ENVIRONMENTAL FEATURES INFLUENCE COMPLEX BEHAVIOR IN SMALL GROUPS OF ANIMALS

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To Joe, my mentor and friend, and my family for showing me that there is more to life than this dissertation.

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Delia S. Shelton

ENVIRONMENTAL FEATURES INFLUENCE COMPLEX BEHAVIOR IN SMALL GROUPS OF ANIMALS

Simple environmental features can shape complex behavior. Identifying key aspects of the environment (e.g., temperature, structure, toxins) that lead to widespread consequences is of central importance in a changing world. The primary objective of my dissertation is to investigate how relatively simple aspects of the environment can influence small groups of animals in profound and complex ways. In the first three chapters, I report on experiments showing how small changes in the environment can affect the expression of behavior at different points in development and can have important physiological consequences for litters of mouse pups. I then report on two sets of experiments showing how subtle changes in the environment can dramatically affect spacing patterns and social dynamics of small groups of adult zebrafish. Together, my results emphasize the ways that subtle changes in the environment can have profound impacts on individuals and small groups. In both lines of work, I have found that a more accurate characterization of the phenomena, infant rodent development and zebrafish social behavior, requires the use of individual and group measures and that temperature, density, and pollutants can have a powerful effect on group responses. These results are important because they show that the physical environment can have profound effects on the phenotype, and that with a changing physical environment or anthropogenic change, dramatic differences may be observed in the behavior of groups.

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Chapter 1:

Introduction

Importance of Studies at the Group-level

A challenge to research is discerning which level of analysis is most illuminating. Studies examining the effect of environmental features on behavior have largely focused on the responses of the extreme levels of organization, such as lower- (e.g., cells, individuals), and higher-level organization (e.g. populations, species). The impact of the environment on mid-level organization (e.g., groups) is expected to be pervasive, as group formations are prevalent amongst almost all animal taxa (Wilson, 1975). For example, approximately 75% of fish species form schools during their development (Shaw, 1978), 50% of bird species form feeding flocks (Lack, 1968), and 2% insects species are social constituting 75% of insect biomass (Hölldobler & Wilson, 2009; Wilson, 1987). We need to know more about the response of groups to the environment to improve our predictive ability, because unique non-additive group-level responses to physical features cannot be simply extrapolated from studying single individuals. Groups, which may contain thousands of individuals and tens of thousands of social links, are too complex to be easily predictable. However, technological innovation, and integration of modeling and empirical techniques have provided evidence that the structure and dynamics of even very complex groups might be derived from a few relatively simple behavioral rules (e.g., alignment, attraction, avoidance) (Katz, Tunstrøm, Ioannou, Huepe, & Couzin, 2011; Vicsek & Zafeiris, 2012). In addition, some consequences of environmental change may be hidden at lower- and higher-levels of organization (Fleeger, Carman, & Nisbet, 2003), but revealed at the group-level, thus group responses may be more sensitive than other levels of organization for detecting the effects of the environment. Making inferences that transpire multiple levels of organization are potentially misleading because individuals, groups, and other levels of organization may respond differently to the environment.

Environment Affects Group Responses

Environments can profoundly impact phenotypic expression of animal groups. For example, temperature influences physiology (Serrat, 2014), small changes in the micro-habitat can impact the expression of multiple phenotypes (Whiteside, Sage, & Madden, 2016),-and toxins can cause widespread consequences for social behavior (Scott & Sloman, 2004). Environments are complex, with many features, including abiotic (e.g., temperature, structure) and biotic (e.g., conspecifics) ingredients. Identifying environmental features that shape behavior is especially important in a changing world. Here, I focus on some abiotic components that are predicted to be affected by anthropogenic change (e.g., temperature, density, pollutants). I use *density* here and throughout to mean the amount of utilized space per individual. For readers interested in a discussion of biotic components influencing group responses, I direct them to literature on parent-offspring interactions (Champagne, Francis, Mar, & Meaney, 2003), conspecific interactions (Modlmeier, Keiser, Watters, Sih, & Pruitt, 2014), and predator-prey interactions (Lima, 1998, 2002).

The amount of available space can profoundly affect the expression of behavior. The enclosure can influence spacing patterns and social behavior. For example, pigs placed in smaller arenas spent more time standing and used the pen area differently than did pigs in larger arenas (Wiegand, Gonyou, & Curtis, 1994). Sheep experiencing a reduction in space allowance showed a decrease in lying time, less synchronized resting and a large increase in the number of displacements (Bøe, Berg, & Andersen, 2006). Domestic fowl exhibit density-dependent changes in spacing patterns, maintaining a closer proximity to their neighbors with less available space per individual (Leone, Christman, Douglass, & Estevez, 2010). The amount of available space can also affect the interactions of individuals with inanimate stimuli. In an intriguing example, the number of flies in an air zone influenced the accuracy at which flies could avoid an aversive odorant, with isolated flies spending considerably less time avoiding an odor than flies tested with more conspecifics present in the same sized air zone or at higher densities (Ramdya et al., 2015).

The shape of the arena can also influence the behavioral repertoire of groups. For example, zebrafish tested in circular arenas show highly polarized shoals (Miller & Gerlai, 2012), whereas those placed in rectangular aquariums show loosely coordinated movement. Pigs in rectangular pens maintained shorter distances between neighbors and formed more smaller social groups than did pigs tested in circular and triangular enclosures (Wiegand et al., 1994). The effect of arena shape on behavior may depend on the perceived environmental geometry. For example, distorting the local environment so that the global geometry is relatively the same lead rats to show little differences in activity, but major changes in the global geometry resulted in significant alterations in the spatial distribution of the rats' activity (Ben-Yehoshua, Yaski, & Eilam, 2010).

Temperature can alter the expression of morphological and behavioral characteristics. For example, developing in the cold leads mice to have shorter tails (Barnett & Dickson, 1984) and lizards to have stubbier appendages (Serrat, King, & Lovejoy, 2008) than do their warm-developing counterparts. The mechanism of action is temperature, as it influences cartilage growth by modulating the levels and delivery routes of hormones and paracrine regulators (Serrat, 2014; Serrat et al., 2008). Temperature also affects the group of animals and consequent social dynamics. For example, temperatures influence aggregations, with individuals exposed to cool temperatures forming tight huddles that either become less compact, or disassociate completely at warmer temperatures (Harshaw & Alberts, 2012). Similarly, in cold conditions, mice closely contact other group members, showing negligible amounts of aggression, but under warmer conditions individuals disperse and sometimes set up territories that they fiercely defend (Batchelder, Kinney, Demlow, & Lynch, 1983). Thus, temperature can cause group and individual phenotypes to change in dramatic ways.

Toxins can also impact group dynamics and responses. For example, pollutants can interfere with the transmission of the signal, such as noise pollution masking auditory signals (Slabbekoorn, 2013), possibly leading individuals to either call louder (Parks, Johnson, Nowacek, & Tyack, 2011) or alter spatial location in relation to the pollution (McLaughlin & Kunc, 2013). Toxins can disrupt social recognition (Ward, Duff, Horsfall, & Currie, 2008), reduce shoaling (Borner et al., 2015), and interfere with communication (Sluijs et al., 2011) through causing deficits in sensory modalities (Ward et al., 2008). Pollutants, through direct action on sensory systems, may have an indirect effect on social interactions, which could reveal a hidden pathway of pollutant action on group dynamics.

The influence of environmental features on behavior may depend on whether the groups are large, composed of familiar adults with coordinated responses, or small, with individuals tested early in life behaving independently. Younger groups, for example, may not have experience with such environmental features, and thus may not have learned or developed the capabilities to respond appropriately (Alberts, 2007, 2008; Leonard, 1974). Smaller uncoordinated groups may be more susceptible to environmental factors with their behavior shaped more by physical factors that elicit taxes (light: Imada et al., 2010, water currents: Capello, Soria, Potin, Cotel, & Dagorn, 2013; temperature: Allan, Domenici, Munday, & McCormick, 2015). In contrast, larger groups may be able to withstand more environmental perturbations, as they have amassed enough critical inertia to be relatively unaffected by environmental fluctuations (Cossins, 2012; Heinrich, 1981). Young individuals may not recognize group members and thus respond more to environmental pressures (cf., Alberts, 2007), whereas individuals in older familiar groups may recognize each other and coordinate group responses. Familiar shoals are more cohesive and more effectively escape predators than do groups of unfamiliar fish (reviewed in Ward & Hart, 2003). Taken together, these studies suggest that the environment can have a powerful influence on group phenotypes.

Group behavior affects the environment

Organisms also modify their environment through niche construction (Odling-Smee, Laland, & Feldman, 2003). Many organisms create nests, burrows, and shelters (like a beaver's dam) that generate suitable micro-climates for certain outcomes (e.g., survival, brood production). For example, overwintering honeybee hives maintain an average temperature of 21.3°C, which is not conducive to successful brood development, but permits survival of colony members (Fahrenholz, Lamprecht, & Schricker, 1989). In the summer months, colony temperature is more tightly regulated to promote brood production (Jones & Oldroyd, 2006). Similarly, clumping male emperor penguins (*Aptenodytes forsteri*) save energy and maintain a constant body temperature, which facilitates successful incubation during frigid winters (C Gilbert, Blanc, Maho, & Ancel, 2008). Caterpillars (*Imbrasia belina*) and woodlice (*Porcellio scaber*) aggregations can generate micro-climatic conditions that minimize water loss, thus permitting them to thrive in environments where they are at risk of desiccation (Broly, Devigne, Deneubourg, & Devigne, 2014; Klok & Chown, 1999). Thus, groups can engineer their environment to make it conducive to certain bio-behavioral outcomes.

Groups can control micro-climatic conditions through huddling, or the active maintenance of an aggregation by individual members. For example, rodent huddles change in surface area with temperature, contracting to reduce heat loss in the cold, and expanding to increase heat dissipation in warm environments (Alberts, 1978; Harshaw & Alberts, 2012). The change in huddle structure in response to temperature is due to individual movements. For example, cooled bees moving from the hive surface inward contract their bodies, thereby decreasing the porosity of the hive (Cossins, 2012; Heinrich, 1981). In warm conditions, bees (*Apis mellifera*) move to the periphery of the hive and increase inter-individual distance, resulting in an expansion of the clump. Similarly, penguins forming huddles in cold climates can generate heat that is more than 20°C above their upper critical limit (Caroline Gilbert, Robertson, Le Maho, Naito, & Ancel, 2006; Pinshow, Fedak, Battles, & Schmidt-Nielsen, 1976). To ensure this generated microclimate does not become lethal, individuals in the huddle rotate positions, such that penguins occupying warmer central positions move to the periphery, permitting cooler more peripherally located individuals to then occupy these newly vacant spaces

(Zitterbart, Wienecke, Butler, & Fabry, 2011). Groups, through individual action, can generate local environments that are radically different from the global conditions.

The ability of the group to engineer the environment may depend on the group's size, experience, and ability to sufficiently clump (e.g., large, experienced, and able to sufficiently clump together). For example, individual bees do not significantly differ from tropical and summer insects in their physiological and behavioral responses to temperatures below 13°C, and extended cold exposure to 6-8°C results in death (Free & Spence-Booth, 1958, 1960; for review see Jones & Oldroyd, 2006). As the size of the colony increases, the hive transitions from exhibiting meager thermoregulatory abilities (characteristic of ectotherms), to showing highly organized group behavioral regulation (Heinrich, 1981). Physical barriers that do not permit group members to interact sufficiently may limit the group's ability to construct a niche that is different from the external environment. For example, Contreras (1984) found that aggregating adult mice can reduce the energy needed to combat cold challenges; when a barrier limited their ability to clump together adequately, mice failed to generate a micro-environment that permitted the same metabolic savings. Previous experience may also influence later group dynamics. For example, younger mouse huddles do not contract as effectively as do older huddles in response to cool temperatures (Harshaw & Alberts, 2012). Thus, individuals forming groups can alter local climatic conditions through engineering organic material, including their own bodies, to create micro-environments, but the ability may be complicated by individual and group characteristics, and relatively fixed physical constraints.

New Directions in Organism-Environment Interactions

The environment affecting the organism and the organism affecting the environment are bi-directional interactions that characterize changing systems. Extending this framework to the group-level serves to further our understanding of system-environment interactions, and helps to characterize physiological and behavioral phenomena. Identifying key characteristics of the environment that influence behavior is an important venture, as we may then begin to predict how animal groups will cope with anthropogenic disturbances.

In my studies of environment-organism interactions, I have manipulated simple features of the environment and examined their impact on group responses. I begin with a presentation of empirical studies that test the impact of environmental features on group responses. I end by reviewing factors that underlie behavioral variation, discussing ways in physical and social contexts can have immediate and longer-lasting impacts.

In the first series of experiments, I placed groups on a flat or a concave structure at different temperatures to examine how this feature of a environment might affect group and individual regulatory behavior. I monitored huddle surface areas of Postnatal Day (P) 2, 4, and 8 mice on flat and concave structures at 22°C and 36°C. I then assessed individual movements of pups as singletons and in the presence of a group to determine the impact of social context on their activity. Next, I placed pups in nests with different amounts of available space (or density), to determine if density was modulating the activity of individual pups. Finally, I exposed litters to different temperatures to determine the impact of temperature on the activity of pups during early development. The goal of the study was to determine the influence of physical and social features on the activity of young mice.

In the second series of experiments, I placed eight-day-old groups in flat, concave or conical enclosures varying in density, shape and types of movements that were permissible. I then used infrared thermography and metabolic measures to link experience in the different enclosures with particular energetic consequences. I then recorded rectal temperatures of pups to examine how enclosure structure influenced individual temperature. The goal of the study was to link enclosure structure with energetic consequences for the group and individual.

In the third study, I tested the influence of temperature on the expression of individual and group movements of young mice. I placed P2, P4, and P8 litters with a marked focal pup and Styrofoam marker in a nest with an ambient temperature of 22°C or 36°C. I used focal animal sampling to quantify the activity of individual pups, and the Styrofoam marker to identify the directional movements of the group. The goal of the study was to determine if temperature influenced the expression of individual and group movements of young mice. I then integrated these empirical findings in a review of the impact of a warming climate on the development, and the behavior and cognition of individuals, groups and families of altricial rodents.

In the fourth study, I examined how elements of the environment influence cohesion and coordination of small, loosely coordinated groups of zebrafish. The goal of the study was to tease apart the influence of simple environmental features (i.e., tank size, density, group size) on spacing and alignment. I exposed groups of 4 and 8 fish to different sized arenas, and manipulated tank dimensions to alter the amount of available space per fish to test the effects of group size, tank size, and density. In each condition, I assessed spacing and orientation at the individual and group levels.

In the final empirical study, I tested the effects of environmental toxins on a sensory system, group dynamics and social behavior. The goal of the study was to determine if a few contaminated fish with possible sensory deficits would then affect the social dynamics and group response of a larger uncontaminated group. First, I treated pairs of fish with a low dose of cadmium or water, and compared their individual responses in a task that assessed visual-motor sensitivity. I then measured the responses of these pairs to a novel stimulus. Next, I returned the pairs to their groups, forming mixed shoals and groups made entirely of water-treated fish, and recorded the group response to the same stimulus. I also recorded measures of shoal cohesion and aggression, to determine how a few, contaminated, fish influence social behavior.

Together, these studies shed light on some of the simple environmental features that profoundly affect behavior. This collection of studies is important because they show that the environment can be powerful force in shaping phenotypes, and its impact may depend on the group's development, size, and composition. The ability to see such effects is also enhanced by studying phenomena at multiple levels, especially at the group level. These results are important with imminence of rapid environmental change, which will likely affect many features of the environment.

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Chapter 2:

Environmental Structure and the Expression of Group and Individual Movements in Young Mice

Abstract

The activities of adult individuals are the result of an interaction between the social and physical environments. Here, we tested whether features of the physical and social environment were also important for determining the activity patterns of young animals. We tested the effects of environmental variables on group behavioral regulation in developing mice (*Mus musculus*). Huddle surface areas of Postnatal Day (P) 2, 4, and 8 mice were monitored on flat and concave structures at 22°C and 36°C. Groups displayed regulated changes in exposed surface area at all ages, but the magnitude of the changes were age-related; all ages showed group behavioral regulation on a concave structure whereas only eight-day-old litters showed the behavior on a flat structure. We then identified key features of the microenvironment that influenced the individual movements from which group behavior arises. We found that the amount of available space (or density) influenced the activity of older, but not younger pups. Temperature influenced the frequency of movements of younger, but not older pups. We found no evidence that the social environment alters the activity of individual pups. Thus, the physical environment is a more salient environmental feature than social context in affecting the movements of young mice. These findings provide insights into how simple environmental features, ones susceptible to anthropogenic change, influence the behavior of infant mice.

Introduction

When animals form groups, the expression of their behavior is a product of their social and physical environments (Bergmüller & Taborsky, 2010; Couzin & Krause, 2003). Ambient temperatures can affect the directional movements of groups, their spacing patterns, and activity of individuals within the group (Cossins, 2012; Gilbert et al., 2010). In cool ambient temperatures, flocks of adult penguins and older bee colonies reduce space between conspecifics, causing the clump to contract with individuals periodically shifting positions within the group (Gilbert, Robertson, Le Maho, Naito, & Ancel, 2006; Heinrich, 1981). The social environment can also affect the spacing and activity of individual members and the group. For example, adult familiar sheep form more integrated herds than do groups composed of unfamiliar individuals; familiar groups of sheep walked farther distances and had longer steps than did groups of unfamiliar sheep (Orihuela, Averós, Solano, Clemente, & Estevez, 2016). Whether social or physical environments are more important in influencing the expression of behavior may depend on whether the group is older and experienced, or young and immature.

The physical environment can influence behavioral repertories and the frequency of particular behavior in the repertoire of animal groups. For example, domesticated animals in larger enclosures are more active, making more movements and covering longer distances than animals in smaller arenas (Averós et al., 2014; Leone & Estevez, 2008). When the amount of available space is reduced, locust collectives become more polarized (all group members facing the same direction; Buhl et al., 2006), and herds of sheep become less synchronized in their resting bouts (all group members not resting at the same time; Bøe, Berg, & Andersen, 2006). The activity pattern of animals in environments with low amounts of available space may depend on the geometry of the enclosure. Pen shape influenced the activity budgets of sheep, with members spending more time lying down in deep pens than in wide enclosures (Bøe et al., 2006). The shape of the enclosure can also influence social interactions, as pigs in rectangular and square pens form more small social groups (1-3 pigs) than do individuals in other-shaped arenas (Wiegand, Gonyou, & Curtis, 1994). Here, we ask if the physical environment affects the expression of behavior in small animal groups.

The social environment can also influence the activities of animals. The presence of other conspecfics may influence developmental transitions or the sequential movement through ontogenetic niches (Alberts, 2008; West & King, 2004), cooperation (Earley, 2010), and activity budgets (Beauchamp, 2013). For example, young trout transition to solid food sooner and consume more of it when in the presence of other fish, than when isolated (Sundström & Johnsson, 2001), and calves in the presence of herd members spent more time motionless and were harder to handle (less docile), than when peers were absent (Grignard, Boissy, Boivin, Garel, & Le Neindre, 2000).

The expression of the behavioral repertoire may depend on familiarity. For example, in the presence of a familiar conspecific, sloth bears show more social behavior than when in the presence of an unfamiliar cage-mate (Forthman & Bakeman, 1992). Similarly, previously-familiarized sheep better integrate into groups showing shorter distances between neighbors than those of herds with unfamiliar sheep (Orihuela et al., 2016). Here, we ask if the presence of siblings influences the expression of movement patterns in young animals.

The effect of the social and physical environment on behavior may depend on whether the group is composed of more experienced, older individuals or younger individuals that behave more independently. For example, young nestlings grew faster in heated nests independent of their parents' rearing condition, whereas the body condition of older nestlings was dependent on the experiences of the parent (Pérez, Ardia, Chad, & Clotfelter, 2008). Similarly, exposure to environmental enrichment influenced the performance of younger rats more than older rats in a spatial cognitive task, likely due to development-dependent changes in brain activity (Sampedro-Piquero, Begega, Zancada-Menendez, Cuesta, & Arias, 2013). Younger animals may be more influenced by the physical factors that elicit taxes (heat: Alberts & Brunjes, 1978; Leonard, 1974; walls: Schank & Alberts, 2000), because they have yet to develop morphological or behavioral competencies that enable more complex social interactions. In contrast, older, more familiar groups may be able to coordinate responses to combat environmental challenges. For example, familiar dogs were able to coordinate their positions to receive a food reward (Bräuer, Bös, Call, & Tomasello, 2012), and familiar colonies of spiders were better able to capture food resources collectively (Laskowski, Montiglio, & Pruitt, 2016).

The departures of the mother from the natal nest leaves mouse pups to develop in the presence of 4-8 siblings, usually within the confines of a nest that helps them maintain proximity to each other and promotes their clumping (Berry, 1970; Smith, 1981). The active maintenance of the clump or huddling is the single most time-intensive behavior of infant rodents. While huddling, pups regulate their exposure to the ambient air temperature. They regulate their exposure to the ambient air temperature by making individual movements such that the huddle contracts when cooled and expands when warmed, is a phenomenon termed “group behavioral regulation” (Alberts, 2007; Harshaw & Alberts, 2012).

The movements of mouse pup aggregates have been studied predominantly with the litters on a flat surface; such structures can provide good visibility and augment some measurements. In general, however, pups in enclosures with a flat surfaces tend to assemble next to one another in formations that are basically two-dimensional (2-D). In contrast, pups in a concave-shaped environment, the sloping walls of the structure facilitate pups piling on top of one another and their movements are thus expressed more in three-dimensional (3-D) space (Alberts, 1978). In concave environments, pups do not just contact each other, they move around each other, under other pups and over them. Individual pups also undergo radical changes during early development, as they transition from blind, deaf, furless and writhing infants to more behaviorally-competent members that seek social interactions (Alberts & May, 1984; Blumberg & Sokoloff, 1998; Schank & Alberts, 2000). Thus, mice might change how they respond to the social and physical environment as they age.

In the present study, we tested whether group and individual movements of mice during early development were more strongly influenced by physical or social environments by varying enclosure structure and the presence of littermates. In this study, we examined the group and individual movements of mouse pups during their first week of life, on flat and concave enclosures, and as singletons and in the presence of littermates. If the physical environment is more important for the expression of individual and group movements, we expect to see a difference in movements of mice in different enclosure structures and at different temperatures. If the social environment is more important, we expect to see differences in the movement of pups in the presence and absence of group members. In the first experiment, we compared the group responses of young pups on a flat and concave structure at different temperatures. In the subsequent experiments, we dissected different elements of the first experiment, carefully testing the effect of social context (Experiment 2), amount of available space (Experiment 3), and temperature (Experiment 4), on the activity of young pups.

Experiment 1: Enclosure structure and the expression of group behavior.

There is considerable variation in the structure of house mouse nests (Lisk, Pretlow, & Friedman, 1969; Shump Jr, 1974); A nest can be relatively flat or take on a dome or concave shape, with gently-sloping walls (Hess et al., 2008). In these enclosures, huddles contract and expand in response to temperature in group behavioral regulation (Alberts, 1978; Harshaw & Alberts, 2012). Studies in mice and rats indicate the onset of group behavioral regulation on flat structures at four-days-old (Harshaw & Alberts, 2012) and 5-days-old (Alberts, 1978), respectively. Yet, pups show other regulatory behavior at younger ages (reviewed in Blumberg & Sokoloff, 1998). Studies suggest that the developmental appearance of group behavioral regulation should be gradual on flat structures (Sokoloff et al., 2000; Alberts, 2007; Harshaw & Alberts, 2012), but the expression of group behavioral regulation on other structures is unknown. Here, we compare the impact of a slightly-concave structure and a flat structure on the expression of group regulatory behavior in prenatal mice. We assess the influence of enclosure structure on the expression of group regulatory behavior in young mice, by exposing litters of different ages to a warm and cool challenge on a flat and concave structure.

Method

Subjects

We used litters of mouse pups (*Mus musculus*). Animals were derived from C57BL/6 stock originally purchased from Jackson Laboratory (Bar Harbor, Maine) and bred in Indiana University's Animal Behavior Laboratory colony. Mothers gave birth and reared pups in standard maternity tubs (27 cm length x 13 cm height x 17 cm width) with food and water available *ad libtum*. We maintained the vivarium on 14:10 h light/dark cycle (lights on at 0700 h) at 22.0 ± 2 °C, and humidity 40%.

Procedure

We began with litters (6 pups/litter) of Postnatal day (P) 2, 4, or 8, with 16 groups per age for a total of 48 litters. For each litter, we removed 6 pups from their home cage, selecting pups of similar body weights. We then assigned half the groups to a concave enclosure (Fig 3B) and the others to a flat structure (Fig. 3A). The gently sloping walls of the concave enclosure facilitated clumping of the pups, whereas the flat structure with walls at 90° served as a typical experimental setting. The structures were circular in shape with equal diameters (8.5 cm) and circumferences (26.7 cm). The concave structure had slope of 128°. The concave structure was 8.5 cm tall, and the height of the flat structure was 1.2 cm. The structures were made out of plaster of Paris and then coated with water-soluble polyeurathane creating a non-conductive surface. The walls of the flat structure were formed of non-conductive mesh.

We placed each litter on its assigned experimental structure in a temperature-controlled chamber. The air temperature of the chamber was controlled through circulating temperature-controlled water through the chamber's walls. Litters were serially exposed to a 36°C (±1°C) warm challenge and a 22°C (±1°C) cool challenge, each for 48mins. We counterbalanced temperature presentations so that cool followed warm for half of the litters. There was a 10 - 15 min transition period between challenges to achieve stabilized temperatures in the chamber for behavioral assessment, monitored by a Type K thermocouple connected to an Omega HH802U thermometer (Omega Engineering, Inc., Stamford, CT).

We took a single snapshot of each litter every 8 mins, yielding a total of 6 snapshots of the litter in each temperature condition using a Sony DXC- 151A video camera, positioned directly above the testing chamber, and Scion Image 1.62a, running on a Power Mac 64 (Mac OS 9.1). Immediately prior or after warm ambient air temperature presentations, we arranged all pups individually under the camera, and captured 24 additional frames at 30s intervals with a 36°C ambient temperature. On average, this procedure yielded 3–4 frames in which pups were not in contact, and these images were used to calculate average area measurements for each pup and thus for litters as a whole (scaled in "pup units"; see below).

Area Measures

We reduced the images of the huddles to quantifiable areas by using ImageJ (Schneider, Rasband, & Eliceiri, 2012). We traced the outlines of the pups, scanned the tracings as images and then measured them in ImageJ (see Harshaw and Alberts 2012 for more detailed methods). In ImageJ, we first repaired lost areas by darkening finely-traced lines so that they were sufficiently pixelated to be detected by ImageJ. We then measured the huddle area using the “Analyze Particles” function, correcting for any enclosed, non-pup areas formed by huddles, using the “Wand” and “Measures” tools, which provide measures of any selected region of an image. To assess contraction of the huddle, we found the huddle area difference, which is the difference between the average huddle area at the warm temperature minus the average huddle area at the cool temperature ($H_{36°C}$-$H_{22°C}$). We obtained an average area for each pup using measurements obtained from the images of individual pups (taken at 36 ± 1°C). We averaged these average measurements for each pup across pups within each litter and divided them by the huddle area to yield an average “pup unit” for that litter. Thus, the maximum size of a litter in pup units would be six, if all pups in the litter were dispersed and making no contact. The maximum value for each pup can, however, exceed one pup unit and groups can thus exceed six, since the metric is based on averages. We report area measurements in pup units, because pup units makes possible direct comparisons within and between ages, independent of variation in body size and camera distance.

Statistical Analysis

We averaged measures of huddle area difference across the six images, and then used this difference value in a two-way ANOVA with Tukey post-hoc tests to examine the effects of enclosure Structure (concave or flat) and Age (P2, P4, or P8) on average huddle area difference. We used Type III sums of squares, an alpha level of .05 and confirmed that the residuals conformed to the normality and homoscedasticity assumptions of the ANOVA. We conducted all statistical analyses in R using the ‘base’ package (R Core Team, 2015).

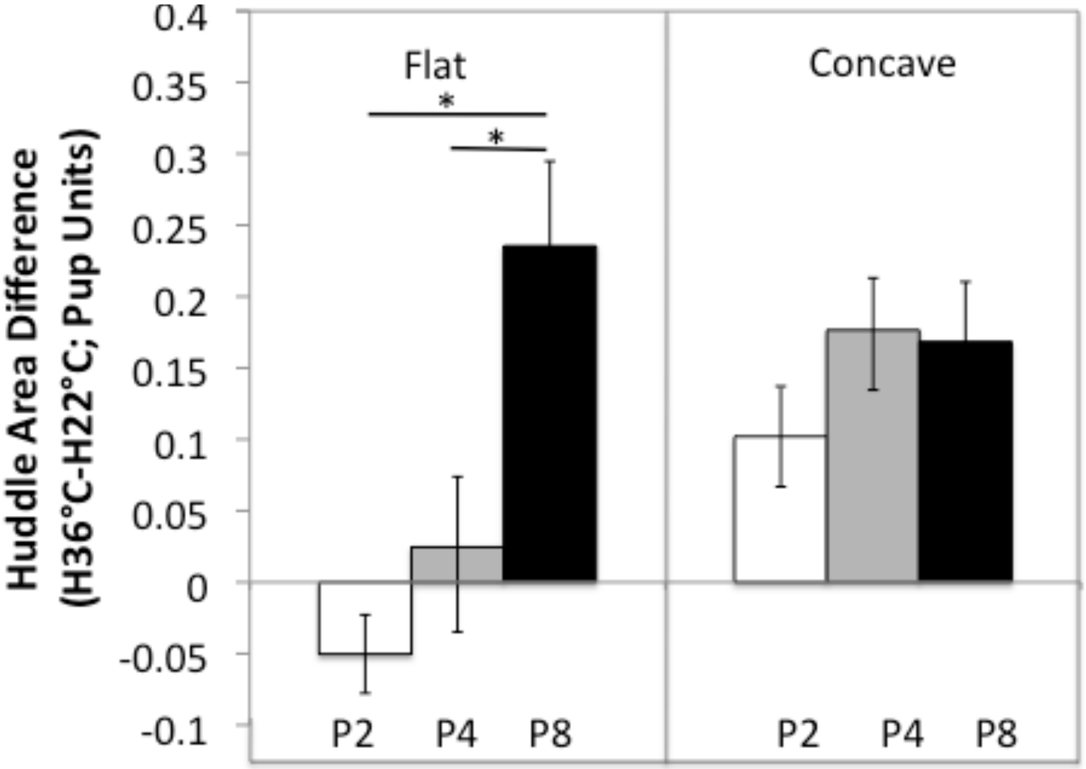

Figure 1. **Environmental structure leads to contrasting expressions of group behavioral regulation across different ages.** On a concave structure, pup groups of all ages showed group regulatory behavior by adjusting group surface area in relation to ambient temperature. In contrast, on a flat structure, only huddles of 8-day-old pups contracted at cool (22°C) temperatures and expanded at warm (36°C) ambient temperatures. Error bars are one standard error. * indicates significant Tukey post-hoc tests at $p < .05$.

