Manuscript Number: GCE-11-330R1

Title: Avian ecologists and physiologists have different sexual preferences

Article Type: Review

Keywords: photoperiodism, quantitative genetics, sex differences, environmental cues, seasonal reproduction, testis, ovary, environmental endocrinology, timing of breeding, micro-evolution

Corresponding Author: Dr. Samuel P. Caro, PhD

Corresponding Author's Institution: Netherlands Institute of Ecology

First Author: Samuel P. Caro, PhD

Order of Authors: Samuel P. Caro, PhD

Abstract: Seasonal timing is studied by ecologists and physiologists alike and it is now widely recognized that further integration of these fields is needed for a full understanding of phenology. This is especially true in the light of the impact of global climate change on living organisms. In studies of avian reproduction, one obstacle to this integration is that ecologists and physiologists do not allocate their research efforts equally to males and females. The physiological orchestration of breeding stages has been studied almost exclusively in males, while in avian ecology and evolutionary biology females are more often considered. This sex bias has severe implications: sexes differ in the way they use external cues to organize their life cycles, but often cue in on each other's physiology and behavior. The simultaneous investigation of both males and females within single studies is thus essential. In this review, I begin by illustrating the sex-bias in studies and attempt to explain its origin. I then provide a number of examples in which focusing on a single sex would have resulted in misleading conclusions. Finally, I review some classical studies of female reproductive physiology that have promoted and developed research on the "forgotten-sex".

Prepared for General and Comparative Endocrinology

Avian ecologists and physiologists have different sexual preferences

Samuel P. Caro

*Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW),
P.O. Box 50, 6700 AB Wageningen, The Netherlands*

Tel: 0031(0)317473451. E-mail: s.caro@nioo.knaw.nl

Abstract

Seasonal timing is studied by ecologists and physiologists alike and it is now widely recognized that further integration of these fields is needed for a full understanding of phenology. This is especially true in the light of the impact of global climate change on living organisms. In studies of avian reproduction, one obstacle to this integration is that ecologists and physiologists do not allocate their research efforts equally to males and females. The physiological orchestration of breeding stages has been studied almost exclusively in males, while in avian ecology and evolutionary biology females are more often considered. This sex bias has severe implications: sexes differ in the way they use external cues to organize their life cycles, but often cue in on each other's physiology and behavior. The simultaneous investigation of both males and females within single studies is thus essential. In this review, I begin by illustrating the sex-bias in studies and attempt to explain its origin. I then provide a number of examples in which focusing on a single sex would have resulted in misleading conclusions. Finally, I review some classical studies of female reproductive physiology that have promoted and developed research on the "forgotten-sex".

Keywords: photoperiodism, quantitative genetics, sex differences, environmental cues, seasonal reproduction, testis, ovary, environmental endocrinology, timing of breeding, micro-evolution

1. Introduction

Animal reproduction is one of the most-studied fields in the biological sciences. In the wild, reproduction takes place in a dynamic environment in which environmental cues and resources show seasonal variation that may be anywhere in the range from unpredictable to predictable. Animals have evolved ecological, behavioral and physiological responses to match their reproduction to the environment. Environmental cues are perceived by sensory systems and transduced at the physiological level, and play a key role in the timing of life history events such as reproduction, molt or pelage change, and migration or hibernation [133]. This timing is in turn often subject to strong selection pressures that favor one particular phenotype over others [4,68]. A great deal of effort has been devoted to measurement of phenotypic performances in a range of environments. Equal effort has been made to decipher the neural and physiological cascades that process environmental cues and regulate reproductive output. However, selection and physiology have historically been studied by different groups of scientists: while ecologists and evolutionary biologists have focused on the ‘ultimate’ fitness consequences of different phenotypes, physiologists have investigated the proximate mechanisms regulating the developmental processes by which they are generated and shaped. Over the years, the two fields have evolved independently and developed their own research agendas with few attempts to share and integrate their respective advances in knowledge [137]. Because of the rapid growth of both disciplines, the gap between them is widening, limiting the understanding of mechanisms and processes that have traditionally been the purview of the other discipline [68,115,137]. Recently, attempts have been made to fill this gap [139]. For example, the ESF/NSF/NSERC-funded E-Bird Research Coordination Network [137] aimed to integrate our understanding of the ultimate and proximate determinants of seasonal reproduction in free-living bird populations.

Although biologists have already recognized the need for integrative, multidisciplinary, studies, differences in the approaches used by ecologists and physiologists have limited the amount of integration. One important constraint is that avian reproductive physiologists have tended to focus on males [14], while ecologists have concentrated on the role played by females in critical reproductive decisions such as mate choice, timing of breeding and brood size [3,53,101,107,110,111]. As a consequence, physiologists have accumulated extensive knowledge on the neurobiology and physiology of male reproduction, but relatively little about that of females (other than in poultry, which are not considered here). The preference

for males in physiological studies is not new. James Wendell Burger noted in 1949 that “most of the experimental work [on seasonal reproduction] has dealt with the male. The female as such, and the reciprocal relationships of the sexes have received little attention” [14]. Since then, there have been a few reviews that have reported the same bias [1,5,6,71,137], but the ways in which this might impact future knowledge and understanding have never been explicitly discussed. Here, I will first illustrate this bias, and then try to explain how it originated and more importantly, how the inclusion of both males and females in single studies on reproduction builds bridges between evolutionary ecology and physiology - bridges that are essential to understanding the proximate mechanisms that animals use in a rapidly changing environment. Finally, I will give some examples of research programs that have successfully integrated females into physiological studies.

2. Literature investigation

To illustrate the difference in the sex-bias of physiological and ecological studies of reproduction, I ran a literature search using the citation database ISI Web of Science® on two sets of five journals that publish research papers on either the reproductive physiology or ecology of birds. This search was performed in July 2011. I deliberately excluded journals that exclusively publish review papers, and broad topic journals in which the same keyword could refer to both physiological and ecological studies. The selected journals in physiology were *General and Comparative Endocrinology*, *Hormones and Behavior*, *Journal of Experimental Zoology part A-Ecological genetics and Physiology* (formerly *Journal of Experimental Zoology*), *Journal of Neuroendocrinology*, and *Physiological and Biochemical Zoology* (formerly *Physiological Zoology*). The selected journals in ecology were *Behavioral Ecology and Sociobiology*, *Ecology*, *Evolution*, *Journal of Animal Ecology* and *Oecologia*. The same query was introduced in the “topic” field for both searches: “*reproducti* AND (bird* OR avian)*”. From the results, I checked each reference found by the Web of Science and manually counted the number of studies that were conducted exclusively in males, exclusively in females or in both sexes. The searches in physiology and ecology journals found 427 and 959 references, respectively. From each of these groups of references, I analyzed the 250 most recent ones. Reviews, and studies conducted exclusively on nestlings or poultry (because the current review concerns reproductive traits in non-domesticated species), were subsequently excluded, leaving 135 physiology and 183 ecology references.

As expected, studies on reproductive traits published in physiology journals concern male birds more often than females (Fig. 1, main panels). Overall, 84% of the physiological studies involved male birds (41% exclusively males + 43% males and females), while only 58% involved females (15% exclusively females + 43% males and females). However, in ecology journals, although studies involving females were predominant (77% of studies involving females, 69% involving males), most studies investigated both sexes (60%, Fig. 1). More strikingly, in both fields, the sex considered was frequently not mentioned in the title, abstract or keywords (16% of studies in the physiology search, 36% of studies in the ecology search, not shown on figure). In those cases, I either knew the sex studied because of sex-specific words in the title or abstract (such as “testis” or “ovary”), or I had to search for this information in the methods of the article. Even more surprisingly, 26 papers did not mention the words “male” or “female” anywhere in the article, so that it was not possible for the reader to know whether the study subjects were males or females (see “?” bar in Fig. 1). Very often, when the sex considered was not reported in the abstract or body of the article, authors tended to extrapolate and generalize by simply citing the name of the species considered. Finally, in many studies that considered both sexes, authors did not consider sex as a factor in their analyses. As a consequence, any sex differences would not be revealed.

