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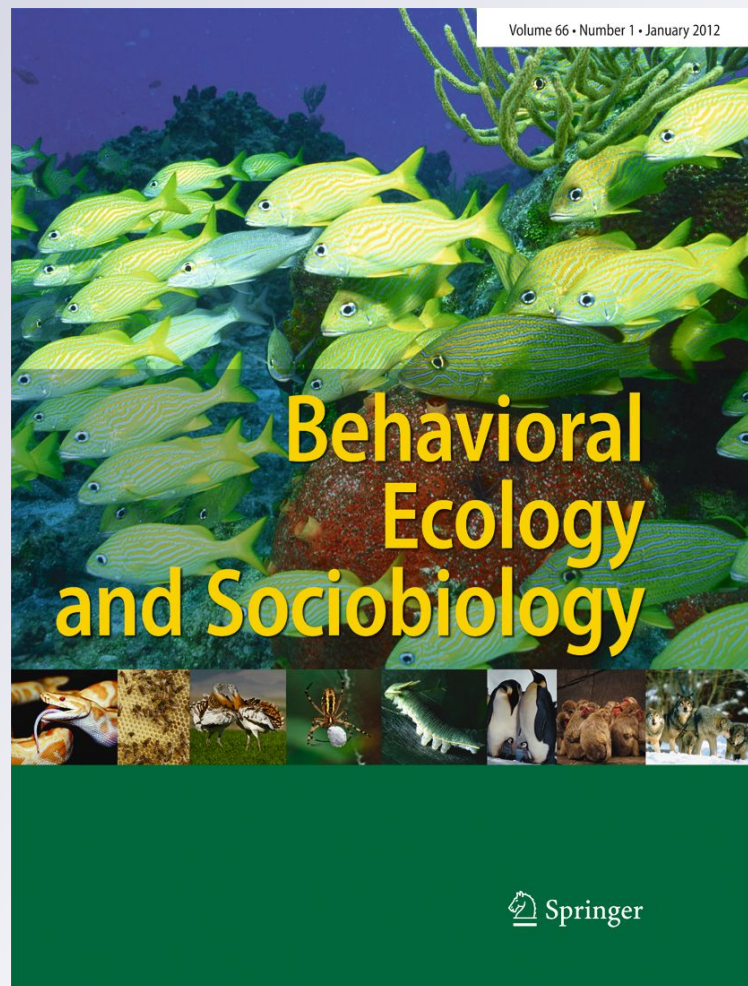Behavioral
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Vortex formation and foraging in polyphenic spadefoot toad tadpoles

**Sepideh Bazazi · Karin S. Pfennig ·
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Abstract Animal aggregations are widespread in nature and can exhibit complex emergent properties not found at an individual level. We investigate one such example here, collective vortex formation by congeneric spadefoot toad tadpoles: *Spea bombifrons* and *Spea multiplicata*. Tadpoles of these species develop into either an omnivorous or a carnivorous (cannibalistic) morph depending on diet. Previous studies show that *S. multiplicata* are more likely to develop into omnivores and feed on suspended organic matter in the water body. The omnivorous morph is frequently social, forming aggregates that move and forage together and form vortices in which they adopt a distinctive slowly rotating circular formation. This behaviour has been speculated to act as a means to agitate the substratum in ponds and thus could be a collective foraging strategy. Here we perform a quantitative investigation of the behaviour of tadpoles within aggregates. We found that only *S. multiplicata* groups exhibited vortex formation, suggesting that social interactions differ between species. The probability of collectively forming a vortex, in response to introduced food particles, increased for higher tadpole densities and when tadpoles were hungry. Individuals inside a vortex moved faster and exhibited higher (by approximately 27%) tailbeat frequencies than those outside the vortex, thus incurring a personal energetic cost. The resulting environmental modification, however, suggests that vortex behaviour may be an adaptation to actively create and exploit a resource patch within the environment.

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Introduction

Collective behaviour is widespread in nature. For example, human crowds, fish schools, bird flocks and insect swarms all exhibit coordinated spatiotemporal formations in which the entire group moves almost as a single unit (Couzin 2009; Sumpter 2006). The striking patterns observed at a group level are often the result of numerous, relatively simple interactions between individuals within the group (Camazine et al. 2001). The collective properties that result from these interactions may be entirely absent at an individual level (Camazine et al. 2001; Torney et al. 2009; Couzin et al. 2002, 2005; Parrish and Edelstein-Keshet 1999; Couzin 2007). Collective behaviour can therefore evolve as a consequence of selection acting on individuals to express behaviours that allow them to obtain fitness benefits by coordinating activity with others (Krause and Ruxton 2002). For example, by regularly adjusting their behaviour to others, individuals within a group

can collectively sense their environment in a way not possible for individuals in isolation (Torney et al. 2009).

Foraging is a key context in which selection may favour individual behaviours that foster collective behaviour. Indeed, foraging as a group can enhance access to resources (Robertson et al. 1976; Creel and Creel 1995; Bertram 1975; Franks and Partridge 1993; Schmidt and Strand 1982), or increase foraging efficiency as a result of the 'many eyes' effect (Powell 1974) whereby individuals can rely, to some degree, on the vigilance of others and thus spend more time feeding (Beauchamp 1998). Further advantages of group foraging specifically arise when resources are not homogeneously distributed but are found in patches within the environment (Giraldeau and Caraco 2000). Individuals in groups can obtain social information derived from the behaviour of others about the location or quality of resources either communicated directly, as is often the case for eusocial insects (Seeley 1995), or through observations of food discovered by others (Beauchamp and Giraldeau 1997; Dall 2005; Danchin et al. 2004; Templeton and Giraldeau 1995).

