Balancing act: An interdisciplinary exploration of trade-offs in reproducing females

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Abstract

Trade-offs resulting from the high demand of offspring production are a central focus of many subdisciplines within the field of biology. Yet, despite the historical and current interest on this topic, large gaps in our understanding of whole-organism trade-offs that occur in reproducing individuals remain, particularly as it relates to the nuances associated with female reproduction. This volume of Integrative and Comparative Biology (ICB) contains a series of papers that focus on reviewing trade-offs from the female-centered perspective of biology (i.e., a perspective that places female reproductive biology at the center of the topic being investigated or discussed). These papers represent some of the work showcased during our symposium held at the 2024 meeting of the Society for Integrative and Comparative Biology (SICB) in Seattle, Washington. In this roundtable discussion, we use a question-and-answer format to capture the diverse perspectives and voices involved in our symposium. We hope that the dialogue featured in this discussion will be used to motivate researchers interested in understanding trade-offs in reproducing females and provide guidance on future research endeavors.

I. Introduction

Understanding trade-offs associated with reproduction has been a central focus within many subdisciplines of biology, dating back to the pioneering work of Lack, Fisher, Williams, and other early scholars (Jönsson and Tuomi 1994; Charnov and Krebs 1974; Stearns 1976). Initially, much of this research revolved around aspects of the life history and easily-quantifiable metrics, such as egg production, for use in theoretical models. Over time, research has expanded to encompass diverse aspects of biology, perspectives, and taxa, highlighting the complexities of reproductive strategies and their associated trade-offs. Although trade-offs have been extensively studied in this context, subsequent research on reproductive physiology and other aspects of organismal biology has been somewhat limited. This limitation may have inadvertently introduced biases and untested foundational assumptions regarding sex that have become paramount to our understanding of trade-offs. Moreover, the operational definitions of what constitutes a trade-off have become myriad and muddy, further complicating efforts to study and interpret these phenomena. As we refine our frameworks and biological constructs, it becomes increasingly important to critically examine and reassess established paradigms to ensure a more comprehensive and unbiased understanding of trade-offs and their implications in evolutionary processes. As discussed in previous symposia held at the Society for Integrative and Comparative Biology (SICB) meetings, "reproductive biology, like many other scientific fields, lags behind changes in our social and cultural climate. This lag is evident in conceptual paradigms, in pedagogy, in language, in peer review, and in research foci." (Orr et al. 2020).

The preceding articles in this volume highlight numerous instances that showcase a female-centered viewpoint regarding trade-offs that occur during reproductive events. Here, we tackle numerous questions that emerged during our symposium ("What do trade-offs mean to reproducing females: An integrative look at whole-organism trade-offs") at the 2024 annual Society for Integrative and Comparative Biology (SICB) conference, along with a few that arose through correspondence after the in-person discussions. We share insights from a group discussion that marked the culmination of the symposium. Attendees, including participants and other SICB members, actively engaged in a forward-looking conversation aimed at shaping the trajectory of future research endeavors. With scholars investigating trade-offs across diverse perspectives and taxa, the symposium provided a platform for fostering essential dialogue among conference attendees. Throughout our discourse, we explored key questions, both formally addressed and arising organically, during discussions afforded by the meeting. Our overarching objective in this paper is to refine terminology and identify knowledge gaps that hold significance for integrative biologists on a broad scale.

The study of trade-offs has a deep history with a valuable framework for considering adaptations and evolution of life histories, yet this research domain requires appreciable refinement. Although we all 'know' what a trade-off is, we too often elide the complexities and nuances of measuring one. Further, we may also overly rely on reductive proxies for traits that do not capture nuances associated with various stages of reproduction in females. Because of these reasons (and others we discuss in this piece), there are many difficulties in our approaches to understanding and interpreting trade-offs, particularly in reproducing females. To this end, below we adopt an informal question-and-answer format to explore these themes and to raise awareness among researchers regarding potential theoretical, semantic, and methodological challenges. Different perspectives are shared, thus allowing a window into some nuances of studying particular taxa and/or types of questions. Questions and replies are grouped by three themes. We start with the historical context with gaps as they relate to the study of trade-offs in general, then present methodological and empirical considerations. We conclude by discussing future directions we would suggest for researchers in the field. We hope that this piece will foster a continued exploration into lesser-understood or under-investigated facets of female biology and more careful consideration of disconnections between what we aim to measure and what we are really measuring. Additionally, we provide a table (Table 1) that defines key variables and highlights nuances deserving special attention in the outlined studies, and another table that summarizes previously identified types of trade-offs as they relate to female reproduction (Table 2) that we hope will motivate and anchor future research endeavors.

II. Where we've been: Gaps in our understanding of trade-offs in reproducing females

In this section, we ask how terminology and untested, background assumptions have shaped our understanding of trade-offs in reproducing females. Many of the emerging themes apply more broadly to understanding, communicating, and measuring trade-offs in all sexes. One such theme that emerged from our discussion revolved around terminology within the literature sometimes being vague, reductionist, or broad. Such reductionist thinking may contribute to assumptions that trade-offs between two variables may manifest in a linear relationship, when in actuality, the relationship between these two variables may be a different function. The authors in this paper also pointed to several other terms within the trade-offs literature that are especially problematic due to multiple definitions that differ slightly by field, which we discuss below as well as in Table 1. We highlight here and throughout this manuscript that trade-offs take different forms (as outlined in Garland et al. 2022), occur at multiple levels of biological organization, may use different types of currency (e.g., energy, nutrients, or time), and are highly context-specific. Other themes that emerged in this section point towards the need to take the nuances of female reproduction in to account when designing experiments, interpreting data, and reporting results to the scientific community. One of the biggest take-aways from this section is the need to report more information regarding the specific methods used to house animals and/or collect data, as these "hidden variables" may contribute to whether trade-offs are observed.

What language, terminology is problematic and/or poorly defined in the field?

T. Orr: I think there are several areas where how we write about trade-offs is problematic. First, it is important to acknowledge that much of the terminology in our field comes from economics à la John Nash. As such the 'currency' discussed is often unclear and treated in a very simplistic way. I won't go into the economics terms here given the lack of space but in terms of a simplistic perspective, I will give a few examples. One is the term 'condition'. In the literature people rarely if ever define what 'good condition' is and it seems to be the norm that 'relatively heavier' means 'good condition' or occasionally something more interesting like 'bactericidal activity' but again this is rarely (if ever) well-defined and almost always treated as one to two easy to measure metrics. This is very interesting if we consider a reproducing female who may undergo a range of physiological changes rendering massive changes in condition! More relevant for this discussion are terms like 'reproductive failure' whereby a female may forego a reproductive event but in the context of trade-offs, this may have been a 'win' in the context of survival and so forth (Tardif et al. 2013). Finally, please people stop saying your animals are non-reproductive! Maybe a female mouse isn't lactating or gravid- but is she ovulating? Is she recovering from a recent reproductive event? As always, I hope that people can define all these terms for readers (Table 1).

K. Hinde: In response to T. Orr's comment above, the way that condition is sometimes discussed predisposes us often to think linearly, when especially regarding condition, we can expect a lot of U-shaped functions (Fairbanks and Hinde 2013).

B. Harris : The terms sex and reproduction also add confusion. For example, when we say sex, what do we mean? How are we assigning sex to an organism – via external genitalia? Gonads? Gametes? Hormonal profile? Chromosomes?. Likewise, when we say reproductive trade-offs, it often is not clear if we mean reproductive behavior (and if so, what type) and/or reproductive physiology (and, again, if so, which measures?). Additionally, many trade-offs deal with stress and the value of reproduction framework – but again, ambiguity is a problem as stress is not well defined across the literature and these frameworks often rely on assumptions about the adaptive value of stress and the "stress response". C. Josefson and I wrote a chapter (in press) about the complicated relationship between stress and reproduction as they are both nuanced and complex terms . Lastly, the use of male-centered terms (e.g., Baker and Hayssen, this volume; Orr et al. 2020) makes female reproduction feel like an afterthought and can leave us with imprecise and incomplete views of reproductive processes.

C. Josefson: Androcentric terminology has absolutely shaped the way we think about female reproduction. We often think of the science we create as being entirely objective, but the fact is that it's hard not to interpret scientific phenomena outside of the framework and norms of the society and cultures we are conducting that science in. One such legacy is that we often view females as being passive, coy, and demure (Gowaty 1992; Campo-Engelstein and Johnson 2014; Nelson 2017; Orr et al. 2020; Hayssen 2020; Baker and Hayssen, this volume); these views are reflected in the way we discuss female reproduction, often from a male-active, female-passive perspective (e.g., we may describe females or their gametes as being impregnated, inseminated, or fertilized; here, males are doing the action, and females are a passive participant in a very active process that is occurring within their own bodies). Another example is the idea that ova are inert and immobile (i.e., the "classic account of fertilization" with ova playing the role of Sleeping Beauty; Schatten & Schatten, 1983). These views have absolutely impacted how we interpret trade-offs in reproducing females.

R. Beltran: Also making sure that gender and sex are distinguished! I would say, too, that we should be very careful about language related to skip breeding. Do we really mean "fail to reproduce" or "choose to terminate"?

A. Liebl: Energetic Cost vs. Effort – an energetic cost would be the energy(kJ, Joules, calories) needed or used by an organism. In contrast, effort would be proportional to the total energy budget the organism has to use. Thus, the effort is what portion of the budget was allocated.

