Sex differences in mate assessment throughout pair bond formation in a monogamous species, Amatitlania nigrofasciata

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Sex differences in mate assessment throughout pair bond formation in a monogamous species, *Amatitlania nigrofasciata*

by

Joseph M. Leese

A Dissertation

Presented to the Graduate and Research Committee

of Lehigh University

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Joseph M. Leese
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Joseph M. Leese
Sex differences in mate assessment throughout pair bond formation in a monogamous species, Amatitlania nigrofasciata

Defense Date

Dr. Murray Itzkowitz
Dissertation Director

Approved Date

Committee Members:

Dr. John Nyby
Committee Member

Dr. Michael Burger
Committee Member

Dr. Ethan Clotfelter
External Examiner
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Abstract

Sexual selection is the evolutionary process driven by competition within and between the sexes for mating opportunities. Its action has primarily been studied within polygamous species demonstrating exaggerated and elaborate sexual dimorphisms. Yet recent theoretical work suggests that sexual selection can and should operate even among monogamous species that generally lack obvious sexual signals. This work sets out to explore this paradox by exploring the action of inter-sexual selection (mate choice) in a serially monogamous cichlid fish. First, the hypothesis was tested that the formation of a pair bond in monogamous systems functions as a mechanism to facilitate mate choice. If supported, this hypothesis predicts that behaviors associated with pair bonding play a role in either advertising an individual’s quality or gathering information about mate quality from potential partners. A second hypothesis was then tested that male courtship behavior is used as a cue in mate choice decisions by females. These hypotheses were tested in a series of behavioral experiments using the convict cichlid, Amatitlania nigrofasciata. Results from the following studies reject the hypothesis that pair bonding is used as a prolonged period of mate assessment in males, but suggest that it may serve that function in females. The second hypothesis that male courtship is used as a cue in mate choice decisions by females was also refuted. These results support sexual selection serving a role in mate choice in this monogamous species, but suggest that the timing and strategy differs between the sexes. Furthermore, these differences seem to reflect a tradeoff between fitness benefits acquired from choosing a high-quality mate versus performing tasks related to parental care.
General Introduction: Sexual Selection and Monogamy

The understanding of differences between males and females remains one of the most stimulating and provocative fields of study in biology. For most vertebrate taxa, species consist of two unique sexes (with a few exceptions of asexuality or hermaphroditism), each of which produces a single type of gamete. This fundamental distinction correlates with a host of additional disparities in morphology, physiology and behavior that scale from the sub-cellular to organismal level.

Many sex differences are thought to have evolved from the differential natural selection on males and females (Darwin 1871; Arnold & Wade 1983; Slatkin 1983; Shine 1988). For example, body size is a sexually dimorphic trait in many species. Size has a significant influence on individual fitness and affects virtually all aspects of an animal’s life including foraging strategies, anti-predator defense, mating decisions and agonistic behaviors. As such, differences in body size suggest differing selection on males and females.

Among various species of raptors (Accipitridae), females are larger than males, primarily as a result of the parental role each sex adopts in caring for the young (Selander 1966; Hughes & Hughes 1986). Females tend to remain with the nest and defend the young, while males are the primary contributor of food for the offspring. Defending the nest from rivals and predators requires a larger optimum body size while hunting necessitates a smaller body size conducive to greater speed and maneuverability.
In many other vertebrate species, fecundity is directly correlated with body size in females, but not males. This is presumably why females are larger than males among certain squamates (with few exceptions among species showing high levels of male-male competition) (Shine 1994). Similar factors likely cause sexual size dimorphism in amphibians (Shine 1979) and some mammals (Ralls 1976) where females are larger than males. In these instances, sexual dimorphism in body size results from the action of natural selection as it contributes to differential survival or fecundity of the sexes, rather than directly impacting the ability to secure mating attempts.

In other cases of sexual dimorphism, disparities between the sexes run in opposition to the action of natural selection. Males are the larger sex in many mammals, despite no obvious direct benefit to either survival or fecundity. Male lions, *Panthera leo*, can be nearly twice as large as their female counterparts and require nearly 30% more calories on a daily basis. Their increased size, however, actually decreases their ability to successfully hunt prey (Schaller 1976). A similar pattern is observed among non-human primates, including gorillas, where males can weigh twice as much as females, yet a vegetarian diet suggests no direct benefit to male fitness through increased foraging ability. Additionally, there is no evidence that large size increases fecundity or decreases predation risk (Fossey 1983).

Darwin’s (1871) theory of sexual selection provided the framework for the evolution of these characteristics in a process separate from natural selection. Darwin stated that the presence of dimorphic traits could be adaptive if they increased mating success, either by outcompeting members of the same sex for access to mates (intra-
sexual competition) or by increasing attractiveness to members of the opposite sex (inter-sexual selection) (reviewed in Andersson 1994).

While Darwin’s general theory has been supported with countless theoretical models and empirical data, a surprising amount of confusion still surrounds how this process operates within certain natural populations (see Clutton-Brock 2007). Historical explanations for the operation of sexual selection involving potential rates of reproduction (Bateman 1948), parental investment (Trivers 1972), mating systems (Emlen and Oring 1977) and their effects on Operational Sex Ratio (OSR) (Clutton-Brock & Parker 1992) often fail to explain satisfactorily the patterns of sex differences observed among many taxa. This is, in part, because Darwin’s theory of sexual selection was initially formed with the purpose of explaining exaggerated characters present in males, but lacking in females, such as the colorful plumage found in birds of paradise (Diamond 1986). In species demonstrating this pattern, exaggerated traits emerged from competition between males and ‘choosiness’ by females, with only one sex (males) essentially evolving under the pressure of sexual selection. Classic examples include the train of male peafowl (Pavo cristatus), the construction of bowers by male bowerbirds (Ptilonorhynchidae), and the presence of antlers in males of some genera of ungulates (Ungulata). Subsequently, sexual selection has been studied mainly in species demonstrating the most robust and drastic dimorphisms reflecting this pattern. In theory, however, sexual selection does not act only on one sex in a given species. Both males and females must find a high-quality mate and compete with members of the same sex for mating opportunities (Kokko & Johnstone 2002). Surprisingly few studies have actually
attempted to address the operation of sexual selection in both sexes within a single species.

The first empirical study of mutual sexual selection appeared less than two decades ago when Jones and Hunter (1993) described sexual selection in the crested auklet (*Aethia cristella*), a monogamous seabird. In this animal, both sexes possess an ornamented crest of feathers and behavioral studies revealed that this trait was utilized in mate choice decisions and had evolved as a direct result of sexual selection. Recent theoretical work predicts that similar findings should occur among other species where mates vary in quality (Johnstone et al. 1996; Kokko & Johnstone 2002; Clutton-Brock 2007), albeit to varying degrees. If individuals do not mate randomly, both sexes can increase their relative fitness by being discerning in their mate choice, thus driving the action of sexual selection. Nevertheless, many animals lack easily identifiable sexual signals, and how mate choice would operate in these species remains poorly understood.

Often, the way in which sexual select acts within a given species correlates to the type of mating system demonstrated (Emlen & Oring 1977, Andersson 1994). Polygyny, the mating system common to most mammals, is marked by sexually selected traits in males, while within polyandrous species; females often develop these signals (Emlen and Oring 1977; Clutton-Brock 2007). While relatively rare, the latter mating system results in a reversal of sex-typical parental roles as well as reversals in morphology. For instance, male ‘pregnancy’ is common among Syngnathids (pipefishes, seahorses and seadragons) (Berglund et al. 1986; 1989), and male incubation and nest defense is frequent in a variety of species of sandpipers, phalaropes and jacanas of the order Charadriiformes.
(Jenni 1974). As such, discerning between the action of natural and sexual selection in sex-role reversed species is more difficult than among polygynous ones.

While representing opposite ends of the spectrum, polygyny and polyandry share an important commonality. In both mating systems, the sexes differ in their relative parental investment as well as the OSR and thus these explanations for the operation of sexual selection are generally supported (Trivers 1972; Emlen & Oring 1977). These theories lose much of their explanatory power, however, when sexual selection among monogamous species are considered. For monogamous animals, sex differences in parental investment are thought to be negligible and furthermore, assuming an equal sex ratio at birth, the OSR is assumed to be nearly equal (Trivers 1972; Emlen & Oring 1977; Clutton-Brock & Vincent 1991). Thus the operation of sexual selection cannot be easily attributed to the dynamics of either of these two factors.

