appear more substantial and recovery slower. We suspect this difference may arise because effects of harvest are different on silty mud (Jensen Spit) than sand substrates (Nemah). Specifically, harvest implements may be more likely to uproot entire plants from mud than sand.

Experimental planting of oysters. Although it is apparent that harvest implements can reduce eelgrass cover, it is yet unclear what other aspect(s) of oyster culture are responsible for the trends identified in the eelgrass surveys. The experimental dredging suggests that eelgrass in some places may recover rapidly (1-2 years) after oyster harvest operations. Additionally, some culture activities could enhance eelgrass presence or productivity, a subject which has become part of a second WRAC project (Ruesink et al. 2005). We planted oysters in small plots to study the response of eelgrass to oysters themselves. One study focused on the effects of adult oysters. In cooperation with Kemmer Oyster Co., we set up 2 x 2 m plots of adult oysters at five densities (0, 5, 10, 20, 30 m^{-2}; 5 replicates of each) in an area of patchy eelgrass near Stackpole Harbor in Willapa Bay. Planting occurred in April 2002. We measured oyster condition and eelgrass growth in July/August 2002 and eelgrass growth again in April and August 2003. Eelgrass cover and density were measured initially and in July, 2002 and August 2003. Eelgrass growth was based on biomass accumulation over one week, assessed as noted above by punching holes at the base of blades (meristem) and then measuring length of new growth from the meristem margin to the holes one week later. Results indicated that neither eelgrass density or growth were affected. A second study focused on the effects of newly planted seed oysters and their growth through time. At the experimental harvest dredge sites described above, we added oyster seed at three planting densities (0, 150 bags/acre, 300 bags/acre; three replicates of each) to 5 x 5 m plots located within both the harvest dredged and un-dredged eelgrass treatment areas in May 2002. Eelgrass cover and density were measured initially and again in July 2002, as well as oyster shell length and eelgrass growth. Unfortunately oysters moved off of the small plots during the winter months and were therefore re-distributed in April 2003 and again in April 2004. Eelgrass cover, density, and growth were measured in August 2003, August 2004 and June 2005. The percent change in eelgrass cover and density on plots planted with both light and heavy oyster treatment densities was different from zero at the Jensen Spit location but not the Nemah location when measured at the conclusion of the experiment (ANOVA, p= 0.025, Figure 4). No significant effect of planted oysters on eelgrass growth was noted in August 2004 (Figure 5).

Objective 2. Compare benthic infauna and epifauna species diversity, density, and biomass across habitat types (eelgrass, mud, bottom-culture oysters, long-line oysters); manipulate habitat and assess response of infauna and epifauna.

Results of infaunal and epifaunal invertebrate studies in Willapa Bay are reported in detail in Hosack (2003) and in Hosack et al. (2005, Appendix 2), but are covered briefly here.

We sampled epibenthic meiofauna in three habitat types (oyster beds, mudflat, eelgrass) in Willapa Bay. All habitat types were represented at each of three locations, chosen in the northern Stony Point/ Cedar River region, which is one of the best oyster growing areas in Willapa Bay. Epibenthic meiofauna represent a major food source for juvenile salmonids. Samples were collected monthly from April to June 2001 at all sites, where free standing fyke nets were also deployed to sample fishes and crabs (see objective 3). We established two diagonal transects across each habitat and took 15 systematic samples for epibenthos using a small epibenthic pump. Samples were taken on the high slack tide in the evening. Samples were sieved (243 μm screen) and preserved in 10% formaldehyde. Results suggest that habitat plays a dominant role in determining meiofaunal community structure. Densities of epibenthos were almost an order of magnitude higher in both structured habitats (eelgrass and oyster) than in unstructured mudflat (Table 1, Mixed effect ANOVA, p = 0.006, Figure 6). Harpacticoid copepods, which are a well documented food source for juvenile salmonids (Simenstad et al. 1982), made up a significant proportion of the epibenthos and therefore were also significantly influenced by habitat structure (Mixed effect ANOVA, p = 0.0025). Canonical correspondence analysis was used to determine the amount of variation in taxonomic composition explained by habitat. To test significance we used a permutation test of model residuals after averaging the data across all sampling intervals at each site. Habitat was coded as a factor with treatment contrasts. Habitat was a significant factor for both meiofauna and harpacticoid copepods explaining 36% and 45 % of the variation respectively (after controlling for the amount of sediment sampled) and the majority of taxa were associated with structured habitat (Figure 7).

In May 2002, we returned to the same sites to sample benthic macro-infauna, which could also play a role as food for salmonid smolts during outmigration in spring. Samples were taken at low tide with a 10.5 cm diameter core to a depth of 10 cm, sieved, stained with rose bengal and fixed in 10% buffered formaldehyde. Results suggest that habitat was marginally significant (Mixed effect ANOVA , p = 0.052) with density in eelgrass higher than those in mud and an intermediate density in oyster habitat. We hypothesize that the recent harvesting activity at one of our oyster locations may have influenced density there and reduced significance, however canonical correlation analyses again suggested that habitat influenced taxa present at the class level (explaining 46% of total variation).

Objective 3: Compare densities of fish and invertebrates, especially juvenile salmonids and Dungeness crabs, across habitat types.

Results of hoop net surveys in Willapa Bay are reported in detail in Hosack (2003) and in Hosack et al. (2005, Appendix 2), but are covered briefly here.

Fish surveys – hoop nets. Results of initial fish and crab studies in Willapa Bay are reported in detail in Hosack (2003) and in the attached manuscript (Hosack et al. 2005), but are covered briefly here. Oyster beds present a significant fish sampling problem because it is impossible to fish with traditional net sampling gear such as beach seines and trawls over such rough and sharp terrain. In the 2000 sampling season, we researched and experimented with various fish sampling techniques including small mesh gill nets, breder traps, beach seines, fyke/hoop nets, and underwater video. Subsequently, we chose small 0.9m depth free standing fyke/hoop nets (7mm mesh) with 0.9 m x 15 m wings as our primary gear for the 2001 season. We began fishing these nets just east of the WDFW Willapa Bay Field Station in Nahcotta in January 2001 to describe the seasonal fluctuations in the estuarine fish community and continued to sample this area on a monthly basis through 2003/4. Nets were deployed and fished for 3 consecutive 24 hour periods and fish sampled at low tide when the nets were uncovered. All fish and crab captured were identified to species, measured and returned live (if possible) to the water.

As noted under objective 2 above, we chose three sites in the northern Stony Point/Cedar River area in Willapa Bay to conduct more extensive epibenthos and fish sampling in multiple habitat types in 2001. At each site we chose 3 sampling locations on eelgrass, bottom cultured oysters and open mud habitat habitats where we deployed the hoop nets. Fyke net mouths were oriented toward the center of the respective habitat away from nearby subtidal channels at approximately +0.33 MLLW. Sites were sampled on each tide run from late March - June 2001 to encapsulate the juvenile salmonid out-migration window and once again in late July. Species richness was not related to habitat or location, but both richness and abundance exhibited a highly significant quadratic temporal trend that peaked in late spring and early summer at both the Nahcotta site and those in the northern part of the estuary (Figure 8). Multivariate canonical correlation analysis indicated that nekton composition was significantly related to location ($p = 0.0056$), but not habitat ($p = 0.34$). Some species however, were positively associated with individual habitats (indicated by the distance of a taxon id from the center of the graph and from each vector in Figure 9). For example tubesnouts (*Aulorhyncus flavidus*), which are known to associate with eelgrass, were caught more often in eelgrass than in open mud or oyster habitats. Other species like juvenile lingcod (*Ophiodon elongatus*) and Dungeness crab (mostly one and two year olds which often filled the nets with densities reaching as high as 1609 crab/net) exhibited a strong seasonal signal, but were captured in similar abundances in all three habitats (Table 2). Juvenile chinook salmon were seasonally most abundant in June and July declining thereafter, but were caught in all three habitats and showed no strong association with habitat. Juvenile coho salmon were seasonally most abundant in April and May, were also caught in all three habitats, but appeared to be slightly associated with eelgrass. We caught very few juvenile chum salmon in our hoop nets during 2001, possibly due to large mesh openings, so 1/8" mesh nets were fished again in April 2002. Chum salmon were captured, but no significant habitat trends were observed.

To address the question of fish utilization of off-bottom long-line culture areas, we fished the fyke nets in paired on-bottom oyster culture areas and long-line culture areas in 2003. Unfortunately, no juvenile salmon were caught in either habitat at the locations and times fished, but use of these habitats by other fish and juvenile Dungeness crab was comparable to that in on-bottom oyster culture areas.

Fish surveys – juvenile salmon. Environmental conditions influence both the abundance and variability of anadromous salmon populations during their juvenile life history phase (Einum et al. 2003) with direct consequences for population viability (Inchausti and Halley 2003). Therefore, research on Pacific salmonids has examined, and linked, salmonid population dynamics to freshwater biological, environmental, and anthropogenic influences prior to smoltification (e.g., Nickelson et al. 1992, Bradford et al. 1997, Sharma and Hilborn 2001). Likewise, extensive research has indicated that the rate of juvenile salmonid mortality in the coastal marine zone shortly after ocean entry depresses the cohort's lifetime survival rate (review in Brodeur et al. 2003).

A number of studies suggest that estuaries function as potential nursery habitats and/or bottlenecks for salmon populations by providing juvenile outmigrating salmonids with refugia from predators, a physiological transition zone from fresh to marine water, and trophic resources (Simenstad et al. 1982, Thorpe 1994). For instance, higher mortality rates of chinook salmon have been recorded in marine waters compared to estuarine and transition zones (Macdonald et al. 1988). The size and habitat quality of estuaries is reported to positively correlate with the survival rates of hatchery chinook salmon (Magnusson and Hilborn 2003). Concern has recently focused on seagrass in the lower estuarine intertidal as critical habitat for juvenile salmonids, since seagrass meadows provide important habitat for juvenile salmonid prey (Webb 1991).

In order to compare the relative importance of intertidal habitats for juvenile Chinook salmon, we examined habitat use by Chinook among the three types of estuarine habitats surveyed with hoop nets above: eelgrass *Zostera marina*, oyster on-ground aquaculture (*Crassostrea gigas*), and unvegetated tideflat. We evaluated habitat utilization using two criteria: (1) juvenile Chinook densities as an indicator of habitat preference and (2) frequency of prey items consumed by habitat relative to the distribution of prey items. We used a novel active gear type to compare the densities and diets of juvenile Chinook salmon within low-intertidal habitats (0-1 m MLLW) in a Pacific Northwest coastal estuary in 2002 and 2003. Additionally, we conducted concurrent neuston tows in 2003 in order to compare dietary preferences with available prey resources.