The action of individuals not only affects their social environment but can ultimately have an effect on the physical environment in which individuals live. Eusocial insects provide some of the most dramatic and well-known examples. Termites build pillars through the deposition of soil pellets immersed with pheromone. The pheromone diffusing from the pellets stimulates other termite workers to add more material to the initial structure, and this leads to the coordinated building of larger pillars (Bonabeau et al. 1998; Bonabeau 1998). Similarly, ant trails are formed from pheromone laid down by and attractive to nest mates (Deneubourg et al. 1989; Franks et al. 1991). In these examples, interactions with a modified environment can have a significant influence on individual behaviour, which influences the environment, which in turn feeds back to affect individuals, and so on. This type of recursive feedback between environmental modification and behaviour is also present among vertebrates, from the formation of human and animal trails (Couzin and Krause 2003; Helbing et al. 1997a, b) to collective excavation and nest-building in birds and mammals (Hansell 2005).

The number and/or density of individuals interacting with one another within an aggregation can also have a major effect on the group behaviour. For example, Buhl et al. (2006) showed that increasing the density of juvenile locusts within a group spontaneously increases the degree of coordination and collective movement exhibited by the group. Furthermore, changes in social interactions amongst individuals can ultimately modify the collective patterns observed in animal groups. For example, the theoretical model of Couzin et al. (2002) showed that changes in the distance over which individuals align with each other has a major impact on the shape and movement of a group.

More recently, in their study of locust migrations, Bazazi et al. (2011) have shown that individual nutritional state, while having minimal influence on individual motion, can modify the way in which individuals interact with one another and therefore affect the group motion. High protein deprivation in cannibalistic locusts results in a stronger and faster response to individuals than low protein deprivation, thereby resulting in coordinated collective motion occurring at a lower density. Therefore, the factors affecting individual behaviour are important in driving the collective behaviours found in animal aggregations.

Anuran tadpoles offer an ideal system for studying collective behaviour during foraging. Many species are known to be gregarious both in the laboratory and in the wild (Duellman and Trueb 1994; McDiarmid and Altig 1999; Wells 2007). Species vary in their group structure, social organization and group movements. Tadpoles may exhibit collective behaviour for a number of reasons, including thermoregulation (Beiswenger 1977), or avoiding, detecting or deterring predators (McDiarmid and Altig 1999; Petranka and Hayes 1998; Skelly and Werner 1990; Wassersug 1973), particularly during metamorphosis when individuals are most vulnerable (Bragg 1965; Beiswenger 1975).

Tadpoles may also aggregate because collective behaviour enhances foraging success. Conspecifics may provide individuals with social information to determine the location and quality of food, as in *Bufo americanus* tadpoles (Sontag et al. 2006). Tadpoles in mobile schools of *Osteocephalus taurinus* move with respect to others within the school, thereby apparently allowing all individuals to regularly come into contact with the substrate where it is most abundant (Duellman and Lescure 1973). Furthermore, social interactions amongst tadpoles have been shown to increase growth of tadpoles (Bragg 1965), as individuals in isolation grew more slowly than those in tadpole groups (in *Rhynophrynus dorsalis* (Foster and McDiarmid 1982) and *Xenopus laevis* (Katz et al. 1981)).

In temporary rain pools, plants and phytoplankton are often scarce (Wells 2007). Thus, the only available food is organic detritus matter found in the mud at the bottom of the ponds, and a large proportion is therefore unavailable to individuals inhabiting such environments, such as amphibian larvae, shrimp and aquatic insects. Tadpoles of spadefoot toads can aggregate into a tight schooling structure appearing like a dense vortex. Although not specifically referring to vortexing behaviour, it has been suggested that moving tadpole schools (of *Bufo*, *Scaphiopus* and other species) can churn up substratum and create a mixture of suspending particles for feeding (Beiswenger 1975; Bragg 1965; Richmond 1947; Stuart 1961), and thus, grouping could be a collective foraging strategy.

Juveniles of spadefoot toads *Spea bombifrons* and *Spea multiplicata* are polyphenic in nature (Pomeroy 1981) and

occur as two behaviourally different morphs: carnivores and omnivores (Pfennig 1990b; Pomeroy 1981), which also differ in their morphology and development (Pfennig 1992). Tadpoles in the omnivorous morph, which feed mainly on organic detritus and live shrimp (Pfennig et al. 1993), frequently aggregate with siblings and exhibit schooling. The carnivorous tadpoles are solitary (Bragg 1965), feed on live animal prey (such as freshwater shrimps or other tadpoles) and develop more quickly than the omnivorous morph (Pfennig 1992). These tadpoles acquire a thyroid hormone from live prey, which induces and maintains the carnivore phenotype (Pfennig 1992).

The species *S. bombifrons* and *S. multiplicata* differ in their tendency to adopt the omnivorous or carnivorous morph, particularly when the two species co-occur (Pfennig and Murphy 2000). When reared together, carnivore production was suppressed in *S. multiplicata* but enhanced in *S. bombifrons* (Pfennig and Murphy 2000). The proportion of carnivorous tadpoles of *S. multiplicata* was higher in high elevation ponds where *S. bombifrons* are absent compared to low elevation ponds where both species are found.

Here we carry out laboratory experiments on spadefoot toad tadpoles to investigate how and why tadpoles exhibit collective vortex behaviour. We perform detailed analyses of tadpole and water movements during vortex formation including quantification of tadpole speed and tailbeat frequency as well as estimate the flow properties (water flow speed and angular velocity) of the resulting vortex. We examine the probability of vortex formation in *S. bombifrons* and *S. multiplicata* tadpoles since populations of *S. multiplicata* tadpoles are more likely, than *S. bombifrons*, to develop into the omnivorous morph and feed on detritus. Therefore, if vortices do provide foraging benefits, such as the stirring up of organic matter from the bottom of ponds, there may be important species-specific differences that relate to their ecology.

