

# Using ecology to inform physiology studies: implications of high population density in the laboratory

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**Newman AE, Edmunds NB, Ferraro S, Heffell Q, Merritt GM, Pakkala JJ, Schilling CR, Schorno S.** Using ecology to inform physiology studies: implications of high population density in the laboratory. *Am J Physiol Regul Integr Comp Physiol* 308: R449–R454, 2015. First published January 14, 2015; doi:10.1152/ajpregu.00328.2014.— Conspecific density is widely recognized as an important ecological factor across the animal kingdom; however, the physiological impacts are less thoroughly described. In fact, population density is rarely mentioned as a factor in physiological studies on captive animals and, when it is infrequently addressed, the animals used are reared and housed at densities far above those in nature, making the translation of results from the laboratory to natural systems difficult. We survey the literature to highlight this important ecophysiological gap and bring attention to the possibility that conspecific density prior to experimentation may be a critical factor influencing results. Across three taxa: mammals, birds, and fish, we present evidence from ecology that density influences glucocorticoid levels, immune function, and body condition with the intention of stimulating discussion and increasing consideration of population density in physiology studies. We conclude with several directives to improve the applicability of insights gained in the laboratory to organisms in the natural environment.

body condition; ecophysiology; glucocorticoids; immune function; stress

*“I would rather sit on a pumpkin and have it all to myself, than be crowded on a velvet cushion”*

– H. D. Thoreau

RESEARCH USING MODEL ORGANISMS in the laboratory is ubiquitous, and the results from these experiments are often translational, with the goal of applying findings to free-living animals and humans. In the wild, the ecological importance of density-dependence is well established; however, the effect of animal density on physiological studies in the laboratory is vastly underappreciated. Notably, lab and field conditions differ in many respects; for example, food availability is often considered to drive density-dependence in the wild, albeit lab animals are typically fed ad libitum. Nonetheless, a recent field experiment using *Tamiasciurus hudsonicus* (American red squirrel) successfully separated the ecological effects of density and resources, demonstrating that even in the absence of resource limitation, high density can significantly influence physiology in natural populations (26). Considering the ecological parameters under which experimental animals are maintained may be crucial in understanding how physiological results reported in lab studies can be translated to organisms living under natural conditions.

Here, using examples from the literature across three taxa (birds, fish, and mammals), we highlight the pervasive corre-

lation between high density and specific physiological parameters, and we emphasize the tendency to overlook animal density in lab studies (Fig. 1 and Supplemental Table S1). Finally, via two case studies of traditional captive animal systems, we demonstrate that even when density is considered, the ecological parameters of the experimental paradigms (e.g., animal density) do not reflect natural conditions and, thus, findings are difficult to interpret. We synthesize the physiological effects of density with the goal of illuminating a critical ecophysiological gap and to demonstrate the feasibility and importance of including density-dependent factors in experimental design and interpretation.

## Literature Investigation

Using a systematic survey of the recent physiological literature, we quantified the extent to which animal density is overlooked in physiological studies. We ran a literature search using the citation database PubMed in October 2014 using the following search terms: [(Title/Abstract)Glucocorticoid OR “body condition” OR “immune function”) AND (Title/Abstract]bird OR avian OR fish OR rat OR mouse OR mice OR hamster) NOT ([All fields]wild)] to examine the most recent 400 manuscripts in 2014. Of the 400 manuscripts, 80 were physiological studies on one of the three primary physiological parameters we assess in our review (glucocorticoid levels, body condition, and immune function); see Supplemental Table S1 for details on each study. We document that nearly 80% of studies do not report animal density either before (i.e., rearing/housing density) or during the experiment, and only 2.5% report density before *and* during the experiment

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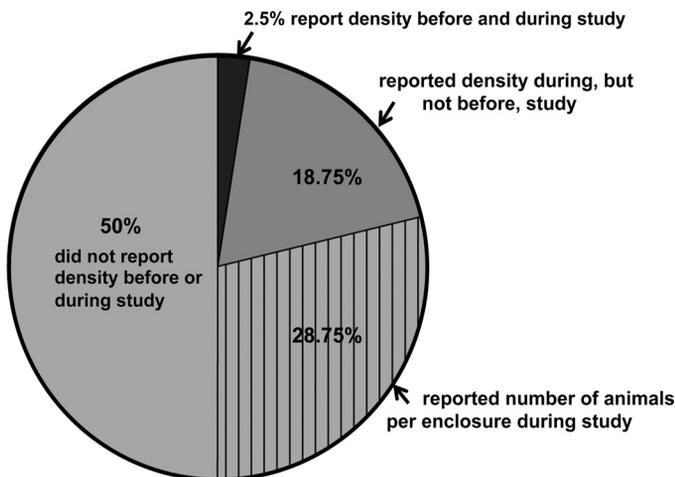


Fig. 1. Only 2.5% of the surveyed literature reported animal density both prior to and during experimentation. Nearly 80% of studies (light gray section: 78.75%) did not report density at any time, although of these studies, 28.75% (section with patterned lines) reported the number of animals per enclosure during the experiment but without cage/aquarium dimensions. Finally, 18.75% reported animal density during the experiment (but not before), including both the number of animals per enclosure and enclosure dimensions. Also, see Supplemental Table S1.

