

THE EFFECTS OF SOCIAL EXPERIENCE ON SEXUAL SELECTION
IN THREESPINE STICKLEBACKS (*GASTEROSTEUS ACULEATUS*)

By

Emily Grace Weigel

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ABSTRACT

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We investigate how social experience can alter traits, and thereby influence sexual selection, using threespine sticklebacks (*Gasterosteus aculeatus* spp.). Specifically, we examine how female and male responses to social conditions affect their reproductive investments and mating success.

We first focus on how mate availability impacts female reproductive investment as measured by the timing and amount of clutches and eggs. Using treatments that mimic high and low availability of reproductive males, we found that Paxton Lake females, although previously shown to adjust their mate choices, did not alter their reproductive investment strategies. Because females must make relatively fixed reproductive investments prior to courtship, but have plastic mate choice, the strength of sexual selection acting on males during mating is subject to change. This work therefore highlights the potential consequences when reproductive investment and female choice operate out of sync.

We focus second on how males allocate resources into several traits, and when and how those traits are assessed in courtship. With Cranby Lake sticklebacks, we show that the sexual signal preferred by females in many populations (red throats) is neither condition-dependent, nor preferred in mating. Males indeed allocate resources to their morphological displays, courtship behavior, and nests, but only a subset of traits matter to females at each stage of the courtship progression. Specifically, females only prefer high male condition early in courtship, and they increasingly prefer more vigorous, display-oriented courtship behavior as courtship progresses. This work suggests that the relative

importance of traits upon which females choose mates is not only a function of whether those traits indicate male quality, but also depends on what stage in courtship traits are assessed.

Third, because population demography is likely to vary spatially and temporally for many organisms, we address how male traits might change in response to the intensity of mate competition. We experimentally manipulated male density ('perceived mate competition'), and examined the effects on male nesting probability, mating traits, and mating success. We found males to display different coloration and build nests differently in response to a two-week exposure to either low or high competition. Given that these changes resulted from such a short-term exposure, this work clarifies how rapid changes in demography affect the courtship and nesting strategies adopted by males. If females are assessing male traits throughout courtship, understanding whether and how male traits change with competitive conditions suggests how mate competition and female choice may interact to shape traits, and how acclimation to social conditions may influence adaptation.

Finally, we inquire how experience and success in mating affect male traits. Because previous experience with other males altered male traits, we were inspired to discover how male traits and mating success respond to prior experience and success in courtship with females. This is particularly important as changes to population demography often alter mating opportunities, competition, and the likelihood of success. Here we show that males alter their body coloration and court more quickly with experience, but males do not alter their nests when they experience mating success. The trait responses to experience suggest a cycle: the male traits used in mating are responsive to prior experiences and success, and these modified traits are used in future mating attempts. Thus, trait variation can be maintained in part by the responses to experiences individuals have across their lifetime.

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INTRODUCTION

Ever since Darwin (1871) proposed that the elaborate traits males possess function in competition with other males to gain the attention of females, we have been striving to understand how male traits are shaped, as well as the female preferences for these traits. In most populations, females are considered the 'choosy' sex. This generally implies that females make a larger investment than males, producing fewer but costlier gametes (eggs) compared to the male's many, cheap sperm (Bateman, 1948; Trivers, 1972). Therefore, females must make decisions about with whom they will mate, and as a consequence, males must compete with other males for mating opportunities. Because some males will enjoy greater reproductive success than others, the competition for mates can drive the evolution of traits that are useful to males both in the context of male-male competition and also in garnering a female's attention (Andersson, 1994).

In the years since Darwin (1871), Bateman (1948), and Trivers (1972) published their seminal works on sexual selection, behavioral ecologists have recognized that sexual selection may be much more complex than imagined by these earlier investigators. For example, Bateman's ideas, although generally true, underestimate investment made by males (Wedell et al., 2002) and largely ignore the importance of social and ecological conditions in the animals' environment (Emlen and Oring, 1977; Endler and Basolo, 1998; reviewed in Andersson, 1994). Evidence is mounting that the quality, quantity, and distribution of potential mates and competitors factor into mate choice decisions (Jennions and Petrie, 1997; Bailey and Zuk, 2009; Hebets and Vink, 2007; Tinghitella et al., 2015). In particular, we are beginning to ask how variables that

change within the lifespans of individual organisms might affect the course of evolution across multiple generations.

There is currently strong interest in increasing our understanding of how population demography affects sexual selection. This is particularly important as population demography is unlikely to remain constant across space or across time within an organisms' lifetime. It is because demography often varies spatially and temporally that the factors that control mate availability, mate competition, and mating success are likely to vary as well. The importance of social information in informing animals' mate choice decisions and mating behavior is increasingly recognized. How, then, do demography and social information affect the expression of traits and mating success?

Organisms that are sensitive to and capable of altering their trait expression in response to social conditions are likely to enjoy fitness advantages. These within-lifetime changes may alter rates of selection on expressed traits, facilitating their rapid spread or damping them out altogether. To understand the evolution of traits, we therefore need to understand to which factors organisms may respond within their lifetimes, in what ways, and to what degree. This is a timely and important area of inquiry, as the rapid environmental changes we are experiencing now are increasing the frequency with which demography and associated social conditions are disturbed (reviewed in Candolin and Wong, 2012).

To answer questions about the roles of various kinds of social experience in sexual selection, I use the stickleback fish (*Gasterosteus aculeatus*), which represent a well-known system for the study of sexual selection (Rowland, 1988; Bakker and

Milinski, 1993; Candolin, 1999a; Kraak and Bakker, 1998; Hendry et al., 2013; Tinghitella et al., 2013; Bolnick et al., 2015). Although my work primarily concerns the sticklebacks of Cranby Lake, I repeatedly draw connections to other populations that live in other freshwater lakes in British Columbia (McPhail et al., 1994; Bell and Foster, 1994; Boughman, 2001). I examine the details of how sexual selection functions in response to specific demographic or social manipulations in this population and then discuss whether my results can be used to compare and contrast them with responses in other populations.

Although Cranby sticklebacks are understudied relative to other populations, much is known about the biology of sticklebacks in general, and this knowledge can aid in our investigations. First, most stickleback fish in the wild live only one year, although some populations may live up to two years, but rarely longer (Baker, 1994). Within this time, and particularly during the breeding season, population density and adult sex ratios vary temporally and spatially (Tinghitella et al., 2013; Tinghitella, Head and Boughman, unpublished data). Males are known to compete strongly for a territory within which to build nests to court females (Lackey and Boughman, 2013). Although populations vary in how they court (Wootton, 1976 and references therein; Ridgway and McPhail, 1987; Foster, 1995; Scotti and Foster, 2007), sticklebacks do follow a characteristic pattern marked by an elaborate courtship dance (Tinbergen 1951), which, if successful, will result in the female spawning eggs within the male's nest. Males are then solely responsible for raising young, which includes oxygenating (fanning) the eggs and guarding fry (Wootton and Wootton, 1984). Males also produce a characteristic red throat coloration, which is relatively muted in Cranby compared to other populations

(Albert et al., 2007). Although exactly which traits are preferred by Cranby females (and to what relative extents) remains untested, females from many populations generally prefer larger, redder males (but see Boughman, 2001), and male color is often, but not always, positively correlated with physical condition (Wootton, 1976; Milinski and Bakker, 1990; Bakker and Milinski, 1993; Bakker and Mundwiler, 1994; Candolin, 1999b; Boughman, 2007). In general, males express a variety of traits to compete for mates, and choosy females must then evaluate male traits to select suitable mates.

Here, I investigate how demography and social experience shape sexual selection. I first address how mate availability impacts female reproductive investment. In Chapter 1, I ask whether the availability of many or few potential mates impacts the timing or amount of clutches and eggs a female produces. I track each female's reproductive state across the season and extract her clutches to determine whether investment varies with mate availability. This work sheds light on whether female reproductive investment is fixed or plastic in the face of changing social conditions. It also draws attention to the potential consequences when reproductive investment and female choice operate out of sync (e.g., when many eggs are invested in less-preferred males).

Next, I turn the focus to males to investigate how and when male traits might be used in reproduction and whether these traits reliably indicate male condition. In Chapter 2, I use no-choice mating trials (i.e., a single female with a single male) to investigate the male traits most favored by females as courtship progresses and determine which traits are linked to male condition. This work determines which male traits are preferred by females in this population, and at which stage in courtship that

preference is expressed. This work also explores the potential function of certain male traits in reproduction, and why they may differ between closely-related species.

I then ask how a male's perception of competition can influence male trait expression and female choice. In Chapter 3, I build on my findings in Chapter 2 to examine how male density (perceived level of mate competition) can affect male traits and mating success. Here I test whether morphological, behavioral, and nesting traits are responsive to competition, and if so, in what ways. This work relies on a temporary exposure to one of two density treatments, followed by individual tank placement, allowing us to tease out what the lingering effects of density may be on male traits and mating success. This work suggests that short-term experiences can affect the variation seen in male traits upon which females will later select.

Finally, I consider that males and females, as a consequence of different levels of mate availability and competition, will likely also vary in their amounts of experience in mating. Modifying one's phenotype in response to experience should be particularly important for males, as they are generally less assured to mate than females (Trivers 1972), and trait changes should have larger effects on their mating success. Thus, in Chapter 4, I ask how mating experience influences male traits and mating success. I build on this to also ask whether past mating success influences the traits expressed and mating success of males in subsequent mating attempts. This work therefore clarifies how mating opportunities and success may differentially impact male traits, and how experience-mediated trait changes may impact the strength of sexual selection on male traits.

In the following chapters, I use ‘we’ to describe the research I completed with the guidance and input of my research advisor, Janette Boughman.

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CHAPTER ONE

No evidence for adjustment of maternal investment under alternative mate availability regimes

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ABSTRACT

Using treatments that mimic high and low availability of reproductive males, it was found that female threespine sticklebacks *Gasterosteus aculeatus* (L.1758), previously shown to adjust their mate choices when male mates were rare, did not alter their reproductive investment strategies. These results suggest that plasticity in investment is perhaps limited by physiological requirements or dependent on relatively extreme mate availability regimes. The probability of becoming reproductive, number of clutches per season (per female), initial clutch size and mass, and the timing of reproduction were all independent of the experience a female had with mate availability. This suggests that pre-copulatory plasticity in reproductive strategies may contribute more to variation in the strength and direction of sexual selection than reproductive investment in offspring.

INTRODUCTION

Life histories of organisms are frequently shaped by trade-offs, whereby the fitness benefits of one trait (or suite of traits) are linked to deficits in another trait (Stearns, 1992). Classic examples of life history trade-offs include whether to invest in growth or reproduction (Gadgil & Bossert, 1970), to care for offspring or court new mates (Lindström, 1998; Bjelvenmark & Forsgren, 2003; among others) and when to reproduce relative to mortality (Zwaan *et al.*, 1995; Flatt, 2011). Because trade-offs are

intrinsically linked to the determinants of fitness, optimizing the costs and benefits of traits in a particular environment is critical. Given that evolutionary trade-offs are fundamentally derived from responses to limited resources, trade-offs also shape the fitness of alternative strategies under fluctuating environmental conditions. This study considers trade-offs that may occur when mates are a limiting resource. Both male and female life histories are shaped by trade-offs between the quantity and quality of current and future reproductive opportunities. Here it is asked whether and how females alter this investment in offspring when mates are rare vs. common.

Population density and mate availability fluctuate dramatically, even within individuals' lifetimes. As a result, experience with and competition for mates can lead to variation in the strength and direction of sexual selection *via* changes in pre- and post-copulatory mechanisms (Forsgren *et al.*, 1996; Hebets, 2003; Cotton *et al.*, 2006; Mück *et al.*, 2013). For example, populations shrinking under high mortality are predicted to increase investment in current reproduction, because survival and therefore future opportunities to mate are uncertain (Gadgil & Bossert, 1970; Stearns, 1992; Stearns, 2000; Cichoń, 2001; Kokko & Rankin, 2006; Kokko & Mappes, 2005). Indeed, empirical evidence supports the theory of early investment in reproduction under high mortality across taxa (Reznick, 1983; Tatar & Carey, 1995; Stearns, 2000) although counter-examples exist (Clutton-Brock, 1984; reviewed in Reznick, 1985). Populations of the two-spotted goby *Gobiusculus flavescens* (Fabricius 1779) shift from early-season male–male competition to late-season female–female competition as males become more scarce, reversing sex roles dynamically (Forsgren *et al.*, 2004; Wacker *et al.*, 2013; Wacker *et al.*, 2014). Similarly, the female preference for large size in this species

disappears late in the season when males are rare (Borg *et al.*, 2006). Hence, male and female life histories are shaped by tradeoffs that depend on both quality and quantity of current and future reproductive opportunities. Therefore, existing evidence demonstrates that fluctuation in within-season mate availability leads to changes in the strength of sexual selection, and can select for phenotypically plastic responses in mating that maximize reproductive success (Stearns, 1992; Roff, 1997; Pigliucci, 2001; DeWitt and Scheiner 2004).

Pre-mating reproductive behaviours like mating competition and mate choice clearly respond to mate availability (e.g. Milinski & Bakker, 1992; Jirotkul, 1999; Kokko & Mappes 2005; Shine *et al.*, 2006; reviewed in Kokko & Rankin, 2006). Whether and how animals vary reproductive *investment* in response to mate availability, however, has received much less attention. In contrast to female choice, current reproductive investment is predicted to increase when future mating opportunities are uncertain (Gadgil & Bossert, 1970; Stearns, 1992; Cichoń, 2001; McNamara *et al.*, 2009). Because females typically invest a great deal in offspring, selection for plastic reproductive investment in response to mate availability should be strong, reflecting the potential loss of fitness if non-adaptive investments are made. Similarly, the greater the reproductive investment by males, the more exaggerated the effects should be on female reproductive success when mates are limited (Trivers, 1972; Emlen & Oring, 1977; Smith, 1977; Halliday, 1978; Patterson *et al.*, 1980; Westneat, 1988; Royer & McNeil, 1993). Indeed, there is growing evidence that mate availability influences female reproductive success (Wedell *et al.*, 2002; Smith & Reichard, 2005; Heubel *et al.*, 2008; Carrillo *et al.*, 2012), particularly in species with significant male investment in

parental care (Borg *et al.*, 2002; Forsgren *et al.*, 2004, Hopwood *et al.* 2015), or nutritional investment (Simmons & Kvarnemo, 2006; Simmons & Kotiaho, 2007; Scharf *et al.*, 2013). For example, previous work in common gobies *Pomatoschistus microps* (Krøyer 1838), has shown that female-biased sex ratios lead to larger first clutches, signifying a trade-off of increased investment early in the season at a cost of producing fewer eggs across the season (Heubel *et al.*, 2008). Although plasticity may come in more forms than previously studied, plastic reproductive investment could facilitate increased reproductive success in rapidly-changing environments, for instance when populations are small, highly fragmented, or experience high mortality.

Threespine sticklebacks *Gasterosteus aculeatus* (L.1758) are an established model system in sexual selection research (Rundle *et al.*, 2000; Boughman *et al.*, 2005; Andersson & Simmons, 2006; Hendry *et al.*, 2013). They offer an opportunity to test the effects of demography on female reproductive investment in a species in which pre-mating behaviour is known to respond to altered demography (Tinghitella *et al.*, 2013). During the summer breeding season, males establish nesting territories on which to build nests, and females search amongst them for mates. Under these circumstances, females, both those not yet gravid and those ready to deposit eggs, are regularly exposed to many males and nesting sites before making mating decisions (Boughman, 2006). Mating occurs throughout the breeding season in this species and occurs only within a male's nest. Males are then responsible for all parental care, which consists primarily of oxygenating eggs and defending fry.

Each time a *G. aculeatus* female develops a clutch, she has the opportunity to make a mating decision. Time is, however, limited; females ovulate all of their mature

oocytes concurrently in any single clutch, thus females must invest an entire clutch with a single male and have limited time to do so (reviewed by McLennan, 2006). Therefore, the rate of accepting a mate in any one encounter is not only dependent on the long-term, perceived availability of mates throughout the breeding season, but also the short-term developmental time of the current clutch. Because body shape and size change dramatically while developing a clutch of eggs, *G. aculeatus* offer an easily observable model in which to manipulate mate availability and quantitatively measure reproductive investment.

In several post-glacial lakes in British Columbia, *G. aculeatus* demography varies within the breeding season. Adult population density decreases through the breeding season, particularly for males (Boughman, Tinghitella, and Head, unpublished data), and there is spatial variation in the operational sex ratio across breeding sites within a given lake (Tinghitella *et al.*, 2013). As the availability of males (proportion of males) can fluctuate by over 30% from location to location in a given lake, females do indeed experience drastic differences in mate availability as they search for mates.

Furthermore, female mating decisions become relaxed at the end of the season (Tinghitella *et al.*, 2013) as residual reproductive value declines (Kokko & Jennions, 2008; Lahti *et al.*, 2009), but only for females who have experienced a female-biased adult sex ratio (ASR). These females spawn more quickly and are more accepting of males, regardless of their advertised quality. Because males also must care for young, fathers are temporarily removed from the pool of potential mates, reducing availability in some areas, adding to the variation in mate availability a female experiences.

Theory predicts that sexual selection on males should be weak when a female's potential mates are rare, but this theory also ignores the possibility that reproductive investment and mating decisions may not be modified in parallel across the breeding season. If female mating decisions are relaxed when mates are rare, but females do not alter their investment in eggs or they increase current investment when the risk of not mating later in the season is high (as in Forsgren *et al.* 2004), then sexual selection will be relatively weak. Lower quality males might sire a sizeable number of offspring if they wait to mate when competition is reduced, avoiding costly male competition and eventually acquiring a mating opportunity with a less choosy female. Alternatively, if investment decreases as mating decisions are relaxed, sexual selection is stronger than originally predicted: lower quality males will sire fewer offspring before the season's close.

Theory also provides specific predictions when females must allocate whole clutches to an individual potential mate. Following the theoretical model in Heubel *et al.* (2008), it is first assumed that females can have many clutches across the season, but have a fixed total budget of eggs. Second, it is assumed that when mates are rare, females developing clutches face uncertain future mating opportunities. Because *G. aculeatus* limnetic females typically have a single season in which to spawn, they can neither delay reproduction to the next season for better mating options nor obtain enhanced fecundity by growing to larger size (unlike other fishes; reviewed in Koslow 1996; Koons *et al.* 2008; Secor 2008). Thus, consistent with life history theory, when mates are rare, reproductive effort should shift to favour investment into the first clutch(es) or to reduce the time between clutches (Gadgil & Bossert, 1970; Stearns,

1992; Cichoń, 2001; Kokko & Rankin, 2006; Kokko & Mappes, 2005; McNamara et al., 2009). This would allow females to capitalise on currently-available mates and avoid the potential costs of waiting too long to spawn. Conversely, when mates are plentiful and females are more assured of future mating opportunities, investment may not shift to favour early clutches. Instead, females may produce many clutches thereby dividing out their resources among many males they will encounter across the breeding season. The partitioning of reproductive effort across clutches could increase not only the genetic diversity of offspring, as different males may be nesting at different points in the season, but also limit the amount of resource competition between siblings by temporally separating clutches.

To test whether females respond to mate availability by altering their reproductive investment, the mate availability (ASR) experienced by female *G. aculeatus* was experimentally manipulated throughout their reproductive lifespans. The following was then measured: (1) the number of clutches produced over the course of the breeding season, (2) the initial clutch size (number of eggs and clutch mass) and (3) the length of time females remained gravid per clutch, across mate availability treatments.

Nearly all research to date has coupled the effects of limited mate availability with seasonal declines in mate availability. Because females encounter variation in mate availability across the breeding season, this study design teased apart different ways by which female reproductive investment may respond. The aim is to provide comprehensive knowledge of whether and how reproductive investment is plastic in response to mate availability, and how investment may modulate the strength of sexual selection in a species that responds behaviourally to mate availability.