In addition vortex formation requires many individuals to coordinate activity since individuals in isolation cannot create or maintain a large-scale rotating flow. Thus, we also examine how the density of tadpoles affects the probability of forming a vortex in response to introduced particulate food. Furthermore, we investigate whether vortices form more readily when tadpoles have been starved than when they are satiated, as would be predicted if this is an adaptation for foraging.

Materials and methods

Tadpole rearing

Adult plains spadefoot toads, *S. bombifrons*, and Mexican spadefoot toads, *S. multiplicata*, were collected from natural populations in the southwestern USA and reared in the laboratory at University of North Carolina, Chapel Hill. To produce tadpoles, adults were bred as described in Pfennig et al. (2007). Several sibships were produced for each species to maximize the number of tadpoles available for experiments. Tadpoles were reared in groups of approximately 100 individuals, placed into separate rectangular tanks (measuring $40 \times 27 \times 14.5$ cm) and filled with 10 L of dechlorinated water (tap water was dechlorinated using AmQuel + deionizing solution). This tadpole density is within the range of natural tadpole populations found in breeding ponds in the field (Pfennig et al. 1991). Rearing tadpoles at similar densities minimizes size differences amongst individuals. Tadpoles were fed ad libitum with Carolina Biological Supply™ *Xenopus* tadpole chow.

Due to limited numbers of adult toads (and therefore sibships) and unavoidable mortality of tadpoles during this vulnerable life stage (Wells 2007), the available sibships from each species were mixed to increase the total number of tadpoles in each species group (the sibships used were similarly sized in terms of numbers of tadpoles). Furthermore, this mimics tadpole groups found in natural populations; aggregations are likely to be composed of a mixture of sibships since many pairs of adult toads may breed in the same pond (Bragg 1965). In our examination of tadpole behaviour and fluid flow during vortex formation, we used vortex-forming tadpole species: *S. multiplicata* (three sibships) and *S. multiplicata–S. bombifrons* hybrid (three sibships). For the examination of group behaviour with respect to density and hunger state, we used one population of *S. bombifrons* (consisting of six sibship groups) and two populations of *S. multiplicata* (*S. multiplicata* 1 consisting of seven sibships and *S. multiplicata* 2 consisting of two sibships). Due to insufficient tadpole numbers, hybrid groups were not examined in group behaviour studies. Tadpoles from *S. multiplicata* 1 and *S. multiplicata* 2 groups could not be mixed as breedings for each group were carried out at different times; thus, tadpole ages would differ by approximately two weeks.

Detailed quantification of vortex properties

In order to capture details of tadpole behaviour and fluid motion during vortex formation and to define a vortex quantitatively, we carried out six experimental trials in which approximately 300 tadpoles of *S. multiplicata* (three trials) or *S. multiplicata–S. bombifrons* hybrid (three trials) species were placed in a tank measuring $85 \times 50 \times 10$ cm. This experimental density was chosen because it lies within the range of densities measured within natural ponds (R. Martin, personal communication based on unpublished data). The tank was filled with 2 cm of dechlorinated water, and two fluorescent lamps (32 W) were placed on two

opposite sides, 32 cm above the tank. All tadpoles had been fed 12 h prior to the experiment. Small trackable floating particles (glitter, approximately 1×1 mm in size) were scattered over the surface of the water. This simple particle velocimetry (Adrian 2005) allowed us to capture the surface fluid motion using two-dimensional tracking (Fig. 1) and effectively simulates food particles to which tadpoles may be exposed. To reduce exposure to potentially toxic and consumable particles, suspended neutrally buoyant particles of the type typically used for particle image velocimetry to facilitate visual tracking were not utilized here (Adrian 1991, 2005; Raffel et al. 1998). The tadpoles were filmed using a digital video camera (SONY EX1) suspended 151 cm above the tank.

After a habituation period of 3 min, a 1-cm^3 block of moistened tadpole chow was crushed and suspended into the water in the centre of the tank, the objective being to induce vortex formation if it is a foraging behaviour. After a further 7 min, filming was stopped and the tadpoles removed from the tank. For each trial, the water in the tank was replaced with fresh dechlorinated water, so as to remove all traces of food and to minimize food odour in the water body. The experimental room temperature was kept at $21 \pm 1°C$ (a temperature within the range found in the natural populations in the field and previously used in tadpole behavioural trials (Pfennig et al. 2007)) with a humidity of 50%. Experimentation was carried out on tadpoles 10 days after hatching. Under the chosen rearing density, tadpoles at this age were large enough in size to facilitate visual tracking.

We analysed 4 s of the video footage of the vortex between 1 and 3 min after it had formed, excluding 4 cm from the edge of the tank (to minimize edge-effect behaviour). During this time window, visual observations revealed the vortex to be stable, exhibiting little variation in its dynamics (structure, size and movement, tadpole

behaviour). We recorded the position and speed of the floating particles in each trial, using tracking software that automatically located the positions of particles in successive time steps and interpolated the identity of a particle using nearest neighbour distances between frames (Fig. 1). We visually located the centre of the vortex at 10 regular time intervals throughout the 4-s videos and interpolated the centre for the whole video (we note that the mean displacement speed of the vortex centre is much slower (6.65 mm/s) than mean particle speed (32.96 mm/s)). We calculated the centre of the vortex as the average coordinates of these interpolated positions. We also calculated the mean absolute angular velocity of the trackable particles from the centre of the vortex. The angular velocity (measured in radian per second) was calculated as: $(r \times v)/|r|^2$, where r is the position vector and v the velocity of the particle. We also compared the mean water speed (averaged across all trials) inside and outside the vortex using a Student's t test.

