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Material Properties of Zooplankton and Nekton from the California Current

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Abstract of the Thesis

Material Properties of Zooplankton and Nekton from the California Current

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This study measured the material properties of zooplankton, Pacific hake (*Merluccius productus*), Humboldt squid (*Dosidicus gigas*), and two species of myctophids (*Symbolophorus californiensis and Diaphus theta*) collected from the California Current ecosystem. The density contrast (g) was measured for euphausiids, decapods (*Sergestes similis*), amphipods (*Primno macropa*, *Phronima sp.*, and *Hyperiid spp.*), siphonophore bracts, chaetognaths, larval fish, crab megalopae, larval squid, and medusae. Morphometric data (length, width, and height) were collected for these taxa. Density contrasts varied within and between zooplankton taxa. The mean and standard deviation for euphausiid density contrast were 1.059 ± 0.009. Relationships between zooplankton density contrast and morphometric measurements, geographic location, and environmental conditions were investigated. Site had a significant effect on euphausiid density contrast. Density contrasts of euphausiids collected in the same geographic area approximately 4-10 days apart were significantly higher ($p < 0.001$). Sound speed contrast (h) was measured for euphausiids and pelagic decapods *(S. similis)* and it varied between taxa. The mean and standard deviation for euphausiid sound speed were 1.019 ± 0.009. Euphausiid mass was calculated from density measurements and volume, and a relationship between euphausiid mass and length was produced. We determined that euphausiid from volumes could be accurately estimated two dimensional measurements of animal body shape, and that biomass (or biovolume) could be accurately calculated from digital photographs of animals. Density contrast (g) was measured for zooplankton, pieces of hake flesh, myctophid flesh, and of the following Humboldt squid body parts: mantle, arms, tentacle, braincase, eyes, pen, and beak. The density contrasts varied within and between fish taxa, as well as among squid body parts. Effects of animal length and environmental conditions on nekton density contrast were investigated. The sound speed contrast (h) was measured for Pacific hake flesh, myctophid flesh, Humboldt squid mantle, and Humboldt squid braincase. Sound speed varied within and between nekton taxa. The material properties reported in this study can be used to improve target strength estimates from acoustic scattering models which would increase the accuracy of biomass estimates from acoustic surveys for these zooplankton and nekton.

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Chapter 1

Material Properties of Northeast Pacific Zooplankon

Introduction:

The California Current is a major eastern boundary current in the Northeast Pacific ocean. The Northern California Current region in particular is characterized by high productivity and seasonal and decadal variability (Brodeur et al., 2003). Ekman driven coastal upwelling of cold nutrient-rich water supports fish production in the Northeast Pacific through bottom up trophic linkages (Ware and Thomson, 2005). Zooplankton in the Northeast Pacific Ocean play the crucial ecological role of transferring energy from the primary producers to higher trophic levels. Northeast Pacific euphausiids are an important food source for many species including several species of Pacific salmon (Brodeur and Pearcy, 1990), myctophids (Tyler and Pearcy, 1975), Pacific hake (Mackas et al, 1997), and many seabirds and marine mammals (Croll et al,. 1998). *Euphausia pacifica* and *Thysanoessa spinifera* are the dominant euphausiid species in the Northeast Pacific region (Gómez-Gutiérrez et al., 2005).

Active acoustic techniques allow researchers to study zooplankton abundances, distributions, and behavior at finer temporal and spatial scales than could be achieved by traditional methods (Foote and Stanton, 2000; Greenlaw, 1979; Simmonds and MacLennan, 2005). Scientific echosounders transmit sound waves into the water and acoustic backscatter is created when those sound waves encounter a target with a different acoustic impedance than the surrounding seawater (Simmonds and MacLennan, 2005). To convert energy into biomass, accurate target strength values are needed for the scatterers in the water column (Simmonds and MacLennan, 2005).

Target strength is determined by the size, shape, orientation, and material properties of the target animal (Chu et al., 2000; Smith et al., 2010; Stanton and Chu 2000; Warren et al., 2002). Target strength can be measured directly in controlled experiments by using a calibrated scientific echosounder and measuring the backscatter of known targets (Foote et al., 1987; Simmonds and MacLennan, 2005). This can be logistically difficult for zooplankton due to their small size. Foote and Stanton (2000) recommended using an acoustic scattering model to estimate target strength for zooplankton if there are no direct target strength estimates available. In order to accurately model acoustic scattering, the material properties of the target organism must be known.

Material properties such as the ratios of the density (g) and sound speed (h) for a target relative to the surrounding seawater, as well as animal length and shape, are important parameters in scattering models (Greenlaw, 1977; Forman and Warren 2010; Smith et al., 2010; Warren and Smith, 2007). These parameters vary between zooplankton taxa (Greenlaw and Johnson, 1982; Stanton et al., 1996), within taxa (Forman and Warren, 2010), seasonally (Køgeler et al., 1987), and geographically (Smith et al., 2010). A small change (2 - 4%) in the material properties used in a scattering model can change the target strength prediction for zooplankton by up to 20 dB (Chu et al., 2000). Demer and Conti (2005) showed that when the scattering model for *Euphausia superba* was improved with updated parameters, the resulting

estimated biomass increased by a factor of 2.5. Smith et al. (2010) suggested it may be necessary to use length and material property measurements from live animals in the geographic area of the study for an accurate scattering model. Scattering models using measured material property values from the geographic area of study produce very different target strength estimates than models using material property values from literature (Smith et al., 2013).

Acoustically targeted net tows are conducted in acoustic surveys in order to ground truth the acoustic data and confirm the composition of zooplankton present in the sampled region. When acoustic scattering models are used to predict biomass, these predictions are often compared to the biomass of animals caught in net tows. To calculate biomass from organisms collected in the net, scientists often use published length and wet weight relationships because taking detailed morphometric measurements at sea is very time consuming. Relationships for euphausiids are often based on measurements made on preserved or frozen animals, and preservation may have an effect on the euphausiids weight (Davis and Wiebe, 1985; Harvey et al., 2012). This study measured the length, width, and height of Northeast Pacific euphausiids and used these data to calculate volume using two different methods. Euphausiid volume and density were used to calculate euphausiid mass, and a new length-to-weight relationship for unpreserved Northeast Pacific euphausiids was produced.

We measured the density (g) and sound speed (h) contrasts for zooplankton in the Northeast Pacific. Material properties for zooplankton in this region have been measured previously, but it was several decades ago (Greenlaw, 1977; Greenlaw & Johnson, 1982). This study is the first to report density contrast values for crab megalopae and larval squid. Environmental conditions (temperature, salinity, density, and fluorescence) and zooplankton morphometric measurements (length, width, and height) were also measured. The effects of environmental and morphometric variables on zooplankton density contrast were investigated. Information from this study could improve the acoustic scattering models for these taxa.

Methods:

We sampled two areas offshore of the Oregon coast during the summer of 2012 (Figure 1). We sampled one region from 27 to 30 July (A1), then a different region (B) from 31 July to 03 August, and resurveyed the first site (A2) from 04 to 10 August (Figure 2). Density contrasts were measured for individual animals from the following taxa: euphausiids (*Euphausia pacifica* and *Thysanonessa spinifera),* amphipods (*Primno macropa*, *Phronima sp.,*and *Hyperiid spp.),* decapods (*Sergestes similis*), chaetognaths, crab megalope, larval fish, siphonophores, larval squid, and medusae (Figure 3). Sound speed contrasts were measured for euphausiids and decapods.

Animal Collection:

Net tows were conducted at 37 stations from the RV *Oceanus* from 26 July 2012 to 10 August 2012. Zooplankton were collected using a 4 m^2 Isaacs-Kidd mid-water trawl (IKMT) (Isaacs & Kidd, 1953). The IKMT consisted of a 1 mm mesh net attached to a rigid v-shaped diving vane with a 1 mm mesh cod-end. The net tows targeted aggregations observed acoustically. The maximum depths of the net tows ranged from 29-550 meters. Conductivity-Temperature-Depth (CTD) data was used to characterize the environmental conditions of regions where net tows were conducted.

When the net was retrieved, the contents were quickly transferred from the cod-end to a large tray (~ 15 L) filled with ambient surface seawater (from the ship's flow through seawater system). Individual zooplankton in the best condition (most viable) were hand-sorted by taxa into smaller containers (~ 1 L) filled with ambient surface seawater. Density and sound speed measurements were made immediately after sorting. Only live zooplankton were measured; most were measured within five hours of collection, but no measurements were made more than ten hours after collection. In some cases with particularly large catches, containers with animals in ambient surface seawater were stored in a refrigerator until density and morphometric measurements could be performed.

Measurements:

Density

The titration method (Smith et al., 2010; Warren and Smith, 2007) was used to measure the density of the animals. The titration method involves using two burets: one with ambient surface seawater and the other with a denser solution. Each solution was titrated into a beaker containing a single organism until the organism reached neutral buoyancy. Warren and Smith (2007) and Smith et al. (2010) used a hypersaline solution as the denser solution, but in this study we used a glycerin mix solution. The density of glycerin is 1.26 g ml^{-1} (ICSC, 2007) and a glycerin mix was created by diluting pure glycerin with ambient surface seawater. A 50/50 glycerin mix was used for all measurements.

Zooplankton were anesthetized in a beaker containing ~ 200 ml of ambient surface seawater and an effervescent tablet which saturated the fluid with carbon dioxide. The temperature and salinity of the ambient surface seawater used in measurements were used to calculate seawater density using the CSIRO MATLAB Seawater Library. The density of the animal is calculated from V_{sw} the volume of seawater (ml), V_m the volume of glycerin mix (ml), ρ_{sw} the density of seawater (g ml^{-1}), and ρ_m the density of glycerin mix (g ml^{-1}) in the beaker (Equation 1). The density contrast is the ratio of animal density to seawater density (Equation 2).

$$\rho_a = \frac{(V_{sw}\rho_{sw})+(V_m*\rho_m)}{(V_{sw}+V_m)} \quad (1)$$

$$g_{animal} = \frac{\rho_{animal}}{\rho_{sw}} \quad (2)$$

This study was the first study to use glycerin instead of a hypersaline solution as the titrant. Preliminary results suggested that the titration using glycerin gave higher density values than when a hypersaline solution was used. We explored these results by conducting an experiment (6 to 13 August 2013) on grass shrimp (*Palaemonetes pugio*) collected from coastal Long Island. We measured the density of 41 shrimp using both methods. The glycerin titration resulted in consistently higher g values than the hypersaline titration. In order to be able to compare data from this study to previous studies, we used the ratio of the mean shrimp g values found using hypersaline solution to the mean shrimp g values found using the glycerin to scale the glycerin-based data. The difference in the measured at sea g value and unity was multiplied by the ratio of the hypersaline-derived g value and glycerin-derived g value for the lab experiments. These adjusted values are reported throughout this manuscript. We do not know the specific reason for

the higher density contrast values from the glycerin method, but it may be due to a difference in osmotic pressure. The glycerin mix used in the titrations had a higher osmotic pressure (167.27 atm) than the hypersaline solution (105.30 atm). This may cause water to be expelled from the animal through osmosis at a faster rate than when the hypersaline solution is used. This would cause the animal to be more dense and result in a higher density contrast value which is what was observed in our data.

Sound Speed

Sound speed measurements were made when net catches allowed for the collection of sufficient biovolumes (~ 10 ml or greater) of monospecific assemblages of organisms. Animals were sorted by hand to remove species other than the one of interest. In this study we made sound speed measurements on euphausiids and the pelagic decapod *Sergestes similis* due to their high abundances in net contents. A series of measurements of received signal level of the APOP (Acoustic Properties of zooPlankton) system were recorded using a digitizing storage oscilloscope (Chu et al., 2000, Chu and Wiebe, 2005) with the chamber containing only seawater (from the ship's flow-through system) and with animals present. Temperature of the ambient seawater was recorded for each trial and salinity was recorded from the ship's flow-through system. Three sets of pings and echoes were typically recorded for both the seawater-only and animals-present trials. Data were analyzed to determine the time-delay between the seawater-only and animals-present trials by an automated MATLAB program. The sound speed contrast (h) is a function of c_a, the sound speed through the zooplankton, c_{sw} the sound speed through seawater, Δ_t the travel time difference between two received waveforms (one with the chamber containing zooplankton and one without zooplankton), Φ the volume fraction (volume of the animals / volume of the acoustic chamber), and t_d the travel time of sound from the transducer to the receiver without zooplankton in the chamber (Equation 3).

$$h = \frac{c_a}{c_{sw}} = 1 + \frac{\Delta_t}{\Phi t_d} \quad (3)$$

Morphometric Measurements

After density contrasts were measured, the specimens were digitally photographed with a length scale for post-cruise measurement of their dimensions. Animals were photographed laterally and dorsally such that length, height, and width could be calculated from the image using a custom MATLAB program (Figure 4). Euphausiid length was measured as the distance from the posterior of the eye to the end of the sixth abdominal segment (Standard Length (SL) 3 in Mauchline, 1980 as cited by Lawson et al. (2006)). Total body length was measured for all other zooplankton taxa. The program recorded the body widths or heights at ten points along the body which provided an approximation of the body shape in both orientations.

We used the morphometric measurements from the MATLAB program to calculate the volume of euphausiids using two different approximations of the animal's shape: cylinder and a truncated cone. The cylinder volume equation used the euphausiids' maximum height (or width) and length measurements to calculate volume. The truncated cone method used all ten height (or width) measurements collected along the euphausiid body. In this method, the volume of each segment was calculated using the equation for the volume of a truncated cone. For the total euphausiid volume, the volumes for all ten segments were added together. The truncated cone

method incorporates the changing shape of the euphausiid along the length of the body. We compared the results of the two methods, and investigated if they had a relationship with density contrast with a linear regression. We used the euphausiid volume and density to calculate mass, and investigated the relationship of euphausiid weight and length with a linear regression. We log (base 10) transformed both variables (euphausiid weight and length) in order to be able to compare our data to previous published values.

Environmental variables

Vertical profiles of environmental parameters were recorded at 22 different locations using a Seabird CTD rosette model SBE 43. The CTD measured temperature, salinity, dissolved oxygen, and fluorescence of the water column with a vertical resolution of 0.05 m. The closest CTD cast (in time and space) to each net tow was used to represent the environmental variables present during that tow. For each environmental variable, a mean water column, mean surface, and in situ values were calculated for data analysis. The mean water column value was calculated by taking the mean of the values measured from the minimum depth to maximum depth of the CTD cast. The surface mean was calculated by taking the mean of each variable from the surface to three meters depth. The depth of three meters was chosen because the mixed layer depth was three meters or greater for all CTD casts. The in situ value was calculated by taking the mean of the variables measured within 10 m around the maximum depth of the associated targeted net tow. For each variable (temperature, salinity, density, fluorescence, and dissolved oxygen) mean water column, mean surface, and in situ values were used to investigate whether environmental conditions affect zooplankton density contrast.

Statistical Analysis

Linear regressions were used to investigate the effect of environmental parameters collected on zooplankton density contrast. In addition, linear regressions were used to identify relationships between zooplankton morphometric measurements, and relationships between density contrast zooplankton morphometrics. A type III ANOVA was used to determine if site has a significant effect on euphausiid density contrast and sound speed. We used a type III, or unbalanced ANOVA because of the imbalance of measurements between each site, however the resulting F statistic and p value were identical regardless of whether a balanced or unbalanced was performed. A Tukey's HSD (honestly significant difference) test was used to look at the significant difference of euphausiid density contrasts between sites.

Results:

Animal Shape

Morphometric measurements of length, height, and width (when possible) were taken on every zooplankter after measuring its density (Table 1). Lengths were recorded for both lateral and dorsal images, however did not differ significantly ($p=0.76$, two-sample t-test). All length values reported in this manuscript are from the lateral images. All length distributions were unimodal except for *Sergestes similis* which was bimodal (Figure 5). Euphausiid lengths ranged from 11.2-27.5 mm with a mean and standard deviation (sd) of 18.1 ± 1.9 mm. The mean and sd of the two length classes of *Sergestes similis* (< 37 mm and > 37 mm) were 29.59 ± 3.09 mm and 41.39 ± 2.58 mm respectively. The lengths for siphonophore bracts, chaetognaths, larval fish,

amphipods, and medusae were variable (Table 1). This may be a result of measuring multiple species or age classes within each taxon.

Ten measurements of height and width at locations along the body were recorded for each zooplankter. Overall, the maximum height and width measurements were variable with length, and all maximum height values were higher than the maximum width values for all zooplankton except for the crab megalope (Table 1). The mean of the maximum height measurement (3.03 ± 0.46 mm) for euphausiids was significantly higher ($p < 0.001$) than the mean maximum width measurement (2.56 ± 0.38 mm). Linear regressions of euphausiid height and length as well as euphausiid width and length resulted in weak correlation values of 0.258 and 0.324 respectively (Figure 6).

