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Measuring the distribution, abundance, and biovolume of zooplankton in an oligotrophic freshwater lake with a 710 kHz scientific echosounder

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Abstract

Acoustic surveys of the distribution and abundance of freshwater zooplankton were conducted in Lake Giles, an oligotrophic freshwater lake. Volume backscatter data from a 710 kHz scientific echosounder were converted to high-resolution spatial and temporal numerical density estimates of small zooplankton. Vertical net tows of a 153 μm mesh closing bongo net at multiple depth intervals provided both identification of the types and sizes (0.5–1.5 mm length) of crustacean zooplankton present in the lake as well as an independent measurement of zooplankton numerical density. Net and acoustic estimates of zooplankton abundance, biovolume, and distribution were very similar. The improved resolution of the high-frequency acoustic sampling provides insight into several aspects of freshwater zooplankton ecology including: separation of migrating and non-migrating zooplankton, high resolution measurements of in situ zooplankton biovolume, calculation of in situ vertical velocities of migrating zooplankton, and fine-scale (sub-meter) horizontal and vertical zooplankton distribution during daytime, nighttime, and vertical migration events. These methods allow for more detailed and accurate estimates of zooplankton distribution than traditional net sampling methods can provide, including determining the total abundance of organisms within a specific habitat. They also provide higher resolution data in both space and time of smaller zooplankton taxa than have been measured previously in freshwater ecosystems.

Pelagic zooplankton play a critical role in freshwater ecosystems as important contributors to water quality and the strength of trophic linkages and are therefore critical to many ecosystem processes. They also provide an excellent model system for understanding fundamental and applied ecological dynamics (Lampert 2011) and genomics (Colbourne et al. 2011). Zooplankton dynamics and their subsequent role in ecosystems are highly dependent on their horizontal and vertical distribution and abundance, as well as environmental factors and processes that also vary spatially and temporally. Conventional net and trap-based sampling methods are typically used to characterize the zooplankton populations in freshwater ecosystems, but are limited in how well they capture these organisms and resolve these processes. Sampling locations within a habitat are often limited to few sites, or in many cases a single site, which makes it difficult to measure the spatial and temporal complexity of zooplankton aggregations and their dynamics. These collection methods are also generally integrative in either space or time making it difficult (or impossible) to resolve fine-scale processes in either dimension. Previous acoustic work with freshwater zooplankton has typically used instruments that are not capable of resolving the smaller species of zooplankton that can often comprise a major portion of the zooplankton biomass.

Yet zooplankton are highly responsive to environmental conditions and capable of habitat selection as a function of ultraviolet and visible light (Leech et al. 2005; Fischer et al. 2006; Williamson et al. 2011), temperature (Kessler and Lampert 2004), food quantity and location within the water column (Kessler and Lampert 2004), vertebrate and invertebrate predation pressure (Dodson 1988; Neill 1990; Nesbitt et al. 1996), individual health (Johnsen and Jakobsen 1987; Van Gool and Ringelberg 1998), and genetic variability (de Meester and Dumont 1989). The complexity of zooplankton habitat selection, migration, and seasonal changes in these behaviors occur on smaller temporal and spatial scales than captured by conventional sampling methods. Real-time, higher-resolution, and less labor-intensive methods are needed to create new insights into the simultaneous vertical and horizontal migration by zooplankton (Armengol et al. 2012) that influences their response to environmental

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change as well as their role in ecosystem dynamics. These include the ways zooplankton respond to climate change that alters phenology and predator-prey dynamics (Winder and Schindler 2004; Manca and DeMott 2009; Winder et al. 2009), lake thermal stratification (Jankowski et al. 2006; Adrian et al. 2009; Wagner and Adrian 2011), as well as the influence of diel vertical migration (DVM) on tradeoffs along vertical habitat gradients (Winder et al. 2004), the interaction of temperature and predation pressure on zooplankton fitness (Pangle and Peacor 2010) and the effect of hypoxic zones on DVM (Vanderploeg et al. 2009a,2009b).

Nets and other direct sampling methods such as Schindler traps or Van Dorn bottles have been used for sampling zooplankton in freshwater and marine habitats for many decades (Hutchinson 1967; Wiebe and Benfield 2003). The benefits of these methods are that physical specimens are collected that allow detailed taxonomic identification, measurement of phenotypic characteristics, and if organisms are viable, further studies of their biology. However, there are limitations with the use of nets as they are integrative samplers, which make it difficult to know where in the net tow path individual organisms were collected. Plankton traps (Schindler 1969), pump sampling (Haney et al. 1990), and continuous plankton samplers (Masson et al. 2001) have led to improved horizontal and vertical resolution. But these systems are labor intensive, and highly limited in their ability to resolve short-term dynamics of zooplankton, particularly during diel vertical or horizontal migrations.

More advanced optical and acoustic techniques have also been used to study freshwater zooplankton (Sprules et al. 1992; Schulze et al. 1995; Holbrook et al. 2006; Rahkola-Sorsa et al. 2014). Optical techniques have the advantage of providing relatively automated data, but they are also limited by their inability to provide high resolution data or any detailed taxonomic identification as well as small sampling volumes. The primary advantage of acoustic assessment of plankton and nekton is that it provides high-resolution data in both space and time compared with traditional trap, net, or optical assessment methods. The downside is that acoustic systems measure scattered energy from objects in the water column and this acoustic measurement must be converted into a biologically useful measure. In environments where a few diverse types or sizes of organisms are present, this conversion process is relatively straightforward. If the amount of energy scattered by an individual scatterer (i.e., the target strength) is known, then measured acoustic backscatter for a volume of water can be converted to estimate the numerical abundance of the scatterers for that volume (Foote and Stanton 2000; Simmonds and MacLennan 2005). However, this is often not the case in many ecosystems. If multiple types or sizes of scatterers are present, then this conversion of acoustic information to ecologically relevant measures is more complex (Warren et al. 2003; Warren and Wiebe 2008; Ressler et al. 2012).

Acoustic backscatter has been used to assess freshwater zooplankton distribution for decades (Shiraishi 1960; Northcote 1964; Teraguchi and Northcote 1966; McNaught 1969). Most freshwater studies to date used acoustic techniques to study fish that could also detect dense aggregations of crustacean zooplankton (copepods, Megard et al. 1997; amphipods, Melnik et al. 1993) or organisms such as the larval midge *Chaoborus* that possess gas vacuoles that make them strong scatterers (Knudsen et al. 2006). In some cases, scattering from zooplankton was strong enough to cause difficulty in measuring the backscatter from the resident fish in the area (Kubecka et al. 2000). Scientists have used the increased spatial and temporal resolution of these acoustic systems to conduct studies that would be impossible using traditional sampling methods including: the vertical migration of amphipods (Trevorrow and Tanaka 1997), spatial heterogeneity of *Daphnia* (Hembre and Megard 2003) or zooplankton community horizontal habitat use (Holbrook et al. 2006). Many studies used multiple frequency systems, which allow some discrimination of the source of the backscatter (Knudsen et al. 2006).

