Some planktonic patches have markedly higher concentrations of organisms compared to ambient conditions and are <5 m in thickness (i.e. thin layers). Conventional net sampling techniques are unable to resolve this vertical microstructure, while optical imaging systems can measure it for limited durations. Zooglider, an autonomous zooplankton-sensing glider, uses a low-power optical imaging system (Zoocam) to resolve mesozooplankton at a vertical scale of 5 cm while making concurrent physical and acoustic measurements (Zonar). In March 2017, Zooglider was compared with traditional nets (MOCNESS) and ship-based acoustics (Simrad EK80). Zoocam recorded significantly higher vertically integrated abundances of smaller copepods and appendicularians, and larger gelatinous predators and mineralized protists, but similar abundances of chaetognaths, euphausiids, and nauplii. Differences in concentrations and size-frequency distributions are attributable to net extrusion and preservation artifacts, suggesting advantages of in situ imaging of organisms by Zooglider. Zoocam detected much higher local concentrations of copepods and appendicularians (53 000 and 29 000 animals m$^{-3}$, respectively) than were resolvable by nets. The EK80 and Zonar at 200 kHz agreed in relative magnitude and distribution of acoustic backscatter. The profiling capability of Zooglider allows for deeper high-frequency acoustic sampling than conventional ship-based acoustics.

KEYWORDS: Zooglider; mesozooplankton; vertical microstructure; patchiness; thin layers
INTRODUCTION

When observed at fine (1–10 m) and micro (<1 m) scales, the vertical structure of planktonic ecosystems is highly patchy (Haury et al., 1978), and thin layers are common. Thin layers have been defined as recurrent and persistent fine-scale features (<5 m in vertical extent) that exhibit elevated concentrations (e.g. three times the ambient concentration) of organisms, chlorophyll or particles (Dekshenieks et al., 2001). These layers and patches can have significant ecological consequences within the planktonic community, such as predatory behavioral changes (Benoit-Bird, 2009), increased encounter rates between predators and prey or between potential mates, differential grazing rates (Menden-Deuer and Grünbaum, 2006), enhanced water column productivity (Rovinsky et al., 1997; Brentnall et al., 2003) and altered carbon cycling (Pinel-Alloul and Ghadouani, 2007; Wilson and Steinberg 2010; Prairie et al., 2015).

Zooplankton vertical structure is currently investigated with three basic approaches: acoustic backscatter, physical collection, and optical imaging. Each sampling method has unique benefits and limitations. Acoustic backscatter methods can approximate biomass, are less susceptible to organismal avoidance and can sample great volumes of water quickly. However, the acoustic sensing of zooplankton is complicated by several factors (e.g. target taxonomic composition, target orientation, material properties of organisms, and frequency-dependence of acoustic backscatter), and targets cannot be identified explicitly (McGehee et al., 1998; Griffiths et al., 2002), unless the acoustic system is complemented with a net or imaging system (Briseño-Avena et al., 2015).

Net tows and plankton pumps physically retain organisms, allow for species-level classification, and with proper preservation the physical specimens can be examined, DNA sequenced, or analyzed for stable isotopes or other properties long after their initial collection date. All types of physical sample collection have associated financial constraints (e.g. ship-time, sample preservation and archiving, and processing time), which severely limit the number of samples that can be obtained and processed. Advances in image processing, including the ZooImage (Grosjean and Denis, 2007), the ZooScan (Gorsky et al., 2010) and the Flowcam (Fluid Imaging Technologies), have helped to improve the post-processing time of net, pump and bottle-collected samples. However, physical collection systems are still hindered by systematic limitations. Pump systems such as CALPS (Pitois et al., 2016) and CUFES (Checkley et al., 1997) are mounted to the hull of a ship and can sample continuously while the ship is underway, but only at a single depth. Like traditional open nets, opening–closing nets give a sample integrated over a horizontal distance and depth range when towed obliquely (MOCNESS; Wiebe et al., 1985) or strictly a depth range when towed vertically (Multi-net; Weikert and John, 1981). Opening–closing nets are superior to traditional nets as they can isolate the vertical component of the plankton community in smaller bins (vertical resolution is generally ≥∼10 m); however, that resolution is not sufficient to resolve the multiple scales of patchiness and predator–prey interactions in the planktonic environment (Møller et al., 2012). Nets can also damage delicate organisms (Hammer et al., 1975; Omori and Hamner 1982), while other organisms dissolve in the preservation solution if not properly treated (Beers and Stewart 1970). Some planktonic organisms, such as euphausiids, exhibit net avoidance behavior (Brinton, 1967; Wiebe et al., 1982), while other zooplankton are extruded through net mesh (Nichols and Thompson 1991; Remsen et al., 2004; Skjoldal et al., 2013) and are thus underrepresented in samples.