To improve our ability to sample juvenile salmonids, we developed an experimental trawl for the 2002 field season (design and construction by Research Nets Inc., Bothell, WA). The net is a two-boat trawl 6.1-m wide at the footline, 9.1-m long, and 1.2-m high. The squashed tow net design allows deployment in the extremely shallow water that is typically found over a tideflat in a coastal estuary at high tide. Additionally, an apron extends a groundline downwards and forward of the net that effectively increases the width (7.9-m) and depth (variable 1.2 – 2.3 m) of the net mouth. The front apron minimizes contact between the net body and substrate while preventing pelagic fishes such as salmon from diving beneath the footline. Sampling trips using this net were conducted monthly in mid-May, June, July, and August, 2002 and again in April/May 2003 (for chum and coho salmon) and July 2003. Diet samples collected in 2002 using gastric levage and numerical counts for each taxa category were recorded. In addition, the wet and dry weights of ingested taxa were pooled into terrestrial and marine categories. Diet samples taken in 2003 were collected from whole stomachs and numerical counts, wet weights, and dry weights were recorded for each taxa category. Four habitats (oyster bed, eelgrass, mudflat, and channel) were sampled over an extensive area of Willapa Bay (Figure 10). Tows were characterized by flow meter, ground distance covered, ground speed, tow duration, depth, and time of day. Temperature and salinity were recorded and neuston samples were taken with a ½ m 220 μm mesh net for comparison of fish diets with prey availability within the shallow water column. Neuston tows were made concurrent to 2003 fish survey tows in order to compare observed diet contents with prey availability at the point of capture.

For univariate tests, mixed-effect models were used to account for the hierarchical and longitudinal sampling design. Models were compared using AIC and likelihood ratio tests and random-intercepts were used where incorporating random structure significantly increased the model fit. Data were transformed to meet assumptions of normality when necessary. For multivariate tests, such as comparison of diet composition between habitats, we used canonical correspondence analysis conducted on site means. Site means were weighted averages which accounted for the different sample sizes observed at each sampling period for a given site. Multivariate tests were performed on Ln(x+1) numerical diet data. Excluded from the analyses were rarely occurring taxa which were only present at a single site for a given species.

Juvenile Chinook densities in 2002 revealed significant patterns with respect to both spatial distribution and time. Densities were significantly different between sampling regions ($p < 0.001$). Estimates were higher in the Stackpole region compared to study sites from the Nemah and Long Island regions ($p < 0.001$, Figure 11). The density of juvenile Chinook had a declining trend from June through September as the cohort completed its seaward outmigration ($p < 0.001$, Figure 11). There was no evidence for a difference in juvenile chinook density between habitats in 2002 ($p = 0.61$). Chinook length and weight did not vary with respect to habitat or region ($p > 0.10$) but both did show a significant positive trend through time ($p < 0.001$, Figure 12).

In 2003, juvenile chum densities (mean FL: 56 mm ± 9.6 sd) were sampled in April and May. Chum densities showed evidence for significant spatial differences ($p = 0.005$) with higher estimates for the northernmost four study sites (North Stackpole) compared to other locations (Figure 13), but did not appear to differ between habitats ($p = 0.77$). Outmigrating juvenile coho were sampled in May, 2003 (mean FL: 129.6 mm ± 23.8 sd), but there was no evidence for differences between habitat or spatial location ($p > 0.60$). Chinook densities in July, 2003 (mean FL: 87.7 mm ± 8.0 sd) may have been moderately related to location ($p = 0.06$), but again there was no evidence for significant differences by habitat ($p = 0.57$). However, these density estimates were confounded by floating eelgrass mats entangling the flow meter for 35% of July, 2003 tows. Separate analyses attempting to control for eelgrass impacted tows or using other covariates such as tow distance and tow duration suggested a weaker location effect ($0.10 < p < 0.15$).

Diet analyses for juvenile Chinook sampled in 2002 showed no relationship between foraging patterns and habitat or proximity to the shoreline after controlling for the latitude and longitude of sample sites ($p > 0.35$). Analyses of spatial and temporal interactions were confounded by an unbalanced design resulting from tows where no fish were caught and thus no diets sampled. A marginally significant location by month interaction effect was present ($p = 0.06$) that may have resulted from this imbalance in sample sizes, although when considered independently both time and location suggested a strong relationship with diet composition.

Juvenile Chinook diets were also sampled in July 2003. Again, no effect of habitat was observed ($p = 0.43$), yet diet composition was weakly correlated with latitude suggesting that foraging patterns may have changed as juvenile Chinook fed in different regions of the bay ($p = 0.08$, Figure 14). Chinook stomach fullness was also weakly related to region ($p = 0.07$), as fullness appeared lower in the most northern Stackpole sites compared to other locations (Figure 15). There was no significant relationship of habitat type or latitude on chum or coho diet composition ($p > 0.10$) or on stomach fullness ($p > 0.10$).

The lack of influence of habitat on juvenile salmonid distribution and diet composition is supported by the finding that most prey items were derived from pelagic rather than benthic sources (Tables 3 and 4). Floating insects were the most important prey taxa for juvenile Chinook and also contributed an important diet component, along with larvaceans, for chum. Coho fed mainly on crab megalopae and larval fish. The only common prey taxa associated with epibenthic or benthic habitats were harpacticoid copepods, an important prey item for chum. Although chum were likely foraging for epibenthos, it is worth noting that *Harpacticus septentrionalis*, the main harpacticoid prey of chum, were also found in the concurrent neuston tows potentially due to rafting eelgrass or suspension into the water column. The majority of prey items consumed by juvenile salmonids in this study were not of benthic or epibenthic origin, despite the capture of specimens over the low intertidal habitats. This finding suggests that juvenile salmonids of these age and size classes that utilize low intertidal habitats may either be foraging pelagically or moving to other habitats (e.g., marsh or wetlands) with greater resources.

Salmon behavior in mesocosms. Large (1.68 m diameter x 1 m high) mesocosms/tanks were constructed at the Hatfield Science Center in 2003 in order to examine juvenile chinook salmon behavior under controlled experimental conditions, focusing on:

1. Different substrate/habitat types

2. Ontogenetic effects – compare responses of chinook of differing ages/sizes.
3. Predation risk - assess changes in habitat preferences and behavior under risk of predation

The goal of this sub-project was to integrate results of field studies with direct observations of salmon-habitat associations in controlled scenarios to provide an overall picture of juvenile salmonid response to habitat modification. The specific goal was to assess how oyster culture affects juvenile salmon-habitat associations. The study focused on chinook (*Oncorhynchus tshawytscha*), which make extensive use of west coast estuaries as juveniles and are the most estuarine-dependent of the salmon species (Groot and Margolis 1991). The aim was to use wild juveniles of different size classes of outmigrating juvenile fall chinook, since behavioral patterns and estuarine experience may differ depending on ontogeny (e.g. Bottom et al. 2001).

The behavioral response of wild juvenile Chinook to a mock predation threat was tested for two size and age classes. In 2003, outmigrating wild fall chinook smolts were captured from the lower Salmon River (Oregon) in August. Smolts averaged 95.6 mm fork length (± 8.3 mm sd). In 2004, wild fall chinook captured in April from the Salmon R. averaged 51.4 mm (± 8.3 mm sd) in fork length. The fish were captured, acclimated to laboratory conditions, and then groups of three fish were exposed to three habitat types (open sand, eelgrass, and oysters) in each mesocosm. Eelgrass was harvested from Yaquina Bay and added to mesocosms at a density of 40 shoots per m^2. Longest blade for eelgrass shoots harvested in fall 2003 averaged 113.6 cm (± 36.2 cm), and mean longest blade lengths per shoot in spring 2004 were 40.9 cm (± 11.1 cm). Live oysters in the fall of 2003 were subtidally cultured 3.5 year olds, and those in 2004 were string cultured 2 year olds. A thin layer of sand covered the mesocosm bottom, and habitat types were randomly reconfigured before each experimental trial. Water depth within the mescosms was set at 55 cm. Three chinook were introduced into each mesocosm and, after approximately 20 hours, a model of a heron was introduced to assess the potential change in habitat preference due to perceived predation risk. The mock predator was presented to the juvenile Chinook for a duration of 60 seconds. The proportion of juvenile Chinook occupying each habitat before and after the mock predation threat was quantified using video cameras in 2003. Both video cameras and direct observation were used in 2004 due to the small size of the younger age class.

The proportion of chinook in each habitat type, before and after predator exposure, was analyzed using a generalized mixed effect model with random intercepts specific to each experimental group and a binomial response distribution. Means were fitted to individual observation periods by using treatment contrasts with the first observation period set as the reference level. In 2004, the small size of fish increased difficulty in detecting their location by video. Therefore, fish locations were documented by direct observation at the start of the heron introduction and at the end of the 60 second heron exposure duration. Additionally, fish locations were observed at the end of each experiment approximately 9 minutes after the heron test. Furthermore, for a subset of trials, fish locations were observed by video for one or two observation periods prior to the mock heron threat. However, not all video observation periods were standardized with respect to the time of the heron introduction and, as for the 2003 analysis, time is treated as an unordered factor with the first observation period set as the reference level.

Reported levels of significance are Bonferroni-corrected p-values based on t-tests of the model coefficients; the coefficient p-values are multiplied by one less than the number of observation periods included in each experiment. In 2003, 5.7% of all possible subsample observations (the number of individual fish at each observation period) were classified as unobserved. Model results were qualitatively unchanged by incorporating unobserved observations as a systematic mud, oyster, or eelgrass preference. Therefore, analyses are conservatively presented using a missing-at-random assumption of the unobserved juvenile Chinook.

Juvenile fall chinook in 2003 initially distributed randomly with respect to habitat (Figure 16). However, during exposure to the mock predator, juvenile Chinook were significantly less likely to be observed in mud than in structured habitat ($p = 0.007$). Within structured habitat, there was an increase in the proportion of juvenile Chinook chosing eelgrass over oyster during and shortly after exposure to the heron ($p < 0.005$). There was evidence for a moderate increased preference for eelgrass during the +11 minute observation period as well ($p = 0.03$). Smolts were again distributed randomly between substrate types approximately 16 minutes following the mock predator exposure. There was a clear and significant increased preference for structured habitat during the predation threat. In addition, evidence suggested that eelgrass preference was increased compared to oyster for a period of >10 minutes following the mock predation threat. These results suggested that juvenile Chinook prefer eelgrass as predator refugia compared to oyster or mudflat habitat.

The smaller age class of juvenile Chinook tested in the spring of 2004 suggested no evidence for a change in habitat preference either prior to or following the mock predation threat ($p > 0.19$, Figure 17). The difference in behavioral response to the mock predation threat may be the result of (1) seasonal changes in the length of eelgrass and the size of oyster clusters used during the experiment and/or (2) ontogenetic shift in predator avoidance behavior. The length of eelgrass used in the fall of 2003 was longer than in spring of 2004, while stem density was kept constant. In 2003, the long blades of eelgrass formed a canopy that perhaps created a preferable habitat for juvenile Chinook when confronted with an avian predator. Additionally, live oyster clusters in 2003 were of larger size than clusters used in the 2004 experiment. These differences between the two experiments may have been sufficient to diminish perceived habitat benefits for juvenile Chinook. Alternatively, the small size class of juvenile Chinook may have been less likely to respond to the predation threat with a change in habitat choice. Perhaps this behavioral difference stems from an ontogenetic shift in the perception of predation threats and/or the relative safety afforded by novel habitats.