MATERIALS AND METHODS

Study population and treatments

At the beginning of the 2011 breeding season, wild limnetic sticklebacks (*Gasterosteus aculeatus*, NCBI Taxonomy ID: 481459) from Paxton Lake, Texada Island in British Columbia were collected using minnow traps. *G. aculeatus* were sexed using well-established differences in body shape (for males and females) and nuptial colour (for males) (McPhail, 1984; McPhail, 1992; Hatfield, 1997) and then transferred to single sex plastic bags at equal densities, which were loaded into coolers for transport by air to Michigan State University. The social conditions for both sexes were, thus, the same prior to placement in treatment tanks.

Immediately upon arrival in the laboratory, *G. aculeatus* were assigned to 283.9 L (75-gallon) replicate tanks in one of two treatments: 16 *G. aculeatus* in a 3:1 (male-biased, early-season) or 1:3 (female-biased, late-season) ratio of males to females in 12 replicate tanks (6 of each treatment). These treatments represent extreme values of sex ratio variation found in the wild, particularly for the female-biased condition (Tinghitella *et al.* 2013). Although male *G. aculeatus* are capable of raising clutches from multiple females across their lifetime, they rarely do. Within each tank, males and females were size-matched by visual examination within sex and uniquely marked with elastomer (Northwest Marine Technology, Shaw Island WA; Jones *et al.*, 2006) along the dorsal side to facilitate individual identification. After 1 day of acclimation, it was confirmed that there were no size differences between treatments or tanks in *G. aculeatus* length (treatment: *t*-test: $t = 0.8084$, $P > 0.05$; tank: ANOVA: $F_{11,191} = 0.892$, $P > 0.05$) and mass (treatment: *t*-test: $t = 0.9998$, $P > 0.05$; tank: ANOVA: $F_{11,191} = 0.103$, $P > 0.05$).

Treatment tanks were visually isolated from one another using opaque, white covers applied to the outside surfaces of the tank. The artificial tank habitat included ceramic caves made of halved flower pots and plastic plants for cover. No materials were added to aid males in building nests; however, males and females could and did otherwise freely court. Makeshift nests were common, but removed to prevent females from spawning. High rates of courtship and male competition still occurred even in the absence of nests (Tinghitella et al 2013). Tanks were maintained with 14-hour day lengths at approximately 18°C, mimicking the natural conditions of their native habitat. All *G. aculeatus* were fed defrosted brine shrimp (*Artemia* sp.) and bloodworms (*Chironomus* sp.) once per day *ad libitum*.

Tracking daily female reproductive investment

To assess daily changes in female reproductive status, each female was visually assessed on a 0-5 gravidity scale, where 0 indicated a non-gravid female and 5 indicates a female ready to release her clutch (as evidenced by an open genital pore and a plump, swollen abdomen; as in Frommen *et al.*, 2012). A gravidity score for each female was recorded blind to the prior day's measurement. This allowed observers to calculate the number of days a given female was gravid, as well as the total number of gravid females per tank each day. Visual assessment of females on the 0 to 5 gravidity scale were made by a single researcher (EGW). Additionally, changes from a gravidity score of 5 to 0 the following day indicated release of a clutch (either because the female dropped the clutch naturally or because the clutch was extracted as described below), and allowed calculation of the total number of clutches each female developed across

the season. These in-tank observations minimized the amount of handling of the *G. aculeatus*.

Tracking reproductive investment in initial clutches

All four females in male-biased tanks and a randomly-determined subset of four females from female-biased tanks were identified as focal individuals (N=48) and used to obtain additional body size and clutch measurements. When these females attained a score of 5 on the gravidity scale, they were weighed to the nearest hundredth of a gram on an OHAUS Scout PRO SPE 202 balance and their body length was recorded to the nearest hundredth of a mm. To obtain standard length, females were photographed using a Canon G-15 digital camera and the distance between digital landmarks at two extremes of the body (the anterior tip of upper lip and caudal border of the hypural plate at the lateral midline) was calculated using the program PAST (<http://folk.uio.no/ohammer/past>) according to established methods (Taylor *et al.*, 2006; Cooper *et al.*, 2011). The female was then gently squeezed to extract her clutch. The clutch was weighed, the number of eggs counted, and the female reweighed post-extraction. Because previous work (Heubel *et al.*, 2008; Carrillo *et al.*, 2012) suggests first clutches are most altered by sex ratio differences, and animal handling and egg extraction could decrease survivorship and reclutching rate (one of the key variables under study), extraction of eggs was conducted only for a female's initial clutch to minimize adverse effects on *G. aculeatus* health and sample size. Note that t-tests conducted following the experiment showed no effect of handling on reproduction (when comparing the 4 focal females to the other females within each female-biased tank; all $p > 0.05$).

Statistical analysis

Because the reproductive investment of multiple females from each replicate tank was assessed, mixed effect model analysis was appropriate to evaluate how perceived availability of mates impacts female reproductive investment. Measures of reproductive investment across mate availability treatments included the number of clutches a female had throughout the breeding season, the number of eggs per initial clutch, initial clutch mass, the number of days spent either nongravid (stage 0), developing eggs (stages 1-4), and fully gravid (stage 5) during the season, and finally the start and stop of seasonal reproduction. Mixed models were conducted within R (R Core Team, 2013, version 3.0.2) using the 'lme4' library (Bates *et al.*, 2013). Treatment (male- or female-bias) was entered as a fixed effect into all models. Intercepts for females (nested within replicate treatment tanks) and number of clutches (for models concerning days gravid) were included as random effects. To account for covariances in daily measures of gravidity, repeated measures were incorporated into models measuring days gravid. Plots of residuals for each response variable were visually inspected to detect deviations from homoscedascity or normality, and generalized linear mixed models were used with a Poisson distribution when appropriate. The fit of the full model (with the fixed treatment effect) was compared against a reduced null model without the fixed effect (only random effects) to test whether the fit of the model decreased significantly ($p < 0.05$) using chi-squared tests (Winter, 2013). Finally, Levene's test was used to test for significant difference in variances between treatments. Data are presented as means $\pm$ SD.

RESULTS

During the summer breeding season of this study, females under both male- and female-biased conditions had an equal likelihood of being reproductive (producing at least one clutch) ($X^2 = 0.112$, $df = 2$, $p > 0.05$). Females in both mate availability treatments had 1.188 ± 1.149 SD clutches on average (range = 0-4). As the focus was on whether and how reproductive investment changed in response to mate availability, for the following analyses, females who were never reproductive ($N=8$) were removed.

Females in male-biased tanks produced 1.47 times the number of clutches produced in female-biased tanks. Despite differences in the number of clutches produced, due to greater variance within treatments, females from both treatments generated an equal number of clutches across the season and the variance did not differ between treatments ($X^2 = 2.148$, $df=2$, $p>0.05$; Levene's Test $F=0.002$ $p>0.05$). Reproductive females (those which had at least one clutch) averaged 1.970 ± 0.825 clutches across the season, regardless of treatment, and further examinations of first clutch mass and first clutch egg number also revealed no difference in investment across treatments (first clutch mass: $X^2 = 0.033$, $df=1$, $p \Rightarrow 0.05$, Levene's Test $F=0.215$ $p \Rightarrow 0.05$ and egg number: $X^2 = 0.033$, $df=1$, $p>0.05$, Levene's Test $F=1.027$ $p \Rightarrow 0.05$; see Fig. 1.1).

Next, the timing of reproductive investment was assessed across mate availability treatments. Variables measured included the number of days females spent in a completely nongravid state (0 on the gravidity scale) both for consecutive days (spans) of nongravidity (time between clutches, or the "interclutch interval") and the total days spent in a nongravid state across the season. This method revealed how clutches

are distributed within a season, and also captures differences in potential 'failed' clutches which may not have developed fully. Interestingly, most days of the reproductive season were spent in a nongravid (0) state (avg 101.880 +/- 12.248 days). Females spent 12.755 +/- 9.352 days between 0 and other states of gravidity (the interclutch interval). When considering differences between mate availability treatments, there was neither a difference in the total number of days spent in a nongravid state (Figure 1.2; $X^2 = 0.967$, $df=1$, $p>0.05$ Levene's Test $F=0.188$ $p>0.05$), nor in interclutch interval length (Figure 1.3; $X^2 = 1.288$, $df=1$, $p>0.05$ Levene's Test $F=0.314$ $p>0.05$). This indicates that females spent little time developing clutches across the season, with the majority of the days spent in a nongravid state.

Next, the number of days a female spent developing a given clutch of eggs, before she was ready to release the clutch (stages 1-4) was examined. The consecutive number of days on which the gravidity score ranged from 1-4 was the measure of time spent developing a clutch. A drop to 0 gravidity before reaching a fully gravid state (5) was considered a "lost" clutch. The treatments did not differ significantly ($X^2 = 0.076$, $df=1$, $p>0.05$, Levene's Test $F=1.067$ $p>0.05$); females spent an average of 9.690 +/- 1.859 days of the season in a state of clutch development, and each span of development was relatively quick, lasting only 2.490 +/- 0.212 days on average.

Then, the number of days spent in a completely gravid state (5 on the gravidity scale) during the 116 day experiment was assessed across sex ratio treatments. Females under both male- and female-biased conditions spent 3.55 +/- 0.64 days across the season in a completely gravid (5) state ($X^2 = 1.955$, $df=1$, $p>0.05$, Levene's Test

$F=0.499$ $p>0.05$), and each span of gravidity lasted < 2 days (1.670 ± 0.181 days; $X^2=0.331$, $df=1$, $p>0.05$).

Finally, the time to first reproductive day (calculated from the day treatments were established) and time to last reproductive day (the end of reproduction for each female) were assessed. Given the importance of diet and time necessary to develop a clutch, females may be physiologically-constrained from modulating clutch numbers, size, or time to development; however, the time of season at which females begin reproducing may not be constrained in the same way. Thus, females may adjust when in the season they become reproductive in response to short term variation in available mates. Therefore, Julian dates were calculated to determine the first day on which each female began developing a clutch (score >0 on gravidity scale) as a measure of the beginning of reproduction for each female. Females, regardless of mate availability, started reproduction in early May and stop reproduction at the end of July with c. 1 week worth of variance during this breeding season experiment ($X^2= 0.736$, $df= 1$, $p >0.05$, Levene's Test $F=0.209$ $p>0.05$; and, $X^2= 0.722$, $df= 1$, $p >0.05$, Levene's Test $F=1.563$ $p>0.05$, respectively); that is, mate availability in female *G. aculeatus* does not appear to determine how early or late in the season a female is likely to become reproductive.

DISCUSSION

Both male and female life histories are shaped by trade-offs between the quality and quantity of current and prospective mates. This experiment investigated whether and how female reproductive investment responds to mate availability, and whether those responses are modified in parallel with known patterns of mate choice. The results show that limited plasticity in reproductive investment may keep females from

mitigating the effects of relaxed choosiness when mates are rare. Indeed, there was no evidence that the number of reproductive females, clutches per season (per female), number or mass of eggs in initial clutches, or the timing of reproduction differed between mate availability treatments.

When males are rare and time is short (females are approaching the end of their reproductive lifespans), female *G. aculeatus* relax their mating decisions (Tinghitella *et al.*, 2013). As female mating decisions are relaxed under low mate availability, but reproductive investment appears fairly canalized, sexual selection should be relatively weakened under these conditions. Because females deposit an entire clutch in the nest of a single male when spawning, all chosen males, regardless of quality, can potentially receive the same reproductive investment because there is limited plasticity in how females allocate reproductive resources. In particular, if female choice is relaxed, allowing lower quality males to spawn, males of lower quality could enjoy the most reproductive success by avoiding costly male competition (particularly at higher-densities) through either 1. nesting in less dense areas, or 2. waiting to nest until other males are providing parental care to eventually acquire a mating opportunity once males become rare. Thus, less-preferred males could reap more benefits simply by being available in the right place (where there are fewer males) and at the right time (when females are ready to spawn).

Plasticity in reproductive investment may also be limited by costs like predation risk and incomplete information about search costs. Specifically, spending more days fully gravid per clutch, or having more clutches, can increase a females' risk of predation (reviewed in Magnhagen, 1991). Because females have incomplete

information about the probability of success in continued mate search (Stephens & Krebs, 1986; Real, 1990; Wiegmann *et al.*, 2010), they experience costs associated with risk (Raiffa 1970; Trimmer *et al.* 2011). Risks should increase with time unmated (as eggs may become unviable), rarity of mates, and prevalence of predators. Although having developed eggs prepares a female should she encounter a rare mate, the risks of unviable eggs and expansive searches could counter selection for plasticity in increasing the expected duration of gravidity per clutch.

Based on observations of short, repeated visits by females to male territories, Dale and Slagsvold (1996) suggested that the number of times a male is encountered on a territory might be an informative signal for females about future mating opportunities, and therefore the risk of continued search (Raiffa, 1970; Trimmer *et al.*, 2011). However, males within this study were not allowed to maintain complete nests in their treatment tanks to prevent females from depositing eggs in them (because females' mating decisions were simultaneously being assessed in a parallel experiment using no-choice mating trials with nesting males; Tinghitella *et al.* 2013). Experience with both males and their nests may be necessary for females to assess and respond to differences in mate availability by adjusting reproductive investments; thus, from the perspective of searching females, although there was a difference in the adult sex ratio between treatments, the operational sex ratio could have been strongly female-biased in both treatments. However, given that the experimental set-up for the study used the same individuals and actually did find an effect of sex ratio treatment on female mating decisions (Tinghitella *et al.* 2013), it is likely that the experimental design was sufficient to produce an effect on female reproductive investment, if one exists. Similarly, had

females within a treatment tank been simultaneously gravid often, the OSRs could have been more variable than the ASRs would suggest. Multiple fully gravid females, however, scarcely occurred within a tank at the same time, thus our sex ratio remained largely consistent for the duration of the experiment.

Although numerous examples exist demonstrating female plasticity in investment in response to her environment, including in response to predation (Giesing *et al.*, 2011), conspecific brood parasitism (Lyon, 1998), and attractiveness or parenting ability of males (Sheldon, 2000; Kolm 2001; Stiver & Alonzo, 2009 (review); Kindsvater *et al.*, 2013; Poisbleau *et al.*, 2013, Soma & Okanoya, 2013), there is limited evidence to suggest plasticity in female reproductive investment due to demography. Perhaps the notable exception of plasticity in reproductive investment shown by Heubel *et al.* (2008) is facilitated by relatively extreme within-season variation in sex ratios in common gobies (*P. microps*). This variation is substantially more dramatic than that observed in *G. aculeatus*, and thus the selection pressure favouring the evolution of plastic reproductive investment may simply be weak. The difference in effects between these species may be exaggerated by the manner in which clutches are partitioned between males. Female *G. aculeatus* must deposit all of their clutch in the nest of a single male, thus they must make investment decisions earlier with uncertainty as to whether any mating will be secured for the clutch under development. *P. microps*, in contrast, could secure a mating with a portion of a clutch, then delay to deposit the remaining eggs with another male. Thus, selection pressures on reproductive investment may differ in these species depending on clutch size, clutch partitioning, and the timing of mate encounters.

Likewise, limitations in observed plasticity may also be due to energetic constraints. Despite ample food, females in this study generally had few clutches, although clutch totals were within range of the expected reproductive output across populations of *G. aculeatus* (Wootton 1976). Therefore differences between clutches, particularly in timing, may be difficult to examine. Adding to this the fact that females are only 30% physiologically-efficient in producing eggs (i.e, resources are not very efficiently converted to eggs; Wootton & Evans, 1976), specific reproductive investment trade-offs and reallocations of energy to other functions would need to be quite large to detectably alter days gravid, clutch sizes, clutch number, or egg number.

Female *G. aculeatus* could also plastically adjust reproductive investment in response to mate availability in other ways than measured in this experiment. For instance, females could alter investment in offspring by varying their quality, not quantity, through increasing cortisol levels and egg size to influence offspring survival (e.g. Giesing *et al.*, 2011). Egg size was not measured here, but increasing egg size is known to positively affect feeding (Knutsen & Tilseth, 1985), swimming abilities (Ojanguren *et al.*, 1996), and survival at both egg and larval stages (Lillelund & Lasker, 1971; Henrich, 1988; Leggett & Deblois, 1994), all of which may translate into fitness advantages in their current environment.

Given that *G. aculeatus* are iteroparous but unlikely to live to a second breeding season, 'waiting' to reproduce likely disfavoured evolutionarily in this species, particularly for females experiencing an excess of mates (male-biased conditions). A more probable strategy might be to modify the final clutch (e.g. in mass, egg number, egg size, etc.), maximizing the terminal investment (Clutton-Brock, 1984). This

experiment did not capture variation in terminal investment, although characteristics of final clutches were not investigated because of the risk involved in extracting clutches.

This study highlights the importance of the interplay between mate availability, female mate choice, and female reproductive investment shaping variation in the strength and direction of sexual selection. Laboratory studies aimed toward estimating investment and behavioural interactions will likely benefit from considering that the demographic effects of mate choice (intersexual selection) and mate competition (intrasexual selection) for both males and females may be tempered by the degree of plasticity in female investment. As Moura & Peixoto (2013) point out in their meta-analysis, responses of females and males may differ from species to species as sex ratios become more male-biased; these responses may be exaggerated in species where males provide care. Recent evidence in burying beetles shows increased care under increased reproductive competition (Hopwood *et al.* 2015), but more evidence is necessary to determine the extent to which this pattern exists across species, and whether it is exaggerated or ameliorated by other alterations in investment. Thus, future work should expand our understanding of how demography influences reproductive strategies of males, in response to mate competition and female choice, particularly in species where males provide care.

APPENDIX

APPENDIX

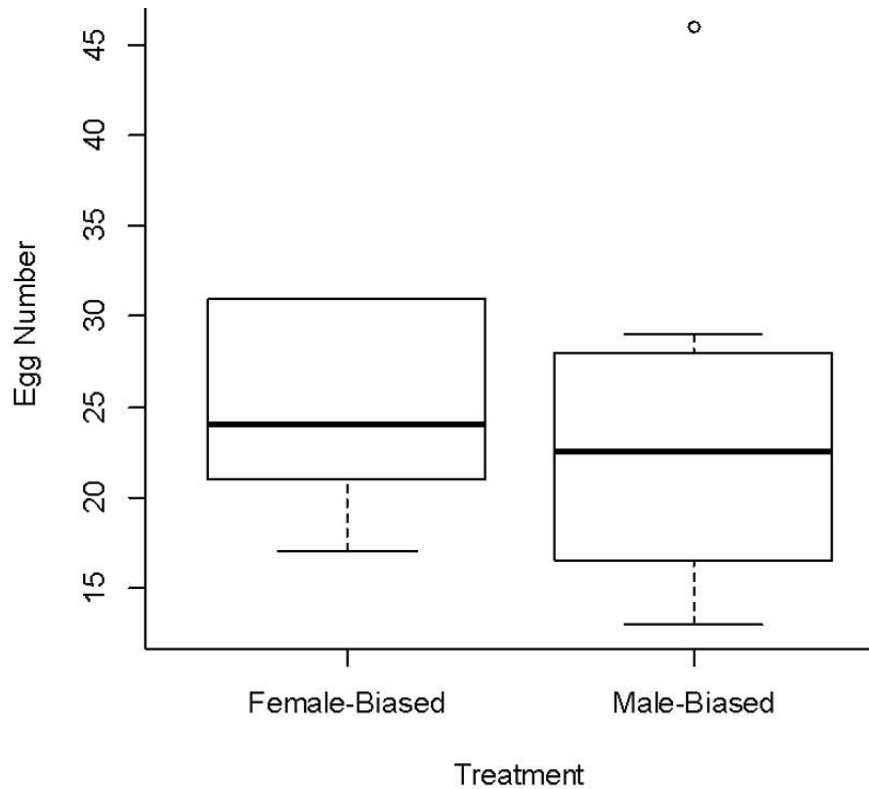


Figure 1.1. Comparison of the number of eggs per initial clutch between female- and male-biased mate availability treatments. The central boxes represent values from the lower to upper quartile (25 to 75 percentile; first and third quantiles), and are intended to give an c. 95% confidence interval for differences in the two datasets. Bold lines represent the median, and extreme values are represented by open circles.