Optical imaging systems can discern the identity or shape profile of organisms; however, the volume sampled is much smaller than acoustic, net and pump-based systems. Imaging systems differ widely in image resolution, capture rate, sample volume, and deployment method. Particle counters (e.g. laser optical particle counter) are only able to discern the rough shape profile of objects within the water column (Herman et al., 2004). The 3D imaging systems utilize either multiple cameras or a single holographic camera to reveal the 3D orientation and identity of an organism in sample volumes ranging from much less than 1 mL to 2 L (Sheng et al., 2003; Wiebe and Benfield, 2003). Several additional imaging systems are also in use for plankton recognition that sample larger volumes of water at slightly lower resolution, e.g. ISIS (Cowen and Guigand, 2008), LOKI (Schulz et al., 2009), SCP (Roberts et al., 2014), UVP (Picheral et al., 2010), Video Plankton Recorder (VPR) (Davis et al., 2003) and ZOOVIS (Trevorrow et al., 2005).

The specific configuration of these instruments on profiling devices or towed bodies can markedly affect the avoidance responses of the targeted zooplankton. Any instrument moving through the water will generate a hydrodynamic disturbance to some degree. For planktonic organisms, this disturbance can induce escape responses if it exceeds an organism-specific shear threshold (Haury et al. 1980; Fields and Yen 1997; Buskey et al., 2002; Bradley et al., 2012). Optical imaging systems have the potential to further influence the behavior of plankton through the illumination needed for imaging. The introduction of light has been shown to lure (Singarajah 1975) and mitigate the escape behavior of zooplankton (Wiebe et al., 2004; Wiebe et al., 2013). Therefore, in situ
instruments should be engineered to minimize the effects of light and hydrodynamic disturbances on the organisms they are observing.

*Zooglider*, a modified *Spray* glider (Sherman et al., 2001), is novel in that it uses a low-power and completely autonomous acoustic (Zonar) and optical imaging system (Zoocam) (Ohman et al., 2019). The Zoocam captures images at 2 Hz, while the Zonar concurrently records acoustic backscatter at two frequencies (200 kHz and 1000 kHz). *Zooglider* resolves both biological (e.g. zooplankton, phytoplankton, marine snow, and chlorophyll-a fluorescence) and physical properties (temperature, salinity, and pressure) at a vertical resolution of ∼5 cm. It is important to note that the Zoocam utilizes a specially designed sampling tunnel that effectively traps organisms and particles, well ahead of the *Zooglider*. The geometry of the sampling tunnel, as well as the placement of the Zoocam on the glider hull, was arrived at after a series of numerical simulations using Solidworks Flow Simulation (Ohman et al., 2019). The design intent was to minimize the effects of shear in simulated flows up to 25 cm s\(^{-1}\) and to shield the organisms from the Zoocam illumination until they are well within the tunnel. Moreover, the wavelength of light was selected to be in the red part of the spectrum where crustacean eyes are relatively insensitive (see Ohman et al., 2019 for details). The efficacy of these design features in natural ocean conditions is evaluated in the present manuscript.

The goal of the present study is to compare *Zooglider* measurements of the plankton assemblage with conventional net-based sampling (MOCNESS) and shipboard acoustic (Simrad EK80) measurements. We sought to determine the comparability between methods and to identify the limitations of each system. We compare the taxon-specific abundances, concentrations and size distributions of organisms detected by the Zoocam in comparison with MOCNESS-collected zooplankton and, separately, the volume backscatter reported by the two acoustic systems.

**METHOD**

For a full description of *Zooglider* engineering details, please see Ohman et al., 2019.

*Zooglider* was deployed near La Jolla Canyon offshore of San Diego, California from 9–16 March 2017. The R/V *Sally Ride* was near *Zooglider’s* last successive reported positions from 11–13 March 2017 (Fig 1). Mean and maximum distances between the active Zonar dives and EK80 track, active Zoocam dives and MOCNESS tows and conductivity–temperature–depth (CTD) Casts and *Zooglider* dives were ∼2.42 km, 1.83 km and 1.40 km and 3.3 km, 2.2 km and 1.45 km, respectively. Distances were calculated using the ship and *Zooglider* GPS at each surfacing. As a safety precaution it was necessary to avoid lowering equipment in close proximity to *Zooglider*.