Fish behavior – acoustic tagging in a large enclosure. In September of 2001 and July of 2002, we used passive acoustics to monitor the fine-scale spatial distribution and behavior of juvenile chinook salmon in Willapa Bay. A Radio Acoustic Positioning and Telemetry (RAPT) system was used in conjunction with large enclosures to resolve habitat preferences at a fine-scale. The RAPT acoustic receiver receives signals from acoustically tagged fish using 10 separate wide-beam hydrophones with overlapping beams. The received signals are synchronized to determine time of arrival for each detected pulse. The arrival time of the pulse at each hydrophone is then used to determine the location of the tag in three-dimensions. A large (~3000^2 m) area of intertidal habitat was enclosed using nets and fence posts. Among the habitat types included in the enclosure were spartina (*Spartina alterniflora*), native eelgrass (*Zostera marina*), non-native eelgrass (*Zostera japonica*), oysters (*Crassostrea gigas*), mudflat, and a sandy beach. Following enclosure set up, juvenile Chinook salmon (100-120 mm) were surgically implanted with acoustic tags and released into the enclosure. In September of 2001, shiner perch and staghorn sculpin were also tagged and released into the enclosure.

The tracks of the fish were overlaid on a habitat map of the enclosure developed from a high-resolution aerial photograph. Visual analyses of these tracks suggest that chinook salmon located structured eelgrass habitat within the enclosure although they spent a large proportion of the time close to the edge of the enclosure. The Animal Movement Analysis Arcview Extension is being used to analyze the data to determine habitat electivity, and to define the basic characteristics of behavior (e.g., rates of movement, turning radii, nocturnal behavior) (Hooge and Eichenlaub 1997).

Dungeness Crab – While the nursery role of complex littoral habitats especially oyster beds for young-of-the-year *C. magister* is well documented (Feldman et al 2000, Dumbauld et al. 2000), the ecology of subsequent age classes within coastal estuarine systems, and in intertidal areas in particular, is less clear. A second study funded in part by this WRAC project was designed to explore the potential energetic incentive for subadult crab to make intertidal migrations and to elucidate their migratory patterns in intertidal eelgrass, oyster, and unstructured habitats. We employed three sampling techniques (trapping, acoustic telemetry, and underwater video) in order to examine various aspects of migratory behavior. We also collected stomach samples from crabs migrating into and out of on-bottom oyster, eelgrass, and unstructured littoral areas during flood and ebb tides in order to examine tidal and habitat specific difference in the diet of migrating crabs.

Baywide trapping surveys were conducted in Willapa Bay in 2002-2004. Fifteen baited traps were placed on eelgrass, on-bottom oyster culture, and unstructured littoral habitats, at 5 sites across the bay. The traps were allowed to soak for 24 hr in 2002 and 12 hrs in 2004, and crabs were subsequent counted, sexed, and measured. In both years, we found significant differences ($P<0.05$) in catches of Dungeness crab, *C. magister* captured in different habitats with the highest catches in unstructured littoral areas and lowest catches in on-bottom oyster culture areas. In contrast we found that catches of rock crab *C. productus* within the same traps were highest in on-bottom oyster culture and lowest in unstructured littoral areas.

In the summer of 2002, thirteen male and female *C. magister* (~100 mm CW) were tagged and simultaneously released. Crabs were tracked manually with acoustic telemetry and their positions obtained on each tide (4 times daily) for the first week, and on each tidal maximum and minimum for an additional week (twice daily); at this point the tag batteries had expired or crabs had emigrated from the study location. We found evidence of tidal patterns in migrations, with crabs regularly undertaking intertidal migrations to littoral areas during nighttime high tides. Selectivity analysis revealed that crabs prefer both subtidal channels and unstructured littoral habitats during nighttime hightides and avoid highly structured habitats such as dense beds of on-bottom oyster culture, eelgrass, stake culture and *Spartina* habitats. Crabs repeatedly traveled more than 0.6 km (one way) into the intertidal, presumably to foraging areas.

Fyke or funnel shaped gates with a camera mounted at the constriction pointing downward were used to record the movement of crabs onto and off of littoral habitats. Observations revealed clear tidal patterns in movement, with the largest migrations corresponding to large tidal exchanges. The magnitude of crab migrations and direction were strongly correlated with water velocity and direction, thus crabs appear to be using water movement over the flats as a cue for tidal migrations. No red rock crabs were observed migrating through our video gates, suggesting that they may be resident within oyster habitats, remaining in littoral areas during periods of tidal exposure.

Finally habitat specific stomach samples were collected from over 300 Dungeness crab in the summer of 2004 Preliminary results indicate that stomach fullness is greatest in crabs collected during ebb tides, and stomachs collected from crabs moving into littoral habitats are often empty. These initial results suggest that crabs are moving into littoral habitats to forage on prey species resident therein.

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SUBMITTED BY:____
Work Group Chair Date

APPROVED:____
Technical Advisor Date

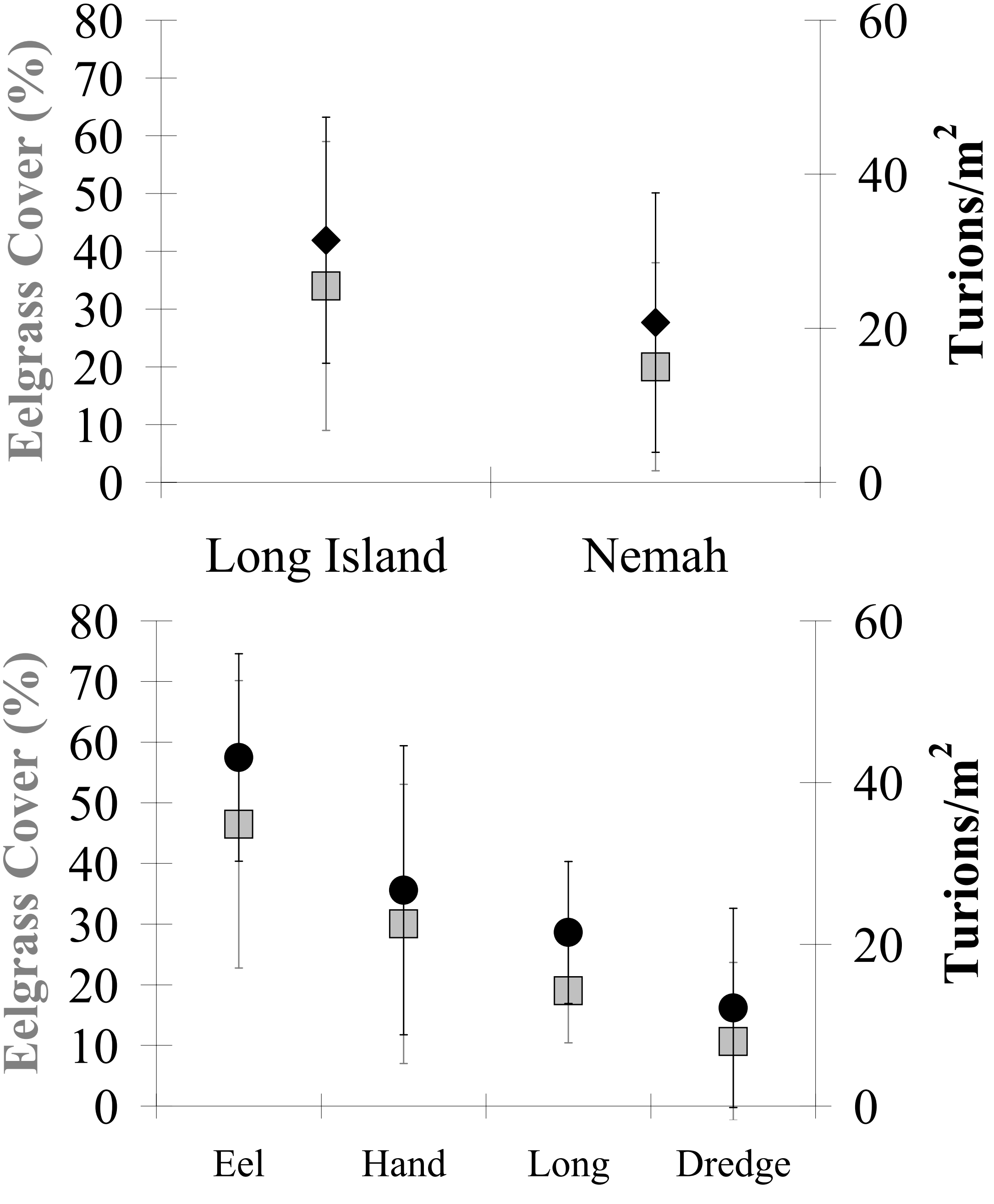


Figure 1. Eelgrass density in May 2004 varied among sites (a) and oyster culture types (b). All cultured areas (Hand-picked, Long lines, Dredged) had lower eelgrass density than uncultured areas (Eel).

