

# *Trophic Ecology of the Pacific sand lance, *Ammodytes hexapterus*, in the San Juan Channel*

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## Abstract

Forage fish, such as the Pacific sand lance (*Ammodytes hexapterus*) occupy a critical intermediate trophic level characteristic of wasp waist food webs. Though Pacific sand lance (PSL) are prey to numerous organisms, little research has been conducted on the trophic ecology of PSL in the San Juan Channel (Salish Sea, Washington, USA). This study examined PSL prey fields in the San Juan Channel (SJC), as well as short term dietary patterns of the PSL during the autumn of 2013. Additionally, this study aims to characterize both long term nutrient assimilation and trophic position of PSL in the SJC using stable isotope analysis (SI). Zooplankton sampling revealed that PSL prey field composition was similar for all sites sampled. Results indicated that zooplankton composition and abundance in the SJC is influenced by environmental drivers including wind and tidal cycle. Gut content analysis revealed that PSL fed preferentially upon calanoid copepods. Secondary prey items included harpacticoid and cyclopoid copepods (20%), and amphipods (13%). There were no significant diel dietary patterns, but there were interannual differences, including the lack of larvaceans and copepod nauplii in 2013 gut contents. The results of SI have not been received at this time.

## Introduction

Forage fish, such as the Pacific sand lance (*Ammodytes hexapterus*), are an important prey source in the Salish Sea. The Salish Sea, which is part of the northern California Current Ecosystem (CCE), exhibits a trophic structure characteristic of eastern boundary upwelling systems (EBUS), known as a “wasp-waist” food web (Fréon *et al.*, 2009). In wasp waist food webs, a limited population of mid-trophic predators applies top-down control on producers, while also exerting bottom-up control on higher trophic levels (Cury *et al.*, 2000) Pacific sand lance (PSL), along with other small, planktivorous schooling fishes, occupy the role of mid-trophic predator in the Salish Sea.

Though they are the known prey item of 40 species of birds, 12 species of marine mammals, and 45 species of fishes, little research has been conducted on the trophic ecology of PSL in the Salish Sea (Robards *et al.*, 2000). Studies have shown that predators, including commercial fisheries species, prefer the lipid-rich tissues of PSL to other prey items, such as

crustaceans (Robards *et al.*, 2000). The availability of PSL, however, is controlled by various environmental factors, all of which are vulnerable to the effects of shifting climate regimes. Within the greater CCE, research has shown that climate change can alter forage fish dynamics, resulting in cascading trophic impacts (Sydemann *et al.*, 2001). Ultimately, PSL abundance corresponds to the availability of their primary prey source, zooplankton (Gurkan *et al.*, 2012). However, environmental instability also impacts zooplankton populations (Reum *et al.*, 2011). Various studies have indicated that zooplankton respond rapidly to physical drivers, exhibiting changes in phenology, range, abundance, and composition (Richardson, 2008). Considering these factors, it is necessary to examine the trophic relationships of PSL in the Salish Sea, and the environmental drivers that influence these relationships, more closely (Robards *et al.*, 1999).

Trophic interactions in EBUS are generally examined through gut content analysis and fisheries catch (Miller *et al.*, 2010). Though these methods offer important ecological information, they are limited in scope, offering only a snapshot of recent feeding activities (Miller *et al.*, 2010). In addition to traditional methods, stable isotope analysis (SI) of  $\delta^{12}\text{C}$ / $\delta^{13}\text{C}$  and  $\delta^{14}\text{N}$ / $\delta^{15}\text{N}$  is now being used to reveal long term nutrient assimilation and trophic position. As part of my research, I utilized both gut content analysis and stable isotope analysis to examine the trophic ecology of PSL within the Salish Sea. The primary goals of my research were to understand short term dietary patterns of PSL in the Salish Sea during the fall of 2013 and to characterize the trophic position of PSL in the San Juan Channel. In investigating these subjects, I set the following objectives:

- 1) Identify and characterize Pacific sand lance prey fields

- 2) Assess PSL diet (gut content) at Jackson Beach
- 3) Characterize long term nutrient assimilation within the San Jun Channel using SI

## **Study Site**

All data analyzed were collected in the San Juan Channel (Salish Sea, Washington, USA) during the autumn (September –November) of 2013 (Fig.1). There were four primary sampling locations, North Station (48.583434°, -123.042960°), South Station (48.426436°, -122.953539°), the Central San Juan Channel sand wave field (~48.512500°, -122.954000°), and Jackson Beach (48.519622°, -123.008197°).

## **Methods**

### PSL Collection at Jackson Beach

During a five week period in the autumn of 2013, PSL were collected at Jackson Beach. Sampling was conducted at dawn and dusk on the dates of October 11, October 14, October 18, and October 21. On November 1, sampling was conducted only at dawn and on September 30 and October 25, sampling was conducted only at dusk. Sampling was attempted on November 4 and November 15, however no sand lance were captured. Collection occurred via small boats, utilizing the seine-haul fishing method. The seine-haul, or beach seine method, employs a vertical fishing net that is leaded on the bottom edge and suspended by buoyed floats on the top edge. The net was deployed between one and four times per sampling effort and hand-dragged inland, where the collected organisms were identified. PSL kept for further analyses were euthanized with a lethal dose of tricaine methanesulfonate (MS-222), a poikilotherm anesthetic. Bycatch; including sculpins, sticklebacks, tubesnouts, shrimp, nudibranchs,

surfperch, salmon, crab, squid, and gelatinous zooplankton, were released immediately after capture.

#### PSL Collection in the San Juan Channel

Pacific sand lance were collected from the San Juan Channel on 7 dates during the fall of 2013; September 26, October 9, October 15, October 22, October 29, November 5, and November 13. Fish were sampled via the R/V Centennial from random locations within the Central San Juan Channel wave field. PSL were collected using a Van Veen grab sampler, a clamshell bucket instrument used to collect ocean sediment. The Van Veen sampler was deployed between 5 and 10 times per cruise. Fish were removed from the collected sediment and euthanized.

#### Laboratory Preparation of PSL

Following euthanasia, fish were either frozen immediately for stable isotope analysis or preserved in a solution of sea water and formalin for gut content analysis. In the laboratory, each fish was measured for fork length and total length (0.1mm), as well as daubed dry of excess water and weighed to the nearest 0.1g. Each specimen was assigned a length designated age class, according to Wylie-Echeverria method (2010, unpublished data). Fish 0-69mm were designated as age young of the year (YOY), 70-109mm were age 1, 110-129mm were age 2, and fish over 130mm were age 3.