Euphausiid Volume and Wet Weight

Morphometric measurements were used to calculate euphausiid volume. The truncated cone method resulted in a krill volume (mean 70.10 ± 24.8 mm^3) that was almost half the volume calculated with the cylinder method (mean 135.11 ± 50.9 mm^3). This was expected however, because the truncated equation incorporates the taper of the euphausiid body. A linear regression of the truncated and cylinder volumes calculated from the two methods showed that they are correlated ($R^2 = 0.861$, $p < 0.001$) (Figure 7A). There was no significant difference ($p = 0.73$, two-sample t-test*)* between the truncated volume calculated using height or width values. Since the resulting volumes were not significantly different regardless of whether height or width values were used, we calculated the volumes using height values for the remainder of analysis. These results support the common assumption in modeling zooplankton body shape that euphausiid cross-sections are circular in shape (Stanton and Chu, 2000)

Euphausiid density and volume were used to calculate euphausiid weight. Euphausiid weight was highly variable and had a mean and sd of 76.90 ± 27.25 mg. The resulting equation from the linear regression of the log transformed weight (W) and length (L) is $W = 0.0527*L^{2.496}$ with an $R^2=0.58$ (Figure 7B). We used equations from the literature and the equation we developed to calculate the weight of euphausiids measured in this study from their measured lengths. We also used euphausiid density and volume to calculate mass. The percent difference between the sum of the calculated mass (from density and volume) and the sum of the weights from regression equations from this study and previous studies was calculated (Table 2).

Density Measurements

Density contrast varied within and between zooplankton taxa (Figure 8). The number of measurements made on each taxon was a result of the net tow composition and animal condition. Euphausiids dominated the composition of most of the net tows and therefore resulted in the greatest number of measurements. The mean and standard deviation (sd) of the ambient seawater in our density measurements were 1.0217 ± 0.0005 g ml^{-1}. The relationship between density contrast and the following variables was investigated for most zooplankton taxa: geographic location, depth collected, morphometrics, fluorescence, and seawater density. Only relationships between g and morphometric variables were investigated for crab megalopae, larval squid, amphipods, and medusae because there were not enough measurements for these taxa to investigate the effect of environmental conditions. Since this study investigated many variables

relationship with density contrast, we will only report relationships with a coefficient of determination greater than 0.60.

Euphausiids: A total of 740 euphausiids were measured. Their g values ranged from 1.018 to 1.084 with a mean and sd of 1.058 ± 0.009. No effect of environmental variables, depth collected, length, or volume was found on euphausiid density contrast. However, euphausiid density contrast did vary by geographic regions with different environmental conditions (Figure 9). Mean euphausiid g was higher at sites A2 and B than at A1. An ANOVA revealed a significant effect of site on density contrast ($p < 0.001$). However, a Tukey HSD test showed that density contrast of A2 and B did not differ significantly ($p = 0.15$). Environmental conditions at the three sites were similar and fluorescence values were low (Table 3). Smith et al. (2010) found a weak negative relationship between Bering Sea euphausiids and mean fluorescence. The fluorescence values in this study were much lower, and density contrasts higher than Smith et al. (2010). To investigate if their proposed relationship held true, we performed a linear regression on the mean euphausiid density contrasts and mean fluorescence values from each site in both studies (our sites: A1, A2, B; Smith et al. (2010) sites: West and East) and found a significant negative relationship between density contrast and fluorescence ($R^2 = 0.92$, $p < 0.001$) (Figure 10).

Shrimp (*Sergestes similis)*: We measured the density contrast of 204 shrimp whose g values ranged from 1.019 to 1.049, with a mean and standard deviation of 1.037 ± 0.005. *Sergestes similis*' length distribution was bimodal, and the two length classes of the shrimp (< 37 mm and > 37 mm) had significantly different g values ($p < 0.001$, two-sample t-test) (Figure 11).

Siphonophore bracts: We measured 108 siphonophore bracts; ninety-one of these were identified as *Lensia sp.* The mean and sd of the density contrast for *Lensia sp.* was 1.012 ± 0.009. The unidentified siphonophores had a density contrast with mean and sd of 1.002 ± 0.004. No effects of environmental or morphometric measurements on siphonophores density contrast were found.

Chaetognaths: The mean density contrast of 94 chaetognaths was 1.013 ± 0.012. No strong correlations between chaetognath g and environmental variables were found.

Larval Fish: Ninety-one larval fish were measured; sixty-five of these larval fish were identified as larval rockfish (*Sebastes sp*). The mean and sd of larval rockfish density contrast was 1.019 ± 0.015, and the mean and sd of the 26 unidentified larval fish was 1.028 ± 0.013.

Larval Squid: Forty-four larval squid were measured. The mean and sd of their density contrast was 1.029 ± 0.02. No relationship between larval squid density contrast and morphometric variables were found.

Amphipods: We measured 38 amphipods; 24 were identified as *Primno macropa* with a mean g and sd of 1.041 ± 0.012; 8 were identified as *Phronima sp*. with a mean g and sd of 1.027 ± 0.006; and 6 were identified as Hyperiid spp. amphipods with a mean g and sd of 1.032 ± 0.006. Density contrast varied among amphipod species (Figure 11). *Primno macropa* density contrast is significantly different than the density contrasts of *Phronima sp.* and *Hyperiid* spp.amphipods. ($p < 0.001$, $p = 0.015$, two-sample t-test). *Phronima sp.* and *Hyperiid spp.* density contrasts were not significantly different ($p = 0.14$, two-sample t-test).

Crab Megalopae: Twenty-five crab megalopae were measured in this study, and the mean ± sd of their density contrast was 1.066 ± 0.006. There was no correlation between crab megalopae density contrast and length.

Medusae: We measured five unidentified medusae. The mean and sd of their density contrasts were 1.002 and 0.0006.

Sound Speed Measurements

We measured the sound speed of seventeen groups of euphausiids and two groups of shrimp. Each group was measured three times, and the mean of these three measurements was used for analysis. The mean and sd of the ambient seawater density in the sound speed measurements was 1.0230 ± 0.0003 g ml^{-1}. Euphausiid sound speed contrast ranged from 0.992 - 1.029 and had a mean and sd of 1.019 ± 0.009. The mean and sd of sound speed contrast for *Sergestes similis* was 1.028 ± 0.001. Site had a significant effect on euphausiid density contrast, but it did not show a significant effect on sound speed contrast ($p = 0.89$). This may be the result of the low sample size.

Discussion:

We used glycerin as the titrant for our density measurements. We chose glycerin because of its high density (1.26 g ml^{-1}), but we found that using glycerin gave a consistently higher animal density values than hypersaline. We think this may be due to the high osmotic pressure of glycerin, but this should be investigated further if glycerin is used for density measurements in the future. Only a handful of studies (Chu and Wiebe, 2005; Greenlaw, 1977; Greenlaw and Johnson, 1982; Foote et al., 1990; Kogeler et al., 1987; Smith et al., 2010) have published g and h values for euphausiids, and even fewer have published values for other zooplankton taxa. The only material property data published for Northeast Pacific zooplankton are over thirty years old (Greenlaw, 1977; Greenlaw and Johnson, 1982). This study provides more recent information on Northeast Pacific zooplankton material properties, and the first density contrast values for the amphipods *Primno macropa*, and *Phronima sp.,* larval rockfish (*Sebastes sp*.), larval squid, and crab megalope. This study also provides information on the morphometrics of zooplankton and calculates volumes for euphausiids.

Zooplankton Morphometric Measurements :

Detailed morphometric measurements of zooplankton are rarely taken at sea because it can be difficult and time consuming. By taking digital photographs (with a scale bar) during the cruise and using a relatively simple post-cruise analysis program, high resolution data of zooplankton morphometrics could be collected. Taking digital photographs of zooplankton at sea and analyzing the picture post cruise is a more efficient use of valuable cruise time than making the same measurements by hand on a moving vessel. We were able to use these data to study the effects of length, width, and height on the density contrasts of zooplankton, and to calculate euphausiid volume. We calculated euphausiid volume using two different equations; for a cylinder and a truncated cone. We found that euphausiid volume calculated using the cylinder method was nearly twice that using the truncated cone method, and they were highly correlated ($R^2 = 0.861$) (Figure 7A). It is interesting that the two methods are not 100% correlated considering they are using height and length values from the same animal. The truncated method

incorporates the taper of the body by using all ten height measurements to calculate volume, and is most likely the source of the observed discrepancy.

It is essential to accurately measure or estimate zooplankton volume because it (or the related biomass) is an ecologically-important parameter. We found a very weak relationships (R^2=0.26, R^2=0.32) between euphausiid length and maximum height and maximum width (Figure 6). The maximum height and width vary for krill of the same length (Figure 6); therefore, using only animal length to estimate euphausiid volume will not be accurate. Calculating volume using the cylinder method requires only two measurements (length and maximum height or width), but this produces an overestimation of animal volume and does not incorporate the taper of the euphausiid body. Volumes from the cylinder method and truncated method are highly correlated (R^2=0.86) (Figure 7A), so one could use the regression equation to convert the measured cylinder volume to the truncated volume. It is relatively simple to take digital photographs of euphausiids collected in a net tow at sea before preservation, and then use these high-resolution measurements on a subset of the animals caught to calculate the volume using both methods and produce a regression equation. Then it would only be necessary to make two measurements (length and maximum height or width) on the rest of the euphausiids caught and calculate animal volumes directly. The benefits of this method include capturing realistic (i.e. non-preserved) values of animal size, shape, and volume information at sea while post-cruise analysis of the images can be used to examine differences in these parameters. Biovolume regressions produced in this manner would be site and species specific which will likely be more accurate than using published values from different geographic regions or species.

The weight-length regression from this study was compared with published equations for euphausiids (Table 2). Our correlation coefficient is lower than the other studies, but this was expected because our mass values were calculated using density values which varied widely among individual euphausiids. However, unlike previous studies, our measurements were from animals that were not preserved or frozen. Greenlaw (1977) found that there was a significant difference (p= 0.01) between the density values of fresh and preserved euphausiids. Preservation or freezing may affect the wet weight-length relationship for euphausiids so our findings may be more representative of the true relationship. Total biomass of the euphausiids measured in this study was calculated using the measured density and calculated volumes (truncated cylinder method) and compared to biomasses from weight-length regression equations from this study and the literature (Table 2). Not surprisingly, the closest biomass estimate was from the regression equation in this study, but interestingly the next closest biomass estimate was from a study (Kim et al., 2009) of *E. pacifica* which is a prevalent species of euphausiid in the Northeast Pacific region. The different weight-length regressions produced biomass estimates that varied by more than 35% so the length to weight conversion process is likely to increase the uncertainty in acoustic estimates of biomass. The photographic measurement method detailed in this study may be a relatively simple and quick way to generate length to weight relationships from live organisms during a cruise which will result in a more accurate estimate of biomass.

Density Measurements

All Euphausiids

Northeast Pacific (NEP) Euphausiid density contrast values measured in this study span a wider range (1.018-1.084) and have a higher mean (1.058) than other published values.

However, all other published euphausiid density contrast values fall within the range of values reported in this study. Greenlaw (1977) only reported density values for fresh and preserved *E. pacifica* (1.063 g ml^{-1} and 1.043 g ml^{-1} respectively) However, Greenlaw and Johnson (1982) showed a density contrast value of 1.037 for fresh *E. pacifica* and cited Greenlaw (1977) in a table. They also reported a g value for preserved euphausiids from Greenlaw (1977), but it is the same number (1.043) as the density which does not make sense. Overall, Greenlaw and Johnson (1982) reported a range of density contrast values for *E. pacifica* (1.035-1.040) and *T. raschii* (1.013-1.050). Kogeler et al. (1987) reported values for *T. inermis, T. raschii*, and *M. norvegica* (1.025-1.049); Foote et al. (1990) reported a mean and standard deviation for *E. superba(* 1.036 ± 0.0067) ; and Chu and Wiebe (2005) also reported values for *E. superba* (1.007-1.036); and Smith et al. (2010) reported values for Bering Sea euphausiids (1.001-1.041).

Smith et al. (2010) found a significant weak negative relationship between Bering Sea euphausiid g and fluorescence (R^2 =0.17, $p < 0.001$). They suggested that material properties may change with food availability; well-fed animals may have different material properties than starved animals. The average water column fluorescence values they reported ranged from 1.7-3.6 mg m^{-3}. However, the values of average water column fluorescence seen in this study were more than an order of magnitude lower and ranged from 0.031-0.132 mg m^{-3}. These values were consistent with fluorescence values reported in this region for the months of July and August (Anderson, 1964). If the relationship of fluorescence and euphausiid g holds true, then it is possible that the higher g values may be a result of a lack of available food in the environment. Although other studies that have reported euphausiid g values do not report fluorescence values, Chu & Wiebe (2005) and Foote et al. (1990) were conducted in the Western Antarctica Peninsula region of the Southern Ocean which is a region of high productivity (Falkowski et al., 1998). Kogeler et al., 1987 sampled from the Barents Sea which is also a productive region (Sakshaug & Slagstad, 1992). Greenlaw and Johnson (1982) made their density measurements on *E. pacifica* offshore of Oregon in the summer, however they only report measurements on 64 organisms (this study measured 740 euphausiids) which could be why our values have a wider range. It is possible that the euphausiids measured by previous studies came from areas of higher productivity, and in turn have lower density contrast values than this study. To investigate this further, we combined data from this study and Smith et al. (2010) and found that there was a significant negative relationship between mean euphausiid density contrast and mean fluorescence (Figure 10). This further supports the relationship proposed in Smith et al. (2010), although more data needs to be collected in order to confirm it.

We did not find a correlation between our euphausiid g values and length. These findings are consistent with Smith et al. (2010) who also did not find a relationship between Bering Sea euphausiid density contrasts and lengths. Our findings do contrast with Chu and Wiebe (2005), who reported a positive linear relationship between Antarctic euphausiids (*E. superba* and *E. crystallorophias*) length, and g values. This may be a result of sampling different species. Euphausiids sampled in Chu and Wiebe (2005) were much larger with a mean length of 36.7 mm while our euphausiids had a mean length 18.1 mm. It is possible that density contrast is more variable in smaller euphausiids.

We found that euphausiid density contrast significantly varied by site. Sites A1 and B are different geographically and temporally (Figure 2), but A1 and A2 only differed temporally (sampled ~5-10 days after A1) (Figure 9). This finding is important because it suggests that

density contrast of zooplankton can vary over time as well as geographically. We don't know if the differences in density contrast of euphausiids at A1 and A2 were a result of environmental conditions changing, different groups of zooplankton present, animal composition changing, or a combination of these things. The fact that there was a significant difference in the density contrast of euphausiids from the same area within the time period of a typical acoustic survey is important. This result should be investigated further in future studies to understand if it is necessary to measure material properties at the same time and geographic location of an acoustic survey to develop an accurate acoustic scattering model.

Other Zooplankton:

There are few studies that report material properties for zooplankton other than euphausiids: Chu et al. (2003), Chu & Wiebe (2005), Greenlaw (1977), Greenlaw & Johnson (1982), Lawson et al. (2004), and Smith et al. (2010). Forman and Warren (2009) and Warren & Smith, (2007) report material properties of coastal species that may be comparable to the taxa in this study. We studied the effect of morphometric and environmental variables on zooplankton g values. Smith et al. (2010) is the only other study that has looked at these relationships with zooplankton other than euphausiids. We found some relationships between zooplankton density contrast and these variables, but only relationships with a correlation coefficient lower than 0.6 were found. More detailed discussion of these findings by taxon is found below.

The range of density contrast values we measured for NEP *Sergestes similis* was 1.019-1.049. Greenlaw (1977) is the only study that contains material property estimates for *Sergestes similis* and they report a density of 1.051 for preserved specimens. They do not report a density contrast (g) value, but the range, mean and sd of density values of our samples were 1.041-1.070 g cm^{-3}, and 1.059 ± 0.005 g cm^{-3}. The value reported in Greenlaw (1977) is close to this study's density values for NEP *Sergestes similis*. They also report finding a significant difference between density values of fresh euphausiids and preserved euphausiids ($p = 0.01$), so their g value may be different since the NEP shrimp were measured immediately after collection. Forman and Warren (2009) reported g values for decapods from coastal Long Island, *(Palaemonetes pugio and Crangon septemspinosa*), and their values ranged from 0.870-1.085. Chu and Wiebe (2005) reported two mean and sd g values for the decapod *Mysid arctomysis*: 1.041 ± 0.008 and 1.024 ± 0.008. Smith et al. (2010) reported that density contrast varies within and between species. The range of NEP decapod g values measured here overlaps with both of these studies. The g values from Forman and Warren (2009) have a much lower range than ours, but this might be a result of density contrast varying by species. Smith et al (2010) found that Bering Sea euphausiid g had a negative relationship with fluorescence, and proposed this could be due to well fed euphausiids containing more lipids which would result in a lower density. Our results show a similar pattern; larger shrimp could be correlated with better fed shrimp which would decrease their density contrast (Figure 11).

The range of the density contrast for siphonophore bracts measured was1.00-1.032. The density contrast for Antarctic siphonophores is 1.02 which falls within our range of values, and is the only other published density contrast for siphonophores (Chu, D. pers. comm. as cited by Lawson et al., 2004). Our wider range of values may be a result of a larger sample size, or different species and location. Since relationships between siphonophore g and both morphometric and environmental variables have not been previously studied, it is not clear whether the nonexistence of these relationships is significant to this study.

