Nearshore larval fishes of Puget Sound

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Abstract

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Puget Sound is home to about 220 fish species that produce pelagic larvae, however little is known about their early life history. Larval fishes were collected from the six basins (Rosario, Whidbey, Admiralty, Central, Hood Canal, South) that comprise Puget Sound's nearshore habitat from April 2011 to February 2012 to explore distribution and abundance patterns within the region. This study marks the most extensive collection of larval fishes to date within Puget Sound both spatially and temporally. A total of 9,317 specimens from 28 families and 71 species were collected. Abundance peaked in spring, particularly April, was lower during summer months, was close to zero during winter, and rose again in early spring. Species counts followed similar seasonal trends. Estimated species richness was above 40 species for most basins, but was significantly lower in Hood Canal and Admiralty. The most specimens collected were from the following species: Pacific Herring (*Clupea pallasii)*, Pacific Sand Lance (*Ammodytes personatus)*, Arrow Goby (*Clevelandia ios)*, and Starry Flounder (*Platichthys stellatus*). In-depth analysis of seasonal and spatial patterns was performed for Pacific Herring, Pacific Sand Lance, and Starry Flounder. These three species have different spawning patterns and larval durations which influence their experience during the larval phase. All three species peaked in abundance during spring, but Pacific Herring was present during summer months, while the other two were absent. Herring was the only species present in more than a few instances in Hood Canal Basin. Herring and Starry Flounder were present in high density in South Basin, while Pacific Sand Lance and Pacific Herring were present in high density in the northern basins of Whidbey and Rosario. This study is the first time many of these seasonal and spatial trends have been described for larvae of these species within Puget Sound. It is clear from this dataset that nearshore habitat is home to a diverse range of larval fish species within Puget Sound. More sampling of the total larval fish community in the region over several years is needed to identify interannual variability and to confirm the results of this study. The general lack of regional larval fish distribution and abundance data impedes further action to protect these hundreds of species who produce pelagic larvae and also means we have insufficient data to plan for future needs of these species, including future vulnerabilities created by changes in climate and habitat.

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Chapter 1

Nearshore larval fishes of Puget Sound: Seasonal and spatial variation in taxonomic composition and distribution among Puget Sound basins

Abstract

The larval stage is a critical and sensitive period of fish development in which habitat requirements and stressors are typically different than those experienced by juveniles and adults. Puget Sound is home to 253 fish species, of which about 220 produce pelagic larvae. The aim of this study is to describe temporal and spatial patterns of distribution and abundance of larval fish species collected within the six basins that comprise Puget Sound from April 2011 through February 2012. A total of 9,317 specimens from 28 families and 71 species was collected. There were clear seasonal patterns in total larval abundance when looking at Puget Sound as a whole and among basins. Abundance peaked in spring, particularly April, was lower during summer months, was close to zero during winter, and rose again in early spring. Seasonal patterns in species richness were similar between basins, but species were not found equally among the six basins of Puget Sound. More than 40 species were recorded from most basins, but species richness in Hood Canal and Admiralty were significantly lower. This collection marks the most extensive sampling of larval fishes to date within Puget Sound. It is clear from the dataset that nearshore habitat is home to a diverse range of larval fish species. More sampling of the total larval fish community in the region over several years is needed to identify interannual variability and expand on the results of this study.

1. Introduction

Most marine fishes produce pelagic larvae. They are a temporary component of the plankton community. This stage is a critical and sensitive period of fish development in which habitat requirements and stressors are typically different than those experienced by juveniles and adults (Miller and Kendall 2009). It is also the period of the life cycle with the highest rate of mortality (>90%) (Hjort 1914, Cushing 1975, Fuiman and Werner 2002). Within the plankton, this group of fishes inhabits ecologically diverse niches and employs a variety of reproductive strategies (Miller and Kendall 2009). While fish larvae are present throughout the year, presence of a particular species is seasonally and regionally dependent. In addition, duration of the larval phase varies greatly from days to months and with regard to species.

Fish larvae have been studied for decades at regions around the globe. However, Koslow and Wright (2016) recognize the need for more extensive sampling and state, "Greater use of ichthyoplankton time series holds promise for vastly improving the ecological monitoring, assessment, and management of the oceans." The embryonic and larval fish stages serve the ecological and evolutionary functions of dispersal and gene pool mixing (Fuiman and Werner 2002). The presence, abundance, and dispersal of larval fishes is mediated by a variety of environmental and oceanographic parameters that vary between regions, and transport of young to unfavorable habitat can result in significantly higher mortality rates (Fuiman and Werner 2002). Regionally specific time series will allow for greater understanding of ecology and management of local species.

As members of the plankton community, larval fishes are highly sensitive to environmental parameters (Kendall and Miller 2009). Assemblages of pelagic fish larvae in coastal regions are associated with environmental parameters such as wind, salinity, and temperature (Lanksbury et al. 2005, Miller and Kendall 2009). Doyle et al. (2009) observed synchrony in larval abundance trends across time-series and similar links to environmental variables (such as temperature, winds and currents) were determined among species in the Gulf of Alaska with similar early life history strategies. Additionally, spawning in many marine fishes is timed to preclude the spring bloom, which produces large quantities of phytoplankton and in turn zooplankton—the primary food sources for larval fishes (Miller and Kendall 2009). The match/mismatch hypothesis states that adult fish must spawn before the spring bloom so that their first-feeding larvae may be matched with appropriate sized prey to fend off starvation (Cushing 1969).

Abundance and distribution of larval fishes has been correlated with many abiotic factors. For example, Syahailatua et al. (2011) noted distinct seasonal differences in ichthyoplankton abundance in samples collected from the separation zone of the East Australian Current with an order of magnitude higher abundance in November than in January. This study also reported fewer fish larvae collected in surface samples relative to subsurface samples. Alternatively, Gruber et al. (1982) reported fish larvae in the Southern California Bight grouped by proximity to shoreline. Furthermore, Busby et al. (2006) reported that Northern Rock Sole (*Lepidopsetta polyxystra*) abundance increased with wind speed in the Eastern Bering Sea shelf. The variety of factors influencing larval fish populations has made it difficult to generalize between regions. To develop an understanding of the ecology of particular species within a region it is necessary to study regional biological and environmental parameters.

A total of 253 fish species have been recorded in Puget Sound, of which about 220 produce pelagic larvae (Pietsch and Orr 2015). Pietsch and Orr (2015) documented occurrence of adult fishes throughout the Juan de Fuca, Puget Sound, and Strait of Georgia. Basic information regarding larval fish distribution and abundance in the region is currently extremely limited (Waldron et al. 1972, Busby et al. 2000, Chamberlin unpublished data). This severe lack of regional data means we have no foundation for designating regions for protection or anticipating future vulnerabilities to environmental forcing. We also have insufficient information on the phenology of taxa within the region, including spawning and hatching times, occurrence, and duration of larvae within the plankton.

A few historical datasets have provided limited information regarding larval fish distribution. Waldron et al. (1972) sampled at sites across The Sound during April of 1967 at a depth of 200 m and collected specimens from the families Scorpaenidae, Pleuronectidae, Gadidae, and Ammodytidae. These four families represented 88 percent of all larvae sampled. Almost double the number of families were collected from sampling along the Pacific Coast as in Puget Sound. Busby et al. (2000) performed day/night dip net sampling at a single site in the San Juan Islands for a decade. They collected specimens from 24 families and 65 species, particularly Cottidae, Stichaeidae, and Plueronectidae. Taxonomic diversity was greatest during spring and varied from year to year. Updated and detailed knowledge of distribution and abundance of Puget Sound fishes during this critical phase of development will contribute to our understanding of seasonal and temporal trends in habitat use and ecology, and associated species' vulnerabilities to environmental forcing. Additionally, comparison with previous studies will allow us to assess changes in taxonomic composition over time. Comparison of spatiotemporal patterns in occurrence of larvae and adults will highlight differences in vulnerabilities to environmental forcing between life stages and species. Knowledge of larval fish occurrence patterns for particular species will allow us to differentiate habitat use between different life stages. Without considering the early life history of these fishes, we are left with an incomplete understanding of their ecology and processes affecting survival and recruitment to the adult stage.

The aim of this study is to explore temporal and spatial patterns in species composition, including diversity (richness and evenness) and abundance of larval fishes, within the six basins that comprise Puget Sound by describing temporal and spatial distribution and abundance of all larval fish species collected from April 2011 through February 2012 and using this information to inform a basic understanding of larval fish habitat use and vulnerabilities to environmental forcing throughout the region. This sampling effort was part of a broad-scale study aimed at describing species composition at multiple trophic levels in Puget Sound nearshore habitat (Greene et al. 2012). This project will help to fill in gaps related to basic larval fish ecology and habitat use in a highly urbanized estuary with persistent human activity. The results of this study will contribute to a broader understanding of Puget Sound's nearshore pelagic foodweb. Knowledge of spatial and temporal distribution patterns of larval fishes in The Sound will allow for assessments of critical early life history habitat for marine fishes that produce pelagic larvae – an ephemeral group including a majority of the region's fish species.

The objectives of this study are to:

1. Document the taxonomic composition of larval fish assemblages in the nearshore zone;
2. Investigate seasonal and spatial variability in these assemblages.

2. Methods

2.1 Region of Study

Puget Sound begins 100 km east of the Pacific Ocean where the Strait of Juan de Fuca branches into two fjord systems, the Strait of Georgia to the north and Puget Sound to the south (Ebbesmeyer et al. 1988) (Figure 1.1a). The Sound has a shoreline length of 2,143 km, an area of 2,632 km^2, and a volume of 168 km^3. This estuary is exceptionally deep with maximum depths of approximately 200 to 300 m depending on the basin (Ebbesmeyer et al. 1988), and is home to 253 fish species (Pietsch and Orr 2015).

The six basins of The Sound, as described by Ebbesmeyer et al. (1988), are Rosario, Whidbey, Admiralty, Hood Canal, Central, and South (Figure 1.1b). These basins are characterized by unique water chemistry parameters (Greene et al. 2012) and delineated by shallow sills (Ebbesmeyer et al. 1988). Water is recycled between basins for months or years before being advected to the Pacific Ocean (Ebbesmeyer et al. 1988, Ahmed, et al 2017). Although there is often vigorous mixing between basins, Admiralty Inlet (as well as north Puget Sound) is always subject to a greater volume of oceanic water than South Puget Sound (Cannon 1983). Circulation within The Sound is governed by intense tidal mixing and restricted water flow at the constrictions between basins. Water from the ocean enters in deeper layers and exits, along with freshwater from river outlets, in shallower waters. The complicated nature of Puget Sound currents makes it difficult to determine the influence of water circulation on larval fish drift patterns, but it is highly likely that there are differences between basins.

Oceanographic parameters vary significantly between Puget Sound basins (Greene et al. 2012). Rosario, Admiralty, Central, and South Basins are characterized by mixed waters and strong currents. Tidal mixing in Rosario Basin mutes seasonal variation in salinity from river outlets (Cannon 1978). Whidbey and Hood Canal Basins are characterized by slower currents and stratified waters. Furthermore, before departing the basin, water recirculates within stratified basins for an order of magnitude longer than it does in well-mixed basins.

Study sites were located throughout the six basins (Figure 1.2). Samples were collected from 79 sites as part of a multi-trophic level analysis of Puget Sound's foodweb (Greene et al. 2012). Sites in each basin were chosen based on proximity to shoreline, depth, geomorphic type (embayment associated with river delta, embayment not associated with river delta, small embayment, and exposed shoreline), amount of exposed shoreline, and level of anthropogenic disturbance. Although sites were selected from the nearshore environment, they were not particularly shallow due to sampling constraints of the vessel which required deeper water. Details of site selection can be found in Greene et al. (2012). The selection of sites was designed to address the three goals of the Greene et al. (2012) study:

1. Identify how foodweb structure differs among the oceanographic basins of Puget Sound;

2. Determine whether particular measurement endpoints of the pelagic ecosystem are sensitive to gradients of land use;

3. Identify a number of potential biological metrics for monitoring ecosystem health.

This study contributes to the first aim of the Greene et al. (2012) goals. Larval fishes are situated low on the trophic web and serve as prey to other fishes (Duffy et al. 2010). They also develop into higher trophic level organisms including planktivores and piscivores. Investigation of seasonal and spatial variability in diversity and abundance will inform the structure of the overall foodweb in the nearshore region of Puget Sound.

2.2 Sample Collection and Processing

Samples were collected for eleven consecutive months from all six basins of Puget Sound. This marks the most extensive larval fish sampling effort within Puget Sound. Plankton samples were collected concurrently with biotic (fish, jellyfish, plankton, birds, microbes) and abiotic samples (pH, dissolved oxygen, salinity, temperature, conductivity, density, photosynthetically active radiation, fluorescence, turbidity) in the nearshore pelagic zone.

Larval fishes were sampled monthly from April through October 2011 and an additional six sites were sampled April 2011 through February 2012 (Table 1.1). Sites were consistent between months, but occasionally sites were not sampled due to inclement weather. Approximately fifteen sites were sampled per basin, except for Admiralty in which approximately seven sites were sampled because there were few embayments and no large river deltas to sample. All sites were in the nearshore pelagic zone and sampled during daylight. Samples were collected using a 500 μm mesh net (1 m diameter x 3 m long). A General Oceanics® model 2030 flowmeter hung in the middle of the net opening to record the volume of water that was sampled and an 18 kg weight was attached at the bottom of the net opening. The net was let out to 24.3 m (which corresponded to an average net depth of 2 to 3 m) and towed for 3 minutes at a speed of 2 kt in an arc to avoid sampling water displaced by the movement of the vessel. The contents were then poured into a 500 μm mesh sieve and large debris was sprayed off and removed. If a large volume of jellyfish was collected, they were sprayed off individually and removed from the sample. The sample was fixed with 5 percent neutral buffered formalin from the beginning of the study through mid-October. Subsequently, samples were fixed in 70 percent ethanol. All fish larvae were transferred to 70 percent ethanol for long-term storage. Fixing larval fishes in formalin and then transferring them to ethanol is standard procedure for taxonomic study (Lavenberg et al. 1984).

