Physiology & Behavior 177 (2017) 155–160

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Environmental structure and energetic consequences in groups of young mice

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ARTICLE INFO

Keywords:
Nest structure
Environment
Infant mice
Energetics
Temperature
Oxygen consumption
Groups

ABSTRACT

Microenvironments can have considerable physiological consequences for the inhabitants by influencing the movements of individual members. The microenvironment 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.

1. Introduction

Microenvironments can affect competitive interactions, social behavior, and influence growth through complex interactions with physiology [1,2]. The impact of the microenvironment depends on the environmental features an animal selects, creates or is deposited (e.g., parents creating nest for offspring, or individuals huddling) [3,4]. Groups can create different geometries with their bodies, thus altering the microenvironment and permitting group members to take advantage of drafts [5,6], vortices [7,8] or zones of low pressure (e.g., cyclists drafting in a peloton; [9], and consequently expend less energy while aggregating [10]). 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 [11], and clumping rodents [12,13] and bees [14] 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 [10,15]. 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 [16], and fish in a diamond lattice formation have high hydrodynamic efficiency than do those in other formations [5,17]. 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 [10,18]. Similarly, termites alter the structure of their

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http://dx.doi.org/10.1016/j.physbeh.2017.04.020
Received 24 January 2017; Received in revised form 22 March 2017; Accepted 19 April 2017

mounds depending on climatic conditions, to regulate temperature within the mound [19,20]. To increase metabolic efficiency, animals may alter the geometry of the group. From a simple, physical perspective, 3-D structures with higher surface area to volume ratios should maintain stable temperatures for longer periods of time than do 2-D structures that have lower surface area to volume ratios [15,21]. Here, we ask if groups of young mice in environments that permit movements in 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 [22] and influence metabolite levels [23], which may lead animals to space themselves evenly to avoid aggressive neighbors [24] and have higher, stress-induced metabolic rates [25,26]. For example, fish under higher stocking densities generate more heat and have higher metabolic rates than do fish under lower density conditions [27]. 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 [28].

Mother mice construct nests that vary in configuration in which they give birth to 4–8 infants [29,30]. The shape of the nest can vary with environmental and intrinsic factors (e.g., climate: [10]; reproductive state: [31]; strain: [32]. 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 [32,33]. There is individual variation in the nests that mice construct [31] with protocols developed to characterize them [34]. 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 preweanlings, radically changing in behavior [35], morphology [36] and physiology [37–39], and taking up more space in the nest as they grow. At birth, altricial rodents lack insulative fur and subcutaneous fat [37]. Initially, autonomic control of vasodilation and vasoconstriction are limited, only becoming effective at combating cold challenges by the end of the second week of life [40]. The ability to shiver is gained by the middle of the first week [41] or even until the third week of life [42]. Huddles show group-level temperature-dependent movements by eight-days-old [39]. Initially, the predominate direction of the huddle is down in response to cool and warm temperatures. Only by postnatal day 8 do huddles show regulated directional movements, downwards in cool (22 °C), and upwards in the warm (36 °C) environment. Thus, the effects of the environment on their physiology may change as they age [39].

In the present study, we tested whether the energetics of young mouse huddles were influenced more by the environmental configuration, amount of available space or the geometry of the space. 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.

2. Method

2.1. 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. In our study, we used 6 pups per litter, a litter size that is typical of C57BL/6 dams [43]. We selected litters of dams that had at least six pups of similar size with an average variance in body mass of pups that composed the huddle of 0.09 ± 0.02 g. If dams had more than six pups per litter, we selected pups that had the most similar body weights. The pups had an average body weight of 4.37 ± 0.04 g. The litters were neither culled nor mixed.

Mothers gave birth and then reared pups in standard maternity tubs (27 cm length × 13 cm height × 17 cm width) with food and water available *ad libitum*. We maintained the vivarium on 14:10 h light/dark cycle (lights on at 0700 h) 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).