#### Zooplankton Collection at Jackson Beach

Zooplankton tows were conducted on at Jackson Beach on the dates of October 18, October 21, October 30, November 1, and November 4. Samples were collected at dawn and dusk, or dawn or dusk, after seining was completed. Tows were taken using a 28 cm diameter,

202µm net on October 18. However for the remaining dates, a net with 28 cm diameter, 150 µm mesh was used to better compare the zooplankton assemblage at Jackson Beach to the assemblage in the San Juan Channel. A total of 9 vertical tows were taken approximately 100 meters from the shore at a depth of 20 meters, and preserved in a solution of formalin and sea water for later analysis. In the laboratory, each sample was filtered through a 118 µm mesh filter, re-suspended in fresh water, and split in half using a Folsom Plankton Splitter. A 15mL aliquot was taken from a single half sample using a Hensen-Stempel Pipette, and analyzed under a compound microscope. Individual zooplankton were identified to class and counted. Zooplankton densities per cubic meter of sea water were calculated using the following formula:

$$\left[ \left( \frac{P * D}{V} \right) * 2 \right] / [(\pi * r^2) * z]$$

Each individual sample was then quantified using the frequency of occurrence method (Ahlbeck *et al.*, 2012) using the following formula:

$$Zooplankton_i^{FO} = \frac{Z_i}{Z_T} * 100$$

Explanations for abbreviations used in the methods equations can be found in the appendix (Table 1).

### Zooplankton Collection in the San Juan Channel

Zooplankton tows were conducted during cruises on the R/V Centennial on 7 dates during the fall of 2013; September 26, October 9, October 15, October 22, October 29, November 5, and November 13. Duplicate vertical tows were taken at both North Station and South Station using a 70 cm diameter, 153 µm mesh net that was deployed to within 10 meters of the ocean floor. All zooplankton samples collected were preserved in a solution of formalin

and sea water for later analysis. In the laboratory, each sample was filtered through a 118  $\mu\text{m}$  mesh filter, re-suspended in fresh water, and split into quarters using a Folsom Plankton Splitter. A 5mL aliquot was taken from a single quarter sample using a Hensen-Stempel Pipette, and analyzed under a compound microscope. Individual zooplankton were identified to class and counted. Zooplankton densities per cubic meter of sea water were calculated using the following formula:

$$\left[ \left( \frac{P * D}{V} \right) * 4 \right] / [(\pi * r^2) * z]$$

Each individual sample was then quantified using the frequency of occurrence method.

#### Gut Content Analysis

The stomachs of 65 PSL were analyzed for gut content. Specimens analyzed were collected at Jackson Beach on the dates of September 30, October 11, October 14, October 18, and October 25. Each stomach, and its contents, was organized by collection date and time. A subsample of stomachs collected on October 14, was also organized by length. After the contents were removed from the stomachs, they were examined under a dissecting scope. Undigested or recognizable zooplankton were identified to class and counted. Zooplankton that were too digested to be identified were not counted. Prey items were then quantified using the frequency of occurrence method, using the following formula:

$$Diet_i^{FO} = \frac{F_i}{F_T} * 100$$

Additional methods for quantifying stomach contents can be found in the appendix.

#### Stable Isotope Analysis

A total of 43 PSL were collected from the San Juan Channel and from Jackson Beach for stable isotope analysis. From the Channel, 10-age 1 fish, 10-age 2 fish, and 3- age 3 fish were

collected. From Jackson Beach, 10- YOY and 10-age 1 fish were collected. After capture and euthanasia, length measurements of each fish were taken to determine an age class. The samples were then frozen at  $-80^{\circ}\text{C}$  to prevent tissue deterioration. In the laboratory, each fish was gently thawed, measured for fork length and total length (0.1mm), daubed dry of excess water and weighed to the nearest 0.1g. Each fish was also assigned a unique identification number and photographed. After dissection, individual fish were placed in labeled Whirl-Paks<sup>®</sup> and re-frozen at  $-80^{\circ}\text{C}$  in preparation for freeze drying. Whole specimens were freeze-dried for 48 hours in a VirTis<sup>®</sup> lyophilizer. After dehydration, muscle tissue was removed from the dorsal posterior end of each specimen and homogenized using a stainless steel mortar and pestle. A subsample of  $0.50 \pm 0.02\text{mg}$  was then prepared for SI of  $\delta^{12}\text{C}/\delta^{13}\text{C}$  and  $\delta^{14}\text{N}/\delta^{15}\text{N}$ . The samples were tested at Washington State University (School of Biological Sciences, P.O. Box 64236, Pullman, WA, 99164-4236, USA).

## **Results**

### Prey Field Analysis

Though zooplankton sampling was conducted at Jackson Beach, it only occurred from October 18 to November 4. Since gut contents could not be compared to zooplankton at Jackson Beach for the entire study period, it was necessary to determine another appropriate prey field. For all three sampling locations, the top five most abundant taxa were the same: calanoid copepod, copepod nauplius, larvacean, polychete larva, and amphipod. These findings are in agreement with previous PEF studies (Dykeman, 2010; Sigley, 2012). The top five most abundant taxa also occurred in similar frequencies across sites for 2013(Fig. 2.). Ultimately, North Station was selected due to its proximity to Jackson Beach. Overall, calanoid copepods

were the most abundant prey item at North Station, 34%, followed by copepod nauplii, 34%. Copepod nauplii were most abundant for the first four cruises, September 26, October 5, October 15, and October 22 (Fig. 3). However, during and following cruise 5 (October 29), there was a dip in nauplii occurrence (52% to 20%), and a spike in calanoid occurrence (16% to 57%). Additionally, there were two peaks in calanoid occurrence during the sampling period. These peaks occurred on the dates of October 15 and October 29.

### Gut Content Analysis

Calanoid copepods were the most frequently consumed prey item, comprising 65% of the diet (Fig. 4). This is in agreement to PEF research conducted by Mary Rood (2010). “Other copepods”, which include harpacticoid and cyclopoid copepods, were the second most consumed prey item, 20%, followed by amphipods, 13%. The occurrence of calanoids varied throughout the study period, peaking on October 14 and October 30. In contrast to previous studies, no nauplii or larvaceans were identified in the gut contents. Previous PEF researchers identified *Coscinodiscus* (phytoplankton) to be a prey item of PSL. In 2013, *Coscinodiscus* composed only 0.05% of the stomach contents analyzed. This value was derived using the frequency of occurrence method, and includes all fish sampled. During the course of this study, *Coscinodiscus* was only found in 2 fish (3 cells total).