In addition, we manually tracked the position and tailbeat frequencies (the number of tailbeats per second) for 4 s, as an estimate of the energy expenditure, using Image J software. We calculated speed, angular velocity and distance from the centre of the vortex for each tadpole. We also recorded the snout-to-vent length (SVL) of tadpoles in the first frame; 1,282 individual tadpoles were tracked in the six experimental trials described above. We performed a statistical analysis employing a linear mixed effects model (LME) examining the relationship between: tadpole tailbeat frequency and tadpole distance from the vortex centre, tadpole SVL and distance from the vortex centre and tailbeat frequency and the tadpole SVL for inside and outside the vortex. Individual nested in experimental trial was treated as a random factor in all LME analyses. The fixed variable species (either *S. multiplicata* or *S. multiplicata–S. bombifrons* hybrid) was also included in the LME but did not
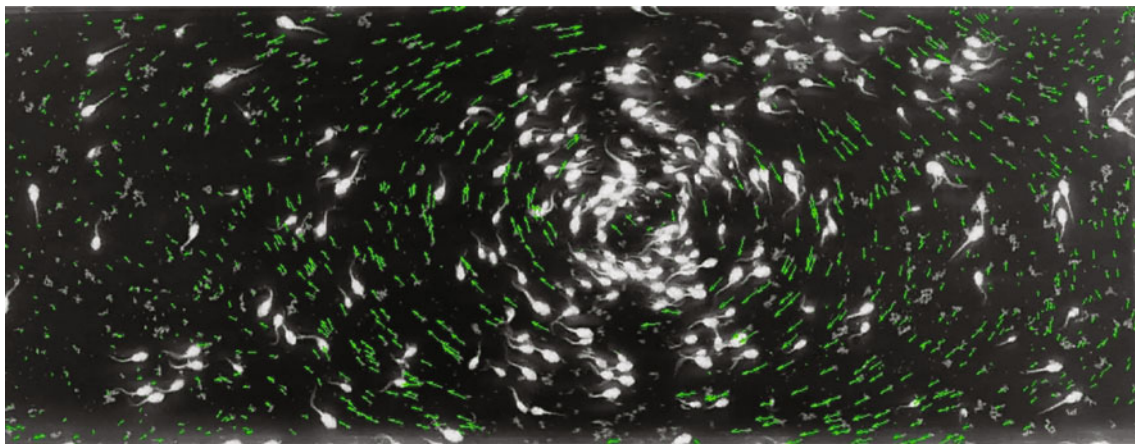

Fig. 1 Automated tracking of passive particles. The tracking software automatically locates the positions of passive particles (shown in *grey*) in successive time steps and interpolates the identity of a particle using nearest neighbour distances between frames. Particles were located using maximum threshold criteria (in pixels) for the object size; hence, tadpoles (*white*) that are above the threshold are not tracked. *Green vector arrows* show the direction of motion of water particles

show a significant effect in any of the analyses and was therefore omitted from the models. We compared the mean true tadpole speed (averaged across all trials) inside and outside the vortex using a Student's t test (data were square-root transformed to achieve normality).

Group behaviour studies

Our study examines the collective behaviour of tadpoles for two species: *S. bombifrons* (one group) and *S. multiplicata* (two groups). As we were interested in the role of density and state dependence on collective behaviour, for each species we examined the behaviour of tadpole aggregates at three densities: 400, 600 and 800 tadpoles, and under two feeding treatments, tadpoles that had been fed between 30 and 60 min prior to the experiment and tadpoles that had been starved for 12 h prior to the experiment, giving at total of six experimental treatments per species group. Tadpoles under each of the feeding treatments were randomly selected for the density treatments. Experimentation began on tadpoles 10 days after hatching. The treatment order of experiments was randomised and trials for each species group took place over 12 days. All tadpoles, both within and between species, used in the experiments were similar in age (±8 h) and did not differ significantly in size or mass (we compared 50 tadpoles in each of the three groups and found no significant differences in the SVL (in millimetres) and mass (in grams) of tadpoles between groups (SVL: ANOVA, $F_{(1, 147)}=0.42$, $p=0.66$; mass: ANOVA, $F_{(1, 147)}=0.14$, $p=0.872$)). Due to the limited number of tadpoles for each species group (despite pooling sibships for each species together), tadpoles used after a trial were replaced into the species group population, from which tadpoles were then randomly selected for the next trial. Tadpoles used in 'starved' treatment trials were not used in further 'starved' treatment trials (since they would have received food within a trial) but could be used for 'fed' treatment trials. Similarly 'fed' treatment tadpoles could be used for 'fed' treatment trials but not 'starved' treatment trials. This protocol allowed us to randomly select tadpoles for each trial, according to the specific treatment, and maximize the number of trials performed for each treatment with the tadpoles available to us.

Tadpoles in each treatment were placed in a white, homogenous square tank (measuring 60×60×15 cm) filled with dechlorinated water to a depth of 2 cm. Trackable water particles were not used in these group behaviour experimental trials (information regarding water flow was not needed in group behavioural trials and repeated exposure to the potentially consumable particles in multiple trials may be harmful to tadpoles). Trials were carried out as outlined above.

Our analyses of vortex formation (described above) allowed us to define a vortex according to the distribution of tadpoles and the mean angular velocity of tadpoles and

trackable particles. The pattern was readily visible to the observer (see Fig. 1), and thus, we could unambiguously record whether a vortex had, or had not, formed (automated tracking analysis in this system was difficult due to the high density and overlap of tadpoles and the absence of trackable particles in these trials). A main effect generalized linear mixed model (GLMM) was used to examine the effect of density and feeding treatment on the probability of forming a vortex, using species group as a random variable. Different density and feeding treatments within a species were also compared using a Wilcoxon rank sum test. We carried out a total of 208 trials. The number of trials for each treatment for each species is shown in Supplementary Table 1.