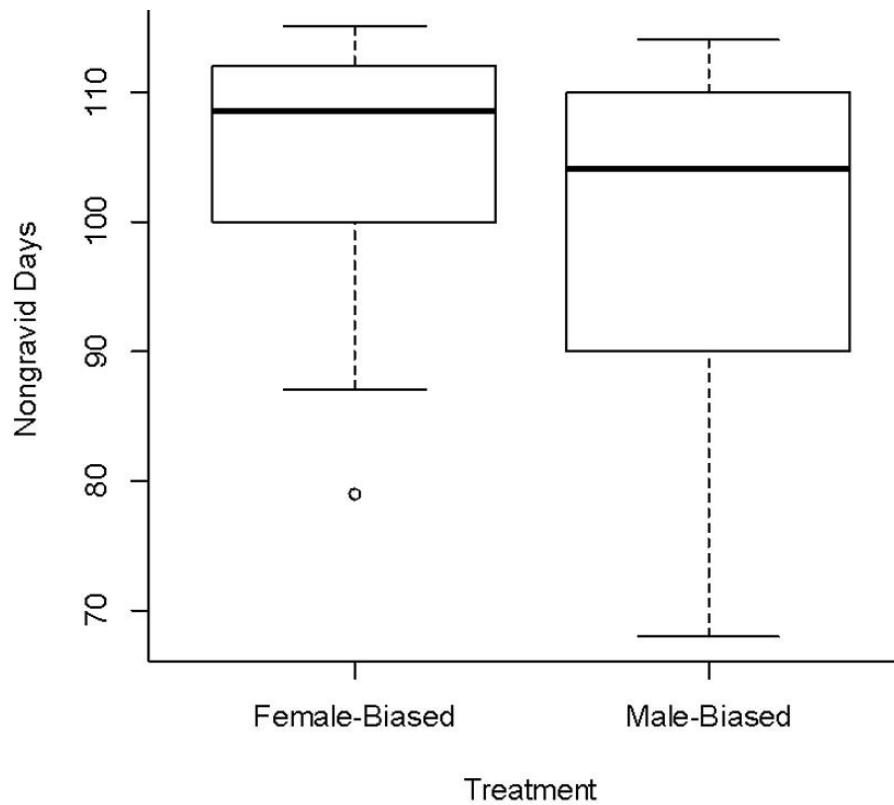


Figure 1.2. Comparison of the number of days spent in a nongravid state between female- and male-biased mate availability treatments. The central boxes represent values from the lower to upper quartile (25 to 75 percentile; first and third quantiles), and are intended to give an c. 95% confidence interval for differences in the two datasets. Bold lines represent the median, and extreme values are represented by open circles.

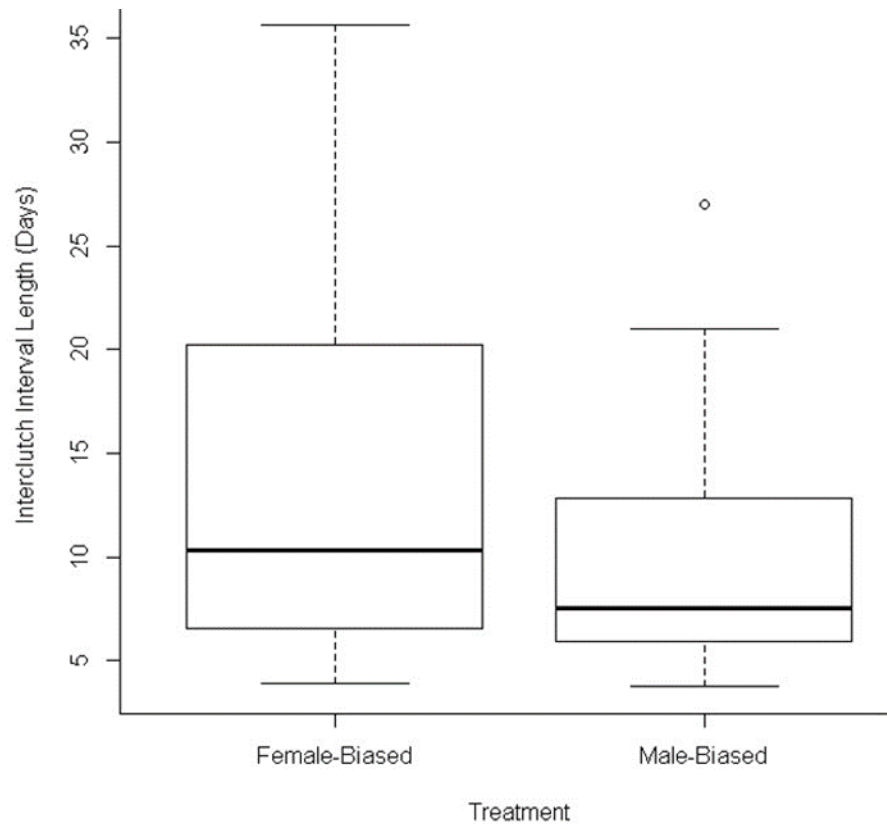


Figure 1.3. Comparison of the interclutch interval length (the number of consecutive days a female is completely nongravid, i.e. neither carrying nor developing a clutch) between female- and male-biased mate availability treatments. The central boxes represent values from the lower to upper quartile (25 to 75 percentile; first and third quantiles), and are intended to give an c. 95% confidence interval for differences in the two datasets. Bold lines represent the median, and extreme values are represented by open circles.

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CHAPTER TWO

Doing the right thing, in the right amount, at the right time: Traits that predict courtship success vary among courtship stages

ABSTRACT

To prepare for reproduction, males can allocate resources into several traits including morphological display, courtship behavior, and external structures built or acquired for mating. However, the value and variation in male traits can depend heavily on how these traits affect mating success and when during courtship traits are assessed by females. Here we show that, although male threespine stickleback (*Gasterosteus aculeatus*) may display a variety of traits, only some of these are directly associated with mating success. Females from this population appear to assess and prefer high male condition only early in courtship, whereas late in courtship, females strongly prefer more vigorous, display-oriented courtship behavior, even more than they prefer the characteristic red nuptial coloration (found to be unrelated to male condition in this population). Our study suggests that the relative importance of several male traits upon which females choose is related not only to whether those traits indicate male quality, but also when during the courtship process traits are assessed.

INTRODUCTION

When females assess potential mates, they often have a variety of traits to consider (Candolin 2003; Hebets and Papaj 2005). When and how to evaluate these traits in courtship is then a challenge, as traits may or may not convey useful information about a mate and may not be accessible at all stages of courtship (Uy and

Safran 2013). Thus, to better understand how male traits evolve, we need to consider their information-signaling utility, as well as when in courtship females may select them.

Exaggerated male traits preferred by females can often result from competition for mates (Kirkpatrick and Ryan 1991; Andersson 1994). These male traits can convey information about a male's quality or condition (Rowe and Houle 1996; Cotton et al. 2004; Tomkins et al. 2004), to which a female attends in order to increase her fitness and that of her offspring (Iwasa et al. 1991; Iwasa and Pomiankowski 1994; Iwasa and Pomiankowski 1999; Houle and Kondrashov 2002). Males of good condition can signal their quality through enlarged, elaborated, and/or elongated versions of traits which females prefer (reviewed in Andersson 1994), whereas males of poor condition may be unable to afford these 'expensive' traits (Pomiankowski 1987; Iwasa et al. 1991; Iwasa and Pomiankowski 1994; Iwasa and Pomiankowski 1999; Cotton et al. 2004). The relative differences in trait expression between high and low-condition males may thus provide females with a direct and reliable indicator of male quality (McLean et al. 2012 and references therein). Although many species display mate preferences for such condition-dependent traits (reviewed in Møller and Alatalo 1999), not all mate choice involves condition-dependent traits, and may instead have mate choice dependent on traits which indirectly signal male quality (reviewed in Andersson 1994).

To produce offspring, males may allocate resources to the expression of several different traits, including morphological display traits to attract females, courtship behaviors to entice them to mate, and structures for mating or raising young. For example, sexually-selected morphological display traits, such as tail length in barn swallows (*Hirundo rustica*) (Møller and Nielsen 1997) or coloration in guppies (Endler

1980), are conspicuous, costly traits thought to signal quality to females (Andersson 1994; Grether 1997; Zuk and Kolluru 1998; as reviewed in Kotiaho 2001). Likewise, courtship behaviors, such as the auditory calls of frogs, lizards, and insects, can function in the location and evaluation of mates (Ryan et al. 1981; Ryan et al. 1982; Kotiaho et al. 1998; Mappes et al. 1996; Hoback and Wagner 1997; Saino et al. 1997; Bailey and Haythornthwaite 1998; Reinhold et al. 1998; Kotiaho 2000; Ophir et al. 2010; Voituren et al. 2012). Finally, males may choose to allocate resources to the acquisition, building, or maintenance of structures used in mating ('extended phenotypes'; Bailey 2012), such as burrows and bowers (Borgia 1985; Kim et al. 2007), or in structures used to care for young, such as nests (Hill et al. 2006; Schaedelin and Taborsky 2006), should a successful mating occur.

Moreover, females may not assess traits simultaneously, but sequentially as courtship proceeds (reviewed in Uy and Safran 2013). Males may need to initially attract a female, and the traits used may differ by the distance at which signals are being assessed (Borgia 1995; Candolin 2003; Hebets and Papaj 2005). For example, conspicuous coloration or calls may be useful to increase a male's detection (Ord and Stamps 2008) and attract females to a male's territory to begin courtship (Tack et al. 2005). However, the traits that function in initial advertisement may differ from the courtship and display traits that convince a female to mate or traits that may signal a male's parental ability post-courtship, such as nest structure (Endler 1992; Bradbury and Vehrencamp 1998). Because females often make mating decisions using male traits, it is useful to assess whether the traits assessed 1) convey different or redundant information about the male (Partan et al. 2005; Uetz et al. 2009; Leonard and Hedrick

2010), 2) are restricted to a particular proximity or stage in courtship at which to convey information for evaluation (Hebets and Papaj 2005), or 3) whether the traits function to gain a female's attention to begin courtship (giving more mating opportunities) versus to communicate quality during courtship. Whether traits are condition-dependent or not, understanding how and at what stage in courtship traits are assessed can provide insight into potential function of male traits in securing matings.

We examined different male traits and their potential utility during courtship in Cranby Lake threespine sticklebacks (*Gasterosteus aculeatus*). Cranby sticklebacks, in contrast to other stickleback populations, are relatively muted in the characteristic red throat coloration (Albert et al. 2007) and are phenotypically highly variable (Robinson 2000). The high amount of variation in male traits and decreased redness in this population is useful in determining the relative importance of various male traits in mating which have been largely ignored compared to red throat coloration. Although Cranby sticklebacks are relatively understudied with respect to female preferences compared to other populations, sticklebacks generally represent a well-known system for the study of sexual selection (Rowland 1988; Bakker and Milinski 1993; Kraak et al. 1999; Candolin 1999; Tinghitella et al. 2013). Because of their well-documented morphology, courtship, and nesting behavior, sticklebacks offer the opportunity to study how females evaluate many different male traits throughout courtship.

Male sticklebacks establish territories on which to build nests, court females, and solely raise young (Wootton 1976). Females from many populations generally prefer larger and redder males, and male color is often, but not always, positively correlated with physical condition (Wootton 1976; Milinski and Bakker 1990; Bakker and Milinski

1993, Bakker and Mundwiler, 1994; Candolin, 1999; Boughman 2007). Although populations vary in how they court (Wootton 1976 and references therein; Ridgway and McPhail, 1987, Foster 1995; Scott and Foster 2007), less is known about courtship in Cranby sticklebacks specifically, and more broadly, about how and when traits in behavior, morphology, and nests are used in stickleback courtship. Evidence suggests that males across populations experience strong sexual selection through female choice, which we will address here, although we acknowledge that other sources of selection may also act on male reproductive traits, including competition with other males or demands of sole parental care (Wootton and Wootton 1984).

To understand which male traits females prefer, and how those preferences may change as courtship progresses, we first confirm the relationship between red throat coloration (the characteristic sexual signal) and male condition in this population. We then ask how other morphological, behavioral, and nesting traits depend on condition and color. Finally, we address which traits are important both in initiating female responsiveness (early courtship) and nest inspections (late courtship). These two stages of courtship serve as early- and late-stage 'checkpoints' through which males must pass on the way to a successful mating. We predict that conspicuous traits (e.g., color, size, and vigorous courtship) should be involved early in courtship to attract females; however, nest characteristics should be important only in late courtship, as nests can be more closely inspected at the stage. Alternatively, traits important early in courtship need not wane in importance as courtship proceeds.

Because males typically possess multiple traits, identifying which traits communicate information about the male, and when during courtship they are

assessed, can provide clues as to their function and relative importance in securing matings.

MATERIALS AND METHODS

Collection of wild fish and laboratory conditions

We collected reproductive sticklebacks (*Gasterosteus aculeatus*) from Cranby Lake, British Columbia at the beginning of the 2012 summer breeding season and transported them to Michigan State University in East Lansing, MI. Once in the laboratory, males and females were placed into separate, visually isolated, 75-gallon (~284 L) holding tanks. Tanks were maintained under summer conditions of 14-hour day lengths at 18°C, and fish were fed defrosted brine shrimp (*Artemia* sp.) and bloodworms (*Chironomus* spp.) once daily throughout the breeding season.

Study design

At two-week intervals throughout the summer 2012 breeding season, 20 males were removed from the male holding tank and placed into individual, 29-gallon tanks and allowed to nest. Each individual tank included a 900-mL tray of sand and 12 g wet-weight *Chara* spp. (nest-building materials), a ceramic pot and plastic plants (for cover), and external, opaque covers (to visually isolate the tanks).

Each male was exposed daily for 10 minutes to a free-swimming, gravid female to encourage nest building. Males were allotted a maximum of 14 days to complete a nest, and males who completed nests (N=85) then underwent courtship trials. No-choice mating trials are standard for measuring mating preferences in sticklebacks (Nagel and Schluter 1998) and allow males and females to interact all the way to spawning. With this method, we measured female preference at various stages in

courtship, as well as the strength of those preferences (Wagner 1998), while excluding male-male competition.

For each courtship trial, a gravid female was released in a nesting male's tank following a 5-minute acclimation period within an opaque holding container. Trials began when the male and female first saw each other and lasted 20 minutes, or until the female entered the male's nest, whichever occurred first. Each female was novel to each male and was removed from a tank at the completion of the trial. All courtship behaviors during the trials were documented using an event recorder (Observer: Noldus Technologies, Wageningen, The Netherlands).

Measuring male traits and their impact on mating success

Morphological traits, such as body size and male nuptial color, are two traits that may be important in mate preferences, as seen in some stickleback species (Head et al. 2013). Thus, we recorded the male's nuptial throat color both directly prior to and immediately following courtship trials using a standardized color index scale developed by our laboratory group (Boughman 2001; Boughman 2007; Lewandowski and Boughman 2008). We then obtained an average color index for the amount of area and intensity of redness for each male. Post-trial, males were immediately weighed (to the nearest 0.01 gram) and photographed in side view using a Canon G-15 digital camera. A second observer placed digital landmarks on the photographs of the males and calculated male centroid size (size) according to established methods (Taylor et al. 2006; Cooper et al. 2011). This observer then calculated the male's condition post-trial as his residual from the regression of male weight on male size.

Male courtship behavior is also comprised of many traits expressed in a complex, conspicuous courtship sequence. Initially, males attract a female via either display (zig-zag 'dancing') or aggressive (chasing, dorsal-pricking, and biting) courtship behaviors. Then males lead females back to the nest (lead), whereupon the male shows the female the nest opening (show). Females may then examine, enter, and deposit eggs within the nest of a preferred male; however, females may terminate courtship with non-preferred males at various points during the courtship sequence (for further descriptions of these behaviors, see Rowland 1994).

To quantify male behavior, we measured the presence and duration of the above courtship behaviors. To correct for trial duration, per-minute rates for all measured behaviors were calculated by dividing by the duration of the trial. We then calculated summary indices to describe a male's overall latency to court, nature of courtship, and courtship vigor. We calculated latency to court as the difference between the start of the trial and the first behavior conducted. We summarized the nature of courtship by first categorizing courtship behaviors as either display-oriented (zig-zags, leads) or aggressive (bites, chases, and dorsal-pricks; as in Kozak et al. 2009). The nature of courtship, an inverse relationship between aggressive and display courtship, was then calculated as proportion of courtship which was display-oriented in nature, where 0 would indicate all courtship was aggressive and 1 all display. To estimate courtship vigor, we summed all courtship behaviors conducted by a male, following Kozak et al. (2009).

Male sticklebacks build and maintain nests in which young are raised; the nest not only serves as a structure conducive to oxygenating embryos, but potentially also an

assessment criterion by females. These ‘tunnel-like’ nests, comprised of surrounding vegetation and sand bound together by a glue (spiggin) secreted by the male, are the structures through which females pass to deposit eggs to be fertilized and later solely reared by the male (for more detail, see Rowland 1994; Wootton 1976). Because of the clear importance of nests in successful fertilization and raising of young, we recorded several nest characteristics that indicate the time and resources invested in constructing and maintaining nests. Nest measurements included the number of days from beginning to completion of nests (“days until nest”; as in Barber et al. 2001) and nesting behaviors (fanning, creeping through, boring) conducted while within one body length of the nest during the courtship trial (as in McKinnon et al. 2012). We recorded nesting behavior within courtship trials as the number of visits a male made to his nest (nesting bouts). Because nests are variable, require energy (both in producing spiggin and gathering materials to construct nests), and have been suggested as indicators of male quality (Rushbrook et al. 2008), we also measured nest weights. To obtain weights, nesting trays were removed and dried, whereupon dry nests were separated from nesting trays and weighed to the nearest 0.01 gram. A second observer who was blind to male identity took nest weight measurements.

Statistical analysis

We considered how male redness and condition are related both to one another and to three categories of potential resource allocation (morphological display traits, courtship behavior, nests and nesting behavior). Significant factors from these models were then combined with evidence from the literature to develop an overall model of

male traits that predict success at different stages of courtship with a female throughout the breeding season.

Because a male's success in courtship depends both on a female's willingness to mate and on ultimately carrying out mating behaviors, we used rates of responsiveness (female receptiveness to mating; the number of times a female followed a male when he led her to the nest; 'early courtship'), and inspection (female preference for this male; the number of times a male showed a female the nest that resulted in a female examining the nest; 'late courtship') to indicate mating success following Kozak et al. (2009). Because females are known under certain conditions to relax choosiness late in the breeding season (Tinghitella et al. 2013), all models also included a time variable (biweekly period in which the courtship trial occurred); we refer to this variable as 'seasonal time'.

Analyses of linear models were conducted in R (R Core Team, 2013, version 3.0.2) using the packages car (Fox and Weisberg 2010), AER (Kleiber and Zeileis 2008) and rsm (Lenth 2009). We visually inspected plots to detect deviations from homoscedascity and normality, and multicollinearity of variables was investigated through Variance Inflation Factor (VIF) of each model. A VIF above 4 was used as the threshold reference value; no tests indicated a VIF above these levels, therefore the redundancies between predictor variables were judged as acceptably low enough to justify their inclusion as separate predictor variables. Models included both predictor variables and their interactions, and models were reduced stepwise by AIC to provide the best model. Note that the count variable of "days until nest" was first analyzed using a generalized linear model with a Poisson link; however, similar results were achieved

with a better model fit (as determined by AIC) using a linear model with Gaussian distribution. To avoid over-fitting the model, select two-way interaction terms were added to the models based on hypothesized relationships between main effects.

Cook's distance was also used for each model to determine if any outliers were disproportionately affecting estimates. No more than two outliers were ever found in any model, and running the models with and without the outliers did not change the significance of effects, so all data points were included in our analyses. Finally, to visualize interactions, graphs were generated following the methods described in Aiken and West (1991, p. 12). In short, to illustrate significant interaction effects, separate regression lines are computed and plotted on an X-Y graph by dividing individuals into two groups; one 1.5 SD above and one 1.5 SD below the mean for a given predictor variable Z.