The mean of the NEP chaetognath g values was 1.013 ± 0.012. Smith et al. (2010) is the only other study that published g values for chaetognaths. Their mean ± sd of Bering Sea (BS) chaetognaths was 1.014 ± 0.007 which is very similar to our measurement. The mean and sd of the density contrast found for all larval fish was 1.021 ± 0.015, and larval rockfish was 1.019 ± 0.015. No other studies have reported g values for larval fish in the Northeast pacific (NEP). Smith et al. (2010) reported g values for larval fish in the Bering Sea with a mean of 1.023, which is close to the NEP g values. Chu et al. (2003) reported g values for cod larvae that had a range of 0.969-1.014. Our values are higher, however this may be because the larvae sampled in this study were a different species than the larvae in Chu et al. (2003). The larvae sampled by Chu et al. (2003) were also smaller than the larval fish sampled in this study (Chu et al. (2003) lengths ranged from 4.48-10.94 mm; our NEP length ranged from 12.26-51.07 mm).

The range of amphipod g values measured in this study was 1.021-1.062. This study is the first to report density values for *Primno macropa*, and *Phronima sp*. There have been other studies which have published values for other species of amphipods. Greenlaw & Johnson (1982) reported g values for four amphipods (*Cyphocaris sp., Gammarus pulex, Parathemisto pacifica*, and *Sciva sp.)* which ranged from 1.055 to 1.088. Chu and Wiebe (2005) published mean g values for *Themisto sp*. amphipods of 1.024 and 1.051, and Smith et al. (2010) published g values for Bering Sea amphipods (*Themisto libellula)* that ranged from 1.001 to 1.029. Our values overlap with some of these published values, but the range of values presented by Smith et al. (2010) are on the lower end of our range. Again, this could be a result of g values changing with species, location, or food availability. We found that density contrast varied significantly with amphipod species in this study (Figure 11). This finding shows that it may be important to have species-specific material property values for amphipods, in order to have an accurate scattering model for amphipods.

The range of g values for the five unidentified Northeast Pacific (NEP) medusae we measured was 1.002-1.003. Two studies have published density contrasts for medusae: Warren & Smith (2007) and Smith et al. (2010), with ranges of 1.004-1.02 and 1.001-1.006 respectively. The NEP medusa density contrasts overlap with the findings of both these studies. This study had a very small sample size (n= 5), so these results may not be a good representation of the medusae in this region.

Currently, no published g values exist for larval squid or crab megalopae. The crab megalopae had the highest mean g value (1.066) of the zooplankton sampled in this study. The mean and sd of the density contrast of larval squid was 1.029 ± 0.019. No effect of length was seen on density contrast of these taxa.

Sound Speed Measurements:

The sound speed was only measured for groups of euphausiids and sergestid shrimp because not all zooplankton taxa were caught in high enough biovolumes for the method. The sound speed contrast for Northeast Pacific (NEP) euphausiids in this study had a mean and sd of 1.019 ± 0.009 and ranged from 0.992 to 1.029. This is higher than the values reported for Bering Sea euphausiids by Smith et al. (2010) who reported a mean and standard deviation of 1.006 ± 0.008. However, the range for NEP euphausiids sound speed contrast in this study agreed with the range Smith et al. (2010) reported for the BS euphausiids (NEP: 0.992-1.029, BS: 0.990-1.017). This study's range of sound speed contrasts for NEP euphausiids also agrees with the

range Greenlaw and Johnson (1982) reported for *E. pacifica* (1.00-1.022). This is important since it is likely that *E. pacifica* was one of the species in the NEP euphausiids we measured. The NEP euphausiid sound speed contrasts in this study are lower than those reported by Kogler et al. (1987) for *Meganyctiphanes norvegica* and a mixture of *T. inermis* and *T. raschii* (1.030 ± 0.01 and 1.026 ± 0.005), Foote (1990) for *E. superba* (1.028 ± 0.002), and Chu and Wiebe (2005) for *E. superba* (1.030 ± 0.004). There was no effect of site on the sound speed contrasts of the NEP euphausiids. This may be a result of a low number of measurements, and may not be representative of the true effect.

The sound speed values we measured for *Sergestes similis* (1.028 and 1.027) are much higher than the two values in Greenlaw and Johnson (1982), the only other study to publish h values for this species (1.006 and 0.997). Forman and Warren (2010) reported sound speed contrasts for two species of decapods (*Palaemonetes pugio* and *Crangon septemspinosa*) from coastal Long Island, and the mean and sd of their values were 0.995 ±0.008 and 0.973 ± 0.046 respectively. These values are lower than the values found in this study for *Sergestes similis*, but this could be because *Palaemonetes pugio* and *Crangon septemspinosa* are different species and live in coastal rather than a pelagic environments. More sound speed measurements on decapods need to be taken to be able to understand how sound speed contrasts change within species, between species, and between environments.

Material property parameters are important in acoustic scattering models, and changes in these parameters can have large effects on the model-estimated target strength. Chu et al. (2000) showed that a 1-2% change in material property values could change the target strength estimate of a scattering model up to 20 dB. Many scientists use material property values from Foote et al. (1990) in their scattering models for euphausiids as well as many other zooplankton. Changes in target strength can significantly alter biomass estimates. Demer and Conti (2005) updated a scattering model for Antarctic krill (*E. superba*) and showed that the target strength estimate from the new model was about 3-7 dB different than the old model depending on krill lengths. This small change in target strength increased the biomass by a factor of 2.5. The density contrast values found in this study differ from the values in Foote et al (1990) by more than 2%, so using values from this study to model NEP euphausiids could significantly affect the target strength estimate. Updated target strength estimates for NEP euphausiids may have significant changes in biomass estimates. Obtaining an accurate euphausiid biomass estimate for the NEP is essential because euphausiids are a crucial link in this ecosystem because they transfer energy from primary producers to higher trophic levels. Using the material property values presented in this study, may result in a more constrained estimate of target strength and biomass estimates for NEP zooplankton.

Conclusion:

This paper reports the first material properties values on Northeast Pacific zooplankton in over three decades. We measured the density contrasts of euphausiids, decapods (*Sergestes similis*), siphonophores, chaetognaths, larval fish, larval squid, amphipods (*Primno macropa, Phronima sp.*), crab megalope, and medusae. Values of g varied within and between taxa as well as among different geographic regions. Density contrast of euphausiids varied with site with the euphausiids sampled at site A1 were significantly lower than euphausiids sampled at sites A2 and B. Euphausiid g values were significantly different for animals from the same geographic region (A) which were sampled approximately 10 days later. We also observed weak

relationships between zooplankton g value and several morphometric and environmental parameters. Sound speed contrast was measured for groups of two zooplankton taxa, euphausiids, and pelagic decapods (*S. similis*). Sound speed contrasts varied within and between taxa, however no effect of location was found for euphausiid sound speed contrast. The material property values in this study may improve estimates of target strength from scattering models for Northeast Pacific zooplankton. More material property data are needed to help us further understand how they are affected by morphometric, geographic, and environmental variables. This study provides material property data for Northeast Pacific zooplankton which can improve acoustic scattering models, which may lead to more accurate estimates of zooplankton biomass in this region.

Chapter 2

Material properties of Pacific hake, Humboldt squid, and two species of myctophids in the California

Introduction:

The California Current (CC) ranges from the coast of British Columbia, Canada (~50^{o}N) to Baja California, Mexico (15-25^{o}N) and is one of the major eastern boundary currents in the Northeast Pacific ocean (Hickey, 1979). The seasonal upwelling of nutrients in the CC ecosystem supports many commercially important fish stocks such as anchovy, hake, mackerel, and sardine through bottom-up trophic linkages (Brodeur et al., 2003; Ware and Thomson, 2005). The CC also supports a community of smaller, deep-dwelling fishes such as myctophids (lantern fish) which contribute significantly to the fish biomass in this region (Brodeur and Yamamura, 2005). The Humboldt squid (*Dosidicus gigas*) has recently expanded its northern range in the CC and has invaded the waters of central California (Zeidburg and Robison, 2007). It has been observed that *D. gigas* are active predators on myctophids and Pacific hake in this region (Field et al. 2007).

Merluccius productus (Pacific hake) is an abundant fish species in the CC, and is both economically and commercially important (Methot and Dorn, 1995; Grover et al., 2002). They are roughly 60 percent of the pelagic biomass in the CC ecosystem, and in 2012 the landings in the United States and Canada were 157 and 47 million metric tons respectively (JTC, 2012; Ware and McFarlane, 1995). In the CC ecosystem, Pacific hake act as a link in the food chain between other commercially important fish such as Pacific herring and marine mammals (Bailey et al., 1982).

Myctophids are one of the most ecologically important and abundant taxonomic groups of mesopelagic fish in the ocean, yet they tend to be undersampled in most field studies because they are smaller than the mesh of large fish trawls(Gjosaeter and Kawaguchi, 1980), and they can avoid plankton sampling gear (Brodeur and Yamamura, 2005). There are approximately 250 species in 33 genera and they inhabit all of the world's oceans except the Arctic (Catul et al., 2011). Myctophids act as an important ecological link from zooplankton such as copepods and euphausiids to larger predators such as tuna and marine mammals (Brodeur and Yamamura, 2005). They can be found throughout the CC region, and they account for the majority of the biomass of fish in the Northeast Pacific region (Gjøsaeter and Kawaguchi, 1980).

Dosidicus gigas is one of the largest species of squid reaching lengths of 2.5 m, and its range in the Eastern Pacific is from 47°S to 40°N (Nigmatullin et al., 2001). Field et al. (2007) examined gut contents of Humboldt squid found in the CC region, and they reported that Pacific hake and the myctophid, *Stenobrachius leucopsarus* were the most frequent species found. Humboldt squid are essential to the CC ecosystem because they are important prey for large fish and marine mammals which transfers energy to higher trophic levels (Gilly et al., 2006). The Humboldt squid fishery is the largest squid fishery in the world, with landings of more than 800,000 tons in 2010 (FAO, 2010) making them an economically important species. Due to the lack of knowledge about the biology and life history of *D. gigas* the fishery started without accurate stock information and has not been managed with consistent methods (Nevdrez-Martinez et al., 2010). *D. gigas* is susceptible to overfishing because their one year lifespan

makes their population sensitive to recruitment variability (Nigmatullin et al., 2001; Goss et al., 2001).

Acoustic technology allows scientists to study nekton at finer temporal and spatial scales than can be achieved by traditional net sampling methods (Simmonds and MacLennan, 2005). Scientific echosounders transmit acoustic energy into the water column which produces backscatter when the acoustic wave encounters a region or object with different acoustic impedance than the surrounding seawater (Foote and Stanton, 2000; Simmonds and MacLennan, 2005). This backscattered energy, in conjunction with acoustic scattering models, can be used to estimate animal biomass (Simmonds and MacLennan, 2005; Misund, 1977). To have an accurate estimate of biomass, a constrained estimate of target strength is needed for the scatterer (Foote et al., 1987). Target strength is a function of the size, shape, orientation, and material properties of the target (Stanton and Chu 2000; Chu et al., 2000). It can be measured empirically; however, few studies are conducted because it is logistically difficult (Foote et al., 1987; Simmonds and MacLennan, 2005). Physics-based mathematical target strength models can estimate target strength by using parameters such as length and material properties to calculate the target strength of an organism (Greenlaw, 1977). Two material properties needed for the target strength models are the animal's density (g) and sound speed (h) contrasts relative to the density of the surrounding seawater (Greenlaw, 1977; Stanton and Chu, 2000; Smith et al., 2010).

Foote (1980) showed that a gas-filled swimbladder in fish can account for 90% or more of the backscatter. Since the swimbladder is such a strong scatterer, scientists have developed target strength models using properties of the swimbladder (reviewed in Simmonds and MacLennan, 2005). These models assume that all the backscatter from the fish is a result of the swimbladder, and do not account for the added backscatter or dampening effect the surrounding fish flesh may have on the target strength of the fish. Fish use swimbladders for buoyancy control, so the size and shape of a swimbladder are not constant. This variability may change how much the swim bladder contributes to the total backscatter, making fish flesh scattering more important. Additionally, not all fish have gas-filled swimbladders. Some fish including certain species of myctophids have a lipid-filled swimbladder (Butler and Pearcy, 1971), and other fish, including commercially important species such as the Atlantic mackeral (*Scomber scumbus*) have no swimbladder at all (Simmonds and MacLennan, 2005).

Knowledge of the acoustic properties of fish flesh may allow for the development of more accurate target strength models for fish. Gorska et al. (2007) modeled the acoustic scattering of groups of Atlantic mackerel and found that the backscatter was dominated by fish flesh at low frequencies (18 and 38 kHz). The material properties for fish flesh used in the scattering model in Gorska et al. (2007) were from calculations and were not measured directly, so empirical material property values would improve the accuracy of these models. Iida et al. (2006) estimated the material properties of fish flesh using an acoustic camera, but this is the first study to measure the material properties of fish flesh. This may be particularly important for Pacific hake because acoustic surveys are the stock assessment method used for this fishery. The target strength value currently used in these assessments is based on only a few *in situ* observations (Williamson and Traynor, 1984; Hesler et al. 2004).

Similarly, data on the material properties of Northeast Pacific myctophids are needed to better understand how they scatter sound. Myctophids, euphausiids, and other micronekton create the deep scattering layer (DSL) (one of the first biological layers observed in acoustics) in

the Pacific (Catul et al., 2011; Dietz, 1948; Hazen and Johnston, 2010). Barham (1966) confirmed that myctophids were part of the deep scattering layer using a submersible. Although empirical target strength measurements have been made for a few species of myctophids (Benoit-bird and Au, 2001; Yasuma et al., 2006; Yasuma et al., 2010), none have been made for myctophids in the Northeast Pacific.

Sampling squid utilizing traditional net methods presents challenges because of their varying abundance, quick movement, and ability to avoid nets (Starr and Thorne, 1998). Goss et al. (2001) suggested that acoustic tools could be used for squid stock assessments even though they are weaker scatterers than fish. Several studies have reported on various acoustic properties of different species of squid: *Todarodes pacificus* (Arnaya et al., 1989; Kang et al., 2005; Kang et al., 2006; Kawabata, 2005), *Ommastrephes bartrami* (Arnaya et al., 1989), *Loligo bleekeri* (Arnaya et al., 1989), *Loligo reynaudii* (Soule et al., 2010), and *Dosidicus gigas* (Benoit-Bird et al., 2008). Many of these studies have focused on target strength, or investigating how the target strength is affected by tilt angle; but only two have focused on material properties of these animals. Kang et al (2006) measured material properties (for the Japanese common squid *Todarodes pacificus)* and Iida et al. (2006) estimated material properties of Japanese common squid mantle using an acoustic camera. No previous studies (that we are aware of) have reported material property values for Humboldt squid.

Benoit-Bird et al. (2008) was the first study to empirically measure target strength of *D. gigas.* They proposed that scattering may be caused by different body parts of the Humboldt squid (the beak, arms, eyes, and braincase) because the target strength changed with the removal of these body parts. Since material property measurements for these body parts did not exist, there was no way to compare the empirical measurements with theoretical target strength predictions from scattering models.

This study measured density (g) and sound speed (h) contrast of nekton collected from two locations in the California Current system: the Oregon coast and Monterey Bay. We measured the material properties of Humboldt Squid (*Dosidicus gigas*) body parts (mantle, braincase, arm, tentacle, eye, beak, and pen); Pacific hake (*Merluccius productus*) flesh; and the flesh of two species of myctophids: the California Lantern fish (*Symbolophorus californiensis)* and the California Headlight fish (*Diaphus theta)* (Figure 12). Density and sound speed contrasts were calculated for body parts of fish and squid specimens. Relationships between the material properties of specimens with geographic location, animal size, and environmental parameters (e.g. fluorescence, temperature, salinity, and density were investigated. Understanding how material property measurements are affected by these parameters could refine target strength estimates from acoustic scattering models. Improving estimates of target strength will make biomass estimates from acoustic surveys more accurate. This is becoming increasingly important because many fisheries use acoustic surveys for stock assessment purposes.

Methods:

Nekton specimens were collected off the coast of Oregon on the *RV Oceanus* from 26 July to 10 August 2012 (Figure 13 A, B). One Humboldt squid and one Pacific hake were collected by jigging with a rod and reel over water depths of 365 m and 354 m respectively. An additional Pacific hake and all myctophids were collected using a 4 m^2 Isaacs-Kidd mid-water trawl (IKMT) in targeted net tows (Isaacs and Kidd, 1953) with maximum depths ranging from

210-350m. The IKMT had a 1 mm mesh net attached to a rigid v-shaped diving vane with a 1 mm mesh cod-end. Eight additional Humboldt squid specimens were also caught by jigging with a rod and reel (maximum depths ranging from 300-500 m) in Monterey Bay on the *RV Fulmar* from 9 to 11 November 2012 (Figure 13, C). Animals caught with jigging were brought on board, and their lengths were recorded (mantle length for squid and standard length for hake). Animals caught in the IKMT were immediately transferred from the cod end into a large (~15L) tray and hand-sorted by species into smaller containers (1L) until they could be measured. Due to the size of our experimental apparatus, the squid and fish were dissected immediately after collection for the density and sound speed experiments. In Monterey Bay, when an abundance of squid specimens were caught at once, the squid were kept alive in a covered holding tank equipped with a flow through surface seawater system until measurements could be performed. A digital photograph of each animal (with a length scale) was taken before all squid and Pacific hake specimens were dissected.

Density measurements (g values):

The density contrast (g) was measured utilizing the titration and pipette methods (Warren and Smith, 2007; Smith et al., 2010; Forman and Warren, 2010). The titration method was used to obtain the specimen's density aboard the research vessels, while the pipette method was used to measure the squid beak and pen pieces density in the laboratory after the cruise. The pipette method was conducted after the cruise because it involved using a microbalance which is difficult to use at sea.