Results and Discussion

We found that enclosure structure influenced the behavior of the pups, which affected the huddle structure, with age-specific differences in the adjustment of group area in relation to temperature. On a concave structure, pups of all ages showed group regulatory behavior by contracting at cool (22°C) temperatures and expanding at warm (36°C) temperatures. The huddles reduced their surface area when exposed to a warm ambient temperature followed by a cool ambient temperature, and increased their surface area when the order of temperatures were reversed. The huddle area difference scores in pup units for P2, P4, and P8 pups were 0.10 (95% CI [0.03, 0.17]), 0.18 (95% CI [0.06, 0.29]), and 0.24 (95% CI [0.14, 0.33]), respectively. In contrast, on a flat structure, only P8 huddles adjusted group surface area in relation to ambient temperature contracting 0.17 (95% CI[0.03, 0.31]), whereas huddles of 2-day-old and 4-day-old pups were similar in size at warm and cool temperatures, -0.05, 95% CI[-0.13, 0.03]) and 0.02 (95% CI[-0.06, 0.11]), respectively. The difference in huddle area difference score on a flat and concave structure for the different ages led to a significant Structure x Age interaction effect ($F_{2, 42} = 4.36$, $p = .02$; Fig. 1).

All litters tested on a concave structure adjusted their area in response to temperature, whereas only eight-day-old litters did so on a flat structure. This difference lead to a significant main effect of Structure ($F_{1\ 42} = 5.10$ $p = .03$). Older litters adjusted their huddle area in response to temperature more than younger litters. This difference lead to a significant main effect of Age ($F_{1\ 42} = 8.49$ $p < .01$). On a concave structure, the difference between the huddle area difference scores of P2, P4, and P8 litters was not significantly greater than zero (p > .80, Tukey; for all pair-wise comparisons). The difference in huddle contraction of P8 and P2 litters on a flat structure was more than a quarter of a pup unit ($p < .01$, Tukey). Similarly, the difference between huddle areas of P8 and P4 litters on a flat structure was a little less than a quarter of pup unit ($p = .01$, Tukey). The difference between the contracted huddles of P4 and P2 huddles was negligible, .07 pup units, and not significantly different from zero ($p = .82$, Tukey).

We found that the concave structure enhanced the infants' capabilities, providing important information on the abilities of infant mice, especially those tested in "standard" laboratory settings, on a flat surface. On a flat structure, the appearance of group behavioral regulation was incremental, whereas in a concave enclosure all ages expressed the ability to adjust the huddle area in response to temperature. The affordances of the concave structure, such as the curved sides, enhanced piling of the pups, which augmented the expression of group behavioral regulation. In a concave enclosure, P4 and P8 huddles contracted nearly a quarter of a pup unit, which may be indicative of a ceiling effect. The structure of the concave structure permitted a maximum contraction of .25 pup units, which may have been prohibitive of demonstrating progressive developmental differences in group behavioral regulation. Harshaw and Alberts (2012) also reported a gradual development of such group regulation, with an earlier onset on a flat structure than I found in the present study; their regime of temperature challenge differed from the one used here. Harshaw and Alberts (2012) also reported larger changes in huddle size than the responses found here, maximum huddle change was 2.2 and .25 pup units for the respective studies. In Harshaw and Alberts (2012), the use of an acclimation period and gradual reduction in ambient temperature may have facilitated the expression of group behavioral regulation in younger litters. Also, differences in the measurements of the huddle used in each study may account for observed findings, as Harshaw and Alberts (2012) used huddle perimeter and huddle area was used in the present study.

Studies examining the phenotypes of infant rodents on a flat structure paint a different picture of rodent competencies than do studies of the same individuals situated in, an enclosure with curved walls. The notion that infant rodents develop homeothermy remains steadfast when examining huddles on a traditional experimental structure, a flat structure (Sokoloff & Blumberg, 2001; Harshaw & Alberts, 2012). In an enclosure with curved walls, a concave structure, P2-P8 litters show a robust reduction of surface area in response to cool ambient temperatures. The accelerated expression of group behavioral regulation by over a week for litters on a concave structure is a testament to the influence of the physical context on development.

Experiment 2: Enclosure Structure, but not Social Context Influences the Expression of Individual Activity

Group behavior is generally thought to arise from the actions and interactions of individuals (Giardina, 2008; Vicsek & Zafeiris, 2012). For example, the collective movement of fish shoals arises from each individual following simple behavioral rules such as attraction, alignment, and repulsion (Hemelrijk & Hildenbrandt, 2008; Viscido, Parrish, & Grunbaum, 2004). Similarly, huddling or the active maintenance of the clump, has been modeled using conditions that relate bouts of activity and inactivity and an individual's preference for objects in the environment (Schank & Alberts 1997). The individual movements that characterize huddling have also been modeled with rules such as turn-towards-heat and phase transitions that characterize the passage of heat between pups bodies (Glancy, Groß, Stone, & Wilson, 2015). These computational views of how group behavior arises are largely dependent on individuals interacting with other individuals, but the physical environment may also play a role.

In young rodents, the complexity of the rules governing collective movement is compounded by development. Initially, infant interactions are strongly guided by their pursuit of warmth (pups are strongly thermotaxic) (Alberts & Brunjes, 1978; Leonard, 1974); as they age individual thermoregulation changes dramatically (Farrell & Alberts, 2007; Hoffman, Flory, & Alberts, 1999; Pfister, 1990) with individuals becoming less dependent on thermal cues (Alberts & May, 1984; Leonard, 1974), and more selective in their contact-dependent interactions (Alberts & Brunjes, 1978; Schank & Alberts, 2000). Here, we test if environmental structure influences the expression of individual behavior during early development in different social contexts by measuring individual activity of young mouse pups on a flat and concave structure in the presence and absence of group members.

Method

Procedure

To determine whether enclosure structure or the presence of littermates affected individual regulatory movements during early development, we tested another 24 litters (6 pups/litter) on P2, P4, and P8. We tested pups on flat and concave structures identical to those described in Experiment 1. Immediately before testing, we removed six pups of similar weight from their home cage. We then selected a pup to serve as the focal individual, and marked it with a line of paint across the point of shoulder for identification and later behavioral scoring. We then placed half of the litters on a flat structure and the others in a concave structure with the focal pups placed in the center of the enclosure and unobscured by littermates. We then placed each enclosure in the temperature-controlled chamber with a temperature of 22°C, filming the subjects with a Logitech digital camera for 1h. Afterwards, we removed the littermates from the structure, and placed the focal pup again in the center of the enclosure. We then returned both pup and the testing arena to the temperature-controlled chamber and filmed for a second hour. We counterbalanced the order of the group and individual sessions so that we tested half of the pups in the context of the litter first and second as individuals, and for the others, individual sessions preceded group testing.

We divided the surface of the structure into four equal-sized quadrats. We then counted the number of times that the marked shoulder of the focal pup crossed into a new quadrant as a measure of activity.

Statistical Analysis

To examine the effects of enclosure Structure (flat, concave), Context (individual, group) and Age (P2, P4, or P8) on the number of line crosses during the trial (or activity), we used a three-way repeated-measures ANOVA with Tukey post-hoc tests. We used Type III sums of squares, an alpha level of .05 and confirmed that the residuals conformed to the assumptions of the ANOVA. We conducted all statistical analyses in R (R Core Team, 2015), using the ‘nlme’ (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2016) package to fit the repeated-measures ANOVA and ‘multcomp’ (Hothorn, Bretz, & Westfall, 2008) package to conduct Tukey post-hoc tests.

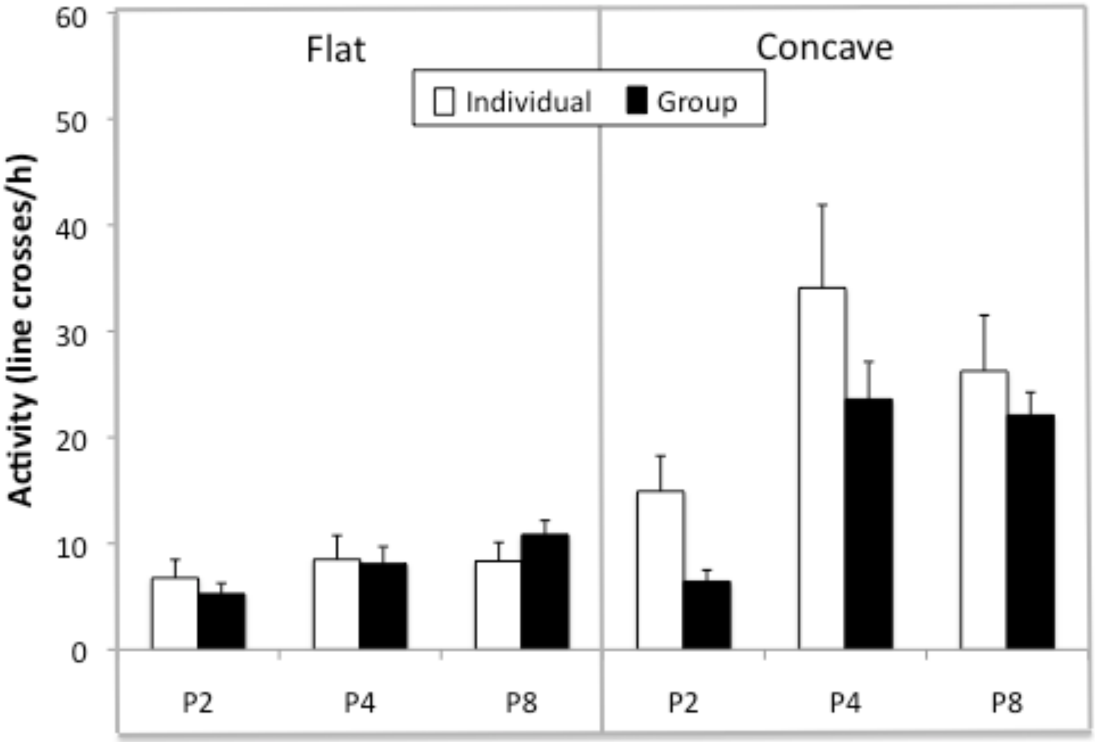

Figure 2. **Enclosure structure, but not social context, influenced the frequency of horizontal transitions in older mouse pups.** In a flat enclosure, all ages had similar activity levels when tested as individuals (white bars) and in the presence of a group (black bars). In a concave-shaped enclosure, pups increased activity with age whether measured as individuals or in the presence of a group. Older pups (P4 and P8) on a concave structure were more active than were younger pups (P2) on a flat structure. Error bars are one standard error.

Results and Discussion

We found that enclosure structure and the age of the pups was associated with the expression of individual movements. Older pups were more active than were younger pups on a concave structure, but not on a flat structure, whether tested as individuals or in a group. When tested as individuals on a concave structure, P4 (M = 34.08, 95% CI[16.88, 51.28]) and P8 (M = 26.25, 95% CI[14.69, 37.82]) pups were 1.8 times more active than were 2-day-old pups (M = 14.92, 95% CI[7.56, 22.27]). On a concave structure, P4 (M = 23.58, 95% CI[15.81, 31.36]) and P8 pups (M = 22.08, 95% CI[17.32, 26.85]) were almost 4 times as active as were P2 pups (M = 6.41, 95% CI[4.03, 8.80]) when tested in the context of littermates. On concave and flat structures, P4 and P8 pups showed a similar number of transitions as individuals and in the context of a group, but pups on a flat structure were less active than were those on a concave structure. On both concave and flat structures, P2 pups had similarly low levels of activity. These differences led to a significant Age x Structure interaction effect ($F_{2, 88} = 11.30$, $p < .01$; Fig. 2).

Pups in a concave enclosure were more active than were pups on a flat structure. On a flat structure, the average activity of pups tested alone for P2, P4, and P8 were 6.75, 8.50, and 8.33 line crosses/h, respectively. The associated 95% confidence intervals for each mean were [2.95, 10.55], [3.53, 13.47]), [4.44, 12.22]. In the context of the group, the average activity of the pups were: 5.25, 8.08, and 10.83 line crosses/h for P2, P4, and P8 pups, respectively. The associated 95% confidence intervals for the means were [3.03, 7.47], [4.50, 11.66], [7.90, 13.76] for P2, P4, and P8 pups, respectively. On a flat structure, all ages showed a low number (< 10) of line crosses. The influence of enclosure structure on activity was driven by the high levels of activity of four- and eight-day-old pups on the concave structure, as they had at least twice the activity levels of counterparts on a flat structure. Two-day-old pups showed meager differences in activity on flat and concave structures. These differences lead to a significant main effect of Structure ($F_{1, 22}$

= 17.10, $p < .01$). The differences between the activity levels of P8 pups on flat and concave structures were greater than zero when pups were tested as individuals ($p < .01$, Tukey), but not as a group ($p = .87$, Tukey). The difference between activity levels of P4 pups on flat and concave structures was greater than zero when pups were tested as individuals ($p < .01$, Tukey) and as groups ($p = .04$, Tukey). The difference between the number of lines crosses of P2 pups on a flat and concave enclosure was not significant whether pups were tested as individuals ($p = 1$, Tukey) or in a group ($p = 1$, Tukey).

Older pups were more active than were younger pups. The difference in activity across ages led to a significant main effect of Age ($F_{2, 88} = 21.13$, $p < .01$). The difference in activity across ages was largely driven by the high levels of activity of P4 and P8 pups on the concave structure, as pups of all ages had similar levels of activity on a flat structure. Four- and eight-day-old litters were at least twice as active as were two-day-old litters on a concave structure ($p < .02$, Tukey, for all comparisons between P2 and older pups). For pups tested on a flat structure, the posthoc comparisons were not statistically significant for any of pair-wise comparisons between any of the ages ($p = 1$, Tukey).

On concave and flat structures, pups displayed meager differences in levels of activity when tested as individuals and in the context of a group. On a flat structure, all ages showed a low number (< 10) of line crosses when tested as individuals and in the presence of littermates. The difference between pups tested on a concave structure as individuals and in a group, though greater than those tested on a flat structure, were small. These differences did not lead to a significant Context x Structure interaction effect ($F_{2, 22}$ = 3.41, $p = .08$). Pups showed similar levels of activity as individuals and in the context of the group, which lead to a main effect of Context that was not statistically significant ($F_{1, 22} = 3.09$, $p = .09$). The Context x Age x Structure, Context x Age, and Context x Structure interaction effects were also not statistically significant ($F_{2, 88} = 0.16$, $p = .86$; $F_{2, 88} = 1.14$, $p = .32$; $F_{2, 22} = 3.41$, $p = .08$, respectively).

The results of Experiment 2 suggest that enclosure structure is more important than the presence of littermates in affecting individual movements. The enclosure structure had age-dependent effects on the expression of individual movements. Younger pups were similarly unaffected by each enclosure structure, whereas older pups showed differences in activity depending on the structure of the enclosure. These findings emphasize the importance of development in influencing the responses to environmental structure, and provide more insights into the contexts that affect the activities of young pups.

Experiment 3: Density, but not Enclosure Shape Influences Activity

The results of Experiments 1 and 2 leave unclear which features of the environment influence the expression of individual movements at different developmental time points. The two enclosure structures had similar diameters and circumferences, but differed in shape and available space. The concave structure had sloping walls and less available space, whereas the flat structure had walls at 90° from the flat floor, and more available space. To determine whether the shape or density of the enclosure influenced a pup's individual movements, we tested pups on a small, flat structure. The small, flat structure had the same shape as the flat structure from Experiments 1 and 2, and densities that were equal to the concave structure of these earlier experiments. If mouse pups showed similar levels of activity on the small and large, flat structures, it would suggest that enclosure shape is the more salient feature affecting individual movements. If mice showed activity levels on the small, flat structure equivalent to those seen on the concave structure, then density would appear to be factor more greatly influencing the individual pup's activity.

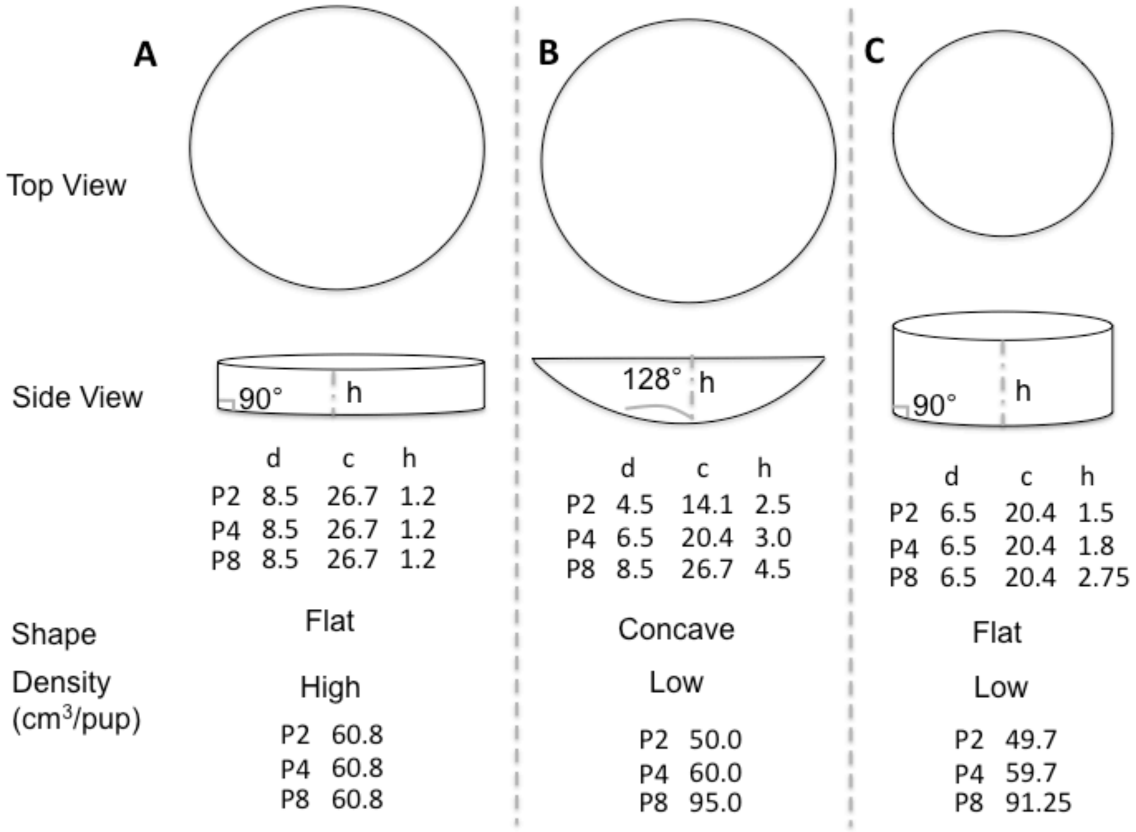

Figure 3. The dimensions used to calculate the density for each of the three treatment conditions for each age: A) flat enclosure with high-density and short height (h); B) a concave enclosure with the same circumference, but with lower density; C) a flat enclosure with same shape as the other flat enclosure, but with a low density. We scaled the measurements, diameter (d), circumference (c), and height (h), to calculate density for each age, so that the available space corresponded to the maximum space used by the

infants in the enclosure. All length measurements are in centimeters and angular measurements are in degrees.

Method

Procedure

To determine if density was influencing the regulatory movements (or activity) of pups in the different enclosures, we used another 68 litters at two, four, and eight days of age, with some litters tested at multiple ages, in a, large flat, concave, or small, or flat enclosure (Fig. 3A, B, C, respectively). The concave and large, flat enclosures were circular and identical to the structures described in the previous experiments. The small, flat structure was circular with a smaller diameter (6.5 cm) and circumference (20.4 cm). The floor of the small flat structure was made of Styrofoam, and the walls were made of mesh; these materials were nonconductive. The structures varied in density with pups in the concave and small flat enclosures having about twice the available space as did pups tested in the large flat enclosure.

For each trial, we selected a focal pup and marked it using the same procedure as described above. We placed the focal pup in the center of the flat-floored enclosure, and on top of littermates in the concave enclosure. The enclosure with pups was then placed immediately in the temperature controlled chamber set at 22°C, and filmed for 1h with a Logitech digital camera. We then scored activity using the same sampling procedure as described above.

Density

Density was defined as the amount of utilized space per individual mouse pup.

$$\text{Density} = \text{Utilized Volume}/\ \text{Number of Animals}$$

The density was calculated by finding the volume of the enclosure and then dividing it by the number of animals in the enclosure. The volume of the enclosure was calculated by finding the maximal space the mouse pups used, or the highest, widest, and longest points the pups reached in the enclosure. The highest points were determined by observing the pups in real time and determining the maximum points reached by the mouse pups. A lower-density enclosure is one where there is more utilized space per pup, or pups had higher peak measurements than pups in enclosures with higher-density, or less utilized space and lower peak measurements for the volume measurements. Because this measure of density was scaled for each age it necessarily takes into account the body size differences that occur across ages.

Statistical Analysis

To examine the effects of enclosure Shape (flat, concave), Context (individual, group) and Age (P2, P4, or P8) on activity (number of line crosses during the trial), we used a mixed-effects model, including an Age x Context interaction term and main effect terms for Shape, Age, and Context. We then used Tukey post-hoc tests to expand significant interaction and main effect terms. We used Type III sums of squares, an alpha level of .05 and visual inspection of the residual plots did not reveal any obvious deviations from homoscedasticity and normality. We conducted all statistical analyses in R (R Core Team, 2015), using the ‘lme4’ package (Pinheiro et al., 2016) to fit the ANOVA and ‘multcomp’ package (Hothorn et al., 2008) to conduct post-hoc comparisons.

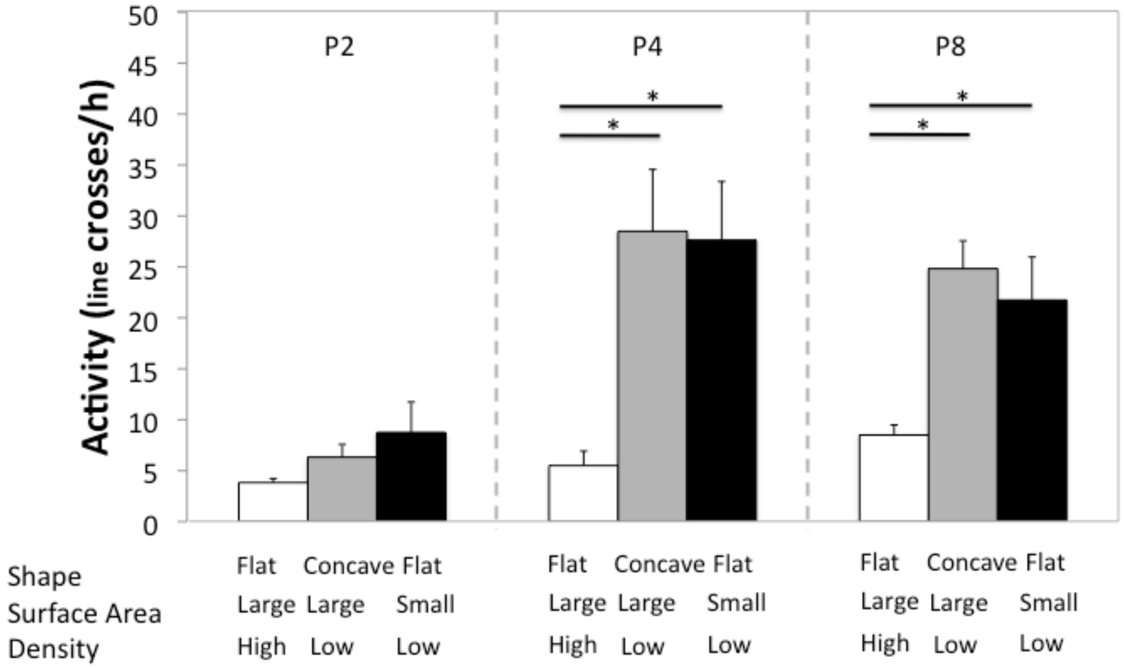

Figure 4. **Density influences activity in older, but not younger pups.** P4 and P8 with more available space (low density) made more transitions than did pups with less available space (high density). P2 pups made a similar number of transitions in higher and lower density conditions. The dashed gray line corresponds to litters tested on a concave structure at low density. The dashed black line indicates groups tested on a small flat structure with high density. The solid black line indicates litters tested on a large flat structure with low density. * indicates significant Tukey post-hoc tests at $p < .05$. Error bars are one standard error.

Results and Discussion

We found that pups with more available space (or lower density) were more active than were mouse pups with less available space (or higher density), especially at four and eight days- of-age. Mice at higher densities showed similar activity levels at all ages P2 (M = 3.83, 95% CI[2.80, 4.87]), P4 (M = 5.50, 95% CI[1.82, 9.18]), P8 (M = 8.50, 95% CI[5.95, 11.05]), whereas mice at lower densities peaked in activity on P4 (Concave High: M = 28.50, 95% CI[12.92, 44.08], Flat High: M = 27.63, 95% CI[14.03, 41.22]), and showed relatively similar levels of activity on P8 (Concave High: M = 24.83, 95% CI[17.83, 31.84], Flat High: M = 21.75, 95% CI[11.75, 31.75]). P4 and P8 pups in lower density enclosures were 5 and 2.5 times as active as were their agemates tested in a higher density enclosure, respectively. The pattern changed with P2 pups, as they showed a similar number of transitions in each test environment. In lower density conditions, older pups were at least three times as active as were the youngest pups tested. This led to a significant Density x Age interaction effect for activity ($F_{2, 12} = 4.11$, $p = .04$; Fig. 4). The difference between P4 and P8 pups at low density and P2 litters at either low or high density was significant ($p < 0.01$, Tukey, for each comparison). The difference in activity for P4 and P8 pups in lower and higher density was significantly different from zero ($p < 0.01$ and $p = 0.03$, Tukey, respectively). For P2 pups, the difference in activity on lower and higher density environments was not statistically significant ($p = 1$, Tukey). The difference between P4 and P8 litters in the higher density condition was also not statistically significant ($p = 1$, Tukey).

Older pups were more active than were younger pups. Four- and eight-day-old pups were at least 1.4 times more active than were two-day-old pups. This difference led to a significant main effect of Age ($F_{2, 12} = 15.46$, $p < .01$). The age-dependent difference in activity was not driven by the activity of pups on lower density enclosures, as posthoc tests were not statistically significant for all age comparisons in this environment ($p = 1$). The implication of these data is that the amount of available space (or density), but not enclosure shape, influences the movements of individual pups. It is likely that the enclosure structures that promote 3-D clumping also augmented activity levels. That is, the greater number of reaction points (e.g., sides of the structure and pup bodies) in a smaller area permitted pups to move more rapidly within the environment.

The shape of the structure did not systematically influence activity. Pups tested on similarly-shaped structures did not have the same levels of activity. Pups tested on the two flat structures had different levels of activity. Pups tested on differently-shaped structures, but with similar density values had similar levels of activity. Pups tested on a low-density flat and low-density concave structure had similar levels of activity. The main effect of Shape was not significant ($F_{2, 41} = 0.03$, $p = .87$).

Density, but not enclosure shape, led to differences in the activity of pups. The effect of density on pup activity depended on age, leading to differences in the activity of older (P4 and P8), but not younger pups (P2). The lack of the influence of density on the individual movements of younger pups suggest that their activity within the group is more fixed or more plausibly influenced by another feature in the environment, such as temperature. Thus, density is a salient environmental feature that influences the individual movements of pups during the latter part of early development.

Experiment 4: Older but not Younger Pups Show Temperature-dependent Activity

Infant rodents develop as and within the context of a litter. These aspects of development are related and distinguishable in certain environmental contexts. As a group, infant rodents form huddles that contract and expand in response to changes in ambient temperature when in a flat and concave structure (Experiment 1; Alberts, 1978; Harshaw & Alberts, 2012). Such group behavioral regulation is accomplished by the movements of individual pups, and depends on environmental structure. The affordances of a concave structure permit pups to pile on top of one another and all ages show group behavioral regulation, whereas in a flat structure only older ages show group behavioral regulation. In Experiment 3, we found that density was a powerful force acting on individual activity with pups under higher density conditions showing a higher frequency of movements than groups in lower density conditions; however, this effect was only seen at older ages.

The effect of density is often confounded by body and air temperature, as animals in higher density conditions tend to be warmer than are groups in lower density conditions. For instance, passerine birds maintained higher body temperatures in less available space than did individuals with more available space (Walsberg, 1990; Wojciechowski, Jefimow, & Pinshow, 2008). Animals that reduce the amount of available space through actively maintaining a clump are able to alter the temperature of the microclimate. For example, cavity-dwelling bats (more bats in a cavity) and voles (more voles in a chamber) with less available space increased the ambient air temperature in comparison to counterparts with less available space (Hayes, Speakman, & Racey, 1992; Willis & Brigham, 2007), thereby indirectly warming (through convection) the inhabitants (Walsberg, 1990). The magnitude of the temperature increase is shown to vary with group size, with more individuals in a cavity (less available space) increasing the temperature more than less individuals in a cavity (more available space) (Angilletta, Cooper, Schuler, & Boyles, 2010; Willis & Brigham, 2007). Here, we test whether ambient temperature influences the expression of individual activity of young pups, by exposing litters of pups to different temperatures while controlling for density.

Method

Procedure

To test whether temperature influences activity during early development, we tested another 80 litters (6 pups/litter) composed of equal number of males and females (3 males and 3 females/ litter) on P2, P4 or P8 at one of four ambient temperatures.

Immediately before testing, we removed six pups from their home cage. A randomly selected male and female pup were marked with white paint for identification. We marked the female pup with a dotted line across the crown of the head, point of shoulder, and around the rump. The male pup was marked with a solid line on the same body areas. We placed all pups into a nonconductive funnel cut horizontally to form a truncated cone, 6.2 cm high, top diameter 7.2 cm with a 30° sloping wall, which enhanced 3-D piling. We standardized the bottom diameter for each age: 2.2 cm, 2.4 cm, and 2.75 cm for P2, P4, and P8, respectively. We covered the bottom of the enclosure with shavings from the litter's home cage.

Prior to recording, we positioned the focal pups on top of the clump. We exposed the groups to one of the following ambient temperatures 11±1 °C, 22±1°C. In addition, groups of P8 litters were exposed to 7.5±1°C. The truncated cone enclosure was located in a temperature controlled doubled-walled glass cylinder (18 cm in height; inner diameter 8 cm). Circulating temperature-controlled water through the cylinder's walls regulated the air temperature inside the enclosure. We recorded the group for 1h using a Logitech digital camera. We then scored activity using the procedure described in Experiment 2.