Traditional echosounder systems operate at 18 kHz, 38 kHz, 120 kHz, and 200 kHz as these are useful frequencies to measure bottom depths, monitor fish schools, and (in some cases) discriminate between fish and other scatterers (Watkins and Brierley 2002; Jurvelius et al. 2008). These systems were designed to have detection ranges of hundreds (and sometimes thousands) of meters, but to achieve these ranges, these systems are limited to detecting only larger organisms or high density aggregations of smaller scatterers. To detect small (length $<$ 2 mm) scatterers, higher acoustic frequencies are needed. High frequency systems (hundreds of kHz and above) can be used to detect both small biological organisms (Holliday and Pieper 1995) and, more commonly, suspended sediments (Lynch et al. 1997) but they are limited to ranges of tens of meters or less.

A variety of high frequency systems have been used to study spatial distributions and dense aggregations ($>10^3$ organisms m^{-3}) of small (1–2 mm length) freshwater zooplankton including a 430 kHz system to measure copepod spatial distribution in Lake Superior (Holbrook et al. 2006) and a 710 kHz system to detect copepod aggregations over a range of 30 m in a shallow coastal bay (Parks et al. 2011). While similar systems, including a 710 kHz echosounder and a 614 kHz Acoustic Doppler Current Profiler (ADCP), have also been used to detect vertical distributions and migrations of zooplankton (Lorke et al. 2004; Knudsen et al. 2006; Rahkola-Sorsa et al. 2014), these studies have focused on the gas-bearing larval insects *Chaoborus flavicans* that are strong backscatterers and large in size (2–10 mm length). To our knowledge, only two previous studies (Roman et al. 2001; Parks et al. 2011) have used acoustic systems to examine vertical distributions or DVM of small ($<$2 mm) zooplankton.

Recent work (Rahkola-Sorsa et al. 2014) did compare optical, net, and a 614 kHz ADCP measures of zooplankton biomass and concluded that "in situ estimation of zooplankton abundances on the basis of ADCP results is not reliable without simultaneous net or optical sampling." High-frequency acoustic surveys in freshwater systems have rarely taken advantage of the temporal resolution of these systems to monitor changes in the distribution of small zooplankton in real-time or the spatial resolution to fully characterize zooplankton distribution over the entire habitat. Unlike ADCPs, scientific echosounders are designed specifically to quantitatively measure the amount of acoustic energy scattered by objects in the water column.

We used a calibrated 710 kHz scientific echosounder to assess abundance, vertical distribution and DVM of small zooplankton in an oligotrophic inland lake. Vertical profiles of zooplankton numerical density were compared with traditional vertically stratified net tow sampling to assess the efficacy of using acoustic backscatter to measure zooplankton biomass and distribution. Acoustic survey transects were conducted across the entire spatial extent of the lake to describe the horizontal variations in zooplankton distribution and how that varied on a diel basis. Here we demonstrate how a high resolution acoustic system can provide novel insights into the dynamics of the zooplankton community in a small freshwater lake including: (1) the resolution of vertically migrating and non-migrating small zooplankton using acoustic sampling, (2) the conversion of acoustic backscatter into accurate estimates of zooplankton biomass, (3) direct observations of upward migration events of small zooplankton, (4) calculation of in situ vertical velocity of zooplankton, and (5) high resolution estimates of the vertical and horizontal heterogeneity of zooplankton densities.

Materials and procedures

The acoustic study was conducted on 17–18 June 2012 and 16 August 2012 in Lake Giles, a 48-hectare, oligotrophic lake located in Northeastern Pennsylvania, USA (41°22′34″N 75°05′33″W) with maximum and mean depths of 24.8 m and 10.1 m, respectively (Fig. 1). The zooplankton community in Lake Giles is dominated by the cladoceran *Daphnia catawba* and the calanoid copepod *Leptodiaptomus minutus*, with low densities of the calanoid *Aglaodiaptomus spatulocrenatus* and the cyclopoid copepod *Cyclops scutifer*.

Direct zooplankton samples were collected from the center of the lake (where depth is maximum) using a 20 cm diameter, 153 μm mesh bongo-style closing plankton net and immediately preserved in ethanol. To compare zooplankton community vertical distribution to acoustic backscatter, we collected zooplankton at midday and midnight at 2 m intervals from 1 m above the lake bottom to the surface on 07 June 2012 and 14–16 August 2012 (inclement weather prevented day and

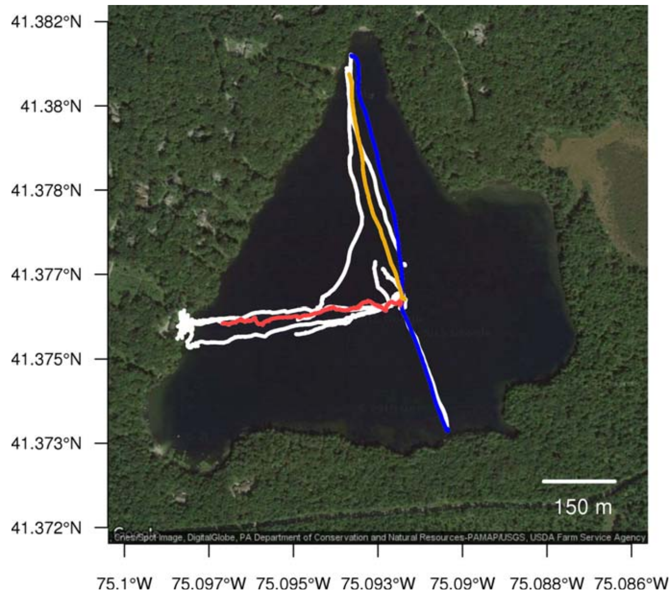

Fig. 1. Lake Giles study site. Acoustic survey tracks are plotted in white with transects 2 (blue), 6 (red), and 9 (gold) highlighted. The intersection of the red and blue transects is the mooring where stationary acoustic backscatter observations and net tow collections were made. North is straight up in this figure.

night sampling within 24 h in August). Net samples were also collected concurrent with acoustic sampling on 17 June and 16 August at specific depth strata (ranging from 2 m to 5 m in thickness) covering depths of 5–20 m of the water column. We assume that net samples from the middle of the lake are representative of the entire lake in terms of species present and their sizes. Individuals were enumerated under a dissecting microscope using a 10 mL Bogorov chamber; cladocerans were identified to genus and copepods to family.