Results

Quantification of vortex properties

The number of tadpoles per unit area decreased sharply with increasing distance from the centre of the vortex, and from approximately 200 mm remained very low (Fig. 2b). The
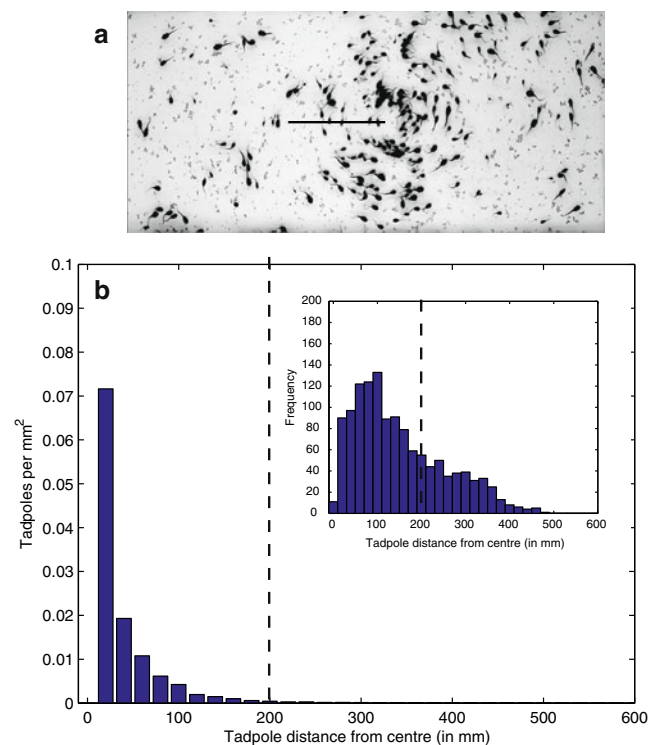

Fig. 2 Tadpole distances and defining vortex size. **a** A snapshot of video footage showing a vortex that was analysed. The *black line* shows a radius of 200 mm. **b** The number of tadpoles per circular area (square millimetres) as a function of distance from the centre (millimetres). The *inset* shows a histogram of the distances of every tracked tadpole in six trials from the centre of the vortex. The *dashed line* shows a distance of 200 mm from the centre, below which we define tadpoles as being inside the vortex. Tadpoles with a distance of greater than 200 mm are considered outside the vortex

distribution of tadpoles in space showed that at the very centre of the vortex, the number of individuals was low, but that it increased with increasing distance from the centre until a peak at approximately 100 mm (Fig. 2 inset). Beyond 100-mm tadpole numbers decreased sharply until 200 mm from the centre and then with a shallower decline until 400 mm from the centre. As can be seen in Fig. 1, the tadpoles in a vortex have a considerable impact on the fluid, tending to move counter-current to the flow. The angular velocity of the tadpoles increased to 1.2 rad/s at approximately 25 mm from the centre, after which the angular velocity decreased at 200 mm and plateaued (Fig. 3a). Similarly, the angular velocity of the water, estimated by the flow properties of floating particles, increased sharply as the distance from the centre of the vortex increased, up to a maximum of 1.2 rad/s then decreased and plateaued at approximately 200 mm from the centre of the vortex (Fig. 3b). We therefore define the area of the vortex as the area of a circle with a radius of 200 mm from the centre, where the number of tadpoles has decreased and angular

velocity is low. We note that our definition of a vortex is for the purposes of examining tadpole behaviour and is based on organismal distribution and behaviour.

Tadpoles inside the vortex had significantly higher tail-beat frequency than those outside the vortex (inside vortex mean tailbeat frequency (per second)—6.31 ± 1.52 (1 SD) and outside vortex mean tailbeat frequency—4.96 ± 1.81 (1 SD); Student's t test: $p < 0.0001$, $df = 1242$, $T = -13.04$). Increasing the distance away from the centre of the vortex resulted in a decrease in the mean tadpole tailbeat rate (LME: $F_{(1,\ 1,237)} = 159.6585$, $p < 0.0001$, Fig. 4a) and an increase in tadpole size (LME: $F_{(1,\ 1,275)} = 8.0814$, $p < 0.005$, Fig. 4b).

In addition, larger tadpoles beat their tails less than smaller tadpoles both inside (LME: $F_{(1,\ 908)} = 77.8056$, $p < 0.0001$, Fig. 4c) and outside the vortex (LME: $F_{(1,322)} = 12.0022$, $p < 0.0001$, Fig. 4c). Such a relationship between tailbeat frequency and body size is as expected since larger mechanical systems operate with characteristically longer timescales (Schmidt-Nielsen 1975; Daniel and Webb 1987). Tadpoles inside the vortex showed significantly higher mean speeds than tadpoles outside the vortex, with a mean speed of 33.02 ± 12.3095 mm/s (1 SD) inside the vortex and a mean speed of 32.33 ± 19.85 mm/s (1 SD) outside the vortex (Student's t test: $p = 0.05$, $df = 566$, $T = 1.96$). The water flow inside the vortex also showed a higher speed than outside the vortex (Student's t test: $p < 0.0001$, $df = 1072$, $T = 16.37$).

Group behaviour results

We calculated the probability of forming a vortex by each species in the six experimental treatments (Fig. 5). *S. bombifrons* never formed a vortex, regardless of the number of individuals in the group or whether or not the tadpoles had been starved prior to the experiment (Fig. 5a). Both *S. multiplicata* groups showed a significant increase in the probability of forming a vortex with increasing tadpole density (Fig. 5b, c). The probability of forming a vortex was strongly influenced by the number of tadpoles in the group and whether or not tadpoles had been fed (GLMM: $p < 0.0001$, $z = 5.48$ and $p < 0.01$, $z = -2.48$, respectively).