Statistical Analysis

We used a two-way ANOVA to test the effects of Temperature (11°C, 22°C), and Age (P2, P4, P8) on activity. We then used Tukey post-hoc tests to expand significant interaction and main effect terms. Next, we used a one-way ANOVA test the effects of colder temperatures (7.5°C, 11°C, 22°C) on the activity of eight-day-old pups. We used Type III sums of squares, which corrects for unbalanced data (Shaw & Mitchell-Olds, 1993) and an alpha level of .05. We also log base-10 transformed activity, as required to obtain residuals that conformed to the homoscedasticity and normality assumptions of the ANOVA.

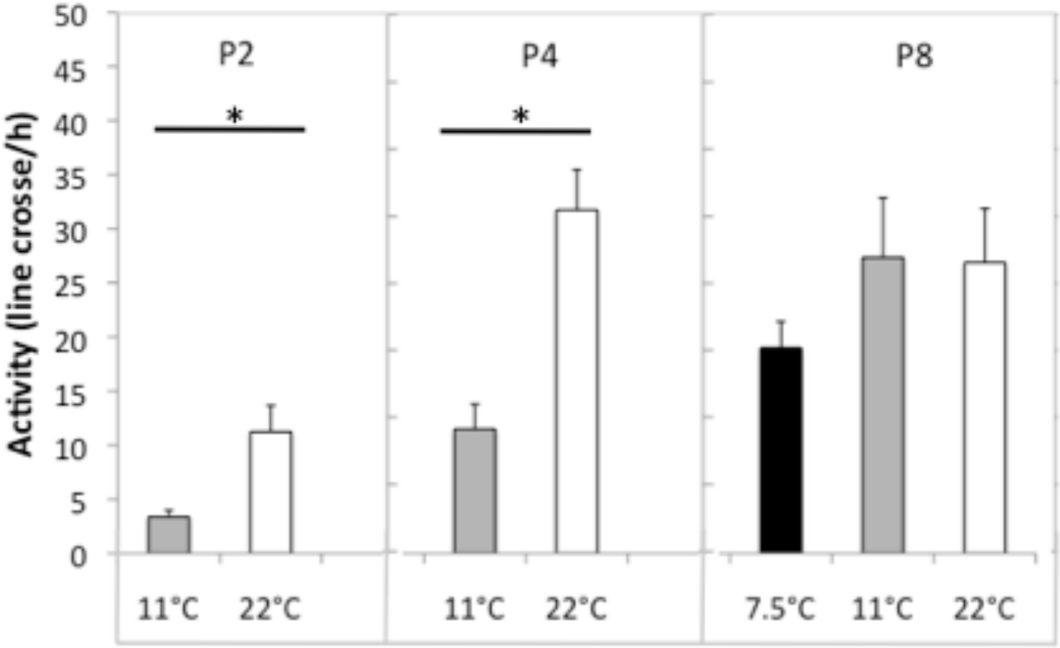

Figure 5. **Younger, but not older ages show temperature-dependent activity.** Activity peaked at 22°C in P2 and P4 huddles, whereas P8 pups showed similar high levels of activity at 11°C and 22°C. Activity in P2 and P4 pups decreased by at least 60% from 22°C to 11°C. P8 litters showed more sustained levels of activity over a broader range of temperatures. * indicates significant Tukey post-hoc tests at $p < .05$. Error bars are one standard error.

Results and Discussion

Younger, but not older pups showed temperature-dependent activity. Temperature had a stronger effect on the activity of the younger (P2 and P4) litters than on the P8 litters. We found that younger pups had higher levels of activity at warmer (22°C: P2, M =11.25, 95% CI[5.55, 16.95]; P4 , M = 31.75, 95% CI[22.98, 40.52]), but not cooler temperatures (11°C: P2, M = 3.38, 95% CI[1.90, 4.85]; P4 , M = 11.50, 95% CI[6.08, 16.92]), whereas older pups had more sustained levels of activity across the tested temperatures (22°C: M = 27.38, 95% CI[14.40, 40.35]; 11°C: M = 26.88, 95% CI[15.10, 38.65]), and even a colder temperature (7.5°C: M = 19, 95% CI[13.21, 24.79]. We found P2 and P4 litters peaked in activity at 22°C and decreased by at least 60% at cooler temperatures. P8 huddles maintained similar levels of activity at 22°C and 11°C, and then possibly decreased in activity at cooler (7.5°C) temperatures. This led to a significant Age x Temperature interaction effect ($F_{2, 42} = 5.06$, $p = .01$; Fig. 5). Two-day-old pups showed a 70% decrease in activity from 22°C to 11°C ($p < 0.01$, Tukey). Similarly, four-day-old pups showed a 64% decrease in activity from 22°C to 11°C ($p < 0.01$, Tukey). Eight-day-old pups showed similar levels of activity at 22 °C and 11°C ($p = 0.34$, Tukey).

Older pups were more active than were younger pups. Eight-day-old pups were more than twice as active as four-day-old pups and around nine times as active as were two-day-old pups at 11°C. At 22°C, four- and eight-day-old pups were the most active, and more than twice as active as were two-day-old pups. This difference led to a significant main effect of Age ($F_{2, 42} = 31.58$, $p < .01$). There was no difference in activity of four- and eight-day-old pups at the highest (22°C) temperature measured ($p = .96$, Tukey). At 11°C, there was a difference between eight- and four-day-old ($p = .045$, Tukey), and eight- and two-day-olds ($p < .01$, Tukey). Four-day-old pups were more active than were two-day-old pups at 22°C ($p < .01$, Tukey) and 11°C ($p < .01$, Tukey).

Pups decreased in activity at cooler temperatures. This difference led to a significant main effect of Temperature ($F_{2, 42} = 22.68$, $p < .01$). Four day-old and two-day-old pups were more active at 22°C than 11°C ($p < .01$, Tukey; for each comparison). The responses of eight-day-old pups were not driving the main effect, because they showed similar levels of activity at 22°C and 11°C ($p = 1$, Tukey). When eight-day-old pups were tested at an even cooler temperature (7.5°C), pups continued to show comparable levels of activity to those of eight-day-old pups tested at 22°C and 11°C. There was no significant main effect of Temperature for eight-day-old pups when tested at the broad range of cool temperatures ($F_{2, 21} = 0.69$, $p = .51$).

Shifts in peak activity levels can be attributed to development of other thermoregulatory mechanisms and perhaps a reduced sensitivity to cold (Blumberg & Sokoloff, 1998; Leonard, 1974; Pfister, 1990). We know from findings in other altricial rodent species, rats, that between 2 and 8 days-of-age, a pup's epidermis thickens, surface area to volume ratio decreases, pelage lengthens and thickens (Hahn, 1956), and locomotor abilities are enhanced (Altman & Sudarshan, 1975). These changes may serve as insulatory buffers against heat loss, increase cold tolerance, and permit P8 pups to remain active at cooler temperatures. It seems possible that the cold challenge of 11°C overwhelmed the P4 and P2 litters resulting in low levels of activity. Ogilvie and Stinson (1966) noted similar immobilization of P2 mice when located on floor temperatures of 16°C or lower. In addition, the locomotor abilities of pups progress in parallel with age (Ogilvie & Stinson, 1966). Poor locomotor ability of P2 pups may account for their attenuated response curve.

Heightened activity of the pups at 22°C may be indicative of pups actively searching for warm spots in the huddle, a limited resource. In other species, the activity pattern of animals in environments have been show to change with the abundance of critical resources. For example, adult herbivores in small pastures were more active, as they searched for high-quality food, than their lower density counter parts with more available food (Mobæk, Mysterud, Loe, Holand, & Austrheim, 2012). Similarly, salmon hatchlings in environments with limited number of shelters become fiercely competitive with fish under higher density conditions losing more weight than do fish under lower density conditions (Finstad, Einum, Ugedal, & Forseth, 2009). In rabbits and rats, young pups compete for more desirable positions in the huddle through actively displacing other individuals (Bautista, Castelán, Pérez-Roldán, Martínez-Gómez, & Hudson, 2013; Sokoloff & Blumberg, 2001). Future studies should determine if young mice compete for different positions within the huddle, and if those ideal positions change with other features of the environment.

General Discussion

We found that the movements of pups depend on the amount of available space (i.e., density) and temperature, but not the presence of group members. Mouse pups in enclosures with less available space were more active than were individuals on structures with more available space. The activity levels of the pups were not affected by the presence of littermates in the different enclosure structures. Thus, the pups were responding more to physical environmental features, adjusting their activity in response to temperature and density. The effect of the physical environment on the movements of the mice varied with the age of the pups. Older pups (P8, P4) showed structure dependent differences in activity, whereas younger pups (P2) had similar levels of activity, independent of the enclosure structure. Temperature strongly influenced the activity of younger pups (P4, P2), but had no measureable effect on older pups (P8), which showed high levels of activity at all temperatures.

The relative amount of available space and temperature were the most salient factors affecting the activity of young mice. Density is clearly important for shaping the behavior of other species as adults. For example, adults are more active (sheep: Mobæk et al., 2012), synchronized (locusts: Buhl et al., 2006), and space themselves closer together (chickens: Leone, Christman, Douglass, & Estevez, 2010) in higher density conditions, than do animals in lower density environments. The relative amount of available space influences the spacing patterns of adult zebrafish, with fish under high-density conditions positioning themselves closer to their neighbors than fish in lower density arenas (Shelton, Price, Ocasio, & Martins, 2015; Chapter 6). Here, we have shown that density and temperature can also be highly relevant to younger animals, influencing their activity in presence of littermates and when tested alone.

Temperature influenced the activity of young mice. Temperature is also important in influencing the behavior of other species that form groups (Gilbert et al., 2010; Gunderson, Leal, III, & Bronstein, 2015). For example, troops of monkeys were more active at night (Fernandez-Duque, 2003) and groomed more (Troisi & Schino, 1987) when the daytime temperature was high than when ambient temperature was cool. In fish, temperature influenced spacing patterns, group dynamics and activity (Colchen, Teletchea, Fontaine, & Pasquet, 2016), and had consequences for growth (Jonsson, Jonsson, & Finstad, 2013), development. (Scott & Johnston, 2012), and survival (Crossin et al., 2008). Future studies should determine if temperature has functional and long-term consequences for altricial mammals.

The influence of the environment on behavioral repertoire depends on the age of the individuals. For example, young rats and hamsters show robust thermotaxis on a thermocline, but the strength of taxic response wanes after the first week of life (Alberts & Brunjes, 1978; Leonard, 1974). As the animals age, features of the environment begin to affect their responses complexly, especially as they become more physically and socially competent (Bateson & Gluckman, 2012; Taborsky & Oliveira, 2012; West-Eberhard, 2003). For example, the body condition of younger swallow nestlings are more influenced by nest box temperature, whereas older nestlings are influenced more by the phenotype of the parents (Pérez et al., 2008). Similarly, older migratory, female songbirds attempt more broods in springs with warmer ambient temperatures than younger, female songbirds (Bulluck, Huber, Viverette, & Blem, 2013), and middle-aged sea-birds are less affected by temperature fluctuations than younger and older birds (Pardo, Barbraud, Authier, & Weimerskirch, 2013). Here, we show that the features of the environment influence their behavior more strongly depend on age, as older, but not younger infants show structure-dependent changes in behavior, and temperature had a more robust effect on the activity levels of younger infants than older infants.

The movements of older animals are sometimes strongly influenced by the social environment. The presence of familiar individuals (Laskowski et al., 2016; Ward & Hart, 2003), their sex (Slocombe & Zuberbühler, 2007), personality (Dyer, Croft, Morrell, & Krause, 2009), their quantity (Beauchamp, 2013; Couzin, Krause, Franks, & Levin, 2005) and social role (Modlmeier, Keiser, Watters, Sih, & Pruitt, 2014; Vital & Martins, 2011) can alter individual behavior and collective action. For example, familiar groups of fish are more cohesive and display more predator evasion tactics than do groups of unfamiliar fish (reviewed in Ward & Hart, 2003). Carolina chickadees manipulate their call structure depending on whether another individual has joined them at a feeding station;

experiments show ‘recruitment calls’ attracted fewer individuals after members joined the initial caller (Mahurin & Freeberg, 2009). Although we found no evidence for the social context influencing the activity of pups during the first week of life, social competence develops (Taborsky & Oliveira, 2012; West & King, 2002), and thus the influence of littermates on individual behavior may be more apparent in ages of pups older than the ones we tested in the present study.

In summary, the goal of the present study was to identify the impact of physical (density, temperature) and social factors (presence of littermates) on individual movements during early development. Our results show that the amount of available space and temperature were salient environmental feature that affected the individual movements of mouse pups, and the strength of the effect was dependent on development. We found no evidence to suggest that the social environment influenced the activity of individual mouse pups. Studies such as this suggest that individual behavior can be influenced by simple environmental features, but their relative impact depends on the age of the individual. Understanding these mechanisms is critical to understanding how individual behavior becomes organized.

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Chapter 3:

Environmental Structure and Energetic Consequences in Young Mice

Abstract

Microenvironments can have considerable physiological consequences for the inhabitants by influencing the movements of individual members. The environment can permit more diverse aggregation patterns or restrict movements to certain dimensions. Here, we tested whether aspects of the microenvironment that influenced aggregation patterns also influenced the energetics of groups of young animals. We tested the effects of enclosure configuration on the group temperature and respiration of infant mice (*Mus musculus*). We monitored the huddle temperature and respiration of groups in flat, concave and conical enclosures, which varied in shape and available space, and consequently the types of movements they permitted. We found that the amount of available space (or density) had a stronger effect on the group temperature than did the shape of the enclosure or types of permissible movements. We found no evidence that density or shape of the arena strongly affected the respiration rate of the group, with groups showing similar levels of oxygen consumption in all treatments. The lower density enclosures conveyed a considerable metabolic savings to groups in comparison to those tested in a higher density enclosure. These findings show density can have a large effect on the energetics of young mice, and provide insights on how simple features of the environment will influence physiology in a changing world.

Introduction

Microenvironments can affect competitive interactions, dominate social behavior, and influence growth through complex interactions with physiology (Huey, 1991; Mathot & Dingemanse, 2015). The impact of the microenvironment depends on the environmental features an animal selects, creates or is deposited in (e.g., parents creating nest for offspring, or individuals huddling) (Day, Laland, & Odling-Smee, 2003; Walsberg, 1985). Groups can create different geometries with their bodies, thus altering the microenvironment and permitting group members to take advantage of drafts (Hemelrijk, Reid, Hildenbrandt, & Padding, 2015; Marras et al., 2015), vortices (Liao, 2007; Portugal et al., 2014) or zones of low pressure (e.g., cyclists drafting in a peloton) (Jeukendrup, Craig, & Hawley, 2000), and consequently expend less energy while aggregating (Gilbert et al., 2010). The amount of available space can also influence the group's ability to manipulate the microenvironment, which can also have functional consequences. The types of physiological advantages achieved in different group formations with variable amounts of available space may depend on whether the formation permits movement in two-dimensional (2-D) or three-dimensional (3-D) space, and the group is not stressed when crowded.

When climatic conditions become challenging, individuals in the group can change their spacing. For example, penguins clump more closely together when exposed to cold challenging conditions (Gilbert, Blanc, Le Maho, & Ancel, 2008), and clumping rodents (Chapter 2; Alberts, 2007; Harshaw & Alberts, 2012) and bees (Heinrich, 1981) increase the space between members creating a less dense group when warmed. Through these individual adjustments members can alter the surface area: volume ratio of the group and their exposure to the external environment, thereby influencing the energy needed to maintain thermal homeostasis (Gilbert et al., 2010; Schmidt-Nielsen, 1984). Here, we ask if groups placed in environments that influence their density show differences in energetic savings.

The structure of the group influences energetic efficiency. For example, birds that flock in a planar V-formation have lower aerodynamic efficiencies than do birds that fly in a non-planar zig-zag formation (Lissaman & Shollenberger, 1970), and fish in a diamond lattice formation have high hydrodynamic efficiency than do those in other formations (Hemelrijk et al., 2015; Weihs, 1973). Individuals can also change the structure of their microenvironment to enhance energetic efficiency. For example, rodents build well-insulated, dome-shaped nests in cold environments, and flat, planar nests in warmer climates, which may permit them to warm and cool their nests efficiently (Gilbert et al., 2010; Glaser & Lustick, 1975). Similarly, termites alter the structure of their mounds depending on climatic conditions, to regulate temperature within the mound (Jones & Oldroyd, 2006; Korb, 2003). To increase metabolic efficiency, animals may alter the geometry of the group. From a simple, physical perspective, 3-D structures with higher surface area:volume ratios should maintain stable temperatures for longer periods of time than do 2-D structures that have lower surface area:volume ratios (Schmidt-Nielsen, 1984, 1997). Here, we ask if groups of young mice in environments that permit movements to 3-D space have a greater metabolic savings than do groups with movements that are restricted to 2-D space.

The effects of enclosure geometry or density on energetics may depend on whether the groups are stressed when crowded or calm under high-density conditions.

Crowding that leads to stress may increase aggression (Van Loo, Mol, Koolhaas, Van Zutphen, & Baumans, 2001) and influence metabolite levels (de las Heras, Martos-Sitcha, Yúfera, Mancera, & Martínez-Rodríguez, 2015), which may lead animals to space themselves evenly to avoid aggressive neighbors (Bazazi et al., 2008) and have higher, stress-induced metabolic rates (Davis & Schreck, 1997; Sloman, Motherwell, O'Connor, & Taylor, 2000). For example, fish under higher stocking densities generate more heat and have higher metabolic rates than do fish under lower density conditions (Medland & Beamish, 1985). Animals that are crowded, but not stressed, may be calm with lower metabolic rates and may clump together freely in the environment. For example, trios of mice and gerbils clumped together and had lower metabolic rates than did three individuals separated by a barrier (Martin, Fiorentini, & Connors, 1980).

Mother mice construct nests that vary in configuration in which they give birth to 4 - 8 infants (Berry & Bronson, 1992; Latham & Mason, 2004). The shape of the nest can vary with environmental and intrinsic factors (e.g., climate: Gilbert et al., 2010; reproductive state: (Bond, Neumann, Mathieson, & Brown, 2002); strain: Gaskill et al., 2012). Mice can build nests with gently sloping walls when provisioned with quality nesting material or when living in colder environments, and flatter nests when construction material is poor or when living in warmer environments (Gaskill et al., 2012; Hess et al., 2008). There is individual variation in the nests that mice construct (Bond et al., 2002) with protocols developed to characterize them (Deacon, 2006). Because there is variation in the nests that mother mice construct, infant mice develop in nests that vary in shape and amount of available space.

Altricial rodents also transition from tiny infants to large pre-weanlings, radically changing in behavior (Williams & Scott, 1953), morphology (Clarke & Still, 2001) and physiology (Blumberg, 2001; Harshaw & Alberts, 2012), and taking up more space in the nest as they grow. Thus, the effects of the environment on their physiology may change as they age.

In the present study, we tested whether the energetics of young mouse huddles was influenced more by the environmental configuration, amount of available space or the geometry of the group. We did so by varying the shape and size of the enclosure, and restricting the movements of the huddle to different dimensions. In this study, we examined the group temperature and metabolic rate of mouse pups on flat, concave, and conical enclosures. If the shape of the nest is more important, then we expected to see differences in temperatures in conical, concave and flat enclosures. If the amount of available space is more important, then we expected to see differences in temperature and metabolism in enclosures with different amounts of available space. If the geometry of the group influences the energetics of the mice, then we expected the temperature of the metabolism to change with the types of movements the animals can do.

Method

Subjects

We used litters of mouse pups (*Mus musculus*). Animals were derived from C57BL/6 stock originally purchased from Jackson Laboratory (Bar Harbor, Maine) and bred in Indiana University’s Animal Behavior Laboratory colony. Mothers gave birth and then reared pups in standard maternity tubs (27 cm length x 13 cm height x 17 cm width) with food and water available *ad libtum*. We maintained the vivarium on 14:10 h light/dark cycle (lights on at 0700h) at 22.0 ± 2 °C, and humidity 40%. We conducted animal care and experiments in accordance with the Indiana University Institutional Animal Care and Use Committee (IACUC).

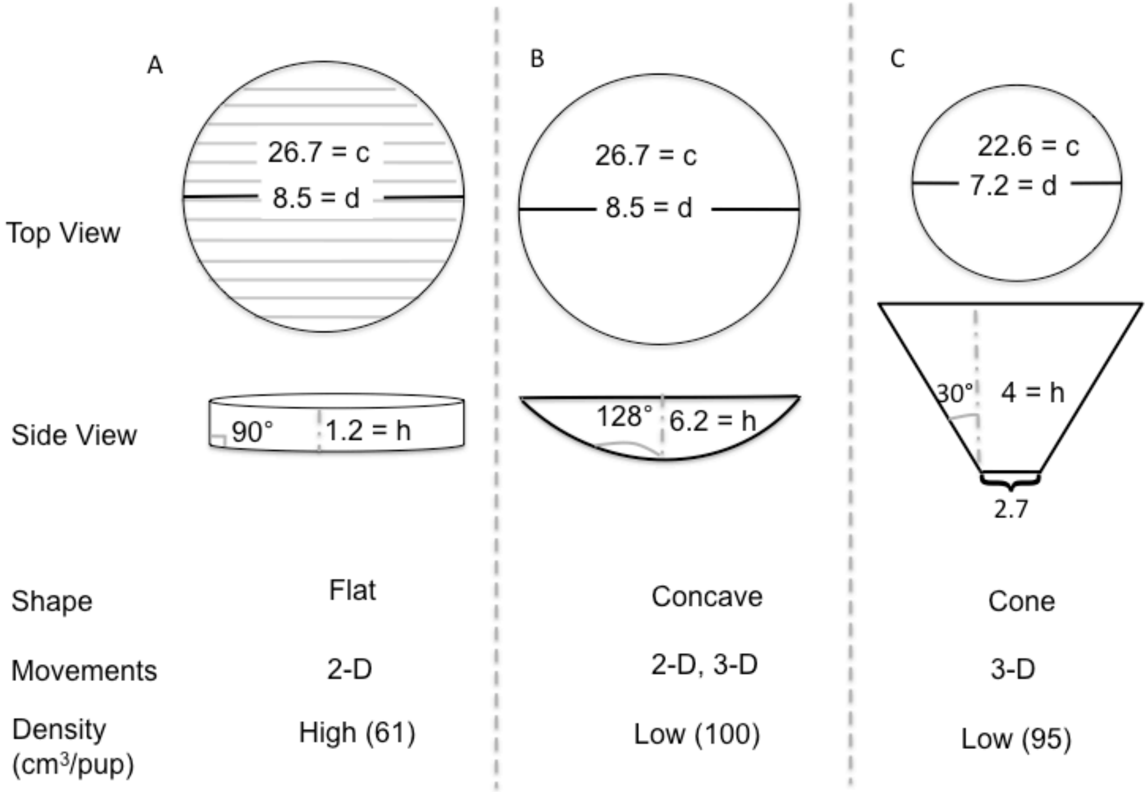

Figure 1. Dimensions of the enclosures for each of the three treatment conditions: A) flat enclosure with high-density and short height (h) that restricts movements to 2-D space; B) a concave enclosure with the same circumference (c) and diameter (d) as the flat enclosure, but with a lower density and gently-sloping walls that permitted movement in 2-D and 3-D space; C) a conical enclosure with low-density and steep-sloping walls and small top surface area that restricted movements to 3-D space. All length measurements

are in centimeters and angular measurements are in degrees. We photographed mice from above.

Procedure

Huddle Surface Temperature

We began with 24 litters (6 pups/litter) of 8 day-old mouse pups. We assigned an equal number of litters (8 groups per condition) to a flat, concave, or conical enclosure (Fig. 1A, B, C, respectively). The steep sides of the conical enclosure required the pups to clump and move in a three-dimensional (3-D) space, whereas the mesh top of the flat surface restricted the movement of the pups to planar or two-dimensional (2-D) space. The intermediate enclosure, or concave enclosure permitted movements in 3-D and 2-D space and had the same diameter as the flat enclosure, but the slope of the walls was less steep. Pups in conical and concave enclosures had nearly 40% more available space than did pups in the flat enclosure.

All enclosures were made of non-conductive materials, and therefore did not alter heat-transfer. The conical structure was made of polypropylene plastic. The concave structure was made out of plaster of Paris and then coated with water-soluble polyeurathane creating a non-conductive surface. The floor of the flat structure was made of Styrofoam and the walls and mesh top were made of polypropylene.

We placed litters on their experimental surface in a temperature-controlled chamber. We exposed litters to a 22°C (±1°C) cool challenge for 56 mins. At the end of the trial, we took a thermograph of the huddle using an ICI 7320 P-Series infrared camera with (IR Flash ver. 2.0 for Windows, Infrared Cameras Inc., Beaumont, TX). Following testing, pups were then returned to their dams.

Analysis of Thermal Images

We calculated huddle surface temperatures from the thermographs using ICR Flash software (Infrared Cameras Inc., Beaumont, TX) and excel. Infrared thermography does not involve handling or otherwise disrupting the subject and avoids problems with heat exchange with the experimenter's hands. We find infrared thermography to be a useful, sensitive measure of body surface temperatures of mouse pups until about 9 days of age, when fur growth begins to interfere with the emissions.

Body surface temperature was measured from each pixel representing a visible area of the pups' bodies, excluding paws and tails. The paws and tails were excluded because they cool rapidly, and infant rodents regulate core areas (Blumberg & Sokoloff, 1998; Cannon & Nedergaard, 2004). The present measurement differs from previous methods that were focused on regional temperatures on pups' bodies (Harshaw & Alberts, 2012; Harshaw, Culligan, & Alberts, 2014); here, we calculated an average surface temperature of the huddle as a single body.

Statistical Analysis

We used a one-way ANOVA with Tukey post-hoc tests to examine the effects of enclosure density and shape (i.e., flat, concave, and conical) on huddle temperature. We confirmed the residuals met the normality and heteroscedasticity assumptions of the ANOVA. We conducted all statistical analyses in R with the "base" package (R Core Team, 2015).

Oxygen Consumption.

To determine whether pups in different nest configurations had different metabolic rates, we tested an additional 17 litters (6 pups/litter) in a sealed respiratory chamber that accommodated the flat test enclosure or the conical enclosure, described earlier. The respiratory chamber was a custom-built, double-walled chamber, constructed of brass on five sides, with a clear Plexiglas lid, with clamps sealing the top against a gasket that was built along the perimeter. We reduced the volume of the chamber by packing it with wood chips to increase the accuracy of the measurements. We tested 9 litters in a flat enclosure and 8 litters in the conical enclosure (described above). We placed groups on either a flat or conical structure inside a double-walled brass chamber (length = 30.48 cm, inner height = 10.48 cm, inner width = 16.51 cm) for 120 min. After a 30 min stabilization period, we recorded oxygen consumption measurements (see below). We maintained the air temperature (Ta) within the chamber at 22°C by pumping temperature-controlled water through the walls of the chamber. Access holes on the lid allowed for the passage of air into and out of the chamber as well as the passage of thermocouple wires. The chamber was filled with clean alpine wood shavings to reduce the volume of air and to minimize pockets of resident air or dead zones.

Oxygen Consumption Measurements

Compressed air passed through a two-stage regulator and split into two lines. One line passed through a digital flowmeter (Omega), was humidified, and was then circulated through the metabolic chamber at 300 ml/min. We dried the exhaust air from the chamber and then drew it through one of two channels of an electrochemical oxygen analyzer (Ametek, Pittsburgh, PA). The second line of air traveled directly from the air cylinder to the second channel of the oxygen sensor. Oxygen concentration in each airstream entered separate chambers and was measured simultaneously, providing a percent difference in concentration at ± 0.003% when animals were not present. We then transformed the percent difference into a measure of oxygen consumption in milliliters of O_2 per kilogram per minute. We did not correct oxygen consumption for respiratory exchange because doing so leads to systematic underestimation of oxygen consumption. After a 30-min stabilization period, we sampled oxygen-consumption twice each minute for 90-min, using a customized data-acquisition system for the Macintosh computer (LabView; National Instruments).

We then used independent sample's t-test (two tailed) with a Welch's correction for unequal variance to compare the metabolic rates of huddles tested in flat and conical enclosures. We conducted all statistical analyses in R with the "base" package (R Core Team, 2015).

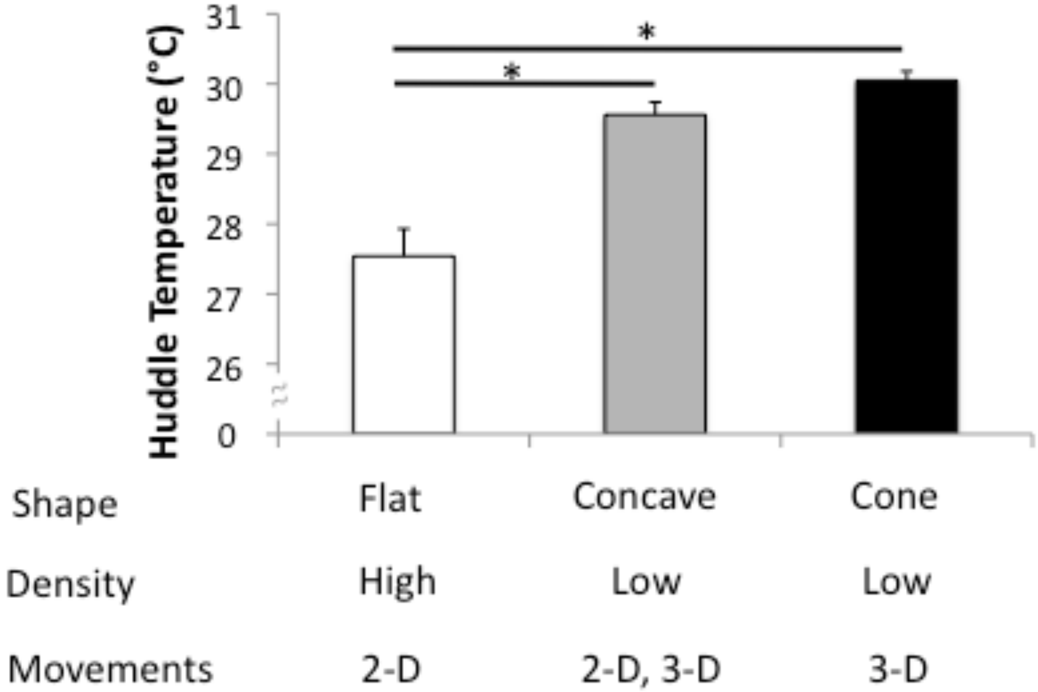

Figure 2. Huddle temperature is more influenced by density than the shape of the nest. Huddles in concave and conical enclosures at high densities were warmer than groups in a flat structure at low densities. Groups had similar temperatures when density was equal. * indicates significant Tukey post hoc comparisons at $p < 0.05$. Error bars are one standard error.