Zooplankton body length and width measurements were taken of a random sample of *Daphnia*, calanoid and cyclopoid copepodids (10 individuals of each species for each sampling date and depth range) from replicate 3–8 m and 15–20 m samples collected on 17 June 2012 and 16 August 2012. These samples were chosen because the large number of individuals collected would provide a reasonable representation of the community size distribution. *Daphnia* length was measured from the anterior to posterior margin of the carapace, excluding the spine and helmet, and copepod length was measured from the anterior portion of the metasome to the metasome-urosome junction. Width measurements for all zooplankton were taken at the broadest portion of the metasome. The zooplankton size measurements were used to convert acoustic backscatter to estimates of zooplankton biovolume.

A 710 kHz EK 60 single-beam scientific echosounder (Simrad-Kongsberg, Norway) was used to collect acoustic backscatter data (volume backscatter strength, S_v) with the transducer deployed at the aft of the survey vessel (3 m row boat) at a depth of approximately 15 cm. Due to the angle of the transom of the vessel, the transducer was oriented at a slight angle (measured from photographs as 7°) toward the bow from the vertical. This offset from the vertical was accounted for in converting acoustic range to depth in the water column. With this deployment geometry and discarding data from the nearfield of the acoustic system (a region where backscatter data are not accurately measured), acoustic data were collected from a depth of 0.5 m from the surface to the lake bottom (maximum of 24.8 m) with a vertical resolution of 5 cm. The transducer (710-30-E) had a beamwidth of 5°. Acoustic data parameters were: 1 Hz ping rate, 256 ms pulse length (resulting in a vertical bin size of 5 cm), and 100 W transmit power.

The echosounder electronics, data acquisition laptop, GPS receiver, and 12 V battery power were enclosed in a customized waterproof case with a waterproof external mouse and keyboard and a viewing port so that data acquisition could be monitored and controlled without opening the case. The acoustic system was calibrated (Foote et al. 1987) using a standard target (38.1 mm Tungsten Carbide sphere) at a depth ranging from 8 m to 12 m midway through the data acquisition. Measured target strength values were within 0.3 dB of the theoretical target strength (-39.6 dB re 1m^2) of the sphere and raw S_v data were corrected using this value. Acoustic backscatter data had a time-varying gain applied to the raw data as part of the instrument processing, but there was no other processing done (i.e., no noise removal, no spike removal) as the data acquisition system (e.g., rowboat, battery-powered echosounder) were acoustically and electrically quiet in terms of system noise. Temperature and salinity profiles were collected from an vertically profiling buoy in the middle of the lake and used to calculate density, soundspeed, and absorption coefficient values which were used in the analysis.

Acoustic surveys were conducted by rowing the vessel along roughly parallel transects on 17–18 June 2012 and 16 August 2012. Sampling surveys were conducted during three different time periods with different light levels (before dusk, dusk, and nighttime) during each day; sunset occurred at 20:39 on June 18 and 20:00 on August 16. Data were collected at a fixed location in the center of lake (with the vessel tied to a moored buoy) during each time period as well as during cross-lake transects such that vertical and horizontal variability in zooplankton distributions could be assessed. While it is possible that aliasing could occur during these transects due to the movement of the zooplankton, we believe this effect will not be significant given that zooplankton horizontal movements are expected to be much slower than the sampling platform over periods of tens of minutes and that horizontal movements of small zooplankton occur in a more random-walk pattern than the (attempted) straight-line movement of the sampling platform.

Since only a single frequency system was used in this study, multiple frequency discrimination of scatterer type was not possible. Based on our observations of the echogram as well as information about the constituent species of zooplankton, fish, and other possible scatterers that were present in the lake at this time of year, we assume that the majority of the scatterers in the water column are small crustaceans (or can be modeled as small crustaceans). Fish, which typically are strong acoustic scatterers, were rarely observed on the echosounder. However, to ensure that their scattering did not bias our results, we used a threshold on the S_v data above the lake bottom, removing any values greater than -60 dB re 1m^2 (Rudstam et al. 2008) before converting acoustic data to zooplankton numerical density. We believe this method will prevent us from accidentally including backscatter from fish targets and will not exclude scattering from zooplankton as even very large aggregations of zooplankton in the lake do not produce backscatter levels that large (e.g., zooplankton densities greater than $\sim 10^7$ animals m^{-3} would be excluded by this threshold). Despite this approach, our acoustic-based calculations of zooplankton biovolume and numerical density will likely be overestimates since we are assuming all scatterers in the water column are zooplankton. The error in this approach for this study will be addressed in the results and discussion sections.

A distorted wave Born approximation scattering model (McGehee et al. 1998) was used to calculate the target strength (TS) at 710 kHz of crustacean zooplankton based on the sizes and shapes of organisms from net sampling. As several scattering model parameter values are unknown for freshwater organisms (specifically the density and sound speed contrasts), values for marine organisms were used (Stanton and Chu 2000). This assumption is another source of possible error in our numerical density estimates as small changes in these parameters can produce large changes in an organism's TS (Chu et al. 2000). Volume backscattering strengths (S_v) were converted to numerical density (N, organisms m^{-3}) estimates by assuming that only a single type of scatterer was present (Smith et al. 2013):

$$S_v = 10\ (\log 10(N)) + \mathrm{TS} \tag{1}$$

Numerical density measurements were converted to acoustically estimated biovolume (mL) using average length and width values of the zooplankton collected during the net tow sampling.

Assessment

Converting acoustic data to biological information

Accurate conversion of acoustic backscatter data to numerical estimates of organisms is dependent on knowing the size and shape of the scattering organism. Net tow data showed that there were three dominant zooplankton taxa in the lake during this study (*Daphnia*, calanoid and cyclopoid copeopds) that had different lengths and widths (Table 1). The mean length-frequency weighted (Warren and Demer 2010) TS at 710 kHz was calculated for each type of scatterer based on simulations of 1000 individuals (of each species) with a random orientation relative to the acoustic wavefront. The *Daphnia* were modeled as an oblate spheroid, whereas the two copepod species were modeled using a shape for a copepod from Chu and Stanton (2000). Additionally, the biovolume of each individual that was modeled was calculated.

