

# Material properties of euphausiids and other zooplankton from the Bering Sea

Joy N. Smith

School of Marine and Atmospheric Sciences, Stony Brook University, 239 Montauk Highway, Southampton, New York 11968

Patrick H. Ressler

Resource Assessment and Conservation Engineering Division, Alaska Fisheries Science Center, National Marine Fisheries Service, 7600 Sand Point Way North East, Seattle, Washington 98115

Joseph D. Warren<sup>a)</sup>

School of Marine and Atmospheric Sciences, Stony Brook University, 239 Montauk Highway, Southampton, New York 11968

(Received 19 August 2009; revised 17 August 2010; accepted 20 August 2010)

Acoustic assessment of Bering Sea euphausiids and their predators can provide useful data for ecosystem studies if the acoustic scattering characteristics of these animals are known. The amount of acoustic energy that is scattered by different marine zooplankton taxa is strongly affected by the contrast of the animal's density ( $g$ ) and sound speed ( $h$ ) with the surrounding seawater. Density and sound speed contrast were measured in the Bering Sea during the summer of 2008 for several different zooplankton and nekton taxa including: euphausiids (*Thysanoessa inermis*, *Thysanoessa raschii*, and *Thysanoessa spinifera*), copepods, amphipods, chaetognaths, gastropods, fish larvae, jellyfish, and squid. Density contrast values varied between different taxa as well as between individual animals within the same species. Sound speed contrast was measured for monospecific groups of animals and differences were found among taxa. The range, mean, and standard deviation of  $g$  and  $h$  for all euphausiid species were:  $g=1.001-1.041$ ;  $1.018 \pm 0.009$  and  $h=0.990-1.017$ ;  $1.006 \pm 0.008$ . Changes in the relationship between euphausiid material properties and animal length, seawater temperature, seawater density, and geographic location were also evaluated. Results suggest that environmental conditions at different sample locations led to significant differences in animal density and material properties.

© 2010 Acoustical Society of America. [DOI: 10.1121/1.3488673]

PACS number(s): 43.30.Sf, 43.30.Ft [KGF]

Pages: 2664–2680

## I. INTRODUCTION

Zooplankton form a key trophic link between primary producers and higher trophic level consumers in pelagic marine ecosystems (Springer and Roseneau, 1985; Brodeur *et al.*, 2002). Euphausiids, along with more variable contributions from calanoid copepods and juvenile walleye pollock, are the most important prey items for the walleye pollock (*Theragra chalcogramma*) stock on the Bering Sea shelf (Dagg *et al.*, 1984; Livingston, 1991; Ciannelli *et al.*, 2004). Ecologically, walleye pollock are a keystone species (Springer, 1992) in the Bering Sea and are important prey for northern fur seals and other marine mammals, as well as for foraging fish and seabirds (Coyle *et al.*, 1992; Sinclair, 1994; Decker and Hunt, 1996). Economically, the pollock fishery is the largest U.S. fishery by mass and makes up over 40% of the global whitefish production (Ianelli *et al.*, 2008). Walleye pollock are broadly distributed throughout the North Pacific, with the largest concentrations found throughout the Eastern Bering Sea (Ianelli *et al.*, 2008). Acoustic surveys along with net trawls are regularly conducted to estimate the abundance

and distribution of the walleye pollock population there (e.g., Honkalehto *et al.*, 2009). These survey data sets may also be used to identify and quantify the abundance of some zooplankton taxa.

Acoustic techniques allow scientists to observe the spatial and temporal variability in zooplankton distributions with a greater resolution and broader areal coverage than traditional net sampling. Acoustic surveys using multiple frequencies (e.g., 38, 120, and 200 kHz) have previously been applied to the study of zooplankton in the Bering Sea (Coyle and Pinchuk, 2002, Honkalehto *et al.*, 2002). However, in order for acoustic backscatter measurements to be used as a quantitative tool for studying these populations, it is necessary to have a well-constrained estimate of target strength (TS) to convert acoustic energy into a particular biological metric (e.g., numerical density, animal taxon, biomass; Simmonds and MacLennan, 2005). TS estimates are available for many species including walleye pollock (Traynor, 1996), other commercially-important fish species (Simmonds and MacLennan, 2005), and Antarctic euphausiids (Demer and Conti, 2005), but such estimates do not exist for Bering Sea zooplankton. In the absence of direct measurements, TS can be estimated through the use of acoustic scattering models for zooplankton (see review by Foote and Stanton, 2000). TS

<sup>a)</sup>Author to whom correspondence should be addressed. Electronic mail: joe.warren@stonybrook.edu

is a function of the acoustic frequency and the size, shape, orientation, and material properties of the animal (Stanton and Chu, 2000; Chu *et al.*, 2000; Warren *et al.*, 2002). If more information is known about the physical characteristics of the target, then more accurate scattering model predictions will result in improved acoustic predictions of biological information (Stanton and Chu, 2000).

The two material properties most important to zooplankton scattering model predictions are  $g$ , the density contrast between a target and the surrounding seawater, and  $h$ , the sound speed contrast between a target and the surrounding seawater (Anderson, 1950). Along with animal length, the density and sound speed contrasts are particularly critical parameters for predicting scattering from crustacean zooplankton because these animals are modeled as weakly scattering fluid-like objects with material properties similar to that of the surrounding seawater. Small changes in these parameters can cause large changes in the predicted scattering from the animal (Stanton *et al.*, 1994; Wiebe *et al.*, 1997; Stanton and Chu, 2000). Modeling by Chu *et al.* (2000) has predicted up to a 20 dB difference in TS for weakly scattering euphausiid-like zooplankton ( $g=1.0357$ ,  $h=1.0279$ ) with only a 2%–4% change in  $g$  and  $h$ . Relatively few studies have measured the material properties of specific zooplankton taxa. The available data show that  $g$  and  $h$  vary significantly among different types of zooplankton (Greenlaw and Johnson, 1982; Foote *et al.*, 1990; Chu and Wiebe, 2005; Warren and Smith, 2007), as well as within the same zooplankton taxon from individual to individual (Forman and Warren, 2010), between geographic regions, and through time both seasonally and as the organisms age (Hagen *et al.*, 1996; K ogeler *et al.*, 1987). For each geographical area under study, it would be ideal to measure parameters such as animal length and material properties of living specimens from different zooplankton groups for use in these scattering models.

This study measured the physical characteristics and material properties of several types of zooplankton from the Bering Sea during the summer of 2008. Animals were grouped into different taxonomic categories for analysis of their size and material properties and the ranges in  $g$  and  $h$  values for each zooplankton taxon were measured. These taxa included *Thysanoessa raschii* and *Thysanoessa inermis*, the two euphausiid species that dominate zooplankton biomass in the Bering Sea (Smith, 1991), along with a less dominant euphausiid species, *Thysanoessa spinifera*. Several other zooplankton taxa were collected and studied including: copepods (*Neocalanus sp.*), amphipods (*Themisto libellula*), chaetognaths (*Sagitta sp.*), gastropods (*Clione limacina*), larval fish (*Theragra chalcogramma*), and jellyfish (*Chrysaora melanaster* and an unidentified species, possibly *Polyorchis penicillatus*). One nektonic taxon, a squid of the family Gonatidae (species undetermined), was also collected and the material properties of some of their body parts were measured. Although squid are not zooplankton, they are often present in the water column and can be significant scatterers of acoustic energy (Greenblatt, 1981; Brierley and Watkins, 1996). Material properties of euphausiids and copepods were compared to previous measurements of these zooplankton in

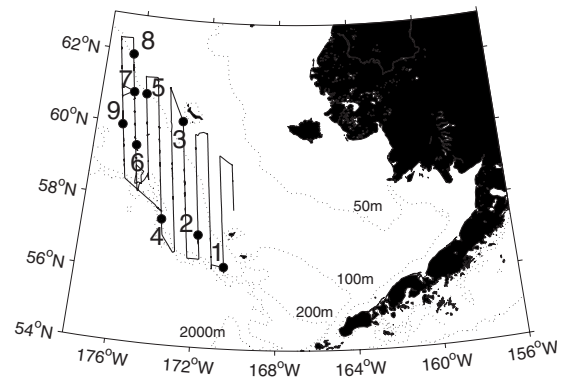


FIG. 1. Cruise trackline (solid line) of the NOAA Ship *Oscar Dyson* from 20 June to 9 July 2008. Methot trawl locations are identified (filled circles) and numbered. Bathymetry contours (dotted lines) are shown for 50, 100, 200, and 2000 m.

the Barents Sea and elsewhere (Greenlaw and Johnson, 1982; K ogeler *et al.*, 1987; Foote, 1990; Chu and Wiebe, 2005). We also examined the influence on  $g$  from animal length, environmental factors (such as seawater temperature and salinity), geographic location, and food availability as measured by fluorescence.

## II. METHODS

### A. Zooplankton collection and husbandry

Live zooplankton were collected using a Methot trawl (MT) at nine stations sampled during an acoustic-trawl pollock survey in the Bering Sea aboard the NOAA ship *Oscar Dyson* from 20 June to 9 July 2008 (Honkalehto *et al.*, 2009; Fig. 1). The Methot trawl is a rigid frame trawl with a mouth area of 5 m<sup>2</sup>, 2 mm by 3 mm oval mesh in the body of the net, and 1 mm mesh in the hard codend (Methot, 1986). In order to obtain live specimens in a minimally-stressed, healthy condition and to maximize the number of animals captured, short duration (average 4.5 min) and shallow (average maximum depth 12.1 m) hauls were conducted at night when zooplankton aggregations in surface waters were expected to be abundant. Upon retrieval of the net, the contents of the codend from each tow were immediately transferred to a large container (~100 l) containing surface seawater. The taxa collected in the Methot trawls included euphausiids, copepods, amphipods, chaetognaths, gastropods, fish larvae, and jellyfish. All zooplankton (or a subset consisting of the most healthy animals depending on the number of animals caught) were then sorted by hand into smaller containers (2 or 34 l) by taxon. They were kept alive until measurements of the physical characteristics and material properties could be made. Animal density measurements were typically made within 6 h (and all measurements were made within 48 h) of the animal being collected.

In addition to the Methot trawl sampling, animals collected in large mid-water trawls conducted as part of the pollock survey (an Aleutian Wing trawl with a mouth area of about 650 m<sup>2</sup>; Honkalehto *et al.*, 2002) were also used in this study. While some jellyfish were caught in the Methot trawl, large jellyfish (e.g., *Chrysaora melanaster* with bell diameters ranging from 15 to 24 cm) and squid specimens

were caught in the mid-water trawl nets, along with pollock and other large nekton. Both the large jellyfish and squid specimens were alive and moving when brought onboard the vessel and were placed in containers (~10 l) of seawater. Unlike the zooplankton captured by the Methot trawl, these animals were likely traumatized or damaged by the net tow collection process. Due to the size of these animals, material property measurements were made on excised parts.

Measurements were taken for a variable number of animals from each taxon depending on the number of animals caught and sorted from each tow. For example, a typical tow contained several hundred to thousands of euphausiids and copepods, while ten to fifty animals from the other taxa were collected. Measurements on individual animals included species identification (when possible), animal length and width, and animal density ( $\rho$ ). Measurements on groups of animals from each taxon included sound speed contrast ( $h$ ). Animals used for the density measurements were separated by taxon and placed into large (34 l) and small (2 l) aerated plastic tanks containing pumped, ambient surface seawater (average temperature 4 °C) in a controlled environment room (air temperature ranging from 2.7–7.2 °C). The large containers held euphausiids and copepods, while the small containers held amphipods, chaetognaths, gastropods, fish larvae, and jellyfish. Animals used in the sound speed experiments were placed in small (2 l) tanks with ambient surface seawater and were then transferred into the chamber used for the  $h$  measurements shortly after the sorting process was completed. The temperature and salinity of the surrounding seawater in the tanks were recorded for each experiment.

## B. Length measurements

Animal lengths were measured for a subsample of animals collected by each Methot trawl. Every animal that had its density measured also had its length recorded. Organisms from a separate subset of animals from each MT had both their lengths and widths measured. Euphausiid lengths were measured from the front of the eye to the end of the telson with the animal positioned as straight as possible. Lengths measured for other zooplankton taxa were total lengths in which the animal was positioned as a straight as possible. Length and width are input variables used in many target strength models (Stanton and Chu, 2000). The relationship between animal length and  $g$  was evaluated through linear regressions for those taxa in which  $g$  was measured for the entire animal (as opposed to pieces of the animal) and where there was a sufficient number of animals collected.