Results

Huddles in low-density structures are warmer than huddles in high-density structures.

We found that huddles in lower density concave (M = 29.56, 95% CI [29.42, 29.70]) or conical (M = 30.05, 95% CI [29.75, 30.35]) enclosures were nearly 2°C warmer than were huddles in a higher-density, flat (M = 27.54, 95% CI [27.24, 27.84]) structure. This led to a statistically significant difference in huddle temperature across the three treatment categories ($F_{2,21} = 14.20$, $p < .01$; Fig. 2). There was no statistically significant difference in temperature between groups in a concave and conical enclosure when they had the same density (*p* = 0.39, Tukey). There was a difference in the temperature of huddles tested in different arena configurations (or flat, concave, conical) when the amount of available space differed (or at a different density; *p* < 0.01, Tukey).

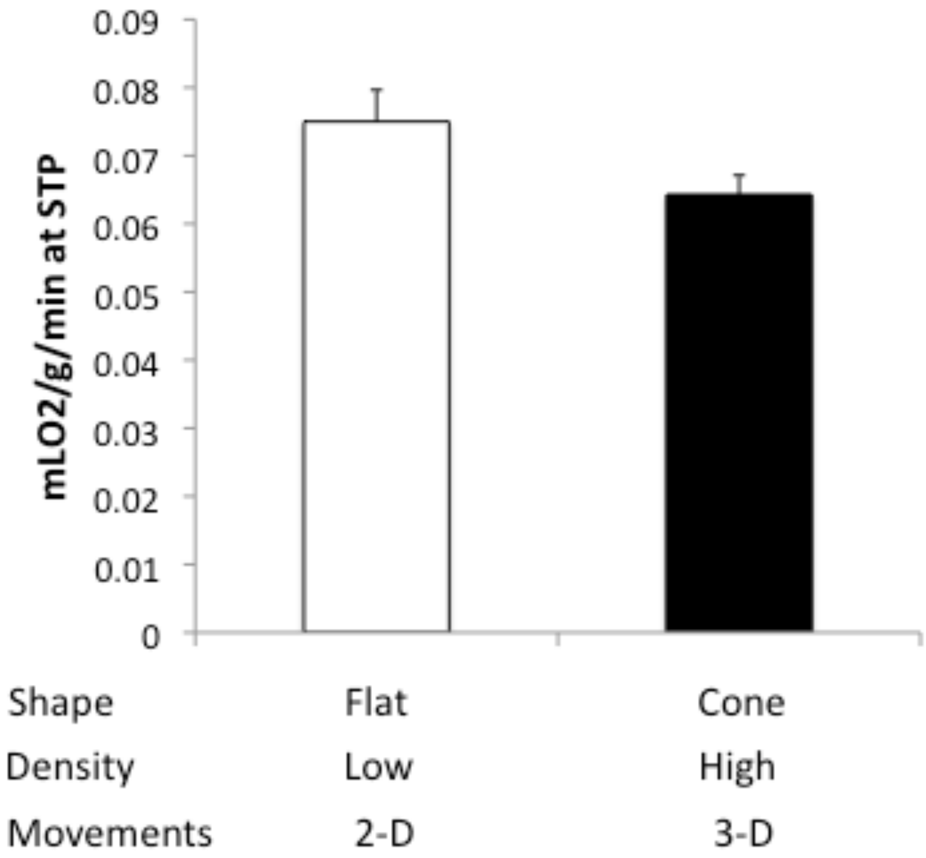

Figure 3. Huddles had similar metabolic rates in enclosures with different shapes and densities. Error bars are one standard error.

Huddles in a low- and high-density enclosures have similar metabolic rates.

We found that huddles tested in flat (M = 0.06, 95% CI [0.05, 0.08]) or concave (M = 0.06, 95% CI [0.05, 0.07]) enclosures had similar metabolic rates. The amount of oxygen consumed per gram at standard temperature and pressure was a little more than 0.06 mL/min (95% CI[-0.01, 0.02]; t(14.28) = 0.72, *p* = 0.48) (Fig. 3).

Discussion

We found that the temperature, but not respiration, of the huddle depends on the amount of available space (i.e., density). Mouse pups in enclosures with less available space were warmer than were individuals in enclosures with more available space. The temperature of the huddle was not affected by enclosure shape. Huddles had similar respiration rates independent of enclosure configuration. Thus, the amount of available space had a stronger effect on the temperature, but not the respiration, of the huddles than did the geometry of the enclosure. That is, while huddles had similar oxygen consumption levels in flat, high-density, enclosures and in conical, low-density, enclosures, huddles in conical low-density enclosures were 2°C warmer. Thus, a low-density enclosure confers 7 % energetic savings in comparison to a low-density structure. The low-density structure led to a considerable energetic savings in comparison to the high-density condition. We found no evidence for the pup movements in different dimensions affected the temperature and metabolic rate of the group, as there was no clear effect of 2-D or 3-D movements on energetics.

Even though our mice were not stressed when crowded, density was the most salient feature affecting the energetics of the group. Density is clearly important in shaping the energetics of crowded and stressed animals (Creel, Dantzer, Goymann, & Rubenstein, 2013; Gilbert et al., 2010). For example, in crowded ants (Cao & Dornhaus, 2008), energetic efficiency decreases with individuals having higher metabolic rates under higher density conditions than animals under lower density conditions perhaps due the effects of crowding on stress (Creel et al., 2013). In contrast, we found that non-stressed mice under higher density conditions had greater energetic savings, were able to maintain higher huddle temperatures while consuming less oxygen, in comparison to mice under lower density conditions. Density has similar effects on other species, which are presumably not stressed when crowded. For example, adult penguins corralled into small groups and spaces showed a 39% reduction in metabolic rate in comparison to isolated birds (Gilbert et al., 2008), and change their huddling intensity, and density of the huddle with temperature (Gilbert, Robertson, Maho, & Ancel, 2007).

Crowding animals that are not stressed can permit them to stay warmer more efficiently. We found that crowded mouse huddles in higher density enclosures were warmer, but had similar metabolic rates as groups in lower density conditions. Thus, the higher density conditions afforded a 7% energetic savings in comparison to pups under lower density conditions. The energetic savings attributed to the different density conditions may arise from the reduction in surface area exposed to cold ambient temperatures. For example, clumping adult mice are estimated to decrease their cold-exposed surface area by 29 - 31% (Canals, Rosenmann, & Bozinovic, 1989), and the amount of cold-exposed surface area decreases when more animals are in contact (Glaser & Lustick, 1975) and are more densely packed (Gilbert et al., 2008). This increased density reduces the exposed surface area of individuals in cooler ambient air temperatures, thereby decreasing the dissipation of heat (Canals et al., 1989; Gilbert et al., 2010). For instance, in adult penguins, bees, and voles, a large percentage of the energetic savings achieved through huddling is due to a reduction in cold-exposed body surface area and to exposure to warmer temperatures inside the clump (Gilbert et al., 2008; Hayes, Speakman, & Racey, 1992; Heinrich, 1981).

The reduced available space may also permit the temperature of the individuals to influence the microclimate more. That is, the ambient temperature around each individual increases due to the dissipation of heat from collective or the body of each individual, and therefore the temperature gradient between the local environment and the body is reduced (Gilbert et al., 2010; Tattersall et al., 2012). The change in microclimate is likely achieved more readily when there is less space in comparison to the number of individuals within it. For example, cavity-dwelling bats (more bats in a cavity) and voles (more voles in a chamber) with less available space increased the ambient air temperature in comparison to counterparts with less available space (Hayes et al., 1992; Willis & Brigham, 2007), thereby indirectly warming (through convection) the inhabitants (Walsberg, 1990). The magnitude of the temperature increase is shown to vary with group size, with more individuals in a cavity (less available space) increasing the temperature more than less individuals in a cavity (more available space) (Willis & Brigham, 2007). In contrast, we found that young crowded mice expended more energy in comparison to their more loosely aggregated agemates. The types of behavioral movements that were permitted (2-D vs. 3-D) in the different types of enclosures clearly had an effect of the energetics. Future studies should identify how movement in space integrates with physiology to lead to such energetic consequences.

In summary, the goal of this study was to test the impacts of simple physical features of the environment (density and enclosure shape) on group temperature and respiration of crowded but not stressed infants. Our results show that the group temperature of young mice was primarily influenced by density. We found no evidence to suggest that density and enclosure shape influenced the respiration rates of young mice. The lower density enclosures conferred energetic savings to young mouse pups in comparison to enclosures with less available space. Studies such as this suggest that the environment can have physiological consequences for its inhabitants. Understanding how these simple environmental features affect young animals may show how they may cope with a changing world.

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Chapter 4:

Development of Behavioral Responses to Thermal Challenges in the Context of Climate Change

Shelton D.S. and Alberts J.R. Development of behavioral responses to thermal challenges in the context of climate change. (invited special issue)

Abstract

The gradual warming of our planet reflects more frequent spikes of high temperatures, so it is pertinent to understand how organisms respond to acute challenges of heat. To understand the consequences of such warming on the life cycle of endothermic animals, it is essential to examine thermal responses during development. These include direct thermal effects on offspring, as well as indirect effects on them, such as those imposed by thermally-associated alterations of maternal behavior. The present paper is a selective review of the existing literature and a report of some new empirical data, aimed at processes of mammalian development, especially those affecting behavior and cognition. We briefly discuss the development of body temperature regulation in rats and mice, and thermal aspects of maternal behavior with emphases on responses to high temperatures. The new data extend previous analyses of individual and group responses in developing rodents to warm and cool ambient temperatures. This literature not only reveals a variety of adaptive specializations during development, but it points to the earlier appearance in young mammals of abilities to combat heat loss, relative to protections from hyperthermia. These relative developmental delays in compensatory defenses to heating appear to render young mammals especially vulnerable to environmental warming. We describe cascading consequences of warming -- effects that illustrate interactions across levels of physiological, neural, and behavioral development.

Key words: altricial rodents, climate change, elevated temperatures, development

Climate Change on Different Timescales

Contemporary climatological data indicate that the Earth has warmed about 0.6°C over the last century (Change, 2001; Nicholls et al., 1996). The rate of warming for the latter half of the last century was twice the rate of the preceding decades. Temperatures of tropical oceans have increased even more dramatically, warming by 1–2°C over the past 100 years (Hoegh-Guldberg et al., 2007). Overall, our planet warmed more in the last century than at any other time during the last 1,000 years.

Characterizing the increase in average global temperatures is key to documenting the magnitude and scope of climate change on a planetary scale, and this is best summarized on timescales of commensurate size –seasons, years, decades, or centuries. Indeed, climate change occurs on multiple timescales, some not readily revealed by averages over long periods. That is, an average annual increase of a 0.5^{o}C in global temperature is composed of days in some locales where temperatures may be 10^{o} or 20^{o}C higher than the organisms there have previously experienced. Likewise, there may be seasons or portions of seasons in which the temperatures are chronically elevated by many degrees more than a 0.5^{o} average. Figure 1 contrasts the kinds of temperature fluctuations and spikes that global warming can comprise.

Organisms and ecosystems do not confront or respond to global averages. Environmental temperatures are experienced in the moment and the relevant timescales are more on the order of hours and days. Thus, while awareness of the gradual shift in global temperature over long timescales has rightfully stimulated much attention and concern, we must keep in mind that it is the local conditions and punctuating events that are the actual challenges faced by organisms.

The present paper is a selective review and discussion of available data and knowledge about the ontogeny of organismal responses to thermal challenge, aimed primarily at providing a foundation for consideration of how individuals, families, and groups may respond to environmental conditions associated with global warming.

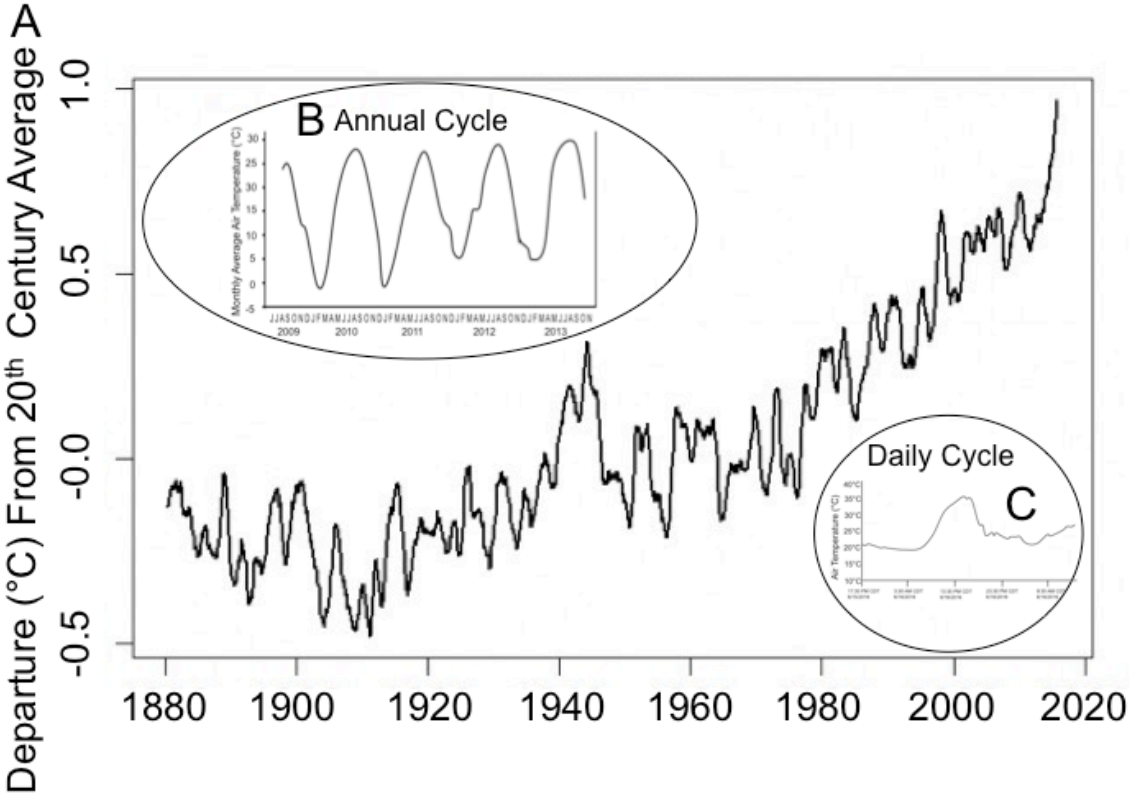

Figure 1. Ambient air temperatures at multiple scales. (A) Global average temperatures from 1880-2015, plotted as difference from the 20th century average overlay (B) annual, and (C) daily temperature cycles. The global temperature data were obtained from the National Aeronautics and Space Administration (GISTEMP Team, 2016; Hansen, Ruedy, Sato, & Lo, 2010). The annual and hourly temperature data were adapted from local temperature reports from the Kentucky Climate Center, Western Kentucky University - Kentucky Mesonet, Lexington, KY, USA; 36.99°N, 86.42°W.

Responses to High Temperatures

Anthropogenic climate change can bring unusually high and low temperatures (the ‘spikes’ depicted in the inset graph of daily temperatures in Fig. 1). Thus, it is pertinent to identify the forms of heat transfer by which animals gain and lose body temperature. Sweating, saliva spreading, gular sac extensions, and panting are examples of tactics used to reduce body temperature through evaporative cooling. Rodents are largely devoid of sweat glands, but they display copious salivation during heat stress (Hainsworth, 1967; Hainsworth & Stricker, 1970). The saliva is spread via grooming movements from the mouth to the body surface, Evaporative cooling occurs with removal of latent heat by liquid vaporization from the body surface (Tattersall et al., 2012).

Bodies gain heat by conduction when environmental surfaces are warmer than the animal’s own surface temperature. In contrast, bodies lose heat by conduction when in or on a cooler surrounding. Conductance is lower in larger bodies; and conductance rate scales allometrically to body size (Schmidt-Nielsen, 1984). In addition, insulation and vascularization can alter conduction. Morphological adaptations that affect conductance are considered ‘specialized surfaces’, and include bird bills, the ears of jack rabbits and elephants, hairless tails of rodents, and the naked legs of camels and ostriches. Physiologically, conductance can be altered by changing blood flow and by vasodilation and vasoconstriction. Behaviorally, animals can regulate conductance by selecting or creating microenvironments that afford body temperature regulation (Huey, 1991; Mathot & Dingemanse, 2015).

The brain is more autonomous than other organs, in the sense that it exerts control over its own temperature, blood flow, and metabolism (Kiyatkin, Kim, Wakabayashi, Baumann, & Shaham, 2015). Neuronal functions of all sorts, e.g., activity of single ion channels, transmitter release and reuptake, action potentials and integrative functions, are temperature dependent. Brain temperature is not uniform; various structures vary in temperature. The regulation of brain temperature is poorly understood and the questions and implications for function and cognition are just emerging. Nevertheless, the picture is already compelling: the brain's function is susceptible to its own temperature. There exist numerous links between pathologies and altered brain temperatures (Kiyatkin et al., 2015). In addition, permeability of the blood-brain-barrier also appears to be temperature-sensitive (Kiyatkin & Sharma, 2009), so a full understanding of thermal-neural relations will likely involve an integrative perspective on brain-body interactions with temperature (Kiyatkin & Brown, 2004).

Developmental Considerations

Infants of endothermic species are typically limited in their capacities for body temperature regulation, relative to their adult counterparts. Smaller size is a major factor, because the correspondingly larger surface area:mass relation means that body heat is lost more rapidly from the infant's smaller body (Schmidt-Nielsen, 1984). A consequence of the "surface law" is that small bodies also gain heat more rapidly from external sources. In the context of global warming, this factor should be kept in mind.

In addition to small size, however, a host of factors associated with altriciality (immaturity) render infants and juveniles thermally vulnerable. At birth, such mammals –

which include the rodent species commonly used in lab research - lack insulative fur and subcutaneous fat (Fig. 2). Autonomic control of vasodilation and vasoconstriction are limited. Shivering is absent until the middle of the first week (Arjamaa & Lagerspetz, 1979) or even until the third week of life (Taylor, 1960). Warming does not produce excessive salivation and saliva spreading until the middle of the second week of life (Pfister, 1990).

In the next sections, we chart some major life history stages of rodents, emphasizing the influence of elevated temperatures on development, including cognition and related processes.

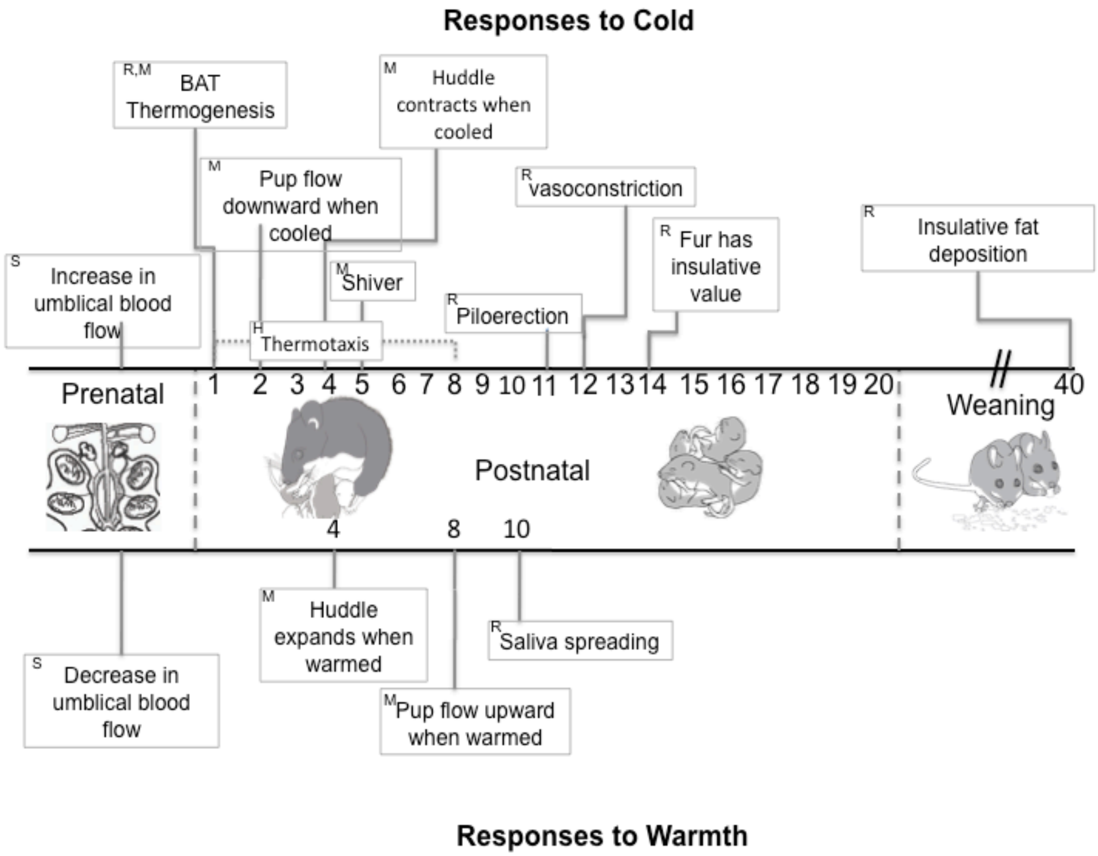

Figure 2. Snapshot view of responses of infant rodents to cool and warm ambient environments and their earliest reported developmental onset. The diagram depicts mostly mice in early postnatal development and rats in later postnatal life with other gaps filled with studies on sheep and hamsters. In each response box, the species from which the information is drawn is indicated in the upper left corner with the initial of the species – mouse (M), rat (R), hamster (H), and sheep (S). The pictures show the four major life history niches that are discussed in the paper: 1) The uterine environment, showing two adjacent fetuses and portion of a third fetus late in prenatal development; 2) Mother-infant interactions, with the mother showing contact-dependent maternal care; 3) A group pups huddling; 4) A group of weanlings converging on a piece of food. References; Responses to cold: Prenatal (Cefalo & Hellegers, 1978; Laburn, Faurie, Goelst, &

Mitchell, 2002; Schroder & Power, 1997); Thermotaxis (Leonard, 1974); BAT thermogenesis (Taylor, 1960); Pup flow this study; Huddle contracts (Harshaw & Alberts, 2012); Shiver (Arjamaa & Lagerspetz, 1979; Taylor, 1960); Piloerection (Pfister, 1990); Vasoconstriction (Conklin & Heggeness, 1971); Fur removal affects metabolism (Hahn, 1956); Insulative fat deposits (Hahn, 1956). Responses to warmth: Prenatal (Cefalo & Hellegers, 1978; Schroder & Power, 1997); Huddle expands (Harshaw & Alberts, 2012); Pup flow (this study); Saliva spreading (Pfister, 1990).

Mammalian Mothers at Elevated Temperatures

Throughout gestation and most of lactation, all the body-building and physiology-maintaining processes in the offspring are accomplished with resources supplied by the mother's metabolism. Generally, lactation is the most energy-demanding phase of the mammalian life cycle. Speakman and associates have advanced a Heat Dissipation Limit (HDL) theory, according to which the maximal capacity to dissipate body heat and thereby avoid hyperthermia sets the upper boundary on metabolism during lactation (Speakman & Król, 2010, 2011). (The HDL theory is a more general, "ecological" framework that accounts for phylogenetic constraints on body size, morphology, and habits -- but we are limiting our discussion of it to the energetics of lactation.)

For our purposes, the HDL theory focuses attention on the limits of the lactating female to dissipate the heat generated by her amplified metabolic activity. For example, lactating rodents may triple their daily food intake to fuel their lactational metabolism (Speakman & McQueenie, 1996). Contrary to some assumptions, this lactational hyperphagia is not bounded by the dam's ability to ingest or to process more food, or by the capacity of her lactational physiology. Instead, the upper boundary is the heat generated by her hypermetabolic state and her capacity to lose heat to the environment and thus avoid hyperthermia. Increased environmental temperature would represent a greater challenge to an already challenged physiological state.

The HDL theory predicts that lactating animals are prone to hyperthermia; there are supporting data. Decades prior to the formulation of the HDL theory, Leon and colleagues reported an extensive series of studies showing that a lactating Norway rats body temperature rises when in contact with a litter of pups (Adels & Leon, 1986; Leon, Croskerry, & Smith, 1978). Throughout the three-week-long "cycle of maternal behavior" rat dams make regular visits to their nest, where they nurse, lick, and huddle with offspring. During the first week or so of the cycle, nest bouts are long, with dams and pups in contact for 80% of each day. But, after the second week, as the litter mass becomes larger and more thermogenic, maternal nest bouts become shorter and more numerous. With further development the bout frequency and bout duration decrease. The best correlate of the termination of a nest bout between mother and pups is a rise in the ventral temperature of the mother's body (Leon et al., 1978). Indeed, manipulations that hastened the rise of dam's ventral temperature shortened nest bouts and, conversely, manipulations that slowed warming of her ventrum increased the time she spent in contact with the litter (Leon et al., 1978). Interestingly, when these investigators manipulated pup temperature independently of that the dam, the mother's nest bout termination remained associated with her ventral temperature, and not the litter temperature, indicating that temperature regulation of the litter is a by-product of the mother’s self-regulation, in concert with her nest building behavior and other factors that alter her body temperature.

There are numerous neuroendocrine correlates of maternal behavior in mammals (Rosenblatt, Mayer, & Giordano, 1988; Stolzenberg & Champagne, 2016). Among these, elevated lactational levels of progesterone have been posited to raise the mothers’ thermal “set point”, thus allowing for the typical, chronic elevation of maternal temperature (Adels & Leon, 1986). Elevated levels of corticosterone are necessary for potentiated maternal heat production (Adels & Leon, 1986). Oxytocin, a neuropeptide especially prevalent in the maternal circulation and in the maternal brain, has thermogenic effects (Chaves, Tilelli, Brito, & Brito, 2013). Interestingly, all three of these hormones, as well as other endocrine correlates of lactation, are associated with alterations in mood, emotion, and related cognitive functioning (Brunton & Russell, 2008; Russell, Douglas, & Ingram, 2001). Thus, it is likely (though untested) that elevated ambient temperature will affect maternal behavior as well as its endocrine substrates– and that such variations in endocrine levels will affect cognitive processes in the mother, her behavior, and mother-offspring interactions. The same elevations in ambient temperature might directly or indirectly alter the developing social behavior and cognitive processes of the young. In Figure 3, we highlight some of the known and possible effects that elevated temperatures may have on maternal care and offspring.

In some studies, natural variation in maternal licking and grooming during the first week postpartum has been used to categorize mother rats (Champagne, Francis, Mar, & Meaney, 2003), as high licking and grooming (high-LG) mothers and low licking and grooming (low-LG) dams. Rat pups that receive high amounts of maternal licking and grooming (or in other studies, frequent nursing with an “arched back” posture) showed increased synaptogenesis in the hippocampus and enhanced learning and memory in adulthood, relative to pups that received low amounts of the same maternal behaviors (Liu, Diorio, Day, Francis, & Meaney, 2000). Similarly, pups that received higher contact-dependent maternal care also show decreased physiological and behavioral responses to stressors, and decreased anxiety (Caldji et al., 1998; Fish et al., 2004). Such alterations in hippocampal anatomy, along with effects on measures of learning and anxiety indicate that neuro-cognitive development is susceptible to variations in maternal behavior (Champagne, 2008; Weaver et al., 2004). Indeed, there is ample evidence from cross-fostering manipulations that these effects derive from mother-offspring behavioral interactions, and are not simply ‘genetically’ inherited (Champagne et al., 2003; Priebe et al., 2005). Thus, contextual factors such as temperature that can affect maternal behavior can be reflected in the developmental outcomes of the next generation.

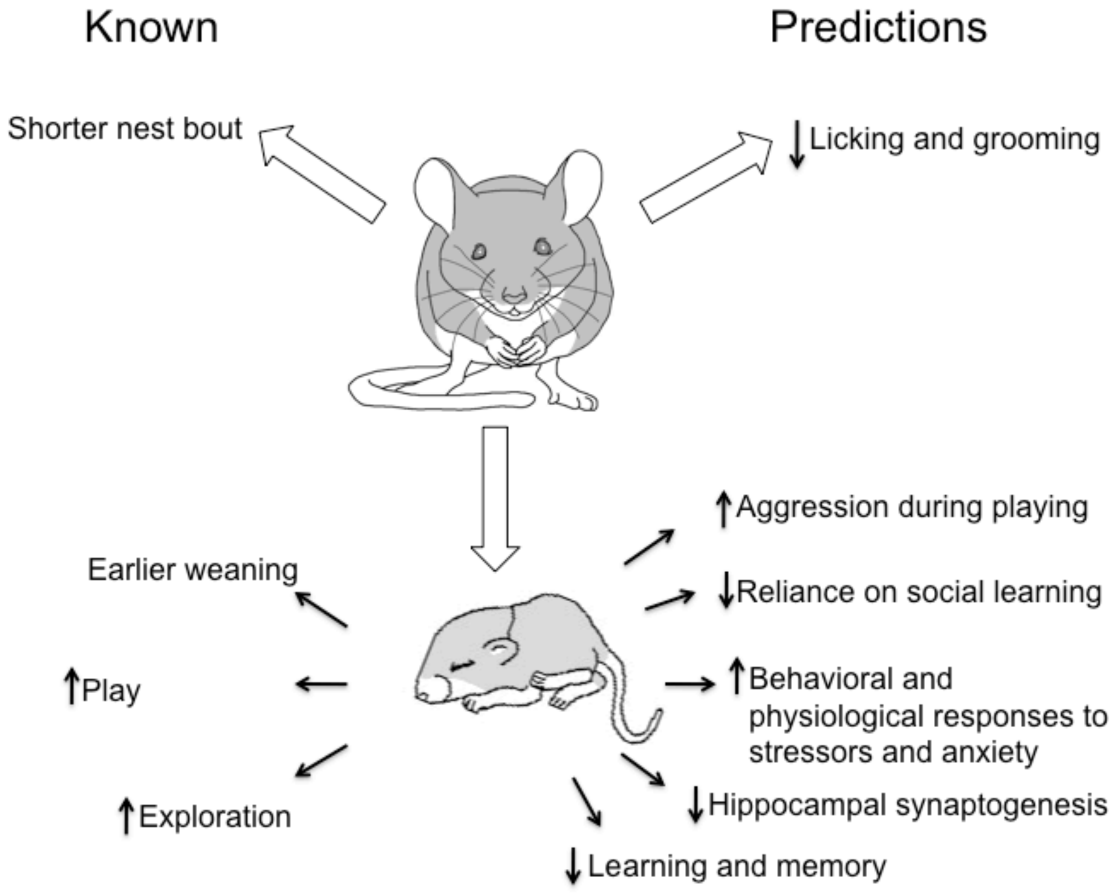

Figure 3. Known and predicted effects of elevated temperatures on maternal behavior and their consequences for infant development. Elevated temperatures are known to decrease maternal nest bouts, and lead to earlier weaning, increased play behavior and exploration in young. We speculate that elevated temperatures that decrease nest bouts will lead to decreases in contact-dependent maternal care such as maternal licking and grooming of the pups. Maternal care is known to have cascading effects on offspring cognition and behavior, such that pups with low-LG mothers are more aggressive during play, and have a lower reliance on social learning (Lindeyer, Meaney, & Reader, 2013), increased behavioral and physiological responses to stressors and

anxiety (Caldji et al., 1998; Fish et al., 2004), decreased hippocampal synaptogenesis (Liu et al., 2000), and retardation in learning and memory (Champagne, 2008; Weaver et al., 2004). We suggests such changes in the cognition, behavior and physiology of offspring may occur in families, mothers and pups, exposed to warmer temperatures.