A total of 294 samples was processed. The specimens were donated to the Fish Collection of the UW Burke Museum of Natural History and Culture in Seattle, WA. Most of the samples collected during April, May, and August were processed for analysis along with a subset of samples collected at 16 sites that represent spatial heterogeneity within each basin for the months of June, July, September, and October. An additional six deep-water sites, collected from April 2011 through February 2012, were all processed. The decision to process most samples from April, May, and August was made based on data that showed these months to be the most productive for larval rockfishes (Greene and Godersky 2012) and also in order to compare results with other samples collected concurrently as part of the Greene et al. (2012) study. April and May were also sampled in one of the few historical datasets of larval fishes from Puget Sound (Waldron 1972). Larval fishes were sorted from the rest of the sample material and identified to species, if possible, using the *Laboratory Guide to Early Life History Stages of Northeast Pacific Fishes* (Matarese et al. 1989) and NOAA's Ichthyoplankton Information System website (http://access.afsc.noaa.gov/ichthyo). The developmental stage and standard length of each specimen was recorded (up to 30 specimens per species per sample). Planktonic fish larvae can be divided into five developmental stages (yolk-sac, pre-flexion, flexion, post-flexion, and transformation) based on physical characteristics (Matarese et al. 1989). Yolk-sac larvae are newly hatched larvae that still absorb nutrients from a yolk-sac. Pre-flexion larvae have absorbed the yolk-sac completely and their notochord has not yet begun to flex dorsally. Flexion larvae have a notochord that has begun to flex but is less than 45 degrees from the notochord axis. Post-flexion larvae have a notochord that has flexed a full 45 degrees from the notochord axis but other anatomical parts are still developing (such as fin rays and spines). Transforming larvae have begun squamation (development of scales), lack larval characteristics, and are fully developed (except reproductive organs). These are the most basic descriptors for each developmental stage, but there are others (Ahlstrom and Moser 1976). Most pelagic larvae hatch in the yolk-sac stage (Matarese et al. 1989). It was not possible to identify all specimens to species because some were degraded and because identification keys are not available for all taxa.

Some taxa are difficult or impossible to identify to species because of morphological similarity across species (Matarese et al. 1989; IIS https://access.afsc.noaa.gov/ichthyo/index.php), e.g. Rockfish of the genus *Sebastes*. The following taxa were assigned to broader taxonomic levels, except where noted, because of such difficulties with identification. Rockfish larvae cannot be identified to the species level and were all designated to the genus *Sebastes*. All Osmerid larvae were identified to family level only. Stichaeid species, except *Anoplarchus insignis* and *A. purpurescens,* were designated only to family level.

2.3 Data Analysis

To estimate total species richness for each of the six basins, a series of species accumulation curves were calculated for each region using the function “specaccum” in the R package “vegan”. This function estimates the total number of taxa in a region based on the number of specimens collected and the number of samples analyzed. This species richness index was calculated using the accumular method called “exact,” and samples were sampled without replacement (Ugland et al. 2003). This method estimates the total species richness within each basin by selecting a site and counting the number of species, then selecting an additional site and adding any new species not already counted to the sum, and repeating this process for all sites. The result of this analysis is a richness index for each basin and for the nearshore environment of Puget Sound as a whole.

The usage is as follows:

```
specaccum(comm, method = "exact", conditioned =TRUE, gamma = "jack1",  ...)
```

where *comm* is equal to the community dataset, *method* is equal the species accumulation method used, *conditioned* means the estimation of the standard deviation is conditional on the empirical dataset for the exact species accumulation curve, *gamma* is the method for estimating the total extrapolated number of species in the survey area using the function “specpool”.

To further explore patterns in species count data a number of other analyses were performed. The number of species at each site was plotted for each sample collected in April in ArcGIS 10.4 on an oceans base map of Puget Sound. The data was displayed as a heat map designating low to high species counts. The total number of species identified for each month was summed for all basins combined. Additionally, the average species count per site was calculated for each month within each basin. Furthermore, the average number of specimens per sample was calculated, by month and by month and basin, as an estimate of relative abundance of all fish larvae collected. Because of flowmeter malfunctions, density estimates were not available and average number of specimens per sample was used instead.

3. Results

A total of 9,317 specimens, 28 families and 71 species was collected. Table 1.3 lists the 30 most numerous species collected and the total number of specimens collected for each. *Clupea pallasii*, *Ammodytes personatus*, *Clevelandia ios*, and *Platichthys stellatus* were the most abundant species. *Sebastes* larvae were frequently collected and may be represented by any of the 26 species present in the region (Pietsch and Orr 2015). Many other species were rarely collected and found only in particular basins (see Table 1.4 for occurrence of species among basins).

There were clear seasonal differences in total larval abundance. When looking at Puget Sound as a whole, the average larval abundance per site peaked in April (73.8 specimens/site), declined through May (22.5 specimens/site) and June (10.3 specimens/site), increased slightly in July (28.0 specimens/site) then decreased to almost zero through December, after which abundance again increased through January (10.0 specimens/site) and February (19.8 specimens/site) (Figure 1.3a). Average abundance for April was 2.5 to more than 7 times higher than any other month. When average abundance was estimated for each basin separately, differences among basins emerged regarding range and seasonal patterns (Figure 1.3b). Variation among basins was particularly evident during summer months. Whidbey and South Basins had the highest abundance and Hood Canal and Admiralty had the lowest. During the summer period, timing of peak abundance differed between basins and temporal patterns were not discernable.

Seasonal patterns in species count were similar between basins. The peak was always in spring (particularly April) and tended to be lower during the summer months. Fall and early winter marked the minimum in species counts, and there was always an uptick in February (Figure 1.3a, Figure 1.4). In April, species counts varied within and between basins and ranged from a minimum of 1 to a maximum of 15 species (Figure 1.5). “Hot Spots” (sites with 13 – 15 species) occurred in all basins in April except Central Basin. These counts should be considered minimum estimates, as many specimens were not identifiable to species and were identified at a higher taxonomic level such as genus or family.

Total species richness varied between basins (Table 1.5). Rosario, Whidbey, Central, and South Basins had similar species richness indexes (44, 44, 48, and 45 taxa respectively), whereas those for Admiralty and Hood Canal were considerably lower (26 and 34 taxa respectively). Reduced sampling effort in these basins could have resulted in underestimated richness, however the slopes of the accumulation curves for these two basins were shallower, suggesting that species were added to the curve at a slower rate and that these basins do have lower total richness (Figure 1.6). Other than a weak pattern in February, when richness appeared to increase from south to north, no distinct latitudinal patterns in richness were observed. Central and South Basins exhibited the largest difference in species richness between spring and summer, however no spatial patterns in species richness were observed during summer months.

4. Discussion

This collection marks the most extensive sampling effort of larval fishes to date within Puget Sound. It seems that despite repeated sampling over the course of a year, only a small number of the resident species with pelagic larval stages were collected, suggesting that more effort is needed to fully characterize pelagic habitat use by larval fishes within Puget Sound. In this study, 28 families and 71 species of larval fishes were identified. This is only about one third of the estimated 220 fish species that produce pelagic larvae that have been recorded within Puget Sound (Pietsch and Orr 2015). Nevertheless, these species were collected while sampling solely in upper few meters of nearshore habitat. In contrast, the paired-townet sampling for this study, which targeted juvenile and adult fishes, caught only 33 fish species in total (Rice et al. 2012).

There are several reasons that larvae of resident fish species that produce pelagic larvae were not collected. Most obviously, the collection effort only targeted waters down to three meters and only in the nearshore zone. Many of the larvae of those species not collected may utilize deeper or offshore habitat not sampled. Additionally, collections were only made at each site once per month, a short sampling window, allowing for rare species to be missed, particularly those with short larval durations. Sampling in winter was particularly limited, covering only six sites, while a large area went without collection. Also, the net was towed at an average of two to three knots, permitting those competent swimmers to escape the net. This relatively slow towing speed is likely the reason that most specimens collected were small, early stage larvae. Finally, many species were not identified as part of this study because identification keys are not available. Specimens may have been collected but not recorded due to lack of information about identification in the larval stage.

In 2011 in Puget Sound, the peak in Chlorophyll *a* production (a proxy measurement for phytoplankton production) occurred in June in most basins, however there was a second peak in Admiralty and Hood Canal Basins (Greene et al. 2012). The seasonal pattern in larval fish species richness and abundance that peaks in spring and falls through winter is a pattern recognized particularly in temperate and sub-arctic latitudes (Doyle et al. 1993, Doyle et al. 2002, Icanberry et al. 1978). On the west coast of the Pacific Ocean, fish spawning occurs in spring prior to the peak in phytoplankton production (Smetacek et al. 1984, Doyle et al. 2002). The spring bloom and zooplankton production are often observed to coincide with the peak in ichthyoplankton production and provide nourishment to fish larvae (Fuiman and Werner 2002). In the current study the peak in phytoplankton was observed after the peak in larval fish abundance (Greene et al. 2012). This was also observed in the San Francisco Estuary in 2004, but productivity was still considered adequate (Bollens and Sanders 2004).

Juvenile fish collected concurrently with the larval catch peaked in richness in June, two months later than for larvae (Rice et al. 2012). The juvenile catch was comprised primarily of salmonids, *Clupea pallasii*, *Gasterosteus aculeatus*, and *Hypomesus pretiosus*. Eggs and larvae may be important food sources for these later stage fishes and thus the presence of early stages may provide a significant ecological function. Ctenophores were also commonly collected with the juvenile catch (Greene et al. 2012) and are common predators of larval fishes (Fuiman and Werner 2002). The juvenile catch revealed higher richness in northern basins compared to southern, a pattern not mimicked in the larval catch.

In November and December, fish larvae were only present in the northernmost basin, Rosario. The larvae caught during these two months were preflexion and flexion larvae of *Leptocottus armatus* and *Hemilepidotus hemilepidotus*. *Hemilepidotus hemilepidotus*, an intertidal benthic species, is known to spawn from October to January in the Gulf of Alaska and Bering Sea (Matarese et al. 1989). Because Puget Sound water is warmer, this species is likely to spawn earlier relative to populations in northern regions. *Leptocottus armatus*, a nearshore shelf demersal species, is known to spawn from October to March in California (Matarese et al 1989). The single specimen caught during December would have been spawned at the early end of this period.

Estimated richness in most basins was between 41 and 45 species, but in two basins, Admiralty and Hood Canal, estimates were significantly lower. These two basins each have distinctly different oceanographic parameters than the other four due to Admiralty's proximity to the Pacific Ocean and connection to the Strait of Juan de Fuca, and Hood Canal's geographic isolation from the rest of The Sound and often hypoxic conditions during the summer months (Newton 2008, Greene et al. 2012). The reasons for lower species counts are likely not the same for Admiralty and Hood Canal Basins.

Depressed species counts in Hood Canal may be due to several different factors. There are fewer described adult fish species recorded from Hood Canal and south Puget Sound than from North Puget Sound and the Strait of Juan de Fuca (Pietsch and Orr 2015). This suggests that Hood Canal may not provide suitable habitat for adult fishes relative to these other areas of Puget Sound. For those species who do spawn in Hood Canal, their eggs and larvae may have poor survival, perhaps due to low oxygen levels.

The effects of hypoxia on the biota of Hood Canal is a common topic in the Puget Sound region. Some marine pelagic larvae are known to have extremely high oxygen consumption (Leis et al. 2011). Additionally, during the yolk-sac phase larvae depend on cutaneous respiration (Fuiman and Werner 2002). August, in particular, is known to have the lowest levels of dissolved oxygen in Hood Canal and is frequently hypoxic (Cope and Roberts 2013). Water chemistry samples collected in 2011 simultaneous with the larval fish samples revealed that Hood Canal had the largest difference in dissolved oxygen concentrations at maximum depth relative to the other basins from April through October (Greene et al. 2012). Values below 5 mg/L of dissolved oxygen are considered biologically stressful (Roberts et al. 2005). During this study, Hood Canal Basin had the most sites with conditions that were biologically stressful at some depth and as shallow as 6 m. An inadequate supply of dissolved oxygen could explain the depressed richness and abundance in this region, particularly at the south end. Northern Hood Canal Basin does appear to have high richness, including three sites in April in which ten or more species were collected.

Admiralty Basin's connection to the Strait of Juan de Fuca and the Pacific Ocean, more saline water, and high flow all may contribute to lower species counts in the basin. In 2011, zooplankton communities in Admiralty and Rosario Basins were represented by more oceanic species relative to other basins (Greene et al. 2011). Additionally, lower species counts in Admiralty may have been due to an association with offshore species which would not have been collected in this study, such as Myctophids, Aulopids, and Stomiids. Finally, strong currents may have transported young larvae, such as those collected in this study, away from nearshore habitat.

Species richness and abundance varied significantly both spatially and temporally in Puget Sound, but it is clear from the dataset that nearshore habitat is home to a diverse range of larval fish species. The four dominant species in this study, *Clupea pallasii*, *Ammodytes personatus, Clevelandia ios*, and *Platichthys stellatus* inhabit different niches as adults and also vary significantly in larval duration within the plankton. Despite co-occurring in the nearshore habitat, the ecology of these species is quite different during the larval phase (Matarese et al. 1989). The Arrow Goby, *Clevelandia ios*, is distributed throughout The Sound and associated with water less than 10 m (Pietsch and Orr 2015). It was collected from May through September and in all basins. Auth and Brodeur (2013) reviewed 60 years of ichthyoplankton reports from the Northern California Current and reported that Clupeids, *Platichthys stellatus*, and *Clevelandia ios* were rare, but that *Ammodytes personatus* was abundant. The Starry Flounder, *Platichthys stellatus*, is an estuarine-associated species (Pietsch and Orr 2015) and that is likely the reason it was commonly collected in Puget Sound, but not from the California Coast.

Genetic analysis to facilitate the identification of morphologically identical species during the larval phase is needed. Proper identification is the first step towards gaining knowledge about habitat use and ecology of these species. Because of the large number of species of *Sebastes* in the region, it is hard to make conclusions regarding abundance, richness, and seasonality based on genus-level data. A sampling effort to collect larval *Sebastes* and fix them in ethanol for molecular identification is crucial for understanding the ecology of these important regional taxa, many of which have been on the decline for decades (NMFS 2016).

Additional sampling of larval fishes in the region over several years is needed to identify interannual variability and to enhance knowledge of early life history habitat and ecology. The general lack of regional larval fish distribution and abundance data means there is a lack of knowledge concerning potential habitat use by fish species within the region as well as sensitivity to environmental forcing. Detailed abundance and distribution data will inform us of potential sensitivity to local environmental forcing in different seasons and areas. Future sampling across the region will provide information on the little known early life history ecology of these species and potential vulnerabilities to environmental forcing and future climate change for the majority of Puget Sound fish species.