Pacific sand lance are crepuscular foragers and may exhibit differential feeding patterns in relation to the time of day. In order to clarify any differences, gut contents were separated and grouped as either dawn or dusk. Overall, calanoids were the dominant prey item consumed at either time of day, comprising 65% (dawn) and 65% (dusk) of the diet (Fig. 5). There were more “other copepods” consumed at dawn, 21%, versus dusk, 17%. Amphipods occurred more

frequently in dusk samples, 15%, than in dawn samples, 10%. A Chi Square test revealed that at both dusk and dawn, PSL consumed significantly higher ( $P \leq 0.05$ ) amounts of copepods, other copepods, and amphipods than expected when compared to abundances of these prey items in the water column.

It has been previously reported that PSL diet changes with age (Inoue *et al.*, 1967; Rood, 2010). In order to better understand age dependent feeding preferences, a subsample of fish (collected October 14, 18:26 -18:36) was separated by age class and analyzed. Fish from this sample were selected because of their mostly undigested and easily identifiable stomach contents. Calanoid copepods were the primary prey item of both age classes. YOY ingested more calanoids than age 1 fish, 73% and 65% respectively. Age 1 fish consumed approximately 7% more “other copepods” than YOY fish, and approximately 3 times more amphipods (Fig. 6) Barnacle cyprids also occurred more frequently in age 1 fish (3%) When strictly considering the frequency of occurrence method, it appeared that calanoid copepods were a more important prey item for YOY fish than age 1 fish. Interestingly, observation showed that age 1 fish were preying upon larger calanoids, and larger prey items in general.

#### Stable Isotope Analysis

I am still awaiting the results of stable isotope analysis.

### **Discussion**

#### The impacts of environmental drivers on zooplankton abundance

There was obvious variability in zooplankton abundance throughout the season, which can be attributed to physical factors, including winds and tides. These phenomena have been examined by previous PEF researchers (Dykeman, 2010; Sigley, 2012), and more extensively by

Jeannette E. Zamon (2002). Zamon's research showed that cool, saline water from the Strait de Juan de Fuca is mixed into the San Juan Channel during flooding tides. With these tides come large aggregates of copepods, which are generally found near or below the pycnocline. In agreement with Zamon's results, calanoid copepod densities during the fall of 2013 were highest on flood tides (Fig. 7).

Wind direction and magnitude also have a strong influence on the availability of copepods in the San Juan Channel. During and following cruise 5 (October 29), there was influx of calanoid copepods at North Station. This influx is correlated with strong southwesterly winds which forced copepods north from the Strait de Juan de Fuca. Earlier in the month of October, strong northeasterly winds forced freshwater from the Fraser Plume toward North Station (Thompson, 2013). As the strength of these winds increased, there was a corresponding increase in copepod nauplii, which peaked on October 22 (Fig. 8). Previous PEF research has revealed a correlation between nauplii abundance and discharge from the Fraser Plume (Abernathy, 2009).

#### PSL response to changing zooplankton abundance

Multiple studies have reported that fluctuations in zooplankton and forage fish populations direct trophic interactions (Kaltenberg *et al.*, 2010). Therefore it is necessary to understand how PSL respond to varying plankton abundance and distribution (Robards *et al.*, 1999). During the period of this study, PSL fed upon 12 taxa of prey, including crustaceans, molluscs, and larval fish. These findings are in agreement with studies conducted recently by Hipfner & Galbraith, (2013), and are comparable to the diets of other species of sand lance in various locations. Though several prey taxa were available, PSL fed primarily upon calanoid

copepods (Fig. 4). Even more, sand lance appeared to increase their feeding as calanoids became more abundant in the water column. This accounts for two peaks in calanoid consumption, occurring on October 14 and October 30 (Fig. 9)

#### *Diel and interannual dietary patterns*

There do not appear to be significant differences in prey choice at dawn or dusk. However, there do appear to be interannual differences. In 2010, larvaceans comprised a significant portion of the diets of PSL collected at Jackson Beach (Rood). However, in 2013, there were no larvaceans found in the diets though larvacean abundances at North Station were similar in both years (16% in 2010, 11% in 2013) (Dykeman, 2010). This was also seen with copepod nauplii. In 2010, nauplii composed 22% of the zooplankton community at North Station. In 2013, nauplii composed a greater percentage of the community, 33%, but there were no nauplii found in the diets. There could be various explanations for these occurrences. Previous research states that prey items such as larvaceans, are only prominent in the diets of Pacific sand lance when calanoids are not available (Purcell & Sturdevant, 2001). However, copepods were abundant in both 2010 and 2013, comprising 52% and 34% of the assemblages, respectively. It is also possible that due to the patchy distribution of zooplankton, certain prey items are not consistently available while sand lance are foraging (Omori *et al.*, 1982). It is also necessary to take in consideration that larvaceans or nauplii may have been present in the gut contents but were unrecognizable due to advanced digestion. However, a larger sample size was analyzed in 2013 than 2010 ( $N_{2010}=20$ ,  $N_{2013}=65$ ) to increase the accuracy of analysis.

## Stable Isotope Analysis

The purpose of conducting stable isotope analysis for this study was to characterize the trophic position of *A. hexapterus* in the San Juan Channel, as well as to better understand prey source and selection (Miller *et al.*, 2012). In doing so, I aim to add to the growing body of knowledge concerning *A. hexapterus* in the Salish Sea and to elucidate differences between the Pacific sand lance population at Jackson Beach and the population in the Central San Juan Channel sand wave field. Research has shown that the stomachs of sand lance collected in the Channel are commonly empty or contain highly digested, and therefore, unquantifiable prey (Pham, 2013; Sisson 2012). Being that gut content analysis is uninformative in this case; stable isotope analysis offers the best opportunity for distinguishing possible differences.

In 2010, Miller *et al.* established the trophic position of PSL in the northern CCE. Though the Salish Sea is part of the CCE, it is an estuarine environment with biological and physical properties distinct from those of the greater NCC. The results presented by Miller do, however, provide a baseline to which the results of this study can be compared.