Development of Behavioral Regulation by Individuals and Groups in Relation to Temperature

From birth to the eve of weaning (Postnatal Day 13), mother mice spent less than half of each day in a nest box with their young (Auclair, König, Ferrari, Perony, & Lindholm, 2014; König & Markl, 1987), leaving the litter to develop in the presence of 4-8 siblings, usually aggregated into a clump or huddle. It is well known that huddling enables adult endotherms to reduce heat loss and increase thermogenic efficiency. Surprising to many researchers, was the finding that immature rats and mice can derive similar benefits from huddling (Alberts, 2007; Blumberg & Sokoloff, 1998). Huddling conserves heat by reducing the exposed surface area of the bodies in the huddle, in effect producing a “single body” with a smaller surface:mass ratio than that of the bodies comprising the huddle.

One early finding is that rat pups interact during huddling in ways that actively regulate the surface area of the group in relation to the surrounding temperature (Alberts, 1978). Strikingly, huddles of rodent pups display group regulatory behavior at early postnatal ages that have long been considered stages when the pups are essentially non-regulatory (Alberts & Schank, 2010; Blumberg & Sokoloff, 1998). Nevertheless, when they can interact as a group, the infants’ regulatory capabilities can be readily seen.

A huddle of pups appears as a nearly constantly seething mass of bodies. But there is order to the turmoil. In a cool environment, pups probe and push into the depths of the group, often displacing littermates that appear on the surface. In a warm environment, pups actively move up to the surface, often covering or burying other pups. These temperature-dependent movements are analogous to convection currents and are termed “pup flow” within the huddle (Alberts, 1978).

In the present study, we measure on three, early postnatal days the individual- and group-level activities of mouse pups at cold and warm temperatures. Specifically, we examine the movements of individual, focal pups within a huddle, and then we examine the direction of pup flow at cold and warm temperatures.

Method

Subjects

We used litters of C57BL/6 mouse pups derived from stock originally purchased from Jackson Laboratory (Bar Harbor, Maine) and bred in the Indiana University’s Animal Behavior Laboratory colony. Mothers delivered and reared pups in standard maternity tubs (27 cm x 17 cm x 13 cm high) with food and water available *ad libtum*. In the vivarium, there was a 14:10 h light/dark cycle (lights on at 0700h) and 22.0 ± 2 °C, and humidity 40% ambience.

Procedure

We used litters (6 pups/litter) on Postnatal day (P) 2, 4, or 8. Using a modified Latin square design such that randomly selected litters were manipulated at one, two or three ages (16 litters were tested at each age for a total of 35 litters). For each litter, we removed 6 pups from their home cage, selecting one pup to serve as the focal subject and marking it with a line of non-toxic paint across the crown of the head, the shoulder, and rump for behavioral scoring. We placed the pups in a conical enclosure with 30^o sloping walls. The bottom diameter was standardized for each age, 22mm, 24mm, and 27.5mm for P2, P4, and P8, respectively. The enclosure was in a temperature-controlled chamber, maintained at 22°C (cool condition) at 36°C (warm condition). We used an overhead Logitech digital camera to record the 1h trial. The video record provided a view of the pups on the upper surface of the clump of bodies contained in the funnel-like enclosure. We divided the circular field into quadrants to provide a metric of activity around the horizontal plane of the huddle. Immediately prior to recording the trial, we placed the focal pup and a styrofoam marker (about 0.7 x 0.6 x 1.5 cm) of negligible weight (0.000018g) on top of the clump unobscured by littermates. This marker was used during video scoring to infer the predominant direction of pup flow, as described in the next section. A thermocouple thermometer (Omega HH806AW, St. Louis, MO) monitored the ambient air temperature.

*Individual-level Behavior***.** The behavior of each focal pup was quantified, using the number of times at least a portion of all three stripes became visible to the camera above the huddle and the number of transitions made by the focal pup between the quadrants on the surface of the funnel for each focal pup. These measures yielded a score of ‘vertical flow’ and ‘horizontal flow’, respectively. The open-source program, CowLog (Hänninen and Pastell, 2009), was used for encoding and storage of these data.

Group-level Behavior. We measured the time that the marker was visible on the huddle surface. The marker served as a directional marker of pup flow. When the marker was on the litter surface for more than 50% of the time, then pup flow was predominantly downwards. When the marker was hidden for more than 50% of the time, pup flow was predominantly upward.

Statistical Analysis. To measure individual-level activity, we summed the frequency of appearances on the huddle surface (vertical flow) and the number of translocations across quadrants (horizontal flow) of each focal pup. To measure group-level activity, we computed the percentage time the Styrofoam marker was hidden below the huddle surface. To examine the effects of Age (P2, P4, or P8) and Temperature (22°C, 36°C) on individual- and group-level responses we ran separate two-way repeated measures ANOVA with Bonferroni adjusted pair-wise comparisons to test the effects of age (P2, P4, P8) and temperature (22°C, 36°C). We used Type III sums of squares to correct for unbalanced data (Shaw & Mitchell-Olds, 1993), and an alpha level of .05. We conducted all statistical analyses in R (R Core Team, 2015), using the 'nlme' (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2016) package to fit the repeated-measures ANOVA and 'multcomp' (Hothorn, Bretz, & Westfall, 2008) package to conduct Tukey post-hoc tests.

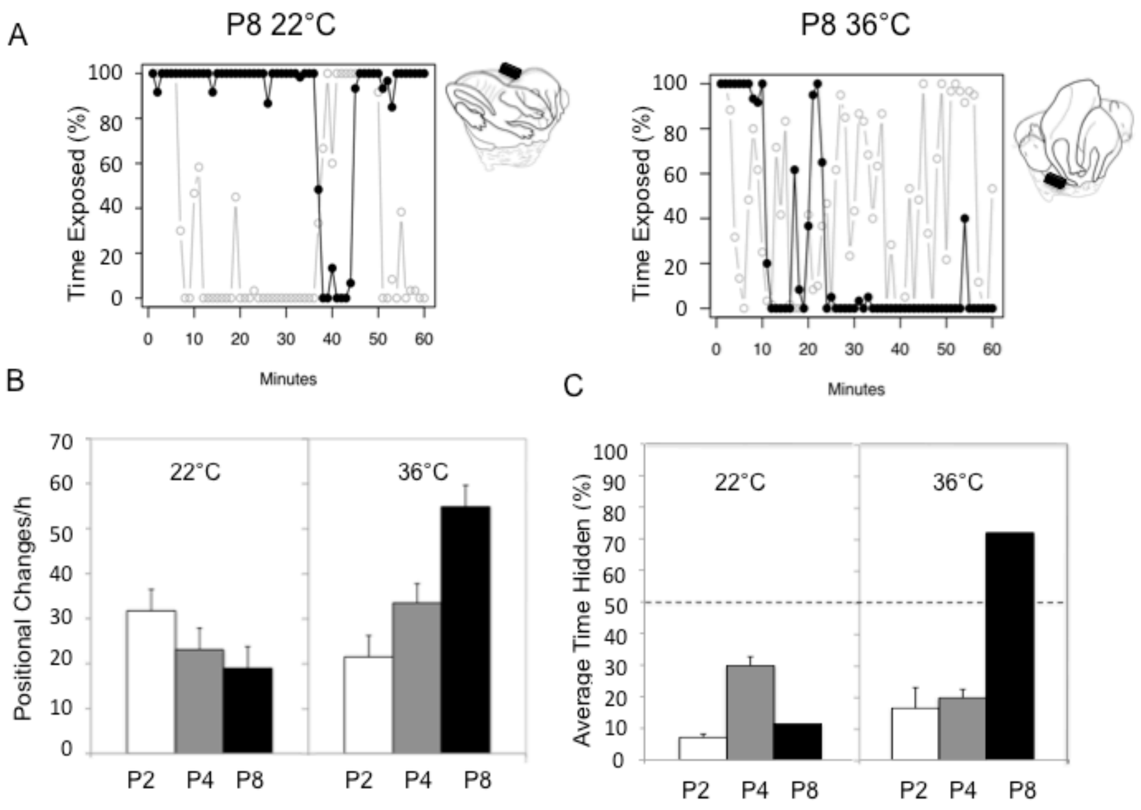

Figure 4. A) Figurative and quantitative depiction of ‘‘pup flow’’ within a huddle of P8 mouse pups in a cool and warm nest. The graphs depict exposures of a marked, focal pup (grey open circles) and the styrofoam marker (black closed circles) on the surface of a huddle during sequential, 1 min intervals. The position of the black marker can be seen in relation to pups’ bodies— in a cool nest the marker floats, and in a warm nest the marker sinks. B) The appearances of a focal pup at all ages in a cool and warm nest. The frequency of the response shows a clear linear developmental relationship in warm and cool nest, but in opposite directions. C) Regulatory movement was confirmed only by P8 huddles, but not by younger groups. The predominant direction of the huddle was downward for P2, P4, and P8 huddles in a cool nest (22°C). In presence of a warm ambient air temperature (36°C), P8 huddles reversed direction and flowed upwards, whereas the direction of younger ages, P2 and P4, was continuously downward into the

warm nest. The directional movements of the huddle as measured by the mean percentage of the time of the marker spent hidden during the one-hour experimental session. Error bars are one standard error.

Results and Discussion

Individual-level behavior at cool and warm temperatures.

In both temperature conditions, pups in all three age groups periodically appeared and disappeared from the huddle surface. The open circles in the two graphs in Figure 4A depict the activities of an 8-day-old focal pup on the huddle surface, showing that the frequency of appearances, and thus the total duration on the surface was greater in the warm condition.

In the cool environment, the average number of appearances/h was 31.75 (95% CI [21.88, 41.62]), 23.13 (95% CI [13.55, 32.70]), 19.00 (95% CI [9.08, 28.91]) for the P2, P4, and P8 groups, respectively (Fig. 4B). In the warm condition, the average number of appearances/h was 21.50 (95% CI [13.98, 29.02]), 33.5 (95% CI [16.53, 50.47]), 54.88 (95% CI [35.92, 73.83]) for the same age groups, respectively. Thus, in the cool environment, there was an age-related decrease in the frequency of appearances on the huddle surface, whereas in the warm environment (36°C) the frequency of appearances on the surface of the huddle increased with age (Temperature x Age interaction effect: $F_{2, 11} = 9.55$, $p < .01$; Fig. 4B). Pups show more positional changes in the warm environment than in a cool environment, which led to a main effect of Temperature ($F_{1, 33} = 6.59$, $p = .02$; Fig. 4B). The main effect of Temperature was driven by the high levels of activity of the older pups in the warm environment; P8 pups were nearly three times more active in the warm nest than in the cool (*p* = .046, Tukey). In contrast, the posthoc tests of P4 and P2 pups between the two ambient temperatures were not statistically significant (p = 1.0, Tukey; for both comparisons). Because the activity of individual pups in the different temperature conditions was similar most of the time, Age was not a statistically significant predictor when tested as a main effect ($F_{2, 11}$ = 2.61, *p* = .12).

Group-level behavior at cool and warm temperatures.

The Styrofoam marker placed on the surface of the huddle at the beginning of each trial, subsequently disappeared from view, but was visible most of the time when huddles were in a cool environment. This can be seen in the left panel of Figure 4A, which shows the percentage time the marker was obscured. The low values of time the marker was hidden indicates a downward "flow" of pup bodies, pushing and burrowing into the clump, leaving the marker floating on the huddle surface. Marker visibility averaged 7.13%, 28.84%, and 11.42% for the P2, 4, and 8 groups, respectively. We list here the 95% confidence intervals for each mean: [-0.21, 14.48], [-11.26, 70.94], and [5.41, 17.43]. When tested at 36^{o} C, P2 and P4, focal pups were hidden for similarly modest percentages of time, 16.37% (95% CI[-2.59, 35.33]) and 19.64% (95% CI[0.21, 39.07]), respectively. Only the P8 pups in the Warm condition were visible most of the time. Thus, only P8 pups manifested regulated, directional flow. The temperature- and age-related differences in pup flow led to a statistically significant Temperature x Age interaction, $F_{2, 11}$ = 10.58, *p* < .01 (Fig. 4C). In a warm environment, the marker was hidden for more time than it was in the cool chamber (main effect of Temperature ($F_{1, 31}$ = 9.99, *p* < .01). This main effect was driven by the differences in marker exposure between P8 huddles tested in the warm and cool conditions ($p < .01$, Tukey). Posthoc comparisons between temperature conditions for P2 and P4 groups were not statistically significant (p = 1.0, Tukey; for both comparisons). In the Cool condition, the marker in the P8 huddles was hidden more than in the P2 huddles, which led to a main effect of Age ($F_{1, 11} = 7.37$, $p < .01$). The main effect was driven by differences between P8 and younger pups (P8-P4: $p < .01$, Tukey; P8-P2: $p < .01$, Tukey) as differences between P4 and P2 huddles were not statistically significant ($p = 1.0$, Tukey).

Some implications of developmental findings in the context of climate change

One striking finding reported here is a developmental onset of regulated directional pup flow by P8 in mouse pups. Initially, during the first postnatal week, the pups' predominant intra-huddle movements were probing and diving downwards into the depths of the huddle, whether the ambient temperature was cool or warm. P8 huddles were the only age group to display temperature-dependent, directionally-regulated pup flow (downwards in the cool (22^{o}C) and upwards in the warm (36^{o}C) environments). This is not to say that pups (or huddles) less than 8-days of age are incapable of behavioral temperature regulation, but they do not respond to a warm challenge by reversing the direction of the pup flow. It is as if they are compelled to respond to the mélange of non-thermal cues in the huddle with the probing, pushing and diving that were their most frequent actions. By P8, however, their responses to the thermal challenge enable them to cease diving and instead, to move upwards, exposing more of their body surface to the surround and, enabling heat loss to the cooler surround.

Thus, if global warming produces warmer nest environments, then infant mice – and other altricial mammals that incorporate socially-based thermoregulatory systems – may be vulnerable to hyperthermia and numerous possible cascading changes in physiology, behavior, and related cognitive developments (Fig. 4).

Weaning in a warmer world

Weaning refers to (a.) the phase in early life when offspring achieve independence from parental provisioning, (b.) the process of shifting to the consumption of solid food after relying on mother's milk, (c.) a more general process of advancing toward independence from parental provisioning (Galef, 1981; Thiels & Alberts, 1991). A pivotal event in weaning (both as a phase and a process) is "leaving the nest". Rodents, like other altricial infants are born into a restricted and protective nest, where they receive parental attention and resources. To advance to the next major phase of their life, they must leave the nest and begin exposure to myriad new experiences and opportunities in the "outside world". Generally, the world outside the nest is more thermally variable, cooler, and challenging. Indeed, there is evidence that a pup's first egression from the nest and therefore the timing of many subsequent and formative milestones, depends on ambient temperature conditions outside the nest. In an earlier study from our laboratory (Gerrish & Alberts, 1996), individual rat dams and their litters residing in a "semi-natural" environment were observed video from P14 - P22. The habitat consisted of a nestbox attached to a larger, open field in which powdered food was available. Ambient temperature of the field was either Warm (30°C), Moderate (2°C), or Cold (10°C); nest temperature was always Moderate. Behavior during 12 continuous hours was monitored and quantified from time-lapse video recordings. The pups' forays into the field and the onset of independent feeding were temperature-related: weaning was earliest in the warmth and increasingly late with decreased ambient temperatures. Among subjects in the Cold condition, there was a positive correlation between duration in the field and duration of feeding. Pups entering the Cold open field left the nest approximately long enough to feed, and then returned to the warm confines of the nest. In contrast, when pups in the Warm open field condition left the natal nest there was not a positive correlation between duration spent in the field and duration spent feeding. This was likely due to pups in warm environments showing additional behaviors outside the nest such as sleeping and playing.

To extend the idea that the thermally-fragile pre-weanling rat is freed by warmth and afforded the ability to foray from the confines of the insulative, natal nest, Gerrish, Onischak, and Alberts (1998) employed a regime in which litters without their dam were exposed on P2 – P14 for 2 h/day to either a Cool, Moderate or Warm ambient temperature (10^{o}C, 21^{o}C, 31^{o}C, respectively). Daily exposures to the Cold condition led to slower growth, delayed maturational markers such eye opening, and less insulative fur, relative to the pups in the Warm and Moderate conditions. Thermogenic capability, measured by oxygen consumption rate when challenged with an 18^{o}C ambience did not differ across groups. Time spent out of the nest and onset of independent feeding was a function of thermoregulatory development.

Ontogenetic Adaptations to Thermal Environments

We have seen that in the young offspring of altricial species, such as mice and rats, thermoregulation is meager and poorly developed. Nevertheless, we have also learned that even the drastically-immature newborn can respond adaptively to thermal challenges with behavioral and physiological responses. Thermogenesis by the specialized organ of brown adipose tissue, combined with contact behavior is one such example (Cannon & Nedergaard, 2004).

A more detailed appraisal of the mammalian infants' repertoire of adaptive responses to thermal stimuli and challenges yields a noteworthy pattern. Over all, the mammalian infant is better equipped with adaptive specializations for regulatory responses to cold challenges than for regulatory responses to heat challenges. Figure 2 provides a general picture of the overall pattern, as seen in the greater number of entries in the upper portion of the diagram, showing compensatory responses to cooling than in the lower portion where responses to warming are displayed. Of course, it difficult to compare precisely different types of mechanism with one another. Hoffman, Flory & Alberts (1999a) devised an operant head-turning procedure whereby 1-, 5- and 11-day-old rat pups in a cool environment could be rewarded with a 20-sec warming of the platform on which they lay. These investigators then established for each age "thermal preferenda" which was the pups' preferred surface temperature on a thermocline when the air temperature was either cool or warm. They then applied these air temperatures as the challenge in the operant setting; the surface preferenda established in the preliminary study were the precise rewards used in the learning task, thus equating the rewards according to the pups' preferences in the context of the two ambient temperatures. The results were dramatic. Whereas 1- , 5- and 11-day-olds learned the head-turning operant for the warm reward in a cool environment, only 5- and 11-day-olds acquired the head-turning response when rewarded with 20-sec cooling of the platform on which they lay. The 1-day-olds did not learn the task to combat heat challenge, although they learned the same operant for the equated reward of warming to the cold challenge (Hoffman, Flory & Alberts 1999b). It was clear in these studies that the 1-day-old pups were capable of using their behavior to serve body temperature homeostasis, for they regularly moved up the thermocline to very warm regions from which they retreated, and settled in areas that were adequately warm or cool to maintain a desirable temperature in a cool or warm environments, respectively (Hoffman et al., 1999a,b).

The 1-day-olds' response patterns consisted of invariant and persistent orientation and turning to the warm stimulus or in its direction, which was likened to a "positive thermotaxis". This thermotaxis was so invariant that it prevented the newborns from learning a reversal of the original operant (Hoffman et al., 1999a). By 5-days of age, the pups' thermotaxis wanes sufficiently that they can respond actively to a cool stimulus and learn an association with it as a reinforcer.

Hoffman et al. (1999b) invoked the concepts of ontogenetic adaptation (Oppenheim, 1980) and developmental niche (Alberts & Cramer, 1988) to frame the newborns' initial lack of learning to a cool stimulus and the subsequent expansion of their learning to include cool reinforcers. In the niche of the newborn, the key stimuli for survival, such as the mother's body, are warm. There are no obvious circumstances in which learning a novel response that could move a pup away from its mother and littermates would be beneficial. The initial existence of the strong, positive thermotaxis and absent reward value of cool stimuli, is consistent with an interpretation of ontogenetic adaptation.

Together with many of the phenomena reviewed earlier, as well as the original data on developmental differences in pup flow reported here, these considerations point toward recognition of the privileged roles of warm stimuli in the organization and establishment of early behavior in immature, altricial, endothermic species. Physiological and behavioral mechanisms, including learning, that serve to maintain thermal homeostasis in the face of cold challenges are the predominant, early-developing capabilities. Thus, thermotaxis, huddling, brown fat thermogenesis and the rewards of warming have developmental onsets prior to saliva spreading for evaporative cooling, pup flow in the upwards direction and cooling as a reinforcer.

Ontogeny of behavior in the context of climate change.

We have discussed some of the ways in which mammalian development is shaped both directly and indirectly by thermal stimuli and environmental temperature. While it is true that infant mammals are buffered and protected from many perturbations, including thermal extremes, we have seen myriad pathways by which thermal conditions can affect developing organisms. We are struck, in particular, by the ways in which young mammals are ill-equipped to adapt to elevated temperatures, the sort of the thermal challenge that is likely to prevail with global warming. This is an immediate message of the present analysis. In addition, we can better see the limits of our knowledge and understanding of these basic processes, so this is also a call for a new generation of empirical questions, the answers to which might help us understand how to adapt to a changing environment or, perhaps better, motivate more vigorous controls of deleterious environmental change.

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Chapter 5:

Density and Group Size Influence Shoal Cohesion, but not Coordination in Zebrafish (*Danio rerio*)

Shelton, D. S., Price, B. C., Ocasio, K. M., & Martins, E. P. (2015). Density and group size influence shoal cohesion, but not coordination in zebrafish (*Danio rerio*). *Journal of Comparative Psychology*, *129*(1), 72.

Abstract

The formations made by gregarious animals can range from loose aggregates to highly synchronized and ordered structures. For very large, coordinated groups, both physical and social environments are important for determining the physical arrangement of individuals in the group. Here we tested whether physical and social factors are also important in determining the structure of small, loosely coordinated groups of zebrafish. We found that even though our fish were not crowded and did not use most of the available space, the distance between individual fish was explained primarily by the amount of available space (i.e., density). Zebrafish in a larger space spread out more and the total dimensions of the shoal were an additive function also of group size. We, however, did not find any impact of social or physical environment on the orientation of individual fish or shoal. Thus, both physical and social factors were important for shoal spatial arrangements, but not individual orientation and shoal alignment.

Keywords: density, group size, shoal cohesion, environmental structure, zebrafish

Introduction

When animals aggregate, their spacing patterns are the result of a complex tug-of-war between approach and avoidance in response to social and physical environmental factors (Bode, Faria, Franks, Krause, & Wood, 2010; Hemelrijk & Hildenbrandt, 2012). In large groups, the social environment can strongly influence spacing and alignment. For example, bird flocks (reviewed in Bajec & Heppner, 2009; Hemelrijk & Hildenbrandt, 2012) and lobster trails (reviewed in Wyatt, 2011) exhibit coordinated movements suggesting that individuals are responding to each other. The physical environment can also impact the spatial distribution and orientation of individuals in a large group. For example, crowding can transform loose groups of locusts into highly organized marches (Buhl et al., 2006). Whether social or physical environments are more important in shaping the spatial structure of the group may depend on whether the group is large and coordinated, or relatively small and loosely interacting.

Groups of animals often balance the competing demands of predator avoidance, information transfer and competition by varying group size dynamically (Focardi & Pecchioli, 2005; Ford & Swearer, 2013). These changes in group size may have important effects on the physical structure of the group (Hemelrijk & Hildenbrandt, 2012). For example, domesticated animals in small groups maintain longer distances between neighbors, whereas larger groups are more compact (reviewed in Estevez, Andersen, & Nævdal, 2007; sheep: Sibbald, Shellard, & Smart, 2000). In addition, individuals may prefer to aggregate in larger groups in the presence of a predator, but prefer smaller groups when food-deprived (Hoare, Couzin, Godin, & Krause, 2004). Very large groups may have improved vigilance and faster information flow (e.g., sandpipers: Beauchamp, 2012), but individuals within those groups are also more likely to compete with each other (marine fish: Stier, Geange, & Bolker, 2013, colobus: Teichroeb & Sicotte, 2012) or to collide during group locomotion (sandpipers: Beauchamp, 2012; locusts: Buhl et al., 2006). To reduce the likelihood of competition and collision, some animals in large groups space themselves in a well-defined pattern to avoid aggressive neighbors (Bazazi et al., 2008), stagger themselves behind a leader (Nagy, Ákos, Biro, & Vicsek, 2010; Yomosa, Mizuguchi, & Hayakawa, 2013), or oscillate among physical positions (Ballerini et al., 2008; Morrell, Ruxton, & James, 2011). Here, we ask whether group size has similar impacts on the physical properties of relatively small aggregations.

Enclosure size and configuration can also strongly influence the shape and behavioral repertoire of animal groups. For example, individuals in larger enclosures tend to disperse farther from their neighbors (chickens: Leone et al., 2010; cows: DeVries, von Keyserlingk, & Weary, 2004), have larger home ranges, and move along the edges of the area (Buijs et al., 2010; Horiuchi & Takasaki, 2012). Mice placed in a flat nest huddled together in a relatively flat, horizontal plane, whereas mice in a concave nest huddled in three dimensions, with pups piling on and crawling under littermates (Shelton & Alberts, 2013). Similarly, locusts crowded in a donut arena showed density-dependent transitions from muddled groups to highly aligned plagues, with evenly-spaced members marching in a single direction (Buhl et al., 2006).

The above impacts may depend critically on whether the groups are large and composed of unfamiliar animals moving in a synchronized fashion (e.g., a “school”) or smaller groups of familiar, loosely-interacting individuals (e.g., a “shoal”). In large groups, for example, social factors may be more important, as individuals copy the behavior of their neighbors (e.g., prairie dogs: Hare, Campbell, & Senkiw, 2014; kangaroos: Pays et al., 2009), creating waves of animals that are all simultaneously vulnerable to predators (Sirot & Touzalin, 2009). In smaller groups, animals may recognize each other as individuals and may thus be more likely to coordinate rather than synchronize behavior, taking turns at vigilance. Familiar shoals are more cohesive and display more predator evasion tactics than do groups of unfamiliar fish (reviewed in Ward & Hart, 2003). The parallel alignment and consistent spacing of large, synchronized groups may have a genetic basis, and thus be relatively fixed (e.g., Greenwood, Wark, Yoshida, & Peichel, 2013). In contrast, the physical properties of smaller, less-coordinated groups may be shaped more directly by physical factors that elicit taxes (light: Bode et al., 2010; Imada et al., 2010; water currents: Capello, Soria, Potin, Cotel, & Dagorn, 2013; Genin, Jaffe, Reef, Richter, & Franks, 2005), or constrain group motion (enclosure shape: Bazazi et al., 2008; Buhl et al., 2006; nest configuration: Shelton & Alberts, 2013).

In the present study, we test whether the physical shape of zebrafish shoals (relatively small groups of familiar, loosely-interacting individuals) are influenced more strongly by social or physical environments, by varying the number of individuals and the size of the arena in which they are tested. Zebrafish display complex social behavior (reviewed in Spence, Gerlach, Lawrence, & Smith, 2008) and are found in shoals of 2-10 fish in the wild (Pritchard, Lawrence, Butlin, & Krause, 2001). Zebrafish are susceptible to social factors, exhibiting strong preferences for shoaling partners with particular phenotypes (Engeszer, Ryan, & Parichy, 2004; Rosenthal & Ryan, 2005) and adopting distinct social roles (Vital & Martins, 2011, 2013), both of which could alter the spacing between members and the cohesion of the shoal. Zebrafish evolved in a wide diversity of natural habitats, including lakes and streams of India, Nepal, and Pakistan (Bhat, 2004), and experience drastic seasonal changes in water velocity and other environmental properties with the Indian monsoons (Bhat, 2004; Sreekantha et al., 2007). Thus, they may also react directly to physical properties such as water flux and amount of available space. In this study, we test for differences in the spacing patterns of zebrafish, while varying group and arena size. If social factors are important in determining the physical properties of the shoal, then we expect to see differences between groups of four and eight fish. If the physical environment is more important, then we expect to see a difference between shoals according to the tank size or amount of available space.

Method

Subjects

We used adult zebrafish from the Scientific Hatcheries strain, an outbred, wildtype strain used recently in other behavioral studies (Moretz, Martins, & Robison, 2007; Oswald & Robison, 2008; Vital & Martins, 2011, 2013). We housed fish in 37.85 L (10-gallon) tanks, maintained at room temperature of 28°C ± 3°C with a 10:14 h light: dark cycle, and fed *ad libitum* commercial flake food (Tetramin Tropical).

Experimental Procedure and Scoring

We formed 20 groups of four fish and 20 groups of eight fish to compare the effects of group size, choosing adults of both sexes from larger groups that had been housed together for at least two weeks before testing. Our experimental arena was slightly smaller than a standard 37.85 L (10-gallon) aquarium and contained 14.5 cm of gently-flowing water (Fig. 1). We tested the effects of physical space by forming 9 additional groups of four fish and placing them in a shortened form of the arena, which reduced the volume of water per fish by half (Fig. 1). We used adjustable collimators to reduce turbulence and to vary the dimensions of the space available to the fish.

After a five-minute acclimation period, we recorded the fish with a Logitech® c525 HD video camera from above. The relatively low water level (14.5 cm) ensured that zebrafish movements were largely restricted to two-dimensions. We took 5 snapshots of each group at 15 s intervals, and used NIH Image J (Schneider, Rasband, & Eliceiri, 2012) to score nearest-neighbor distance (NND), the distance between each fish and its closest neighbor. We also scored shoal area and perimeter by creating a minimum convex polygon from the positions of the outermost fish, and measured shoal length as the distance between the two farthest fish. Finally, we scored individual orientation by marking the nose and midsection of each fish, drawing a line through the points, and measuring the angle of the fish in relation to the water current. For NND and individual orientation, we analyzed individual measures of four fish from each group (all fish from groups of four and four randomly-selected fish for groups of eight) to maintain equal sampling variances in the treatments. To measure shoal orientation, we found the long axis of the shoal and recorded its angle in relation to the water flow. Because motivation can affect orientation (sharks: Gardiner & Atema, 2014; mottled sculpin: Coombs & Grossman, 2006), we fed the fish prior to the beginning of each trial.