Choosing the 250 most recent references led to different time spans for the literature searches in physiology and ecology (time span for physiology: June 2003 – July 2011; for ecology: March 2007 – July 2011). Restricting the search in physiology to the same time span as the search in ecology reduced the number of references analyzed to 82 but did not change the observed pattern (37% exclusively males, 48% males and females, 12% exclusively females and 3% unknown).

The search terms used were broad, and different sex-biases might have been found if narrower search terms had been used. I noticed, for example, that many of the references that investigated both sexes studied seabirds, stress responses, or both. When I excluded those studies from the physiology references, the proportion of studies performed *exclusively* on males rose to 55%. To see whether the sex-biases observed with the general searches are a general property of each of the two fields, I ran a second set of searches using different, more discipline-specific, keywords to find studies on photoperiodism and quantitative genetic heritability. These new searches do not aim at directly comparing physiology with ecology as the keywords used are very different, and therefore hardly comparable. Instead, they should

be taken as examples of how the sex-bias might vary *within* each discipline. Photoperiodism and heritability of traits are key topics for avian physiologists and ecologists, respectively. The central tenant that day length plays a key role in regulating timing of breeding has been widely accepted for almost 100 years since the pioneering work of Rowan [95,96], and his investigations have been very influential in driving neuroendocrinological research on the control of seasonality. Photoperiod is still considered as the major environmental cue containing long-term predictive information about the optimal period for initiating and terminating breeding [29,100,133], and is therefore one of the most studied topics in avian physiology. In comparison, heritability studies have become common in evolutionary ecology over recent decades, because of the development of computational resources and statistical methods such as the “animal model”, now allowing us to analyze multigenerational pedigrees and reliably estimate the additive variance that underlies heritability [50,60,124]. Finally, heritability studies on physiological mechanisms in birds are still extremely rare [54,115, but see 35,87], so the keyword “heritability” almost never refers to physiological studies which might contaminate the results.

For this second literature investigation, I used the following search terms in the “topic” field: “*photoperiodism AND (bird* OR avian)*”, and “*heritabilit* AND (bird* OR avian)*”. Because these keywords are discipline-specific (see above), I did not restrict the array of journals in which the searches were performed. Although heritability is often used as a keyword, many studies did not contain quantitative genetic analyses *per se*. I therefore restricted the analysis to the papers that reported some heritability measurements. The “photoperiodism” and “heritability” searches found 87 and 417 references, respectively. From the 417 references resulting from the “heritability” search, I analyzed the 250 most recent ones. Reviews and poultry science were again subsequently excluded from the results.

The results of this more specific set of searches emphasize the pattern found in the first search: the vast majority of studies of photoperiodism concern exclusively the males (see inserts in Fig. 1). Overall, 91% of the references that I analyzed involved male birds (70% exclusively males + 21% males and females), while only 26% involved females (5% exclusively females + 21% males and females). In studies investigating quantitative genetic heritabilities of traits, the proportion of studies investigating both males and females rose from 60% to 71% (Fig. 1). The number of studies that did not mention the sex of birds involved in the title, abstract or keywords was higher for both search terms than in the first

searches (55% of studies on photoperiodism, 48% of studies on heritability, not shown on figure).

3. Reasons for the disequilibrium

There are many practical reasons for favoring males in studies of avian reproduction. Firstly, male birds have developed a wide array of secondary sexual characters that make them generally brighter and louder than females. Males often sing routinely from a visible song post in order to acquire and defend a territory or attract females, and they generally have bright plumage and behave aggressively towards competitors [136]. These secondary sexual characteristics are thought to have evolved due to sexual selection [3]. The selection pressures underlying these traits are outside the scope of this paper, but one of their major consequences is that males are generally much easier to detect than females. For the field biologist, males are therefore easier to locate, to record and/or to catch [49,58,121] and their secondary sexual morphological and behavioral characters are generally easily quantifiable. This first practical reason is however valid for both the field physiologist and the field ecologist who study birds, and should therefore not explain why the observed bias in favor of males only occurs in the studies of breeding physiology.

Physiological traits are also often easier to quantify in males than females. Although this may not initially have been the factor resulting in higher research effort on males, it may now be so. Hundreds of studies have addressed the relationship between male traits and androgens (especially testosterone), but few have investigated the role of steroids in females. There has been a recent upsurge in the number of studies investigating the role of testosterone in females [e.g. 27,51,72,84], but how 17β -estradiol (one of the main female gonadal steroids) influences the reproductive activity in free-living birds is poorly known [49]. In fact, testosterone is rather simple to assay compared to 17β -estradiol, which often requires higher plasma volumes or sensitive assays [49]. The extensive knowledge accumulated on the effects of testosterone, combined with the ease to assay it, might still currently encourage scientists to focus on males rather than on females and reinforce the male-bias in avian breeding physiology. Another reason for favoring males over females resides in the male brain. Male songbirds show a high level of seasonal neuroplasticity associated with song learning and production [83]. The volumes of some telencephalic nuclei that control song behavior are significantly larger in spring than in fall and generally correlate with the amount of singing activity and the

testosterone concentrations [see review in 7, but see 20,67]. These song-control nuclei are also present in the females of many species (not all), but their seasonal variations are much smaller and in general it is hard to correlate these variations to song behavior.

Finally, wild male birds often show apparently complete sexual development in captivity, while females often do not. Providing artificial spring-like environmental conditions to captive male songbirds causes a cascade of reactions resulting in the activation of their hypothalamo-pituitary-gonadal (HPG) axis [36]. Even if reproductive hormone levels rarely reach the concentrations naturally observed in the field, the testes grow normally and reach a full, active size within the time-frame observed in the wild. Females however are more reluctant to breed in captivity; their gonadal development rarely reaches the final maturation stages [28,30,37,58,70,78,81,125,128,132]. Several authors have concluded that females require a wider range of environmental cues to achieve complete reproductive development than do males [58,81,128], and that the set of environmental cues available in captivity is generally too limited for full female gonadal maturation [6,10].

As well as practical reasons, some people have suggested that the sex-bias might arise from the different perspectives of male and female biologists [e.g. 43] and thus be sociological in origin. However, the preponderance of male authors in early studies of avian reproductive ecology [see references cited in 61] argues against the difference in the proportion of studies on females between physiological and ecological studies being caused in this way. The aim of this article is to focus on the practical reasons and solutions for a sex-bias in physiological studies, and I therefore will not discuss a possible sociological bias further here.

