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Experimental evidence for concentration-dependence and intra-specific variation of movement behaviour in American lobster (*Homarus americanus*) larvae

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Abstract

Predicting dispersal paths of marine larvae with extended pelagic durations, such as American lobster (*Homarus americanus* (Milne Edwards, 1837)), requires understanding the cues to which larvae respond, and how that response reflects changes in larval behaviour. If larvae respond to conspecific presence by varying their movement, this behaviour can bias laboratory estimates of environmental responses. We tested whether larvae actively decreased their local intraspecific density by measuring how the vertical distribution of larvae changed under high versus low concentrations of conspecifics. We observed weak increases in vertical dispersion at higher concentrations in both newly-hatched larvae and in post-larvae, but not in intermediate larval stages. We also tested for differences in horizontal swimming behaviour in high and low concentrations, by fitting a novel random walk model that allowed us to model both larval interactions and persistent turning behaviours. We showed substantial reduction in diffusive behaviour under high concentration conditions resulting from more frequent turns by each larva, but no evidence for consistent avoidance of conspecifics. Our study is the first to demonstrate concentration-dependent behaviours in lobster larvae.

Keywords: *Homarus americanus*, American lobster, movement ecology, concentration dependence, larval dispersal

Introduction

Many marine benthic invertebrates are either sedentary or only move a few kilometers throughout their adult lives. Upon maturation, these meroplanktonic species produce planktonic larvae that disperse over much longer distances than adults (Pineda et al. 2007). The larval stage, therefore, plays a critical role in connecting distant populations, allowing species to respond to changing habitat conditions, recover from localized population losses, and spread to new habitats (Sale et al. 2006). As such, predicting how these populations will change over time requires understanding how larvae disperse between source and settlement sites. Predicting dispersal and resulting connectivity requires understanding how larvae will react to environmental cues, including the presence of conspecific larvae.

Water movement itself heavily influences a larva's path through the water column. Therefore, most attempts to predict marine larval settlement patterns have focused on understanding current patterns, assuming larvae act as passive drifters. However, increasing evidence (Metaxas 2001; Metaxas and Saunders 2009) demonstrates that larval swimming behaviour can significantly affect its path while dispersing, either by altering its vertical position in the water column (and thus changing the horizontal current regime it encounters), or by swimming horizontally through current discontinuities, such as fronts where different water masses meet. Although a larva may swim slowly relative to the currents it moves through, the ability to switch behaviours in response to changes in surrounding conditions can result in substantial control over its path (e.g. Fiksen et al. 2007).

Most larval dispersal behavioural studies focus on larval response to external abiotic cues such as temperature (e.g. Rooney and Cobb 1991); salinity (Anger 2003), or light levels (Thorson 1964). However, in addition to abiotic factors, the survival of an individual during dispersal to settlement depends on its biotic environment, including food, predators, and competitors.

Larval conspecifics comprise a potentially important part of an individual larva's biotic environment. During dispersal, nearby conspecifics may help protect an individual from predation (e.g. sea urchins and sea stars, Roy et al. 2012) or attract nearby predators (e.g. planktivorous fish, McNaught and Hasler 1961; Gliwicz et al. 2006). Further, nearby conspecifics may compete for resources during dispersal (e.g. Fortier and Harris 1989) and for resources or settlement sites if they eventually settle in close proximity (e.g. barnacles, Connell 1985). All these factors add concentration-dependence to dispersal, because neighboring larvae may affect the probability that an individual propagule will survive until settlement.

In species such as American lobster (*Homarus americanus* (Milne Edwards, 1837)), where larvae of all stages, at least under laboratory conditions, readily attack conspecifics (Herrick 1909), aggregation presumably offers little benefit. Further, as in many other meroplanktonic species, settlement competent postlarval lobster encounter increased

mortality and shelter limitation with increased settlement densities over small scales (Wahle and Incze 1997; Steneck 2006), meaning that individual larvae should benefit by moving away from one another so as to avoid settling near competitors.

Given that marine currents can aggregate larvae during transport (Siegel et al. 2008), an individual may potentially increase its probability of survival to settlement through behaviours that reduce aggregation. Four broad types of behaviour could reduce aggregation. First, individual random movements spread aggregations through a diffusion-like mechanism (Harrison et al. 2013). Second, consistent inter-individual differences in behaviour, such as differences in mean swimming direction or responses to environmental cues, may spread larvae (Vikebø et al. 2007). Third, larvae could actively increase their local rate of diffusion (the rate at which they spread apart) when near conspecifics, by either moving more rapidly or by turning less frequently (Kareiva and Shigesada 1983) when near other larvae. Finally, larvae could simply swim away from nearby conspecifics.

Any of the last three behavioural mechanisms will affect interpretation of larval behaviour from small-scale experiments. First, if larvae exhibit strong inter-individual differences in behaviour, experimental results can only inform large scale (km or greater) dispersal predictions if the experiment includes sufficient larval behavioural diversity to capture this variability. Second, if larvae move away from one another at high concentrations through either diffusive or directional movement, estimates of larval response to a given cue will depend on larval concentrations used in the trials to estimate that cue. Even species that would rarely experience high concentrations under field conditions may exhibit these behaviours under high concentration laboratory conditions, because larvae may treat one another as potential predators and actively avoid interaction.

