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Review

Lab and field experiments: Are they the same animal?

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ABSTRACT

To advance our understanding of biological processes we often plan our experiments based on published data. This can be confusing though, as data from experiments performed in a laboratory environment are sometimes different from, or completely opposite to, findings from similar experiments performed in the "real world". In this mini-review, we discuss instances where results from laboratory experiments differ as a result of laboratory housing conditions, and where they differ from results gathered in the field environment. Experiments involving endocrinology and behavior appear to be particularly susceptible to influence from the environment in which they are performed.

As such, we have attempted to promote discussion of the influence of housing environment on the reproductive axis, circadian biology and behavior, immune function, stress biology, neuroplasticity and photoperiodism. For example, why should a rodent species be diurnal in one housing environment yet nocturnal in another? Are data that are gathered from experiments in the laboratory applicable to the field environment, and vice-versa? We hope not only to highlight the need for experiments in both lab and field when looking at complex biological systems, but also to promote frank discussion of discordant data. Perhaps, just as study of individual variation has been gaining momentum in recent years, data from variation between experimental arenas can provide us with novel lines of research.

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> The great tragedy of Science — the slaying of a beautiful hypothesis by an ugly fact.
>
> Thomas H. Huxley (1825–1895)

Introduction

The vast majority of experiments involving vertebrates are performed in a laboratory environment. The main reason behind this is straightforward: to control as many environmental variables as possible, facilitating interpretation of the resulting data. In general, this type of experiment tends to yield relatively "clean" data and allows us to investigate the effects of a specific manipulation. If, after careful statistical analysis, we can reject our initial null hypothesis, then we have a foundation upon which we can devise further experiments. Hopefully in time we are able to gain some insight into the biological workings of our model organism, whether we are studying behavioral, physiological or molecular mechanisms, or a combination of all three. What happens if we take our initial experiment out to the model organism's natural environment, where there are numerous uncontrollable physical and socio-

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environmental factors? We would hope that our initial findings are the same as those gathered in the laboratory, or at least leaning in the same direction. Often they are not. This review attempts to highlight a few of the many instances where results from laboratory experiments differ to some degree from their field counterparts (see Table 1 for examples of this).

Ricklefs and Wikelski (2002) state that understanding life-history traits requires an understanding of how individuals respond to particular environments, and studies of such should, "integrate behavior and physiology within the environmental and demographic contexts of selection." In the same vein, "an animal that is not in its natural habitat is out of context…(animals) only make sense when they are living in the natural environments in which they evolved and to which they have become adapted," (Eric Pianka, *pers. comm.*). Sometimes the differences in data from lab and field experiments are extreme; at other times they are more subtle and more easily overlooked/explained away. Whatever the degree of difference, each raises questions about how we should interpret lab data, and to what extent we should use them as generalizations about what happens in the real world. The reverse is also true: we might miss important factors in field experiments because of data "noise" from uncontrolled factors. This review is neither intended to be critical of either laboratory or field experiments, nor to give the impression that one type of experiment is more pertinent than another. Rather, we hope to highlight the need for experiments in both lab and field when looking at complex biological systems. We should perhaps view the laboratory and field experimentation arenas as being complementary to one another, rather than as independent entities.

Gonadotropins, gonadal and adrenal steroids

When an animal is taken out of its natural environment it is common to observe changes in its basic endocrine milieu. The endocrine system is a chemical communication network that has evolved to be sensitive to environmental perturbations. It is intended for the relay of external and internal messages to specific tissues and elicits appropriate chemical and behavioral responses. A change in an individual's immediate environment can thus induce a quantitatively or qualitatively different endocrine response. For example, free-living male kestrels (*Falco tinnunculus*) have 3-fold higher androgen concentrations during the breeding season than in captivity, while the gonadotropin luteinizing hormone (LH) during the winter is elevated in captive males and females as compared to their free-living conspecifics (Meijer and Schwabl, 1989).

Even changes from a natural to a captive diet can alter behavior and physiology. For instance, most lab chow for rodents contains soy. Soybean isoflavone extracts can affect growth and reproductive system development in growing rats (Guan et al., 2008). Phytoestrogens found in soy can also reduce female sexual behavior in rodents (Kudwa et al., 2007), as well as decrease cloacal protuberance growth rate in songbirds (cloacal protuberance is an androgen-dependent sperm storage and non-intromittent copulatory organ) (Corbitt et al., 2007).

What happens when a natural environment is simulated within a captive setting? Territorial interactions can be differentially androgen-dependent in wild and captive male dusky-footed woodrats (*Neotoma fuscipes*; Caldwell et al., 1984; Monaghan and Glickman, 1992; Glickman and Caldwell, 1994). In their natural habitat, castrated and intact male dusky-footed woodrats fought with equal intensity over self-built twig shelters, suggesting that territorial behavior in this species is independent of gonadal steroids. However, when offered a shelter in captivity, intact males fought more intensely than castrated males, demonstrating that territorial behavior may be dependent on gonadal steroids only in this type of artificial situation (Caldwell et al., 1984; Monaghan and Glickman, 1992; Glickman and Caldwell, 1994). The reasons for this differential response to gonadal steroids remain unclear.