T. Orr: People often discuss physiological 'costs' as synonymous with 'energy' traded off without any consideration of nutrients, free radicals, and so forth. Something could be cheap energetically but truly costly in other ways and these are not always a 1:1 relationship (Bell 1980; Harshman and Zera 2007). In some regards, I think the classical works (e.g. Stearns 1976; Williams 1966) did a better job with this than we are doing nowadays!

K. Hinde: Personally, I really appreciated how Clutton-Brock (1991) addressed trade-offs in his book, especially the differences in precision of measurement of maternal care, maternal effort, and maternal investment.

W. Saltzman: Indeed, "Costs of reproduction" is too vague (although this isn't problematic when deliberately used in a very broad sense).

J. Fornara: The term “trade-off” covers a lot of processes and is really quite ambiguous. It covers both behavioral processes (e.g., parental favoritism in offspring provisioning, Van de Walle 2020, Brode et al. 2021) and physiological processes (e.g., the energetic costs of mounting an immune response; Lochmiller and Deerenberg 2000). It is also unclear whether all inverse relationships should be regarded as trade-offs. For example, activation of the hypothalamic-pituitary-adrenal (HPA) axis results in suppression of the hypothalamic-pituitary-gonadal (HPG) axis—is this a trade-off? (Acevedo-Rodriguez et al. 2018). Trade-offs are commonly discussed in the context of time/energy budgets (i.e., investing in Trait A necessarily divests from Trait B), but do they also extend to cost-benefit scenarios (i.e., investing in Trait A provides a benefit but also directly causes a costly change in Trait B; Zera and Harshman 2001)?

C. Josefson: Trade-off is often talked about as being one thing, but really, there are many different types of trade-offs that exist and can be a result of constraints on various aspects of the organism (morphological, physiological/metabolic, biomechanical, genetic) and can apply to so many levels of biological organization, from the cellular/molecular to whole-organism to ecosystems (Garland et al. 2022). We also have so many types of scientists studying trade-offs from different perspectives shaped by their fields (e.g., animal science, anthropology, ecology and evolution, physiology) and sometimes specific words, like cost or demand or investment, may not translate exactly to another field. In reality, we’re all essentially studying some of the same topics.

Specific to my own work and the work of some of the other authors on this paper, I study trade-offs as they relate to lactation. I often read work from animal or dairy science, ecological and evolutionary physiology, molecular and cellular biology, anthropology, etc. Even though many groups from these various disciplines have the same core questions (e.g., what factors constrain lactation performance), they may have different approaches, emphasize different aspects of their work, or interpret their data within a different context. I think that standardizing language across these fields and taking a multidisciplinary approach is key to really getting at understanding this complex topic, but it can sometimes be a little intimidating to step outside of one’s own field.

D. Misra: "Yes," in my opinion, given the word "trade-off" and how broadly it can apply to different organisms from vertebrates to invertebrates. Research needs to make clear how trade-offs affect both current and future reproduction, since animals are always trying to change in order to withstand natural selection and procreate more times. To be more specific, trade-offs can be assessed and studied at multiple biological levels, including the genotype, phenotype, and intermediate structures (Stearns 1989). This is an undeniable and well acknowledged fact. However, the significance of each step in advancing our comprehension hasn't always been highlighted. Instead of focusing just on phenotypic correlations, physiological trade-offs, or genetic relationships, one must also comprehend how these metrics work together to reveal possible evolutionary responses.

A. Liebl: I said this above, but currency in reproduction: what's important? Number? Size? Developmental length? All?

K. Hinde: I think a big part of this is the consideration of timescales. In nutritional ecology, there is a recognition of nutrient balancing across time instead of being framed as a trade-off between prioritizing this nutrient now and that nutrient later. Timescale of investigation may exaggerate or obscure some aspects of trade-offs (such as milk synthesis and mobilizing skeletal calcium), so are things considered trade-offs that are a sequence that becomes balanced over time?

C. Josefson: I could not agree more (see my comment in the next section). We often use data that may provide a snapshot of a physiological (or other) state, but may not speak to any overall changes or whether the observed changes in physiological (or other) proxies are transient/ephemeral or truly are costs to fitness. For example, if energy is re-allocated towards another process, and we are able to measure that, what other information would be required to call it a true cost to fitness? Some of W. Hood's work (Zhang and Hood 2016; Hood et al. 2018) gets at this question and why *when* samples we take (e.g., after the reproductive event) can be re-assessed so as to answer this question.

Are there any examples of faulty or poorly supported background assumptions that are commonly made in studying trade-offs?

C. Josefson: In my introduction to the symposium, I talked about an assumption that we should apply methods that were validated in males to understand the same variable in the context of reproducing females. For example, one of my previous papers (Josefson et al. 2020) looked at how reproduction might alter the antibody response curve, finding that there was a temporal difference between when males are assumed to peak and when females at various stages of reproduction did peak. There are certainly other examples where methods may not be appropriate or other large assumptions that we make in research. Do you have any that are specific to your studies or your field?

A. Liebl: The idea of sexual conflict exists and that reproduction isn't always beneficial for a female is usually diminished in teaching (e.g. love darts or forced copulation). Fitness is about reproduction AND survival.

N. Place: Particularly for female mammals, having elevated glucocorticoids shouldn't automatically be equated with stress (MacDougall-Shackleton et al. 2019). It's stressful when there's not enough food to support the offspring, but the timing of lactation is generally beautifully coordinated with times when resources are in abundance and can support the energetic demands of lactation. With regard to the stress hyporesponsive period during lactation, rats were shown by others to have a blunted glucocorticoid response to stress during lactation (e.g., Stern et al. 1973; Lightman and Young 1989; Lightman 1992; Brunton et al. 2008; Josefson and Skibiel 2021), but G.J. Kenagy and I did not see a blunted glucocorticoid response in free-living yellow-pine chipmunks (*Tamias amoenus;* Kenagy and Place, 2000).

W. Saltzman: A common assumption is that stress of all kinds inhibits reproduction or that there can be trade-offs between reproducing and responding to stress. While there certainly is evidence for this, I think it might be less frequent or less pronounced than is often assumed.

B. Harris: I trained in ecology/evolution/organismal biology but I've been collaborating with a lot of people that have a biomedical focus. I've learned that different fields have very different assumptions about what experimental variables are important when conducting animal work. These assumptions can have huge implications in interpretation of experimental outcomes and can matter if we build hypotheses about female trade-offs from these data. I know a lot of us in our community think about animal housing and husbandry as these variables matter. Recent work has highlighted how important "hidden variables", such as types of cages, single vs. social grouping, the bedding, if nesting or enrichment supplies are provided, the room temperature and light cycle etc., are to experimental outcomes (see Butler-Struben et al, 2022; Holloway and Lerner, 2024). Work in Brian Trainor's lab (and others) found that housing mice with corncob bedding alters estrogen signaling and behavior (Trainor et al. 2013)! This can obviously impact data relating to reproductive trade-offs.

If we're thinking about energetic trade-offs in the lab, considering the temperature of the room and the animals' ability to build nests to thermoregulate matters as AAALAC-determined temperatures (see The Guide) for rodents are below the thermoneutral zone for most species. Housing temperature can absolutely impact reproductive efforts and the interpretation of life history strategies (Gubernick et al., 1993). Another consideration that has been brought up to me recently, which I never really thought about and came out of our teaching workshop (Harris et al., this volume): I do report sex of my animals, but I never report how I defined the sexes. In rodents, it's usually by looking at the anogenital distance and I don't genotype them; for frogs we dissect them and look at gonads. I think it's really important to tell our readers, especially when we are talking about females, what criteria we using to classify what is a female. We should identify this information in the methods to decrease ambiguity in determining sex differences and other differences; and because this may not be obvious to readers who work with different taxa or come from biomedical backgrounds.

For my work in stress biology, we spend a great effort in determining ecologically relevant challenges or stressors to which we can expose our animals. We also consider the nature of stressors – for example acute vs. chronic, single vs. multiple stressors, etc. and balance what would be ideal vs. what is possible. We often do things out of necessity, especially in the lab, as single variables that we can manipulate but we know that's not accurate, especially with climate change, so what is the role of multiple challenges and multiple constraints and how do those then play a role in reproduction and trade-offs? And that is really hard to get at in the lab, and in the field, but especially in the lab because all of my mice are pathogen-free and have food and water.

Overall, being clearer and more explicit in our methods, and leaving less information up to assumptions, seems critical; using the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (Percie du Sert et al., 2020) in our publications could help.

C. Josefson: I agree completely with B. Harris. I similarly don't always state how I sexed offspring, just that they were sexed (usually either by anogenital distance or by anogenital distance and then confirmation of gonads during dissections). There's absolutely variation in external genital morphology. There are numerous ways to define sex, and they don't always match with one another (Nelson 2017), so I think being clear on the methods you used is critical, especially when everything hinges on being able to discern males from females so that you can measure sex as a biological variable.

T. Garland (in response to B. Harris): We should also note that studies in environmental toxicology have shown that the combined effects of multiple low-level stressors can be worse than expected from studies of individual stressors (interactive effects) (Jaeger et al. 1999; Daskalakis et al. 2013).

