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The association between male-biased sex ratio and indicators of stress in red-spotted newts

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HIGHLIGHTS

- Female activity did not differ in response to chemical cues from one vs three males.
- Females increase activity with increasing stress hormone to chemical cues of three males.
- Females in male-biased enclosures had higher CORT and testosterone release rates.
- Sexual conflict in male-biased environments can reduce female fitness.

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ABSTRACT

In populations with a male-biased operational sex ratio, coercive mating by males can have fitness consequences for females. One component of reduced fitness for females in populations with a male-biased OSR may be greater activation of the stress response, resulting in higher corticosterone release rates (CORT; a glucocorticoid stress hormone in amphibians). We test the hypothesis that a male-biased sex ratio affects female activity and release rates of CORT and testosterone (T) in male and female red-spotted newts (*Notophthalmus viridescens*). First, we evaluated if chemical cues from a male-biased sex ratio affect activity and CORT release rates in females. We predicted that females exposed to chemical cues of three males would be less active and have higher CORT release rates than those exposed to chemical cues of one male. Second, we measured CORT release rates of red-spotted newts in field enclosures with either a male-biased or a female-biased sex ratio. We predicted that females in the male-biased treatment would have higher CORT and T release rates than those in a female-biased treatment, owing to higher levels of male harassment. We also predicted that males would have higher CORT and T release rates in male-biased treatments due to higher levels of male-male competition. Females were not less active in response to chemical cues from more males over fewer males, but there was a positive relationship between female activity and CORT when they were exposed to the cues of three males. We also found that females, but not males, in the male-biased sex ratio treatment had higher CORT and T release rates than those in the female-biased treatment. Our results support the hypothesis that a male-biased sex ratio leads to a higher stress response, which may underlie the observed decrease in immune function and body condition in previous work exposing female red-spotted newts to a male-biased sex ratio. This study furthers our understanding of the mechanistic basis for costs associated with a male-biased sex ratio in a pond-breeding amphibian.

1. Introduction

How males and females of the same species maximize fitness often differs, which sets the stage for conflicts of interest during male-female interactions. This conflict is referred to as sexual conflict when males and females interact in a mating context [1]. Sexual conflict occurs if the individual fitness of one sex is increased as a function of a trait that alters the behavior of the other sex, at an expense to the latter's own fitness. Sexual conflict can lead to a decrease in the fitness of one sex through a number of different actions, such as mating harassment, coercive behaviors, or injury during mating. Sexual selection favors traits that increase an individual's ability to differentially access mates

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and to be successful in syngamy. Sexual selection is stronger when the asymmetries in male and female mating success are larger, which is influenced by the degree of operational sex ratio bias (OSR; [2–4]). Although the processes of sexual selection and sexual conflict have traditionally been presented as opposing explanations for mating behaviors, Kokko and Jennions [1] argue that sexual selection and sexual conflict are conceptually linked in a number of ways, including the observation that there are likely no biologically plausible conditions under which either exists without the other.

The outcome of sexual conflict is important because in addition to effects on evolutionary trajectories, it can have significant effects on population-level processes [e.g., reviewed in 5,6]. For example, if females are on the winning side in sexual conflict, population fitness should be higher than if males are on the winning side because females are more likely to control demography; the more females in a population, the higher number of offspring that are produced [1,7]. Understanding the mechanisms by which sexual conflict plays out can help us understand which sex might be in the "winning role" in a population. For example, males of many species engage in coercive behaviors, including harassment of solo and mating females, and these behaviors could lead to a reduction in female fitness, and/or costly modifications in female behavior [8–17]. Coercive behaviors are expected to be higher in frequency in populations when the OSR is male-biased, as has been found in several taxa (*Aquarius*: [18,19]; *Thamnophis*: [19]; *Gambusia*: [20]).

The OSR may vary within a species temporally and at different scales. At a broader scale, a highly male-biased OSR in a population is expected to lead to greater variation in reproductive success, and therefore an increase in the opportunity for sexual selection [2–4, but see 21]. Male sexual harassment increases with a more male-biased OSR, which can detrimentally impact almost every aspect of female behavior and life history [reviewed in 8]. Females in male-biased OSR populations may respond both behaviorally and physiologically to male coercive behaviors, and these responses have implications for female health and therefore population fitness. Similar to the indirect effects of predator risk on prey ("ecology of fear"), which include morphological or behavioral responses that can be costly (e.g., reduced survival, growth or reproduction) [reviewed in 22], breeding females may also experience indirect effects from the risks of male harassment. For example, when female seed beetles (*Callosobruchus maculates*) are both harassed by or could mate with more than one male, they had decreased life spans and reduced reproductive rates relative to monandrous females [23]. In the Atlantic molly (*Poecilia mexicana*), male mating behavior often consists of constant female harassment by males and attempted forced copulations, and female feeding rates are lower when the female is accompanied by a male due to increased vigilance to potential harassment [24]). In a viviparous lizard (*Lacerta vivipara*), a male-biased OSR affects immediate female body condition and reduces lifetime reproductive rate [25].