Previous studies report a wide range of behavioural responses to conspecific concentrations in planktonic organisms, in both field and laboratory studies. Larval sea urchins and sea stars (Roy et al. 2012) and copepods (Hamner and Carleton 1979) and a wide variety of taxa other display aggregation behaviours. In contrast, *Daphnia* move out of areas of high conspecific concentration, possibly to avoid predators (Gliwicz et al. 2006). Fish larvae in the field (Fortier and Harris 1989) and *Daphnia* populations in laboratory experiments (Lampert 2005) vertically position themselves in an ideal free distribution to exploit available prey.

Even in the absence of conspecific avoidance behaviour, consistent behavioural variability between individuals can increase variation in final settlement sites (Fraser et al. 2001; Bowler and Benton 2005). Behavioural differences between larvae can influence large-scale population dynamics and patterns of connectivity (Phillips et al. 2008; Clobert et al. 2009), effects that would otherwise be missed in large-scale dispersal simulations assuming identical larval behaviours (e.g. Katz et al. 1994; Incze et al. 2010).

For most meroplanktonic species, few data exist either on movement in response to conspecifics or intraspecific variation in larval movement behaviours. For this study, we focused on concentration responses and behavioural variability in larvae of American lobster. Effective management of this commercially important species, fished across the North American Atlantic coast from Newfoundland, Canada to the mid-Atlantic U.S.,

requires understanding factors that affect their dispersal. Dispersal from offshore stocks may stabilize and increase yields in inshore stocks (Fogarty 1998), and knowing how dispersal connects populations can help predict how management actions in one region will affect distant populations (Fogarty 1995; Chassé and Miller 2010). Our study tested whether lobster larvae alter their vertical or horizontal movement behaviour at different conspecific concentrations. We hypothesized that larvae will increase inter-individual distances at higher concentrations, because larvae actively move to reduce their local concentration and thus the potential for intra-specific competition or predation.

Female lobsters brood their eggs for 9–12 months before releasing hatchlings as batches of free swimming larvae (Ennis 1995). The larvae develop over several weeks (Annis et al. 2007), depending on temperature (MacKenzie 1988), through three larval stages (I–III) and one post-larval, pre-settlement stage (stage IV). Throughout this developmental period, they occur in the water column, dispersing over a wide range distances before setting (Incze et al. 2010; Chassé and Miller 2010). Behavioural studies suggest that all four lobster larval stages are behaviourally competent (Ennis 1986; Stanley et al. 2016) can actively mediate their vertical position in the water column (Ennis 1975a). Generally, only the post-larvae are considered strong horizontal swimmers (Ennis 1986; Cobb et al. 1989).

Whereas the concentrations of larval lobsters used in laboratory conditions are rarely observed *in situ* (Harding et al. 2005; Fogarty 1983; Harding et al. 1982), mothers nonetheless release larvae in batches of a few hundred (Ennis 1975b) to a few thousand individuals at a time (personal observation). Experimental tests of larval behaviour typically utilize relatively high concentrations (e.g. Ennis 1975a; Boudreau et al. 1992; Stanley et al. 2016). Understanding how accurately laboratory results predict behaviour *in situ* requires quantifying how experimental concentrations affect observed behaviours.

This study consisted of two sets of experiments. In the first, we tested how larval concentration in the water column affects vertical distributions at each developmental stage. We hypothesized that larvae increase vertical dispersion at higher concentrations. We also tested whether vertical dispersion varied consistently among larvae from the same mother, as a measure of intraspecific variation in movement behaviour.

Our second experiment recorded horizontal swimming behaviour of small groups of postlarvae at low and high concentrations. We then developed a novel random walk model to estimate between-treatment and inter-individual variability in diffusion rates and inter-individual attraction or repulsion, while accounting for directional and turning rate persistence. As many meroplantonic larvae have been observed to show persistent looping behaviour (e.g. Stanley et al. 2016), this model may be useful more generally as a tool to model larval behaviour under experimental conditions.

We finally determined overall patterns of larval clustering, by testing whether the distribution of distances between larvae in each video frame clustered more or less than null model of non-interacting movement. We hypothesized that increased conspecific avoidance and higher activity rates by larvae in the high concentration treatment would increase diffusion rates and inter-individual spacing relative to the low concentration treatment.

Methods and Materials

Larval rearing

Fishermen collected egg-bearing female American lobsters (*Homarus americanus*) using commercial traps, from Port au Choix (n=5) in Newfoundland, Canada in June of 2010, under Fisheries and Oceans Canada experimental license NL-1339-12. The females ranged in size from 72 – 136 mm in length. The females were held in individual tanks at the Ocean Sciences Centre of Memorial University, Newfoundland and Labrador with continuous flow ambient sea water (7 – 15 C), and fed twice weekly meals of squid. We used a reversed 12 hour light / 12 hour dark light cycle, with light on from 7 pm to 7 am, because hatching typically occurs at the transition from light to darkness (Ennis 1995).

Each morning we collected larvae using a fine mesh net, and then maintained larvae from each maternal origin in separate four-litre holding containers for their first two days in a shared water bath of filtered ambient sea water (7 – 15 C). On the third day post-hatching, we transferred larvae to shared 50-l plankton kreisel tanks filled with filtered sea water. Larvae in the kreisel tanks were maintained at concentrations of less than 50 individuals $\cdot l^{-1}$, and on a constant 12 hour dark/light cycle. Larvae in both types of tank were fed live enriched *Artemia salina* ad libitum, and bubbled vigorously to reduce cannibalism. For larval trials, we removed stage II through IV larvae from the kreisel tanks by net and sorted them to stage. No special permissions or permits were required for larval rearing or experiments.