W. Saltzman: Something I've been thinking about a lot is that in many rodent species and some other mammalian taxa, females are very frequently concurrently pregnant and lactating. This is something we know very little about, both mechanistically, in terms of what's going on at the neuroendocrine and physiological levels, and what this overlapping reproduction means for females. We might think "Why shouldn't all female mammals do this; it's obviously a way of increasing reproductive rate." But I assume that there are important trade-offs in terms of female condition and offspring quality. And, again, that's been studied surprisingly little, especially given that very commonly studied rodents, like mice and rats, do this in their natural environments but are usually prevented from doing so under laboratory conditions. So, I think there are likely to be trade-offs there that have barely been investigated. Species should be studied under multiple conditions. Even in the lab, manipulating environmental and/or organismal conditions can provide important insights into trade-offs.

W. Hood: Laboratory mice often mate within 24 hours of birth and as a consequence, they can gestate while lactating. Maria Johnson's work with Speakman suggested that females on average present higher reproductive performance in reproductive bouts following a pregnancy that was concurrent with lactation (Johnson et al. 2001). Which really makes you wonder what the constrains are on performance.

K. Hinde: Further among dairy cows with overlapping gestation and lactation, we see that fetal daughters can influence lactation performance by increasing milk output in an already-established lactation in response to a son (Hinde et al. 2013).

E. Hunt: On a macroevolutionary scale, there's a large amount of work to be conducted to simply quantify the morphology of female reproductive tracts since many studies have historically focused on male morphology, in part due to challenges imaging female genitalia, as well as presumptions about the dominant role of males. Historically it's been presumed that female genitalia simply correspond to male genitalia. But, evidence is now emerging that female genitalia evolves faster and is what's driving the changes in males, for instance the Simmons and Fitzpatrick 2019 paper. Reproductive tract shapes have only been investigated in a handful of clades; waterfowl (Brennan and Prum 2012), snakes (Showalter et al. 2014), sharks (Hedrick et al. 2019) and marine mammals (Orbach et al. 2021). Yet, recent advances in scanning techniques mean that we are no longer limited by female tracts being internal and therefore being more challenging to measure. Once we can quantify patterns of female reproductive morphology we can start to think about the biomechanical trade-offs and functional constraints on reproductive strategies and the interplay between behavior and form. This will help us identify the mechanisms driving genital divergence to shed light on species isolation and the role of sexual and natural selection.

B. Harris: This is really interesting to me to think about how traits in one sex can be a selective force on the other sex to determine evolutionary trajectories. Another point to add is, how do organisms determine or sense their residual reproductive value? So many hypotheses about reproductive trade-offs hinge on residual reproductive value (see Harris, 2020). How do animals "know" or sense this? Are there cues and do these cues change across reproductive stages and across sexes and ages? How are animals picking up on this? Are there signals?

N. Place: We might have tried to look at that. We had found that Syrian hamsters become less choosey for a mate as they get older (Place et al. 2014). However, when we used a chemical (4-vinylcyclohexene diepoxide, VCD) to accelerate ovarian aging by reducing their reserve of follicles, they maintained their preference for dominant over subordinate males (Roosa and Place 2015).

A. Litmer: Thinking about methods, there's a lot of lizard studies that are looking at maternal effects on offspring, thinking about climate change, looking at different environmental conditions, and what the females do in response to such variables. However, such studies nearly always fail to look at energetics and food consumption rates, and how such behavioral components (such as willingness to eat) changes. Instead, most studies of this type focus on things like female body temperature and then offspring quantity, lay date, and phenotypes. I think considering behavioral compensations that may happen during reproduction and when environments change is important mechanistically; the energy intake and assimilation certainly impacts the number of offspring a female can produce, and even offspring size. Therefore, the underlying mechanism may be more related to behavioral shifts in consuming food or physiological shifts in allocation of energy, than simply temperature influence clutches.

D. Costa: Probably because I worked on longer lived animals, the question about quality in reproductive success is quite important for trade-offs and compensation. What I'm thinking here is, if animals breed every year but produce smaller offspring, they may not have any offspring survive or live long enough to produce offspring of their own, whereas the alternative would be to skip breed, so they don't produce as many offspring but those offspring might be of higher quality. And that could also vary widely across individual females. It's important to remember that within these populations, not all females are equal.

R. Beltran: We've done a lot of work in our elephant seal system to measure and understand differences in quality among individual females. We've found extensive intraspecific variation in traits ranging from life history timing (Beltran et al. *in press* Proceedings B), at-sea behavioral strategies (Beltran, Hernandez et al. Ecology Letters 2023), and offspring quality (Condit et al. *in press* Canadian J Zoology). We're starting to understand the immense role that environmental fluctuations, like ocean conditions, play in mediating these trade-offs (Beltran et al. *in review*). These seals can live so long – adult females can have lifespans exceeding 20 years – that understanding lifelong trade-offs is challenging, but possible through long-term monitoring of individually flipper-tagged seals.

T. Garland: Just pointing out how litter size has the ability to affect many traits of offspring, but most papers don't even report the litter size into which their subjects were born, even if they know it. A paper that highlights this is Parra-Vargas et al. 2023.

G. J. Kenagy: I will make some comments in two stages. The first is, sort of a tribute to T. Garland's talk, that trade-offs are very complex and he reviewed very articulately the range of trade-offs which include everything from a nice trade-off to no trade-off (Garland et al. 2022). And that we need to recognize the difference. In the sense of my own interest in female reproductive effort particularly, at moments of income breeding, energy expenditure, the fact that females producing 3 or 4 or 5 young can all show the same rate energy expenditure and that this then, because it's not a trade-off can only be accounted for by the expectation that the females producing the most young are doing so because of experiments and efficiency. In a quality home range, there is more food, so it is easier to come by food in a smaller area and the struggling mothers that are only producing 3 young, but have the same daily energy expenditure, are no part of a trade-off that we like to see, but a surprising kind of trade-off. And it's sometimes hard to believe your results and pay attention and try to explain them in terms of what you measured and not being disappointed because you don't get a trade-off.

K. Hinde: One thing I think about a lot in mammals is the difference in maternal investment in their daughters' condition in many species may exert a greater influence on those daughters reproductive performance than their son's reproductive performance. For example in Cercopithecines, baboons and macaques, females initiate reproduction at much younger ages than do males (Pittet et al. 2017; Turcotte et al. 2022. With that longer reproductive time horizon for sons, they have more time to compensate for deficits in maternal endowment or squander what their mama gave them. With that shorter time horizon for daughters, much more of the variance in daughter's condition at reproductive maturity may be explained by her condition at weaning and her mother's condition (Johnson 2006).

J. Fornara: When we talk about trade-offs, I think there is often an underlying assumption that we are talking about only two variables (i.e., investment in Trait A divests from Trait B and vice versa). While this simplified approach might be useful when the two variables in question explain most of the observed variation in a given phenotype, it seems likely that we increase the risk of false negatives if we fail to account for trade-offs that might exist between n number of variables. Therefore, we must ensure that our theoretical and empirical frameworks can expand to accommodate added complexity (e.g., see examples with path analysis in Garland et al. 2022).

P. Lopes: Because I think a lot about disease, I think sometimes diseases or infections might push animals to extreme situations or almost like a terminal investment situation. The decisions that you make right now are really important if you may not survive. You could see disease systems as putting animals on the spot, pushing animals to their limits.

L. Powers: I agree that disease really puts constraints on physiological processes, like reproduction. It seems really important to me to consider how reproduction impacts immune response to disease, and vice-versa.

C. Josefson: I agree with P. Lopes and B. Harris in their above comments! I think reproduction-immune trade-offs are one topic within this field where as biologists, we really need to question what underlying assumptions we are making when we are designing our experiments. For example, immune molecules may have a different function in the context of female reproduction than they do outside of that. Additionally, the role of reproduction in shaping what optimal defenses (Viney et al.2005) is really intriguing to me, especially for lactating individuals (or other taxa where maternal immune programming, sometimes via passive transfer of antibodies, is possible) because the optimal defense must also take the developing neonate in to consideration. I think this also highlights a huge question in ecological immunology: when are immune challenges (as opposed to ecologically-relevant, actively-replicating pathogens) informative and what are their limits in the context of understanding optimal defenses/trade-offs during reproduction?

III. Where we're at: Current approaches and methodological considerations for understanding trade-offs in reproducing females

As discussed in the previous section, understanding and contextualizing the many different types of trade-offs at multiple levels of biological organization can prove difficult. We continue the discussion on the challenges of studying trade-offs, as well as the challenges associated with studying female reproduction, in this section below. Although both of these topics can be methodologically challenging, there is room for creativity in experimental design. This creativity may be demonstrated in the organisms or taxa used in an experiment, as the unique properties of reproduction in certain species can be leveraged to understand the contexts under which trade-offs can be observed or the various constraints on reproduction. We point to the need for careful consideration of the proxies we may choose to measure in our experiments. For example, when measuring physiological or hormonal variables, careful attention should be paid to ensure that the proxies chosen are ecologically-relevant, accurately reflect the hypothesis being tested, and are representative of the complex traits or physiological processes being measured. In addition, we highlight the utility in publishing negative results from empirical studies that do not find evidence for trade-offs as well as the need for studies that assess maternal trade-offs during reproduction across the lifespan and their potential impact on offspring phenotype in to adulthood.

Are there nuances of reproductive trade-offs in certain clades that may be worth highlighting as revealing something fundamental to the field and/or that cause 'noise' when trying to understand broader patterns?

A. Liebl: Yes- I think post-natal care should be considered more. Additionally, I think many lab studies eliminate too much noise (e.g., *ad libitum* food and constant environment-restricted competition, etc.) which is actually fundamental to trade-offs.