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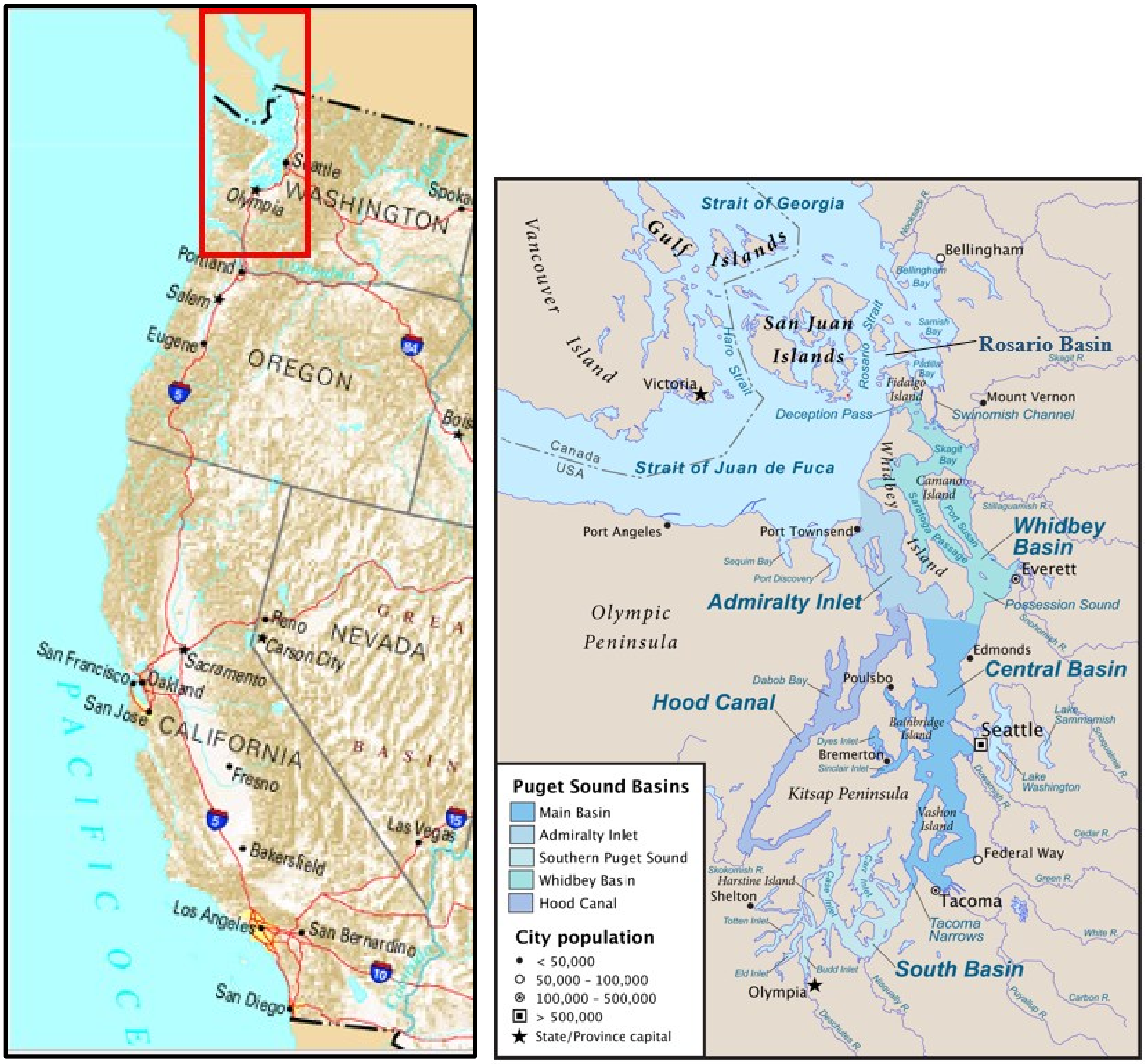


a) b)

Figure 1.1 Map of Puget Sound. a) Location in reference to the west coast of USA and b) local map with basins delineated. Figures from Wikipedia Commons. a) Modified from Public Domain, https://commons.wikimedia.org/w/index.php?curid=12997. b) Edited by Pfly. Origianal data from Geobase and The National Map.

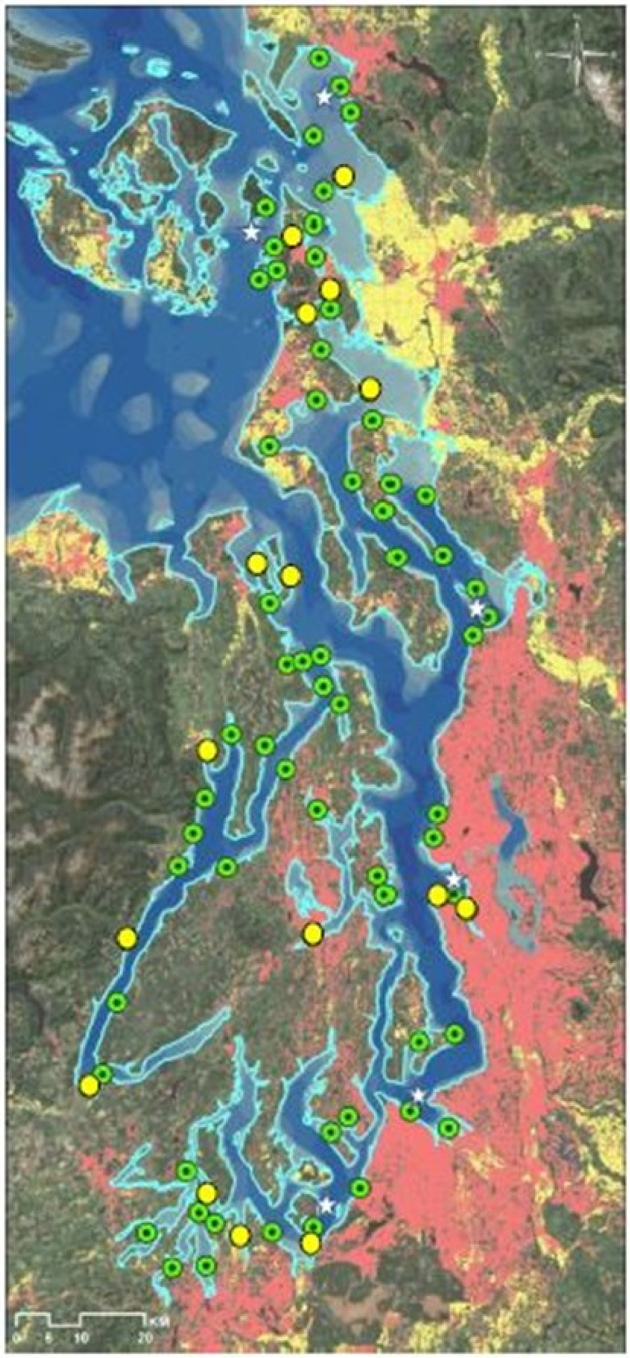


Figure 1.2. Map depicting sites where plankton samples were collected for this study (circles), as well as the six sediment disposal sites where samples were collected for Greene and Godersky (2012) (white stars). Yellow circles indicate 16 sites chosen as subsample for comparison across months.

Table 1.1 Sites sampled each month within each basin (1 of 3).

Basin	Site	Year 2011									Year 2012	
		April	May	June	July	August	September	October	November	December	January	February
	Hood Head	X	X			X						
	Nodule Point	X	X	X	X	X	X					
	Oak Bay	X				X						
Admiralty	Port Gamble	X				X						
	Port Ludlow	X				X						
	Port Townsend	X	X	X	X	X	X	X				
	Tala Point	X	X									
	South of Foulweather Bluff	X										
	Total	8	4	2	2	6	2	1				
	Alki	X	X	X	X							
	Burien	X										
	Clam Bay	X										
	Commencement Bay	X	X	X	X	X	X	X	X		X	X
	Duwamish 1	X	X	X	X	X	X	X				
	Duwamish 2	X		X		X						
	Eagle Harbor	X		X		X						
	Elliot Bay	X	X	X	X	X	X	X	X		X	X
Central	Liberty Bay					X						
	Meadow Point	X										
	Port Blakely	X		X		X						
	Puyallup 1	X				X						
	Puyallup 2	X				X						
	Quartermaster Harbor	X		X								
	Robinson Point	X										
	Shilshole	X				X						
	Sinclair Inlet	X	X	X	X			X				
	Total	16	5	9	5	10	3	3	2		2	2
	Dabob Bay	X		X	X	X	X	X				
	Dosewallips 1	X										
	Duckabush	X				X						
	Hamma Hamma	X										
	Jackson Cove	X				X						
	North Dewatto Bay	X				X						
Hood Canal	Pleasant Harbor	X										
	Seabeck Bay	X				X						
	Skokomish	X	X	X		X						
	Tarboo Bay	X	X									
	Thorndyke Bay	X				X						
	Union	X				X						
	Vinland	X	X	X		X		X				
	Total	13	3	3	1	9	1	2				

Table 1.1 Sites sampled each month within each basin (2 of 3).

Basin	Site	Year 2011									Year 2012	
		April	May	June	July	August	September	October	November	December	January	February
	Allen Island	X	X			X						
	Bellingham Bay	X	X	X		X	X	X		X	X	X
	Burrows Bay	X	X			X						
	Chuckanut Bay	X	X			X						
	Deadman Bay	X				X						
	Deepwater Bay		X			X						
	Fairhaven					X						
	Fidalgo Bay	X	X			X						
	Guemes Channel		X		X	X	X	X				
Rosario	North Samish Bay	X				X						
	Nooksack 1	X				X						
	Nooksack 2		X			X						
	Nooksack 3	X	X			X						
	Rosario Strait	X	X		X	X	X	X	X	X	X	X
	Samish	X	X		X	X	X	X				
	Samish Island	X	X									
	Ship Harbor	X	X			X						
	Sunset Beach	X	X									
	Three Rocks	X				X						
	Total	15	10	1	3	17	4	4	1	2	2	2
	Anderson Island	X	X		X	X	X	X	X		X	X
	Briscoe	X										
	Budd Inlet	X						X				
	Chambers Creek	X				X						
	Eld Inlet					X						
	Henderson Inlet	X	X	X	X	X	X					
	Hunter Point	X				X						
South	North Fox Island	X				X						
	Nisqually 1	X	X	X	X	X	X	X				
	Nisqually 2					X						
	Oro Bay					X						
	South Salom Point	X				X						
	Squaxin Island	X				X	X	X				
	Totten Inlet	X	X			X						
	Wollochet Bay	X										
	Zangle Cove	X				X						
	Total	13	4	2	3	13	4	4	1	0	1	1

Table 1.1 Sites sampled each month within each basin (3 of 3).

Basin	Site	Year 2011									Year 2012	
		April	May	June	July	August	September	October	November	December	January	February
Whidbey	Bells' Beach	X	X			X						
	Camano Head	X	X			X						
	Crescent Harbor		X			X						
	Elger Bay	X	X			X		X				
	Holmes Harbor	X										
	Hoypus Point	X	X	X	X	X	X					
	Lone Tree	X	X			X						
	Mukilteo		X			X						
	Onamac Point		X			X						
	Penn Cove		X			X						
	Port Gardner			X	X	X	X	X	X	X	X	
	Port Susan 1		X			X						
	Port Susan 2	X	X					X				
	Similk Bay	X	X	X	X	X	X	X				
	Skagit 1	X	X	X	X	X	X	X				
	Skagit 2		X			X						
	Snohomish 1	X	X			X						
	Snohomish 2	X	X			X						
	Utsalady	X	X			X						
	Total	12	17	4	4	17	4	5	1	1	1	0
Total for All Basins Combined		77	47	21	18	72	18	20	5	3	6	5

Table 1.2 Total number of specimens collected for the 30 most numerous species and the genus *Sebastes*.

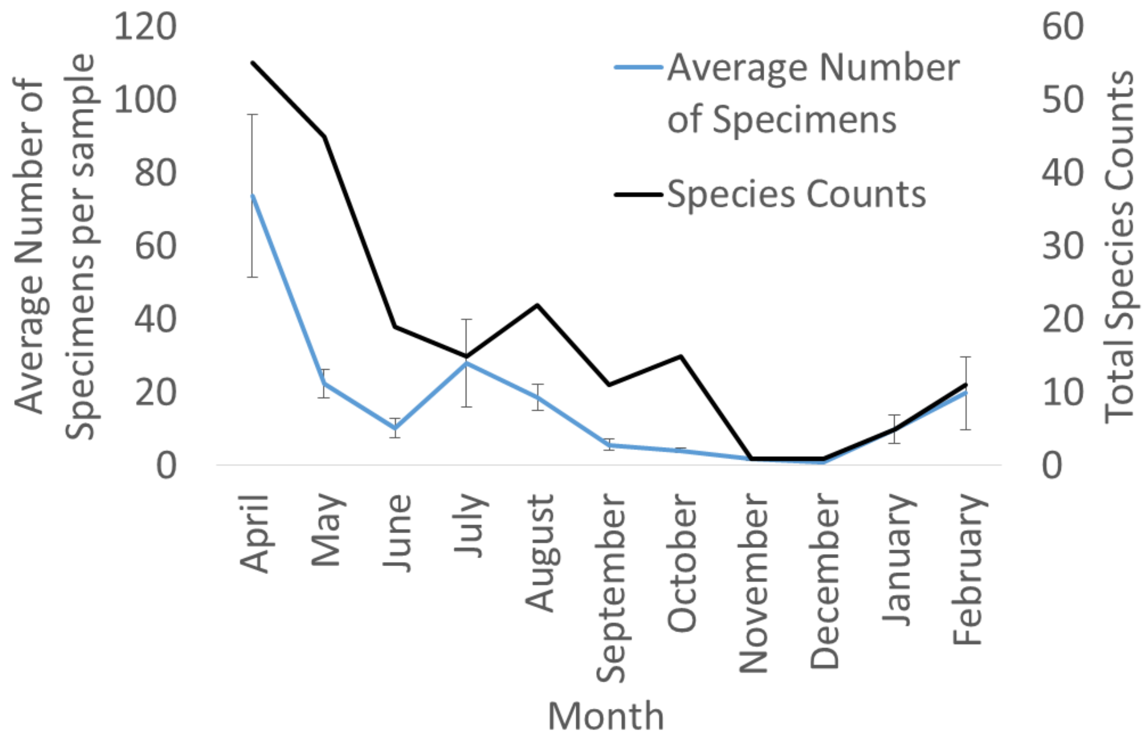
Taxa	Common Name	#
Clupea pallasii	Pacific Herring	2822
Ammodytes personatus	Pacific Sand Lance	1082
Clevelandia ios	Arrow Goby	802
Sebastes spp.	Rockfishes	539
Platichthys stellatus	Starry Flounder	386
Anoplarchus purpurescens	High Cockscomb	335
Lepidogobius lepidus	Bay Goby	290
Parophrys vetulus	English Sole	217
Citharichthys sordidus	Pacific Sanddab	178
Anoplarchus insignis	Slender Cockscomb	151
Cottus asper	Prickly Sculpin	145
Engraulis mordax	Northern Anchovy	140
Citharichthys stigmaeus	Speckled Sanddab	115
Leptocottus armatus	Pacific Staghorn Sculpin	115
Gadus macrocephalus	Pacific Cod	88
Myoxocephalus polyacanthocephalus	Great Sculpin	79
Gadus chalcogrammus	Walleye Pollock	68
Artedius harringtoni	Scalyhead Sculpin	62
Hippoglossoides elassodon	Flathead Sole	57
Psettichthys melanostictus	Sand Sole	55
Microgadus proximus	Pacific Tomcod	53
Lepidopsetta bilineata	Rock Sole	51
Pleuronichthys coenosus	C-O Sole	47
Artedius fenestralis	Padded Sculpin	37
Sardinops sagax	Pacific Sardine	26
Ronquilus jordani	Northern Ronquil	25
Odontopyxis trispinosa	Pygmy poacher	23
Pholis laeta	Crescent Gunnel	20
Scorpaenichthys marmoratus	Cabezon	16
Syngnathus leptorhynchus	Bay Pipefish	15
Malacocottus kincaidi	Blackfin Sculpin	13
Other		1265
Total		9317

Table 1.3 Species present in each basin, listed in phylogenetic order (1 of 2).