2.2. Procedure

2.2.1. 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 min. We maintained the temperature at 22 °C to control the experimental environment. In addition, a 22 °C ambient air temperature is within the range of ambient temperatures recommended for housing laboratory rodents by the US National Research Council [44]. A 22 °C ambient air temperature is the ambient temperature of the colony where these mice were housed. At the end of the trial, we took a thermograph of the huddle using an ICI 7320 P-Series infrared camera with 320 × 240 pixel resolution, 27 mk Thermal Sensitivity and IR Flash ver. 2.0 for Windows (Infrared Cameras Inc., Beaumont, TX). The camera was 70–75 cm above the surface of the tested enclosures. Following testing, pups were returned to their dams.

2.2.2. 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 associated with a temperature representing a visible area of the pups' bodies,

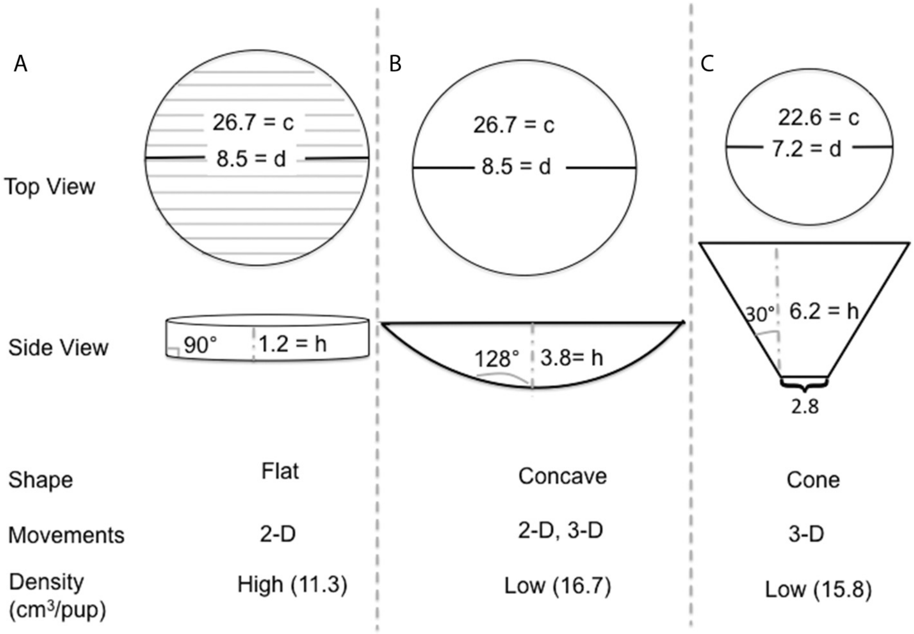

Fig. 1. Dimensions of the enclosures for each of the three treatment conditions: A) flat enclosure with high-density and short height (h) that restricts movements to 2-D space; B) a concave enclosure with the same circumference (c) and diameter (d) as the flat enclosure, but with a lower density and 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 thermographed mice from above.

excluding paws and tails. The paws and tails were excluded because they cool rapidly, and infant rodents regulate core areas [45,46]. The present measurement differs from previous methods that were focused on regional temperatures on pups' bodies [13,47]; here, we calculated an average surface temperature of the huddle as a single body.

2.2.3. 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 [48].

2.3. 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. We used similar selection criteria for litters and pups as described above. 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 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 (T_a) 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, thereby increasing the accuracy of the measurements.

2.3.1. 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 gram 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).

2.3.2. Statistical analysis

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 [48].

3. Results

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

We found that huddles in lower density concave (mean ± SE: 29.56 ± 0.18 °C) or conical (30.05 ± 0.13 °C) enclosures were > 2 °C warmer than were huddles in a higher-density, flat (27.54 ± 0.39 °C) structure. This led to a statistically significant difference in huddle temperature across the three treatment categories ($F_{2,\,21} = 14.20, p < 0.01$; 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