4. Why is it so important to consider both sexes?

Restricting biological studies to a single sex, or extrapolating conclusions obtained from one sex to the other, may lead to false conclusions because males and females may differ in the proximate cues used to orchestrate their breeding cycle, and the behavior of each sex may act as a cue for the other [6]. One example of the importance of considering both sexes is given by the recent comparative study of seasonal sexual development in two Corsican blue tit (*Cyanistes caeruleus*) populations. The laying dates of these populations differ by about a month despite their close geographic proximity (25km). This divergence in laying dates is

selected by a difference in the phenology of their prey [13], and would be orchestrated by a genetic differentiation of their photoperiodic sensitivity: birds breeding late would respond to longer photoperiods than birds breeding early in the spring [21,62-64,see review in 115]. However, whether the responses of males and females to photoperiod is the same is not known, because no physiological measurements were made. Subsequent field studies have shown that males actually start their development into breeding condition at the same time in both environments [18,20,22]; results that are thus inconsistent with the differential photoperiodic threshold hypothesis. In contrast, female gonadal development was well differentiated between the two populations [19] and analyses of the respective additive genetic influences of males and females on laying dates have demonstrated that genes expressed in males did not have any effect on laying dates. This suggests that the differentiation of the breeding periods, and potentially the differentiation of the photoperiodic sensitivities, is exclusively driven by the females [19].

In the above example, if the study of the proximate physiological mechanisms had been limited to males, and the ecological data not been available, the erroneous conclusion that Corsican blue tits do not respond to the heterogeneity of their environment would have been drawn from the physiological study. One adaptive reason for the physiological difference between males and females is that, for males, being reproductively active early in the breeding season brings a fitness benefit by allowing time for seeking additional (extra-pair) partners [6], with potentially lower energetic (and therefore fitness) costs associated with maintaining an active reproductive machinery than in females [24,57,119,123] (although more studies of these costs in males and females are desirable, see [113]). Relatively high costs of breeding in females might explain why their phenology is more closely tied to environmental variation than in males, and why they drive the differentiation between populations, such as the example described above. While it was believed some decades ago that, because males are of the first to show reproductive activity (territory acquisition and defense), they would also be more sensitive to environmental cues [e.g. 82 p. 217], it now appears that females are at least as sensitive as males. One example comes from work by Wingfield and colleagues [129-131] showing a latitudinal cline in the sensitivity to temperature cues in white-crowned sparrows, with a steeper cline in females than males. The lower sensitivity of males to some cues facilitates physiological studies, especially in captivity, but does not provide insights into complex life-history strategies related to environmental conditions. A difference between males and females in sensitivity to environmental cues might also explain why females breed

less freely in captivity. These examples demonstrate how important it is to take account of both sexes in integrative studies. In order to shed light on the rules governing adaptive responses to environmental variation, and understand whether and how organisms can match their life-histories to environmental change [1,114], it is essential to investigate males and females concurrently, using an integrative approach mixing ecology, physiology, quantitative genetics, and wild and captive studies [17,41,115,137].

There are other examples of the importance of considering both sexes. Natural selection on traits in one sex may cause an indirect evolutionary response in the other. Thus sexual morphological, physiological or behavioral characters measured in males may well reflect selection on females [6,52,76]. For example, Ketterson, Nolan and colleagues manipulated plasma levels of testosterone in dark-eyed juncos (*Junco hyemalis*) and assessed the consequences in terms of survival, mating success and offspring produced [55,56,94]. Males implanted with testosterone (T-males) had decreased body mass, immune function and survival but produced more extra-pair offspring than control males (C-males). In addition, treatment of young males increased their ability to attract older, more experienced, females, even if this did not necessarily lead to higher reproductive performance [94]. Overall, T-males had higher fitness than C-males, which raises the question of why natural counterparts of T-males are not more common. McGlothlin and Ketterson [76] suggested that the evolution of very high levels of testosterone in males may be constrained by a genetic correlation across the sexes. If testosterone levels are genetically correlated between males and females, selection for higher concentrations of testosterone in males, would also induce higher testosterone levels in females, which has detrimental effects on female body mass, immune function, timing of breeding and molt [27,140]. Therefore, the net cost of high testosterone levels in females might counterbalance selection for higher testosterone levels in males [76]. From these examples and the available literature, we should remember that (1) males and females differ in most of the cues they use to orchestrate their life-history stages [6]; (2) the behavior of each sex acts as a cue for the other [26,40,66,80,79,81] and, (3) females often play critical roles in determining fitness-related traits such as mate-choice or the timing of breeding. As a consequence, future research on bird natural history and breeding biology should systematically investigate both sexes.

5. Reducing the sex-bias in physiological studies

The literature review above suggests that males and females are reasonably equitably represented in ecological studies (Fig. 1), so the main challenge is in reducing the bias towards males in physiological studies. Previous sections have identified several obstacles to the use of females in such research. Given that most of these obstacles still exist, there is no simple and universal solution that will eliminate the problem and it is mainly to individual researchers to find the best options for their specific questions and study systems. Nevertheless, this section attempts to identify approaches that are most likely to be productive in alleviating the sex-bias, and reviews some remarkable examples of research on female reproductive physiology.

In the field, females are often more difficult to observe or catch than males. However, the females of some species (particularly tropical ones [38]), are wholly or partially responsible for the defense of a territory (e.g. spotted antbird, *Hylophylax n. naevioides* [46]; splendid fairy-wren, *Malurus splendens* [91]; European robin, *Erithacus rubecula* [105]), facilitating research on females. The same behaviors are observed in sex-role reversed species, such as jacanas (Jacanidae), sandpipers (Scolopacidae) and coucals (Centropodidae). In these species, the females compete for males who then raise the offspring. Females are generally larger than males and display male-typical behaviors such as territory defense and singing [32,39,42,85,86]. Females of such species are therefore conspicuous and good models for studying the neuroendocrine mechanisms underlying some behaviors such as resource-defense aggression and, more generally, behavioral sex differences [32,45,118]. But even in species with conventional sex roles, where territorial males are much more conspicuous than females, simply increasing observation or catching effort, or diversifying the techniques used, are affordable and efficient solutions [e.g. 51,104]. In the 1960s and the 1970s for example, females sparrows of the genus *Zonotrichia*, especially the white-crowned sparrow (*Zonotrichia leucophrys*), were the subject of extensive research [e.g. 37,58,69,81,128]. This effort now seems to have faded away for no apparent reason. The females from these studies were however caught in the same way as males, generally using mist-nets, sometimes combined with simulated territorial intrusion, and baited traps [see also 34,45,59,89,97,134]. Finally, females of hole-nesting passerines can be easy to catch as they often roost and breed in nestboxes [e.g. 93], creating another relatively easy opportunity for collecting data on females in these species.

In captivity, wild-caught female birds often do not breed. It has been argued that these females may imprint on their habitat characteristics early in life and later require the full range of environmental stimuli to complete egg formation when raised in captivity [117]. Several solutions exist for those who want to address proximate questions in captive female birds. Firstly, some species of birds are easier to raise in captivity than others. This is of course the case for species in which domesticated strains have been created, such as the canary (*Serinus canaria*), zebra finch (*Taeniopygia guttata*) and ring dove (*Streptopelia risoria*). These readily breed in relatively small cages and have been used in a wide array of behavioral and physiological studies including neurobiology, (neuro)endocrinology and metabolism [e.g. 11,26,48,65,66,92,103,120,122]. However, although domesticated and wild strains of the same species share many life-history characters [106], the selection pressures exerted during the domestication processes are different from those faced in the wild [138]. As a consequence, domesticated strains are not appropriate in studies aimed at linking physiological mechanisms to evolutionary adaptations, unless a proper validation of the results using non-domesticated individuals is added to the study. Caution is therefore required in generalizing results obtained from domesticated strains of these species. Among non-domesticated species, European blackbirds (*Turdus merula*), warblers (Sylviidae), stonechats (Muscicapidae), tits (Paridae) and European starlings (*Sturnus vulgaris*) are examples of birds that breed in captivity, especially when hand-reared (see below) and housed in sufficient space in aviaries with access to a choice of mates, nestboxes and/or natural earth floors [12,21,31,33,47,77,90,98,108,109,112,116, G.E. Bentley and A. Dawson, pers.comm.].