T. Garland: Sure. Turtles/tortoises are a good example because an individual has a uniquely constrained amount of space (Congdon and Gibbons 1987; Iverson et al. 1993). Most other individual organisms can expand their abdomen to accommodate somewhat larger and/or more numerous offspring during gestation. An individual turtle cannot, although the available space does increase with any growth that occurs after sexual maturity. And, of course, over evolutionary timescales the size and shape of turtle shells can change, diversifying within the context (constraints) of other aspects of their biology, such as mode of locomotion (Stayton et al. 2018).

A. Litmer: I think with regards to animals that shed, this may be part of their life history and represent a huge source of energy use. Shedding could also be associated with chemical signaling for reproduction in snakes – but often ignored. I also think considering things like filial cannibalism and why it occurs could be also useful for the future.

K. Hinde: We should look at the findings from Elinor Karlsson's Zoonomia project (2020), as they looked at genes for unique behaviors, like hibernation. This would be a really cool tool to revisit what is known about those species and those adaptations in terms of reproductive trade-offs in females.

L. Powers: Bats end up "off the charts" in regressions of many life history traits vs. body size (litter size, neonate body mass relative to maternal body mass, gestation length, life span) (Kurta and Kunz 1987; Jones and MacLarnon 2001). Humans also have an inordinately long gestation length (Purvis and Harvey 1995). Presumably, this is to accommodate prenatal development of unusual morphology (wings or brain size) to some needed point before parturition. However, some obvious examples of taxa with extreme morphologies (giraffes, for example) don't exhibit extended gestation length. I'd be interested to see what other clades buck the trend (especially animals outside of mammals, and perhaps even plants, although I know nothing of their germination and early development).

T. Orr: As an evolutionary biologist, I think we should expend extra effort to study transitional species and/or those who are sole living representatives of certain clades. Monotremes, red pandas (*Ailurus fulgens*), Mountain beavers (*Aplodontia rufa*) all come to mind. But so too do groups like the subterranean rodents that vary enormously in lifespan, sociality (mating systems), and litter size. I think focal studies in a clade with natural variation (see also Clark et al. 2023) can get at these issues as can a broader brush study focusing on a larger suite of taxa with care to sample species at key steps of presumed transitions.

M. Meuti: There are also some invertebrates that do heavily invest in parental care (e.g., tsetse flies that give birth to a mature larvae that can weigh more than them; burying beetles that provision their young with food), so it could be interesting to consider energetic trade-offs across vertebrates and invertebrates that do and do not invest in parental care.

What you do with negative data, when you don't find a trade-off (or nothing is statistically significant)?

N. Place: This seems like an important point. Studies that measure hormone response or give hormones as treatments need to consider their ecophysiological relevance. What's really to be understood by an animal's response to a dose of hormones that is out of the range they would ever experience naturally? Along the same lines, what's the point of trying to study hormone response under conditions that are far from what the animal would experience in nature in concert with those hormone fluctuations (e.g. Constant temperature that is hotter at night and cooler during the day than they would actually experience; photoperiod that is constant year-round; constant uniform diet.)?

Especially from an endocrinological perspective, potential trade-offs associated with hormones are tested either by stimulating their endogenous production, supplementing with exogenous sources of hormones, or blocking or removing the source of the target hormone(s). I think we need to be more nuanced as to what we consider to be physiological levels of hormones. For example, I administered testosterone implants to chipmunks and measured levels that were physiological in terms of concentration (Place 2000). However, the implants did not truly reflect physiological levels because they were administered outside of the breeding season and the levels were tonic rather than periodic. Therefore, the implants produced supraphysiological levels, even though the concentrations measured within range of breeding males. Investigators need to be cognizant of this and be aware that stimulatory hormone challenge tests can elicit endogenous hormone concentrations that are super physiological. These comments might be applicable to studies of trade-offs associated with elevated testosterone or elevated glucocorticoids, for example.

T. Garland: This also relates to experimental design and trade-offs in studying trade-offs. Statistical power is a huge issue. Within a single species, sex, and age class, you have a relatively low range of variation as compared with looking among populations or among species. This alone will lower statistical power for a given sample size. In the life history trade-off literature, it has been noted that trade-offs are much more commonly detected among species than among individuals. This is not unique to studying trade-offs. It's about experimental design in general when one has limited resources, which is always the case. It's about how generalizable one's results may be if they study, say, one inbred strain of mice (or one *Drosophila* sp.) and look among individuals, or an outbred strain and look among individuals, or 10 inbred strains and look among strains.

J. Fornara: I think a lot of evolutionary biologists study trade-offs because they are a mechanism that can explain variation in behavior, morphology, physiology, etc. A classic example from life history theory is the trade-off between number of offspring per brood and offspring size/quality, which contributes to diversity in brood/litter size across the tree of life (Einum and Fleming 2000). In cases where we don't find evidence of a trade-off, we must consider what else could be driving variation within/among species (e.g., genetic, developmental, or physiological constraints). The 'Y-model' developed by van Noordwijk and de Jong (1986) predicts that energetic trade-offs are most likely to occur when individuals are equally successful at acquiring resources (e.g., have similar foraging efficiencies) but vary in how they allocate those resources to different processes/activities (Reznick et al. 2000). Conversely, the Y-model suggests null results should occur when there is high variance in acquisition but low variance in allocation (Reznick et al. 2000, but see Roff and Fairbairn 2007). While these predictions provide a useful framework for experimental design (deciding what to measure) and interpretation of results, testing them empirically has proven challenging and remains an active area of research (Garland et al. 2022).

As with any other field, it is difficult to make progress when you don't know what other research groups have already tested. Hence, editors reviewing papers that report negative results should consider that null results can lay the foundation for future review papers/meta-analyses that are important for elucidating broad-scale trends. At an individual level, we should also continue to report null results in conference presentations and discuss them with our collaborators, but I also think it is crucial to have these data available in an open-access forum (e.g., a peer-reviewed journal).

A. Litmer: I would add that finding non-significant results is incredibly interesting and informative. Such findings offer more room for exploration into how robust processes are to different changes, and what that could mean for fitness. Even more, non-significant findings can indicate that some traits may not have an adaptive purpose. Instead, some traits may simply be a byproduct of something else, or have a neutral effect.

K. Hinde: Report it and try to consider what that may mean to highlight questions for future directions.

B. Harris: I agree with A. Litmer and K. Hinde and think all results should be published! My lab often gets results that do not align with our original predictions and I think publishing these findings provides a great opportunity to discuss how experimental design and choices (as T. Garland said) matter, a discussion of the trade-offs made to study trade-offs, if you will.

What do you see as some of the biggest methodological or empirical challenges, specific to female reproduction and trade-offs?

T. Garland: One might be having to handle/manipulate gravid or lactating females, which may lead to litter loss, etc., and so the observation affects the system. Like the Heisenberg uncertainty principle.

A. Litmer: I think in general understanding constraints imposed by natural systems is challenging, and likely incredibly important. There are so many biotic and abiotic variables that can matter – yet we usually study these things in a controlled setting. Methodologically, this is really tough, but would likely offer the most insight as to what happens in natural systems with regards to female trade-offs.

C. Josefson: Agreed with both of the above points. I study how environmental conditions, such as chronic stress, impacts lactation performance. I've been using laboratory organisms because of how easy they are to work with and how much control over the environment I am able to have. Yet, we know that there are some drawbacks to using laboratory-reared and artificially-selected species to understand questions rooted in ecological and evolutionary theory (e.g., Abolins et al. 2017). Being able to recapture the same individuals over a period of lactation is not an easy feat and may not be feasible for many mammalian species. Catching a female and collecting samples provides snapshot data, which can be very informative, but there's a lot of huge variables we may not know about her (e.g., age, how many days/weeks in to pregnancy/lactation, how many offspring does she have, etc.).

So, as it's been stated before in this discussion, a trade-off exists in how we can study trade-offs: do we use laboratory organisms where we have more control and know more about their reproductive bout (but they might not necessarily have the same responses or constraints as wild conspecifics due to their natural history or environment), or do we relinquish some of that control in favor of measuring factors in a natural context? T. Garland discussed this above and in his previous work (see Garland and Rose 2009) and I think it warrants repeating: using different strains (or types) of artificially-selected species, such as dogs or mice, can be a powerful tool for understanding trade-offs (Jimenez 2016; Jimenez 2021; Josefson and Hood 2023).

P. Lopes: It is hard to come up with a unifying measure of cost that would be comparable across taxa. This could maybe be quantifying the energetic costs of producing one offspring from fertilization to fledging, but this sounds very hard to do.

C. Josefson (in response to P. Lopes): Along those same lines, even within the same species or taxa, coming up with a unifying measure of cost across all stages of reproduction. Each stage may comprise vastly different types of trade-offs or use different currencies that are being traded off. Methodologically, this may also prove difficult, as we may not be able to overtly observe certain stages of reproduction. For example, various species may undergo reproductive delays, which are often underexplored (see Orr and Zuk 2014), and may be missed if the researcher is not actively looking for evidence of delays.

J. Fornara: I think a huge challenge is just getting buy-in from the public, and even from colleagues who don't study sex differences. It is easy to forget how limited some people's understanding of female reproductive processes can be, even among AFAB (assigned female at birth) individuals regarding their own reproductive health (Fowler et al. 2023). If the average layperson doesn't have a fundamental understanding of female biology, it can be hard to convince people outside of our field that this kind of work matters (with important implications for the public's trust in science, funding support, etc.).