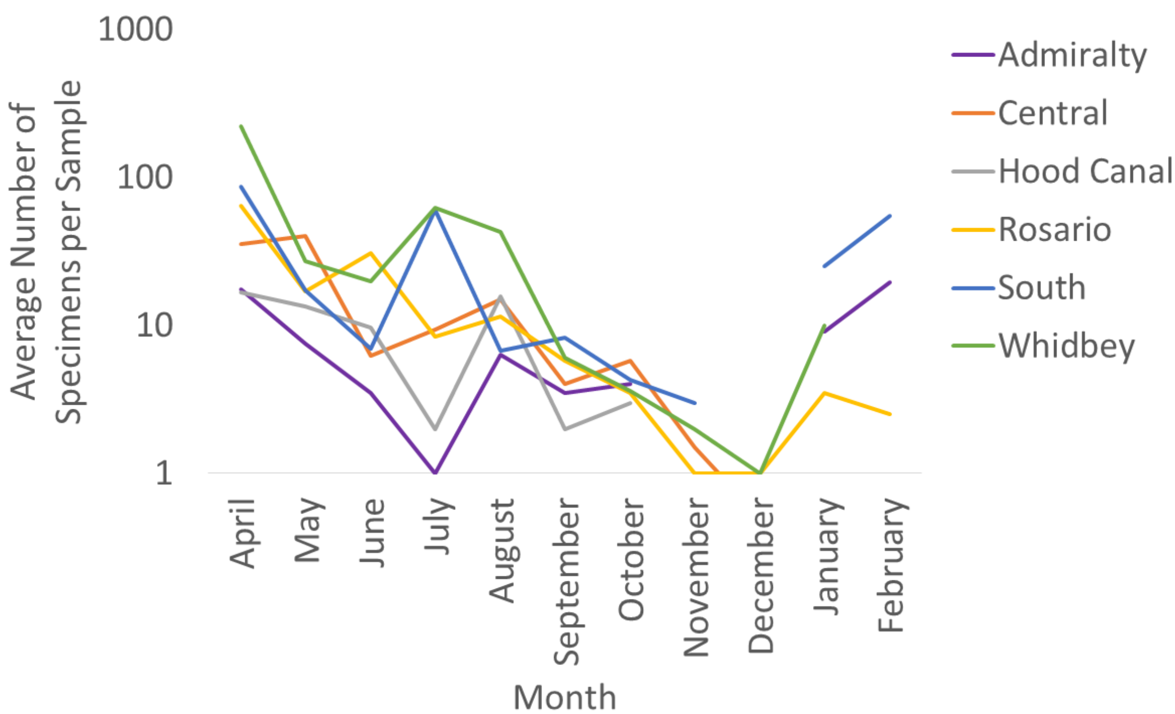
Family	Species	Basin					
		Rosario	Whidbey	Admiralty	Hood	Central	South
Engraulidae	*Engraulis mordax*	X	X		X	X	
Clupeidae	*Clupea pallasii*	X	X	X	X	X	X
	Sardinops sagax		X		X	X	X
Bathylagidae	*Leuroglossus schmidti*	X					
Osmeridae	Osmeridae spp.	X	X	X	X	X	X
Salmonidae	*Oncorhynchus keta*		X				
Merlucciidae	*Merluccius productus*		X		X		
Gadidae	*Gadus chalcogrammus*	X	X	X	X	X	X
	Gadus macrocephalus	X	X			X	X
	Microgadus proximus	X	X		X	X	X
Bythitidae	*Brosmophycis marginata*		X			X	
Gasterosteidae	*Aulorhynchus flavidus*		X				
Syngnathidae	*Syngnathus leptorhynchus*	X	X		X	X	X
Scorpaenidae	*Sebastes* spp.	X	X	X	X	X	X
Hexagrammidae	*Hexagrammos decagrammus*	X		X			
	Oxylebius pictus			X		X	
	Ophiodon elongatus					X	
Cottidae	*Artedius fenestralis*	X	X			X	X
	Artedius harringtoni	X	X	X		X	X
	Artedius lateralis	X	X			X	X
	Artedius meanyi	X			X		
	Ascelichthys rhodorus					X	
	Chitonotus pugetensis		X		X		X
	Clinocottus acuticeps				X	X	X
	Clinocottus recalvus		X				
	Cottus asper	X	X		X	X	X
	Enophrys bison	X			X		X
	Hemilepidotus hemilepidotus	X					
	Icelinus borealis	X					
	Leptocottus armatus	X	X	X	X	X	X
	Myoxocephalus polyacanthocephalus	X	X	X			X
	Oligocottus maculosus				X		X
	Radulinus asprellus			X		X	X
	Ruscarius meanyi	X			X	X	
	Scorpaenichthys marmoratus	X	X	X	X	X	X
Hemitripteridae	*Nautichthys oculofasciatus*		X			X	X
Agonidae	*Bathyagonus alascanus*	X					
	Bathyagonus infraspinatus			X			
	Odontopyxis trispinosa	X	X	X		X	X
	Podothecus acipenserinus	X				X	X
	Xeneretmus latifrons			X			
Psychrolutidae	*Malacocottus kincaidi*	X					X
Liparidae	*Liparis fucensis*	X		X		X	X
	Liparis gibbus	X					
Stichaeidae	*Anoplarchus insignis*	X	X	X			
	Anoplarchus purpurescens	X	X	X	X	X	X
	Stichaeidae sp.	X	X			X	
Bathymasteridae	*Ronquilus jordani*	X	X		X	X	X
Cryptacanthodidae	*Cryptacanthodes aleutensis*	X			X		
Pholidae	*Pholis laeta*	X	X			X	X
	Xererpes fucorum						X

Table 1.3 Species present in each basin, listed in phylogenetic order (2 of 2).

Family	Species	Basin					
		Rosario	Whidbey	Admiralty	Hood	Central	South
Ammodytidae	*Ammodytes personatus*	X	X	X	X	X	X
Gobiesocidae	*Gobiesox maeandricus*						X
Gobiidae	*Clevelandia ios*	X	X	X	X	X	X
	Lepidogobius lepidus	X	X	X	X	X	X
	Rhinogobiops nicholsii		X		X	X	
Scombridae	*Scomber japonicus*		X				
Stromateidae	*Peprilus simillimus*					X	
Paralichthyidae	*Citharichthys sordidus*	X	X		X	X	X
	Citharichthys stigmaeus	X	X	X	X	X	X
Pleuronectidae	*Hippoglossoides elassodon*	X	X		X	X	X
	Hippoglossus stenolepis					X	X
	Isopsetta isolepis					X	X
	Lepidopsetta bilineata	X		X	X	X	X
	Lepidopsetta polyxystra					X	
	Lyopsetta exilis	X	X		X	X	X
	Microstomus pacificus						X
	Parophrys vetulus	X	X	X	X	X	X
	Platichthys stellatus	X	X	X	X	X	X
	Pleuronichthys coenosus		X	X	X	X	X
	Psettichthys melanostictus	X	X	X	X	X	X



a)



b)

Figure 1.3. Average number of larval fish specimens per sample for a) each month and b) each month and basin

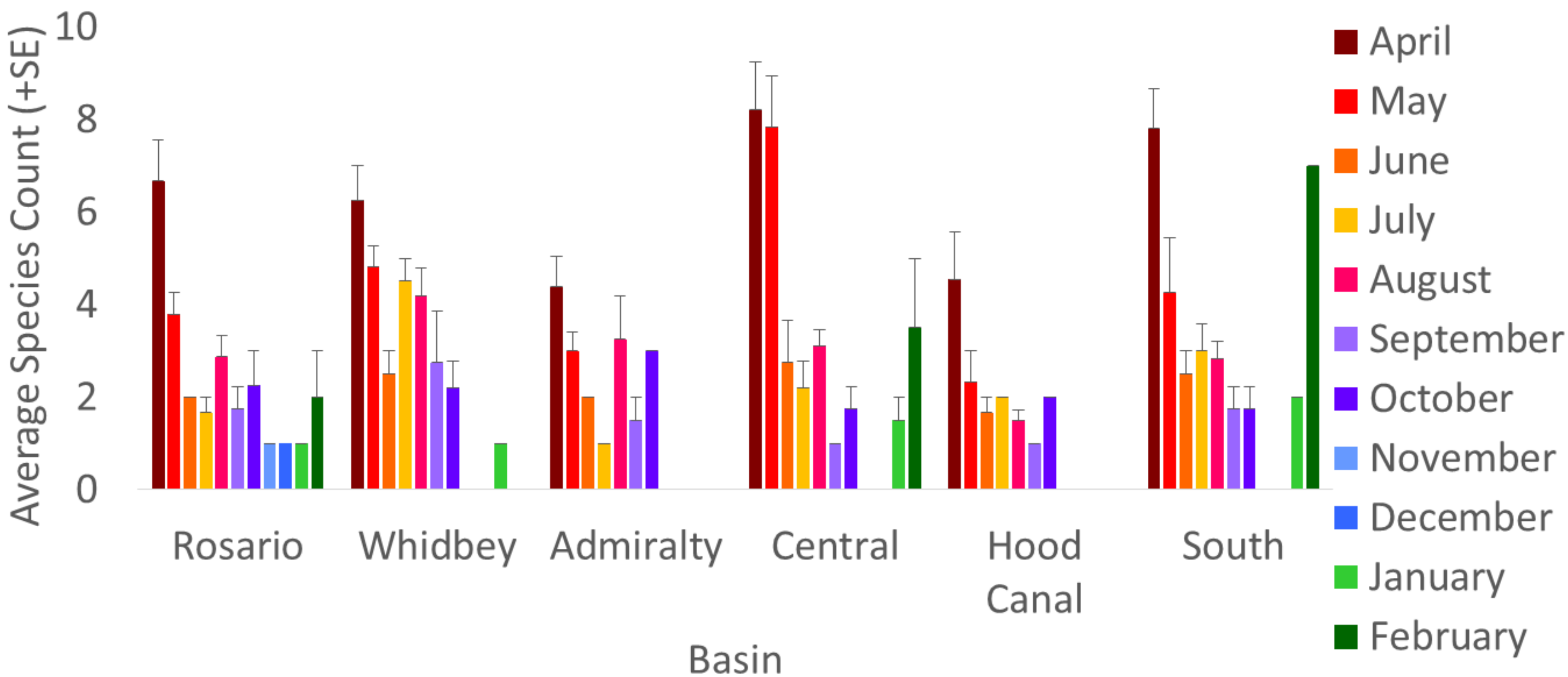


Figure 1.4. Estimated average species count per site plus standard error for each month within each basin.

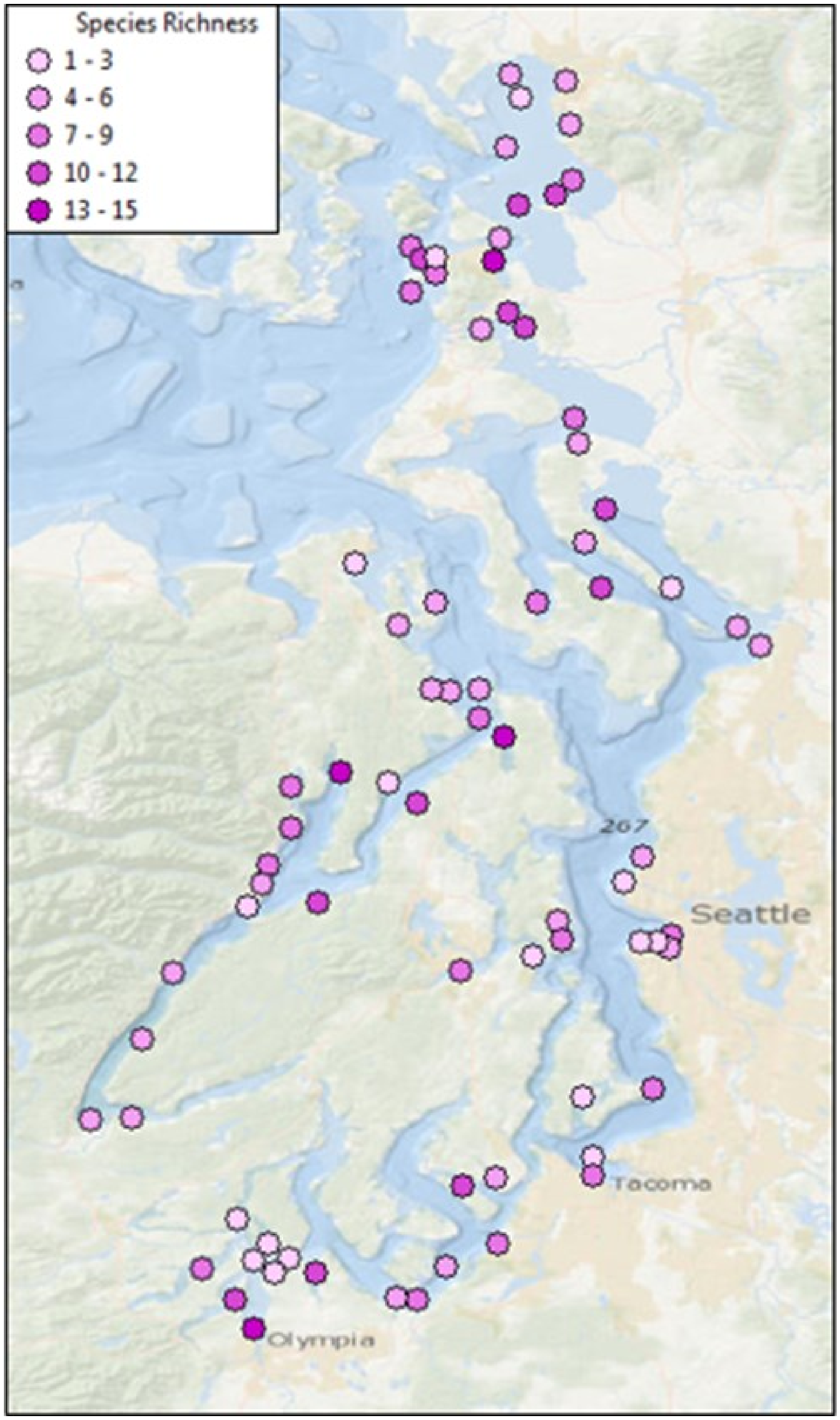


Figure 1.5. Heat map of species counts (ranging from 1 to 15 species) per sample during April 2011. This map was created using ArcGIS® software by Esri. ArcGIS® and ArcMap™ are the intellectual property of Esri and are used herein under license. Copyright © Esri. All rights reserved. For more information about Esri® software, please visit www.esri.com.