(Fig. 1). In some cases (23/80: 28.75%), the authors reported the number of animals per cage during experimentation, but did not provide cage dimensions. Not surprisingly, most studies were using rodent models (mouse: 29/80; rat: 38/80), yet there appears to be an interesting bias in that mouse-based studies are the least likely to report animal density (1/29 reported density during the study), whereas 9/38 studies using rats reported density, 2/6 studies using birds reported density, and 5/7 studies using fish reported density. In fact, most studies report that animals were purchased from a supplier and “housed in standard conditions”, yet our survey revealed that even when studies do report the number of animals per cage and the dimensions of the cage (17/80 papers), the size of the cage varies across studies, as does the number of animals housed per cage, and there is no mention of the number of cages or animals per room. Not only is animal density an important factor that is overlooked, but a “standard condition” for housing does not appear to exist.

#### Physiological Impacts of High Density

**Glucocorticoids.** One mediator of stress, or allostatic load (60), is the hypothalamic pituitary adrenal (HPA) axis and the associated release of glucocorticoids (GCs). Increases in GCs are known to alter vertebrate behavior and physiology and can have profound effects on individual fitness (e.g., 89, 93). It is worth noting that the relationship between HPA axis dynamics and fitness is complicated and there are inconsistent findings among studies on wild animal systems (9, 11). Nonetheless, population density, and the associated conspecific interactions and competition, also affects GC levels (24, 26), and these effects are evident across taxa and experimental paradigms. Many field studies report a positive correlation between population density and GC levels. This was the case for wild populations of *Tamiasciurus hudsonicus* (American red squirrel; Ref. 26) *Microtus pennsylvanicus* (meadow voles; Ref. 10), and *Rhombomys opimus* (Midday gerbils; Ref. 88). Furthermore, simulated territorial intrusions, which temporarily

increase perceived local density, increase GC levels in a variety of wild bird species (51, 70, 104); also in captive *Sturnus vulgaris* (European starlings), several studies report an increase in GCs and cardiovascular stress response in intruders (30, 69). In captivity, some lab studies have reported effects of density, albeit they are uncommon. For example, studies on captive *Mus musculus* (house mice) (78), *Rattus norvegicus* (Wistar rats) (14), and wild-caught *Peromyscus leucopus* (white-footed mice) report positive relationships between cage density and GC levels (102). There is also strong evidence from studies on *Gallus gallus domesticus* (chickens) that plasma GCs increase at higher cage densities (27, 59, 73) and likely play a role in lower egg quality from stressed hens (27, 74). Likewise, in cultured fish species, high stocking density was shown to elevate circulating GCs (2, 38).

**Immune function.** The tightly woven relationship between the immune system and HPA axis suggests that high population densities, and the resulting increased GC levels, can influence immune function (28, 41, 68, 88, 95). Further, as the number of neighbors in a population increases, so too does the spread of pathogens and the outbreak of disease (18, 95). This increased allocation of physiological resources to antipathogen defense can have negative impacts on survival, reproduction, and fitness (18, 65). Measures of immune defense range widely across studies and taxa (e.g., 41, 66, 68); thus, we refer generally to “immune function” for this review.

Immune function varies across vertebrate life-history stages and seasons, often in parallel to local conspecific density. For example, altricial nestlings are typically more vulnerable to immune challenges than their adult counterparts, in part, due to nest confinement and close association with nest-mates (66, 94, 97). Similarly, adult individuals, such as *Myodes glareolus* (Bank voles), show reduced immune function during the breeding season (high population density) compared with other seasons of relatively low population density (91). Even wild animals adapted to living at high population densities, such as colonial birds and cooperative breeding mammals, experience increases in individual pathogen loads and impaired immune function that can also reduce offspring immune defense (5, 13, 65). For instance, *Spheniscus magellanicus* (Magellanic penguin) and *Rhombomys opimus* (Greater gerbils) show greater social stress and impaired immune function in high population densities because of increased social contact (12, 88, 96). Finally, captive studies investigating the effects of social crowding on laboratory mice, farmed chickens, and farmed fish, also report a negative relationship between density and immune function (29, 34, 41, 44, 108).

**Body condition.** Although widely used, the term body condition is not often explicitly defined. Broadly, it is used to refer to the physical condition of an animal, frequently with reference to protein or fat reserves (20, 31, 63); here, we also include body mass and growth to allow for a more comprehensive analysis of density-dependent effects on physical condition.

Controlled laboratory experiments have revealed that generally, higher population densities result in decreased body condition across taxa. The relationship between body condition and population density is one that is highly studied in fish due to the implications for aquaculture stocks. Stocking experiments of *Barbodes gonionotus* (Java barb; Ref. 37), and *Etroplus suratensis* fingerlings (Pearlspot; Ref. 7), revealed that diminished stocking densities yielded fish with comparably

better growth profiles. This trend has been observed in the majority of laboratory fish studies, including *Oreochromis niloticus* L. (Nile tilapia; Ref. 35) *Gadus morhua* L. (Atlantic cod; Ref. 56), and *Oncorhynchus mykiss* (rainbow trout; Ref. 76). Captive mammal experiments have presented similar findings. Rabbits caged at lower densities showed higher body weights and heightened daily weight gain (75), and sheep maintained at lower population density birthed lambs with better body condition, as well as higher weight gain rates (64). The results are similar for laboratory colonies of *Taeniopygia guttata* (zebrafinch), where birds housed at low density gained more mass and produced a higher number of heavier offspring than those housed at high density (82).