The *Sally Ride* was equipped with a five-frequency (18, 38, 70, 120 and 200) Simrad EK80, which was active for the duration of the cruise. The Zonar was active for 14 dives that corresponded in time and space with the *Sally Ride’s* EK80. The Zonar was in listening mode for one dive off station for the purpose of background noise estimation. The Zoocam was active for nine dives from 11–13 March 2017. *Zooglider* dives were made at 3-h intervals continuously while on station near La Jolla Canyon. Each dive was to a depth of ∼400 m, and data were collected solely during the ascent portion of the dive.

Two-day and two-night 1-m\(^2\) Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) tows were conducted from 400 m to the surface. The MOCNESS had 10 202-μm nets and was equipped with a front mounted CTD, Chl-a fluorometer, transmissometer and a calibrated flow meter. The 202-μm mesh size was chosen as *Zooglider* was initially designed to target mesozooplankton ranging in size from 0.45–20 mm. CTD casts were conducted ∼0.5 km away from the *Zooglider’s* last surfacing to collect water for extracted Chl-a vertical distributions. Each MOCNESS tow began ∼2 km south of the *Zooglider’s* last reported location and was towed with the same heading as the *Zooglider*. The goal of each tow was to maintain a speed over ground of 0.75–1.0 m s\(^{-1}\) and a MOCNESS net angle of 45°. Net 0 of the MOCNESS was open for the descent and beginning ascent of the tow and was closed at 400 m. The MOCNESS was towed obliquely from 400 m to the surface, and nets were tripped sequentially at predetermined depths that were consistent for all four tows. For all tows, nets 1–9 sampled consistent depth intervals (∼400-350-250-200-150-100-60-40-20-0 m) from 400 m to the surface. The smaller depth intervals near the surface (nets 7–9) were used to better define the structure of the upper layers of the water column. MOCNESS samples from nets 1–9 were immediately rinsed then preserved in a 1.8% solution of formaldehyde buffered with sodium tetraborate for post-processing on land.

**Data analysis**

MOCNESS samples were processed using the ZooScan flatbed scanner and ZooProcess software (Gorsky et al., 2010). Each net sample was passed through three sieves (5 mm, 1 mm and 0.202 mm) for size fractionation. Each size fraction was then subsampled, using a Folsom splitter or Stempel pipette, into smaller aliquots based on the amount of material present within the sample.
The aliquots were then scanned, imaged, segmented, and cropped into individual regions of interest (ROI) using ZooProcess. A total of 68 geometric features (e.g., area, min/mean/max intensity, etc.) were calculated for each ROI. The pixel resolution of the ZooScan is 10.6 μm pixel⁻¹, and the minimum threshold for a ROI to be counted and cropped is 0.45 mm equivalent circular diameter (ECD). The measured ROIs were pre-sorted into 26 categories by a Random Forest algorithm; then classifications of 100% of the images were manually confirmed. Each confirmed ROI was scaled by appropriate aliquot factors and the volume of water filtered in situ to obtain organismal densities as number m⁻³.

The Zooglider CTD and fluorescence measurements were collected at different frequencies than the Zoocam images: 8 s and 0.5 s, respectively; so the CTD and fluorescence data were linearly interpolated using the Zoocam image timestamps. The Zoocam images were 1.2 MB (960 x 1280 pixels), with an image resolution of 40 μm pixel⁻¹ and a sample volume of 250 mL image⁻¹. The raw Zoocam images were flat fielded to allow for consistent illumination across the frame (Ohman et al. 2019). The flat-fielded images were passed through a dual-pass image detection and segmentation algorithm based on Canny (1986) in order to identify ROIs within each image. Each ROI had 70 geometric features calculated and embedded in XMP format within the image, together with the interpolated physical data from Zooglider (Ellen, 2018). The threshold for a ROI to be cropped and saved was 0.45 mm ECD. The 0.45 mm threshold was found to be the smallest identifiable target size after several thousand frames of testing. The cropped ROIs were manually sorted into 57 categories.

