



Partial diel vertical migration over strong environmental gradients structured by physiology, not adult size or genotype, in a freshwater zooplankton

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Abstract *Daphnia* and other zooplankton often harbour substantial intraspecific diversity in migration behaviour. One example is *partial diel vertical migration* (DVM), wherein a portion of the population migrates vertically at night while another portion remains deep in the water column. This behaviour is widespread among aquatic invertebrates and can strongly influence interspecific competition. However, the mechanisms maintaining partial DVM in zooplankton are poorly understood. Here, we take an observational approach to identify the likely mechanisms maintaining partial DVM in a natural population of *Daphnia pulex*. The *Daphnia* at our study site show intraspecific diversity in body hemoglobin (Hb) concentration. This variation serves as a marker for differential use of a deep low-oxygen layer, which allows us to concurrently examine the relationships between individual state, genotype, and migration

behaviour within the population. We found that migration behaviour within Hb-rich and Hb-poor *Daphnia* was not related to individual size. Furthermore, Hb-rich and Hb-poor individuals were present within all commonly found genotypes. Thus, partial DVM in the population is neither a state-dependent behaviour based on size, nor the result of a genetic polymorphism. Characterizing partial DVM provides a more precise picture of aquatic ecosystems which can strongly influence estimates of population growth and trophic interactions.

Keywords Partial migration · *Daphnia* · Zooplankton · Diel vertical migration · Intraspecific diversity · State-dependent behaviour · Genetic polymorphism

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Introduction

Diel vertical migration (DVM) of zooplankton is a nearly ubiquitous feature of marine and freshwater environments and likely constitutes the largest synchronized migration in the world in terms of biomass (Hays, 2003). Among freshwater zooplankton, individuals typically avoid visual predators such as juvenile fish by migrating into the light-poor hypolimnion during the day and returning to the epilimnion at night to feed in warmer waters (Zaret & Suffern,

1976; Stich & Lampert, 1981). While predator-avoidance is considered the ultimate cause of DVM, proximate factors such as gradients in food, temperature, oxygen, light, and UV radiation strongly influence the depth and timing of zooplankton migrations (reviewed by Ringelberg, 2009). Although much is known about the causes of DVM at the population level, a growing body of work has shown that zooplankton species harbor substantial heterogeneity in depth distribution and migration behaviour. Much of this evidence comes from high-resolution studies of zooplankton vertical distributions that identify different migratory strategies among groups of individuals within the same population (Hansson & Hylander, 2009; Berge et al., 2014; Euclide et al., 2017; Meyer & Nelson, 2019).

A widespread pattern of behavioural diversity within zooplankton populations occurs when a portion of the population undergoes diel vertical migration, while another portion of the population does not. This pattern is common in freshwater *Daphnia* (Prepas & Rigler, 1978; Boronat & Miracle, 1997; Hanazato et al., 1989; Salonen & Lehtovaara, 1992; Tessier & Leibold, 1997; Winder et al., 2004; Duffy, 2010), but also exists in populations of marine copepods (Hays et al., 2001; Cottier et al., 2006) and mysid shrimp (Ogonowski et al., 2013; Euclide et al., 2017). This intraspecific behavioural diversity is puzzling because zooplankton such as *Daphnia* can rapidly adjust their vertical position across environmental gradients to optimize individual fitness (Jensen et al., 2001; Kessler & Lampert, 2004; Larsson & Lampert, 2012; Hansson et al., 2016). These environmental conditions, such as predation pressure, temperature, oxygen, and food availability, are important for the fitness of all individuals and often vary greatly down a water column. As such, a naïve expectation based on theory and laboratory experiments is that most individuals in a population will respond similarly to a particular environment and adopt a single behaviour that optimizes fitness (Lampert et al., 2003; Kessler & Lampert, 2004).

In fish and aquatic invertebrates, the co-occurrence of vertically migrating and non-migrating individuals within a population has recently been described as a form of *partial migration* (Mehner & Kasprzak, 2011; Ogonowski et al., 2013; Mehner, 2014; Euclide et al., 2017). Partial migration, whereby a portion of a population resides in an area year-round while another

portion migrates seasonally, is a well-documented phenomenon among birds, fish, and other taxa (reviewed by Chapman et al., 2011), and provides a useful framework for understanding within-population diversity in DVM (Chapman et al., 2011; Lundberg, 1988). Paralleling this work in seasonally migrating populations, Vos et al. (2002) discuss alternative hypotheses to explain partial DVM in zooplankton. First, behavioural diversity may reflect consistent migratory strategies that vary among genotypes (e.g. De Meester & Dumont, 1988; Boronat & Miracle, 1997). Second, independent of genotype, individuals may adjust their migratory behaviour to account for changes in the state of an individual such as reproductive status, body condition, ontogenetic stage, or body size (e.g. Uye et al., 1990; Sekino & Yamamura, 1999; Hays et al., 2001). Alternatively, Larsson and Lampert (2012) found that the vertical distribution of zooplankton in a laboratory setting resembled an ideal free distribution, suggesting that a frequency-dependent hypothesis may also explain partial DVM. The extent to which these three hypotheses explain partial DVM in zooplankton has not been widely studied and remains poorly understood, particularly for natural populations.