Volume backscattering data (dB re 1 m^{-1}) were converted to numerical densities of scatterers (# m^{-3}) using Eq. 1. There were differences between the relative abundances of *Daphnia* and the two copepod species in the net tows depending on the month, time period, and depth strata, however the average (s.d.) relative abundance (averaging over all net tow measurements, $n = 22$) of the species were: 2.9 (2.6) *Daphnia* L^{-1}, 6.9 (6.8) calanoid copepods L^{-1}, and 2.6 (2.5) cyclopoid copepods L^{-1}. We used these relative abundances to calculate (in the linear domain) the total TS (-117.5 dB re 1m^2) of a mixed aggregation (2.9 *Daphnia*, 6.9 calanoid copepods, 2.6 cyclopoid copepods) of these organisms that was then used to determine the total number of organisms in a scattering volume. The total number of organisms was then re-apportioned (using the relative abundances) to the number of *Daphnia*, calanoid copepods, and cyclopoid copepods per volume. These data were then converted to estimates of zooplankton biovolume by multiplying by the mean volume of each scatterer type.

Biovolume was calculated using the organism's shape as defined in the acoustic modeling section (i.e., either a oblate spheroid or a copepod-specific body shape) with the measured length and width as the major and minor axes. This volume was used to determine the equivalent spherical radius (ESR) of an scatterer with the same volume. Then the ESR value and numerical densities from both net and acoustic methods were used to generate biovolume estimates. While this approach may result in errors in estimates of numerical densities given that the relative abundances of the three types of zooplankton varied with depth and month. One advantage of the acoustic estimation process is that estimates of biovolume for a given level of scattering (S_v) are less affected by errors in the specific species composition of the zooplankton. For example, a given level of backscatter could be the result of either few *Daphnia* (as these animals are larger and have a higher TS) or many copepods (which are smaller and have a lower TS). However, since numerical density and volume of the animal are inversely related, the estimates of biovolume from acoustic data will not vary as much as the numerical densities (i.e., a few *Daphnia* or many small copepods can produce the same S_v and biovolume values).

Acoustic estimation of zooplankton biovolume

Concurrent net and acoustically derived measures of total integrated water column biovolume from sampling during August were similar for both day and night samples (Table 2). It is important to note that, particularly for night-time sampling, the lack of acoustic data in the near-surface layer will miss organisms located within the top 0.5 m of the water column a region that can easily be sampled with nets. Similarly, net tows often do not sample close to the bottom (to avoid sediment contamination), which is not an issue for acoustic sampling. To assess how much of the upper-water column organisms may be missed, we compared acoustic and net measurements for the near surface (0–4 m) in August when both methods were used concurrently. The acoustic estimates were based on data collected from the near-surface (0.5 m depth) to 4 m and then extrapolated to calculate the abundance over the entire depth range (0–4 m depth). Acoustic estimates of biovolume in this region were very similar (within a few percent) to net-derived biovolumes (Table 2). Given that the differences in biovolume between the two methods were much smaller than the differences in biovolumes that occurred in both time and space, we believe these data support the use of acoustically derived zooplankton biovolume. We also note that the acoustic system generally has a much greater sampling volume than the vertical net hauls, with the exception of depths very near the surface.

Direct observations of migrating and non-migrating zooplankton layers

The June acoustic sampling observed vertical migration occurring during the dusk to night transition (from 21:08 to 21:50 on 17 June, Fig. 2). Migration had already begun when sampling commenced (e.g., diagonal streaks in Fig. 2). In addition to the upward-migrating organisms, a surface layer of scatterers (between 5 m depth and the surface) did not appear to vertically migrate. The abundance of organisms in this near-surface static layer may have decreased slightly over the course of the sampling period, however it is difficult to determine this since the vertically migrating organisms began to occupy the same depth ranges as the static layer. The direct observation of the migration event allowed us to investigate the timing of the migration and observe organisms that may have emerged from near or in the sediments and are often missed with traditional net sampling.

Net samples collected at 21:34 and 22:04 on 18 June show more than a doubling in the density of *Daphnia* and calanoid copepods in the top meter of the water column over that period (1.9–5.5 org L^{-1} and 7.9–20.5 org L^{-1}, respectively). The difference in distribution between the *Daphnia* and calanoid copepods from net sampling suggest that during the acoustic survey we observed *Daphnia* species migrating from deeper in the water column to the surface within approximately 30 min while the calanoid copepods

Table 1. Physical and acoustical properties of zooplankton sampled by net on 18 June 2012. Animal size is reported as mean and standard deviation. TS were calculated on a length-frequency weighted basis using the distorted wave Born approximation model (McGehee et al. 1998) and the measured lengths of animals. ESR is the equivalent spherical radius of the zooplankton.

Organism	Length (mm)	Width (mm)	TS at 710 kHz (dB re: 1 m^2)	Biovolume (mL)	ESR (mm)
Daphnia	1.1 ± 0.2	0.7 ± 0.2	−111.3	3.4 E − 4	0.43
Calanoid copepod	0.7 ± 0.2	0.2 ± 0.1	−129.8	3.3 E − 5	0.19
Cyclopoid copepod	0.5 ± 0.1	0.2 ± 0.05	−137.0	1.5 E − 5	0.15

Table 2. Comparison of depth-integrated zooplankton biovolumes (mL m^{-2}) from net and acoustic sampling in August 2012 and the sampling volumes for each system. Acoustically estimated biovolumes were generated using median acoustic backscatter strength for the depth range sampled with nets to calculate numerical densities of scatterers. These data were converted to biovolumes using taxa specific numerical abundances for the three zooplankton types. Net data are the mean of two samples and acoustic data are the mean of several hundred samples. Trends between day and night biovolumes were consistent with both methods and there was excellent agreement in total integrated biovolume over the entire water column during both day and night sampling.

Depth interval (m)	Net sampling date	Net-measured integrated bio volume (mL m^{-2})	Net sample volume (m^3)	Acoustic sampling date	Acoustically estimated integrated biovolume (mL m^{-2})	Acoustic sample volume (m^3)
0–20	14 August - Day	14.6	0.6	16 August - Day	14.6	10.0
0–20	16 August - Night	19.1	0.6	16 August - Night	21.4	10.0
0–2*	16 August - Night	4.1	0.06	16 August - Night	2.2	0.009
0–4	16 August - Night	4.5	0.12	16 August - Night	4.7	0.07

*Depth interval for the acoustic sample was 1–2 m.

were likely the static layer observed in the upper 5 m (Fig. 2).

We examined the June 17 migration event more closely by dividing our sampling observations into three 7–10 min periods representing the early dusk (21:03–21:11), peak migration (21:13–21:23), and late dusk (21:28–21:35) periods (sunset was at 20:39). It is important to note that the acoustic measurements do not include the upper 0.5 m of the water column because of the transducer depth and nearfield range where no data are collected. As a result upward migrating organisms may be underestimated near the surface. While differences in the vertical distribution of scatterers are evident (Fig. 3), the total number of scatterers measured acoustically over the water column was very consistent (~10 million organisms) varying by less than 10%. This suggests that horizontal movement of these organisms during the migration period is either not significant or isotropic (i.e., organisms exiting the acoustic beam are replaced by organisms from outside the beam). However the vertical velocities of these organisms are quite fast.