RESULTS

Is red throat coloration related to male condition?

Stickleback males of many populations use condition-dependent red nuptial coloration to attract mates. However, redness in this population from Cranby Lake was not predicted by male condition nor size, but only by seasonal time (slope $\pm$ SE = 0.46 $\pm$ 0.136, $t_{3,80} = -3.37$, $p = 0.0012$, $R^2 = 0.12$). Specifically, males were redder earlier in the season.

How do other male traits relate to a male's condition and throat coloration?

Courtship behavior

Males varying in color and condition do not differ in courtship vigor, but do court females differently (nature of courtship and latency to court). Both color and condition

affect the latency to court (Table 1.1), and these variables interact in their effects (Figure 2.1). More colorful males are faster to court when larger, but dull males are faster to court when smaller. Males in better condition perform more display-oriented courtship (Table 1.1). Neither seasonal time, male size, male condition, male color, nor interactions between these variables were found to influence how vigorously males court females (all $t_{6,77} < -1.17$, $p > 0.24$, $N=85$).

Nests and nesting behavior

High-condition males have heavier nests (17.4 ± 6.57 , $t_{6,74} = 2.65$, $p = 0.0097$, $R^2 = 0.077$, $N=85$), and the interaction between condition and color also significantly predicted nest weight (6.7 ± 2.33 , $t_{6,74} = 2.88$, $p = 0.0052$, $R^2 = 0.090$, $N=85$; Figure 2.2). Neither seasonal time, male size, male condition, male color, nor their interactions predicted days until nest (All $t_{6,73} < -1.37$, $p > 0.17$, $N=85$) or number of nest bouts during courtship (All $t_{6,77} < -1.66$, $p > 0.10$, $N=85$).

Which traits predict courtship success?

For models of female responsiveness (early courtship) and inspection rate (late courtship), the nature of courtship and courtship vigor appear to be the strongest factors predicting success with females, and females appear to be evaluating males on many characteristics early in courtship, but only a few factors as courtship proceeds (vigor and the nature of courtship; see Table 1.2 and Figure 2.3).

DISCUSSION

Male mating traits

Our results suggest that several traits are involved in mate selection, but not all preferred traits depend on condition. Both the nature of courtship and vigor were

preferred in mating. Yet condition was found to predict the nature of a male's courtship, not how vigorously he courted. Specifically, males in better condition were not more vigorous, but did perform the preferred display-oriented courtship behaviors more often. Display-oriented courtship may therefore provide females with some information on the male's condition or quality, and females indeed most strongly prefer this trait throughout courtship, consistent with a direct benefits model of sexual selection.

Contrary to many populations, male Cranby sticklebacks do not have condition-dependent red throats, nor do females have a preference for redness in courtship. In general, Cranby sticklebacks are not as red (Albert et al. 2007; Boughman 2007), likely due to a combination of both diet (Schluter and McPhail 1992; Frischknecht 1993) and unfavorable light conditions for red expression in these lakes (Albert et al. 2007). Because the water in Cranby Lake is more red-shifted, red mating signals do not contrast well against the water and are less visible because females have low perceptual sensitivity to red (Boughman 2001). Thus, the expression of red coloration may not be as effective in ensuring mating success. This aligns with predictions of both condition-dependent models, where an indicator trait should be both informative and preferred by females to evolve, and also sensory drive, which suggests biased sensory perception drives male trait evolution (reviewed in Andersson 1994). If the trait is not easily detected, both models are applicable and suggest the trait should not be used in mate selection. Furthermore, if primarily female choice acts to maintain the relationship between redness and condition, then the lack of relationship here may help to explain the variability of color preferences across stickleback populations (Boughman 2001) and their reliability as a condition-dependent trait (Wootton 1976; Milinski and Bakker 1990;

Bakker and Milinski 1993, Bakker and Mundwiler 1994; Boughman 2007; Candolin 1999).

Other signals, such as courtship behaviors, may be more reliable indicators of quality. Females should use these quality-indicative traits to assess males for the fitness-increasing benefits (direct or indirect) they provide to their offspring. Here, females prefer vigor and display-oriented courtship. The reliance on behavior may also be due to the fact that behaviors should convey information about a male's present condition, rather than the past condition reflected in more fixed traits, such as size (Sullivan 1994). Even where behavior does not directly represent male quality, behaviors likely also encode some information about the male (such as age or courtship experience; Weigel, Chapter 4) or enhance the detection of his other signals (Hailman 1977; Smith 1977; Endler 1992; Rowland et al. 1995), which helps to explain why mating success based on behavior is so common across species (Gibson and Bradbury 1985; Andersson 1991). More broadly, as suggested by Candolin (2003), and supported in a variety of species (Zuk et al., 1990; Houde 1997; Møller et al. 1998), this reliance on multiple traits could mean the selection of higher-quality males in female mate choice (Møller and Pomiankowski 1993).

Several other morphological, behavioral, and nesting traits do not reflect a male's condition or offer a direct advantage in mating (see Table 2). Although some extended phenotypes, such as nests, have been suggested to signal information useful in mate assessment (Barber et al. 2001), other selective pressures, such as those from parental care or survival, may be more responsible for the variation seen in these traits. Future studies are needed to clearly establish whether extended phenotypes reflect condition

and whether they come at a cost to, or enhance other aspects of, courtship, survival, and parental care.

Stages of courtship

Our results suggest that some traits may function early in courtship to initially attract a female, but other traits are more critical at later stages of courtship and potentially in raising young. Females responded to only a few traits and grew more discriminating as courtship proceeded. Early in courtship, females responded to condition, the nature of courtship, and vigor (plus interactions), but late in courtship they only responded strongly to the nature of courtship and vigor. Because these behaviors are likely assessed at the same distance from the male and for similar duration, assessment based on these different aspects of courtship behavior suggests redundancy in these signals (Partan et al. 2005; Uetz et al. 2009; Leonard and Hedrick 2010) rather than differing propagation properties of signals in our system (Hebets and Papaj 2005). However, neither explanation rules out the possibility that the information garnered from early signals could simply have been used and no longer aid in further narrowing the pool of potential mates, or that females may also need more time to evaluate behavior than is required for other traits, such as size (Sullivan 1994). Two consequences of sequentially evaluating traits could be either to switch traits as more useful information becomes available (perhaps at a closer range or because the male starts a new type of signal), or to appear more discriminating if signals lose their information-providing utility as courtship proceeds.

Our work is consistent with findings in several species where early, typically long-range courtship is governed by different traits than is later, short-range courtship. These

studies include dark-eyed juncos and field crickets (*Gryllus lineaticeps*), where many male traits are assessed differently during long- and short-range courtship (reviewed in Reichard et al. 2011), and squirrel tree frogs and house crickets, whose courtship relies on acoustic signals early, but later switches to visual signals (Taylor et al. 2007; Stoffer and Walker 2012). As suggested by Uy and Safran (2013), the sensory environment is likely to couple the signal's function in courtship with the stage in courtship and range at which it can be assessed; our results add that the duration of expression (particularly important for behavior) may play a role in signal use.

Many male traits may not be preferred by females directly, but lead to more mating opportunities because of their function in male competition. For example, quickly beginning to court may help males gain access to females when in the presence of rivals (i.e. in the face of male competition), which is typical in the wild but not captured in the no-choice design of our study (Rowland 1988). If males must compete for a female's attention, quickly initiating courtship to avoid interruption can be a mechanism by which courtship success is achieved. Because male competition can change how females select mates (Trail, 1985; Zimmerer and Kallman 1988; Greenfield 1994; Morris et al. 1995; Galeotti et al. 1997; Petersson et al., 1999), future studies should consider the role of courtship latency in gaining courtship opportunities under male-male competition.

Our study demonstrates that females assess many male traits to differing degrees as courtship progresses. Behavior, not the red throat coloration characteristic of many stickleback populations, appears to be the strongest target of selection by females throughout the courtship process. By studying many male traits and the stages

in courtship at which they may be assessed, we can gain insight into the functional importance of traits.

APPENDIX

APPENDIX

Table 1.1. Significant predictors of two metrics of courtship behavior from multiple regression models ($df=6,77$).

	<i>Slope $\pm$ SE</i>	<i>T</i>	<i>p</i>	<i>Partial R²</i>
<i>Latency to Court</i>				
Condition	5.18 $\pm$ 1.110	4.66	0.000013	0.19
Color	1.78 $\pm$ 0.880	2.04	0.045	0.036
Color: Size	0.028 $\pm$ 0.0128	-2.19	0.031	0.042
<i>Nature of Courtship</i>				
Condition	-0.013 $\pm$ 0.00570	-2.22	0.030	0.059

Table 1.2. Male traits that predict female responsiveness and inspection rate. Multiple regression models with estimates and SE scaled by 100 ($df=68$). Significant findings are bolded.

		Responsiveness (Early in Courtship)				Inspection Rate (Late in Courtship)			
		<i>Slope ± SE</i>	<i>t</i>	<i>p</i>	<i>Partial R²</i>	<i>Slope ± SE</i>	<i>t</i>	<i>p</i>	<i>Partial R²</i>
Morphology									
	Size	0.08 ± 0.043	1.8	0.080	0.013	0.041 ± 0.047	0.9	0.38	0.0029
	Condition	7.2 ± 2.30	3.1	0.0028	0.039	4.80 ± 2.49	1.9	0.058	0.014
	Color	2.4 ± 1.27	1.7	0.097	0.011	2.07 ± 1.37	1.5	0.14	0.0086
Courtship Behavior									
	Vigor	37.4 ± 13.50	2.7	0.0075	0.031	33.68 ± 14.70	2.3	0.025	0.02
	Latency to Court	-0.002 ± 0.0015	-1.5	0.150	0.009	0.00034 ± 0.0016	0.2	0.83	0.00018
	Nature of Courtship	8330 ± 1060	7.8	<0.001	0.250	7430 ± 1150	6.4	<0.001	0.16
Nest Characteristics									
	Nest weight	-0.04 ± 0.026	-1.4	0.170	0.008	-0.016 ± 0.029	-0.6	0.58	0.0012
	# Nest Bouts	-0.55 ± 0.93	-0.6	0.560	0.0014	-0.270 ± 1.01	-0.3	0.78	0.00027
Interactions									
	Color:Size	-0.03 ± 0.018	-1.7	0.089	0.012	-0.03 ± 0.02	-1.5	0.14	0.0087
	Nature of Courtship:Vigor	-35820 ± 18300	-2.0	0.054	0.016	-2215 ± 19700	-0.11	0.91	0.000048
	Vigor:Condition	-87 ± 35.9	-2.4	0.018	0.024	-72.24 ± 38.80	-1.9	0.067	0.013
Seasonal Time		0.15 ± 0.19	0.76	0.45	0.002	0.20 ± 0.21	0.95	0.35	0.00

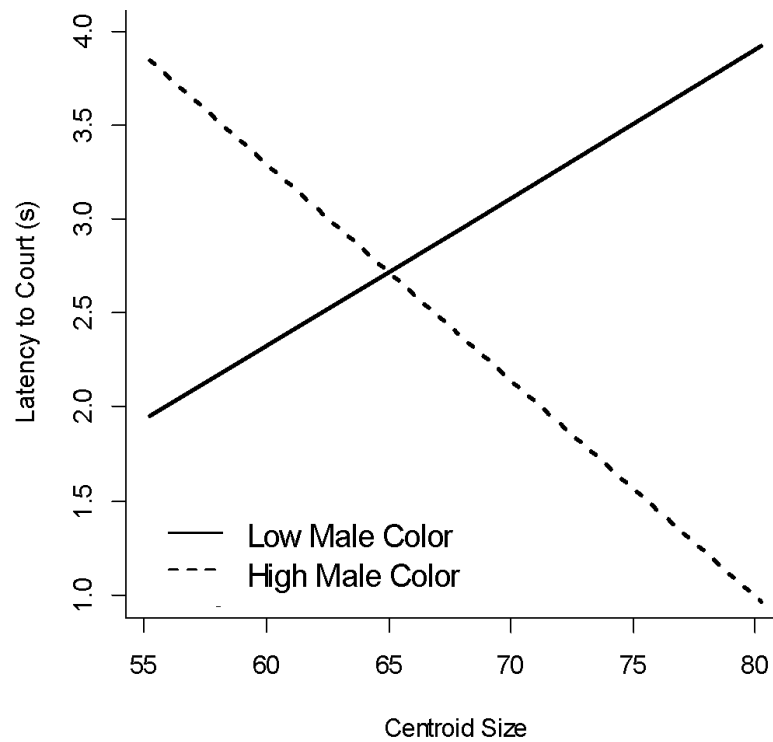


Figure 2.1. More colorful males are faster to court when larger, but dull males are faster to court when smaller. Regression lines for male color represent categories of high and low coloration at 1.5 standard deviations above and below the mean (respectively).

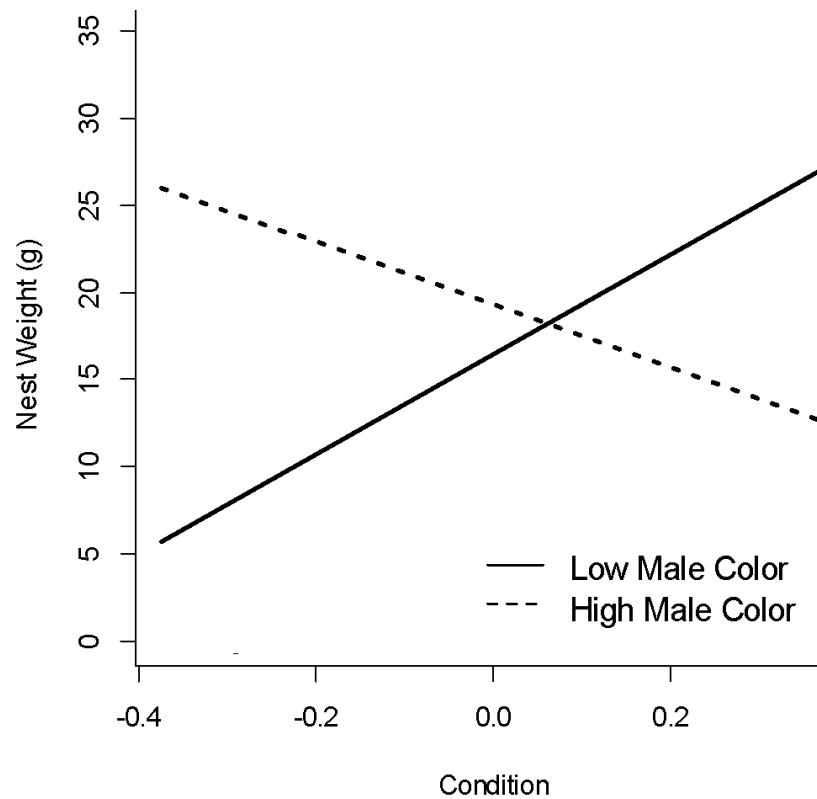


Figure 2.2. As condition improves, low color males have heavier nests, whereas high color males have lighter nests. Regression lines for male color represent categories of high and low coloration at 1.5 standard deviations above and below the mean (respectively).

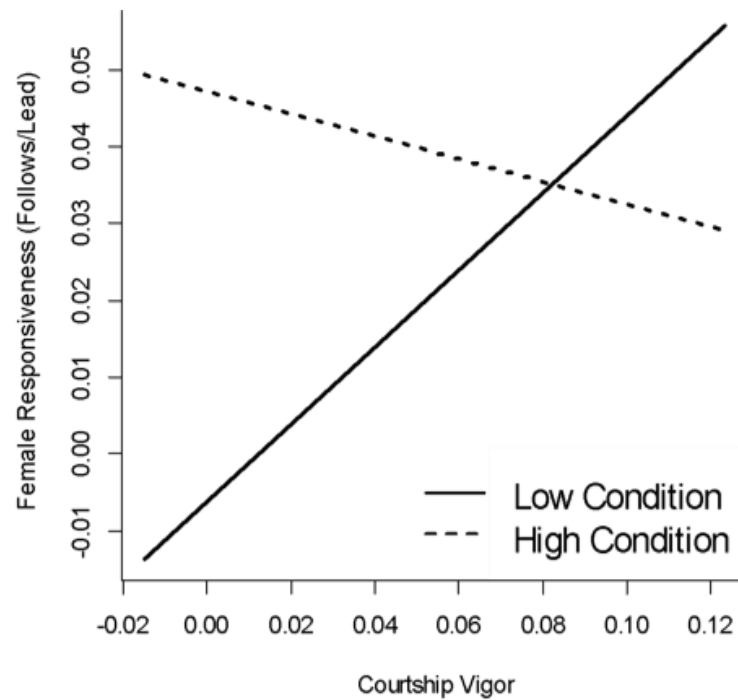


Figure 2.3. Females respond differently to males based on interactions between male condition and vigor: Increased vigor leads to more responsiveness (follows/lead) for low-condition males, but less responsiveness for high-condition males. Regression lines for male condition represent categories of high and low condition at 1.5 standard deviations above and below the mean (respectively).

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CHAPTER THREE

Short term shifts in population density influence male reproductive strategies

ABSTRACT

Population demography, although not a constant feature of populations, can change the traits favored by sexual selection. Using a captive population of threespine sticklebacks, we experimentally manipulated the male density (and thereby competition perceived by males), and examined the effects on male nesting probability, mating traits, and mating success. Males from low density were more colorful and built larger nests, whereas males from high density were less conspicuously colored and built smaller nests. Although there was no main effect of density on mating success, density interacted with vigor to favor vigorous males from low density. Given that these changes resulted from short, two-week long density treatments, this work clarifies how rapid changes in demography can affect the courtship and nesting strategies adopted by males, and how those strategies are shaped by female mate choice.

INTRODUCTION

Population demography is important to how populations function and can alter both natural and sexual selection, yet the demography of any given species can vary widely over time and space. For example, the number of available mates, competitors, and their densities are all factors that can change within an organism's lifetime and impact fitness. Individuals who respond appropriately to the demographic conditions of their social environment may have a fitness advantage.

In particular, the ability to perceive and respond to changes in density can aid individuals in competing for resources, including mates (Emlen and Oring 1977; Kokko

and Rankin 2006; Kaiser et al. 2013; Clutton-Brock and Huchard 2013; Holveck et al. 2015). Density-induced competition can alter individual fitness (e.g., Grant 1997; Rodenhouse et al. 1997; Sutherland and Norris 2002; reviewed in Candolin and Wong 2012). However, responses can also manifest in the physical and behavioral traits individuals display (e.g. Møller et al. 2006; Candolin and Selin 2012; Guyer et al. 2012; Sprenger et al. 2011; Niemela et al. 2012) and are likely to be important for survival and reproduction, particularly if density affects not just mate availability (i.e., sex ratio), but also other resources used in mating, such as territory.

Because males and females experience differing evolutionary pressures, responses to altered densities may differ by sex. Females may, for example, relax their mating requirements (Tinghitella et al. 2013) or change their mate search strategies (Lehmann 2007) when opportunities to mate are rare. Males, in contrast, may change both how they compete with other males and how they attract females (reviewed in Moura and Peixoto 2013).

Males may use a variety of traits to compete with rivals and attract females. Conspicuous and often costly morphological display traits are used to attract females (Møller and Nielsen 1997; Endler 1980). Courtship behaviors, such as mating calls, are also frequently used both to attract females and during courtship itself, as are locomotor displays (Bradbury and Vehrencamp 2011). In some taxa, males may also build structures as such as burrows and bowers for mating (Borgia 1985; Kim et al. 2007) or nests used to care for young (reviewed in Schaedelin and Taborsky 2009). These are considered extended phenotypes, which are external to the animal but can relay information about their builder that is useful during courtship (Dawkins 1999; Bailey

2012). As such, these extended phenotypes can impact courtship, parental care, or both.

