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ORIGINAL ARTICLE

Diverse taxa of zooplankton inhabit hypoxic waters during both day and night in a temperate eutrophic lake

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As the frequency and intensity of hypoxic events increase in both fresh and marine waters, understanding the ecological effects of hypoxia becomes more important. The extant literature reports varying effects of hypolimnetic hypoxia on the vertical distribution and diel vertical migration (DVM) of zooplankton, with some but not all taxa reported to avoid hypoxic waters. We studied the vertical distribution and DVM of diverse zooplankton taxa throughout three seasons over 2 years (2014 and 2015) in Lacamas Lake, WA, USA. We observed hypoxia ($<2 \text{ mg L}^{-1}$ dissolved oxygen) in the hypolimnion of Lacamas Lake during five of six sampling periods, with zooplankton populations often exhibiting 'h-metric' values (defined as the proportion of a zooplankton population residing within hypoxic waters) ranged from 0.14 to 1.00, with an overall mean of $h = 0.66$. Moreover, we observed a lack of DVM in most zooplankton taxa on most occasions. Our findings indicate both community-level and taxon-specific zooplankton tolerances to hypoxia, although the exact mechanisms at play remain to be fully elucidated. Nevertheless, the common residency in hypoxic waters and the lack of DVM by diverse zooplankton taxa that we observed likely have implications for food web dynamics in Lacamas Lake and other water bodies.

KEYWORDS: vertical distribution; diel vertical migration; h-metric; hypolimnetic hypoxia; freshwater

INTRODUCTION

Hypoxia [defined as dissolved oxygen (DO) $< 2 \text{ mg L}^{-1}$] in subsurface waters of lakes, reservoirs and coastal oceans is becoming increasingly common as eutrophication and rising global temperatures increase chemical and thermal stratification in aquatic systems (Helly and Levin, 2004; Mallin *et al.*, 2006; Ficke *et al.*, 2007; Jenny *et al.*, 2016). Strong oxygen and temperature stratification of the water column can result in bottom water hypoxia, which can act as a sub-lethal displacement mechanism, thereby forcing vertebrate (Roberts *et al.*, 2009; Arend *et al.*, 2011) and invertebrate taxa (Eby and Crowder, 2002) with relatively high oxygen demands into shallower depths where DO concentrations are more favorable and may alter predator–prey interactions and food web dynamics (Ekau *et al.*, 2010). Bottom water hypoxia has been associated with large fish kills or ‘dead-zones’ (Rabalais *et al.*, 2002; Stauffer *et al.*, 2012), and planktivorous and piscivorous fishes have been observed to avoid hypoxic waters in marine and estuarine systems such as the Gulf of Mexico (Rabalais *et al.*, 2002; Pierson *et al.*, 2009; Zhang *et al.*, 2009) and Puget Sound, WA (Parker-Stetter and Horne, 2009), as well as freshwater lakes such as Lake Erie (Vanderploeg *et al.*, 2009a, b).

The effects of hypolimnetic hypoxia on zooplankton vertical distribution and migration have also been reported; however, much of this information concerns marine and estuarine taxa, with relatively few studies of freshwater zooplankton. For instance, during seasonal hypoxia events in Chesapeake Bay and the northern Gulf of Mexico, the lack of DO in near-bottom waters compressed the vertical distribution of zooplankton, pushing zooplankters to shallower depths (Kimmel *et al.*, 2009; Elliot *et al.*, 2012; Roman *et al.*, 2012; Pierson *et al.*, 2017). Similar avoidance of hypoxic waters has been observed in some demersal and pelagic fishes in a number of freshwater systems subject to seasonal hypoxia (Ludsin *et al.*, 2009; Zhang *et al.*, 2009; Breitburg *et al.*, 2018). Yet, in other instances, some species of zooplankton have been observed occupying hypoxic waters [e.g. Gulf of Mexico (Zhang *et al.*, 2009) and Puget Sound, WA (Sato *et al.*, 2016)].

Hypolimnetic hypoxia is relatively common in temperate lakes and reservoirs and often follows a seasonal pattern, whereby DO levels in deep waters begin to diminish in early spring due to increased biological oxygen demand near the lake bottom (Diaz, 2001). Thermal stratification, often beginning in late spring/early summer, increases surface temperatures and inhibits vertical mixing of the water column, exacerbating hypolimnetic oxygen reduction. DO concentrations often continue to

decline throughout the summer, reducing hypolimnetic oxygen concentrations to hypoxic levels (Diaz, 2001; Jenney *et al.*, 2016). Cooler temperatures in the autumn result in the collapse of the thermocline and allow for vertical mixing of the water column over winter months.

Whereas DO concentrations below 2 mg L^{-1} can act to exclude large planktivorous predators, for many zooplankters such exclusion often occurs at lower DO concentrations, allowing hypoxic waters to be utilized as predation refugia. Indeed, several zooplankton taxa have been observed occupying hypoxic waters during periods of high predation (Lass *et al.*, 2000; Vanderploeg *et al.*, 2009b; Goto *et al.*, 2012), often exhibiting species-specific DO tolerances (Stewart and George, 1987; Kizito and Nauwerck, 1995; Galkovskaya and Mityanina, 2005; Goto *et al.*, 2012). For example, in Lake Erie, *Daphnia mendotae* consistently avoided hypoxic waters (Goto *et al.*, 2012), whereas *Daphnia longiremis* regularly utilized hypoxic hypolimnetic waters during daylight periods when oxygen concentrations were as low as 1.0 mg L^{-1} (Field and Prepas, 1997; Vanderploeg *et al.*, 2009b). Additionally, the copepod *Diacyclops thomasi* and the cladoceran *Bosmina longirostris* in Lake Erie and *Daphnia pulex* and *B. longirostris* in Amisk Lake (Alberta, Canada) have been observed to inhabit hypoxic waters with DO concentrations as low as 1.2 mg L^{-1} (Field and Prepas, 1997; Vanderploeg *et al.*, 2009b).

Diel vertical migration (DVM) of zooplankton is widespread in both freshwater and marine systems and may be affected by a range of extrinsic factors including predation, prey availability, light, temperature or chemical gradients, turbulence and advective flows (e.g. Bollens and Frost, 1989a, b; Lampert, 1993; Williamson *et al.*, 2011). Normal DVM, in which zooplankton reside at deeper depths during the day and shallower depths during the night, can have important implications for predator–prey interactions and the vertical flux of material and energy in pelagic ecosystems (Bollens *et al.*, 2011). DVM may also be affected by the upward expansion of hypoxic conditions in the hypolimnion, reducing the vertical extent of DVM to the point that some zooplankton populations are in direct contact with predators for extended periods (Galkovskaya and Mityanina, 2005; Pothoven *et al.*, 2009; Vanderploeg *et al.*, 2009a, b; Goto *et al.*, 2012). The reduction of optimal habitat can have detrimental effects on individual growth rates, survival and reproduction of zooplankton by reducing foraging opportunities, increasing predation and increasing metabolic rates, which in turn may lead to significantly lower population growth rates and thus lower abundances of zooplankton in regions that experience moderate to severe hypoxia (Goto *et al.*, 2012; Elliott *et al.*, 2013).

Thus, the extant literature reports varying results regarding the effects of seasonally variable hypolimnetic hypoxia on zooplankton distribution and vertical migration in temperate lakes, reservoirs and coastal waters. Our study attempted to shed further light on this issue through a multi-year, multi-season examination of the vertical distribution and DVM patterns of a diverse freshwater zooplankton community in response to seasonal hypolimnetic hypoxia in a temperate, eutrophic lake (Lacamas Lake, WA, USA). Our investigation addressed the following overarching research question: what effect, if any, does hypolimnetic hypoxia have on the vertical distribution and DVM of diverse zooplankton taxa? Specifically, we tested the two following hypotheses in our study:

- 1) Hypolimnetic hypoxia in Lacamas Lake causes vertical compression of the habitable range of the water column such that most zooplankton avoid the hypolimnion, and
- 2) Hypolimnetic hypoxia leads to reduced amplitude of DVM by zooplankton within the epilimnion and metalimnion.

METHOD

Study site

Lacamas Lake is a small (1.3 km²) temperate monomictic impoundment of Lacamas Creek located in Washington state, USA (45.618379°N, 122.427720°W; Fig. 1). Lacamas Lake receives drainage from the surrounding 17 383 ha watershed, which consists primarily of agricultural lowlands dedicated to farming and ranching, and rural residential properties. Non-point source pollution in the form of agricultural and residential runoff is the single greatest contributor to the high levels of organic and inorganic N and P found in the lake, which since 2004 has resulted in Lacamas Lake being listed by the US Environmental Protection Agency as an impaired water body (Deemer *et al.*, 2011). This eutrophic reservoir is highly modified from its original state and subject to seasonal hypolimnetic hypoxia that is exacerbated by long hydraulic residence time and strong thermal and chemical stratification during summer and autumn months (Mueller and Downen, 1999; Deemer *et al.*, 2011).

An annual drawdown event takes place each September that decreases lake depth by ~2 m, from a maximum depth of 18 m during high-pool (pre-drawdown) stages to 16 m at low-pool (post-drawdown), and decreases lake volume by roughly 50% (Deemer *et al.*, 2011). This drawdown event is used to facilitate annual maintenance on the impoundment dam located at the southern-most point of

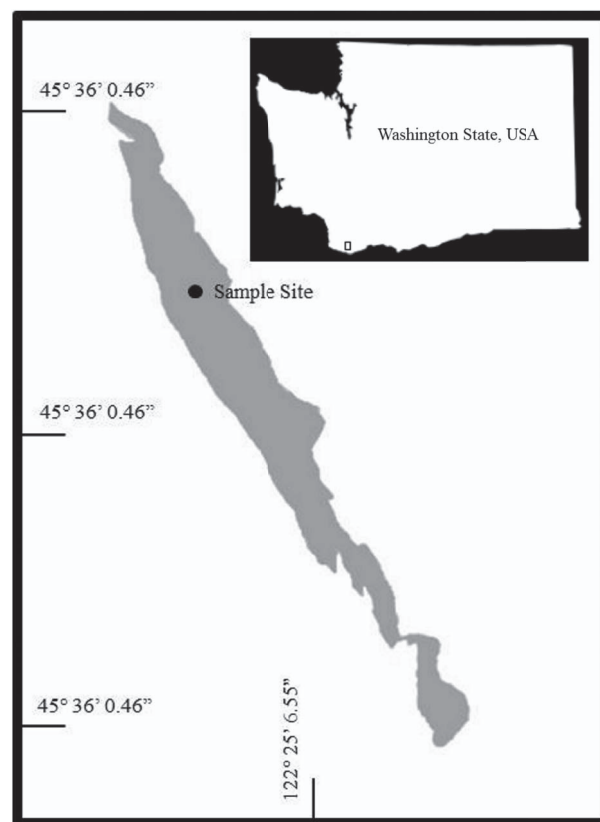


Fig. 1. Map of Lacamas Lake, located in southwestern Washington state, USA. Filled circle shows the location of the sampling site.

the lake. Previous research has shown that this annual drawdown event has little to no effect on chlorophyll or DO concentrations in the center of the lake (Perkins *et al.*, 2019). Though the majority of the lake has an average depth of less than 9 m, an elongate, basin-like region of the lakebed dominates the northwest portion of the reservoir; this is the region of maximum lake depth (18.3 m at full pool), as well as the location of our sampling site (Fig. 1).