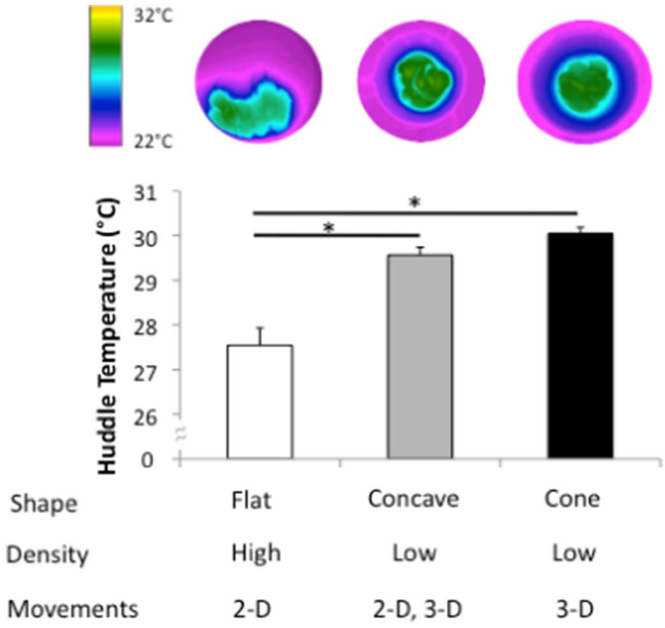

Fig. 2. Huddle temperature is more influenced by density than the shape of the nest. Huddles in concave and conical enclosures at high densities were warmer than groups in a flat structure at low densities. Groups had similar temperatures when density was equal. Representative thermographs for each treatment are above each column. A color gradient shows which temperatures correspond to each color with the coolest temperatures in magenta at 22 °C and the warmest colors in yellow at 32 °C. Cooler temperatures are in * indicates significant Tukey post hoc comparisons at $p < 0.05$. Error bars are one standard error. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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).

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

We found that huddles tested in flat (mean ± SE: 0.065 ± 0.005 ml O_2/g/min at STP) or concave (0.059 ± 0.005 ml O_2/g/min at STP) enclosures had similar metabolic rates. The amount of oxygen consumed per gram at standard temperature and pressure was around 0.06 ml/min ($t(14.28) = 0.72$, $p = 0.48$; Fig. 3).

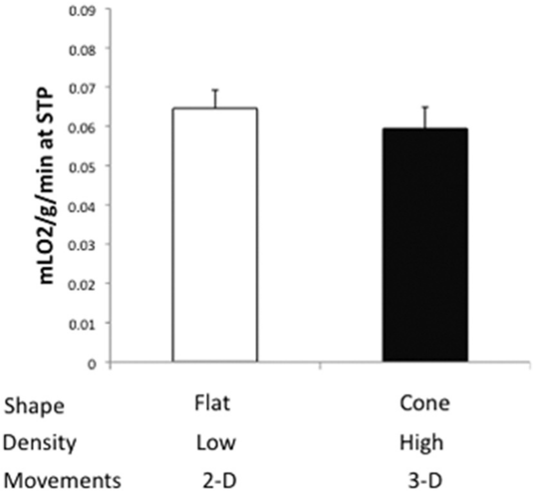

Fig. 3. Huddles had similar metabolic rates in enclosures with different shapes and densities. Error bars are one standard error.

4. 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 around a 7% energetic savings in comparison to a high-density enclosure. Huddles in low-density enclosures respire less for each degree of each heat they produce. The low-density structure led to a considerable energetic savings in comparison to the high-density condition.

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 [10,49]. For example, in crowded ants [50], energetic efficiency decreases with individuals having higher metabolic rates under higher density conditions than animals under lower density conditions perhaps due to the effects of crowding on stress [49]. 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 [11], and change their huddling intensity, and density of the huddle with temperature [51].

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 around 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% [52], and the amount of cold-exposed surface area decreases when more animals are in contact [18] and are more densely packed [11]. This increased density reduces the exposed surface area of individuals in cooler ambient air temperatures, thereby decreasing the dissipation of heat [10,52]. 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 [11,14,53].

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 [10,54]. 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 [53,55], thereby indirectly warming (through convection) the inhabitants [56]. 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) [55]. 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 may have had an effect on the energetics. Future studies should identify how movement in space integrates with physiology to lead to such energetic consequences.

5. Conclusions

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.

Acknowledgements

We thank Jeffrey Alberts, Emília Martins, Laura Hurley, Meredith West, Christopher Harshaw, Joseph Leffel, Cathleen Rodda, Alison Ossip-Klein, Jesualdo Fuentes-G, Delawrence Sykes, Stephanie Campos and David Noakes for helpful discussions and comments on earlier versions of this manuscript. This material is based on work supported by the NIMH grant RO1-MH082019 to JRA, NICHD grant RO1-HD082203 to CH and JRA, NSF Graduate Research Fellowship and NSF Postdoctoral Fellowship [grant number 1611616] to DSS, internship awards to DSS on an NSF Integrative Graduate Education and Research Traineeship in "Dynamics of Brain–Body–Environment Systems in Behavior and Cognition" [DGE 0903495] to RDB, and the Oregon Hatchery Research Center.