Males may alter reproductive traits in response to changes in male density, which can reflect both altered operational sex ratio and general population density. Density affects the level of competition, and thus, males may perceive more intense competition at high density than at low density. Because male density may reflect the perceived intensity of competition for mates, if males respond to perceived competition, then we might expect to see changes in expression of traits which would enhance a male's competitiveness. Morphologically and behaviorally, we might expect males to increase their display intensity to attract females or defeat rivals, or conversely, reduce their display to decrease negative male-male aggression (Bertin and Cezilly 2005). Similarly, the use and size of extended phenotypes (bowers or nests) could either be altered to increase success with females or simply to maintain the nest or bower if males compete by destroying their opponents' structures (Bolton et al. 2013). Because several traits may be affected by density, examining how male traits may change together, rather than in isolation, elucidates how combinations of traits, trait means, and trait variation may respond to density at a population-level. Ultimately, selection on these male traits, both through mate selection and male competition, might be altered by the density of competitors present (Kokko and Rankin 2006; Shuster and Wade 2003, p. 174).

Thus far, studies that have addressed density-induced changes in traits have considered the impact of density directly. That is, the impact of density on traits is measured concurrent with density experience. However, a male may perceive density at one time-point and exhibit a delayed response at a later time-point. Past experiences

can influence later behavior, as in the case of pre-mating density experience and future mate guarding behavior (Oku 2009), but it remains to be seen how changes in traits displayed before, during, and after mating are affected by perceived density.

Investigating the perception of density and delayed or lingering responses, rather than perception and responses concurrently, can help to identify when mismatches occur between the environment and an organism's current phenotype, which was formed in response to prior experience. This is particularly important and timely, as rapid environmental changes are increasing the frequency with which demography is disturbed (reviewed in Candolin and Wong 2012), increasing the frequency at which phenotypic mismatches might occur.

Here we examine how male traits in a population of threespine stickleback fish (*Gasterosteus aculeatus*) change in response to male density; we experimentally modify demography to manipulate the perceived level of mate competition. Threespine stickleback males are under strong sexual selection and produce a distinctive nuptial coloration, engage in competitive and courting behaviors, and build nests for raising young (Wootton 1976). Although these traits are common among sticklebacks, variation exists both between and among populations in morphology, particularly redness (Boughman 2001), behavior (reviewed in Ward and McLennan 2008), and nests (Wootton 1976). Additionally, population density is known to vary both temporally and spatially (Tinghitella et al. 2013; Tinghitella, Head and Boughman, unpublished data), and males are known to compete strongly for territory and mating opportunities (Lackey and Boughman 2013a). The temporal and spatial variation in levels of competition suggest that selection might strongly favor responsiveness to changes in density. We

posit that a male's traits will be modified in response to density experienced with potential effects on male reproductive success in attracting females.

To test our ideas, we investigate the potential induced differences in suites of morphological-, behavioral-, and extended-phenotypic traits in response to a temporary shift in male density, and whether those differences alter mating success. To set the stage, we first address the question of which morphological, behavioral, and extended-phenotypic traits predict which males build nests, and whether this varies across the breeding season or with perceived mate competition. We then ask which traits predict mating success, and whether the density experienced alters the suite of traits that do so.

MATERIALS AND METHODS

Collection of wild fish and laboratory conditions

At the beginning of the 2013 breeding season, we collected reproductive sticklebacks (*Gasterosteus aculeatus*) from Cranby Lake, British Columbia (49.7 N, -124.5 W). We then transported the collected fish to Michigan State University in East Lansing, MI, whereupon males and females were placed into separate, visually-isolated, 75-gallon holding tanks. The fish acclimated to laboratory conditions for 48 hours before assignment to treatment tanks. We maintained all tanks under conditions of 14 hours of simulated daylight and 10 hours of darkness at 18°C and fed the fish defrosted brine shrimp (*Artemia* sp.) and bloodworms (*Chironomus* spp.) once daily.

Study design

Establishing density treatments

We removed individual males from the 75-gallon holding tank and randomly assigned them to a treatment tank at either 'high' density (1 fish/gal, or ~ 41 fish/m²) or 'low' density (~ 0.5 fish/gal, ~ 20 fish/m²). These treatments fall within the ranges of density found in wild stickleback populations (Gislason et al. 1998; Wootton and Smith 2000), although densities are known to fluctuate strongly within and between years (Wootton and Smith 2000). Males in the two treatments did not initially differ in size, condition, or coloration (all $t < 0.024$, all $p > 0.36$). Males were not provided nest building materials while in treatment tanks, but they were free to interact, and they exhibited male-male competition behaviors, including territoriality, chasing, tussling, and biting. After a 2-week exposure to their density treatment, we then placed individual males into 29-gallon tanks in which to build nests for courtship trials ($N = 238$; with $N_{\text{High}} = 114$, $N_{\text{Low}} = 124$).

Enticing males to build nests

Each male's 29-gallon tank contained nest building materials (a 900-mL tray of sand and 12 g wet-weight of the aquatic plant *Chara* spp.) and a ceramic pot and plastic plants for cover. We used removable opaque white covers to visually isolate male tanks from one another, and we encouraged nest building by enticing males daily with a free-swimming, gravid female for 10 minutes. We recorded male courtship and nesting behavior following the introduction of the female and visually surveyed tanks for signs of nest building (holes in the sand, use of plant material, signs of glue, and sand removed from nests and tray box). Once we identified a nest as complete, either because the

male swam through the nest or the nest had a visible opening, males underwent a courtship trial the following day.

Conducting courtship trials

Courtship in sticklebacks is complex and comprised of many steps. Males engage in display (zig-zag 'dancing') or aggressive behaviors (chasing, dorsal-pricking, and biting) to attract females, and then males lead females back to the nest. Once a male shows a female his nest, she may examine it, whereupon she may either enter the nest or abandon the male (for further descriptions of these behaviors, see Rowland 1994). Because females may choose to terminate courtship with a male at any step during this process, males that are considered preferable by females should proceed further in courtship, and some males may receive no response from the female at all.

In this study, males that had built nests ($N=121$ with $N_{\text{High}}=52$, $N_{\text{Low}}=62$) were used in no-choice courtship trials within each nesting male's 29-gallon tank. No-choice mating trials are standard for measuring mating preferences in sticklebacks (Nagel and Schluter 1998) and allow for the measurement of female preference, while excluding male-male competition. Each male was used only once, and females were always unfamiliar to males to ensure that familiarity did not alter female preferences. A single observer (EGW) scored all mating trials.

In each courtship trial, we allowed the gravid female to acclimate within an opaque holding container for 5 minutes before being released into a male's tank to freely interact with the male. Trials began when the male and female first saw each other and lasted 20 minutes, or until the female entered the male's nest, whichever

occurred first. We used an event recorder (Observer 2007: Noldus Technologies, Wageningen, The Netherlands) to document all courtship behaviors that occurred.

We recorded the number, time of occurrence, and duration of each courtship behavior, correcting for trial duration by using per-minute rates, calculated by dividing counts by the duration of the trial. We then characterized male courtship style by calculating summary indices to describe a male's overall latency to court, nature of courtship, and courtship vigor. We calculated latency to court as the difference between the start of the trial and the first courtship behavior conducted. To calculate the nature of courtship, we first categorized behaviors as either display-oriented (zigzags, leads) or aggressive (bites, chases, and dorsal-pricks; as in Kozak et al. 2009). We then calculated the nature of courtship as the proportion of courtship that was display-oriented in nature, where 0 indicated all courtship was aggressive and 1 was all display. Finally, we summed all courtship behaviors conducted by a male and again corrected for trial duration to estimate courtship vigor (Kozak et al. 2009).

Our measures of female choice included rates of female approach, response (a measure of motivation to mate; calculated as the number of times a female followed a male per times he led her to the nest), and nest inspection (the number of times a female inspected a male's nest per times he showed her the nest). As an overall measurement to describe a male's success in courtship, we also used preference score, which measures both how attractive a male is to a female and how far courtship progresses (on a scale from 0-4, from 0 = no response to 4 = attempted spawning; Kozak and Boughman 2009; Kozak et al. 2009).

Gathering male phenotypic data

Morphological traits, such as body size and male nuptial color are important in mate preferences of some stickleback populations and species (Nagel and Schluter 1998; Boughman 2001; Boughman et al. 2005). Therefore each individual male's traits were examined at several time-points in the experiment: directly after the male was moved from the density treatment to the nesting tank (after density exposure), once a male had completed his nest (one day before the male's courtship trial), and following the courtship trial. We photographed and recorded the nesting male's nuptial throat color using a standardized color scoring method (Boughman 2001; Boughman 2007; Lewandowski and Boughman 2008) that closely matches reflectance data (Albert et al. 2007; Boughman, 2007). We also collected eye color, body color, and darkness following established methods (Lewandowski and Boughman 2008; Lackey and Boughman 2013b), as well as each male's weight (to the nearest 0.01 gram) and length (to the nearest 0.01 mm). To determine male condition, we calculated the residuals of a regression of male weight on length.

Generating nest measurements

Male sticklebacks build and maintain tunnel-like nests in which females deposit eggs and young are raised. In addition to providing a structure conducive to oxygenating and protecting embryos, nests may also serve as an assessment criterion by females. Thus, in addition to recording the time in days that it took males to begin building and to complete nests (Barber et al. 2001), we also recorded the frequency and duration of nesting behavior during courtship trials, including fanning, creeping through,

and boring, conducted while within one body length of the nest (McKinnon et al. 2012). Each visit to a nest denoted a nesting bout.

Following the completion of courtship trials, nests were carefully removed from tanks to dry, weighed to the nearest 0.01 g, and photographed from above at a standard height and with a ruler. Photos were later analyzed blindly with regard to treatment conditions using IMAGEJ (Abràmoff et al. 2004) to extract both the overall and exposed nest area and perimeter. Overall nest area and perimeter reflect the area and outline of the entire nest, whereas exposed area and perimeter reflect only the portion of the nest not covered by sand.

Statistical analysis

We considered four general categories in which males can experience changes in response density: morphological and color traits, nesting behavior, courtship behavior, and mating success. Within each category, we visually inspected each variable to detect deviations from homoscedascity, normality, and homogeneity of covariance. Variables were standardized by calculating z-scores for use in statistical tests. We also included the predictor variable of seasonal time, divided into months during the breeding season, which was incorporated in the models to account for known seasonal changes in both female choosiness (Tinghitella et al. 2013) and male coloration (Weigel, Chapter 2; Candolin 2000a).

All analyses were conducted in R (version 3.0.2) using the packages car (Fox and Weisberg 2010), lmSupport (Curtin et al. 2014) and multcomp (Hothorn et al. 2008). To investigate whether nesting rates are affected by density, we used linear models to determine the variables (and their interactions) that most influence which males build

nests. The model included density, time of season, male throat color, eye color intensity, body color intensity, body darkness, condition, and length as predictors.

We next conducted multivariate analyses to investigate whether density affects the morphology, behavior, nests, or mating success of nesting males. Significant MANCOVA models were then followed with univariate models examining each predictor, with significance determined using Bonferroni corrections for the number of predictors tested in the MANCOVA (Wright 1992). Significant results were examined individually post hoc using Tukey adjustments on all pairwise comparisons. In an effort to maximize power and find the best model, we reduced models stepwise using AIC to include only variables and interactions hypothesized to be of biological importance. Finally, we used Levene's test to determine whether trait variation differed between the two density treatments for any of these traits, and the significant results are reported below.

When considering male morphological traits in aggregate, we chose two models: The first model, "Initial Response," addresses the initial morphological changes that might have occurred directly after density exposure but before nesting. The Initial Response model uses the measurements taken directly after the male was removed from the density treatment. The second model, "Changed Response," addresses how morphological factors might further change after males have nested and courted. The Changed Response model examines the change in each variable that occurs between measurements made directly after density exposure (but prior to nesting) and the final measurements taken after courtship trials. In both morphology models, our predictors were seasonal time and density, and response variables were male condition, length,

throat color index, eye intensity, body color, and body darkness. Models for male behavior and nesting also used the predictors of seasonal time and male density and added the morphological response variables above as predictors. The response variables for the behavior model included the nature of courtship, latency to court, and courtship vigor. The response variables for the nest model of days until nest completion (pre-building lag (in days) + construction days), nest weight, nest perimeter, nest area, exposed nest area, and exposed nest perimeter.

To determine whether the density males experienced affected their mating success, we ran two models: a MANCOVA model and a generalized linear model. In both models, we considered a male's success in courtship as predicted by density treatment, but also including male traits which differ between treatments and/or traits widely supported in the literature as factors in female choice. The MANCOVA model used response variables of rates of female approach, response, and nest inspection. The predictors were male throat color index, body color, body darkness, body length, condition, vigor, the nature of courtship, nest area, days to construct a nest, seasonal time, and density treatment, as well as 2-way interactions with density (reduced stepwise by AIC to only the interactions of density with vigor, nature of courtship, and body length). The second linear model included only the preference score, a measure that includes eventual spawning, as a response to these predictors.

RESULTS

Which males nest?

Approximately half of the males introduced to individual tanks completed nests, and males are most likely to build nests at midseason ($t_{2,235} = 4.56$, $p < 0.001$). Nesting is

best predicted by intense coloration (Table 2.1), not male condition, length, or density experienced (all $t_{1,233} < 1.38$, $p > 0.17$). Note that males with redder throats were more likely to nest, whether the intensity, area, or brightness of red coloration increased (see Table 2.1), thus throat color is hereafter collapsed to a single variable, color index (color area + intensity; as in Boughman, 2001; Boughman 2007; Lewandowski and Boughman 2008), but models using all throat color variables individually and in combination yielded similar results.

Do male trait values differ between nesting males who experienced high versus low density?

We established which males nest, and then used MANCOVA models to ask whether nesting male traits differed by density. As females could only select among those males who build nests, density-induced differences in trait values and variation should be important to the eventual outcomes of female choice. Here we examined how density affected three broad trait categories: male morphology, behavior, and nest construction.

Morphology

Within the “Initial Response” model, seasonal time and density treatment significantly predicted changes in male morphology (Pillai’s Trace, Seasonal Time: $F_{12,700} = 2.72$, $p = 0.0006$; Density Treatment: $F_{8,102} = 5.50$, $p < 0.0001$). In contrast, our “Changed Response” model showed only seasonal time to cause significant morphological changes from the time males exited the density treatment to the time they courted the female (Pillai’s Trace, Seasonal Time: $F_{16,192} = 1.99$, $p = 0.015$).

With respect to specific morphological traits among nesters, males from low density treatments had more colorful bodies, whereas high density males were darker along their dorsal side (Figure 3.1). However, density did not affect male condition, length, throat color index, or eye color intensity (all $F_{1,233} < 1.10$, $p > 0.27$). Thus, among nesting males, whether males experienced high or low density affected only the *body* coloration they displayed.

In addition, although male condition was not affected, density did affect both the mean initial weight of males immediately after leaving the density treatment ($t_{112} = -2.07$, $p = 0.041$) and the change in weight observed between leaving the density treatment and completing courtship trials with females ($t_{102} = 2.09$, $p = 0.039$). Males were on average heavier after leaving the low density treatment (Low density: 1.33 g; High Density: 1.22 g), but then lost weight during the nesting and courtship period (1.31 g), whereas high density males gained weight during nesting and courtship (1.33 g), ending up at weights equal to those of low density males.

Behavior

Behavior while constructing nests did not differ by any model predictor (all $F_{3,98} < 1.30$, $p > 0.10$). Considering behavior during courtship trials, we found that males generally courted differently based only on their condition (Pillai's Trace, $F_{4,101} = 2.96$, $p = 0.023$). No one single courtship variable was responsible for the condition-dependent courtship change (all $F_{1,116} < 3.58$, all $p > 0.061$), although the strongest effects seemed to be driven by more display-oriented courtship as condition improves.

Nests

Nesting traits were significantly predicted by density treatment (Pillai's Trace, $F_{7,94} = 2.22$, $p = 0.039$), seasonal time (Pillai's Trace, $F_{112,700} = 1.69$, $p < 0.0001$), and male condition (Pillai's Trace, $F_{7,94} = 2.79$, $p = 0.011$). Nests were built faster and with bigger area and perimeter by males from low density (Figure 3.2). Total time to construct nests varied with both time of season and density treatment (Figure 3.3). Nests were heavier later in the season (June: $t_{6,114} = 4.69$, $p < 0.0001$; and July, $t_{6,114} = 2.96$, $p = 0.004$). The exposed area of the nest also increased across the season, marginally so by June ($t_{6,114} = -1.94$, $p = 0.055$), and significantly by July ($t_{6,114} = -2.22$, $p = 0.028$). Moreover, males of better condition had more exposed nests ($t_{6,114} = 2.78$, $p = 0.006$) which were heavier ($t_{6,114} = 1.99$, $p = 0.049$).

Does density affect mating success?

We measured trait impacts on mating success in two ways: a composite MANOVA model based on rates of approach, response, and nest inspection by females; and a linear model predicting preference score (how far males progress in courtship, including spawning; Table 2.2). Density is coded such that negative values refer to low density; for example, males from high density have a greater composite mating score in the MANOVA, whereas males from low density have greater preference scores in the linear model. Our models revealed that mating success is predicted by courtship behaviors, as well as the interactions of courtship behavior with density. These models also revealed that rates of female responses to males and whether a stage of courtship was reached were affected by different factors. In particular, vigorous males who experienced high density treatments had high rates of female responses

throughout courtship; however, vigorous males who experienced low density treatments had higher preference scores than less-vigorous males from the same treatment (see Table 2.2).

How does the variation in male traits compare between treatments?

We examined the above traits with respect to differences in variance between treatments, as detected by Levene's test, and report the significant results here. Among morphological traits, male growth, as measured by change in length, was more variable at low density ($F_{116}=6.60$, $p=0.012$), and throat color index was more variable among males who experienced high density prior to construction of a nest ($F_{117}= 4.03$, $p=0.047$). The number of days required to construct a nest was significantly more variable among males from high density ($F_{119}= 6.01$, $p= 0.016$). Finally, the latency of response of a female to a male was significantly more variable among males who experienced low density ($F_{119}=6.24$, $p=0.015$). Variation in all other male traits and rates of success were not significantly different between treatments (all $F_{119}<1.99$, $p>0.160$).

DISCUSSION

Density should affect sexual selection by increasing same-sex competitive interactions (Emlen and Oring 1977; Eshel 1979; Kokko and Rankin 2006). Consistent with this expectation, we found that prior experience with either high or low density altered a variety of male traits known from other studies to be important to male competition and mate choice, including coloration (Baube et al. 1995), behavior (reviewed in Bell and Foster 1994), and nest construction (Barber et al. 2001; Ostlund-Nilsson and Holmlund 2003). Moreover, while different traits were impacted to different

degrees, suites of traits often responded to the density treatments similarly and in complementary ways.

Males experienced body coloration changes differently depending on density treatment. Males from high density treatments were both less colorful and darker on their dorsal side than those that experienced low density treatments, both before constructing nests and during courtship. As prior work has shown that experience with other males or females alters male throat color (Guderley 2009), our study suggests male body color may also communicate information in this population, perhaps about the perceived level of competition. For example, when competition was perceived to be low, males displayed brighter coloration, which may be ideal for attracting mates at a distance (McLennan, 2007). Alternatively, when the level of perceived competition was high, darker dorsal coloration may serve, as it does in many fish, to make males less conspicuous (Price et al. 2008; Johnsen 2002), which could potentially reduce aggressive interactions during competition for nesting territory, decrease egg-cannibalism while raising young (FitzGerald 1991), or decrease predation (Stevens and Merilaita 2009), all of which may be high in dense areas. Directional color plasticity for background-matching has been found in other populations of sticklebacks (Clarke and Schluter 2011). However, it is also possible that the darker coloration that males display is not to avoid competition, but instead to enhance their competitive ability (Tinghitella et al. 2015) and references therein). Darkened coloration in this case could signal increased aggressiveness, as it does in tortoises and many birds (Gonzalez et al. 2001; Mafli et al. 2011). Responses may also vary among individuals, where quality males, who are better able to compete, may increase signaling, whereas lower quality may

decrease signal coloration (Candolin 2000b). Future studies would be needed to determine whether body color is condition-dependent in this population, and how male-male competition, female choice, and predation integrate to influence rapid body color changes.