## C. Material properties measurements

### 1. Density

The density ( $\rho$ ) of individual animals from each taxon was measured and the density of the surrounding seawater was calculated from the temperature and salinity measurements. These values were then used to calculate the density contrast ( $g$ ). Animal density was measured for the following: euphausiids, copepods, amphipods, chaetognaths, gastropods, fish larvae, jellyfish and squid body parts. Density measurements for most taxa were made on live organisms, so

their movement had to be suppressed in order to accurately monitor any changes in buoyancy. Individual zooplankton were placed in a small container holding approximately 50 ml of seawater and 1 drop of clove oil until they stopped moving. Density measurements for fish larvae and most jellyfish were not conducted on live animals; however, the measurements were taken shortly after their death. Fish larvae typically did not survive the Methot trawl, while large jellyfish (*Chrysoara melanaster*) had to be cut into small pieces in order to fit into the equipment used to measure animal density. For squid, separate density measurements were recorded for different parts of the body (mantle, pen, beak) after each part was removed from the rest of itself. Beaks were stored in seawater while the pens and mantle pieces were frozen. The density of all three body parts of the squid were measured separately in the laboratory after the cruise. In the cases where live animals of a taxon were not measured, we assume that any changes in density as a result of decomposition or time elapsed between capture and measurement were negligible (except where noted for the squid specimens).

Two different methods were used to measure the density of an animal: the titration method onboard the ship and the pipette method on land. The titration method (Warren and Smith, 2007) for all density measurements, except those of squid material, involved placing the animal in a beaker containing a known volume of ambient sea water ( $V_{sw}$ ). A solution of higher-salinity seawater was created by dissolving Instant Ocean (Aquarium Systems, Inc., Mentor, OH) into ambient seawater. This saltier water was then titrated into the beaker until the buoyancy of the animal was altered and the animal began to rise. The volume of the hypersaline solution required to make the animal float was recorded ( $V_{hs}$ ). For some individual animals that were positively buoyant, fresh water was titrated into the container until the buoyancy of the animal changed and the animal began to sink. Prior to each measurement, the temperature, salinity, and conductivity of the water were recorded (YSI 65, YSI Inc.). The temperature and salinity of the ambient, fresh, and hypersaline seawater solutions were used to calculate the solution density using the CSIRO MATLAB Seawater Library. With the information collected, the density of the individual animal was calculated based on the following equation:

$$\rho_{animal} = \frac{\rho_{sw}V_{sw} + \rho_{hs}V_{hs}}{V_{sw} + V_{hs}}, \quad (1)$$

where  $\rho_{sw}$  is the density of seawater ( $\text{g ml}^{-1}$ ),  $V_{sw}$  is the volume of seawater water used initially to hold the organism (ml),  $\rho_{hs}$  is the density of the solution ( $\text{g ml}^{-1}$ ), and  $V_{hs}$  is the volume of solution used in the titration (ml). Once the density of the animal was measured,  $g$ , the ratio of the animal density to the density of the seawater, was calculated using the following equation:

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

The maximum potential error that can result from calculating animal density has previously been estimated for material

property measurements of different zooplankton species collected from Antarctic waters (Chu and Wiebe, 2005). The maximum potential error associated with the equation used to calculate the density of Bering Sea euphausiids [Eq. (1)] was also estimated with the equation:

$$\begin{aligned} \delta\rho_{animal} = & \left( V_{sw} \frac{\rho_{sw}}{V_{sw} + V_{hs}} \right) \left( \frac{\delta V_{sw}}{V_{sw}} + \frac{\delta\rho_{sw}}{\rho_{sw}} + \frac{\delta V_{sw} + \delta V_{hs}}{V_{sw} + V_{hs}} \right) \\ & + \left( V_{hs} \frac{\rho_{hs}}{V_{sw} + V_{hs}} \right) \left( \frac{\delta V_{hs}}{V_{hs}} + \frac{\delta\rho_{hs}}{\rho_{hs}} \right. \\ & \left. + \frac{\delta V_{sw} + \delta V_{hs}}{V_{sw} + V_{hs}} \right). \end{aligned} \quad (3)$$

The estimated uncertainty of the instrumentation for each parameter was  $\delta\rho_{sw}=4 \times 10^{-5}$  g ml<sup>-1</sup>,  $\delta\rho_{hs}=4 \times 10^{-5}$  g ml<sup>-1</sup>,  $\delta V_{sw}=0.05$  ml and  $\delta V_{hs}=0.05$  ml. The maximum potential

error for the animal density was  $\delta\rho_{animal}=0.0129$ . As the name implies, the maximum potential error gives the greatest possible value of error, however this value is likely to be an overestimation if the uncertainty from the instrumentation is independent and random, as it is in this study. Therefore, a more realistic estimate of the combined uncertainty can be made through quadrature addition. This error analysis technique is based on the principle that measurements from two independent instruments (for example, x and y) will have a normal distribution (thus, an associated  $\sigma_x$  and  $\sigma_y$ ) around their true value (X and Y). The associated error from summing x and y would then be governed by statistical rules and be described by  $\sqrt{[(\sigma_x)^2 + (\sigma_y)^2]}$  (Taylor, 1982). Applying the quadrature technique to Eq. (1), the error estimated through quadrature was calculated using Eq. (4):

$$\begin{aligned} \delta\rho_{animal} = & \rho_{animal} \sqrt{\left( \frac{\rho_{sw}}{V_{sw}\rho_{sw} + V_{hs}\rho_{hs}} - \frac{1}{V_{sw} + V_{hs}} \right)^2 \delta V_{sw}^2 + \left( \frac{V_{sw}}{V_{sw}\rho_{sw} + V_{hs}\rho_{hs}} \right)^2 \delta\rho_{sw}^2} \\ & \cdot \sqrt{\left( \frac{\rho_{hs}}{V_{sw}\rho_{sw} + V_{hs}\rho_{hs}} - \frac{1}{V_{sw} + V_{hs}} \right)^2 \delta V_{hs}^2 + \left( \frac{V_{hs}}{V_{sw}\rho_{sw} + V_{hs}\rho_{hs}} \right)^2 \delta\rho_{hs}^2}. \end{aligned} \quad (4)$$

The estimated uncertainty for each parameter remained the same for both error analysis equations, ( $\delta\rho_{sw}=4 \times 10^{-5}$  g ml<sup>-1</sup>,  $\delta\rho_{hs}=4 \times 10^{-5}$  g ml<sup>-1</sup>,  $\delta V_{sw}=0.05$  ml and  $\delta V_{hs}=0.05$  ml), however the calculated error through quadrature was approximately two orders of magnitude lower than the error calculated using the maximum potential error equation and had a value of  $\delta\rho_{animal\_quad}=1.1721 \times 10^{-4}$ . Since the error estimated through quadrature was small, fluctuations in the g measurements were likely the result of variability from animal to animal instead of error from the instrumentation.

The titration method was used to measure the density of squid beak pieces, but instead of a hypersaline solution being used as the titrant, glycerine ( $\rho=1.173$  g ml<sup>-1</sup>) was used since the beaks were substantially more dense than the hypersaline solution. Squid beaks were excised from the surrounding tissue, stored in seawater, and brought back to the laboratory. There was no visible deterioration or dissolution of the squid beaks from when they were collected and when they were measured. The jaw was divided into top and bottom portions and the density of each piece was measured. Two measurements were taken for each squid beak piece and the mean percent difference between the trials was 0.46%. We had hoped to also measure the density of the squid pen in addition to the beak and mantle; however, the pens deteriorated even more than the squid mantle by the time the density measurements were taken, so no results for the pens are reported.

The pipette method (Warren and Smith, 2007; Forman and Warren, 2010) was used to measure squid mantle den-

sity, since this material was too dense to be properly measured using the titration method. Squid mantles were cut into multiple pieces which were frozen, transferred back to the laboratory, then thawed. The mass of a piece of mantle was measured after excess water had been removed and then the tissue was placed in a graduated cylinder containing a known volume of seawater. The amount of water displaced by the mantle was extracted and weighed. The density of the mantle was calculated using the following equation:

$$\rho_{animal} = \frac{m_a}{\left( \frac{m_d}{\rho_{sw}} \right)}, \quad (5)$$

where  $m_a$  is the mass of the animal (g);  $m_d$  is the mass of water displaced by the organism (g), and  $\rho_{sw}$  is the density of the seawater (g ml<sup>-1</sup>). The density of each mantle piece was measured twice and the mean percent difference between trials was 1.55%. Similar to the titration method, g was calculated using Eq. (2). As the squid mantle measurements were made on frozen and thawed samples, the mantle was slightly deteriorated by the time the measurement was taken. Considering the interest in material properties of squid (Kang et al., 2004), we present these data with the caveat that our measurements may be biased due to degradation of the tissue.

## 2. Sound speed

The sound speed contrast ( $h$ ) was measured for euphausiids, copepods, jellyfish, amphipods, and gastropods.

Animals were placed in a small chamber (PVC t-tube), with a volume of either 26 or 84 ml depending on the size and number of the animals being measured. Two 500 kHz transducers (one transmitter, one receiver) were clamped on either end of the containers. The time it took for a sound wave to travel from one end of the container to the other was recorded when the compartment contained only seawater and when it was full of a mixture of zooplankton and seawater. Knowing the difference in travel time, along with the container volume and zooplankton volume,  $h$  [the ratio of sound speed through zooplankton tissue ( $c_a$ ) compared to sound speed through seawater ( $c_{sw}$ )] was calculated with the following equation:

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

where  $\Delta t$  is the travel time difference between two received waveforms (one waveform where the chamber was filled with animals and one waveform where the chamber was only filled with seawater),  $\Phi$  is the volume fraction [ $\Phi = V_{\text{animal}}/V_{\text{tube}}$  where  $V_{\text{animal}}$  is the volume (ml) of the zooplankton and  $V_{\text{tube}}$  is the volume (ml) of the PVC-tube chamber], and  $t_d$  is the travel time (s) of sound from transducer to receiver with an empty chamber (Greenlaw and Johnson, 1982; K ogeler *et al.*, 1987; Chu *et al.*, 2000; Chu and Wiebe, 2005; Warren and Smith, 2007). The animal volume ( $V_{\text{animal}}$ ) was measured using the displacement method in which a known volume of water filled the chamber, animals were then placed inside the chamber, and the volume of water displaced by the animals was measured. The maximum potential error attributed to the instrumentation was estimated from Eq. (6) using the following:

$$\delta h_{\text{max}} = h \left( \frac{\delta \Delta t}{\Delta t} + \frac{\delta \Phi}{\Phi} + \frac{\delta t_d}{t_d} \right), \quad (7)$$

while the error estimated through quadrature was calculated with:

$$\delta h_{\text{quad}} = \sqrt{\left( \frac{1}{\Phi t_d} \right)^2 \delta \Delta t^2 + \left( \frac{-\Delta t}{t_d \Phi^2} \right)^2 \delta \Phi^2 + \left( \frac{-\Delta t}{\Phi t_d^2} \right)^2 \delta t_d^2}. \quad (8)$$

The uncertainties corresponding to each variable were estimated as  $\delta \Phi = 0.04$ ,  $\delta \Delta t = 5.44 \times 10^{-8}$  s, and  $\delta t_d = 1.62 \times 10^{-7}$  s. The volume fraction uncertainty was estimated using the uncertainty of the graduated cylinder used to measure the volumes of the animals and chamber (0.5 ml). The typical volume of animals in the chamber was 20 ml which results in an uncertainty in the volume fraction of 0.04. The uncertainty of  $\Delta t$  was determined from the resolution of the cross correlation technique used to determine the time shift in the two waveforms. The uncertainty of  $t_d$  was the difference between the maximum and minimum time delay for the transmitted waveform as it traveled from transmitter to receiver for each trial. The maximum potential error calculated for all zooplankton groups was  $\delta h_{\text{max}} = 0.1922$  while the error calculated through quadrature was  $\delta h_{\text{quad}} = 1.5 \times 10^{-3}$ . Similar to the error analysis used for  $g$ , the maximum potential error calculated for  $h$  was likely to be an overestimate of the

true error since the uncertainties of each parameter were independent and random. Thus, a more reasonable estimate of our measurement uncertainty was obtained from the quadrature method using Eq. (8).

The criteria for determining which zooplankton groups would be measured for  $h$  depended on the volume of each zooplankton group caught in the net tow. The volume of zooplankton had to be enough to fill (or nearly fill) the measurement chamber in order to get an accurate measurement of sound speed contrast.