D. Misra: Understanding the total balance where not only reproductive cycle but also there are various pathways associated with the processes In addition to hormone regulation, other factors that affect female reproduction include energy levels, behavioral patterns, and the activation of different immunological pathways to protect the children. The physiological, biochemical, and metabolic pathways that are linked to better under the trade-offs must be taken into account.

T. Orr: I would say that the nuances of female reproduction can make it tricky. As I mentioned before at least before menopause (if relevant) a female is always doing something she is never 'non-reproductive' she is recovering and so on. Being sure to keep that in mind is important. Thinking back on the advice W. Hood gave me as a graduate student you must study mid-lactation to be able to compare across taxa well. I think this standard time point needs to be considered in all studies to allow for cleaner and more meaningful comparisons.

W. Saltzman: It's easy to quantify or manipulate hormone levels, but it is more difficult to quantify or manipulate receptor concentrations or affinities, although this can be done to some extent using pharmacological or other, more invasive methods. However, receptor concentrations and sensitivity play a crucial role in determining hormonal effects. So, for example, you might jack up circulating concentrations of hormones, such as glucocorticoids, estrogen, or testosterone, but if there's a limited number of receptors, then altering hormone levels might have limited effects. Similarly, blocking receptors might have little effect if hormone concentrations are very low. In addition, there can be cross talk between different hormones and their receptors. For example, oxytocin and vasopressin can bind to each other's receptors, although with different affinities. These sorts of things, in addition to possible variation in binding proteins and hormone clearance/inactivation, can make it difficult to interpret hormonal data and manipulations. There is so much more to the endocrine system than just hormone concentrations in the blood.

A. Liebl: I think we need to be better at considering *ad libitum* food and what that means for trade-offs and how it's going to play a role. Also, defining what exactly the "currency" of reproduction is and what is being traded off.

B. Harris: What proxies are we using for fitness and how long are we following animals – are we looking at single or select reproductive bouts or attempting to get at lifetime fitness? Do we consider what conditions the animals have been under and how to best measure the impact of those conditions – do we expect immediate impacts on fitness (proxies) or would we expect a longer-term cumulative impact? Lastly, at what level of reproduction are we asking our questions – for example gonadal physiology? Circulating hormones? Offspring produced/survived? Maternal physiological and behavioral investment?, and what are the species- and life history stage-appropriate dependent variables we should use to answer those questions.

T. Garland: This also relates to experimental design and trade-offs in studying trade-offs (see above comments). Obviously, lower sample size reduces statistical power, and I am sure this is an issue in many studies. Until recently, it was thought that an inbred strain of mice will have less phenotypic variation than an outbred one, and this should also reduce the power to detect a trade-off versus an outbred strain of mice. However, a recent meta-analysis shows that not to be true (Tuttle et al. 2018)! You also need to control age, sex, etc., either experimentally or by use of covariates and cofactors in statistical analyses. It also relates to issues of generalizability of findings. Any result you find in an inbred strain or a highly specialized organism (Naked mole rats? Horned lizards?) is going to be less generalizable than something found in an outbred strain or a more generalist species (Wild house mice? Cockroaches? House flies?) (Clark et al. 2023). These review papers note huge problems with low sample size in studies of selection in the wild: Hoekstra et al. 2001, Kingsolver et al. 2001.

K. Hinde: As a lactation biologist studying non-human primate dyads, it can be really hard to measure some variables without the experimental design affecting the outcomes being measured. I hypothesize that there is a methodological trade-off between precision of measurement and perturbation of the system in socially housed monkeys. (PUN INTENDED, I regret nothing.)

C. Josefson: For sure! To tack onto that, we need to also question whether as biologists, are we actually measuring what we intend to measure? Are you selecting the most appropriate variable(s) and exploring whether being female and/or in a specific reproductive stage itself is influencing those variables? Are the nuances associated with female reproduction being captured by your experimental design? Are the data being interpreted correctly? For example, just because two traits are negatively correlated, that doesn't necessarily point to a trade-off.

W. Saltzman (to C. Josefson): I think you mentioned this in your talk, but reproduction is sometimes seen as a sort of monolithic state, so people may talk about pregnancy and lactation (or other components of reproductions, for non-mammals) as if they are identical in terms of demands, trade-offs, etc. I think careful attention to the different stages of reproduction is necessary for understanding the relevant nutritional demands, energetics, morphological constraints, time budgets, etc.

C. Josefson: I think that’s one of the biggest things, and it’s kind of why I wanted to do this symposium and showcase some excellent work currently being done on this topic. Too many papers lump females at vastly different reproductive stages (e.g., pregnancy versus lactation or nest-building versus incubation versus caring for new hatchlings). Each of these stages has different consequences for mother and her offspring as well as different physiological milieux that support these processes. It may not be appropriate to combine certain stages of reproduction, depending on what you are trying to assess! Along those same lines, females of a species are often reproductive in some capacity, either because they are actively engaged in supporting offspring pre- or post-nataly or because their ovaries are actively cycling. So, is it appropriate or accurate to call females who have not undergone ovarectomies “non-reproductive”?

A. Litmer: I appreciate the responses here, especially those of A. Liebl and C. Josefson. I think a major challenge is understanding female trade-offs is quantifying mechanisms in the field. We can control many variables in the lab to identify true mechanistic links, but we often leave out interactive environmental components. In nature, researchers are challenged to determine important variables, such as how much food a female has eaten, energy use, and behavioral changes. Another factor to think about in addition to allocation of energy is allocation of time. Time allocation is especially important now, with a lot of projections suggesting that under future climate scenarios, time suitable for activities may be restricted. However, the question remains as to what time and activity restrictions would mean in different environments or taxonomic groups. We should be asking, “how does allocation of time, or amount of available time, influence critical behaviors like foraging or mate search, and how might that shift?”, instead of simply asking whether time of activity will shift.

B. Fitzwater.: Following what others said, I have read a lot of literature where the authors estimate that there could be fitness consequences on offspring into their adulthood, but then they never measure it. Usually in the discussion, there is a basic explanation of “this could be occurring but we are not sure.” It is important that we first define what fitness we are examining and then keep following these loose ends by measuring the offspring’s fitness over time.

C. Josefson: I could not agree more with Brooke’s statement! I am very cautious to assign any sort of valence to changes we see during offspring development, as we don’t know whether any changes we find are transient/ephemeral or fixed. We also have no clue *how* those changes may impact offspring when they are in adulthood. For example, I am interested in the developmental effects of maternal stress and have found changes associated with the hypothalamo-pituitary-adrenal axis in offspring of chronically-stressed mothers. Are these changes temporary? Are they beneficial (e.g., are they programming offspring for an anticipated environment; Bruener 2008)? Are they detrimental (e.g., do they always lead to a disease outcome or consequences to fitness; Monaghan 2008)? How much does environmental matching play a role in tipping the scales between when developmental programming of the adult phenotype is beneficial versus detrimental?

M. Meuti: I agree with the others who commented similarly throughout this discussion; fitness is a product of both survival and reproduction. Related to an earlier comment, we do need to consider trade-offs across the lifetime, including variables related to lifetime reproductive performance, as well as how the offspring of mothers fare in to adulthood.

D. Misra: Females of different species and creatures exhibit a wide range of life histories, including the number of children and the frequency of reproduction (gestation period, active cycle). This involves a broad range of studies to obtain a general understanding across different taxa. Additionally, the physiological changes that occur in females during reproduction vary throughout taxa. These elements may be crucial to comprehending trade-offs in a larger framework.

IV. Where we're going: Looking towards the future of studying trade-offs from the female-centered perspective

Trade-offs in their many forms are of broad interest to scientists belonging to many different fields and subdisciplines within biology, leading to a vast amount of literature on the topic. Yet, many gaps exist in our understanding of whole-organism trade-offs, especially in the context of female reproduction, and there is much left to be explored. In this section, we discuss specific areas to focus future research endeavors and how understanding trade-offs in reproducing females extends beyond organismal biology.

What biases in taxa studied are there and how might this be problematic? Where are our biggest gaps? Where would the best investments time/effort-wise (e.g., more diverse taxa or should we really dig into studying certain model species)? If so, what would you suggest?

A. Liebl: Mammals and birds are the best studied. Even some insects are decently studied. I've been surprised this week with how limited knowledge in herps is (even reproductive anatomy is unknown in females, particularly surprising when male genitalia is used to identify species!)

T. Garland: Somebody should do a tally of trade-off papers and count studies by taxon. I think that birds are overrepresented due to the common use of banding and nest boxes, for studies of life history traits, which is great, but is not so possible with most other vertebrates.

A. Litmer: Lizards, especially those in genus *Sceloporus*, are great model systems for life history and trade-offs, as well as potential trade-offs in locomotor performance (Albuqerque et al. 2015; Scales and Butler 2016). *Sceloporus* lizards have historically be studied with regards to life history, due to their wide geographic distribution and documented variation in life history as a result of environment and genetics. Using organisms which are closely related, yet experience different environments and life history phenotype can help us understand mechanisms influence such traits. However, in herpetology specifically, we have major gaps in knowledge regarding snakes, and many reptiles and amphibians in general. Reptiles and amphibians are often most sensitive to environmental change and represent a highly diverse group of organisms. Focusing on determining life history and trade-offs in such groups would offer great insight and assist in understanding such sensitive, yet ecologically important, taxonomic groups. I suggest conducting more thorough research studies across large geographic ranges of closely-related organisms when possible, such as *Sceloporus* lizards, for fundamental and theoretical approaches. I would suggest studying more snakes, crocodilians, or amphibians for filling gaps in knowledge.

