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 long pelagic durations, such as American lobster (*Homarus americanus*), 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. Further, we found that larvae from different mothers consistently differed in vertical distribution, which may indicate maternal effects on dispersal behavior. 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

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Introduction

Many marine benthic invertebrates spend their adult lives as either sedentary individuals or moving slowly across the seafloor. 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,

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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*, where cannibalistic larvae of all stages readily attack any conspecifics they detect (Herrick, 1909), aggregation presumably offers little benefit. Further, as in many other meroplanktonic species, larval 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 this 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)

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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 behavioral 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.

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 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). Consistent behavioural differences between larvae from different parents are paticularly interesting, because heritable variability in dispersal traits can shape large-scale population dynamics and patterns of connectivity (Phillips et al., 2008, Clobert et al., 2009), effects that would be missed in large-scale dispersal simulations assuming identical larval behaviours (e.g. Katz et al., 1994, Incze et al., 2010). Heritable variation in dispersal behaviours have been observed in a wide variety of taxa (Zera and Brisson, 2012) but remains largely unstudied in larval studies of meroplanktonic species.

For most meroplanktonic species, little data exist either on movement in response

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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). 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.

Female lobsters brood their eggs for 9-12 months before releasing hatchlings as free swimming larvae, up to 2000 larvae at a time (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 upwards of 100 km before settling. Behavioural studies suggest that all four lobster larval stages can actively mediate their vertical position in the water column (Ennis, 1975), however, the post-larvae are also strong horizontal swimmers (Ennis, 1986, Cobb et al., 1989).

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 potentially heritable variation in dispersal behaviours.

Our second experiment recorded horizontal swimming behaviour of small groups of

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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, 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 the null model. 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 the ports of Port au Choix and Red Harbour in Newfoundland, Canada in June of 2010, under Fisheries and Oceans Canada experimental license NL-1339-12. The females were held in individual tanks at the Ocean Sciences Center 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 mother 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-

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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 *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 by eye. 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, 1975).

For each of the stage I trials, all larvae in a given trial came from a single mother. This strategy allowed 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. However, as daily larval mortality was high, our larval pool was reduced to individuals from two of the mothers for the second and third days for a single trial each, resulting in 8 trials for these stages.

Larvae were equilibrated for half an hour at 15 °C, then placed in two 120 cm tall

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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® 25 w '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, 2015). 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 rel-

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ative 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 to determine a pseudo F-ratio, then permuted the labels on each observation and reran the process multiple times. We used these permutations to calculate a null distribution of pseudo F-ratios for model p-values (Anderson, 2001). The permutation method used 10000 permutations for each analysis to test how frequently the observed difference in dissimilarities between treatments would arise assuming no relationship between the treatment and observed dissimilarity. Within each developmental stage, we regressed dissimilarity on three different factors:

1. Time period, to determine whether systematic within-stage differences occurred between the distribution of larvae between observation times.

2. Identity of the mother, to test for systematic 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. Concentration of larvae in the tank, to determine whether increasing intraspecific encounter rates produced systematic effects on the vertical distribution of larvae.

Because the analysis of variance of dissimilarities only tested whether the two treatments differed and not the factors responsible for that difference, we used mixed-effect

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modelling to determine how treatments differed. Given that we were testing to determine 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, varying from zero if every individual was found in the same depth stratum, to a maximum of $ln(n^{-1})$ if there are n strata and the same number of individuals occurred at every stratum in the tank (Lande, 1996). Shannon diversity of a given tank i was defined as:

$$E_i = -\sum_j p_{i,j} ln(p_{i,j}) \quad (1)$$

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 for mixed effect modelling, version 1.1-7 (Bates et al., 2014), assuming a normal distribution in residual Shannon diversity with equal variances for each maternal source and treatment within an age class. Treatment effects were compared using parametric bootstrapping to determine the distribution of differences between levels. The parametric bootstrap procedure estimates uncertainty in a parameter (Bates et al., 2014) by first assuming an appropriate model has been chosen (i.e., correctly specified errors and estimates of the predictor values), and then simulating new data from that model many times. For each simulation, we assumed the same numbers of each fixed factor (mother, time step, and concentration treatment) as observed, and that each value would have the same mean as the estimated model. We then added a new random effect and residuals from individual-level observations to this mean value. For each replicate simulation of the data, we refit the full model and used

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the resulting distribution of estimates for each fixed effect to build confidence intervals for that parameter. Here we used percentile confidence intervals: for a given parameter, the 95% CI encompassed the range from the 2.5% percentile to the 97.5% percentile of the distribution of parameter estimates from the simulations.

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. We chose to use the flume because it provided a suitably large experimental "arena" surrounded by water of the same temperature. Screen barriers (100 um 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® 25 w) 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 $\sim$ 10 pixels mm^{-1} at 30

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frames per second (fps).

Prior to analysis, we reformatted videos as uncompressed avi files using the software Avidemux 2.6.8. Each video was then broken into three ten minute parts and sub-sampled to 10 fps using Matlab ® R2014a, because the original files were too large to process as single blocks.

Files were loaded into the image processing software ImageJ ® (Schneider et al., 2012) (see Fig. 2A for the video processing applied to a sample frame). We then cropped videos to remove side walls and lighting artifacts at the video edges (Fig. 2B). We thresholded each frame to set any pixel with a saturation value below 148 (where saturation ranged from 0 to black to 255 for white) as black and all other pixels to white. This approach removed the light background (Fig. 2C). We used the ImageJ CASA plugin, designed to follow sperm movement in videos (Wilson-Leedy and Ingermann, 2007), to detect tracks and extract coordinates of individual larvae in each video. Because the software was unable to track larvae perfectly and larvae often moved to the edge of the frame outside the cropped area, we could not associate individuals with a single unique path. However, the software often tracked larvae for several minutes at a time (Fig. 2D). After files were processed in CASA, we excluded any paths in the 40-pixel region on the bottom edge of the frame from further analysis because lighting artifacts created too many spurious paths. Any paths that passed through this region were split into new paths at the point where they entered. Finally, we removed any paths recorded for fewer than 100 frames (10 seconds) or with maximum displacement from their start point of 50 pixels or less ($\sim$ 5 cm), to remove potentially spurious paths.

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

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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). This approach can account for a wide range of different behaviours and inter-individual interactions, and can be used to predict population-level parameters such as average rate of diffusion in a population under a given set of conditions (Turchin, 1998, Méndez et al., 2014).

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. The long-term rate at which mean-squared displacement (MSD) of an individual from its starting point increases with time provides one measure of increase in spread. MSD typically increases linearly with time, assuming finite variance of step lengths. The slope of the time-MSD relationship represents the diffusion coefficient (Méndez et al., 2014). 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

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bias or turning angle bias (Kareiva and Shigesada, 1983). We showed previously that this method effectively captures patterns of mean squared displacement in larval lobsters under similar experimental conditions (Stanley et al., 2016). We sub-sampled each path to one frame per second, to reduce correlation between turn angles in each step and estimated the diffusion coefficient for path i as:

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

Here, $\sigma_{l,i}^2$ denotes the sample variation of step distances ($\text{cm}^2 \cdot \text{second}^{-1}$), $\bar{l}_i$ was the mean step distance ($\text{cm} \cdot \text{second}^{-1}$), 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., 2014). 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. Our null expectation was that individuals at both high and low concentrations would have similar diffusion rates. Deviations away from this null model indicated how larval behaviour changed under different conspecific concentrations.