## D. Other parameters measured

### 1. Biomass density

The wet weight of all zooplankton collected by each Methot trawl (MT) was measured and the total volume of water filtered by the MT was calculated by multiplying the area of the opening of the MT and the distance sampled. Both the wet weight and total volume filtered were used to calculate zooplankton biomass density ( $g \text{ m}^{-3}$ ).

### 2. Environmental variables

Animal density contrast is a function of both the density of the animal and the density of the surrounding seawater. Since both temperature and density vary vertically and horizontally (e.g., across frontal regions) in the ocean, changes in  $g$  as a function of these parameters were examined. The average water temperature of the containers holding zooplankton was 4 °C. Several experiments were conducted in which this temperature was manipulated to see if it affected the animal density contrast ( $g$ ). Water temperature was cooled by placing the container with the live animals in an ice bath. Subsequently, temperatures warmed naturally through time as the water adjusted to the surrounding air temperature. Water temperatures ranged from 0.6 to 6.3 °C, which lies within the range of water temperatures that zooplankton may experience throughout the year within the Bering Sea (Coyle *et al.*, 2008). Experiments in which water temperature was manipulated were conducted only for euphausiids since they were the main zooplankton group of interest and most common animal in the Methot trawl catches. We also recorded the changes that occurred in the salinity and density of the water the euphausiids were kept in over the course of the experiments. The influence of ambient water conditions (temperature, salinity, and density) on  $g$  was evaluated.

Fluorescence data were collected from a WETStar (WETLabs) sensor on a Conductivity-Temperature-Depth (CTD) rosette deployed at multiple stations during the cruise. Seven of the MT locations (MT01, MT02, MT04, MT05, MT07, MT08, MT09, Fig. 1) had CTD profiles conducted either immediately before or after the MT, while two MT locations (MT03, MT06) did not. Chlorophyll-a (chl-a) concentration ( $mg \text{ m}^{-3}$ , based on a factory calibration of chl-a fluorescence from the WETStar sensor) was used as a proxy of zooplankton prey (phytoplankton) at each location. Pearson correlations were computed between euphausiid  $g$  and the chl-a concentration at the water depth from which the

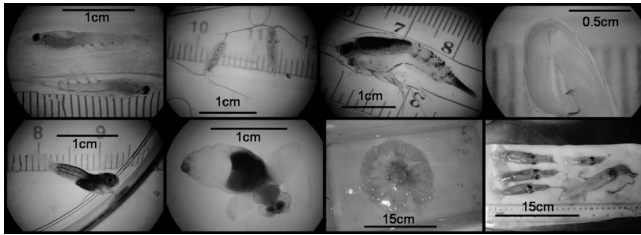


FIG. 2. Representative photographs of taxa collected and measured for their material properties. Top row (from left to right): euphausiid, copepod, amphipod, and chaetognath. Bottom row (from left to right): fish larvae, gastropod, jellyfish, and squid.

zooplankton were collected. Pearson correlations were also computed between  $g$  and the integrated chl- $a$  over the entire water column.

### 3. Statistical analysis

Since the variables that potentially have an effect on  $g$  may also be inter-related, a principal component analysis was performed in order to reduce the variables with redundant behavior into several factors which account for the variance in the observed data. The relationships among variables and between groups of variables and  $g$  were also explored. Second, a linear regression was performed to evaluate the effect of length on  $g$ . Finally, factorial ANOVAs were used to determine whether or not particular variables affected  $g$  by themselves or whether or not those variables interacted with one another to simultaneously affect  $g$ .

## III. RESULTS

Total zooplankton biomass density in Methot catches varied from  $2.5 \text{ g m}^{-3}$  (MT04, the largest value observed) to  $0.03 \text{ g m}^{-3}$  and  $0.05 \text{ g m}^{-3}$  (MT06 and MT07, respectively) and were dominated by euphausiids, copepods, and parts of gelatinous zooplankton. Not all taxa were collected at each station; of all the zooplankton taxa that were collected and measured (Fig. 2) euphausiids were most common and present in all MTs. However, the relative abundance of the three euphausiid species differed with location (Fig. 3). The two dominant euphausiid species were *T. inermis* and *T. raschii*, while *T. spinifera* were only present in MT04.

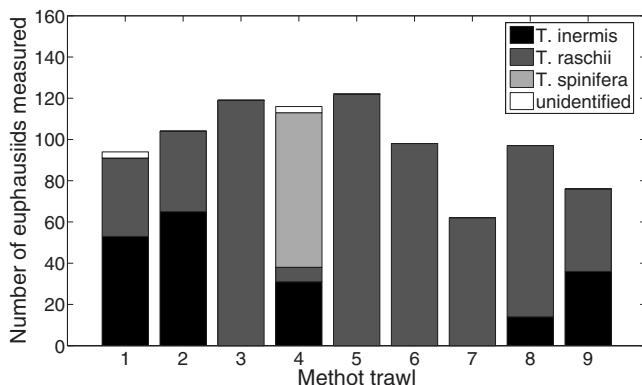


FIG. 3. Species composition of euphausiids caught in Methot trawls that were measured for their physical (length) or material ( $g$ ,  $h$ ) properties.

The average and standard deviation of  $g$  and animal length for euphausiids were calculated for each species; whereas the average and standard deviation of  $h$  were calculated for each MT (Table I). Environmental parameters measured for the ambient water the euphausiids were kept in during the experiments (a function of both natural environment and experimental manipulation; Table I), and for the water column at each location were also reported (Table II). These data are representative of environmental conditions and material properties for euphausiids during summer in the Bering Sea.

## A. Length distributions

Animal lengths varied both within and between different taxa (Table III). There were a sufficient number of specimens to determine the length distribution for euphausiids, copepods, and amphipods. The length distributions of euphausiids (for all species combined and each separate species) were roughly similar, with smaller animals being more abundant than larger animals (Fig. 4, Table III). The mean length (mm) and standard deviation (sd) for all euphausiid species combined as well as for each species was as follows: all ( $18.2 \pm 5.0$ ), *T. inermis* ( $19.2 \pm 2.4$ ), *T. raschii* ( $17.2 \pm 2.5$ ), and *T. spinifera* ( $20.6 \pm 2.4$ ). The length distribution of copepods was also unimodal with a mean and standard deviation of  $8 \pm 0.45$  mm, although the range in lengths was much narrower compared to euphausiids. The length distribution of amphipods was bimodal. There were two size classes of amphipods: small ( $<20$  mm) and large ( $>20$  mm). Amphipods were found only in MT05 and MT08. With the exception of one animal, all of the small amphipods were collected from MT05 while large amphipods were collected at both MT05 and MT08. The bell diameter of the larger jellyfish species (*Chrysaora melanaster*) ranged from 150 to 240 mm with a thickness of 15 to 30 mm. They were drastically larger than the smaller, unidentified jellyfish (a bell-shaped hydromedusae) which had a bell diameter ranging in length from 20 to 31 mm with a bell thickness ranging from 5 to 9 mm.

## B. Density

The density contrast varied among and within the different taxa (Fig. 5). A more detailed examination of the density contrast results for each taxon is presented below.

### 1. Euphausiids

Euphausiids were collected at all MT stations and the densities of 448 euphausiids were measured. As a group (all species),  $g$  ranged from 1.001 to 1.041 with a mean and sd of  $1.018 \pm 0.009$  (Table III). Pairwise scatter plots suggest a positive relationship between location (MT) and both  $g$  and the animal density for all euphausiids: both  $g$  and animal density increased in samples taken further to the west (Figs. 1 and 6). Similarly, we also examined the effects of temperature, salinity, and density of the water in which the animals were kept during the experiments on  $g$  (Table I, Fig. 7); however, the relationships were weak for these variables. A

TABLE I. Mean and standard deviation (sd) of material properties, physical features, and environmental variables for euphausiids at each MT. Density contrast ( $g$ ) values are presented for all euphausiid species combined as well as for each individual species. The sd for all water density measurements was negligible ( $<0.001$ ). The average volume fraction ( $\Phi$ ) of the chamber used for the  $h$  measurements is also reported for each MT.

MT	Taxonomic group	Animal length (mm)		Density contrast		Sound speed contrast ( $h$ )	Temperature ( $^{\circ}\text{C}$ )	Salinity (ppt)	Water density ( $\text{g ml}^{-1}$ )	$\Phi$
		Length	$n$	$g$	$n$					
MT01	All euphausiids	$19.6 \pm 9.1$	94	$1.005 \pm 0.002$	61	1.008	$4.4 \pm 2.4$	$32.1 \pm 0.1$	1.025	0.94
	<i>T. inermis</i>	$20.1 \pm 2.5$	53	$1.006 \pm 0.002$	29					
	<i>T. raschii</i>	$18.7 \pm 1.8$	41	$1.004 \pm 0.002$	15					
MT02	All euphausiids	$20.4 \pm 2.5$	104	$1.007 \pm 0.005$	54	1.002	$5.5 \pm 0.5$	$32.9 \pm 0.5$	1.026	1.00
	<i>T. inermis</i>	$20.3 \pm 2.6$	65	$1.007 \pm 0.005$	38					
	<i>T. raschii</i>	$20.6 \pm 2.1$	39	$1.009 \pm 0.005$	16					
MT03	All euphausiids	$15.3 \pm 1.7$	117	$1.019 \pm 0.007$	67	0.996	$4.7 \pm 0.8$	$30.4 \pm 0.4$	1.024	0.63
	<i>T. raschii</i>	$15.3 \pm 1.7$	117	$1.019 \pm 0.007$	67					
MT04	All euphausiids	$20.0 \pm 3.3$	118	$1.017 \pm 0.007$	58	1.006	$4.0 \pm 1.3$	$33.4 \pm 0.9$	1.026	0.94
	<i>T. inermis</i>	$19.4 \pm 1.3$	31	$1.018 \pm 0.004$	20					
	<i>T. raschii</i>	$19.0 \pm 1.0$	8	$1.016 \pm 0.003$	3					
	<i>T. spinifera</i>	$20.5 \pm 2.4$	76	$1.017 \pm 0.005$	32					
MT05	All euphausiids	$16.3 \pm 1.3$	122	$1.024 \pm 0.006$	72	1.012	$4.1 \pm 2.0$	$33.0 \pm 1.2$	1.026	0.85
	<i>T. raschii</i>	$16.3 \pm 1.3$	122	$1.024 \pm 0.006$	72					
MT06	All euphausiids	$17.5 \pm 1.4$	98	$1.023 \pm 0.006$	48	n/a	$3.9 \pm 2.6$	$33.2 \pm 1.4$	1.026	n/a
	<i>T. raschii</i>	$17.5 \pm 1.4$	98	$1.023 \pm 0.006$	48					
MT07	All euphausiids	$16.9 \pm 1.1$	62	$1.027 \pm 0.003$	12	0.990	$4.9 \pm 0.0$	$32.7 \pm 0.0$	1.027	0.77
	<i>T. raschii</i>	$16.9 \pm 1.1$	62	$1.027 \pm 0.003$	12					
MT08	All euphausiids	$19.1 \pm 3.0$	98	$1.022 \pm 0.008$	48	1.006	$3.3 \pm 2.4$	$34.5 \pm 0.3$	1.027	0.71
	<i>T. inermis</i>	$17.9 \pm 2.2$	14	$1.018 \pm 0.006$	10					
	<i>T. raschii</i>	$19.4 \pm 3.1$	83	$1.024 \pm 0.008$	38					
MT09	All euphausiids	$19.0 \pm 2.3$	77	$1.019 \pm 0.006$	27	1.014	$2.6 \pm 2.2$	$32.1 \pm 0.4$	1.026	1.00
	<i>T. inermis</i>	$18.8 \pm 1.9$	36	$1.016 \pm 0.004$	17					
	<i>T. raschii</i>	$19.3 \pm 2.8$	41	$1.024 \pm 0.005$	10					

principal component analysis (PCA) examined variation among these explanatory variables: MT, species, animal length, temperature, salinity, and water density. The PCA was conducted using only euphausiids with data for all variables, thus only 429 animals were used. The components were orthogonally rotated so correlations between the vari-

ables and the components could be more easily interpreted. The results indicated that PC1, PC2, and PC3 explained 39%, 23%, and 17% respectively (total 79%) of the total variance. The major loadings on each primary component varied: PC1 [location (MT), salinity (ppt), and water density ( $\text{g ml}^{-1}$ )], PC2 [location, temperature ( $^{\circ}\text{C}$ ), and animal

TABLE II. Average water column properties for MT stations, based on nearby CTD casts. No data were available for MT03 and MT06.