## **Acknowledgements**

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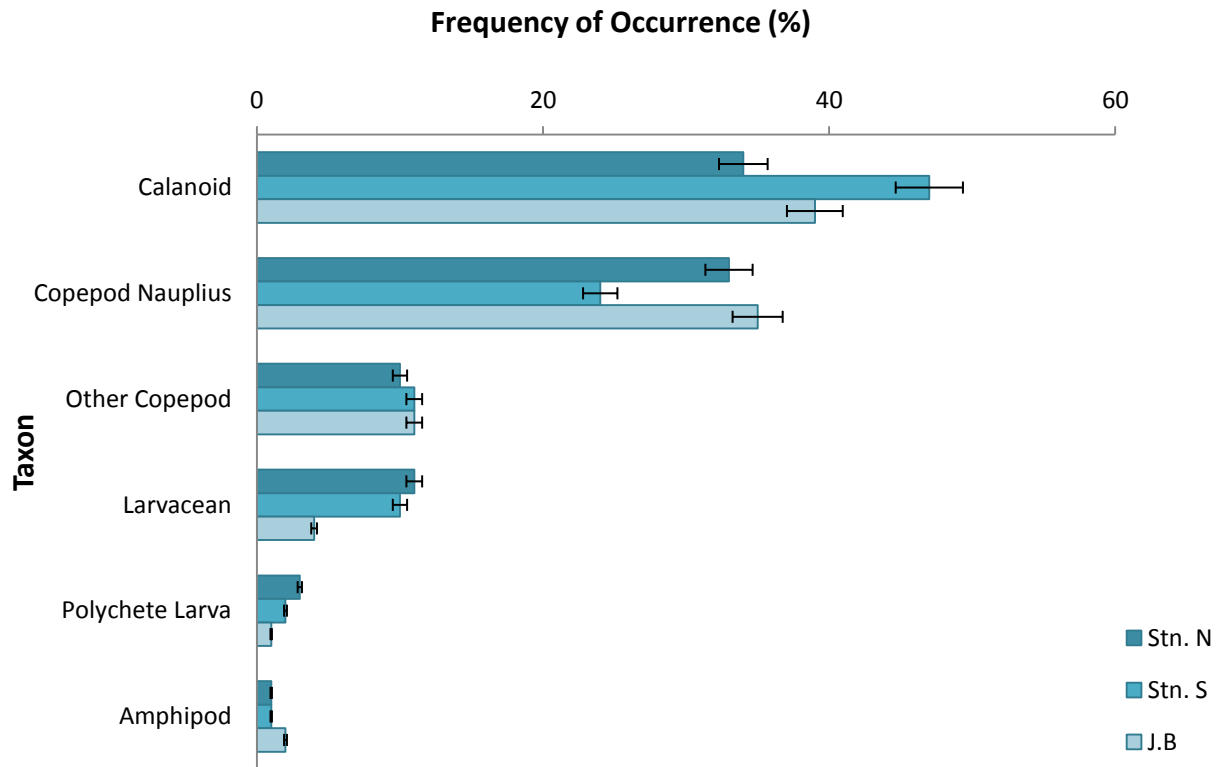
## Appendix

Table 1: Legend for formulas listed in the methods section.

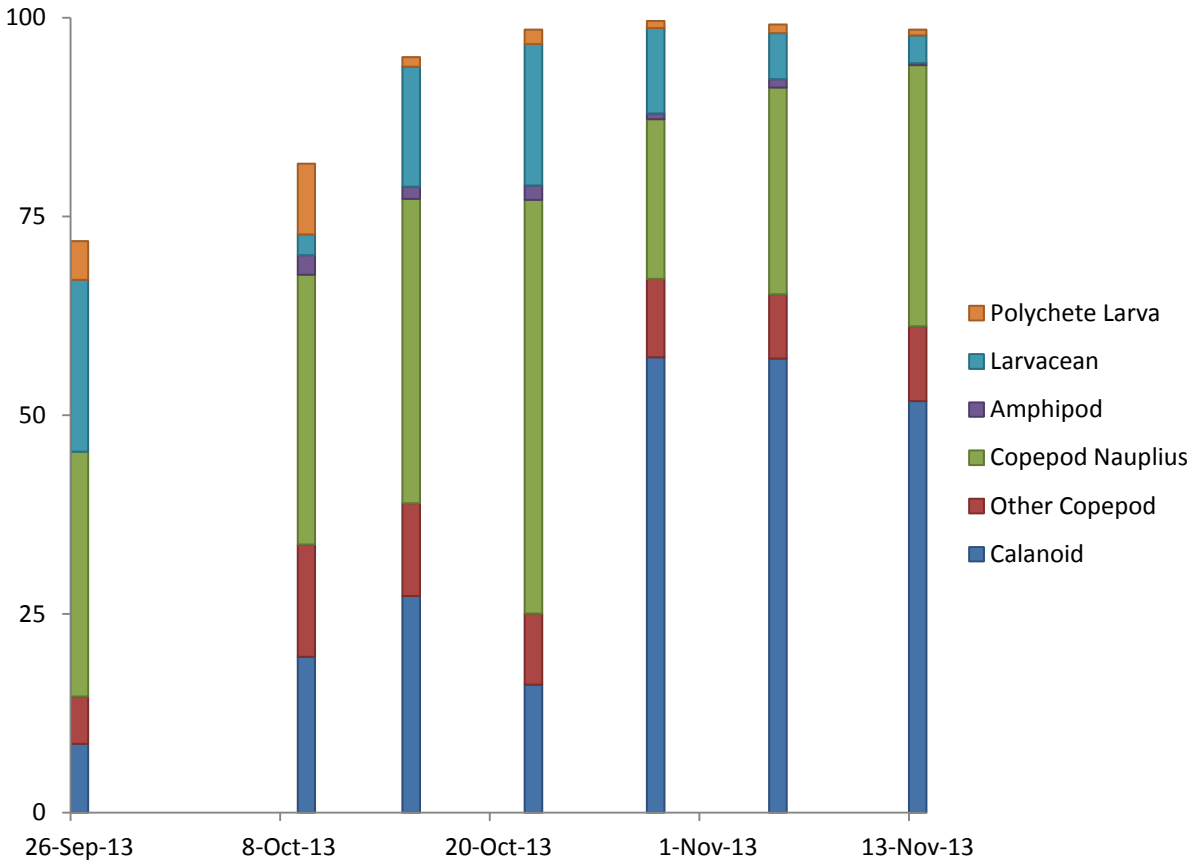
P	Prey items per taxonomic group
D	Diluted volume
V	Aliquot (mL)
r	Radius of the net
z	Depth
FO	Frequency of occurrence
$Z_i$	All individuals in a taxonomic group
$Z_t$	Total zooplankton in entire sample
$F_i$	Individual fish
$F_t$	Total fish sampled