Random walk models of intra-individual attraction or repulsion

To test whether conspecifics attracted or repelled individual larvae, we fit random walk models to each larval path. We used an autocorrelated random walk model where the angle of the step each larva took from one frame to the next persisted (based on the angular correlated random walk model of Shimatani et al., 2012). The random walk model included three key parameters: w_p, w_c and κ (see Appendix A 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

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346 $w_p = 1$ corresponded to the case where a larva moved in loops with the same turning
347 speed over time. We included this parameter to model the persistent looping behaviour
348 observed in larval movement paths (Fig. 2).

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

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

357 We fit the three models to all larvae with a path consistently recorded for three min-
358 utes or more, to ensure paths sufficiently long to produce a reliable estimate of model
359 coefficients. We sub-sampled larval paths to one frame per second, to reduce the correla-
360 tion of turn angles with steps further in the past. For each path, we estimated all three
361 models using maximum likelihood. We logit-transformed w_c and w_p, and log-transformed
362 κ prior to fitting, to ensure the parameters were unbounded to avoid issues with bounded
363 optimization. The Nelder-Mead algorithm in the *optim* function for R 3.1.2 (R Develop-
364 ment Core Team, 2015) estimated the maximum likelihood value for each model for each
365 path. We estimated standard errors for each parameter as the diagonal of the inverse
366 Hessian of the negative log-likelihood for that fit (Bolker, 2008).

367 We used a permutation test to determine if each path was better fit by attraction,
368 repulsion, or the null model (no interactions). For each path, we calculated the difference
369 in log-likelihood between the null and each interaction model. We then generated a
370 distribution of likelihood differences by shuffling the order of observations of angles to the
371 centroid 500 times while retaining the same order of absolute and relative angles, then re-

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fit the null model and both interaction models to the new paths. For each simulated path, we calculated the difference in log-likelihood between the null and interaction models. We then compared what fraction of simulated paths had a log-likelihood difference greater than that observed in the data, giving us a p-value of observing that large a deviation when the null model was true. 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 (Appendix A). We finally tested whether possible attractive or repulsive behaviours detected in the movement paths resulted from bounding larvae within a fixed arena where they could not move far from one another (Appendix B).

Variation in larval spatial distribution

If individuals actively moved 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 compared the distribution of distances between larvae observed in each frame to the distribution of distances drawn from a null model: the set of all observed larval locations. More weight at short distances in the observed distribution compared with the null model would indicate clustering, whereas more weight at long distances would indicate over-dispersion (Bonetti and Pagano, 2005).

For each frame where our program detected more than one larva, we calculated the distance from each larva to every other larva in the frame. This calculation provided our observed distribution of inter-individual distances. To determine the probability of a given observed distance in the absence of clustering or avoidance, we generated a null distribution of distances. We drew 1000 samples of larval coordinates randomly from those observed across all videos and calculated the distance between each pair of draws.

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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. 3). The bimodally phototactic zero-day old stage I larvae either moved to the top or bottom of the tank (Fig. 3A). One-day old larvae almost always occupied the bottom 10 cm, only occasionally moving to higher depth strata (Fig. 3B). 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. 3C-E). Finally, stage IV returned to a bimodal distribution, with the bulk of the larvae at the top or bottom of the tanks (Fig. 3F). 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. 4). However, we observed a significant between-treatment difference in diversity only for

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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 (0.1 - 0.5, 95% bootstrap percentile CI) units for zero day olds, 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. 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). This correlation may indicate heritable or maternal effects on larval vertical dispersion.

We did not detect any significant effect of measurement time (first versus last 15 minutes) 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 seconds in the high concentration treatment, and 28 seconds 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 cm^2sec^{-1} with substantial intra-individual variability around this mean (0.01 cm^2sec^{-1} to 1800 cm^2sec^{-1}) (Fig. 5A). Furthermore, diffusion rates differed substantially between high and low concentration treatments, but not in the direction originally hypothesized: diffusion rates

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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. 5B-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 Appendix B for details). However, several paths fitted showed poor fit, with substantial long-term auto-correlation 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. 6), 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.

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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, a-directional 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 diffusion rates between concentration treatments, this result did not translate into differences in the overall spatial clustering of larvae between treatments (Fig. 7). 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

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494 scale up to overdispersion in their horizontal distribution, which was close to random in
495 both high and low concentrations.

496 We observed decreasing vertical clustering at higher concentration in our experimen-
497 tal water columns, as we originally hypothesized. This decrease may indicate repulsive
498 movement in early stage I larvae and post-larvae. However, the effect was weak relative to
499 inter-tank variation in vertical distribution within each stage. We observed the strongest
500 response to concentration in newly hatched larvae. Given the large numbers released at
501 time of hatching (Ennis, 1995), moving away from conspecifics may be a mechanism to
502 avoid cannibalism or predators attracted to aggregations. This strategy would also ex-
503 plain the absence of a concentration response in our one-day old larvae: 24 hours should
504 allow sufficient time to dissipate small-scale clustering of larvae from the same hatching.

505 These results also highlight the importance of measuring individual larval behaviours
506 as well as aggregate distributions in behavioural movement experiments. We observed
507 large differences in vertical overdispersion in larvae from different mothers, and this effect
508 persisted for at least one day; larvae from mothers that produced overdispersed zero-day
509 old larvae also tended to overdisperse as one-day old larvae. The large degree of variabil-
510 ity in vertical dispersion among larvae from different mothers has interesting implications
511 for lobster dispersal. We showed significant variability in the degree of variation in verti-
512 cal distribution among larvae from different mothers, and consistent variability, at least
513 between newly hatched and one-day old larvae.

514 Although we did not design our experiments to test for heritability, and we only
515 examined behavioural variation over a very small vertical range, our results nonetheless
516 suggest a useful direction for further work. Oceanographic models incorporating larval
517 behaviour demonstrate that larvae starting from the same point but at depths differing by
518 only a few meters can settle at very different locations (Hinckley et al., 1996, Fiksen et al.,
519 2007, Vikebø et al., 2007, Paris et al., 2011, Phelps et al., 2015). Heritable variation in

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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 Appendix B). 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, 1975) or thermocline location (Boudreau et al., 1992). The experiments demonstrating these responses have

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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 m^3 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.

Both our own and prior larval experiments measured aggregate distributions of larvae across a water column, rather than tracking individual larvae. As such, 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, 1975, Boudreau et al., 1992). 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.

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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, Chassé and Miller, 2010, White et al., 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 as they move (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, in review).

However, even simple behavioural responses, such as increasing diffusive swimming (Harrison et al., 2013) or changes in vertical distribution (Fiksen et al., 2007), may substantially affect how 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.

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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 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

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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. Katz et al., 1994, Incze et al., 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

	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

Table 1: Analysis of variance of dissimilarities between vertical distributions of larvae within each stage.