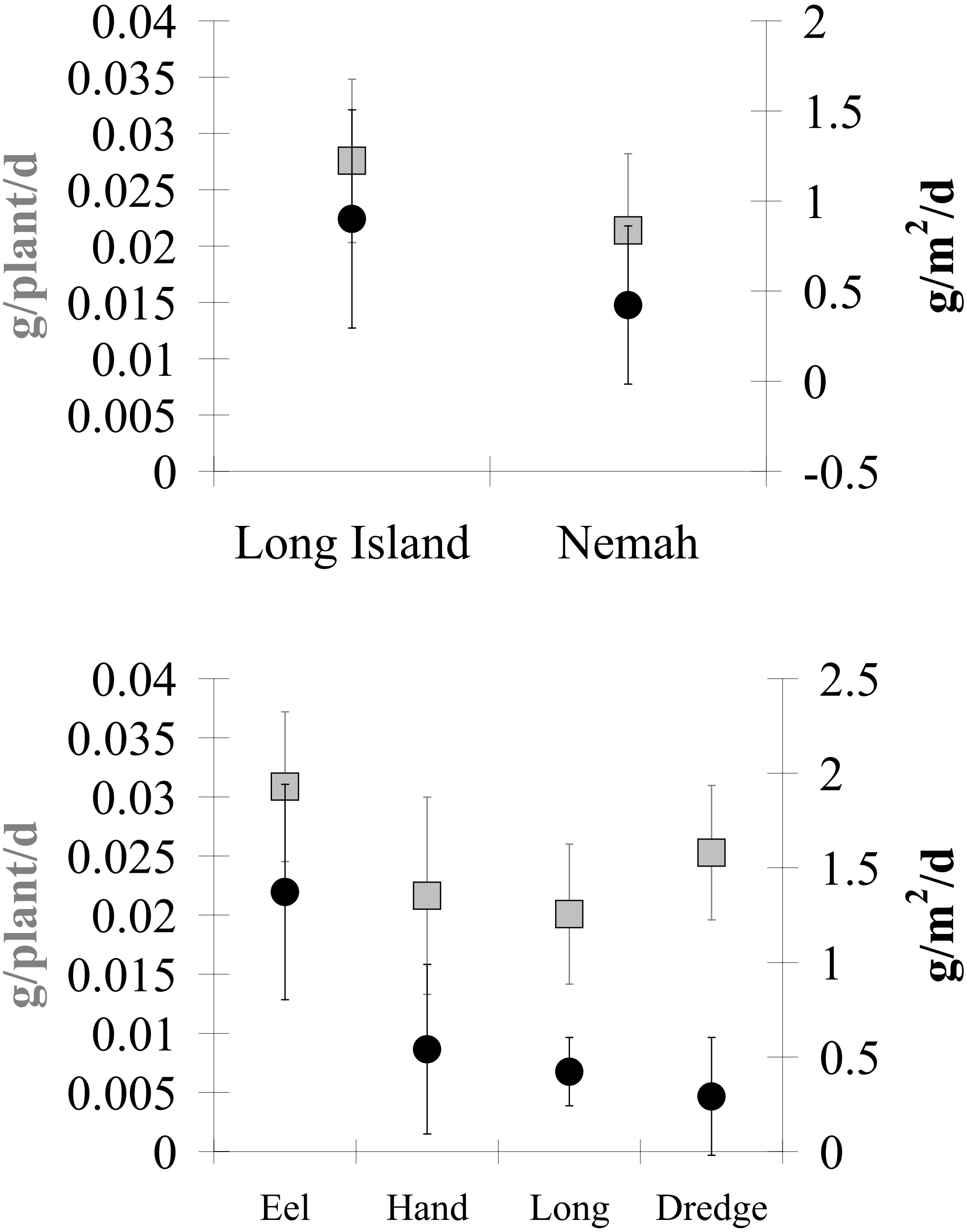


Figure 2. Eelgrass growth varied among sites (a) and oyster culture types (b). Individual plant growth rates (g/plant/d) were lowest in long line culture, while aerial productivity (a combination of density and growth measures, $g/m^2/d$) was lowest in dredged culture.

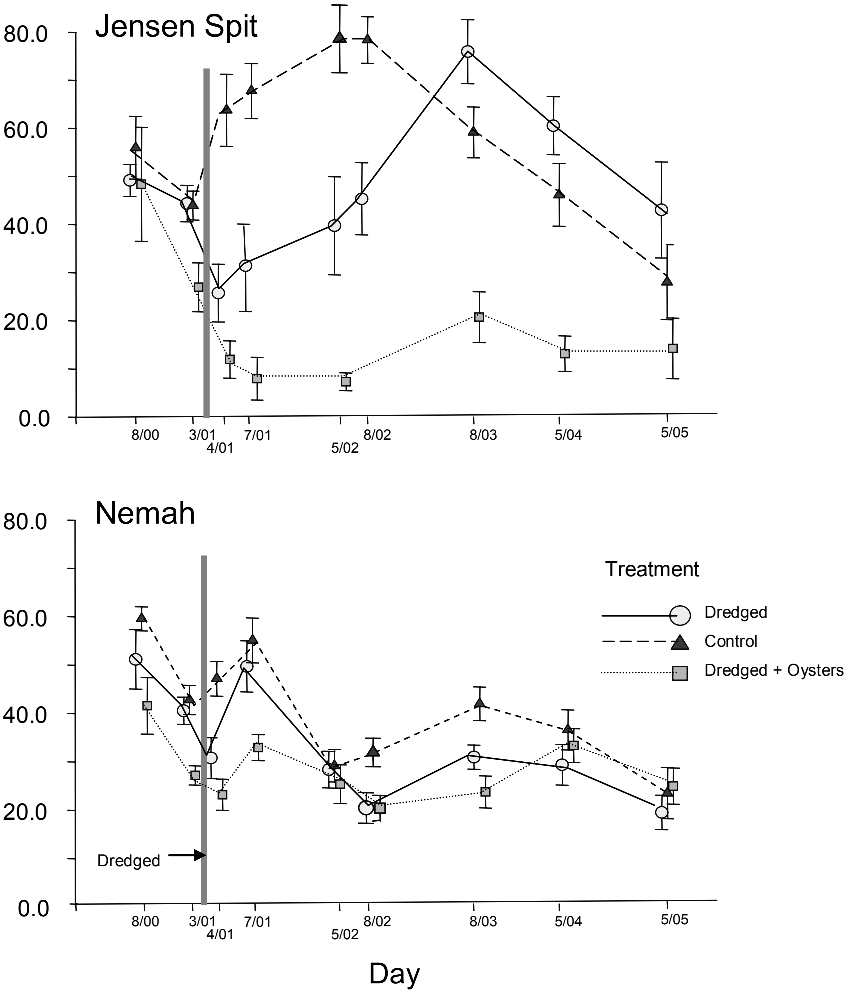


Figure 3. Eelgrass (*Zostera marina*) stem density measurements taken from Aug 2000 – May 2005 at two experimental sites in Willapa Bay, Washington where a harvest dredge experiment was conducted in March 2001. Note decline in eelgrass density associated with the harvest disturbance when compared with the control plot, fairly rapid recovery of the eelgrass treatment at the Nemah location, and slower recovery at the Jensen Spit site. Bars represent standard error.

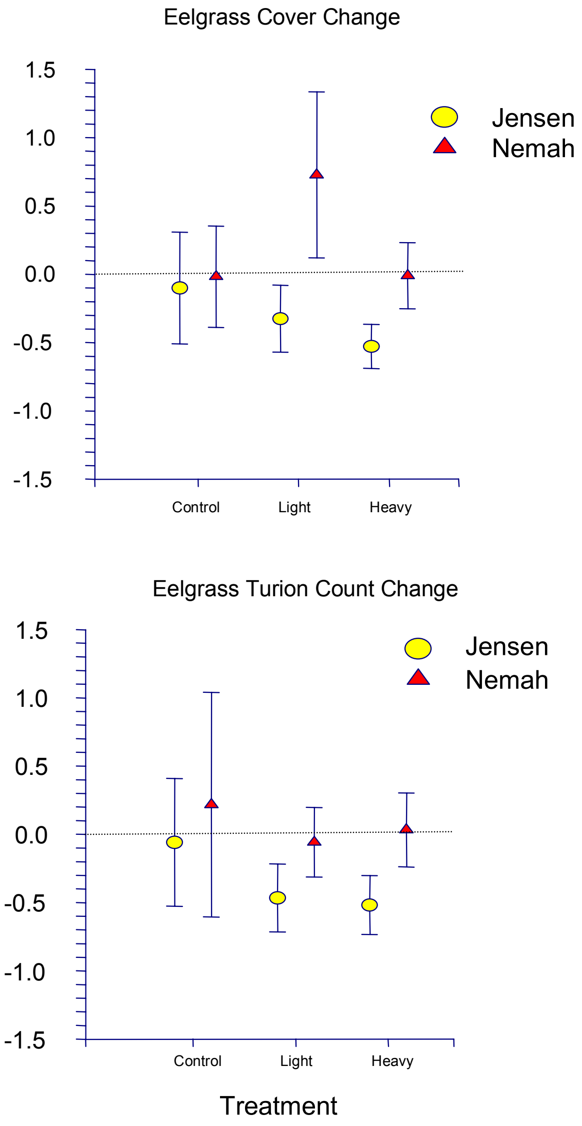


Figure 4. Results of oyster addition experiment. Oysters were added to plots located within treated (harvest dredge) and untreated (no harvest) areas at two locations in 2002. Oysters were added at two rates (light = 150 bags seed/acre, heavy = 300 bags seed/acre). A trend towards reduced eelgrass cover and density was observed at the Jensen Spit location, but not at the Nemah location (results from June 2005 at end of experiment).

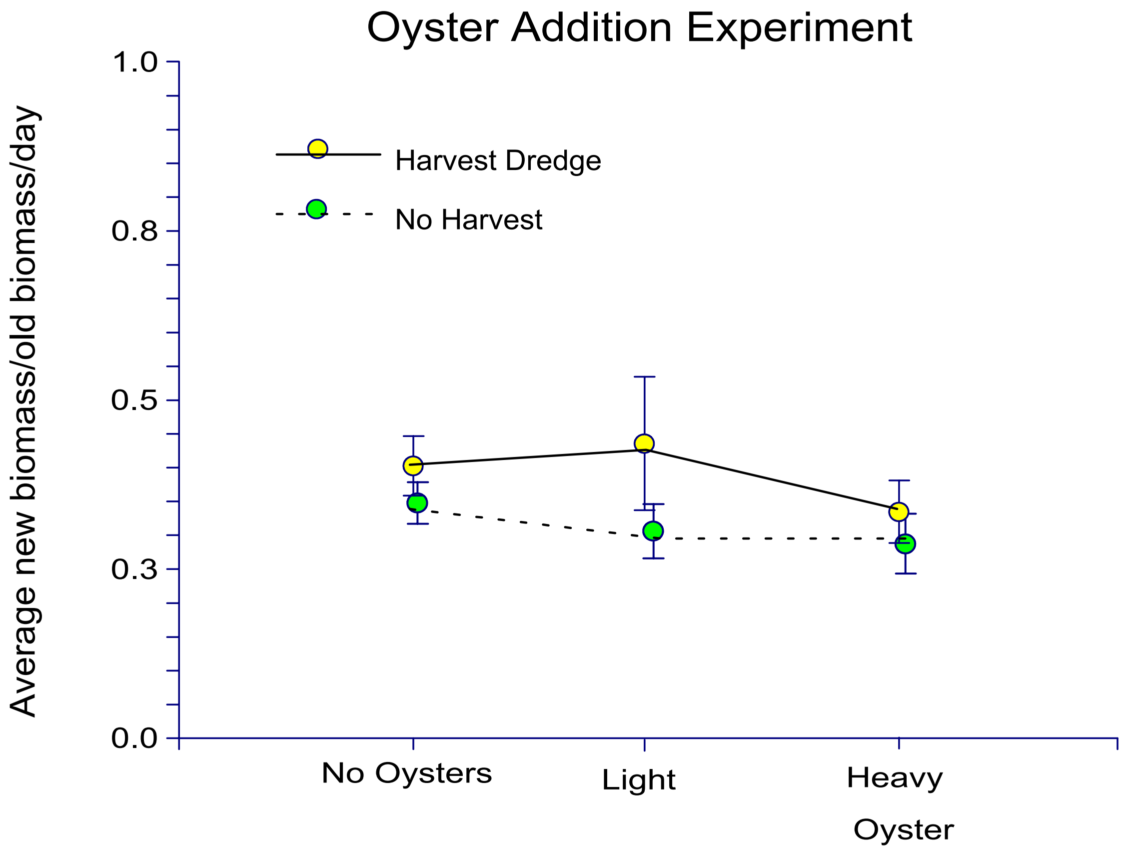


Figure 5. Results of oyster addition experiment. Oysters were added to plots located within treated (harvest dredge) and untreated (no harvest) areas at two locations in 2002. Oysters were added at two rates (light = 150 bags seed/acre, heavy = 300 bags seed/acre). Average growth measured over several days by punching holes in the blades (here represented as an average daily gain in biomass corrected by plant size by measuring old biomass) was not significantly affected by the presence of oysters.