Another important factor to consider is the influence of social interactions on the physiology and resulting behavior (or vice-versa) of an organism. Wingfield et al. (1990) proposed the Challenge Hypothesis which emphasized the impact of social stimuli on hormonal and behavioral responses. Simplified, the Challenge Hypothesis predicts that circulating testosterone should be higher in birds during periods of social instability, whether that instability is a result of a territorial intrusion or progression from one life-history stage to the next (i.e., transition from non-breeding to establishment of breeding territories). Initially over 20 species of birds were examined, encompassing six orders and 14 families, representing various mating systems and breeding strategies. Results generally demonstrated that testosterone concentrations were many times higher 1) in response to social instability, and 2) in free-living, breeding birds compared to those in captivity. Behaviors that are believed to lead to greater fitness – such as territory and mate acquisition and defense – are responsive to and themselves influence circulating androgen concentrations. These behaviors are either altered or cease completely when lab animals are maintained in solitary or artificial group sizes or sex ratios. For example, wild, paired male cowbirds (*Molothrus ater*) had higher concentrations of testosterone than unpaired males early in the season, with testosterone peaking during social hierarchy formation and mate acquisition (Dufty and Wingfield, 1986a). However, paired males kept in captivity and exposed to long days had a more rapid increase and longer maintenance of testosterone, enhanced gonadal growth and longer maintenance of enhanced gonadal size than unpaired males, though maximal gonadal weights were lower than those of free-living males (Dufty and Wingfield, 1986b).

Stress

Environmental factors and social interactions (or lack thereof) in captive versus wild settings can yield differences in the stress response. We define stress here as that which occurs in association with unpredictable and life-threatening perturbations in the environment and invokes an "emergency life-history stage" (Wingfield et al., 1998; Wingfield and Sapolsky, 2003). Glucocorticoids such as corticosterone (CORT) and cortisol, are steroid hormones commonly measured to quantify the stress response.

Baseline and stress-induced plasma glucocorticoid concentrations can change when an organism is brought into a captive setting. In a study examining baseline CORT in wild and captive white-throated sparrows (*Zonotrichia albicollis*) and white-crowned sparrows (*Zonotrichia leucophrys*) during the non-breeding season, both species had baseline plasma CORT levels that were 2–3 times higher than wild birds, even after being held in captivity for 35 days to acclimate (Marra et al., 1995). Wild and captive brown-headed cowbirds do not differ in baseline CORT concentrations; however, birds housed in solitude as opposed to being housed with other conspecifics have elevated corticosterone levels, regardless of photoperiod (Dufty and Wingfield, 1986b). In contrast, there are reports of corticosterone concentrations being higher in free-living birds (Romero and Wingfield, 1999; Wingfield and Kitaysky, 2002; Cyr and Romero, 2008) and mammals (Kunzl and Sachser, 1999). For example, Cyr and Romero (2008) found fecal glucocorticoid concentration differences between captive and wild female European starlings (*Sturnus vulgaris*) in response to stress. In this study, experimentally-induced chronic stress did not alter captive female fecal glucocorticoid concentrations during the day or night, though induced stress was associated with higher concentrations in the field.

In sum, the social and physical environment can influence an organism's endocrine status.

Table 1
Examples of result discrepancies between data collected from wild animals in the field and captive animals in the lab.