In *S. multiplicata* 1, tadpole groups of 600 and 800 showed a significantly higher probability of forming a vortex than groups of 400 for starved tadpoles (for 400 ($n = 10$) and 600 ($n = 9$), $p < 0.01$, ranksum = 120.5; and for 400 and 800 ($n = 8$), $p < 0.01$, ranksum = 107, respectively, Fig. 5b), and groups of 800 showed a significantly higher probability than groups of 400 for fed tadpoles (for 400 ($n = 10$) and 800 ($n = 10$), $p < 0.01$, ranksum = 70, Fig. 5a). Similarly tadpoles of *S. multiplicata* 2 in groups of 800 were more likely than groups of 400 to form the vortex both when starved (for 400 ($n = 11$) and 800 ($n = 16$), $p < 0.01$, ranksum = 110, Fig. 5c) and fed (for 400 ($n = 16$) and 800 ($n = 14$), $p < 0.01$,
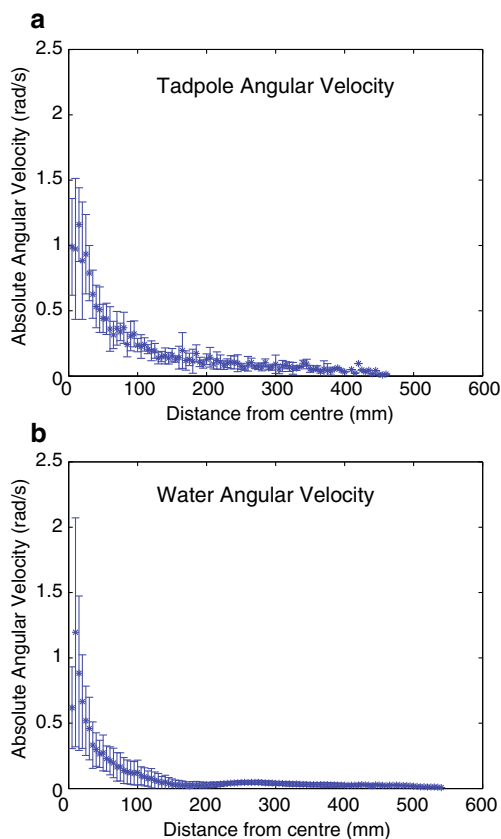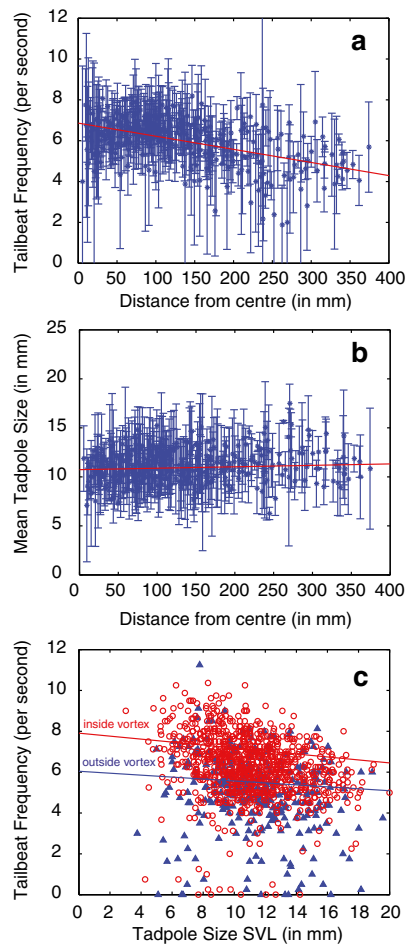

Fig. 3 Angular velocity. **a** The mean absolute angular velocity of tadpoles during a vortex calculated every 5 mm from the centre of the vortex for each trial and averaged across all experimental trials. *Error bars* show ±1 SD. **b** The mean absolute angular velocity of the water particles during a vortex calculated every 5 mm from the centre of the vortex for each trial and averaged across all experimental trials. *Error bars* show ±1 SD

Fig. 4 Tadpole behaviour during vortex formation. **a** The mean tail-beat frequency (per second) of a tadpole as a function of its mean position from the centre of the vortex (across all time steps) was calculated for each trial and averaged across six experimental trials. The line of best fit is also shown. *Error bars* show ±1 SD. **b** The mean size of a tadpole (measured as snout-to-vent length (*SVL*) in milli-metres) as a function of its mean position (across all time steps) from the centre of the vortex was calculated and averaged for all trials analysed. The line of best fit is also shown. *Error bars* show ±1 SD. **c** The mean tailbeat frequency (per second) of a tadpole as a function of its body size (SVL in millimetres) for tadpoles that spent all their time inside the vortex (*red circles*) and outside the vortex (*blue triangles*). The lines of best fit for each group are also shown. For **a–c**, means calculated using a single datum are not shown for visual purposes, but these data were used in statistical analyses

ranksum=256, Fig. 5c) and were more likely to form a vortex than groups of 600 when fed (for 600 (*n*=14) and 800 (*n*=14), *p*<0.05, ranksum=168, Fig. 5c).

For *S. multiplicata* species, where a vortex had formed, starved groups showed a higher probability of forming a vortex than fed groups overall, with a significant difference in *S. multiplicata* 1 group of 600 (starved (*n*=9) and fed (*n*=11), *p*<0.05, ranksum=119.5, Fig. 5b). *S.multiplicata* 1 tadpoles were generally more likely than *S.multiplicata* 2 tadpoles to form a vortex (except fed tadpoles in a

group of 400 where no vortices were observed), with a sig-nificant difference between species groups in starved tadpoles in groups of 600 (*S. multiplicata* 1 (*n*=9) and *S.multiplicata* 2 (*n*=21), *p*<0.05, ranksum=190.5).