Contrary to expectations, we did not observe significant differences in mean red throat coloration between males from high density versus ones from low density, although the variation in red throat coloration was greater in males who experienced high density prior to nesting (but variation differences disappeared after exposure to females). The greater variation in red throat coloration among males who perceived more mate competition (i.e. who experienced high density) is perhaps because red serves as a threat signal among males (Baube 1997; Maynard-Smith and Harper 2003). Red throat coloration is important in male competition for territories (Bakker and Sevenster 1983) and influences the frequency and intensity of antagonistic attacks between males (Pelkewijk and Tinbergen 1937; Tinbergen 1948; Rowland 1982; Rowland 1994; Rowland et al. 1995; Baube 1997). Under competition, better-quality males tend to be brighter, and lower quality males may decrease color expression to reduce the risk of fights with superior males (Candolin 2000b). Our results agree with prior findings that Cranby females have weak preference for red coloration (Boughman 2001), however variation in red coloration appears to be strongly affected by male-male competition in this population.

Although we expected perceived male competition to result in appreciable differences in behavior (Andersson 1994), it did not. The same behaviors predict mating success, but the strength of that relationship varies with density. At both densities,

females prefer more vigorous males. Specifically, vigorous males from high density had higher response rates from females than did less vigorous males from high density. More vigorous males from low density treatments, in contrast, had higher preference scores than did less vigorous males from low density. Therefore, males from high density were able to elicit responses from females more often compared to males from low density, but males from low density were able to progress further with females in courtship than could males from high density. Courtship behavior between treatments might therefore have interacted with differences in other traits, such as male color, to produce these different outcomes. For example, males who are vigorous and antagonistically-colored, versus colored for advertisement, may experience different responses from females. These responses could result in different levels of mating success, but this remains to be tested. Given that studies across species have established that male-male competition can change how females select mates (Trail 1985; Zimmerer and Kallman 1988; Greenfield 1994; Galeotti et al. 1997; Petersson et al. 1999), our work provides evidence that prior experience with male-male competition may impact female choice later on, even when females are not directly exposed to males competing.

We found that aspects of nests and nest building behavior were strongly affected by differences in male density exposure. Nests of males who experienced high density were constructed more slowly and were of smaller size, which suggests risk mitigation: potential vigilance towards male competitors may slow nest building, and smaller nests are perhaps both less likely to be noticed by competitors and less costly if destroyed. It is important to note here, however, that these changes resulted from a 2-week exposure

to a certain density. The two-week duration represents about only one-eighth of the breeding season, but a male could build a nest, successfully court a female, and raise young within this time (Wootton 1976). Furthermore, demography is expected to change substantially across the breeding season (Tinghitella et al. 2013), which may render a nest vulnerable if no longer ideally structured under new levels of competition. Because males are expected to mate across the breeding season, the mismatch of nests to the competitive environment could have consequences for the mating success of males and survival of young. Previous studies have shown within-individual repeatability of nest construction under constant conditions, suggesting a strong genetic component (Rushbrook et al. 2008), but sensitivity to environmental variation also suggests potential rapid responses to altered environments (Raeymaekers et al. 2009; Tuomainen and Candolin 2011; Wong et al. 2012). Future studies should examine within-individual consistency in nests when environments—both social and physical—change.

Regardless of nest structure, males were just as likely to construct a nest following exposure to either male density regime. Once constructed, nests may provide females with additional details about a male's quality, as supported by our findings that nest weight and area are impacted by condition. This is supported by prior work which found that males not under immune stress build “neater” nests more quickly (Barber et al. 2001), and that nests may serve as honest signals of genetic and physical quality in an environment (Raeymaekers et al. 2009). It is also likely that nest structure may provide important information for parental care, as in the related 15-spine stickleback, *Spinachia spinachia* (Ostlund-Nilsson 2000; Ostlund-Nilsson 2001). Females in our

study did generally prefer smaller nests, consistent with expectations from these other findings. Although recent evidence in burying beetles has shown increased parental care under increased reproductive competition (Hopwood et al. 2015), more evidence is necessary to determine whether parental care responds to competition in sticklebacks and whether this occurs in parallel with variation in nest structure. These questions are intriguing, particularly given that males in this species are sole caregivers (Wootton 1976).

Given our experimental design, an interesting hypothesis follows from our observations regarding male competition in nesting. Acquiring a territory is essential before males can build a nest (Wootton 1976), and construction also requires that a minimum healthy body condition is achieved (Rushbrook et al. 2007). Our males, by virtue of being in a laboratory setting, likely all developed enough body condition to attempt building nest. However, in the wild, male competition for territory and its defense would mean only a subset of males (much smaller than the half of males who constructed nests in our experiment) would be able to initially construct a nest, and the nest structure would likely reflect the competitive conditions under which it was built. In response to initial housing with rivals and subsequent nesting in isolation, males built nests that reflected the perceived competition they experienced, even in the absence of current, direct competition. Given the importance of male competition in establishing nesting territories (Wootton 1976), we therefore hypothesize that direct competition from rivals may determine who is successful in initially building a nest, but the perceived level of competition experienced then dictates how quickly and large build it. Direct competition by rival males may prevent other males from securing a territory or may

result in their nests being destroyed. However, nest size or the pace at which it is constructed may be controlled by the availability of supplies and the likelihood of a nest being destroyed once built, which may be signaled to males by the level of competition they perceive. Our work suggests that aspects of nest construction, such as pace and compactness, may be subject to some level of control by interactions with other males prior to nesting, even in the absence of direct competitive actions such as the destruction of nests or takeover of territory by rival males.

We found that the seasonal timing of nest construction was also important in determining nest characteristics. Nests were most likely to be constructed at midseason, with increasing weight and area as the season progressed. Because male density decreases toward the end of the mating season in the wild (Tinghitella et al. 2013), increased area may be possible at late season, as fewer males remain on the nesting grounds defending territories. This same logic applies to our low density treatment: when fewer competitors exist, nests should be larger. Because low density is likely to exist late in the season in the wild, this may also be when males are approaching the end of their lives. Because males build nests faster both at late season and at low density, there is some support for the idea that male competition or natural responses to light cycle changes (Wootton and Wootton 1984) may control how the speed and size at which males build nests to avoid missed mating opportunities later in life (Cluttonbrock 1984). Females may then benefit if they relax their mate choice on other traits, like redness (Tinghitella et al. 2013), and instead use other traits, such as nests, to assess males later in the season. Because females prefer more compact nests, as previously suggested by Barber et al. (2001), females may be able to assess

variation in nest size later in the season to detect which mates are of better condition and thus more likely to survive to raise their young. This relationship remains to be explicitly tested; however, our findings provide some empirical support that female mate choice decisions take nest area into account.

Changes in male traits in response to perceived mate competition have important implications for sexual selection. First, the delayed changes displayed in male traits and nests after density exposure can mean a mismatch between a male and his current social environment. The once-favorable changes in male's phenotype may now be disfavored under new selection pressures when conditions, such as male competition or mate availability, change during his lifetime. For example, larger nests built under low competition may be more vulnerable if competition increases. Secondly, when an individual's traits change plastically, such changes may alter the perception of other traits in that individual. For instance, if body color darkens, red coloration may be accentuated (Flamarique et al. 2013), which may modify how males are perceived, in both male competition and female choice. Third and ultimately, when male traits respond intra-generationally and evolutionarily to selection, the variation in traits reflects both the organism's genes and their expression in a particular environment (here, defined by degree of male competition). Because evolution acts on phenotypes, the degree to which a trait reflects underlying genes is reduced in the face of trait plasticity. Therefore, responding to perceived changes in mate competition may allow for rapid acclimation but also ultimately retard trait adaptation.

Population demography plays a major role in determining which traits are favored by sexual selection, but demography often varies. We have shown that the perceived

level of mate competition can alter the expression of multiple traits and alter female choice, even when females are not directly exposed to such density differences themselves. Therefore, if females select on traits that change at different perceived male densities, sexual selection may favor males who not only have the preferred traits inherently, but also those that can best adjust traits to cope with competition. Of course, increasing density of rivals is only one of many ways in which population demography may change. Interactions and experiences with the opposite sex may also modulate responses to density changes, so more work is needed to determine whether mating experience and success can alter traits. Another area of interest is the question of the point during an organism's lifetime at which such experiences exert the strongest effects. Understanding the various ways organisms can respond to demographic changes is critical to elucidating whether and how organisms can cope with new social conditions consequent to environmental change.

APPENDIX

APPENDIX

Table 2.1. Significant morphological predictors, all color-related, from a linear model of male nest building ($df= 1, 233$)

<i>Morphology of Nest-Building Males</i>			
	<i>Slope $\pm$ SE</i>	<i>t</i>	<i>p</i>
<i>Throat Color</i>			
Throat Intensity	0.09 $\pm$ 0.030	3.17	0.002
Throat Area	0.11 $\pm$ 0.030	3.57	0.0004
Throat Brightness	0.80 $\pm$ 0.027	2.95	0.004
<i>Eye Color Intensity</i>	0.15 $\pm$ 0.036	4.30	< 0.0001
<i>Body Color Intensity</i>	0.13 $\pm$ 0.023	5.75	< 0.0001
<i>Body Darkness</i>	0.37 $\pm$ 0.064	5.74	< 0.0001

Table 2.2. Predictors of male mating success as measured by composite MANOVA using rates of approach, responsiveness, and nest inspection (Pillai's Trace, $df = 3, 94$); and a linear model of preference score ($df = 15, 93$). Negative coefficients for density and interactions indicate that the relationship is stronger for males from low than high density. Main effects, when not significant, are included to aid the interpretation of interactions. Significant results are bolded.

	MANOVA (Composite)		Linear Model (Includes Spawning)		
	<i>F</i>	<i>p</i>	<i>Slope ± SE</i>	<i>t</i>	<i>p</i>
<i>Density Treatment</i>	0.30	0.825	-4.27 ± 2.56	-1.67	0.099
<i>Nature of Courtship</i>	0.43	< 0.0001	9.54 ± 2.07	4.58	< 0.0001
<i>Vigor</i>	11.47	< 0.0001	0.97 ± 0.59	1.65	0.102
<i>Nest Area</i>	0.82	0.483	-0.007 ± 0.004	-2.03	0.046
<i>Condition</i>	1.53	0.212	0.44 ± 0.57	0.77	0.443
<i>Body Length</i>	0.31	0.820	-0.06 ± 0.04	-1.34	0.184
<i>Throat Color Index</i>	0.16	0.923	-0.05 ± 0.07	-0.77	0.442
<i>Body Color Intensity</i>	0.91	0.441	0.005 ± 0.06	0.086	0.932
<i>Body Darkness</i>	1.21	0.311	-0.11 ± 0.16	-0.68	0.499
<i>Seasonal Time</i>	0.44	0.848	0.67 ± 0.59	1.14	0.259
<i>Body Length * Density Treatment</i>	0.35	0.787	0.09 ± 0.05	1.77	0.080
<i>Vigor * Density Treatment</i>	4.62	0.005	-1.31 ± 0.60	-2.19	0.031
<i>Nature of Courtship * Density Treatment</i>	8.99	< 0.0001	-4.55 ± 2.30	-1.99	0.051

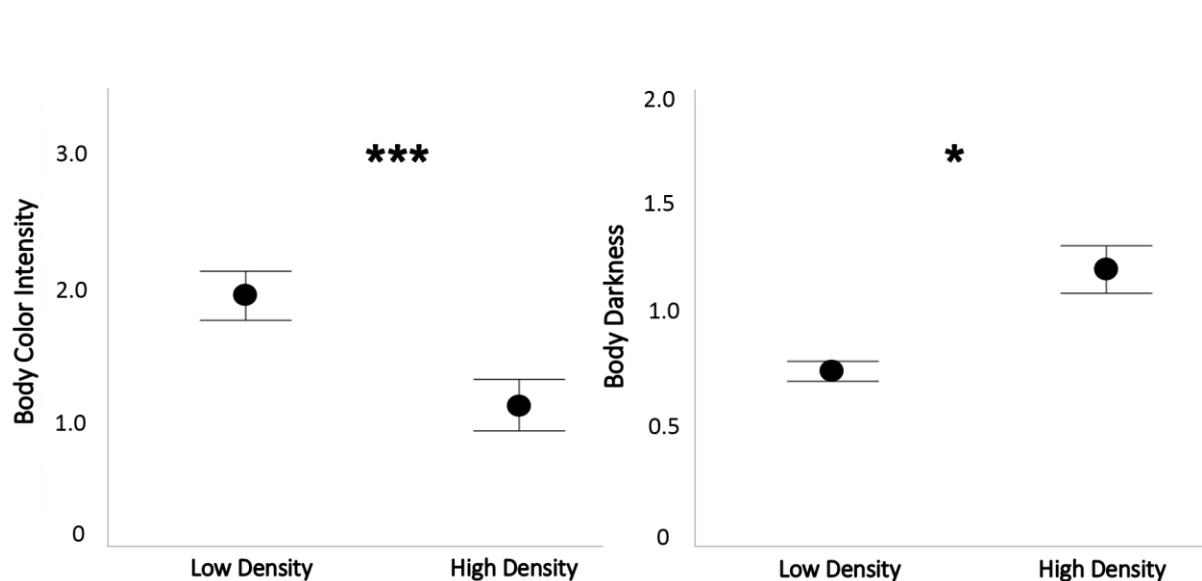


Figure 3.1. After experiencing low density, males have more colorful bodies ($t_{1,233}=5.28$, $p<0.0001$), whereas after experiencing high density, males are darker along their dorsal surface. Points indicate mean $\pm$ SE. Asterisks denote significance at $*p<0.05$ and $***p<0.0001$.

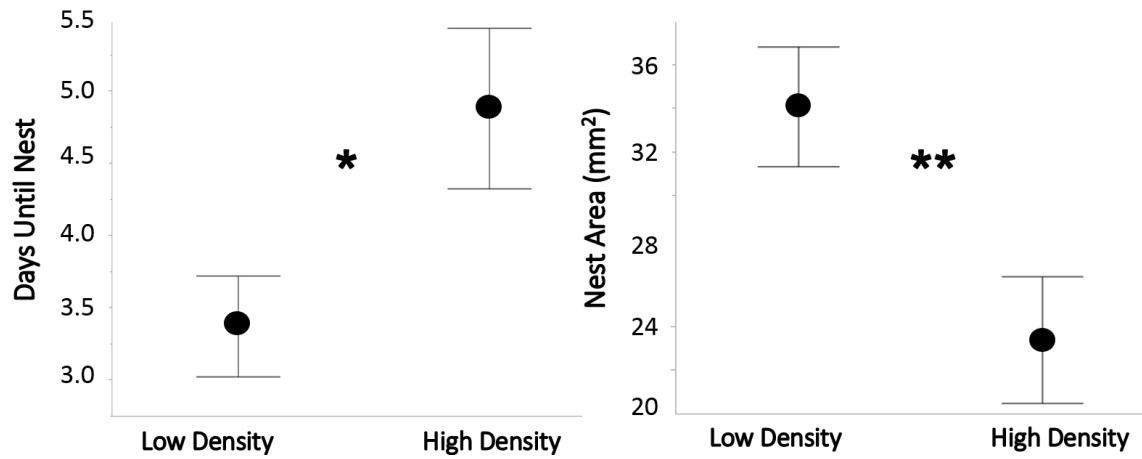


Figure 3.2. Males build nests faster and larger after experiencing low than high density. Males who experienced low density took fewer days to build nests ($t_{6,114} = -2.06$, $p = 0.042$). Their nests were greater in both perimeter and area ($t_{6,114} = 3.21$, $p = 0.002$ and $t_{6,114} = 2.67$, $p = 0.009$, respectively), although the exposed nest area and perimeter (portion of the nest uncovered by sand) was unaffected (all $t_{117} < 0.38$, $p > 0.705$). Asterisks denote significance at $p < 0.05$ (*) and $p < 0.01$ (**).

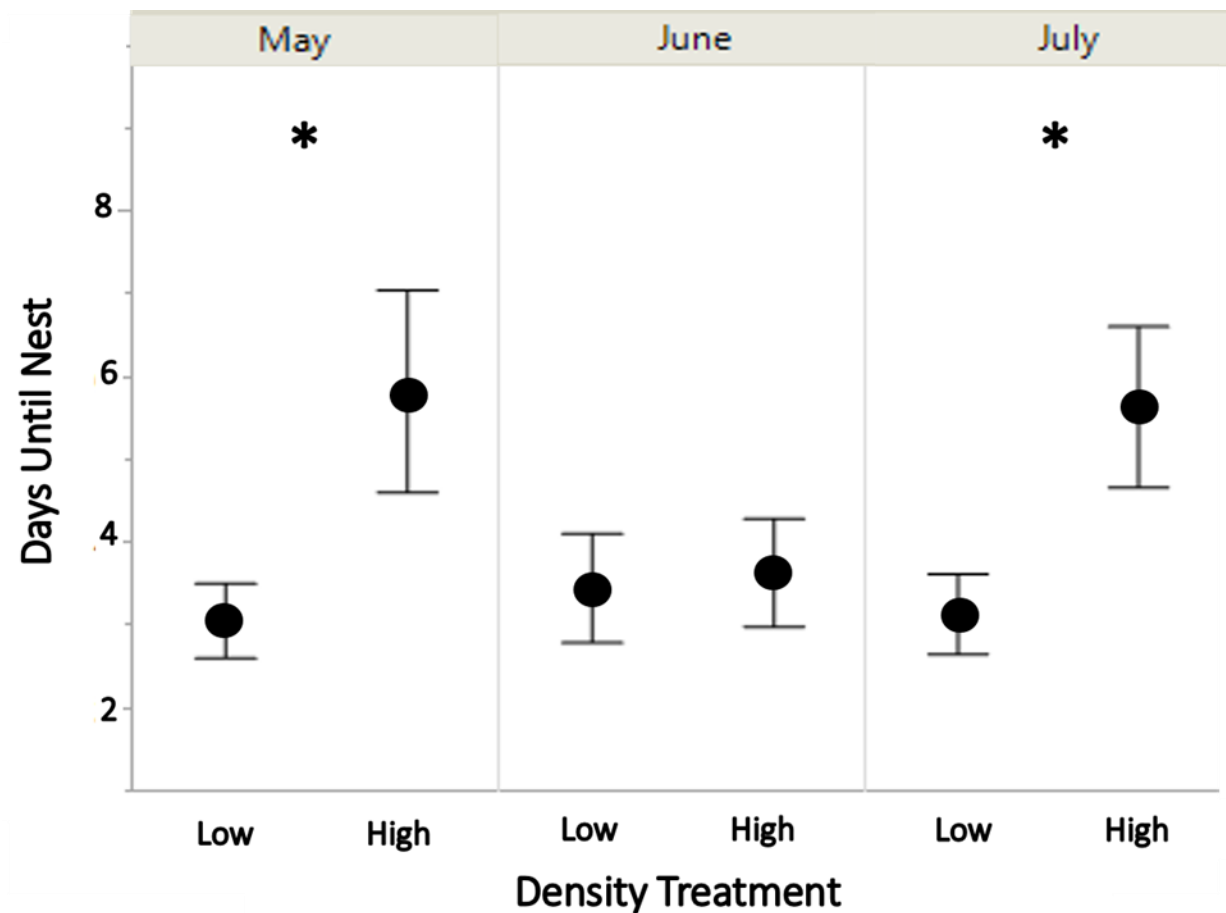


Figure 3.3. At early and late season, males who experienced high density took longer to build nests than males from low density. This effect was primarily driven by the lag to begin nest construction, rather than the days spent in construction. Lags were longer in May ($t_{6,114} = -2.30$, $p = 0.023$) and July ($t_{6,114} = -2.60$, $p = 0.011$), but males from both high and low density build nests equally quickly at midseason. Points indicate mean $\pm$ SE. Asterisks denote significance at $p < 0.05$

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CHAPTER FOUR

Courtship Experience and Success Affect Male Traits

ABSTRACT

Mating opportunities and experience are likely to vary for males both among and within populations. Differential experience may lead to variation in traits used in sexual selection and thereby advantage males that are most responsive to courtship experiences. Here we show that, with increasing courtship experience, male threespine sticklebacks (*Gasterosteus aculeatus*) alter their body and eye coloration but tend to decrease their latency to court regardless of whether their courtship was previously successful. However, in response to prior courtship success associated with spawning, males intensify both their nest care and their color displays. Animals are likely to have differential opportunities to acquire experience during their lifetimes. Our study demonstrates how trait values can change as a result of experience, which thus modifies the variation on which sexual selection can act. Furthermore, our study suggests that selection may act on plasticity in response to experience itself.