The titration method uses two burets, one with surface seawater and the other with a solution with a greater density than seawater and titrating each liquid into a beaker containing an individual specimen until the specimen reaches neutral buoyancy. Previously, Warren and Smith (2007) and Smith et al. (2010) used a hypersaline solution as the denser solution, but this study used a glycerin mix solution. We used glycerin because it had a high density (1.26 g ml^{-1}, ICSC, 2007) and creating a hypersaline solution dense enough for measuring nekton body parts is difficult as salt would precipitate out of solution causing the hypersaline solution density to fluctuate during the titration. We needed to dilute the glycerin (create a glycerin mix) in order for the viscosity to be low enough to flow through the burets. The glycerin mix was created by diluting pure glycerin with surface seawater, a 50% glycerin and 50% ambient seawater mix was used for all measurements. If the specimen was buoyant in seawater before the density measurements, we titrated freshwater until the specimen achieved neutral buoyancy.

For hake, pieces of muscle were cut into roughly 2 x 2 x 1 cm (length, width, thickness) pieces for measurements. For myctophids, the gut contents, head, and tail were removed and then the body was cut into multiple pieces for measurement. The size of the myctophid body pieces varied depending on the body size of the animal. Humboldt squid were dissected with a knife and several body parts including the mantle, arm, tentacle, braincase, eye, pen, and beak were removed for measurements. Squid mantle was cut into ~3 x 3 x 1 cm pieces for measurement. Arms and tentacles were cut into pieces with dimensions of ~3 x 2 x 1 cm with slight variations of width and thickness depending on the position of the cut along the tapered arm or tentacle as well as variations due to squid size. On the *RV Oceanus*, the braincase was cut into several small pieces roughly 3 x 2 x 1cm with width and thickness varying between pieces due to the shape of the braincase, however on the *RV Fulmar*, the braincase was cut in half and measured with rough

dimensions of 3 x 3 x 2. The eyes of the squid were measured whole and were approximately 4 cm in diameter.

The squid beak and pen pieces had a density that was very close to or larger than the density of glycerin as they did not float in pure glycerin. They were measured using the pipette method in the lab after the cruise. The squid pen extends almost the entire mantle length of the animal and is not uniformly shaped, so it was cut into pieces with lengths of 3-5 cm and stored in seawater until they could be measured. All surrounding tissue was removed from the beaks by soaking them in seawater and the top and bottom jaws were separated and stored in seawater for measurement back at the lab. All other measurements were taken immediately after animals were collected, and body parts were stored in surface seawater after dissection until they could be measured. No measurements were made more than 5 hours after the squid were collected.

The density of the specimens ρ_a was calculated using Equation 4 where V_0 is the volume of seawater initially in the beaker, V_{sw} is the volume of seawater added to the beaker, ρ_{sw} is the density of seawater, V_g is the volume of glycerin mix added, and ρ_g is the density of glycerin mix.

$$\rho_a = \frac{(V_0+V_{sw})\rho_{sw}+V_g\rho_g}{V_0+V_{sw}+V_g} \quad (4)$$

This study used glycerin as titrant, and preliminary results suggested that using glycerin gave larger density values than using a hypersaline solution. Given that previous studies have used a hypersaline solution as a titrant, we conducted an experiment on grass shrimp (*Palaemonetes pugio*) collected from coastal Long Island. Forty-one grass shrimp were measured once with both the hypersaline solution, and the 50% glycerin solution. We found that using glycerin as the titrant resulted in consistently higher density values than the hypersaline solution. Due to these findings, we used the ratio of the mean shrimp g values found with hypersaline to the mean shrimp g values found with glycerin to scale the glycerin-based data to comparable values. To scale the data in this study, the difference in the measured at sea g value and unity was multiplied by the ratio of the hypersaline-derived g value and glycerin-derived g value for the lab experiments. The adjusted values are reported in this manuscript. The exact cause of the higher density values when using glycerin as a titrant is not known, however, it may be due to osmotic pressure. We calculated the osmotic pressure for our glycerin solution and hypersaline solution and found that the glycerin solution was higher (167.27 atm) than the hypersaline solution (105.30 atm). The higher osmotic pressure of glycerin may cause water to diffuse from the specimen at a faster rate than when a hypersaline solution is used. This would result in the specimen becoming more dense which supports our observations.

The pipette method (Warren and Smith, 2007; Forman and Warren, 2010) was used to measure the Humboldt beak and pen pieces. For this method, the body part was placed in a graduated cylinder filled with a known volume of seawater and the mass of the body part was recorded (m_{part}). Seawater was then removed from the graduated cylinder with a pipette until the volume of seawater was equal to the original volume in the graduated cylinder. The removed water was weighed on a microbalance, and the mass was recorded (m_{rw}). Temperature and salinity of the seawater was recorded, and the density of the seawater (ρ_{sw}) was calculated using the CSIRO MATLAB Seawater Library. This method was performed three times on each body

part and the mean was used for data analysis. These measurements were then used to calculate the density of the body part according to Equation 5.

$$\rho_{animal} = \frac{m_{animal}}{V_{animal}} = \frac{m_{animal}}{\left(\frac{m_{rw}}{\rho_{sw}}\right)} \quad (5)$$

Sound speed measurements (h values):

Sound speed contrast (h) was measured using the APOP (Acoustic Properties of zooPlankton) system (Chu et al., 2000). The body parts were put in an acoustic chamber and two broadband transducers (350–650 kHz, Materials Systems, Inc.) were attached at each end of the chamber. The sound speed was recorded for the chamber containing only seawater, and again with the animal parts added to the chamber. For this method, the acoustic chamber needed to be mostly filled with animal parts, so it required several of the same type of part. Due to this requirement, the sound speed was only measured for Pacific hake muscle tissue, Humboldt squid mantle, and Humboldt squid braincase. Three consecutive sets of one hundred pings were collected for each group of specimens measured. The sound speed contrast was calculated for each set of a hundred pings, and we report the mean of the three consecutive sets as the sound speed contrast for the specimen group. The sound speed contrast (h) was calculated using Equation 6 where c_a is the sound speed through the animal parts, c_{sw} is the sound speed through seawater, Δ_t is the travel time difference between two received waveforms (one with the empty chamber and one with the chamber containing the animal parts), Φ is the volume fraction (volume of the animals parts / volume of the acoustic chamber), and t_d is the travel time of sound from the transducer to the receiver without animal parts in the chamber.

$$h = \frac{c_a}{c_{sw}} = 1 + \frac{\Delta_t}{\Phi t_d} \quad (6)$$

Morphometric measurements:

A digital photograph of the specimens with a length scale was taken before the sound speed measurements and after the density measurements; these photographs were used for post-cruise dimension measurements. Body parts or tissue pieces were photographed laterally and dorsally such that length, width, and height (or thickness) could be calculated from the photographs using a custom MATLAB program. Similar images were also taken for Humboldt squid and Pacific hake prior to their dissection, and these pictures were also analyzed to obtain length and height measurements of the whole animal after the cruise.

Environmental variables:

On the *RV Oceanus* and *RV Fulmar*, environmental variables were recorded using a Seabird Conductivity-Temperature-Depth (CTD) sensor model SBE 43 and SBE 19 plus respectively. The CTD was deployed at 22 location on the *RV Oceanus*, and 4 locations on the *RV Fulmar*. The CTD measures temperature, salinity, dissolved oxygen, and fluorescence of the water column. Relationships between the material properties of specimens and animal length, temperature, salinity, dissolved oxygen, fluorescence, and geographic location were investigated. Statistical analyses such as linear regressions and t-tests were used to determine if these relationships were statistically significant. Unless otherwise noted, values are reported as mean and standard deviations throughout this manuscript.

Results:

Nekton Density Contrast:

Pacific hake

Two Pacific hake were collected off the coast of Oregon on the R/V Oceanus. The first hake was collected in a net trawl and had a standard length of 28.9 cm. The second hake was collected by jigging and had a standard length of 40 cm. Twenty hake flesh pieces were measured and their density contrast was 1.029 ± 0.004 with a range of 1.023-1.036 (Table 1, Figure 14). Since only two hake specimens were collected, we did not investigate the relationship between hake length and density contrast, although we note that there was no significant difference ($p = 0.647$, two-sample t-test) between the density contrasts of the two hake specimens collected.

Myctophids

Myctophids were collected in targeted net trawls off the coast of Oregon. Myctophids were dissected, and myctophid flesh pieces were measured. Digital photographs were not taken of all myctophids used for measurements before dissection, but a small sample of measured myctophids (n =11) were measured and their lengths ranged from 5.4-7.9 cm. A total of 64 myctophid flesh pieces were measured: 20 were California Lantern fish, 26 were California Headlight fish, and 18 were unidentified (Table1, Figure 14). The two species of myctophids had significantly different density contrasts ($p < 0.001$, two-sample t-test) and the density contrast of Pacific hake flesh was significantly greater than myctophid fish flesh ($p < 0.001$, two-sample t-test).

Humboldt squid

Nine Humboldt squid were collected; one off the coast of Oregon (mantle length of 28 cm) on the *R/V Oceanus* and eight in Monterey Bay (mantle lengths ranging from 41 to 53 cm) on the *R/V Fulmar*. The density contrast (g) varied among the squid body parts with the beak and pen being the densest parts of the squid (Table 1; Figure 15 and 16). The density contrasts of two body parts (tentacles, eyes) were significantly different (higher ($p < 0.001$, two sample t-test) and lower ($p < 0.001$, two sample t-test) respectively) than the mantle, arms, braincase and eye (Figure 15).

Six beaks were measured, but in order to displace enough water for the method they were measured in two groups. The two groups had density contrasts 1.46 ± 0.046 and 1.26 ± 0.021. Due to the method, we could not investigate the effect of squid size (mantle length) and environmental parameters on beak density contrast. Density was measured for 7 pens, and they had a density contrast with a range of 1.085-1.235 with a mean and standard deviation of 1.151 ± 0.057. No relationship between pen density contrast and mantle length was found.

The squid collected in Oregon was smaller (28 cm ML) than the squid collected in Monterey Bay (41-56 cm ML).We found that the density contrast for the mantle of the squid collected in Oregon was significantly lower ($p = 0.001$, two-sample t-test) and the braincase density contrast was significantly higher ($p < 0.001$, two-sample t-test) than the squid collected

in Monterey Bay (Figure 17). No other body parts of the squid showed a significant difference between the two study regions.

Nekton Sound Speed Contrast

The sound speed contrast (h) was measured for Pacific hake flesh pieces, myctophid flesh pieces, Humboldt squid mantle, and Humboldt squid braincase (Table I). We measured the sound speed of flesh pieces from the two hake collected. After the first hake was dissected, two groups of flesh pieces were measured twice. Hake flesh pieces from the second specimen and Humboldt braincase pieces were measured were qualitatively separated by the firmness of the flesh (pliable and firm).

Environmental variables

The number of specimens measured was a result of net tows and jigging. The small number of hake collected limited investigations on the effect of environmental conditions on hake flesh density contrast. The environmental conditions differed between Oregon and Monterey Bay (Figure 18). Fluorescence had the most striking difference, with the surface fluorescence being much higher in Monterey Bay than in Oregon (Figure 18). The effect of environmental conditions on the density contrast of myctophid flesh and Humboldt squid body parts was investigated and no significant relationships were found. Since the sound speed contrast data are limited, the effects of environmental conditions on sound speed were not investigated.

Discussion:

Very few studies have measured the density and sound speed of nekton. Density (g) and sound speed (h) contrasts are crucial parameters for acoustic scattering models which are often used to convert acoustic data to biomass estimates. Knowledge of how these values vary with species, animal length, and environmental conditions could improve the accuracy of acoustic scattering models for nekton. This was the first study to examine how material properties of Pacific hake, myctophids, and Humboldt squid were affected by animal length and environmental conditions. Effect of environmental parameters on density contrast has been documented for zooplankton taxa (Smith et al. 2010), but no effect on myctophid density contrast was found in this study. This may be because fish have a more complex body structure than zooplankton, and their flesh is not as susceptible (or rapidly adjusting) to fluctuations in the environment.

No studies (that we are aware of) have directly measured the material properties of fish flesh. Forman and Warren (2010) reported material property values for whole fish: *Fundulus majalis* and *Fundulus heteroclitus*. Iida et al. (2006) estimated material properties of fish (*Theraga chalcogramma* and *Sebastes thompsani*) from an acoustic camera, and reports density contrasts for fish flesh. Combining the data from this study with previously published work shows that material property values can vary widely between species (Figure 19).

Pacific hake flesh density contrast values overlap with the higher ranges of values reported by Forman and Warren (2010), but are lower than the estimate by Iida et al. (2006). The density contrast of all myctophids measured in this study agrees with the range of values Warren and Forman (2010) reported for fish, but is also lower than the estimate by Iida et al. (2006). The

differences seen between the fish density contrast values in this study and previous studies could be due to a number of factors including species differences or the difference between whole fish and fish flesh. Additionally, most of the fish measured in Forman and Warren (2010) were smaller (< 5 cm) than the fish measured in this study, and they were collected from a coastal rather than pelagic ecosystem.

The mean density contrasts of flesh from California Lantern fish (*Symbolophorus californiensis*) (0.9992 ± 0.005) and California headlight fish (*Diaphus theta*) (1.013 ± 0.005) were significantly different from each other ($p < 0.001$). Even though these two fish are different species, they are very similar in that they both are triglyceride-storing lantern fish (Neighbors and Nafpaktitis, 1982). Neighbors and Nafpaktitis (1982) reviews the lipid compositions, water contents, swimbladder morphologies, and buoyancies of nineteen midwater fishes including both *Symbolophorus californiensis* and *Diahus theta.* Neighbors and Nafpaktitis (1982) reported that *Diaphus theta* was less dense than *Symbolophorus californiensis*, but they measured whole fish, not just fish flesh. The other components of the fish body such as skull, spine, and swimbladder would have important effect on the overall buoyancy of the fish.

Pacific hake density contrasts were significantly different than myctophid density contrast. Combined with the finding that the two myctophid species were significantly different from each other, our results suggest that fish flesh varies with species. This is particularly important because it means it may be necessary to have species-specific knowledge of density contrast to have the most accurate scattering model.

Acoustic surveys are used throughout the world for fisheries stock assessments (Simmonds and MacLennan, 2005). We found that Pacific hake density contrast was significantly different than the density contrasts from both myctophid species, and both myctophid species were significantly different from each other. Our data suggest it may be important to use species specific material property values in scattering models to achieve the most accurate estimate of target strength. To investigate this further, more fish flesh material property data are needed to understand how the density values change with species. Chu et al. (2000) reported that a small change (2-4%) in material property values could have a very large effect on target strength estimates from scattering models. In order to understand how the values reported in this study would affect target strength, we used a similar approach as Smith et al. (2012) and investigated how target strengths would change with different material property values. Pacific hake flesh contribution to target strength was 15 dB greater than California headlight and 31dB greater than California lantern fish flesh contribution. This shows the importance of using material property values for specific fish species, as not doing so for these species could yield errors in biomass estimates that are as great as three orders of magnitude.

Fish sound speed contrasts (h) varied within and between taxa (Table 1). The Pacific hake values herein overlap with both the Forman and Warren (2010) and Iida et al. (2006) values (Figure 20). Rigid and pliable flesh differed in sound speed contrast which suggests that hake flesh sound speed may vary within the animal. The sound speed contrast of unidentified myctophids was within the range of h values found in Forman and Warren (2010), but is lower than the estimate by Iida et al. (2006) (Figure 20). The difference in this study's sound speed contrast for fish flesh could be because we measured different species than both Forman and Warren (2010) and Iida et al. (2006).

The density contrast values were variable between the different body parts of Humboldt squid (Figure 15 and Figure 16). The Humboldt squid mantle density is the most similar to the value found in Kang et al. (2006), but is lower than the estimate by Iida et al. (2006) (Figure 19). Since the mantle covers the majority of the body of the squid, it is not surprising that we found similar density contrast values between the mantle pieces in this study and the whole Japanese flying squid in Kang et al (2006). However, our density contrast values are much lower than the estimate by Iida et al. (2006), but this could be due to the fact that the data in Iida et al. (2006) were estimated from an acoustic camera, and not a direct measurement. In order to understand the significance of the difference between our estimates and previous studies, we calculated how much the target strength estimate would change if only material properties were changed. We found an 11% difference in numerical abundance when the density contrast for Japanese flying squid instead of Humboldt squid was used in a scattering model which would be important if acoustic surveys were to be used for stock assessment purposes. More data need to be collected on different species of squid in order to develop species-specific acoustic scattering models for squid.

Benoit-bird et al. (2008) proposed that the braincase of the squid is a significant source of acoustic scattering for Humboldt squid because it is composed of dense cartilage and contains statoliths which contain aragonite crystals. The range of density contrast of Humboldt squid braincase pieces was 1.012-1.057 with a mean of 1.025. We found that the mantle and braincase density contrasts from our Oregon squid were significantly different than the density contrasts from the Monterey squid (Figure 17). A relationship between age and mantle length for Humboldt squid has not been defined, however it is accepted that length is positively correlated with age. One possible explanation for this finding could be that squid body composition changes with maturation. Markaida et al. (2004) investigated the age, growth, and maturation of Humboldt squid. They found that age of maturation was affected by size and sex, so mantle length was not an absolute way to determine if the squid was mature. Differences in environmental conditions could also be the cause of the difference in the Oregon and Monterey squid. Environmental conditions, especially fluorescence, differed between locations (Figure 18). Further investigation of whether size (or age or maturation) or environmental conditions affects Humboldt squid density contrast is needed to confirm what cause the difference of mantle and braincase density contrasts between our two study sites.