**Figure 1: Study sites in the San Juan Channel (Salish Sea, Washington, USA). Fish were collected at Jackson Beach and at the sand wave field. Zooplankton were collected at North Station, South Station, and Jackson Beach.**



**Figure 2: Abundances of the most frequently occurring zooplankton at North Station (N), South Station (S), and Jackson Beach (JB). Zooplankton abundances amongst stations were similar overall. Error bars represent a value of 5 percent.**



**Figure 3: Abundances of the most frequently occurring zooplankton at North Station for the duration of the sampling period (Sept 26, 2013- Nov, 13, 2013). Copepod nauplii were the most frequently occurring zooplankton until Oct. 22, after which calanoid copepods become the most abundant.**

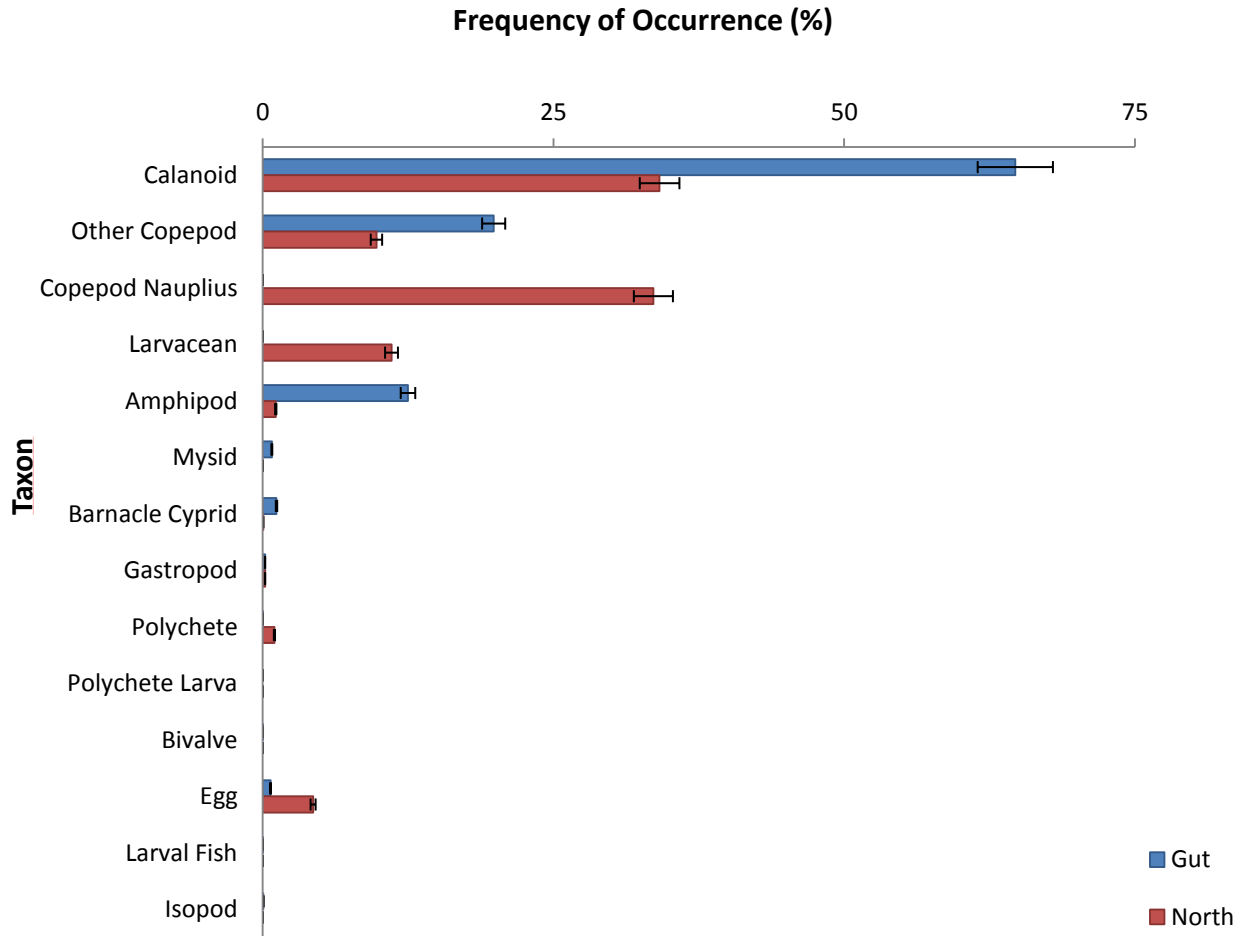
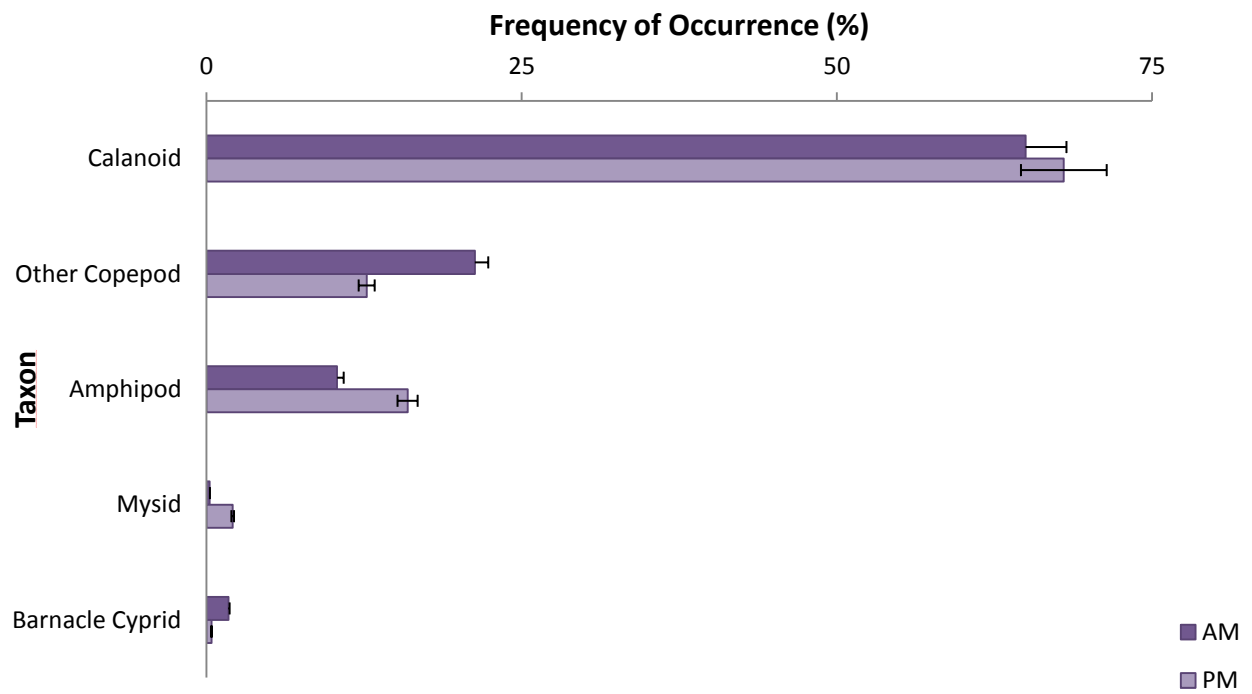
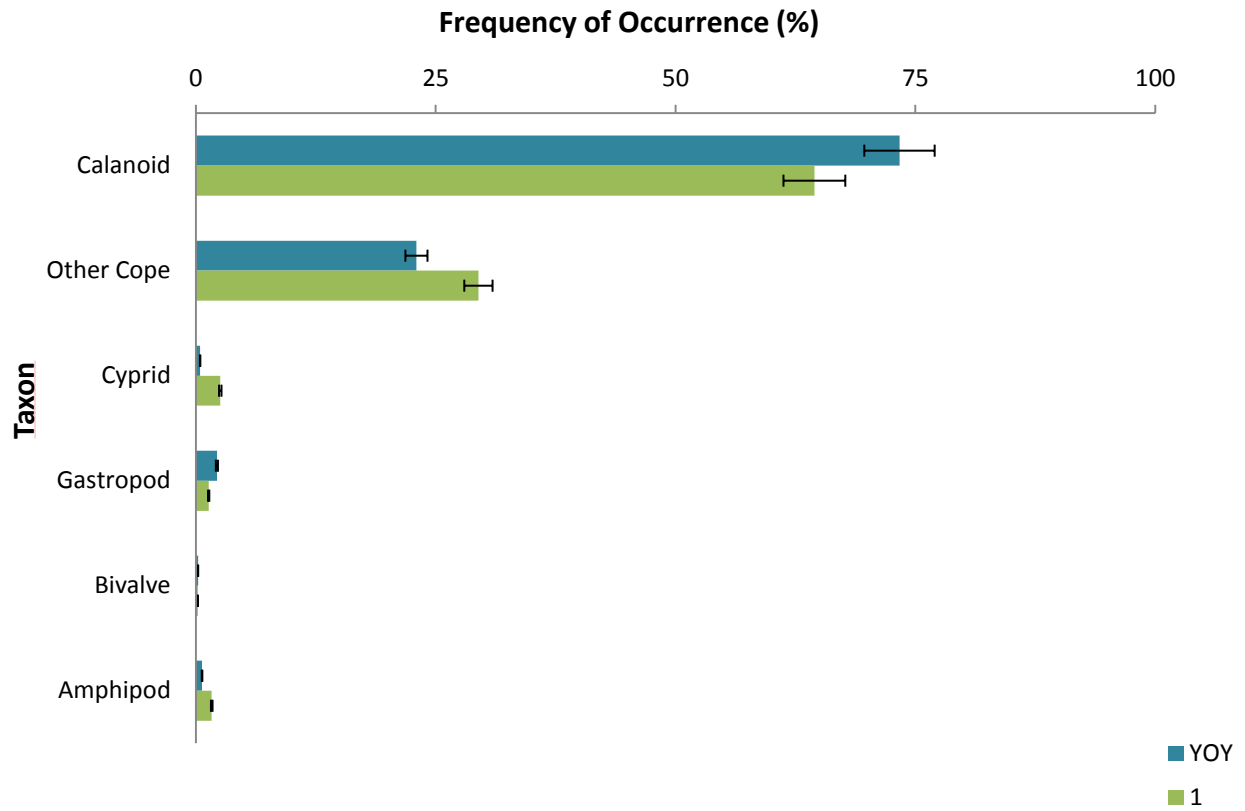