INTRODUCTION

Males and females of many species often mate more than once in their lifetimes. At each breeding attempt, males generally compete with other males for a female's attention, and the female tends to choose males possessing the traits she prefers (reviewed in Andersson 1994). In addition to fighting off rivals, males exhibit a variety of traits to attract females during courtship. For example, to attract females, males exhibit classic morphological display traits like large tails (Møller and Nielsen 1997) or colorful

bodies (Endler 1980). Likewise, male behaviors, such as the mating calls found in many amphibians and insects, can be used by females to locate and evaluate prospective mates (Ryan et al. 1982; Mappes et al. 1996; Hoback and Wagner 1997; Reinhold et al. 1998; Ophir et al. 2010; Voituren et al. 2012). In addition to their morphological traits and emitted behaviors, males may also alter their environment by constructing structures such as burrows, bowers, craters, or nests which may be used in courtship and/or raising young (Borgia 1985; Hill et al. 2006; Schaedelin and Taborsky 2006; Kim et al. 2007). These 'extended phenotypes' can also function in the evaluation of males by females, as they are variable within populations and may indicate male quality (reviewed in Bailey 2012).

The traits males exhibit during courtship do not necessarily remain constant across an individual's lifetime. Individuals often gain increasing experience in courtship as they age, and with each experience, the potential exists for new information to be acquired about courtship. For example, the success or failure of a courtship attempt may shape trait expression and impact future mating attempts. If males can modify their own traits in response to such newly-acquired information, they may be able to rapidly acclimate to novel conditions and thus enhance their fitness (Baldwin 1896; Osborn 1896; Robinson and Dukas 1999; Dukas 2013).

Courtship experience in particular has been shown to affect how males court. Responses to experience have been shown to help males reduce their time and effort in pursuing females (Dukas et al. 2012), target the females most likely to accept them as mates (Dukas 2004; Dukas 2009; Dukas 2008), and better compete against more-naïve males, thereby increasing their fecundity (Dukas et al. 2006). Experienced males may

be better at courting generally, as they can cue onto receptive females (Siegel and Hall 1979; Ejima et al. 2005) and adjust their courtship to suit the females (King and West 1983; Patricelli et al. 2002).

Most work to date has focused on direct comparisons of behavior between experienced and naïve males. This is understandable, as behavior is thought to change faster than other traits, such as morphology (West-Eberhard 2003), and has widely been shown to respond to external stimuli (reviewed in Snell-Rood 2013). However, males may show responses to experience in traits other than behavior, and these trait responses may strengthen with additional experience. For example, with experience, males may not only increase their behavioral display traits, but also their morphological display traits, such as color, to attract females. Similarly, males may modify their extended phenotypes to increase success with females. Given that such effects may also strengthen with positive feedback from courtship success, studying males in serial courtship can elucidate how population means and trait variation might change in response to experience during the lifetimes of individual animals in ways that may in turn affect evolutionary processes.

Because experience tends to be coupled with increasing age in the wild, it is difficult to determine how male age and experience might interact to shape trait expression. In particular, individuals need not gain experience consistently across their lifetime; some experiences may be restricted to specific phases of life, yet they may still impact how behaviors and traits are expressed in the future. Particularly because male mating traits can change at different rates with age (Miller and Brooks 2005), there is a need to decouple experience from age to understand how mating traits change. Here

we designed an experiment allowing us to address how increasing courtship experience affects males independent of their age.

We conducted an experiment in which our goal was to determine how courtship experience and success affect both a male's phenotype and his future success in courtship. To investigate the effects of courtship experience and success, we examined threespine sticklebacks (*Gasterosteus aculeatus*) from Cranby Lake using four successive no-choice courtship trials. We staggered the beginning of the animals' courtship experiences across the breeding season to help control for male age (as sticklebacks are generally annual and only survive for a single breeding season; Baker, 1994). We then tracked how a suite of male traits change with courtship experience and courtship success and whether past courtship success begets future success.

The biology of the threespine stickleback is relatively well-studied, particularly with respect to sexual selection (Rowland 1988; Bakker and Milinski 1993; Kraak et al. 1999; Candolin 1999; Tinghitella et al. 2013). Male sticklebacks establish territories for constructing nests to court (Wootton 1976). Larger, redder males are generally preferred across populations (Wootton 1976; Milinski and Bakker 1990; Milinski and Bakker 1992; Bakker and Mundwiler 1994; Candolin 1999; Boughman 2007), and because males are responsible for all parental care (Wootton and Wootton 1984), nests have also been suggested to factor into female mate-choice decisions (Barber et al. 2001). Across the breeding season, males may be visited by several females searching for suitable mates, thus providing males with ample opportunities for experience with courtship. Stickleback population density is known to vary temporally and spatially in nature (Tinghitella et al. 2013; Tinghitella, Head and Boughman, unpublished data),

potentially creating variation among individuals in the amount of courtship they experience. Finally, given that female sticklebacks are known to alter their mate choice decisions with their own courtship experience (Kozak et al., 2013), it seems reasonable to expect that male sticklebacks might likewise be able to respond.

Male sticklebacks are under strong sexual selection through female choice, which should select for males displaying the traits females most prefer. Variation exists within and between populations in male traits, particularly in regard to their morphology, color (Boughman 2001), behavior (reviewed in Ward and McLennan 2008), and nest characteristics (Wootton 1976). We were therefore interested to learn whether and how the traits that males display during courtship are affected by experience within the males' lifetime, because changes in male traits as a result of experience might modify how selection acts on male traits over generations.

MATERIALS AND METHODS

Study population

We used minnow traps to collect reproductive sticklebacks (*Gasterosteus aculeatus*) from Cranby Lake, British Columbia in summer 2013. We separated fish by sex using well-established differences in body shape (for males and females), the presence of eggs (females), and nuptial color (males) (McPhail 1984; McPhail 1992; Hatfield 1997), and we transported them to Michigan State University in East Lansing, MI. Fish were placed into single-sex 75-gallon holding tanks for 48 hours to acclimate to laboratory conditions. Each tank contained cover (plastic plants and ceramic half-pots) and was maintained under summer conditions of 14-hour day lengths and 18°C. Fish

were fed defrosted brine shrimp (*Artemia* sp.) and bloodworms (*Chironomus* spp.) once daily.

Encouraging males to create and maintain nests

At two-week intervals across the summer breeding season, males were introduced to individual 29-gallon nesting tanks and allowed a maximum of 14 days to complete a nest. Each tank contained nest-building materials (a 900-mL tray of sand and 12 g wet-weight of *Chara* spp.), cover (ceramic pot and plastic plants), and opaque boards along the external perimeter of the tank to visually isolate the males. To encourage nest building, a free-swimming gravid female was introduced to each tank for 10 minutes daily. We identified completed nests by a visible nest opening and/or a male swimming through a nest.

Twenty four hours after each male completed a nest, he underwent his first courtship trial, and subsequent trials were conducted at 3-day intervals. Trial date was used as our proxy for male age, as these annual fish generally complete one breeding season before dying (Wootton and Smith 2000). In order to encourage nest maintenance, on each of the two days between trials, males were presented for 10 minutes with females placed into their nesting tanks in 0.47-L glass jars with mesh lids. These jars allowed for visual and olfactory communication between the fish but prevented physical interaction; the use of in-jar females ensured that the males continued to see females and to maintain their own nests once built.

Courtship trials

A total of 121 males built complete nests and underwent four no-choice mating trials at 3-day intervals. No-choice mating trials allow males and females to interact in

the absence of male-male competition, in order to focus exclusively on male-female interactions (Wagner 1998). No-choice trials have become standard for measuring mating preferences in sticklebacks (Nagel and Schluter 1998).

During each courtship trial, a gravid female was placed in an opaque holding container in a nesting male's 29-gallon tank to acclimate for 5 minutes. After acclimation, the female was released from the holding container. Trials began when the male and female first viewed one another and ended after 20 minutes, or when a female entered the male's nest, whichever occurred first. Nesting males were always novel to females. Females that entered nests were prevented from depositing eggs within the nest by gently tapping on the female's tail until she exited the nest before depositing eggs.

A single observer (EGW) documented all courtship and nesting behaviors using an event recorder (Observer: Noldus Technologies, Wageningen, The Netherlands). Changes in male behaviors due to experience may be found not only in which behaviors a male emits, but also in the duration of each activity. Therefore we measured the frequency and duration of display-oriented behaviors (zig-zag 'dancing' and leads to the nest) and aggressive behaviors (chasing, dorsal-pricking, and biting), as well as male nest-fanning and showing the nest to the female. Given that females may or may not choose to approach and follow a male and subsequently examine, enter, or deposit eggs within his nest, we also recorded female behaviors in response to males at several points during the courtship sequence (for further descriptions of these behaviors, see Rowland 1994). Our measures of female choice included responsiveness (the number of times a female followed a male when he led her to the nest; a measure of motivation)

and preference score, which measured how far courtship progressed (on a scale of 0-4, ranging from no response to attempted spawning), and thus how strongly females were attracted to any given male (Kozak and Boughman 2009; Kozak et al. 2009).

We calculated per-minute rates for all measured behaviors to correct for trial duration as well as summary metrics to capture a male's overall latency to court, the nature of courtship, and courtship vigor. We calculated the latency to court as the difference between the start of the trial and the first courtship behavior emitted by the male. We also developed a metric to characterize the overall nature of courtship on a 0-1 scale describing the relative proportion of courtship activity that was display-oriented in nature, where 0 indicates all courtship behaviors were aggressive, and 1 indicates all behaviors were displays. Finally, we calculated courtship vigor as the total number of all courtship behaviors emitted by a male, corrected for trial duration, as in Kozak et al. (Kozak et al. 2009).

We also calculated two variables to investigate how previous mating success, here defined as a female inspecting or entering the male's nest, might modulate the effect of prior courtship experience in the absence of mating. Previous courtship success was assessed in four different ways. First we inquired whether a male had gotten a female to 1) inspect his nest in the previous trial, or 2) enter his nest ('spawning') in the previous trial (previous success). We then considered all prior successes (additive success) by adding up across all prior trials whether 3) inspection or 4) 'spawning' occurred. Note that, although we interrupted the courting pair at the last second, when a female entered a male's nest we referred to this as 'spawning' because

all other naturally-occurring elements of female reproduction occurred except egg deposition.

Male phenotypic data

Phenotypic traits, such as body size and male nuptial color, are important for the reproductive success of males in some stickleback species (Nagel and Schluter 1998; Boughman 2001; Boughman et al. 2005). To document changes in these traits that might occur with exposure to females, we took several phenotypic measurements before and after each courtship trial, as well as directly prior to initial placement in the 29-gallon nesting tank.

During each individual courtship trial, we observed males in-tank (to avoid excessive handling of the fish) and took color measurements directly before and after each trial. We scored each nesting male's average nuptial throat color index (amount and area of throat coloration), body color intensity, eye color intensity, and body darkness following methods established previously (Boughman 2001; Boughman 2007; Lewandowski and Boughman 2008; Lackey and Boughman 2013). These measurements were made on a 0-5 scale to describe the intensity of color display on distinct parts of male's bodies.

Nest measurements

Following McKinnon et al. (2012), we recorded all male behavior (fanning, creeping through, boring, etc.) directed toward the nest. Because fanning is crucial to egg survival, and boring and gluing behaviors by the male are crucial to nest construction and maintenance (von Hippel 2000), we divided nest-oriented behavior into two categories: nesting bouts denoted the number of visits the male made to fan his

nest, and nest care was a composite score that included counts of depositing spiggin, boring at the sides of the nest, as well as all movements to swim through the nest (which may alter its shape). To determine whether males changed their nests between trials, we visually observed and recorded the nest location, depth, orientation, amount of plant material used (1-5 scale, where 1 is sparse plant use and 5 full plant use throughout the nest), and the visibility/exposure of the nest immediately before each courtship trial. We noted any and all changes to the nest as described above and categorized nests for the following trial as 'changed' or 'unchanged' if at least one of the above changes had been made. Note that, even though changes were considered in a binary fashion, regardless of the degree of nest change, males were still found to maintain nests 76% of the time across trials.

Statistical analysis

We asked how courtship experiences and previous courtship success affected male traits (display traits, courtship behavior, nests, and nesting behavior). We then asked whether past experience or courtship success led to future courtship success.

We conducted mixed-effect, repeated measures model analyses in R (R Core Team, 2013, version 3.0.2) using the packages car, lme4, lmerTest, optimx, and languageR. We used plots to visually detect deviations from homoscedascity and normality. Multicollinearity of variables was investigated through the Variance Inflation Factor (VIF) of each model. A VIF exceeding 4 (our threshold reference value) was not found, and therefore predictor variables were included in model as independent predictors (O'brien 2007). We standardized all variables and also used Cook's distance to search for outliers that might have disproportionately affected estimates. Although

rare, when detected, models with and without the outliers were run and showed no differences in the significance of effects. Therefore we included all data points in our analyses.

We ran models to inquire whether and how male traits change in response to experience. Our “experience” models used trial number, trial date (our proxy for male age), and the interactions between these two variables as predictors and used each male trait as a response variable. In regard to morphology, we examined possible changes in male throat, eye, and body coloration, as well as body darkness. In regard to behavior, we examined the latency to court, courtship vigor, the overall nature of courtship, and the nature of the first action directed by the male toward the female. We also measured how two specific nesting behaviors, nest bout rate and nest care rate, changed between consecutive trials, and whether a male changed or rebuilt his nest (by nest placement, visible amount of material used, and/or orientation as identified through visual inspection and confirmed through photographs).

We next conducted models to inquire whether and how male traits change in response to courtship success (accounting for courtship experience). We used the same response variables in our “success” models as in the “experience” models described above. Our predictors included trial number (to now account for the effects of courtship experience), trial date, previous courtship success, and the interaction between trial number and date to document responses in male traits to previous courtship success. Previous courtship success was assessed in two different ways: 1) whether a male had gotten a female to inspect his nest in the previous trial, or 2) whether a male had gotten a female to spawn in the previous trial (previous success).

We then also considered all (additive) prior successes by adding up across all prior trials whether 3) inspection or 4) spawning was achieved (e.g., 0-3 are possible prior success values).

We then inquired whether past courtship success predicted future courtship success. These models used trial number, trial date, previous courtship success, and the interaction between trial number and date to predict response rate, inspection rate, and preference score. As in our “success” models above, we ran separate models for each of the four measures of courtship success.

We examined the residual variation of each significant response variable in the experience model post-hoc to determine whether the variation could be explained by other male traits that we measured. The predictors in these models included male condition, size, behavior variables (nature of courtship, vigor, latency to court), and color variables (throat color, eye color, body color, and body darkness). In order to reduce the number of variables, Principle Components Analysis (PCA) was done on the color variables for models predicting behavior. Scree plots and loadings showed that the color variables could be combined into the first principle component, which explained 37 percent of the variation in color. Stepwise AIC was used to further reduce these models to find the set of variables that best explained change in male traits after experience.

To correct for multiple comparisons across our models, we used False Discovery Rate (FDR), and report both the initial and corrected p-value below; we used stepwise AIC to find the best models where appropriate. All graphs were constructed using the package ggplot2 in R.

RESULTS

How does courtship experience affect male traits?

As the number of prior courtship experiences increased, males increased their throat and eye coloration, increased their vigor, and decreased their latency to court (Figure 4.1). However, males did not change aspects about their nest or its care, the nature of their courtship, or body coloration in response to courtship experience (all $t < 2.286$, all $p > 0.13$). Courtship latency and courtship vigor also varied, not only with male experience, but also with male age, represented by trial date; however this variation was not significant after FDR correction (Table 3.1).

Several variables that responded to experience showed much residual variation, so we conducted post hoc analyses to understand what contributed to that variation. We examined the residual variation in each of the four significant variables (latency to court, courtship vigor, throat color, and eye color) in response to the suite of male trait variables shown in Figure 4.1. The residual variation in latency to court was correlated with courtship vigor (slope $\pm$ SE = -0.264 ± 0.067 , $t = -3.927$, $p < 0.0001$). The residual variation in vigor was correlated with latency to court (slope $\pm$ SE = -0.0043 ± 0.001 , $t = -4.01$, $p < 0.0001$) and the nature of courtship (slope $\pm$ SE = -0.0067 ± 0.002 , $t = -3.537$, $p < 0.0001$), but male color also trended toward statistical significance (slope $\pm$ SE = 0.0023 ± 0.001 , $t = 1.74$, $p = 0.083$). Males who were more vigorous generally started courting more quickly, courted more aggressively, and were often more colorful. The residual variation in throat color was correlated with eye and body color (slope $\pm$ SE = 0.1130 ± 0.040 , $t = 2.831$, $p = 0.005$; and slope $\pm$ SE = 0.1090 ± 0.039 , $t = 2.767$, $p = 0.006$, respectively), as well as the latency to court (slope $\pm$ SE = -0.0450 ± 0.022 , $t = -$

2.081 $p = 0.038$). Residual variation in eye color was best explained by darkness and throat color (slope $\pm$ SE = -0.0780 ± 0.034 , $t = -2.294$, $p = 0.020$; and slope $\pm$ SE = 0.4560 ± 0.040 $t = 11.516$, $p < 0.001$, respectively).

How does courtship success affect male traits?

We considered the effects of mating success on male traits in four different ways. We first evaluated the effect of success in previous trials (previous success) based on whether a male had gotten a female to 1) inspect his nest in the previous trial, or 2) spawn in the previous trial. We then considered all prior successes (additive success) by adding up across all prior trials whether 3) inspection or 4) spawning was reached. A summary of the significant findings for each stage can be found in Table 3.2.

First, we examined the response of traits to previous inspection success. Here, prior success led to an increase in red throat coloration during the next courtship attempt (Table 3.2). However, nest bout rate, body color, and body darkness did not significantly change with previous inspection success (all $t < 0.179$, $p > 0.054$, all adjusted $p = 0.197$). None of the other male traits were affected by previous courtship success (all $t < 1.584$, all $p > 0.114$, all adjusted $p > 0.251$). We then examined male traits that responded to previous spawning success. Between trials, males merely returned their nests to the state which they appeared during the earlier successful trial (Table 3.2). In addition, the difference in the rate of nest care between trials merely trended toward statistical significance (slope $\pm$ SE = $2.593e-04 \pm 1.37e-04$, $df = 369.7$ $t = 1.892$, $p = 0.059$, adjusted $p = 0.651$). None of the other male traits were significantly altered (all $t < 1.48$, all $p > 0.138$, all adjusted $p > 0.759$).

Next, we examined trait responses to additive inspection success. With additive inspection events, males increased their throat and body color (Table 3.2). However, none of the following variables changed significantly between trials: nest bout and care rates, latency to court, the nature of courtship, courtship vigor, nest changes, eye color, or body darkness (all $t < 1.247$, all $p > 0.105$, all adjusted $p = 1$). Finally, we examined trait responses to additive spawning success. Males increased their nest care rate but made no changes to their nests (Table 3.2). No other male traits were significantly affected by additive courtship success (all $t < -1.624$, all $p > 0.105$, all adjusted $p > 0.385$).

Does past courtship success predict future courtship success?

In investigating trait changes in response to success, we considered previous courtship success in four different ways: previous inspection success, additive inspection success, previous spawning success, and additive spawning success. We then determined how female behaviors (responsiveness, inspection rate, and preference scores) changed in relation to these four ways of measuring prior courtship success. We found that previous success (measured by inspection and spawning) did not affect future success as measured by female responsiveness, inspection, or preference score (all $t < 1.647$, all $p > 0.101$, adjusted $p > 0.302$). Nor did additive success affect future success in any measure (all $t < 1.564$, all $p > 0.119$, all adjusted $p > 0.357$). Past success apparently does not guarantee future success.