Calculation of in situ vertical velocity of zooplankton

The direct observation of upward migration gives us the ability to calculate in situ vertical velocities of the zooplankton scatterers. Vertical velocities were calculated from the acoustic data by tracking the movement of scattering volumes (most likely containing multiple zooplankton) as well as calculating the change in mean scattering layer vs. time. These methods produced similar values (0.8–1.0 cm s^{-1} and 0.5 cm s^{-1}, respectively) of vertical (upward) velocity during the DVM event observed (Figs. 2 and 3). If we assume a vertical velocity of 0.5 cm s^{-1} then an individual could migrate nearly the entire depth of the water column (approx. 20 m) in less than 70 min. We measured an upward shift in the center of mass of the vertical zooplankton distribution, which shoaled from 9.3 m (early dusk) to 9.1 m (peak migration) to 7.3 m (late dusk).

High resolution observations of the spatial heterogeneity of zooplankton densities

Acoustic backscatter from the August survey also shows thin layers of zooplankton at higher densities than the net tow data suggests. While it is possible that these thin layers represent aggregations of zooplankton moving in or out of the beam pattern, the acoustic distributions in Fig. 4 represent average backscatter over a 10 min window so that is less likely. Instead the observations of thin, high-density layers demonstrate a limitation of the net sampling, which integrates the organisms over the collection interval and obscures fine-scale aggregations and depth selection.

Cross-lake transects taken at midday and at night with the echosounder showed a high degree of spatial heterogeneity of backscatter and provide horizontal resolution that would not be possible with traditional sampling methods. A north to south transect was sampled both during the day and at night. As expected, zooplankton were concentrated

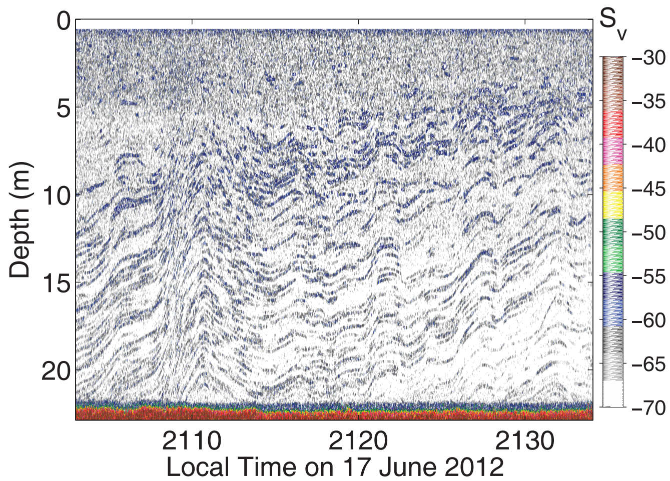

Fig. 2. Acoustic backscatter data collected during the dusk to night transition on 17 June. Rapid changes in the vertical distribution of organisms in the water column can easily be seen as deep organisms move upward and darkness increases. Note the distinct layer of zooplankton in the epilimnion (between 1 m and 5 m depth) that remained in the near-surface waters throughout this period. The red in the echogram represents the lake bottom, white represents very little (or no) backscatter, blues, greens, yellows, and red correspond to increasingly strong backscatter. Echogram has been thresholded with water column values greater than −60 dB removed to eliminate echoes from fish. The dark blue layer just above the red bottom could be scattering from a soft, muddy bottom, or from a dense layer of zooplankton concentrated on the bottom. The surface nearfield region where no valid acoustic data are collected is shown in white.

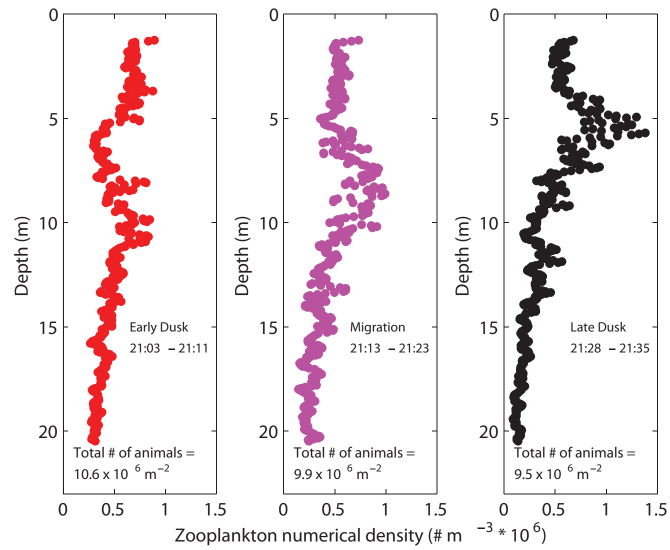

Fig. 3. Vertical distribution of acoustically measured zooplankton (combined *Daphnia*, calanoid copepods, and cyclopoid copepods) numerical densities in the water column sampled on 17 June 2012 averaged over three different local time periods: early dusk (21:03–21:11), peak migration (21:13–21:23), and late night (21:28–21:35). Vertically integrated zooplankton abundance totals for the water column for the three periods are within 10% of each other, and the decrease in abundance with time may be due to animals moving into the near-surface layer which is not sampled acoustically.

between depths greater than 7 m and a few meters above the bottom during the day, although there were some zooplankton that remained at or near the surface during these hours (Fig. 5). There was also substantial variation in horizontal distribution with more zooplankton found on the northern side of the lake basin. At night, a portion of this transect was resurveyed (Fig. 5) which found that the vertical distribution of zooplankton had changed with most individuals now being found concentrated in two layers: one between 4 m and 7 m and another layer near the surface.

During both day and night sampling of this transect, there was a marked decrease in zooplankton abundance near the edges of the lake (when bottom depths were shallower than approximately 7 m). This shore avoidance by pelagic zooplankton has been well-documented (Siebeck 1969) and is thought to be the result of predator avoidance near-shore (Gliwicz and Rykowska 1992). Our data further suggest that the observed shore-avoidance may also be the result of the zooplankton movements being concentrated in the vertical rather than the horizontal dimension. In order for zooplankton to take advantage of a deep refuge from daytime visual predation they must be positioned in portions of the lake where depths exceed the attenuation of light, whether it be low levels of visible light to avoid visual predators, or low levels of UV radiation to avoid the negative effects of UV damage. At night, upward migration would cause them to remain concentrated in the deeper areas of the lake.