Vertical movement experimental trials

We first tested whether increasing larval concentration resulted in larvae in an aggregate change in larval phototactic response, by larvae spreading out in the water column. To measure how concentration effects changed through larval age, we tested larvae at several developmental stages: zero-day old (10 trials), one day old (8 trials) and two day old (8 trials) stage I larvae, stage II (4 trials), stage III (4 trials) and stage IV larvae (5 trials). Availability of larvae determined the number of trials per stage. We separated stage I larvae by day because phototactic behaviour shifts rapidly post-hatching (Ennis 1975a) and a previous experiment noted significant intra-ontogenetic variation in behaviour (Stanley et al. 2016).

For each of the stage I trials, we obtained all larvae in a given trial from a single mother, thus allowing us to test whether larvae from the same mother exhibited consistent patterns of concentration-dependent vertical movement. It also ensured that, as would be expected under natural conditions, a given larva's closest neighbor would come from the same mother until sufficient time passed for diffusion to mix larvae from different parents. We tested five mothers, with two trials per mother for zero-day old larvae, for 10 total trials. However, because daily larval mortality was high, we were only able to complete two trials per mother from three of our mothers for one and two day old larvae; we only obtained sufficient larvae for one trial per day per mother for the other two mothers, for a total of eight trials for each of those stages.

Larvae were equilibrated for half an hour at 15 °C, then placed in two 120 cm tall plexiglass tanks (Fig. 1A) filled with filtered sea water, and held at 15 °C. Overhead lighting lit tanks equally, both to maintain a constant light environment and to induce phototactic behaviour. We used broad spectrum (Exo-Terra® 440 lumen 'day light') bulbs positioned 30 cm above each tank to approximate daylight lighting. We selected one of each pair of replicate tanks at random for the high concentration treatment and the other for the low concentration treatment. In the low concentration tank, we placed either 20 larvae (stage I) or 10 larvae (stage II–IV), in contrast to 40 larvae (stage I) or 20 larvae (stage II–IV) in the high concentration tank.

Larval counts for high and low concentration treatments were chosen to balance the desire to match low larval concentrations typically encountered in the wild while maintaining sufficient numbers of larvae in the tank to generate a reliable estimate of distribution. We used different counts of larvae for different stages to account for the fact that lobster typically release stage I larvae in groups that occur at much higher concentrations than the other stages in the wild (Harding et al. 1982), and the difficulty in maintaining large numbers of post-stage I larvae.

Larvae were placed at the top of the tank and allowed to move freely in the columns for 15 minutes. We then counted the number of larvae visible in each 10 cm vertical segment of the tank (Fig. 1A). We repeated this count at 30 minutes to determine whether the vertical distribution of larvae within had equilibrated. This strategy yielded four sets of observations for each trial: two sets of counts for the left tank and two for the right.

Statistical analysis of vertical movement

All statistical analyses were conducted in R 3.1.2 (R Development Core Team 2008). To determine whether larvae were distributed similarly in comparable tanks, we used a permutation-based analysis of variance of dissimilarities among tanks. For each stage tested, we transformed the observed set of larval counts into a dissimilarity matrix of relative abundances, by dividing counts in each 10 cm segment by the total number of larvae observed in that observation, then calculating the Bray-Curtis (BC) dissimilarity between all pairs of samples. We used the Bray-Curtis dissimilarity as it equally weights categories with both high and low abundances when calculating how dissimilar two samples are, and treats all pairs of samples with no shared counts as equally dissimilar (McCune et al. 2002). We then used the *adonis* function from the *vegan* package (Oksanen et al. 2013) to determine the fraction of variance in between-observation dissimilarities explained by our experimental treatments. This function calculated the fraction of the sum of squared dissimilarities between observations explained by group membership then permuted the labels of each observation 10000 times and recalculated dissimilarities. We used these permutations to calculate a null distribution of dissimilarities (Anderson 2001). Within each developmental stage, we regressed dissimilarity on three different factors:

1. Time period (whether an observation was from the first or last 15 minutes of the trial).

2. Identity of the mother, to test for differences in larval distribution associated with maternal origin. We only tested this effect for stage I larvae because subsequent stages were reared in pooled tanks.
3. Larval concentration.

We then used a mixed-effect model to determine how treatments differed. Given that we were testing whether higher larval concentration caused larvae to spread out vertically, we used Shannon diversity of counts within each tank at a given time point as the outcome variable. Shannon diversity measures the degree of spread among individuals at different depth stratum in the system (Lande 1996). Shannon diversity of a given tank i was defined as:

$$E_i = - \sum_j p_{i,j} \ln(p_{i,j})$$

where $p_{i,j}$ denotes the fraction of total larvae in tank i found at height j .

We used a linear mixed effect model to fit variation in diversity within each stage (Bolker 2008), treating time period, mother, and the concentration treatment as fixed effects, and trial as a random effect to account for the repeated measures structure of the data. We ran these tests using the lme4 package, version 1.1-7 and significance was calculated using a parametric bootstrapping with percentile intervals (Bates et al. 2015).