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Figures

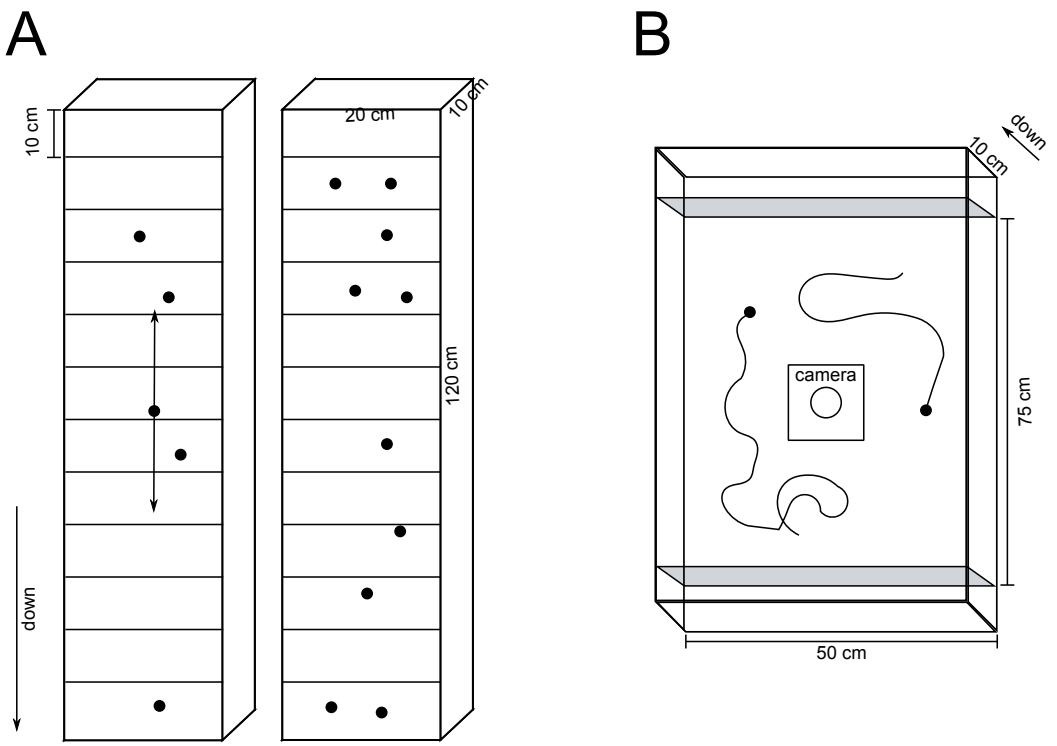

Figure 1: Experimental set-up for vertical (A) and horizontal (B) swimming trials. A) Paired 20 x 10 x 120 cm experimental tanks, marked in 10 cm increments. B) Top-down view of flume arena for horizontal movement trials. Experimental area was in a 50 cm wide flume, with 10 cm deep water. Arena ends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

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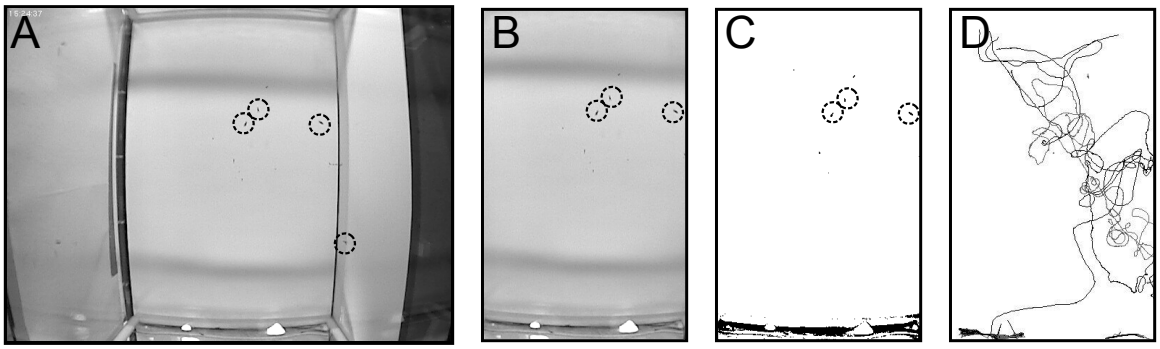

Figure 2: Example of video processing of horizontal movement videos. Dotted circles indicate larval positions. A) Original frame from a low-density concentration treatment, with four larvae visible (fifth larvae obscured in this frame). The same frame after B) cropping and C) thresholding. D) final paths, extracted by CASA program.

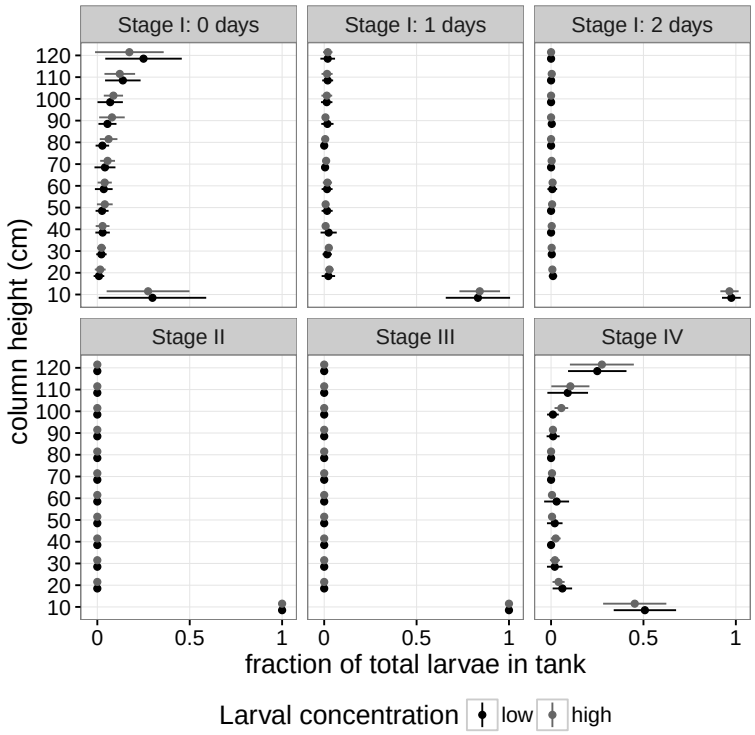

Figure 3: Mean vertical distribution of larvae in experimental tanks, ± 1 st. dev. The value at a given column height indicates the mean fraction of total larvae in the tank, found between that height and the next 10 cm increment.

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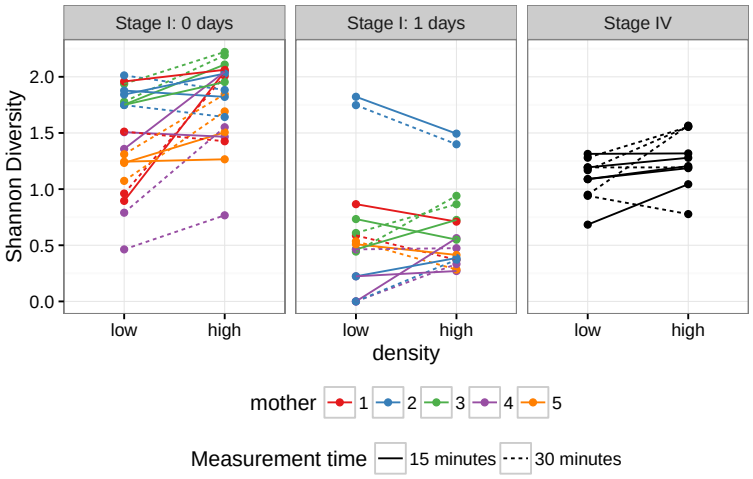

Figure 4: Shannon diversity (unitless) of distribution of larvae in vertical tank experiments. Each point denotes Shannon diversity of counts of larvae per 10 cm segment in a given tank, at a given time. Lines connect tanks measured at the same time with high and low concentrations of larvae. Dashed lines indicate diversity measured in the first 15 minutes, and solid lines denote measurements from the end of the trial.