It seems paradoxical, then, that some theoretical work suggests that monogamous species, especially those demonstrating bi-parental care, are expected to show similar, if not greater, levels of mate choosiness as polygamous systems (Trivers 1972; Johnstone et al. 1996; Kokko & Johnstone 2002; Lee et al. 2008). Unique to monogamy is the fact that both parents generally contribute more than genetic material to their offspring. Food provisioning, predator defense, and social learning are all benefits garnered from parental care and these tasks are frequently shared by the sexes (Clutton-Brock 1991). As the parental contribution toward offspring survival increases, so too do the potential costs and benefits of mating with a given partner. In the most extreme case, a mistake in mate choice could result in desertion and force the remaining individual to take on all parental
duties leading to drastic reductions in fitness (Grafen & Sibly 1978; Lazarus 1990; Choudhury 1995). This pattern is well documented in birds (reviewed in Bart & Tornes 1989) and fishes (Keenleyside 1978; Keenleyside & Bietz 1981; Balshine-Earn 1997). In a monogamous mammal, the Djungarian hamster (*Phodopus campbelli*), pups raised by single females had a 50% reduced rate of survival than their counterparts raised by both parents (Wynne-Edwards 1987). Consequently, the degree of parental contribution by both parents within monogamous species predicts a high incentive to be discerning when selecting a mate (Johnstone et al. 1996; Deutsch & Reynolds 1995).

Given that the potential fitness benefits of choosing a high-quality mate are expected to be similar (or higher) among monogamous species than those showing single or no parental care at all, it remains unclear why these animals generally lack the drastic ornaments observed in other species. One possible, yet unlikely, explanation is that monogamous species mate randomly and thus signals used in mate choice decisions have not evolved. An alternative hypothesis is that monogamous species select mates based on behavioral cues rather than morphological phenotypes. This hypothesis seems more plausible for two reasons. First is that, as mentioned above, mates of monogamous individuals contribute more than genetic material to their offspring. Morphological signals, like bright coloration and long tails, while being influenced by environmental factors, often reflect a strong genetic component. In contrast, behavioral phenotypes are generally regulated by a more complex interaction of genes and environment that may serve as a more honest indicator of mate quality, especially when that mate must contribute parental care to offspring. A long tail may not indicate a male’s ability to
provide food for his young as well as constructing an elaborate bower that requires time, energy and an ability to successfully navigate the surrounding environment. A second reason that behavioral phenotypes may be used in mate choice decisions among monogamous species is that in monogamy, both sexes should be choosy. The drastic ornaments observed among polygamous species almost appear exclusively in a single sex because one sex is considered ‘choosy’ and the other sex, competitive, when finding mates. When both sexes are choosy and competitive, the traits and characters selected for should appear in both sexes. Many displays of behavior can be expressed by both males and females and/or involve interactions between the two.

These behavioral phenotypes are widespread among monogamous animals and many species are famous for the mutual behaviors they engage in prior to mating (Huxley 1914; Lack 1940; Lehrman 1964; Bastock 1967; reviewed in Wachtmeister 2001). The Western grebe (*Aechmophorus occidentalis*) serves as an exemplary model of this. Their ritualized displays include vocal duets, preening, shaking, diving and a ‘rushing’ behavior in which both sexes run noisily across the surface of the water for 10-20 m at a time (Neuchterlein & Storer 1982). Other monogamous animals show similar behavioral displays as well as shared activities including territorial defense and nest building. These activities occur prior to reproduction, and selective partner preference, along with the suite of behaviors, is collectively referred to as the formation of a pair bond. Pair bonds can form days to weeks prior to actual reproduction and last for an animal’s entire lifetime.
The formation of a pair bond has been hypothesized to serve many functions, not least of which is a prolonged period during which individuals continuously assess their partner’s quality (Zahavi 1975; Andersson 1994). In this way, the behaviors associated with pair bonding can serve as a proxy for elaborate sexual signals to facilitate mate choice. Furthermore, if these behaviors are under the action of sexual selection, the adaptive significance of pair bonding could differ between the sexes. Sex differences in pair bonding have long been assumed in the field of behavioral ecology, but experimentally testing for these differences is difficult due to the complex interdependence of the behavior of the two individuals.

Mate removal can be used as a technique to address this issue. In a species of duetting wren (*Thryothorus nigricapillus*) mate removal experiments have explored the function of courtship song in males and females. Levin (1996) showed that no differences exist in the courtship song between new or old mates and demonstrated that male song was tightly liked to mating status (paired/unpaired), while female song was not, suggesting that this courtship behavior has different roles in the sexes. In a similar manner, a study in the monogamous convict cichlid (*Amatitlania nigrofasciata*) showed that immediately after losing a partner, females can successfully reproduce with a new mate following an abbreviated pair bonding period while males cannot (Bockelman & Itzkowitz 2008).

In such studies, it is difficult to determine the contribution that prolonged mate assessment played as an evolutionary driving force in the formation of pair bonds. The following experiments attempt to address this issue by exploring the mate assessment
process of both males and females of a serially monogamous species after formation of a pair bond. If the maintenance of a pair bond serves as a prolonged period of mate assessment, bonded males and females should continue to visit and court other potential mates until reproduction occurs. Additionally, the amount of time spent in a pair bond might influence this behavior as well as other factors like the relative difference in quality between potential mates.

These dynamics were explored in a series of laboratory experiments, controlling for intra-sexual competition and with a focus on identifying sex differences in behavior. Another study focusing specifically on the importance of male courtship in female mate choice decisions was conducted by experimentally reducing the display of certain types of behavior in males. Taken as a whole, these studies shed light on the ultimate function of pair bond formation, and more generally on the action of sexual selection within monogamous species. Specific hypotheses and methods are described in the following chapters.
Animal Model: *Amatitlania nigrofasciata*

An appropriate model species for the study of sex differences in mate assessment throughout pair bonding is the convict cichlid, *Amatitlania nigrofasciata*. These fish are a serially monogamous, bi-parental species endemic to freshwater streams and lakes of Central America (Bussing 1987). The system has been studied extensively in the laboratory setting and in the field due to its unique social system, which allows for empirical studies of aggression, monogamy, mate choice and parental care (McKay 1977; McKay 1986; Keenleyside et al. 1990; Wisenden 1994a, 1994b, 1995; Wisenden et al. 1995; Gagliardi-Seeley & Itzkowitz 2006). More recently there has been interest in the neuroendocrine mechanisms underlying these behaviors (Earley et al. 2006; van Breukelen 2008; Wong et al. 2008; Oldfield & Hofmann 2010).

Adults form size-assortative pairs in which males are usually 10.0 – 20.0 mm larger than their female partner (McKay 1986; Wisenden 1995). In the field, males and females first form pairs and then secure a nest site in a small cave or crevice where females deposit adhesive eggs. Several days after spawning, fertilized eggs hatch into young that are still absorbing their egg sac and unable to swim. The young emerge from the nest as free swimming fry between four and five days post-hatching.

Both male and female parents provide parental care to the young for 4-6 weeks after hatching. Parental behaviors include nest defense, anti-predatory behaviors, and retrieval behaviors, as well as providing food for the young by fin digging and leaf lifting (Keenleyside et al. 1985; Wisenden 1994a; 1994b; 1995; Wisenden et al. 1995; Zworykin et al. 2000). Both sexes have been shown to be capable of performing all parental tasks;
however sex-typical roles are often observed, with females providing direct care and males responsible for indirect care primarily through defense (Keenleyside 1985; Keenleyside et al. 1990; Lavery & Reebs 1994; Wisenden 1995; Itzkowitz et al. 2005; Snekser & Itzkowitz 2009).

When the young reach independence, the pair bond dissolves and adults may form new pair bonds and attempt another breeding bout. In an extensive field study, twenty-eight percent of breeding males raised multiple broods in a single field season, while only five percent of females attempted a second brood (Wisenden 1995). In the laboratory under constant conditions, females spawn multiple times within a few weeks with the same or different partners (Wisenden 1993, pers. obs.). The limiting factors that hinder females from producing multiple broods in natural settings are unclear; however energy and/or nest availability likely play a role (Lavery & Keiffer 1994; Gumm & Itzkowitz 2007).

The following experiments focus on male and female mate assessment after pair bond formation has occurred. Prior to forming a pair bond, both sexes engage in unique mate searching tactics and courtship behaviors (Santangelo & Itzkowitz 2004; Santangelo 2005). These behaviors are difficult to observe in the field due to intra-sexual aggression (Santangelo & Itzkowitz 2006). In the laboratory, a choice apparatus that restricts individuals to specific arenas allows for observations of courtship and preference in the absence of intra-sexual competition (Santangelo & Itzkowitz 2004; Santangelo 2005; Santangelo & Itzkowitz 2006; Gagliardi-Seeley et al. 2008).
A key feature of convict cichlid mate choice is that both males and females prefer the larger of two mates when given a choice (Noonan 1983; Keenleyside et al. 1985; Nuttal & Keenleyside 1993; Beeching & Hopp 1999; Gagliardi-Seeley et al. 2008). Large size may confer fitness benefits in many ways. Among teleost fish, large females are usually more fecund (Gross & Sargent 1985; Sargent et al. 1986), although some evidence suggests that female convict cichlids adjust egg production based on external factors so that size does not necessarily equate to fecundity in this species (Wisenden 1993). Large male convict cichlids are more adept at territory defense (Gagliardi-Seeley & Itzkowitz 2006) and pairs containing large males are more successful in securing territories from pairs containing smaller males (Draud & Lynch 2002). For both sexes, choosing the largest available mate likely increases fitness, and size can serve as a proxy for mate quality.