DISCUSSION

The goal of this experiment was to determine whether and how courtship experience and prior success change both a male's phenotype and his current success in courtship. We found that courtship experience altered male color, vigor, and latency

to court. Mating success, in contrast, discouraged males to alter their nests. However, past success in courtship did not predict future success.

Trait changes in response to experience

With increasing courtship experience, the throats and eyes of males became more colorful. Even though Cranby males have duller throats than do males from other stickleback populations (Boughman 2001), they nonetheless became redder with courtship experience. This aligns with previous work showing that male throat coloration intensifies in response to social experience with conspecifics (Guderley 2009). Becoming redder, even in Cranby's red-shifted waters (Albert et al. 2007), should create a greater contrast between the male's throat and the water, thereby facilitating detection of the male's signal by conspecifics. Given that male redness does not function in female choice in this population (Boughman 2001), it may be that increasing redness generally serves to communicate a male's courting experience, competitive ability, and nesting status to other males. Indeed, in other populations, redness functions in competition among males, both in territory acquisition (Bakker and Sevenster 1983) and antagonistic interactions (Rowland 1982; Rowland 1994; Rowland et al. 1995; Baube 1997). Because throat color increases along with eye color (an important combination of cues in stickleback signaling; Flamarique et al. 2013), it is likely that these color changes are mediated by the same mechanisms. Although coloration is dynamic in fish and responsive to many cues (Kodric-Brown 1998), a good candidate mechanism here might be melanocyte concentrating hormone, as it has previously been shown to darken both fish skin and eyes (Sköld et al. 2015).

With increasing courtship experience, males courted more quickly. Quicker courting with experience has also been demonstrated in fruit flies (Dukas 2005), where experienced males identified and courted receptive females faster than did naïve males. In our experiment, however, individual males were tracked through a series of courtship experiences, and thus increases in speed detected were detected within males, rather than between groups of males. Experience evidently has compounding effects such that each experience results in ever-faster courting. Furthermore, males saw only gravid females, which can be visually differentiated from nongravid females at a distance (Rowland 1989). Thus, faster identification of appropriate females cannot account for males courting more quickly. However, given the strong competition males face in the wild (Rowland 1988), courting more quickly might be a way for males to beat the competition before it starts. Parsons (1973) suggested that, across many species, quick-courting individuals should have a selective advantage, which has been subsequently supported by studies conducted in both lab and field (Parsons 1973; Markow 1988; Markow and Sawka 1992; Stoltz et al. 2008).

Vigor, a male trait commonly preferred by females (Gibson and Bradbury 1985; Andersson 1991), also tended to increase with experience. Evidence suggests that female sticklebacks prefer more intense, natural amounts of courtship vigor (Rowland 1995). Thus, males who learn to court more vigorously may have increased mating success with courtship experience. Vigor may also function to signal parental quality (Knapp and Kovach 1991), or may simply indicate younger males who are likely to survive to care for young, which is critical to offspring survival in this species (Wootton and Wootton 1984).

Increased vigor and decreased courtship latency appear to be related. Changes in both vigor and latency to court suggest males may become more sexually aroused with experience (Andretic et al. 2005). Because sexual arousal has been shown to reinforce cues in stimulus-response work (Jenkins and Rowland 2000), arousal may also help males learn how vigorously and quickly court.

Because males in other species have been shown to learn from mating experience (Dukas 2004; Dukas et al. 2006; Dukas 2008; Dukas 2009) and alter their behavior mid-courtship-attempt to suit females (Patricelli et al. 2002), it is important that future studies consider how learning, and potentially also arousal, may change as males age. Interestingly, our results also show multiple elements of stickleback courtship to be plastic. Given that stickleback courtship behaviors have been considered to be a classic example of instinctive behaviors or fixed action patterns (Tinbergen 1951), it is somewhat surprising that we find so much variation in response to experience. Although the triggers to emission of courtship behaviors might be fixed in this species, it is clear that experience can affect the intensity or frequency of the ensuing behavioral and morphological displays. In any case, this classic example of a putative fixed action pattern clearly shows plasticity.

Trait changes in response to courtship success

Male traits can evidently change in response to courtship success, and these changes depend on the level of success experienced. It appears that reaching the stage of nest inspection by the female tends to intensify male coloration, and that attempted spawning within the nest discourages changes to nests. Achieving nest inspection, but not quite achieving spawning, implying that males were almost successful in courtship,

may induce males to 'try harder' to gain subsequent encounters by increasing color and the vigor of courtship and courting more quickly. Given that nest inspection is the female's last behavior before actually entering the male's nest, the evaluation of nests likely plays a role in mate choice, as suggested by Barber et al. (2001). Having a female enter the nest may indicate to the male that his nest structure was approved. This may explain our finding that, if a male was successful in the past with his nest, he is unlikely to modify it, and why unsuccessful males change their nests.

Male traits may also change when courtship success is experienced additively across encounters. Multiple nest inspections increase male coloration for advertisement in future courtship attempts, but repeated spawnings encourage the maintenance of nests. The odor and the structural integrity of a nest is likely altered by females repeatedly entering it. Thus, with increasing spawning, males tend to 'patch up' and care for nests by depositing glue, smoothing the tunnel, and tidying edges. These behaviors may function either to make the nest more attractive (through nest compactness or added odor) for further courting, or to prepare the nest for fatherhood. If a male continues courting, caring for a nest previously deemed 'good enough,' may help him garner additional clutches before he switches entirely into his parental mode (reviewed in Mayer and Páll 2006). If males are preparing to be parents, then the purpose of the nest shifts from a structure for mating to one for rearing young, such that nest care and tending behaviors are needed to increase offspring survival (Sevenster 1961; Sargent and Gebler 1980). Because nest structure is repeatable within individuals (Rushbrook and Barber 2008), explicit tests are needed to disentangle whether males

increase nest care to repair nests for further courtship, to correct flaws in nest structure that might affect survival of their young, or both.

Does past success predict future success?

In many species, repeated encounters with potential mates affect sexual selection, particularly when animals search for mates sequentially. Our results indicate that past success in courtship does not guarantee future success, suggesting that females vary in their mate choice decisions. This is consistent with several studies showing that female preferences vary based on conditions intrinsic and extrinsic to the female (reviewed in Jennions and Petrie 1997; Cotton et al. 2006; Bell et al. 2009). In particular, although all females in our study were kept in a single-sex tank and were novel to each male, the potential still exists for the females' prior experiences in the wild to have affected their mate choice decisions. Females in many species have mate preferences that can be influenced by both potential and prior mates (Shelly and Bailey 1992; Jennions and Petrie 2000; Wong et al. 2004; Kokko and Mappes 2005), and experience with high-quality males and their signals can increase a female's acceptance criterion in several of these species (Janetos 1980; Reid and Stamps 1997; Rebar et al. 2011; Fowler-Finn and Rodriguez 2012), including in sticklebacks (Bakker and Milinski 1991; Milinski and Bakker 1992; Kozak et al. 2013). The various experiences of females help to explain variation in acceptance of males with different females across mating attempts, particularly for species with complex displays (Coleman et al. 2004).

Another potential explanation for why males are not consistently successful is that perhaps males don't court, or display the same traits, consistently. Some evidence

exists to suggest that males tailor their courtship to the individual females with which they interact, not only in sticklebacks (Kozak et al. 2009), but also in other taxa (Meffert and Regan 2002). In particular, if our gravid females varied in abdominal distention (a sign of fecundity; Fletcher and Wootton 1995), males might have varied in their courtship behavior directed toward the females (Kraak and Bakker 1998). If males consistently favored more distended females, but these females were presented at random to males and across trials, responses to females could have masked behaviors which could have led to consistent success across trials if our sample size was not large enough to overcome this noise. Nonetheless, modifying courtship behavior is said to be a learned response acquired from prior courtship attempts (Jenkins and Rowland 1997), which would persist and potentially be adaptive for males even if its positive effects on fitness (here, courtship success) are only occasional (Dukas and Visscher 1994; Dukas 1998). Furthermore, the traits expressed in our males did not change consistently together across trials. If male traits change inconsistently, they may experience inconsistent mating success.

Our data show that males respond differently due to both the nature of success and experience. Experiences prior to spawning appear to lead to many strong responses in traits for future courtship, and spawning appeared to only discourage changing of nests. These different responses suggest that experiences prior to spawning may generally aid a male in developing and displaying desired traits for future courtship attempts. However, we did not see consistent mating success across trials among our males. This suggests that selection may be weaker on later courtship attempts if a male has been previously successful, compared to fish that have yet to

succeed. In nature, stickleback males vary strongly in reproductive success, where many males are unable to mate (often due to an inability to secure nesting grounds), and very few males are able to sire offspring from several clutches. Therefore repeated success may only be experienced by a select few males. Traits may change to help secure at least one successful siring, after which responses may decrease (as suggested by Whoriskey and FitzGerald 1994) or be redirected to the nest for rearing (to counter selection against offspring), rather than wooing additional females.

Population size and the densities of potential mates and competitors are well known to affect sexual selection (Emlen and Oring 1977). Our experimental paradigm permitted males to interact with four different females over time, which may represent a male stickleback's lifetime total of courtship experiences under intensive mate competition in the wild. In sparse populations, mating opportunities may be even rarer. However, in populous areas, mating opportunities should vary widely between males because of both competition and encounter rates. If mating opportunities vary between individuals, and these individuals respond to mating experiences, then variation should result from differences in mating experience between males. Demographic parameters may also affect whether males are more likely to modify their behavior based on a single prior experience or a series of such experiences. Learning by males based on their experience should theoretically affect not only the individual trait values expressed by males, but also the overall behavioral variation present in their larger populations. Thus, learning may help to increase and maintain the trait variation upon which selection can act in nature.

Modification of traits in response to experience should allow individuals to adjust to unique or changing circumstances in the habitats where they live; these might vary significantly over ecological and evolutionary time. Understanding individuals' responses to experience helps explain the distribution of traits upon which females choose their mates. Furthermore, because suites of traits may not all change together, acclimation through adjustment of certain traits can alter the trait combinations males display, potentially affecting other aspects of fitness, such as male-male competition and parental care. When male traits change within lifetimes, rather than across generations, the rate at which sexual selection acts can be altered. In particular, selection may be slowed, as the phenotypes males display are a product of both their genes and differential experience with females (assuming the experience males garner is unrelated to their genotype). Because females may prefer males with good genes (which would nullify this assumption), the effects of plasticity on sexual selection may operate differently depending on the underlying nature of female choice (i.e., whether good genes or runaway, etc.) across species. Our study illustrates the contribution of experience, not merely to variation among males in their behavioral traits, but also in their morphological traits and their nest-oriented behavior, and thus to the extended phenotypes they express.

APPENDIX

APPENDIX

Table 3.1. Male traits that varied significantly with male age (trial date).

	<i>Estimate ± SE</i>	<i>df</i>	<i>t</i>	<i>Pr(>t)</i>	<i>FDR Corrected p</i>
Latency to Court	0.011 ± 0.0047	118.9	2.41	0.017	0.187
Vigor	-2.50e-04 ± 1.12e-04	136.6	-2.25	0.026	0.286

Table 3.2. Male trait changes in response to courtship success, as represented by inspection and spawning, both in the previous courtship trial (“Previous Success”) and additively (“Additive Success”). Significant findings from repeated measures mixed models are displayed, and non-significant results are denoted with “—” ($df=232$). P -values are noted before and after FDR correction using asterisks, where $p<0.05^*$, $p<0.01^{**}$, and $p<0.001^{***}$, and ‡ for trending results ($0.05<p<0.10$).

	Inspection		Spawning	
	<i>Previous Success</i>	<i>Additive Success</i>	<i>Previous Success</i>	<i>Additive Success</i>
Nest Characteristics				
Nest Bout Rate	—	—	—	—
Nest Care Rate	—	—	—	$2.261e-04 \pm 9.95e-05$ $t = 2.27^* / --$
Changed Nest	—	—	-0.879 ± 0.43 $t = -2.06^* / --$	-0.244 ± 0.0021 $t = -113.60^{***} / ***$
Morphology				
Throat Color Index	0.746 ± 0.28 $t = 2.60^{**} / ‡$	0.460 ± 0.18 $t = 2.52^* / --$	—	—
Body Color	—	0.126 ± 0.060 $t = 2.12^* / --$	—	—
Body Darkness	—	—	—	—
Eye Color	—	—	—	—
Courtship Behavior				
Latency to Court	—	—	—	—
Nature of Courtship	—	—	—	—
Courtship Vigor	—	—	—	—
Nature of First Event	—	—	—	—

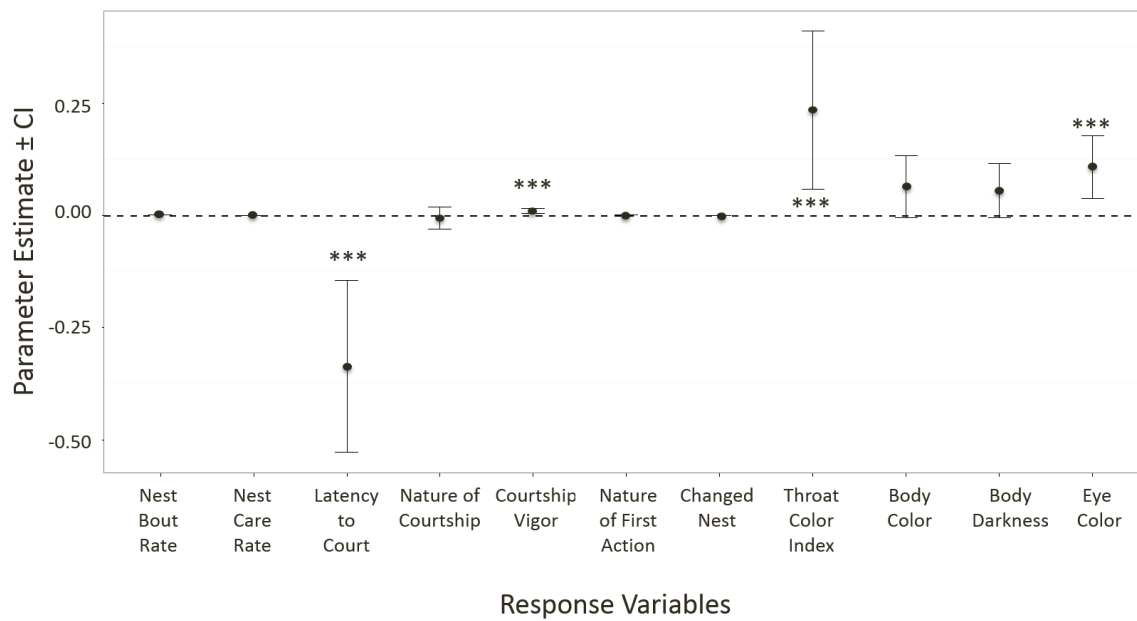


Figure 4.1. Standardized parameter estimates and confidence intervals for the effect of courtship experience on male traits. Male traits that responded significantly to courtship experience are denoted by asterisks (***, where after FDR adjusted $p < 0.001$).

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CONCLUSION

Through this series of studies, we have demonstrated the role of social experience in altering sexual selection. In particular, we have examined how different forms of experience modify traits in males and females, and how experience ultimately influences mating success. This work strengthens our understanding of how within-lifetime responses to social conditions can affect how sexual selection shapes traits.

In Chapter 1, we provide evidence that female reproductive investment need not change in concert with reproductive behavior, or at all. Our work reveals that limited plasticity in reproductive investment may keep females from mitigating the effects of relaxed choosiness when mates are rare. When female choice relaxes, but their reproductive investment remains constant, less-preferred males may profit. The fixed nature of investments can combine with plastic behaviors to lead to the persistence of less-preferred male traits and ultimately slow the rate of selection on them. This work underscores the importance of investigating responses to male availability in both reproductive investment and behavior by females to determine the ultimate outcomes of sexual selection. It also raises the question of whether male trait investments act in parallel with those of females (e.g., decrease in female preference for red when male expression of red also decreases) to mitigate conflicts between female investment and behavior. If so, to which traits does this apply, and how often does this occur?

In Chapter 2, we show that males are evaluated by females based on several different traits and to different degrees as courtship progresses. Although males allocate resources into several morphological, behavioral, and nesting traits, only a subset of these are indicative of male condition and are ever preferred by a female. In contrast to

several populations of stickleback, Cranby Lake sticklebacks lack both a condition-dependent relationship for red throat coloration (the preferred sexual signal in many populations) and a female preference for it in mating. This highlights the extent of population divergence in condition-dependent sexual signals within a rapidly-evolving species and helps to clarify that red is not only difficult to detect given the ecology of Cranby Lake, but it is also an uninformative signal of male condition to females in that population. Interestingly, we show that preferences in this population depend more on male behavior, which may be both a more visible and more reliable indicator of male quality than nesting or morphological traits. This work therefore suggests that the relative importance of traits upon which females choose is based both on baseline female preference for the trait (quality-indicating or not) and the time at which the traits are assessed. If some traits act as initial advertisements, and others to secure mates, future work is needed to investigate how ecology may disrupt the detection of signals used at all stages of mating. This will further our understanding of how sexual signals may be altered, lost, or exchanged for alternatives which may be more reliable in the habitat occupied by that particular organism.

In Chapter 3, we test how male traits might change in response to mate competition. We find that males tended to express traits that correspond with the perceived male density to which they are temporarily exposed. Males under conditions of high competition have risk-mitigating darker coloration, which helps to avoid negative interactions with other males, and they build smaller nests, which require less investment to build and are less costly if destroyed. Conversely, males under conditions of low competition build larger nests and have far more colorful bodies, suggesting that

these males optimize their potential for detection by females rather than preparing to face rivals. The rapid and lingering manner in which males respond to the short-term shifts in demography is particularly telling, as demography is likely to vary spatially and temporally for these and many other organisms. This work therefore illuminates how mate competition and female choice may shape traits, and how acclimation to social conditions may influence rates of adaptation to female preferences. Future work should determine the permanence of these effects as well as whether or not a critical window of exposure is required.

In Chapter 4, we address how a male's experience and success in mating affect the traits he displays to females. We find that experience in mating is not predictive of future success and that the male traits that change in response to experience are not the same as those that respond to mating success. In particular, the phenotypic traits that allow for advertisement to females (such as coloration) increase with courtship experience, as does a male's quickness to begin courting. However, a male's nest, the trait assessed latest in courtship, is very likely to remain unchanged when a male was previously successful in courtship. This response in nests can indicate, as we learned in Chapter 3, that females indeed assess nests, and males receive the cue to keep suitable nests structurally the same when they enjoy mating success (i.e., females enter the nest). Additionally, we find that mating success operates additively to alter the expression of multiple traits as experience accrues. However, a single, immediately-prior, successful mating attempt can still change some male traits (coloration and nest care). These responses indicate that male traits respond to experience and success, and expression of these traits may differ between sequential mating attempts. This has

important implications in regard to determining not only male trait values in the population, but also how females formulate relative preferences among mates. If experiences with the opposite sex change male traits and female preferences, then selection can fluctuate in strength over time and space, particularly as opportunities to mate vary and experiences (and potential successes) accumulate. Although some work has been done to determine how female preferences and searching strategies change with exposure to males, future studies should examine whether demography-induced changes in female preferences keep pace with or lag behind commensurate changes in male traits (i.e., whether a within-lifetime arms race exists between the male traits that change with experience and female preferences for them).

Sexual selection is undergoing a renaissance as we discover more about how within-lifetime variation, including modifications in physical or social environments, can influence rapid responses in animals. This is particularly timely and important, as rapid environmental changes induced by human activity are increasing the frequency and urgency with which organisms need to respond. The studies here contribute to our understanding of how variation in demographic parameters and social experience affect the physical and behavioral traits displayed by males and females. They also help predict the changes to sexual selection that should result when demography and social experience vary.