Integrative and Comparative Biology

Integrative and Comparative Biology, volume 57, number 2, pp. 195–206
doi:10.1093/icb/icx016

Society for Integrative and Comparative Biology

SYMPOSIUM

The Physiology of Exercise in Free-Living Vertebrates: What Can We Learn from Current Model Systems?

Kang Nian Yap,[1,*] Mitchell W. Serota[*] and Tony D. Williams[*]

*Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British V5A 1S6, Canada, Columbia

From the symposium "The Ecology of Exercise: Mechanisms Underlying Individual Variation in Movement Behavior, Activity or Performance" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2017 at New Orleans, Louisiana.

[1]E-mail: knyap@sfu.ca

Synopsis Many behaviors crucial for survival and reproductive success in free-living animals, including migration, foraging, and escaping from predators, involve elevated levels of physical activity. However, although there has been considerable interest in the physiological and biomechanical mechanisms that underpin individual variation in exercise performance, to date, much work on the physiology of exercise has been conducted in laboratory settings that are often quite removed from the animal's ecology. Here we review current, laboratory-based model systems for exercise (wind or swim tunnels for migration studies in birds and fishes, manipulation of exercise associated with non-migratory activity in birds, locomotion in lizards, and wheel running in rodents) to identify common physiological markers of individual variation in exercise capacity and/or costs of increased activity. Secondly, we consider how physiological responses to exercise might be influenced by (1) the nature of the activity (i.e., voluntary or involuntary, intensity, and duration), and (2) resource acquisition and food availability, in the context of routine activities in free-living animals. Finally, we consider evidence that the physiological effects of experimentally-elevated activity directly affect components of fitness such as reproduction and survival. We suggest that developing more ecologically realistic laboratory systems, incorporating resource-acquisition, functional studies across multiple physiological systems, and a life-history framework, with reproduction and survival end-points, will help reveal the mechanisms underlying the consequences of exercise, and will complement studies in free-living animals taking advantage of new developments in wildlife-tracking.

Introduction

Exercise can be defined as physical activity that involves movement supported by sustained locomotor performance, increased cardiac output, and increases in energy expenditure above basal levels (Piersma 2011; Booth et al. 2012). As a consequence, the term can theoretically be applied to most activities or behaviors exhibited by free-living animals, for instance, migration, foraging, finding mates and escaping predators (though there is currently little evidence that free-living animals do "exercise"; Halsey 2016). All of these behaviors are essential for successful reproduction and survival. Thus, it would be predicted that individuals with higher "exercise capacity," or ability to cope with the costs of exercise (i.e., reduced fecundity or survivorship due to exercise), would have higher fitness. Furthermore, there has been a long-standing interest in the physiological and biomechanical mechanisms that underpin individual variation in exercise performance (Arnold 1983; Lailvaux and Husak 2014; Storz et al. 2015). Nevertheless, to date, much work on physiology of exercise has been conducted using captive animals in laboratory settings that are often quite different from the animal's ecology, for example, wheel-running in mice, wind tunnel flights in birds, and treadmill endurance tests in lizards (see below for references). Recent technological advances in methods for wildlife tracking or bio-logging (Wilmers et al. 2015) are giving biologists an unprecedented ability to track the behavior of free-living animals 24/7. However, this technology also provides

Advance Access publication June 28, 2017

physiologists and endocrinologists with an unprecedented opportunity to investigate the physiological mechanisms underlying individual variation in movement behavior, activity, or performance in free-living animals. This raises the question of how we should approach the integration of physiology and behaviour in the context of movement in free-living animals, for example, what physiological traits should we measure?

Here we review current, laboratory-based, model systems for exercise or activity to ask if these can inform us about common physiological markers underlying high exercise capacity (Halsey 2016) or the physiological costs underlying reduced fecundity and/or survival due to exercise, that might provide relevant "targets" for mechanistic analysis of routine activities in free-living animals. We review studies using wind or swim tunnels to mimic migration in birds and fishes, manipulation of exercise associated with non-migratory activity in birds, locomotion in lizards and wheel running in rodents. Our main objectives are first to identify common physiological signatures of individual variation in exercise capacity and/or costs of exercise, focusing on suites of functionally-related traits, for example, those underpinning aerobic or metabolic capacity, intermediary metabolism or energy supply, oxidative stress, and immune function. A table summarizing a list of common physiological markers will be provided at the end of this review (Table 1). Second, we consider how physiological responses to exercise or activity might be influenced by (1) the nature of the exercise (i.e., voluntary or involuntary, intensity, duration, and aerobic scope of activity) performed by animals in each model system and (2) resource acquisition and food availability, in the context of routine activities in free-living animals. Finally, we consider evidence that the physiological effects of experimentally-elevated activity affect components of fitness such as reproduction and survival.

Migration in birds and fishes

Migration is ubiquitous across many taxonomic groups, including birds, fish, reptiles, insects, and mammals (Bowlin et al. 2010), although much of what we know about migration comes from migratory birds and fish. Large-scale migrations often involve long-distance movements lasting from a few days to weeks (Bowlin et al. 2010; Guglielmo 2010) requiring metabolic scopes of $8\times$ basal metabolic rate (BMR) in birds (Piersma 2011) and $7–15\times$ standard metabolic rate (SMR) in salmonids (Morash et al. 2013). Recent technological advancements, for example, small data loggers, digital telemetry, wind tunnel, and swim tunnel respirometry, have advanced the study of physiological aspects of animal migration not only in the field, but also under controlled laboratory conditions. Though there are some conflicting results between laboratory- and field-based studies, laboratory based migration studies have largely been successful at providing a parallel for long-distance migration in natural settings (Bowlin et al. 2010), for example, energy expenditure of birds flying in wind tunnels is consistent with data gathered from transoceanic flights by shorebirds (Piersma 2011).

Migration can therefore be extremely energetically demanding compared to other activities such as lactation and thermoregulation (in terms of metabolic scope) and it is not surprising that a range of physiological changes associated with aerobic capacity and fuel metabolism sometimes result from this high-intensity exercise (Palstra and Planas 2011; Piersma 2011; Pierce and McWilliams 2014; Butler 2016). Given that considerable variation exists within and among species in migratory behavior and distance, we might expect to see some differences in physiological traits mediating these aspects of migration (Terrill 1990). Nevertheless, many migratory birds and fish appear to up-regulate a common suite of physiological traits associated with aerobic and metabolic capacity in preparation for and during migration, increasing hematocrit, muscle myoglobin, and activity of metabolic enzymes (Palacios et al. 1984; Wingfield et al. 1990; Morash et al. 2013). Laboratory studies that simulate migration in fish using a swim tunnel have confirmed some of the field observations with increased citrate synthase (CS) activity (Morash et al. 2014), increased vascularization and angiogenesis, as well as myogenesis (Palstra et al. 2014). Fuel use or metabolism is probably the most researched aspect of migration physiology and field data suggest that most migratory birds and salmonids use predominantly lipids to fuel migration (Weber 2009; Guglielmo 2010; Butler 2016). Migrants up-regulate lipoproteins such as muscle fatty-acid binding proteins to increase transport of fuel to working muscles to sustain the exercise necessary for migration (Weber 2009; Guglielmo 2010). Again, similar findings have been reported in laboratory studies, for example, fish show an increase in lipoprotein lipase activity in response to endurance training in a swim tunnel (Weber et al. 2016).