In freshwater *Daphnia*, several studies have identified migratory and non-migratory groups based on variation in body hemoglobin (Hb) concentration (Prepas & Rigler, 1978; Landon & Stasiak, 1983; Salonen & Lehtovaara, 1992; Duffy, 2010). For many *Daphnia* species, body Hb concentration is plastic in response to changing oxygen availability and allows for greater time spent in oxygen-poor conditions (Fox, 1955; Green, 1956). Though individuals may increase or decrease Hb concentrations throughout their life, this process takes several weeks (Kring & O'Brien, 1976; Landon & Stasiak, 1983). Thus, for freshwater *Daphnia*, body Hb concentration provides an effective indicator of an individual's consistent occupation of depths with very low oxygen. Furthermore, in a recent field study simulating naturally-occurring migration behaviours, Meyer & Nelson (2019) found no fitness differences between Hb-poor individuals migrating to the epilimnion at night and Hb-rich individuals remaining in the hypolimnion, despite vast differences in environmental conditions between Hb-behaviour groups. They also observed poor fitness outcomes for Hb-poor subpopulations switching to a deep migration behaviour, suggesting that rapid behavioural

switching among groups is limited by individual Hb production (i.e. state-dependent behavior) (Kring & O'Brien, 1976; Meyer & Nelson, 2019). However, whether the Hb-migration association within *Daphnia* species is also reinforced by genotypic differentiation around Hb regulation remains unclear. Leibold et al. (1994) found that for lab populations of *Daphnia*, acclimation to varied environmental conditions had a much stronger influence on vertical distribution than genotypic variation, though they suggest the strength of natural selection (and thus genotypic differentiation) should increase when multiple ecologically relevant traits are correlated.

Here, we take an observational approach to identify patterns of behavioural diversity associated with variation in ontogeny, adult size, and colour (i.e. individual state) in a natural population of *Daphnia pulicaria* Forbes, 1893. Further, we investigate whether behavioural diversity is reinforced by a genetic polymorphism associated with Hb regulation. Our study site is a relatively deep (max 30 m) meromictic lake in eastern Ontario in which prior sampling has identified the co-occurrence of pale individuals and deep-dwelling red individuals during summer and fall (Gittens & French *unpublished data*). The lake has strong environmental gradients in temperature, oxygen, and food that create significant fitness trade-offs associated with spending time in the epilimnion versus hypolimnion (Meyer & Nelson, 2019). First, we characterize intraspecific patterns of diel vertical migration in the population associated with variation in ontogeny and colour. The behaviour of juvenile *Daphnia* is potentially useful for identifying mechanisms that maintain behavioural diversity because there are fewer state differences (e.g. in reproductive status (Table S1) and colour (*personal observation*)) among juveniles than adults. We compare the depth profiles of juveniles to those of adults to gain insight into how age and other state-differences influence habitat selection (encompassing depth selection and DVM). Second, we compare adult size-by-depth distributions between pale and red individuals. Third, we use protein electrophoresis to determine whether genotypes segregate by colour. Hb concentration is a strong indicator of an individual's integrated time spent in the oxygen-poor hypolimnion, thus a genotype-by-colour comparison provides a more precise link to consistent behavioural patterns than a genotype-by-depth comparison. Since previous

work has identified heritable differences in Hb production among *Daphnia* (Green, 1956; Weider & Lampert, 1985) and demonstrated equal fitness between Hb-migration groups (Meyer & Nelson, 2019), we expect that the state-dependent behaviour in this population is reinforced by an association between genotype and hemoglobin upregulation.

Methods

Lake sampling

The study site is a 15-hectare lake located near Queen's University Biological Station (44° 34' 2.64" N, – 76° 19' 28" W) known as Round Lake. The lake is oligotrophic (Reavie et al., 2006) with a mean depth of 12 m and a maximum depth of 30 m. Limnological parameters such as Chlorophyll α (Chl- α), particulate organic carbon (POC), dissolved oxygen (mg/l) and temperature (°C) gradients at the deepest point in the lake were monitored throughout the summer in 2012. Dissolved oxygen and temperature profiles were taken using a YSI probe (model 560A) starting at 0 m and continuing at 1 m intervals to a maximum depth of 20 m. Chl- α samples were collected on July 17 and August 11, 2012. Water samples were collected and stored in brown 1 l Nalgene bottles using a water pump, starting at 1 m and continued sequentially at 1 m intervals to a maximum depth of 25 m. To measure Chl- α , either 100 ml, 150 ml, or 200 ml from each sample was filtered onto 25 mm GF/F filters and then frozen. The frozen samples were thawed at a later date and Chl- α was extracted overnight in 25 ml of methanol and analyzed using a TD-700 fluorometer. POC samples were collected using a water pump on August 11, 2012, starting at 1 m and continued sequentially at 1 m intervals to a maximum depth of 25 m, and water was stored in clear 1 l Nalgene bottles. To measure POC, either 100 ml or 400 ml of water was filtered onto 25 mm GF/F filters that were pre-combusted at 400°C for four hours. POC filters were analyzed using a Thermo Fisher Scientific Elemental Analyzer (FlashEA 1112).