A second way of getting around the problem that wild-caught females hardly adapt to captive settings is to increase their responsiveness by injecting them with exogenous hormones such as steroids or Gonadotropin Releasing Hormone (GnRH). These techniques have mostly been used in studies investigating behavioral responses of females to male song [2,8], but have also been helpful in physiological studies [27,51,74,73,79,80,102,127,135]. The major drawback of hormone implants is that they alter the normal physiology of the female by creating an artificial hormonal environment [15,23], and this restricts the array of applications. GnRH injections however do not induce supra-physiological levels of hormones because they only release the amount of hormone that is stored in the endocrine glands in a similar fashion as a social stimulus would do [16,51,75].

Finally, one of the best solutions for increasing the probability of observing natural-like behaviors in captivity by females of wild origin is to hand-rear them. For example, female white-crowned sparrows brought into captivity as nestlings and hand-raised readily ovulated in captivity under conditions in which females taken from the wild as adults have never been reported to lay [10]. These results suggest that hand-reared chicks may more easily display the species-typical concentrations of GnRH, gonadotropins and gonadal steroids when kept in captivity and therefore allow the precise investigation of the respective roles of photoperiod and supplemental cues in the final maturation of female HPG axis. Baptista and Petrinovich also reported that egg-laying intervals were much shorter if the females were provided with nesting material, suggesting that the wider the set environmental cues available, the more natural the female reproductive output [10]. Thus, both hand-rearing and enrichment of the captive environment seem to have positive effects on the expression of reproductive phenotypic characters in female birds. Hand-rearing birds or keeping them in large aviaries increases both the manpower and budget necessary for the experiments, and this could affect the sample sizes. However, a reduced sample size is sometimes better than holding pairs in small cages and applying different treatments to separate rooms, within which all the pairs receive the same treatment. The resultant pseudoreplication can lead to a considerable loss of statistical power, even when analysed correctly.

The physiology of female birds has been much less studied than that of males. I will not re-discuss the reasons for the male-bias here, but it should be remembered that the inequality may have reinforced the bias by influencing the techniques that are currently available. While there are commercial kits available for testosterone, nothing is available for measuring estradiol in birds. Ironically, an extremely sensitive method for assaying 17β -estradiol has recently been described, but this was developed to measure estrogens in the male brain [25], which are critical for the activation of male reproductive behaviors through the local aromatization of testosterone [9]. Another complication related to female physiology is that the rapid maturation of the ovarian follicles, mainly occurring during egg-yolk deposition, is sudden and transient and can easily be missed during sampling. Although it requires careful validation, the analysis of hormones in droppings allows precise tracking of temporal variation in reproductive hormone levels and hence the ability to assess the reproductive stage of females [44,88]. Sockman and Schwabl [103] for example demonstrated that 17β -estradiol and progesterone levels in female canaries can be followed on a daily basis by analysing fecal hormones. So in summary more effort should be put into developing sensitive and reliable

methods for assessing female reproductive physiology. This would inevitably lead to positive effects on the balance between male- and female-oriented studies. The best demonstration for this comes from the field of acoustic communication, where avian studies have historically been male-oriented [99]. This bias was largely due to the lack of a method for assessing female response to song playback [99], and persisted until estradiol implants were developed and started to be widely used in this field.

6. Conclusions

1. Despite the importance of females in adaptive decision-making, many physiologists still focus their research exclusively on males. As discussed above, there are several reasons that have motivated, and might still encourage, researchers to concentrate their research effort on males.
2. Contrary to what has sometimes been stated in the literature, females seem to be at least as sensitive to environmental cues as males. This, combined with possibly higher fitness costs associated with variation in some life-history traits in females, may explain why they often drive critical decisions such as mate-choice, timing of breeding or clutch size. Restricting studies to males undoubtedly leads to extrapolations and generalizations, and we have seen examples of how the sole consideration of males can lead to conclusions that are not fully relevant on an ecological point of view. The goal of this review is not to encourage researchers to switch from studying males to females, but rather to consider both sexes.
3. There are several different kinds of solution to the difficulties of studying physiology in females. This includes increasing observation or catching effort in the field, enriching the environment in captive setting, hand-rearing birds, and improving the techniques available for assessing female reproductive physiology.
4. Ultimately, the knowledge of the female reproductive physiology will be critical for understanding micro-evolution. There is growing interest from the public opinion and the politics for the current and future consequences of the human-related environmental perturbations. A first step toward the understanding of these ecological consequences involves comprehension of how the environment is perceived by the organisms and transduced at the physiological level [1,6,115,126]. This is important because selection mechanisms act through the cascade of physiological processes that precede critical life-history traits such as timing of

breeding, molt or migration. If we already differ in the sex we study, it will not be possible to unify the frameworks of our different disciplines, which is critical for predicting how populations respond to environmental change.

Acknowledgements

This manuscript originated from a breakout session entitled “Which sex to study in which field?” at the “Keeping Track of the Seasons: The ecology and physiology of annual cycles in mammals and birds” workshop in 2008 in Leiden, The Netherlands. The workshop was organized by Alistair Dawson, Barbara Helm, Marcel E. Visser and funded by the Lorentz Centre and the Dr. J.L. Dobberke foundation. I thank the other participants of this session for their constructive comments. I also thank George E. Bentley, C. (Kate) M. Lessells, Marcel E. Visser and two anonymous reviewers for their very constructive comments on an earlier version of this manuscript. SPC was supported by a NWO-VENI and a WBI.World (Wallonie-Bruxelles International) grants.