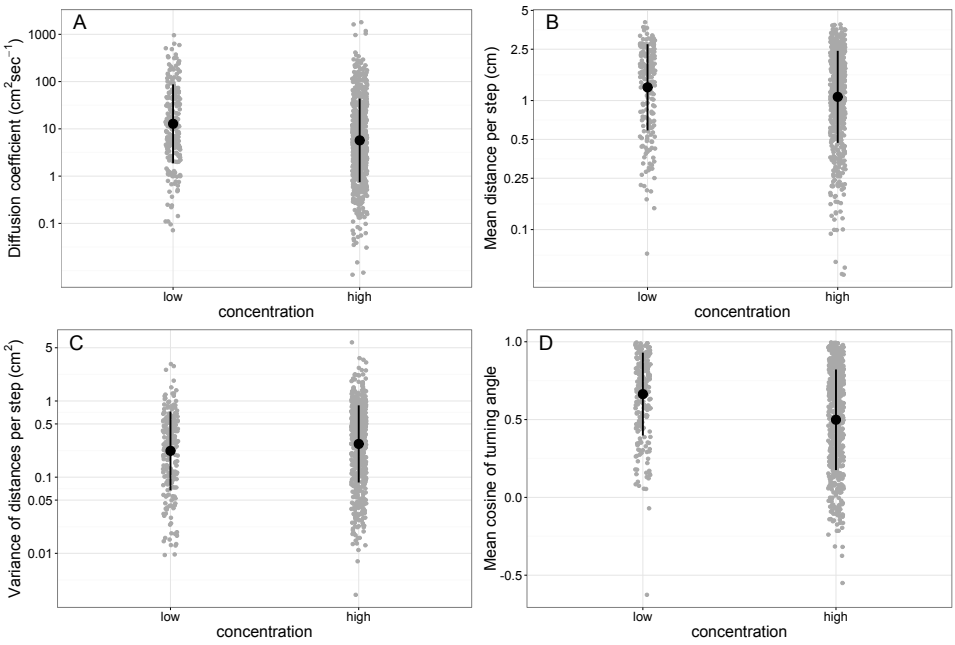

Figure 5: Estimated long-term horizontal diffusion rate (A) and components affecting the estimate of the diffusion rate (B-D) for each path observed in videos. Each grey point represents an estimate for a path, and the black point and lines are the (geometric) means and standard deviations.

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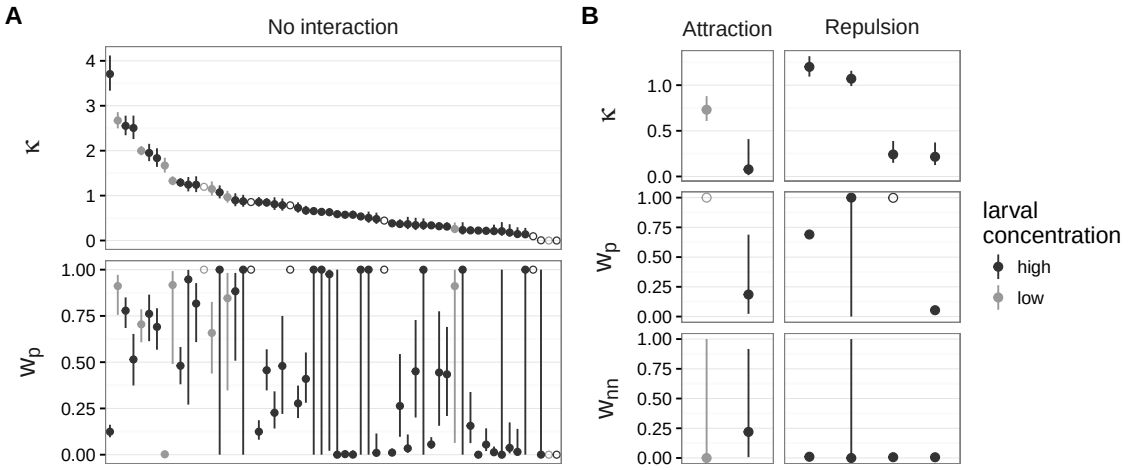

Figure 6: Random walk model parameter estimates ± 1 s.e., inverse transformed to each parameter's original scale. Each point represents one larval path. Error bars across the whole interval indicate a parameter that was not able to be estimated with high precision given the data. Hollow circles indicate 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.

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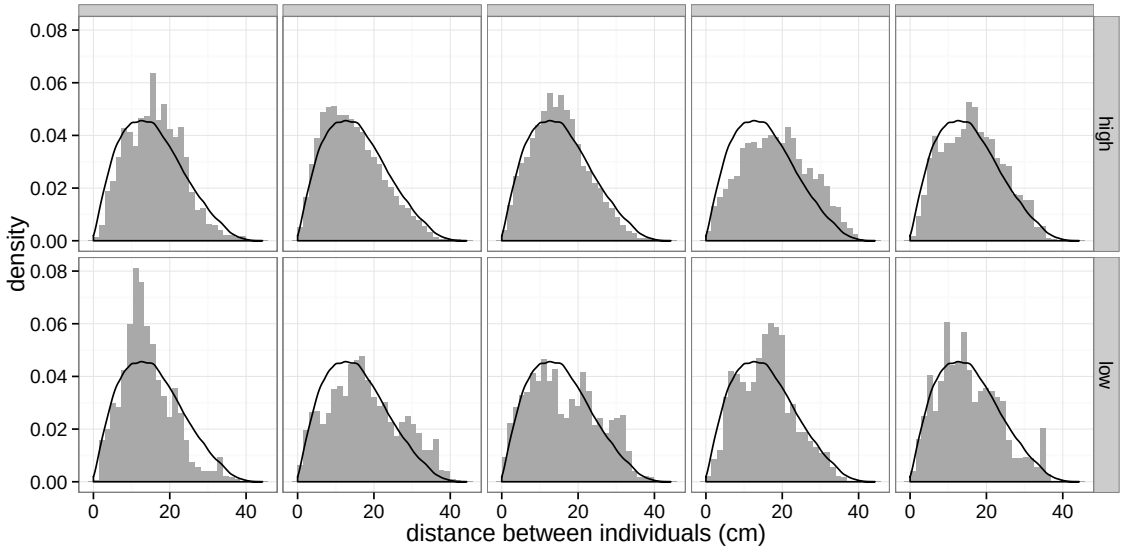

Figure 7: Probability distributions of distances between individuals present in the same frame (grey histogram) versus the null distribution (black line). Lower concentration at short distances relative to the null indicates under-dispersion, with individuals further apart than expected. Lower concentration 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.

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Appendix A: Circular auto-regressive models with larval interaction and turning angle persistence

The random walk models we used were a modified form of the circular auto-regressive model described by Shimatani et al. (2012). The models were designed to account for three features of larval lobster movement:

1. Directional persistence: larvae tended to move in a relatively constant direction (see Fig. 2D);
2. Turning persistence: Larvae frequently looped, constantly turning in a single direction for several frames (see Fig. 2D);
3. Attraction or repulsion to neighbouring larvae;

The circular auto-regressive model assumed that, for each time-step t, individual i had a preferred absolute $\bar{\theta}_{i,t}$ direction (from 0 to 2π), where absolute direction was measured from the the x-axis, counter-clockwise (Fig. A1A). The actual direction of step t, $\theta_{i,t}$, was distributed around $\bar{\theta}_{i,t}$ following a Von Mises (circular normal) distribution: $\theta_{i,t} \sim VonMises(\bar{\theta}_{i,t}, \kappa_i)$, where κ_i denotes the concentration parameter for individual i. If $\kappa_i \to \infty$, then larva i always move in its preferred direction, whereas if $\kappa \to 0$, the angle of each step for larva i was entirely random.