Diel vertical migration of colour phenotypes was characterized by comparing the depth distribution of *D. pulicaria* between day and night. The migration study was done on September 28, 2012. Samples were collected using a 19.7 l plexiglass Schindler trap with

an 80 µm mesh, starting at a depth of 1 m and continued sequentially at 1 m intervals to a maximum depth of 25 m. Based on previous data (Gittens & French *unpublished data*), samples were taken at 4 pm and 4 am because they represent the largest difference in vertical distribution at that time of year. Samples were held in insulated coolers (4–8°C) and processed live within a couple hours of sampling using a dissecting microscope. Individuals were size classed using pre-measured counting circles (S1: < 1.0 mm; S2: 1.0 mm–1.4 mm; S3: 1.4 mm–1.85 mm; S4: 1.85 mm–2.3 mm; S5: > 2.3 mm) and assigned a colour as either being *pale* with no redness, or *red* with detectible redness based on visual inspection. The colour of individuals reflects their body Hb concentration (Fig. S2).

To characterize the patterns of diel vertical migration, the data were parsed into juvenile and adult classes using the mean size of first reproduction of 1.4 mm for the related species *D. pulex* (Nisbet et al., 2004). This corresponds to juveniles being S1 and S2, and adults being S3, S4 or S5, which aligns well with observed reproductive statuses of *Daphnia* in Round Lake (Table S1). Evidence for diel vertical migration in the adults was evaluated statistically using a Generalized Additive Model (GAM). The covariates were depth (D), sampling time (T) and colour (C) with zooplankton density (Y) as the response variable (Fig. 2b). We used GAMs because they do not assume a prior shape to the abundance over depth. Sampling time was treated as a factor, and the interaction between sampling time and the smooth function over depth provides the statistical test for diel vertical migration. The model can be heuristically written as $Y = a + s(D)*T$ where $s()$ denotes the smooth spline model. The analysis was conducted for adult densities independent of colour, and then separately for each colour class. The error distribution for the model was an over-dispersed Poisson, and the interaction between depth and time was tested using quasiAICs. To evaluate the extent that adult migration could be accounted for by adult size, we further divided the data into small adults (S3) and large adults (S4 & S5) and statistically tested whether the size distribution depended on colour (Fig. 3). The test was a logistic regression with proportion of small versus large adults (P) as a function of depth, colour, and sample time. The full model is heuristically written as $P = a + D*T*C + D^2$ with an over-dispersed

Binomial and logit link function. The interaction between depth, time and colour was tested using quasiAICs.