References

- [1] E. Adkins-Regan, Do hormonal control systems produce evolutionary inertia?, *Phil. Trans. R. Soc. B* 363 (2008) 1599-1609.
- [2] R.C. Anderson, Operant conditioning and copulation solicitation display assays reveal a stable preference for local song by female swamp sparrows *Melospiza georgiana*, *Behav. Ecol. Sociobiol.* 64 (2009) 1185-1194.
- [3] M. Andersson, *Sexual selection*. University Press, Princeton, 1994.
- [4] J.R. Baker, The evolution of breeding seasons, in: G.R. de Beer (Ed.), *Evolution, Essays on Aspects of Ev. Biology Presented to Prof. E.S. Goodrich on His 70th Birthday*. Oxford University Press, London, New York, 1938, pp.
- [5] G.F. Ball, J. Balthazart, Neuroendocrine mechanisms regulating reproductive cycles and reproductive behavior in birds, in: D.W. Pfaff, A.P. Arnold, A.M. Etgen, S.E. Fahrbach, R.T. Rubin (Eds.), *Hormones, Brain and Behavior*. Academic Press, San Diego, 2002, pp. 649-798.
- [6] G.F. Ball, E.D. Ketterson, Sex differences in the response to environmental cues regulating seasonal reproduction in birds, *Phil. Trans. R. Soc. B* 363 (2008) 231-246.
- [7] G.F. Ball, L.V. Riters, J. Balthazart, Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones, *Front. Neuroendocrinol.* 23 (2002) 137-178.
- [8] B. Ballentine, J. Hyman, S. Nowicki, Vocal performance influences female response to male bird song: an experimental test, *Behav. Ecol.* 15 (2004) 163-168.
- [9] J. Balthazart, A. Foidart, Brain aromatase and the control of male sexual behavior, *J. Steroid Biochem. Mol. Biol.* 44 (1993) 521-540.
- [10] L.F. Baptista, L. Petrionovich, Egg production in hand-raised white-crowned sparrows, *Condor* 88 (1986) 379-380.
- [11] G.E. Bentley, N.C. Audage, E.K. Hanspal, G.F. Ball, T.P. Hahn, Photoperiodic response of the hypothalamo-pituitary-gonad axis in male and female canaries, *Serinus canaria*, *J. Exp. Zool. A* 296A (2003) 143-151.
- [12] P. Berthold, U. Querner, Genetic Basis of Migratory Behavior in European Warblers, *Science* 212 (1981) 77-79.
- [13] J. Blondel, P.C. Dias, P. Perret, M. Maistre, M.M. Lambrechts, Selection-based biodiversity at a small spatial scale in a low-dispersing insular bird, *Science* 285 (1999) 1399-1402.
- [14] W.J. Burger, A review of experimental investigations on seasonal reproduction in birds, *Wilson Bull.* 61 (1949) 211-230.
- [15] B.E. Byers, D.E. Kroodsma, Female mate choice and songbird song repertoires, *Anim. Beh.* 77 (2009) 13-22.
- [16] K.E. Cain, E.D. Ketterson, Competitive females are successful females; phenotype, mechanism, and selection in a common songbird, *Behav. Ecol. Sociobiol.* Online First (2011).
- [17] R.M. Calisi, G.E. Bentley, Lab and field experiments: Are they the same animal?, *Horm. Behav.* 56 (2009) 1-10.
- [18] S.P. Caro, J. Balthazart, D.W. Thomas, A. Lacroix, O. Chastel, M.M. Lambrechts, Endocrine correlates of the breeding asynchrony between two Corsican populations of blue tits (*Parus caeruleus*), *Gen. Comp. Endocrinol.* 140 (2005) 52-60.
- [19] S.P. Caro, A. Charmantier, M.M. Lambrechts, J. Blondel, J. Balthazart, T.D. Williams, Local adaptation of timing of reproduction: females are in the driver's seat, *Func. Ecol.* 23 (2009) 172-179.
- [20] S.P. Caro, M.M. Lambrechts, J. Balthazart, Early seasonal development of brain song control nuclei in male blue tits, *Neurosci. Lett.* 386 (2005) 139-144.

- [21] S.P. Caro, M.M. Lambrechts, J. Balthazart, P. Perret, Non-photoperiodic factors and timing of breeding in blue tits: Impact of environmental and social influences in semi-natural conditions, *Behav. Proc.* 75 (2007) 1-7.
- [22] S.P. Caro, M.M. Lambrechts, O. Chastel, P.J. Sharp, D.W. Thomas, J. Balthazart, Simultaneous pituitary-gonadal recrudescence in two Corsican populations of male blue tits with asynchronous breeding dates, *Horm. Behav.* 50 (2006) 347-360.
- [23] S.P. Caro, K.B. Sewall, K.G. Salvante, K.W. Sockman, Female Lincoln's sparrows modulate their behavior in response to variation in male song quality, *Behav. Ecol.* 21 (2010) 562-569.
- [24] S.P. Caro, M.E. Visser, Temperature-induced elevation of basal metabolic rate does not affect testis growth in great tits, *J. Exp. Biol.* 212 (2009) 1994-1998.
- [25] T.D. Charlier, K.W.L. Po, A.E.M. Newman, A.H. Shah, C.J. Saldanha, K.K. Soma, 17 β -Estradiol levels in male zebra finch brain: Combining Palkovits punch and an ultrasensitive radioimmunoassay, *Gen. Comp. Endocrinol.* 167 (2010) 18-26.
- [26] M.F. Cheng, The role of vocal self-stimulation in female responses to males: Implications for state-reading, *Horm. Behav.* 53 (2008) 1-10.
- [27] E.D. Clotfelter, D.M. O'Neal, J.M. Gaudioso, J.M. Casto, I.M. Parker-Renga, E.A. Snajdr, D.L. Duffy, V. Nolan, E.D. Ketterson, Consequences of elevating plasma testosterone in females of a socially monogamous songbird: evidence of constraints on male evolution?, *Horm. Behav.* 46 (2004) 171-178.
- [28] A. Dawson, Plasma-luteinizing hormone and prolactin during circannual rhythms of gonadal maturation and molt in male and female European starlings, *J. Biol. Rhythms* 12 (1997) 371-377.
- [29] A. Dawson, Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability, *Phil. Trans. R. Soc. B* 363 (2008) 1621-1633.
- [30] A. Dawson, A.R. Goldsmith, Effect of gonadectomy on seasonal changes in plasma LH and prolactin concentrations in male and female starlings (*Sturnus vulgaris*), *J. Endocrinol.* 100 (1984) 213-218.
- [31] P.J. Drent, K. van Oers, A.J. van Noordwijk, Realized heritability of personalities in the great tit (*Parus major*), *Proc. R. Soc. Lond. B Biol. Sci.* 270 (2003) 45-51.
- [32] M. Eens, R. Pinxten, Sex-role reversal in vertebrates: behavioural and endocrinological accounts, *Behav. Proc.* 51 (2000) 135-147.
- [33] M. Eens, R. Pinxten, R.F. Verheyen, Song learning in captive European starlings, *Sturnus vulgaris*, *Anim. Beh.* 44 (1992) 1131-1143.
- [34] M.M. Elekonich, J.C. Wingfield, Seasonality and hormonal control of territorial aggression in female song sparrows (Passeriformes : Emberizidae : *Melospiza melodia*), *Ethology* 106 (2000) 493-510.
- [35] M.R. Evans, M.L. Roberts, K.L. Buchanan, A.R. Goldsmith, Heritability of corticosterone response and changes in life history traits during selection in the zebra finch, *J. Evol. Biol.* 19 (2006) 343-352.
- [36] D.S. Farner, Annual rhythms, *Ann. Rev. Physiol.* 47 (1985) 65-82.
- [37] D.S. Farner, B.K. Follett, J.R. King, M.L. Morton, A quantitative examination of ovarian growth in the white-crowned sparrow, *Biol. Bull.* 130 (1966) 67-75.
- [38] B.C. Fedy, B.J.M. Stutchbury, Territory defence in tropical birds: are females as aggressive as males?, *Behav. Ecol. Sociobiol.* 58 (2005) 414-422.
- [39] A.J. Fivizzani, L.W. Oring, Plasma steroid-hormones in relation to behavioral sex-role reversal in the spotted sandpiper, *Actitis macularia*, *Biol. Reprod.* 35 (1986) 1195-1201.
- [40] M.B. Friedman, Interactions between visual and vocal courtship stimuli in neuroendocrine response of female doves, *J. Comp. Physiol. Psychol.* 91 (1977) 1408-1416.