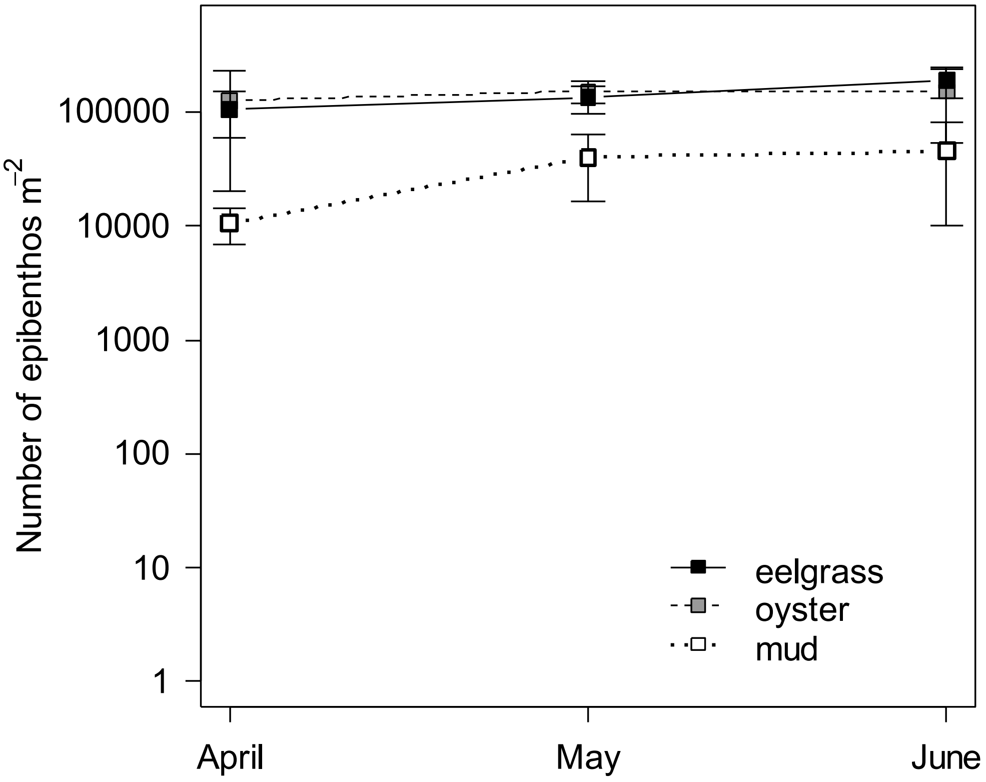


Figure 6. Densities of epibenthic meiofauna sampled in three habitats in Willapa Bay (m^{-2}; mean ± SE). Note the logarithmic scale. Meoifauna abundance was almost an order of magnitude higher over structured habitats than open mud.

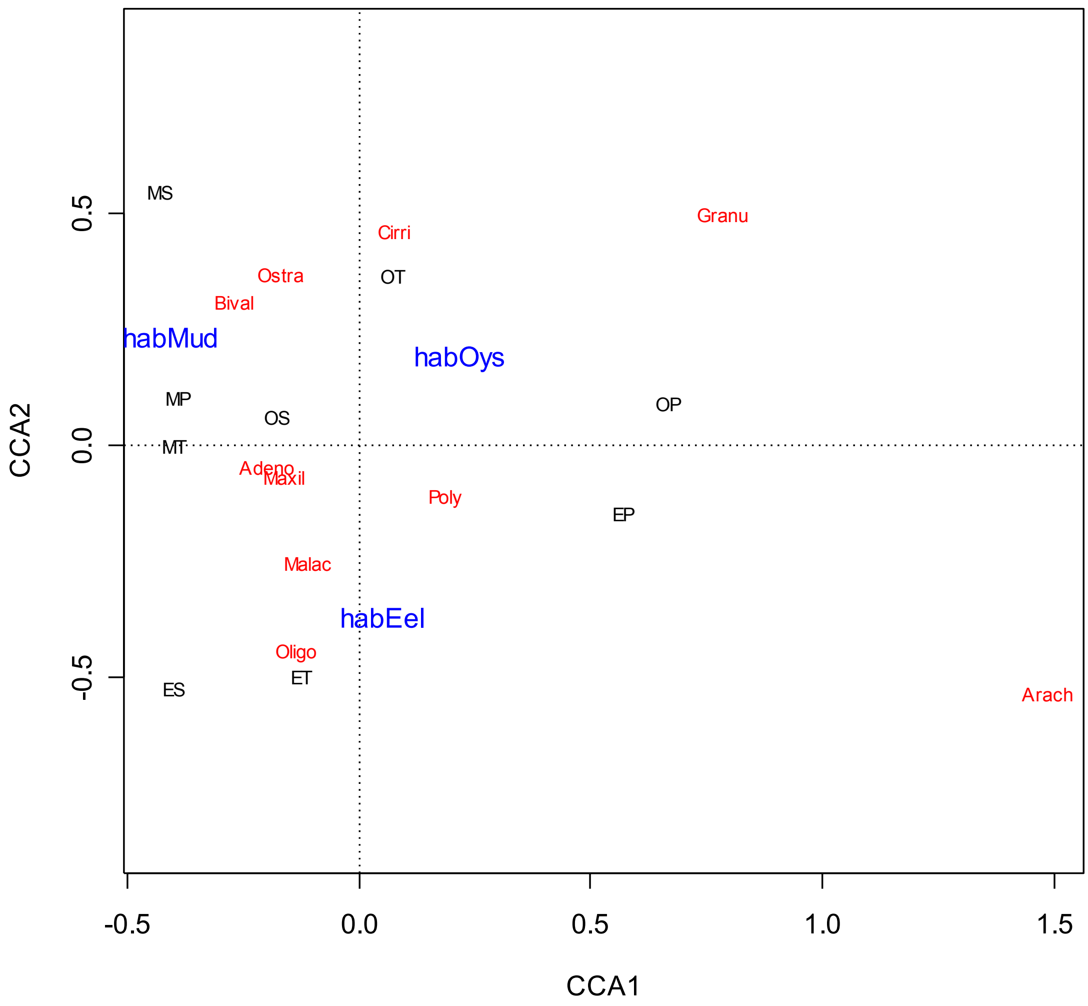
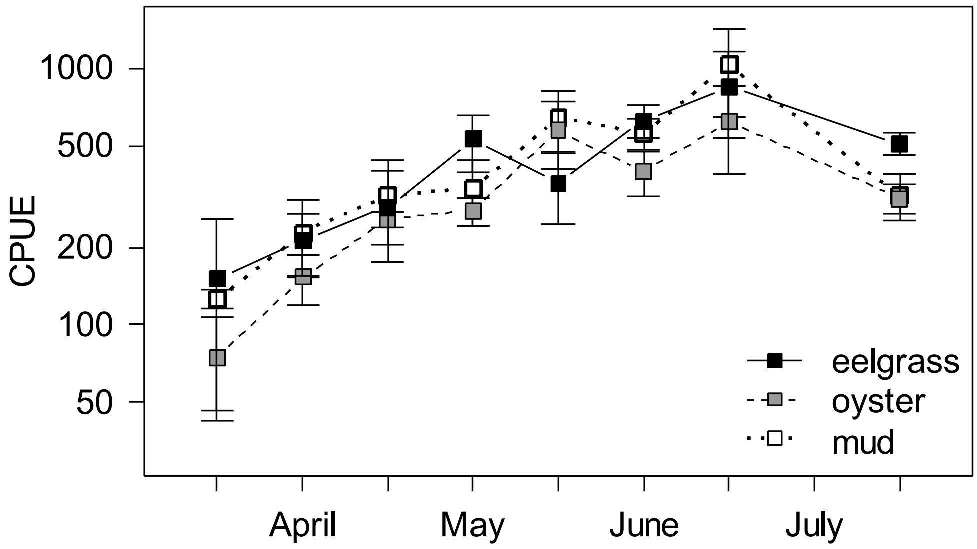


Figure 7. CCA biplot based on ln(x+1) transformed densities of epibenthic meiofauna. Species and site scores are scaled by the square root of their eigenvalues. Taxa are identified by the first five letters of their taxonomic class (see Table 3). Sites are identified by habitat (first letter; Eelgrass, Mudflat, Oyster) and location (second letter; Toke Point, Stony Point, Pine Island).



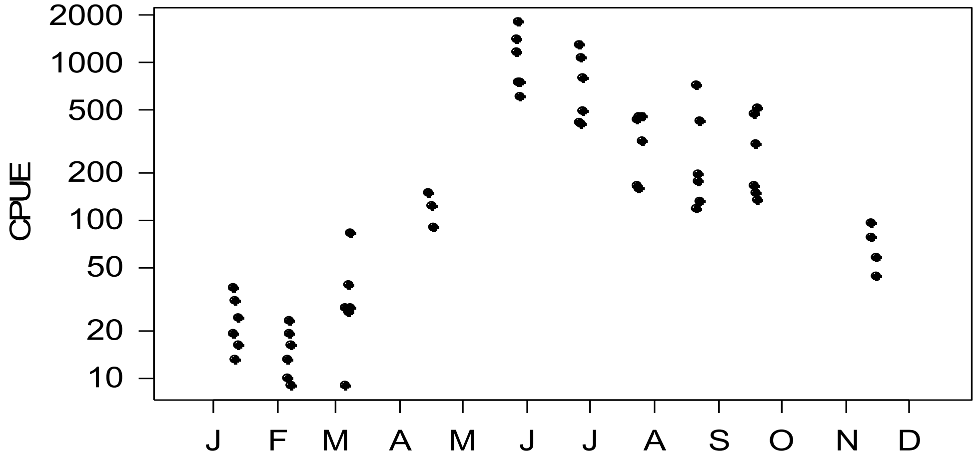


Figure 8. Means (±SE) of nekton catch per unit effort in fyke nets for each habitat during spring 2001 (top) and time series of fish and crab abundance from two sites deployed at +0.5m MLLW in mixed eelgrass and oyster habitat located off the WDFW lab in Nahcotta, Washington, 2001 (bottom). Note the logarithmic scale and seasonal peak in catch during June and July.