An additional cross-lake transect running east to west was surveyed at dusk (Fig. 5). The spatial distribution of zooplankton was similar to that of the north-south transect with zooplankton being concentrated from 4 m to 7 m, as well as in a thin layer near the surface. This transect ended before water depths were as shallow as in the other night time transect, but these data suggest that night-time distributions, while influenced by bathymetry, are isotropic. This is in contrast to the day-time transect data which showed a latitudinal gradient in zooplankton distribution at depth. Possible explanations for this gradient could be differences in light penetration or water clarity, physical mixing processes, or spatially variable predation pressure.

These data show that high frequency acoustics allow for three-dimensional synoptic sampling of zooplankton distribution and abundances in small lakes. Vertical distributions of zooplankton measured acoustically were very similar to those collected by stratified net tows, although some of the discrepancies in the two methods may be due to the higher vertical resolution of the acoustic sampling. Vertically integrated biovolume estimates for both day and night sampling periods were very similar (within 10%) from the acoustic and net tow samplings. Sampling coverage in this study was limited by vessel speed (rowing), time required for net and hydrographic sampling, and battery (and personnel)

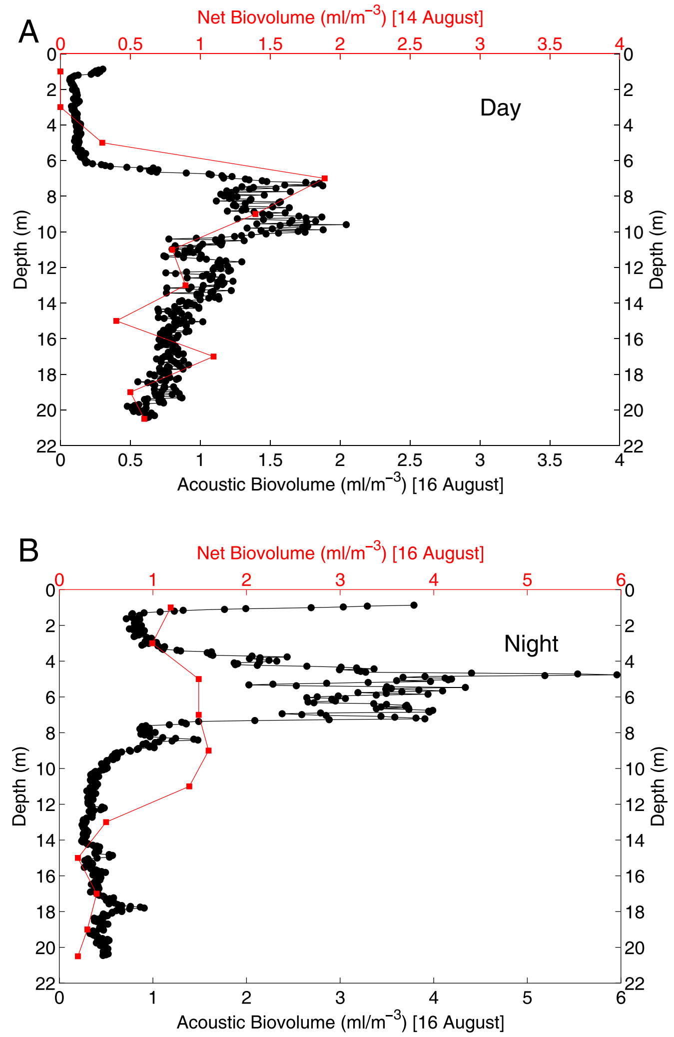

Fig. 4. Vertical distributions of zooplankton biovolume during day (left) and night (right) measured with stratified vertical net tows (red; day samples were collected from 12:00 to 13:00 on 14 August and night samples from 23:55 to 01:12 on 16–17 August) and acoustic backscatter (black; day; 16:51–16:54 and night; 21:42–21:52: 16 August). The higher resolution of the acoustic data is evident and suggests that high abundance layers of lake zooplankton can be very thin, a feature that is missed by the integrative vertical net tow sampling.

limitations. But it is certainly possible for a two-person team to provide comprehensive descriptions of the zooplankton distribution for a lake this size within a few hours, and to repeat these surveys for daytime, nighttime, and transition periods to investigate a variety of different ecosystem processes. Whether this is truly a synoptic measure will depend on the movements of the animals (either vertically or horizontally) during the sampling period. During day and night conditions, lake-wide surveys would be synoptic (assuming that horizontal movements of the zooplankton are much smaller than that of the moving vessel), however the time frame of diel vertical migration events (~1 h) are shorter than the lake-wide survey duration so care must be taken when sampling near dawn or dusk. If we assume zooplankton vertical velocities during migration events (~20 m h^{-1}) are similar to their potential horizontal movement, our sampling vessel is moving several orders of magnitude faster (~3000 m h^{-1}) suggesting that aliasing from the movement of zooplankton themselves is not a large issue. However under certain weather conditions, horizontal advective movements of zooplankton may be much faster.

Discussion

Resolving small zooplankton using acoustic sampling

The acoustic survey data from the high frequency (710 kHz) echosounder was able to measure the distribution of small zooplankton including *Daphnia* (1 mm length) and small copepods (0.5 mm length), which exhibited different DVM behaviors and daytime depth selection. Previous acoustic work in freshwater lakes using frequencies from 192 kHz to 200 kHz were unable to detect backscatterers smaller than 1.3 mm in length (Trevorrow and Tanaka 1997; Hembre and Megard 2003; Knudsen et al. 2006). Using a 1.3 mm length cutoff for the zooplankton community in our study system, we would detect only 20% of the *Daphnia* and none of the cyclopoid or calanoid copepod populations. Thus, a lower frequency instrument would miss ~ 90% of the zooplankton biomass in our study lake, or would only detect dense aggregations of organisms, making distribution and biomass estimates inaccurate. Lower frequency instruments (200 kHz or lower) have been used to detect the movement of *Chaoborus* (Haney et al. 1990; Knudsen et al. 2006), but these are larger organisms (≥1 cm) that possess air chambers that act as strong scatterers and make them much easier to detect at low frequencies.

In the past, marine biologists have used higher frequency acoustics to resolve small zooplankton. Most notable is the study of Roman et al. (2001) where the authors were able to resolve small copepods (down to 0.225 mm in length) using a six frequency system with 285 kHz, 420 kHz, 700 kHz, 1100 kHz, 1850 kHz, and 3000 kHz channels in the Chesapeake Bay. This study provided accurate vertical distribution of the zooplankton community including very small zooplankton in relation to several biogeochemical and physical parameters. While this study had similar fine-scale resolution in the vertical dimension (cm-scale), the vertical information is the result of vertical casts of the instrument through the water column. Therefore the horizontal resolution of their data was quite coarse, consisting of 11 vertical profiles over a range of 70 km. While the higher frequencies of this system are better at detecting very small zooplankton, these MHz (and greater) frequencies have limited range and are not capable of collecting fine-scale horizontal data from surveys of larger areas, as we were able to do in this study.