Horizontal movement behaviour experiment

Our second set of experiments tested whether larvae altered their horizontal movement behaviour as a function of different conspecific concentrations. We recorded larval movement in an experimental arena in a 50 x 75 cm region of a recirculating flume (Fig. 1B), with 10 cm deep water maintained at 15 °C, without flow. Screen barriers (100 µm mesh) blocked off the two open ends in order to confine larvae to the arena. The arena was lit with four broad spectrum daylight incandescent lamps (Exo-Terra® 440 lumen) placed in the corners of the experimental arena, 75 cm above the surface of the water, to maintain a constant and homogeneous lighting environment. Larvae were allowed to adjust to the experimental temperature (typically within 5 °C of rearing temperature) for 30 minutes prior to recording.

We only tested the horizontal concentration response of stage IV larvae, because previous studies demonstrated that earlier developmental stages have little control over their horizontal (as opposed to vertical) position (Ennis 1995). We recorded five trials for each treatment, adding five larvae to the arena for the low concentration trials and ten larvae for the high concentration trials.

We recorded larval movement using an overhead digital camera (Axis 221 Day and Night Network Cameras, model no. 0221-01-04, Axis Communications, Lund, Sweden), placed in the centre of the arena, 200 cm above the surface of the water. The camera recorded larvae for 30 minutes in grey scale with a resolution of ~ 10 pixels mm^{-1} at 30 frames per second (frames/s).

Each video was broken into 10 minute sections and frames were processed and analyzed sequentially for larval movement using ImageJ (Schneider et al. 2012) the Computer Assisted Sperm Analysis plugin (Wilson-Leedy and Ingermann 2007), using the same method described in Stanley et al. (2016). Refer to Supplementary Fig. A1 for an example of video processing and Supplementary Section A for a more detailed description of video processing.

Random walk modelling of horizontal behaviour

We used a set of correlated random walk models to estimate inter-individual differences in behaviour, and to determine how individual larvae may change their behaviours in response to conspecifics in the horizontal movement trials. The random walk models treated each individual movement path as a stochastic process: the direction and length of move in a given period of time were treated as random variables, which may depend on the previous movements in the path or on an individual's local environment (Okubo and Levin 2001).

We used two types of random walk models to determine whether stage IV larvae changed their horizontal behaviour with concentration. The first set of models estimated changes in the rate that individuals spread out in the water column, by measuring diffusion coefficients. The second set of models measured whether nearby larvae attracted or repelled individual larvae. In the first set of models, we hypothesized that individuals would change their behaviour to increase their effective diffusion rate at higher conspecific concentrations. For the second set of models, we hypothesized that individuals would move away from one another to increase local dispersion.

Calculating horizontal diffusion coefficients

Dispersers patchily distributed in space could decrease encounter rates with other dispersers by increasing their spreading rate whenever encountering other larvae. Therefore, for each path observed in each video, we estimated the long-term diffusion coefficient for that individual, assuming it followed a correlated random walk with no directional bias or turning angle bias (Kareiva and Shigesada 1983). We showed previously that this method effectively captures patterns of displacement in larval lobsters under similar experimental conditions (Stanley et al. 2016). We sub-sampled each path to one frames/s, to reduce correlation between turn angles in each step, and estimated the diffusion coefficient for path i as:

$$D_i = \sigma_{l,i}^2 + \bar{l}_i^2 + 2\bar{l}_i^2 \left(\frac{c_i}{1 - c_i} \right)$$

Here, $\sigma_{l,i}^2$ denotes the sample variation of step distances ($\text{cm}^2 \cdot \text{s}^{-1}$), $\bar{l}_i$ was the mean step distance ($\text{cm} \cdot \text{s}^{-1}$), and c_i denotes the mean cosine of path i . We compared diffusion coefficients between the high and low concentration treatments with a mixed effect model, using the lme4 package (Bates et al. 2015). We treated the concentration treatment as a fixed effect and used paths nested in video as a random effect to control for between-video heterogeneities.

Random walk models of intra-individual attraction or repulsion

To test whether conspecifics attracted or repelled individual larvae, we fit random walk models with aggregation to each larval path (based on Shimatani et al. 2012). The model included three key parameters: w_p , w_c and κ (see Supplementary Section B for the mathematical details on the model).

The parameter w_p determined whether larvae tended to continue moving in the same direction as the last step or to turn at the same rate as the previous step; $w_p = 0$ corresponded to the case where a larva continued travelling in the same direction, whereas $w_p = 1$ corresponded to the case where a larva moved in loops with the same turning speed over time. This parameter captures the looping behaviour observed in larval movement paths (Supplementary Fig. A1).

The parameter w_c determined how strongly a given larva was repelled (or attracted) from the common centroid of the other larvae present in the flume. When $w_c = 0$ a larva tended to keep moving in the same path predicted by w_p regardless of the location of other larva. When $w_c = 1$, a larva tended to move toward (away) from the common center of the other larvae, regardless of its behaviour in the last step.

The final parameter, κ , measured the random variation around the mean predicted step given by w_p and w_c . If $\kappa = 0$, a larva always moved in the predicted direction. When $\kappa \rightarrow \infty$, a larva chose the direction of each step at random.