To ascertain whether Zooglider and ship-based instruments were sampling the same water parcel, we compared ship-based and Zooglider mounted CTD profiles, as potential density (σθ), and chlorophyll-α in vivo fluorescence among the Zooglider, MOCNESS and CTD fluorometers, as well as the extracted chlorophyll-α from the CTD-rosette Niskin bottle samples. Water samples were filtered onto GFF filters, extracted in 90% acetone and analyzed with acidification on a Turner 10 AU fluorometer.

Eight taxa were compared between the MOCNESS samples and the Zooglider in situ images: Appendicularia, chaetognaths, Oithona (copepod), other Copepoda, euphausiids, gelatinous predators (Cnidaria and
Ctenophora), mineralized protists (Acantharia, Collo-
daria, Foraminifera, and Phaeodarea) and nauplii. These
taxa were chosen because they had the greatest numbers of
organisms within both the MOCNESS and Zooglider
data sets.

The MOCNESS tows and Zooglider dives were divided
into day and night samples to minimize expected diel
differences in organismal concentrations. The day sam-
ples included two MOCNESS tows and five Zooglider
dives, while the night samples included two MOCNESS
tows and four Zooglider dives. Total abundances (No. m⁻³)
for the eight classes of organisms were vertically inte-
grated from 400 m to the surface for both the Zooglider
and MOCNESS data. These abundances were compared
using a two-sample paired t-test (ttest2, MATLAB). No
difference was observed when the total abundance data
were dichotomized by time of day for both the MOC-
NESS and Zooglider, thus all day and night sampling
was pooled for each sampling system and reanalyzed for
differences in the total abundances.

Vertical distributions of the concentration of organ-
isms (No. m⁻³) were generated for the day and night
sampling of all eight taxa, for both sampling systems. The
Zooglider vertical distributions were binned at two different
levels: the same depth intervals as sampled by the MOC-
NESS nets and 25 cm. The first binning was done for a
side-by-side comparison between the two systems, while
the 25 cm bin shows the finer vertical structure resolvable
by the Zooglider. While Zooglider is capable of resolving
5 cm bins, the vertical structure of the less abundant taxa
was hard to discern when viewed at full resolution, but
more apparent at the 25 cm bin size. To emphasize the
fine vertical structure of each taxon in the upper part of
the water column, the graphs at the 0.25 cm bin size
were truncated to 0–200 dBar. For the MOCNESS net depth
intervals, the vertical distributions were compared using a
two-sample paired t-test (ttest2, MATLAB).

In addition to the computer-generated geometric
measurements, the width of each ROI was manually
measured in ImageJ in order to make direct comparisons
between organism sizes from Zoocam and ZooScan. Care
was taken to not measure the moveable parts of each
organism such as grasping spines, setae, tentacles and
antennae. It was necessary to measure these widths man-
ually, as the ROIs had several characteristics that hindered
consistent computer-generated width measurements (e.g.
pose, existence of appendicularian houses and relatively
transparent features of the organism). The measured
widths (w) were used to generate taxon-specific normal-
ized probability distributions for both the MOCNESS
and Zooglider data sets. Each probability distribution used
a bin width of 40 μm (the largest pixel resolution between
the ZooScan and Zoocam images). The normalized
frequency distributions were then compared using a
Kolmogorov–Smirnov test (ktest2, MATLAB). The
40 μm bin width resulted in probabilities well below 0.01
at the upper size range of each taxa size distribution.
When such small probabilities were found, the smallest
probability values were summed into one size class as
avoiding artificially increasing the number of size classes
being compared.

The width distribution data were subdivided into three
size categories: small (w ≤ 0.28 mm, the diagonal of the
net mesh), medium (0.28 < w ≤ 1 mm) and large organ-
isms (w > 1 mm). These size categories were combined
with the vertical distribution data to view taxon-specific
size differences by depth. These MOCNESS and Zooglider
size-dependent concentrations by depth were compared
using a two-sample paired t-test (ttest2, MATLAB).

Active acoustic analysis focused on 200 kHz as this
was the only common frequency between the EK80 and
Zonar. Both instruments were calibrated using a standard
tungsten carbide reference sphere (Fouote et al., 1987). The
EK80 transmitted at a rate of 23 kHz with a 1.024-ms
pulse length. EK80 acoustic backscatter was analyzed
in Myriax Echowview 8 software. Background noise was
removed following De Robertis and Higginbottom (2007),
with a signal-to-noise threshold of 10 dB, which limited
the depth of analysis to 200 m for comparison between
instruments. Zonar data were processed following
Ohman et al. (2019). The Zonar used a 5 kHz sampling
rate with a 6-ms pulse length. Backscatter data were
analyzed over a range of 3–8.1 m from the Zonar
transducer face. For both instruments, average profiles
of mean volume backscattering strength (Sv, dB re
1 m⁻³); details in Ohman et al., 2019) were calculated
for the time period of the upcast of each dive in 10-
m vertical bins and compared via regression analysis (r²
from polyfit and polyval, P-value from fitlm and analysis
of variance, MATLAB) for daylight and night dives
separately.