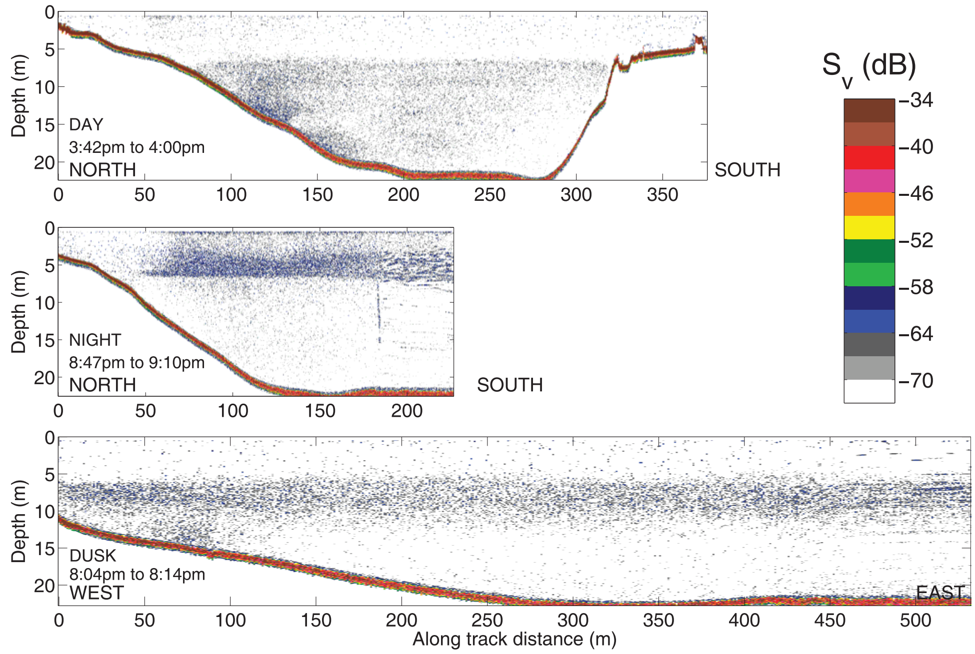

Fig. 5. Cross-lake acoustic survey transects of Lake Giles on 16 August 2012. Top panel: Transect #2 (blue in Fig. 1) occurred during daytime (15:42–16:00) hours and found zooplankton to be located deeper, but with substantial horizontal differences in the spatial distribution of zooplankton. Middle panel: Transect #9 (gold in Fig. 1) is a similar survey line except it was conducted at night (20:47–21:10) and found that the zooplankton had vertically migrated toward the surface. Note the distinctly low density of zooplankton near the north shore in both top and middle panels. Bottom panel: Transect #6 (red in Fig. 1) from the Western edge to mid-lake occurred during dusk (20:04–20:14) and captured the distribution of zooplankton before their migration was complete. Echogram has been thresholded with water column values greater than −60 dB removed to eliminate echoes from fish. Vessel speed was not always constant during the transect which results in a change in the appearance of the echogram (due to more pings being collected per meter in some areas), such as from 180 m to 250 m in the middle panel and 430–550 m in the lower panel.

Converting acoustic backscatter into accurate estimates of zooplankton biomass

Estimates of zooplankton numerical densities and vertical distributions from traditional net and grab samplers followed by laboratory counts, while providing valuable information on species composition, sex ratios and developmental stages, are time consuming because microscopy is required to enumerate individuals. In addition, traditional samples are not able to resolve the high level of horizontal and vertical variability that is apparent from this study. The accurate estimation of zooplankton biomass and distribution is a valuable step toward providing high-resolution data, both spatial and temporal, on zooplankton population size, depth selection and DVM behaviors.

Several studies have converted acoustic backscatter into biologically relevant measurements (Megard et al. 1997; Hembre and Megard 2003; Holbrook et al. 2006). However, these studies were unable to detect small organisms (<1.3 mm) and therefore could not convert backscatter into estimates of zooplankton abundance that encompassed the majority of the zooplankton community. Further, these studies used areal estimates or estimated zooplankton biomass at specific depth intervals. Rahkola-Sorsa et al. (2014) noted that their 614 kHz ADCP data were better correlated with *Chaoborus* and medium-sized zooplankton (300–500 mm). Using the 710 kHz frequency echosounder we were able to both accurately estimate zooplankton biovolume and provide high-resolution information on the zooplankton depth distribution (Table 2; Figs. 2–5).

Due to the use of a single frequency echosounder in this study, we were unable to discriminate between different-sized scatterers. Therefore the acoustic-estimates are an aggregate measure of all scatterers in the water column (other than fish). Because of these assumptions, we expected the acoustic method to overestimate zooplankton biovolume relative to net sampling (which it did). Some of this error is likely due to inaccuracies in the modeling of the zooplankton TS which relies on several different assumptions (e.g., material properties, size and orientation distribution). However, the vertical distributions of organisms and integrated biovolumes during both day and night in August were similar. This suggests that the acoustic sampling is measuring zooplankton scatterers and not other organisms such as fish. An additional explanation for the acoustic estimates being larger than net sampling is the inclusion of the small cyclopoid and calanoid copepods whose scattering may be close to the detection limit of our system. If copepods are excluded from the biovolume calculations, then the acoustic estimates of biovolume will decrease by 1/4 to 1/3, making them smaller than the net tow results. This suggests that we are effectively sampling the small zooplankton (0.5–1.5 mm in length) in this lake. Further work in determining the scattering characteristics of these small copepods at 710 kHz is needed.

Direct observations of upward migration events of small zooplankton and calculation of in situ vertical velocity of zooplankton

One distinct advantage of acoustic sampling is the significant increase in the number of measurements made in a given time period relative to stratified net tows. For instance, the full water column net sampling that occurred in August took roughly 75 min to collect two vertical profiles (one from each bongo net) with 10 points per profile with a depth resolution of 2 m over the 20 m sampling range. In this same time period, the acoustic system collected 4500 replicate vertical profiles with 425 points per profile with a depth resolution of 5 cm. This increased sampling allows acoustic measurements to better characterize the environment, particularly for patchily-distributed organisms, or dynamic processes or environments.