We fit the three models to all larvae with a path consistently recorded for three minutes or more, to ensure paths sufficiently long to produce a reliable estimate of model coefficients. We sub-sampled larval paths to one frames/s, to reduce the correlation of turn angles with steps further in the past. For each path, we estimated all three models using maximum likelihood. We logit-transformed w_c and w_p , and log-transformed κ prior to fitting, to ensure the parameters were unbounded to avoid issues with bounded optimization. The Nelder-Mead algorithm in the *optim* function for R 3.1.2 (R Development Core Team 2008) estimated the maximum likelihood value for each model for each path. We estimated standard errors for each parameter as the diagonal of the inverse Hessian of the negative log-likelihood (Bolker 2008).

We used a permutation test to determine whether attraction, repulsion, or the null model (no interactions) produced a better fit for each path. For each path, we calculated the difference in log-likelihood between the null and each interaction model, then randomized the order of observations of angles to the centroid 500 times and re-fit the null model and both interaction models to the simulated paths. For each simulation, we calculated the difference in log-likelihoods, giving a null distribution of log-likelihood differences in the absence of larval interactions. For each path, we also tested how well the best fit model captured the movement dynamics of that individual, using several goodness-of-fit tests (Supplementary Section C).

Variation in larval spatial distribution

If individuals actively moved away from one another, then at any given point in time larvae in higher concentration treatments should be more dispersed than expected from the overall distribution of larval locations. To test for this pattern, we calculated the distance from each larva to every other larva in each frame. We then generated a null distribution of distances by drawing 1000 samples of larval coordinates randomly from those observed across all videos and calculated the distance between each pair of draws. In this case, a greater number of observations at short distances in the observed distribution compared with the null model would indicate clustering (Bonetti and Pagano 2005).

Results

Vertical distribution of larvae

At each stage, the aggregate distribution of larvae in the vertical column was similar for the high and low concentration treatments, and was consistent with patterns of phototactic behaviour previously observed for *H. americanus* larvae (Hadley 1908) (Fig. 2). The bimodally phototactic zero-day old stage I larvae either moved to the top or bottom of the tank (Fig. 2A). One-day old larvae almost always occupied the bottom 10 cm, only occasionally moving to higher depth strata (Fig. 2B). Two-day old stage I larvae, as well as stage II and III larvae, occurred almost exclusively in the bottom 10 cm of the tanks (Fig. 2C–E). Finally, stage IV returned to a bimodal distribution, with the bulk of the larvae at the top or bottom of the tanks (Fig. 2F). We restricted the remaining analyses to zero and one day old stage I and stage IV larvae given the lack of variation in the distribution of larvae between tanks for the other stages.

Although larval distribution varied among trials, we detected systematic variation by treatment only in the zero-day old and one-day old larvae (Table 1). In both zero and one day olds, maternal origin affected distribution most, explaining 63% of the variance in dissimilarities for zero day old and 23% of the variation in one day old larvae. The concentration of larvae in the tank only minimally influenced vertical distribution, explaining less than 10% of the variation for all treatments with a statistically significant effect (at the 0.05 level) only for zero-day old larvae. Finally, we observed a weak effect ($R^2 < 5\%$ for all treatments) of time of measurement on vertical distribution of larvae, which may indicate that larval distribution had not stabilized before the end of the trial. However, this effect was significant only at the 0.05 level for the zero-day old larvae.

Shannon diversity for each tank varied substantially between individual tanks (Fig. 3A). However, we observed a significant between-treatment difference in diversity only for zero-day old stage I larvae and for stage IV larvae, both in the predicted direction (higher diversity in the high concentration treatment). On average high concentration diversity exceeded that in low concentration treatments by 0.3 units (0.1 - 0.5, 95% bootstrap percentile CI) for zero day old larvae, and 0.2 units (0.05 - 0.3, 95% bootstrap percentile CI)

higher in Stage IV high concentration treatments compared to low. One-day old larvae showed no significant effect (0.8 - 1.6 times).

As with overall distribution, Shannon diversity also varied strongly between larvae from different mothers in zero-day old larvae, differing by up 0.7 units, an effect size roughly two and half times larger than the effect of increasing concentration (Fig. 3A). Maternal source had no significant effect on diversity in one-day old larvae. However, the estimated diversity effects for each mother correlated strongly between zero-day old and one day old larvae ($r=0.9$). Diversity of location varied with the size of the mother (measured as carapace length), with lower diversity in larvae from larger mothers than from smaller mothers (Fig. 3B).

We did not detect any significant effect of measurement time on average tank diversity for any of the three stages examined.

Horizontal movement of state IV larvae

We observed a total of 223 paths in the low concentration treatment, and 629 paths in the high concentration treatment. Paths were tracked for a median of 26 s in the high concentration treatment, and 28 s in the low concentration treatment. These values varied substantially, with several paths in both treatments lasting for the entire ten-minute period of the video segment.

Overall, we observed a mean estimated long-term diffusion rate of $42 \text{ cm}^2 \cdot \text{s}^{-1}$ with substantial intra-individual variability around this mean ($0.01 \text{ cm}^2 \cdot \text{s}^{-1}$ to $1800 \text{ cm}^2 \cdot \text{s}^{-1}$ (Fig. 4A). Furthermore, diffusion rates differed substantially between high and low concentration treatments, but not in the direction originally hypothesized: diffusion rates were 4.5 times lower (1.2 - 16 times, 95% bootstrap percentile CI) in the high concentration treatment, compared to the low concentration treatment. Differences in the mean cosine of turning angles drove this pattern, as opposed to mean or variance of step length per second (Fig. 4B–D). This result indicates that larvae travelled at similar speeds in both treatments, but followed more tortuous paths in high concentration conditions.