Gonadotropins, gonadal and adrenal steroids		
Species	Field data	Captive data
Kestrels (*Falco tinnunculus*)	Male androgens higher during breeding season Male and female LH lower in winter (Meijer and Schwabl 1989)	Male androgens lower during breeding season Male and female LH higher in winter (Meijer and Schwabl 1989)
Dusky-footed woodrats (*Neotoma fuscipes*)	Castrated and intact males fight over shelters with equal intensity Territorial behavior may be androgen-independent (Caldwell et al., 1984; Monaghan and Glickman, 1992; Glickman and Caldwell, 1994)	Intact males fight more intensely over shelters than castrated males Territorial behavior may be androgen-dependent (Caldwell et al., 1984; Monaghan and Glickman, 1992; Glickman and Caldwell, 1994)
20 species of birds (6 orders, 14 families)	T higher in response to social instability in breeding birds (Wingfield et al., 1990)	T lower in response to social instability in breeding birds (Wingfield et al., 1990)
Cowbirds (*Molothrus ater*)	Paired male T higher early in breeding season Male T peaks during social hierarchy formation and mate aquisition (Dufty and Wingfield 1986a)	Paired males had more rapid T increase and longer maintenance, enhanced gonad growth and size maintenance than unpaired Maximal gonadal weights less than wild males Males housed in solitude had higher CORT than those housed with conspecifics (Dufty and Wingfield 1986b)
White-throated sparrows (*Zonotrichia albicollis*)	Baseline CORT lower in non-breeding season (Marra et al., 1995)	Baseline CORT higher in non-breeding season (Marra et al., 1995)
White-throated sparrows (*Zonotrichia leucophrys*)	Baseline CORT lower in non-breeding season (Marra et al., 1995)	Baseline CORT higher in non-breeding season (Marra et al., 1995)
European starling (*Sturnus vulgaris*)	Female fecal glucocorticoids altered by induced stress (Cyr and Romero, 2008)	Female fecal glucocorticoids not altered by induced stress (Cyr and Romero, 2008)
Brain morphology and function		
Species	Field data	Captive data
Black-capped chickadee (*Poecile atricapillus*)	No photoperiodic effects in HVC and Area X (Smulders et al., 2006)	Photoperiodic effects in HVC and Area X (MacDougall-Shackleton et al., 2003) Minimal to no seasonal differences in song nuclei, though breeding birds had slightly larger HVC and RA as a proportion of their telencephalic volume than non-breeding birds (Phillmore et al., 2006)
Nuttall's white-crowned sparrow (*Zonotrichia leucophrys nuttali*)	Greater HVC and RA volumes in spring than fall (Brenowitz et al., 1998)	No seasonal change in HVC or RA in wild males captured at 30 days and put in captivity (Baker et al., 1984)
Dark-eyed juncos (*Junco hyemalis*)	Hippocampal volume larger than captive birds, independent of T treatment (Smulders et al., 2000)	Hippocampal volume smaller than wild birds, independent of T treatment (Smulders et al., 2000)
Long–Evans rats		Cortical thickness was greater in those housed in enriched environments versus standard (Diamond et al., 1987)
Harlan Sprague Dawley mice		Dentate gyrus cells more abundant and hippocampal granule cell layer larger if housed in enriched environment versus standard (Kempermann et al., 1997)
Photoperiodic responsiveness		
Species	Field data	Captive data
Golden hamsters (*Mesocricetus auratus*)		Melatonin-dependent gonadal regression under short photoperiod (Reiter, 1980; Goldman and Darrow, 1983) Wheel-running prevents melatonin-induced gonadal regression and reverses short-day anestrus (Borer et al., 1983; Pieper et al., 1988)
Canary (*Serinus canaria*)	Robust photoperiodic responses (Leitner et al., 2003)	Photoperiod non-responsiveness in some domesticated strains (Bentley et al., 2000)
Circadian rhythms		
Species	Field data	Captive data
Naked mole rat (*Heterocephalus glaber*)	Temporally structured activity (Urrejola et al., 2005)	Short bursts of activity over a 24 h cycle (Urrejola et al., 2005fs)
Cururus (*Spalacopus cyanus*)	Diurnal (Reig, 1970; Rezende et al., 2003; Urrejola et al., 2005)	Nocturnal or arrhythmic activities (Begall et al., 2002; Rezende et al., 2003)
Tucos (*Ctenomys sociabilis*)	Diurnal (Lacey et al., 1997)	Nocturnal (Lacey, pers comm.)
Unstriped nile rats (*Arvicanthis niloticus*)	Diurnal (Blanchong et al., 1999)	Diurnal, but nocturnal if supplied running wheel and dependent on parentage and sex (Blanchong et al., 1999)
Brown-throated three-toed sloths (*Bradypus variegatus*)	Sleep 9.63 h per day (Rattenborg et al., 2008)	Sleep 15.85 h per day (`Galvão deMoura Filho et al., 1983)
Degus (*Octodon degus*)	Crepuscular (Fulk, 1976; Labyak et al., 1997; Kenagy et al., 2002)	Diurnal, nocturnal, crepuscular. Will alter diurnal activity if supplied running wheel. (Labyak et al., 1997; Kas and Edgar, 1998, 1999)

(continued on next page)

Table 1 (*continued*)

Gonadotropins, gonadal and adrenal steroids		
Species	Field data	Captive data
Immune function		
Species	Field data	Captive data
Wistar rats		High CORT and decrease in body weight in juveniles given immune challenge; CORT low and body weight maintained when housed in enriched environment (Mlynarik et al., 2004)
Sprague–Dawley rats		Greater neurodegeneration and forelimb use deficit after cortical impact, though increased recovery capacity, in rats raised in an enriched environment as compared to controls (Zoklowski et al., 2004)
Superb fairy-wrens (*Malurus cyaneus*)	Greater immune response in males with naturally high T than males with basal T concentrations (Peters, 2000)	Decreased immune response in males with T implants as compared to controls (Peters, 2000)

We consider that it is important to bear these findings in mind when interpreting results from organisms observed in either a lab or field environment.

Brain morphology and function

The environment in which an animal is raised and/or housed can affect brain morphology and function. Captive experimental manipulations of rodent housing environments have resulted in differences in brain morphology. Diamond et al. (1987) examined the effects of lab conditions on rodent brain by varying their captive environment, with treatments consisting of a standard lab environment, an enriched lab environment and a crowded, enriched lab environment. Crowding in rodents was already known to have deleterious effects, such as destructive behavior, retardation of sexual maturity and increased infant mortality (Calhoun, 1973) which enrichment could abate, if not totally alleviate (Calhoun, 1962; Christian and Davis, 1964). Enrichment in the study by Diamond et al. (1987) included running wheels, ladders, tunnels, mazes and various other objects. Cortical thickness, which has been associated with certain enriched versus impoverished environmental conditions (Diamond, 1976; Diamond et al., 1972, 1976), was greater in the two enriched groups as compared to the standard control group. The authors posited that enrichment in a laboratory setting may mitigate the stress of crowding. Kempermann et al. (1997) later demonstrated that neurogenesis in the dentate gyrus of the rodent hippocampus can occur differentially according to laboratory environment. Mice housed in an enriched, or environmentally complex, laboratory environment had a greater abundance of dentate gyrus cells and a larger hippocampal granule cell layer than littermates housed in standard cages. Thus, enriched environments could promote survival of neural hippocampal precursor cell progeny, higher granule cell numbers and a larger hippocampal volume. The authors tested performance using a Morris water maze and found a significant enhanced performance in the enriched environment group, implying that the neural changes influence behavioral performance.