Table 1.4. Species richness index for each basin and for the nearshore environment of Puget Sound as a whole (All) based on species accumulation curves.

Basin	Number of Species
Rosario	43
Whidbey	41
Admiralty	25
Hood	33
Central	45
South	44
All	70

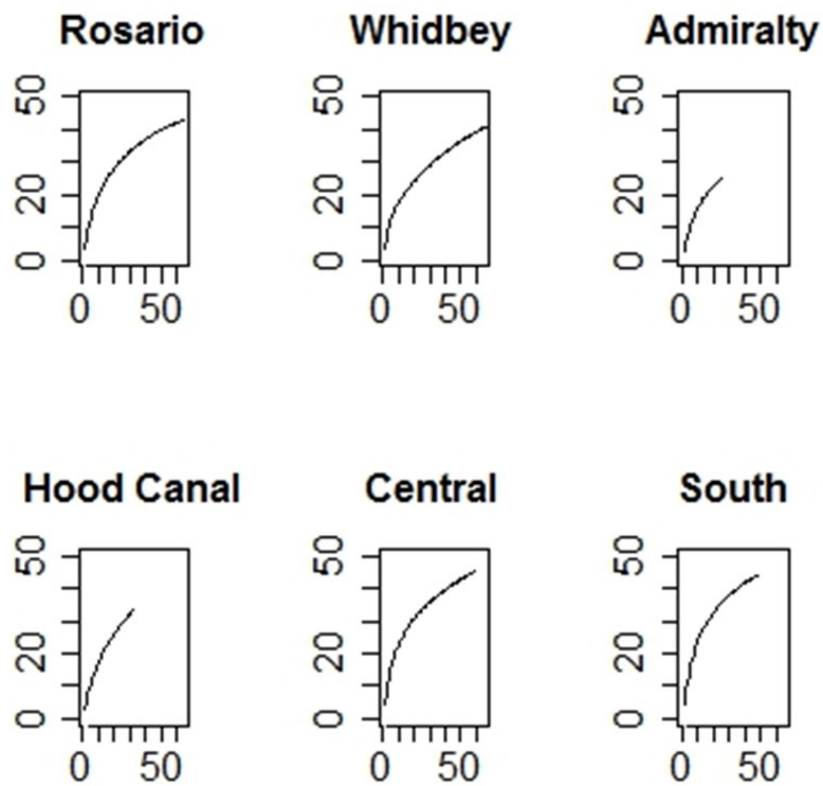


Figure 1.6. Species accumulation curves estimating total richness in each basin for all months sampled.

Chapter 2

Nearshore larval fishes of Puget Sound: Seasonal and spatial variation in occurrence and abundance of three species, *Clupea pallasii,* Pacific Herring; *Ammodytes personatus,* Pacific Sand Lance; and *Platichthys stellatus,* Starry Flounder.

Abstract

Early life history habitat and ecology of marine fishes is variable among species. Early life history is critically important because variation in survivorship has a marked effect on populations. *Clupea pallasii* (Pacific Herring), *Ammodytes personatus* (Pacific Sand Lance), and *Platichthys stellatus* (Starry Flounder), were chosen as focal species for this study because they were abundant in the dataset, prevalent in the concurrent juvenile catch, and each experiences this phase differently due to variation in spawning patterns and ontogenetic development. The objectives of this project are to describe the spatial and temporal dynamics of the larvae of the focal species in Puget Sound nearshore habitat. A total of 2,822 specimens of *Clupea pallasii* was caught during this study, far more than any other species. Specimens were present from April through October 2011 and in 19.4 percent of all samples. A total of 1,091 *Ammodytes personatus* larvae was collected, making it the second most abundant species caught during this study. *Ammodytes personatus* was present in 18.7 percent of all samples and was only present in the months of April and May 2011 and January and February 2012. A

total of 386 specimens of *Platichthys stellatus* was caught during this study, making it the fourth most abundant species in the dataset. It was present in 21.8 percent of samples and in the months of April, May, and June 2011 and February 2012. This is the first time a detailed analysis of early life history patterns has been reported for these species within Puget Sound and this analysis confirmed that nearshore habitat in Puget Sound is valuable year-round and in all six basins as rearing habitat for fish larvae of multiple species. Future research should focus on more sampling to corroborate the trends noted in this study and to investigate interannual variation in larval fish patterns within the plankton. Continued effort to investigate larval fish habitat and early life history ecology for species of interest within Puget Sound will allow for management of species of commercial and ecological importance.

1. Introduction

Early life history habitat and ecology of marine fishes is diverse and species specific. Additionally, ontogeny varies widely even among closely related species (Matarese et al. 1989). For example, duration of egg and larval phases can range from days to months. Spawning and hatching season can occur at any time of year (depending on the species) and can even occur multiple times a year. Size and swimming ability vary widely among species, and even from day to day, as larvae grow and develop (Fisher et al. 2000). Together, early life history habitat and ontogeny of marine fishes influence the ecology of individual fish species and result in different vulnerabilities to the environment.

Early life history is critically important because variation in survivorship has a marked effect on populations. Early life history of fishes is a period of exceptionally high mortality such that few individuals recruit to the juvenile and adult stage. Moreover, mortality is highly sensitive to environmental conditions that govern predation, starvation, and advection. Hjort (1914) hypothesized that timing of first feeding was a critical period whereby starvation would follow if prey was not available. Cushing (1990) elaborated on this hypothesis and developed the match/mismatch hypothesis that stated that the overlap of larval timing with favorable environmental conditions was necessary for survival. Consequently fish populations are subject to highly variable recruitment due to variable environmental conditions during the larval phase.

Understanding ecology and habitat is important for a number of reasons. One, observations of when and where larval fishes occur reveal the life history strategies utilized by a particular species. Sinclair and Tremblay (2011) hypothesized that the period of settlement to juvenile habitat for Atlantic Herring is fixed and that the spawning period of individual populations is based on relative habitat quality in larval retention zones, and the time necessary to develop prior to metamorphosis governed by that habitat quality. Two, this information provides a foundation for mechanistic understanding of recruitment variability. Namely, understanding species ecology and habitat characteristics reveals vulnerabilities and strengths affecting survival (Doyle and Mier 2012). For example, Pepin and Myers (1991) reviewed stock data and information on the duration of the larval stage of fishes and concluded that recruitment variability increases with larval duration. Three, understanding ecology and habitat allows us to better predict species' exposure to environmental changes and thereby aid ecological forecasting (Daewel et al. 2011).

Below I detail known information of the biology and ecology of fish larvae of three species in Puget Sound, WA. These species, *Clupea pallasii* (Pacific Herring), *Ammodytes personatus* (Pacific Sand Lance), and *Platichthys stellatus* (Starry Flounder), were chosen for a number of reasons. All three species were abundant in Puget Sound larval fish sampling (Chapter 1; Greene et al. 2012), thereby affording high statistical power to detect spatial and temporal patterns of occurrence. These species were also prevalent in the concurrent juvenile sampling (Rice et al. 2012). *Clupea pallasii* occurred in 58 percent of the juvenile catch, *Ammodytes personatus* in 22 percent, and *Platichthys stellatus* in 8 percent. While these species all spend their early life as pelagic larvae, each experiences this phase differently due to variation in spawning patterns and ontogenetic development. Two of them (Pacific Herring and Pacific Sand Lance) are forage fishes that play important roles in the Puget Sound foodweb by acting as prey for a number of fish, marine mammals, and seabirds (Penttila 2007). The term "forage fishes" describes small, plentiful fishes that serve as prey for higher trophic level animals (Penttila 2007). Starry Flounder provides a useful contrast to Herring and Sand Lance because it is a flatfish, occupies benthic habitat, and has a short larval phase. Despite its ubiquity, little is known about Starry Flounder within Puget Sound, particularly regarding its early life history. The dispersal of benthic fish species is most often carried out during the pelagic phase which lends further justification for knowledge of early life history patterns (Leis 2010). This study will lay a foundation for understanding the early life history of *Platichthys stellatus* in the Puget Sound region. It is considered an indicator species for estuary habitat because it readily accumulates contaminates (Emmett et al. 1991). Below I provide a review of the biology and ecology of each of these three focal species as background to understanding their early life history ecology in Puget Sound.

1.1 Species Reviews

Clupea pallasii, a pelagic forage fish found throughout the eastern North Pacific Ocean, is prevalent in Puget Sound (Pietsch and Orr 2015). The southern end of its range is the San Francisco Estuary in California State and the northern end is in the Arctic (Feyrer et al. 2004, Pietsch and Orr 2015). *Clupea pallasii* inhabits nearshore and intertidal habitat, plentiful in Puget Sound due to its elongate geomorphology and many spreading inlets. As a whole, Herring populations within The Sound are considered moderately healthy, but some stocks are in decline (Stick et al. 2014). The primary differentiation between healthy stocks and unhealthy stocks appears to be genetic (Stick et al. 2014). Currently, there is no commercial fishery for Herring in The Sound, and only a small sport bait fishery is legally open, but throughout the 1900s there were a variety of active commercial fishery operations (Stick et al. 2014).

The Herring spawning period typically extends from January through April in Puget Sound (except the Cherry Point and Elliott Bay Stocks which start later around March and run through June) (Stick et al. 2014). Decades of observations on spawning habitat and egg deposition of *Clupea pallasii* in Puget Sound have shed little light on the details of the larval stage. Spawning habitat is not considered to be limited in Puget Sound (Shelton et al. 2014). However, in 2015, the Puget Sound Partnership designated the Pacific Herring a Vital Sign ecosystem indicator for the region and noted that spawning biomass is decreasing and is below the 25 year mean (Hamel et al. 2015). Vital Sign indicators "provide insight into ecosystem health and recovery progress of Puget Sound. This set of measurements tracks a variety of different conditions related to Puget Sound recovery (Hamel et al. 2015)."

Herring eggs are demersal and adhere to eel grass beds and other marine vegetation in the intertidal zone (Stick et al. 2014). Eggs range in size from 1.3-1.7 mm in diameter (Matarese et al. 1989). Herring eggs in Puget Sound incubate for 10 to 14 days before hatching (Penttila 2007). Pacific Herring larvae hatch at a standard length of 8.5 to 9.0 mm in the Strait of Georgia (Arai and Hay 1982). Their larvae spend about three months in the plankton (Stick and Lindquist 2009). The yolk-sac is absorbed by one week of age at which time they must begin feeding (Penttila 2007).

Little is known of the ecology of the Pacific Sand Lance in Puget Sound (Penttila 2007). Its range extends across the Pacific Ocean from the Bering Sea in the north, west to the Sea of Japan, and to California in the south (Pietsch and Orr 2015). Adult *Ammodytes personatus* bury themselves in substrate for part of the day. Penttila (2007) noted that Pacific Sand Lance larvae are common in nearshore waters in winter months. Routine stock assessments are not performed for Sand Lance in Puget Sound (Penttila 2007). There has never been a commercial fishery for Pacific Sand Lance in Puget Sound, and there is currently a ban on harvest in recognition of their integral ecological role as forage for other species (Penttila 2007).

Pacific Sand Lance spawning occurs from November through February (Moulton and Penttila 2001). Records of spawning habitat have existed since 1989, but no routine assessment is performed (Penttila 2007). Throughout The Sound approximately ten percent of nearshore habitat is used annually for Sand Lance spawning (Penttila 2007). Sites are used multiple times throughout the season for batch spawning (Penttila 2007). In Puget Sound, Pacific Sand Lance spawning beds are widely dispersed along nearshore beach habitat which has made it difficult to pinpoint discrete spawning populations (Penttila 2007).

Sand Lance spawn adhesive eggs on gravel and sand beaches, and incubation lasts about a month (Penttila 2007). In contrast, the incubation time is more than two months in the northern Gulf of Alaska (Robards et al. 1999), likely due to lower water temperature which slow developmental rates. Eggs are small, less than 1 mm in diameter (Doyle and Mier 2012). Larvae hatch at about 5 mm in diameter (Hart 1973). The duration of the pelagic larval phase of *Ammodytes hexapterus* (a closely related species to the north) is quite long, more than two months (Robards et al. 1999). First feeding does not begin until the yolk-sac is absorbed after approximately 12 days (Yamashita and Aoyama 1985).

The Starry Flounder, *Platichthys stellatus,* is common in the North Pacific Ocean coastal waters and river systems and occurs in a wide range of depths from the surface down to 600 m (Orcutt 1950). This euryhaline species can be found upriver or in oceanic water and is common in estuarine environments. Puget Sound is an especially deep estuary (Ebbesmeyer et al. 1988) that provides habitat suitable to Starry Flounder during all stages of its lifecycle (Orcutt 1950). It is present throughout Puget Sound (Pietsch and Orr 2015). In the mid-1990s, Palsson et al. (1996) reported that the stock was being overfished in North Puget Sound. In Puget Sound, commercial fisheries catch 68,000 to 272,150 kg of Starry Flounder per year (Palsson et al. 1996). The trawl fishery for Starry Flounder in South Puget Sound ended in 1989 (Palsson et al. 1996). Historically, catch from trawls in Washington and Oregon combined was significantly greater than in California, but by the 2000s commercial catch had dwindled in all three regions.

Platichthys stellatus spawning season in Washington and British Columbia occurs from February through April near fresh water (Hart 1973, Emmett 1991). Spawning is known to occur in shallow waters (<16 m) near freshwater input (Orcutt 1950). During broadcast spawning, buoyant eggs are released to the pelagic environment (Orcutt 1950, Ralston 2006). Less than three to five days after fertilization, the larvae hatch. Duration of the larval stage can last from one to three months, depending on water temperature and genetics (Policansky 1982). Larvae hatch at about 2 mm in length (Orcutt 1950). The yolk-sac is depleted by about four days post-hatch, but depends on water temperature (Orcutt 1950).