Inter-individual interactions

We detected substantial variation in inter-individual interactions between larvae, with intraspecific attraction more common than repulsion. Of the 64 individual paths (54 from high concentration and 10 from low concentration treatments) that exceeded 3 minutes, an attraction-driven random walk model fit two paths best (one from low concentration, one from high), whereas a repulsion-driven model fit four others best (all from the high-concentration treatment), with the null model best fitting the remainder.

In general, goodness-of-fit tests showed that the random walk model captured the movement dynamics of each path (see Supplementary Section C for details). However, several paths fitted showed poor fit, with substantial long-term autocorrelation of turn angles remaining in the model residuals. This finding indicates that our intraspecific interaction models may not have captured all the features of the fitted larval paths. Even

with these caveats in mind, examination of parameter estimates for individual models offers some utility, as they illustrate average trends in larval movement.

Although parameter estimates within each model class varied substantially among paths (Fig. 5), we typically observed a small degree of intra-specific attraction or repulsion even for superior non-null models ($w_c < 0.5$). Further, attraction or repulsion paths also produced lower estimates of κ than models paths best fit by the null model. As κ determined the between-step variability of movement, this result suggests that larvae exhibiting attraction or repulsion also take more tortuous paths.

For all three models, w_p was bimodally distributed, with values typically either close to one or zero. This result points to two types of behaviour: persistent cycling (w_p close to zero) or constant straight-line movement (w_p close to one). This result matches the two types of movement behaviour previously described in stage IV lobster larvae, where larvae switch between a directional “claws together” swimming mode, and a claws apart, adirectional mode (Cobb et al. 1983). This result is also consistent with a positive correlation between our estimates of w_p and κ ($r = 0.22$), implying more variability in turn angles of larvae not traveling directionally.

Evidence for overall horizontal larval clustering

Although we observed significant differences in larval diffusion rates between high and low concentrations, this result did not translate into differences in the overall spatial clustering of larvae between treatments (Fig. 6). Neither low nor high-concentration treatments showed evidence for either further or closer spacing of larvae than expected, given the observed distribution of larval locations across all trials. This result indicates an essentially random distribution of larvae within each frame across all trials.

Discussion

Our study demonstrated intra-individual variability in *H. americanus* larval behaviour under different encounter rates. We also demonstrated that newly hatched larvae and postlarvae increase vertical dispersion in response to higher concentrations of larvae in the water column. Finally, we demonstrated more diffusive horizontal behaviour at lower concentrations of postlarvae than at higher concentrations, and detected both attractive and repulsive horizontal responses to other larvae in a small subset of individuals measured. However, the individual variability and response of larvae to conspecifics did not scale up to overdispersion in their horizontal distribution, which was close to random in both high and low concentrations.

We observed decreasing vertical clustering at higher concentration in our experimental water columns, as we originally hypothesized. This decrease may indicate repulsive movement in early stage I larvae and postlarvae. However, the effect was weak relative to inter-tank variation in vertical distribution within each stage. We observed the strongest response to concentration in newly hatched larvae. Given the large numbers released at time of hatching (Ennis 1995), moving away from conspecifics may be a mechanism to

avoid cannibalism or predators attracted to aggregations. This strategy would also explain the absence of a concentration response in our one-day old larvae: 24 hours should allow sufficient time to dissipate small-scale clustering of larvae from the same hatching.

These results also highlight the importance of measuring individual larval behaviours as well as aggregate distributions in behavioural movement experiments. We observed differences in overdispersion in larvae from different mothers, and this effect persisted for at least one day: larvae from mothers that produced overdispersed zero-day old larvae also tended to overdisperse as one-day old larvae.

Although we did not design our experiments to test for heritability, and we only examined behavioural variation over a very small vertical range, we nonetheless observed consistent differences between larvae from the same mother in vertical distribution and dispersion in the water column. We also observed lower dispersion in larvae from larger mothers (as measured by carapace length). Although our sample size (five mothers) was too low to test for consistent maternal or heritable effects on larval movement, our results suggest a useful direction for further work. Further, a related study showed substantial variation in horizontal movement behaviour among larval lobsters from different maternal origins (Stanley et al. 2016). Oceanographic models incorporating larval behaviour demonstrate that larvae starting from the same point but at depths differing by only a few meters can settle at very different locations (Hinckley et al. 1996; Fiksen et al. 2007; Vikebø et al. 2007; Paris et al. 2011; Phelps et al. 2015). Heritable variation in vertical movement of lobsters could potentially affect large-scale patterns of connectivity between lobster populations.

We also detected a net change in horizontal diffusion rates from low to high concentration treatments, but a change opposite to what we had predicted. We anticipated that diffusion rates would increase at higher concentrations as a non-directional mechanism of increasing distances among individuals. Instead, we observed a decrease in diffusion. The difference in mean cosines of larval path, rather than either the mean or variance of step length, drove between-treatment differences. This result indicates that larvae moved at similar speeds in both treatments but turned more frequently at higher concentrations, perhaps altering their paths when encountering another larva.