A potential source of disagreement between the Zonar
and EK80 is the difference in the volume each instrument
insinifies (Guichen et al., 2014; Moline et al., 2015). For
comparison, we calculated the volume insinified between
7–8 m from the Zonar transducer (the widest insinified
radius used from that instrument) and in 1-m deep
bins from 7–200 m from the EK80 transducer using
equation 1.

\[ V = \left( \pi r_2^2 \frac{h_2}{3} \right) - \left( \pi r_1^2 \frac{h_1}{3} \right) \]

where V is the insinified volume in m³, r is the insinified
radius in m, h is the distance from the transducer in m,
the subscript 1 denotes the values for the shallower bound
of the bin and the subscript 2 denotes the deeper bound
of the bin. We calculated \( r \) using equation 2, where \( \Psi \) is the equivalent beam angle of the transducer in radians (0.17 rad for the Zonar and 0.12 rad for the EK80).

\[
\begin{align*}
\omega & = \Psi \times h. \\
\end{align*}
\]

For analysis of the difference in insonified volumes between the two systems, we considered the ratio of EK80 sampling volume to the Zonar sampling volume as a function of depth.

RESULTS

The potential density profiles (Fig. 2a) from the CTD casts, MOCNESS tows and Zooglider dives correspond well, showing a relatively mixed layer from 10 m to 30 m. The extracted chlorophyll-\( \sigma \) values from the CTD casts agree with the \textit{in vivo} fluorescence measured by the CTD, MOCNESS and Zooglider fluorometers (Fig. 2b), with all sampling methods showing a sharply defined subsurface chlorophyll maximum between 30 m and 40 m, thus suggesting that we sampled similar water parcels with each instrument.

The vertically integrated abundances for all eight taxa are shown in Fig. 3. Significantly higher vertically integrated abundances were found for Zooglider relative to MOCNESS samples for other copepods \( (P < 0.001) \), Oithona \( (P < 0.001) \), appendicularians \( (P < 0.01) \), mineralized protists \( (P < 0.01) \) and gelatinous predators \( (P < 0.05) \). No difference was found for chaetognaths, euphausiids and nauplii \( (P > 0.20) \).

The vertical distributions for all taxa, when binned at MOCNESS net depth intervals (Fig. 4), show relatively consistent patterns of distribution by depth; however, the concentrations measured by Zooglider were typically much greater than the MOCNESS concentrations. Significant differences were observed between the MOCNESS and Zooglider concentration profiles for both the day and night profiles of other copepods \( (P < 0.05) \), Oithona \( (P < 0.01) \), appendicularians \( (P < 0.05) \), mineralized protists \( (P < 0.001) \) and gelatinous predators \( (P < 0.05 \text{ day}, \ P < 0.01 \text{ night}) \). No differences were detected for chaetognaths, euphausiids and nauplii \( (P > 0.05) \).

When the dives are examined individually at 0.05-dBar (5 cm) vertical intervals, markedly higher maximum concentrations were observed for all taxa, e.g. 53 000 other copepods m\(^{-3}\) and 29 000 appendicularians m\(^{-3}\) (not shown). However, as stated in the methods, at that resolution the relative scarcity of the other taxa makes it difficult for their vertical structure to be resolved from so few dives.
because of abundant zero counts. When the vertical distributions for the Zooglider are instead binned at 0.25 dBar, the vertical microstructure becomes more clearly apparent, and the maximum concentrations remain greatly elevated relative to the MOCNESS measurements for all taxa (Supplementary Fig. 1). Chaetognaths appear to be relatively evenly distributed with respect to depth, while the other taxa typically show elevated concentrations between 0–75 dBar.

Normalized size distributions for the body widths of organisms for all eight taxa are shown in Fig. 5. Significant differences were found between the MOCNESS and Zooglider size distributions for mineralized protists \( (P < 0.01) \), gelatinous predators \( (P < 0.01) \), euphausiids \( (P < 0.05) \) and nauplii \( (P < 0.05) \). Other copepods, Oithona, appendicularians and chaetognaths showed no difference \( (P > 0.05) \) in size distributions. The vertical dotted line in Fig. 5 represents 0.28 mm, the diagonal measurement of the MOCNESS net mesh size.