Discussion

We investigated collective vortex formation in spadefoot toad tadpoles. Tadpole behaviour and fluid flow, both inside and outside the vortex, were quantified. We studied two species of spadefoot toad tadpoles, *S. bombifrons* and *S. multiplicata*. We examined differences between these spe-cies in the vortexing behaviour of tadpole groups at different densities and under different feeding treatments.

Our group level behavioural results reveal differences between species in the probability of forming a vortex under our experimental conditions. *S. bombifrons* never formed a vortex (Fig. 5a), whereas *S. multiplicata* did exhibit vortex-ing behaviour. The existence of group level differences suggests that the social interactions amongst tadpoles differ in *S. bombifrons* and *S. multiplicata*. Social interactions in tadpole groups may be strongly influenced by individual tadpole motion in response to each other and/or as a response to the fluid flow generated by swimming individuals. When alone *S. multiplicata* tadpoles have previously been found to spend more time grazing than actively swimming and *S. bombifrons* tadpoles tend to spend more time swimming (Pomeroy 1981; Pfennig et al. 2007; Pfennig and Murphy 2000). In addition, many aspects of tadpole biology, such as tail morphology (Arendt 2010) and muscle fibre composition (Arendt and Hoang 2005), have been shown to explain differ-ences in swimming capacity in different species of spadefoot toad tadpoles. Such features relating to motion may have important consequences on social interactions, and it may be profitable to consider these in future work exploring species differences in social behaviour.

For the vortex forming *S. multiplicata*, the probability of forming a vortex increased with increasing density (Fig. 5b, c), highlighting the importance of the frequency of individ-ual interactions in vortex formation. Furthermore, starved tadpoles were more likely to form a vortex (Fig. 5b), sug-gesting that hunger in tadpoles can therefore encourage vortex formation. The differences in feeding treatments are the clearest at an intermediate density (600 individuals). At lower (400) and higher (800) densities, it may be that the strong influence of density tends to overshadow the effects of pre-experimental feeding. Tadpoles are known to cease feeding when food concentrations are below a certain threshold, perhaps to prevent energy loss that would occur whilst foraging for scarce resources (McDiarmid and Altig 1999). Our experimental results suggest that vortex forma-tion may also be triggered by a state-dependent response to
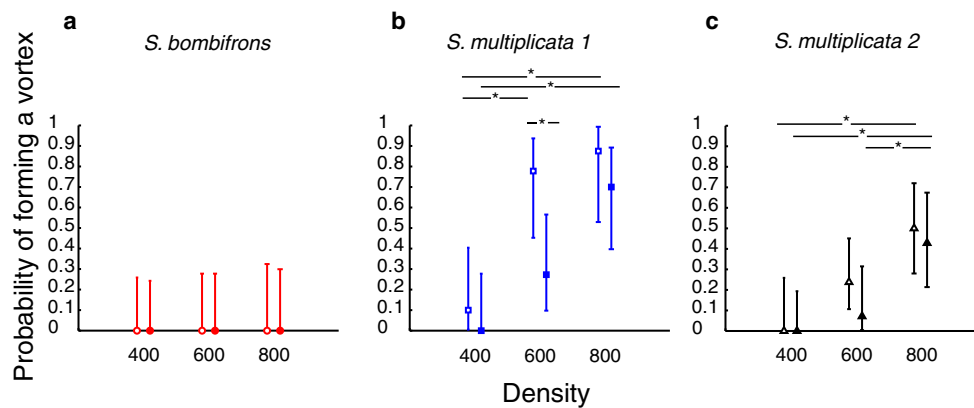
Fig. 5 The probability of forming a vortex in a group. The mean probability of forming a vortex for the different species groups: *S. bombifrons* (**a**), *S. multiplicata* 1 (**b**) and *S. multiplicata* 2 (**c**). Each *panel* shows the results of the six experimental treatments: three densities (400, 600 and 800 tadpoles), and for tadpoles that had been starved (*open symbols*) and fed (*filled symbols*) prior to the experiment. *Error bars* show 95% confidence intervals. The results of different treatment groups were compared statistically (Wilcoxon sum rank test). Significant differences between groups ($p<0.05$) are shown,

food in combination with a threshold density. Hungry animals tend to be more active than animals that have recently fed, which could lead to increases in local interactions. The interactive effect of nutrition and density on group motion has also been observed in locusts (Bazazi et al. 2011) where the threshold density for collective motion decreases with increasing protein deprivation.

Our examination of the structure of vortex formation showed that there were more tadpoles per unit area inside the vortex (defined as a circular area with a radius of less than 200 mm) than outside the vortex and that tadpoles moved faster and beat their tails more quickly when inside the vortex. Larger tadpoles beat their tails less than smaller tadpoles, as expected; this scaling relationship between body size and tailbeat frequency has previously been shown in tadpoles (McDiarmid and Altig 1999; Wassersug and Hoff 1985) and is similar to that found in fish and other animals (Bainbridge 1958; Biewener 2003; Hunter and Zweifel 1971; Schmidt-Nielsen 1975; Vogel 2008). The rate of water flow was also greater inside the vortex (Fig. 3b). The angular velocity of the fluid increased up to 1.2 rad/s at approximately 25 mm and then decreased for greater distances from the centre of the vortex. Therefore, at short distances from the centre (approximately 25 mm), fluid rotates quickly but the speed of rotation decreases with increasing distance from the centre. We suggest that a higher density of tadpoles (resulting from tadpoles aggregating around food) can result in faster water flow due to individuals beating their tails. We hypothesize that the increased flow can orient tadpoles, as they swim into the flow and move faster thereby creating more flow through positive feedback. Thus, a change in an individual's motion alters its local environment, which also affects the behaviour of other individuals. Evidence of such behaviour, known as rheotaxis, has previously been found in tadpoles (Simmons

et al. 2004) and observed in fish (Baker and Montgomery 1999; Kanter and Coombs 2003; Montgomery et al. 1997).