Gonadal steroid differences in wild versus captive animals may be the cause or result of changes in the brain. Seasonally-breeding vertebrates, for example, experience various neurochemical and neuroanatomical changes according to the stage of their reproductive cycle. Nottebohm (1981) first noticed that certain brain regions involved in vocal control of male canaries, such as the HVC, robust nucleus of the arcopallium (RA), and Area X, are larger in the spring than in the fall. During the spring, the testes secrete higher amounts of androgens and males sing more frequently (Nottebohm and Nottebohm, 1978). The elevation of testosterone induces rapid and sequential growth of the song control system (Tramontin et al., 2003). Seasonal volumetric growth of HVC largely involves increased recruitment of new neurons and their survival (Tramontin et al., 2000; Thompson and Brenowitz, 2005). Increases in the volumes of RA and Area X involve increases in neuronal size and spacing, with no apparent change in neuron number. Both RA and Area X are efferent targets of HVC and depend upon HVC for trophic support (Nottebohm, 1981). However, this volumetric growth of song nuclei may be affected by lab and field settings. For example, captive studies of the seasonally breading black-capped chickadee (*Poecile atricapillus*) revealed photoperiodic effects in HVC and Area X (MacDougall-Shackleton et al., 2003), but wild-caught conspecifics did not exhibit such change (Smulders et al., 2006). Phillmore et al. (2006) found minimal to no seasonal differences in captive black-capped chickadee song control nuclei, although captive breeding birds did have slightly larger HVC and RA as a proportion of their telencephalic volume than non-breeding captive birds. Phillmore et al. (2006) and Smulders et al. (2006) suggest one possibility for this lack of nucleus volume change is that captive birds lack exposure to the year-round vocalizations and social interactions of their wild counterparts, although these hypotheses have yet to be tested.

Synaptogenesis within the song control system may result in the ability to learn new syllables each year and increase song repertoire. However, this effect of learning can be muted in captivity, possibly due to low levels of circulating androgens (Baker et al., 1984; Brenowitz et al., 1998). Captive animals are generally documented as having lower testosterone concentrations as compared to their wild-caught conspecifics (Wildt et al., 1993; Wingfield, 1980, 1983; Wingfield and Farner, 1980; Wingfield and Moore, 1987). Baker et al. (1984) asked whether Nuttall's white-crowned sparrow (*Zonotrichia leucophrys nuttali*) exhibits volume increases in specific song control nuclei. Males were exposed to song in the wild when they were juveniles and then captured when they were 30 days old. Birds were then kept in captivity and housed under long days, in which they were exposed to recorded song, and short days, in which recorded song was not played. These captive birds gave evidence of seasonally fluctuating androgen levels in captivity, as seen from cloacal protuberance and testes weight, though hormone concentrations were not assayed. Histological examination revealed no seasonal change in the volumes of the song control nuclei HVC and RA (Baker et al., 1984). The authors claim their results to be consistent with the hypothesis that seasonal changes in volume of HVC and RA may be specifically related to the capacity to learn new syllables each breeding season (which Nuttall's white-crowned sparrows do not). However, when Brenowitz et al. (1998) examined the same subspecies of white-crowned sparrow in the wild, they found that birds caught in the spring had significantly greater HVC and RA volumes than birds caught in the fall. The results of the study by Baker et al. (1984) may have been a result of low circulating steroid levels in captive birds or differences in social cues (Tramontin et al., 1999). The fact that gonadal steroid-dependent secondary sexual characteristics such as cloacal protuberance were measurable implies to us that circulating gonadal steroids were at sufficiently high concentrations to cause neural changes. Thus, some aspect of captive housing is likely to have influenced volumetric changes of song control nuclei via some unknown mechanism.

Neuroanatomical changes in wild dark-eyed juncos (*Junco hyemalis*) were thought to also be androgen-dependent, but observations from birds in captive conditions suggest otherwise (Smulders et al., 2000). In the field, dark-eyed juncos treated with testosterone feed their young less often than sham-treated controls, sing more (Ketterson et al., 1992; Chandler et al., 1994) and have larger home ranges (Chandler et al., 1997). The hippocampus, which is involved in the processing spatial and relational information in birds and mammals (O'Keefe and Nadel, 1978; Bingman, 1990; Wood et al., 1999), was thus thought to be influenced by testosterone. In the lab, hippocampal volume was reduced compared to free-living birds but was, however, independent of testosterone treatment (Smulders et al., 2000). Smulders et al. (2000) propose that environmental stressors in captivity may have been attributed to increased corticosterone, which could decrease hippocampal volume. However, work by Klukowski et al. (1997) on captive juncos and by Schoech et al. (1999) on free-living juncos implies that captive juncos might have lower corticosterone concentrations than free-living juncos (but note that this is a result of inter-assay interpretation). Perhaps spatial and behavioral deprivation, which can alter brain function and morphology (Rosenzweig and Bennett, 1996), may have influenced hippocampal volume changes. Smulders et al. (2000) conclude, "laboratory studies alone may not accurately reflect the actual neural processes that underlie behavior in the wild, and thus field studies may be indispensable in characterizing brain-behavior relations."