Field studies in fish, mammals, and birds have found negative relationships between relative abundance and body condition, though are confounded by relative decreases in food availability. Experiments on wild *Pterois volitans* (red lionfish) revealed a linear decrease in body length, and an exponential decrease in body weight after a manipulated population increase (4). Similarly, young *Sardinops melanosticta* (Japanese sardine; Ref. 45) and *Salmo salar* (Atlantic salmon; Ref. 43) showed negative relationships between body size and density. Further, several mammalian species, including *Aquus asinus* (feral donkey; Ref. 20), *Arctocephalus forsteri* (fur seal; Ref. 8), *Capreolus capreolus* (roe deer; Ref. 32), and *Lynx canadensis* (lynx; Ref. 109), have decreased body condition under high-population density conditions. This trend has also been recognized in wild birds such as *Larus audouinii* (Audouin's gulls; Ref. 90), *Anser caerulescens atlanticus* (snow geese; Ref. 85), *Stercorarius parasiticus* (Arctic skua; Ref. 80), *Spheniscus magellanicus* (Magellanic penguins; Ref. 96), and *Branta bernicla nigricans* (Black Brant geese; Ref. 92).

*Implications of density for lab experiments: two case studies.* Scientists have long used lab animals to investigate complex hypotheses across the physiological and biomedical fields. Prior to experimentation, these animals are typically reared and/or housed in captive colonies for which population density is often neither reported, nor is density routinely considered during experimentation (Fig. 1). Understanding the ecological parameters for lab animals and the implications on individuals may be critical in translating results to free-living organisms. Calisi and Bentley (16) also allude to the important differences between laboratory and field studies in their poignant review, where they highlight a number of incidences across vertebrate taxa, where the results from the laboratory differ from their field counterparts; however, the influence of density has not yet been addressed.

### Lab Mouse

Although the great majority of laboratory studies do not report the population density of mouse colonies, several experiments have looked directly at the impact of cage density on physiological outcomes and highlight the importance of considering animal density. Laboratory studies reveal that mice show a consistent decrease in reproductive output at high population density, which has been attributed to smaller reproductive organs, decreases in fecundity, and the inhibition of juvenile maturation (21–23, 25, 56, 88, 98–101).

Glucocorticoids, immune function, and body condition are three possible mechanistic links between high population den-

sity and decreased fitness. Laboratory studies clearly show that as cage density increases, GCs increase, while immune function and body condition decrease [(GCs: Refs. 42, 78, 102) (immune function: Refs. 34, 78, 102) (body condition: Ref. 1)]. Many of the early lab studies were criticized, as the experiments were only performed on captive mice (48, 55), albeit subsequent field studies on wild mice found that as population density increases reproductive output decreases (34, 58, 67, 101) and GCs generally increase (46); however, more field research is required to understand the effect of density on immune function and body condition and to determine whether laboratory results are biologically relevant to wild populations.

Despite the studies mentioned above, densities experienced by lab mice are extremely high compared with natural populations. The home range for a wild mouse ranges from 1000 m<sup>2</sup> to 7500 m<sup>2</sup> depending on the species (62). The average cage size for lab mice is ~0.05 m<sup>2</sup> with up to eight mice per cage to simulate high density, affording each mouse ~1/20,000th of its natural area. This ecological discrepancy may explain some phenomena observed only in laboratory populations, such as the Bruce Effect, which is a pregnancy disruption where a female will absorb/abort her fetus when introduced to an unknown male. The Bruce Effect has been reported for at least 12 species in the laboratory, including several species of mice but has never been observed in the wild (reviewed in Ref. 107). Laboratory artifacts such as this highlight the potential difficulty in interpreting results in the absence of ecologically relevant population densities.

### Rainbow Trout (*Oncorhynchus mykiss*)

Aquaculture science is an ecologically and economically important applied version of highly controlled lab studies on captive animals. *O. mykiss* is one of the most commonly farmed freshwater species, and although a vast amount of effort is invested in elevating stocking density (SD) to maximize yield, there are well-described negative effects of high SD on physiology and fitness. For example, high SD is related to elevated plasma GCs (81) and altered HPA axis responsivity to acute stressors (83), and it is associated with indices of chronic stress, such as increased brain serotonergic activity (53). Further, it has been shown that repeated acute stress can reduce *O. mykiss* gamete quality, leading to decreased survival rates of progeny (17).

The effects of SD on *O. mykiss* body condition also reveal consistent negative consequences of high density. Specific growth rate, often correlated with reductions in food conversion efficiency, decreases with increasing SD (52, 61, 103), and body condition factor and hepatosomatic index, used as proxies for nutritional status, also decrease at high SD (54, 79). In the majority of these studies, fish were fed ad libitum to ensure differences among density groups were not caused by food shortages. Unfortunately, reports of *O. mykiss* immune function are both scarce and inconsistent (e.g., 46, 71, 81), highlighting the necessity to better understand density-dependent effects on farmed fish physiology.