References

[1] R.B. Huey, Physiological consequences of habitat selection, Am. Nat. 137 (1991) S91–115.

[2] K.J. Mathot, N.J. Dingemanse, Energetics and behavior: unrequited needs and new directions, Trends Ecol. Evol. 30 (2015) 199–206, http://dx.doi.org/10.1016/j.tree.2015.01.010.

[3] R.L. Day, K.N. Laland, F.J. Odling-Smee, Rethinking adaptation: the niche-construction perspective, Perspect. Biol. Med. 46 (2003) 80–95.

[4] G.E. Walsberg, Physiological consequences of microhabitat selection, Habitat. Sel. Birds Acad. Press. N. Y. (1985) 389–413.

[5] C.K. Hemelrijk, D. Reid, H. Hildenbrandt, J. Padding, The increased efficiency of fish swimming in a school, Fish Fish. 16 (2015) 511–521, http://dx.doi.org/10.1111/faf.12072.

[6] S. Marras, S.S. Killen, J. Lindström, D.J. McKenzie, J.F. Steffensen, P. Domenici, Fish swimming in schools save energy regardless of their spatial position, Behav. Ecol. Sociobiol. 69 (2015) 219–226, http://dx.doi.org/10.1007/s00265-014-1834-4.

[7] J.C. Liao, A review of fish swimming mechanics and behaviour in altered flows, Philos. Trans. R Soc. B Biol. Sci. 362 (2007) 1973–1993, http://dx.doi.org/10.1098/rstb.2007.2082.

[8] S.J. Portugal, T.Y. Hubel, J. Fritz, S. Heese, D. Trobe, B. Voelkl, et al., Upwash exploitation and downwash avoidance by flap phasing in ibis formation flight, Nature 505 (2014) 399–402.

[9] A.E. Jeukendrup, N.P. Craig, J.A. Hawley, The bioenergetics of world class cycling, J. Sci. Med. Sport 3 (2000) 414–433, http://dx.doi.org/10.1016/S1440-2440(00)80008-0.

[10] C. Gilbert, D. McCafferty, Y. Le Maho, J.-M. Martrette, S. Giroud, S. Blanc, et al., One for all and all for one: the energetic benefits of huddling in endotherms, Biol. Rev. 85 (2010) 545–569, http://dx.doi.org/10.1111/j.1469-185X.2009.00115.x.

[11] C. Gilbert, S. Blanc, Y. Le Maho, A. Ancel, Energy saving processes in huddling emperor penguins: from experiments to theory, J. Exp. Biol. 211 (2008) 1–8, http://dx.doi.org/10.1242/jeb.005785.

[12] J.R. Alberts, Huddling by rat pups: ontogeny of individual and group behavior, Dev. Psychobiol. 49 (2007) 22–32, http://dx.doi.org/10.1002/dev.20190.

[13] C. Harshaw, J.R. Alberts, Group and individual regulation of physiology and behavior: a behavioral, thermographic, and acoustic study of mouse development, Physiol. Behav. 106 (2012) 670–682, http://dx.doi.org/10.1016/j.physbeh.2012.05.002.

[14] B. Heinrich, The mechanisms and energetics of honeybee swarm temperature regulation, J. Exp. Biol. 91 (1981) 25–55.

[15] K. Schmidt-Nielsen, Scaling: Why is Animal Size so Important? Cambridge University Press, 1984.

[16] P.B.S. Lissaman, C.A. Shollenberger, Formation flight of birds, Science 168 (1970) 1003–1005.

[17] D. Weihs, Hydromechanics of fish schooling, Nature 241 (1973) 290–291, http://dx.doi.org/10.1038/241290a0.

[18] H. Glaser, S. Lustick, Energetics and nesting behavior of the northern white-footed mouse, *Peromyscus leucopus noveboracensis*, Physiol. Zool. 48 (1975) 105–113.