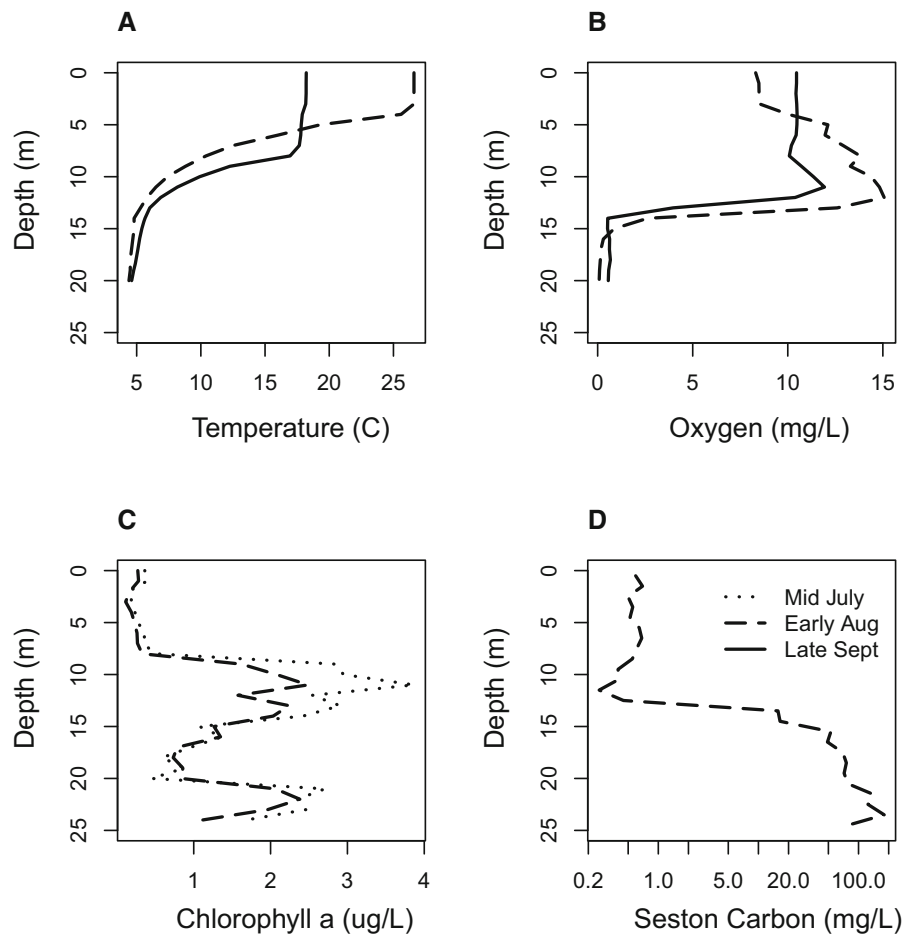
Cellulose acetate electrophoresis

To evaluate the relationship between the observed phenotype and the genotypic identity of individual *Daphnia*, we scored allozymes at five loci for 351 individuals collected during 4am and 4 pm samplings on October 20, 2012. Adult *Daphnia* were colour indexed, photographed, measured (from the top of the eye to the base of the tail spine), and stored in a – 80°C freezer. Frozen individuals were analyzed using cellulose acetate electrophoresis at aldehyde oxidase (AO), lactate dehydrogenase (LDH), phosphoglucosmutase (PGM), malate dehydrogenase (ME), and glucose-6-phosphate isomerase (PGI). *D. pulex* were prepped for electrophoresis by placing individuals in 6 µl of distilled water in Super Z applicator 12 well plate (Helena Laboratories), and ground with a glass cover slip. All frozen individuals were compared against two controls of known laboratory genotypes. The controls were either a heterozygote or homozygote at each of the five loci tested; this was done to ensure accurate genotyping of the unknown individuals. The homogenates were then applied to cellulose acetate electrophoresis gel plates (76 mm × 76 mm, Helena Laboratories) and ran at a 200 V for 20 min using a VWR power supply. All electrophoresis chemicals, buffers, and stains follow Hebert and Beaton (1989). PGM and AO were co-stained, staining individuals for PGM first and AO second (Hebert & Beaton, 1989). Independence between colour and genotype was evaluated using a Chi square analysis performed in the R software environment (R Core Team, 2016). Genetic distance was calculated as the number of loci that differ between each multiloci genotype using the *dist.gene()* function in the R package *ape* (Paradis et al., 2004).

Results

Round Lake is meromictic with strong environmental gradients in temperature, oxygen, and available food (Fig. 1). The lack of seasonal turnover below about 18 meters generates a strong oxygen gradient. Using 3 mgO₂/l as a rough estimate for the onset of oxygen

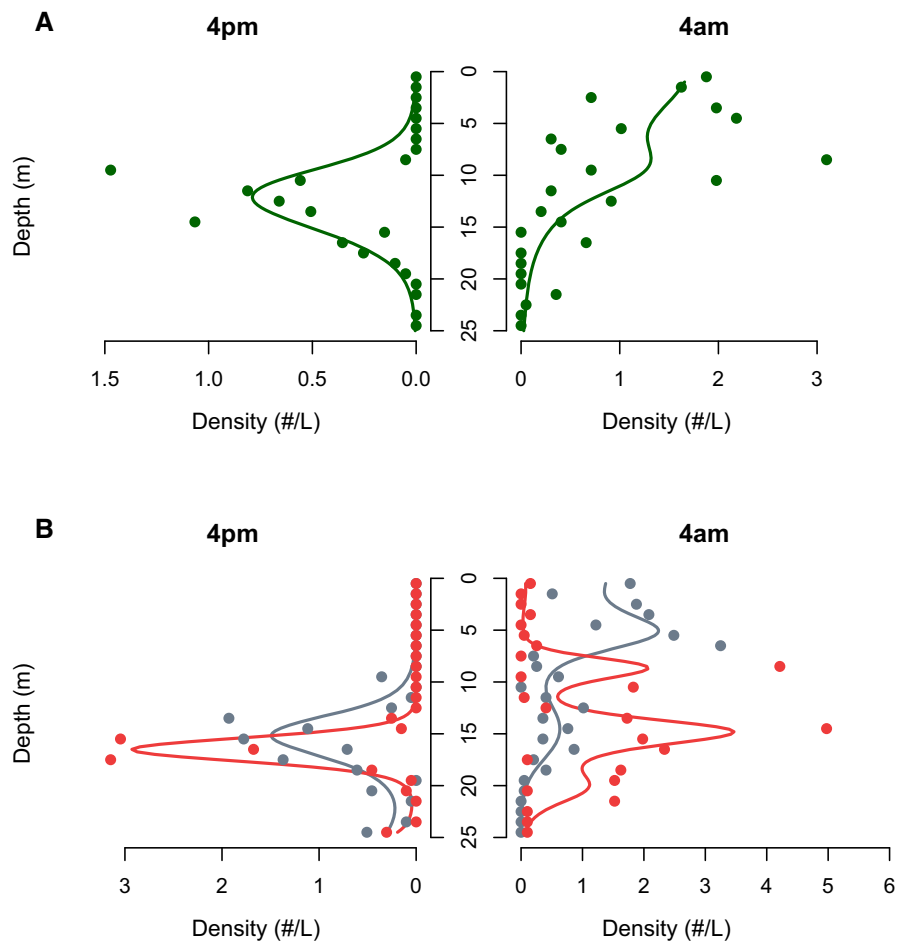
Fig. 1 Depth profiles of several biotic and abiotic variables in July (dotted line), August (dashed line) and September (solid line) 2012. **A** Temperature. **B** Oxygen. **C** Chlorophyll α . **D** Seston carbon