M. Meuti: I think that there are lots of invertebrate taxa that can and should be studied to understand trade-offs. Admittedly, most of these species do not invest in parental care, but they still exhibit trade-offs in egg size and egg number, and also need to make important decisions about survival and reproduction. Of course, *Drosophila* have often been used as models for the study of life history trade-offs from a variety of perspectives (e.g., Hiraszumi 1961; Mueller and Ayala 1981; Nunney 1996; Rose et al. 2005; Burger et al. 2008).

T. Orr: Absolutely we should be studying the diversity from insects and herps! I am always a fan of comparative studies but that said it is wonderful to be able to draw on data from well-studied taxa be it a fruit fly or a lab mouse. The Krogh Principle is king here (Krebs 1975). If you are studying certain questions there is quite likely a 'best' model. It is just important we don't try to generalize too much. An example of this would be the commonly held belief that the spines of the genitalia of some mammals induce ovulation. Fun fact: that's just a cat thing! In other groups like the Eulipotyphla (shrews and moles), the exact converse is true (taxa with spines lack induced ovulation while those without tend to have induced ovulation) (Orr and Brennan 2016). Had no one studied the shrews and moles we would continue to believe these structures only serve one function.

P. Lopes: I don't think we can focus on a single species for studying female reproductive trade-offs because there are so many modes of female reproduction. For example, focusing on mammals, lactation seems to bring very unique costs, since lactation generally inhibits ovulation and also tends to make parental care maternally biased. In contrast, avian reproduction, for example, will likely impose more balanced costs between males and females, and so the reproductive trade-offs experienced by females here are likely different from those experienced by mammalian females.

R. Beltran: From my perspective, we need a lot more work on free-ranging animals (e.g., long-term, individual-based monitoring programs, see St. Kilda Soay Sheep Project, McKenna-Ell et al. 2023). Fundamentally, trade-offs are strongly influenced by environmental conditions in wild systems. Resource pulses like masting events and phytoplankton blooms can cause massive population pulses in wild animals, suggesting that constraints on trade-offs look fundamentally different when resources are less limiting. So, lab studies can lay important groundwork for understanding allocation strategies at the organismal scale, but it is also important to study how those studies scale to the highly variable conditions present in nature. Resource pulses and extreme climate conditions set up ideal natural experiments for measuring whole-organism trade-offs in the wild, but it takes careful studies of uniquely identifiable animals studied before, during, and after these events to truly understand their effects.

K. Hinde: Indeed, some of the very best information about trade-offs come from agricultural species such as cows (Ollion et al. 2016) and other livestock (Douhard et al., 2021) that have been under extraordinary amounts of artificial selection, so it tells us about plasticity and flexibility in these systems, but how they reflect more naturally constrained systems is less clear.

C. Josefson: I agree with the above sentiments that there isn't one species or even taxa that will encompass everything we want to study. Although laboratory organisms may not be informative about the *contexts* that shape when trade-offs appear or what they may look like in wild organisms (e.g., trade-offs resulting from ecological circumstances, sexual versus natural selection; Table 2), they can be an excellent tool in understanding more proximate mechanisms that might underlie certain types of trade-offs, such as those shaped by functional conflicts, allocation constraints, shared biochemical pathways, or antagonistic pleiotropy (Table 2).

What do you think are the biggest questions that we have unanswered in this field as it relates to the female-centered perspective? What do you think we need to focus on most?

P. Lopes: I think one thing to consider from this roundtable is that there are many ways to be a reproducing female. We saw in the symposium talks and associated manuscripts that bats have to fly and lactate at the same time or that certain mouse species can lactate and gestate at the same time. And then animals with external fertilization or that lay eggs, and so with different types of trade-offs, obviously depending on how the reproduction happens.

L. Powers: Researchers really need to stop and think about the potential effects of sex differences and reproductive biological processes when they are designing their studies. This is especially crucial when doing physiological studies. They need to think about how the reproductive physiological and behavioral processes that are taking place during reproductive cycles might affect the physiological response to treatments (or other input variables). If they don't have the resources to include enough subjects to represent major different reproductive states (for example: pregnancy, offspring rearing, spermatogenesis, periods of reproductive dormancy, mating behavior) they should design studies to include the stages of reproduction they think are most crucial to examine, determine reproductive stage part of their methods, explicitly state what stage the subjects were in during the study, and acknowledgement of the limitations of the conclusions that can be drawn because certain reproductive stages were not included in the study.

A. Litmer: When considering females, it is important to know the whole organism's energy budget and quantify energetic demand versus effort. Specifically, it is useful to know what proportion of a female's energy (or time) budget is being allocated to certain processes, as opposed to just considering variables such as clutch or offspring size, or even fecundity, as an indicator of "effort" or "energetic cost". We should consider if females are allocating more of their budget proportionally, or if they simply have a bigger budget overall. Such attention to female reproductive effort in relation to life history strategy would offer incredibly insightful information, especially for studies interested in comparing populations or life history strategies. I would also emphasize quantifying caloric (energetic) consumption and assimilation in lab-based trials, which provides an indication of the resources an organism has to allocate. Often, energy budgets, and energy allocation, underlies trade-offs and life history traits, yet we don't quantify such variables.

T. Garland: One important area might be how behavioral and physiological trade-offs change across ontogeny in general or, as others have suggested here, across the stages of reproduction. Young organisms that are growing rapidly and have not yet reached sexual maturity are in a different physiological "space" than those that are actively reproducing (including lactating) or into the post-reproductive phase of life. If they are social, then they may also be in a different behavioral "space." For example, in a social species, other individuals might help an individual thrive even if their physical abilities were failing, and thus alleviate some trade-offs that would otherwise occur. In general, different kinds of trade-offs are likely to be crucial at different life stages, and behavioral compensation may be common to alleviate trade-offs (e.g., see Bauwens and Thoen 1981; Clobert et al. 2000). More generally, compensatory mechanisms likely play a common role in the biology of trade-offs, and they also affect our ability to detect trade-offs (Oufiero and Garland 2007; Husak and Swallow 2011; Garland et al. 2022).

J. Fornara: When females are observed performing "male-typical" courtship or reproductive behaviors, I think there is a tendency to dismiss these observations as "flukes" rather than considering how these behaviors might serve a functional role in females. For example, [male] scientists initially hypothesized that female singing behavior in North American passerines was caused by overproduction of male-typical hormones (e.g., androgens; Byers and King 2000, Catchpole and Slater 2008). Over the past two decades, work by Naomi Langmore (1998), Karan Odom (2014), Victoria Austin (2021), and others has demonstrated that song is widespread (and serves a biological function) in many female birds, but the female-centered perspective is still slow to gain momentum. This misconception that so-called "male-typical" behaviors are irrelevant for females marks a significant barrier to progress in research related to trade-offs in reproducing females since behavior often mediates fitness trade-offs. Addressing this issue head-on by empirically testing the mechanisms and function of "male-typical" behaviors in female animals is a critical next step in our field. Furthermore, we must acknowledge that classifying behaviors, physiology, etc. as "male-typical" or "female-typical" biases what we pay attention to and measure in our studies, which could cause us to overlook important aspects of an organism's biology (Byers and King 2000; Langmore 1998; Odom and Benedict 2018; Austin et al. 2021).

K. Hinde: I think the literature has gotten away from some of the germinal texts in this field of trade-offs around parental investment and the literature gets blurry around effort, care, constraints, and costs, and then assuming trade-offs without really integrating constraints, costs, and how costs are paid across scales (time, whole organism), and relatively few papers effectively bring together theoretical modeling with empirical evidence. (We see the same thing around arm-waving about adaptation without demonstrating heritability, variance, and impact on fitness, the Darwinian Trifecta). I think it would be important to highlight both Gold Standard aspirations, how to triangulate understanding from aggregating results in the literature that fall short of the gold standard but collectively provide a nice understanding, and what questions remain that would benefit from gold-standard research effort and what that would look like.

C. Josefson: I love K. Hinde's point above. We all have Gold Standard aspirations that would be lovely to study, but in reality, methodologies are constrained by so many factors. Even still, understanding the contexts in which trade-offs are and are not present – and what they may look like – is important in understanding patterns at many levels of biological organization. I think part of this is also being explicit with where limitations to your study exist so that readers can more easily fit your work in with the larger patterns demonstrated by the literature.

A. Liebl: I agree with T. Garland that trade-offs work at different scales at different times and we need to be better about acknowledging that. Also, in some species we literally know NOTHING about female reproduction (currently working with someone looking at the anatomy of female chameleons, which we have no previous knowledge of!!!!) and that we need to start somewhere. Understanding integral steps to help us get to the ultimate understanding of trade-offs (or conflict!) is useful!

B. Harris: I want to also add that I think the idea of taking a step back and assessing underlying assumptions to/of our biological questions, frameworks, and paradigms would be an excellent start. The book Biology and Feminism (Nelson, 2017) really got me thinking about this and I feel it is an excellent exercise and could provide rich opportunities for hypothesis testing in research on female trade-offs.