Like lab mice, farmed *O. mykiss* are kept at astonishingly high densities compared with the natural environment. While measures of natural fish density are notoriously difficult to obtain given the three-dimensional underwater environment, density estimates for natural Alaskan populations of *O. mykiss* (>25-cm fork length) range from 1.4 fish/ha to 50.5 fish/ha

(15), whereas *O. mykiss* are typically farmed at densities resulting in 30–40 kg/m<sup>3</sup> or higher (36), which, for comparison, if an average trout weighs 0.01 kg/cm of fork length (25 cm FL fish = 0.25 kg), results in >100 small fish/m<sup>3</sup>.

In general, high density increases GC levels, inhibits immune function, decreases body condition, and reduces reproductive capacity. Work with captive animals has been fundamental in our understanding of many physiological processes and in agricultural practices; however, even when including density as a factor in laboratory experiments, the animals are housed in such tight quarters, the ecological relevance is difficult to extract. Nonetheless, the purpose of these case studies is not to discourage the use of captive animals, but rather to draw attention to the subtle, yet important, effects density may play in explaining variability measured in experimental results.

### Perspectives and Significance

This review highlights a critical gap in physiology: the potential for population density to have profound influences on physiological experiments using captive populations is underappreciated. To increase applicability of laboratory results to free-living animal and human physiology, we propose several specific directives. 1) Although logistically difficult, if not largely impossible given current infrastructure, ideally, population density of lab animals should reflect natural population densities. It is worth considering that we would not conduct clinical trials on humans confined to similar conditions that we do lab mice and expect the results to be broadly meaningful to the public at large. 2) When this is not feasible, as is often the case due to a range of logistics, the effects of density should be experimentally evaluated, such that treatment groups from a range of densities be compared, so that one can either confirm that density is an important factor, or to be confident in ruling it out in related experiments. 3) Not only is intracage density important, but the number of cages per experimental room should be a factor as animals in the room are interacting via scent and vocalizations. 4) While design considerations are extensive for new or renovated lab space, similar considerations of space and design should be granted to researchers in designing animal facilities, both for independent principal investigators and also for collective vivaria that serve multiple researchers and their respective simultaneous experiments. 5) Finally, density should be accounted for in a statistical manner during data analysis, emphasizing the responsibility of suppliers to make available the specific information regarding the housing density and ecological parameters of their stock populations. Experimental results may be inherently affected by the ecological conditions of the animal prior to and during experimentation and a substantial degree of the variation around physiological measurements may be explained by the animal's housing density throughout its life history.

While we focus on evidence from a variety of vertebrate taxa, important advances in density-dependent effects on physiology and fitness are being made using invertebrate models in the laboratory (6). Also, although there is substantial evidence for a relationship between high-density, compromised physiological measures and decreased fitness, we do acknowledge that these trends do not apply across species, *carte blanche*. For example, circulating GCs were not correlated with higher breeding densities in Icterids (blackbirds; Ref. 3), and Char-

bonnel et al. (19) report in wild populations of *Arvicola scherman* (fossorial water voles) an inverse relationship between density and GCs when compared across two years. Further, there are several lab studies that describe the relationship between social isolation, or being singly housed, and increased GC levels, compromised immune function, and decreased body condition (e.g., 49). Further, phenomena such as the “Allee Effect” can have physiological consequences at very low densities. Importantly, in *Melospiza melodia* (song sparrows), the ability to respond to a novel immune challenge decreases with homozygosity and corresponding low population densities (72, 86). Furthermore, the negative relationship between high population density and decreased body condition is not universal, with high density promoting better body condition in *Lepomis gibbosus* (pumpkinseed sunfish; Ref. 39), *Dicentrarchus labrax* (European seabass; Ref. 77), and *Salvelinus alpinus* (Arctic charr; Ref. 105).

Another important consideration that we do not address here in detail is that the effect of animal density on physiology may be sex-specific. When designing studies and interpreting results, it is critical to consider that males and females may differentially respond to environmental and experimental conditions. In some cases, males and females respond similarly to population density (e.g., wild mice; Ref. 40), while in other cases, the sex difference can be profound (e.g., 47, 84, 106) and dependent on the sex of conspecifics in the same enclosure.

In closing, we hope to stimulate discussion and awareness around the influence of animal density on experimental design and outcome. It is clear that density plays an important role in shaping animal behavior and physiology and should be considered as a key parameter in physiological studies.

### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

### AUTHOR CONTRIBUTIONS

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### REFERENCES

- Anderson A, Werboff J, Les EP. Effects of environmental temperature-humidity and cage density on body weight and behavior in mice. *Experientia* 24: 1022–1023, 1968.
- Barcellos LJG, Nicolaiewsky S, De Souza SMG, Lulhier F. The effects of stocking density and social interaction on acute stress response in Nile tilapia *Oreochromis niloticus* (L.) fingerlings. *Aquaculture* 30: 887–892, 1999.
- Beletsky LD, Orrians GH, Wingfield JC. Steroid hormones in relation to territoriality, breeding density and parental behavior in male yellow-headed blackbirds. *Auk* 107: 60–68, 1990.
- Benkwitt CE. Density-dependent growth in invasive lionfish (*Pterois volitans*). *PLoS One* 8: e66995, 2013.
- Begon M, Harper JL, Townsend CR. *Ecology: Individuals, Populations and Communities*. Oxford, UK: Blackwell, 1986.
- Betini GS, Griswold CG, Norris DR. Density-mediated carry-over effects explain variation in breeding output across time in a seasonal population. *Biol Lett* 9: 20130582, 2013.
- Biswas G, Ghoshal TK, Natarajan M, Thirunavukkarasu AR, Sundaray JK, Kailasam M, De D, Sukumaran K, Kumar P, Ponniah AG. Effects of stocking density and presence or absence of sail base on growth, weight variation survival and body composition of pearlspot, *Etroplus suratensis* (Bloch) fingerlings. *Aquac Res* 44: 1266–1276, 2013.