As in other systems, mate choice in convict cichlids is likely based on cues other than size alone. Both sexes demonstrate courtship behaviors (brushes, quivers, and tail beats); however, there is evidence that neither sex prefers mates that perform more courtship behaviors or that court at a higher rate (Santangelo & Itzkowitz 2004). Whether other factors, like prior experience with a partner or reproductive status, might influence mate assessment and choice is unknown.

In contrast to other monogamous systems, there is little evidence that convict cichlids will abandon a bonded partner prior to spawning when presented with a potential mate of higher quality (but see Triefenbach & Itzkowitz 1998; van Breukelen & Draud 2005). Pair bonds dissolve during the parental care stage, but commonly only males
desert their partner at this point (Wisenden 1994b; Lamprecht & Rebhan 1997; Jennions & Polakow 2001).

Whether mate loss occurs due to divorce, desertion, or predation, females and males respond differently. When mate loss occurs just prior to spawning, females are likely to spawn with a new male immediately, while males require a longer period of time before spawning with another female (Bockelman & Itzkowitz 2008). It is unclear if this pattern is a result of differences in reproductive physiology or difficulties in dissolution and formation of a pair bond.

The experiments in this proposal build on the current body of literature to explore further the factors involved in mate assessment throughout pair bond formation. The influences of courtship, size, reproductive status, and past experience are examined in the absence of intra-sexual competition to determine if there are sex differences in mate assessment and if these sex differences follow the expected predictions of current theories of sexual selection.
Dissertation Outline

In Chapter II, sex differences in the function of pair bonding were studied by giving both male and female convict cichlids a choice of potential mates at varying stages of pair bond formation. It was hypothesized that females, but not males, would alter the way they interacted with potential mates based on the length of time in a pair bond. Focal subjects were first paired and then placed in a choice apparatus allowing them access to both their partner and a novel member of the opposite sex. Clear sex differences were observed in behavior, both in the time spent with potential mates as well as behaviors directed toward them. Females preferred their partner over another male at all stages of pair bonding, while males showed this pattern only early in pair bond formation.

Contrary to the initial hypothesis, the stage of pair bonding had a similar effect on both males and females, with increased partner preference immediately after pair bond formation. While both sexes visited novel individuals throughout pair bonding, these visits appeared to be a reflection of territorial aggression rather than continuous mate assessment.

In Chapter III, a second set of experiments using a similar methodology were conducted to further delineate the finding that pair bond formation does not act as a prolonged period of mate assessment. At 4 stages of pair bonding, focal individuals were given a choice between their current partner and another potential mate of higher quality (larger size). Here, the sex differences observed in Chapter 2 were no longer present. Duration of pair bonding had no effect on male or female behavior, and focal individuals did not show a time-based preference for either their partner or the novel individual at
any stage of pair bonding. Observations of courtship and aggression suggest that the similar patterns of mate searching between the sexes are likely the result of separate behavioral mechanisms.

Finally, in Chapter IV, the role of courtship behaviors in mate assessment was explored by observing the effects of reduced male courtship upon the ways in which males and females interacted. It has been hypothesized that the expression of specific types of courtship behavior in monogamous species is more important than discrete morphological characteristics when selecting a suitable mate. Males were treated with an androgen receptor blocker, flutamide, which subsequently decreased their courtship. Females, however, did not discriminate between flutamide-treated and control males. This result suggested that courtship behavior facilitates aspects of reproduction independent of mate choice in this species.

These studies suggest that the function of pair bonding differs between the sexes, and that these differences primarily reflect divergence in the way natural selection acts between the sexes, rather than resulting from the effects of sexual selection via the ability to attract and secure mates. This conclusion is discussed in Chapter V, with implications for the operation and expression of sexual selection in other monogamous species.
Sex differences in the function of pair bonding

Abstract

Many monogamous animals engage in a period of pair bonding prior to their ultimate mate choice (reproduction). One hypothesis about the function of this behavior is that pair bonding allows individuals to continuously assess both their partner as well as other individuals. For example, pair bonded individuals could visit and interact with other potential mates and abandon their current partner if they encounter an individual of higher quality. This hypothesis was tested in the convict cichlid, Amatitlania nigrofasciata, by providing males and females with a choice between their partner and a novel individual of the opposite sex at various times after pair bond formation. Clear sex differences were observed in behavior, with females spending significantly more time with their partner, while males spent more time with a novel female. The stage of pair bonding, however, affected this pattern, with both sexes showing decreased partner preference with increased time in a pair bond. Observations of courtship and aggression indicate that, at least within this paradigm, neither sex actively courts a novel individual after a pair bond is formed. The time males spent with other females appears to be a reflection of territorial aggression. This suggests that the pair bond and associated behaviors that occur prior to mating serve an alternative function to acting as a prolonged period of mate assessment.
Introduction

Monogamous mating systems have been described within certain animal taxa for centuries; however the term ‘pair bond’ was not applied to these associations until the mid 1900’s. ‘Pair bond’ has since become the common nomenclature to describe the selective affiliation some individuals show toward a specific member of the opposite sex (Lack 1940). Initially, this term was applied exclusively to birds, but was quickly adopted by comparative psychology and ethology to describe associations in other taxonomic groups as well, including humans. The formation and maintenance of a pair bond is now a defining characteristic of monogamous species (social monogamy), more so than sexual monogamy where a single male and female mate exclusively, and is marked by the display of various types of mutual behavior between partners (Orians 1969; Kleiman 1977; Wittenberger & Tilson 1980; Wickler & Seibt 1983). These behaviors occur for varying lengths of time prior to mating and often continue into parental care.

Among birds, monogamy is the predominant mating system and nearly 90% of species form pair bonds prior to copulation (Lack 1940; Clutton-Brock 1991). The behaviors that these species show during pair bond formation vary, but generally include mutual defense of a territory, construction of a nest, and ritualized displays of courtship. In zebra finches, *Taeniopygia guttata*, unfamiliar birds begin forming pair bonds almost immediately when introduced in the laboratory. Males court females by singing, and if the female accepts the male, together they begin to search for a suitable nest site. During the next several days, the pair will often maintain tactile contact and engage in allopreening (Morris 1954; Silcox & Evans 1982). In ring doves, *Streptopelia risoria*,...
males and females also begin pair bond formation almost immediately after introduction to a cage containing nest building materials. The first day of pair bonding consists mainly of the male strutting, bowing and cooing at the female. If the female accepts, the pair signals the selection of a nest site with a distinctive call performed by both sexes and construction of the nest begins (Lerhman 1958; 1964). In Bewick’s swans, *Cygnus columbianus bewickii*, similar behaviors occur during the breeding season, but males and females also remain paired throughout the non-breeding season. Partners remain in spatial proximity during the winter and assist each other in finding food and engaging in aggressive encounters with rivals (Scott 1980). Year round pair bonds are present in several other avian species, especially among large birds of prey (Lack 1940).

Apart from birds, however, monogamy is relatively rare and may only occur in 3-5% of mammals and fish (Kleiman 1977; Dunbar 1984; Gross & Sargent 1985; Clutton-Brock 1991). Monogamy is even less frequent among the remaining vertebrate taxa and virtually non-existent among invertebrates. When pair bonds form in taxa other than birds, similar suites of behavior are observed. Territories are often secured and defended, nests constructed and partners engage in ritualized courtship behaviors. Within mammals, monogamy is common among canids (wolves, jackals and foxes) where pairs usually maintain spatial proximity, mate exclusively with their partner and ‘nest’ in caves (Kleiman 1977). Monogamy is found in rodent species as well, such as prairie voles, *Microtus ochrogaster*, where males and females show a time-based preference for their partner, share the duties of nest building and food caching, and engage in mutual grooming (Thomas & Birney 1979). Behaviors that appear to be fundamental to pair
bonding for monogamous mammals are tactile stimulation and vocalizations, although scent marking and olfactory stimulation are also key components (Ralls 1971; Eisenberg & Kleiman 1972; Thomas 2002).