Given that we detected behavioral differences in average diffusion rates between high and low concentrations and the presence of both attraction and repulsion to conspecifics, three factors may explain random aggregate distributions. First, the bulk of larvae appeared not to move toward or away from conspecifics, and approximately equal numbers of larvae were apparently attracted to or repelled from one another. Therefore, averaging out combined effects of some larvae moving towards one another while others avoided each other should not affect the aggregate distribution. Second, larvae may not move toward or away from conspecifics, or our models might have missed such movement (see Supplementary Section C). Third, the tank walls act as a boundary, preventing over-dispersion. Even with higher diffusion rates in the low concentration treatment, the larvae could not spread out further because they could not leave the experimental arena.

Implications for experimental lobster research

Lobster larvae of all stages demonstrate strong behaviour responses to a range of environmental cues, such as vertical responses to light levels (Ennis 1975a), thermocline location (Boudreau et al. 1992), and horizontal responses to light and temperature (Stanley et al. 2016). The experiments demonstrating these responses have typically relied on measuring large numbers of larvae together in a single tank, to build up aggregate measures of responses.

Our work shows that estimates of behavioural responses based on aggregated experimental measurements may underestimate the strength of larval response to environmental cues. Larval experiments typically utilize much higher concentrations than would typically occur in the wild. Surface tows across the lobster's range rarely detect more than 100 postlarvae per 1000 m³ of water (Wahle and Incze 1997; Incze et al. 2000), and earlier larval stages are rarely detected at abundances more than an order of magnitude higher (Harding et al. 2005; Fogarty 1983; Harding et al. 1982). As such, laboratory tests may conflate larval response to a given cue with response to high concentrations of conspecifics. However, our results also indicate that this problem may arise primarily in experiments on very young larvae, or for measurements of horizontal diffusivity.

Our vertical experiments measured aggregate distributions of larvae across a water column, rather than tracking individual larvae. These experiments could not test for consistent differences in movement behaviour among individual larvae. For instance, although larvae often show a characteristic pattern of vertical distribution in response to light at each stage, these distributions vary considerably (Ennis 1975a; Boudreau et al. 1992; Stanley et al. 2016). Because most studies measure vertical distributions (as counts of total numbers of larvae observed at different heights), they cannot determine whether individual larvae vary in vertical position over time, or if that variation reflects differences in which depth stratum each larva would generally choose to occupy in response to light. For instance, Vikebø et al. (2007) used individual based models of larval cod dispersal to show that the interaction between small consistent inter-individual differences in movement behaviour and complex ocean currents can result in larvae following radically different dispersal paths. This result highlights the need to measure intraspecific variability in movement in addition to aggregate patterns in future work.

Variable movement and connectivity

Techniques for modelling realistic patterns of larval transport have advanced substantially over the last two decades, incorporating complex patterns of marine currents (e.g. Xue et al. 2008; White et al. 2010; Chassé and Miller 2010) and larval behaviour (e.g. Incze et al. 2010). However, these models do not account for interactions among dispersers.

Many physical ocean processes aggregate dispersers at a wide range of scales (see Martin (2003) for a review). These mechanisms can keep larvae together for long periods, meaning that larvae travelling in water packets with high concentrations of conspecifics may also compete for suitable environments at settlement and thereafter. Further, these

physical mechanisms could concentrate propagules of multiple species, potentially clustering both food sources (Olson and Olson 1989) and predators (Godø et al. 2012). In this sense, the plankton could act as a dynamic meta-community, with multiple species interacting in patches that constantly break up and rejoin through the action of ocean currents and organism movement. These aggregation mechanisms can substantially increase the strength of density-dependent processes affecting the fitness of dispersing larvae (Pedersen and Guichard 2016).

Larval transport reflects a complex interaction between ocean circulation, the vertical position of dispersing larvae, and the duration of dispersal (Levin 2006; Moksnes et al. 2014). Biophysical simulations of larval transport ideally incorporate both biological and physical factors. The accuracy of biophysical models depends on how well the physical and biological variables within the model reflect natural processes (Metaxas and Saunders 2009). There is mounting evidence that larval American lobsters, and larval decapods in general, do not simply disperse passively and can vary their movement behaviour substantially in response to local oceanographic and ecological conditions. This variation in behaviour can have a strong influence on the outcome of dispersal. For example, vertical migration of larvae over a tidal cycle can alter dispersal outcomes relative to larvae maintaining a fixed position (selective tidal stream transport; DiBacco et al. 2001). Simulations of larval Atlantic cod dispersal demonstrated that small differences in vertical position coupled with vertically stratified circulation significantly changed predicted dispersal kernels (Fiksen et al. 2007).

Although a comprehensive review of how larvae vary behaviour in response to their local conditions is beyond the scope of this discussion (see Queiroga and Blanton (2004) for a review), we note that decapod larvae generally exert some control over their horizontal and vertical movement, and in response to multiple cues such as temperature, depth, and food availability. Larvae presumably vary their movement to improve their own fitness, through foraging, predator avoidance, and habitat selection. If environment-fitness relationships correlate strongly within species, larval behaviour should generally aggregate larvae more strongly than passive marine currents in the absence of concentration-dependent avoidance or inter-individual variation in responses to cues ("bet hedging", sensu Crean and Marshall (2009)).