W. Saltzman: I agree that it's important to acknowledge that trade-offs are likely to differ among species and among phases of reproduction. In addition, trade-offs are likely to vary with both environmental conditions (e.g., which resources are limiting) and physiological state of the animal. For social species, social environment can be very important in both enhancing the severity of constraints proving on reproduction or alleviating potential trade-offs. Examples include parental/alloparental care, disease transmission and competition among females, and we know female aggression can vary with reproductive state.

D. Misra: The reproductive stages and the energy metabolism affect. I feel that there is a limitation in knowledge about how biosynthesis pathways may change when in the context of a reproducing female. Trade-offs also can be multi-faceted; for example, in humans, they may involve biological, social, and cultural factors or in other species. Important phases of reproduction, such as pregnancy, nursing, and postpartum, call for extra consideration of metabolic and other physiological issues. Other pathways that are primarily focused on the growth and nourishment of the fetus emerge once the mother begins to breastfeed. Furthermore, as phenotypic plasticity in trade-offs involves environmental cues and changes quickly among populations, it requires greater attention.

What are the societal/other benefits of studying trade-offs?

Aid to Society:
T. Garland: One possible benefit could relate to teaching. Trade-offs are all around us in daily life (e.g., electric cars versus those with internal combustion engines). At present, we just don't have the charging infrastructure to make electric cars really viable in a lot of places. But this trade-off is context dependent, because some places (e.g., wealthy neighborhoods) do have the chargers, and will change over time as countries build the charging networks. Lots of trade-offs exist in health care and treatment strategies. Many of these are biological in origin, but they also involve economics, morals (e.g., funding for abortion), etc. I see that increasing societal understanding of all types of trade-offs will give us benefits in terms of personal finance, better-educated voters, and so forth. A lot of resources on the web illustrate how to teach trade-offs in the context of economics. Some examples are: *https://fte.org/teachers/teacher-resources/lesson-plans/rslessons/trade-offs-and-opportunity-cost/* *https://keydifferences.com/difference-between-trade-off-and-opportunity-cost.html* and *https://www.linkedin.com/pulse/difference-between-trade-off-curves-opportunity-cost-bhavya-mangla/*

Coming back to biology, lots of teachable examples are available, such as shapes of bird wings or beaks (e.g., Milne 2008; Herrell et all. 2009; Krishnan 2023).

K. Hinde: I think about this all the time with regard to adolescent lactation in long-lived mammals with low extrinsic mortality risk (like humans and other large primates). There are likely adaptations for limiting milk synthesis capacity so that young mothers aren't stunted/compromised across a reproductive career, which means that young primiparous mothers may have challenges in milk synthesis that no amount of extra food, sustained pumping, etc. can overcome. We have to tailor health care to understand that there are going to be limitations in a woman's capacity as a function of adaptations for trade-off priorities that can't necessarily be circumvented, but don't necessarily predict ability to lactate on subsequent offspring (Pittet et al. 2017; Pittet and Hinde 2023).

Conservation and Ecology:
B. Fitzwater: Reproductive trade-offs can impact more than just reproduction, such as vertebrate susceptibility to parasites as a result of sex hormone concentration (Zuk and McKean 1995; Folstad and Karter 1992; Moore and Wilson 2002). This has implications for organism health and survival, and variation in reproductive trade-offs between males and females could therefore result in different needs for medical treatment, conservation efforts, and our overall understanding of a species' ecology and life history. For example, if differential dietary needs as a result of specific reproductive trade-offs between males and females cause them to utilize different habitats, this may introduce more variables that could impact the sexes. Differential habitat use between the sexes has been observed and could have conservation implications (van Toor and Safi 2011). For example, pregnant females/females that lay eggs may do so in locations that are different from where males are typically observed, differential susceptibility to pathogens and parasites, variation in predator susceptibility, etc.

A. Litmer: I think trade-offs and life history provide insight into population dynamics, and populations are often the unit of conservation research and management. Subsequently, we can understand certain life stages that may be more critical for conservation, or certain abiotic or biotic components that are having the greatest influence on organisms. Knowing more about trade-offs and the variation that occurs over time and space can also inform more population-specific conservation approaches, because trade-offs vary even among closely related organisms.

L. Powers: Studying reproductive trade-offs can help us create models to predict how species of concern will respond to changes to the environment (such as climate change, or proposed construction or changes in land use by humans). This will provide land managers at state and federal agencies to make more accurate environmental impact statements when new human land use is proposed. We could improve models to predict when spillover of pathogens from reservoir host species if we knew more about how hosts reproductive costs affect immune function, and what the conditions are that will result in energetic trade-offs between reproduction and immune function.

M. Meuti: It will help us to identify "winners" and "losers" in the face of climate change, such that which species that are able to minimize trade-offs associated with reproduction will be likely to win, while those that face severe reproductive trade-offs will likely lose without help and protection.

R. Beltran: Understanding how resources limit allocation is fundamental when we have control over the abundance and distribution of resources that are in a system and available to animals. An obvious example is human-wildlife conflict.

A. Liebl: Reproduction is fundamental to population growth and stability, which means trade-offs are as well.

W. Saltzman: It might be able to provide relevant information for 1) captive breeding programs and 2) human reproduction, especially under sub-optimal conditions.

D. Costa: I just wanted to point out how important understanding your resource space is. When things are good, these trade-offs are probably not going to be apparent, there's not going to be a cost. When things are bad, these trade-offs are going to be very profound and we've done studies where we've put tracking devices on animals that created a drag effect. In a good year it made absolutely no difference, there was no change in the females. In the El Niño year, it was just devastating. This also becomes important for climate change, and our ability to understand how these animals respond is completely related to all the resources available.

T. Garland: That's a great example. This also relates to experimental design and trade-offs in studying trade-offs. Context is hugely important.

B. Fitzwater (in response to D. Costa): That is a great point about how reproductive trade-offs can be more apparent in "bad" years vs "good" years. This could also have implications for anthropogenic factors in the environment and climate change. For example, females in an ecosystem that is facing greater anthropogenic threats or climate change may not have previously experienced a measurable degree of trade-offs. However, as the selection pressures change, do we see measurable trade-offs? It would be interesting from an evolutionary perspective to see if there is plasticity for making these reproductive trade-off decisions. I also want to mention that this can be very important when studying reproductive trade-offs in behavior. Could resource availability impact how choosy a female may be? How might resource availability impact maternal care, particularly in species with high maternal care, such as octopuses that invest so fully into caring for their eggs that they die afterward and therefore trade-off subsequent reproduction events (Cortez et al. 1995; Robison et al 2014)?

V. Conclusion

Despite working on different research questions using diverse taxa across various contexts and applications, our multidisciplinary team of authors agree on one thing: studying trade-offs is difficult, requiring attention to detail. This roundtable highlights some of these difficulties in our past, current, and future understanding of trade-offs both broadly and within the context of reproducing females. This discussion emphasizes myriad considerations to make when understanding, testing, and communicating work on trade-offs using a female-centered perspective that captures the nuances associated with the various stages of female reproduction.

Across the questions posed in this discussion, several key themes emerged. We urge researchers to question the background assumptions that underlie their research and use methods that are specifically tailored to the hypothesis that is being tested and the organism that is being used. These assumptions may impact the proxies (e.g., assessing not only a hormone of interest, but also other hormones and/or receptors involved in the pathway), sampling regime (e.g., choosing repeated measures or sampling after a reproductive event has ended rather), and conditions used in experiments that measure trade-offs. Because trade-offs are likely to be context-specific and linked to many biotic and abiotic factors that are beyond our control as researchers, we suggest including as much detail as possible when communicating results from experimental or observational approaches. Important details may include the conditions the animals were in (e.g., food or nutrient availability, approximate temperature, etc.), how data were collected (e.g., how body condition or offspring sex was determined), or information about the reproductive bout (e.g., measures of offspring quality, litter size, sex ratio, etc.). Further, we highlight the importance of testing similar hypotheses under differing conditions and in diverse taxa and the need to publish null data where trade-offs were not observed so that researchers can avoid redundant work and understand the context under which trade-offs and costs of reproduction may be present.