Sample collection

Zooplankton samples were collected during three periods of varying hypoxia—spring (non-hypoxia to weak hypoxia), summer (strong hypoxia) and autumn (moderate hypoxia)—in both 2014 and 2015. A boat was anchored at a sampling site located near the center of the lake (Fig. 1) at least 1 hr prior to the start of sampling, and the use of white light was restricted to avoid triggering any possible zooplankton behavioral responses (e.g. Benoit-Bird *et al.*, 2010). Over a 24-hr period, triplicate zooplankton samples were collected at midday (beginning precisely at 12:00 p.m.) and midnight (beginning precisely at 12:00 a.m.) at six discrete depths (1, 3, 5, 7, 9 and

15 m) using a 30-L Schindler–Patalas trap (Wildco 34A-10; Schindler, 1969) with a retrieval rate of $\sim 1 \text{ m s}^{-1}$. The design of the Schindler–Patalas trap allows for the isolation and concentration of 30 L of water collected at a discrete depth; the trap is initially lowered through the water column with top and bottom ‘doors’ open, and once the desired sampling depth is reached, the two doors are allowed to close via the force of gravity and remain closed during retrieval due to water pressure pushing on the top of the trap (Schindler, 1969). Once at the surface, each sample was concentrated through a 63- μm cod end, immediately transferred to a 250-mL jar and then fixed with a 5–10% concentration of buffered formalin. Additionally, a YSI85 multimeter was used to measure water temperature and DO concentrations at 1-m intervals at the beginning of each midday and midnight sampling period. Water transparency was measured by deploying a Secchi disk during midday, and weather conditions were noted.

Zooplankton sample analysis

Identification and enumeration of preserved mesozooplankton (75–2000 μm) were performed using an inverted light compound microscope (Nikon TS1000) at $20\times$. Each sample was filtered through a 75- μm sieve, then rinsed and re-diluted to a volume of 50–200 mL, depending on the density of the sample. A 75- μm sieve was chosen to maintain continuity with our laboratory group’s protocols for previous investigations, in which we utilized 75- μm tow nets and sieves for mesozooplankton enumeration in other, nearby freshwater systems. The 1-mL aliquots were taken with a Stempel pipette and the zooplankton counted, without replacement, until a minimum of 300 specimens per sample were enumerated. Specimens were identified to the lowest possible taxonomic level and life stage using Haney *et al.* (2013) and Hudson and Lesko (2003). Abundances were calculated as $\# \text{ L}^{-1}$.

Determination of zooplankton vertical distribution relative to hypolimnetic hypoxia

We defined the depth at which DO concentration fell below 2 mg L^{-1} as the hypolimnetic hypoxia threshold (HHT). To determine the proportion of the sampled population of each zooplankton taxon residing above or below the HHT, we first calculated Weighted % Water Column Total (WCT) abundances using the following formula:

$$\text{Weighted \% WCT} = \frac{A_i * Z_i}{\sum (A_i * Z_i)} * 100$$

where A_i is abundance at a specific depth (i) and Z_i is the depth stratum represented by that specific depth (i.e. from the midpoint between sample depth i and the sample above it, to the midpoint between sample depth i and the sample below it). This vertical weighting was necessitated by the fact that the vertical distance between samples was not the same (i.e. 2 m between the five shallowest samples, but 6 m between the two deepest samples).

We then calculated the proportion of the sampled population of each taxon above (h_a) and below (h_b) the HHT by using the following equations:

$$h_a = \frac{\sum Z_a * \bar{x}_a}{\left(\sum Z_a * \bar{x}_a\right) + \left(\sum Z_b * \bar{x}_b\right)} \quad \text{and}$$

$$h_b = \frac{\sum Z_b * \bar{x}_b}{\left(\sum Z_a * \bar{x}_a\right) + \left(\sum Z_b * \bar{x}_b\right)}$$

where $\bar{x}_a$ is the mean abundance above the HHT, $\bar{x}_b$ is the mean abundance below the HHT, Z_a is the height of the depth stratum of water above the HHT and Z_b is the height of the depth stratum of water below the HHT. We defined the proportion of the population residing below the HHT, h_b (i.e. *within* hypoxic waters), as the **h-metric**.

In addition, we calculated the weighted mean depth (WMD) of each population of zooplankton during each midday and midnight vertical series of samples as follows:

$$\text{WMD} = \frac{\sum A_i (D_i * Z_i)}{\sum (A_i * Z_i)}$$

where A_i is abundance at sample depth midpoint, D_i is sample depth midpoint and Z_i is height of the depth stratum (Bollens *et al.*, 1993).

For subsequent statistical analyses, only those sets of triplicate vertical series in which a minimum total of $n = 100$ individuals per taxon were observed during any one midday or midnight sampling period were used. Taxa with fewer than 100 individuals observed were not statistically analyzed. Student’s t -tests ($df = 2$) were performed using R software to test for significant diel (i.e. day vs. night) differences in the WMDs for each taxon in any given season. A significant difference ($P < 0.05$) between mean midday and mean midnight WMDs for any one taxon and date was interpreted as the presence of DVM. Regression analysis was performed using R software to examine the relationship between the proportion of the population of a given taxon residing in hypoxic water against the vertical extent of the HHT.

Beyond determining the vertical extent (amplitude) of DVM by comparing midday and midnight WMDs, the strength of DVM can also be measured by calculating

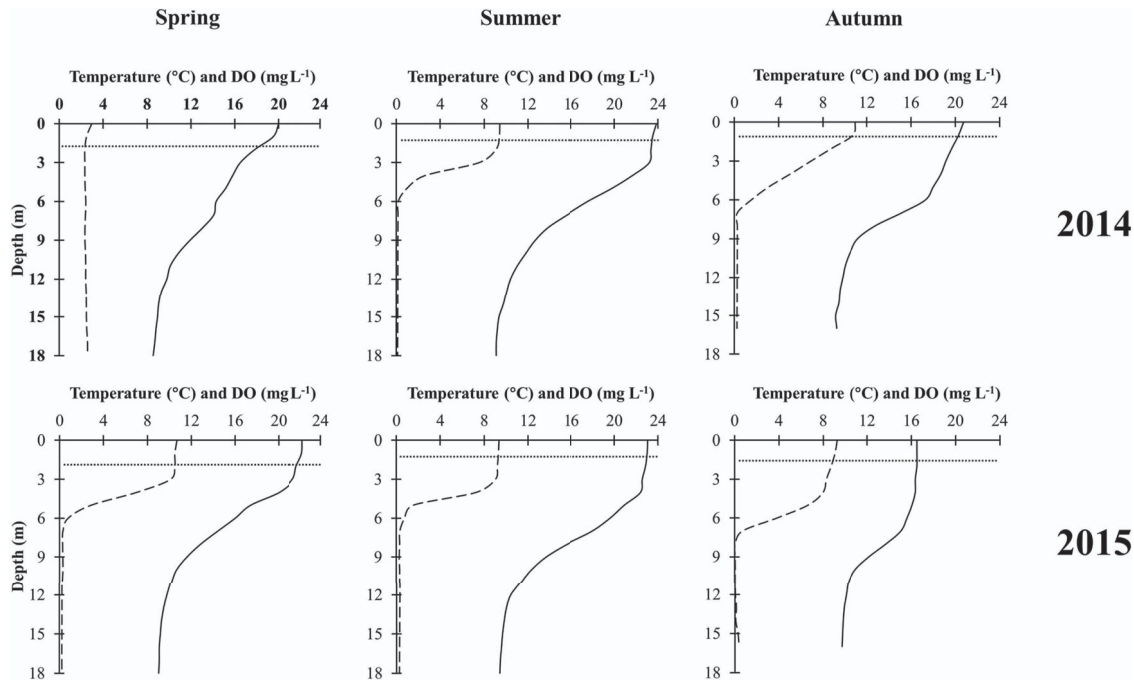


Fig. 2. Vertical profiles of water temperature (°C) and DO concentration during six sample periods at Lacamas Lake, WA, USA. Dashed lines indicate DO concentrations, solid lines indicate temperature and horizontal dotted lines indicate Secchi disk depths.

the proportion of a population (V) migrating across a given depth over a diel cycle (*sensu* Bollens and Frost, 1989a; Bollens *et al.*, 1992). In our study, we used the HHT as the reference depth across which DVM might occur on any given sampling date, and V values were calculated as the difference between the proportion of a population residing above the HHT (h_a) at midnight and the proportion of the same population residing above the HHT at midday.

RESULTS

Environmental conditions

The vertical profiles of temperature and DO concentration, as well as Secchi depth, varied between seasons and years in Lacamas Lake (Fig. 2). Lake clarity varied only slightly between seasons, with Secchi depths ranging between 1.0–1.5 m for all 2014 dates and 1.0–1.6 m for all 2015 dates (Fig. 2). Hypolimnetic hypoxia below 6 m was a fairly consistent phenomenon across seasons and years, with the exception of spring 2014, during which hypoxia was not observed (Fig. 2).

Zooplankton composition, h-metrics and DVM

Zooplankton community composition exhibited some interannual and seasonal variability, with only 6 of 19

taxa present during every season sampled: the cladoceran *D. mendotae*, cyclopoid copepodid stages I–III and IV–V and the rotifers *Keratella* spp., *Polyathra* spp. and *Trichocerca similis* (Figs 3, 4, S1 and S2). All other taxa were observed in at least two seasons throughout the 2-year study period. The vast majority of zooplankton taxa were found broadly distributed throughout the water column during each sample period.

Most zooplankton taxa exhibited h-metric values >0.50 (Figs 5 and 6), indicating that more than 50% of the individuals in these populations were found within the hypoxic zone, although there was some seasonal variability and species-specific differences. For instance, the highest h-metric values were observed during summer of both years, ranging from 0.51 to 1.00, while h-metric values were somewhat lower and more variable during autumn and spring (0.14–0.98 and 0.41–0.91, respectively; Figs 5 and 6). During summer and autumn 2014, 17 of 17 taxa and 7 of 12 taxa (respectively) exhibited h-metric values >0.50 . In spring and summer 2015, all 14 taxa observed exhibited h-metric values above $h=0.50$, while in autumn 2015, 7 of 10 taxa exhibited h-metric values >0.50 . The absence of hypoxia during spring 2014 precluded us from calculating h-metric values for that season. Species-specific differences in h-metric values were also notable. Averaged across our five sampling periods, taxon-specific h-metric values ranged from a maximum of 0.89 for