Despite the plethora of descriptions of behaviors associated with pair bond formation and maintenance, evolutionary explanations for this process remain poorly understood (Kleiman 1977; Wittenberger & Tilson 1980; Reynolds 1996; Wachtmeister & Enquist 2000). Often, the display of a pair bond continues into stages of parental care and is rationalized by the need for bi-parental care to successfully raise offspring where a single parent could not (Clutton-Brock 1991). In some instances, however, specific behaviors occurring after pair bond formation, but prior to fertilization remain enigmatic when they do not appear to directly contribute to offspring care. The construction of a nest has clear direct benefits for young, but vocal duetting among song birds may not. Multiple hypotheses have attempted to explain the biological significance of these behaviors, but rarely have these hypotheses been tested experimentally.

The most simplistic explanation for pair bond behaviors is that they must contribute to parental care in some way, albeit indirectly. The display of mutual behaviors prior to mating could help pairs coordinate in preparation for the shared duties required after young are born. If supported, this hypothesis predicts that pairs showing greater displays of courtship behavior or spending a longer duration in a pair bond would be better parents and therefore experience increased fitness. The benefits of increased coordination has demonstrated among monogamous animals that raise subsequent broods with the same partner (Cooke et al. 1981; Choudhury & Black 1994). A second
hypothesis is that the formation of a pair bond is necessary for the synchronization of reproductive physiology between the sexes (Pfaff 2002). All vertebrate taxa show a conservation of the hypothalamic-pituitary-gonadal (HPG) axis, which regulates the production of gametes. In males, this system typically operates continuously. In contrast, females of many species do not constantly produce mature gametes (Blaustein & Erskine 2002). Some species, including humans, have evolved regular cycles of fertility with eggs maturing based on either external or internal triggers (Conaway 1971). But for the majority of monogamous species, females do not produce or release mature ova until stimulated by specific behaviors performed by their partners. Ring doves show this pattern (Lehrman 1964) as well as cats and several species of rabbits (Conaway 1971).

A third hypothesis is that the ultimate function of pair bonding serves as a prolonged period of mate assessment during which both sexes gather information about the quality of both their partner and other potential mates prior to copulation (Andersson 1994). In this case, the formation of a pair bond would be the direct result of sexual selection rather than natural selection, and be driven by competition both between and within the sexes for increased mating success. This hypothesis is supported by the high incidence of extra-pair copulation and divorce observed among avian species (Choudhury 1995).

These hypotheses are clearly not mutually exclusive. This is perhaps why few studies have attempted to delineate between them. It is likely that behaviors associated with pair bond formation function in all the ways listed above. Nevertheless, the possibility exists that the function of pair bond formation differs among different
monogamous species that differ in ecological niche and life history. Furthermore, if driven by sexual selection, behaviors associated with pair bond formation could serve different purposes in the two sexes within a given species. Here, I set out to determine if the latter hypothesis, that pair bond formation provides a prolonged period of continuous assessment of mate quality, is supported in the convict cichlid fish, *Amatitlania nigrofasciata*. Pair bonded males and females were provided with a choice of alternative mates at varying times after pair bond formation. In each instance, one potential mate was a pair-bonded partner of the focal fish and the other was a novel, size-matched individual. If the hypothesis is supported, focal individuals should continuously visit and court other potential mates throughout pair bonding. In natural populations, this behavior could be masked by mate guarding tactics and/or intra-sexual competition, so a choice paradigm was employed to control these influences.
Methods

Experimental subjects consisted of lab raised individuals bred from a mix of both wild caught and commercially available convict cichlids. The behavior of animals in the lab is similar to that of natural populations observed in Costa Rican streams (pers. obs.). All animals were housed in single sex 473l tanks maintained at 25 ± 3 °C on a 14/10 hr light/dark cycle and fed commercially available food pellets (Finfish Starter, Ziegler Bros Inc.) ad libitum. After testing, subjects were returned to separate stock tanks to avoid pseudoreplication.

Two separate experiments were conducted in this study (male choice and female choice), but using the same general methodology so as to make direct comparisons between the sexes possible. All procedures were identical for male and female choice experiments except where noted.

At the start of each replicate, a male and female convict cichlid was added to a 76l “home aquarium” (Figure 2.2A). Males within each replicate were larger by approximately 10 mm total length than their female counterparts, as is typical of patterns observed in the field (Wisenden 1995). Total length, standard length, height, width, and body mass were recorded prior to testing (for descriptions, see Figure 2.1). A clear plastic divider was placed approximately 10 cm from one end of the aquarium creating an intruder compartment to which a small conspecific juvenile was added. The presence of an intruder facilitates pair bond formation in a related cichlid (Itzkowitz & Draud 1992). Each aquarium contained an air stone and a terra cotta flower pot to be used as a nesting site. All pairs were monitored daily for the formation of a pair bond. A pair bond was
considered formed on the first day that individuals were observed together in the nest site or simultaneously attacking the juvenile intruder (Draud & Lynch 2002).

In each pair, a single animal was selected as the focal subject. In the male choice experiment, focal subjects were male and in the female choice experiment, focal subjects were female. Focal subjects were assigned to one of 4 treatment groups associated with a specific time point after pair bond formation. These time points did not refer to evenly spaced intervals after the first day of pair bonding and were labeled as 1) early, 2) mid, 3) late and 4) post. All treatment groups consisted of 10 replicates with the exception of the mid-pair bonding group in the male choice experiment in which N = 9. Individuals were size-matched across treatment groups (Tables 2.1-2.4). Subjects in the ‘early’ pair bond treatment group were moved to a choice aquarium on the first day of pair bond formation. Subjects in the ‘mid’ treatment group were moved into a choice aquarium 4 days after pair bond formation. Four days was considered an approximate half-way point from pair bond formation to spawning based on a previous study of spawning time (Bockelman & Itzkowitz 2008). As female convict cichlids near spawning, their ovipositor descends from the cloacal opening and becomes apparent as a visual protuberance (Bockelman & Itzkowitz 2008; pers. obs.) The appearance of the ovipositor was used as a general indicator that a pair was nearing spawning and focal individuals in the ‘late’ treatment group were moved to a choice tank when the ovipositor descended. The appearance of the ovipositor occurred at an average of 10 days after the start of replicates in the male choice experiment and 11 days in the female choice experiment. The final treatment group, ‘post’, refers to individuals tested post spawning. In these replicates, males and
females were allowed to successfully spawn in their home tank. Within 24h of spawning, the fertilized eggs were removed from the home tank and the focal individual was moved to a choice tank an additional 24 h later.

At the specific time-point for a given treatment, the focal subject was removed from the home aquarium and placed into a “choice aquarium” (Figure 2.2B). The choice aquarium (265 l) contained 2 opaque plastic partitions creating 3 equal-sized compartments. The partitions contained holes allowing the focal individual free access to the entire aquarium, while limiting visual contact between the outer compartments. Each of the outer two compartments contained a flower pot as a potential nesting site.

After introduction to the neutral compartment, the focal individual was given 1h to acclimate to the new aquarium. At the conclusion of acclimation, a potential mate was placed in each of 2 clear plastic box enclosures in the neutral compartment beginning a 4h visual comparison period (for explanation, see Gagliardi-Seeley et al. 2008). Each plastic box contained holes allowing for water flow and chemical communication, but limited physical interactions and visual communication of the fish in the boxes. One of the potential mates was the current partner of the focal subject, while the other was a size-matched (±3mm) individual pulled from stock populations that had not previously interacted with the focal subject. This animal will be referred to throughout as a ‘novel’ potential mate.

At the conclusion of the comparison period, each potential mate was moved to one of the outer compartments. In the female choice experiment, males were too large to move through the holes in the dividers and were thus restricted to their compartment. In
the male choice experiment, it was necessary to tether the potential female mates to their nest sites. Tethering was done by inserting a small microfilament loop through the epaxial musculature of the female and attaching this loop to another piece of microfilament secured to the flower pot. All surgical procedures were conducted in accordance with Lehigh IACUC protocol #86 and caused minimal distress to the animals. Tethered females could move throughout their compartment, interact with males and spawn successfully (Santangelo & Itzkowitz 2004; Santangelo 2005; pers. obs.)

Behavioral Observations

Focal subjects remained in the choice tank for a 72 hr period after introduction. The position of the focal individual in the 3 compartments was observed 5 times daily and 30-min video recordings were taken 24 and 48 hrs after the placement of potential mates in the outer compartments. Behavioral data for each of the 3 fish in the choice aquaria was gathered from video recordings using JWatcher behavioral event recorder software (UCLA & Macquarie University). The number of courtship (brushes, tailbeats, quivers) and aggression (bites, chases, displays) behaviors were totaled directed toward either the partner or novel individual (for detailed descriptions of behaviors, see Appendix A). The number of visits of the focal individual to each compartment as well as total time spent in each compartment was also recorded.