Three factors could affect $\bar{\theta}_{i,t}$ in the models: the absolute angle of the previous step, $\theta_{i,t-1}$, the relative angle of the previous step, $\psi_{i,t-1}$ (how much the angle of the previous step deviated from the step before it; $\psi_{i,t-1} = \theta_{i,t-1} - \theta_{i,t-2}$; Fig. A1A)), and the angle to the centroid of the locations of other larvae detected in the previous time step, $\rho_{i,t-1}$ (Fig. A1B). We chose movement toward or away from the centroid as a metric of attraction or repulsion because moving away from the centroid would maximize the mean distance

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between that larva and all others. We used the link function, L, described in Shimatani et al. (2012) to combine the interacting effects of these different drivers:

$$L(x, a, w) = a + 2 \cdot tan^{-1}(w(tan(\frac{x-a}{2})) \tag{3}$$

This function interpolated between two angles, x and a. If $w = 1$, then $L(x, a, 1) = x$, if $w = 0$, $L(x, a, 0) = a$, and when $0 < w < 1$, L would have a value between a and x.

We fit three different models to each path:

1. The null model of no interactions:

$$\begin{aligned} \bar{\theta}_{i,t} &= L(\theta_{i,t-1}, \theta_{i,t-1} + \psi_{i,t-1}, w_{p,i}) \\ \theta_{i,t} &\sim VonMises(\bar{\theta}_{i,t}, \kappa_i) \end{aligned} \tag{4}$$

2. An attraction model, where a larva tends to move in the direction of the centroid of the larvae around it:

$$\begin{aligned} P_{i,t} &= L(\theta_{i,t-1}, \theta_{i,t-1} + \psi_{i,t-1}, w_{p,i}) \\ \bar{\theta}_{i,t} &= L(P_{i,t}, \rho_{i,t}, 1 - w_{c,i}) \\ \theta_{i,t} &\sim VonMises(\bar{\theta}_{i,t}, \kappa_i) \end{aligned} \tag{5}$$

3. A repulsion model, where a larva tends to move in the opposite direction of the centroid:

$$\begin{aligned} P_{i,t} &= L(\theta_{i,t-1}, \theta_{i,t-1} + \psi_{i,t-1}, w_{p,i}) \\ \bar{\theta}_{i,t} &= L(P_{i,t}, \rho_{i,t} - \pi, 1 - w_{c,i}) \\ \theta_{i,t} &\sim VonMises(\bar{\theta}_{i,t}, \kappa_i) \end{aligned} \tag{6}$$

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In all three models, the parameter w_p determined how strongly the larval path is biased towards either its previous course or by relative angle of its previous step (that is, its tendency to loop); If w_p approached one, the the larva continued in the same direction, and if it approached zero, it continued turning at the same rate (Fig. A1 C,E,G).

The parameter w_c in models 1&2 determined how strongly previous movement determines the path of a larva as opposed to the direction to the centroid (c) of the other larvae present. When w_c approached zero, the direction towards the centroid would not affect direction of larval movement. When w_c approached one, the larva tended to move directly towards the centroid for model 2, or directly away from it, for model 3 (Fig. A1 D,F,H).

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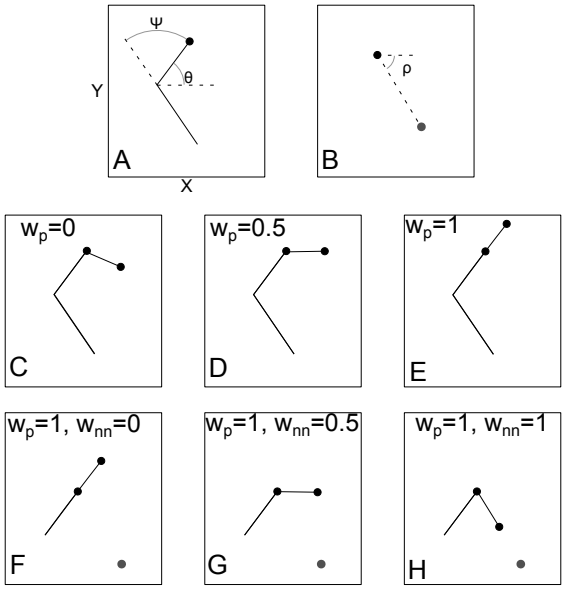

Figure A1: Parameters of the random walk model with intra-specific interactions. Solid lines denote steps in a larval movement path. A) Movement angles for step i: The absolute angle from the x axis (θ), and relative angle(ψ), the difference between the current and last absolute step angles. B) Absolute angle to the point of attraction (grey circle) of the rest of the larvae present (ρ). This angle would represent the centroid of the other observed larvae in an attractive model, or the opposite direction of the centroid in a repulsive model. C-E) effect of varying the persistence parameter, w_p, on the expected angle of the next step. Increasing w_p leads to angles more biased towards the absolute angle of the last step, and decreasing it leads to angles more biased towards the absolute plus relative angle of the last step (maintaining constant turning rates). F-G) Effect of varying the attraction parameter (w_c) on the expected angle of the next step (assuming $w_p = 1$ for simplicity). When w_c is zero (F) the expected angle points in the direction the larva would travel if the other larvae were absent. As w_c increases (G,H), the expected angle is pulled toward the point of attraction.

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Appendix B: Goodness-of-fit tests of horizontal movement models

Model selection procedures, such as likelihood ratio tests or information criteria, will tell which of a set of candidate models best fit the observed data, for some measure of fit (Johnson and Omland, 2004). However, even the best fitting model of a set of may be poorly specified. Goodness-of-fit tests are an important compliment to model selection, as they tell us which aspects of the data generating process our model is not effectively capturing (Mayo, 1996). Goodness-of-fit testing measures the absolute degree to which a given model accurately captures specific features of the data. Goodness-of-fit tests generally work by generating new sets of simulated data using the fitted model, and measuring how frequently the given feature would occur (Spanos, 2011).

We used several goodness-of-fit tests to determine how accurately our attraction and repulsion models were capturing patterns in larval movement. We first tested how likely it would be to observe the fitted summed likelihood for each model, as a simple test of overall goodness-of-fit. We then tested whether the residual absolute and relative angles for each path were well-described by a Von-Mises distribution with the fitted κ parameter, to determine if the models were missing substantial directional biases. We tested how well the best fit model for each path explained the observed patterns of angular autocorrelation of absolute and relative angles for each path. If the model is accurately capturing the temporal dependence in movements, there should not be any substantial correlation between residual angles from one time-step to the next. Finally, we tested whether our model would falsely detect attractive behaviours if individual larvae were merely turning away from the boundary of the test chamber.