Density contrast varied within and among different body parts of squid. The beak and pen were the densest body parts with values of 1.360 ± 0.14 and 1.151 ± 0.057 respectively. The density contrast of the tentacle pieces of the squid was higher than the arms (Table I).The arms and tentacles of the Humboldt squid both contain sucker rings which are more rigid than most squid tissue. The difference in the density contrast between these two body parts could be due to the tentacle pieces containing a higher number of sucker rings than the arms. Although the number of rings on tentacles and arms was not investigated in this study, the tentacles are used for prey capture and it is likely they contain more sucker rings.

Kang et al. (2006) and Iida et al (2006) both report sound speed values for Japanese flying squid. The range of sound speed contrasts for measured mantle and braincase pieces were lower than both previous studies (Figure 20). These differences could be due to sound speed changing between species, or that a whole squid was measured in Kang et al. (2006) and the value in Iida et al. (2006) was an estimate from an acoustic camera. The difference in sound

speed of the two types of braincase pieces suggests that sound speed may vary within the braincase itself. More sound speed data are needed to investigate further how sound speed changes within and between species of squid.

Species-specific material property data for nekton are limited, and scientists often use material property values from the closest species available. We found that the difference in material properties for the hake and myctophids had a large effect on the numerical abundance if these values were to be used to convert acoustic data to biomass. We also found that even though the density contrast value for Japanese Flying squid reported in Kang et al. (2006) was similar to the value we reported for Humboldt squid mantle, it would change the numerical abundance estimate by 11%. This would be important if the biomass estimate was being used for stock assessment purposes. Overall, in order to have an accurate target strength estimate, accurate species-specific material property values are required.

Conclusion:

This study reports the first measured material property values for Pacific hake flesh, myctophid fish flesh, and Humboldt squid body parts. Our results show that density and sound speed contrasts for fish flesh vary within and between taxa. We found that there was a significant difference in the density contrasts of two species of myctophids, *Symbolophorus californiensis* and *Diahus theta*. The density contrasts vary between the different body parts of the Humboldt squid, and the density contrasts of the mantle and braincase were significantly different between the squid collected in Oregon and Monterey Bay. It is unclear if this difference is due to a difference of squid size or environment. Future studies are needed to investigate the effect of environmental conditions and animal length on the material properties of these nekton. The material property data in this study may help improve the target strength estimates of Pacific hake, myctophids, and Humboldt squid. This study could also help improve the scattering models for fish species without swim bladders where their backscatter will be dominated by their flesh and other body components. Improving target strength estimates could constrain biomass estimates from acoustic surveys for these nekton.

Tables

Table 1: The mean and sd of length, maximum height, maximum width, density contrast (g), and sound speed contrast (h) for all zooplankton taxa and species sampled. The number taxa sampled is n.

		Length (mm)	Max Height (mm)	Max Width (mm)	Density Contrast	Sound Speed Contrast	
Zooplankton Taxon	**n**	**Mean ± sd**	**Mean± sd**	**Mean± sd**	**Mean± sd**	**n**	**Mean± sd**
Euphausiids	740	18.1 ± 1.86	3.03 ± 0.456	2.56 ± 0.383	1.058 ± 0.009	17	1.019 ±
Sergestes similis: All	204	30.9 ± 7.76	4.72 ± 0.850	3.73 ± 0.645	1.037 ± 0.005	2	1.028 ±
-large (>37mm)	22	41.4± 2.58	6.45 ± 0.508	4.89 ± 0.339	1.031 ± 0.005	n/a	n/a
-small (<37mm)	182	29.6 ± 3.09	4.51 ± 0.611	3.59 ± 0.530	1.038 ± 0.004	n/a	n/a
Siphonophores: All	108	22.4 ± 2.70	9.53 ± 3.71	4.34 ± 1.22	1.011 ± 0.009	n/a	n/a
Lensia sp.	91	21.8 ± 1.41	8.18 ± 1.37	4.12 ± 0.943	1.012 ± 0.009	n/a	n/a
Unidentified	16	24.6 ± 2.97	16.3 ± 3.65	n/a	1.002 ± 0.004	n/a	n/a
Chaetognaths	94	47.5 ± 5.16	4.97 ± 1.25	n/a	1.006 ± 0.012	n/a	n/a
Larval Fish: All	91	22.5 ± 7.62	6.55 ± 2.03	n/a	1.021 ± 0.015	n/a	n/a
- *Sebastes sp.*	65	20.5 ± 4.27	6.33 ± 1.00	2.316 ±	1.019 ± 0.015	n/a	n/a
-Unidentified	26	27.7 ± 11.1	7.103 ± 3.45	n/a	1.028 ± 0.013	n/a	n/a
Larval Squid	44	24.2 ± 7.51	12.1 ± 3.25	n/a	1.029 ± 0.019	n/a	n/a
Amphipods: All	38	15.0 ± 7.70	4.24 ± 1.32	3.42 ± 1.31	1.037 ± 0.011	n/a	n/a
- *Primno macropa*	24	12.5 ± 1.93	3.59 ± 0.77	2.66 ± 0.500	1.041 ± 0.011	n/a	n/a
- *Phronima sp.*	8	28.7 ± 2.97	6.16 ± 1.15	5.22 ± 0.836	1.027 ± 0.006	n/a	n/a
-Hyperiid spp.	6	6.87 ±	4.27 ± 0.572	n/a	1.032 ± 0.006	n/a	n/a
Crab Megalope	25	9.20 ± 4.82	3.51 ± 0.540	4.57 ± 0.490	1.066 ± 0.006	n/a	n/a
Unidentified Medusae	5	33.3 ± 4.17	33.0 ± 5.07	n/a	1.002 ± 0.006	n/a	n/a

Table 2: A summary of wet weight (g) and length (mm) regression equations from the literature and their correlation coefficients. The mass presented here is the summation of the weights calculated using the regression equation and the lengths of euphausiids measured in this study. The summation of euphausiid mass calculated in this study (from density and volume) was 56.0 g. The difference column is the percent difference of the mass calculated from the regression equations and the euphausiid mass calculated from density and volume values in this study.

Publication	**n**	**Type of Euphausiids**	**Method**	**Equation**	**R^2**	**Mass (g)**	**Difference (%)**
Davis & Wiebe, 1985	93	Atlantic Euphausiids	Preserved Specimens	$WW= 0.0138*L^{3.071}$	0.99	76.71	36.9
Wiebe et al., 2004	100	Antarctic Euphausiids	Preserved Specimens	$WW= 0.0055*L^{3.206}$	0.98	45.40	19.0
Kim et al., 2009	67	*E.pacifica*	Preserved	$WW=0.0082*L^{3.130}$	0.99	54.16	3.3
Harvey et al., 2012	530	*T. raschii*	Frozen/ Thawed	$WW=0.012*L^{2.98}$	0.97	51.13	8.7
	248	*T. inermis*	Frozen/ Thawed	$WW=0.009*L^{3.02}$	0.95	43.10	23.0
	186	*T. longipes*	Frozen/ Thawed	$WW=0.009*L^{3.06}$	0.99	48.45	13.5
This study	740	Pacific Euphausiids	Calculated from density and volume.	$WW= 0.0527*L^{2.496}$	0.58	54.69	2.4

Table 3: The range of mean water column temperature, salinity, density, and fluorescence values for the three sampling sites A1, B, and A2. Mean water column environmental variables were calculated by taking the average of the values measured from the minimum to maximum depth of the CTD cast.

Site	Temperature (°C)	Salinity	Density (g/ml)	Fluorescence (mg/m^3)
A1	7.62-8.16	33.46-33.58	1.027-1.027	0.032-0.058
B	7.76-8.34	33.47-33.65	1.027-1.027	0.032-0.038
A2	5.46-8.82	33.07-34.09	1.026-1.030	0.031-0.132

Table 4: A summary of the mean and standard deviation (sd) of animal length, density contrast (g), and sound speed contrast (h) for all nekton taxa and body parts measured. The number of individual animals (n) and number of body parts measured (m) (from n individual animals) are provided. Mantle length and standard length are reported for squid and fish respectively. The superscript "p" denotes pliable tissue, and "f" denotes firm tissue. Sound speed measurements were on myctophids that were not identified to species, but are likely to be a mix of the two species present.

		Body Length (cm)			**Density Contrast (g)**	**Sound Speed Contrast (h)**	
Species	**n**	**Mean ± sd, range**	**Body part**	**n**	**mean ± sd**	**n**	**h**
Humboldt Squid (*D.gigas*)	9	43.9 ± 6.9, 28-53	Mantle	48	1.027 ± 0.003	3	1.027,1.018,1.023
			Braincase	29	1.025 ± 0.133	2	0.937^{p},1.028^{f}
			Arm	38	1.029 ± 0.004	n/a	n/a
			Tentacle	25	1.037 ± 0.007	n/a	n/a
			Eye	9	1.014 ± 0.005	n/a	n/a
			Pen	7	1.151 ± 0.057	n/a	n/a
			Beak	2	1.360 ± 0.14	n/a	n/a
Pacific Hake (*M. productus*)	2	28.9,40.0	Flesh pieces	20	1.029 ± 0.004	4	0.988^{p},0.988^{f}, 0.986^{p},1.027^{f}
California Lantern Fish (*Symbolophorus californiensis*)		n/a	Flesh pieces	26	0.9992 ± 0.005	1	1.015
California Headlight fish (*Diaphus theta*)		n/a	Flesh pieces	20	1.013 ± 0.005		

Figures:

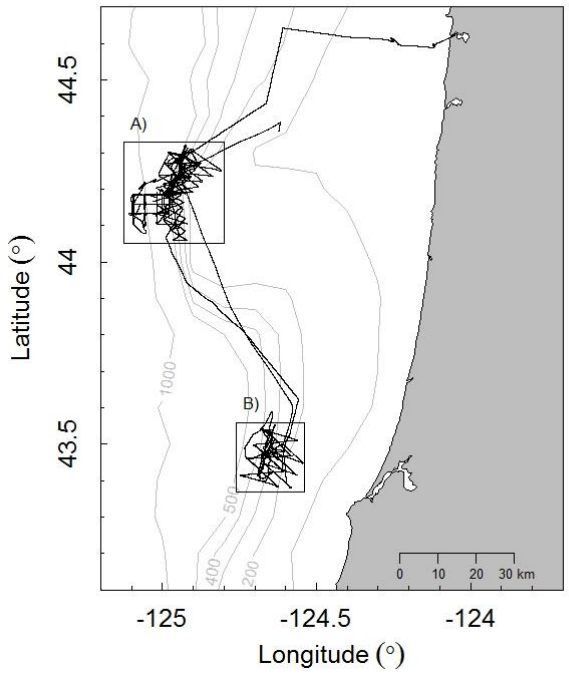

Figure 1: Cruise trackline (solid black line) of the RV Oceanus from 26 July 2012 to 10 August 2012. Region A and Region B are outlined. Bathymetry contours (grey lines) are shown at 100, 200, 300, 400, 500, and 1000m.

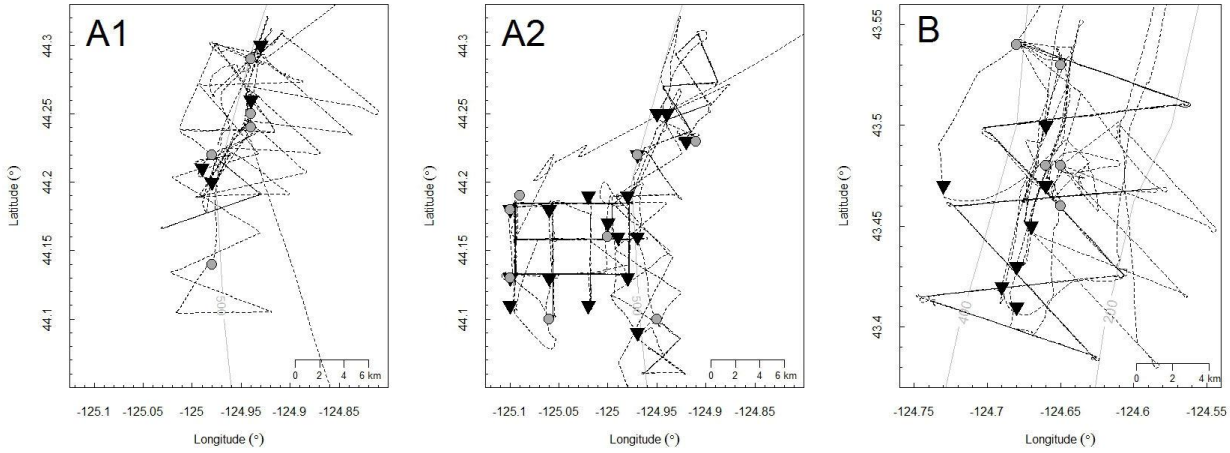

Figure 2: Sampling during the cruise took place within three surveys (Site A (27 July-30 July 2012), Site B (31 July-03 August 2012), and second visit to site A (04 August-10 August 2012) and are referred to as A1, B, and A2 respectively. The ships trackline is in black, net tows (black triangles) and CTD casts (grey circles). Bathymetry contours (grey lines) are shown for 200, 400, and 500m.

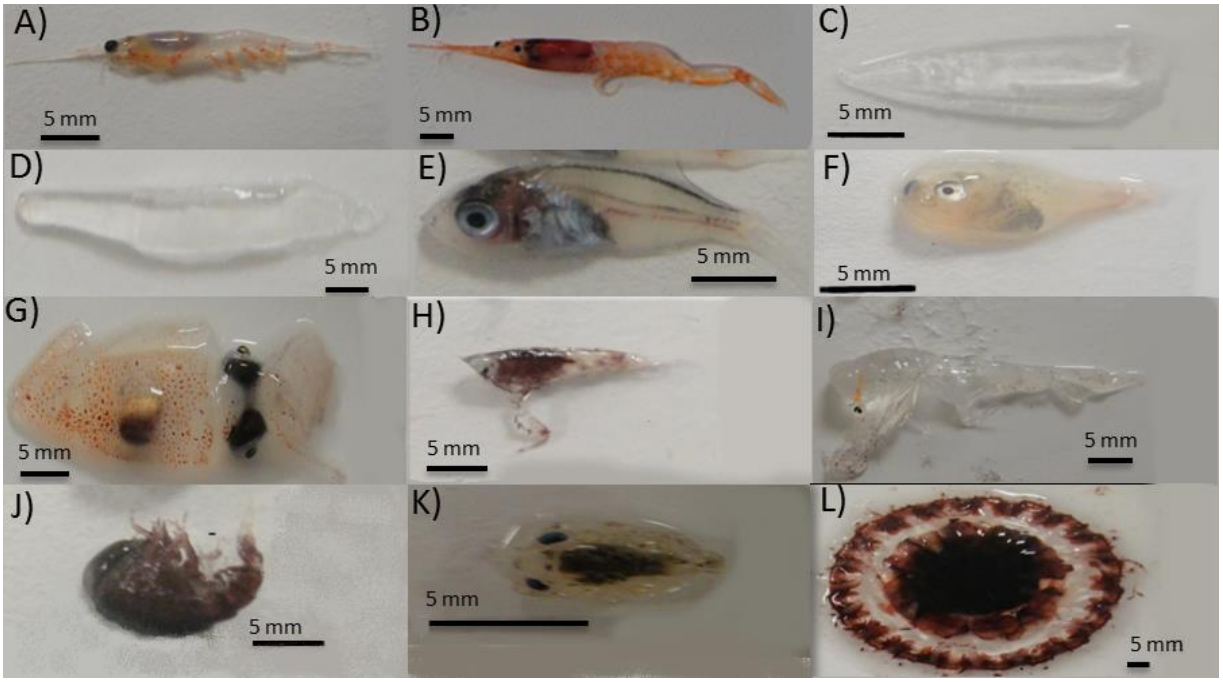

Figure 3: Photographs of Zooplankton Sampled. A) euphausiid, B) *Sergestes similis*, C) siphonophore, D) chaetognath, E) larval rockfish (*Sebastes sp.),*F) UID larval fish, G)larval squid, H) *Primno macropa,* I) *Phronima sp., J)* Hyperiid spp. amphipod, K) crab megalops, L) UID medusa.

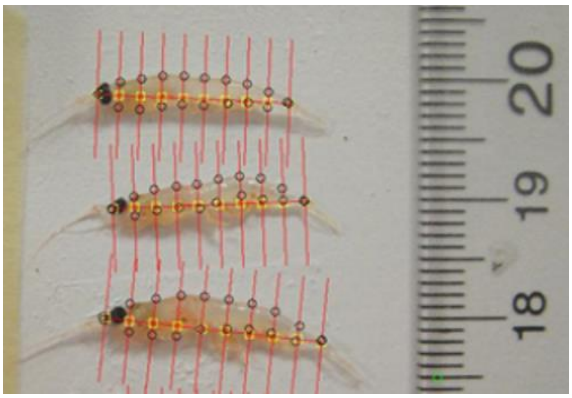

Figure 4: An example digital image showing how the morphology (body shape and size) of animals were measured with a custom MATLAB program. The user clicks the endpoints for the body length measurement and the program generates 10 equally-spaced perpendicular guidelines in red which the user uses to measure the height (or width) along the body length. The black circles show where the user clicked the perimeter of the animal's body. The scale in the image is in cm.