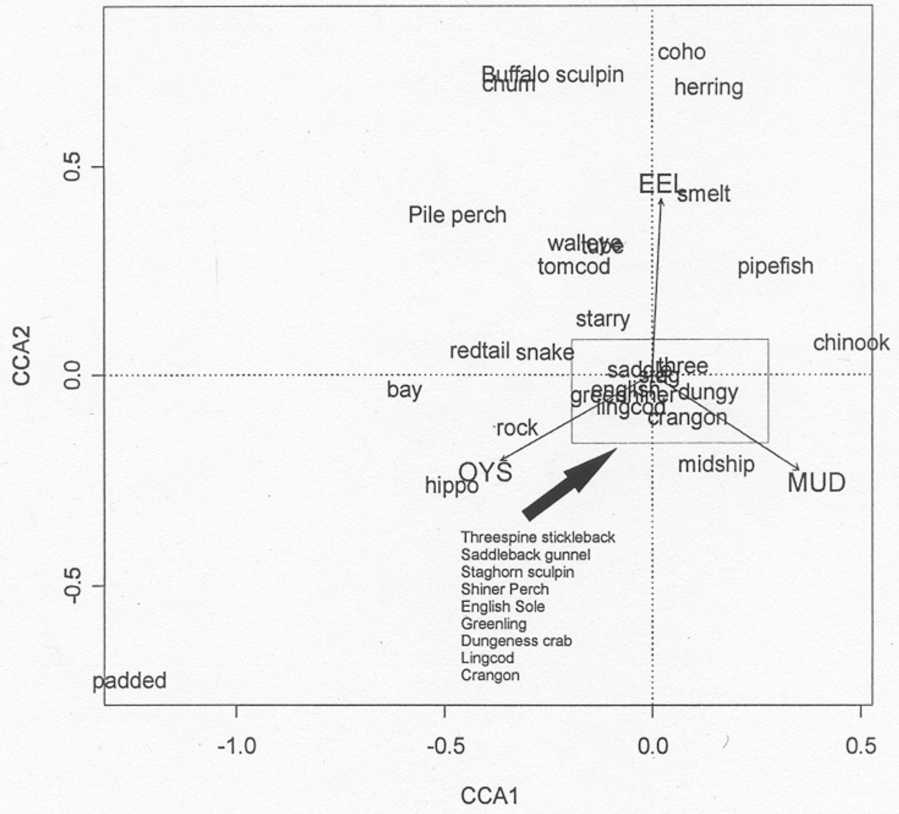


Figure 9. Canonical correspondence analysis biplot of nekton catch per unit effort in fyke nets placed over three habitats (oyster, eelgrass, open mud) in 2001. Species plotted close to habitat vectors are positively associated with that habitat.

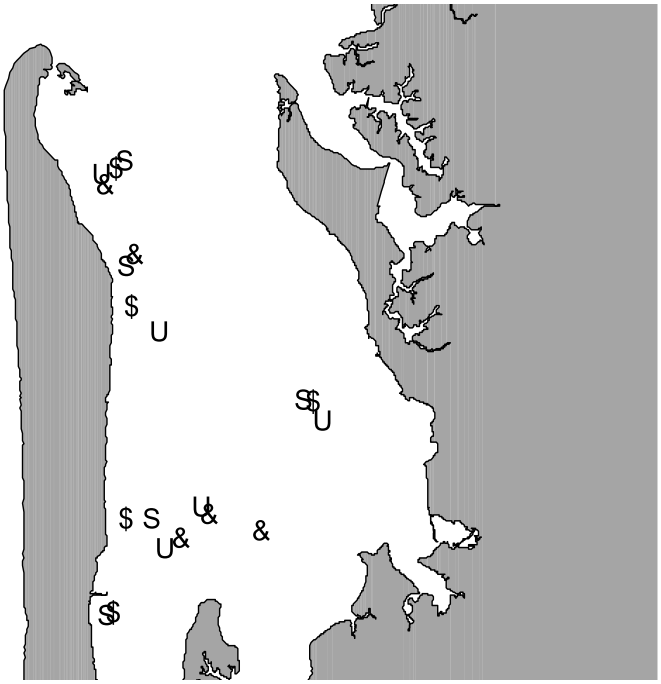


Figure 10. Tow net sampling sites in Willapa Bay for 2002 and 2003; *eelgrass* = open squares, *channel* = open circles, *oyster* = filled pentagons, *mudflat* = filled triangles. The three clusters of points above were grouped by region (from north to south) as Stackpole, Nemah, and Long Island.

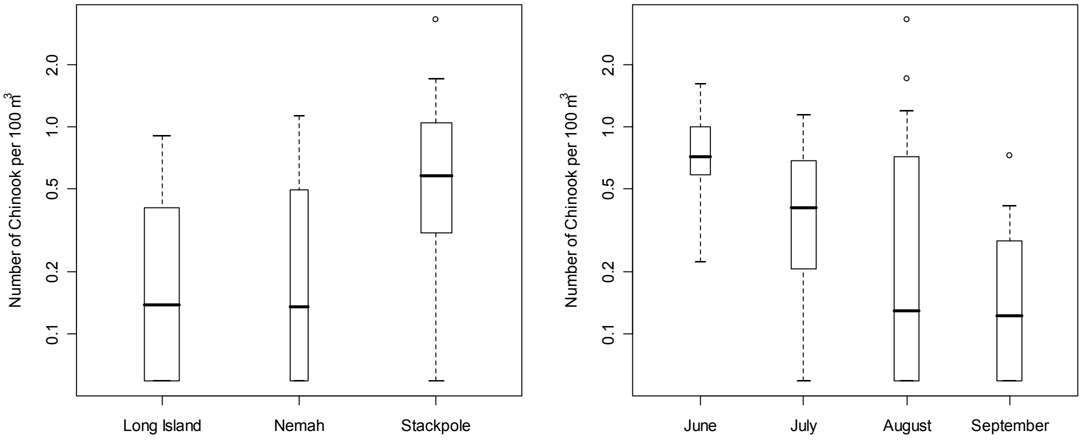


Figure 11. Densities of Chinook from 2002. Solid dark lines indicate medians, boxes represent approximately the 1st and 3rd quartiles, and horizontal lines represent the most extreme outlying observation falling within 1.5 times the interquartile distance from the median; outliers beyond this range are represented as points. Box widths are proportional to the square root of the corresponding sample size.

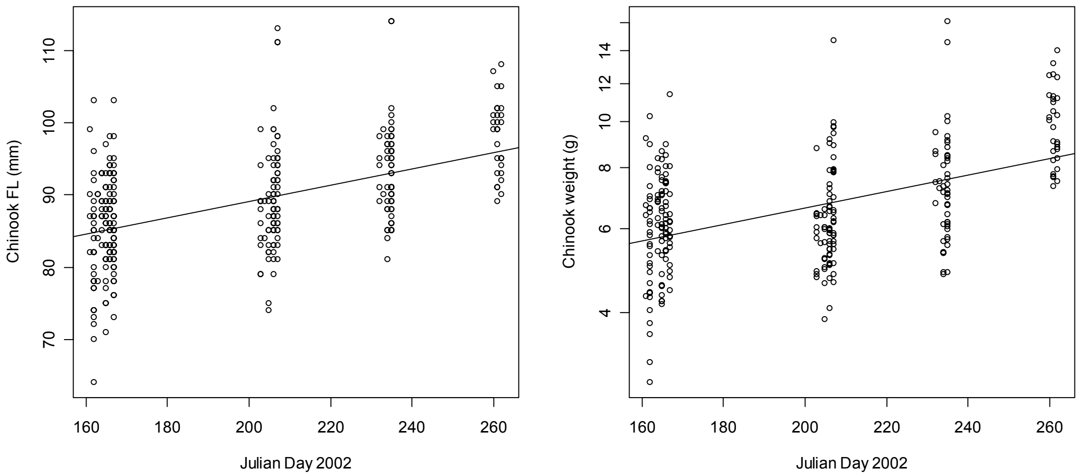


Figure 12. Length and weight of juvenile Chinook from Willapa Bay, 2002. Trend lines correspond to estimates from the best fitting linear models.

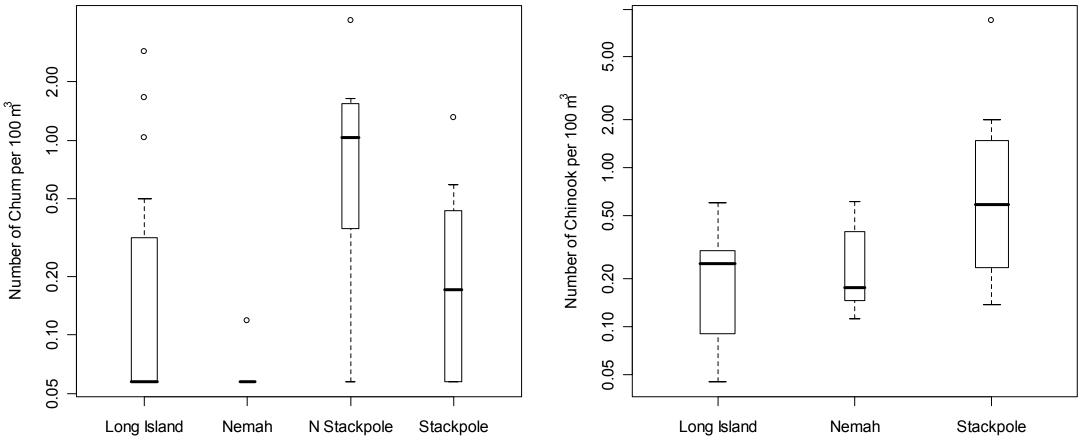


Figure 13. Densities of chum and Chinook from 2003. Solid dark lines indicate medians, boxes represent approximately the 1st and 3rd quartiles, and horizontal lines represent the most extreme outlying observation falling within 1.5 times the interquartile distance from the median; outliers beyond this range are represented as points. Box widths are proportional to the square root of the corresponding sample size.

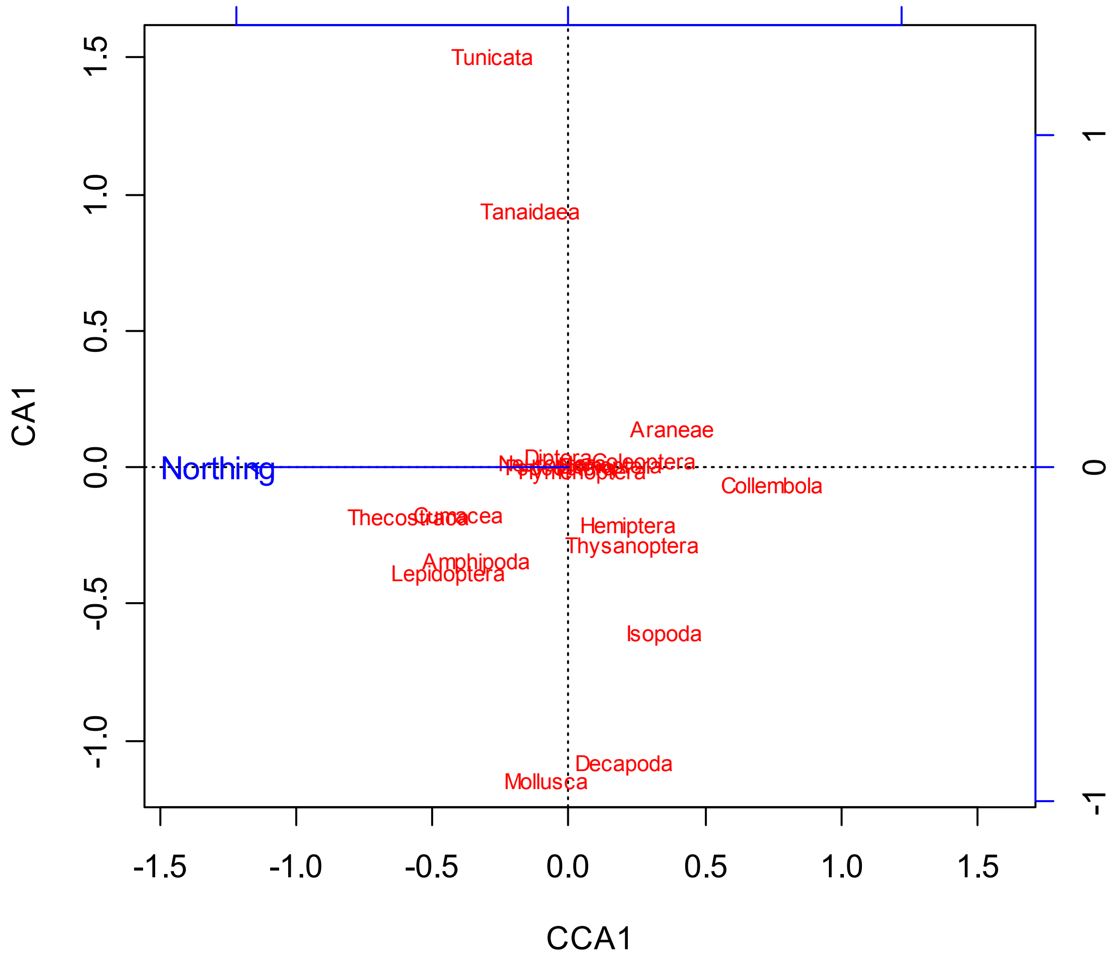


Figure 14. Species composition of juvenile Chinook diets sampled from Willapa Bay, 2003, was weakly correlated with latitude. Canonical Correspondence Analysis showed that the relative abundances of the orders Amphipoda, Cumacea, Lepidoptera, and Thecostraca were greater for northern sites compared to other taxa, whereas Collembola relative abundances were higher at southern sites.