Statistical Analysis

Data taken on the position and movement of focal subjects were analyzed separately from measures of aggression and courtship, as time could be spent in any of the 3 compartments, whereas measures of aggression and courtship could only be
observed when potential mates were available to interact with. Apart from the positional observations of focal subjects taken throughout the 72h period, behavioral measures used in the final analysis were obtained only from video recordings taken 24h after introduction to the choice aquarium. The percent of observations in and visits to a given compartment were compared using a GLM mixed-model ANOVA with three levels of a within-subjects factor (partner, neutral or novel) and two between-subjects factors of treatment group (early, mid, late or post) and sex (male or female). Planned pair-wise comparisons were conducted within each sex at each time point treatment for percent of observations and visits between the partner and the novel individual using paired t-tests. Time spent with potential mates was converted into a measure of strength of preference by subtracting the time spent with the novel individual from the time spent with the partner. Strength of preference was compared between sexes and across time point treatments using a 2x4 factorial ANOVA. Planned pair-wise comparisons were conducted post hoc within each sex using Fisher’s Least Square Differences.

Behaviors directed toward potential mates were grouped together into 2 variables as a total measure of either aggression or courtship. Aggression and courtship toward potential mates were compared in a General Linear Model repeated-measures ANOVA similar to above, but with only 2 levels of the within-subjects factor (partner/novel). All statistical tests were conducted using SPSS, were two-tailed and alpha was set at 0.05.
Results

The sexes differed in the strength of preference for their partner ($F_{1,71} = 18.27$, $p < 0.001$) and this was influenced by the stage of pair bonding as well ($F_{3,71} = 4.56$, $p = 0.006$). Females preferred their partner more than did males at all 4 stages of pair bonding (Fig. 2.3). Males actually showed a negative partner preference at most time points, indicating that males spent more time with novel individuals than their partner. The only exception to this pattern was the early treatment group. This pattern was reflected in females as well, with a stronger preference for a partner early in pair bonding. There was not a significant interaction of focal sex with stage of pair bonding on the strength of preference ($F_{3,71} = 0.294$, $p = 0.83$).

Males showed a significantly higher preference for their partner in the early treatment group than in mid ($p = 0.04$), late ($p = 0.03$) or post ($p = 0.05$) groups (Fig. 2.3). The latter three groups were not significantly different from each other (mid vs. late: $p = 0.90$; mid vs. post: $p = 0.84$; late vs. post: $p = 0.74$). Females showed a similar pattern; however, the preference demonstrated early in pair bonding was not significantly different from that at the mid pair bonding treatment ($p = 0.14$), but was different from measurements at late ($p = 0.05$) and post ($p = 0.01$). For females there were no significant differences among the last 3 time point treatments (mid vs. late: $p = 0.58$; mid vs. post: $p = 0.27$; late vs. post: $p = 0.58$, Fig. 2.3).

There were several significant main effects as well as interactions of factors influencing the percent of observations and the visits to a specific compartment by focal individuals (Table 2.5). The most notable of these is a significant main effect of sex on
the number of visits to compartments ($F_{1,71} = 35.412, p < 0.001$). Males were more likely to move between compartments than females, regardless of the stage of pair bonding.

Males showed no differences in the number of visits to their partner or novel female at any stage of pair bonding (early: $t = 2.025, p = 0.07$; mid: $t = -0.185, p = 0.44$; late: $t = -0.557, p = 0.59$; post: $t = -0.164, p = 0.87$; Fig. 2.4A). They also showed no significant difference in the percent of observations with either female early ($t = -0.441, p = 0.67$), mid ($t = -0.979, p = 0.36$), or late ($t = -1.50, p = 0.17$) in pair bonding (Figure 2.5A). In the post spawning treatment group, males showed a significantly higher percentage of observations with the novel female than the partner ($t = -3.096, p = 0.01$).

Within the female choice experiment, females showed significant differences in the number of visits ($t = 3.881, p < 0.01$) and percent of observations ($t = 8.143, p < 0.001$) between their partner and a novel male early in pair bond formation as well as at the mid treatment group (visits: $t = 3.280, p = 0.01$; observations: $t = 3.274, p = 0.01$). Females did not show differences in these behavioral measures at the late (visits: $t = 1.593, p = 0.15$; observations: $t = 1.403, p = 0.19$) or post (visits: $t = 0.152, p = 0.88$; observations: $t = 0.502, p = 0.63$) time points.

The display of stereotypic aggression and courtship behavior were relatively sparse, with only a few individuals displaying behavior during the 20-min video recordings (Table 2.6). The statistical results of the GLM repeated measures ANOVA comparing these behaviors are given in Table 2.7. There were significant effects of focal sex ($F_{1,71} = 22.345, p < 0.001$) and potential mate ($F_{1,71} = 20.020, p < 0.001$) on levels of aggression with males performing an average of $9.58 \pm 2.03$ more aggressive acts than
females. Aggression was overwhelmingly directed at novel individuals with a mean of 9.16 ± 2.19 behaviors compared to only 0.35 ± 0.18 acts of aggression directed toward partners. The significant effect of potential mate on courtship behavior ($F_{1,71} = 10.758, p = 0.002$) reveals that when performed, courtship was directed toward the current partner. Main effects and interactions of all other factors on courtship behavior were non-significant.
Discussion

Both male and female convict cichlids visited novel potential mates after pair bond formation. The pattern of time spent with these novel individuals differed greatly between the sexes and was affected by the time the focal individual spent in a pair bond. Both sexes showed the greatest time-based preference for their partner early after pair bond formation. While females continued to show a partner preference throughout the pair bond, males did not. Males spent more time with a novel individual at later stages of pair bonding.

This result could be interpreted as males pursuing other mating opportunities apart from their partner. The same could be said of the females as well, albeit to a lesser degree. On the other hand, behavioral observations suggested time spent with novel individuals was more likely the result of territorial aggression. Almost no courtship behavior was performed toward novel individuals and scarcely any aggression was directed toward partners. Thus while males spent more time with novel females, they would chase, bite and harass this female, often returning to the compartment containing their partner.

Studies of pair bond formation in mammals, especially voles, indicate that once a pair bond has formed, individuals show a time-based preference for their partner. This observation, however, might only reflect an appropriate strategy for animals with a certain ecological niche (Crews & Moore 1986). For some species, like the convict cichlid, once a pair has formed, partners often divide specific tasks and may spend little time in close proximity. For example, one individual often remains to defend the nest.
while the other forages (Wisenden 1994a; Itzkowitz et al. 2001; 2002). Sex differences in nest defense can contribute to spatial separation of the bonded pair. Defending the nest site from conspecific intruders is crucial for fitness in this species. In the field, the availability of suitable nest sites is limited and thought to be the most rate limiting factor in brood production (Wisenden 1995; Gumm & Itzkowitz 2007). As such, intruders must be attacked, but generally a single individual will engage in this aggression, while the partner remains vigilant for trespassers. Territorial aggression can be directed toward intruders of both sexes as both could usurp a nest site or feed on unguarded young after they hatch (pers. obs.). When these factors are considered, the time spent away from partners by males in this paradigm is indicative of territorial aggression rather than active pursuit of extra-pair mating attempts.

For both sexes, focal individuals showed a stronger partner preference early in pair bond formation than later, which has interesting implications. Proximity and tactile stimulation appear to play a role in the formation of a pair bond, but once established, the need for physical proximity decreases. This could be explored on a smaller time scale of hours to determine at what time point individuals start to show greater separation from their partner. It would also be interesting to determine if there are sex differences in this time-point, or a conflict between the sexes in when to spend time together and when to separate into parental roles.

A difficulty in interpreting the results of this study is the lack of behavior observed from the video recordings. Clear displays of courtship behavior (brushes, tailbeats and quivers) were infrequent and only observed in a few focal individuals. This
finding, however, is similar to rates of courtship behavior published elsewhere 
(Santangelo & Itzkowitz 2004; Santangelo 2005) as well as observations of natural 
populations of this species (pers. obs.). With these behaviors occurring so infrequently, 
they seem more likely to play a role in the continued coordination of the pair, rather than 
acting as signals of mate quality. This finding corroborates that of Santangelo & 
Itzkowitz (2004) that neither male nor female convict cichlids prefer individuals that 
demonstrate more courtship behaviors or that court at a higher rate.