Supplementary Fig. 2 shows the vertical distributions from Fig. 4 subdivided into three size groups based on organismal body width: small \( (0–0.28 \text{ mm}) \), medium \( (0.28–1.0 \text{ mm}) \) and large \( (>1.0 \text{ mm}) \) with respective \( P \)-values, \( P_S \), \( P_M \) and \( P_L \). For the small size class, significant differences in concentrations between the MOCNESS and Zooglider \( (P < 0.05) \) were shown for other copepods \( (\text{day only}) \), Oithona and mineralized protists. The medium size class was significantly different for Oithona \( (P < 0.001 \text{ day}; P < 0.05 \text{ night}) \), appendicularians \( (P < 0.05) \) and mineralized protists \( (P < 0.01) \). The large size classes showed significant differences \( (P < 0.05 \text{ day}; P < 0.01 \text{ night}) \) for gelatinous predators and euphausiids. The remaining size classes showed no differences in concentrations between the MOCNESS and Zooglider.

Average day and night vertical profiles for acoustic volume backscatter at 200 kHz from the EK80 and Zonar are shown in Fig. 6A and B, respectively. The two instruments generally agree in pattern and magnitude of acoustic backscatter, although agreement was markedly better at night \( (\text{r}^2 = 0.58, P < 0.001) \) when depth variability of scatters was lower than during the day \( (\text{r}^2 = 0.21, P < 0.05) \). When comparing the volume backscatter of the two instruments within the upper 200 m, the volumes sonified are substantially different, with the surface-mounted EK80 sonifying \( \sim 350 \) times the volume of the Zonar at a depth of 200 m (Fig. 6C).

**DISCUSSION**

As to be expected, there were subtle variations in both the physical and biological properties as sampled by the multiple Zooglider dives, MOCNESS tows and CTD profiles. However, to properly address the potential influence of these variations in water column properties, as well as zooplankton patchiness on a broad spectrum of spatial scales \( (\text{e.g. Haury et al., 1978}) \), on the organisms sampled many additional profiles and transects would be necessary and are beyond the scope of the present study. The general correspondence in potential density suggests that
water parcels sampled by the CTD, MOCNESS and Zooglider had similar physical properties. The agreement in chlorophyll-a profiles suggests that the water parcels sampled by both the Zooglider and the instruments aboard the R/V Sally Ride bore similar biological characteristics. These similarities are supported by the proximity of the Zooglider to the R/V Sally Ride and minimal time difference between dives and MOCNESS tows (±3 h).

Zooglider and MOCNESS agreed on the abundances of taxa relative to one another within the water column (i.e. other copepods, appendicularians, and Oithona as the most abundant). However, there were stark differences between the MOCNESS and Zooglider measurements with regards to total abundance, concentrations and size distributions for many of the taxa. Zooglider showed significantly higher vertically integrated abundances and local concentrations for five of the eight taxa compared to the MOCNESS. There were significant differences between the two systems in the size distributions for four of the eight taxa. It should be recalled that Zooglider images organisms alive, in situ, while the MOCNESS/ZooScanned samples reflect both net capture and preservation artifacts, which could account for some of the size differences.

Previous studies have yielded similar results to Zooglider, concerning taxon-specific discrepancies in abundance, when comparing optical imaging systems to nets. The VPR showed discrepancies in numerical concentrations for medusae, appendicularians and copepods by factors of 360, 16.4 and 2.9, respectively (Benfield et al., 1996). The Shadowed Image Particle Profiling and Evaluation Recorder revealed that a 162-μm mesh net significantly underestimated the abundance of appendicularians (300%), doliolids (379%), protists (522%) and ctenophores/cnidarians (1200%), but no significant differences in chaetognaths, copepods or euphausiids were detected (Remsen et al., 2004). These differences in taxon abundance, or lack thereof, are primarily attributable to differences in net extrusion or robustness of different organisms and in particular whether they are fragile, soft-bodied or hard-bodied taxa. We believe that these results cannot be explained by light attraction of organisms because (i) red light was used to which the organisms are insensitive, (ii) the light source is recessed well inside the sampling tunnel and is difficult to discern and (iii) Zooglider’s ascent speed exceeds the sustained swimming capacity of small copepods (Wong 1988; Yen 1988) and most other...
Fig. 5. Comparison of normalized size distributions of body widths for Zooglider (gray) and MOCNESS (black) samples, by taxon. The vertical dotted line represents 0.28 mm (the diagonal of the MOCNESS mesh size); (* = P < 0.05; ** = P < 0.01). For ease of viewing mineralized protists and gelatinous predators, probabilities were pooled for body widths exceeding 4 mm and 6 mm, respectively.

zooplankton (De Robertis et al., 2003; Seuront et al., 2004; Genin et al., 2005).