stress (Kring & O'Brien, 1976; Heisey & Porter, 1977), depths below 13 meters are expected to be an oxygen stressed environment. The thermocline during the summer is between 5 and 10 m depth with predominantly cold water ($< 8^{\circ}\text{C}$) below 10 m. Thus, the lower parts of the water column form an environment with temperatures expected to reduce *Daphnia* fitness (Orcutt & Porter, 1983). There is a chlorophyll- α peak between 8 and 15 m depth, and high levels of seston carbon below 13 m, which means that the lower parts of the water column form an environment with comparatively high food abundance. The lake contains at least 13 species of fish (Table S2) and *Chaoborus*, which are invertebrate predators of *Daphnia*. The Secchi depth is 6–7 m during the summer, suggesting that the epilimnion and metalimnion are likely to be environments with high daytime predation risk from visual predators.

The zooplankton *Daphnia pulicaria* undergo strong diel vertical migration (Fig. 2). Juveniles are found around a depth of 10–15 m during the day and move upwards to spread out over the epilimnion and metalimnion during the night. The difference in day and night depth profiles has strong statistical support ($\Delta\text{QAIC} = 33.2$). The relative abundance of colour morphs at the time of sampling was roughly equal with 46% of the adults being pale, and 54% being red. Overall, adults are found around a depth of 10–20 m during the day, but the upward migration is different for each colour phenotype. Within each colour phenotype there is strong statistical support for the difference between day and night depth profiles (pales: $\Delta\text{QAIC} = 63.9$; reds: $\Delta\text{QAIC} = 21.3$). Moreover, a model comparison including both time and colour indicated strong statistical support for different nighttime depth profiles for each colour phenotype ($\Delta\text{QAIC} = 27.4$). There is a difference in the

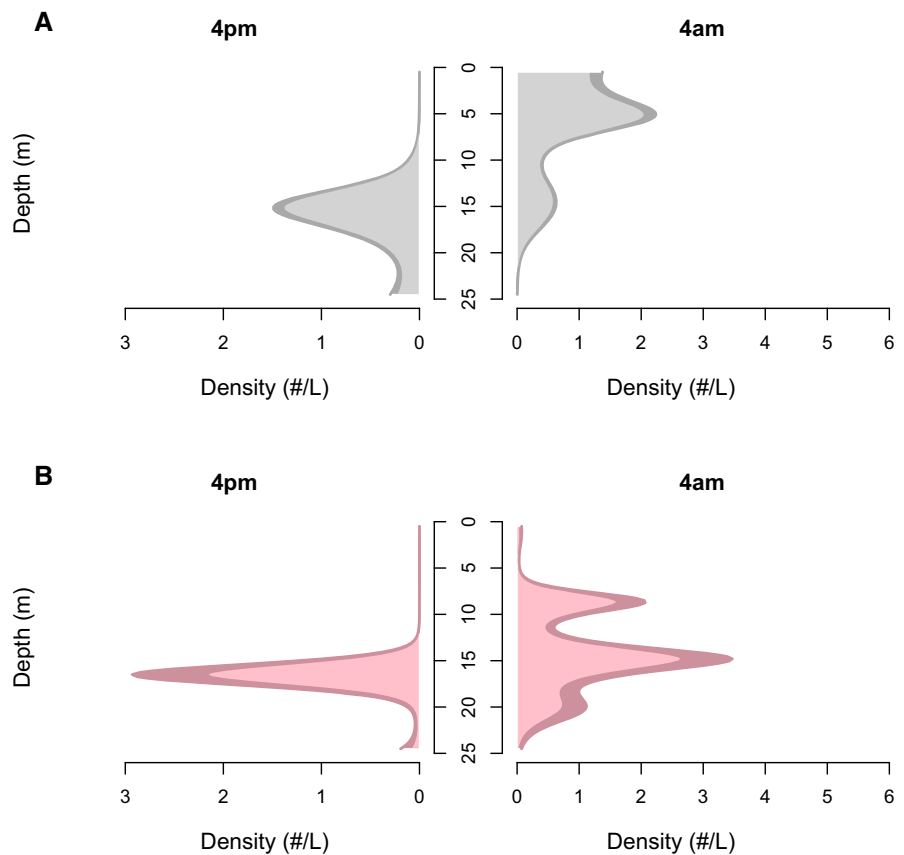
Fig. 2 Diel vertical migration of *Daphnia pulicaria* in late summer 2012. Raw data are shown as circles, and the best fit statistical model (a Generalized Additive Model with over-dispersed Poisson error distribution) is shown as lines. **A** Juvenile migration. **B** Adult migration for both pale colour morphs (gray symbols and lines) and red colour morphs (red symbols and lines)