Are there common physiological markers of "costs" associated with the high metabolic demand imposed by migration (i.e., exercising at or close to

Table 1 Summary of physiological markers associated with exercise in different model systems in relation to intensity and duration of exercise

			Physiological markers			
Model systems	**Exercise intensity**	**Exercise duration**	**Aerobic capacity**	**Fuel metabolism**	**Oxidative stress**	**Immune function**
Migration in birds and fish	8 × BMR (birds) 7–15 × SMR (fish)	Days	↑ Hematocrit ↑ Myoglobin ↑ Metabolic enzyme activity	↑ Fatty-acid binding proteins ↑ Lipoprotein ↑ Lipoprotein lipase activity	↓ Anti-oxidant capacity ↑ DNA damage	↓ Constitutive immune function
Exercise associated with non-migratory activity in birds	3–4 × BMR	Weeks	↑ Muscle CS activity	↑ CPT ↑ HOAD ↑ Fatty-acid binding proteins	↑ Plasma oxidative damage ↓ Thiol concentration ↓ Biochemical integration of antioxidant defenses ↓ MDA[a]	No data
Endurance and sprint speed in lizards	10 × SMR	Seconds to minutes	↑ Hematocrit ↑ Heart CS activity ↑ Liver pyruvate kinase	No data	No data	↓ Immune function
Wheel-running activity in rodents	2–6 × BMR	Days to weeks depending on training regime	↑ Hematocrit ↑ CS activity ↑ Muscle capillarity ↑ Mitochondrial volume and number ↑ Vascular endothelial growth factor	↑ Insulin-stimulated glucose uptake[b] ↑ Liver and muscle glycogen level[c] ↓ Glucose oxidation[c] ↑ Plasma membrane fatty-acid binding protein[c]	↓ Superoxide-dismutase-2 activity[b] ↓ Catalase activity[b] ↓ Lipid peroxidation[c] ↓ Protein carbonyls[c] ↓ TBARS[c]	↓ Immune function[b] Dose-dependent effect on immune function[c]

[a]Only observed under escape flight training regime.
[b]Observed in artificially selected mice.
[c]Observed in endurance exercise trained mice.

maximal aerobic capacity for days and sometimes weeks) (Piersma 2011)? European starlings, *Sturnus vulgaris*, showed a decrease in constitutive immune function immediately after an endurance flight in a wind tunnel experiment (Nebel et al. 2012). Similarly, Chinook salmon, *Oncorhynchus tshawytscha*, suffer from loss of immune functions during spawning migration, possibly due to diminished energy reserves or hormonal changes associated with spawning (Dolan et al. 2016). Wilson et al. (2014) found that spawning migration causes decreased antioxidant capacity and higher oxidative DNA damage in various tissues in pink salmon. However, although it has been documented that exercise associated with migration causes reduced spawning success and increased mortality in salmon (Berman and Quinn 1991) and birds (Baduini et al. 2001), more research is needed to causally link observed mortality to markers of physiological costs such as oxidative damage and immune suppression.

With the exceptions of migratory flights of short-hop migrant species, almost all long-distance migratory flights and spawning migrations are associated with fasting (Dickhoff et al. 1997; Guglielmo 2010; Piersma 2011; Eliason and Farrell 2016). Thus, some physiological adjustments observed in preparation for, and during migration, could be due to food deprivation, changes in activity level and intensity, or a combination of both factors. For instance, several long-jump migrant species exhibit facultative reduction of the digestive organs as an energy saving mechanism (Piersma et al. 1993, 1999) though this physiological adjustment could also be due to lack of food ingestion per se. Conversely, the observed increase in fatty-acid binding protein levels in migratory birds (Weber 2009; Guglielmo 2010) and up-regulation of plasma lipoproteins in salmonids (Magnoni et al. 2006) are most likely a direct response of increased activity level. However, changes in fuel selection in migratory birds and fish, probably involve demands of both exercise and osmoregulation: for example, Guglielmo (2010) suggested that while birds catabolize proteins and lipids in muscle during migration (Jenni and Jenni-Eiermann 1998), they also generate metabolic water under dehydrating conditions. It is currently unclear whether birds that engage in activity such as foraging for food, exercising at lower-intensity but for longer duration than migrating birds (Drent and Daan 1980), use the same fuel mixture for exercise, or upregulate lipid metabolism enzymes (e.g., lipoprotein lipase).

Despite the high metabolic demand of migration, associated with a wide range of physiological changes, evidence of "cost" is scarce, mainly because lab studies have not often investigated "fitness" endpoints such as reproduction and survival. To address this particular caveat, researchers could design lab experiments that investigate carry-over effects (processes in one season affecting processes in subsequent season) of migration on reproduction, for example, simulating migratory flight in wind tunnels (for birds) and spawning migration in swim tunnels (for fishes) and then allowing individuals to breed to assess reproductive success. Findings from these lab studies can be used to complement field studies that are often limited by researchers' ability to obtain detailed physiological measurements from large samples of animals.

Manipulation of exercise associated with non-migratory activity in birds in a laboratory setting

Birds exhibit a wide range of non-migratory behaviors that impose elevated metabolic demand at a lower (3–4 × BMR) but more sustained level (3–4 weeks) than during migration (e.g., foraging, chick provisioning during parental care; Piersma 2011). One method used in early studies to manipulate foraging effort involved increasing chaff/seed ratio so birds had to work harder or longer to maintain intake rates (Deerenberg and Overkamp 1999; Lemon 1993, Wiersma and Verhulst 2005), though this method had problems—birds continuously increase the chaff:seed ratio by consuming seeds and dropping chaff in the mix, making it a challenge to maintain the experimentally-targeted food ratio (Koetsier and Verhulst 2011). More recently, a number of automated systems have been developed to manipulate flight activity in captive birds (Nudds and Bryant 2000; Koetsier and Verhulst 2011;Costantini et al. 2012). Energy expenditure of birds subjected to increased exercise using these techniques generally resemble those of "hard working" birds feeding chicks in the field (Wiersma et al. 2005). Other lab studies confirm that birds trained to work harder show significantly higher exercise capacity post-training and compared to sedentary controls (Butler and Turner 1987; Zhang et al. 2015b).