Circadian rhythms

Circadian rhythms are endogenous physiological oscillations which occur within a period of approximately 24 h and persist under constant conditions. In mammals, these rhythms are coordinated by the body's master clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus and can be maintained by a combination of genetic, cellular, and neural regulatory mechanisms (Kriegsfeld and Silver, 2006). Circadian rhythms, such as sleep/wake cycles and hormone production and secretion, are adaptive in that they aid in the synchronization of an organism's internal environment with its fluctuating external environment (Rusak and Zucker, 1979). For example, DeCoursey et al. (2000) found that a significantly higher proportion of SCN-lesioned free-living eastern chipmunks (*Tamias striatus*) were killed by weasel predation as compared to surgical and intact controls. This is presumably an effect of temporally inappropriate activity, as chipmunks may have been more active when predators were present as a result of the loss of their SCN. Authors questioned if nighttime restlessness in SCN-lesioned chipmunks may have alerted predators to their whereabouts more easily than the behavior of controls, although this has yet to be tested fully.

Many times, mechanistic studies of circadian rhythms take place in controlled lab settings. Multiple reports in mammals suggest circadian rhythms can differ in captivity from those in a natural setting (Nevo et al., 1982; Labyak et al., 1997; Blanchong et al., 1999; Rezende et al., 2003; Urrejola et al., 2005; Rattenborg et al., 2008; Lacey, *pers. comm.*). For example, naked mole rats (*Heterocephalus glaber*) exhibit short bursts of activity over a 24 h cycle in captivity compared to more temporally structured behavior in the field (Urrejola et al., 2005). One change in circadian rhythmic activity commonly reported is the shift from diurnal activity in the wild to nocturnal activity in captivity. Cururus (*Spalacopus cyanus*) are naturally diurnal (Reig, 1970; Geiser et al., 2000; Rezende et al., 2003; Urrejola et al., 2005) but exhibit nocturnal or arrhythmic activities in captivity (Begall et al., 2002; Rezende et al., 2003). Tuco-tucos (*Ctenomys sociabilis*) are also naturally diurnal (Lacey et al., 1997) but often become more nocturnal when brought into a lab setting (Lacey et al. *pers. comm.*). Blanchong et al. (1999) report that unstriped nile rats – also named African grass rats – (*Arvicanthis niloticus*) exhibit diurnal activity in the field but are both diurnal and nocturnal in the lab. Nocturnal activity could be brought about by supplying a running wheel in the animal's enclosure as well as being dependent on parentage and sex. Degus (*Octodon degus*) also will switch from diurnal activity to nocturnal activity when supplied a running wheel in the lab (Kas and Edgar, 1998, 1999). This species exhibits crepuscular behavior in the field (Fulk, 1976; Labyak et al., 1997; Kenagy et al., 2002) but diurnal, nocturnal or crepuscular activity in captivity (Labyak et al., 1997), a pattern also found in blind mole rats (*Spalax ehrenbergi*; Nevo et al., 1982; Ben-Shlomo et al., 1995). More recently, Rattenborg et al. (2008) reported that brown-throated three-toed sloths (*Bradypus variegatus*) living in tropical rainforest canopies sleep about 6 h less than their captive conspecifics. These examples raise the question of how circadian rhythms can be influenced by the housing environment. What influence do "abnormal" social cues have on patterns of rhythmicity? How can something as alien to a rodent's natural environment as a running wheel shift something as apparently hard-wired as circadian rhythmicity? Perhaps circadian rhythms are more plastic than has been considered. Are some of the phenomena that can be observed in the lab under specific conditions (e.g., "splitting" of circadian rhythms into 2 activity bouts per 24 h cycle under constant light in some species) simply a representation of the plastic and adaptable nature of the underlying rhythm? These examples also raise the question of whether commonly-used lab strains of rodents would even exhibit the same rhythmic behaviors in the wild as in the lab. All these are intriguing questions that are waiting to be tested.