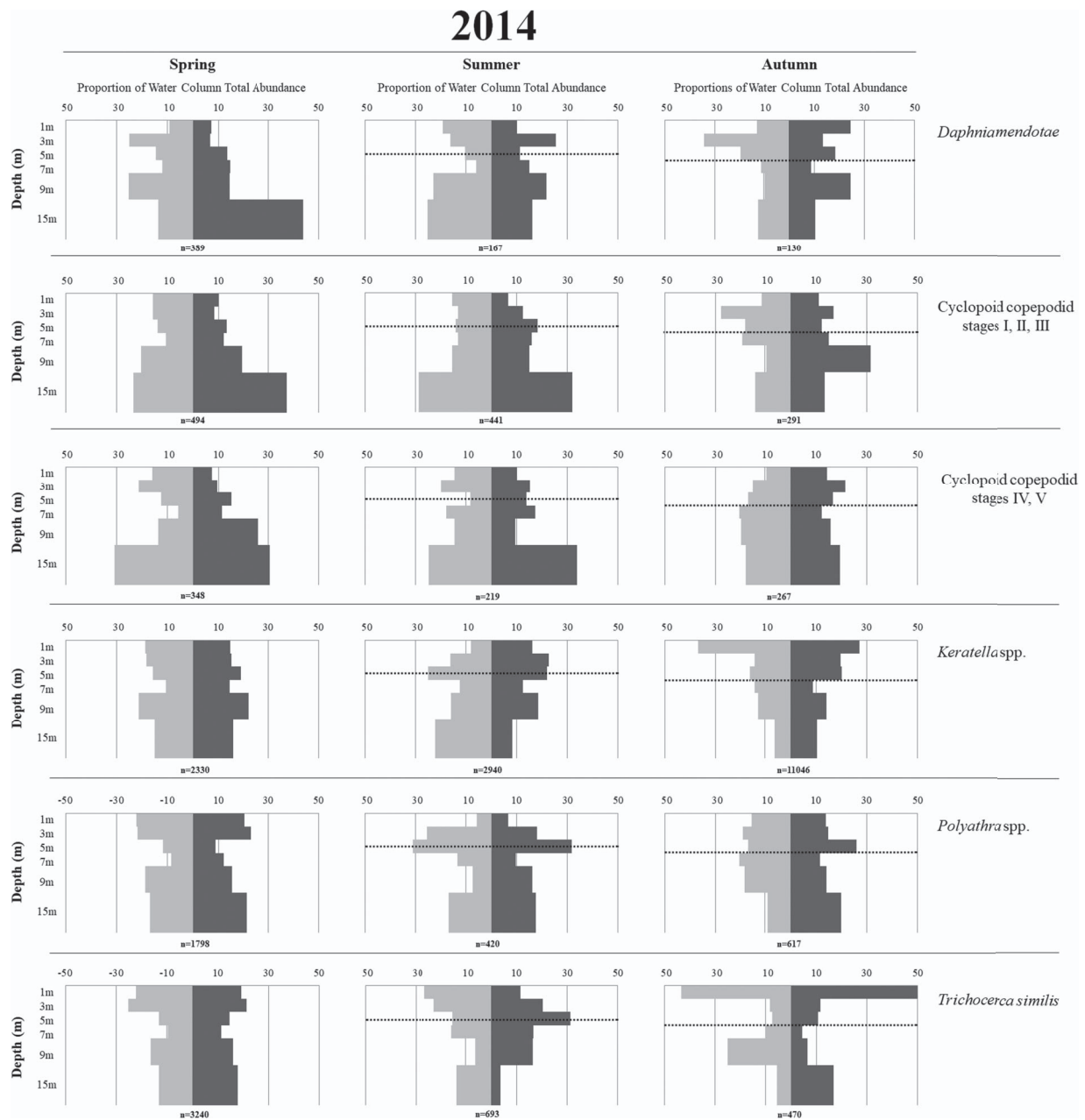


Fig. 3. Vertical distributions of zooplankton during 2014, plotted as proportion of water column total abundance, of six select taxa during midday (gray bars) and midnight (black bars). Dotted horizontal lines indicate the depth of the HHT for the sample season, and n is the number of specimens identified during each season (from three replicate midday and three replicate midnight vertical series of samples).

Kellicottia bostoniensis to a minimum of 0.52 for *T. similis* (Figs 5 and 6).

Regression analysis of the proportion of the population of a given taxon residing in hypoxic water (h-metric) against the vertical extent of the HHT showed differences between taxa (Fig. 7). Rotifers, specifically *Keratella* spp. ($R^2 = 0.557$, $P = 0.013$, $F = 10.05$), *Polyarthra* spp. ($R^2 = 0.938$, $P > 0.001$, $F = 121.3$) and *T. similis*

($R^2 = 0.672$, $P = 0.004$, $F = 16.41$), as well as the cladoceran *D. mendotae* ($R^2 = 0.423$, $P = 0.042$, $F = 5.866$), exhibited statistically significant positive relationships between their h-metric and the vertical extent of the HHT. In contrast, cyclopoid copepodids of stages I–III ($R^2 = 0.154$, $P = 0.263$, $F = 1.452$) and stages IV–V ($R^2 = 0.021$, $P = 0.687$, $F = 0.175$) showed no such significant relationship (Fig. 7).

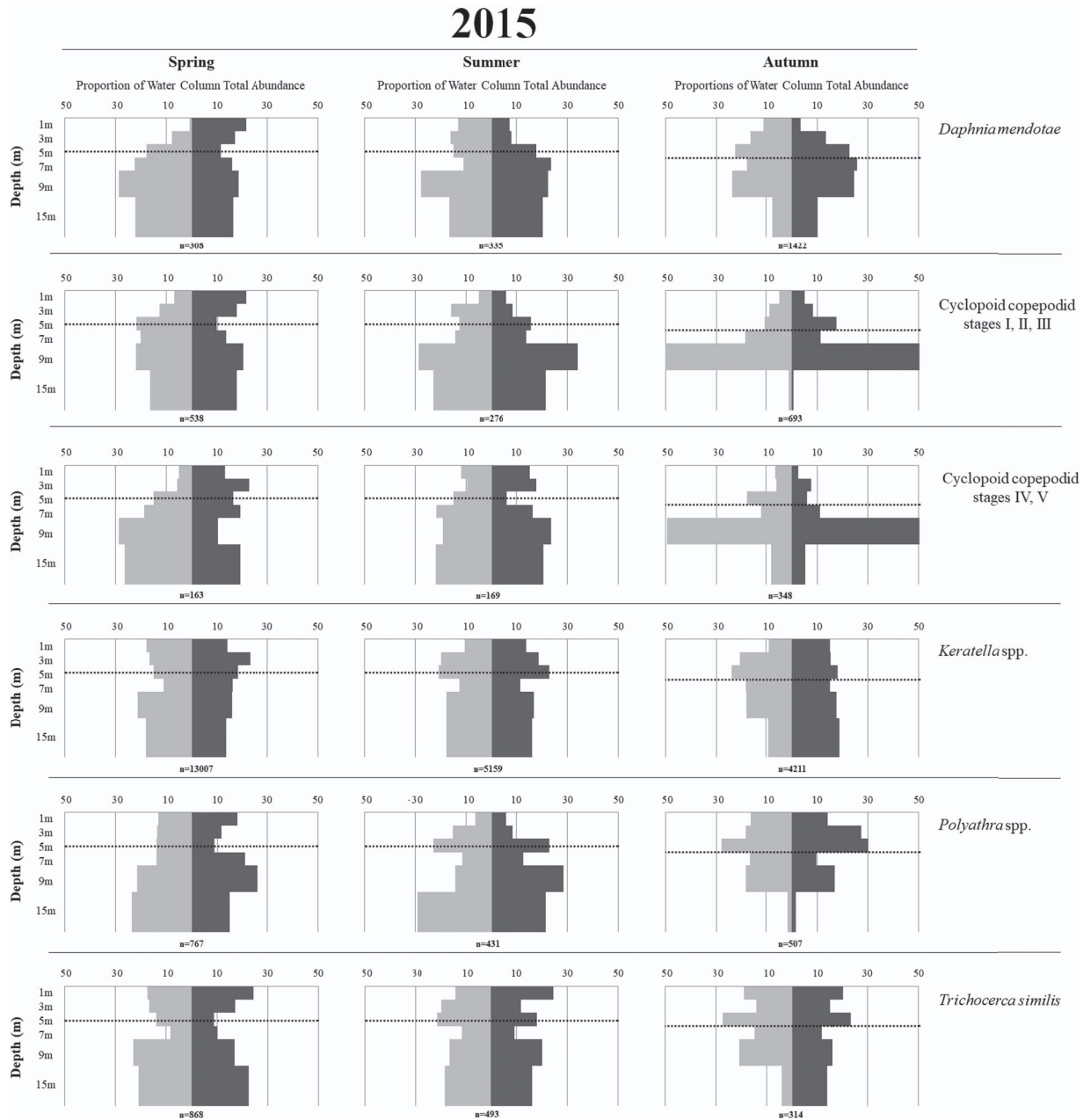


Fig. 4. Vertical distributions of zooplankton during 2015, plotted as proportions of water column total abundance, of six select taxa during midday (gray bars) and midnight (black bars). Dotted horizontal lines indicate the depth of the HHT for the sample season, and n is the number of specimens identified during each season (from three replicate midday and three replicate midnight vertical series of samples).

With respect to DVM, only two taxa on two occasions were observed to exhibit statistically significant DVM behaviors during the entirety of our study: cyclopoid copepodids (I–III) during spring 2014 and *Keratella* spp. during summer 2014 (Table I). Similarly, V values were very low across all seasons and taxa, with values less than 0.10 on 41 of 66 occasions and values less than 0.20 on 61 of 66 occasions, indicating consistently weak or non-existent DVM (Table II).

DISCUSSION

We observed strong hypolimnetic hypoxia over multiple seasons in Lacamas Lake in 2014 and 2015. A strong thermocline can act to suppress vertical mixing (Rabalais et al., 2002; Friedrich et al., 2014) and is often a key factor contributing to the development of hypolimnetic hypoxia (Rabalais et al., 2007; Kidwell et al., 2009). The strong thermocline (5–7 m) that we observed in Lacamas Lake

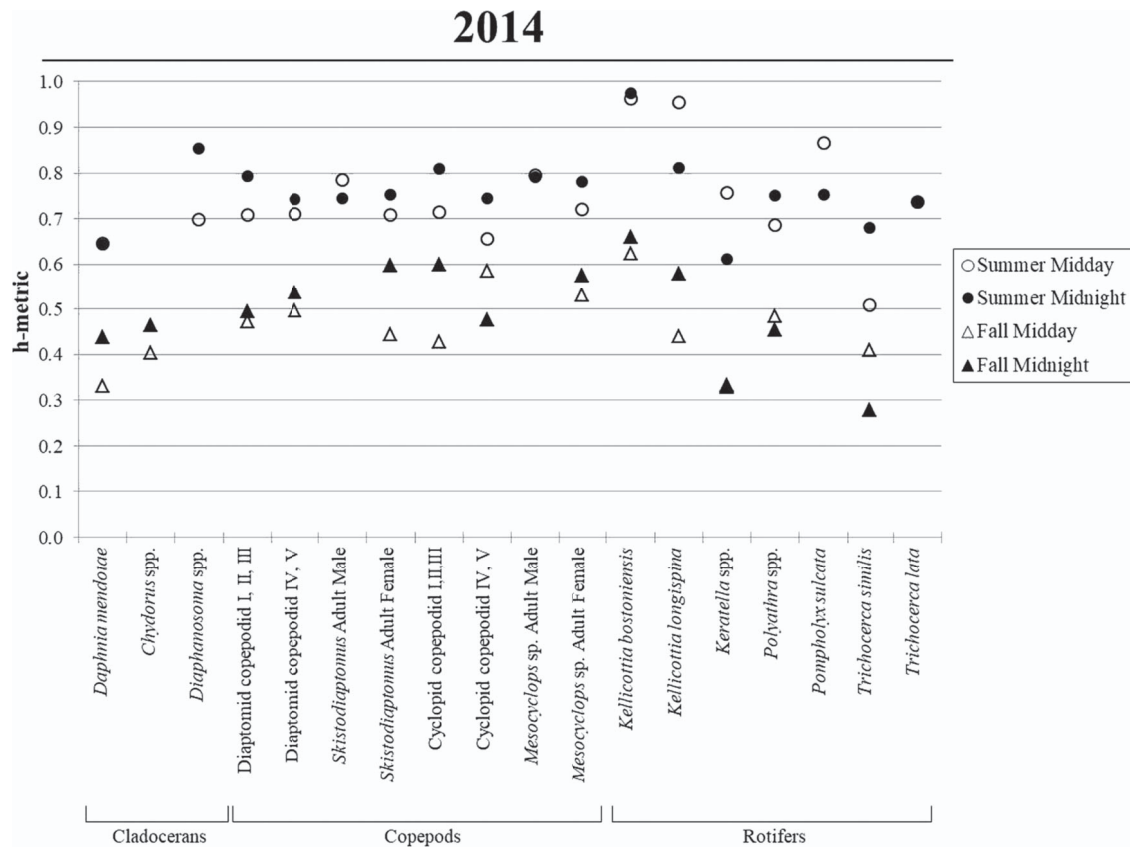


Fig. 5. Mean h-metrics for 18 zooplankton taxa observed during 2014 in Lacamas Lake, WA, USA.

from late spring to early autumn in both 2014 and 2015 likely had a significant effect on the severity of hypoxia observed in the lake, similar to the conditions observed by Zhou *et al.* (2013) in Lake Erie.