demographics of size within each colour phenotype with pales comprising mostly small adults (91.3% S3) and reds comprising comparatively more large adults (74% S3). However, there was equivocal support for models comparing the proportional distribution of small (S3) versus large (S4-S5) adults across depth for each time of day ($\Delta\text{QAIC} < 2$) indicating that there is no evidence that the day and night depth distribution for each morph was caused by the movement of a particular size class (Fig. 3, see Supplementary Information for depth profiles of each size class by proportion (Fig. S3) and density (Fig. S4)). Partial migration of the zooplankton population in Round Lake has been observed across multiple years (Meyer & Nelson, 2019; Gittens & French *unpublished data*), indicating that the phenomenon occurs regularly and is likely the result of the environmental gradients in the lake.

To evaluate the relationship between colour and genotype, individuals from the lake were sampled for image analysis (to record colour) and then genotypes using protein electrophoresis. Using five electrophoretic loci, we identified 22 multi-locus genotypes. We found both pale and red phenotypes for all multi-locus genotypes that had more than four individuals (Fig. 4). Since both phenotypes are observed, we can conclude that these genotypes do not distinguish hemoglobin up-regulation. An analysis looking at the relative abundance of phenotypes within each genotype found that colour phenotype and genotype were not independent ($\chi^2 = 21.4$, $df = 5$, $P < 0.001$). Thus, even though all non-rare genotypes had both pale and red individuals, the relative abundance of each colour phenotype differed among the genotypes.

Fig. 3 Best fit statistical depth-density profiles for pale (A) and red (B) colour morphs. The shading within each depth-density profile represents the relative abundance of small adults (light gray/light red) versus large adults (dark gray/dark red) revealing that both size classes are found across the full depth distribution



Discussion

Our study reveals strong intraspecific diversity in Hb concentration and diel vertical migration (DVM) behaviour in a natural population of the freshwater zooplankton, *Daphnia pulicaria*. In late summer, the population comprises at least two behavioural groups, made visible by variation in individual colour. The majority of pale individuals undergo DVM from the hypolimnion to the epilimnion at night, while red individuals remain in the hypolimnion during the day and night. Individual colour correlates strongly with body Hb concentration (Fig. S2), demonstrating that the migratory pale individuals are Hb-poor, while non-migratory red individuals are Hb-rich. Prior work suggests this Hb-migration association is common among *Daphnia* populations (e.g. Prepas & Rigler, 1978; Landon & Stasiak, 1983; Salonen & Lehtovaara, 1992; Duffy, 2010; Meyer & Nelson, 2019), however the mechanisms promoting and maintaining this physiology-behaviour association have received

little attention. In this study, we characterized additional phenotypic correlations with migration behaviour as well as genotypic correlations with Hb regulation. As an observational study, these tests are limited in their capacity to identify the precise mechanism maintaining behavioural diversity. However, based on the phenotypic and genotypic structure of the population, several statements can be made regarding the likelihood of particular mechanisms, and some mechanisms can be excluded.

We first considered the role of ontogenetic stage in shaping individual migration behaviour by sampling the depth profiles of juvenile *Daphnia*. Juvenile *Daphnia* in Round Lake are rarely Hb-rich, even when sampled from oxygen-poor regions of the hypolimnion (*personal observation*). Thus, depth selection among juveniles precedes the development of intraspecific variation in Hb concentration. Since juveniles exhibit greater uniformity in individual state (e.g. Hb concentration, reproductive status) we expected greater uniformity in depth selection among

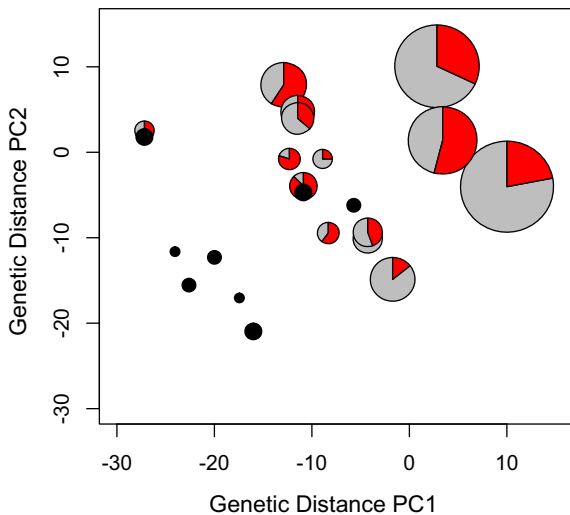


Fig. 4 Genetic distance among individuals based on protein electrophoresis. Each circle represents a unique multilocus genotype. The area of the circle is proportional to the number of individuals in each genotype. Black circles denote genotypes with less than 4 individuals. If there are 4 or more individuals, the colour of each circle reflects the relative abundance of pale colour morphs (grey area) and red colour morphs (red area)