Doyle and Mier (2012) studied fish larvae from the Gulf of Alaska and proposed that exposure profiles are linked to early life history strategies and indicative of a response to environmental forcing. The three focal species of this study have different exposure/response profiles. Herring spawning begins in April, egg and larval duration are considered short, their young larvae are competent, and they become sexually mature at two to four years (Matarese et al. 1989). For Herring, there is less risk of hatching when food is sparse because larvae are present along with the spring phytoplankton bloom, but more risk of encountering predators (Doyle and Mier 2012). In contrast, Sand Lance spawning beings in November, egg and larval duration is extended, young larvae are relatively weak, and it becomes sexually mature at about two years (Robards et al. 1999). A major risk for early stage Sand Lance larvae is drift into unfavorable habitat and exposure to a wide variety of conditions due to its extended larval phase in the pelagic environment. Starry Flounder early life history ecology is characterized by spawning that begins in February, small quickly developing eggs and larvae, and larvae that transform at a very small size. Like all flatfish, one eye migrates to the across the head to the other side, a process that is energetically taxing (Matarese et al. 1989). This phenology is associated with an environmental exposure profile based on spending a short time in the plankton which results in less exposure to pelagic environmental conditions. These exposure/response connections are dictated by habitat and ontogeny, which in concert determine an individual species' vulnerabilities to environmental forcing.

1.2 Objectives

The objectives of this project are to describe the spatial and temporal patterns of occurrence and abundance of the larvae of *Clupea pallasii*, *Ammodytes personatus*, and *Platichthys stellatus* in Puget Sound nearshore habitat. Specifically, the aim is to document species level differences in occurrence by month and across Puget Sound basins and describe patterns of abundance by developmental stage to identify early life history habitat locations of importance. For each species, spatio-temporal trends in frequency of occurrence and mean abundance of larvae in samples will be quantified, as well as variation in larval fish size.

2. Methods

2.1 Region of Study and Sample Collection and Processing

The samples that are reported on in this paper were collected from April 2011 through February 2012 at multiple stations within each of Puget Sound's six basins. This collection marks the largest larval fish collection effort in The Sound to date. For description of the region of study and sample collection and processing, see chapter 1.

2.2 Data Analysis

The frequency of occurrence (percent of samples in which it was present) of each species was calculated and then plotted in a bar chart for each month of the study. Then, the mean abundance (average number of specimens per sample), proportion of specimens collected at each life stage, and average standard length of specimens in each month was calculated and plotted. The yolk-sac stage can range in duration from days to weeks, thus the presence of a yolk-sac may indicate a recent hatching event. For species with a short yolk-sac phase, such as *Platichthys stellatus*, presence of a yolk-sac is more likely to indicate a recent hatching event.

The density of larvae of each focal species at each site in April (for which flow meter data was available) was calculated to better understand spatial heterogeneity and abundance of each species within The Sound during the period of peak abundance in the dataset. Density (#/1000 m^3) was estimated by dividing the number of specimens by the volume of water sampled for each individual plankton sample using the following equation:

$$Density = 1000 * (\#\ of\ specimens)/\pi r^2 d \quad (1)$$

where *r* is equal to the radius of the net opening (0.5 m) and *d* is equal to the distance towed in meters as estimated by the flowmeter. The density estimate is based on 1000 meters because density was generally low and for congruence with historical data sets such as Waldron (1972).

Density estimates were plotted in ArcMAP using a heat map for relative density. The heat map was split into five brackets that were manually chosen. This method was used because there was a large range in density estimates and most of the samples had relatively low density. The five brackets were chosen to best represent the full density range as well as the most common density estimates. For samples for which flow meter data was unavailable a triangle was used to designate that the species of interest was present. For comparison, a square was used to designate samples in which fish larvae but not the species of interest were collected.

Presence/absence data for each species was included in a Generalized Linear Model (GLM). This analysis helped to determine in which months or basins there were significant differences in the presence of larvae for each species. A GLM is a type of univariate regression model that is appropriate for use if response variables have a non-normal distribution that is within the exponential family, including binary response variables such as presence/absence data (McCullagh and Nelder 1989). This statistical analysis is a combination of linear, logistic, and Poisson regression models and uses iteratively reweighted least squares method of maximum likelihood estimation of the model parameters. Five models were tested for best fit with the following independent variables: (1) intercept only, (2) basin, (3) month, (4) basin and month, and (5) month and basin plus their interaction. The Akaike Information Criterion (AIC) was calculated for each model and the ΔAIC was calculated to compare between models and determine the best fit model. The model with the lowest AIC was considered the best fit model and any model with at a ΔAIC score of less than four could also be considered a good fit.

To explore the results of the GLM analysis further, the coefficients and their standard errors were calculated for the best fit model for each species. The coefficients are log-odds ratios that estimate the predicted difference from one month or basin to another. The log-odds ratio estimates the likelihood of occurrence divided by the likelihood of absence. A negative value for a coefficient suggests absence where as a positive value suggests presence. A determination of the likelihood that a species would be present in a specific basin was made based on visually assessing the coefficients and their standard errors.

3. Results

3.1 Pacific Herring

A total of 2,822 specimens of *Clupea pallasii* was caught during this study, far more than any other species. Specimens were present from April through October 2011 and in 19.4 percent of all samples. Specimens were absent from samples collected in September and November through February. It is likely that larger larvae evaded the net and were present but not captured in other months. During April, the month of peak abundance for *Clupea pallasii*, specimens were caught in over half (54.4 percent) of samples and the average abundance (average number of specimens per sample) was almost six times greater than any other month (average of 63.8 specimens/sample in April versus 11.3 specimens/sample or less for each other month) and higher than for any other species collected (Figure 2.1a). In the subsequent months both frequency of occurrence and abundance decreased significantly.

All larval developmental stages of Herring, besides transformation stage, were collected. In April samples, yolk-sac, pre-flexion, flexion, and post-flexion stages were all collected, suggesting that the specimens were the product of multiple spawning events. During this month flexion stage larvae were most often collected, followed by pre-flexion, then post-flexion, then yolk-sac larvae. In the following month, only flexion larvae and post-flexion larvae were collected. In June, yolk-sac larvae again appeared in the sample collection, along with pre-flexion, and flexion larvae, indicating an additional spawning event. After this month no yolk-sac or post-flexion larvae were collected except one instance of a specimen with a yolk-sac in October in Rosario Basin. Presence of this specimen suggests a late spawning event in Rosario Basin.

In April, when all larval developmental stages were present, including yolk-sac and pre-flexion larvae, the average standard length was quite small, 4.9 mm, reflecting the young larvae that were present (Figure 2.2a). In May, when only later stage larvae were present, the average length jumped to 19.6 mm. When yolk-sac larvae were again present in June the standard length dipped down to 7.4 mm and then rose to an average of 11 mm for July and August when flexion and post-flexion larvae were present. In October, when yolk-sac, flexion and post-flexion larvae were present, the average standard length was 6.7 mm.

Herring was present in all basins in April, but larvae were not evenly distributed (Figure 2.3a). In basins for which density estimates were available, density ranged from 4.98 to 8,068.74 larvae/1000 m^3. In a quarter of the samples density was higher than 100 larvae/1000 m^3, suggesting typically low density at most sites. "Hot Spots," regions with a density of more than 500 specimens/1000 m^3, were present in Rosario, Whidbey, and South basins, indicating that habitat in both north and south Puget Sound is utilized by Herring larvae. While density estimates were not available for Hood Canal Basin, Herring was present along most of the canal, except at the conjunction with Admiralty Basin which was notably devoid of Herring. Central Basin was characterized by low density and appears, along with Admiralty Basin, to be less utilized by Herring as early life history habitat.

The GLM analysis supported the presence/absence patterns documented for *Clupea pallasii* in this study. *Clupea pallasii* was likely to be absent in Admiralty Basin and more likely to be present in April relative to any other month, particularly in comparison to fall and winter months. Likelihood in October, however, was similar to summer months. Model 4, which included both "month" and "basin" as variables, had the lowest AIC, indicating that it was the best fit of the models tested (Table 2.1). No other model was considered equally well-fit. The standard errors for the basins were relatively small suggesting that the coefficients were accurate. The standard errors for the months, however, were as much as two orders of magnitude larger than the coefficients. This was due to the many instances in which Herring was not present. Because of the large number of "zero" data points, it was not possible to calculate the lower bounds for the standard error.

3.2 Pacific Sand Lance

A total of 1,091 *Ammodytes personatus* larvae was collected, making it the second most abundant species caught during this study. *Ammodytes personatus* was present in 18.7 percent of all samples. Pacific Sand Lance was only present within the catch in the months of April and May 2011 and January and February 2012. It is likely that larger larvae evaded the net and were present but not captured in other months. In April 2011 and January and February 2012 Sand Lance was relatively abundant (frequency of occurrence ranged from 50 to 60 percent of samples in those months), and in May 2011 frequency of occurrence of larvae was significantly lower at 12.8 percent (Figure 2.1b).

During April, all stages were present and in May only flexion and later stage larvae were present (Figure 2.2b). Yolk-sac larvae were only present in April and accounted for only 0.4 percent of specimens, indicating that spawning occurred much earlier. Sampling after October 2011 was significantly reduced and yolk-sac larvae were not collected during this period. In 2012, larvae were present in January and February and all specimens during these months were in the pre-flexion stage. Spawning would have occurred from November through February and limited sampling during winter months did not collect any yolk-sac larvae. The length data corroborated the developmental stage data with larger larvae represented in the months when later stage larvae were present. During January and February, when only pre-flexion larvae were present, the standard length rose only slightly from one month to the next.

In April, Sand Lance density ranged from 3.87 to 740.00 larvae/1000 m^3, a span of more than two orders of magnitude (Figure 2.3b). Most sites had fairly low density, and “hot spots” in which density was more than 380 specimens/1000 m^3 occurred in only the northernmost basins, Rosario and Whidbey. Only two Sand Lance larvae were recorded in Hood Canal and were collected in April. Sand Lance larvae were absent from southern Hood Canal and the inlets of South Basin during April, suggesting that Sand Lance do not utilize this area for spawning and/or rearing habitat. Density estimates were not available for Hood Canal Basin due to malfunction of the flowmeter.

A combination of both “month” and “basin” best predicted the presence of Sand Lance larvae (Table 2.1). Model 4, which included both “month” and “basin” as variables had the lowest AIC score (158.1). Model 3, which included only “month” as an independent variable, was the second best fit model with a ΔAIC score of 23.7 (Table 2.1). Generally only ΔAIC scores of less than four are considered comparably well-fit.

Based on the results of the GLM analysis, *Ammodytes personatus* was likely to be present in Admiralty relative to other basins and likely to be absent summer through early winter (June through December) relative to April. It was almost as likely to be present in May, January, and February as in April. The standard errors for the basin coefficients were relatively small suggesting that the coefficients were accurate. The standard errors for the month coefficients, however, were as much as two orders of magnitude larger than the coefficients. This was due to the many instances in which Sand Lance was not present and resulted in many “zero” values in the dataset. These results give credence to the abundance analysis which suggests that Sand Lance larvae are associated with winter and spring months and utilize northern Puget Sound as early life history habitat.

3.3 Starry Flounder

A total of 386 specimens of *Platichthys stellatus* was caught during this study, making it the fourth most abundant species in the dataset. It was present in 21.8 percent of samples and in the months of April, May, and June 2011 and February 2012. Based on frequency of occurrence estimates, May (followed by April) was the period of peak abundance and individuals were present in 54.3 and 44.3 percent of samples, respectively (Figure 2.1c). The average number of specimens caught per sample was highest in April with an average of 6.6 specimens per sample, followed by May with 5.8 specimens per sample (Figure 2.2c). Starry Flounder was also present in June 2011 and February 2012 but in much lower numbers. It is likely that larger larvae evaded the net and were present but not captured in other months.

In April and May all stages of larvae except the transformation stage were present, but by June only later stage larvae were present. After this period, Starry Flounder was absent from samples until February 2012 when pre-flexion larvae appeared. Spawning must have occurred prior to February 2012 and yolk-sac larvae were not collected likely due to limited sampling during winter months. The average standard length of specimens varied little over the sampling period (3.8 to 6.1 mm) and was highest in June when only flexion larvae were present. This reflects the short duration of Starry Flounder larvae within the plankton.

Density in April ranged from 3.88 to 370.24 specimens/1000 m^3 (Figure 2.3c). South Basin had the highest density of Starry Flounder for the month and was the only basin with “hot spots” (density of more than 185 specimens/1000 m^3). These “hot spots” occurred in the inlets of South Basin. Starry Flounder is an estuary-associated species and nearby Nisqually River may influence the high density of larval Starry Flounder in the area. All other basins were relatively depauperate in *Platichthys stellatus* and when they were present it was at low density (<70 specimens/1000 m^3). Only two Starry Flounder specimens were recorded in Hood Canal throughout the study and occurred in April, suggesting that spawning adults and/or larvae do not utilize the region for habitat.

A combination of both “month” and “basin” best predicted the presence of Flounder larvae (Table 2.1). Model 4, which included both “basin” and “month” as variables, had the lowest AIC score indicating it was the best fit model. *Platichthys stellatus* was likely to be present in Rosario, Whidbey, Central, and South basins relative to Admiralty and was less likely to be present in Hood Canal. Relative to April, it was likely to be absent July through December and in February and it was about as likely to be present in May, June, and January, reflecting the abundance data which showed Starry Flounder to be absent from the dataset in late summer and fall months. The standard errors for the basin coefficients were relatively small suggesting that the coefficients were accurate. The standard errors for the month coefficients, however, were as much as two orders of magnitude larger than the coefficients. This was due to the many instances in which larvae were not present, which resulted in many “zero” values in the dataset.

4. Discussion

The aim of this study was to describe the spatial and temporal patterns in occurrence and abundance of the larvae of *Clupea pallasii*, *Ammodytes personatus*, and *Platichthys stellatus* in Puget Sound nearshore habitat. Additionally, it aimed to document species-level differences in occurrence by month and across Puget Sound basins and to describe patterns of abundance by developmental stage to identify early life history habitat “hot spots". This is the first time a detailed analysis of early life history patterns has been reported for these species within Puget Sound and this analysis confirmed that nearshore habitat in Puget Sound is valuable year-round and in all six basins as pelagic habitat for fish larvae of multiple species. Upon review, each species had distinct spatial and temporal patterns of occurrence and abundance. This means that species have different exposure to environmental forcing with different implications for management. The results described here lay the groundwork for future studies on the early life history ecology of marine fishes in Puget Sound and highlight the importance of species-specific research to understand the ecology of different fish populations.

The results of this study revealed seasonal and distributional differences among the three species, which in turn result in different environmental exposure. Environmental factors of importance include water temperature, food availability, predation, and advection to favorable or unfavorable habitat (Fuiman and Werner 2002). Thus, the finding of distinct spatio-temporal habitat use implies that exposure to these forcing factors are likely to also be distinct. Hence, species-specific research will be useful to reveal the main causes of variation in mortality during early ontogeny and how those vary through space and time. Recognizing spatial and temporal patterns of abundance allows us to understand which environmental influences may be most relevant to a particular species' early life history survival.