Location	Top 5 m water temperature ( $^{\circ}\text{C}$ )	Average water column temperature ( $^{\circ}\text{C}$ )	Average water column salinity (ppt)	Average water column $\sigma_t$ ( $\text{kg m}^{-3}$ )	Average water column chlorophyll-a ( $\text{mg m}^{-3}$ )
MT01	5.8	3.8	32.3	25.9	4.0
MT02	5.4	3.4	32.4	26.1	3.1
MT04	6.3	3.5	32.8	25.7	6.0
MT05	5.5	-0.4	32.0	26.1	3.6
MT07	5.3	0.3	32.1	26.0	1.8
MT08	5.7	0.1	32.1	26.0	3.0
MT09	3.9	0.8	32.4	26.3	1.7

TABLE III. Range, mean, and standard deviation (sd) of animal length, density contrast ( $g$ ), and sound speed contrast ( $h$ ) for different zooplankton taxa; regardless of location. The number of animals measured ( $n$ ) for  $g$  from each taxon is also presented along with the number of animal groups (nn) for which  $h$  was measured. The volume fraction ( $\Phi$ ) of animals relative to the chamber used for the  $h$  measurements is averaged for each zooplankton taxon. Data presented for *Chrysaora melanaster* is from pieces of their bell, not the whole animal.

Zooplankton taxon	n	Animal length (mm)		Density contrast ( $g$ )		nn	Sound speed contrast ( $h$ )		$\Phi$
		Range	Mean $\pm$ sd	Range	Mean $\pm$ sd		Range	Mean $\pm$ sd	
Euphausiids (all species)	448	12–27	18.2 $\pm$ 5.0	1.001–1.041	1.018 $\pm$ 0.009	12	0.990–1.017	1.006 $\pm$ 0.008	0.88
<i>T. inermis</i>	114	15–26	19.2 $\pm$ 2.4	1.001–1.027	1.011 $\pm$ 0.007	n/a	n/a	n/a	n/a
<i>T. raschii</i>	282	12–27	17.2 $\pm$ 2.5	1.001–1.041	1.021 $\pm$ 0.008	n/a	n/a	n/a	n/a
<i>T. spinifera</i>	32	17–27	20.6 $\pm$ 2.4	1.004–1.029	1.017 $\pm$ 0.005	n/a	n/a	n/a	n/a
Copepods	90	7–9	8.0 $\pm$ 0.45	0.995–1.015	1.005 $\pm$ 0.006	3	1.003–1.010	1.007 $\pm$ 0.004	0.88
Amphipods (all)	66	8–32	21.3 $\pm$ 8.3	1.001–1.029	1.010 $\pm$ 0.006	n/a	n/a	n/a	n/a
Small (<20 mm)	22	8–14	10.0 $\pm$ 1.7	1.001–1.009	1.005 $\pm$ 0.002	n/a	n/a	n/a	n/a
Large (>20 mm)	44	23–32	26.9 $\pm$ 2.4	1.002–1.029	1.013 $\pm$ 0.006	3	0.990–1.007	1.001 $\pm$ 0.009	0.59
Chaetognaths	14	13–35	25.6 $\pm$ 6.4	1.007–1.026	1.014 $\pm$ 0.007	n/a	n/a	n/a	n/a
Gastropods	17	9–23	14.9 $\pm$ 4.1	1.008–1.031	1.016 $\pm$ 0.006	1	n/a	1.008 $\pm$ 0.0	0.88
Fish larvae	15	20–33	23.8 $\pm$ 3.3	1.008–1.039	1.023 $\pm$ 0.008	n/a	n/a	n/a	n/a
Jellyfish (all)	40	20–95	49.0 $\pm$ 25.3	1.001–1.006	1.003 $\pm$ 0.001	n/a	n/a	n/a	n/a
<i>Chrysaora melanaster</i>	36	35–95	53.0 $\pm$ 28.0	1.001–1.006	1.003 $\pm$ 0.001	8	0.996–1.007	1.002 $\pm$ 0.004	0.97
Unidentified	4	21–31	24.0 $\pm$ 4.5	1.004–1.005	1.005 $\pm$ 0.001	n/a	n/a	n/a	n/a
Squid beak	30	25–17	5.0 $\pm$ 3.1	1.125–1.180	1.149 $\pm$ 0.013	2	1.010	1.010 $\pm$ 0.0	0.51

length (mm)], and PC3 (species). These results suggest that spatial location and correlated changes in physical water properties (density, salinity, and temperature) were responsible for most of the variance among explanatory variables, while animal length and species had measurable but weaker contributions.

Average  $g$  was slightly different among species (Table III), but the uneven distribution of species by station (Fig. 3) makes it difficult to separate species effect from that of location. There was a similar trend in changes in  $g$  with location for both *T. inermis* and *T. raschii*, the euphausiid species found at more than one station (Fig. 8). There was no strong relationship between euphausiid length and  $g$  for any species or for all species combined, as there was a large variability in

$g$  between different animals of the same length (Fig. 9). Linear regressions between animal length and  $g$  indicated a very weak negative relationship for all euphausiids ( $R^2=0.062$ ) and for each species individually (*T. inermis*— $R^2=0.014$ ; *T. raschii*— $R^2=0.004$ ; *T. spinifera*— $R^2=0.063$ ).

The seawater surrounding the animals during their measurements was maintained at the ambient temperature of the seawater at the collection site. Occasionally, the temperature of the water in the container holding the animals was lowered (by placing the container in an ice bath) or raised (by exposing the container to warmer, ambient air) to provide a greater experimental temperature range. To evaluate the complexity of multiple factors impacting  $g$ , two-way factorial ANOVAs were used to determine the relationship be-

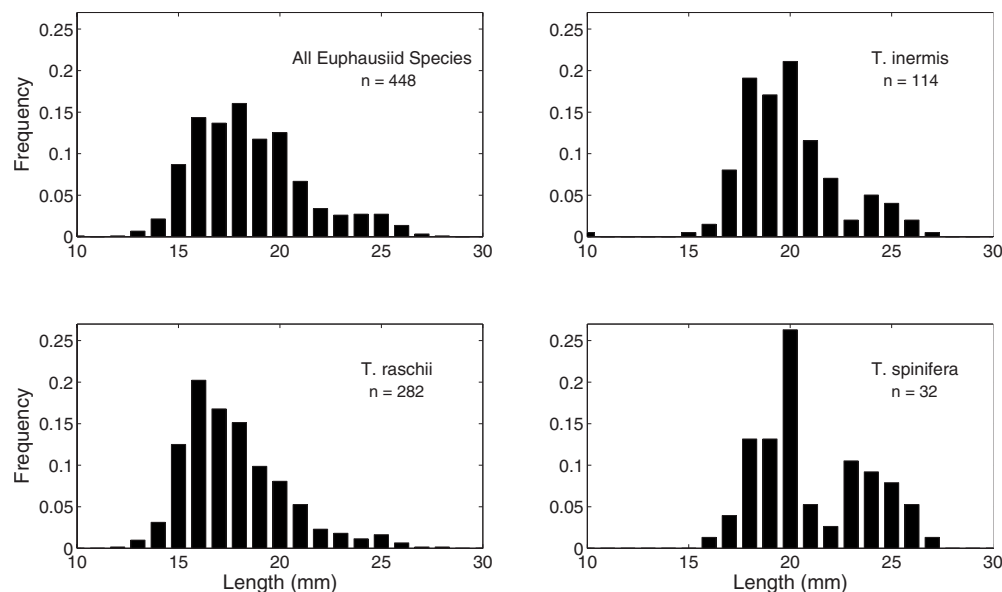


FIG. 4. Length-frequency distributions for all euphausiids (upper left), *T. inermis* (upper right), *T. raschii* (lower left), and *T. spinifera* (lower right).

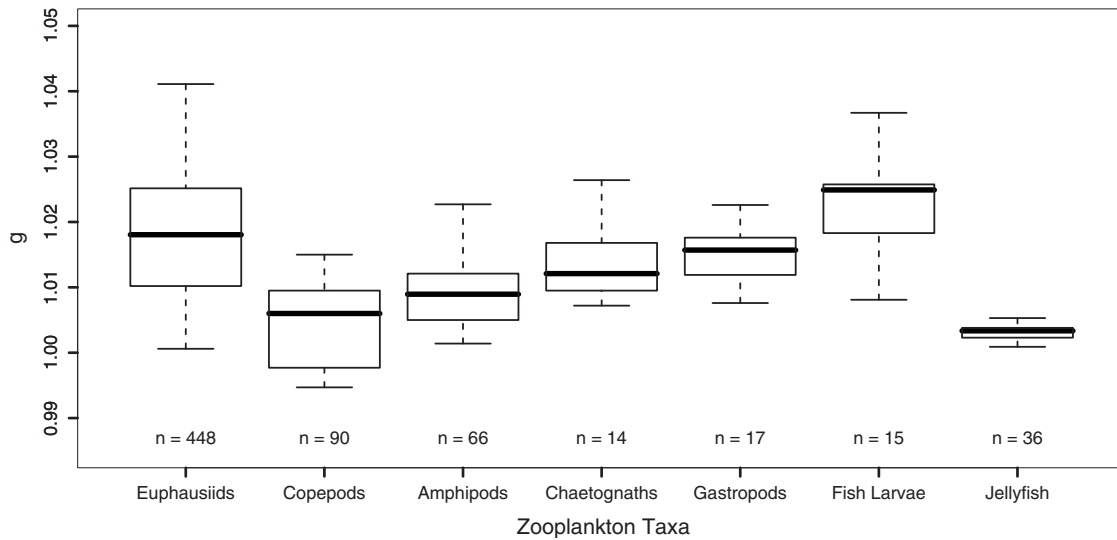


FIG. 5. Density contrast of each zooplankton taxon. The lower line of the box represents the 1<sup>st</sup> quartile, the thick black line is the median, and the upper line of the box is the 3<sup>rd</sup> quartile while the whiskers are the maximum and minimum values. Circles represent outliers. The zooplankton taxa are all fluid like and subsequently have lower values of  $g$ . Euphausiids have the largest range in  $g$  values while jellyfish (data from two species combined) have the smallest range in  $g$  values.

tween location, temperature, and the interaction between the two variables on animal density, water density, and  $g$ . These statistical analyses were performed on each species separately. *T. inermis* were exposed to water temperatures ranging from 0.5 to 6.3 °C and *T. raschii* were exposed to similar temperature ranges of 0.5 to 6.4 °C. Results for *T. inermis* indicated that location, temperature, and the interaction between the two variables had a significant effect on the animal density, the water density, and  $g$  ( $p < 0.001$  for all tests): animal density, water density, and  $g$  increased at stations located further to the west and with colder ambient water temperatures, and ambient water temperature was related to station location.

The relationship between location and water density on  $g$  was analyzed through two-factorial ANOVAs. This analysis was applied to *T. inermis* and *T. raschii* separately and the results differed for each species. *T. inermis* were exposed to water densities ranging from 1.0252–1.0282  $g\ ml^{-1}$ . Results from the two-factorial ANOVA for *T. inermis* indicated that location, water density, and the interaction between the two

variables had a significant effect on  $g$  ( $p < 0.001$  for all three results). *T. raschii* were kept in water with densities with a larger range (1.0234–1.0282  $g\ ml^{-1}$ ) and yet only location had a significant effect on  $g$  ( $p < 0.001$ ).