Effect of hypoxia on vertical distributions of diverse zooplankton taxa

We observed large proportions of diverse zooplankton taxa occupying hypoxic waters during each season in which hypolimnetic hypoxia was present in Lacamas Lake. Indeed, one of our most notable findings was that every zooplankton taxon we sampled had some proportion of its population residing within hypoxic waters at some point during our study and that much of these zooplankton populations were consistently observed within the hypoxic zone (mean $h = 0.45$; Table III). Several investigators have observed zooplankton to reside in hypoxic waters in freshwater lakes (Field & Prepas, 1997; Davidson *et al.*, 1998; Vanderploeg *et al.*, 2009b; Pothoven *et al.*, 2012), estuaries (Taylor *et al.*, 2007; Ludsins *et al.*, 2009) and marine systems (Zhang *et al.*, 2009; Sato *et al.*, 2016). Conversely, other studies have

shown that many zooplankton exhibit strong aversion to hypoxia and rarely, if ever, venture into hypoxic waters (Stewart and George, 1987; Field and Prepas, 1997; Galkovskaya and Mityanina, 2005; Goto *et al.*, 2012). Thus, there appears to be considerable variation in DO avoidance between zooplankton taxa.

The tolerance of zooplankton to hypoxic waters may vary by taxon and location (Table V). Multiple studies have shown that severe hypoxia ($DO \leq 0.5\text{--}1.0\text{ mg L}^{-1}$) leads to strong avoidance by zooplankton (Vanderploeg *et al.*, 2009b; Zhang *et al.*, 2009; Pothoven *et al.*, 2012; Elliott *et al.*, 2013a; Table V). Yet we observed the cladoceran *D. mendotae* to reside in extremely hypoxic waters with DO levels as low as 0.09 mg L^{-1} . Several studies point to the production and utilization of hemoglobin in *Daphnia* spp. as a mechanism for tolerating hypoxia (Weider and Lampert, 1985; Kimura *et al.*, 1999; Smirnov, 2014). While hemoglobin may stave off the early, detrimental effects of hypoxia (Kring and O'Brien, 1976), there are lower limits ($0.5\text{--}1.0\text{ mg L}^{-1}$) below which hypoxia can become lethal to *Daphnia* spp. (Weider and Lampert, 1985). It is important to note that negative individual growth rates for *Daphnia* spp. have

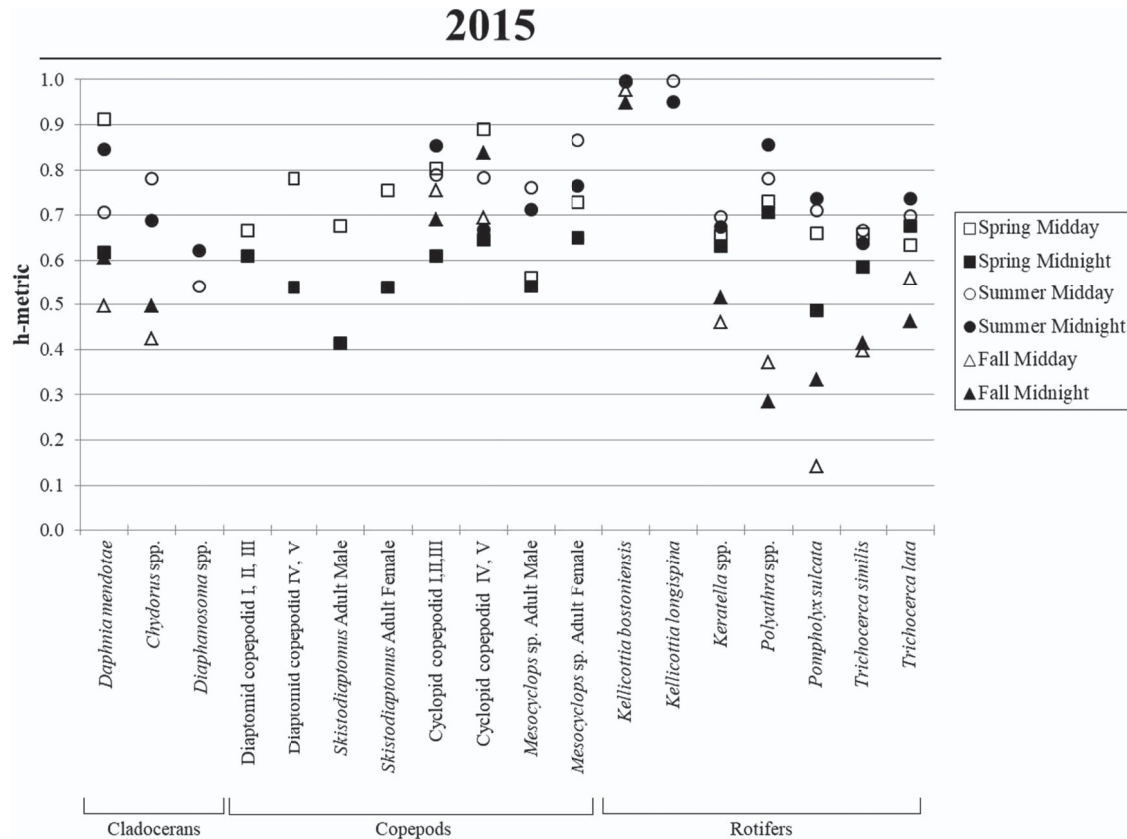


Fig. 6. Mean h-metrics for 18 zooplankton taxa observed during 2015 in Lacamas Lake, WA, USA.

been observed in hypoxic environments (Hanazato and Dodson, 1995; Hanazato, 1996), though other studies have attributed those same negative individual growth rates to poor food quality (Lass et al., 2000). While some previous studies have found *D. mendotae* to reside in moderately hypoxic waters, our observations in Lacamas Lake appear to be the first to show substantial portions ($h = 0.52$) of *D. mendotae* inhabiting severely hypoxic waters (≤ 0.09 mg L⁻¹ DO).

Similarly, our observations of rotifers residing in hypoxic waters ($h = 0.14$ – 1.00) differ from previously reported studies (Table V). Unlike the large proportions we observed, other studies have reported only small proportions of rotifer populations in hypoxic waters. For instance, Kizito and Nauwerck (1995) reported finding a larger group of rotifers (*Keratella tropica*, *Conochiloides natans* and *Asplanchna sieboldii*) in low DO waters of Lake Nyahirya, western Uganda, though only small proportions of those populations were found residing within waters with DO < 1.0 mg L⁻¹. Other taxa of rotifers appear to avoid hypolimnetic hypoxia altogether, e.g. the rotifers *Ascomorpha* spp., *Keratella* spp., *Trichocerca* spp, *Pompholyx sulcata* and *Polyathra vulgaris* in Lake Kruglik,

Belarus (Galkovskaya and Mityanina, 2005) and *Kellicottia* spp. and *P. vulgaris* in Priest Pot, UK (Stewart and George, 1987).

We also observed a substantial proportion of all copepod populations in Lacamas Lake residing within hypoxic waters, albeit with some slight taxon (order)-specific variation (mean h-metric values of cyclopoids ranged from 0.69 to 0.70 and calanoids ranged from 0.62 to 0.65). Previous studies of copepods, much like rotifers, have been mixed (Table V), finding either avoidance (Roman et al., 1993; Field and Prepas, 1997; Keister et al., 2000) or occupancy of hypoxic waters (Vanderploeg et al., 2009a, b; Kimmel et al., 2010; Hirche et al., 2014). Copepod avoidance of hypoxia has been observed in several estuaries by Roman et al. (1993), Keister et al. (2000) and Pierson et al. (2017). Likewise, in a number of freshwater impoundments subject to seasonal hypoxia, Doubek et al. (2018) found copepod populations exhibited compression of their vertical distribution within oxygenated surface waters above the hypolimnetic hypoxia.

However, reports of copepod residency within hypoxic waters are also common (Table V). Taylor et al. (2007) observed large proportions of the copepod assemblage in

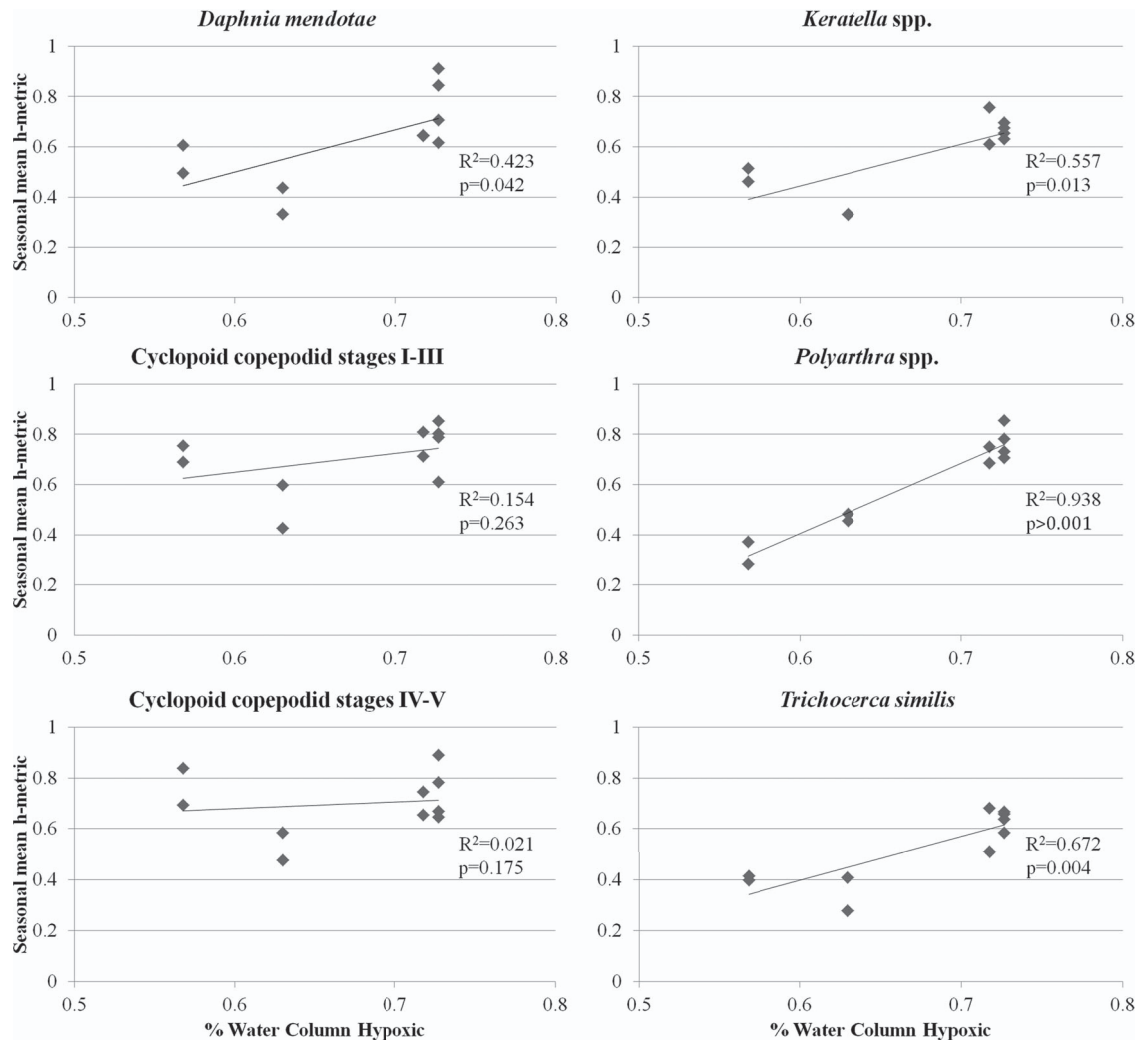


Fig. 7. Regressions comparing mean h-metric values to the percent (%) water column hypoxia for six representative taxa for all sample dates ($n = 10$) during which hypolimnetic hypoxia was present in Lacamas Lake, WA, USA.