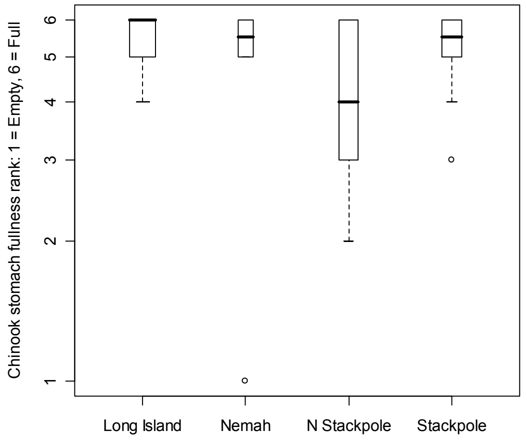


Figure 15. Stomach fullness indices for juvenile Chinook, July, 2003. Solid dark lines indicate medians, boxes represent approximately the 1st and 3rd quartiles, and horizontal lines represent the most extreme outlying observation falling within 1.5 times the interquartile distance from the median; outliers beyond this range are represented as points. Box widths are proportional to the square root of the corresponding sample size.

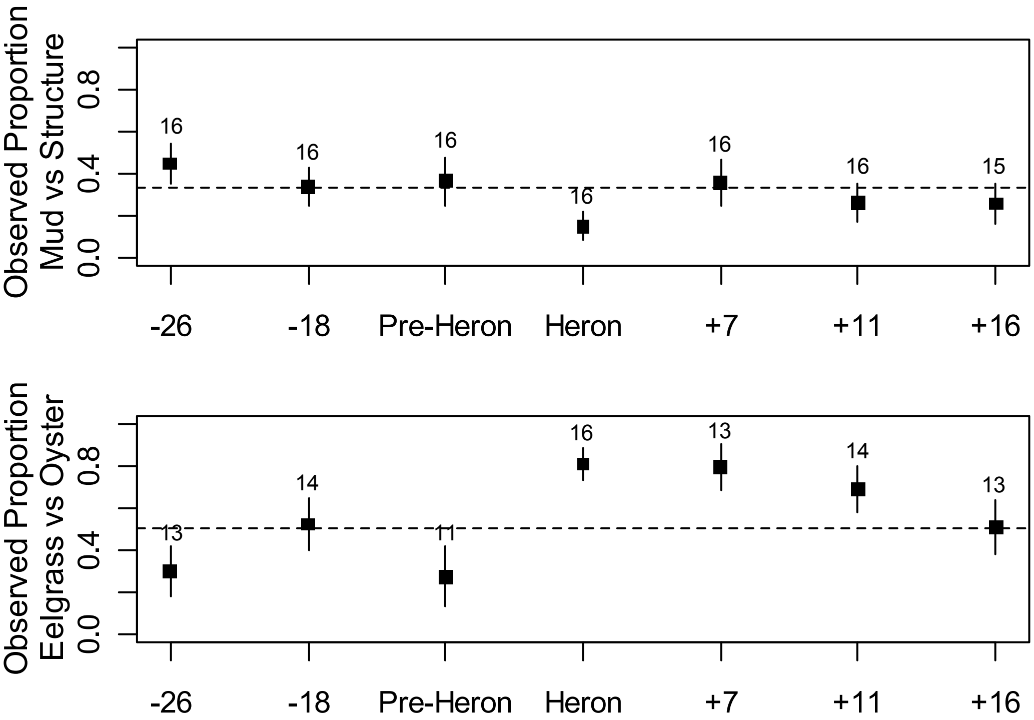


Figure 16. Mean observed proportions of Chinook by observation period (± SE) where preference for eelgrass, oyster, and mud was tested simultaneously. Mean time in minutes from the mock heron introduction is shown for each observation period. The "Pre-Heron" observation period occurred immediately before the mock heron introduction. Dotted lines show the expected proportion if juvenile Chinook were distributed randomly between habitats. *Top:* Proportion observed in mud habitat versus the two structured habitats eelgrass and oyster. *Bottom:* For fish choosing structured habitat, the proportion observed in eelgrass versus oyster. The number of occurrences where fish were observed is shown above each sample mean. The x-axis is not drawn to scale.

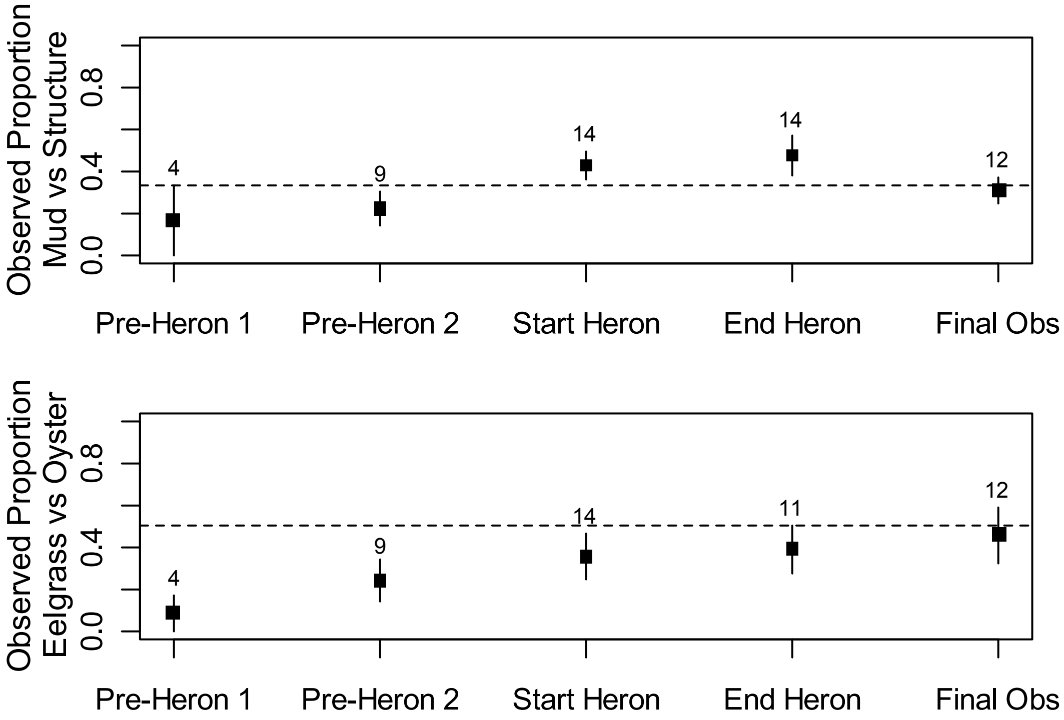


Figure 17. Mean observed proportions of Chinook by observation period (± SE) where preference for eelgrass, oyster, and mud was tested simultaneously. Dotted lines show the expected proportion if juvenile Chinook were distributed randomly between habitats. *Top:* Proportion observed in mud habitat versus the two structured habitats eelgrass and oyster. *Bottom:* For fish choosing structured habitat, the proportion observed in eelgrass versus oyster. The number of occurrences where fish were observed is shown above each sample mean. The x-axis is not drawn to scale.

Table 1. Epibenthic meiofauna densities. Values are based on site means derived from the average of subsamples taken during three spring tide series in late April, late May, late June, 2001, in northern Willapa Bay, Washington.

Identified taxa	Class	**Eelgrass** mean ± sd *n* = 3	**Mud** mean ± sd *n* = 3	**Oyster** mean ± sd *n* = 3
Adenophora	Adenophora	32,223.66 ± 8,875.04	7,955.38 ± 4,094.55	39,058.85 ± 16,136.73
Acarina	Arachnida	410.50 ± 301.56	17.61 ± 10.26	531.16 ± 542.28
Bivalvia	Bivalvia	591.21 ± 216.20	395.40 ± 44.45	695.85 ± 619.60
barnacle cyprid	Cirripedia	104.61 ± 72.13	74.89 ± 60.10	229.12 ± 90.81
barnacle nauplii	Cirripedia	1,000.56 ± 980.89	338.23 ± 254.13	472.87 ± 260.01
Foraminifera	Granuloreticulosea	917.71 ± 1,070.85	130.96 ± 112.92	3,741.85 ± 1,493.86
Collembola	Insecta	18.18 ± 1.04	5.11 ± 3.40	22.97 ± 38.02
Caprellidae	Malacostraca	66.90 ± 15.75	1.47 ± 2.55	31.85 ± 18.61
Corophium spp.	Malacostraca	24.18 ± 19.20	0.74 ± 1.28	66.27 ± 43.61
Photis spp.	Malacostraca	991.95 ± 1,321.88	9.48 ± 1.38	115.46 ± 113.06
Pontogenia rostrata	Malacostraca	138.16 ± 62.30	2.21 ± 2.21	2.28 ± 2.40
other Amphipoda	Malacostraca	16.62 ± 21.82	1.47 ± 2.55	0.69 ± 1.19
Cumella vulgaris	Malacostraca	4,325.08 ± 2,845.37	1,393.17 ± 629.19	2,278.37 ± 645.62
Nippoleucon hinumensis	Malacostraca	86.98 ± 59.79	22.77 ± 28.15	250.11 ± 366.37
other Cumacea	Malacostraca	23.40 ± 21.62	66.81 ± 58.40	69.03 ± 71.12
Leptochelia dubia	Malacostraca	542.32 ± 451.14	38.98 ± 23.71	1,148.17 ± 695.57
other Tanaidacea	Malacostraca	7.72 ± 9.53	0.71 ± 1.23	2.95 ± 5.11
Eurytemora spp.	Maxillipoda	1,392.63 ± 670.59	273.37 ± 51.04	875.43 ± 809.83
other Callanoida	Maxillipoda	2,876.61 ± 976.32	1,461.55 ± 438.65	2,512.68 ± 1,173.84
Cyclopoida	Maxillipoda	30.18 ± 38.46	0.00 ± 0.00	23.23 ± 14.69
Harpacticoida	Maxillipoda	77,751.96 ± 27,265.73	12,723.89 ± 7,717.30	68,360.35 ± 42,041.38
Poecilostomatoida	Maxillipoda	933.08 ± 330.12	340.18 ± 170.95	1,242.74 ± 831.80
Copepoda nauplii	Maxillipoda	1,492.77 ± 426.30	152.62 ± 138.95	207.96 ± 38.46
Oligochaeta	Oligochaeta	2,158.10 ± 1,079.11	656.90 ± 30.58	1,479.00 ± 1,278.80
Ostracoda	Ostracoda	1,178.72 ± 581.09	2,357.45 ± 3,725.46	4,786.76 ± 1,195.80
Polychaeta	Polychaeta	5,709.93 ± 4,528.78	833.37 ± 655.62	6,493.69 ± 4,443.54
Total density		139,961.90 ± 32,048.13	30,088.10 ± 16,078.99	140,661.30 ± 68,952.41

Table 2. Nekton CPUE based on site means.