We suggest that due to the increased water flow generated, vortex formation may act as a foraging strategy. Further work is needed to definitively show this. Maximizing growth rate is a driving force for selection in spadefoot toad tadpoles since they need to grow as large as possible during their relatively short juvenile stage and reach adulthood before the temporal pools in which they live dry up (McDiarmid and Altig 1999). Mobile aggregations have been frequently observed in anuran larvae species that are specialized for suspension feeding (Wassersug 1973), and previous work on *X. laevis* tadpoles has shown individuals in schools exhibit more even spacing at higher densities (Katz et al. 1981), which can allow better access to food and minimize competition. Furthermore, tadpole aggregations and schools of *Spea*, *Scaphiopus* and *Bufo* observed in nature tend to form when pond productivity is low and tadpole densities are high (Wells 2007).

The fluid flow prevents local depletion of resources; thus, there is a constant stream of new food particles entering regions where tadpoles are feeding. Vortex structures of the type demonstrated here have the potential to create secondary flows characterised by an upwelling at the centre (Schlichting and Gersten 2000). In shallow waters, this will result in the disturbance of nutrient particles located on the pond bottom and subsequently transport them from an initially wide area towards the surface centre of the vortex. Much work on group foraging has examined how animals exploit patchy resources (Beauchamp and Giraldeau 1996; Giraldeau and Caraco 2000; King et al. 2009; Vickery et al. 1991). Here we give an example of how coordinated collective behaviour may allow animals to actively modify their habitat in order to localize distributed or otherwise unavailable resources.

Vortex formation has also been observed in a variety of other animals often in response to changes in environmental conditions. Motile bacteria, *Bacillus subtilis*, in a fluid film create vortices in response to an increase in substrate thickness (Sokolov et al. 2009); daphnia show vortex motion above critical densities in response to light (Ordemann et al. 2003); army ants can form circular columns when isolated from the main swarm raid (Franks et al. 1991; Gotwald 1995; Schneirla 1971) and torus formation is frequently exhibited by pelagic fish species such as barracuda and jack (Parrish and Edelstein-Keshet 1999). Despite the visual similarity of such behaviour, the mechanisms at work and functional reasons for them can be very different. For example, torus formation in fish allows continual motion needed for respiration (Couzin et al. 2002), and bacteria form vortices when constrained to high densities due to mechanical principles related to their mode of locomotion and the viscosity of their fluid environment (Sokolov et al. 2009). Army ants mill when they have been separated from the colony, such as during rainstorms (Franks et al. 1991; Gotwald 1995; Schneirla 1971) and unless 'reconnected' will be doomed to die of exhaustion. Consequently although these patterns may appear similar, from a biological perspective they may be very different. Understanding the adaptive significance of these collective behaviours has been limited largely due to the large number of individuals in the group and the limitations of tracking software available, particularly in three dimensions.

Vortex formation in tadpoles appears to be energetically costly to participants, and their collective behaviour can create a public good since many individuals could gain the benefits of feeding from the reorganization of resources. Therefore there remain important questions regarding the role of kin structure. Spadefoot toad tadpoles can recognize kin from non-kin using chemical cues (Pfennig 1990a), and thus, we would expect preferential investment of tadpoles in vortex behaviour when among relatives (Pfennig 1990a). In our investigation, *S. multiplicata* 1, which contained more kin groups, was more likely than *S. multiplicata* 2 to form a vortex. Additional experiments with populations containing mixed and pure kin groups would be required to determine whether such a relationship holds with a larger sample size. Vortex formation may be occurring due to local clustering as kin associate with one another. Tadpoles within the same kin group could be tagged using elastomers (Arendt 2009) and tracked to see whether kin association affects group motion. Tracking tagged individuals could also reveal whether all individuals are equally likely to contribute to this costly behaviour, or whether cheaters (individuals who benefit from the group action without paying the energetic cost) are present in such populations. An increase in the net availability of food to all tadpoles might result in more efficient feeding, but nevertheless this still sets the scene

for exploitation, especially among non-kin. Increased feeding efficiency is a commonly regarded kin-selected benefit of aggregating (Beiswenger 1975) but is yet to be tested rigorously in spadefoot toad tadpoles or other anuran larvae (Roche 1993; Waldman 1991).

In addition to examining how individuals differ in their participation of a vortex, monitoring tagged individuals can reveal important information regarding the relationship between the spatial position tadpoles adopt and their feeding rate. The growth of tadpoles in groups that do and do not exhibit vortices can also be compared in order to determine whether individuals in vortex-forming groups have higher growth rates. Furthermore, such growth benefits should be explored in natural populations, where tadpoles can experience harsher environmental conditions than those found in the laboratory. This therefore presents even higher selection pressures for rapid development and can strongly influence the dynamics of tadpole aggregations (McDiarmid and Altig 1999).

Moreover, it is important to identify the key features of individuals that give rise to the different social interactions (manifested as a difference in vortexing behaviour) between species. The presence of vortex formation in *S. multiplicata* and hybrids but not *S. bombifrons* uncovers the possibility that there may be a relatively direct genetic influence on this collective behaviour. Understanding how individuals interact with one another will ultimately help elucidate the mechanisms of group motion in spadefoot toad tadpoles and provide further insights into collective behaviour in nature.

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