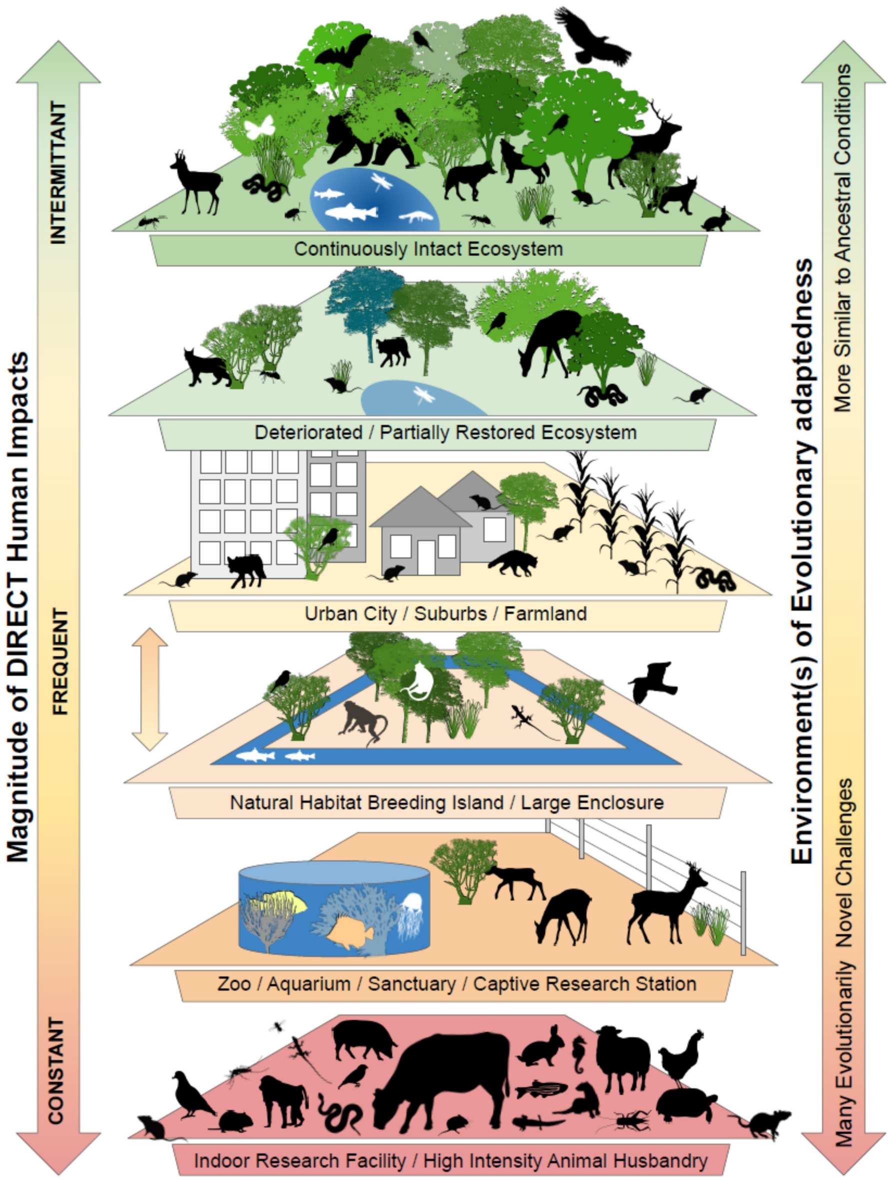

Figure 1. Too often animal research is simplified into a captive/wild dichotomy, but climate change, resource extraction, hunting pressure, and other anthropogenic disturbances indirectly or directly impact nearly every terrestrial, marine, and aquatic ecosystem. The captive/wild dichotomy is not merely overly simplistic, but obscures the extent that animals are responding to challenges and opportunities substantially altered from the ancestral conditions that shaped adaptations for reproductive trade-offs within and across time (Rodrigues et al., 2023). Further, depending on the spaces being considered, the magnitude of the perturbations may overwhelm physiological sensitivities that influence reproductive mechanisms implicated in trade-offs. Images of biological organisms sourced from phylopic.org; thank you to the artists for putting these silhouettes into the public domain. Silhouettes are not arranged to accurate scale.

Authorship Contributions

Initial conception for the symposium, and thus, the roundtable discussion, as well as securing funding, were by CJ and TJO. All authors contributed to the writing and editing of the final manuscript, especially in the parts indicated by their initials. Compilation of responses and final edits were done by CJ, BMF, and TJO. Tables were completed by BMF, TJO, TG, and CJ. Figure by KH.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Table 1. Definitions for Problematic Terms. Here, we provide a few suggested definitions, empirical considerations, and further reading; terms: allocation, condition, constraint, cost, fitness, model organism, non-reproductive, optimal, trade-off. This is not a comprehensive list and we direct readers to the paper by Baker and Hayssen (this volume) in this edition for additional terminology relative to female-centric biology in general.

Term	**Suggested definition(s)**	**Empirical considerations**	**Recommended reading**
Acquisition	In the present context, the process or act of acquiring a resource (e.g., energy). Also used in behavior and neuroscience to refer to acquiring information, developing a response to stimuli, etc.	Variation may exist in the amount of resources an individual is able to acquire. Therefore, measuring the amount of resources an individual acquires, albeit difficult, may be important to the question being asked. Proxies, such as resource availability, are sometimes used, as it can prove difficult to measure total resources acquired. Similarly, resource acquisition can be experimentally altered by limiting resource availability (e.g.., restricting nutrients and/or calories).	Reznick et al. 2000; Van Noordwijk and De Jong 1986.
Allocation	Devoting a resource to something, such as energy or time being 'allocated' to reproductive effort.	As with the discussion of 'costs' below, what an animal is allocating can be diverse and you may or may not capture it in your measurements. If you don't find a relationship between energy and time, then what an animal is allocating could be unmeasured, such as nutrients.	Reznick et al. 2000; Van Noordwijk and De Jong 1986.
Condition	Physiological (and perhaps behavioral) health.	Not measured by one variable at time. No single variable can indicate 'good condition' and this should be acknowledged. Further, authors are encouraged	Labocha and Hayes 2012; Labocha et al. 2014

		to be cognizant that some variables do not have clear predictions: ex. is a high hematocrit an indication of 'bad' condition, perhaps due to dehydration?	
Constraint	Often discussed relative to allocation, whereby as described by Garland et al. (2022) "a limit exists for the total amount of a resource that is available (e.g., energy, time, space, essential nutrients)"		Ardia et al. 2011; Garland et al. 2022
Cost	Any resource (time, energy, nutrient, opportunity) that is depleted through an event.	Clearly explain what is considered a 'cost' and what assumptions are being made.	Harshman and Zera 2007
Fitness	Difficult to broadly define fitness, "there does not seem to be any comprehensive definition" (Hamilton 1964). One common definition is "lifetime reproductive success," but the broadest definition applicable to reproductive trade-offs could be "an organism's capacity to pass on its genes to the next generation"	Authors need to 1) clearly define what measure of fitness they are referring to and 2) whether they measured it and if so, how it was measured.	Hendry et al. 2018; Byerly and Michod 1991; Kimbrough 1980
Model organism	Model organisms are species that are used in research due to desirable traits (e.g.,	A model organism needn't be just what is standardly used in biology and biomedicine (giant-squid, mouse, rat, fruit-fly, and	Clark et al. 2023; Krebs 1975; Alfred and Baldwin 2015.

	short generation time, annotated genome, etc.) to study a biological phenomenon. For any question, there is an organism best suited to address it (see Krogh Principle).	so on), but can include diverse species that have traits that can aid in testing specific hypotheses.	
'non-reproductive'	An animal that currently cannot undergo a reproductive event and that is not recovering from a previous event.	Specify what stages are meant. If an animal is not pregnant nor lactating but might be recovering: report.	Hayssen and Orr 2017
Optimal	Best solution given a set of optimality criteria (e.g., net energy gain) and a series of restrictions. This may not be the 'best of all situations,' and multiple equally good solutions may exist.		See any of the classics (Charnov and so on). Park and Smith 1990; Viney et al. 2005; Taylor and Thomas 2014
Trade-offs	"…one trait **cannot** increase without a decrease in another." (Garland et al. 2022)	Many types of trade-offs are known. Be cautious to consider what category you are trying to study (see Garland et al. 2022).	Garland et al. 2022, Glazier 2009; Taylor and Thomas 2014; Grubb 2016.

Table 2. Types of trade-offs and examples within reproductive contexts.
The types of trade-offs listed were as described by Garland et al. 2022.

Category of Trade-Off	**Definition from Garland et al. 2022**	**Female reproduction example**
Allocation constraints	"Occur when a limit exists for the total amount of a resource that is available."	Producing many small offspring vs a few large ones, or simply producing many offspring vs only a few (Koch and Meunier 2014; Kindsvater and Otto 2014); Proposed energetic trade-offs between reproduction and simultaneously occurring processes (self-maintenance, immune defense, etc.).
Functional conflicts	Often biomechanical, "features that enhance performance of one task decrease performance of another."	Simplex uterus and big litters; gravid females or females with attached young having diminished locomotor capabilities (C-start, sprint speed etc.), decreased local innate immune defense in the eutherian female reproductive tract that accommodates the developing young but decreases defense against pathogens (Wigby *et al.* 2019)
Shared biochemical pathways	Pathways may share molecules that can have either beneficial or deleterious effects on other traits.	Estrogen interacts with many receptors, including aspects of the immune system (Harding and Heaton 2022) stress hyporesponsive period during lactation may be mediated by hormones (e.g., prolactin, oxytocin) that support milk synthesis and ejection and pro-maternal behaviors (Slattery and Neumann 2008).

Antagonistic pleiotropy	"Genetic variants that increase one component of Darwinian fitness simultaneously decrease another, causing a negative additive genetic correlation between the two components."	Menopause; estrogen aids in reproductive success but can also lead to higher parasite susceptibility (Vom Steeg and Klein 2017; Zhang et al. 2018); traits that may favor increased reproductive performance early in life lead to decreased longevity (Williams 1957; Austad and Hoffman 2018).
Ecological circumstances (selective regime)	Context-dependent, factors within the environment may impact Darwinian fitness; this can also fluctuate with changes in environmental conditions, such as seasonality.	Gravid females may be more susceptible to predation and therefore must alter their behavior (Ibáñez et al. 2015); females engaged in maternal care must partition time away from other tasks (e.g., foraging).
Sexual vs. natural selection	Secondary sexual characteristics may improve reproductive success but could also negatively affect other aspects, such as survival, parasite susceptibility, or energetic costs.	Ornamented female pipefish may face higher predation risk (Fuller and Berglund 1996); cryptic female choice may interact with immunity Drayton et al. 2013).