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Global goodness of fit tests

We tested overall goodness-of-fit by comparing the observed log-likelihood of our model to that of paths simulated from the fit parameters. The fitting procedure for each path i was:

1. Determine the number of points in the path (n), and the fitted values of κ_i, $w_{p,i}$ from the model that best fit path i, as well as $w_{c,i}$ for those models best fit by either attraction or repulsion;

2. Simulate 100 new paths, using the fitted parameters from 1 and the observed sequence of nearest neighbour angles for path i;

3. Fit a null (Eq. 4), attraction (Eq. 5), and repulsion (Eq. 6) model to each simulated path, j;

4. For each simulated path, j, determine which model best fits using AIC;

5. Extract the summed log-likelihood for the true path, L_i, and for each simulated path, L_j;

6. Calculate the p-value as the fraction of simulated paths with log-likelihoods lower than the observed paths ($L_j < L_i$).

This procedure tells us how frequently we would see a log-likelihood as low as that observed, if the model were true. If the overall model fit very poorly, each individual path would have a low log-likelihood relative to the simulated paths. We observed that for all paths, the p-value of observing the actual log-likelihood was greater than 0.05, meaning that no individual path was extremely unlikely to occur (Fig. B1).

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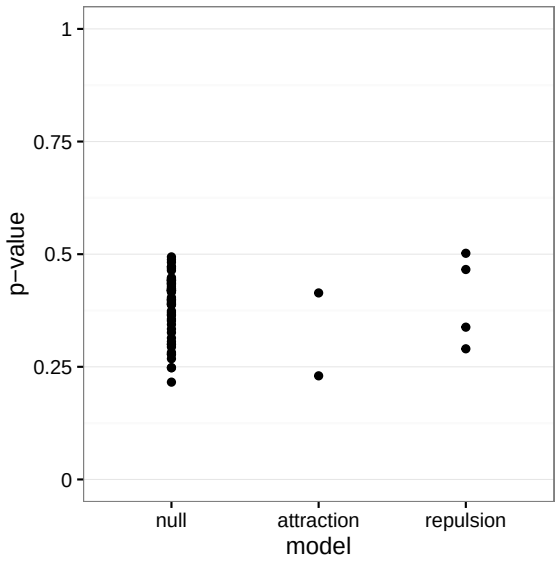

Figure B1: P-values of log-likelihoods of random walk models. Each point is the p-value of observing a log-likelihood at least as small as what was observed for a given path, assuming the model was true. P-values calculate as per text.

Testing for misspecification of the Von Mises distribution

There are, however, many ways in which this model could fit poorly and still pass the previous test. Our next set of tests looked at how well the distribution we assumed for the residuals, the Von Mises distribution, fit the observed pattern of steps. The Von Mises distribution is a symmetrical unimodal distribution, where the probability density of angles around the mean angle is determined purely by the concentration parameter κ (Shimatani et al., 2012). If this accurately described the path data, the distribution of residuals for each path should follow this distribution. To test this, we extracted the residuals for both absolute and relative angles for each path, i, using the following procedure:

1. Extract the estimated values of κ_i, $w_{p,i}$ and $w_{c,i}$ from the model that best fit path i;

2. Use the fitting equations (Eq.4, 5, or6) as well as the observed sequence of angles

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939 to the centroid of other larvae to calculate what the mean angle for each step t for
940 path i would be: $\bar{\theta}_{i,t}$;

941 3. Calculate the residual of the absolute angles as $\epsilon_{i,t,abs} = \theta_{i,t} - \bar{\theta}_{i,t}$;

942 4. Calculate the residual of the relative angles as $\epsilon_{i,t,rel} = \theta_{i,t} - \theta_{i,t-1} - \bar{\theta}_{i,t}$;

943 5. Remap each set of residuals so that all residual angles lie between $-\pi$ and π,
944 using the two-argument arctan function: $\epsilon_{i,t,abs} = atan2(sin(\epsilon_{i,t,abs}), cos(\epsilon_{i,t,abs}))$,
945 $\epsilon_{i,t,rel} = atan2(sin(\epsilon_{i,t,rel}), cos(\epsilon_{i,t,rel}))$;

946 6. For each residual, calculate how likely it is to occur using the Von Mises distribution
947 centered at zero with a dispersion parameter κ_i;

948 The Von Mises distribution fit well for most paths for both absolute (Fig. B2) and
949 relative (Fig. B3) distributions of residuals. No path showed signs of multimodal or
950 strongly skewed residuals for either distribution. However, for both absolute and relative
951 residuals, the distributions were more concentrated around zero, with longer tails than
952 the Von Mises distribution predicted, given the estimated value of κ. This indicates that
953 the actual step angle distribution may be more long-tailed than our model suggests.

954 Testing for unexplained autocorrelation in turn angles

955 Our third set of goodness-of-fit tests look at how well these models capture the time series
956 dependence in the pattern of turn angles in our data. One of the standard tools to do this
957 for time series is the auto-correlation function, which measures how strongly correlated
958 data points in the time series are as a function of the time lag separating the points are.
959 If the observed values of a given model show strong auto-correlation at multiple lags but
960 the residuals of a given model fit to that data do not show any strong auto-correlations,
961 this is evidence that the model is effectively capturing the dependence structure in the

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time series. Model residuals should be unpatterned in a well-specified model (Spanos, 2011).

However, the standard auto-correlation function is not usable for angular data, as angles wrap around: while the Pearson correlation would assume that $-\pi$ and π are different values, they are equivalent angles. Instead, we make use of the function proposed by Fisher and Lee (1983), $\rho_T(\theta_1, \theta_2)$ to calculate the circular correlation between the two sets of angles, θ_1 and θ_2. This function preserves most of the basic properties of the Pearson correlation coefficient: it ranges between -1 and 1 and is zero only when there is no linear dependence between θ_1 and θ_2. Further, positive values of ρ_T indicate that angles in the two sets increase together, and negative values indicate that higher angles in one set are associated with lower angles in the other (see Fisher and Lee (1983) for more details on this function).

For a given time series of angles i, and a given lag value, τ , the angular auto-correlation value for the series was the angular correlation between each angle and the angle τ steps ahead in the series: $\rho_T(\theta_{i,t}, \theta_{i,t+\tau})$. We calculated the angular auto-correlation function for the first 20 lags for the following time series: the observed absolute and relative angles of each path, and the residual absolute and relative angles (as calculated above).

For each series of residuals, we calculated the 95% confidence intervals for the auto-correlation value at a given lag by drawing 1000 sets of n points (where n is the number of steps in the series) from a Von Mises distribution with zero mean angle and κ equal to the value estimated for that series. For each simulated series, we calculated the estimated autocorrelation function. For a given lag value τ for a given series, the 95% CI for the autocorrelation parameter, assuming no dependence, was the range from the 2.5% to the 97.5% quantile of the autocorrelation values estimated for the random data at that lag. Any observed residual autocorrelation value outside of this CI was unlikely to be the

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result of random fluctuations in the data, and thus represented a pattern not captured by our model.

For the series of absolute angles, the fitted model generally captured the pattern of dependence in the turn angles (Fig. B4). While the original series of turn angles showed strong patterns of auto-correlation, often up to lags of 20 steps (indicating directional persistence, Fig. B4 solid lines), the residuals for most paths showed no pattern or weak patterns of auto-correlation. However, for several of the paths that were best fit by the repulsion model (Fig. B4 green lines), the model was not able to accurately capture the pattern of dependencies between steps, indicating that these paths showed stronger directional persistence than the fitted model predicted, over longer time horizons. This indicates that the attraction model may be misspecified, missing longer-term patterns of directional persistence.