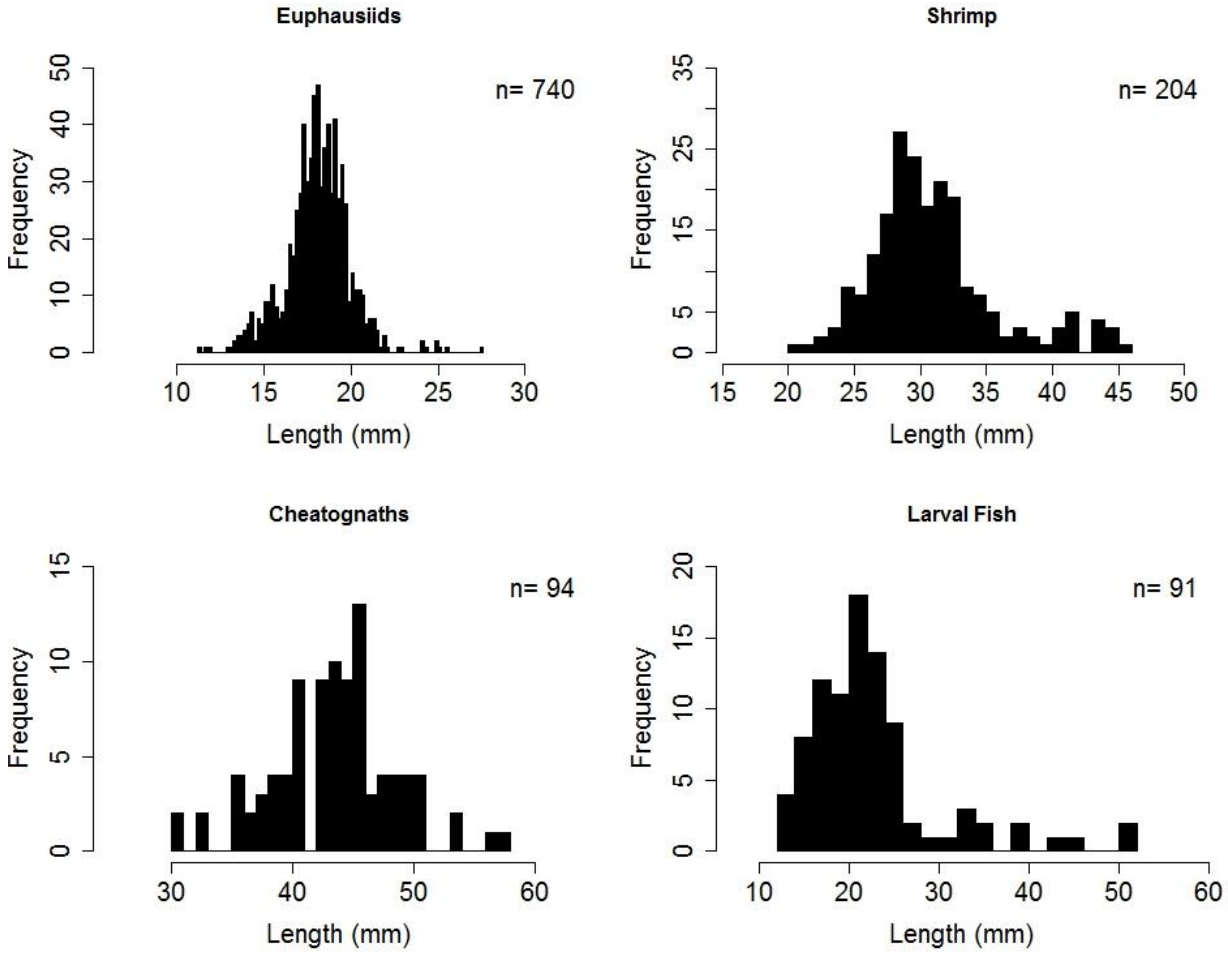

Figure 5: Length distributions for four different zooplankton taxa sampled. The length distribution was bimodal for shrimp (*Sergestes similis)*, but unimodal for the rest of the zooplankton taxa.

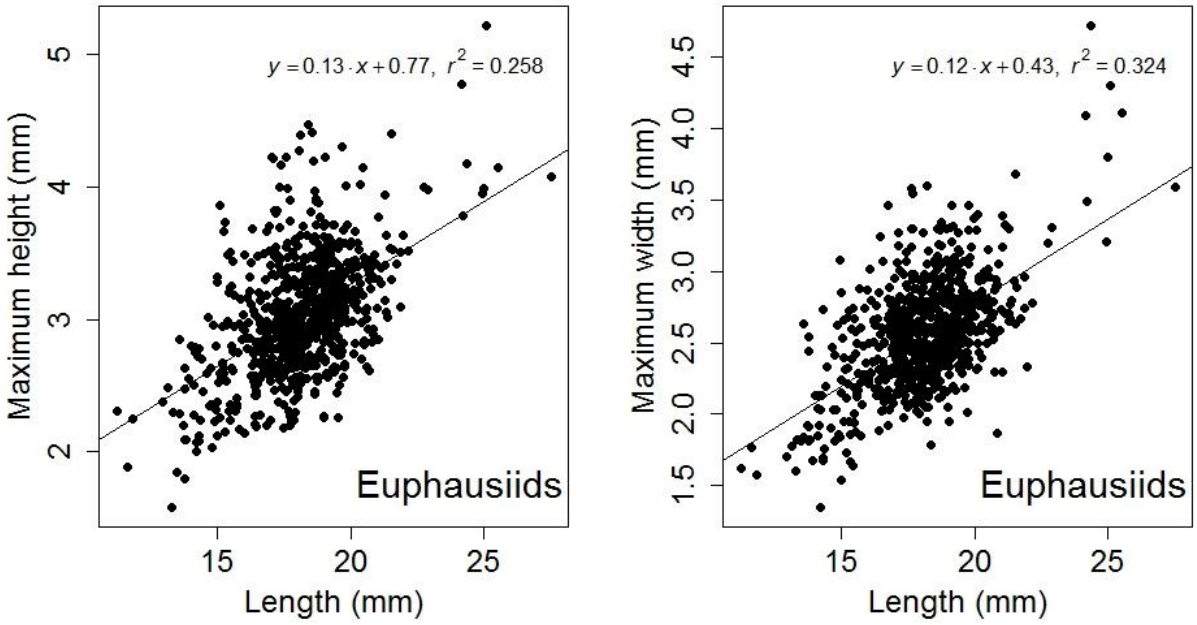

Figure 6: Euphausiid maximum height and maximum width varied with krill length. The regression equations and correlation coefficients are shown.

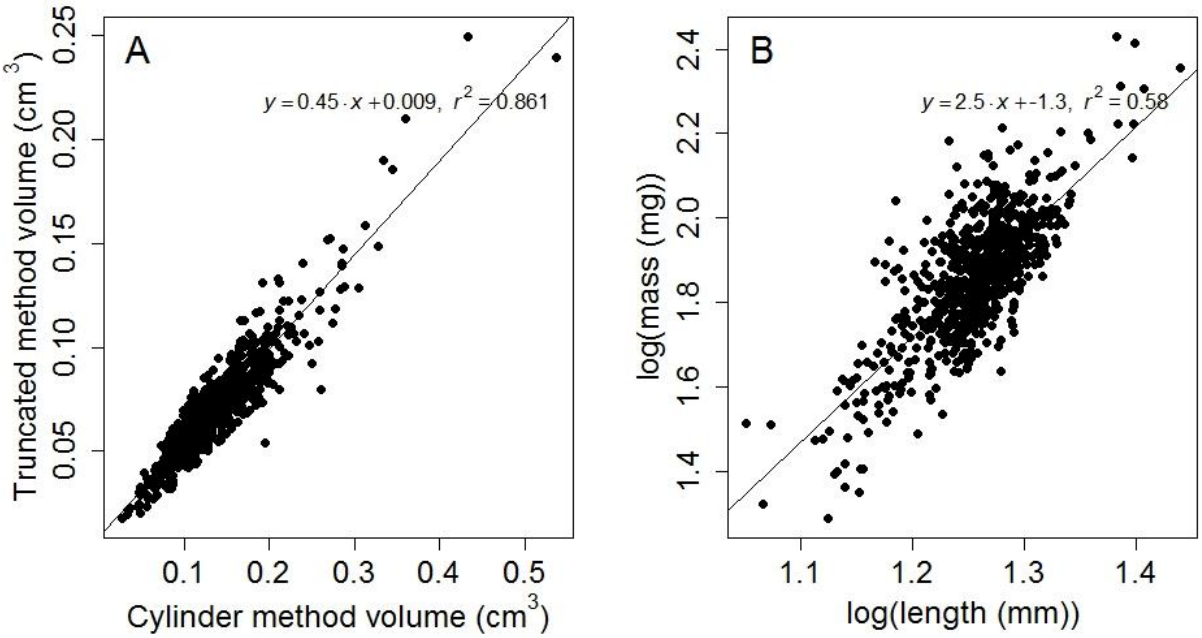

Figure 7: A) Linear regression of euphausiid volume calculated with the truncated and cylinder method. The volumes calculated with the cylinder method were greater than the volumes calculated with the truncated method by almost a factor of two. B) Linear regression of Euphausiid log mass and log length. Mass was calculated from measured density and volume. The regression equations and correlation coefficients are shown.

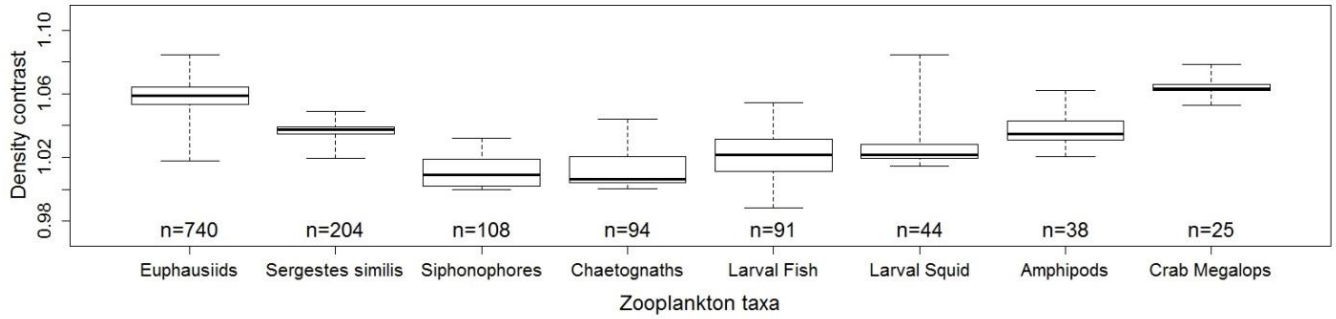

Figure 8: Density contrast for seven different zooplankton taxa. The lower line of each box represents the 1st quartile, the middle bolded line represents the median, and the top line of the box represents the 3rd quartile. The whiskers of the plot represent the minimum and maximum values.

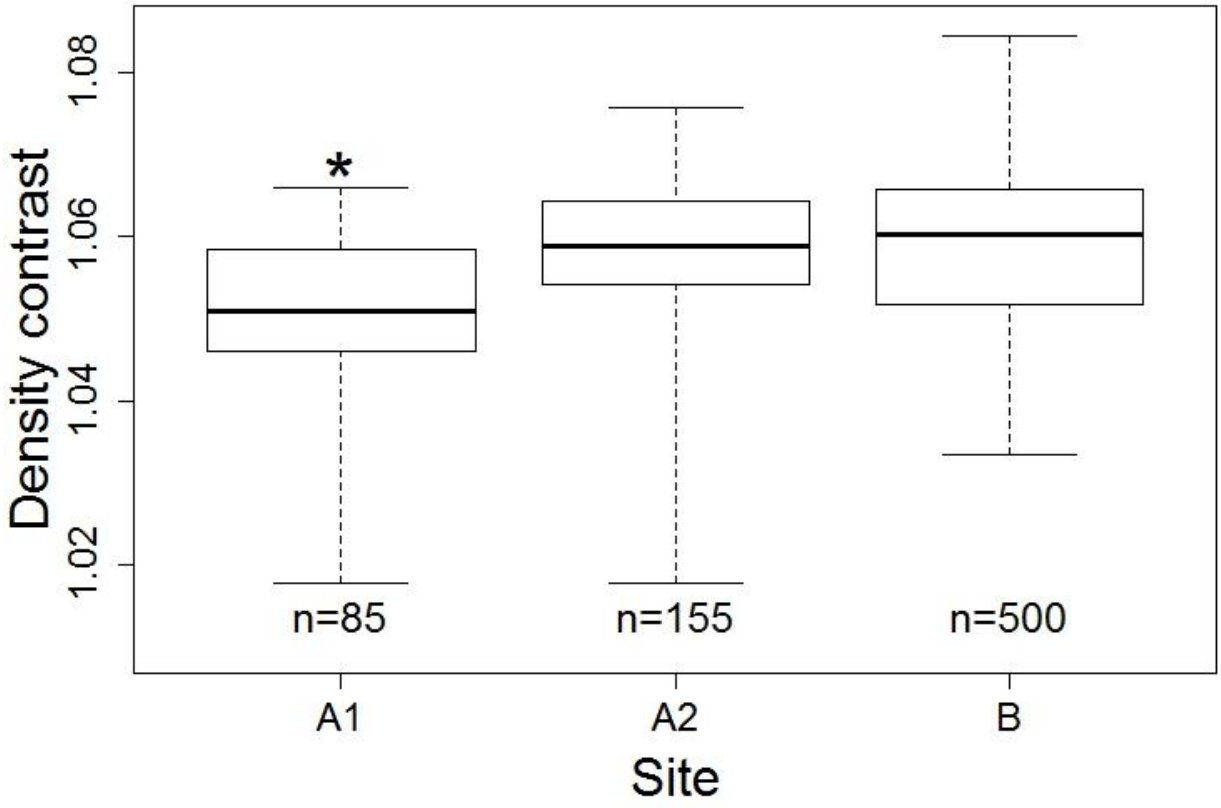

Figure 9: Euphausiid density contrast at the three different sample sites, A1, B, and A2. The lower line of each box represents the 1st quartile, the middle bolded line represents the median, and the top line of the box represents the 3rd quartile. The whiskers of the plot represent the minimum and maximum values. The asterisk (*) indicated that site A1 was significantly lower than the other sites. Euphausiid density contrasts at A2 and B were not significantly different.

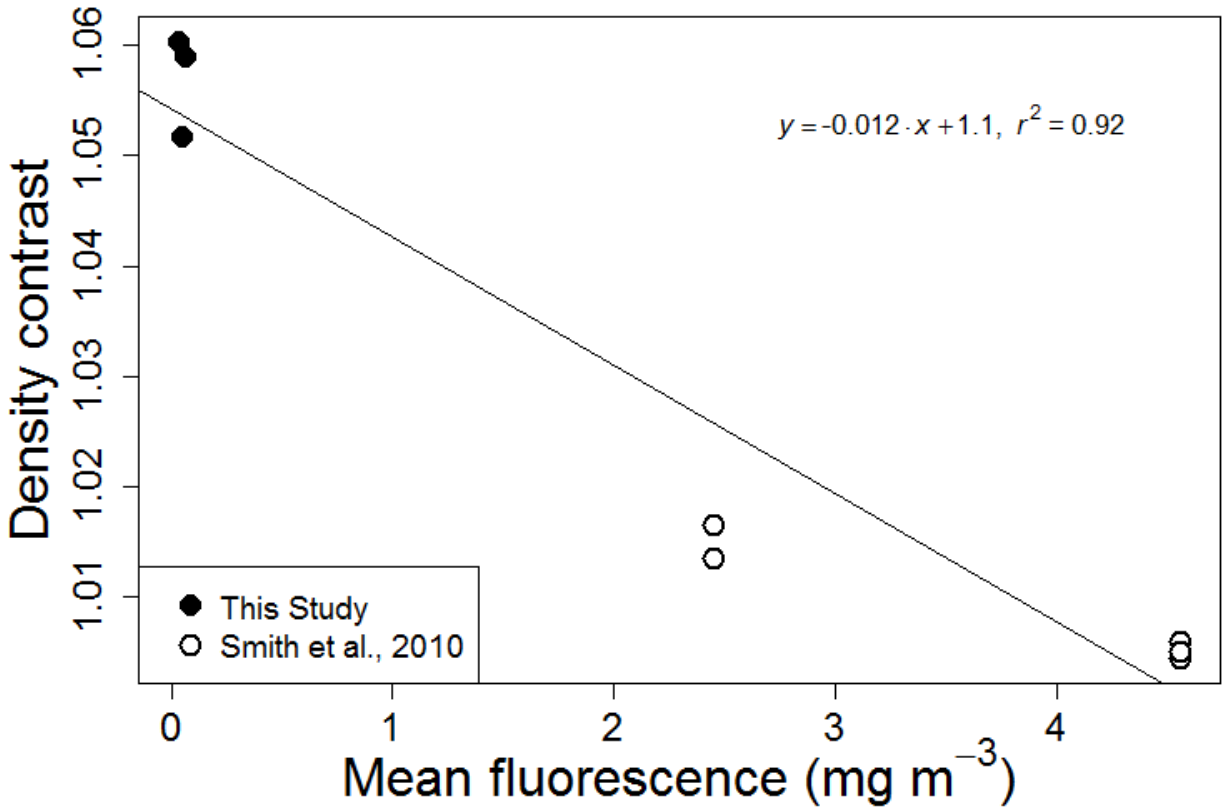

Figure 10: Linear regression of euphausiid g values and mean water column fluorescence at sites from this study and Smith et al., 2010. For this study we plotted average euphausiid density contrast and mean fluorescence values from sites A1, A2, and B. We plotted mean euphausiid (*T. inermis T. raschii T. spinifera)* g values against the mean fluorescence values of 2.45 mg m^3 and 4.56 mg m^3 from the east and west sites reported in Smith et al., 2010.