The results from the PCA and previously described two-factorial ANOVAs have indicated that location influences  $g$ . Furthermore, a piecewise multiple comparison procedure (Dunn's Method) indicated that the western locations (MT05, MT06, MT07, MT08, MT09) were significantly different from the eastern locations (MT01, MT02, MT03, MT04) for both *T. inermis* and *T. raschii* ( $p < 0.05$  for all pairwise comparisons between eastern and western sites). The  $g$  values of *T. inermis* and *T. raschii* were grouped into eastern and western sites and a Kruskal-Wallis one-way ANOVA on ranks was evaluated between the two groups for both species. Results from both ANOVAs indicated that there was a significant difference in  $g$  between the eastern and western sites ( $p < 0.001$ , for both species) with the animals

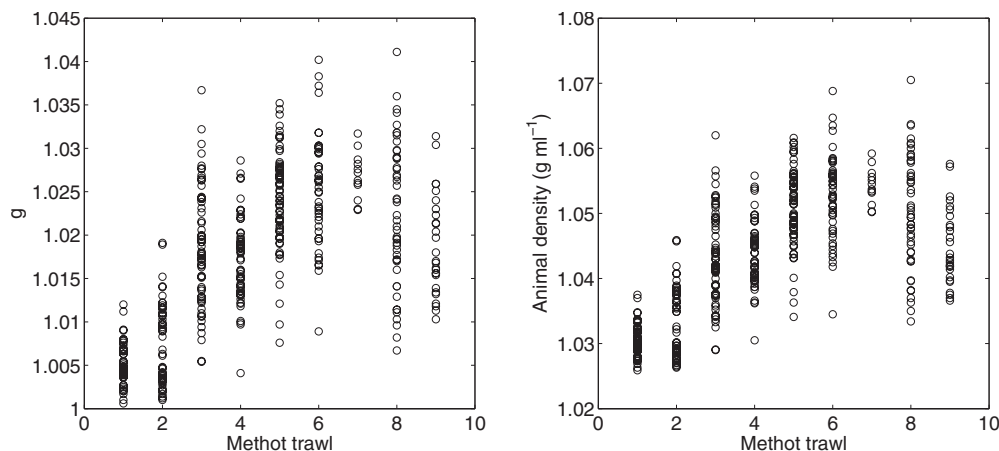


FIG. 6. Pairwise scatter plots of  $g$  and animal density as a function of location for all euphausiids.

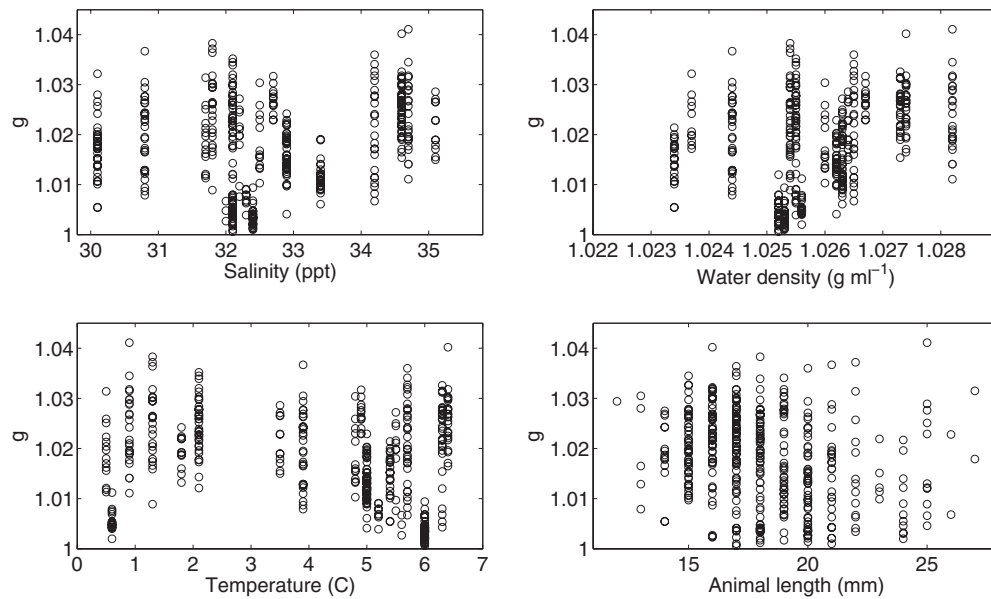


FIG. 7. Pairwise scatter plots for ambient salinity, water density, temperature, and animal length against  $g$ . Location, salinity, and water density are components of PCI and location, temperature and animal length are components of PC2. Although the relationship is weak, as PCI increases so does  $g$ . On the contrary, as PC2 increases,  $g$  decreases.

from western sites having a significantly lower  $g$  than those from the eastern sites (Fig. 10).

Water column properties at each MT station (rather than the properties of the ambient water in which the animals were kept) indicate that the western stations were characterized by generally colder and denser water with lower chlorophyll concentrations (Table II). Since location was shown to have an effect on  $g$ , we examined the hypothesis that differences in chl-a between the sites explained the differences in  $g$  with location that were observed. The maximum chl-a concentration varied at each location and occurred at different depths (ranging from 16.9 m to 50.0 m). The results from the Pearson correlation between  $g$  and the chl-a measured at the same depth at which the zooplankton were collected indicated that there was a weak relationship between the two variables ( $r^2=0.025$ ,  $p<0.05$ ). Euphausiids migrate vertically and move throughout the water column over the

course of a diel cycle (Schabetsberger *et al.*, 2000); thus, a Pearson correlation was also computed between  $g$  and chl-a integrated over the entire water column which found a significant relationship ( $r^2=0.172$ ,  $p<0.001$ ). The correlation between chl-a and  $g$  was negative, that is  $g$  decreased as chl-a increased. Finally, the chl-a data were divided into the same two areas (eastern and western) and t-tests were used to determine whether there was a significant difference in chl-a between the two areas. There was no significant difference between the eastern (MT01, 02, 04) and western (MT05, 07, 08, 09) locations for the chl-a measured at the depth from which the zooplankton were collected ( $p=0.06$ ). However, there was a significant difference between the integrated water column chl-a concentration for the eastern and western sites ( $p<0.05$ ). Chl-a was higher in the eastern MTs compared to the western MTs (Fig. 11). Furthermore, the mean

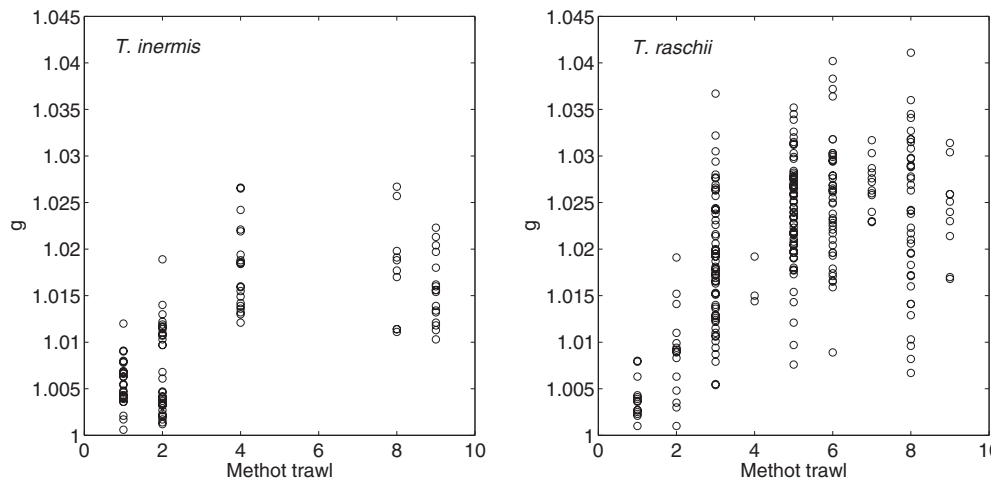


FIG. 8. Density contrast for *T. inermis* and *T. raschii* for each MT. Overall  $g$  values are lower for the East region (MT01, 02, 03, 04) compared to the West (MT05, 06, 07, 08, 09).

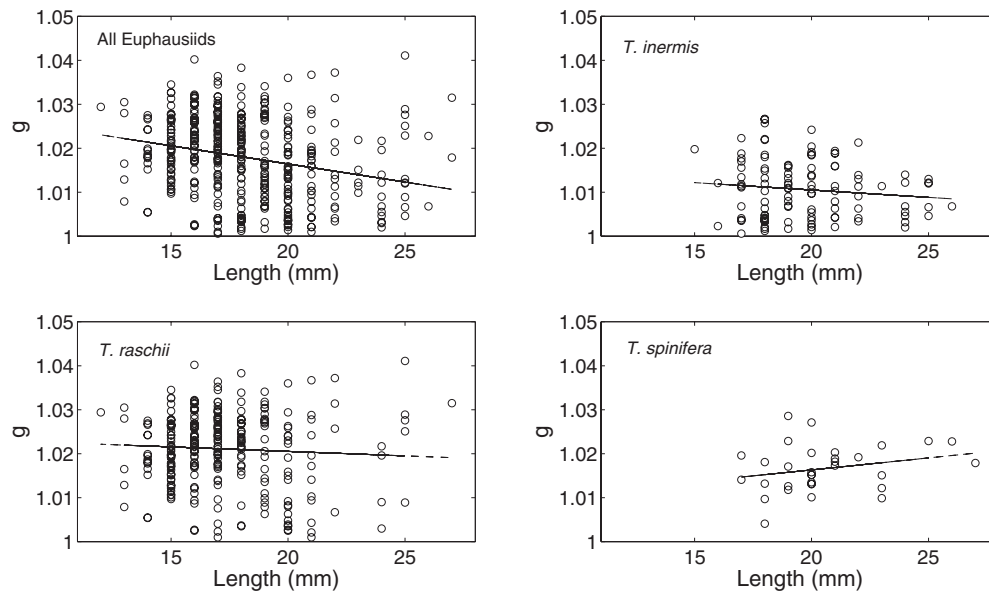


FIG. 9. Density contrast ( $g$ ) as a function of animal length for all euphausiids ( $n=448$ ), *T. inermis* ( $n=114$ ), *T. raschii* ( $n=282$ ), and *T. spinifera* ( $n=32$ ). There is a large range in  $g$  values for all animal lengths. A linear regression showed that there is little correlation between the two variables for all euphausiids and each species. All species:  $R^2=0.062$ ;  $g=-0.000825 \cdot \text{Length}(\text{mm})+1.03$ ; *T. inermis*:  $R^2=0.014$ ;  $g=-0.000336 \cdot \text{Length}(\text{mm})+1.02$ ; *T. raschii*:  $R^2=0.0035$ ;  $g=-0.000203 \cdot \text{Length}(\text{mm})+1.02$ , and *T. spinifera*:  $R^2=0.063$ ;  $g=-0.000545 \cdot \text{Length}(\text{mm})+1.01$ .

integrated chl-a of the western sites was  $2.45 \text{ mg m}^{-3}$  while the mean of the eastern sites was nearly double that at  $4.56 \text{ mg m}^{-3}$ .

## 2. Other zooplankton groups

Along with euphausiids, other abundant zooplankton taxa in the Methot trawl catches included copepods and amphipods, however there were relatively few chaetognaths, gastropods, fish larvae, jellyfish, and squid specimens collected. Material properties and length measurements for each zooplankton group were measured, however relatively small data sets did not allow for environmental and spatial analysis to be conducted as was done for the euphausiid data.

*a. Copepods* Ninety copepods (*Neocalanus sp.*) were analyzed from MT02 ( $n=18$ ), MT04 ( $n=31$ ), and MT09 ( $n=41$ ).

Density contrast ranged from 0.995 to 1.015 with a mean and sd of  $1.005 \pm 0.006$  (Table III). A linear regression showed little relationship ( $R^2=0.007$ ) between copepod length and  $g$ . The regression fit was likely affected by the small range in copepod length and by the presence of both positively ( $g < 1$ ) and negatively ( $g > 1$ ) buoyant copepods.

*b. Amphipods* A total of 66 amphipods (*Themisto libellula*) were collected from MT05 and MT08. Twenty-two amphipods were classified as small ( $< 20 \text{ mm}$  length) and 44 amphipods were classified as large ( $> 20 \text{ mm}$  length). The density contrast of small amphipods ranged from 1.001 to 1.009 with a mean and sd of  $1.005 \pm 0.002$  (Table III). The density contrast of large amphipods ranged from 1.002 to 1.029 with a mean and sd of  $1.013 \pm 0.006$  (Table III; Fig. 12). A linear regression showed that a weak correlation ( $R^2=0.33$ ) existed between amphipod length and  $g$ . Density contrast varied both between and within the small and large amphipods. The large amphipods had a greater range in  $g$  than the smaller animals and  $g$  was significantly different between the two groups (t-test,  $p < 0.001$ ).