Species Common name (*Scientific name*)	**Eelgrass** mean ± se *n* = 3	**Mud** mean ± se *n* = 3	**Oyster** mean ± se *n* = 3
American shad (*Alosa sapidissima*)*	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04
arrow goby (*Clevelandia ios*)*	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00
bay goby (*Lepidogobius lepidus*)	0.42 ± 0.36	0.08 ± 0.04	0.96 ± 0.46
bay pipefish (*Syngnathus griseolineatus*)	0.42 ± 0.18	0.29 ± 0.04	0.13 ± 0.07
buffalo sculpin (*Enophrys bison*)	0.21 ± 0.04	0.00 ± 0.00	0.08 ± 0.04
cabezon (*Scorpaenichthys marmoratus*)	0.04 ± 0.04	0.00 ± 0.00	0.13 ± 0.13
chinook (*Oncorhynchus tshawytscha*)	0.54 ± 0.36	0.75 ± 0.47	0.08 ± 0.04
chum (*Oncorhynchus keta*)	0.08 ± 0.04	0.00 ± 0.00	0.04 ± 0.04
coho (*Oncorhynchus kisutch*)	0.88 ± 0.13	0.08 ± 0.04	0.13 ± 0.13
crangonid shrimp (*Crangon* spp.)	42.63 ± 20.28	62.38 ± 27.44	34.54 ± 11.47
crescent gunnel (*Pholis laeta*)	0.00 ± 0.00	0.08 ± 0.08	0.00 ± 0.00
cutthroat (*Oncorhynchus clarki clarki*)*	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00
Dungeness crab (*Cancer magister*)	54.92 ± 22.55	154.71 ± 134.19	37.17 ± 13.28
English sole (*Parophrys vetulus*)	25.58 ± 8.62	25.54 ± 20.51	37.54 ± 17.16
ghost shrimp (*Neotrypaea californiensis*)*	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00
graceful crab (*Cancer gracilis*)*	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00
hermit crab (Paguroidea)	0.17 ± 0.08	0.13 ± 0.13	0.13 ± 0.07
hippolytid shrimp (Hippolytidae)	0.08 ± 0.08	0.04 ± 0.04	0.21 ± 0.15
kelp greenling (*Hexagrammos decagrammus*)	2.08 ± 1.10	1.00 ± 0.54	1.83 ± 0.86
lingcod (*Ophiodon elongatus*)	4.17 ± 2.55	2.50 ± 0.25	5.25 ± 0.64
midshipman (*Porichthys notatus*)	0.38 ± 0.26	0.83 ± 0.11	1.08 ± 0.72
northern anchovy (*Engraulis mordax mordax*)	0.17 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
northern kelp crab (*Pugettia producta*)*	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04
Pacific herring (*Clupea harengus pallasi*)	4.42 ± 3.92	0.13 ± 0.07	0.08 ± 0.04
Pacific staghorn sculpin (*Leptocottus armatus*)	123.63 ± 20.22	71.42 ± 13.18	91.71 ± 21.26
padded sculpin (*Artedius fenestralis*)	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.00
pile perch (*Rhacochilus vacca*)	0.17 ± 0.11	0.00 ± 0.00	0.17 ± 0.11
pygmy rock crab (*Cancer oregonensis*)*	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
red rock crab (*Cancer productus*)	0.92 ± 0.44	0.67 ± 0.30	1.88 ± 0.63
redtail perch (*Amphistichus rhodoterus*)	0.21 ± 0.21	0.04 ± 0.04	0.33 ± 0.33
saddleback gunnel (*Pholis ornata*)	11.58 ± 3.59	7.96 ± 1.92	9.75 ± 2.55
sandab (Bothidae)	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.04
shiner perch (*Cymatogaster aggregata*)	70.29 ± 19.07	43.58 ± 12.35	65.58 ± 8.19
snailfish (Cyclopteridae)*	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04
snake prickleback (*Lumpenus sagitta*)	2.88 ± 2.38	0.75 ± 0.63	3.21 ± 1.48
starry flounder (*Platichthys stellatus*)	0.33 ± 0.15	0.17 ± 0.04	0.29 ± 0.04
surf smelt (*Hypomesus pretiosus*)	1.96 ± 1.83	0.17 ± 0.04	0.13 ± 0.13
threespine stickleback (*Gasterosteus aculeatus*)	90.08 ± 48.88	73.33 ± 38.90	40.46 ± 11.02
tomcod (*Microgadus proximus*)	0.29 ± 0.23	0.08 ± 0.08	0.21 ± 0.21
tube snout (*Aulorhynchus flavidus*)	2.13 ± 0.47	0.54 ± 0.25	1.04 ± 0.11
walleye perch (*Hyperprosopon argenteum*)	0.17 ± 0.11	0.04 ± 0.04	0.08 ± 0.04
unidentified cottid (Cottidae)*	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04

Table 3. Percent occurrence, percent numerical counts, percent wet weight, and percent Index of Relative Importance (IRI) for juvenile Chinook, chum, and coho outmigrants, Willapa Bay, 2003.

	Chinook				Chum				Coho			
	n = 47				n = 50				n = 16			
Prey Taxa	% O	% N	% W	% IRI	% O	% N	% W	% IRI	% O	% N	% W	% IRI
Acarina	0.2	0.0	0.0	**0.0**	1.3	0.1	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Adenphorea	0.2	0.1	0.0	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Amphipoda	7.7	4.1	5.7	**4.0**	6.0	0.7	5.5	**1.5**	10.3	18.8	2.4	**5.8**
Araneae	5.0	0.5	1.2	**0.4**	0.9	0.0	0.2	**0.0**	0.0	0.0	0.0	**0.0**
Calanoida	0.0	0.0	0.0	**0.0**	9.4	1.8	1.5	**1.2**	0.0	0.0	0.0	**0.0**
Coleoptera	8.2	2.4	6.0	**3.6**	0.0	0.0	0.0	**0.0**	5.1	1.0	0.0	**0.1**
Collembola	2.2	0.2	0.0	**0.0**	0.9	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Cumacea	1.2	0.1	0.0	**0.0**	8.1	0.5	2.5	**1.0**	5.1	7.7	0.6	**1.1**
Decapoda	1.2	0.3	2.9	**0.2**	3.0	0.2	0.2	**0.0**	25.6	41.1	44.7	**58.1**
Dermaptera	0.2	0.1	3.5	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Diplostraca	0.0	0.0	0.0	**0.0**	0.9	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Diptera	11.1	62.3	18.3	**46.8**	17.9	8.5	33.2	**29.6**	15.4	8.2	0.3	**3.5**
Euphausiacea	0.0	0.0	0.0	**0.0**	0.4	0.0	0.2	**0.0**	0.0	0.0	0.0	**0.0**
Fish	0.0	0.0	0.0	**0.0**	3.0	0.2	13.8	**1.7**	17.9	6.3	51.9	**27.6**
Harpacticoida	0.0	0.0	0.0	**0.0**	12.3	9.3	6.5	**7.7**	0.0	0.0	0.0	**0.0**
Hemiptera	8.4	1.5	5.3	**3.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Homoptera	9.6	3.0	8.4	**5.8**	6.0	1.7	6.7	**2.0**	0.0	0.0	0.0	**0.0**
Hymenoptera	10.6	7.4	40.0	**26.4**	0.4	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Isopoda	2.4	0.3	0.2	**0.1**	0.0	0.0	0.0	**0.0**	2.6	0.5	0.0	**0.0**
Larvacea	0.0	0.0	0.0	**0.0**	13.6	72.2	24.1	**52.1**	0.0	0.0	0.0	**0.0**
Lepidoptera	1.2	0.1	1.8	**0.1**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Mollusca	0.7	0.1	0.2	**0.0**	0.4	0.0	0.0	**0.0**	2.6	0.5	0.0	**0.0**
Mysida	0.0	0.0	0.0	**0.0**	0.9	0.2	3.1	**0.1**	0.0	0.0	0.0	**0.0**
Neuroptera	1.4	0.1	0.1	**0.0**	0.4	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Odonata	0.2	0.0	0.4	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Oligochaeta	0.2	0.0	0.1	**0.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Poecilostomatoida	0.0	0.0	0.0	**0.0**	0.9	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Psocoptera	11.1	7.4	4.2	**6.8**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Tanaidaea	1.7	0.2	0.1	**0.0**	0.4	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Thecostraca	5.0	1.6	0.5	**0.5**	11.5	4.5	2.3	**3.1**	5.1	4.3	0.0	**0.6**
Thysanoptera	6.5	2.6	0.2	**1.0**	0.4	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Trematoda	0.0	0.0	0.0	**0.0**	1.3	0.1	0.0	**0.0**	10.3	11.6	0.0	**3.1**
Tunicata	3.6	5.7	0.9	**1.3**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**

Table 4. Percent numerical counts for all neuston tows taken concurrently with fish sampling tows, Willapa Bay, 2003.

Neuston

Taxa	% N
Acarina	0.8
Adenophorea	14.1
Amphipoda	0.4
Araneae	< 0.1
Bivalvia	< 0.1
Calanoida	61.2
Chaetognatha	0.2
Coleoptera	< 0.1
Collembola	< 0.1
Cumacea	0.1
Cyclopoida	0.1
Decapoda	2.2
Diplostraca	1.9
Diptera	0.7
Fish	0.2
Foraminiferida	1.7
Gastropoda	< 0.1
Harpacticoida	6.5
Hemiptera	< 0.1
Homoptera	< 0.1
Hydroida	< 0.1
Hymenoptera	< 0.1
Isopoda	< 0.1
Kinorhyncha	< 0.1
Larvacea	0.6
Ostracoda	0.1
Phoronida	< 0.1
Phthiraptera	< 0.1
Poecilastomatoida	< 0.1
Polychaeta	1.8
Psocoptera	< 0.1
Pycnogonida	< 0.1
Scyphozoa	0.3
Tanaidacea	< 0.1
Theocostraca	6.8
Thysanoptera	< 0.1