Is there evidence from laboratory studies in birds that sustained, but lower-intensity exercise (3–4 × BMR) is associated with up-regulation of physiological traits, and are these the same traits that are modified during high-intensity exercise (e.g., migration)? In relation to traits associated with aerobic capacity, pectoralis muscle CS activity, an indicator of cellular aerobic capacity, increases in birds that are

trained to fly non-stop between two perches located 6 m apart for 45 min each day over 30 days (Zhang et al. 2015a). This finding is consistent with changes in free-living birds preparing for migration (Guglielmo et al. 2002) and cold-acclimatized birds in winter (Vézina and Williams 2005). In addition, similar to migratory birds, captive birds that are exercise trained also up-regulate a suite of physiological traits associated with fatty-acids transport and oxidation, for example, carnitine palmitoyl transferase (CPT), β-hydroxyacyl CoA-dehydrogenase (HOAD) and fatty-acid binding proteins (Zhang et al. 2015b). Hence, sustained work at lower-intensity but for longer duration appears to involve similar physiological changes to those associated with higher aerobic scope activities such as migration and cold-acclimatization.

Does sustained, lower-intensity exercise generate physiological costs? Traditionally, many studies have focused on the energetic costs of exercise (but see Nilsson 2002; Veasey et al. 2001; Simons et al. 2014) although the idea of non-energy based mediators of carry-over effects has been increasingly recognised (Zera and Harshman 2001; Williams 2012). For example, Costantini et al. (2012) showed that increased flight activity (distance: 165.8 m × 3 days) in zebra finches, *Taeniopygia guttata*, leads to increased plasma oxidative damage and decreased thiol concentration in red blood cells. Costantini et al. (2013) then investigated biochemical integration of the blood redox system (i.e., how different antioxidants interact synergistically and/or competitively and how antioxidants respond to changes in levels of oxidative damage). They found that increased flight activity caused a reduction in biochemical integration among different components of blood antioxidant defences. However, contrary to the findings by Costantini et al. (2012), Larcombe et al. (2010) found that levels of malondialdehyde (MDA) in budgerigars, *Melopsittacus undulates*, decreased after 9 weeks of escape flight training (flown six times on 1 day per week), suggesting that the type of exercise training and the duration of training play important roles in mediating exercise induced oxidative stress. Hard work may depress the immune system in mammals (Pedersen and Hoffman-Goetz 2000; Nieman 2000), but the idea has yet to be tested experimentally for sustained, low-intensity exercise in birds in a laboratory setting.

Are the physiological correlates and costs of sustained, low-intensity exercise observed in lab-based studies ecologically relevant? Furthermore, are there discrepancies among different lab-based studies utilizing similar technique to manipulate exercise? Despite the fact that researchers studying birds in lab setting managed to simulate exercise intensity and duration typical of free-living birds (3–4 × BMR) we need to be cautious about extrapolating these findings to "hard-working" free-living birds (e.g., brood provisioning birds) (Drent and Daan 1980). First, while some lab based studies in zebra finch have found an increase in daily energy expenditure (DEE) with increased exercise (Wiersma and Verhulst 2005), other studies using the same species and European Starling either found no change in DEE (Lemon 1993), or a decrease in DEE (Bautista et al. 1998) respectively, with increased exercise. Wiersma and Verhulst (2005) suggested that these discrepancies might be due to the design of the reward schedule, which ties into the animal's foraging motivation. Furthermore, many of the studies that utilize automated systems to manipulate exercise in birds (e.g., Costantini et al. 2012; Nudds and Bryant 2000; Zhang et al. 2015b) did not include elements of resource acquisition and food availability, and often involved forced exercise (see Fonseca et al. 2014). The techniques for manipulation of foraging effort with resource acquisition developed by Koetsier and Verhulst (2011) and Wiersma et al. (2005) overcome this issue and could prove valuable in future studies.

Physiological costs incurred due to sustained, low intensity exercise in laboratory systems can lead to downstream effects of reduced survival and/or reproduction. Increased exercise intensity at 2–3 × BMR for 2–5 weeks can delay onset of reproduction, though other metrics of reproductive output such as clutch size, brood size, egg mass, and fledgling number do not seem to be affected (Deerenberg and Overkamp 1999; Wiersma and Verhulst 2005; Simons et al. 2014). However, these studies did not comprehensively assess physiological response to increased foraging effort, making it difficult to causally link indicators of physiological costs to delayed reproduction. Wiersma and Verhulst (2005) found that birds exposed to increased foraging cost invested less in somatic maintenance though it was not shown that this lead to decreased survival. Reichert et al. (2014) looked at effects of brood size manipulation on telomere dynamics and survival in captive zebra finch. Although enlarged brood size led to greater telomere loss in parents, they did not detect any effects of brood size manipulation on short-term survival (Reichert et al. 2014). Reichert et al. (2014) speculated that the ad libitum feeding conditions might have prevented detection of a significant negative effect on adult survival, again highlighting the importance of considering resource acquisition as a component of lab studies. Briga et al. (2017)

addressed the issue of resource acquisition by directly manipulating foraging effort in zebra finch using the technique developed by Koetsier and Verhulst (2011) and found that birds raised in larger brood size suffered higher mortality when subjected to increased exercise in adulthood, although actuarial senescence did not seem to be affected.

Endurance and sprint speed in lizards

Locomotion as a form of exercise has been studied extensively in lizards (Irschick and Losos 1998; Le Galliard et al. 2004; Husak et al. 2006), and is viewed as an integral contributor to reproductive success and survival (Irschick and Garland 2001; Lailvaux and Husak 2014). In terms of intensity of exercise, many lizard studies distinguish two components of locomotion: sprint speed and endurance, both of which are ecologically relevant. Lizards may need a high sprint speed in order to escape predators (Irschick and Losos 1998), or in some cases to forage, but lizards may also require endurance to patrol their territory or to forage more widely (Le Galliard et al. 2013). Thus, sit-and-wait predators can sprint after their prey at a high-intensity for a short period of time (10 × SMR), while widely foraging lizards will work at a lower metabolic scope for a longer period of time (Nagy et al. 1984). Although maximal aerobic capacity might also play a more important role in foraging and patrolling a territory given these activities involve intermittent bouts of intense movement (John-Alder 1984; John-Alder et al. 2009), the research effort on maximal aerobic capacity has been limited to date for these types of behavior. Hence, we will focus on sprint speed and endurance for the purpose of this review. Perhaps surprisingly, early studies of exercise training in lizards, including training for treadmill endurance, maximum run time, and maximum burst speed often failed to lead to an increase in running performance or changes in metabolic correlates or performance (Gleeson 1979; Garland et al. 1987; O'Connor et al. 2011). Husak et al. (2015) suggested that this lack of response could be due to (1) an insufficiently intense training regimen, (2) a training regimen too intense resulting in skeletal joint degradation, and (3) a lack of specialized exercise, that is, endurance versus speed. Using a training regimen specific for either endurance or sprinting, Husak et al. (2015) found that endurance, but not sprint speed, did improve and that this was associated with metabolic changes (increased hematocrit and increased size of fast glycolytic muscle fibers). Another possible reason for inconsistent results on effects of training on performance and physiology is that many studies used different species, sometimes distantly related, the discrepancies regarding physiological and performance can sometimes be confounded by phylogeny (Huey et al. 2001).