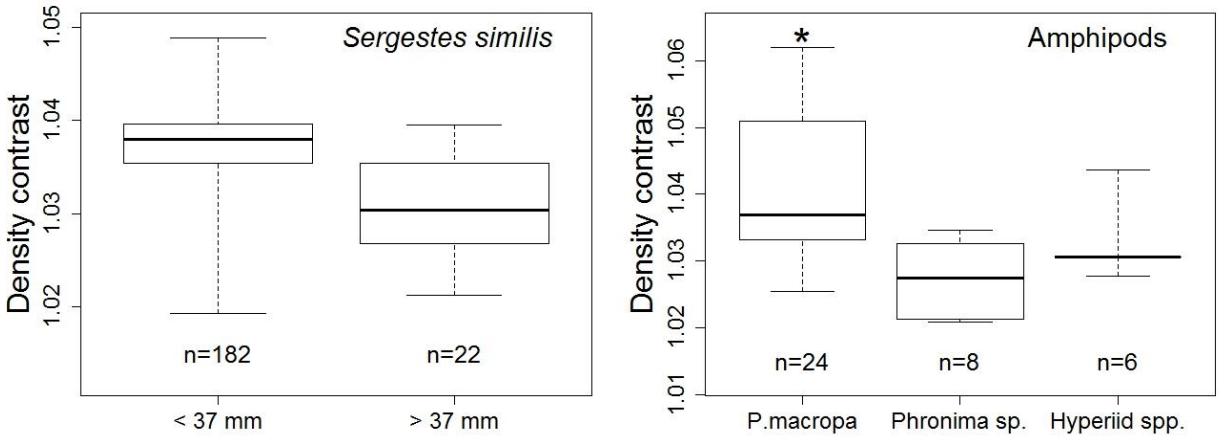

Figure 11: Density contrast varied within and among zooplankton taxa. The density contrasts were significantly different between the two length classes of Sergestid shrimp. Density contrast varied significantly between the species of amphipods sampled in this study. The asterisk (*) indicates that *Primno macropa* density contrast was significantly higher than the density contrasts of *Phronima sp.* and *Hyperiid spp.* amphipods. *Phronima sp.* and *Hyperiid spp.* density contrasts were not significantly different.

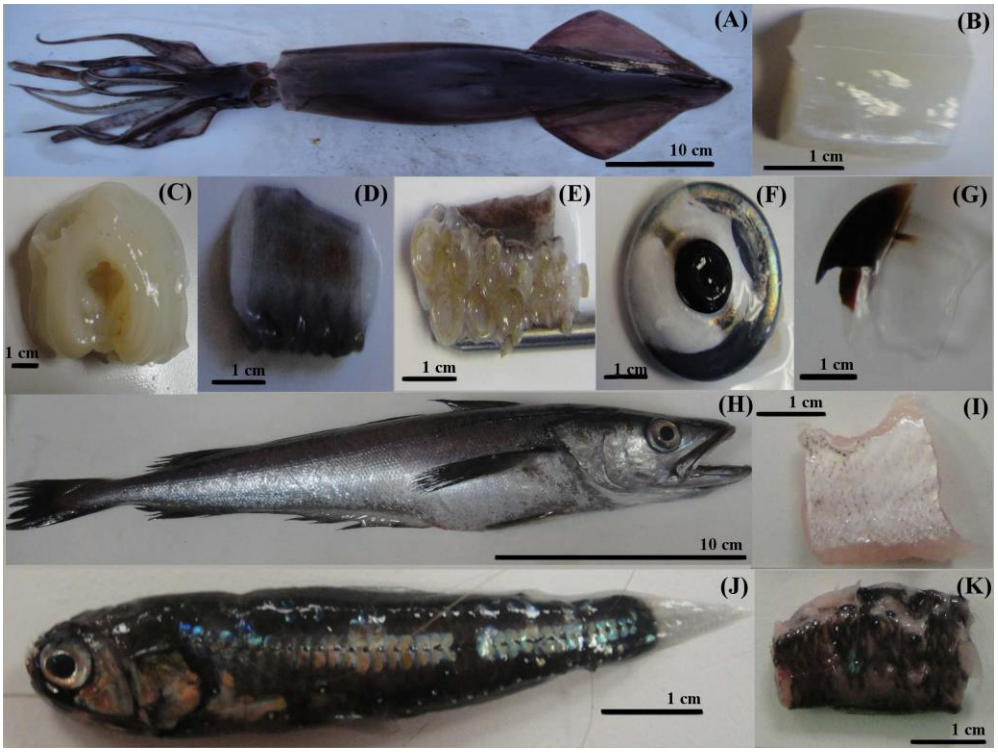

Figure 12: Photographs of nekton (and their body parts) sampled: A) Humboldt squid (Dosidicus gigas), B) Humboldt squid mantle, C) Humboldt squid braincase, D) Humboldt squid arm, E) Humboldt squid tentacle, F) Humboldt squid eye, G) Humboldt squid beak, H) Pacific hake (Merluccius productus), I) hake flesh J) myctophid (*Symbolophorus californiensis)*, K) myctophid flesh.

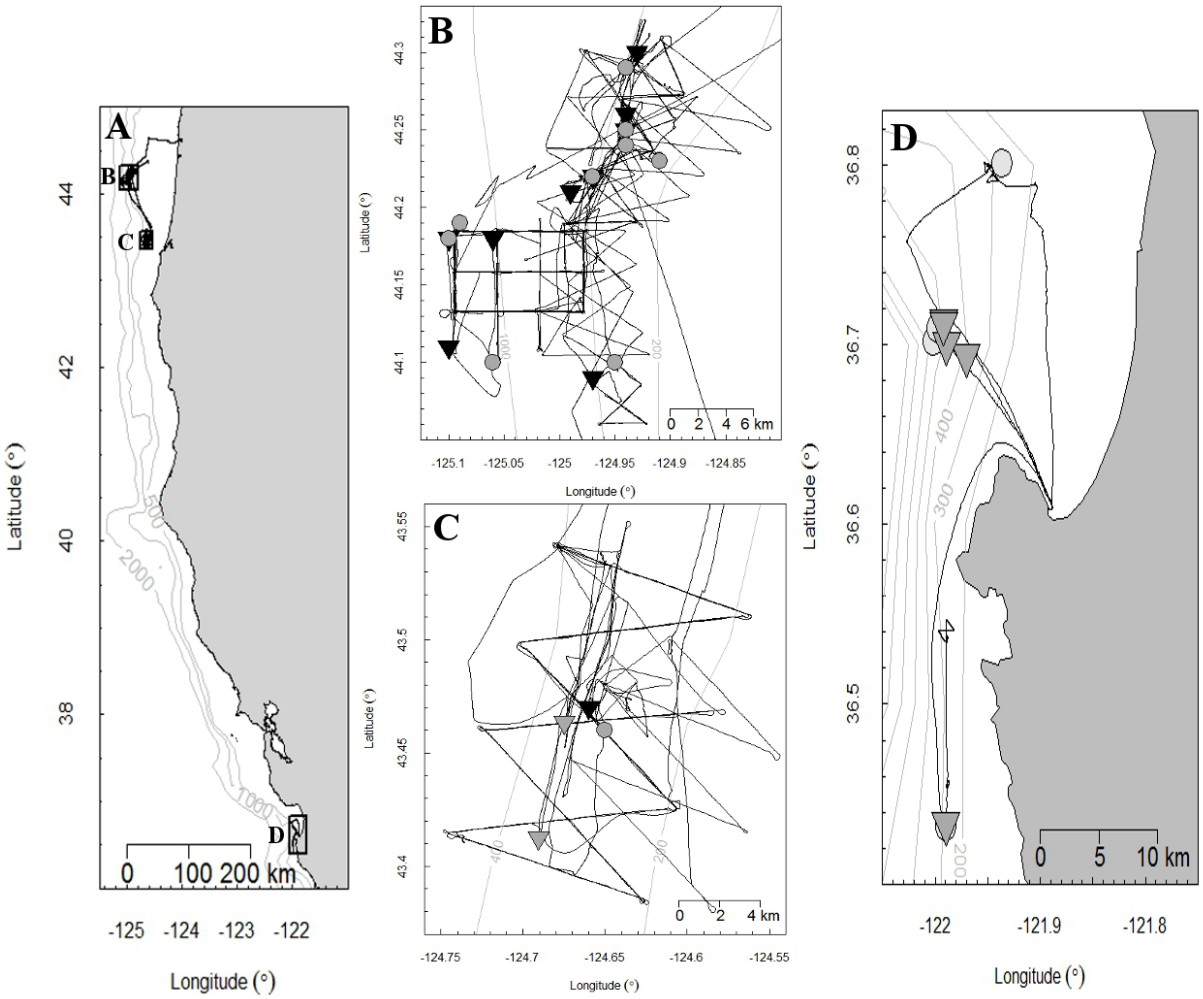

Figure 13: Nekton were sampled in two regions in the California Current (A). These regions include: coastal Oregon from 26 July to 10 August 2012 (B, C); Monterey Bay from 9-11 November 2012 (C). The ship tracks are black lines and bathymetry lines are grey with the depth labeled in meters. Black triangles are where nekton were collected in net tows. Grey triangles are where specimens were collected through jigging. Grey circles are locations where CTD casts were conducted.

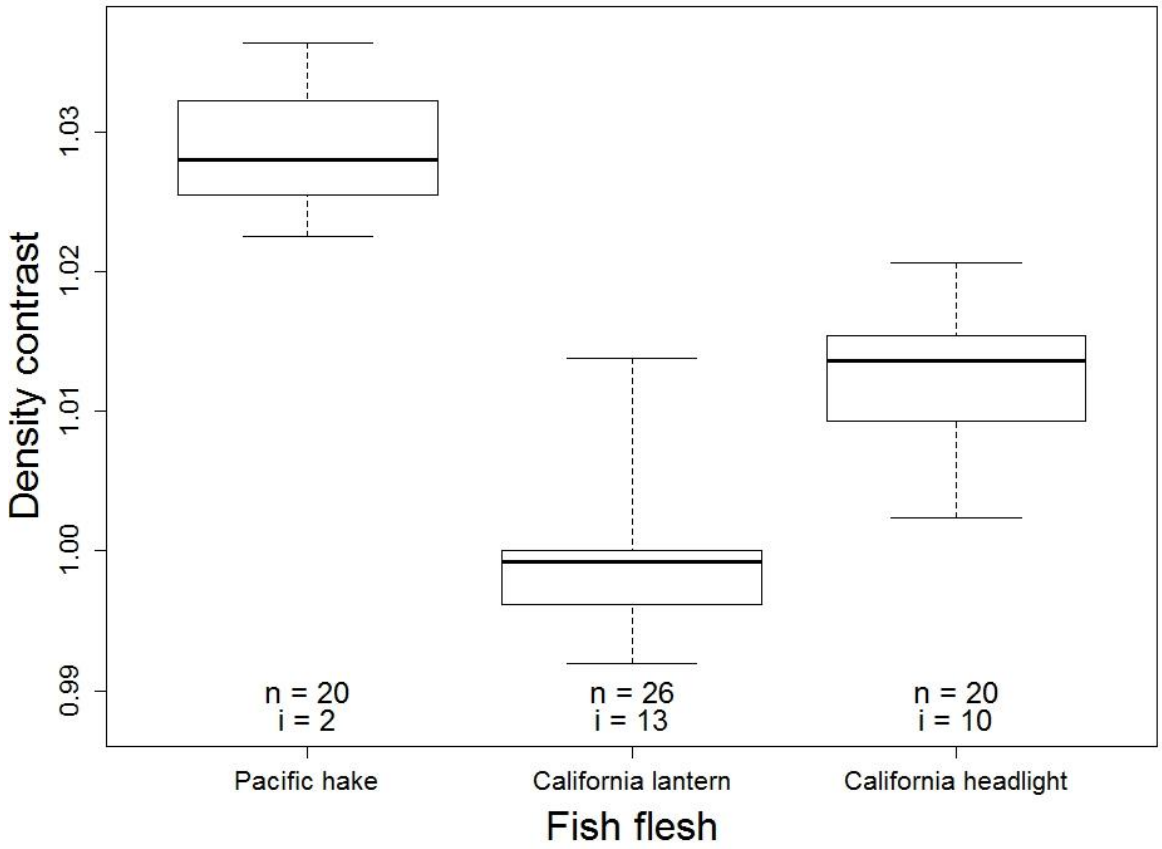

Figure 14: There was a significant variation between Pacific hake and myctophid density contrast ($p < 0.001$). Density contrast also varied significantly between the two species of myctophids measured in this study ($p < 0.001$). For all box and whisker plots in this manuscript: the lower line of each box represents the 1st quartile, the middle bolded line represents the median, the top line of the box represents the 3rd quartile, and the whiskers of the plot represent the minimum and maximum values. The number of measurements is noted by n and the number of individuals measured is noted by i.

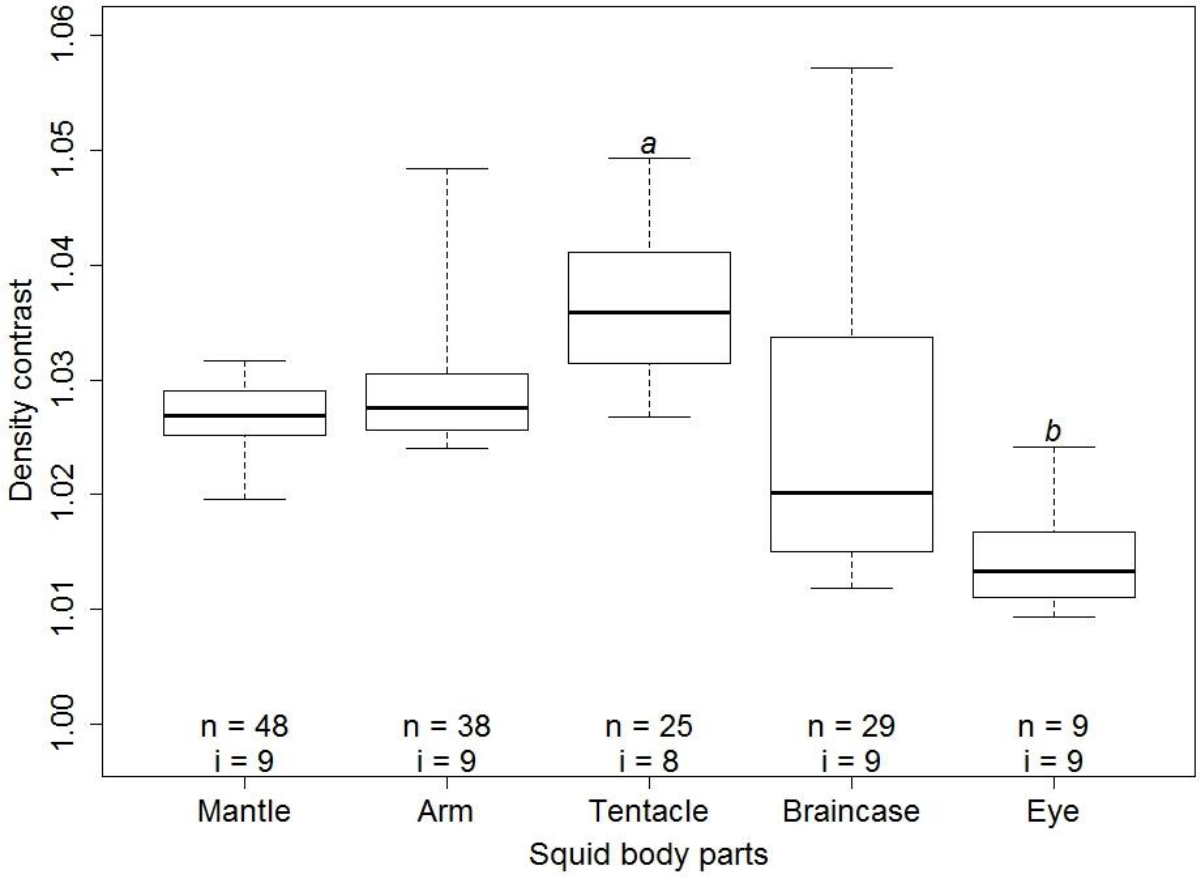

Figure 15: Density contrast varied among the different body parts of Humboldt squid. The mean density contrasts of the tentacle and eye were significantly different ($p < 0.001$, two-sample t-test) (significance indicated by *a*,*b*) than the other squid body parts. The number of measurements is noted by n and the number of individuals measured is noted by i.