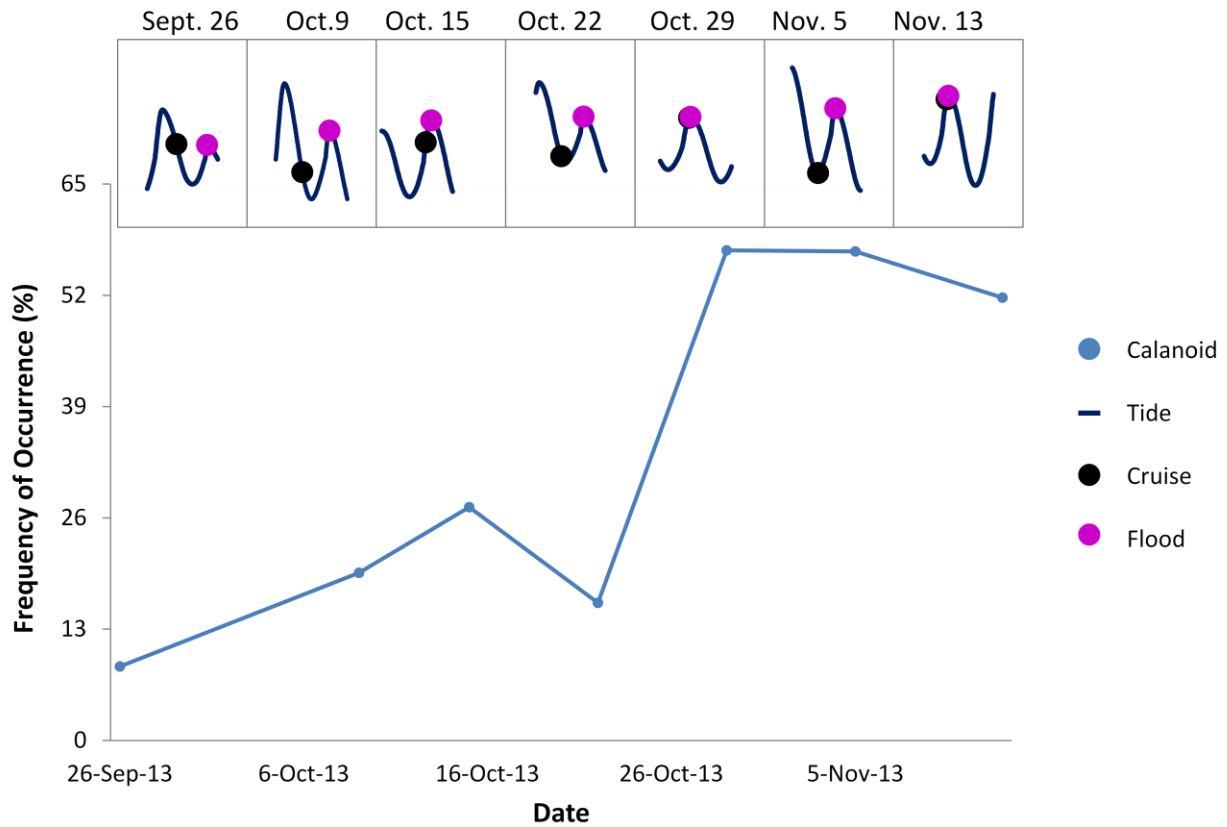
Figure 4: Zooplankton abundance at North Station (red) in comparison to PSL gut content (blue) for the autumn of 2013. PSL fed preferentially upon calanoid copepods, followed by “other copepods” (harpacticoid and cyclopoid), and amphipods. Though both larvaceans and copepod nauplii were abundant in the water column, neither one of these prey items was found in the gut contents. Error bars represent a value of 5 percent.  $N_{fish}=65$ .