[19] J.C. Jones, B.P. Oldroyd, Nest thermoregulation in social insects, in: S.J. Simpson (Ed.), Adv. Insect Physiol. vol. 33, Academic Press, 2006, pp. 153–191.

[20] J. Korb, Thermoregulation and ventilation of termite mounds, Naturwissenschaften 90 (2003) 212–219, http://dx.doi.org/10.1007/s00114-002-0401-4.

[21] K. Schmidt-Nielsen, Animal Physiology: Adaptation and Environment, Cambridge University Press, 1997.

[22] P.L.P. Van Loo, J.A. Mol, J.M. Koolhaas, B.F.M. Van Zutphen, V. Baumans, Modulation of aggression in male mice: influence of group size and cage size, Physiol. Behav. 72 (2001) 675–683, http://dx.doi.org/10.1016/S0031-9384(01)00425-5.

[23] V. de las Heras, J.A. Martos-Sitcha, M. Yúfera, J.M. Mancera, G. Martínez-Rodríguez, Influence of stocking density on growth, metabolism and stress of thick-lipped grey mullet (*Chelon labrosus*) juveniles, Aquaculture 448 (2015) 29–37, http://dx.doi.org/10.1016/j.aquaculture.2015.05.033.

[24] S. Bazazi, J. Buhl, J.J. Hale, M.L. Anstey, G.A. Sword, S.J. Simpson, et al., Collective motion and cannibalism in locust migratory bands, Curr. Biol. 18 (2008) 735–739, http://dx.doi.org/10.1016/j.cub.2008.04.035.

[25] L.E. Davis, C.B. Schreck, The energetic response to handling stress in juvenile coho salmon, Trans. Am. Fish. Soc. 126 (1997) 248–258.

[26] K.A. Sloman, G. Motherwell, K.I. O'Connor, A.C. Taylor, The effect of social stress on the Standard Metabolic Rate (SMR) of brown trout, *Salmo trutta*, Fish Physiol. Biochem. 23 (2000) 49–53, http://dx.doi.org/10.1023/A:1007855100185.

[27] T.E. Medland, F.W.H. Beamish, The influence of diet and fish density on apparent heat increment in rainbow trout, *Salmo gairdneri*, Aquaculture 47 (1985) 1–10, http://dx.doi.org/10.1016/0044-8486(85)90003-1.

[28] R.A. Martin, M. Fiorentini, F. Connors, Social facilitation of reduced oxygen consumption in *Mus musculus* and *Meriones unguiculatus*, Comp. Biochem. Physiol. A Physiol. 65 (1980) 519–522, http://dx.doi.org/10.1016/0300-9629(80)90072-9.

[29] R.J. Berry, F.H. Bronson, Life history and bioeconomy of the house mouse, Biol. Rev. 67 (1992) 519–550.

[30] N. Latham, G. Mason, From house mouse to mouse house: the behavioural biology of free-living *Mus musculus* and its implications in the laboratory, Appl. Anim. Behav. Sci. 86 (2004) 261–289, http://dx.doi.org/10.1016/j.applanim.2004.02.006.

[31] T.L.Y. Bond, P.E. Neumann, W.B. Mathieson, R.E. Brown, Nest building in nulligravid, primigravid and primiparous C57BL/6J and DBA/2J mice (*Mus musculus*), Physiol. Behav. 75 (2002) 551–555, http://dx.doi.org/10.1016/S0031-9384(02)00659-5.

[32] B.N. Gaskill, C.J. Gordon, E.A. Pajor, J.R. Lucas, J.K. Davis, J.P. Garner, Heat or insulation: behavioral titration of mouse preference for warmth or access to a nest, PLoS One 7 (2012) e32799, http://dx.doi.org/10.1371/journal.pone.0032799.

[33] S.E. Hess, S. Rohr, B.D. Dufour, B.N. Gaskill, E.A. Pajor, J.P. Garner, Home improvement: C57BL/6J mice given more naturalistic nesting materials build better nests, J. Am. Assoc. Lab. Anim. Sci. 47 (2008) 25.

[34] R.M. Deacon, Assessing nest building in mice, Nat. Protoc. 1 (2006) 1117–1119, http://dx.doi.org/10.1038/nprot.2006.170.

[35] E. Williams, J.P. Scott, The development of social behavior patterns in the mouse, in relation to natural periods, Behaviour (1953) 35–65.