8. **Bradshaw JA, Davis LS, Lalas C, Harcourt RG.** Geographic and temporal variation in the condition of pups of the New Zealand fur seal (*Arctocepalus forsteri*): evidence for density dependence and differences in the marine environment. *J Zoo* 252: 41–51, 2000.
9. **Bonier F, Martin PR, Moore IT, Wingfield JC.** Do baseline glucocorticoids predict fitness? *Trends Ecol Evol* 24: 634–642.
10. **Boonstra R, Boag PT.** Spring declines in *Microtus pennsylvanicus* and the role of steroid hormones. *J Anim Ecol* 61: 339–352, 1992.
11. **Breuner CW, Patterson SH, Hahn TP.** In search of relationships between the acute adrenocortical response and fitness. *Gen Comp Physiol* 157: 288–295.
12. **Brinkhof MWG, Heeb P, Kölliker M, Richner H.** Immunocompetence of nestling great tits in relation to rearing environment and parentage. *P R Soc Lond B* 266: 2315–2322, 1999.
13. **Brown C, Brown MB.** Coloniality in the cliff swallow: the effect of group size on social behavior. Chicago, IL: Chicago University Press, 1996.
14. **Brown KJ, Grunberg NE.** Effects of housing on male and female rats—crowding stresses males but calms females. *Physiol Behav* 58: 1085–1089, 1995.
15. **Burr J.** A summary of abundance and density estimates for selected lake trout populations in the Alaska range, and an examination of trends in yield. *Alaska Department of Fish and Game 92-1*: 1–25, 1992.
16. **Calisi RM, Bentley GE.** Lab and field experiments: Are they the same animal? *Horm Behav* 56: 1–10, 2009.
17. **Campbell PM, Pottinger TG, Sumpter JP.** Stress reduces the quality of gametes produced by rainbow trout. *Biol Reprod* 47: 1140–1150, 1992.
18. **Carey C, Cohen N, Rollins-Smith L.** Amphibian declines: an immunological perspective. *Dev Comp Immunol* 23: 459–472, 1999.
19. **Charbonnel N, Chaval Y, Berthier K, Deter J, Morand S, Palme R, Cosson J.** Stress and demographic decline: a potential effect mediated by impairment of reproduction and immune function in cyclic vole populations. *Physiol Biochem Zool* 81: 63–73, 2008.
20. **Choquenot D.** Density-dependent growth, body condition, and demography in feral donkeys: Testing the food hypothesis. *Ecology* 72: 805–813, 1991.
21. **Christian JJ.** The adreno-pituitary system and population cycles in mammals. *J Mammal* 31: 247–259, 1950.
22. **Christian JJ.** Adrenal and reproductive responses to population size in mice from freely growing populations. *Ecology* 37: 258–273, 1956.
23. **Christian JJ.** Phenomena associated with population density. *Proc Nat Acad Sci* 47: 428–449, 1961.
24. **Creel S, Danzter B, Goymann W, Rubenstein DR.** The ecology of stress: effects of the social environment. *Funct Ecol* 27: 66–80, 2013.
25. **Creigh SL, Terman CR.** Reproductive recovery of inhibited male prairie deer mice (*Peromyscus maniculatus bairdii*) from laboratory populations by contact with females or their urine. *J Mammal* 69: 603–607, 1988.
26. **Dantzer B, Newman AEM, Boonstra R, Palme R, Boutin S, Humphries MM, McAdam AG.** Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. *Science* 340: 1215–1217, 2013.
27. **Davami A, Wineland MJ, Jones WT, Ilardi RL, Peterson RA.** Effects of population size, floor space, and feeder space upon productive performance, external appearance and plasma corticosterone concentration of laying hens. *Poult Sci* 66: 251–257, 1987.
28. **Daynes RA, Araneo BA, Hennebold J, Enioutina E, Mu HH.** Steroids as regulators of the mammalian immune response. *J Invest Dermatol* 105: 14S–19S, 1995.
29. **Demers NE, Bayne CJ.** The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. *Dev Comp Immunol* 21: 363–373, 1997.
30. **Dickens MJ, Nephew BC, Romero LM.** Captive European starlings (*Sturnus vulgaris*) in breeding condition show an increased cardiovascular stress response to intruders. *Physiol Biochem Zool* 79: 937–943, 2006.
31. **Dobson FS.** Body mass, structural size, and life-history patterns of the Columbian ground squirrel. *Am Nat* 140: 109–125, 1992.
32. **Douhard M, Gaillard JM, Delorme D, Capron G, Duncan P, Klein F, Bonenfant C.** Variation in adult body mass of roe deer: early environmental conditions influence early and late body growth of females. *Ecology* 94: 1805–1814, 2013.
33. **Drost CA, Fellers GM.** Density cycles in an island population of deer mice, *Peromyscus maniculatus*. *Oikos* 60: 351–364, 1991.
34. **Edwards EA, Dean LA.** Effects of crowding of mice on humoral antibody formation and protection to lethal antigenic challenge. *Psychosom Med* 31: 19–24, 1977.