While the hypothesis that pair bond formation serves as a prolonged period of 
mate assessment cannot be entirely rejected based on these results, there clearly is no 
evidence to suggest that this is indeed the function of this behavior. Given the sex 
differences observed and the consistency in behavior at various stages in pair bonding, it 
seems much more likely that mating decisions occur at the onset of pair bond formation 
and that behaviors taking place prior to spawning contribute more to the synchronization 
of reproductive physiology and coordination of shared activities. Mate abandonment after 
pair bond formation is probably rare among convict cichlids and likely happens only 
when forced through intra-sexual competition (van Breukelen & Draud 2005).
Table 2.1: Mean ± SD size measurements (mm) of focal males in male choice experiment. Males were size matched among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>52.21 ± 4.45</td>
<td>68.49 ± 4.30</td>
<td>22.81 ± 0.75</td>
<td>9.92 ± 0.61</td>
<td>5.66 ± 0.69</td>
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<tr>
<td>Mid</td>
<td>54.46 ± 2.90</td>
<td>69.78 ± 3.05</td>
<td>23.58 ± 1.33</td>
<td>10.19 ± 0.81</td>
<td>6.30 ± 1.15</td>
</tr>
<tr>
<td>Late</td>
<td>54.18 ± 2.54</td>
<td>69.36 ± 3.85</td>
<td>23.30 ± 1.34</td>
<td>10.08 ± 0.61</td>
<td>6.25 ± 0.71</td>
</tr>
<tr>
<td>Post</td>
<td>53.75 ± 3.60</td>
<td>69.02 ± 3.57</td>
<td>23.06 ± 1.62</td>
<td>9.69 ± 0.77</td>
<td>5.98 ± 1.11</td>
</tr>
</tbody>
</table>
Table 2.2: Mean ± SD size measurements (mm) of focal females in female choice experiment. Females were size matched among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>42.24 ± 1.83</td>
<td>54.54 ± 2.25</td>
<td>18.36 ± 0.97</td>
<td>8.23 ± 0.91</td>
<td>3.18 ± 0.43</td>
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<tr>
<td>Mid</td>
<td>42.95 ± 2.13</td>
<td>53.85 ± 3.29</td>
<td>18.25 ± 1.05</td>
<td>8.33 ± 0.46</td>
<td>3.00 ± 0.55</td>
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<tr>
<td>Late</td>
<td>42.86 ± 2.94</td>
<td>54.01 ± 3.23</td>
<td>18.15 ± 1.32</td>
<td>8.19 ± 1.01</td>
<td>3.05 ± 0.65</td>
</tr>
<tr>
<td>Post</td>
<td>43.35 ± 2.68</td>
<td>55.13 ± 3.17</td>
<td>18.30 ± 0.71</td>
<td>8.10 ± 0.64</td>
<td>3.02 ± 0.47</td>
</tr>
</tbody>
</table>
Table 2.3: Mean ± SD size measurements (mm) of partner and novel females provided to males. Females were size-matched within each replicate and among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>47.63 ± 4.35</td>
<td>58.99 ± 2.80</td>
<td>20.39 ± 1.72</td>
<td>9.05 ± 0.88</td>
<td>4.04 ± 0.66</td>
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<tr>
<td>Novel</td>
<td>46.64 ± 2.12</td>
<td>59.05 ± 2.57</td>
<td>19.76 ± 1.36</td>
<td>8.70 ± 0.73</td>
<td>3.94 ± 0.69</td>
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</tr>
<tr>
<td>Partner</td>
<td>47.24 ± 3.21</td>
<td>60.04 ± 3.47</td>
<td>20.16 ± 1.46</td>
<td>9.09 ± 1.09</td>
<td>4.17 ± 0.82</td>
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<td>Novel</td>
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<td>59.61 ± 2.18</td>
<td>20.36 ± 1.42</td>
<td>9.10 ± 0.75</td>
<td>3.87 ± 0.51</td>
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<td></td>
</tr>
<tr>
<td>Partner</td>
<td>47.01 ± 3.41</td>
<td>59.28 ± 3.86</td>
<td>19.75 ± 1.22</td>
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<td>19.87 ± 1.43</td>
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<tr>
<td>Post</td>
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<tr>
<td>Partner</td>
<td>46.47 ± 2.14</td>
<td>58.93 ± 2.95</td>
<td>20.06 ± 0.79</td>
<td>8.99 ± 0.61</td>
<td>4.04 ± 0.56</td>
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<tr>
<td>Novel</td>
<td>46.60 ± 2.95</td>
<td>59.18 ± 3.21</td>
<td>18.86 ± 3.47</td>
<td>8.80 ± 0.93</td>
<td>3.89 ± 0.79</td>
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</table>
Table 2.4: Mean ± SD size measurements (mm) of partner and novel males provided to females. Males were size-matched within each replicate and among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL (g)</th>
<th>TL (g)</th>
<th>H (g)</th>
<th>W (g)</th>
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<tr>
<td>Early</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Partner</td>
<td>49.48 ± 2.39</td>
<td>64.30 ± 3.12</td>
<td>21.58 ± 1.22</td>
<td>9.18 ± 0.89</td>
<td>4.83 ± 0.48</td>
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<tr>
<td>Novel</td>
<td>49.60 ± 3.54</td>
<td>64.29 ± 4.07</td>
<td>22.26 ± 2.07</td>
<td>9.47 ± 0.97</td>
<td>4.83 ± 1.04</td>
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<tr>
<td>Partner</td>
<td>49.90 ± 3.16</td>
<td>63.12 ± 3.82</td>
<td>21.58 ± 1.16</td>
<td>9.49 ± 0.71</td>
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<tr>
<td>Novel</td>
<td>50.15 ± 2.62</td>
<td>63.27 ± 3.88</td>
<td>21.79 ± 1.24</td>
<td>9.26 ± 0.69</td>
<td>4.72 ± 0.82</td>
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<tr>
<td>Late</td>
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<tr>
<td>Partner</td>
<td>50.28 ± 2.68</td>
<td>64.13 ± 3.94</td>
<td>22.64 ± 2.05</td>
<td>9.68 ± 0.97</td>
<td>4.51 ± 1.27</td>
</tr>
<tr>
<td>Novel</td>
<td>50.01 ± 4.70</td>
<td>64.48 ± 5.48</td>
<td>22.39 ± 1.32</td>
<td>9.78 ± 0.79</td>
<td>4.97 ± 0.56</td>
</tr>
<tr>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>50.91 ± 3.92</td>
<td>65.68 ± 4.30</td>
<td>21.90 ± 1.09</td>
<td>9.13 ± 0.83</td>
<td>4.96 ± 0.64</td>
</tr>
<tr>
<td>Novel</td>
<td>51.77 ± 3.87</td>
<td>65.68 ± 3.70</td>
<td>22.66 ± 2.38</td>
<td>9.99 ± 0.95</td>
<td>5.31 ± 1.15</td>
</tr>
</tbody>
</table>
Table 2.5: Results of a GLM repeated measures ANOVA showing main effects and interactions of factors on the percent of observations and number of visits of focal individuals to the three compartments in the choice aquarium.

<table>
<thead>
<tr>
<th>Effect</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Observations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment</td>
<td>5.125</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Focal sex</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Stage of pair bonding</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Compartment*Focal sex</td>
<td>22.991</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Compartment*Stage of pair bonding</td>
<td>2.660</td>
<td>0.02</td>
</tr>
<tr>
<td>Focal sex*Stage of pair bonding</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Compartment<em>Focal sex</em>Stage of pair bonding</td>
<td>0.459</td>
<td>0.84</td>
</tr>
<tr>
<td>Visits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment</td>
<td>30.691</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Focal sex</td>
<td>35.412</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stage of pair bonding</td>
<td>0.609</td>
<td>0.61</td>
</tr>
<tr>
<td>Compartment*Focal sex</td>
<td>16.204</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Compartment*Stage of pair bonding</td>
<td>1.371</td>
<td>0.23</td>
</tr>
<tr>
<td>Focal sex*Stage of pair bonding</td>
<td>0.795</td>
<td>0.50</td>
</tr>
<tr>
<td>Compartment<em>Focal sex</em>Stage of pair bonding</td>
<td>0.504</td>
<td>0.81</td>
</tr>
</tbody>
</table>
Table 2.6: Mean ± SE number of aggression and courtship behaviors directed toward potential mates in male and female choice experiments.

<table>
<thead>
<tr>
<th></th>
<th>Stage of pair bonding</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Mid</td>
<td>Late</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression toward partner</td>
<td>1.60 ± 1.38</td>
<td>0.11 ± 0.10</td>
<td>0.50 ± 0.31</td>
<td>0.50 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>Courtship toward partner</td>
<td>2.70 ± 4.52</td>
<td>0.11 ± 0.10</td>
<td>0.50 ± 0.34</td>
<td>1.30 ± 1.09</td>
<td></td>
</tr>
<tr>
<td>Courtship toward novel</td>
<td>0.00 ± 0.00</td>
<td>0.11 ± 0.10</td>
<td>0.00 ± 0.00</td>
<td>0.20 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression toward partner</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.10 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Aggression toward novel</td>
<td>0.10 ± 0.10</td>
<td>0.00 ± 0.00</td>
<td>0.10 ± 0.10</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Courtship toward partner</td>
<td>1.70 ± 1.17</td>
<td>0.40 ± 0.31</td>
<td>2.20 ± 1.17</td>
<td>0.20 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Courtship toward novel</td>
<td>0.10 ± 0.10</td>
<td>0.00 ± 0.00</td>
<td>0.20 ± 0.20</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7: Results of a GLM repeated measures ANOVA comparing main effects and interactions of factors on measures of aggression and courtship toward potential mates.