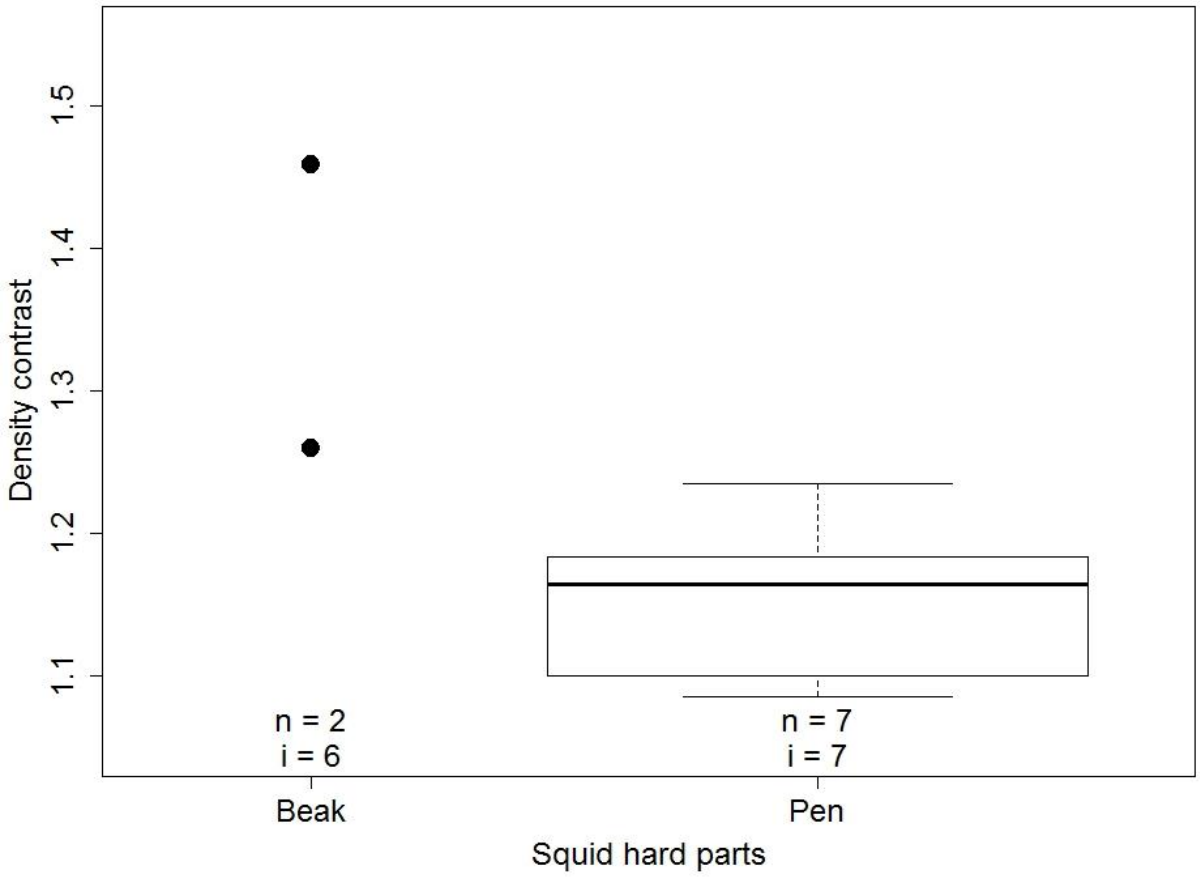

Figure 16: Squid beaks and pens were the densest of the Humboldt squid body parts measured. The number of individual squid measured is noted by i and the number of measurements made is noted by n. There are only two beaks measurements because they did not displace enough water in the pipette method to be measured alone and had to be measured in groups of 3. Only six Humboldt squid beaks were collected. In the pipette method, each measurement was repeated three times, and the mean and standard deviation of those measurements for the two groups of beaks are shown. The measurements plotted for the pen are the mean of the measurements of seven pens collected from seven individual squid.

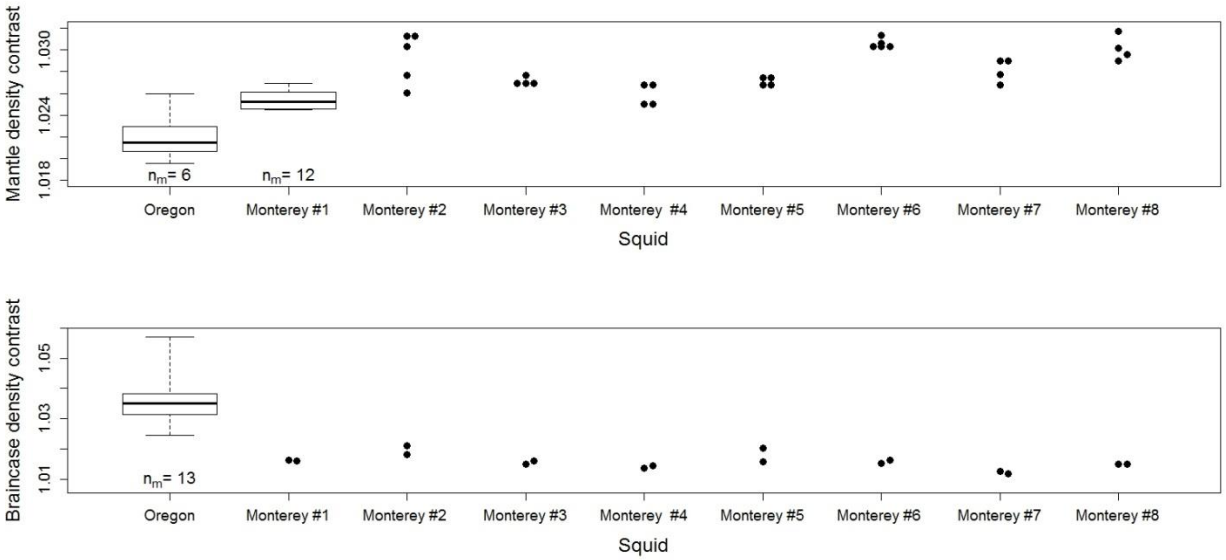

Figure 17: Density contrast of Humboldt squid mantle and braincase of the squid caught in Oregon mantle was significantly lower (two-sample t-test, $p = 0.001$) and density contrast for the braincase was significantly higher (two-sample t-test, $p < 0.001$ than the squid collected in Monterey bay. The Oregon squid was the smaller (28 cm ML), than the Monterey (41-53 cm ML). It is unclear if these differences are a result of size, environmental variables, or location.

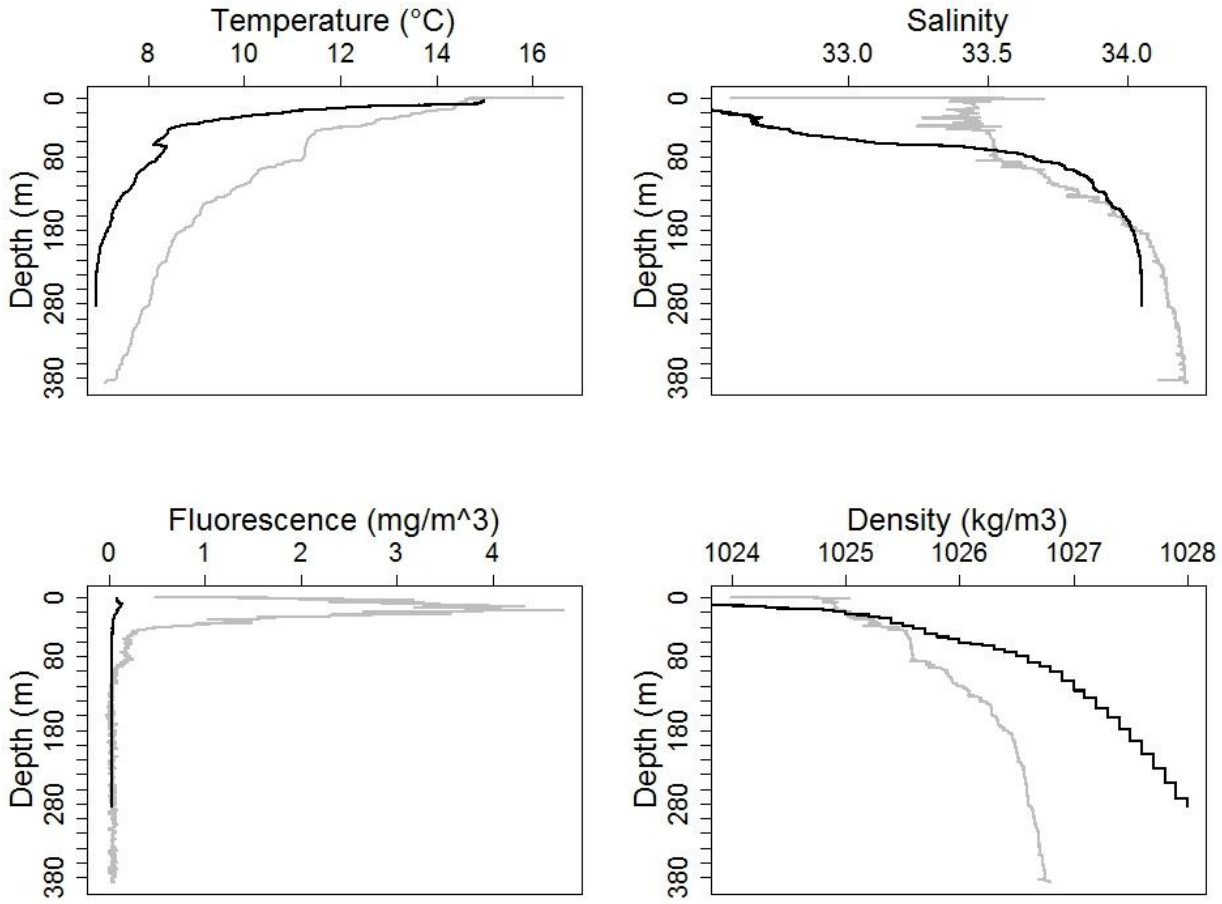

Figure 18: Vertical profiles of environmental data from CTD casts in Oregon (black line) and Monterey Bay (grey line). The environmental conditions, particularly fluorescence, differed between the study sites.

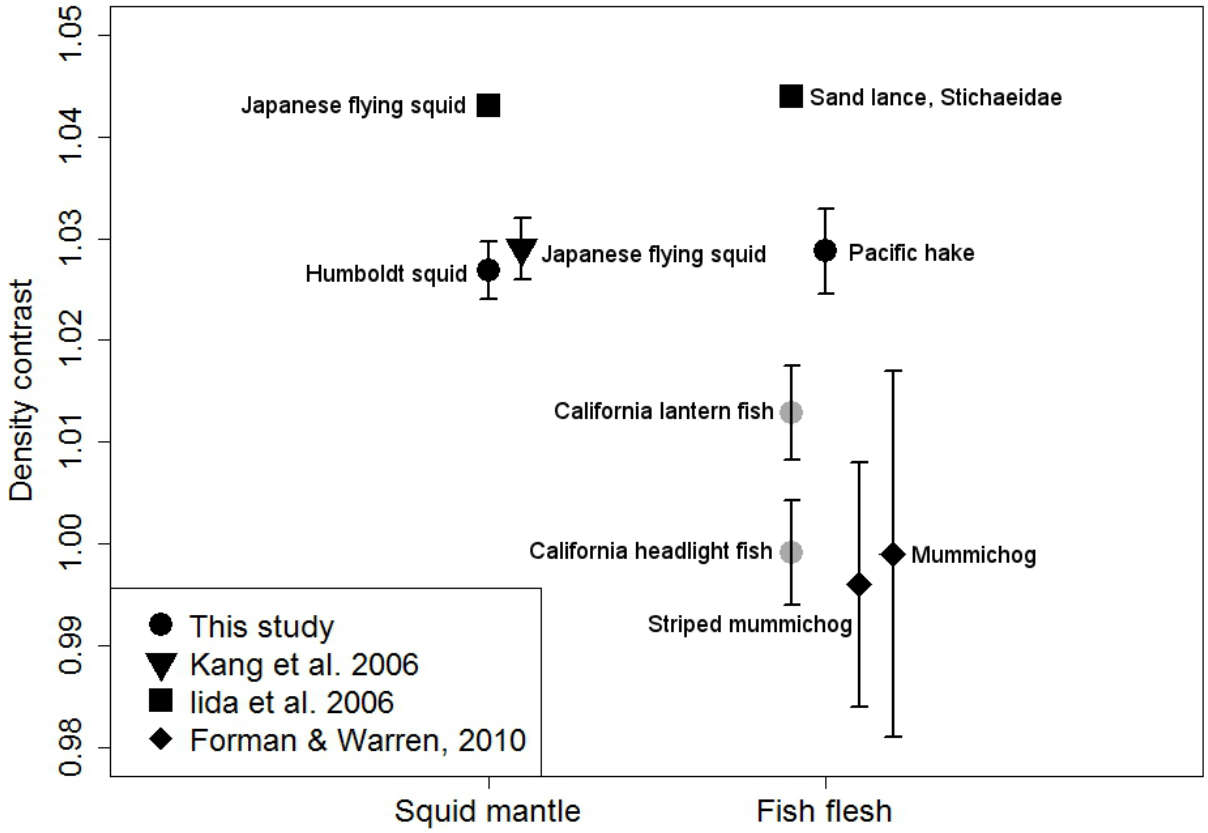

Figure 19: Comparison of published values of density contrast for squid mantle and fish flesh to values measured in this study. For fish flesh, the black and grey circles are Pacific hake and myctophid values respectively. The name of the species sampled in each study is next to the symbol on the plot.

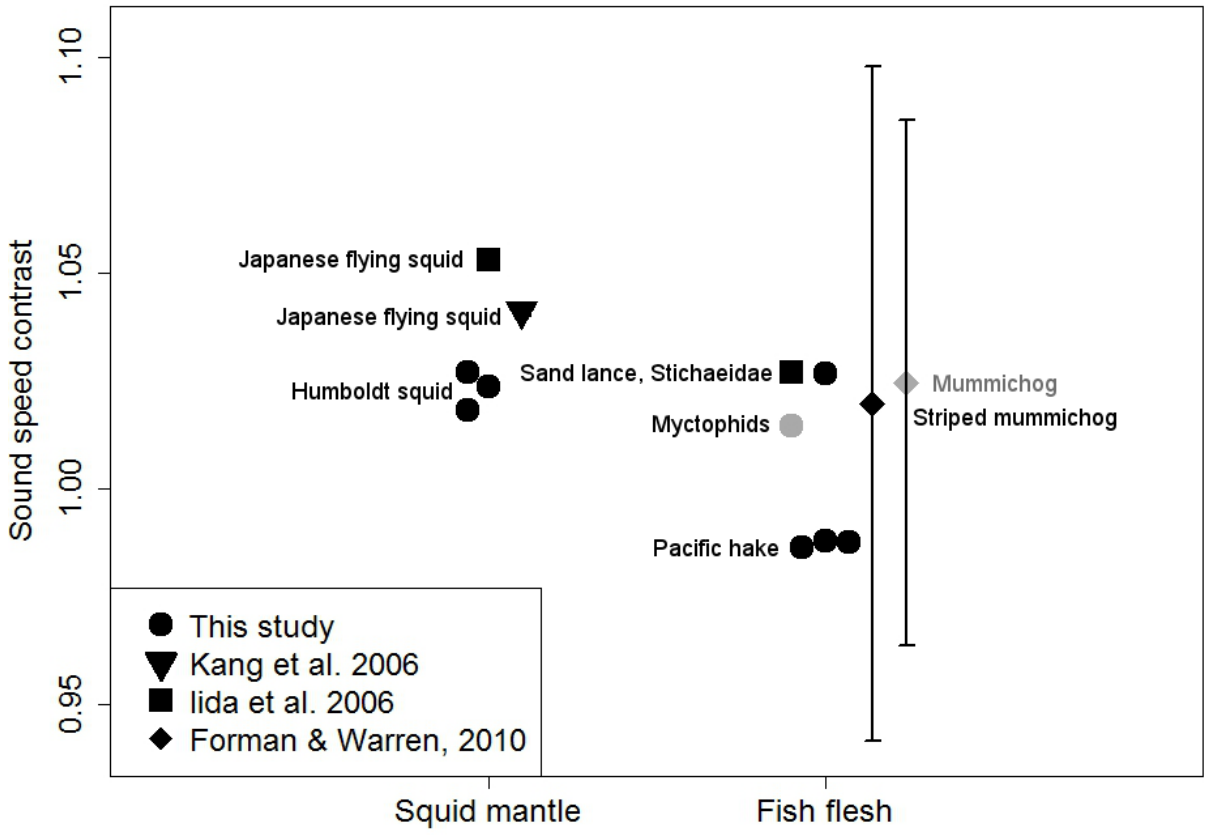

Figure 20: Comparison of published values of sound speed contrast for squid mantle and fish flesh to values measured in this study for fish flesh, the black and grey circles are Pacific hake and myctophid values respectively. The name of the species sampled in each study is next to the symbol on the plot.

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