**Figure 5: Frequency of occurrence of top prey items occurring in PSL gut contents at both dawn and dusk. Calanoid copepods were the most frequently consumed prey item at either time of day, followed by other copepods, and amphipods. Both mysids and amphipods were more abundant at dusk, but not significantly. Error bars represent a value of 5 percent.**



**Figure 6: Age dependent feeding behaviors for PSL collected on October 14, 2013 (18:26 -18:36). Age YOY fish consumed more calanoid copepods, however age 1 fish consumed larger copepods. Error bars represent a value of 5 percent.  $N_{fish}=10$**



**Figure 7: Calanoid copepod abundance in comparison to the timing of flood tides. During the autumn of 2013, calanoid copepods were most abundant when sampling occurred close to flood tide, particularly on Oct. 15, Oct. 29, and Nov. 13. Calanoid abundance was high on Nov. 5, however this is likely due to high tidal exchange and strong southwesterly winds.**

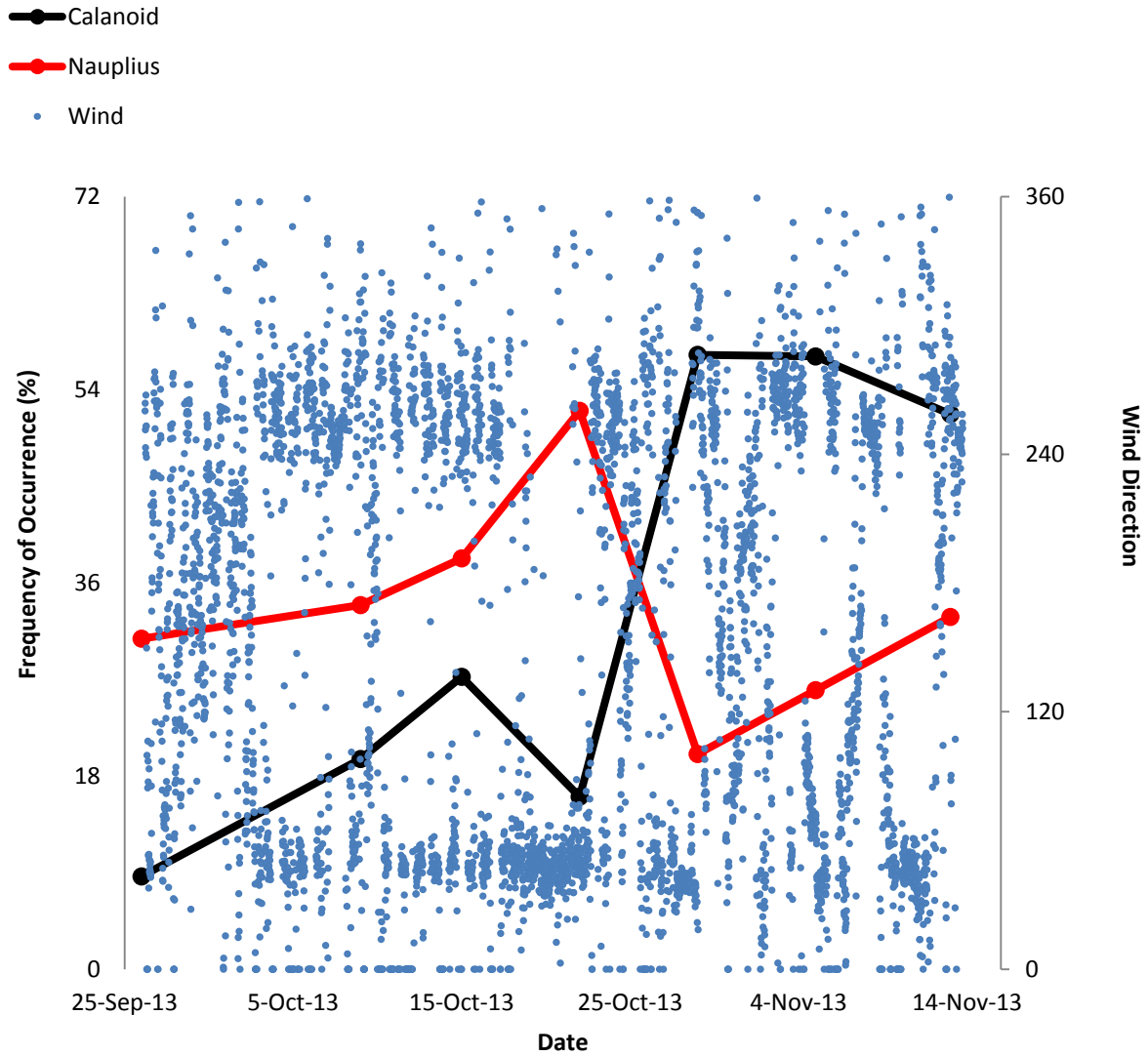
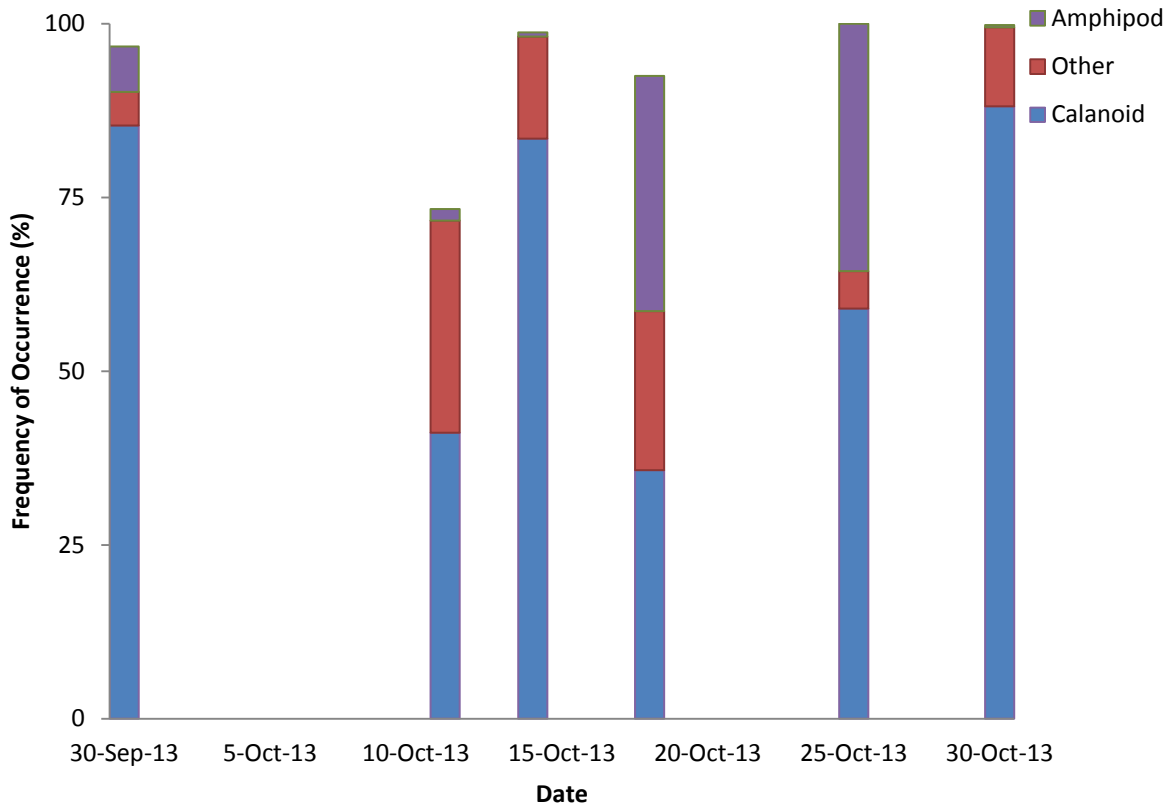