Photoperiodic responsiveness

Many mammalian species are photoperiodic. That is, their reproductive system responds to changing photoperiod so as to time parturition to the time of year most conducive to raising young (Baker, 1938). Over 30 rodent species are characterized as "long-day" breeders (Prendergast et al., 2001). During exposure to short, winter-like day lengths, an increased duration of the nocturnally-secreted pineal hormone melatonin causes a suite of physiological changes associated with reproductive quiescence. There is a decrease in synthesis and release of the neuropeptide gonadotropin-releasing hormone (GnRH), with consequent reductions in circulating gonadotropic hormones (luteinizing hormone, LH; follicle-stimulating hormone, FSH), gonadal regression and reduction in sex steroids and reproductive behaviors (see Goldman and Nelson, 1993 for review, Prendergast et al., 2001). For some time in mammalian photoperiodic research, a dogma stood that the costs of breeding at other times of year were too great for this strategy to be maintained over evolutionary time. Despite the dogma, in many laboratory experiments involving photoperiodic rodents such as microtine voles, mice (*Peromyscus* spp.) and Siberian hamsters (*Phodopus sungorus*), subsets of the experimental populations did not regress their reproductive systems under short, winter-like photoperiods (see Prendergast et al., 2001 for review). This phenomenon was generally considered to be a lab artifact, and thought most likely to be a result of constant food availability, or generally "less harsh" conditions in the laboratory compared to the animals' natural setting (see Nelson, 1987). As a result, the so-called "non-responders" to changing photoperiod were identified as animals with gonad sizes that fell outside 2 standard deviations above the mean (during exposure to short photoperiods), and excluded from data analysis. Sample-sizes in experiments were increased to account for this phenomenon, although approximately 30% of individuals in some of these species can now be classified as photoperiodic non -responders (Hoffmann, 1978; Puchalski and Lynch, 1986; Prendergast et al., 2001). Eventually, Lynch and colleagues clearly demonstrated that photoperiodic non-responsiveness was heritable in Siberian hamsters (Lynch et al., 1989; Kliman and Lynch, 1992). Thus it is likely that 30% of individuals in wild populations of these species retain the ability to breed year-round, regardless of photoperiod. However, as Prendergast et al. (2001) state

in their review, the highly inbred laboratory animals of these species might be, "a very peculiar animal model that may be different from the same species living in the wild". The fitness payoffs of photoperiodic non-responsiveness in the wild have yet to be determined, and of course these benefits will vary according to the prevailing environmental conditions other than photoperiod (Prendergast et al., 2001).

Even within a laboratory environment, the individual housing conditions can affect the reproductive response to photoperiod or even melatonin treatment (melatonin is the pineal hormone which is secreted at night and which, in photoperiodic mammals, is responsible for gonadal regression during short photoperiods via an unknown link to the GnRH system). Traditionally, rodents have been supplied with exercise wheels within their cages as a form of environmental enrichment, or as a way of measuring nocturnal activity levels via electrical contacts between the rotating wheel and the stationary cage. In golden hamsters, wheel-running prevents melatonin-induced gonadal regression (Pieper et al., 1988) and reverses short-day-induced anestrus (Borer et al., 1983), most likely via increased gonadotropin secretion (Pieper et al., 1995). In contrast, the absence of exercise wheel-running can also cause a loss of photoperiodic responsiveness in a PNRj line of Siberian hamsters (Freeman and Goldman, 1997). Thus it is possible that somewhat unexpected factors can influence the expression of physiological and behavioral traits in a given environment. How the brain integrates the seemingly disparate stimuli of exercise and ambient photoperiod to influence reproductive status in a non-intuitive direction (exercise stimulating the reproductive axis, for example) is a matter for debate.

Many avian species are also photoperiodic. It is noteworthy that males of strictly photoperiodic wild bird species tend to exhibit much more uniform gonadal responses to changing day length than the mammalian species already discussed, both in the laboratory and the wild. A possible reason for this uniform response in males is that the costs of maintaining an active reproductive system year-round are simply too high for this strategy to remain viable within a population. Females of these species often show a muted gonadal response to changing photoperiod in lab conditions. Presumably this is because they do not receive sufficient supplementary information and/or fulfillment of their dietary requirements (Wingfield et al., 1990). Ovarian and follicular development in captive European starlings (*Sturnus vulgaris*) (Dawson, 1997) and several sparrow species (*Zonotrichia* spp) (King et al., 1966; Wingfield, 1984; Wingfield and Farner, 1993; Dawson et al., 2001) is often only 20–30% of its maximum in the wild in response to identical day lengths. Thus, at the gonad level, one might say that 50% of the population (females) shows a dramatically muted response to changing photoperiod in the laboratory. Having said that, many of the other physiological changes associated with the photoperiodic reproductive response to changing photoperiod do occur in lab-housed females, and these are often reduced to a lesser degree than ovarian development. These include changes in the gonadotropin-releasing hormone system, changes in circulating hormones such as luteinizing hormone, testosterone and prolactin, and feather molt at the termination of the reproductive period (Dawson, 1997; Bentley et al., 2000). Thus in wild birds, the underlying neurobiology and physiology of reproductive responsiveness to photoperiod is similar to that seen in the lab (see Leitner et al., 2003), but some aspect of the laboratory environment prevents full expression of the response at the level of the gonads. As a result, females housed in a laboratory environment often will not exhibit behaviors that depend upon high concentrations of circulating gonadal steroids. An example of this lack of steroid-dependent behavior in the laboratory is the copulation solicitation display which females usually only will exhibit in captivity if their circulating estradiol is supplemented with a subcutaneous implant (Moore, 1983; Maney et al., 1997). It may be that female expression of the full suite of endocrine responses to photoperiod depends on the birds' prior experience. Baptista and Petrinovich (1986) describe an experiment on female white-crowned sparrows which were collected as nestlings in the wild and hand-reared in the laboratory. They readily ovulated in captivity when housed in a situation under which ovulation had never been reported in wild-caught adult females. They suggest that young female white-crowned sparrows undergo an imprinting process during their first year in terms of what stimuli supplementary to photoperiod are required for ovulation. When hand-reared in captivity they "imprinted" on substitute supplementary stimuli and therefore would ovulate under these conditions. Kern (1972) studied morphological differences between ovaries from free-living and captive white-crowned sparrows. This author's main findings were that the ovaries of captive birds lack pre- and postovulatory follicles; they contain more atresias; the stroma is dense and poorly vascularized year-round; the membrana granulosa of developing follicles becomes proliferative earlier in the spring and is always less active mitotically than in wild birds; thecae do not develop beyond formative stages; thecal gland cells become abundant earlier in spring and stromal gland cells are less numerous. Kern concluded that many of these differences suggest that white-crowned sparrows do not breed in captivity because of an abnormal synthesis and/or secretion of hypophysial gonadotropin. How many environmental cues are involved in the final maturation of the ovaries and how the brain integrates all of these cues into an endocrine response is not known, nor is how early imprinting might influence the interpretation of a laboratory versus field environment.