Does exercising for short bursts at a high-intensity (i.e., sprinting) or running at a lower-intensity for a longer period of time (i.e., endurance) induce physiological costs in lizards, and are the same traits affected? Much research on lizard locomotion has focused on morphology, rather than physiology per se. Vanhooydonck et al. (2015) found a trade-off between power output and fatigue resistance using data from isolated muscle tissue in 17 lacertid lizard species, though this trade-off was not apparent at the whole organism level. At the intraspecific level too, effects of exercise training on morphology and physiology has produced equivocal results. After training for submaximal exercise at 1 km/h on a motorized treadmill for 5 days a week and 8 weeks in total, *Amphibolurus nuchalis* showed decreased heart and thigh muscle mass, but increased liver mass, hematocrit, liver pyruvate kinase, and heart CS activity (Garland and Else 1987;Garland et al. 1987). Meylan et al. (2013) found that *Zootoca vivipara*, when mounting an immune response did not compromise their treadmill endurance. However, Garland et al. (1987) observed that some lizards exhibit swelling and partial immobilization of hind limbs and muscle fiber necrosis in response to endurance training, potentially due to depression of immune system. Furthermore, green anole lizards, *Anolis carolinensis*, when subject to endurance training for 2 days a week and 9 weeks in total, suppressed reproduction and immune function, and immune suppression was associated with increased corticosterone (Husak et al. 2016).

Are laboratory-based systems used to investigate exercise in lizards, and the physiological changes revealed by these techniques, ecologically relevant? Given that the vast majority of lizard locomotion research uses treadmills and circular racetracks, we must first consider whether varying environments affect performance (Irschick and Garland 2001). Lizards in nature may choose to vary their speed and duration of activity to increase their efficiency (Christian et al. 1997; Irschick and Jayne 1999). In addition, differing substrates might alter the performance of lizards (Sathe and Husak 2015; Vanhooydonck et al. 2015). In a field experiment, *Uma scoparia* moved about their habitat at two preferred speeds depending on the environment and incline of the surface (Jayne and Irschick 2000). Moreover, while sprint speed may be important for

escaping predators and obtaining prey, there may be a trade-off between speed and maneuverability which may affect the lizard's fitness (Wynn et al. 2015). Therefore, one could postulate that the use of forced exercise on treadmills and circular tracks in laboratory studies may inaccurately represent individual performance of free-living animals (Irschick and Garland 2001).

Finally, do the physiological costs associated with endurance and sprint training in lizards lead to negative effects on fitness? Endurance in juvenile *Lacerta vivipara* predicted survival (Le Galliard et al. 2004) and Clobert et al. (2000) found that individual *Lacerta vivipara* with low endurance at birth tended to have reduced activity and growth rate, and a higher parasite load, although they experienced lower predation risk as assessed by tail loss. Conversely, individuals with high endurance at birth had higher activity and growth rates, lower parasite load, but higher incidence of tail loss though endurance at birth was not correlated with survivorship. In some lizards, sprint speed predicts juvenile survival (Miles 2004; Husak 2006), though not adult survival (Husak 2006; Irschick et al. 2008). Sprint speed also predicts mating success and the number of offspring in some lizards (Husak et al. 2006). Nevertheless, given that increasing aerobic performance through training in the laboratory has proven to be difficult, data on exactly how physiological changes mediate the effect of exercise on reproduction and/or survival in lizards are largely lacking (Husak et al. 2015). It is also important to note that it is unclear if lizards undergo training-like situations in the wild. If most of the within-individual variation in locomotor performances in the wild are caused by hormonal fluctuations, for example, then the effect of training over fitness is not important ecologically (John-Alder et al. 2009).

Wheel-running activity in rodents

Wheel-running in captive rodents is perhaps the most well-studied model system for exercise in non-human animals (Sherwin 1998; Garland et al. 2011; Meijer and Robbers 2014; Mason and Wurbel 2016) utilizing two main experimental approaches: endurance training (voluntary and involuntary) and artificial selection on voluntary wheel-running activity. Involuntary endurance training in rodents often involves using electrical stimulation on treadmills to force animals to exercise (Even et al. 1998; Chappell et al. 2007). In contrast, training paradigms for voluntary exercise involves giving animals access to a running wheel and allow them to run willingly (Sherwin 1998; Novak et al. 2012). Artificial selection experiments have identified changes in suites of morphological and physiological traits, as well as the underlying neuroendocrine mechanisms, associated with increased exercise performance (Garland 2003; Rhodes et al. 2005; Rhodes and Kawecki 2009; Swallow et al. 2009). These varied experimental approaches result in considerable variability in metabolic scope associated with wheel-running exercise but this generally ranges from 2 to 6 × BMR (Even et al. 1998; Koteja et al. 1999; Garland et al. 2011) so this would represent sustained, relatively low intensity activity (cf. migration).

Most studies of rodents, using either endurance training or artificial selection, have shown an increase in exercise capacity (Conley et al. 1985; Willis et al. 1988; Swallow et al. 1998; Hoydal et al. 2007; de Araujo et al. 2016), so do we see up-regulation of similar physiological traits underlying this increased exercise? Once again traits underlying aerobic capacity such as CS activity, hematocrit, muscle capillarity, mitochondrial volume, and number, and exercise-induced vascular endothelial growth factor expression were up-regulated in response to both endurance training (Holloszy and Coyle 1984; Fentz et al. 2015; Hedrick et al. 2015;) and artificial selection for voluntary wheel-running (Houle-Leroy et al. 2000; Garland 2003; Swallow et al. 2005; Audet et al. 2011) (Table 1). In contrast, aside from higher insulin stimulated glucose uptake by muscles (Dumke et al. 2001; Garland 2003), there were few adjustments in fuel metabolism in mice selected for high voluntary wheel running activity. However, endurance trained rats and mice do show increased muscle and liver glycogen levels (de Araujo et al. 2016), decreased glucose oxidation (Even et al. 1998), and increased plasma membrane fatty-acid binding protein (Fentz et al. 2015). Thus, despite the common response of increasing exercise performance, physiological traits underlying high activity level seemed to be modulated differently in endurance training versus artificial selection.

Does a higher activity level, due either to endurance training or selection for high voluntary wheel running activity, generate physiological costs in rodents? As with metabolic changes (above), responses in immune function and oxidative state seem to be different depending on the specific training paradigm. Female mice selected for voluntary wheel-running exhibit reduced activities of the antioxidant enzymes superoxide-dismutase-2 and catalase, compared to control lines (Thomson et al. 2002).