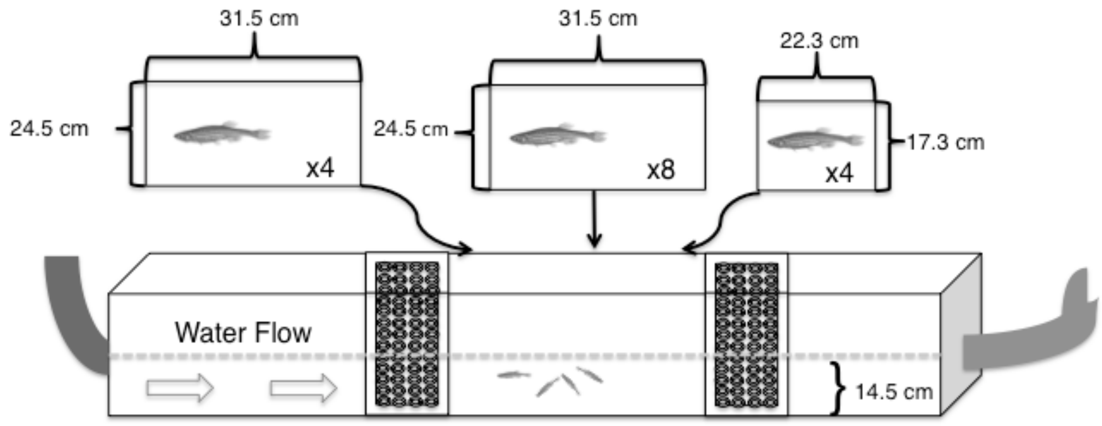

Figure 1. Dimensions of the observational areas for each of three treatment conditions: a group of four fish in a large tank (2.8L/fish), a group of eight fish in the same size tank but higher density (1.4L/fish), and a group of four fish in a smaller tank and consequently a higher density (1.4L/fish). The experimental arena was a fluvial tank with a unidirectional water flow system and two collimators designating an observation area and minimizing turbulence. We photographed the fish from above, keeping the water level low to restrict the movements of the fish to 2-dimensional space.

Analysis

We averaged measures of each parameter across the five images taken of each group, and then used an ANOVA with Tukey post-hoc tests to examine the effects of group and arena size (4 fish large tank 2.8 L/fish, 8 fish large tank 1.4 L/fish, or 4 fish small tank 1.4 L/fish) on average NND, shoal perimeter, shoal area, and shoal diameter. We used Type III sums of squares, which correct for unbalanced data (Shaw & Mitchell-Olds, 1993), and an alpha level of .05. We also log 10-transformed NND and shoal area, as required to obtain residuals that conformed to the assumptions of the ANOVA. To assess the effects of social and physical structure on orientation, we calculated the mean vector length, ρ of each individual and group. A vector length of 1 results from perfect concordance for all phase angles (a highly polarized collective of fish or a consistently responding shoal), whereas a vector length of 0 represents an asynchronous group of individuals and randomly oriented shoal. We conducted all statistical analyses in R (Team, 2012), using the 'base' package and 'circular' package as needed (Lund, Agostinelli, & Agostinelli, 2013).

Results

Zebrafish Aggregated, Using Much Less than the Total Available Space

Fish in this study clumped together in the relatively large testing arena (Fig. 1). The testing arena (771.75 cm^2) was at least 7 times larger than the average shoal area of the largest group size under the highest density condition (M = 104.8, 95% CI [91.00, 118.60]). Similarly, groups of four fish with similar available space had a mean shoal area (M = 23.2, 95% CI [15.91, 30.49]) that was over 15 times smaller than the area of the testing arena (385.79 cm^2). Groups of four fish in the same larger tank (but half the density) had even smaller shoal area to tank size ratio, 23 times smaller (M = 41.9, 95% CI [37.53, 46.27]).

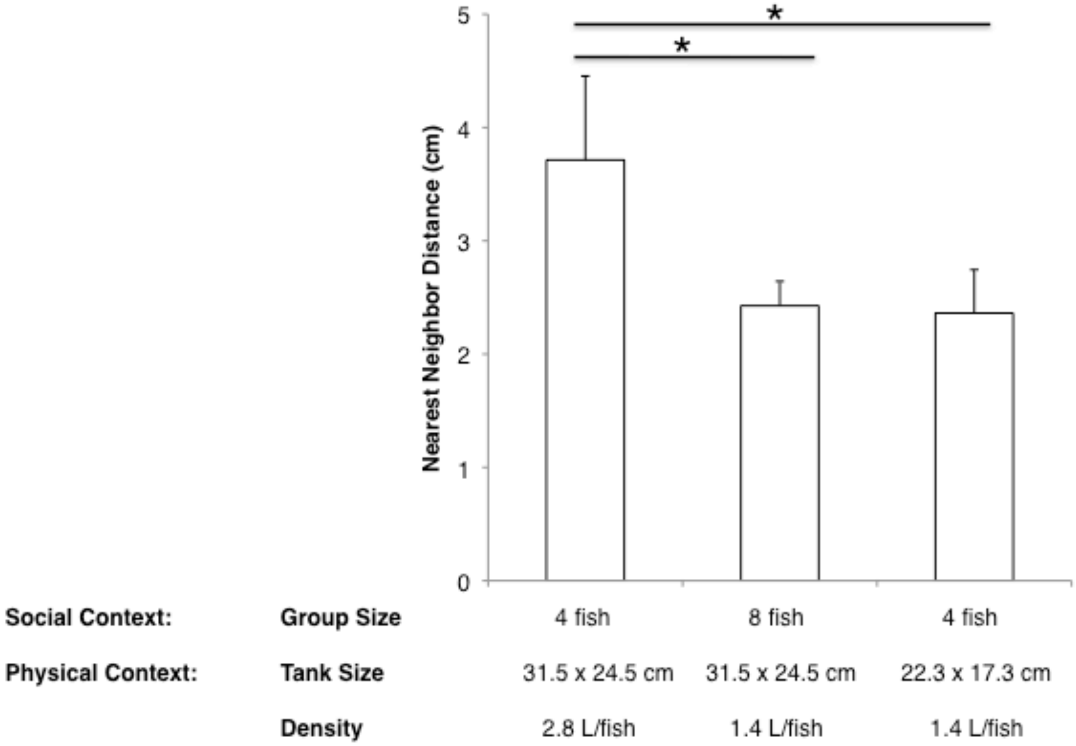

Figure 2. Nearest Neighbor Distance is more influenced by the physical environment than the social structure. Groups of four fish in a relatively large arena (2.8 L / fish) were more dispersed than groups of eight fish in the same size space but at a higher density (1.4 L / fish) and groups of four fish in a smaller arena (1.4 L / fish). Groups of four and eight fish were similarly dispersed when density was equal. Error bars are one standard error. *Corresponds to significant Tukey post-hoc comparisons at $p < .05$.

Both Physical and Social Factors Influenced Shoal Cohesion

Groups of four fish in a large space (2.8 L / fish) maintained nearly twice the distances between neighbors (M = 3.7 cm, 95% CI [-.14, 7.54]) than did groups of four or eight fish in a smaller space (M = 2.4 cm and 2.4 cm, 95% CI [.58, 4.22] and 95% CI [-.19, 4.99], respectively). This resulted in a significant difference in NND across the three treatment categories ($F_{2, 46} = 8.32$, $p = .003$, $\eta^2 = .27$; Fig. 2). There was no significant difference between the groups of four and eight fish under the same available space conditions (*p* = .90, Tukey). There was a significant difference between groups of four fish tested in larger and smaller arenas (or at different densities; *p* < .01, Tukey).

Groups of eight fish maintained a shoal perimeter (M = 36.1, 95% CI [31.45, 40.75]) that was nearly twice the shoal perimeter of groups of four fish (M = 20.0, 95% CI [14.98, 25.02]) under similar density conditions and almost 25% larger than groups of four fish (M = 26.6, 95% CI [22.05, 31.15]) with double the available space. This led to a significant difference in shoal perimeter across all treatment conditions ($F_{2, 46} = 21.85$, $p < .0001$, $\eta^2 = .49$; Fig. 3). The difference between groups of four and eight fish tested under similar available space conditions was significant (*p* < .0001, Tukey). There was a significant difference between groups of four fish tested in larger and smaller arenas (or at different densities; *p* = .04, Tukey). Similarly, there was a significant difference between groups of eight fish and four fish tested in the same size arena (but consequently at different densities; *p* < .0001, Tukey). The pattern was consistent and nearly identical for shoal area and shoal diameter measures.

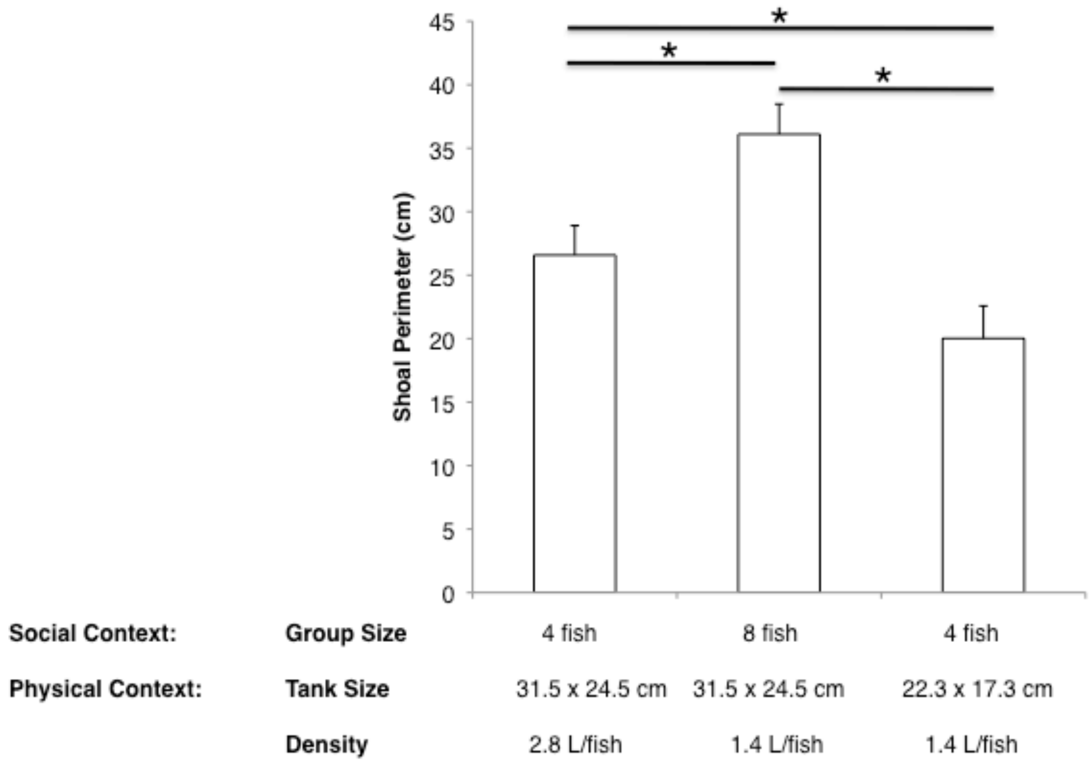

Figure 3. Social and physical environments affect shoal perimeter. Groups of eight fish in a relatively large arena (1.4. L / fish) were more dispersed than groups of four fish under the same density conditions, but smaller space (1.4 L / fish) and groups of four fish in a larger arena and at half the density (2.8 L / fish). Groups of four fish at higher densities had smaller shoal perimeters than groups of four fish at lower densities. Error bars are one standard error. *Corresponds to significant Tukey post-hoc comparisons at $p < .05$.

Social and Physical Environment Do Not Enhance Individual or Shoal Polarity

In all treatment conditions, individual fish oriented at random in response to the water flow, showing no signs of aligning with the direction of water flow (Fig. 4). We found no evidence of enhanced synchrony among individual fish (ρ ranged from 0.01 to

0.28; Fig. 4a). Groups also did not appear to orient with respect to the flow (ρ ranged from 0.98 to 0.99; Fig. 4b) in any of the social or physical conditions.

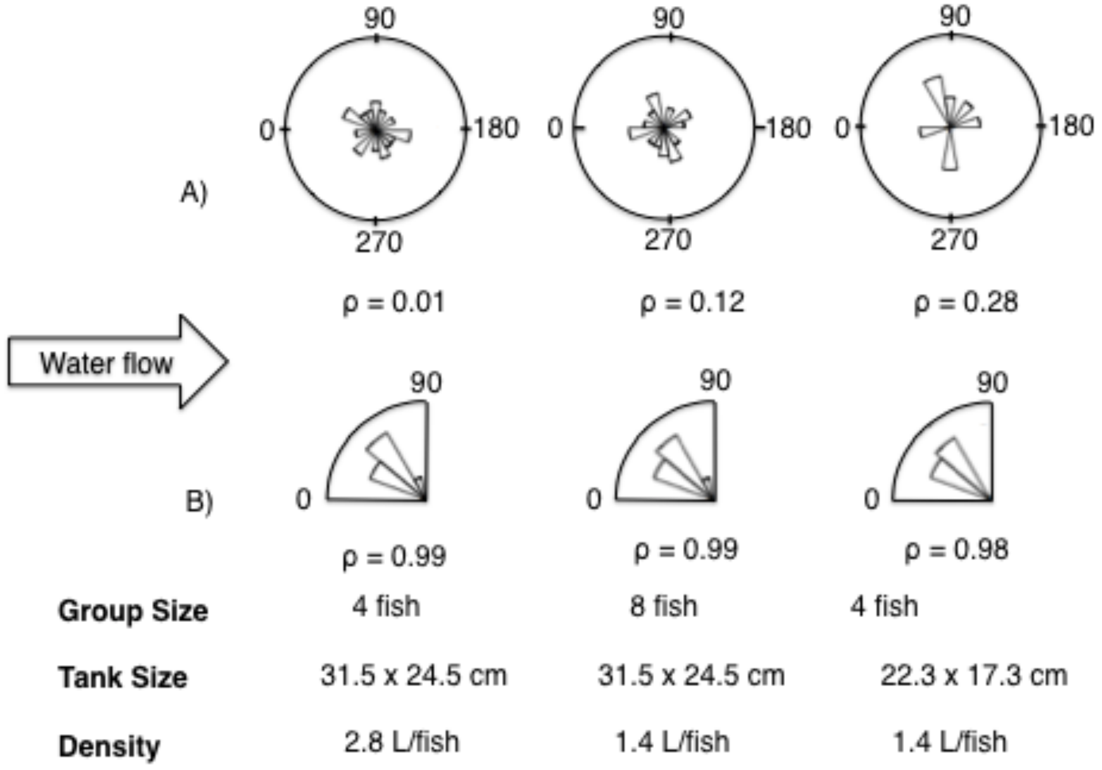

Figure 4. Individuals randomly orient and shoals maintain consistent polarity independent of social and physical contexts. The direction of the water flow in relation to the orientation of the fish and shoals is depicted by the arrow. The synchrony of individual fish and the polarity of the shoal are indicated by ρ. In all tank and group sizes, individual fish orient randomly (A). The shoal's polarity varies independently of tank size and number of fish within the shoal (B). We set each bin to encompass an angular range of 18 degrees (so 0–18 degrees, 18–36 degrees, etc.).

Discussion

We found that although the distance between individuals in a shoal depends primarily on the relative amount of available space, shoal dimensions depend also on group size. Although our experimental arena was much larger than the dimensions of the shoal, zebrafish spread themselves out more when there was more available space, indicating that the fish adjusted their proximity to neighbors according to the relative amount of space (i.e., density). Groups of four and eight fish tested at the same relative densities maintained the same distance between neighbors, with the groups of eight fish taking up roughly twice the amount of total space as a consequence. Because groups of four fish in a larger arena spread out more (larger NND), they occupied an intermediate amount of total space – not as much as groups of eight fish in the same sized arena, but more than groups of four fish in a smaller arena. We found no obvious differences in orientation or synchrony of individuals or shoals.

Even though our animals were not crowded, the relative amount of space was the most significant factor affecting spatial distributions in our study. Density is clearly important for shaping the spatial distribution of other species when in crowded conditions. For example, as the amount of allowable space per individual increases, domesticated rabbits transition from avoiding pen members to increasing proximity to conspecifics (Buijs et al., 2011). In chickens, groups under higher density conditions had smaller distances between neighbors than groups with more space per individual, irrespective of group size (Leone et al., 2010), and assumed spatial positions that were indicative of social attraction (Febrer, Jones, Donnelly, & Dawkins, 2006). Here, we show that density can also be highly relevant to space use when animals are not obviously crowded, clumping together in such a way that they occupy a small portion of the available space.

In large groups, spatial arrangement of individuals within the group can show substantial variation and complexity. For example, in large starling flocks the inner structure of the group is less dense than the border, as birds on the periphery push inwards to maintain cohesion (Ballerini et al., 2008; Cavagna, Queirós, Giardina, Stefanini, & Viale, 2013). Large schools and flocks also tend to have complex structures with pseudopodia, and pockets of high or low density (reviewed in Bajec & Heppner, 2009; Hemelrijk & Hildenbrandt, 2012). In contrast, we found that the shoal area, shoal perimeter, and shoal diameter of groups of eight zebrafish were nearly double that of groups of four fish under similar density conditions, suggesting that the arrangement of individuals in each of these relatively small groups were additive.

Animals that form very large groups also sometimes vary distance between individuals dynamically to balance the benefits of grouping with the need to minimize resource competition. Flocks of barnacle geese, for example, land as a tight, synchronized group, but then slowly expand in total dimension as individuals along the edges of the flock begin exploring (Carbone, Thompson, Zadorina, & Rowcliffe, 2003). Similarly, individuals along the periphery of large deer herds can venture so far away that the group fissions, separating the exploring individuals from others at the center of the herd that are tightly synchronized (Focardi & Pecchioli, 2005). As discussed by Hamilton (1971), perceived predation risk is also a major factor influencing whether groups are tightly cohesive and synchronized (e.g., starlings: Carere et al., 2009; cranes: Ge, Beauchamp, & Li, 2011). Although we found no evidence for social or physical factors impacting the alignment or synchrony of zebrafish shoals, additional studies are needed to determine whether individual behavior or social roles depend on group or enclosure size, or whether synchrony is simply less variable in smaller groups.

Our study also highlights the importance of using both individual and group measures to characterize social behavior. Although individual metrics (e.g., NND) are often used to approximate group properties (Miller & Gerlai, 2007; Parrish, Viscido, & Grünbaum, 2002; Buijs et al., 2011), our measures of NND suggested that the structure of zebrafish groups depended only on the available space, and not group size. It was only when we considered also measures of the group as a whole (e.g., shoal diameter) that we saw the effects of group size. We conclude that a better and more accurate characterization of zebrafish shoals involves both individual and group measures, as the individual and whole are dynamically linked (reviewed in (Parrish & Edelstein-Keshet, 1999). Future studies should explore under what context individual metrics sufficiently characterize the group and in which conditions group-level measures are needed to describe the group more accurately.

In summary, the goal of this study was to identify the impact of social (group size) and physical factors (tank size and density) on individual and group spatial distributions and orientation. Our results show that the spatial distribution of zebrafish in small groups is primarily determined by density, followed by group size, and that these effects can vary substantially depending on the combination of metrics. We found no evidence to suggest that group size had a fundamental impact on how individual fish spaced themselves in relation to neighbors or oriented in response to the water currents. In contrast, the dimensions of the shoal were clearly affected by enclosure size and number of fish in an additive manner. Studies such as this suggest that complex spacing patterns of small animal groups can be generated by simple mechanisms. Understanding these mechanisms is critical to understanding how complexity and order can arise.

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Chapter 6:

A Few Cadmium-Treated Fish Affect Group Dynamics and Social Behavior in Zebrafish

Abstract

In social animals, a few animals can influence the behavior of the majority. Such effects occur as a result of robust healthy individuals, and contaminants could also induce some individuals to have similar profound impacts on group responses. Here, we asked whether pollutants could affect the sensory systems of a few treated fish that then influences the group responses and social behavior of a larger group of untreated fish. We found that groups containing contaminated individuals were more likely to stay in the vicinity of a novel stimulus than were control groups, even though most of the group members had not been exposed to the pollutant. Delving deeper into the underlying behavioral mechanisms, we found that contaminated fish exhibited more aggressive and investigatory behavior and responded less to a moving visual stimulus in an optomotor assay. Weak displays of social behavior (advances, mouth contacts), but not more active behavior (chases), were detected in pairs containing contaminated versus control fish. Thus, a few contaminated individual can have profound effect on the social behavior and group responses of a larger uncontaminated group.

Introduction

The impact of a few individuals in a social group can be profound (reviewed in Modlmeier, Keiser, Watters, Sih, & Pruitt, 2014), especially for learning (Vital & Martins, 2011), foraging success (Dyer, Croft, Morrell, & Krause, 2009), and social interactions (Flack, Girvan, de Waal, & Krakauer, 2006). Here, we ask whether the impact of pollutants on a few individuals extends also to indirect effects on the larger social group. Environmental pollutants can have direct effects on physiology and development, leading to major abnormalities (Hayes et al., 2002). In addition, pollutants can have extended effects, for example, by impacting sensory systems in ways that then have consequences for later social interactions (Halfwerk & Slabbekoorn, 2015). On a larger scale, exposure to pollutants can have effects through ecological cascades, such as through trophic transfer of microplastics (S. L. Wright, Thompson, & Galloway, 2013), or transgenerational transfer of toxic effects (Crews, 2010). Here, we ask whether pollutants have an additional, indirect, effect on social animals by influencing the behavior of social groups as a whole. Here, we expose a few individual animals in a larger social group to a pollutant and examine the consequences of that treatment on the behavior of the group as a whole.

Certain individual characteristics are associated with specific impacts on the group. Within these social groups, some individuals with distinct, often healthy phenotypes influence the interaction with conspecifics more than others (e.g. “gatekeeper”, “keystone”, “dominant”), and thus may have a special impact on group dynamics and performance (reviewed in Modlmeier, Keiser, Watters, Sih, & Pruitt, 2014). Individuals may choose the direction of group travel, control access to a resource, or speed the transmission of diseases. In some cases, these individuals are larger, more dominant, or otherwise phenotypically-distinct from other group members (Fischhoff et al., 2007; McComb et al., 2011). In many groups, for example, animals that are more bold approach potential food sources more readily than do others in the group (e.g., Dyer, Croft, Morrell, & Krause, 2009; Kurvers, Nolet, Prins, Ydenberg, & Oers, 2012), and may thus have a disproportionate effect on the direction of group movement. Also, "superspreaders" are the few individuals that are responsible for the majority of pathogen transmission events, remaining tolerant to factors that exacerbate disease symptoms (Gopinath, Lichtman, Bouley, Elias, & Monack, 2014). Not all individuals in groups are as healthy, and resistant to external factors as others, as some animals are more susceptible to environmental pollutants (Bridges & Semlitsch, 2000; Sih, 2013). For example, first-time breeding female birds are more susceptible to pollutants than are other birds (Brasso & Cristol, 2007), and dominant fish accumulate more cadmium than do controls (Sloman et al., 2003). In the current study, we ask whether a few contaminated individuals can impact group behavior.

Behavior serves as a link between physiological and ecological processes and may be ideal for studying multiple pathways of pollutant action (Sih, Ferrari, & Harris, 2011; Wong & Candolin, 2015). Exposure to pollutants can have widespread and long-lasting consequences for behavioral development (Colborn, vom Saal, & Soto, 1993), antipredatory behavior (Hazelton et al., 2014; Pelli & Connaughton, 2015), learning (Golub, 2002), communication (Sluijs et al., 2011) and other complex behavior (Scott & Sloman, 2004; Zala & Penn, 2004), and through its effects on the epigenome, can exert influences on the behavior of subsequent generations (Jirtle & Skinner, 2007). For example, frogs exposed to neuro-endocrine disruptors during early development are de-masculinized and have altered growth rates and immune responses (Hayes et al., 2006), which has cascading effects on reproduction and disease resistance (Hayes, Khoury, et al., 2010) and is implicated in amphibian declines (Hayes, Falso, Gallipeau, & Stice, 2010). These effects are often a product of low doses and the direct action of the pollutant. We also know that secondary action of the pollutant can have equally profound consequences, as contaminated individuals can interact with conspecifics even after the pollutant is removed from the environment, thereby extending the impact of the pollutant (reviewed in Fleeger, Carman, & Nisbet, 2003; Scott & Sloman, 2004). For example, male ibises experimentally exposed to a contaminant showed typical de-masculinization, which affected interactions with un-exposed conspecifics (Frederick & Jayasena, 2010), and Siamese fighting fish exposed to fluoxetine became less bold when presented with novel stimuli (Dzieweczynski, Kane, Campbell, & Lavin, 2015) and less aggressive in territorial defense during certain reproductive phases (Forsatkar, Nematollahi, Amiri, & Huang, 2014). Here, we ask whether a few animals exposed to pollutants can impact the behavior of a larger social group.

Sensory systems are a gateway for social interactions. They guide coordination and synchronization in collective movement (Bode, Faria, Franks, Krause, & Wood, 2010; Partridge & Pitcher, 1980), influence the propagation of information (Strandburg-Peshkin et al., 2013), inform mate choice (Ryan & Cummings, 2013), and help to communicate social status (Fernald, 2014). In coordinated and synchronized animal groups, vision is important for assessing the behavioral movements of their neighbors (S. B. Rosenthal, Twomey, Hartnett, Wu, & Couzin, 2015), determining spacing patterns

(Gerlai, 2014), and informing collective antipredator defense (Kim et al., 2009). Visual cues are also important for determining shoaling preferences, as fish prefer others with similar stripping patterns (G. G. Rosenthal & Ryan, 2005), color and tail beating frequencies (Polverino, Phamduy, & Porfiri, 2013). In a contamination event, sensory systems are prime targets, as they are in direct contact with the external environment. When a sensory deficit is induced by a pollutant, it can disrupt social recognition (Ward, Duff, Horsfall, & Currie, 2008), reduce shoaling (Borner et al., 2015), and alter communication (Sluijs et al., 2011). Contaminants can also block channels of communication by altering the ability of an animal to receive the signal due to shifts in receptor sensitivity. For example, increased turbidity in lakes was linked to changes in signaling colors, and also to shifts in visual physiology (Seehausen et al., 2008). Here, we test whether the effects of a few toxin-treated individuals on group responses could be caused by changes in vision.

Impairments in sensory systems may also alter interactions with familiar stimuli in the environment. The change in behavior may occur through alteration in risk-assessment (Ferrari et al., 2012), impeding recognition (Dixson, Munday, & Jones, 2010), or disruption of learning and memory (Lu, Li, Qiao, Yan, & Yang, 2008). For example, coral reef fishes exposed to pollutants that interfered with visual and olfactory systems showed atypical anti-predatory responses, moving closer to threatening stimuli (Ferrari et al., 2012), and an inability to distinguish between habitats (Munday et al., 2009), even choosing locations that they previously avoided (Dixson et al., 2010). Pollutant exposure can also lead animals to change their approach-avoidance responses. For example, natural populations of fish exposed to an ecotoxicological agent were more bold and active than were unexposed fish, approaching food sources more often and avoiding social interactions more than did untreated fish (Brodin, Fick, Jonsson, & Klaminder, 2013). In the present study, we assessed if contaminated individuals could influence a larger group's response to a novel stimulus.

Specifically, we tested whether pollutants could have indirect effects on group behavior even when only a few of the group members have been exposed to toxins. Zebrafish form small groups (Suriyampola et al., 2015) with individuals of different phenotypes (D. Wright, Rimmer, Pritchard, Butlin, & Krause, 2003) and associated social roles (Vital & Martins, 2011), and aspects of their social behavior are influenced by environmental factors (Shelton, Price, Ocasio, & Martins, 2015). Zebrafish are native to areas experiencing rising levels of pollution. One of the pollutants accumulating most rapidly is cadmium due to anthropogenic mobilization (Goering, Waalkes, & Klaassen, 1995; Satarug et al., 2003; Satarug, Garrett, Sens, & Sens, 2011). Cadmium is a ubiquitous sensory modifier, with severe effects on vision at low doses (Avallone et al., 2015). Vision is a key sensory modality for zebrafish, as it informs shoaling behavior, neighbor preferences, anti-predatory strategies, and general approach-avoidance responses (Fleisch & Neuhauss, 2006). Through direct action of cadmium on the visual system, cadmium may have an indirect effect on social interactions, which could reveal a hidden pathway of pollutant action on collective behavior. In this study, we treated a few members of a larger group with low doses of cadmium, and asked whether this exposure to pollutants influenced group response to a novel stimulus. Along the way, we explored the specific behavior patterns affected by a low-dose cadmium treatment, and used an optomotor assay to assess the impact of cadmium on vision. To assess the direct action of cadmium through the visual system, we also examined the optomotor response of cadmium- and water-treated fish. We then examined how exposure to pollutants affected different aggregative and aggressive behavior.

Method

Subjects

We used zebrafish from the wild-type, outbred, Scientific Hatcheries strain bred by Aquatica Biotech (Florida, USA). This strain has been used in several recent studies (Moretz et al., 2007; Shelton, Price, Ocasio, & Martins, 2015; Vital & Martins, 2011, 2013). We housed the fish in standard conditions: 18.9 L (5.5 gallon) aquaria, 28^{o} C, 10:14 hour light/dark cycle, and ad libitum flake food.

Procedure

Group Performance

We began by forming 36 groups of adult zebrafish (6 fish per group), and allowing them to become familiar with each other over one week (7 days). We then tested each group twice in an experimental arena consisting of a 38 L (10 gallon) aquarium with gravel substrate and a buried filter. To create a novel stimulus, we also buried a 100 mL Eppendorf tube in the gravel substrate on one side of the arena, attaching it to clear fishing line so that it could be pulled abruptly from the substrate. At each stimulus presentation, we pulled the string, gently lifting and shaking the attached tube for 30 s, before removing it from the aquarium. We could then score the number of fish that approached or stayed away from this novel stimulus.

Before testing, we selected two similar-sized fish (one male and one female) from each group for treatment. We exposed half of these pairs to a low dose of cadmium (0.0001 mg/L) by placing each pair in a 1 L beaker of cadmium-water overnight for a total of 17 h. Concentrations in our study were lower than usually observed in polluted natural waters. Cadmium is found at concentrations of 0.0001–0.028 mg/L in tributaries of the Ganga river, India (Kaushik et al., 2009; Singh, Singh, & Mohan, 2005), and even higher during short-term, episodic contamination events. As a control, we placed the remaining half of the pairs in 1000 ml beakers of water, also for 17 h. The following morning, we rinsed each of the 36 pairs of fish in fresh water to remove any unbound cadmium, and placed them into the experimental arena for a 1 h acclimation period. After the 1 h acclimation period, we tested the direct impact of cadmium treatment by measuring the response of each treated pair to the novel stimulus. We then reburied the novel stimuli and added the remaining four fish to re-form each group of six. On the following day (after 24 h), we tested the indirect effect of cadmium treatment by repeating the experimental assay, gently removing the novel stimulus to test the behavioral response of each full group of six fish.