Despite the tenet that even if the gonadal response is muted in the laboratory, but the underlying neurobiology and physiology is relatively unchanged as compared to the wild, domestication of some bird species appears to have reduced the response to changing day length, even at the level of the brain. Canaries (*Serinus canaria*) in the wild exhibit robust photoperiodic responses (Leitner et al., 2003), as do many of the domesticated strains. However some domesticated strains appear to have lost the ability (or need) to alter their reproductive status according to the prevailing photoperiod, even at the level of the gonadotropin-releasing hormone (GnRH) system (Bentley et al., 2000).

Perfito et al. (2008) considered cues other than solely photoperiod as sources for reproductive timing. The opportunistically breeding Lesser Sundas zebra finch (*Taeniopygia guttata guttata*) lives in an Australian environment that experiences unpredictable pulses in food. This species can breed year-round, both in the lab and the field. Using a semi-domesticated strain of males to examine whether long days or food availability was the prevailing factor or stimulate gonadal growth, Perfito et al. (2008) found that extended feeding times or increased food abundance during long days, not just the long days themselves, is the driving permissive cue for gonadal growth. These results beg the question of what cues are needed in a captive environment, be it photoperiod, food availability, etc., to truly mimic an organism's natural environment? If studying reproductive timing in Lesser Sundas zebra finches, we now know it is incorrect to assume photoperiodic treatments in a captive setting completely mimic reproductive cues in the wild (Perfito et al., 2008), thus permitting a more revealing analysis of reproductive mechanisms in this opportunistically breeding species. Another example showing how possible factors that can influence results and their interpretation can be found in Fig. 1.

Immune function

Variation in environmental conditions can accompany variation in immunocompetence. Factors such as photoperiod, temperature, food availability and social environment may create energetic tradeoffs in order to maintain critical physiological processes. For example, immune function may become enhanced during winter months to aid in survival during climatic and nutritionally harsh times (Sinclair and Lochmiller, 2000). It may also become enhanced because a long-day breeder does not have reproductive energetic

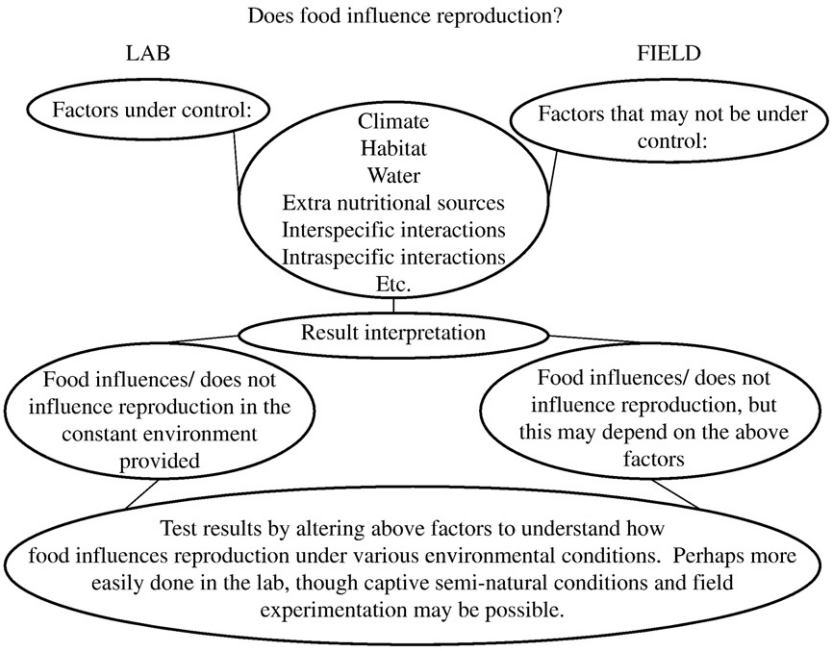

Fig. 1. An example of possible factors that can influence results and their interpretation in lab and field experiments. While a lab setting may yield cleaner data, a field setting may yield more biologically relevant data. Both testing realms can reveal important separate though relatively equal aspects. Thus, both can bring about a more comprehensive understanding of the question at hand.

requirements in the short days of winter and thus can allocate more energy towards others physiological processes such as immune function (Greenman et al., 2005). Social environment can also affect immune function: Weil et al. (2007) reported that housing adult male Siberian hamsters (*Phodopus sungorus*) with ovariectomized females prevented short-day enhancement of delayed-type hypersensitivity immune responses, independently of photoperiod-mediated reproductive changes (Weil et al., 2007). How the social environment is translated into an immune signal is not known.