<table>
<thead>
<tr>
<th>Effect</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential mate (partner/novel)</td>
<td>20.020</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Focal sex</td>
<td>22.345</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stage of pair bonding</td>
<td>0.228</td>
<td>0.88</td>
</tr>
<tr>
<td>Potential mate*Focal sex</td>
<td>19.908</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Potential mate*Stage of pair bonding</td>
<td>0.185</td>
<td>0.91</td>
</tr>
<tr>
<td>Focal sex*Stage of pair bonding</td>
<td>0.229</td>
<td>0.88</td>
</tr>
<tr>
<td>Potential mate<em>Focal sex</em>Stage of pair bonding</td>
<td>0.168</td>
<td>0.92</td>
</tr>
<tr>
<td>Courtship</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential mate (partner/novel)</td>
<td>10.758</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Focal sex</td>
<td>0.002</td>
<td>0.96</td>
</tr>
<tr>
<td>Stage of pair bonding</td>
<td>1.741</td>
<td>0.17</td>
</tr>
<tr>
<td>Potential mate*Focal sex</td>
<td>0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>Potential mate*Stage of pair bonding</td>
<td>1.684</td>
<td>0.18</td>
</tr>
<tr>
<td>Focal sex*Stage of pair bonding</td>
<td>1.314</td>
<td>0.28</td>
</tr>
<tr>
<td>Potential mate<em>Focal sex</em>Stage of pair bonding</td>
<td>0.889</td>
<td>0.45</td>
</tr>
</tbody>
</table>
Figure 2.1: All animal subjects were measured for standard length (SL), total length (TL), height (H), width (W) and mass (M).
Figure 2.2: Diagrams of experimental aquaria. Subjects were initially paired in ‘home’ aquaria (A) and later transferred to a separate choice aquarium (B). Openings in the opaque dividers were offset to decrease visual contact between the outer compartments.
Figure 2.3: Mean (± SE) strength of preference of focal individuals for potential mates at various stages after pair bond formation. Positive values indicate a time-based preference for a partner; negative values indicate a time-based preference for the novel individual. Letters indicate significant differences in pair-wise comparisons conducted between each time-point treatment within each sex for females (a,b) and males (c,d).
Figure 2.4: Mean (+SE) number of visits to compartments containing the partner (solid) and novel (open) individuals by males (A) and females (B) at four stages of pair bonding. Note difference in scales.
Figure 2.5: Mean percent observations (+ SE) in compartments containing either the partner (solid) or novel individual (open) averaged over the 3 day experimental period for male choice (A) and female choice (B) experiments.
The influence of body size on mate preference throughout pair bonding

Abstract

Body size can be used as an indicator of mate quality in a variety of vertebrate species. Both male and female convict cichlids, *Amatitlania nigrofasciata*, prefer the larger of available mates when provided a choice. It is unclear whether this preference occurs only at the beginning of pair bond formation, or if it is continuously assessed throughout pair bonding. Here, pair bonded individuals were given the choice between their partner and another potential mate that was 15-20% larger in body size. Both sexes were tested at 4 times after pair bonding for measures of preference and courtship behavior. Neither sex showed a preference for large mates at any time after pair bond formation. Nor did they show a preference for their current partner. These results were based on averages for each group, but rather than suggesting that specific individuals showed no preference for either potential mate, this finding indicates that some focal individuals preferred the larger of the two mates and the others preferred their current partner. These data support that body size influences mate preference, but suggest that it is less likely to play a significant role in mate choice after individuals have formed a pair bond.
Introduction

Body size is one of the most easily recognizable characteristics of animals and is tightly linked to ecological niche specialization and a host of life history tradeoffs. The influence of body size can be observed in every behavioral dynamic of biological systems including locomotion, foraging strategies, anti-predator defense, dispersal, mating behavior and fight tactics. As such, body size is susceptible to a complex network of selection pressures. Moreover, for many animals body size is not rigidly fixed, but fluctuates with ontogeny and is influenced by both genetic and environmental factors (Shine 1990).

It is not surprising then that body size is commonly used as an indicator of mate quality (Kodric-Brown & Brown 1984; Ryan 1990; Andersson 1994). Body size can serve as a proxy for many factors related to fitness including age, health, sexual maturity, experience and resource holding potential. Individuals typically prefer the larger of available mates in a wide range of species of fish (Berglund et al. 1986; Sargent et al. 1986; Gagliardi-Seeley et al. 2008), birds (Price 1984; Møller 1990) reptiles (Censky 1997; Shine et al. 2001) mammals (Berger 1989; Charlton 2007), and invertebrates (Gwynne 1982; Aquiloni & Gherardi 2008).

In monogamous systems, both sexes should be choosy, and when body size varies, the larger of available mates should be preferred. In a natural setting where individual encounter rates are high, this selection process often results in size-assortative mating (reviewed in Jennions & Petrie 1997). That is, large males tend to pair with large females and small males pair with small females.
Size-assortative pairing clearly occurs in the convict cichlid, *Amatitlania nigrofasciata* (Wisenden 1994a). The resulting pair bonds, however, do not necessarily equate to reproduction. Convict cichlids have been known to ‘divorce’ or dissolve pair bonds prior to reproduction (Wisenden 1994b; van Breukelen & Draud 2005). In natural settings, it is largely unclear how or why pair bonds dissolve. In laboratory settings, divorce has been shown to occur only because of intra-sexual competition (van Breukelen & Draud 2005). That is, if a paired male loses an agonistic encounter with a rival, the female will leave her partner for the dominant male. Females that stay with their partner after losing an aggressive encounter are also likely to lose their nest site, and be forced to forgo reproduction for a given breeding season. Thus there is a high fitness advantage to divorcing a losing partner in cases of intra-sexual competition.

Whether individuals divorce a partner in the absence of intra-sexual competition is unknown in convict cichlids. Here, males and females were provided a choice of their current partner or a novel individual of larger size at various stages after pair bond formation. While both sexes benefit from mating with the larger of two partners, it was hypothesized that females would be more likely than males to abandon a current partner for a larger mate after forming a pair bond. This hypothesis is based on female convict cichlids being capable of spawning with another male shortly after losing a partner, while males require an extended period of pair bonding to spawn with a new mate (Bockelman & Itzkowitz 2008). Thus, males are more likely to incur a larger fitness cost by switching to a new partner than females. Additionally, it was predicted that both sexes would be
less likely to abandon their current partner for a novel mate as the time spent in a pair bond increased.
Methods

General methodologies and experimental design mirror those in Chapter 2 with a single modification. Male and female convict cichlids were again used as focal individuals (for sizes, see Tables 3.1 & 3.2) in separate experiments in which they were provided with a choice of potential mates at a given stage after forming a pair bond. As in Chapter 2, one potential mate was always the current partner of the focal individual, while the other was a novel individual obtained from stock populations. The single alteration is that the novel individuals in this set of experiments were 15-20% larger than the partner (Tables 3.3 & 3.4). This size difference influences mate choice decisions in both female (Noonan 1983; Gagliardi-Seeley et al. 2008, Beeching et al. 2003) and male (Nuttall & Keenleyside 1993; Beeching & Hopp 1999) convict cichlids.

After introduction into the choice apparatus, behavioral observations were recorded as described in Chapter 2 and identical statistical analyses were used.
Results

When comparing partner preference between male and female choice experiments, there was no significant main effect of sex ($F_{1,71} = 0.672, p = 0.42$) or stage of pair bonding ($F_{3,71} = 0.082, p = 0.97$). Males and females showed a similar strength of preference for their partner at all stages of pair bonding (Fig. 3.1). Not only did they show similar strengths of preference, they also showed no significant preference for either their partner or a novel individual (1-sample t-test, test value = 0; males: early: $t = 1.299, p = 0.23$; mid: $t = 0.724, p = 0.49$; late: $t = 0.909, p = 0.39$; post: $t = -0.745, p = 0.48$; females: early: $t = -0.235, p = 0.82$; mid: $t = 0.438, p = 0.67$; late: $t = -0.318, p = 0.76$; post: $t = 0.759, p = 0.47$). There was no significant interaction of focal sex with stage of pair bonding on the strength of preference ($F_{3,71} = 0.899, p = 0.45$). Pair-wise comparisons were not conducted due to the lack of significant main effects.