In addition, this study found profound differences in the duration of the larval period. For example, Herring larvae were recorded within the Puget Sound nearshore plankton community for an extended period of time, April through October 2011 but excluding September, suggesting they adopt a "bet hedging" strategy to cope with unpredictable environmental conditions in time and space (Lambert and Ware 1984). This "bet hedging" strategy allows for mortality of certain cohorts due to unfavorable environmental conditions and "bets" that others will find favorable conditions and survive by employing batch spawning in which multiple cohorts of larvae are produced over several months. This idea is supported by the data which indicates at least two peak spawning times.

Sand Lance are known to spawn from November through February in Puget Sound. Although samples were collected during spawning season, very few yolk-sac larvae were collected. The few yolk-sac larvae collected were from April 2011 and would have been incubating in the sediment for several months before hatching. The larvae were collected from Rosario and Whidbey Basins and suggest that these basins are utilized as spawning habitat for Sand Lance. They were likely spawned during the latter half of the spawning period in January or February of 2011. Spawning events during November 2011 through February 2012 were not reflected in the dataset, likely due to limited sampling. Eggs spawned in January and February 2012 would have still been incubating during the collection period.

Starry Flounder spawning events were indicated by the presence of yolk-sac stage larvae in April and May 2011. Duration of the larval stage is very short for Starry Flounder with yolk-sac absorption occurring after about four days. Thus, these larvae spawned and hatched within the month they were collected. Yolk-sac larvae were collected from Rosario, Whidbey, Central, and South Basins, but not Admiralty or Hood Canal. This suggests that spawning is dispersed throughout most of Puget Sound basins, despite high density occurring only in South Basin.

The nearshore habitat of Puget Sound is clearly important to larval fishes as rearing habitat. With that in mind, it is necessary to take into account larval duration, seasonality, and distribution that is unique to each species when considering the value of nearshore habitat to fish larvae. For instance, Flounder larvae were observed in high concentrations in South Basin and were rarely observed in Hood Canal suggesting that South Basin provides valuable habitat for Flounder larvae whereas Hood Canal is rarely utilized by this species as early life history habitat. When it comes to evaluating the health of fish stocks within Puget Sound, fish larvae and their unique characters need to be acknowledged. It is necessary to recognize the unique patterns of abundance and distribution that characterize each species.

One of the starkest differences between species patterns in the dataset was the almost complete absence of Starry Flounder and Pacific Sand Lance larvae from Hood Canal Basin relative to the strong presence of Pacific Herring. Hood Canal is well known for hypoxic conditions during summer months (Cope and Roberts 2013). Values below 5 mg/L of dissolved oxygen are considered biologically stressful (Roberts et al. 2005). During 2011, Hood Canal Basin had the most sites with conditions that were biologically stressful at some depth and as shallow as 6 m (Greene et al. 2012). These low oxygen conditions could contribute to the absence of spawning adults or the poor viability of fish eggs and larvae. However, Starry Flounder and Pacific Sand Lance larvae are associated with winter and spring seasons when dissolved oxygen levels are higher (Cope and Roberts 2013). Herring spawn at two locations within Hood Canal (Stick et al. 2014). The Quilcene Bay stock, located in northern Hood Canal, is the largest Herring spawning stock in Puget Sound. Spawning occurs there from January through April and adults are known to migrate offshore during summer months, the period with the lowest dissolved oxygen concentrations. Spawning of Herring in south Hood Canal occurs from January through March. Adults of Starry Flounder have been recorded in Hood Canal and Pacific Herring have been recorded in the basin in large schools (Pietsch and Orr 2015). However, spawning information for Starry Flounder and Pacific Sand Lance has not be reported for Hood Canal. The results of this study do not indicate that Hood Canal is used as spawning or early life history habitat for Starry Flounder or Pacific Sand Lance.

Future research should focus on more sampling to corroborate the trends noted in this study, and to investigate interannual variation in larval fish patterns within the plankton. Knowledge of reproductive characteristics and habitat conditions during early life history is important and mostly undocumented throughout Puget Sound. During the most active periods, directly after spawning, it is recommended that the sampling period be more frequent than in this study, weekly or more often would be ideal for capturing temporal dynamics that were likely missed under the current sampling regime. For example, some locations were "hot spots" for more than one species. In Whidbey Basin, Burrows Bay was a "hot spot" for both Herring and Sand Lance larvae in April but Flounder were not present. It is possible that the lack of freshwater output near Burrows Bay was the reason that Flounder were not associated with the site. In South Basin, however, sites near Budd and Totten Inlet were "hot spots" for both Herring and Flounder but not Sand Lance. Further sampling could confirm if timing and location of spawning varies from year to year.

Herring larvae were present in high density in Rosario, Whidbey, and South Basins in April. Additionally, it was the only one of the three focal species with any significant presence in Hood Canal during April. In contrast, it was found rarely found in Admiralty Basin and only at low density in Central Basin. *Clupea pallasii* larvae are rarely caught in Gulf of Alaska coastal waters (Doyle and Mier 2012) and were collected at very low density in deep water of the Strait of Georgia (Arai and Hay 1982). Its absence from deep waters in the nearby Strait of Georgia may be a reflection of its preferred spawning habitat within the intertidal zone. Gruber et al. (1982) did not report the capture of any *Clupea pallasii* larvae during their sampling of the Southern California Bight, an inshore region south of the California Current. Additionally, Auth and Brodeur (2013) reported that Clupeids were rare in sampling projects from the California Current that spanned from the years 1940 to 2012. The absence or low density of Pacific Herring larvae from other NE Pacific waters highlights the importance of the Puget Sound region for spawning and rearing of this species.

Sand Lance larvae were present at high density in Rosario and Whidbey Basins in April and noticeably absent from most of Hood Canal and the inlets of South Basin. They were present, however at moderate density in the northern end of South Basin and to some degree in

Admiralty Basin. This pattern of high density in some regions and absence in others is in contrast to records from the Gulf of Alaska shelf region in which Pacific Sand Lance were ubiquitous (Doyle and Mier 2012). Gruber et al. (1982) did not report the capture of any *Ammodytes personatus* during their sampling of the Southern California Bight. However, further north, Brodeur et al. (2008) reported a catch dominated by Pacific Sand Lance larvae from nearshore stations off the Oregon Coast. While the Pacific Sand Lance is by no means ubiquitous throughout the sound, high density areas in April highlight the importance of northern Puget Sound, in particular, as important early life history habitat for this species.

Starry Flounder was collected at high density in the inlets of South Basin and found at low density throughout the other Puget Sound basins during April. It was noticeably absent from Hood Canal, except at a single site in April. The Nisqually Estuary is associated with large numbers of Starry Flounder (Fresh et al. 1979). The Nisqually River flows into South Basin just east of the inlets. South Basin is the primary early life history habitat for Starry Flounder identified in this study. Gruber et al. (1982) did not report the capture of any *Platichthys stellatus* during their sampling of the Southern California Bight. Additionally, Auth and Brodeur (2013) reported that Starry Flounder were rarely collected in sampling projects from the California Current that span from the years 1940 to 2012. However, it was collected off the Oregon coast by Mundy (1984) and Laroche et al. (1982). It was also collected in about 18 percent of samples from the Gulf of Alaska by Doyle et al. (2009). The present study expands on the known early life history habitat of Starry Flounder throughout the Northeast Pacific Ocean.

It is highly recommended that the month of March be added to the sampling period because data are currently lacking and the relative importance of this month is currently unknown. Given the importance of April, March may also be a period of peak abundance for larval fish species. Larvae were present in both April 2011 and February 2012 for both Sand Lance and Starry Flounder, suggesting that larvae are also present within the plankton during March. While Herring were present at high abundance during April 2011, it was not collected during February 2012. The importance of March for Herring larvae is particularly unknown given its absence from the dataset during February.

Offshore sampling should be conducted to compare with nearshore patterns and to assess the offshore region's value as larval fish rearing habitat within Puget Sound. This study collected samples in deep water (tens to hundreds of meters), but in close association with nearby land. Sampling in the middle of the basin will allow us to compare the importance of offshore habitat with what we now know of nearshore habitat. Now that some spatial and temporal patterns have been recognized, further sampling focused specifically on the period and location where larvae of a specific species are known to occur can elucidate more detailed patterns of larval duration, distribution, abundance, and associated relevant environmental factors.

This study is the most comprehensive to date regarding the early life history of these three species in Puget Sound. In 1967, Waldron collected early life history data of fishes at sites across Puget Sound during the months of April and May (Waldron 1972). Abundance and distribution of Pacific Sand Lance and Starry Flounder are different than in the Waldron study. This may reflect a population shift or differences between the current sample locations and those in the Waldron study. For example, Pacific Sand Lance was a commonly caught species during the Waldron study however, half the specimens were collected at one site in Hood Canal. This stands in contrast to the results of the current study in which Sand Lance was rarely collected in the Hood Canal Basin. Starry Flounder was the third most abundant Plueronectid and Pacific Sand Lance was the fifth most abundant taxa collected in the Waldron study. Both of these species were collected more frequently during the current study. Further collections will be necessary to confirm a baseline abundance and distribution for each of these species.

Investigations of environmental patterns associated with larval fish abundance and distribution will allow for better understanding of the mechanisms governing the distribution and survival of larval fish species in estuarine habitats. As climate change alters seasonal and weather patterns and we continue to populate and use Puget Sound shorelines, larval fish species will not all react equally to these disturbances. Understanding details of individual species early life history can help us gauge the relative importance of different environmental factors during early ontogeny. Continued effort to investigate larval fish habitat and early life history ecology for species of interest within Puget Sound will also contribute to regional assessment and management of commercially and ecologically important fishes and the ecosystem in which they reside.

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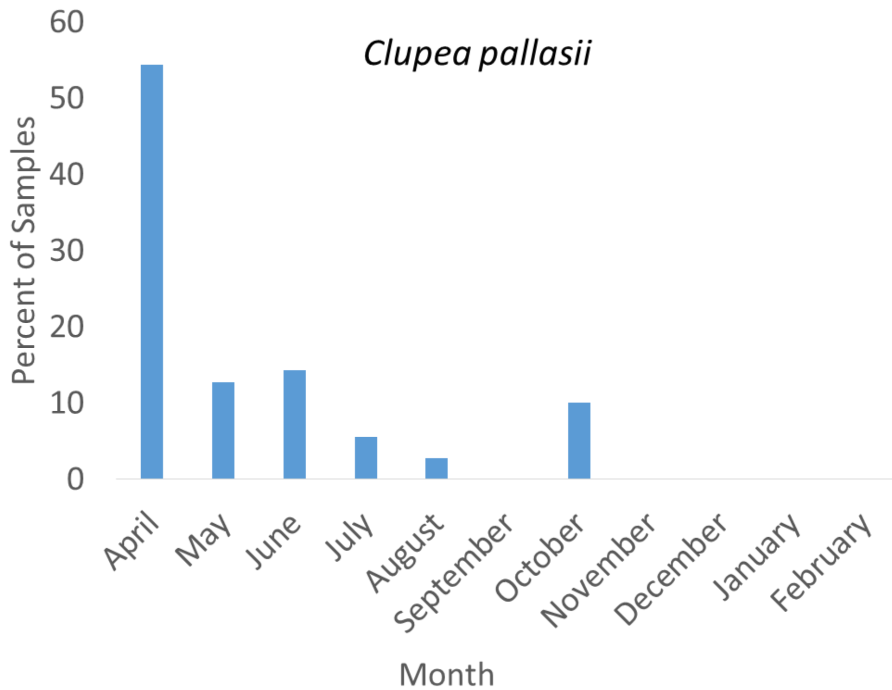
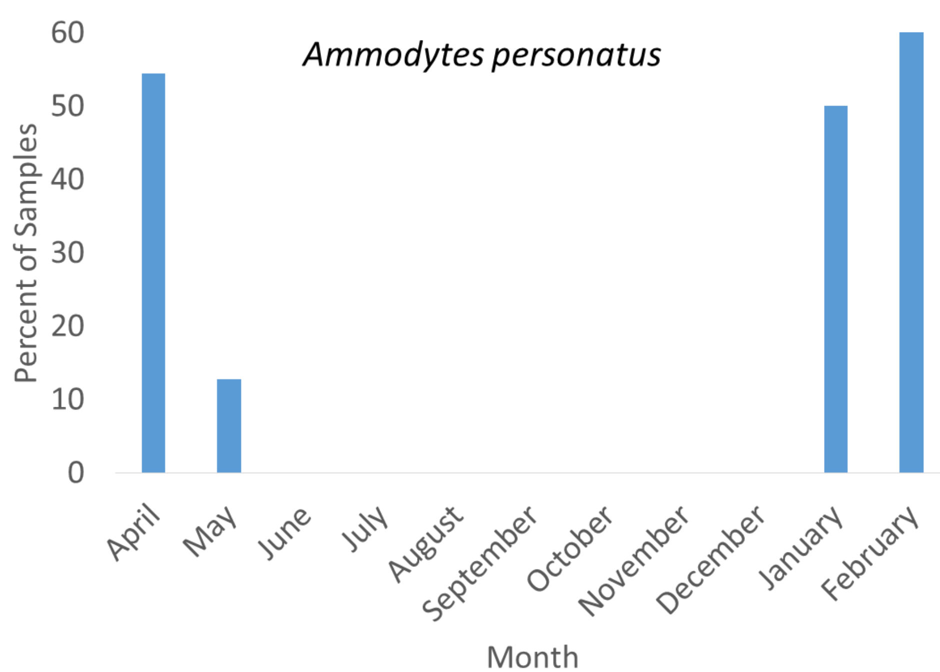


a)



b)

Figure 2.1. Percent of samples in which species was present by month for a) Pacific Herring; b) Pacific Sand Lance; c) Starry Flounder (1 of 2).