the Neuse River estuary occupying hypoxic waters. Similarly, in Chesapeake Bay (Elliot *et al.*, 2013), Puget Sound (Keister and Tuttle, 2013), the Gulf of Mexico (Pierson *et al.*, 2009; Kimmel *et al.*, 2010) and the eastern Pacific Ocean (Hirche *et al.*, 2014), sizable portions of the copepod assemblage were found within hypoxic waters. Populations of copepods in Amisk Lake in Alberta, Canada (Field and Prepas, 1997), as well as adult and copepodid *D. thomasi* in Lake Erie (Vanderploeg *et al.*, 2009b), have been observed to occupy hypoxic waters. Our observations of large proportions of copepod populations residing within the hypoxic zone in Lacamas Lake ($h = 0.62$ – 0.70) are similar to those reported by Taylor *et al.* (2007) and Ludsin *et al.* (2009), though they contrast with the relatively low proportions presented by Roman *et al.* (1993), Field and Prepas (1997) and Keister *et al.* (2000).

In addition to the wide diversity of zooplankton taxa that we observed residing in hypoxic water, we observed seasonal variation in the proportion of zooplankton populations occupying hypoxic waters. Specifically, larger proportions of zooplankton occupied hypoxic waters during summer, when the HHT was found closer to the surface. This was also observed by Pierson *et al.* (2009) in Chesapeake Bay and the Gulf of Mexico, who found zooplankton inhabited hypoxic waters only when hypoxia comprised 30–60% of the total water column. However, our higher h-metric values (i.e. increased proportions of zooplankton inhabiting hypoxic waters) in the summer cannot be attributed to the shallowing of the HHT alone, as zooplankton WMDs were often deepest during summer compared to other seasons for most zooplankton taxa (Table IV). Why this summertime increase in

Table I: Midday and midnight mean WMDs for all taxa observed during six sample periods at Lacamas Lake, WA, USA

Taxon	2014						2015					
	Spring Day	Summer Night	Autumn Day	Autumn Night	Spring Day	Spring Night	Summer Day	Summer Night	Autumn Day	Autumn Night	Spring Day	Spring Night
<i>Daphnia mendotae</i>	6.8	9.6	7.5	6.8	5.5	5.9	8.6	6.4	7.3	7.7	6.3	7.2
Juvenile <i>Daphnia</i>	6.5	9.2			5.3	6.8						
<i>Chydorus</i> spp.									6.4	7.1	7.0	7.1
<i>Diaphanosoma</i> spp.			7.1	7.0					7.1	7.0		
Diaptomid copepodid I, II, III			7.8	8.1	6.3	6.6	7.1	6.9				
Diaptomid copepodid IV, V			7.8	8.7	7.0	7.3	8.4	6.1				
<i>Skistodiaptomus</i> adult male			8.5	7.8			7.1	6.0				
<i>Skistodiaptomus</i> adult female	8.5	10.4	7.3	7.0	6.1	7.7	8.0	5.5				
Cyclopoid copepodid I, II, III	7.4*	9.1*	7.7	8.5	6.0	7.1	7.4	6.3	7.8	8.2	7.2	7.1
Cyclopoid copepodid IV, V	7.8	8.7	7.4	8.5	7.1	6.9	8.8	6.4	7.5	7.3	7.5	8.2
<i>Mesocyclops</i> sp. adult male			8.5	7.9			6.5	5.0	7.3	7.9		
<i>Mesocyclops</i> sp. adult female	7.9	8.1	8.0	8.0	6.7	7.4	7.1	5.8	7.2	8.5		
<i>Kellicottia bostoniensis</i>			9.4	9.4	6.9	7.4			9.4	11.4	8.5	8.8
<i>Kellicottia longispina</i>			9.7	8.7	6.6	7.8			9.2	10.7		
<i>Keratella</i> spp.	6.4	6.8	7.5**	5.7**	4.6	5.3	6.8	6.2	6.9	6.5	6.2	7.0
<i>Polyarthra</i> spp.	6.3	6.6	6.6	6.9	6.1	6.9	7.8	7.0	7.5	7.8	5.3	4.9
<i>Pompholyx sulcata</i>	6.5	6.5	8.5	8.2			7.1	5.5	5.7	7.0	5.4	4.8
<i>Trichocerca similis</i>	5.6	6.4	5.6	5.4	4.7	4.9	7.2	6.7	6.7	6.4	5.5	6.2
<i>Trichocerca lata</i>	7.4	7.1	7.6	7.9			6.6	6.6	7.3	8.1	6.8	6.7

Shaded areas indicate minimum sample size ($n \geq 100$) not met for that season. Bold numbers indicate occurrence of WMD above HHT. Asterisks indicate significant difference between midday and midnight WMDs for any sampling date (* $P < 0.05$; ** $P < 0.01$)

Table II: V values for all taxa present during hypoxic conditions in Lacamas Lake

Taxon	2014			2015	
	Summer	Autumn	Spring	Summer	Autumn
<i>Daphnia mendotae</i>	0.00	-0.11	0.29	-0.14	-0.11
<i>Chydorus</i> spp.				0.09	-0.07
<i>Diaphanosoma</i> spp.	-0.16			-0.08	
Diaptomid I, II, III	-0.09	-0.02	0.06		
Diaptomid IV, V	-0.03	-0.04	0.24		
<i>Skistodiaptomus</i> adult male	0.04		0.26		
<i>Skistodiaptomus</i> adult female	-0.04	-0.15	0.22		
Cyclopoid I, II, III	-0.10	-0.17	0.19	-0.06	0.06
Cyclopoid IV, V	-0.09	0.11	0.25	0.12	-0.14
<i>Mesocyclops</i> sp. adult male	0.00		0.02	0.05	
<i>Mesocyclops</i> sp. adult female	-0.06	-0.04	0.08	0.10	
<i>Kellicottia bostoniensis</i>	-0.01	-0.04		0.00	0.03
<i>Kellicottia longispina</i>	0.14	-0.14		0.05	
<i>Keratella</i> spp.	0.15	0.00	0.02	0.02	-0.05
<i>Polyarthra</i> spp.	-0.07	0.03	0.02	-0.07	0.09
<i>Pompholyx sulcata</i>	0.11		0.17	-0.03	-0.19
<i>Trichocerca similis</i>	-0.17	0.13	0.07	0.03	-0.02
<i>Trichocerca lata</i>	0.00		0.04	0.04	-0.10
HHT	5 m	6 m	5 m	5 m	7 m

V values are the difference in the proportion of a population residing above the HHT at night and the proportion of the same population residing above the HHT during the day. Positive values represent 'normal' diel migration (up at night, down during day); negative values indicate 'reverse' diel migration (down during night, up during day). Shaded areas indicate minimum sample size ($n \geq 100$) not met for that taxon-season

zooplankton WMDs occurred, coincident with a period of shallowing of the HHT, remains to be fully elucidated. Additional comparisons of Shannon's diversity index (H') and species richness in both oxic and hypoxic waters in Lacamas Lake showed no relationship between diversity and hypoxia (data not shown). However, our regression

analysis indicated that some taxa (*D. mendotae*, *Keratella* spp., *Polyarthra* spp. and *T. similis*, specifically) exhibit greater occupancy of hypoxic waters when the extent of hypolimnetic hypoxia in the water column is greatest, whereas for some other taxa (cyclopoid copepodid stages I–III and IV–V), the extent of occupancy in hypoxic

Table III: Mean density (L^{-1}), h -metric and V values, averaged across all seasons and all years, for each taxon present in the study, listed in descending order of abundance

Taxon	%assemblage	Density ($\#L^{-1}$)	Mean h -metric	Mean V value
<i>Keratella</i> spp.	44.46	141.75	0.47	0.02
<i>Pompholyx</i> sulcata	10.16	32.38	0.49	0.00
<i>Kellicottia</i> longispina	8.22	26.21	0.55	0.01
<i>Trichocerca</i> lata	5.96	19.00	0.54	−0.03
<i>Trichocerca</i> similis	5.22	16.63	0.44	0.01
<i>Chydorus</i> spp.	4.88	15.55	0.20	0.00
<i>Polyarthra</i> spp.	4.18	13.33	0.51	0.00
<i>Kellicottia</i> bostoniensis	4.07	12.98	0.59	0.00
Cyclopoid copepodid I, II, III	2.77	8.82	0.59	−0.01
<i>Daphnia</i> mendotae	2.36	7.51	0.52	−0.01
Cyclopoid copepodid IV, V	1.40	4.46	0.58	0.04
<i>Mesocyclops</i> sp. adult female	1.31	4.18	0.59	0.01
<i>Mesocyclops</i> sp. adult male	1.14	3.63	0.46	0.03
<i>Skistodiaptomus</i> adult female	0.78	2.48	0.32	0.00
Diaptomid copepodid I, II, III	0.74	2.37	0.31	−0.01
<i>Skistodiaptomus</i> adult male	0.72	2.30	0.38	0.04
Diaptomid copepodid IV, V	0.71	2.26	0.32	0.03
<i>Diaphanosoma</i> spp.	0.25	0.79	0.23	−0.04
Grand means		17.59	0.45	0.01

Table IV: Mean h -metric (midday and midnight combined) and mean WMDs (midday and midnight combined) for all zooplankton taxa present during hypoxic conditions in Lacamas Lake

Taxon	2014				2015					
	Summer		Autumn		Spring		Summer		Autumn	
	h -metric	WMD	h -metric	WMD	h -metric	WMD	h -metric	WMD	h -metric	WMD
<i>Daphnia</i> mendotae	0.64	7.13	0.38	5.70	0.76	7.53	0.78	7.50	0.55	6.73
Juvenile <i>Daphnia</i>			0.43	6.08						
<i>Chydorus</i> spp.							0.73	6.77	0.46	7.03
<i>Diaphanosoma</i> spp.	0.78	7.04					0.58	7.04		
Diaptomid copepodid I, II, III	0.75	7.96	0.48	6.45	0.64	6.97				
Diaptomid copepodid IV, V	0.73	8.25	0.52	7.16	0.66	7.25				
<i>Skistodiaptomus</i> adult male	0.76	8.14			0.54	6.51				
<i>Skistodiaptomus</i> adult female	0.73	7.14	0.52	6.94	0.65	6.74				
Cyclopoid copepodid I, II, III	0.76	8.12	0.51	6.56	0.71	6.86	0.82	7.97	0.72	7.16
Cyclopoid copepodid IV, V	0.70	7.95	0.53	7.00	0.77	7.62	0.73	7.41	0.77	7.85
<i>Mesocyclops</i> sp. adult male	0.79	8.19			0.55	5.76	0.74	7.58		
<i>Mesocyclops</i> sp. adult female	0.75	7.97	0.55	7.10	0.69	6.42	0.81	7.84		
<i>Kellicottia</i> bostoniensis	0.97	9.38	0.64	7.17			1.00	10.39	0.96	8.63
<i>Kellicottia</i> longispina	0.88	9.22	0.51	7.19			0.97	9.95		
<i>Keratella</i> spp.	0.68	6.56	0.33	4.95	0.64	6.52	0.69	6.70	0.49	6.58
<i>Polyarthra</i> spp.	0.72	6.79	0.47	6.49	0.72	7.38	0.82	7.64	0.33	5.10
<i>Pompholyx</i> sulcata	0.81	8.32			0.57	6.31	0.72	6.34	0.24	5.13
<i>Trichocerca</i> similis	0.60	5.48	0.34	4.79	0.62	6.92	0.65	6.54	0.41	5.84
<i>Trichocerca</i> lata	0.74	7.75			0.66	6.58	0.72	7.70	0.51	6.74

Shaded areas indicate minimum sample size ($n \geq 100$) not met for that taxon season

waters is independent of the vertical extent of hypoxia (Fig. 7).