35. **El-Sayed AFM.** Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus* L.) fry. *Aquac Res* 33: 621–626, 2002.
36. **Ellis T, North B, Scott AP, Bromage NR, Porter M, Gadd D.** The relationships between stocking density and welfare in farmed rainbow trout. *J Fish Biol* 61: 493–531, 2002.
37. **Faizul MIM, Christianus A.** Salinity and stocking density effects on growth and survival of *Barbodes gonionotus* (Blooker, 1850) fry. *Can J Fish Aquat Sci* 8: 419–424, 2013.
38. **Fox HE, White SA, Kao MF, Fernald RD.** Stress and dominance in a social fish. *J Neurosci* 17: 6463–6469, 1997.
39. **Fox MG.** Growth, density, and interspecific influences on pumpkinseed sunfish life histories. *Ecology* 75: 1157–1171, 1994.
40. **Harper JM, Austad SN.** Fecal corticosteroid levels in free-living populations of deer mice and southern red-backed voles. *Am Midl Nat* 152: 400–409, 2004.
41. **Heckert RA, Estevez I, Russek-Cohen E, Pettit-Riley R.** Effects of density and perch availability on the immune status of broilers. *Poult Sci* 81: 451–457, 2002.
42. **Hunt C, Hambly C.** Faecal corticosterone levels suggest that separately housed male mice are not more stressed than grouped mice. *Physiol Behav* 87: 519–526, 2006.
43. **Imre I, Grant JWA, Cunjak RA.** Density dependent growth of young-of-the-year Atlantic salmon *Salmo salar* in Catamara Brook, New Brunswick. *J Anim Ecol* 74: 508–516, 2005.
44. **Khansari DN, Murgu AJ, Faith RE.** Effects of stress on the immune system. *Immunol Today* 11: 170–175, 1990.
45. **Kim JY, Kim S, Choi YM, Lee JB.** Evidence of density-dependent effects on population variation of Japanese sardine (*Sardinops melanosticta*) off Korea. *Fish Oceanogr* 15: 345–334, 2006.
46. **Kindschi GA, Smith CE, Koby RF Jr.** Performance of two strains of rainbow trout reared at four densities with supplemental oxygen. *Prog Fish-Cult* 53: 203–209, 1991.
47. **Kleszczynska A, Kulczykowska E.** Stocking density influences brain arginine vasotocin (AVT) and isotocin (IT) levels in males and females of three-spined stickleback (*Gasterosteus aculeatus*). *Gen Comp Endocrinol* 183: 14–16, 2013.
48. **Krebs CJ, Myers JH.** Population cycles in small mammals. *Adv Ecol Res* 8: 267–607, 1974.
49. **Krugel U, Fischer J, Bauer K, Sack U, Himmerich H.** The impact of social isolation on immunological parameters in rats. *Arch Toxicol* 88: 853–855, 2014.
50. **Lambert Y, Dutil JD.** Food intake and growth of adult Atlantic cod (*Gadus morhua* L.) reared under different conditions of stocking density, feeding frequency, and size-grading. *Aquaculture* 192: 233–247, 2001.
51. **Landys MM, Goymann W, Raess M, Slagsvold T.** Hormonal responses to male-male social challenge in the blue tit *Cyanistes caeruleus*: single-broodedness as an explanatory variable. *Physiol Biochem Zool* 80: 228–240, 2007.
52. **Larsen BK, Skov PV, McKenzie DJ, Jokumsen A.** The effects of stocking density and low level sustained exercise on the energetic efficiency of rainbow trout (*Oncorhynchus mykiss*) reared at 19°C. *Aquaculture* 324: 226–233, 2012.
53. **Laursen DC, Silva PIM, Larsen BK, Höglund E.** High oxygen consumption rates and scale loss indicate elevated aggressive behaviour at low rearing density, while elevated brain serotonergic activity suggests chronic stress at high rearing densities in farmed rainbow trout. *Physiol Behav* 122: 147–154, 2013.
54. **Leatherland JF, Cho CY.** Effect of rearing density on thyroid and interrenal gland activity and plasma and hepatic metabolite levels in rainbow trout, *Salmo gairdneri* Richardson. *J Fish Biol* 27: 583–592, 1985.
55. **Lee AK, McDonald IR.** Stress and population regulation in small mammals. *Oxf Rev Reprod Biol* 7: 261–304, 1985.
56. **Lidicker WZ.** Social behaviour and density regulation in house mice living in large enclosures. *J Anim Ecol* 45: 677–697, 1976.
57. **Lombardi JR, Whitsett JM.** Effects of urine from conspecifics on sexual maturation in female prairie deer mice. *J Mammal* 61: 766–8, 1980.
58. **Lusk SJG, Millar JS.** Reproductive inhibition in a short season population of *Peromyscus maniculatus*. *J Anim Ecol* 58: 329–41, 1989.
59. **Mashaly MM, Webb ML, Youtz SL, Roush WB, Graves HB.** Changes in serum corticosterone concentration of laying hens as a response to increased population density. *Poult Sci* 77: 32–40, 1984.
60. **McEwen BS, Wingfield JC.** What is in a name? Integrative homeostasis, allostasis and stress. *Horm Behav* 57: 105–111.