- [41] L. Fusani, V. Canoine, W. Goymann, M. Wikelski, M. Hau, Difficulties and special issues associated with field research in behavioral neuroendocrinology, *Horm. Behav.* 48 (2005) 484-491.
- [42] N. Geberzahn, W. Goymann, C. Muck, C. ten Cate, Females alter their song when challenged in a sex-role reversed bird species, *Behav. Ecol. Sociobiol.* 64 (2009) 193-204.
- [43] P.A. Gowaty, Principles of females' perspectives in avian behavioral ecology, *J. Avian Biol.* 28 (1997) 95-102.
- [44] W. Goymann, Noninvasive monitoring of hormones in bird droppings: physiological validation, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels., *Ann. N.Y. Acad. Sci.* 1046 (2005) 35-53.
- [45] W. Goymann, A. Wittenzellner, I. Schwabl, M. Makomba, Progesterone modulates aggression in sex-role reversed female African black coucals, *Proc. R. Soc. B Biol. Sci.* 275 (2008) 1053-1060.
- [46] M. Hau, S.T. Stoddard, K.K. Soma, Territorial aggression and hormones during the non-breeding season in a tropical bird, *Horm. Behav.* 45 (2004) 40-49.
- [47] B. Helm, E. Gwinner, L. Trost, Flexible seasonal timing and migratory - Behavior results from stonechat breeding programs, in: U. Bauchinger, W. Goymann, S. JenniEiermann (Eds.), *Bird Hormones and Bird Migrations: Analyzing Hormones in Droppings and Egg Yolks and Assessing Adaptations in Long-Distance Migration*. New York Acad Sciences, New York, 2005, pp. 216-227.
- [48] R.A. Hinde, E. Steel, B.K. Follett, Effect of photoperiod on oestrogen-induced nest-building in ovariectomized or refractory female canaries (*Serinus Canarius*), *J.Reprod.Fertil.* 40 (1974) 383-399.
- [49] K.E. Hunt, J.C. Wingfield, Effect of estradiol implants on reproductive behavior of female Lapland longspurs (*Calcarius lapponicus*), *Gen. Comp. Endocrinol.* 137 (2004) 248-262.
- [50] A. Husby, D.H. Nussey, M.E. Visser, A.J. Wilson, B.C. Sheldon, L.E.B. Kruuk, Contrasting patterns of phenotypic plasticity in reproductive traits in two great tit (*Parus major*) populations, *Evolution* 64 (2010) 2221-2237.
- [51] J.M. Jawor, J.W. McGlothlin, J.M. Casto, T.J. Greives, E.A. Snajdr, G.E. Bentley, E.D. Ketterson, Testosterone response to GnRH in a female songbird varies with stage of reproduction: implications for adult behaviour and maternal effects, *Func. Ecol.* 21 (2007) 767-775.
- [52] H. Jensen, I. Steinsland, T.H. Ringsby, B.E. Saether, Evolutionary dynamics of a sexual ornament in the house sparrow (*Passer domesticus*): The role of indirect selection within and between sexes, *Evolution* 62 (2008) 1275-1293.
- [53] A.G. Jones, N.L. Ratterman, Mate choice and sexual selection: What have we learned since Darwin?, *Proc. Natl. Acad. Sci. USA* 106 (2009) 10001-10008.
- [54] B. Kempenaers, A. Peters, K. Foerster, Sources of individual variation in plasma testosterone levels, *Phil. Trans. R. Soc. B* (2008).
- [55] E.D. Ketterson, V. Nolan, Hormones and life histories - an integrative approach, *Am. Nat.* 140 (1992) S33-S62.
- [56] E.D. Ketterson, V. Nolan, Jr., J.M. Casto, C.A. Buerkle, E. Clotfelter, J.L. Grindstaff, K.J. Jones, J.L. Lipar, F.M.A. McNabb, D.L. Neudorf, I. Parker-Renga, S.J. Schoech, E. Snajdr, Testosterone, phenotype and fitness: a research program in evolutionary behavioral endocrinology, in: A. Dawson, D.M. Chaturvedi (Eds.), *Avian endocrinology*. Narosa Publishing House, New Delhi, 2000, pp. 19-40.
- [57] J.R. King, Energetics of reproduction in birds, in: D.S. Farner (Ed.), *Breeding biology of birds*. National Academy of Sciences, Washington, D.C., 1973, pp. 78-120.

- [58] J.R. King, B.K. Follett, D.S. Farner, M.L. Morton, Annual gonadal cycles and pituitary gonadotropins in *Zonotrichia leucophrys gambelli*, *Condor* 68 (1966) 476-487.
- [59] E. Kriner, H. Schwabl, Control of winter song and territorial aggression of female robins (*Erithacus rubecula*) by testosterone, *Ethology* 87 (1991) 37-44.
- [60] L.E.B. Kruuk, Estimating genetic parameters in natural populations using the 'animal model', *Phil. Trans. R. Soc. B* 359 (2004) 873-890.
- [61] D. Lack, *Ecological adaptations for breeding in birds*. Methuen, London, 1968.
- [62] M.M. Lambrechts, J. Blondel, M. Maistre, P. Perret, A single response mechanism is responsible for evolutionary adaptive variation in a bird's laying date, *Proc. Natl. Acad. Sci. USA* 94 (1997) 5153-5155.
- [63] M.M. Lambrechts, P.C. Dias, Differences in the onset of laying between island and mainland Mediterranean blue tits *Parus caeruleus*: phenotypic plasticity or genetic differences?, *Ibis* 135 (1993) 451-455.
- [64] M.M. Lambrechts, P. Perret, J. Blondel, Adaptive differences in the timing of egg laying between different populations of birds result from variation in photoresponsiveness, *Proc. R. Soc. Lond. B Biol. Sci.* 263 (1996) 19-22.
- [65] D.S. Lehrman, The reproductive behavior of ring doves, *Scientific American* 211 (1964) 48-54.
- [66] S. Leitner, R.C. Marshall, B. Leisler, C.K. Catchpole, Male song quality, egg size and offspring sex in captive canaries (*Serinus canaria*), *Ethology* 112 (2006) 554-563.
- [67] S. Leitner, C. Voigt, L.M. Garcia-Segura, T. Van't Hof, M. Gahr, Seasonal activation and inactivation of song motor memories in wild canaries is not reflected in neuroanatomical changes of forebrain song areas, *Horm. Behav.* 40 (2001) 160-168.
- [68] C.K. Lessells, Neuroendocrine control of life histories: what do we need to know to understand the evolution of phenotypic plasticity?, *Phil. Trans. R. Soc. B* 363 (2008) 1589-1598.
- [69] R.A. Lewis, Reproductive biology of the white-crowned sparrow. II. Environmental control of reproduction and associated cycles, *Condor* 77 (1975) 111-124.
- [70] B. Lofts, R.K. Murton, Photoperiodic and physiological adaptations regulating avian breeding cycles and their ecological significance, *J. Zool.* 155 (1968) 327-394.
- [71] B. Lofts, R.K. Murton, Reproduction in birds, in: D.S. Farner, J.R. King (Eds.), *Avian Biology*. Academic Press, New York and London, 1973, pp. 1-107.
- [72] I. Lopez-Rull, D. Gil, Elevated testosterone levels affect female breeding success and yolk androgen deposition in a passerine bird, *Behav. Proc.* 82 (2009) 312-318.
- [73] D.L. Maney, E. Cho, C.T. Goode, Estrogen-dependent selectivity of genomic responses to birdsong, *Eur. J. Neurosci.* 23 (2006) 1523-1529.
- [74] D.L. Maney, R.D. Richardson, J.C. Wingfield, Central administration of chicken gonadotropin-releasing hormone-II enhances courtship behavior in a female sparrow, *Horm. Behav.* 32 (1997) 11-18.
- [75] J.W. McGlothlin, J.M. Jawor, T.J. Greives, J.M. Casto, J.L. Phillips, E.D. Ketterson, Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged, *J. Evol. Biol.* 21 (2008) 39-48.
- [76] J.W. McGlothlin, E.D. Ketterson, Hormone-mediated suites as adaptations and evolutionary constraints, *Phil. Trans. R. Soc. B* 363 (2008) 1611-1620.
- [77] T. Meijer, U. Nienaber, U. Langer, F. Trillmich, Temperature and timing of egg-laying of European starlings, *Condor* 101 (1999) 124-132.
- [78] T. Meijer, H. Schwabl, Hormonal patterns in breeding and nonbreeding kestrels, *Falco tinnunculus* : field and laboratory studies, *Gen. Comp. Endocrinol.* 74 (1989) 148-160.
- [79] M.C. Moore, Hormonal response of free-living male white-crowned sparrows to experimental manipulation of female sexual behavior, *Horm. Behav.* 16 (1982) 323-329.