It is important to note the possible interactive effects of temperature and DO, e.g. the temperature-dependence of oxygen solubility in water and thus its availability to organisms (Benson and Krause, 1980), which can affect the respiration rates of copepods (Ikeda *et al.*, 2007). For instance, Pierson *et al.* (2017) found life stage and sex differences in vertical distribution of copepods when the effects of temperature and hypoxia were

considered together, with males found more commonly in the cooler hypolimnion in the presence of hypoxia and females and copepodids found more frequently in the more oxygenated epilimnion (Pierson *et al.*, 2017). This was attributed to physiological differences, in which copepodids and egg-laying females required greater rates of development than males (Kessler, 2004; Pierson *et al.*, 2017). Pierson *et al.* (2009) calculated the expected respiration of copepods at different temperatures yet were unable to confidently attribute differences in vertical

Table V: Published studies of zooplankton tolerances to hypoxia, organized from greatest (top) to least (bottom) tolerance

Reference	Body of water	Taxa	Tolerance of hypoxia
Ludsin <i>et al.</i> (2009)	Chesapeake Bay, USA	mixed assemblage	Tolerance $\geq 0.2 \text{ mg L}^{-1}$
Taylor <i>et al.</i> (2007)	Neuse River, USA	mixed assemblage	Tolerance $\geq 0.2 \text{ mg L}^{-1}$
Stewart and George (1987)	Priest Pot, UK	<i>Keratella</i> spp.	Tolerance $\geq 0.5 \text{ mg L}^{-1}$
		<i>Polyarthra vulgaris</i>	Tolerance $\geq 0.5 \text{ mg L}^{-1}$
Galkovskaya and Mityanina (2005)	Lake Kruglik, Belarus	<i>Kellicottia longispina</i>	Tolerance $\geq 0.5 \text{ mg L}^{-1}$
		<i>Polyarthra vulgaris</i>	Tolerance $\geq 0.5 \text{ mg L}^{-1}$
Vanderploeg <i>et al.</i> (2009b)	Lake Erie, USA	<i>Bosmina</i> spp.	Tolerance $\geq 1.0 \text{ mg L}^{-1}$
		<i>Mesocyclops</i> copepods	Tolerance $\geq 1.0 \text{ mg L}^{-1}$
		<i>Daphnia longiremis</i>	Tolerance $\geq 1.2 \text{ mg L}^{-1}$
		<i>Diacyclops thomasi</i>	Tolerance $\geq 1.2 \text{ mg L}^{-1}$
Field and Prepas (1997)	Amisk Lake, Canada	<i>Diacyclops thomasi</i>	Tolerance $\geq 1.5 \text{ mg L}^{-1}$
		<i>Daphnia longiremis</i>	Tolerance $\geq 1.7 \text{ mg L}^{-1}$
		<i>Daphnia pulex</i>	Tolerance $\geq 1.9 \text{ mg L}^{-1}$
Doubek <i>et al.</i> (2018)	Beaverdam Reservoir, USA	<i>Daphnia</i> spp.	Tolerance $\geq 2.0 \text{ mg L}^{-1}$
		<i>Diaptomus</i> copepods	Tolerance $\geq 2.0 \text{ mg L}^{-1}$
		<i>Mesocyclops</i> copepods	Tolerance $\geq 2.0 \text{ mg L}^{-1}$
Keister <i>et al.</i> (2000)	Chesapeake Bay, USA	mixed assemblage	Tolerance $\geq 2.0 \text{ mg L}^{-1}$
Roman <i>et al.</i> (1993)	Chesapeake Bay, USA	<i>Arcatia tonsa</i>	Tolerance $\geq 2.0 \text{ mg L}^{-1}$
		<i>Oithona colcarva</i>	Tolerance $\geq 2.0 \text{ mg L}^{-1}$
Keister and Tuttle (2013)	Hood Canal, USA	<i>Arcatia</i> spp.	Tolerance $\geq 2.1 \text{ mg L}^{-1}$
Kimmel <i>et al.</i> (2010)	Gulf of Mexico	mixed assemblage	Tolerance $\geq 2.1 \text{ mg L}^{-1}$
Pierson <i>et al.</i> (2017)	Chesapeake Bay, USA	<i>Arcatia tonsa</i>	Tolerance $\geq 2.6 \text{ mg L}^{-1}$
Goto <i>et al.</i> (2012)	Lake Erie, USA	<i>Daphnia mendotae</i>	Tolerance $\geq 4.0 \text{ mg L}^{-1}$

distribution of zooplankton to respiration rates; rather, they attributed the increased occupation of hypoxic water to the extent of hypoxia within the water column. These findings are in agreement with our results, which show greater h-metric values during seasons in which greater proportions of the water column are hypoxic, at least for most (four of six) abundant taxa (Table IV, Fig. 7).

The differences observed by Pierson *et al.* (2009), as well as those observed within our study, could be attributed to adaptation within a localized population. It is possible, given the long-term (at least 35 years) presence of seasonal hypoxia in Lacamas Lake (Mueller and Downen, 1999), and the relatively short generation time of zooplankton, localized adaptation may have occurred. Similar scenarios have been suggested previously (Decker *et al.*, 2003; Dam, 2013; Elliott *et al.*, 2013b).

Effects of hypoxia on zooplankton DVM

Given the common and widespread occurrence of DVM in freshwater and marine zooplankton worldwide, we were surprised to find that the vast majority of zooplankton taxa in Lacamas Lake did not exhibit DVM (only two very weak instances over 2 years, as measured by both the small amplitudes and small proportions of the populations migrating). One interpretation of the lack of differences in midday versus midnight vertical distributions is our limited sample size ($n=3$ replicates) and thus the limited statistical

power to discern such differences, should they be present. However, variation in DVM behavior as a result of hypoxia has previously been observed by other investigators. Doubek *et al.* (2018) reported the interruption of zooplankton DVM in the presence of hypoxia, although it was coupled with compression of vertical distributions within surface waters. Alternatively, Pierson *et al.* (2017) reported both amplitudinal and directional changes of DVM whereby zooplankton exhibited reverse DVM during normoxic conditions and normal DVM during periods of hypoxia. Additionally, periods of hypoxia resulted in changes of DVM amplitude when hypoxic conditions were potentially lethal (Pierson *et al.*, 2017). Given the lack of vertical compression of zooplankton distributions that we observed in Lacamas Lake, there is likely a different driver responsible for the lack of DVM.

DVM is recognized as a complex behavioral response to a number of different cues and adaptive pressures, including predation, ultraviolet (UV) radiation and prey resources, depending on the system (Bollens and Frost, 1989a; Lampert, 1989; Williamson *et al.*, 2011). More specifically, avoidance of visual predators has been shown to be a strong driver of zooplankton DVM in temperate eutrophic systems (Bollens and Frost, 1989a, b; Bollens *et al.*, 1992; Lampert *et al.*, 2003), with avoidance of UV radiation likely playing a larger role in oligotrophic systems (Williamson *et al.*, 2011). With respect to Lacamas Lake, we know of only two studies that examined fish

populations (Mueller and Downen, 1999; Hutton, 2002), and these authors observed relatively low abundances of adult and juvenile planktivorous Largemouth bass, bluegill and perch, which were found to predominantly occupy littoral waters. While low abundance of visual predators may help explain the near absence of zooplankton DVM observed in Lacamas Lake, it fails to explain the substantial proportions of the zooplankton community residing at depth within hypoxic waters (as discussed above). There are no data available to us on abundance of large invertebrate predators in Lacamas Lake, but if present in surface waters, this may help to explain the common occurrence of zooplankton residence in hypolimnetic hypoxic waters. Nevertheless, if predation pressure in surface waters is sufficient to cause zooplankton to seek refuge in deeper hypoxic waters, then one would expect it to be sufficient to trigger DVM as well (down to depth at night, and up during the day to feed in surface waters), although this is not what we observed in Lacamas Lake.

Avoidance of UV radiation is another factor that has been suggested to contribute to DVM in zooplankton (Donson, 1990; Williamson *et al.*, 2011). In oligotrophic waters where turbidity is low, UV radiation can penetrate to considerable depths (greater than 10 m), increasing exposure risks to zooplankters and inducing vertical migration to depths below which UV radiation becomes negligible (Williamson *et al.*, 2011). Conversely, UV penetration is diminished in highly turbid systems and avoidance of UV via DVM becomes unnecessary. The eutrophic conditions and accompanying high turbidity (Secchi depths of 1.0–1.6 m) observed at Lacamas Lake indicate that UV penetration was likely very low (1–1.5 m), and this may help explain the absence of zooplankton DVM in Lacamas Lake, but not the regular occurrence of zooplankton residence in deep (>6 m) hypoxic hypolimnetic waters.

Prey availability, and the assumption that it is greatest in the epilimnion, is often an implicit component of DVM theory, though the question of subsurface food availability and its effects on DVM have been addressed previously (Williamson *et al.*, 1996). Similar to the absence of predation, without a disparity of food resources between surface waters and those at depth, vertical migration is unnecessary (Gliwicz and Pijanowska, 1988). Observations of the vertical distribution of chlorophyll in Lacamas Lake, made contemporaneously to ours, found subsurface chlorophyll maxima of 30–60 $\mu\text{g L}^{-1}$ to frequently occur at 2–5 m, just above the thermocline, but with low concentrations of chlorophyll ($\sim 1 \mu\text{g L}^{-1}$) to occur in the hypolimnion (Perkins *et al.*, 2019). It has been observed previously in Lake Eymir, Turkey, that food resources at depth were likely responsible

for the absence on DVM in *D. pulex* (Muluk and Beklioglu, 2005). Moreover, microbes (not previously investigated in Lacamas Lake) have been suggested to be an available food resource for zooplankton at depth (Bennet *et al.*, 1990; Arndt, 1993; Williamson *et al.*, 1996). Similarly, larger zooplankters (e.g. copepods and cladocerans) can feed upon smaller rotifers (Williamson and Magnien, 1982; Williamson and Butler, 1986; Williamson, 1987; Lapesa *et al.*, 2002), which generally occurred at depth in Lacamas Lake. Thus, there may have been adequate food in the hypolimnion of Lacamas Lake that made it unnecessary for the zooplankton to migrate into the epilimnion, thereby manifesting as an absence of DVM.

A final caveat on the limitations of our sampling design and protocols is in order. Although we consistently observed large proportions ($h > 0.50$) of diverse zooplankton taxa to reside in hypoxic waters, and not to undertake DVM, our sampling frequency (12 hrs) does not allow us to preclude the possibility that individual zooplankters are making higher frequency forays (*sensu* Leising *et al.*, 2005) throughout the water column. Such higher frequency sampling of the vertical distribution of zooplankton is recommended in the future.