We recorded each pair or group of six fish from above with a Logitech c525 HD web camera. We also counted the number of “advances”, “chases”, and “mouth contacts” by any fish in the shoal during the 30 s immediately prior to the presentation of the novel stimulus. We defined an “advance” as any episode in which one fish rapidly approached another individual, “chases” were any occurrence in which one fish accelerated towards a fleeing fish, and “mouth contacts” were episodes of direct mouth to body contact between two fish. All three are likely forms of aggression or socially investigative behavior, with "advances" being less aggressive than "chases", which in turn are less aggressive than are "mouth contacts". A fish receiving a “mouth contact” was physically moved by the sender in some instances. To measure the response to the novel stimulus, we counted the number of fish on each side of the experimental arena (same or opposite side from the stimulus) at 10 or 15 s after stimulus presentation, and measured also the nearest-neighbor distance (minimum distance between any two fish in the group) and the diameter of the group (maximum distance between any two fish in the group) at that single instance in time. We then used independent sample’s t-test (two-tailed) to compare groups of fish containing cadmium- and water-treated individuals in terms of the average number of fish on the stimulus side of the aquarium, nearest-neighbor distance, group diameter, and number of advances, chases, and mouth contacts. When data were not equal in variance, we used Welch’s correction for unequal variance, and when data were not normally distributed and thus did not conform to the t-test assumptions, we used a non-parametric Mann-Whitney *U* test instead. We conducted all statistical analyses in R with the “base” package (R Core Team, 2015).

Optomotor assay

To determine whether our weak cadmium treatment had an impact by influencing vision or visual response behavior, we treated a second set of 70 fish and tested their response in an optomotor assay. As above, we first placed 35 pairs of fish (36 males and 34 females) in 1000 ml beakers containing either cadmium (0.001 ppm) or water for 17 – 20 h overnight. We then tested visual-motor response by placing each fish in a small plastic container (7.7 cm diameter) in shallow water (3 cm water height), and suspending that container in the center of a rotating visual stimulus. The stimulus was a repeating pattern of black and white vertical bars printed on paper and affixed to a circular drum that rotated at speeds powered and controlled by a motor (Bodine 24v). We tested each fish at 5 different spatial frequencies (8, 10, 12, 14, 16 rotations/min), created by changing the speed of the rotating drum. We presented each fish with each of the 5 frequencies presented in random order during a series of consecutive 2-min trials. We lit the arena with a bright light (5,600K lumens- Brightest Setting).

A human observer scored behavior during the optomotor assay dichotomously as either (0) no response or (1) a response. We operationally defined a response as the fish making 2 full circular turns within 20 s in the direction of the stimulus. If the fish failed to make 2 consecutive circular turns, we scored it as not having responded. We used Repeated-Measures Logistic regressions to test whether cadmium- and water-treated fish differed in their visual-motor response. We conducted all statistical analyses using the R statistical package (R Core Team, 2015). We used the ‘glmer’ function of the ‘lme4’ package to fit a repeated-measures logistic regression (Bates, Mächler, Bolker, & Walker, 2014).

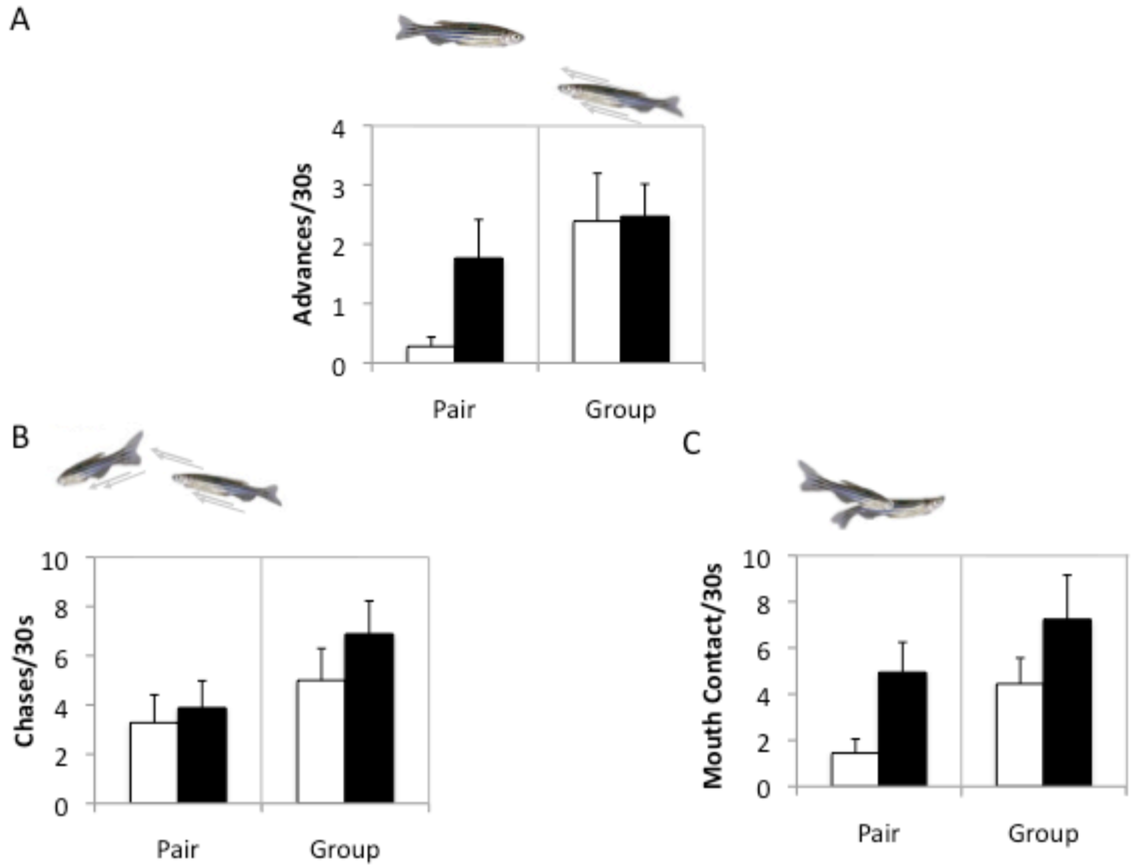

Figure 3. **Cadmium-treated pairs and groups are more socially investigative than controls.** A) Pairs and groups with cadmium-treated fish (black bars) display more bites than those with water-treated fish (white bars). B) Cadmium-treated fish (black bars) show more advances in the pair condition than water-treated fish (white bars), but the difference disappears when fish are tested in groups. C) Pairs and groups with cadmium-treated fish (black bars) show more chases than those with control fish (white bars). The drawings depict the behaviors scored for each figure. Error bars represent one standard error.

Results

Cadmium-treated fish influence social dynamics.

Cadmium treatment was linked to a difference in advances and mouth contacts. We observed larger differences in the cadmium and water treatments in the pairs of fish than in the groups of fish (Fig 3). The pattern was slightly different for chases, with subtle differences in the number of chases in pairs of cadmium- and water-treated fish, and bigger differences for larger groups with cadmium- and water-treated fish. Cadmium-treated zebrafish pairs (M = 1.76, 95% CI [0.38, 3.14]) showed six times more advances than did control pairs (M = 0.28, 95% CI [-0.05, 0.61]) in the 30s before the novel stimulus presentation (95% CI [0.08, 2.89], *t*(17.88) = 2.22, *p* = 0.04). The difference between cadmium-treated (M = 2.47, 95% CI [1.32, 3.62]) and control fish (M = 2.39, 95% CI [0.69, 4.09]) was considerably reduced in the group condition in comparison to the pair condition (95% CI [-1.90, 2.07], *t*(2.07) = 0.08, *p* = 0.93). Similarly, cadmium-treated zebrafish pairs (M = 4.94, 95% CI [2.18, 7.71]) showed nearly 3.5 times more mouth contacts than did water-treated pairs (M = 1.44, 95% CI [0.20, 2.69]) (95% CI [0.53, 6.46], *t*(22.33) = 2.44, *p* = 0.02). Groups of six fish, including two cadmium-treated zebrafish (M = 7.24, 95% CI [3.18, 11.29]) also engaged in more mouth contacts than did groups containing only water-treated fish (M = 4.44, 95% CI [2.08, 6.81]), although the difference was not statistically significant (95% CI [-1.77, 7.35], *t*(25.98) = 1.26, *p* = 0.22). The number of chases displayed by cadmium- (M = 3.88, 95% CI [1.56, 6.21]) and water-treated fish (M = 3.28, 95% CI [0.89, 5.66]) was about the same in the pair condition (95% CI [-2.60, 3.81], *t*(33) = -0.38, *p* = 0.70). In the larger group, shoals with cadmium fish (M = 6.88, 95% CI [4.04, 9.72]) showed slightly more chases than groups with water-treated fish (M = 5.00, 95% CI [2.27, 7.73]), but the difference was not statistically different from 0 (95% CI [-1.91, 5.67], *t*(32.86) = 1.01, *p* = 0.32).

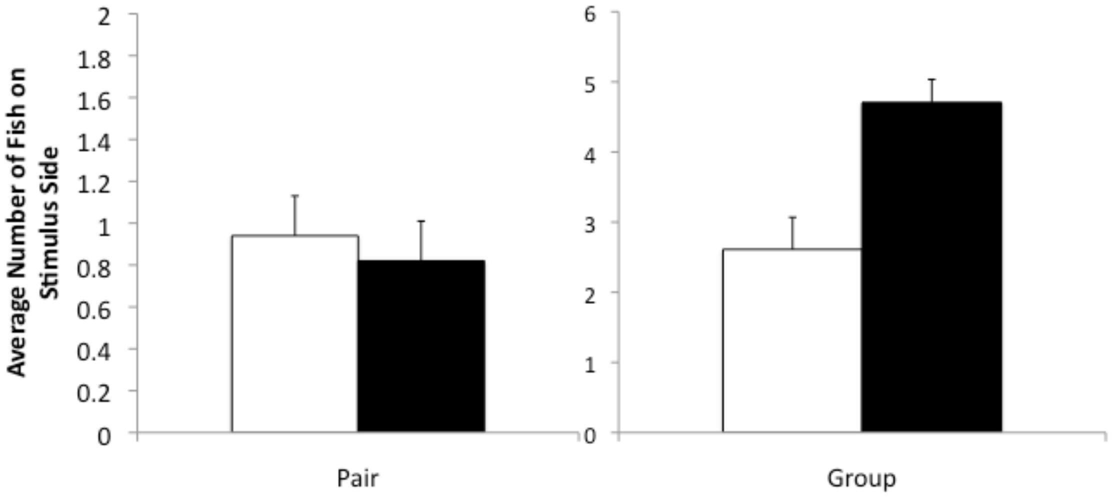

Figure 1. **Cadmium effect is hidden in pairs, but revealed in the group.** The average number of cadmium (black bars) and control (white bars) pairs on the stimulus side were at chance levels. In groups, cadmium shoals approached the stimulus more than controls. Cadmium groups (black bars) overwhelming found on side of the stimulus, whereas controls (white bars) were on the stimulus side at chance levels. Error bars represent one standard error.

Cadmium-treated pairs affects group responses.

Groups containing cadmium-treated fish remained near the area of the novel stimulus presentation, whereas control groups did not. At 10 s after the novel stimulus was removed from the arena, we found about five of the six fish (M = 4.71 fish, 95% CI [4.01, 5.40]) containing cadmium-treated fish were on the stimulus side of the arena, whereas the number of fish on the stimulus side of the arena in control groups hovered around chance levels (M = 2.61 fish, 95% CI [1.64, 3.58]) (95% CI [0.94 – 3.25]; *t*(30.47) = 3.71, $p < 0.01$) (Fig. 1). We found no evidence of a direct effect of cadmium treatment on response to the novel stimulus. We found no difference between the number of fish on the same side as the novel stimulus when we compared the responses of only the pair of zebrafish that had actually received treatment and the water-treated pair (95% CI [-0.68 – 0.43]; *t*(32.852) = -0.44, $p = 0.66$). In both treatment conditions, the number of fish on the same side as the novel object was at chance levels (cadmium: M = .82 fish, 95% CI [0.41, 1.24]; water: M = 0.94 fish, 95% CI [0.55, 1.34] (Fig. 1).

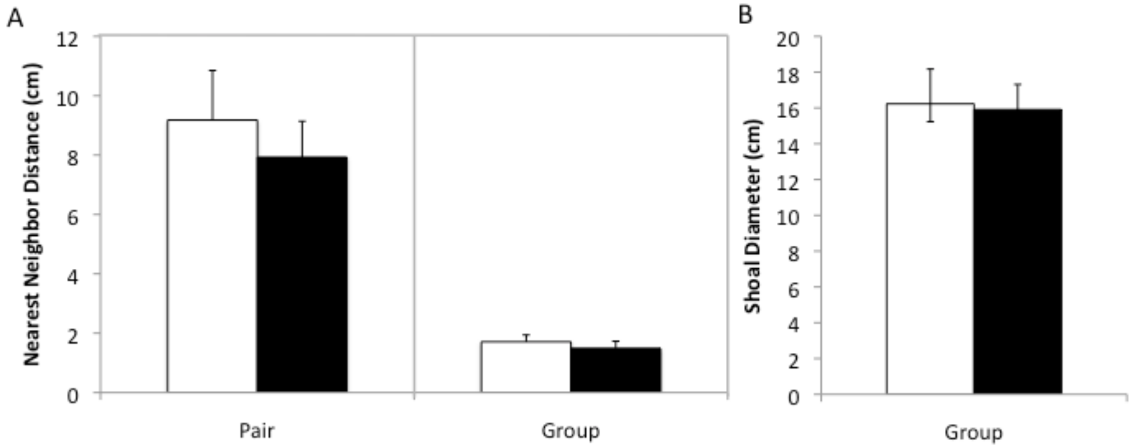

Figure 2. **Pairs and groups with cadmium and water-treated fish are equally cohesive.** A) Pairs with cadmium (black bars) and water-treated (white bars) fish had similar nearest neighbor distances, and groups with pairs of fish exposed to the experimental treatment had relatively short distances between their neighbors. B) Shoals with pairs of cadmium- and water-treated fish had similar shoal diameters. Error bars represent one standard error.

Cadmium treated pairs did not affect shoal cohesion.

Cadmium treatment did not have a clear impact on group cohesion (Fig. 2). Groups of six fish in both treatment conditions were equally cohesive (cadmium: M = 11.33 cm, 95% CI [3.43, 19.24]; water: (M = 15.66 cm, 95% CI [6.72, 24.59]), with short NND (95% CI [-0.90 – 0.45], *t*(32.81) = -0.67, *p* = 0.51) and compact shoals (cadmium: M = 15.91 cm, 95% CI [12.93, 18.88]; water: (M = 16.23 cm, 95% CI [12.13, 20.32]) (95% CI[-5.21 – 4.57], *t*(30.55) = -0.13, *p* = 0.89). Similarly, cadmium did not directly affect pair spacing patterns, as pairs of cadmium- (M = 7.93 cm, 95% CI [5.36, 10.50]) and water-treated fish (M = 9.19 cm, 95% CI [5.66, 12.71]) had similar nearest-neighbor distances (95% CI [-4.01 – 2.77], *U* = 151, *p* = 0.96).

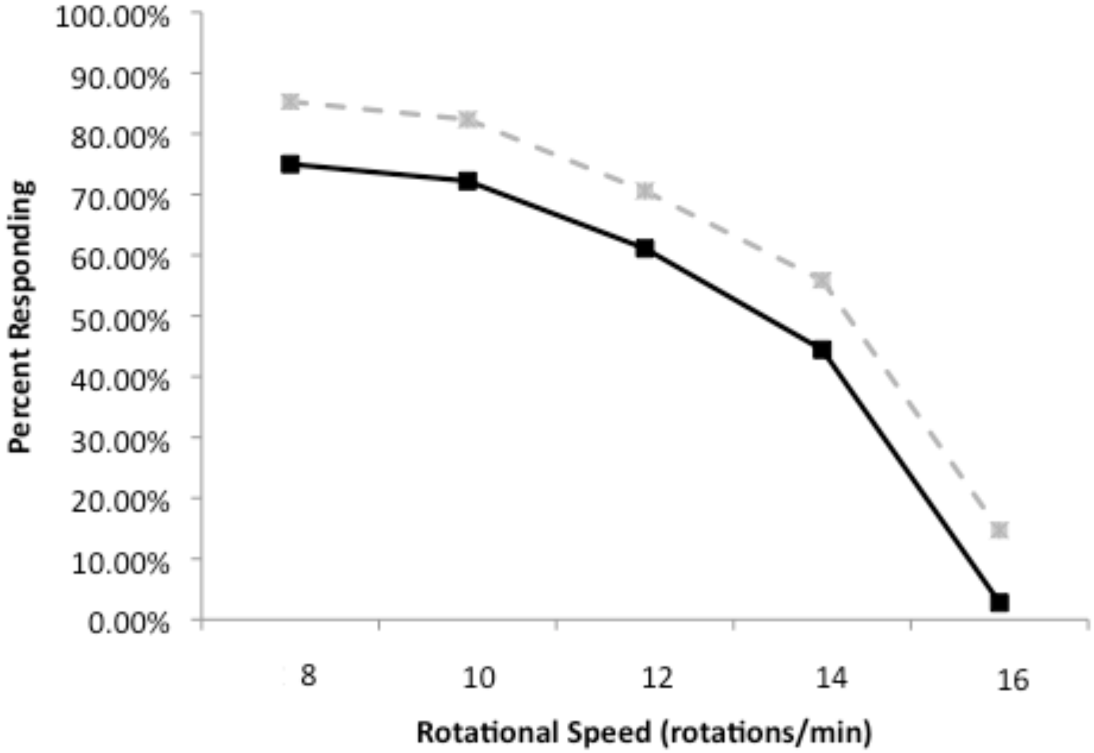

Figure 4. **Cadmium-treated fish show a possibly attenuated optomotor response in comparison to water-treated fish.** At all spatial frequencies the cadmium-treated fish (black line) show around a 10% lower response rate than water-treated fish (dashed gray line). As the spatial frequencies become smaller the percentage of fish responding decreases in parallel.

Cadmium-treated fish have somewhat attenuated optomotor responses.

Cadmium-treated fish responded somewhat less to the optomotor stimulus at all spatial frequencies than did the water-treated fish (Fig. 4), although the main effect of cadmium was not quite statistically significant in our repeated-measures logistic regression (main effect of cadmium: slope estimate = -0.8 ± 0.44, z = -1.8, p = 0.07). The logistic regression also confirmed the OMR procedure, finding that zebrafish responded significantly to the OMR stimulus (intercept estimate = 5.5 ± 1.05, $z = 5.2$, $p < 0.01$) and gave a more robust response at lower than at higher spatial frequencies (main effect of frequency: slope estimate = -0.9 ± 0.18, $z = -5.3$, $p < 0.01$).

Discussion

Here, we demonstrate that fish in shoals with cadmium-treated fish can be affected by pollutants without direct exposure to the heavy metal. These results suggest that the effect of toxicological agents can be hidden in certain social contexts but revealed in others. Remarkably, comparing the pair and group responses, we found the effect of the pollutant was hidden in pairs and revealed in the larger groups. A few polluted individuals can have profound effects on social groups. Zebrafish groups containing a few cadmium-treated fish remained in the vicinity of a novel object more than did groups with water-treated fish. Groups with a few cadmium-treated fish showed differences in their social dynamics in comparison to groups with water-treated fish. These effects can provide greater insight into the effects on pollutants, especially those not captured with traditional toxicology studies where single individuals are often the subject of manipulation and analysis.

In the present study, only a fraction of the individuals were exposed to a pollutant, but this resulted in a change of behavior in the majority, sometimes disproportionately so (e.g., Fig. 3a). The presence of the cadmium-treated fish altered the social dynamics (e.g., advances, chases, mouth contacts) with groups with contaminated fish being more socially investigative or aggressive than controls. It was unclear how the presence of the cadmium influenced the intricacies of the social dynamics. This is the case because the observer did not know if the cadmium-treated fish were initiating more of the social interactions, or were more often the targets of social investigation, because treated fish were not obviously identifiable in larger groups. Further studies are needed to describe details of the social dynamics, to better understand the specific mechanisms by which a few contaminated individuals influence the responses of the majority. For example, did the cadmium-treated fish become more bold and aggressive in the presence of group members, thereby coercing their group members and directing the group responses, or did the other members display heightened levels of aggression and direct more social-investigative behavior towards the contaminated fish? Social network analyses may be good tools for understanding the social architecture of animal groups (Croft, Madden, Franks, & James, 2011; Modlmeier et al., 2014). The presence of impaired individuals may also dilute the risk for the healthy members, perhaps, emboldening uncontaminated individuals (the majority) them to engage in more risky behavior or approaching the novel stimulus. For example, male guppies will engage in vigorous courtship behavior in the presence of a predator when there are more vigilant females inspecting the predator (Magurran & Nowak, 1991), and female mosquitofish forage more efficiently when harassment by solitary males is reduced by aggregating with more females (Pilastro, Benetton, & Bisazza, 2003). Instead, exposure to cadmium may have altered perception of treated fish, and consequently affected social interactions. For example, exposure to trace metals can alter dyad interactions and dominance hierarchies, because exposed individuals are less aggressive during contests, assume a subordinate role (Sloman, 2007), and hierarchies form faster in contaminated groups than unexposed groups (Sloman et al., 2003).

We found pollutants through direct action on a few treated fish can influence the responses and social interactions of the larger uncontaminated group. The effects of pollutants are not restricted to those directly exposed, but can act through extended and indirect effects. In the present study, the pollutant likely affected un-treated fish through influencing the interactions of contaminated and water-treated fish. In other species, pollutants have had extended and indirect effects on parental behavior (Forsatkar et al., 2014), agonistic behavior (Vuori, 1994), predator-prey interactions (Weis, Smith, Zhou, Santiago-Bass, & Weis, 2001) and other ecological outcomes (Boyd, 2010). For example, contaminated individuals pursue different behavioral tactics during encounters depending on their role, intruder or resident, thereby influencing the duration and intensity of the agnostic interaction (Vuori, 1994). The present work is linked with the keystone species concept, where some individuals have a disproportionate effect on the functioning of the community, which has been extended to encompass diverse interactions at multiple levels of organization, from individual to ecosystem (Modlmeier et al., 2014; Mouquet, Gravel, Massol, & Calcagno, 2013). The idea that the pollutant had an extended effect on the responses untreated fish is related to other observed indirect effects of pollutants on ecosystem functioning, or trophic cascades through consumer-resource interactions (Fleeger et al., 2003; Frank, Petrie, Choi, & Leggett, 2005). For instance, some sediment burrowing insects avoid burrowing in contaminated sediment, and consequently increase their exposure to sediment biting-insects thereby altering predator-prey dynamics (Hinkle-Conn, Fleeger, Gregg, & Carman, 1998). Here, we found that cadmium can also have an extended impact by influencing the investigative behavior of a larger social group of mostly-untreated individuals.

In the present study, adult zebrafish responded to optomotor assay with the response increasing in robustness at lower spatial frequencies. The responsive adult zebrafish moved in the same direction as the moving stimulus typical of a positive optomotor response, which contrasts with other reports that argue zebrafish switch from showing a positive to a negative optomotor response early in life (Bak-Coleman, Smith, & Coombs, 2015). Cadmium-treated zebrafish showed a somewhat attenuated optomotor response in comparison to uncontaminated fish. Thus, the pollutant likely affected untreated fish through influencing the interactions of contaminated and water-treated fish, perhaps through altering the visual system. In other species, environmental stimuli have affected social interactions through influencing sensory systems. For example, animals that normally aggregate in coordinated clumps, disperse when light levels are low (Kowalko et al., 2013), or is their ability to see impaired by debris (Borner et al., 2015). Animals learn how to interact with some stimuli via social interactions (Zentall & Galef Jr, 2013), but those experiences may be disrupted by sensory impairments (Wong & Candolin, 2015), and thus interfere with an organism's ability to mount an appropriate behavioral response. For example, rats rendered anosmic fail to learn about novel food sources from demonstrator rats (Galef & Wigmore, 1983). Here, we found that cadmium may have possibly affected the visual system of the zebrafish, and consequently affected social interactions and group responses. We found that contaminated fish in larger uncontaminated groups influenced the interaction of the group with novel stimuli. Perhaps, the presence of the cadmium treated fish influenced the tendency of the larger social group to investigate objects in their environment. In other species, pollutant exposure has been shown to influence exploratory behavior and personality traits. For example, the offspring of gestating-mother mice that consumed a pesticide had an increased propensity to explore novel environments (Palanza, Morellini, Parmigiani, & vom Saal, 2002) and deficient diets have been shown to affect boldness, with birds having micronutrient deficiencies being less bold (Noguera, Metcalfe, Surai, & Monaghan, 2015). The pollutant may have also interfered with the behavioral repertoire of the fish thereby inducing an abnormal behavioral interaction with the novel stimulus. In other species pollutants have been shown to induce aberrant behaviors in contaminated individuals (reviewed in Zala & Penn, 2004). Thus, there are several avenues by which cadmium exposure may have influenced the response of the zebrafish to the novel stimulus, and future studies to should uncover the mechanisms of action.

Our results add to the growing literature that shows a few individuals can radically alter the group responses. Here, we integrate aspects of anthropogenic change and social roles to highlight the profound effect of a few contaminated individuals can have on social dynamics and group responses. We found that a few contaminated individuals influenced the response of the larger group to novel stimuli and increased social investigative behavior among group members, which was possibly driven by differences in the visual system. We emphasize sensory and social mechanisms, but other studies should rule out other nonsocial mechanisms that may have led to differences between cadmium- and water-treated fish. Understanding the impact of pollution on social dynamics is critical to understanding how future anthropogenic change may influence animal behavior.

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Chapter 7:

Concluding Remarks

Defining Physical and Social Features

Animal groups such as bird flocks (Carere et al., 2009), fish schools (Hemelrijk & Hildenbrandt, 2012), and insect swarms (Eriksson et al., 2010) present opportunities to link individual behavior with dynamic group-level properties (Becco et al., 2006). Understanding these interactions has allowed us to move past reductionistic techniques without losing a central question in many biological disciplines, which is understanding complexity. That is, how do biological phenomena arise from the action and interactions of their components, or in the case of my dissertation how does group behavior arise from the interaction of individuals, which is often modified by the environment.

The components of the environment that influence group behavior are classically divided into physical and social factors. Of these two factors the physical environment is easier to define. Physical features are sometimes described as an inanimate, abiotic stimuli, whereas social features are animate, biotic stimuli (Soberon & Peterson, 2005). In other cases, the social stimuli are characterized as a special feature of the environment, and defined by exclusion, or as the elements that are left over or cannot be characterized as physical (Alberts, 2012). Still others may prescribe a more quantitative framework where the relative importance of particular elements in the environment can be quantified using mathematical formulas or ratios to determine the relative importance of the physical and social environment (Capello, Soria, Cotel, Deneubourg, & Dagorn, 2011; Lynch & Walsh, 1998; Soberon & Peterson, 2005).

Experimental impact of Physical and Social Features

In my dissertation, I defined physical features as abiotic stimuli and social features as a biotic stimuli. I examined the influence of the physical and social environment on behavior. One of the key environmental features I examined was density, or the number of animals per utilized volume. It is important to note that although it may appear there is a difference between the calculation of density between the mouse and zebrafish experiments, the measures are equivalent. This is the case because zebrafish fully utilized the available space in their enclosures, which are their tanks. Mouse pups were unable to fully utilize the available space, which necessitated quantifying the dimensions of the utilized space for each group.

In some studies I found a clear effect of the physical environment. For example, I found that found, in Chapter 2, Environmental Structure and the Expression of Group and Individual Movements in Young Mice, that temperature had a more pronounced effect on the activity of younger than on that of older pups. In Chapter 3, Environmental Structure and Energetic Consequences in Young Mice, I found that environments that structure the group's geometry can have significant metabolic consequences for the group. In other studies I found a clear effect of the social environment. For example, in Chapter 6, A Few Cadmium-Treated Fish Affect Group Dynamics and Social Behavior in Zebrafish, I found that contaminated fish can affect the social dynamics and group responses of the larger uncontaminated groups, but not in pairs. In the majority of studies, I found that the physical features interacted with social features to affect behavior. For example, in Chapter 4, Development of Behavioral Responses to Thermal Challenges in the Context of Climate Change, I found the age of the mice influenced their response to cold and warmth. The appearance of responses to combat cold challenges has an earlier onset than responses to hot temperatures. In small groups of adult animals, I found that social and physical features influenced individual and group behavior. In Chapter 5, Density and Group Size Influence Shoal Cohesion, but Not Coordination in Zebrafish (*Danio rerio*), I found that the amount of available space, or density influenced the spacing patterns, but not the polarization of the group.

Reexamining the Distinction between Physical and Social Features

The interrelated influence of different physical and social features on behavior leads to a deeper reflection on what distinguishes these classes of environmental stimuli. Is the difference based on: 1) animation, or movement; 2) experience with these features; or 3) individual characteristics and group composition. My findings suggest that each of these aspects maybe important in distinguishing social from the physical environment. For example, the movements of the youngest pups were low in all contexts. The pups changed their responses with different physical features (temperature), but not social features (presence of littermates, density). In contrast, pups in older age groups that moved more, varied their responses with an interrelated social and physical feature, or density. Groups with cadmium-treated fish responded differently to physical features than shoals with only water-treated fish. A unifying distinction between individual and group responses to physical and social features maybe responsiveness, or the level of activity that was elicited by these two stimuli. Younger mouse pups are less responsive (i.e., moved less) than older mouse pups to nearly all stimuli, and cadmium-treated fish are less responsive to visual stimuli in their environment than water-treated fish, as measured by the optomotor response assay.

The responsiveness of individuals can also be modified in several ways, including exposure to contaminants (e.g., cadmium), and by varying their environmental (e.g., nest structure). When I changed how litters of mouse pups could respond to their environment, by restricting their movements to two-dimensions (2-D) or permitting movements in three-dimensions (3-D), it altered their activity levels and had consequences for their metabolics.

Testing Predictions

The ability of mouse pups to respond to the environment might be altered pharmacologically by dosing the animals with curare, which would immobilize them or decrease their responsiveness. When the mouse pups are immobilized and tested in a flat and concave enclosure, if responsiveness is influencing the difference in physiology, then I expect that the huddles will show similar metabolics independent of enclosure structure. If surface area:volume ratio is having the largest effect on metabolics, then I expect that the huddles in the concave enclosure will have a lower metabolic rate than the huddles in the flat enclosure.