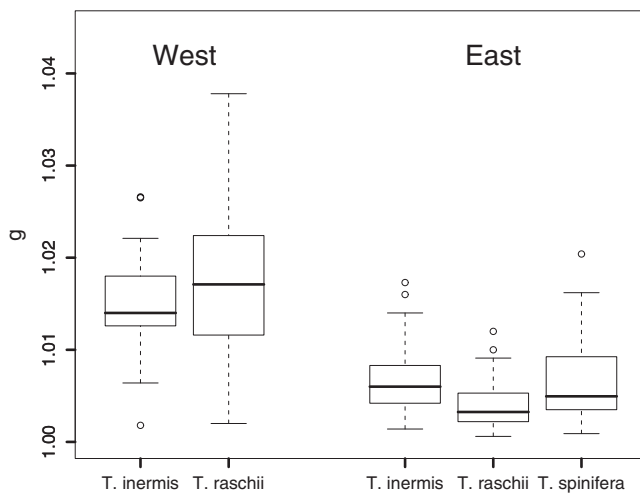


FIG. 10. Density contrast ( $g$ ) distributions for different euphausiid species between the East (MT01, MT02, MT03, MT04) and West (MT05, MT06, MT07, MT08, MT09) locations. Density contrast was significantly different between the western and eastern sample sites for euphausiids ( $p < 0.001$ ).

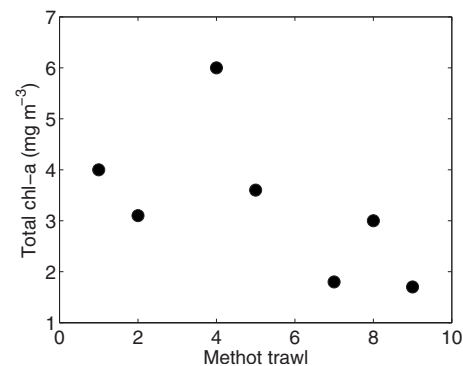


FIG. 11. Chlorophyll-a integrated over the water column varied with Methot trawl location. Sites in the western region (MTs 5 and higher) had lower levels of chl-a than eastern sites (MTs 4 and lower).

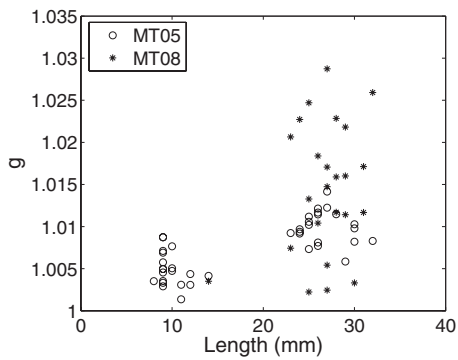


FIG. 12. Amphipod density contrast ( $g$ ) was weakly correlated with animal length. Amphipods from Methot trawl 05 were split between small (<20 mm) and large (>20 mm) animals, whereas animals from Methot trawl 08 were almost all large animals. The large animals showed a greater range in  $g$  values. Density contrast was significantly different between the small and large amphipods (t-test,  $p < 0.001$ ). Results from a linear regression showed that  $R^2 = 0.33$  and  $g = 4.35 \times 10^{-4} \text{ Length}(\text{mm}) + 1$ .

*c. Chaetognaths* Although they were found in small numbers in multiple MTs, a total of 14 chaetognaths (*Sagitta* sp.) all from MT09 were measured. Their density contrast ( $g$ ) values ranged from 1.007 to 1.026 with a mean and sd of  $1.014 \pm 0.007$  (Table III). Chaetognaths had the highest correlation of all taxa between length and  $g$  ( $g = -7.16 \times 10^{-3} \cdot \text{Length}(\text{mm}) + 1.03$ ;  $R^2 = 0.503$ ).

*d. Gastropods* Seventeen gastropods (*Clione limacina*) collected from two MTs (13 from MT06, 4 from MT07) had a  $g$  ranging from 1.008 to 1.031 with a mean and sd of  $1.016 \pm 0.006$ . A linear regression between length and  $g$  produced a low coefficient of determination ( $R^2 = 0.03$ ).

*e. Fish larvae* Fifteen larval fish (*Theragra chalcogramma*) were collected and measured (6 from MT06, 9 from MT07). Their  $g$  values ranged from 1.008 to 1.039 with a mean and sd of  $1.023 \pm 0.008$  (Table III). A linear regression between length and  $g$  produced a low coefficient of determination ( $R^2 = 0.00062$ ).

*f. Jellyfish* Two species of jellyfish were measured, *Chrysaora melanaster* and an unidentified hydromedusa. *Chrysaora melanaster* were too large in size (bell diameters ranged from 15 to 24 cm) for density measurements with the available equipment, so the bells were cut into smaller pieces ( $n = 36$ ) and measurements performed immediately after this. The mean length, width, and thickness of the pieces were 49 mm, 25 mm, and 8 mm respectively. The density contrast ranged from 1.001 to 1.006 with a mean and sd of  $1.003 \pm 0.001$  (Table III). The density contrast was calculated for the entire body of the unidentified jellyfish species ( $n = 4$ ), and ranged from 1.004 to 1.005 with a mean and sd of  $1.005 \pm 0.001$  (Table III).

*g. Squid* The density of the squid mantle was measured in a laboratory using the pipette method, while the densities of the squid beaks were measured in the laboratory using the titration method, but with glycerine as the titrate instead of a hypersaline solution. As noted previously, measurements on the squid pens were not possible due to deterioration of the samples. Seven pieces of squid mantle were measured and  $g$  ranged from 1.023 to 1.116 with a mean  $\pm$  sd of  $1.073 \pm 0.030$  (Table III). The mantle pieces were slightly

deteriorated, so  $g$  may differ from that of live tissue. Fifteen squid beaks were collected and separated into upper and lower jaw pieces for a total of 30 density measurements. Their  $g$  ranged in value from 1.125 to 1.180 with a mean of  $1.149 \pm 0.013$  (Table III). The squid beaks showed no evidence of deterioration.

### 3. Sound speed

Sound speed contrast data were collected for those zooplankton taxa abundant enough to fill a 26 ml or 84 ml PVC T-tube measurement chamber. The sound speed contrast was measured for groups of animals from the same taxon in each haul. When a particular taxon was abundant within the MT, several different groups of those animals were measured for their  $h$  value. In total,  $h$  was measured for 11 separate groups of euphausiids representing all MTs except MT06. The number of group measurements of  $h$  for each taxon were: copepods (3), amphipods (3), gastropods (1), jellyfish (*Chrysaora melanaster*, 8), and squid mantle (2). Three trials were conducted for each group and the results were averaged. The mean percent difference in  $h$  between trials was 0.076%. As a taxon, euphausiids had both the greatest number and the widest range of  $h$  measurements (range 0.990 to 1.017; mean 1.006, sd 0.008; Table III). The two squid mantle measurements were the most consistent with  $h = 1.010$ . Copepods, amphipods, and jellyfish also displayed a range in  $h$  values (Table III). Location was found to be an important factor influencing  $g$ , hence euphausiids (the taxon with the most measurements of  $h$ ) were separated into eastern (MT01, MT02, MT03, MT04) and western (MT05, MT07, MT08, MT09) groups and a t-test was used to evaluate the difference in  $h$  between the two groups. Unlike the density data, there was no significant difference in  $h$  of groups of euphausiids from the eastern ( $n = 4$ ) and western ( $n = 7$ ) sites, although this result may have been affected by the small sample size for the sound-speed measurements.

## IV. DISCUSSION

Although there are a small number of previous studies on the material properties of several of the taxa we measured, there have been no such measurements on specimens from the Bering Sea. There are also several relatively common zooplankton groups (such as chaetognaths) which have had no information reported about their material properties at all prior to this study. A major contribution of this study are measurements of zooplankton material properties from this ecosystem; these data (Table I) can be used in scattering models to provide more accurate estimates of zooplankton biomass or abundance.

Little is known about how material properties might be affected by or related to factors such as animal length, geographic location, surrounding environmental conditions, age, gender, fecundity, feeding state, or numerous other factors which are likely to affect the body composition of these organisms and thus their density and sound speed (Chu *et al.*, 2000; Chu and Wiebe, 2005; Forman and Warren, 2010). Therefore, we also examined the effects of various parameters on the material property measurements, focusing prin-

cipally upon euphausiid  $g$ , the zooplankton group and material property for which we had the most observations. The variables considered were species, length, water temperature, water density, location, and water column chl-a concentration as a proxy of zooplankton prey abundance. Principal component analysis determined how these different explanatory variables were related to one another. Separate analyses were used to further examine specific relations between a given variable (or variables) and  $g$ .

### A. Density contrast

All of the explanatory variables examined appeared to contribute to the variance in  $g$  to some degree. The confounding effects and interactions between these variables makes it difficult to reach any definitive conclusions about direct relationships between a single variable and animal density, however these data do provide insight into some of these relationships and offer avenues for future investigations. Both euphausiid density and  $g$  were greater at western MT locations than at eastern stations and increased with increasing water density and salinity and decreasing temperatures. These relationships occurred for both ambient (at the MT station) or experimental (in the tank the animals were maintained) conditions. However, if all other factors are constant and the animals are not altering their own density, increasing ambient water density should cause a decrease, not an increase, in  $g$ . We suspect that the increase in  $g$  was due to an increase in animal density at the western stations (Fig. 6) which was greater than any increase in ambient water density; as  $g$  was significantly different between the western and eastern sample sites for euphausiids ( $p < 0.001$ ). These western sample sites were characterized by colder water and lower chlorophyll concentrations.

Water temperature changes both spatially and seasonally and can alter the material properties of zooplankton throughout the year (Forman and Warren, 2010). Changes in lipid composition were suggested by K ogeler *et al.* (1987) to explain the differences in  $g$  between seasons. Many zooplankton increase their lipid composition in colder months as a means to store energy through winter (Campbell and Dower, 2003). Female euphausiids alter their lipid composition throughout egg reproduction (Smith, 1991). Such changes in lipid composition are likely triggered by temperature changes, though the exposure time to different temperatures in our experiments (several hours to a day) may have been too short to allow the animals to adjust their body composition. Long term exposure to the ocean temperatures at the locations from which the euphausiids were collected (average water column temperature for the stations in the eastern group was 2.6 °C compared to 0.4 °C for the west) could partially explain the difference in animal density between MTs (Fig. 6).

It is also possible that zooplankton that are well-fed have different material properties than those animals without a sufficient food supply. The concentration of phytoplankton varies both spatially and seasonally, and we used chl-a concentration as a crude indicator of the abundance of phytoplankton, the primary prey for euphausiids in these waters.

Chl-a had a significant correlation with animal density and  $g$  ( $r^2=0.17$ ) with western MT sites having less chlorophyll than eastern sites. The  $r^2$  value is fairly low, but the mean chl-a values differ by a factor of two (western region = 2.45 mg m<sup>-3</sup>; eastern region = 4.56 mg m<sup>-3</sup>). This difference may be substantial when considering the difference in surface chl-a values between a spring bloom (3.5–6.0 mg m<sup>-3</sup>) and summer months (0.8–1.0 mg m<sup>-3</sup>) where nutrient consumption limits phytoplankton production for the middle and outer shelf of the Bering Sea (Iida and Saitoh, 2007). The difference in mean chl-a concentrations between the eastern and western sites is not as large as the difference between a spring bloom and the subsequent decline for a similar region. However, the difference might be great enough to potentially influence the material properties of euphausiids. We did not examine the specific mechanism responsible for this relationship; it may be the result of the phytoplankton prey being less dense than the euphausiids and as the euphausiids eat the less dense material they themselves become less dense, or it may be that well-fed animals are storing energy as lipids which will affect their density relative to the surrounding seawater. Information on euphausiid gut contents, gut volume, and phytoplankton densities would need to be measured to determine the differences in  $g$  for euphausiids that are well- and poorly-fed. Future studies examining material properties of zooplankton should include an examination of lipid content in order to better resolve this issue.

We found differences in  $g$  for the same species due to changing environmental conditions over relatively small distances (10–100 s km). Differences due to environmental conditions that vary with location may explain the differences between our data for *T. inermis* and *T. raschii* from the Bering Sea and those of K ogeler *et al.* (1987) for the same species of euphausiids collected off Norway. Greenlaw and Johnson (1982) also measured the density contrast of *T. raschii* in two widely separated locations; the density contrast of *T. raschii* from Norway ranged from 1.013 to 1.018 while  $g$  ranged from 1.045 to 1.050 in Saanich Inlet, British Columbia. Results reported by Greenlaw and Johnson (1982) for *T. raschii* in Norway are similar to our  $g$  estimates for the same species in the Bering Sea, while their results for  $g$  of *T. raschii* from British Columbia are similar to K ogeler *et al.* (1987)'s results from the Barents Sea. Variation of material properties among different locations, environmental conditions, and times of year is not yet well understood.