Figure 8: Calanoid copepod and copepod nauplii abundance in relation to wind direction and magnitude. As wind magnitude increases from the north east, copepod nauplii abundance increases, peaking on Oct. 22, 2013. Conversely, as wind magnitude increases from the southwest, calanoid abundance increases.



**Figure 9: Frequency of occurrence of top zooplankton found in PSL gut contents throughout the study period. Calanoid copepods were the most frequently consumed prey item, followed by other copepods and amphipods. Increased calanoid consumptions correspond to peaks in calanoids abundance at North Station.**

**Additional equations for quantifying gut content (Ahlbeck *et al.*,2012):**

*Frequency of occurrence (FO)*

The proportion of stomachs containing each prey type was calculated and expressed as a percentage of the total number of stomachs.

$$\text{Diet}_i^{\text{FO}} = \frac{N_{\text{fish},i}}{N_{\text{fish}}} \times 100$$

*Dominance method (DM)*

The proportion of stomachs dominated by mass by a certain prey type was calculated and expressed as a percentage of the total number of stomachs.

$$\text{Diet}_i^{\text{DM1}} = \frac{N_{\text{fish},i,w}}{N_{\text{fish}}} \times 100$$

In numerical method 2 (NM2), the stomach contents of all fish in the sample were pooled before percentages of different prey types were calculated (Hyslop 1980). The impact of each fish on the result differed among fish and was proportional to the number of prey in the stomach.

$$\text{Diet}_i^{\text{NM2}} = \frac{\sum_{j=1}^{N_{\text{fish}}} N_{ij}}{\sum_{j=1}^{N_{\text{fish}}} N_{\bullet j}} \times 100$$

Numerical method 3 (NM3) was basically the same as NM2, but before pooling prey from different stomachs, prey numbers were divided by the mass (length<sup>3</sup>) of the fish. This reduced the greater impact that larger fish, with on average more prey in their stomach, may have had.

$$\text{Diet}_i^{\text{NM3}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \frac{N_{ij}}{L_j^3}}{\sum_{j=1}^{N_{\text{fish}}} \frac{N_{\bullet j}}{L_j^3}} \times 100$$

### *Mass method (MM1–MM6)*

As with the NM, diets based on prey mass could be calculated in different ways. In mass method 1 (MM1), mass percentages of the digested ( $w$ ) prey items found in the stomach were first calculated for each fish separately and then averaged over all individuals in the sample. This meant that each fish impacted the result equally.

$$\text{Diet}_i^{\text{MM1}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \left( \frac{w_{ij}}{w_{\bullet j}} \times 100 \right)}{N_{\text{fish}}}$$

In mass method 2 (MM2), the stomach contents of all fish in the sample were pooled before mass percentages of different prey were calculated (Hyslop 1980). The impact of each fish on the result differed among fish and was proportional to the mass of prey in the stomach.

$$\text{Diet}_i^{\text{MM2}} = \frac{\sum_{j=1}^{N_{\text{fish}}} w_{ij}}{\sum_{j=1}^{N_{\text{fish}}} w_{\bullet j}} \times 100$$

Mass method 3 (MM3) was basically the same as MM2, but before pooling prey from different stomachs, prey masses were divided by the mass ( $\text{length}^3$ ) of the fish (adjusted pooled analysis; Hyslop 1980).