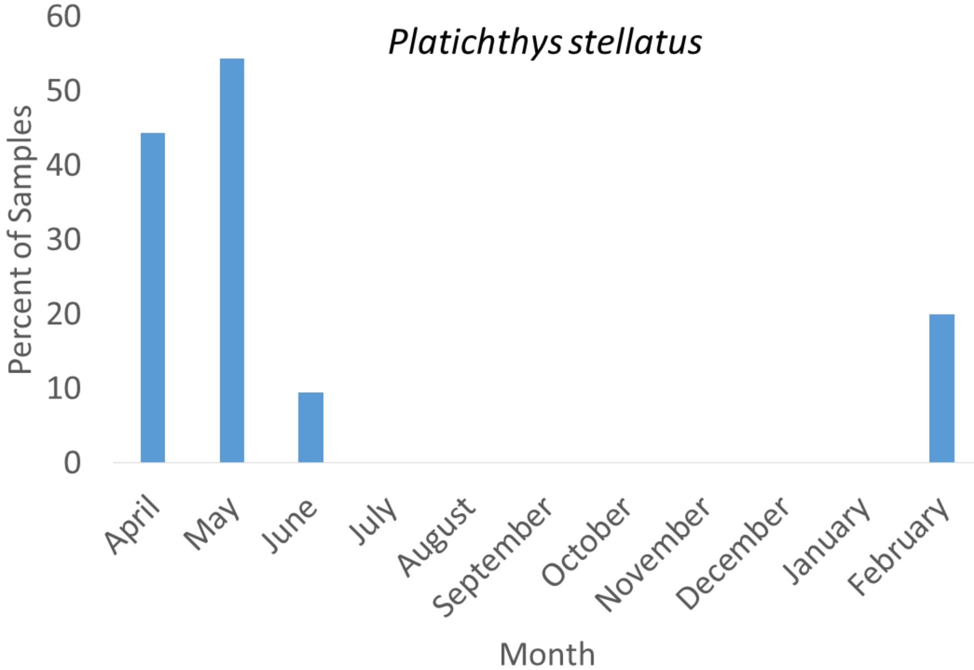


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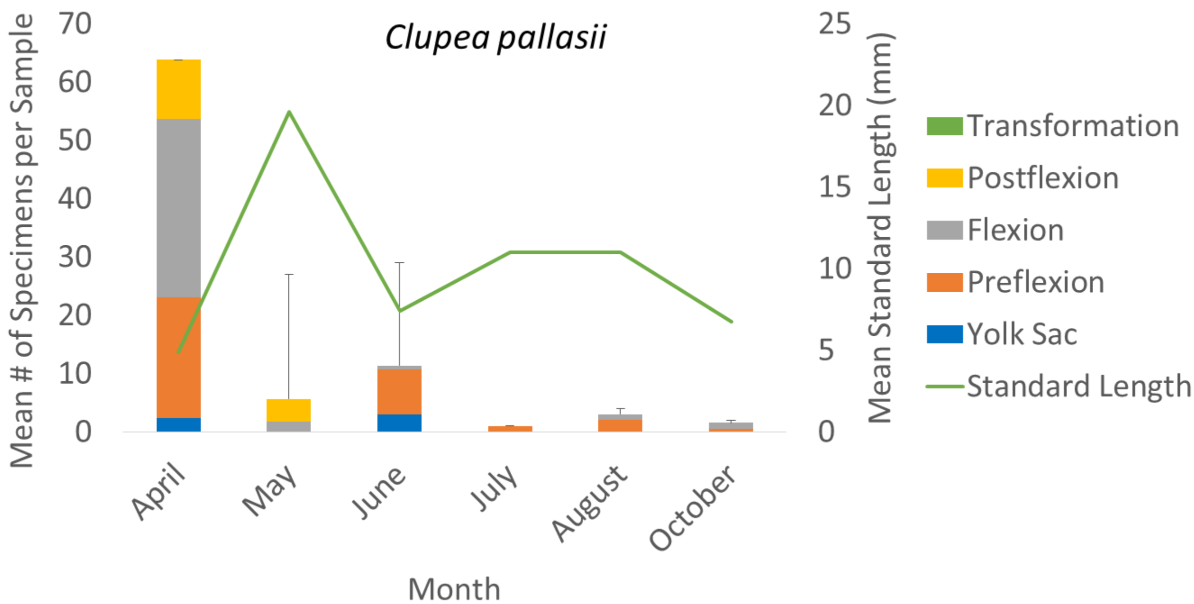
Figure 2.1. Percent of samples in which species was present by month for a) Pacific Herring; b) Pacific Sand Lance; c) Starry Flounder (2 of 2).



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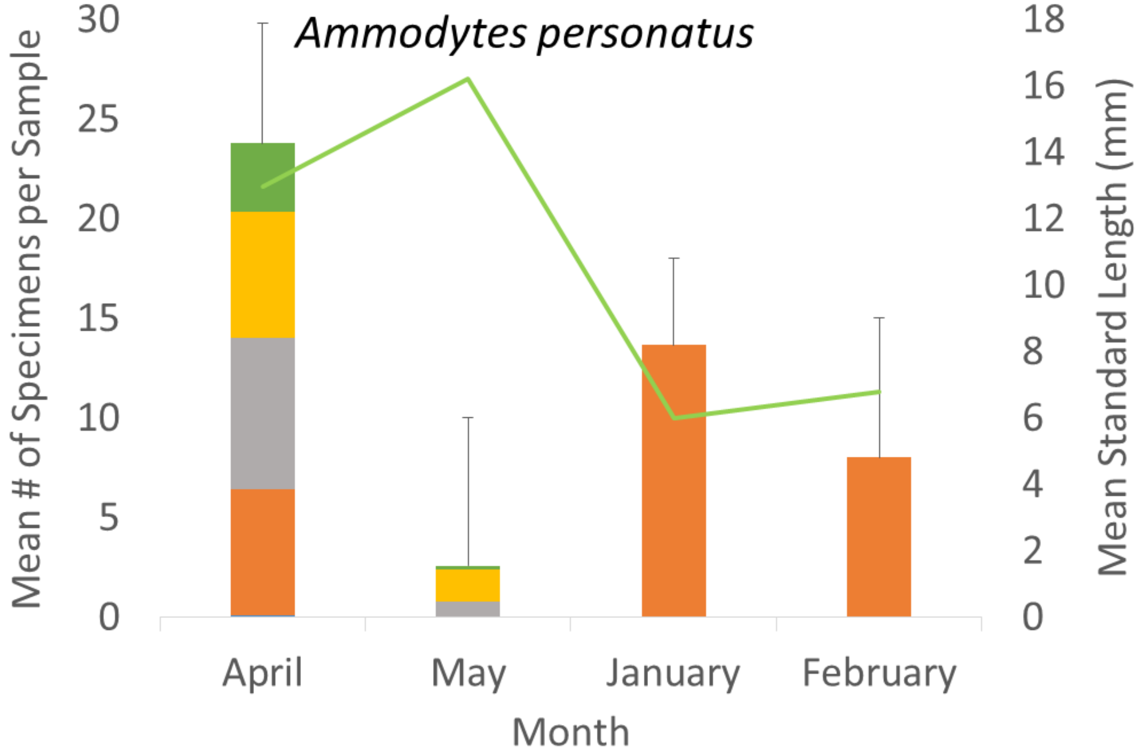
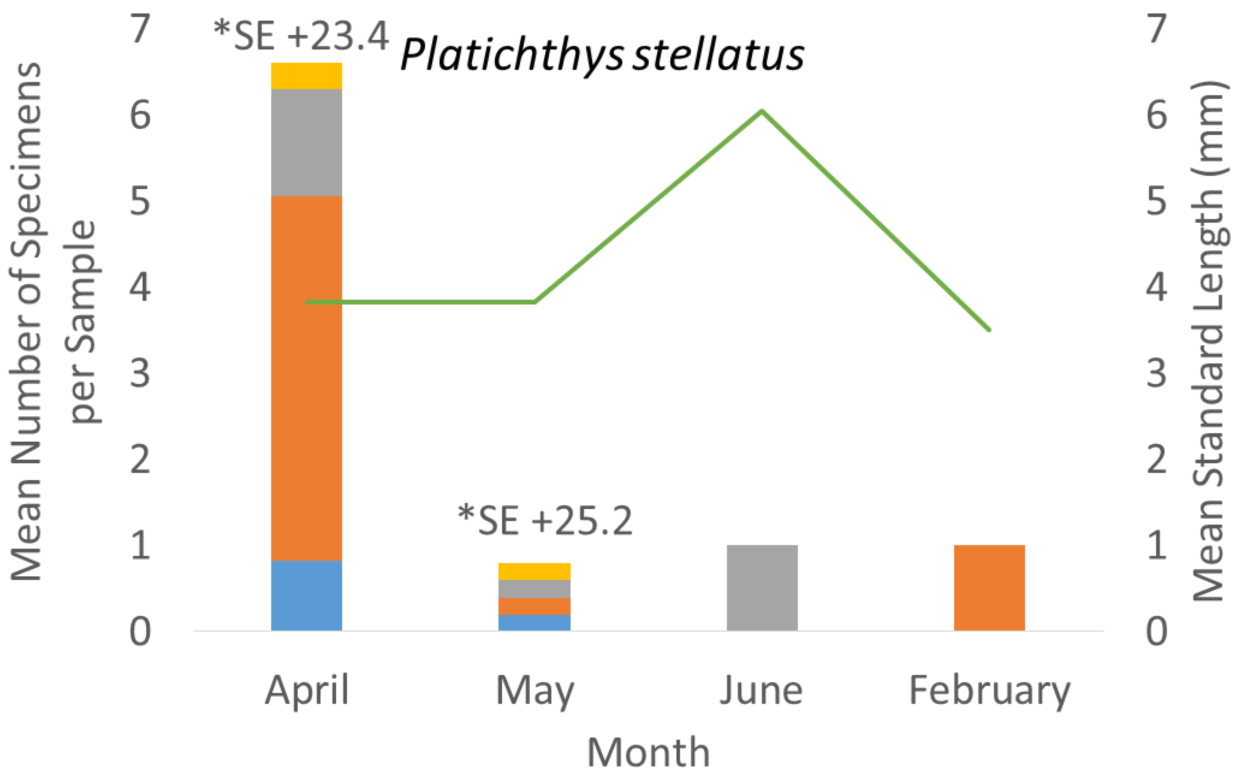


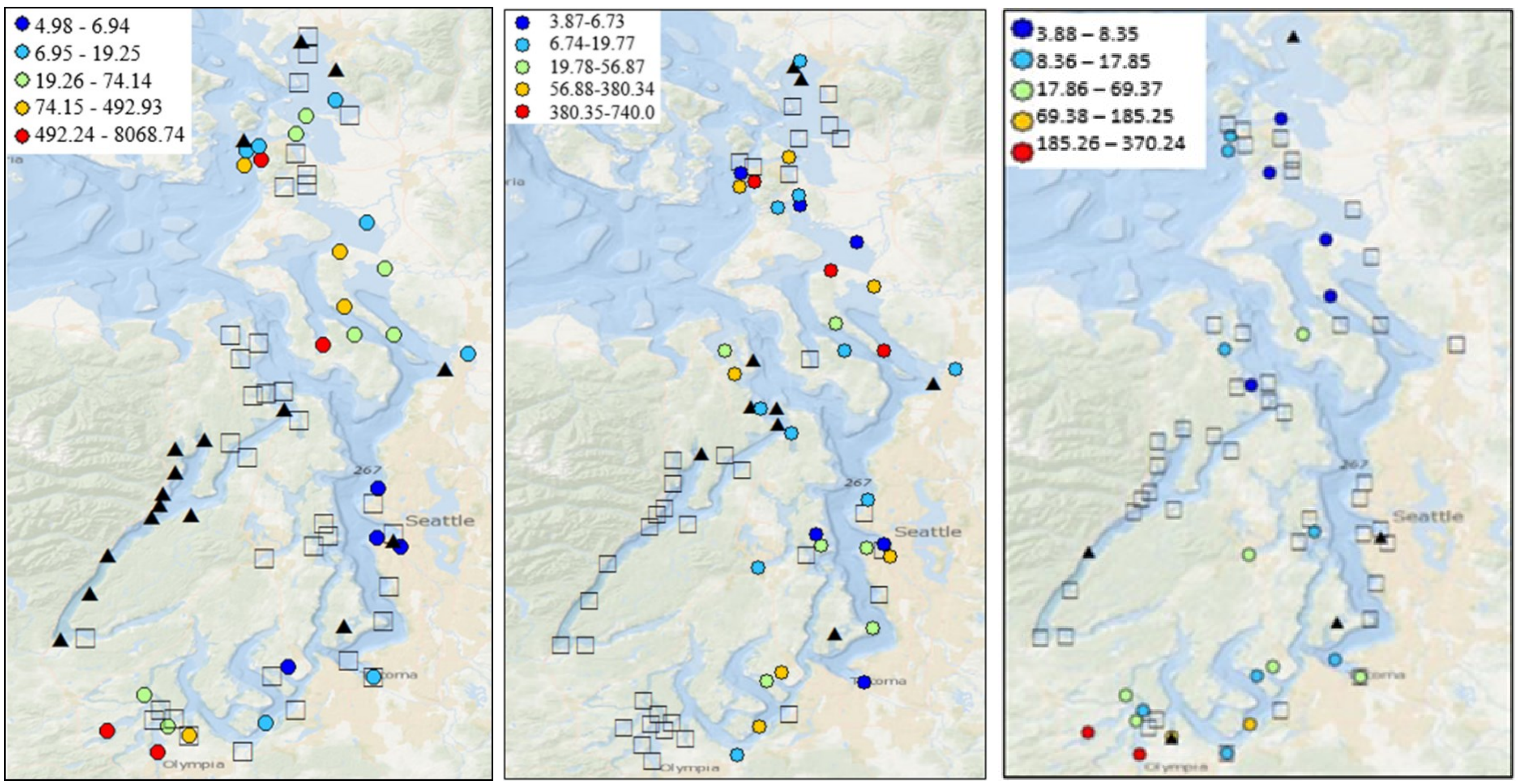
b)

Figure 2.2 Larval abundance by developmental stage (bars) and average standard length (line) by sample month for a) Pacific Herring; b) Pacific Sand Lance; c) Starry Flounder. Standard lengths based on measurement of up to 30 specimens per sample. Error bars indicate maximum number of specimens caught in a single sample for each month (1 of 2).



c)

Figure 2.2 Larval abundance by developmental stage (bars) and average standard length (line) by sample month for a) Pacific Herring; b) Pacific Sand Lance; c) Starry Flounder. Standard lengths based on measurement of up to 30 specimens per sample. Error bars indicate maximum number of specimens caught in a single sample for each month (2 of 2).



a) b) c)

Figure 2.3. Density estimates for each species during April for (a) Pacific Herring; (b) Pacific Sand Lance; (c) Starry Flounder. Colored circles represent density (#/1000m^3), triangles represent presence when density estimates were unavailable, and squares represent sites where larval fishes were present but not the species of interest.

Table 2.1. Aikike Information Criterion scores for the five generalized linear models tested to predict the presence of Pacific Herring, Pacific Sand Lance, and Starry Flounder.

Model Description	Pacific Herring		Pacific Sand Lance		Starry Flounder	
	AIC	ΔAIC	AIC	ΔAIC	AIC	ΔAIC
Null	291.2	74.0	285.4	127.3	310.1	113.8
Basin	291.9	74.8	281.7	123.6	301.4	105.1
Month	223.0	5.9	181.8	23.7	213.3	17.0
Basin + Month	217.2	0.0	158.1	0.0	196.3	0.0
Basin + Month + Basin:Month	275.9	58.7			252.3	56.0