Swimming speeds for *Daphnia* have been measured from 0.5 cm s^{-1} to 1.0 cm s^{-1} (this study and O'Keefe et al. 1998, respectively). Using a conservative swimming speed of 0.5 cm s^{-1}, we estimate that *Daphnia* could swim more than the entire depth of the water column (over 22 m) during the course of the 75-min sampling period required for the traditional net tows. Wong et al. (1986) measured average swimming speeds of *L. minutus* in 18–20°C water as 0.86 mm/s. At these speeds *L. minutus* could migrate 3.8 m during the 75 min required to net sampling. While a much smaller distance than estimated for *Daphnia*, it is important to note that *L. minutus* are generally found in the epilimnion of our study lake, show smaller migration amplitudes, weaker migration behavior and that this distance still exceeds the resolution of most net sampling. Given the estimates of possible migration amplitudes we can conclude that any attempt at real-time observation of vertical migration with traditional net tows may be inaccurate or misleading.

High resolution observations of the vertical and horizontal heterogeneity of zooplankton densities

Net or other integrative sampling methods can obscure fine-scale variation in zooplankton depth selection as evidenced by the thin, high-density layers of scatterers observed in this study (Fig. 3). We know that zooplankton are capable of detecting and responding to subtle biotic and abiotic cues including light (Smith and Macagno 1990; Storz and Paul 1998), temperature (Kessler and Lampert 2004) and fish kairomones (Dodson 1988; De Meester 1993) suggesting that current sampling methods are still orders of magnitude coarser than necessary to understand critical issues of the spatial and temporal distribution. Increased resolution of the vertical habitat selection of the zooplankton, coupled with high resolution data of temperature, dissolved oxygen, light or chlorophyll *a* concentrations will allow for in situ observation of these subtle responses on a scale that has not been reported in other freshwater studies, but have been observed in marine studies (Benoit-Bird et al. 2010; Moline et al. 2010).

Spatial heterogeneity of zooplankton is a commonly reported phenomenon (De Stasio 1989; Tessier and Horwitz 1990) and patchiness or horizontal movement in response to biotic cues such as vertebrate predation pressure or the presence of floating and submerged plants (Masson et al. 2001; Castro et al. 2007) has been well documented. However, it is difficult and labor-intensive to examine horizontal movement or patchiness with traditional sampling methods at the whole lake scale so few studies exist. Organisms can also move substantial distances during the time required to sample horizontal distributions. For example, De Stasio (1989) reported that horizontal sampling at four locations along two offshore to nearshore transects took approximately 2 h to complete. At the swimming velocities that we have estimated here, that is more than enough time for zooplankton to traverse the entire water column as well as move a similar horizontal distance.

Differences in total zooplankton biomass between day and night samples are commonly reported (Tessier 1983; De Stasio 1993), however we observed similar biomass estimates from day to night in August (Table 2; Fig. 3). This pattern is consistent with a more in-depth analysis that we have performed in Lakes Giles on these so-called diel deficits as well as with both our net and acoustic data. Others have suggested that these deficits may be caused by horizontal migration that is driven by active predator avoidance, passive convection-driven movements or a combination of the two (Masson et al. 2001; Armengol et al. 2012). The acoustic technology provides the ability to examine horizontal distribution and diel horizontal movement of zooplankton, which is a potentially important ecological phenomena that has not been examined in depth and is thus not well understood.

Comments and recommendations

Fine-scale distribution and abundance of small zooplankton were measured using a high frequency acoustic echosounder. While the sampling platform (small rowboat) used did not allow for full synoptic coverage of the lake, information on rapid changes in the diel vertical migration of zooplankton were observed while the vessel was stationary and information about the horizontal distribution of the organisms was collected during survey transects. Given that the boat was human-powered, there is some difficulty in resolving whether the differences observed spatially are a function of temporal changes (as the survey occurred) or spatial changes. Weather conditions were favorable during the sampling with a relatively flat-water surface, which suggests that wind-driven circulation was unlikely to be advecting the zooplankton within the lake. Studies which seek to use acoustics to map spatial distributions of zooplankton may need to be limited to time periods (mid-day and night) when the vertical movement of the organisms is thought to be small. During the DVM transition periods, it may be necessary to collect acoustic data from a fixed location to properly sample the environment without the risk of aliasing errors. That is, a mobile platform measuring organisms that are also moving runs the risk of multiple-counting or missing individuals depending on the relative scales and directions of the movements of the survey platform and organisms.

Using only a single frequency makes it very challenging to differentiate between different types of zooplankton, unless they are spatially or behaviorally segregated. It is important to collect direct samples of the zooplankton during acoustic studies in lakes, as these data are necessary to accurately convert acoustic data into biological measures (e.g., numerical densities or biovolume) and to provide a priori information in interpreting the acoustic data. In terms of biovolume estimates, a 10% increase in the mean length of the zooplankton produced a roughly 25% increase in the integrated acoustically measured biovolume. Given that dramatic changes can occur in acoustically derived biovolume estimates, careful consideration must be given to potential sources of error when using acoustic data for quantitative or absolute measures of zooplankton biovolume. Relative or qualitative differences can be derived from the acoustic data with much more confidence in their accuracy. So, if acoustic data are to be used as a quantitative measure, then direct sampling of taxa, size and shape, and material properties of the organisms also need to be conducted. We used density and sound speed contrasts in our scattering model from studies of marine zooplankton. The validity of using these measurements for freshwater organisms is a critical assumption of this study that needs to be further tested by making material property measurements on freshwater zooplankton.

Despite the above limitations, acoustic surveys in lake ecosystems provide a unique tool to measure and observe the ecology of zooplankton with a resolution in space and time that no other technique can provide. The increased spatial and temporal resolution can improve our understanding of both biotic and abiotic factors that drive zooplankton habitat use and thus important trophic interactions within freshwater ecosystems. For example, near-shore to off-shore gradients in young of the year fish and invertebrate predators may influence horizontal movements and distribution of zooplankton at a whole lake scale, while short-term changes in the underwater light environment caused by cloud cover or turbidity pulses may influence vertical depth distributions. Further, many recent advances in our understanding of freshwater environments have been driven by the use of high-frequency and high-resolution sensors (e.g., Staehr et al. 2010; Jennings et al. 2012).

While we have learned a great deal from these studies it has been challenging to link our fine-scale understanding of physical processes or the ecology of producers to the ecology of consumers, such as zooplankton, due to the limited resolution and frequency of manual zooplankton sampling. The high resolution (vertically, horizontally, and temporally) and increased spatial area sampled by acoustic methods can provide this critical link and increase our understanding of the effects of a broad range of environmental changes such as eutrophication, invasive species, episodic events such as wildfires or extreme precipitation events, or parasite epidemics that may cause important but subtle or short-lived changes in zooplankton habitat use or migration behavior.

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