Similarly, Downs et al. (2013) found that mice selected for high mass-independent metabolic rate have suppressed immune function, as measured by cytokine production in response to injection with lipopolysaccharide. Conversely, most studies that employ an endurance training approach have found positive effects of exercise on oxidative stress: decreased lipid peroxidation (Costa et al. 2014), decreased thiobarbituric acid-reactive substances (TBARS; Boveris and Navarro 2008; Oharomari et al. 2015), and decreased protein carbonyls (Oharomari et al. 2015). Gholamnezhad et al. (2014) also found that moderate exercise training results in a decrease in susceptibility to viral infection, that is a positive effect on immune function, but that "overtraining" caused immune suppression, characterized by prolonged IL-6 and TNFα elevation (Gholamnezhad et al. 2014). This again highlights the fact that the nature, intensity and duration of the training paradigm need to be considered in interpreting physiological correlates of exercise in these laboratory systems.

Given the centrality of wheel-running to mammalian models of exercise there has been debate about whether wheel-running behaviour in the lab reflects locomotion of free-living animals in the wild, that is whether this behavior is ecologically relevant. Some studies suggested that domestication of house mice has involved minor differences in overall physiology and behavior (Dohm et al. 1994; Richardson et al. 1994; Garland 2003; Rezende et al. 2005). Indeed, Meijers and Robbers (2014) placed running wheels in animals' natural habitat and found that free-living wild mice, shrews, rats (as well as snails, slugs and frogs) used the running wheels. However, the act of wheel running itself is not a goal-oriented activity and can become a self-perpetuating behavior that has the capacity to reach obsessive levels (Novak et al. 2012) and could be considered an "abnormal," maladaptive behavior (Sherwin 1998). The neurobiological profile of mice selected for wheel running does share features of Attention Deficit Hyperactivity Disorder (ADHD) indicating, perhaps, that mice become "addicted" to exercise (Rhodes et al. 2005; Garland et al. 2011; Kolb et al. 2013). Fonseca et al. (2014) showed that rats subjected to exercise contingent training protocol, where individuals need to perform exercise to acquire food, expressed significantly different physiological and morphological changes (e.g., lower adiposity, reduction in body mass, smaller liver, and heart masses) compared to mice subjected to non-exercise-contingent training protocol. Careful experimental design that addresses food-exercise contingency is needed in studies linking exercise, food, and physiology going forward.

Despite the vast body of literature on wheel-running activity in rodents, relatively few studies have investigated fitness consequences of wheel-running activity, despite some reported effects on the reproductive axis (e.g., Klomberg et al. 2002). Female golden hamsters housed with functional running wheels had significantly larger litters than those housed with non-functional wheels and Gebhardt-Henrich et al. (2005) attributed this to "improved well-being" due to the presence of functional wheels, for example less stereotypical bar-mouthing behavior. Endurance training, either forced or voluntary, improved survival in both rats and mice (Holloszy 1993; Boveris and Navarro 2008) but although exercise increased average longevity in rats, maximum lifespan was unaltered (Holloszy 1993). Given the role of oxidative stress in ageing and senescence (Selman et al. 2012) and findings that showed attenuation of oxidative stress response due to exercise (see above), it is conceivable that exercise could actually have positive effects on reproduction and survival in free-living animals.

Conclusion

A multitude of laboratory-based, model systems have been used to investigate "exercise" encompassing a wide range of intensities of activity. Nevertheless, our review indicates that there are some common physiological markers of increased exercise across systems, such as upregulation of traits underlying aerobic capacity (hematocrit, metabolic enzyme activity) in response to increased activity level (Table 1). However, specific changes in traits associated with fuel metabolism through training seem to be dependent on the intensity of the exercise and study system. In terms of costs of exercise, increased oxidative stress (DNA damage, lipid peroxidation, suppressed antioxidant enzyme activity) appears to be a common response (Table 1), though seemingly with the exception of training in rodents, and a decline in innate immune function (though not adaptive immune function) occurs in response to increased exercise across study systems. Nevertheless, we suggest that more ecologically relevant study systems to understand exercise in free-living animals should be developed. In the wild, free-living animals may choose to vary their speed and duration of activity to increase efficiency (e.g., Irschick and Jayne 1999). Moreover, resource acquisition is often the main goal of activity in the wild, but this is often absent from lab-based systems (Fonseca et al. 2014). As mentioned before, physiological adjustments observed in exercising animals could occur in response

to food deprivation (fasting), changes in activity level and intensity, or a combination of both factors. Finally, few laboratory studies directly link physiological correlates of high activity levels with downstream effects of reduced survival and reproduction; though these links are often assumed. Therefore, developing more ecological relevant laboratory systems (e.g., Fonseca et al. 2014; Briga et al. 2017), explicitly incorporating both functional studies across multiple physiological systems and a life-history framework, with reproduction and survival end-points, will help reveal the mechanisms underlying the consequences of exercise. The knowledge we gain from these laboratory based studies can then be used to complement studies in free living animals. In other words, new ground and space-based tracking can be used to field test what is learned from captive animals.

Acknowledgments

This article is based on a talk given by K.N.Y., M.W.S. and T.D.W. in the "Ecology of Exercise" symposium at the 2017 Society for Integrative and Comparative Biology meeting in New Orleans, Louisiana, USA. The authors thank Shaun Killen and Ryan Calsbeek for co-organizing the symposium.

Funding

We thank the National Science Foundation (Grant number IOS-1637178), Company of Biologists, British Ecological Society, Loligo systems and Society for Integrative and Comparative Biology's Divisions of Animal Behaviour, Comparative Biomechanics, Comparative Physiology and Biochemistry, Ecology and Evolution, and Vertebrate Morphology for funding the symposium. The authors are funded by a Natural Sciences and Engineering Council of Canada Discovery and Accelerator Grant to T.D.W. (Grant numbers 155395-2012- RGPIN and RGPAS/429387-2012).

References

Arnold S. 1983. Morphology, performance and fitness. Am Zool 23:347–61.

Audet GN, Meek TH, Garland TJ, Olfert IM. 2011. Expression of angiogenic regulators and skeletal muscle capillarity in selectively bred high aerobic capacity mice. Exp Physiol 96:1138–50.

Baduini C, Lovvorn J, Hunt G. 2001. Determining the body condition of short-tailed shearwaters: Implications for migratory flight ranges and starvation events. Mar Ecol Prog Ser 222:265–77.

Bautista L, Tinbergen J, Wiersma P, Kacelnik A. 1998. Optimal foraging and beyond: how starlings cope with changes in food availability. Am Nat 152:543–61.

Berman C, Quinn T. 1991. Behavioral thermoregulation and homing by spring chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in the Yakima River. J Fish Biol 39:301–12.

Booth FW, Roberts CK, Laye MJ. 2012. Lack of exercise is a major cause of chronic diseases. Compr Physiol 2:1143–211.

Boveris A, Navarro A. 2008. Systemic and mitochondrial adaptive responses to moderate exercise in rodents. Free Radic Biol Med 44:224–9.

Bowlin MS, Bisson I, Shamoun-Baranes J, Reichard JD, Sapir N, Marra PP, Kunz TH, Wilcove DS, Hedenstrom A, Guglielmo CG, et al. 2010. Grand challenges in migration biology. Integr Comp Biol 50:261–79.