Even though the euphausiid species we examined are of the same genus and have a roughly similar morphology, there were enough differences between each species to suggest an effect on  $g$ , though we were not able to conclusively separate a species effect from that of sample location. K ogeler *et al.* (1987), working in the Barents Sea, examined the material properties of three euphausiid species (*Thysanoessa inermis*, *T. raschii*, and *Meganycitiphanes norvegica*) over the course of a year. The *Thysanoessa* species they measured are also the dominant euphausiids on the Bering Sea shelf (Smith, 1991). Although K ogeler *et al.* (1987) did not test for any significant difference in density contrast values among the three euphausiid species, they showed that  $g$  values were

slightly different among the species and that  $g$  fluctuated with season. The density contrast of *T. inermis* and *T. raschii* fluctuated in a similar pattern in which the maximum  $g$  occurred in February and the minimum  $g$  occurred in mid-November for *T. inermis* and mid-December for *T. raschii*.

The  $g$  values that we report are generally smaller and have a wider range, and yet they overlap with the range of measurements described in K ogeler *et al.* (1987) and Greenlaw and Johnson (1982) for the same euphausiid species [this study: 1.001 to 1.041; K ogeler *et al.* (1987): 1.022 to 1.054; Greenlaw and Johnson (1982): 1.013 to 1.050]. Comparing  $g$  values between euphausiid species is also appropriate considering that the material properties of a particular species has often been applied to modeling the scattering of other species. The  $g$  values we obtained for *Thysanoessa spp.* appear to be somewhat lower than  $g$  values reported for *Euphausia superba* by other studies [1.021 to 1.040, Greenlaw and Johnson (1982); 1.021 to 1.040, Chu and Wiebe (2005)]. Data on *E. superba* from these studies (as well as Foote, 1990) are often used for scattering models for any euphausiid-like animal, even though there are large differences between different euphausiid species particularly in their size (adult *E. superba* are roughly two to three times as large as *T. inermis* or *T. raschii*).

The density contrast of our copepods ranged from 0.995 to 1.015 with a mean of  $1.005 \pm 0.006$ . The mean copepod  $g$  value from our results lies within the range of copepod density measured by K ogeler *et al.* (1987). However, the copepods examined in our study (*Neocalanus sp.*) are larger (7 to 9 mm in length) compared to the copepods examined in K ogeler *et al.* (1987) (*Calanus finmarchicus*, 2.2 to 3.0 mm and *Calanus hyperboreus*, 3.5 to 5.5 mm in length). The density contrast of copepods measured by K ogeler *et al.* (1987) ranged from 0.999 (February through September) to 1.003 (in late-February) for *Calanus hyperboreus*; for *Calanus finmarchicus*,  $g$  ranged from 0.996 at the end of July to 1.010 in late-February. Unlike the measurements of euphausiids, both our study and that of K ogeler *et al.* (1987) measured copepods with positive and negative buoyancy ( $g < 1$ ): approximately a quarter of our copepods were less dense than the seawater, while K ogeler *et al.* (1987) showed that copepods were less dense than seawater the majority of the year and only became denser immediately prior to spawning in March. Copepods with both positive and negative buoyancy have also been reported elsewhere for the same genera and species. *Calanus sp.* found in Antarctic waters were all measured to have densities less than seawater (Chu and Wiebe, 2005), but none of the copepods measured by Greenlaw and Johnson (1982), including representatives of the species *Calanus finmarchicus*, were less dense than seawater. It is possible that the majority of the copepods measured in this study had recently spawned or otherwise did not contain sufficient lipid stores to be less dense than water.

Our examination of the effect of animal length on  $g$  revealed that for most zooplankton taxa in this study there was little or no correlation between the two variables. Amphipods ( $R^2=0.33$ ) and chaetognaths ( $R^2=0.50$ ) were the only taxon with a coefficient of determination greater than

0.10 between length and  $g$ . Though our results indicated no significant relationship between euphausiid length and  $g$ , we calculated a linear regression between animal length and  $g$  for euphausiids in order to compare to the linear regressions for the same species found in K ogeler *et al.* (1987). Their euphausiids (*T. inermis* and *T. raschii*) ranged in length from 10 to 25 mm while our euphausiids ranged in length from 12 to 27 mm. They provided multiple regression equations [ $g=a \cdot \text{Length}(\text{mm})+b$ ] for each month that data were collected. The slopes for their linear regressions were all small negative numbers (ranging from  $-0.01 \times 10^{-3}$  to  $-2.5 \times 10^{-3}$  with a mean slope of  $-1.27 \times 10^{-3}$ ) and their coefficients of determination ranged from  $R^2=0.01$  to  $R^2=0.90$ . We calculated a similar slope of  $-0.93 \times 10^{-3}$  for the linear regression between euphausiid length and  $g$  and a small coefficient of determination ( $R^2=0.06$ ). Greenlaw and Johnson (1982) examined euphausiids of the genus *Thysanoessa* (but from unspecified locations) and demonstrated a positive relationship between animal length and the density contrast, but their data represented a very small range (15 to 18 mm) of lengths. Chu and Wiebe (2005) also reported a positive correlation between euphausiid length and  $g$ , however all of their animals were larger (25–52 mm) and of a different species (*Euphausia superba*) than the euphausiids in our study.

## B. Sound speed contrast

While density contrast was measured for individual zooplankton,  $h$  was measured on groups of animals (Table III). Unfortunately the sound speed contrast could not be measured for all zooplankton groups since not enough individuals of some taxa were collected. Overall,  $h$  was fairly close to unity for all zooplankton groups for which measurements were made and in some cases it was less than one, which signifies that sound travels slower through the group of zooplankton compared to the surrounding seawater. It is likely that these bulk measurements, containing multiple sizes and species for each taxon, are intrinsically more variable than measurements made on individuals of a single type and size. The sound speed contrast for euphausiids ranged from 0.990 to 1.017 with a mean and standard deviation of  $1.006 \pm 0.008$ , lower than  $h$  measured for two of the same species (a mixture of *T. inermis* and *T. raschii*) by K ogeler *et al.* (1987), who reported a mean of  $1.026 \pm 0.005$ . Greenlaw and Johnson (1982) measured  $h$  for *T. raschii* ranging from 1.032 to 1.045, which is also higher than the  $h$  measurements collected for euphausiids from the Bering Sea. Similarly, Bering Sea euphausiid  $h$  measurements were lower than the average  $h$  value measured for *E. superba* by Chu and Wiebe (2005) which was  $1.030 \pm 0.004$  and by Foote (1990) which was  $1.0279 \pm 0.0024$ . Euphausiid  $h$  did not exhibit the east-west spatial pattern observed for euphausiid  $g$ . Bering Sea copepod  $h$  measurements were lower than the results K ogeler *et al.* (1987) reported for a different genus, species, and animal size of copepod: our average  $h$  for *Neocalanus sp.* copepods was  $1.007 \pm 0.004$ , while K ogeler *et al.* (1987) for several species of the genus *Calanus* showed a mean  $h$  of  $1.027 \pm 0.007$ . Greenlaw and Johnson (1982) report similar  $h$  values to this study ranging from 1.006 to 1.012, even

though they measured a smaller copepod species (*Calanus plumchrus*).

### C. Sources of uncertainty

Measurements of both the density and sound speed contrast of animals have several sources of uncertainty. Because the titration method involved adding a saltier solution to the seawater surrounding the animal, it is possible that the resulting osmotic pressure could have altered the density of the animal. We observed that a change in the buoyancy occurred over several minutes if the animal was allowed to remain in the solution after the saltier solution had been added. In this study, the measurement needed for estimation of animal density using the titration method was completed within approximately 30 s of the saltier solution being added, which we believe minimized the chance of osmotic adjustment by the animal. In addition, the titration method was performed multiple times on each animal; the density measurements were similar between iterations and had a mean percent difference of 0.41%. This small mean percent difference, with no systematic change in animal density after repeated iterations, suggests that there was no bias due to osmotic effects in density measurements using the titration method.

Another possible source of uncertainty with this method is that seawater bound to the animal by surface tension will bias the measurement toward unity, since the titration method measures the density of the animal along with any seawater that is attached to the animal by surface tension. This factor will increase in importance as animal volume decreases and thus is most likely to impact the smallest animals we measured, the copepods. However, other studies have used similar density measurement methods on even smaller copepods than those in this study (Køgelier *et al.*, 1987; Greenlaw and Johnson, 1982; Greenlaw, 1977). The density contrast measurements also may be underestimated due to seawater viscosity. Viscous friction at the interface between the animal and seawater could potentially affect the accuracy of the density measurements, however any such effect is difficult to quantify.

The  $h$  measurements were likely biased minimally (toward unity) considering the difficulty in entirely removing excess water from around the animals and the difficulty in completely filling the chamber with animals (i.e.,  $\Phi$  is always less than 1). In other words, interstitial water between the animals being measured when using displacement volume to estimate volume fraction [Eq. (6)] may explain some of the variation in  $h$  for each zooplankton taxon. It is preferable that animals be uniformly distributed within and appear to fill the horizontal section of the measurement tube (Foote, 1990). Køgelier *et al.* (1987) reported that the maximum volume fraction within their measurement chamber was 65%, but the mean volume fraction in this study ranged from 63% to 100% with a mean of 89%. Indirect methods of measuring volume fraction involve measuring the resistivity of a group of animals and then calculating its theoretical volume fraction; Chu *et al.* (2000) showed that this method can significantly reduce the error in calculating  $h$ , but this technique has not yet been widely used.

### D. Effect on predictions of target strength (TS)

Material properties appear to be a function of the environment as well as a function of the physical parameters of the various zooplankton groups. It is very difficult to understand the relationship of each parameter on  $g$  considering many of the factors are inter-related (e.g., location, water temperature). The importance of these relationships is that very small differences in  $g$  and  $h$  (0.01 or less for each) that may result from differences in, for example, location, species, or animal size can produce different TS values that will result in vastly different estimates of numerical abundances of animals when used in conjunction with acoustic backscatter survey data. Forman and Warren (2010) showed that using the lowest, average, and highest values of  $g$  and  $h$  that they measured for coastal zooplankton can result in differences in population estimates of up to three orders of magnitude. Given the wide range of  $g$  and  $h$  values in the literature and the fact that bioacousticians often use material property values from species other than the ones they are studying (primarily because the data they need do not exist), it is imperative that more material property measurements are made. For example, many studies use the values reported for *Euphausia superba* by Foote *et al.* (1990) or more recently Chu and Wiebe (2005) for any fluid-like crustacean, even when the animal being studied is either much smaller in size or a completely different family or order of animal.

Chu and Ye (1999) show that the differential backscattering cross section ( $\sigma_{bs}$ ) is proportional to the square of the sum of the deviations of  $g$  and  $h$  from unity [ $\sigma_{bs} \propto (\Delta h + \Delta g)^2$  where  $\Delta h = h - 1$  and  $\Delta g = g - 1$ ]. Since  $\sigma_{bs}$  is related to target strength by the equation  $TS = 10 \log \sigma_{bs}$  (Stanton *et al.*, 1996), we can calculate how much TS will change when different  $g$  and  $h$  values are used. Both  $g$  and  $h$  are proportional to the backscattering cross-section; however, we cannot be certain that  $g$  and  $h$  are correlated given that  $h$  can only be measured on groups of animals while  $g$  is measured for individual animals. For instance, euphausiids measured in this study had a range in  $g$  from 1.001 to 1.041 and a range in  $h$  from 0.990 to 1.017, which would result in TS estimates that vary by as much as 16 dB. As a difference in TS of 10 dB corresponds to an order of magnitude difference in the numerical density of a scatterer, the variation in  $g$  and  $h$  from our study needs to be taken into account to produce zooplankton population estimates that are accurate enough for studies of ecosystem processes.