juveniles compared to adult *Daphnia*. During the day, juveniles occupied slightly shallower waters than adults, forming peak abundances in the upper hypolimnion between 10 and 15 m (Fig. 2). Here, the water is cold but oxygen-rich, and food is abundant (Fig. 1). However, juvenile profiles showed little qualitative difference in pattern or uniformity compared to pale adults. During the day, juveniles occupied roughly the same range of depths as pale adults, with many occupying cold and oxygen-poor waters between 10 and 15 m (Figs. 1b, 2). Like pale adults, juveniles also migrated upwards at night to spread out across the metalimnion and epilimnion. This result demonstrates that, despite the fact that many juveniles will ultimately become red adults, the population of juveniles undergoes a shallow migration mostly resembling that of pale adults.

Interestingly, the migration of juveniles included a notable proportion of juveniles that remained in the far colder water below 10 m in the same manner as pale adults (Fig. 2). This result is puzzling given that phenotypic variation among juveniles is low and individual growth rate is closely tied to temperature (Orcutt & Porter, 1983). There is evidence from previous work that acclimation to different

temperature regimes can cause sister juveniles to ‘choose’ different vertical migration behaviours (Leibold et al., 1994). Thus, one explanation may be that variation in temperature during early development influences juvenile depth selection, such that juveniles hatched from red adults occupy colder waters. Alternatively, juvenile depth selection may reflect the trade-off between food availability and favourable temperatures present across the epilimnion and metalimnion, suggesting that even among individuals with very little phenotypic variation, behavioural variation may be promoted by trade-offs in environmental conditions. Previous work has reported a similar response to trade-offs between favourable food conditions and favourable temperature and oxygen conditions. In a highly controlled laboratory experiment, Larsson and Lampert (2012) reported a bimodal distribution of *Daphnia* split between the warm, well oxygenated, but food-poor epilimnion, and a cold, food-rich and oxygen-poor hypolimnion. Under similar environmental conditions, asynchronous migrations have also been observed in natural populations of *Daphnia* (Salonen & Lehtovaara, 1992; Winder et al., 2004). Moreover, Winder et al. (2004) successfully induced a population of *Daphnia* to undergo partial DVM in a controlled laboratory experiment by simulating the naturally occurring trade-off between food availability and temperature at their study site. In that study, partial migration was induced in the presence, but not the absence, of fish, suggesting that predator avoidance as well as a trade-off between food and temperature is important for promoting partial DVM. Studies reporting both partial DVM and data regarding the environmental gradients at the study site are limited. However, these few examples demonstrate that a specific set of environmental conditions may be important in promoting partial DVM in zooplankton by facilitating different strategies of equal fitness. Our results contribute to this discussion by demonstrating that intraspecific behavioural diversity can occur over strong environmental gradients even in the absence of any distinguishable phenotypic variation.

The depth distribution of adult *Daphnia* did not appear to be strongly influenced by individual size in our study. Though a greater proportion of large individuals was observed among non-migrating red adults (26% compared to 9% among pale adults), large adults were observed throughout the entire night-time

depth distributions of both red and pale *Daphnia* (Fig. 3). We found equivocal support for models comparing the proportional depth distribution of small and large adults within pale and red adults ($\Delta\text{QAIC} < 2$), indicating that movement of different adult size classes cannot explain the migration behaviour of each colour phenotype. Thus, among adult *Daphnia* we did not find support for a state-dependent partial DVM based on individual size. This result was unexpected given that larger individuals are at greater risk of predation by planktivorous fish (Vinyard & O'Brien, 1975; Wright & O'Brien, 1982). One possible explanation is that the threat from visual predators is reduced at night, such that variation in size among adult *Daphnia* does not strongly influence individual migration behaviour. It is also possible that *Daphnia* reach a threshold size during adulthood, at which point variation in size becomes less important for predator avoidance. However, this scenario is unlikely because *Daphnia pulicaria* in this study did not exceed 2.3 mm in length and Vinyard and O'Brien (1975) reported a steady increase in predation pressure for *Daphnia magna* Straus, 1820 up to 4 mm in length.