The harder-bodied copepods are less likely to experience significant shrinkage due to preservation, thus any size discrepancies are most likely attributable to the sampling process. The majority of the *Oithona* that were captured by both systems were small, below 0.28 mm in body width, which is the open dimension of the diagonal of the net mesh (vertical dotted line; Fig. 5). It is likely that although the size distributions did not differ significantly, many *Oithona* were extruded through the MOCNESS 202-μm mesh with the added force of the water flowing through the net. Presumably, the *Oithona* that were captured by the net were more likely to be oriented orthogonal to the mesh opening. Similar reasoning applies to the other copepods category. As the discrepancy in concentration continues to persist into the medium size category of other copepods (0.28–1 mm), it is likely that some copepods exceeding 0.28 mm in body width were also extruded but to a lesser extent. This is not the first study to find such conclusions. Di Mauro et al. (2009) showed that a 220-μm mesh underestimated the copepod *Oithona nana* by 96.29%, harpacticoid copepods by 96.52%, and copepodites (stage I–III) of small calanoids by 99.7% when compared to a 67-μm mesh. Copepods with prosome lengths less than 550 μm were most efficiently sampled by a 64-μm mesh off the central coast of California (Hopcroft et al., 2001).

The higher abundances and concentrations of appendicularians and gelatinous predators detected by Zooglider are also attributable to net extrusion; however, due to the softer bodies of these particular taxa, it is likely that the size range for extrusion may be higher than that of the harder-bodied copepods. Di Mauro et al. (2009) found that the soft-bodied appendicularian *Oikopleura dioica* was significantly underestimated for trunk lengths <500 μm with 220-μm mesh nets. Furthermore, appendicularians and gelatinous predators (here cnidarians and ctenophores) are more susceptible to degradation via net collection and formaldehyde-induced shrinkage and distortion (Nishikawa and Terazaki 1996; Beaulieu et al., 1999), which in turn makes those degraded samples more difficult to identify and count for abundance estimates.

Soft-bodied zooplankton are not the only organisms that are distorted by net collection and preservation. The fragile pseudopodia and spines of mineralized protists are often destroyed or degraded by the processes of net collection, rinsing and fixation at sea. In the case of acantharians, their strontium sulfate spines are well known to dissolve in preservatives if sufficient strontium chloride is not added (Beers and Stewart 1970). Evidence of such
sample degradation was clearly observed in the MOCNESS samples, as no mineralized protists (also including phaeodarians, foraminifers, and colloidarians) retained their spines or pseudopodia. This degradation can render mineralized protists too small to be saved by our 0.45-mm ECD threshold and hinder their accurate classification, which would account for the size, concentration and abundance differences seen by Zooglider. In contrast to the degradation associated with net samples, Zooglider images organisms in their natural posture within the water column, with delicate structures intact (Ohman et al., 2019; Gaskell et al., 2019). Accordingly, mineralized protists along with soft-bodied appendicularians and gelatinous predators are generally larger than their shrunken and broken preserved counterparts, which accounts for differences in size between the MOCNESS and Zooglider samples.

The abundance of nauplii did not differ between the two sampling approaches, although smaller nauplii made up a larger proportion of the MOCNESS samples compared to Zooglider samples. We believe this size discrepancy is due to the difference in pixel resolution between the two systems. The smaller appendages of many nauplii were more readily identifiable within the MOCNESS samples at the ZooScan resolution of 10.6 μm pixel$^{-1}$, while many possible nauplii were labeled ‘unsure’ due to the Zoocam resolution of 40 μm pixel$^{-1}$ and therefore not included in the nauplii data.