CONCLUSION

We determined the midday and midnight vertical distributions of the zooplankton assemblage in Lacamas Lake under conditions of strong hypolimnetic hypoxia during summer and autumn, a condition that is becoming increasingly common in temperate freshwater lakes and reservoirs (Jenny *et al.*, 2016). Zooplankton responses to this hypoxia were fairly consistent across diverse taxa. Contrary to our original hypothesis, we found that the majority of zooplankton populations, rather than being constrained by hypolimnetic hypoxia to residing within the epilimnion, were broadly distributed throughout the water column, with large proportions (h -metric values > 0.50) residing within hypoxic waters. Additionally, the vast majority of zooplankton taxa in Lacamas Lake did not exhibit DVM, or were only very weakly migratory (as measured by both small amplitude of migration and small proportion of the population migrating). This lack of diel migratory behavior did not take the form we hypothesized—rather than being constrained by hypolimnetic hypoxia to residing only in the epilimnion during both day and night, zooplankton usually remained at depth during both day and night.

While zooplanktivorous fish predation is recognized as a common factor contributing to both hypoxic residency and DVM of zooplankton, our results are somewhat

contradictory with respect to a role for predation. On the one hand, the relatively low abundance of planktivorous fish in Lacamas Lake supports the lack of zooplankton DVM yet does not support the observed residence of most zooplankton in hypoxic waters. Similarly, the availability of prey at depth may contribute to the lack of DVM observed, though the majority of assumed food resources (measured as chlorophyll) are generally distributed at shallower depths in Lacamas Lake (Perkins *et al.*, 2019); thus prey availability alone does not support hypoxic residency by zooplankton (although the role of other food resources, e.g. microbes, remains an open question). Whether the diel residence in deeper water was an attempt by zooplankton to utilize hypoxic waters as refuge from predators or whether it was some other cue or process that drove zooplankton depth selection remains to be determined. Nevertheless, our results add to the current body of literature describing zooplankton responses to hypolimnetic hypoxia and provide additional evidence of the ability of a range of freshwater zooplankton taxa to not only tolerate low ($<2 \text{ mg L}^{-1}$) DO concentrations, but also to reside within the hypoxic zone for prolonged periods. This pattern of consistent residence in hypoxic waters by zooplankton has implications for our understanding of planktonic food webs in Lacamas Lake specifically, and for other temperate lakes and reservoirs more generally.

DATA ARCHIVING

The samples and data collected for this study will be archived with Washington State University's College of Arts and Sciences. Additionally, this work will be archived with the Aquatic Ecology Laboratory at Washington State University.

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REFERENCES

- Arend, K. K., Beletsky, D., DePinto, J. V., Ludsins, S. A., Roberts, J. J., Rucinski, D. K., Scavia, D., Schwab, D. J. *et al.* (2011) Seasonal and interannual effects of hypoxia on fish habitat quality in central Lake Erie. *Freshw. Biol.*, **56**, 366–383.
- Arndt, H. (1993) Rotifers as predators on components of the microbial food web (bacteria, heterotrophic flagellates, ciliates) – a review. *Hydrobiologia*, **255**, 231–246.
- Bennet, S. J., Sanders, R. W. and Porter, K. G. (1990) Heterotrophic, autotrophic, and mixotrophic nanoflagellates: seasonal abundances and bacterivory in a eutrophic lake. *Limnol. Oceanogr.*, **35**, 1821–1832.
- Benoit-Bird, K. J., Moline, M. A., Schofield, O. M., Robbins, I. C. and Waluk, C. M. (2010) Zooplankton avoidance of a profiled open-path fluorometer. *J. Plankton Res.*, **32**, 1413–1419.
- Benson, B. B. and Krause, D. (1980) The concentration and isotopic fractionation of gases dissolved in freshwater in equilibrium with the atmosphere. 1. Oxygen. *Limnol. Oceanogr.*, **25**, 662–671.
- Bollens, S. M. and Frost, B. W. (1989a) Zooplanktivorous fish and variable diel vertical migration in the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.*, **34**, 1072–1083.
- Bollens, S. M. and Frost, B. W. (1989b) Predator-induced diel vertical migration in a planktonic copepod. *J. Plankton Res.*, **11**, 1047–1065.
- Bollens, S. M., Frost, B. W., Thoreson, D. S. and Watts, S. J. (1992) Diel vertical migration in zooplankton: field evidence in support of the predator avoidance hypothesis. *Hydrobiologia*, **234**, 33–39.
- Bollens, S. M., Osgood, K., Frost, B. W. and Watts, S. D. (1993) Vertical distributions and susceptibilities to vertebrate predation of the marine copepods *Metridia lucens* and *Calanus pacificus*. *Limnol. Oceanogr.*, **38**, 1827–1837.
- Bollens, S. M., Rollwagen-Bollens, G., Quenette, J. and Bochdansky, A. B. (2011) Cascading migrations and implications for vertical fluxes in pelagic systems. *J. Plankton Res.*, **33**, 349–355.
- Breitbart, D., Levin, L. A., Oschlies, A., Gregoire, M., Chavez, F. P., Conley, D. J., Arcon, V., Gilbert, D. *et al.* (2018) Declining oxygen in the global ocean and coastal waters. *Science*, **359**, eaam7240.
- Dam, H. G. (2013) Evolutionary adaptation of marine zooplankton to global change. *Ann. Rev. Mar. Sci.*, **5**, 349–370.
- Davidson, N. L., Kelso, W. E. and Rutherford, D. A. (1998) Relationships between environmental variables and the abundance of cladocerans and copepods in the Atchafalaya River Basin. *Hydrobiologia*, **379**, 175–181.
- Decker, M. B., Breitbart, D. L. and Marcus, N. H. (2003) Geographical differences in behavioral responses to hypoxia: local adaptation to an anthropogenic stressor? *Ecol. Appl.*, **13**, 1104–1109.
- Deemer, B. R., Harrison, J. A. and Whiting, E. W. (2011) Microbial dinitrogen and nitrous oxide production in a small eutrophic reservoir: an in situ approach to quantifying hypolimnetic process rates. *Limnol. Oceanogr.*, **56**, 1189–1199.
- Diaz, R. J. (2001) Overview of hypoxia around the world. *J. Environ. Qual.*, **30**, 275–281.
- Dodson, S. (1990) Predicting diel vertical migration of zooplankton. *Limnol. Oceanogr.*, **35**, 1195–1200.
- Doubek, J. P., Campbell, K. L., Doubek, K. M., Hamre, K. D., Lofton, M. E., McClure, R. P., Ward, N. K. and Carey, C. C. (2018) The effects of hypolimnetic anoxia on the diel vertical migration of freshwater crustacean zooplankton. *Ecosphere*, **9**, e02332.

- Eby, L. A. and Crowder, L. B. (2002) Hypoxia-based habitat compression in the Neuse River Estuary: context-dependent shifts in behavioral avoidance thresholds. *Can. J. Fish. Aquat. Sci.*, **59**, 952–965.
- Ekau, W., Auel, H., Portner, H. O. and Gilbert, D. (2010) Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). *Biogeosciences*, **7**, 1669–1699.
- Elliot, D. T., Pierson, J. J. and Roman, M. R. (2012) Relationship between environmental conditions and zooplankton community structure during summer hypoxia in the northern Gulf of Mexico. *J. Plankton Res.*, **34**, 602–613.
- Elliot, D. T., Pierson, J. J. and Roman, M. R. (2013a) Copepods and hypoxia in Chesapeake Bay: abundance, vertical position, and non-predatory mortality. *J. Plankton Res.*, **35**, 1027–1034.
- Elliot, D. T., Pierson, J. J. and Roman, M. R. (2013b) Predicting the effects of coastal hypoxia on vital rates of the planktonic copepod *Acartia tonsa* Dana. *PLoS One*, **8**, e63987.
- Ficke, A. D., Myrick, C. A. and Hansen, L. J. (2007) Potential impacts of global climate change on freshwater fisheries. *Rev. Fish Biol. Fish.*, **17**, 581–613.
- Field, K. M. and Prepas, E. E. (1997) Increased abundance and depth distribution of pelagic crustacean zooplankton during hypolimnetic oxygenation in a dep, eutrophic Alberta lake. *Can. J. Fish. Aquat. Sci.*, **54**, 2146–2156.
- Friedrich, J., Janssen, F., Aleynik, D., Bange, H. W., Boltacheva, N., Cagatay, M. N., Dale, A. W., Etiope, G. *et al.* (2014) Investigating hypoxia in aquatic environments diverse approaches to addressing a complex phenomenon. *Biogeosciences*, **11**, 1215–1259.
- Galkovskaya, G. A. and Mityanina, I. F. (2005) Structure distinctions of pelagic rotifer plankton in stratified lakes with different human impact. *Hydrobiologia*, **546**, 387–395.
- Gliwicz, M. Z. and Pijanowska, J. (1988) Effect of predation and resource depth distribution on vertical migration of zooplankton. *Bull. Mar. Sci.*, **43**, 695–709.
- Goto, D., Lindelof, K., Fanslow, D. L., Ludsins, S. A., Pothoven, S. A., Roberts, J. J., Vanderploeg, H. A., Wilson, A. E. *et al.* (2012) Indirect consequences of hypolimnetic hypoxia on zooplankton growth in a large eutrophic lake. *Aquat. Biol.*, **46**, 217–227.
- Hanazato, T. (1996) Combined effects of food shortage and oxygen deficiency on life history characteristics and filter screens of *Daphnia*. *J. Plankton Res.*, **18**, 757–765.
- Hanazato, T. and Dodson, S. I. (1995) Synergistic effects of low oxygen concentration, predator kairomone, and a pesticide on the cladoceran *Daphnia pulex*. *Limnol. Oceanogr.*, **40**, 700–709.
- Haney, J. F., Aliberti, M. A., Allan, E., Allard, S., Bauer, D. J., Beagan, W., Bradt, S. R., Carlson, B. *et al.* (2013) *An image-based key to the zooplankton of North America: version 5.0*, University of New Hampshire Center for Freshwater Biology (cfb.unh.edu).
- Helly, J. J. and Levin, L. A. (2004) Global distribution of naturally occurring marine hypoxia on continental margins. *Deep Sea Res Part 1*, **51**, 1159–1168.
- Hirche, H. J., Barz, K., Anyon, P. and Schulz, J. (2014) High resolution vertical distribution of the copepod *Calanus chilensis* in relation to the shallow oxygen minimum zone off northern Peru using LOKI, a new plankton imaging system. *Deep Sea Res Part 1*, **88**, 63–73.
- Hudson, P. L. and Lesko, L. T. (2003) Free-living and parasitic copepods of the Laurentian Great Lakes: keys and details on individual species. In *US Geological Survey: Great Lakes Science Center*, MI, Ann Arbor.
- Hutton, R. (2002) *Lacamas Lake Restoration Program. Washington State Dept. of Ecology, final report*, Appendix G.
- Ikeda, T., Sano, F. and Yamaguchi, A. (2007) Respiration in marine pelagic copepods: a global-bathymetric model. *Mar. Ecol. Prog. Ser.*, **339**, 215–219.
- Jenny, J., Francus, P., Normandeau, A., LaPointe, F., Perga, M., Ojala, A., Schimmelmann, A. and Zolitschka, B. (2016) Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. *Glob. Chang. Biol.*, **22**, 1481–1489.
- Keister, J. E., Houde, E. D. and Breitburg, D. L. (2000) Effects of bottom-layer hypoxia on abundances and depth distributions of organisms in Patuxent River, Chesapeake Bay. *Mar. Ecol. Prog. Ser.*, **205**, 43–59.
- Keister, J. E. and Tuttle, L. B. (2013) Effects of bottom-layer hypoxia on spatial distributions and community structure of mesozooplankton in a sub-estuary of Puget Sound, Washington, U.S.A. *Limnol. Oceanogr.*, **58**, 667–680.
- Kessler, K. (2004) Distribution of *Daphnia* in a trade-off between food and temperature: individual habitat choice and time allocation. *Freshw. Biol.*, **49**, 1220–1229.
- Kidwell, D. M., Lewitus, A. J., Jewett, E. B., Brandt, S. and Mason, D. M. (2009) Ecological impacts of hypoxia on living resources. *J. Exp. Mar. Biol. Ecol.*, **381**, S1–S3.
- Kimmel, D. G., Boicourt, W. C., Pierson, J. J., Roman, M. R. and Zhang, X. (2009) A comparison of the mesozooplankton response to hypoxia in Chesapeake Bay and the northern Gulf of Mexico using the biomass size spectrum. *J. Exp. Mar. Biol. Ecol.*, **381**, S65–S73.
- Kimmel, D. G., Boicourt, W. C., Pierson, J. J., Roman, M. R. and Zhang, X. (2010) The vertical distribution and diel variability of mesozooplankton biomass, abundance, and size in response to hypoxia in the northern Gulf of Mexico USA. *J. Plankton Res.*, **32**, 1185–1202.
- Kimura, S., Tokishita, S., Ohta, T., Kobayashi, M. and Yamagata, H. (1999) Heterogeneity and differential expression under hypoxia of two-domain hemoglobin chains in the water flea, *Daphnia magna*. *J. Biol. Chem.*, **274**, 10649–10653.
- Kizito, Y. S. and Nauwerck, A. (1995) Temporal and vertical distribution of planktonic rotifers in a meromictic crater lake, Lake Nyahirya (western Uganda). *Hydrobiologia*, **313**, 303–312.
- Kring, R. L. and O'Brien, W. J. (1976) Effects of varying oxygen concentrations on the filtering rate of *Daphnia pulex*. *Ecology*, **57**, 808–814.
- Lampert, W. (1989) The adaptive significance of diel vertical migration of zooplankton. *Funct. Ecol.*, **3**, 21–27.
- Lampert, W. (1993) Ultimate causes of diel vertical migration of zooplankton: new evidence for the predator-avoidance hypothesis. *Ergeb. Limnol.*, **39**, 79–88.
- Lampert, W., McCauley, E. and Manly, B. F. J. (2003) Trade-offs in the vertical distribution of zooplankton: ideal free distribution with costs? *Proc. R. Soc. Lond.*, **270**, 765–773.
- Lapesa, S., Snell, T. W., Fields, D. M. and Serra, M. (2002) Predatory interactions between a cyclopoid copepod and three sibling rotifer species. *Freshw. Biol.*, **47**, 1685–1695.
- Lass, S., Boersma, M. and Spaak, P. (2000) How do migrating daphnids cope with fish predation risk in the epilimnion under anoxic conditions in the hypolimnion? *J. Plankton Res.*, **22**, 1411–1418.