Even simple behavioural responses, such as increasing diffusive swimming (Harrison et al. 2013) or changes in vertical distribution (Fiksen et al. 2007), may substantially reduce how strongly ocean currents cluster larvae. Spatial scales and patterns of clustering vary by taxa depending on relative swimming ability (Daigle et al. 2014), indicating that species-specific and concentration-dependent behaviours may be driving patterns of spatial clustering. The response of individual movement rates to the presence of conspecifics, as revealed by our study, may influence connectivity of adult populations over time and merits further research.

Scaling from laboratory behaviour to behaviour to patterns of large-scale dispersal

Our study demonstrates that newly hatched lobster larvae increase their vertical dispersion in the presence of higher concentrations of conspecifics. Furthermore, we demonstrate that stage IV larvae increase the rate at which they change direction at higher concentrations (although this increase did not affect the average degree of over-dispersion in the experimental tanks). However, we do not suggest our study offers an accurate estimate of the magnitude of these effects in the field.

The main issue with scaling these responses to the field is that even our low concentration treatments likely greatly exceed natural concentrations, and we measured responses over very short timescales relative to the time scale of dispersal. Furthermore, we conducted our experiments in a well-lit environment over short distances, providing larvae strong visual cues on locations of other larvae. While this was necessary to be able to track the larvae with video, given the cannibalistic tendencies of lobster larvae, their behavioural responses may have reflected larvae alternately hunting one another and moving away to avoid predation. Lobster larvae are not purely visual predators given that they obtain much of their food at night (Juinio and Cobb 1992), however they do visually detect, pursue, and attack potential prey (Herrick 1909). Strong anti-predator responses also occur in postlarvae treated with predator scent (Boudreau et al. 1993). Although no study has measured rates of visual predator avoidance in *H. americanus*, we observed many pairs of post-larvae engaging in chase and evasion behaviour in our horizontal trials (personal observation).

Summary

We have shown that lobsters change their movement in the presence of conspecifics, which future studies of larvae behaviour should consider. Future work should focus on understanding how larvae change their behaviours across a range of concentration, how the presence of conspecifics changes larval responses to other environmental cues, and how to incorporate these responses into large-scale models of larval connectivity (e.g. Incze et al. 2010; Chassé and Miller 2010). Our approach of tracking individual larvae and using random-walk models to assess behavioral response to outside stimuli, holds substantial promise for understanding what factors drive larval movement across scales, and measuring variation in behavioural responses to environmental cues.

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Tables

Analysis of variance of dissimilarities between vertical distributions of larvae within each stage.

	Treatment	df	F statistic	R ²	Pr(>F)
Stage I: 0 day old	time period	1	3.14	0.03	0.04
	mother	4	15.24	0.61	<0.01
	concentration	1	3.05	0.03	0.04
Stage I: 1 day old	time period	1	0.72	0.02	0.5
	mother	4	2.01	0.23	0.05
	concentration	1	0.76	0.02	0.5
Stage IV	time period	1	0.79	0.04	0.5
	concentration	1	1.39	0.07	0.2

Figures

Fig. 1. Paired 20 x 10 x 120 cm experimental tanks, marked in 10 cm increments. (B) Top-down view of flume arena for horizontal movement trials. Experimental area was in a 50 cm wide flume, with 10 cm deep water. Arena ends were blocked off by mesh barriers, 75 cm from each other (in grey). An overhead camera recorded larval paths for 30-minute periods.

Fig. 2. Mean vertical distribution of larvae in experimental tanks, ± 1 st. dev. The value at a given column height indicates the mean fraction of total larvae in the tank, found between that height and the next 10 cm increment.

Fig. 3. Shannon diversity (unitless) of distribution of larvae in vertical tank experiments. Each point denotes Shannon diversity in a given tank at a given time. (A) Diversity vs. treatment by stage and mother. Lines connect tanks measured at the same time with high and low concentrations of larvae. Dashed lines indicate diversity measured in the first 15 minutes, and solid lines denote measurements from the end of the trial. (B) Diversity vs.

maternal carapace for stage I larvae. Lines indicate best-fitting linear regression slope of diversity on maternal size.

Fig. 4. Estimated long-term horizontal diffusion rate (A) and components affecting the estimate of the diffusion rate (B--D) for each path observed in videos. Each grey point represents an estimate for a path, and the black point and lines are the (geometric) means and standard deviations.

Fig. 5. Random walk model parameter estimates ± 1 s.e., inverse transformed to each parameter's original scale. Each point represents one larval path. Error bars across the whole interval indicate a parameter that was not able to be estimated with high precision given the data. Hollow circles indicate a parameter with a zero value in its Hessian entry, preventing calculating any standard error for it. (A) Estimates for paths best fit by the model without larval interactions. (B) Estimates for paths best fit by interaction models.

Fig. 6. Probability distributions of distances between individuals present in the same frame (grey histogram) versus the null distribution (black line). Lower concentration at short distances relative to the null indicates under-dispersion, with individuals further apart than expected. Lower concentration at high distances than the null represents clustering, with individuals in the same frame occurring closer together than expected. Top: distributions for the five low concentration videos. Bottom: distributions for the high-concentration videos.

A

B

column height (cm)

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Larval concentration • low • high