- [80] M.C. Moore, Effect of female displays on the endocrine physiology and behaviour of male white-crowned sparrows, *Zonotrichia leucophrys*, J. Zool., Lond. (A) 199 (1983) 137-148.
- [81] M.L. Morton, M.E. Pereyra, L.F. Baptista, Photoperiodically induced ovarian growth in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*) and its augmentation by song, Comp. Biochem. Physiol. A-Physiol. 80 (1985) 93-97.
- [82] R.K. Murton, N.J. Westwood, Avian breeding cycles. Oxford University Press, London, 1977.
- [83] F. Nottebohm, A brain for all seasons - Cyclical anatomical changes in song control nuclei of the canary brain, Science 214 (1981) 1368-1370.
- [84] D.M. O'Neal, D.G. Reichard, K. Pavilis, E.D. Ketterson, Experimentally-elevated testosterone, female parental care, and reproductive success in a songbird, the dark-eyed junco (*Junco hyemalis*), Horm. Behav. 54 (2008) 571-578.
- [85] L.W. Oring, A.J. Fivizzani, M.A. Colwell, M.E. Elhalawani, Hormonal changes associated with natural and manipulated incubation in the sex-role reversed wilson phalarope, Gen. Comp. Endocrinol. 72 (1988) 247-256.
- [86] L.W. Oring, A.J. Fivizzani, M.E. Elhalawani, Testosterone-induced inhibition of incubation in the spotted sandpiper (*Actitis macularia*), Horm. Behav. 23 (1989) 412-423.
- [87] J.Q. Ouyang, M. Hau, F. Bonier, Within seasons and among years: When are corticosterone levels repeatable?, Horm. Behav. 60 (2011) 559-564.
- [88] R. Palme, Measuring fecal steroids - Guidelines for practical application, Annals of the New York Academy of Sciences 1046 (2005) 75-80.
- [89] H. Parn, K.M. Lindstrom, M. Sandell, T. Amundsen, Female aggressive response and hormonal correlates - an intrusion experiment in a free-living passerine, Behav. Ecol. Sociobiol. 62 (2008) 1665-1677.
- [90] J. Partecke, T. Van't Hof, E. Gwinner, Differences in the timing of reproduction between urban and forest European blackbirds (*Turdus merula*): result of phenotypic flexibility or genetic differences?, Proc. R. Soc. Lond. B Biol. Sci. 271 (2004) 1995-2001.
- [91] R.B. Payne, L.L. Payne, I. Rowley, Kin and social relationships in splendid fairy-wrens: recognition by song in a cooperative bird, Anim. Beh. 36 (1988) 1341-1351.
- [92] N. Perfito, J.M.Y. Kwong, G.E. Bentley, M. Hau, Cue hierarchies and testicular development: Is food a more potent stimulus than day length in an opportunistic breeder (*Taeniopygia g. guttata*)?, Horm. Behav. 53 (2008) 567-572.
- [93] J. Pinowski, B. Pinowska, N. Chernetsov, J. Romanowski, K. Sierakowski, Nest construction during autumn display and winter roosting in the tree sparrows *Passer montanus*, Acta Ornithol. 43 (2008) 185-195.
- [94] W.L. Reed, M.E. Clark, P.G. Parker, S.A. Raouf, N. Arguedas, D.S. Monk, E. Snajdr, V. Nolan, E.D. Ketterson, Physiological effects on demography: A long-term experimental study of testosterone's effects on fitness, Am. Nat. 167 (2006) 667-683.
- [95] W. Rowan, Relation of light to bird migration and developmental stages, Nature 115 (1925) 494-495.
- [96] W. Rowan, On photoperiodism, reproductive periodicity, and the annual migrations of certain birds and fishes, Proc. Boston Soc. Nat. Hist. 38 (1926) 147-189.
- [97] M.I. Sandell, Exogenous Testosterone Increases Female Aggression in the European Starling (*Sturnus vulgaris*), Behav. Ecol. Sociobiol. 62 (2007) 255-262.
- [98] S.V. Schaper, A. Dawson, P.J. Sharp, P. Gienapp, S.P. Caro, M.E. Visser, Increasing temperature, not mean temperature, is a cue for avian timing of reproduction, Am. Nat. in press (2011).

- [99] W.A. Searcy, Measuring responses of female birds to male song, in: P.K. McGregor (Ed.), Playback and studies of animal communication. Plenum Press, New York, 1992, pp. 175-189.
- [100] P.J. Sharp, Photoperiodic regulation of seasonal breeding in birds, *Ann. N.Y. Acad. Sci.* 1040 (2005) 189-199.
- [101] B.C. Sheldon, L.E.B. Kruuk, J. Merila, Natural selection and inheritance of breeding time and clutch size in the collared flycatcher, *Evolution* 57 (2003) 406-420.
- [102] B. Silverin, Behavioral, hormonal, and morphological responses of free-living male pied flycatchers to estradiol treatment of their mates, *Horm. Behav.* 25 (1991) 38-56.
- [103] K.W. Sockman, H. Schwabl, Daily estradiol and progesterone levels relative to laying and onset of incubation in canaries, *Gen. Comp. Endocrinol.* 114 (1999) 257-268.
- [104] L. te Marvelde, M.L. Webber, A.B. van den Burg, M.E. Visser, A new method for catching cavity-nesting birds during egg laying and incubation, *J. Field Ornithol.* 82 (2011) 320-324.
- [105] J. Tobias, Asymmetric territorial contests in the European robin: The role of settlement costs, *Anim. Beh.* 54 (1997) 9-21.
- [106] B. Tschirren, A.N. Rutstein, E. Postma, M. Mariette, S.C. Griffith, Short- and long-term consequences of early developmental conditions: a case study on wild and domesticated zebra finches, *J. Evol. Biol.* 22 (2009) 387-395.
- [107] M. van de Pol, D. Heg, L.W. Bruinzeel, B. Kuijper, S. Verhulst, Experimental evidence for a causal effect of pair-bond duration on reproductive performance in oystercatchers (*Haematopus ostralegus*), *Behav. Ecol.* 17 (2006) 982-991.
- [108] E. Van den Steen, M. Eens, A. Covaci, A.C. Dirtu, V.L.B. Jaspers, H. Neels, R. Pinxten, An exposure study with polybrominated diphenyl ethers (PBDEs) in female European starlings (*Sturnus vulgaris*): Toxicokinetics and reproductive effects, *Env. Poll.* 157 (2009) 430-436.
- [109] E. Van den Steen, M. Eens, A. Geens, A. Covaci, V.M. Darras, R. Pinxten, Endocrine disrupting, haematological and biochemical effects of polybrominated diphenyl ethers in a terrestrial songbird, the European starling (*Sturnus vulgaris*), *Sci. Tot. Env.* 408 (2010) 6142-6147.
- [110] H.P. van der Jeugd, R. McCleery, Effects of spatial autocorrelation, natal philopatry and phenotypic plasticity on the heritability of laying date, *J. Evol. Biol.* 15 (2002) 380-387.
- [111] A.J. van Noordwijk, J.H. van Balen, W. Scharloo, Genetic variation in the timing of reproduction in the great tit, *Oecologia* 49 (1981) 158-166.
- [112] K. van Oers, G. de Jong, P.J. Drent, A.J. van Noordwijk, A genetic analysis of avian personality traits: Correlated, response to artificial selection, *Behav. Genet.* 34 (2004) 611-619.
- [113] F. Vezina, K.G. Salvante, Behavioral and physiological flexibility are used by birds to manage energy and support investment in the early stages of reproduction, *Curr. Zool.* 56 (2010) 767-792.
- [114] M.E. Visser, Keeping up with a warming world; assessing the rate of adaptation to climate change, *Proc. R. Soc. B Biol. Sci.* 275 (2008) 649-679.
- [115] M.E. Visser, S.P. Caro, K. van Oers, S.V. Schaper, B. Helm, Phenology, seasonal timing and circannual rhythms: towards a unified framework, *Phil. Trans. R. Soc. B* 365 (2010) 3113-3127.
- [116] M.E. Visser, L.J.M. Holleman, S.P. Caro, Temperature has a causal effect on avian timing of reproduction, *Proc. R. Soc. B Biol. Sci.* 276 (2009) 2323-2331.
- [117] M.E. Visser, M.M. Lambrechts, Information constraints in the timing of reproduction in temperate zone birds: great and blue tits, in: N.J. Adams, R.H. Slotow (Eds.), *Proc. 22 Int. Ornithol. Congr., Durban. BirdLife South Africa, Johannesburg, 1999*, pp. 249-264.