Our model did not do as well in predicting the time-dependence of relative turn angles (Fig. B5). Many of the original series showed strong (if non-linear) patterns of autocorrelation of relative turn angles, indicating complex patterns of persistent turning. While for most time series, the residual relative turn angles showed weaker auto-correlation than the original series (Fig. B5 dashed lines), for several series the model was not able to account for any of the autocorrelation in relative turn angles (Fig. B5 row 3, columns 5&6). Further, the model induced autocorrelation in residual relative turn angles that was not present in the original data for several paths fit by the attraction model (Fig. B5 blue lines). This indicates that these models may be missing longer-scale patterns of persistent turning, and the attraction model may be mi-specified.

Estimating boundary effects on random walk simulations

Our random walk models estimated how strongly individuals may have been moving toward or away from one another based on their direction of travel relative to the centroid

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of other larvae present in the frame. However, our experimental chamber was bounded, and thus all larvae would eventually have to turn toward the centre at some point in their paths. This in turn may have biased our model select procedure toward attraction-driven models. We used a simulation test to determine how this boundary effect might have affected our results.

To do this, we simulated paths from a model that incorporated both directional and turning angle persistence, but without any intraspecific attraction or repulsion (hereafter our null model). We simulated two sets of paths for this test. The first set of paths were unbounded (that is, each random walker was allowed to go in any direction for any distance). For each of the 64 paths we had previously observed, we simulated 10 new paths with the same number of steps as the observed one, and with w_p and κ set equal to value estimated for that path using our null model (equation 4). The second set of paths were modified from the first set, but with a boundary condition imposed: any time a path reached the limit of the experimental arena (-140 and +140 units in the x-direction, or -210 and +210 units in the y-direction), the path was reflected, by reversing the direction of movement at the point where the path crossed the boundary. For each simulated path, we then fit null, attraction, and repulsion models, and determined which model fit best using AIC.

For both the bounded and unbounded set of simulated paths, $\tilde{6}5\%$ were best fit by the null model, $\tilde{1}7\%$ were best fit by a model with attraction, and $\tilde{1}7\%$ were best fit by a model with repulsion. This indicates that the boundary itself is likely not biasing our estimates toward attraction models.

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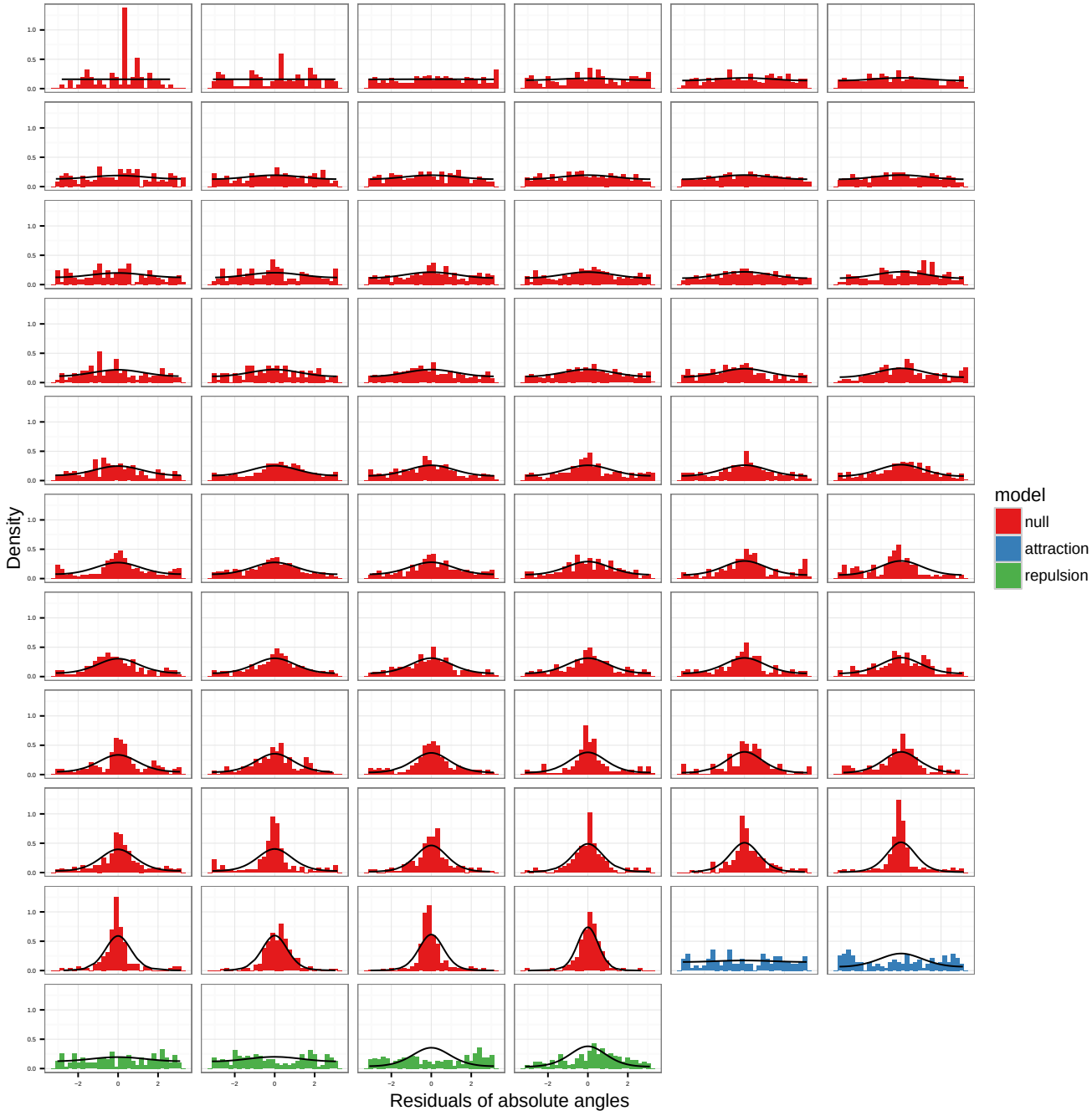

Figure B2: Probability density of observed and predicted residuals of absolute angles for each path. Bars are the histogram of observed residuals, black lines denote the predicted distribution of residuals. Histograms are coloured by which model best fit the path. Within each model, paths are sorted in order of increasing κ.