Briga M, Koetsier E, Boonekamp JJ, Jimeno B, Verhulst S. 2017. Food availability affects adult survival trajectories depending on early developmental conditions. Proc R Soc B Biol Sci 284:20162287.

Butler PJ. 2016. The physiological basis of bird flight. Philos Trans Biol Sci 371:20150384.

Butler P, Turner D. 1987. Maximum oxygen uptake and capillarity and enzyme-activity in the gastrocnemius-muscle of trained and untrained tufted ducks, *Aythya fuligula*. J Physiol 394:131.

Chappell MA, Garland TJ, Robertson GF, Saltzman W. 2007. Relationships among running performance, aerobic physiology and organ mass in male Mongolian gerbils. J Exp Biol 210:4179–97.

Christian K, Baudinette R, Pamula Y. 1997. Energetic costs of activity by lizards in the field. Funct Ecol 11:392–7.

Clobert J, Oppliger A, Sorci G, Ernande B, Swallow J, Garland TJ. 2000. Trade-offs in phenotypic traits: endurance at birth, growth, survival, predation and susceptibility to parasitism in a lizard, *Lacerta vivipara*. Funct Ecol 14:675–84.

Conley KE, Weibel ER, Taylor CR, Hoppeler H. 1985. Aerobic activity estimated by exercise vs cold-exposure: endurance training effects in rats. Respir Physiol 62:273–80.

Costa AE, Silva JL, Simoes MJ, Nouailhetas VL. 2014. Morphofunctional alterations of the nonpregnant murine uterus in response to intense and exhaustive exercise are not related to oxidative stress. J Appl Physiol 116:604–10.

Costantini D, Monaghan P, Metcalfe NB. 2013. Loss of integration is associated with reduced resistance to oxidative stress. J Exp Biol 216:2213–20.

Costantini D, Mirzai N, Metcalfe NB. 2012. An automated system to control and manipulate the flight activity of captive birds. Behav Ecol Sociobiol 66:1195–9.

de Araujo GG, Papoti M, dos Reis Ivan Gustavo M, de Mello MA, Gobatto CA. 2016. Short and long term effects of high-intensity interval training on hormones, metabolites, antioxidant system, glycogen concentration, and aerobic performance adaptations in rats. Front Physiol 7:505.

Deerenberg C, Overkamp G. 1999. Hard work impinges on fitness: an experimental study with zebra finches. Anim Behav 58:173–9.

Dickhoff WW, Beckman BR, Larsen DA, Lee-Pawlak B. 1997. Physiology of migration in salmonids. Mem Fac Fish Hokkaido Univ 44:14–17.

Dohm MR, Richardson CS, Garland TJ. 1994. Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. Am J Physiol 67:1098–108.

Dolan BP, Fisher KM, Colvin ME, Benda SE, Peterson JT, Kent ML, Schreck CB. 2016. Innate and adaptive immune responses in migrating spring-run adult chinook salmon, *Oncorhynchus tshawytscha*. Fish Shellfish Immunol 48:136–44.

Downs CJ, Brown JL, Wone B, Donovan ER, Hunter K, Hayes JP. 2013. Selection for increased mass-independent maximal metabolic rate suppresses innate but not adaptive immune function. Proc R Soc B Biol Sci 280:20122636.

Drent RH, Daan S. 1980. The prudent parent - energetic adjustments in avian breeding. Ardea 68:225–52.

Dumke C, Rhodes J, Garland TJ, Maslowski E, Swallow J, Wetter A, Cartee G. 2001. Genetic selection of mice for high voluntary wheel running: effect on skeletal muscle glucose uptake. J Appl Physiol 91:1289–97.

Eliason EJ, Farrell AP. 2016. Oxygen uptake in pacific salmon *Oncorhynchus* spp.: when ecology and physiology meet. J Fish Biol 88: 359–88.

Even PC, Rieth N, Roseau S, Larue-Achagiotis C. 1998. Substrate oxidation during exercise in the rat cannot fully account for training-induced changes in macronutrients selection. Metab Clin Exp 47:777–82.

Fentz J, Kjobsted R, Kristensen CM, Hingst JR, Birk JB, Gudiksen A, Foretz M, Schjerling P, Viollet B, Pilegaard H, et al. 2015. AMPKα is essential for acute exercise-induced gene responses but not for exercise training-induced adaptations in mouse skeletal muscle. Am J Physiol Endocrinol Metab 309:900–14.

Fonseca IAT, Passos RLF, Araujo FA, Lima MRM, Lacerda DR, Pires W, Soares DD, Young RJ, Rodrigues LOC. 2014. Exercising for food: bringing the laboratory closer to nature. J Exp Biol 217:3274–82.

Garland TJ. 2003. Selection experiments: an under-utilized tool in biomechanics and organismal biology. Exptl Biol Rev 2003:23–56.

Garland TJ, Else PL. 1987. Seasonal, sexual, and individual variation in endurance and activity metabolism in lizards. Am J Physiol 252:439–49.

Garland TJ, Else PL, Hulbert AJ, Tap P. 1987. Effects of endurance training and captivity on activity metabolism of lizards. Am J Physiol 252:450–6.

Garland TJ, Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, Acosta W, Drenowatz C, Maciel RC, van Dijk G, et al. 2011. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. J Exp Biol 214:206–29.

Gebhardt-Henrich SG, Vonlanthen EM, Steiger A. 2005. How does the running wheel affect the behaviour and reproduction of golden hamsters kept as pets? Appl Anim Behav Sci 95:199–203.

Gholamnezhad Z, Boskabady MH, Hossein M, Sankian M, Khajavi RA. 2014. Evaluation of immune response after moderate and overtraining exercise in wistar rat. Iran J Basic Med Sci 17:1–8.

Gleeson TT. 1979. The effects of training and captivity on the metabolic capacity of the lizard *Sceloporus occidentalis*. J Comp Physiol 129:123–8.

Guglielmo CG. 2010. Move that fatty acid: fuel selection and transport in migratory birds and bats. Integr Comp Biol 50:336–45.

Guglielmo C, Haunerland N, Hochachka P, Williams T. 2002. Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a migratory shorebird. Am J Physiol Regul Integr Comp Physiol 282:1405–13.

Halsey LG. 2016. Do animals exercise to keep fit? J Anim Ecol 85:614–20.

Hedrick MS, Hancock TV, Hillman SS. 2015. Metabolism at the max: how vertebrate organisms respond to physical activity. Compr Physiol 5:1677–703.

Holloszy JO. 1993. Exercise increases average longevity of female rats despite increased food intake and no growth retardation. J Gerontol 48:97–100.

Holloszy JO, Coyle EF. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol Respir Environ Exerc Physiol 56:831–8.

Houle-Leroy P, Garland TJ, Swallow JG, Guderley H. 2000. Effects of voluntary activity and genetic selection on muscle metabolic capacities in house mice *Mus domesticus*. J Appl Physiol 89:1608–16.