The association between body Hb concentration and migration behaviour suggests that if genotypic variation in Hb regulation exists in the population, it may also lead to variation in migration behaviour between genotypes. That is, heritable variation in Hb regulation may be an underlying mechanism of behavioural differentiation in this system. There are strong gradients in oxygen concentration in Round Lake, and previous work has shown that genotypes of *Daphnia* can differ in oxygen tolerance and Hb production in response to temperature and oxygen flux (Green, 1956; Weider & Lampert, 1985; Cambroner et al., 2018). To address this potential mechanism, we examined the relationship between colour and genotype to determine whether individual Hb concentration is associated with a genetic polymorphism in the population. The relative abundance of red and pale individuals in the population was not independent of genotype, which suggests that some genotypes are more prone to increased Hb concentration and non-migratory behaviour than others. However, red and pale *Daphnia* were present in all commonly found genotypes (Fig. 4), indicating that intra-genotype variation in Hb regulation, and thus likely migration behaviour, is common in the population. This result is somewhat surprising because

there is a rich literature describing the genetic basis of migration behaviour in *Daphnia*. For instance, genotypes within a population can show heritable variation in body size (Tessier & Leibold, 1997), phototaxis (Dumont et al., 1985; De Meester & Dumont, 1988), response to predator cues (De Meester, 1993), and depth preference (King & Miracle, 1995; Sell, 1998; Ringelberg et al., 2005), which are all important components of migration behaviour. However, the relationship between genotype and behaviour seems to vary greatly between lakes (Geedey et al., 1996). Moreover, there can be substantial plasticity in depth selection and migration behaviour between and within clones in the same population (Spaak & Ringelberg, 1997; Ringelberg et al., 2005), making the role of genotype in maintaining partial DVM unclear. Our results suggest that genotype plays at least a small role in an individual's Hb regulation and migration behaviour, but that partial DVM and Hb regulation in this population is not structured by a genetic polymorphism.

Our study provides a snapshot of genotypic structure and behaviour over strong environmental gradients that are established during summer. However, like many lakes, there are large fluctuations in food, temperature, and oxygen conditions between seasons in Round Lake (Gittens, 2014). As a result, there may be stronger selection for behaviourally plastic genotypes capable of adopting a range of behaviours. Variation in adaptive phenotypic plasticity in Hb production has been observed between clones of *Daphnia magna* from different latitudes (Yampolsky et al., 2014), indicating that selection for highly plastic genotypes around Hb production could be occurring in Round Lake. If all genotypes have been 'selected' for behavioural plasticity, and fitness is roughly equal between migration strategies (Meyer & Nelson, 2019) then both Hb-poor migrating and Hb-rich non-migrating individuals should be expected amongst all common genotypes. It should be noted that selection for a range of behaviours within a genotype does not necessarily guarantee behavioural plasticity throughout an individual's life. Recent theory has suggested that highly behaviourally flexible individuals can over time become inflexible, even when environmental conditions shift (Dingemanse & Wolf, 2010). For instance, behaviourally plastic individuals may adopt temporally stable behaviours when the success of a chosen behaviour is increased with repeated use (i.e.

with experience) (Wolf et al., 2008). Physiological responses such as shifts in digestive enzyme production in birds (Afik & Karasov, 1995) or Hb-regulation in zooplankton may act in similar ways to promote temporally stable behaviours among otherwise highly flexible individuals. Meyer and Nelson (2019) found no difference in the fitness outcomes of Hb-rich and Hb-poor *Daphnia* undergoing their respective natural migration behaviours. Thus, it is possible that many individuals are capable of obtaining equal fitness outcomes via either DVM behaviour, but that behavioural switching is limited later in life by fitness costs associated with Hb-regulation (Meyer & Nelson, 2019).

Diel vertical migration is one of the most studied animal movement phenomena, and the ecological importance of zooplankton diel vertical migration at the population level is well documented. For instance, vertical migrations can strongly affect oceanic carbon cycling (Longhurst & Harrison, 1989), dispersal patterns (Emsley et al., 2005), predator behaviour (reviewed by Hays, 2003), and phytoplankton dynamics and community composition (Reichwaldt & Stibor, 2005; Haupt et al., 2009), among other phenomena. However, to our knowledge only one study to date has examined the ecological consequences of intraspecific variation in the diel vertical migrations of zooplankton. Duffy (2010) presents strong evidence that non-migratory individuals within a natural population of *Daphnia dentifera* Forbes, 1893 experience different seasonal dynamics and levels of interspecific competition than do migratory individuals. Clearly, characterizing intraspecific diversity in diel vertical migration provides a more precise picture of aquatic ecosystems. Though further empirical evidence is lacking in zooplankton, it is likely that the ecological consequences of population-wide DVM will be at least modulated by the presence of within-population variation in behaviour, especially under shifting environmental conditions. Thus, understanding how such diversity forms and is maintained is not only a rich biological question, but may be important for predicting the ecological and evolutionary consequences of environmental change. Our study revealed partial DVM in a natural population of zooplankton that was not explained by state-dependent variation in adult size, or by genotypic variation, leaving open the possibility that these behaviours represent frequency-dependent behaviours of equal fitness that are

reinforced by increasing variation in Hb concentration between behavioural groups (Meyer & Nelson, 2019). The role of seasonal change in environmental gradients in maintaining frequency-dependent behaviours may be particularly important for this system. Whether characterizing such variation in zooplankton will lead to better predictive models of nutrient cycling, community assembly, or interspecific interactions remains to be seen, but this may be a productive avenue for future work in aquatic and behavioural ecology.

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Data availability The datasets generated and analysed during the current study are available in the DRYAD repository.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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