In a controlled lab setting, enriched environments are positively associated with immunocompetence (Diamond, 1964, 1967; Rosenzweig, 1966). Mlynarik et al. (2004) gave captive male juvenile Wistar rats an immune challenge (*Escherichia coli* lipopolysaccharide in increasing amounts) which resulted in high levels of corticosterone and decreases in body weight. However, rats housed in enriched conditions had low levels of corticosterone and maintained body weight, despite the immune challenge. In a related study by Kozlowski et al. (2004), Sprague–Dawley rats raised in enriched conditions and then given a controlled cortical impact suffered greater neurodegeneration and deficit in their forelimb use than controls raised in standard environments. However, the enriched group demonstrated an increased capacity for recovery. Studies such as these emphasize the need to be cautious when interpreting results for clinical use, as results vary depending on the captive lab conditions.

Given the topics already covered in this mini-review, it is of no surprise that the immunocompetence of captive organisms can also differ from their free-living counterparts. The evolution of host resistance is speculated to, in part, be the result of a continuous arms race between pathogens and their hosts (Hamilton and Zuk, 1982). Secondary sexual characteristics can be costly to maintain and thus can serve as an honest signal of quality. Testosterone, which can mediate these secondary sexual characteristics, often has a negative correlation with aspects of immune function (Folstad and Karter, 1992). Hence, a trade-off may exist between immunocompetence and the expression of elaborate traits. To test, Peters (2000) examined the primary antibody response of captive control and testosterone

Pros and cons of different experimental arenas

	Pros	Cons
Lab:	controlled setting allows for clearer understanding of direct cause and effect	biological and physiological response in an unnatural setting may not be biologically relevant to organism
Field:	natural biological and physiological responses occur	difficult to understand direct cause and effect in an uncontrolled setting due to complex environmental interactions
Semi-natural:	semi-controlled setting, such as an outdoor, size-appropriate enclosure in the organism's natural environment, may yield natural biological and physiological responses; social, nutritional, etc. factors can be mostly controlled	some factors, like climate, light, noise, etc. may still confound results and hinder clear interpretations

Fig. 2. Pros and cons of experimenting in the lab, field or semi-natural environment.

implanted male superb fairy-wrens (*Malurus cyaneus*) to sheep red blood cell (SRBC) immunizations. Peters (2000) also examined a cross-section of free-living males with elevated or basal testosterone, as determined by their plumage phase. Captive males implanted with testosterone had a decreased SRBC antibody response, which in itself lends support to the immunocompetence handicap hypothesis.

However, free-living males with naturally elevated testosterone had an increased likelihood to respond to SRBCs than males with basal testosterone concentrations, which is contrary to Folstad and Karter's (1992) hypothesis. The free-living males of this study had undergone early molting and nuptial plumage development, which is associated with an increase in testosterone concentrations. Early molting and high testosterone may be a sign of high quality and good endurance, which females show preferences for (Dunn and Cockburn, 1999). Peters (2000) posits that these particular wild males exhibited an increased SRBC response because they were of high quality beforehand. While this hypothesis may be the reason behind this discrepancy, we must also consider the possible social and environmental factors denied to captive animals which may play a role in their immunocompetence.

Summary

It seems clear that experimental arena and design can influence the outcome of certain experiments. We expect that there are equally as many examples of experiments that are repeatable in the lab and the wild, although it is not the goal of this review to highlight such studies. Context-dependent rejection of null hypotheses might well influence the direction of research programs, and just as lines of research that are not applicable to an organism's natural environment might be developed and sustained as a result of housing context, opportunities for investigating behavioral or physiological phenomena in the wild might be missed. We highlighted only a small subset of examples to emphasize the need for experiments in both lab and field when looking at complex biological systems, or at least caution when interpreting a study's results. Pros and cons exist for both lab and field experiments (Fig. 2), therefore understanding the subject or behavior in question may be best attempted by a combination of both settings. By comprehending how and why these various results occur may lead to an even greater understanding of the question at hand. Of course, specific phenomena might have no relation to what occurs in an animal's natural setting but may help us elucidate mechanisms of physiology and behavior. As such, lab models remain very powerful tools for investigation and in no way do we wish to imply otherwise.

Semi-natural environmental settings might be the one of the best ways to study an organism's physiology and behavior. This type of experimental arena allows an animal to remain in and be exposed to its natural setting, yet still allows for some control by the researcher. To deprive an animal of its ecology may deprive the researcher of a true result, or introduce an artefactual one. It is difficult, if not mostly impossible, to control various confounding social and environmental factors, therefore we must remain ever cognizant of these issues and open-minded when designing experiments and interpreting their outcomes. As mentioned in the Introduction, we assume that there are many more examples of discrepancies between lab and field data that have not been published, simply because investigators find them hard to explain. Perhaps, just as study of individual variation has been gaining momentum in recent years, data from variation between experimental arenas can provide us with unprecedented lines of research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.yhbeh.2009.02.010.

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