- Leising, A. W., Pierson, J. J., Cary, S. and Frost, B. (2005) Copepod foraging and predation risk within the surface layer during night-time feeding forays. *J. Plankton Res.*, **27**, 987–1001.
- Ludsin, S. A., Zhang, X., Brandt, S. B., Roman, M. R., Boicourt, W. C., Mason, D. M. and Costantini, M. (2009) Hypoxia-avoidance by planktivorous fish in Chesapeake Bay: implications for food web interactions and fish recruitment. *J. Exp. Mar. Biol. Ecol.*, **381**, S121–S131.
- Mallin, M. A., Johnson, V. L., Ensign, S. H. and MacPherson, T. A. (2006) Factors contributing to hypoxia in rivers, lakes, and streams. *Limnol. Oceanogr.*, **51**, 690–701.
- Mueller, K. W. and Downen, M. R. (1999) *1997 Lacamas Lake Survey: The Warmwater Fish Community of a Highly Eutrophic Lowland Lake*, Research Publication, Washington Department of Fish and Wildlife.
- Muluk, C. B. and Beklioglu, M. (2005) Absence of typical diel vertical migration in *Daphnia*: varying role of water clarity, food, and dissolved oxygen in Lake Eymir, Turkey. *Hydrobiologia*, **537**, 125–133.
- Parker-Stetter, S. L. and Horne, J. K. (2009) Nekton distribution and midwater hypoxia: a seasonal diel prey refuge? *Estuar. Coast. Shelf. S.*, **81**, 13–18.
- Perkins, K. R., Rollwagen-Bollens, G., Bollens, S. M. and Harrison, J. A. (2019) Variability in the vertical distribution of chlorophyll in a spill-managed temperate reservoir. *Lake Reserv. Manag.* doi.org/10.1080/10402381.2019.1566935.
- Pierson, J. J., Roman, M. R., Kimmel, D. G., Boicourt, W. C. and Zhang, X. (2009) Quantifying changes in the vertical distribution of mesozooplankton in response to hypoxic bottom waters. *J. Exp. Mar. Biol. Ecol.*, **381**, S74–S79.
- Pierson, J. J., Slater, W. L., Elliot, D. and Roman, M. R. (2017) Synergistic effects of seasonal deoxygenation and temperature truncate copepod vertical migration and distribution. *Mar. Ecol. Prog. Ser.*, **575**, 57–68.
- Pothoven, S. A., Vanderploeg, H. A., Hook, T. O. and Ludsin, S. A. (2012) Hypoxia modifies planktivore-zooplankton interactions in Lake Erie. *Can. J. Fish. Aquat. Sci.*, **69**, 2018–2028.
- Pothoven, S. A., Vanderploeg, H. A., Ludsin, S. A., Hook, T. O. and Brandt, S. B. (2009) Feeding ecology of emerald shiners and rainbow smelt in central Lake Erie. *J. Great Lakes Res.*, **35**, 190–198.
- Rabalais, N. N., Turner, R. E., Sen Gupta, B. K., Boesch, D. F., Chapman, P. and Murrell, M. C. (2007) Hypoxia in the northern gulf of Mexico: does the science support the plan to reduce, mitigate, and control hypoxia? *Estuaries Coast.*, **30**, 753–772.
- Rabalais, N. N., Turner, R. E. and Wiseman, W. J. Jr. (2002) Gulf of Mexico hypoxia, a.k.a. “the dead zone”. *Annu. Rev. Ecol. Syst.*, **33**, 235–263.
- Roberts, J. J., Hook, T. O., Ludsin, S. A., Pothoven, S. A., Vanderploeg, H. A. and Brandt, S. B. (2009) Effects of hypolimnetic hypoxia on foraging and distributions of Lake Erie yellow perch. *J. Exp. Mar. Biol. Ecol.*, **381**, S132–S142.
- Roman, M. R., Gauzens, A. L., Rhinehart, W. K. and White, J. R. (1993) Effects of low oxygen waters on Chesapeake Bay zooplankton. *Limnol. Oceanogr.*, **38**, 1603–1614.
- Roman, M. R., Pierson, J. J., Kimmel, D. G., Boicourt, W. C. and Zhang, X. (2012) Impacts of hypoxia on zooplankton spatial distributions in the Northern Gulf of Mexico. *Estuaries Coast.*, **35**, 1261–1269.
- Sato, M., Horne, J. K., Parker-Stetter, S. L., Essington, T. E., Keister, J. E., Moriarty, P. E., Li, L. and Newton, J. (2016) Impacts of moderate hypoxia on fish and zooplankton prey distributions in a coastal fjord. *Mar. Ecol. Prog. Ser.*, **560**, 57–72.
- Schindler, D. W. (1969) Two useful devices for vertical plankton and water sampling. *J. Fish. Res. Bd. Can.*, **26**, 1948–1955.
- Smirnov, N. N. (2014) *Physiology of the Cladocera*, Elsevier, Amsterdam.
- Stauffer, B. A., Gellene, A. G., Schnetzer, A., Seubert, E. L., Oberg, C., Sukhatme, G. S. and Caron, D. S. (2012) An oceanographic, meteorological, and biological ‘perfect storm’ yields a massive fish kill. *Mar. Ecol. Prog. Ser.*, **468**, 231–243.
- Stewart, L. J. and George, D. G. (1987) Environmental factors influencing the vertical migration of planktonic rotifers in a hyper-eutrophic tarn. *Hydrobiologia*, **147**, 203–208.
- Taylor, J. C., Rand, P. S. and Jenkins, J. (2007) Swimming behavior of juvenile anchovies (*Anchoa* spp.) in an episodically hypoxic estuary: implications for individual energetics and trophic dynamics. *Mar. Biol.*, **152**, 939–957.
- Vanderploeg, H. A., Ludsin, S. A., Cavaletto, J. F., Hook, T. O., Pothoven, S. A., Brandt, S. B., Liebig, J. R. and Lang, G. A. (2009a) Hypoxic zones as habitat for zooplankton in Lake Erie: refuges from predation or exclusion zones? *J. Exp. Mar. Biol. Ecol.*, **381**, S108–S120.
- Vanderploeg, H. A., Ludsin, S. A., Ruberg, S. A., Hook, T. O., Pothoven, S. A., Brandt, S. B., Lang, G. A., Liebig, J. R. et al. (2009b) Hypoxia affects spatial distributions and overlap of pelagic fish, zooplankton, and phytoplankton in Lake Erie. *J. Exp. Mar. Biol. Ecol.*, **381**, S92–S107.
- Weider, L. J. and Lampert, W. (1985) Differential response of *Daphnia* genotypes to oxygen stress: respiration rates, hemoglobin content and low-oxygen tolerance. *Oecologia*, **65**, 487–491.
- Williamson, C. E. (1987) Predator-prey interactions between omnivorous diatomid copepods and rotifers: the role of prey morphology and behavior. *Limnol. Oceanogr.*, **32**, 167–177.
- Williamson, C. E. and Butler, N. M. (1986) Predation on rotifers by the suspension-feeding calanoid copepod *Diaptomus pallidus*. *Limnol. Oceanogr.*, **31**, 393–402.
- Williamson, C. E., Fischer, J. M., Bollens, S. M., Overholt, E. P. and Breckenridge, J. K. (2011) Toward a more comprehensive theory of diel vertical migration: integrating ultraviolet radiation and water transparency into the biotic paradigm. *Limnol. Oceanogr.*, **56**, 1603–1623.
- Williamson, C. E. and Magnien, R. E. (1982) Diel vertical migration in *Mesocyclops edax*: implications for predation rate estimates. *J. Plankton Res.*, **4**, 329–339.
- Williamson, C. E., Sanders, R. W., Moeller, R. E. and Stutzman, P. L. (1996) Utilization of subsurface food resources for zooplankton reproduction: implications for diel vertical migration theory. *Limnol. Oceanogr.*, **41**, 224–233.
- Zhang, H., Ludsin, S. A., Mason, D. M., Adamack, A. T., Brandt, S. B., Zhang, X., Kimmel, D. G., Roman, M. R. et al. (2009) Hypoxia-driven changes in the behavior and spatial distribution of pelagic fish and mesozooplankton in the northern Gulf of Mexico. *J. Exp. Mar. Biol. Ecol.*, **381**, S80–S91.
- Zhou, Y., Obenour, D. R., Scavia, D., Johengen, T. H. and Michalak, A. M. (2013) Spatial and temporal trends in Lake Erie hypoxia, 1987–2007. *Environ. Sci. Technol.*, **47**, 899–905.