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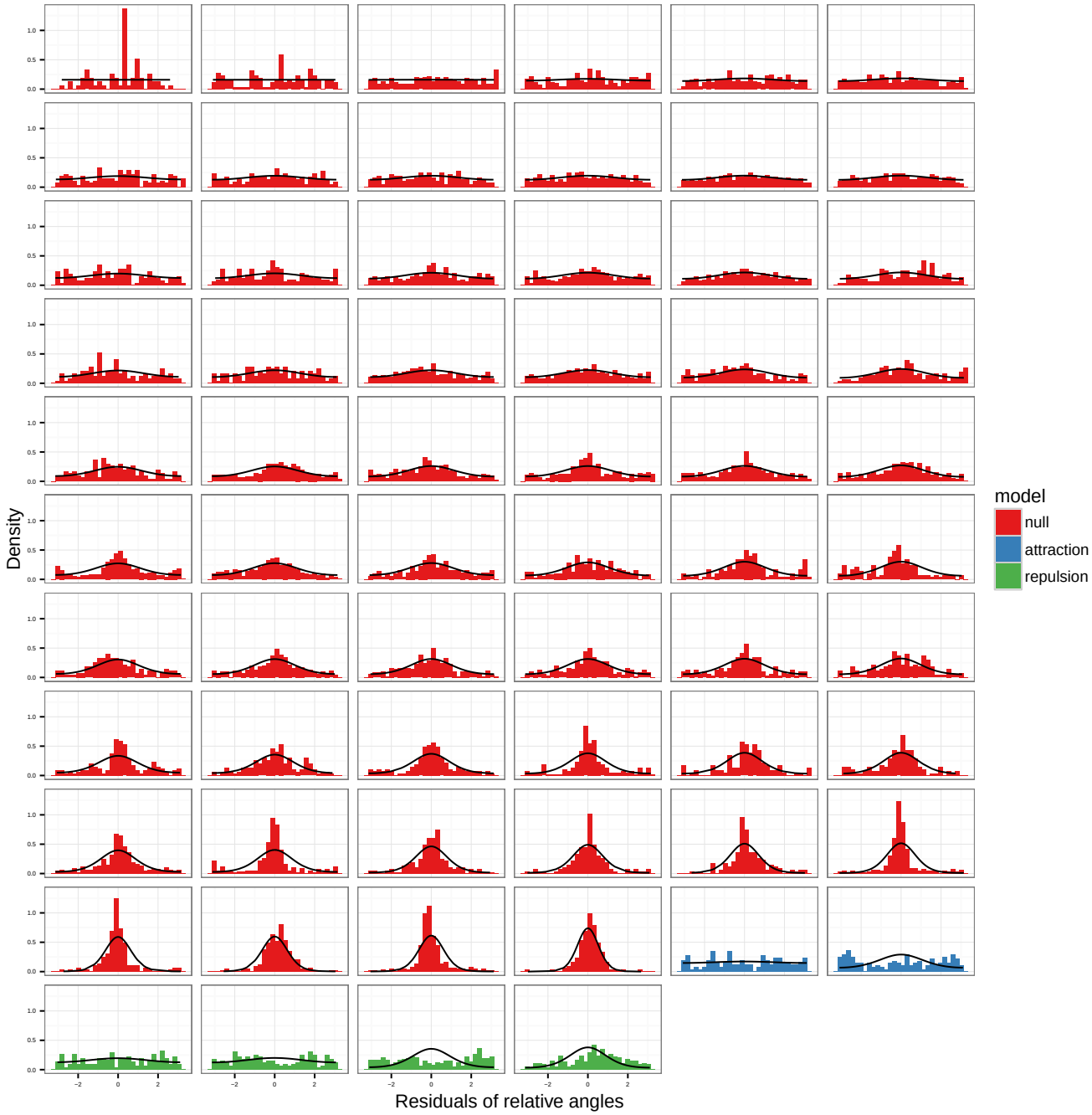

Figure B3: Probability density of observed and predicted residuals of absolute angles for each path. Colours, lines, and histograms are as in Fig. B2.

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Figure B4: Autocorrelation functions of absolute turn angles. Solid lines represent the angular autocorrelation of the movement paths themselves, and the dashed lines represent the residuals of the absolute angles. The grey ribbon is the 95% CI assuming no temporal dependence of angles. Lines are coloured by which model best fit the path, and within models, are sorted in order of increasing κ.

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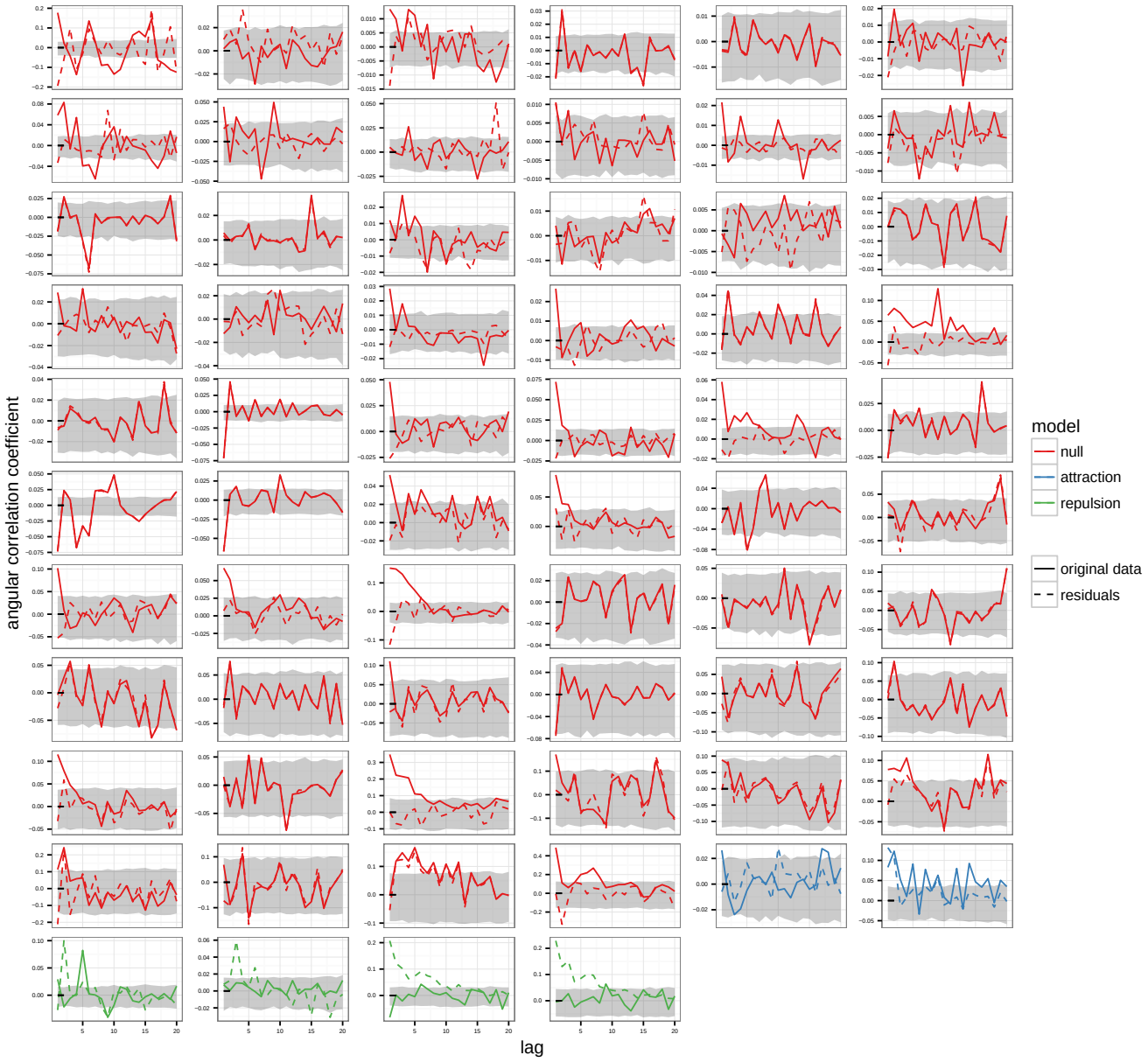

Figure B5: Autocorrelation functions of relative turn angles. Solid lines represent the angular autocorrelation of the movement paths themselves, and the dashed lines represent the residuals of the absolute angles. Colours, lines, and histograms are as in Fig. B2.

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