[36] K.A. Clarke, J. Still, Development and consistency of gait in the mouse, Physiol. Behav. 73 (2001) 159–164, http://dx.doi.org/10.1016/S0031-9384(01)00444-9.

[37] M.S. Blumberg, The developmental context of thermal homeostasis, in: E.M. Blass (Ed.), Dev. Psychobiol. Springer US, 2001, pp. 199–228.

[38] C.J. Gordon, Thermal physiology of laboratory mice: defining thermoneutrality, J. Therm. Biol. 37 (2012) 654–685, http://dx.doi.org/10.1016/j.jtherbio.2012.08.004.

[39] D.S. Shelton, J.R. Alberts, Group behavior of mouse litters and the development of regulated pup flow, Dev. Psychobiol. 54 (2012) 766.

[40] P. Conklin, F.W. Heggeness, Maturation of tempeature homeostasis in the rat, Am. J. Physiol. Content 220 (1971) 333–336.

[41] O. Arjamaa, K.Y.H. Lagerspetz, Postnatal development of shivering in the mouse, J. Therm. Biol. 4 (1979) 35–39.

[42] P.M. Taylor, Oxygen consumption in new-born rats, J. Physiol. 154 (1960) 153–168, http://dx.doi.org/10.1113/jphysiol. 1960.sp006570.

[43] F.A. Verley, D. Grahn, W.P. Leslie, K.F. Hamilton, Sex ratio of mice as possible indicator of mutation rate for sex-linked lethals, J. Hered. 58 (1967) 285–290.

[44] National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory, Environment, Housing, and Management, National Academies Press, US, 2011.

[45] M.S. Blumberg, G. Sokoloff, Thermoregulatory competence and behavioral expression in the young of altricial species—revisited, Dev. Psychobiol. 33 (1998) 107–123.

[46] B. Cannon, J. Nedergaard, Brown adipose tissue: function and physiological

significance, Physiol. Rev. 84 (2004) 277–359, http://dx.doi.org/10.1152/physrev.00015.2003.

[47] C. Harshaw, J.J. Culligan, J.R. Alberts, Sex differences in thermogenesis structure behavior and contact within huddles of infant mice, PLoS One 9 (2014) e87405, , http://dx.doi.org/10.1371/journal.pone.0087405.

[48] R Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2015.

[49] S. Creel, B. Dantzer, W. Goymann, D.R. Rubenstein, The ecology of stress: effects of the social environment, Funct. Ecol. 27 (2013) 66–80, http://dx.doi.org/10.1111/j.1365-2435.2012.02029.x.

[50] T.T. Cao, A. Dornhaus, Ants under crowded conditions consume more energy, Biol. Lett. 4 (2008) 613–615, http://dx.doi.org/10.1098/rsbl.2008.0381.

[51] C. Gilbert, G. Robertson, Y.L. Maho, A. Ancel, How do weather conditions affect the huddling behaviour of emperor penguins? Polar Biol. 31 (2007) 163–169, http://dx.doi.org/10.1007/s00300-007-0343-6.

[52] M. Canals, M. Rosenmann, F. Bozinovic, Energetics and geometry of huddling in small mammals, J. Theor. Biol. 141 (1989) 181–189, http://dx.doi.org/10.1016/S0022-5193(89)80016-5.

[53] J.P. Hayes, J.R. Speakman, P.A. Racey, The contributions of local heating and reducing exposed surface area to the energetic benefits of huddling by short-tailed field voles (*Microtus agrestis*), Physiol. Zool. (1992) 742–762.

[54] G.J. Tattersall, B.J. Sinclair, P.C. Withers, P.A. Fields, F. Seebacher, C.E. Cooper, et al., Coping with thermal challenges: physiological adaptations to environmental temperatures, Compr. Physiol. John Wiley & Sons, Inc., 2012.

[55] C.K.R. Willis, R.M. Brigham, Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat, Behav. Ecol. Sociobiol. 62 (2007) 97–108, http://dx.doi.org/10.1007/s00265-007-0442-y.

[56] G.E. Walsberg, Communal roosting in a very small bird: consequences for the thermal and respiratory gas environments, Condor 92 (1990) 795–798, http://dx.doi.org/10.2307/1368707.