Theory predicts that females can evolve counterstrategies to reduce the costs of male coercion, or to reduce the frequency of mating. These counterstrategies are in the form of morphology [reviewed in 8], as well as behaviors. For example, female guppies (*Poecilia reticulata*) that are not in mating condition associate with more attractive females in response to increases in male harassment [26]. Female red-spotted newts (*Notophthalmus viridescens*) avoid large groups of males [27], and spend more time in refuge habitat in male-biased sex ratio treatments [28]. Aside from changes in behavior, physiological changes can occur alongside reductions in fitness and observable countermeasures to an overabundance of a particular sex. The endocrine systems of animals are dynamic, such that rapid changes in circulating hormones arise from the various social and ecological environments that individuals may encounter, and the fitness correlates of these changes may vary across contexts [29–31].

The red-spotted newt is a pond-breeding amphibian that exhibits complex courtship and mating behaviors, including amplexus [32,33], and breeding populations are often male-biased [28,33–35]. Grayson et al. [28] showed that a male-biased OSR in the red-spotted newt affects female body condition and decreases total lymphocyte and leukocyte abundance in blood, a potential indication of physiological stress. As the adaptive immune system is sensitive to elevated glucocorticoids [GC; 36], these effects on female condition and immunity may be proximally linked to an increase in corticosterone (CORT; a primary GC in birds, amphibians and reptiles) during amplexus, which may occur at greater rates in male-biased OSR populations. Further, high reproductive effort by males is expected to be associated with higher CORT levels in males [e.g., the CORT-Adaptation Hypothesis 37,38]. Higher rates of amplexus and aggressive male behaviors may also be costly to females in terms of loss of time foraging, increased risk of predation, and the potential for injuries, which could manifest as an increase in CORT levels in amplectant females relative to those not in amplexus. Indeed, amplexus has been associated with an increase in CORT release rates in both male and female red-spotted newts [39].

In addition to effects on CORT, population sex ratios during the mating season could also affect release of the steroid hormone, testosterone (T). For example, in the bank vole, *Clethrionomys glareolus*, (T) levels can influence both intra- and inter-sexual selection [40]. However, there is not a significant relationship between T and OSR [40]. Testosterone is the primary androgen hormone stimulating secondary sexual characteristics but at the same time can be immunosuppressive (and thus may be negatively associated with CORT) due to the negative feedback between the endocrine and immune systems [41]. The immunosuppressive effects of sexual hormones may be adaptive as resources can be redirected toward mating [42]. Yet, most studies of male amphibians have found a positive correlation for CORT and T [43–46], but in some species CORT and T are inversely correlated [47]. Little is known about the relationship between CORT and reproductive hormones such as T in female amphibians [but see 48,49]. Harvey et al. [48] found that in explosive breeding spadefoot toad, *Scaphiopus couchii*, T and estradiol increase in amplexed females but CORT did not change across the reproductive cycle of females.

In this experiment, we tested the hypothesis that a male-biased sex ratio will affect female activity and release rates of CORT and T in male and female red-spotted newts. We first conducted a lab experiment to evaluate how sex ratio affects activity and CORT levels in females. We exposed females to water-borne chemical cues of either one male or three males and we measured pre and post-experiment water-borne CORT release rates. We predicted that females exposed to cues of three males would have a higher CORT response and lower activity than those exposed to the cues of one male. In the second experiment, newts were placed in field enclosures in groups of four at either a male- or female-biased (3 males: 1 female or 3 females: 1 male) sex ratio for seven days and we measured post-experiment water-borne hormone release rates. We predicted that males, as well as females, exposed to a male-biased sex ratio will have higher CORT and T.

2. Materials and methods

2.1. Study site

This research was conducted at Mountain Lake Biological Station (MLBS; Giles County, Virginia, USA) from June—July 2015. We conducted a behavioral experiment to test for effects of male chemical cues on female activity and CORT levels in a laboratory room at MLBS. We also performed an experiment to evaluate the effects of biased sex ratio on male and female CORT and T in a field experiment in Riopel Pond (0.65 ha). Riopel Pond contains a large population of red-spotted newts [estimated newt population = 7600–12,700 individuals; 28,50].

2.2. Effects of male chemical cues on female activity and CORT

To acquire male chemical cues, we collected male newts (N = 16 total males) from the adjacent pond and immediately placed either one male or three in a container (12 × 18 × 31 cm) with 1 L of spring