Chaetognaths were sampled with similar vertically integrated abundances, depth-specific concentrations and size distributions by the two methods. We presume that chaetognaths are less likely than fragile cnidarians, ctenophores and appendicularians to be damaged by net collection or to be extruded through the net mesh. The MOCNESS and Zooglider captured similar abundances of euphausiids, with slightly larger body widths recorded by Zooglider. However, the very largest specimens we found were detected in MOCNESS net samples, albeit at very low abundances ($<0.0001$ animals m$^{-3}$). This size difference may be attributed to a relatively low abundance of large euphausiids within the water column, coupled with the discrepancies in sample volume between Zooglider and MOCNESS, or perhaps to avoidance behavior (cf., Brinton, 1967). However, the euphausiids in Zoocam images are in natural postures and do not exhibit abdominal flexure typically associated with avoidance. Furthermore, the Zoocam utilizes a sampling tunnel that was designed specifically to minimize hydrodynamic disturbances that may trigger escape responses (Ohman et al., 2019).

Zooglider was able to discern much greater concentrations and abundances of several taxa. When viewed
at small scales (<1 m) maximum concentrations for other copepods and appendicularians reached 53,000 and 29,000 animals m\(^{-3}\), respectively. The persistence and extent of these high concentrations will ultimately determine their effect on the planktonic community, a topic we will address in future publications.

The dual frequency Zonar records acoustic backscatter from smaller (1000 kHz) and larger zooplankton (200 kHz) and other organisms. However, the only acoustic frequency held in common between the Zonar (200 kHz) and other organisms. However, the only acoustic frequency held in common between the Zonar and EK80 was 200 kHz; hence comparisons could only be made for the larger component of the acoustic backscatter. At 200 kHz, the vessel-mounted EK80 and Zooglider-mounted Zonar generally agree in magnitude and overall distribution of backscatter when averaged over all day and all night dives. Agreement was better at night when scatterers migrated to the surface and their distributions were less variable. The differences may be attributable to the difference in volume insonified between instruments. The detection probability for rare but strong scatterers would be higher for larger sampling volumes. An acoustic beam insonifies an approximately conical volume of water that widens with increasing distance from the instrument. The vessel-mounted EK80 only samples from the surface, and therefore the sampling volume increases proportionally with depth, while the Zonar sampling volume remains constant. Thus, the larger rare, strong scatterers will be better represented in the EK80 backscatter data. However, the EK80 200 kHz has an effective depth sampling limit of 200 m due to a decline in the signal-to-noise ratio (SNR) in deeper depths. Conversely, the profiling glider-mounted Zonar permits the effective sampling of much deeper water than vessel-mounted echosounders (Guihen et al., 2014; Moline et al., 2015; Powell and Ohman 2015). The acoustic systems were not compared with the imaging and net collections in this study as that would require information regarding taxon-specific acoustic scattering models, frequency-dependent acoustic target strength and orientation of the organisms insonified (Briseño-Avena et al., 2015).

CONCLUSION

Zooglider captures greater numbers of smaller-sized organisms (i.e. copepods and appendicularians) and larger-sized organisms (i.e. mineralized protists, medusa, siphonophores and ctenophores) compared to the MOCNESS. Comparable abundances and similar size distributions are found for other taxa (chaetognaths, euphausiids and nauplii). A combination of net extrusion, net-induced damage and preservation effects all contribute to these abundance and size discrepancies. Zooglider was able to resolve elevated concentrations of copepods and appendicularians, to 53,000 and 29,000 animals m\(^{-3}\), respectively. The Zonar agrees with the EK80 in magnitude and overall distribution of acoustic backscatter at 200 kHz. The profiling nature of the Zooglider allows it to sample much deeper than vessel-mounted echosounders without losing sample resolution due to a decline in SNR. Zooglider’s acoustic and optical sensing systems, in combination with its autonomy and endurance, make it uniquely capable to sample zooplankton distributions with minimal disruption to the organisms.

SUPPLEMENTARY DATA

Supplementary data can be found at Journal of Plankton Research online.

ACKNOWLEDGEMENTS

The Instrument Development Group (R. Davis, J. Sherman, K. Grindley, B. Rieneman, E. Goodwin and D. Vana) is to be credited with the design and manufacture of Zooglider. We thank UC Ship Funds for research vessel time and the crew of the R/V Sally Ride. E. Tovar Zooscaned the MOCNESS samples. J. Ellen assisted with post-processing of the Zoocam images and J. Trickey assisted with EK80 processing.

FUNDING

Gordon and Betty Moore Foundation (3576 and 5479); CCE-LTER (NSF OCE-16-37632); DoD SMART fellowship; UC Ship Funds for the award of ship-time.

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