Hoydal MA, Wisloff U, Kemi OJ, Ellingsen O. 2007. Running speed and maximal oxygen uptake in rats and mice: practical implications for exercise training. Eur J Cardiovasc Prev Rehabil 14:753–60.

Huey R, Pianka E, Vitt L. 2001. How often do lizards "run on empty"?. Ecology 82:1–7.

Husak JF. 2006. Does speed help you survive? A test with collared lizards of different ages. Funct Ecol 20:174–9.

Husak JF, Ferguson HA, Lovern MB. 2016. Trade-offs among locomotor performance, reproduction and immunity in lizards. Funct Ecol 30:1665–74.

Husak JF, Fox SF, Lovern MB, Van Den Bussche RA. 2006. Faster lizards sire more offspring: sexual selection on whole-animal performance. Evolution 60:2122–30.

Husak JF, Keith AR, Wittry BN. 2015. Making Olympic lizards: the effects of specialised exercise training on performance. J Exp Biol 218:899–906.

Irschick D, Garland T. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. Annu Rev Ecol Syst 32:367–96.

Irschick D, Jayne B. 1999. A field study of the effects of incline on the escape locomotion of a bipedal lizard, *Callisaurus draconoides*. Physiol Biochem Zool 72:44–56.

Irschick D, Losos J. 1998. A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. Evolution 52:219–26.

Irschick DJ, Meyers JJ, Husak JF, Le Galliard J. 2008. How does selection operate on whole-organism functional performance capacities? A review and synthesis. Evol Ecol Res 10:177–96.

Jayne B, Irschick D. 2000. A field study of incline use and preferred speeds for the locomotion of lizards. Ecology 81:2969–83.

Jenni L, Jenni-Eiermann S. 1998. Fuel supply and metabolic constraints in migrating birds. J Avian Biol 29:521–8.

John-Alder HB. 1984. Seasonal variations in activity, aerobic energetic capacities, and plasma thyroid hormones (T3 and T4) in an iguanid lizard. J Comp Physiol B 154:409–19.

John-Alder HB, Cox RM, Haenel GJ, Smith LC. 2009. Hormones, performance and fitness: natural history and endocrine experiments on a lizard (*Sceloporus undulatus*). Integr Comp Biol 49:393–407.

Klomberg KF, Garland TJ, Swallow JG, Carter PA. 2002. Dominance, plasma testosterone levels, and testis size in house mice artificially selected for high activity levels. Physiol Behav 77:27–38.

Koetsier E, Verhulst S. 2011. A simple technique to manipulate foraging costs in seed-eating birds. J Exp Biol 214:1225–9.

Kolb EM, Kelly SA, Garland T. 2013. Mice from lines selectively bred for high voluntary wheel running exhibit lower blood pressure during withdrawal from wheel access. Physiol Behav 112:49–55.

Koteja P, Swallow JG, Carter PA, Garland TJ. 1999. Energy cost of wheel running in house mice: Implications for coadaptation of locomotion and energy budgets. Physiol Biochem Zool 72:238–49.

Lailvaux SP, Husak JF. 2014. The life history of whole-organism performance. Q Rev Biol 89:285–318.

Larcombe SD, Coffey JS, Bann D, Alexander L, Arnold KE. 2010. Impacts of dietary antioxidants and flight training on post-exercise oxidative damage in adult parrots. Comp Biochem Physiol B Biochem Mol Biol 155:49–53.

Le Galliard J, Paquet M, Cisel M, Montes-Poloni L. 2013. Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. Funct Ecol 27:136–44.

Le Galliard J, Clobert J, Ferriere R. 2004. Physical performance and Darwinian fitness in lizards. Nature 432:502–5.

Lemon W. 1993. The energetics of lifetime reproductive success in the zebra finch *Taeniopygia guttata*. Physiol Zool 66:946–63.

Magnoni LJ, Patterson DA, Farrell AP, Weber J. 2006. Effects of long-distance migration on circulating lipids of sockeye salmon (*Oncorhynchus nerka*). Can J Fish Aquat Sci 63:1822–9.

Mason G, Wurbel H. 2016. What can be learnt from wheel-running by wild mice, and how can we identify when wheel-running is pathological? Proc R Soc B Biol Sci 283:1824.

Meijer JH, Robbers Y. 2014. Wheel running in the wild. Proc R Soc B Biol Sci 281:1786.

Meylan S, Richard M, Bauer S, Haussy C, Miles D. 2013. Costs of mounting an immune response during pregnancy in a lizard. Physiol Biochem Zool 86:127–36.

Miles DB. 2004. The race goes to the swift: fitness consequences of variation in sprint performance in juvenile lizards. Evol Ecol Res 6:63–75.

Morash AJ, Vanderveken M, McClelland GB. 2014. Muscle metabolic remodeling in response to endurance exercise in salmonids. Front Physiol 5:452.

Morash AJ, Yu W, Le Moine CMR, Hills JA, Farrell AP, Patterson DA, McClelland GB. 2013. Genomic and metabolic preparation of muscle in sockeye salmon *Oncorhynchus nerka* for spawning migration. Physiol Biochem Zool 86:750–60.

Nagy K, Huey R, Bennett A. 1984. Field energetics and foraging mode of Kalahari lacertid lizards. Ecology 65:588–96.

Nebel S, Bauchinger U, Buehler DM, Langlois LA, Boyles M, Gerson AR, Price ER, McWilliams SR, Guglielmo CG. 2012. Constitutive immune function in European starlings, *Sturnus vulgaris*, is decreased immediately after an endurance flight in a wind tunnel. J Exp Biol 215:272–8.

Nieman D. 2000. Exercise effects on systemic immunity. Immunol Cell Biol 78:496–501.

Nilsson J. 2002. Metabolic consequences of hard work. Proc R Soc B Biol Sci 269:1735–9.

Novak CM, Burghardt PR, Levine JA. 2012. The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward. Neurosci Biobehav Rev 36:1001–14.

Nudds R, Bryant D. 2000. The energetic cost of short flights in birds. J Exp Biol 203:1561–72.

O'Connor JL, McBrayer LD, Higham TE, Husak JF, Moore IT, Rostal DC. 2011. Effects of training and testosterone on muscle fiber types and locomotor performance in male six-lined racerunners (*Aspidoscelis sexlineata*). Physiol Biochem Zool 84:394–405.

Oharomari LK, Garcia NF, de Freitas EC, Júnior AAJ, Ovídio PP, Maia AR, Davel AP, de Moraes C. 2015. Exercise training and taurine supplementation reduce oxidative stress and prevent endothelium dysfunction in rats fed a highly palatable diet. Life Sci 139:91–96.

Palacios L, Palomeque J, Riera M, Pages T, Viscor G, Planas J. 1984. Oxygen-transport properties in the starling, *Sturnus vulgaris* L. Comp Biochem Physiol A Physiol77:255–60.