- [118] C. Voigt, W. Goymann, Sex-role reversal is reflected in the brain of African black coucals (*Centropus grillii*), *Dev. Neurobiol.* 67 (2007) 1560-1573.
- [119] G.E. Walsberg, Avian ecological energetics, in: D.S. Farner, J.R. King, K.C. Parkes (Eds.), *Avian Biology*. Academic Press, New York, 1983, pp. 161-220.
- [120] S. Ward, J.R. Speakman, P.J.B. Slater, The energy cost of song in the canary, *Serinus canaria*, *Anim. Beh.* 66 (2003) 893-902.
- [121] M. Wikelski, M. Hau, J.C. Wingfield, Seasonality of reproduction in a neotropical rain forest bird, *Ecology* 81 (2000) 2458-2472.
- [122] T.D. Williams, Experimental (Tamoxifen-Induced) manipulation of female reproduction in zebra finches (*Taeniopygia guttata*), *Physiol. Biochem. Zool.* 73 (2000) 566-573.
- [123] T.D. Williams, Mechanisms underlying the costs of egg production, *Bioscience* 55 (2005) 39-48.
- [124] A.J. Wilson, D. Reale, M.N. Clements, M.M. Morrissey, E. Postma, C.A. Walling, L.E.B. Kruuk, D.H. Nussey, An ecologist's guide to the animal model, *J. Anim. Ecol.* 79 (2010) 13-26.
- [125] J.C. Wingfield, Environmental and endocrine control of reproduction in the song sparrow, *Melospiza melodia*. I. Temporal organization of the breeding cycle, *Gen. Comp. Endocrinol.* 56 (1984) 406-416.
- [126] J.C. Wingfield, Comparative endocrinology, environment and global change, *Gen. Comp. Endocrinol.* 157 (2008) 207-216.
- [127] J.C. Wingfield, J.W. Crim, P.W. Matfoks, D.S. Farner, Responses of Photosensitive and Photorefractory Male White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) to Synthetic Mammalian Luteinizing Hormone Releasing Hormone (Syn-LHRH), *Biol. Reprod.* 21 (1979) 801-806.
- [128] J.C. Wingfield, D.S. Farner, The annual cycle of plasma irLH and steroid hormones in feral populations of the white-crowned sparrow, *Zonotrichia leucophrys gambelii* *Biol. Reprod.* 19 (1978) 1046-1056.
- [129] J.C. Wingfield, T.P. Hahn, D.L. Maney, S.J. Schoech, M. Wada, M.L. Morton, Effects of temperature on photoperiodically induced reproductive development, circulating plasma luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha*, *Gen. Comp. Endocrinol.* 131 (2003) 143-158.
- [130] J.C. Wingfield, T.P. Hahn, M. Wada, L.B. Astheimer, S. Schoech, Interrelationship of day length and temperature on the control of gonadal development, body mass, and fat score in white-crowned sparrows, *Zonotrichia leucophrys gambelii*, *Gen. Comp. Endocrinol.* 101 (1996) 242-255.
- [131] J.C. Wingfield, T.P. Hahn, M. Wada, S.J. Schoech, Effects of day length and temperature on gonadal development, body mass, and fat depots in white-crowned sparrows, *Zonotrichia leucophrys pugetensis*, *Gen. Comp. Endocrinol.* 107 (1997) 44-62.
- [132] J.C. Wingfield, R.E. Hegner, A.M. Dufty, G.F. Ball, The Challenge Hypothesis - Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies, *Am. Nat.* 136 (1990) 829-846.
- [133] J.C. Wingfield, G.J. Kenagy, Natural regulation of reproductive cycles, in: P.K.T. Pawg, M.P. Schreibman (Eds.), *Vertebrate endocrinology: fundamentals and biomedical implications*. Academic Press Inc., New York, 1991, pp. 181-241.
- [134] J.C. Wingfield, D.M. Lewis, Hormonal and behavioural responses to simulated territorial intrusion in the cooperatively breeding white-browed sparrow weaver, *Plocepasser mahali*, *Anim. Beh.* 45 (1993) 1-11.

- [135] J.C. Wingfield, D. Monk, Behavioral and hormonal responses of male song sparrows to estradiol-treated females during the non-breeding season, *Horm. Behav.* 28 (1994) 146-154.
- [136] J.C. Wingfield, M.C. Moore, Hormonal, social, and environmental factors in the reproductive biology of free-living male birds, in: D. Crews (Ed.), *Psychology of Reproduction: An evolutionary perspective*. Inc. Englewood Cliffs, New Jersey, Prentice-Hall, 1987, pp. 148-175.
- [137] J.C. Wingfield, M.E. Visser, T.D. Williams, Integration of ecology and endocrinology in avian reproduction: a new synthesis, *Phil. Trans. R. Soc. B* 363 (2008) 1581-1588.
- [138] R.A. Zann, *The zebra finch: a synthesis of field and laboratory studies* Oxford University Press, Oxford, 1996.
- [139] A.J. Zera, L.G. Harshman, T.D. Williams, Evolutionary endocrinology: The developing synthesis between endocrinology and evolutionary genetics, *Annu. Rev. Ecol. Evol. Syst.* 38 (2007) 793-817.
- [140] D.A. Zysling, T.J. Greives, C.W. Breuner, J.M. Casto, G.E. Demas, E.D. Ketterson, Behavioral and physiological responses to experimentally elevated testosterone in female dark-eyed juncos (*Junco hyemalis carolinensis*), *Horm. Behav.* 50 (2006) 200-207.

Figure caption

Figure 1: Relative abundances of studies in reproductive physiology (left panel) and ecology (right panel) performed exclusively on males (M), on both males and females (M+F), exclusively on females (F) and studies where the sex considered is not mentioned in the paper (?). Literature references were obtained from ISI Web of Science[®]. The main panels refer to the same literature search (keywords: reproducti* AND (bird* OR avian)) performed in two sets of journals publishing papers in physiology and ecology, respectively. The inserts refer to two additional searches using different and more discipline-specific keywords (keywords: photoperiodism AND (bird* OR avian); and keywords: heritability* AND (bird* OR avian)). See text for details.

Physiology

Ecology