Since location appeared to be a significant factor influencing  $g$  for euphausiids in the Bering Sea, the variation in TS was calculated using the ranges in  $g$  and  $h$  found in the eastern and western groups. TS estimates could vary by 19.5 dB for eastern euphausiids and 16.7 dB for western euphausiids. These large dB ranges are the maximum differences that could occur within a population based on data collected in the Bering Sea; the likely range of TS estimates based on the combinations of mean  $g$  and  $h$  observed in our data from each MT may be substantially smaller. In addition to estimating the maximum possible difference in TS due to  $g$  and  $h$  measurements of euphausiids in the Bering Sea, a more likely estimate of the variability in TS can be found by

computing the proportional effect on the backscattering cross-section ( $\sigma_{bs}$ ) from the average  $g$  and  $h$  for each MT (using the equation presented in [Chu and Ye, 1999](#)). Using this method, the estimated difference in the range of TS values (between 95% confidence bounds approximated as twice the standard error of the mean) was 5.7 dB for all euphausiids, 9.0 dB for the East, and 4.8 dB for the West. The larger range for the East region is the result of  $g$  values being very close to unity, where small changes in  $g$  will produce larger variations in TS. The actual TS distribution for a population of animals may need to be calculated on an individual-by-individual basis to determine the impact of the variability in material properties on numerical estimates of animal abundance.

## V. CONCLUSIONS

This study presents material property values for Bering Sea zooplankton. We measured the material properties for euphausiids, copepods, amphipods, chaetognaths, gastropods, fish larvae, jellyfish, and body parts of squid. In those cases where there were prior measurements of the same taxa using specimens collected from different regions, our measurements showed significant variation; these differences suggest that uncertainty in scattering model predictions should be reduced if material property values are specific to each target taxon in the location being studied. Potentially, if an environment is relatively stable from year to year, the material properties of a particular zooplankton group may also be stable. The only way to verify this is to measure the material properties for a specific location and group of animals during the time period of interest. If material properties are shown to be stable or predictable, then less frequent measurements of material properties would be needed. With improved knowledge of material properties, including what factors cause these properties to change, as well as other scattering model inputs such as animal orientation ([Demer and Conti, 2005](#); [Warren et al. 2002](#)); more accurate estimates of zooplankton abundance and distribution could be made from acoustic surveys which would improve our ability to understand the status and trends of these populations.

## ACKNOWLEDGMENTS

This project was supported by the Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, and the North Pacific Research Board's Bering Sea Integrated Ecosystem Research Program. The captain, crew, and scientists aboard the NOAA ship *Oscar Dyson* provided excellent logistical support and sampling assistance. Abigail McCarthy helped us greatly with the set up and maintenance of the on board aquaria as well as with the trawl sampling operations. We acknowledge the taxonomic advice of Elaina Jorgensen (squid) and Claudia Mills (hydromedusae). We would like to thank Alex De Robertis and Neal Williamson for their comments and suggestions on this manuscript.

Anderson, V. C. (1950). "Sound scattering from a fluid sphere," *J. Acoust. Soc. Am.* **22**, 426–431.

Brierley, A. S., and Watkins, J. L. (1996). "Acoustic targets at South Georgia and the South Orkney Islands during a season of krill scarcity," *Mar.*

*Ecol.: Prog. Ser.* **138**, 51–61.

Brodeur, R. D., Wilson, M. T., Ciannelli, L., Doyle, M., and Napp, J. M. (2002). "Interannual and regional variability in distribution and ecology of juvenile pollock and their prey in frontal structures of the Bering Sea," *Deep-Sea Res., Part II* **49**, 6051–6067.

Campbell, R. W., and Dower, J. F. (2003). "Role of lipids in the maintenance of neutral buoyancy by zooplankton," *Mar. Ecol.: Prog. Ser.* **263**, 93–99.

Chu, D., Wiebe, P., and Copley, N. (2000). "Inference of material properties of zooplankton from acoustic and resistivity measurements," *ICES J. Mar. Sci.* **57**, 1128–1142.

Chu, D., and Wiebe, P. H. (2005). "Measurements of sound-speed and density contrasts of zooplankton in Antarctic waters," *ICES J. Mar. Sci.* **62**, 818–831.

Chu, D., and Ye, Z. (1999). "A phase-compensated DWBA representation of the bistatic scattering by weakly scattering objects: Applications to zooplankton," *J. Acoust. Soc. Am.* **106**, 1732–1743.

Ciannelli, L., Brodeur, R. D., and Napp, J. M. (2004). "Foraging impact on zooplankton by age-0 walleye pollock (*Theragra chalcogramma*) around a front in the southeast Bering Sea," *Mar. Biol. (Berlin)* **144**, 515–526.

Coyle, K. O., Hunt, G. L., Jr., Decker, M. B., and Weingartner, T. J. (1992). "Murre foraging, epibenthic sound scattering, and tidal advection over a shoal near St. George Island, Bering Sea," *Mar. Ecol.: Prog. Ser.* **83**, 1–14.

Coyle, K. O., and Pinchuk, A. I. (2002). "The abundance and distribution of euphausiids and zero-age pollock on the inner shelf of the southeast Bering Sea near the inner front in 1997–1999," *Deep-Sea Res., Part II* **49**, 6009–6030.

Coyle, K. O., Pinchuk, A. I., Eisner, L. B., and Napp, J. M. (2008). "Zooplankton species composition, abundance and biomass on the eastern Bering Sea shelf during summer: The potential role of water-column stability and nutrients in structuring the zooplankton community," *Deep-Sea Res., Part II* **55**, 1775–1791.

Dagg, M. J., Clarke, M. E., Nishiyama, T., and Smith, S. L. (1984). "Production and standing stock of copepod nauplii, food items for larvae of the walleye pollock *Theragra chalcogramma* in the southeastern Bering Sea," *Mar. Ecol.: Prog. Ser.* **19**, 7–16.

Decker, M. B., and Hunt, G. L., Jr. (1996). "Murre foraging at the frontal system surrounding the Pribilof Islands, Alaska," *Mar. Ecol.: Prog. Ser.* **139**, 1–10.

Demer, D. A., and Conti, S. G. (2005). "New target-strength model indicates more krill in the southern ocean," *ICES J. Mar. Sci.* **62**, 25–32.

Foote, K. G. (1990). "Speed of sound in *Euphausia superba*," *J. Acoust. Soc. Am.* **87**, 1405–1408.

Foote, K. G., Everson, I., Watkins, J. L., and Bone, D. G. (1990). "Target strengths of Antarctic krill (*Euphausia superba*) at 38 and 120 kHz," *J. Acoust. Soc. Am.* **87**, 16–24.

Foote, K. G., and Stanton, T. K. (2000). "Acoustical methods," in *ICES Zooplankton Methodology Manual*, edited by R. Harris, P. Wiebe, J. Lenz, H. Skjoldal, and M. Huntley (Academic, London), pp. 223–258.

Forman, K. A., and Warren, J. D. (2010). "Variability in the density and sound-speed of coastal zooplankton and nekton," *ICES J. Mar. Sci.* **67**, 10–18.

Greenblatt, P. (1981). "Sources of acoustic backscattering at 87.5 kHz," *J. Acoust. Soc. Am.* **70**, 134–142.

Greenlaw, C. F. (1977). "Backscattering spectra of preserved zooplankton," *J. Acoust. Soc. Am.* **62**, 44–52.

Greenlaw, C. F., and Johnson, R. K. (1982). "Physical and acoustical properties of zooplankton," *J. Acoust. Soc. Am.* **72**, 1706–1710.

Hagen, W., Van Fleet, E. S., and Kattner, G. (1996). "Seasonal lipid storage as overwintering strategy of Antarctic krill," *Mar. Ecol.: Prog. Ser.* **134**, 85–89.

Honkalehto, T., Patton, W., de Blois, S., and Williamson, N. (2002). "Echo integration-trawl survey results for walleye pollock (*Theragra chalcogramma*) on the Bering Sea shelf and slope during summer 1999," NOAA Technical Memorandum No. NMFS-AFSC-125, U.S. Department of Commerce, Seattle, WA.

Honkalehto, T., Williamson, N., Jones, D., McCarthy, A., and McKelvey, D. (2009). "Results of the echo integration-trawl survey of walleye pollock (*Theragra chalcogramma*) on the U.S. and Russian Bering Sea shelf in June and July 2008," NOAA Technical Memorandum No. NMFS-AFSC-194, U.S. Department of Commerce, Seattle, WA.

Ianelli, J. N., Barbeaux, S., Honkalehto, T., Kotwicki, S., Aydin, K., and Williamson, N. (2008). "Assessment of the walleye pollock stock in the eastern Bering Sea," NPFMC Bering Sea and Aleutian Islands Stock As-

- essment and Fishery Evaluation (SAFE) Document, Anchorage, AL, pp. 47–136.
- Iida, T., and Saitoh, S. I. (2007). “Temporal and spatial variability of chlorophyll concentrations in the Bering Sea using empirical orthogonal function (EOF) analysis of remote sensing data,” *Deep-Sea Res., Part II* **54**, 2657–2671.
- Kang, D., Iida, K., Mukai, T., and Hwang, D. (2004). “Acoustic target strength of Japanese common squid, *Todarodes pacificus*, and important parameters influencing its TS: Swimming angle and material properties” in OCEANS ‘04 MTS/IEEE/TECHNO-OCEAN ‘04, pp. 364–369.
- Køgel, J. W., Falk-Petersen, S., Kristensen, Å., Pettersen, F., and Dalen, J. (1987). “Density- and sound speed contrasts in sub-Arctic zooplankton,” *Polar Biol.* **7**, 231–235.
- Livingston, P. A. (1991). “Walleye pollock,” NOAA Technical Memorandum No. NMFS-F/NWC-207, U.S. Department of Commerce, Seattle, WA.
- Method, R. D. (1986). “Frame trawl for sampling pelagic juvenile fish,” CalCOFI Report No. 27, Southwest Fisheries Center, La Jolla, CA.
- Schabetsberger, R., Brodeur, R. D., Ciannelli, L., Napp, J. M., and Swartzman, G. L. (2000). “Diel vertical migration and interaction of zooplankton and juvenile walleye pollock (*Theragra chalcogramma*) at a frontal region near the Pribilof Islands, Bering Sea,” *ICES J. Mar. Sci.* **57**, 1283–1295.
- Simmonds, J., and MacLennan, D. (2005). *Fisheries Acoustics: Theory and Practice*, 2nd ed. (Blackwell, Oxford, UK).
- Sinclair, E. (1994). “Prey selection by northern fur seals (*Callorhinus ursinus*) in the eastern Bering Sea,” *Fish. Bull.* **92**, 144–156.
- Smith, S. L. (1991). “Growth, development and distribution of the euphausiids *Thysanoessa raschi* (M. Sars) and *Thysanoessa inermis* (Kroyer) in the southeastern Bering Sea,” *Polar Res.* **10**, 461–478.
- Springer, A. M. (1992). “A review: Walleye pollock in the North Pacific—How much difference do they really make?” *Fish. Oceanogr.* **1**, 80–96.
- Springer, A. M., and Rose, D. G. (1985). “Copepod-based food webs: Auklets and oceanography in the Bering Sea,” *Mar. Ecol.: Prog. Ser.* **21**, 229–237.
- Stanton, T. K., and Chu, D. (2000). “Review and recommendations for the modeling of acoustic scattering by fluid-like elongated zooplankton: Euphausiids and copepods,” *ICES J. Mar. Sci.* **57**, 793–807.
- Stanton, T. K., Chu, D., and Wiebe, P. H. (1996). “Acoustic scattering characteristics of several zooplankton groups,” *ICES J. Mar. Sci.* **53**, 289–295.
- Stanton, T. K., Wiebe, P. H., Chu, D., Benfield, M. C., Scanlon, L., Martin, L. V., and Eastwood, R. L. (1994). “On acoustic estimates of biomass,” *ICES J. Mar. Sci.* **51**, 505–512.
- Taylor, J. R. (1982). *An Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements* (University Science Books, Mill Valley, CA), pp. 52–74.
- Traynor, J. J. (1996). “Target-strength measurements of walleye pollock (*Theragra chalcogramma*) and Pacific whiting (*Merluccius productus*),” *ICES J. Mar. Sci.* **53**, 253–258.
- Warren, J. D., and Smith, J. N. (2007). “Density and sound speed of two gelatinous zooplankton: ctenophore (*Mnemiopsis leidyi*) and lion’s mane jellyfish (*Cyanea capillata*),” *J. Acoust. Soc. Am.* **122**, 574–580.
- Warren, J. D., Stanton, T. K., McGehee, D. E., and Chu, D. (2002). “Effect of animal orientation on acoustic estimates of zooplankton properties,” *IEEE J. Ocean. Eng.* **27**, 130–138.
- Wiebe, P. H., Stanton, T. K., Benfield, M. C., Mountain, D. G., and Greene, C. H. (1997). “High-frequency acoustic volume backscattering in the Georges Bank Coastal Region and its interpretation using scattering models,” *IEEE J. Ocean. Eng.* **22**, 445–464.