61. McKenzie DJ, Höglund E, Dupont-Prinet A, Larsen BK, Skov PV, Pederson PB, Jokumsen A. Effects of stocking density and sustained aerobic exercise on growth, energetics and welfare of rainbow trout. *Aquaculture* 338: 216–222, 2012.
62. McNab BK. Bioenergetics and the determination of home range size. *Amer Nat* 133–140, 1963.
63. Milner JM, Stein A, Irvine RJ, Albon SD, Langvatn R, Ropstad E. Body condition in Svalbard reindeer and the use of blood parameters as indicators of condition and fitness. *Can J Zool* 81: 1566–1578, 2003.
64. Møbæk R, Mysterud A, Holand Ø, Austrheim G. Temporal variation in density-dependent body growth of a large herbivore. *Oikos* 122: 421–427, 2013.
65. Møller AP, Erritzøe J. Host immune defense and migration in birds. *Evol Ecol* 12: 945–953, 1998.
66. Møller AP, Martín-Vivaldi M, Merino S, Soler JJ. Density-dependent and geographical variation in bird immune response. *Oikos* 115: 463–474, 2006.
67. Morris DW. Density-dependent habitat selection: testing the theory with fitness data. *Evol Ecol* 3: 80–94, 1989.
68. Nelson RJ, Fine JB, Demas GE, Moffatt CA. Photoperiod and population density interact to affect reproductive and immune function in male prairie voles. *Am J Physiol Regul Integr Comp Physiol* 270: R571–R577, 1996.
69. Nephew BC, Romero LM. Behavioral, physiological, and endocrine responses of starlings to acute increases in density. *Horm Behav* 44: 222–232, 2003.
70. Newman AEM, Soma KK. Aggressive interactions differentially modulate local and systemic levels of corticosterone and DHEA in a wild songbird. *Horm Behav* 60: 389–396, 2011.
71. North BP, Turnbull JF, Ellis T, Porter MJ, Migaud H, Bron J, Bromage NR. The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 255: 466–479, 2006.
72. O'Brien SJ, Evermann JF. Interactive influence of infectious disease and genetic diversity in natural populations. *Trends Ecol Evol* 3: 254–259, 1988.
73. Onbasilar EE, Aksoy FT. Stress parameters and immune response of layers under different cage floor and density conditions. *Livestock Prod Sci* 95: 255–263, 2005.
74. Quart MD, Adams AW. Effects of cage design and bird density on layers. *Poult Sci* 61: 1606–1613, 1982.
75. Paci G, Prezioso G, D'Agata M, Russo C, Dalle Zotte A. Effect of stocking density and group size on growth performance, carcass traits and meat quality of outdoor-reared rabbits. *Meat Sci* 93: 162–166, 2013.
76. Papoutsoglou SE, Papaparaska-Papoutsoglou E, Alexis MN. Effects of density on growth rate and production of rainbow trout (*Salmo gairdneri* Rich.) over a full rearing period. *Aquaculture* 66: 9–17, 1987.
77. Papoutsoglou SE, Tziha G, Vrettos X, Athanasiou A. Effects of stocking density on behavior and growth rate of European sea bass (*Dicentrarchus labrax*) juveniles reared in a closed circulated system. *Aquacult Eng* 18: 135–144, 1998.
78. Peng X, Lang CM, Drozdowicz CK, Ohlsson-Wilhelmer BM. Effect of cage population density on plasma corticosterone and peripheral lymphocyte populations on laboratory mice. *Lab Anim* 23: 302–306, 1989.
79. Person-Le Ruyet J, Labbé L, Le Bayon N, Sévere A, Le Roux A, Le Delliou H, Quémener L. Combined effects of water quality and stocking density on welfare and growth in rainbow trout (*Oncorhynchus mykiss*). *Aquat Living Res* 21: 185–195, 2008.
80. Phillips RA, Furness RW, Stewart FM. The influence of territory density on the vulnerability of Arctic skuas *Stercorarius parasiticus* to predation. *Biol Conserv* 86: 21–31, 1998.
81. Pickering AD, Pottinger TG. Crowding causes prolonged leucopenia in salmonid fish, despite interrenal acclimation. *J Fish Biol* 30: 701–712, 1987.
82. Poot H, ter Maat A, Trost L, Schwabl I, Jansen RF, Gahr M. Behavioural and physiological effects of population density on domesticated Zebra finches (*Taeniopygia guttata*) held in aviaries. *Physiol Behav* 105: 821–828, 2012.
83. Procarione LS, Barry TP, Malison JA. Effects of high rearing densities and loading rates on the growth and stress response of juvenile rainbow trout. *N Am J Aquacult* 6: 91–96, 1999.
84. Rabin BS, Lyte M, Epstein LH, Caggiola AR. Alteration of immune competency by number of mice housed per cage. *Ann NY Acad Sci* 496: 492–500, 1987.
85. Reed A, Plante N. Decline in body mass, size, and condition of greater Snow Geese, 1975–94. *J Wildl Manage* 61: 413–419, 1997.
86. Reid JM, Arcese P, Keller LF. Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): direct and inter-generational effects. *P Roy Soc Lond B* 270: 2151–2157, 2003.
87. Rocha JS, Bonkowski MS, Masternak MM, Franca LR, Bartke A. Effects of adult onset mild calorie restriction on weight of reproductive organs, plasma parameters and gene expression in male mice. *Anim Reprod* 9: 40–51, 2012.
88. Rogovin K, Randall JA, Kolosova I, Moshkin M. Social correlates of stress in adult males of the great gerbil, *Rhombomys opimus*, in years of high and low population densities. *Horm Behav* 43: 132–139, 2003.
89. Romero LM, Wikelski M. Stress physiology as a predictor of survival in Galapagos marine iguanas. *Proc Roy Soc B* 277: 3157–3162, 2010.
90. Ruiz X, González-Solís J, Oro D, Jover L. Body size variation in Audouin's Gull *Larus audouinii*; a density-dependent effect? *IBIS* 140: 431–438, 1998.
91. Saino N, Canova L, Fasola M, Martinelli R. Reproduction and population density affect humoral immunity in bank voles under field experimental conditions. *Oecologia* 124: 358–366, 2000.
92. Sedinger JS, Lindberg MS, Person BR, Eichholz MW, Herzog MP, Flint PL. Density dependent effects on growth, body size and clutch size in Black brant. *Auk* 115: 613–620, 1998.
93. Sheriff MJ, Krebs CJ, Boonstra R. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *J Anim Ecol* 78: 1249–1258, 2009.
94. Sorci G, Soler JJ, Møller AP. Reduced immunocompetence of nestlings in replacement clutches of the European magpie (*Pica pica*). *Proc R Soc London Biol Sci* 264: 1593–1598, 1997.
95. Svensson E, Sinervo B, Comendant T. Density-dependent competition and selection on immune function in genetic lizard morphs. *Proc Nat Acad Sci USA* 98: 12,561–12,565, 2001.
96. Tella JL, Forero MG, Bertelotti M, Donazar JA, Blanco G, Ceballos O. Offspring body condition and immunocompetence are negatively affected by high breeding densities in a colonial seabird: a multiscale approach. *Proc R Soc London Biol Sci* 268: 1455–1461, 2001.
97. Tella JL, Scheuerlein A, Ricklefs RE. Is cell mediated immunity related to the evolution of life-history strategies in birds? *Proc R Soc London Biol Sci* 269: 1059–1066, 2002.
98. Terman CR. A study of population growth and control exhibited in the laboratory by prairie deer mice. *Ecology* 46: 890–895, 1965.
99. Terman CR. Populations of prairie deer mice: a test of pheromone influence. *Ecology* 49: 1169–1172, 1968.
100. Terman CR. Weights of selected organs of deer mice from asymptotic laboratory populations. *J Mammal* 50: 311–320, 1969.
101. Terman CR. Studies of natural populations of white-footed mice: reduction of reproduction at varying densities. *J Mammal* 74: 678–687, 1993.
102. Thomason CA, Hedrick-Hopper TL, Derting TL. Social and nutritional stressors: agents for altered immune function in white-footed mice (*Peromyscus leucopus*). *Can J Zool* 91: 313–320, 2013.
103. Trenzado CE, Morales AE, de la Higuera M. Physiological effects of crowding in rainbow trout, *Oncorhynchus mykiss*, selected for low and high stress responsiveness. *Aquaculture* 258: 583–593, 2006.
104. Van Duyse E, Pinxten R, Darras VM, Arckens L, Eens M. Opposite changes in plasma testosterone and corticosterone levels following a simulated territorial challenge in male great tits. *Behaviour* 141: 451–467, 2004.
105. Wallace JC, Kolbeinshavn AG, Reinsnes TG. The effects of stocking density on early growth of Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* 73: 101–110, 1988.
106. Weintraub A, Singaravelu J, Bhatnagar S. Enduring and sex-specific effects of adolescent isolation in rats on adult stress reactivity. *Brain Res* 1343: 83–92, 2010.
107. Wolff JO. Laboratory studies with rodents: facts or artifacts? *BioScience* 53: 421–427, 2004.
108. Yin Z, Lam TJ, Sin YM. The effects of crowding stress on the nonspecific immune response in fancy carp (*Cyprinus carpio* L.). *Fish Shellfish Immunol* 5: 519–529, 1995.
109. Yom-Tov Y, Yom-Tov S, MacDonald D, Yom-Tov E. Population cycles and changes in body size of the lynx in Alaska. *Oecologia* 152: 239–244, 2007.