Palstra AP, Rovira M, Rizo-Roca D, Ramon Torrella J, Spaink HP, Planas JV. 2014. Swimming-induced exercise promotes hypertrophy and vascularization of fast skeletal muscle fibres and activation of myogenic and angiogenic transcriptional programs in adult zebrafish. BMC Genomics 15:1136.

Palstra AP, Planas JV. 2011. Fish under exercise. Fish Physiol Biochem 37:259–72.

Pedersen B, Hoffman-Goetz L. 2000. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev 80:1055–81.

Pierce BJ, McWilliams SR. 2014. The fat of the matter: how dietary fatty acids can affect exercise performance. Integr Comp Biol 54:903–12.

Piersma T. 2011. Why marathon migrants get away with high metabolic ceilings: towards an ecology of physiological restraint. J Exp Biol 214:295–302.

Piersma T, Gudmundsson G, Lilliendahl K. 1999. Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. Physiol Biochem Zool 72:405–15.

Piersma T, Koolhaas A, Dekinga A. 1993. Interactions between stomach structure and diet choice in shorebirds. Auk 110:552–64.

Reichert S, Stier A, Zahn S, Arrivé M, Bize P, Massemin S, Criscuolo F. 2014. Increased brood size leads to persistent eroded telomeres. Front Ecol Evol 2:9.

Rezende EL, Chappell MA, Gomes FR, Malisch JL, Garland T. 2005. Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. J Exp Biol 208:2447–58.

Rhodes JS, Gammie SC, Garland TJ. 2005. Neurobiology of mice selected for high voluntary wheel-running activity. Integr Comp Biol 45:438–55.

Rhodes JS, Kawecki TJ. 2009. Behavior and neurobiology. In: Experimental evolution: concepts, methods, and applications of selection experiments. Berkeley (CA): University of California Press. pp. 263–300.

Richardson CS, Dohm MR, Garland Jr T. 1994. Metabolism and thermoregulation in crosses between wild and random-bred laboratory house mice (*Mus domesticus*). Physiol Zool 67:944–75.

Sathe EA, Husak JF. 2015. Sprint sensitivity and locomotor trade-offs in green anole (*Anolis carolinensis*) lizards. J Exp Biol 218:2174–9.

Selman C, Blount JD, Nussey DH, Speakman JR. 2012. Oxidative damage, ageing, and life-history evolution: where now?. Trend Ecol Evol 27:570–7.

Sherwin C. 1998. Voluntary wheel running: a review and novel interpretation. Anim Behav 56:11–27.

Simons MJP, Briga M, Leenknegt B, Verhulst S. 2014. Context-dependent effects of carotenoid supplementation on reproduction in zebra finches. Behav Ecol 25:945–50.

Storz JF, Bridgham JT, Kelly SA, Garland TJ. 2015. Genetic approaches in comparative and evolutionary physiology. Am J Physiol Regul Integr Comp Physiol 309:R197–214.

Swallow JG, Carter PA, Garland TJ. 1998. Artificial selection for increased wheel-running behavior in house mice. Behav Genet 28:227–37.

Swallow JG, Rhodes JS, Garland TJ. 2005. Phenotypic and evolutionary plasticity of organ masses in response to voluntary exercise in house mice. Integr Comp Biol 45:426–37.

Swallow JG, Hayes JP, Koteja P, Garland TJ. 2009. Selection experiments and experimental evolution of performance and physiology. In: Garland T Jr, Rose MR, editors. Experimental evolution: concepts, methods, and applications of selection experiments. Berkeley (CA): University of California Press. pp. 301–51.

Terrill S. 1990. Evolutionary aspects of orientation and migration in birds. Experientia 46:395–404.

Thomson S, Garland T, Swallow J, Carter P. 2002. Response of Sod-2 enzyme activity to selection for high voluntary wheel running. Heredity 88:52–61.

Vanhooydonck B, Measey J, Edwards S, Makhubo B, Tolley KA, Herrel A. 2015. The effects of substratum on locomotor performance in lacertid lizards. Biol J Linn Soc 115:869–81.

Veasey J, Houston D, Metcalfe N. 2001. A hidden cost of reproduction: the trade-off between clutch size and escape take-off speed in female zebra finches. J Anim Ecol 70:20–24.

Vézina F, Williams T. 2005. Interaction between organ mass and citrate synthase activity as an indicator of tissue maximal oxidative capacity in breeding European Starlings: implications for metabolic rate and organ mass relationships. Funct Ecol 19:119–28.

Weber J. 2009. The physiology of long-distance migration: extending the limits of endurance metabolism. J Exp Biol 212:593–7.

Weber J, Choi K, Gonzalez A, Omlin T. 2016. Metabolic fuel kinetics in fish: swimming, hypoxia and muscle membranes. J Exp Biol 219:250–8.

Wiersma P, Salomons H, Verhulst S. 2005. Metabolic adjustments to increasing foraging costs of starlings in a closed economy. J Exp Biol 208:4099–108.

Wiersma P, Verhulst S. 2005. Effects of intake rate on energy expenditure, somatic repair and reproduction of zebra finches. J Exp Biol 208:4091–8.

Williams TD. 2012. Hormones, life-history, and phenotypic variation: opportunities in evolutionary avian endocrinology. Gen Comp Endocrinol 176:286–95.

Willis WT, Dallman PR, Brooks GA. 1988. Physiological and biochemical correlates of increased work in trained iron-deficient rats. J Appl Physiol 65:256–63.

Wilmers CC, Nickel B, Bryce CM, Smith JA, Wheat RE, Yovovich V. 2015. The golden age of bio-logging: how animal-borne sensors are advancing the frontiers of ecology. Ecology 96:1741–53.

Wilson SM, Taylor JJ, Mackie TA, Patterson DA, Cooke SJ, Willmore WG. 2014. Oxidative stress in Pacific salmon (*Oncorhynchus spp.*) during spawning migration. Physiol Biochem Zool 87:346–52.

Wingfield JC, Schwabl H, Mattocks PW. Jr. 1990. Endocrine mechanisms of migration. Berlin Heidelberg: Springer. pp. 232–56.

Wynn ML, Clemente C, Nasir AFAA, Wilson RS. 2015. Running faster causes disaster: trade-offs between speed, manoeuvrability and motor control when running around corners in northern quolls (*Dasyurus hallucatus*). J Exp Biol 218:433–9.

Zera AJ, Harshman LG. 2001. The physiology of life history trade-offs in animals. Annu Rev Ecol Syst 32:95–127.

Zhang Y, Carter T, Eyster K, Swanson DL. 2015a. Acute cold and exercise training up-regulate similar aspects of fatty acid transport and catabolism in house sparrows (*Passer domesticus*). J Exp Biol 218:3885–93.

Zhang Y, Eyster K, Liu J, Swanson DL. 2015b. Cross-training in birds: cold and exercise training produce similar changes in maximal metabolic output, muscle masses and myostatin expression in house sparrows (Passer domesticus). J Exp Biol 218:2190–200.