Group and Individual Measures Provide Richer, and Sometimes Different Perspectives

Our study also highlights the importance of using both individual and group measures to characterize animal groups. In Chapter 5, Density and Group Size Influence

Shoal Cohesion, but Not Coordination in Zebrafish (*Danio rerio*), our measures of nearest neighbor distance have suggested that the structure of zebrafish groups depend only on the available space, and not group size. When we also considered measures of the group (e.g., shoal diameter), we then saw the effects of group size. We conclude that a better and more accurate characterization of infant rodent development and zebrafish social behavior involves both individual and group measures, as the individual and whole are linked dynamically.

In the literature, individual measures are used to characterize group phenomena, with the assumption that individual measures predict group measures. Our study shows that relationship between individual and group measures are variable. For example, in Chapter 5, Elevated Ambient Temperatures: Altering Developmental Pathways and Phenotypes, the conclusions drawn from individual- and group-level behavioral measures were similar. Our measures of individual activity suggested that young pups respond similarly to cold and warm temperatures, whereas older mouse pups show differences in their response to cold and warm temperatures. When we examined a group measure of their responses to cold and warm temperatures, we found a similar pattern – older, but not younger ages showed regulatory responses to cold and warm temperatures. In Chapter 5, Density and Group Size Influence Shoal Cohesion, but Not Coordination in Zebrafish (*Danio rerio*), we found that individual and group measures of shoal cohesion showed that different factors were important. When using individual behavioral measures of cohesion we found that density, but not group size were important, whereas when we use group behavioral measures we found that group size and density were important. The variable relationships between individual and group measures suggests that prior to conclusions being drawn about group dynamics from solely individual measures, the relationship between individual and group measures should be established.

Future Outlook and Recommendations

With a rapidly changing world, small changes in the environment may have huge impacts on the development, behavior, and physiology of groups. Identifying the simple features of the environment that most affect physiology and behavior may help determine which environmental factors are necessary and sufficient for ensuring the expression of typical behavioral repertoires and those that may lead to altered responses. Through identifying the elements of the environment that have the largest effect on physiology and behavior we maybe be able to predict responses to environmental change or develop intervention plans so that functioning of animal groups are minimally impacted. In addition, understanding when and under what conditions individual behavioral measures predict the responses of groups, will help us to better understand and characterize group phenomena. Moreover, identifying the relationship between individual and group level processes will permit us to better understand multi-level organizations. Further, studying the contributions of the physical and social environment to the expression of behavior mirror an age-old dichotomy, the nature-nurture debate, or the relative contributions of the genes and the environment to the expression of a phenotype. Much like the nature-nurture debate is a false dichotomy the social-physical environment is also a false dichotomy. The social and physical environment are interrelated, and relative – dependent on key characteristics of the individuals, perhaps, on their responsiveness.

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Eriksson, A., Jacobi, M. N., Nyström, J., & Tunstrøm, K. (2010). Determining interaction rules in animal swarms. *Behavioral Ecology*, *21*(5), 1106–1111. http://doi.org/10.1093/beheco/arq118

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EDUCATION

September 2016
Ph.D Psychological and Brain Sciences & Evolution Ecology and Behavior, minor Cognitive Science
Indiana University, Bloomington, IN, USA
Committee: Emília P. Martins, Jeffrey R. Alberts, Laura Hurley, Meredith West

2011
Poynter Institute Teaching Research Ethics Certificate
Indiana University, Bloomington, IN, USA
Director: Kenneth Pimple

2009-2010
Alternative Teacher Certification Program
Prairie View A&M University, Prairie View, TX, USA
Secondary Education, General Science Teacher

2005-2009
Bachelor's of Science (B.S.) Animal Behavior, minor Spanish
Southwestern University, Georgetown, TX, USA
Thesis: *Stay in School or Drop Out*, *What is a Fish to Do?: Multiple Selection Pressures on Teleost Fish.*
Adviser: Jesse E. Purdy

2003-2005
Texas Academy of Mathematics and Sciences (TAMS)
University of North Texas, Denton, TX, USA
High School Diploma

POSITIONS

2016-2019	National Science Foundation Postdoctoral Fellow
2010-2015	National Science Foundation Predoctoral Fellow
2010-2015	National Science Foundation IGERT Fellow in the Dynamics of Brain-Body-Environment Systems
2009-2010	Science Teacher, Houston Independent School District
2007-2008	UNCF·MERCK Science Initiative Scholar
2005-2009	Dixon Scholar, Southwestern University

PUBLICATIONS

(† contributed equally; undergraduate co-authored works marked with+)

PEER-REVIEWED PAPERS

1. Dzieweczynski T.L., +Bessler A.M., **Shelton D.S.,** and Rowland W.J., (2006). Effect of a Dummy Audience on Male–Male Interactions in Siamese fighting fish, *Betta splendens*. *Ethology*, 112(2), 127-136.
2. *†*Dillon G.M, *†***Shelton D.**, McKinney A.P., Caniga M., Marcus J.N., Ferguson M.T., Kornecook T.J., and Dodart, J.C., (2009). Prefrontal cortex lesions and scopolamine impair attention performance of C57BL/6 mice in a novel 2-choice visual discrimination task, *Behavioral Brain Research*, 204, 67-76.
3. **Shelton D.S**., +Price B.C., +Ocasio K.M., and Martins E.P. (2015) Social and physical environment influences shoal cohesion, but not coordination in zebrafish (*Danio rerio*). *Journal of Comparative Psychology, 129*, 72-77.
4. **Shelton D.S.**, Atagi, E., Keene, J. R., and Ross, T (2015). Netlogo Boomshakalaka model. Indiana University, Bloomington, IN. http://ccl.northwestern.edu/netlogo/models/community/Boomshakalaka_shelton-atagi-keene-ross
5. Suriyampola, P.S., **Shelton, D.S.,** Shukla, R., Roy, T., Bhat, A. and Martins, E.P. (2016). Zebrafish social behavior in the wild. *Zebrafish*, 13(1), 1-8.
6. **Shelton D.S.** and Alberts J.R. Development of behavioral responses to thermal challenges in the context of climate change. (invited special issue in Animal Cognition, submitted).
7. Suriyampola, P. S., Sykes, D. J., Khemka, A., **Shelton, D. S.**, Bhat, A., Martins, E P. Social plasticity in complex environments: effects of water flow on social behavior of zebrafish. *Behavioral Ecology and Sociobiology* (accepted)
8. **Shelton D.S.**, +Austin Z.M., +Khemka A., and Martins, E.P. Hidden in pairs, revealed in groups: deficient individuals influence zebrafish group behavior. (in prep).
9. **Shelton D.S.** and Alberts, J.R., Nest structure influences the development of group regulatory behavior in mice. (in prep).
10. +Myers M.A.,+Kim N., +Cahela, J., **Shelton D.S.**, and Alberts J.R. Temperature-dependent spatial and social centrality. (in prep).

CONFERENCE PROCEEDINGS

11. **Shelton, D.S**. and Alberts, J.R. (2013). Ontogenesis of group regulatory behavior in mouse litters. *Integrative and Comparative Biology,* 53:E370.
12. +Price B.C., **Shelton, D.S.**, and Martins, E.P. (2013). Group-size-dependent cohesion of zebrafish (*Danio rerio*) in the presence of disturbances. *Integrative and Comparative Biology* 53:E353.

BOOK CHAPTERS

13. **Shelton D.S.** (2014). Locating and securing funding. In: Smith. M.J.T., Browne, M.M, Johnson, K, & Peck, W. (Ed.s), GPS for Graduate School: Students Share their Stories. ISBN:15573536470
14. **Shelton D.S.** and Martins, E.P. Behavioral variation, adaptation, and evolution. In: Call, J., Burkhardt, G., Pepperberg, I., Snowdon C., and Zentall T. (Ed.s), APA Handbook of Comparative Psychology. (in press)

OTHER WORKS

15. **Shelton D.S.** (2013). Shared goals. *Bulletin of the Museum of Zoology,* 3(1), 6.
16. **Shelton D.S**. (2015). CISAB Ambassadors in the Student Conference Exchange Program. *Center for the Integrative Study of Animal Behavior Bulletin, 17(1)*, 11.
17. **Shelton, D.S.** (2016). A review of primer effects by murine pheromone signaling: pheromonal influences on reproductive conditions. *Journal of Ethology*, 1–1.

HONORS/FELLOWSHIPS

2016	Diversity Dissertation Year Fellowship, Indiana University – I declined
2015	Heller Fellowship, Psychological and Brain Sciences, Indiana University
2012	ExxonMobil Bernard Harris Summer Science Camp Alumni Award
2010-2015	Indiana University Graduate Scholars Fellowship – I declined
2009	Southwestern University Animal Behavior Student of the Year
2005-2009	Paideia Scholar

GRANTS

EXTERNAL AWARDS

2015	NSF Travel Award - $700
2013	Society for Integrative and Comparative Biology Broadening Participation- $500
2013	Charlotte Mangum Housing Award
2012	NIH (NICHD) and Sackler Institute - $450
2009	Project Grad Innovative Grant - $1,000
2008	Animal Behavior Society Charles H. Turner Travel Award
2007	UNCF·MERCK Departmental Grant - $10,000
2005-2006	Animal Behavior Society Charles H. Turner Travel Award

FELLOWSHIP SUPPLEMENTS

2015	NSF IGERT Dissertation Improvement Grant - $4758
2015	NSF IGERT Travel Award - $2930
2015	NSF IGERT Travel Award - $2425
2014	NSF IGERT Travel Award - $1629
2013	NSF IGERT Travel Award - $2953
2012	NSF IGERT Travel Award - $580

INTERNAL AWARDS (IU – INDIANA UNIVERSITY, SU – SOUTHWESTERN UNIVERSITY)

2015	Center for Integrative Study for Animal Behavior Travel Award, IU - $400
2015	Provost Travel Award, IU - $800
2013	Provost Travel Award, IU - $700
2013	Biology Department Travel Award, IU - $250
2012	Indiana University Women in Science Program, IU - $550
2012	Animal Behavior Lab Travel Award, IU - $400
2012	Center for Integrative Study for Animal Behavior Travel Award, IU - $360

2011	Animal Behavior Laboratory Travel Grant, IU - $500
2010	Animal Behavior Laboratory Travel Grant, IU - $400
2005	Paideia Scholar Research Grant, SU- $1,000.
2006-2007	King Creativity Fund, SU - $2,140

PROFESSIONAL AFFLIATIONS

- Society for Integrative and Comparative Biology 2011
- International Society for Developmental Psychobiology 2010-2013
- Center for the Integrative Study of Animal Behavior 2010-present
- Alliance for Graduate Education and the Professoriate (AGEP)- Midwest Cross Roads Alliance 2010-Present
- Southwestern Psychological Association (SWPA) 2007-2009
- Southwestern Comparative Psychological Association (SCPA) 2007-2009
- Animal Behavior Society (ABS) 2005-present

TEACHING EXPERIENCE

2013	Guest Lecture, Social Behavior: Group Structure and Cooperation, in undergraduate/graduate Animal Behavior course, April 9, 2013. ~50 students. Indiana University-Bloomington
2011	Lecturer, Indiana University-Bloomington *BioEthics*: Center for Integrative Study of Behavior Mentor: Emilia Martins, Ph.D.
2009-2010	Science Teacher at Recognized High School in Houston Independent School District *Integrated Physics and Chemistry*: Science Department Supervisor: Carolyn Brown, M.A.

ADDITIONAL EXPERIENCE

2009	**University of Ghana: Oceanography and Fisheries** *Legon, Ghana (West Africa*) Research Assistant –Academic School Term Fisheries research ● aquatic sampling ● coastal surveying
2008	**INBIO Parque** *Heredia, Costa Rica (Central America)* Research Assistant –Academic School Term Managed butterfly colony ● Herpetological husbandry
2007, 2008	**Merck Research Laboratories** *Boston, MA, USA* Research Associate- Summer Intern Drug discovery ● Operant conditioning ● Immunohistochemistry ● Alzheimer's models ● High throughput behavioral phenotyping

2005-2008 **Aquatic Animal Laboratory- Southwestern University**
Georgetown, TX, USA
Research Assistant –Academic School Term
Classical conditioning ● Cephalopod husbandry ● Animatronics ● Pharmacology ● Saltwater system management

2005-2006 **Alliance of Graduate Education and the Professoriate (AGEP)**
Rice University, Houston, TX, USA
Research Associate- Summer Intern
Carnivore enrichment ● Stereotypical behavior in captive environment ● Public outreach
2004
NSF REU- Center for Integrative Study of Animal Behavior (C.I.S.A.B.)
Indiana University, Bloomington, IN, USA
Research Associate-Summer Intern
Audience effect ● tropical fish ● video-play back

MENTORING

Mentored 18 undergraduates and 4 high school students in full-time summer research programs or multi-week academic year internships. The students were from diverse backgrounds, 15 women, 12 people of color, 2 first generation college students, and one veteran.

UNDERGRADUATES

2015-2016	Mary Myers- current research assistant
2015-2016	Nahrie Kim- current research assistant
2015	Xenia Davis- NIH MARC Scholar, won best poster presentation at Annual Biomedical Conference for Minority Students for her summer research project through the NSF REU at Indiana University
2015	Samantha Schwindel- research assistant, Hutton Honors Scholar, Indiana University
2015	Stephanie McQueen- research assistant, Indiana University
2014-2015	Anuj Khemka- research assistant, Indiana University
2014	Zoe Austin- NSF REU; OK Louis Stokes Alliance for minority participation scholar, graduate student, Indiana University
2014	Jason Cahela- NIH MARC scholar
2014	Meital Shacaf- IFLE scholar, Indiana University
2013-2014	Erik Wegner-Clemens- veterinary student
2014	Karen Ocasio- NSF REU, Jr. High School Biology teacher, applying to graduate school in Fall 2015
2012-2013	Devin Jacobs- conference presentation, 1st place poster at Animal Behavior Conference

2012-2013	Brittany Price- NSF REU- conference presentation, Broadening Participation Travel Award, Charlotte Mangum Award, CISAB travel award, honor’s thesis at North Carolina State University, post-baccalaureate at Wright State University
2012	Patrick Sweeny- military veteran
2012	Gray Stephenson- summer undergraduate research assistant, biology major, founder of a green start-up company
2012	Dakota Scheu- applied for J.D., Environmental Law, 2nd Place Poster in Animal Behavior Seminar
2011	Lauren Green- Professional Experience in Radio Communication
2011	Ticia Watson- NSF REU, Master’s in Public Health Florida International University, Ali-Zaidi Award for Academic Excellence

HIGH SCHOOL STUDENTS

2013	Roger Morris- Bloomington North high school research assistant, pursuing economics at Indiana University
2013	Andy Morris- Bloomington North high school research assistant
2013	Hannah Fox-Teague- Holland Summer Scholar, high school research assistant
2011-2012	Moonju Lee- high school student, pursuing B.S. in Neuroscience at Rice University

JR. HIGH SCHOOL STUDENTS

2015	Dolores Shelton – Brenham Jr. High summer research assistant

SERVICE

2011-Present	Volunteer with Bethel Homework Help, contributing to the efforts that were honored by the 2014 *Be More Engaged Award* from the City of Bloomington Community and Family Resources Department for encouraging literacy.
2011-2013	Indiana University, Mechanisms of Behavior (Graduate Student Representative)
2010-2016	Student Member, CISAB Animal Behavior conference planning committee. Chair of program committee (2013-2014), peer reviewing and compiling abstracts.
2007-2008	Southwestern University Animal Behavior Society (Student Representative)
2005-2008	Empowering Blacks and Others to Never Yield (E.B.O.N.Y.) - Dollas Committee head– 2005-2006; Secretary 2006-2007; Multicultural Council Representative -2007-2008
2006-2008	Theatre for Social Justice (T.S.J.)- 2006-2008
	Students Helping Admissions Recruit Prospects (SHARP), Southwestern University

PRESS

1. National AGEP News - GPS for Graduate School: Students Share their Stories (1/26/2015)
   http://blogs.mtu.edu/agep/2015/01/26/gps-for-graduate-school-students-share-their-stories/
2. CISAB - segment in Indiana University REU program in Animal Behavior (7/17/2013)
   http://www.indiana.edu/~animal/reu/REU.php
3. Southwestern University Newsroom - Spanish Department Alumni Stories (9/24/2013)
   http://www.southwestern.edu/live/news/8504-delia-shelton-class-of-2009
4. Southwestern University - King Creativity Scholars: I am a real shark! (4/19/2007)
   http://www.southwestern.edu/live/news/2510-i-am-a-real-shark/academics/kcf/news.php

RELEVANT SKILLS

Laboratory: Fish, Exotic and Agriculture animal husbandry, video play-back techniques, animal trapping, animatronics, animal tracking software, Med-associates software, electrical wiring, western blot techniques, small and large animal surgeries, fluvial tank construction and design.

Computer: PC, Mac, Windows, Microsoft Word, PowerPoint, Excel, SPSS, SAS, Sigma Plot, Photoshop, iMovie, Event Recorder Software, JMP 6.0, Odyssey (2.1), Invitrogen iBlot, & hand held GPS, Python, Netlogo, Matlab, R, CowLog, Image J, Zotero, IR Flash, Ethovision.

Field Research: Ghana, Costa Rica, India, PADI Diver certified

Languages: Spanish (intermediate proficiency), Twi & Ewe (beginning proficiency)

PRESENTATIONS

(high school and undergraduate co-authored works marked with+)

INVITED TALKS

1. **Shelton, D.S.** (2016, April). Environmental features influence the behavior of zebrafish shoals. Talk presented in Fisheries and Wildlife Departmental Seminar, Oregon State University, Corvallis, OR, USA.
2. **Shelton, D.S.** (2015, September). Collective Behavior in Huddles and Puddles: simple environmental mechanisms shape complex behavior in two model organisms. Talk presented at Psychology seminar, University of Tennessee at Knoxville, TN, USA.

3. **Shelton, D.S.** (2015, September). A tale of scales and tails: environmental mechanisms and social roles in mice and fish. Talk presented at Biology seminar University of Windsor, Windsor, Ontario, Canada.
4. **Shelton, D.S.** (2015, July). Zebrafish behavior shaped by the natural world. University of Exeter, Exeter, Southwest England, United Kingdom.
5. **Shelton, D.S.** (2015, June). Splash into environmental mechanisms of zebrafish behavior. Carleton University, Ottawa, Ontario, Canada.
6. **Shelton, D.S.,** +Austin, Z.M., +Khemka, A., +Shachaf, M., and Martins, E.P. (2015, February). Individuals influence the responses of zebrafish shoals. Talk presented at the Keck Center for Behavioral Biology, North Carolina State University, Rayleigh, NC, USA.
7. **Shelton. D.S.** (2014, October) The importance of social relationships for zebrafish and graduate school. Talk presented at University of Peradeniya, Kandy, Sri Lanka.
8. **Shelton D.S.**, and Martins E.P. (2014, August). Martins lab ethoinformatics. Integrated Behavior Ontology for the Behavioral Science Community in Two Workshops-August 7-8 2014, Princeton, N.J., USA. Organizers: Anne Clark, Susan Margulis, Peter Midford, and Cynthia Parr.
9. **Shelton D.S.**, Ghazinejad, A., and, Ekbia, H. (2011, April). UmWeltian empirical studies and developmental situated-embodied agents: Computational models of group behavior. Talk presented at Epistemology of Modeling and Simulation, Pittsburgh, PA, USA.

TALKS

10. Suriyampola P.S., +Iruri-Tucker, A.A., +Enriques, A., **Shelton D.S.**, and Martins E.P. (2016, August). Does group size improve synchronized motion in social groups? Talk presented at Animal Behavior Society Conference, Columbia, MO, USA.
11. **Shelton D.S.**, +Austin Z.M., +Khemka A., and Martins E.P. (2016, February). Cadmium-exposed individuals direct group behavior. Association for School for Public and Environmental Affairs Student Conference, Indiana University, Bloomington, IN, USA.
12. **Shelton D.S.**, +Austin Z.M., +Khemka A., and Martins E.P. (2015, August). Hidden in Pairs, Revealed in Shoals: indirect effects of cadmium on zebrafish group behavior. Talk included in Symposium organized by Thomas Bugnyar, Andrea Griffin, Sabine Tebbich at the International Ethological Conference, Cairns, New South Wales, Australia.
13. Suriyampola P.S., **Shelton D.S.**, Sykes D.J., Bhat A., and Martins E.P. (2015, August). Out of the wild: Social behavior of wild and domesticated zebrafish (*Danio rerio*) in response to habitat alteration. Talk presented at International Ethological Conference, Cairns, New South Wales, Australia.
14. +Davis, X. +Kim, N., **Shelton, D.S.,** and Alberts, J.R. (2015, July). Individual variation in thermal physiology and maternal care. Talk presented at Center for Integrative Study of Animal Behavior, Indiana University, Bloomington, IN, USA.

15. **Shelton D.S.**, +Austin Z.M., +Khemka A., and Martins E.P. (2015, June). Hidden in Pairs, Revealed in Groups: cadmium influences group responses in zebrafish. Talk presented at Animal Behavior Society Conference, Anchorage, AK, USA.
16. Suriyampola P.S., **Shelton D.S.**, Sykes D.J., Bhat A., and Martins E.P. (2015, June). A tale of two strains: Social behavior of wild and domesticated zebrafish (*Danio rerio*) in response to habitat alteration. Talk presented at Animal Behavior Society Conference, Anchorage, AK, USA.
17. +Austin, Z.M. +Khemka, A., +Shachaf, M., **Shelton, D.S**., and Martins, E.P. (2015, April). Cadmium affects the responsiveness of zebrafish shoals. Talk presented at National Conference on Undergraduate Research Conference, Eastern Washington University, Cheny, WA, USA.
18. Suriyampola, P.S., **Shelton, D.S.**, Sykes, D.J., and Martins, E.P. (2015, March). Water flow influences the group behavior of zebrafish. Talk presented at Midwest Ecology and Evolution Conference, Bloomington, IN, USA.
19. **Shelton, D.S.** (2014, November). Social roles in small animal groups. Talk presented at Biology Club Undergraduate Research Night, Indiana University, Bloomington, IN, USA.
20. **Shelton D.S.**, +Cahela J.P., and Alberts J.R. (2014, August). Physiological consequences of 2-D and 3-D huddles in infant mice. Talk presented at Animal Behavior Society conference, Princeton University, Princeton, NJ, USA.
21. +Austin, Z.M. Khemka, A., Shachaf, M., **Shelton, D.S.**, and Martins, E.P. (2014, July). Cadmium alters the social dynamics of zebrafish. Talk presented at Center for Integrative Study of Animal Behavior, Indiana University, Bloomington, IN, USA.
22. +Cahela, J.P., **Shelton, D.S.**, and Alberts (2014, July). The effects of thermal physiology on the location of individuals within a group. Talk presented at Center for Integrative Study of Animal Behavior, Indiana University, Bloomington, IN, USA.
23. **Shelton D.S.**, +Price B.C., +Jacobs D., +Wegner-Clemens E., Alberts J.R., and Martins E.P. (2013, March). Collective behavior in model organisms. Talk presented at University of Malaya and Universiti Teknologie Malaysia-Shah Alam, Kuala Lumpur, Malaysia.
24. **Shelton, D.S** (2014, April). Geometry of collective behavior in huddles and puddles. Talk presented at seminar series in Evolution, Ecology and Biology, Indiana University, Bloomington, IN, USA.
25. +Price, B.C., **Shelton, D.S.**, and Martins, E.P. (2014, April). Larger shoals disrupt rheotaxis performance in zebrafish (*Danio rerio*). Talk presented at Thomas Jefferson Scholars seminar, North Carolina State University, Rayleigh, NC.
26. **Shelton, D.S.**, +Price, B.C., +Wegner-Clemens, E., and Martins, E.P. (2013, August). Geometry of collective detection in zebrafish. Talk presented at International Ethological Conference, New Castle, England, UK.
27. +Ocasio, K. M., **Shelton, D.S.**, P+rice, B.C., and Martins, E.P. (2013, July). Are two heads better than one?: differences between individual and group responses to water currents. Talk presented at Center for Integrative Study of Animal Behavior, Indiana University, Bloomington, IN, USA.

28. **Shelton, D.S.**, +Price, B.C., +Jacobs, D., +Wegner-Clemens, E., Alberts, J.R., and Martins, E.P. (2013, March). Collective behavior in model organisms. Talk presented at University of Malaya and Universiti Teknologie Malaysia-Shah Alam, Kuala Lumpur, Malaysia.
29. **Shelton D.S.** (2012, December). Research opportunities for undergraduates. Talk presented at Center for Integrative Study for Animal Behavior. Bloomington, IN, USA.
30. **Shelton, D.S**, +Price, B.C., and Martins, E.P. (2012, September). Rheotaxis and group cohesion is modulated by group size in zebrafish. Talk presented at Psychological and Brain Sciences Developmental Seminar.
31. **Shelton, D.S.**, +Price, B.C., and Martins, E.P. (2012, September). Group size influences group cohesion. Talk presented at Psychological and Brain Sciences, Bloomington, IN, USA.
32. +Price, B.C. **Shelton, D.S.**, and Martins, E.P. (2012, July). Group effects on rheotaxis in zebrafish (*Danio rerio*). Talk presented at Center for Integrative Study for Animal Behavior REU Showcase, Bloomington, IN, USA.
33. +**Shelton, D.S.** et al. (2008, March). Stay in school or drop out, what is a fish to do? Talk presented at Southwestern Comparative Psychological Association, Georgetown, TX, USA.
34. **Shelton D.S.**, Dillon, G.M., Dodart J-C. (2007, July). The development of an attention assay. Talk presented at Neuroscience Drug Discovery Meeting, Boston, MA, USA.
35. **Shelton D.S.**, Dzieweczynski T.L, and Rowland W. (2004, July). *Betta* be aggressive: using dummy fish to control audience behavior while examining audience effects in male Siamese fighting fish (*Betta splendens*). Talk presented at CISAB-REU Symposium, Indiana University, Bloomington, IN, USA.

POSTERS

36. +Kim, N. S., +Myers, M. A., +Cahela, J. P., **Shelton, D.S.**, and Alberts, J.R. (2016, April). The effects of body temperature, sex, and weight on spatial positioning. Poster presented at Animal Behavior Conference, Indiana University, Bloomington, IN, USA
37. +Myers, M. A., +Kim, N. S., +Cahela, J. P., **Shelton, D.S.**, and Alberts, J.R. (2016, April). Body temperature and social centrality in mouse pups. Poster presented at Animal Behavior Conference, Indiana University, Bloomington, IN, USA.
38. +Iruri-Tucker, A.A., Suriyampola, P.S., +Enriques, A., +Blackwell, G., **Shelton, D.S.**, Caceres, J., and Martins, E.P. (2016, April). The more the merrier: larger groups are better at responding to fluctuating environments. Poster presented at Animal Behavior Conference, Indiana University, Bloomington, IN, USA.
39. +Davis, X. +Kim, N., **Shelton, D.S.,** and Alberts, J.R. (2015, November). Individual variation in pup thermal physiology and maternal care. Poster presented at 2015 Annual Biomedical Research Conference for Minority Students (ABRCMS), Seattle, Washington, USA.
40. +Khemka, A., +Austin, Z., +Shachaf, M., **Shelton, D.S**., and Martins, E.P. (March, 2015). Hidden in Pairs, revealed in aggregation: cadmium influences

zebrafish behavior. Poster presented at Animal Behavior Conference, Indiana University, Bloomington, IN, USA.

41. **Shelton, D.S.**, +Price, B.C., +Ocasio, K.M., and Martins, E.P (2014, April). Spatial structure of zebrafish shoals is influenced more by the physical environment than the social environment. Poster presented at Animal Behavior Conference, Indiana University, Bloomington, IN, USA.
42. **Shelton D.S.** and Alberts J. R. (2013, January). Ontogenesis of group regulatory behavior in mouse litters. Poster session presented at Society for Integrative and Comparative Biology, San Francisco, C.A., USA.
43. +Fox-Teague, H., +Ocasio, K.M., **Shelton, D.S.**, and Martins, E.P. (2013, July). Testing the waters: My first research experience. Poster presented at Jim Holland Summer Science Research Program, Indiana University, Bloomington, IN, USA.
44. +Jacobs, D.S., **Shelton, D.S.,** and Alberts, J.R. (2013, April). Energetic consequences of 2D and 3D huddling in mice. Poster session presented at Animal Behavior Conference, Bloomington, IN, USA.
45. **Shelton, D.S.** and Alberts, J. R. (2013, January). Ontogenesis of group regulatory behavior in mouse litters. Poster session presented at Society for Integrative and Comparative Biology, San Francisco, C.A., USA.
46. +Price B.C. **Shelton D.S.**, and Martins E.P. (2013, January). Group size dependent cohesion of zebrafish (*Danio rerio*) in the Presence of Disturbances. Poster presented at Society for Integrative and Comparative Biology, San Francisco, CA, USA.
47. **Shelton D.S.** and Alberts J.R. (2012, October). Group behavior of mouse litters and the development of regulated pup flow. Poster presented at the international conference of International Society for Developmental Psychobiology, New Orleans, LA, USA.
48. **Shelton, D.S.**, and Alberts, J.R. (2012, April). Dynamics of temperature dependent pup flow in developing mice. Poster presented at IGERT Showcase 3rd Annual Symposium, Bloomington, IN, USA.
49. **Shelton, D.S.** and Alberts, J.R. (2011, November). Development of temperature dependent pup flow in mice. Poster presented at the international conference of International Society for Developmental Psychobiology, Washington, D.C., USA.
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52. **Shelton, D.S**. et al (2008, August). Under the Bottom and Over the Top: Defensive strategies of mummichogs (*Fundulus heterocilitus*). Poster presented at Animal Behavior Society (ABS) 45th Annual International Conference, Snowbird, Utah, USA.
53. +**Shelton D.S.** et al. (2008, July). Stay in School or drop out, What is a fish to do? Antipredatory strategies of teleost fish. Poster presented at UNCF~Merck Fellows Symposium, Philadelphia, PA, USA.

54. **Shelton D.S.** +Christian L., and +Decker H. (2007, February). I Am A Real Shark! Poster presented at Southwestern Undergraduate Creative Works and Research Symposium, Georgetown, TX
55. **Shelton D.S.**, +Myer D. et al., (2007, April). Landscaping the history of war and peace. Poster presented at Southwestern University Paideia Symposium, Georgetown, TX, USA.
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58. **Shelton, D.S.**, Mathews-Borak, C., and Meffert, L.M. (2006, July). The behavioral effect of several enrichment conditions in Indo-Chinese tigers (*Tigris corbetti*). Poster presented at Rice University AGEP, Houston, TX.
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