There were several significant main effects as well as interactions of factors influencing the percent of observations and the visits to a specific compartment by focal individuals (Table 3.5). Further analyses within each sex suggest that the significant main effects were due to the observations in and visits to the neutral compartment. Focal individuals of both sexes were less likely to spend time in the neutral compartment than in a compartment containing a potential mate. Males were more likely to move between compartments than females, regardless of the stage of pair bonding (main effect of Focal Sex on visits: $F_{1,71} = 55.126, p < 0.001$).

In paired comparisons within the male choice experiment, males showed no differences in the number of visits to their partner or novel female at any stage of pair
bonding (early: \( t = -0.484, p = 0.64 \); mid: \( t = 1.890, p = 0.10 \); late: \( t = -1.076, p = 0.31 \); post: \( t = 0.583, p = 0.57 \); Fig. 3.2A) or in the percent of observations with either female (early: \( t = 1.476, p = 0.17 \); mid: \( t = 1.061, p = 0.32 \); late: \( t = 1.319, p = 0.22 \); post: \( t = -0.486, p = 0.64 \) Fig. 3.3A).

Females also showed no significant differences in the number of visits or percent of observations between their partner and a novel male at any stage of pair bonding (visits: early: \( t = -0.246, p = 0.81 \); mid: \( t = -0.246, p = 0.81 \); late: \( t = -0.318, p = 0.76 \); post: \( t = 1.677, p = 0.13 \); percent of observations: early: \( t = -0.736, p = 0.48 \); mid: \( t = 0.830, p = 0.43 \); late: \( t = -0.098, p = 0.92 \); post: \( t = 0.225, p = 0.83 \); Fig. 3.2B and Fig. 3.3B).

The statistical results of the GLM repeated-measures ANOVA for aggression and courtship behaviors are given in Table 3.7. The display of stereotypic aggression and courtship behavior were relatively sparse, with only a few individuals displaying behavior during the 20 min video recordings (Table 3.6). There were significant effects of focal sex \( (F_{1,71} = 15.271, p < 0.001) \) and potential mate \( (F_{1,71} = 5.816, p = 0.02) \) on levels of aggression. There was also a significant main effect of potential mate on courtship \( (F_{1,71} = 11.256, p = 0.001) \), with only a single instance of a focal individual courting a novel potential mate. Main effects and interactions of other factors on courtship behavior were non-significant.
Discussion

As is true of many vertebrates convict cichlids use body size to inform mate choice decisions. In previous studies, both males and females have been shown to prefer the larger of potential mates when given a choice. These studies, however, did not examine already pair bonded subjects. When individuals are provided with a choice after they are paired, neither males nor females show a preference for the larger of two mates. This outcome occurred at all time points after forming a pair bond.

These results are intriguing when compared with those of the previous chapter. In Chapter 2, clear sex differences in partner preference were observed throughout pair bonding. These sex differences appeared to reflect the different parental roles each sex adopts after pairing, rather than a likelihood of divorce from a current partner. Males showed a negative partner preference at all time points except for early in pair bonding, but rather than actively pursuing other mating opportunities, behavioral observations showed that males were leaving partners to engage in territorial aggression. The data here complement and support that conclusion. If males were leaving their partner to engage in territorial aggression, the fact that novel females in the present study were as large as focal males makes males less likely to engage agonistically. In this species, both sexes are highly aggressive, especially after formation of a pair bond and when providing parental care. While aggression between members of the same sex is most common, this is not always true of convict cichlids. When inter-sexual aggression is observed, the winner is almost exclusively the larger individual, regardless of sex (pers. obs.). While males may have been treating novel females as intruders, the females’ large size allowed
them to defend themselves, essentially forcing males to spend less time in the compartment with the novel female. The levels of aggression directed toward novel females support these conclusions (Table 3.6). A significant effect of sex on aggression levels was due to males showing higher levels of aggression relative to females. This is despite a lack of sex difference in the time spent with either potential mate (Fig. 3.1)

In contrast, focal females were always smaller than males in this experimental design as well as that in Chapter 2. As such, females were unlikely to win agonistic encounters with any male. The presence of a focal female in the novel compartment, then, is more likely to suggest a willingness to divorce a current partner for a mate of higher quality. At each time point tested, females did not show a mean positive preference for either their partner or the large novel male. This reflects some females in each sample preferred the larger of the two males and some preferred their partner.

As with the previous experiment using the same design, these results must be considered with the caveat that in monogamous systems, both sexes are expected to be choosy. Measuring an individual’s preference, therefore, can be difficult based on the receptivity of the other individual. If the levels of receptivity of potential novel males are similar across these experiments and that in Chapter 2, it is reasonable to assume that the increased preference for large males truly reflects a change in female preference rather than male receptivity. A second qualification is that the lack of preference observed for females in this paradigm does not actually suggest that specific individuals do not form a preference for a given mate. Based on the movement data (Fig. 3.2B), females were not going back and forth between males and seemed to remain in a given compartment.
When linked with the strength of preference data, this movement data suggests that some females preferred their partner and the rest preferred the large novel male. Mate choice decisions are highly susceptible to fluctuations in environmental conditions and can change with season, predation rates, or with the preferences of other individuals in the population (Jennions & Petrie 1997). The ‘lack of preference’ here suggests that variation exists within females for when and how individuals make decisions regarding mate choice.

These data support the growing understanding that for most animals, mate choice decisions are informed by multiple signals incorporating different sensory modalities (Ryan 1990; Candolin 2003). Clear fitness benefits have been demonstrated for both male and female convict cichlids that choose the larger of two mates, but the benefits and/or costs of mate switching have not been well described. The factors determining these costs and benefits are likely to be dynamic and would need to be considered for any specific individual in order to predict when it might pay to abandon a current pair-bonded partner in order to mate with an individual of higher quality.
Table 3.1: Mean ± SD size measurements (mm) of focal males in male choice experiment. Males were size matched among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>57.84 ± 1.38</td>
<td>73.83 ± 1.54</td>
<td>23.45 ± 0.83</td>
<td>10.10 ± 0.33</td>
<td>7.55 ± 0.55</td>
</tr>
<tr>
<td>Mid</td>
<td>57.54 ± 0.94</td>
<td>73.25 ± 1.06</td>
<td>25.71 ± 0.71</td>
<td>10.72 ± 0.41</td>
<td>8.61 ± 0.55</td>
</tr>
<tr>
<td>Late</td>
<td>57.83 ± 0.95</td>
<td>73.79 ± 1.04</td>
<td>24.45 ± 0.71</td>
<td>10.29 ± 0.28</td>
<td>7.88 ± 0.46</td>
</tr>
<tr>
<td>Post</td>
<td>58.26 ± 1.58</td>
<td>73.43 ± 1.63</td>
<td>24.18 ± 0.53</td>
<td>10.42 ± 0.32</td>
<td>8.00 ± 0.50</td>
</tr>
</tbody>
</table>
Table 3.2: Mean ± SD size measurements (mm) of focal females in female choice experiment. Females were size matched among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>44.10 ± 0.57</td>
<td>55.62 ± 0.66</td>
<td>18.31 ± 0.29</td>
<td>8.52 ± 0.26</td>
<td>3.41 ± 0.17</td>
</tr>
<tr>
<td>Mid</td>
<td>44.38 ± 1.31</td>
<td>55.39 ± 1.37</td>
<td>18.45 ± 0.44</td>
<td>8.53 ± 0.34</td>
<td>3.28 ± 0.21</td>
</tr>
<tr>
<td>Late</td>
<td>45.06 ± 0.40</td>
<td>56.46 ± 0.47</td>
<td>18.63 ± 0.24</td>
<td>8.99 ± 0.23</td>
<td>3.57 ± 0.14</td>
</tr>
<tr>
<td>Post</td>
<td>45.06 ± 0.65</td>
<td>56.96 ± 0.88</td>
<td>18.15 ± 0.31</td>
<td>8.25 ± 0.24</td>
<td>3.51 ± 0.14</td>
</tr>
</tbody>
</table>
Table 3.3: Mean ± SD size measurements (mm) of partner and novel females provided to males. Females were size-matched within each replicate and among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>49.69 ± 1.29</td>
<td>63.16 ± 1.55</td>
<td>20.62 ± 0.48</td>
<td>9.68 ± 0.31</td>
<td>5.33 ± 0.35</td>
</tr>
<tr>
<td>Novel</td>
<td>57.62 ± 1.14</td>
<td>73.10 ± 1.35</td>
<td>24.37 ± 0.68</td>
<td>11.43 ± 0.41</td>
<td>8.39 ± 0.47</td>
</tr>
<tr>
<td>Mid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>50.01 ± 0.91</td>
<td>63.00 ± 1.09</td>
<td>20.42 ± 0.45</td>
<td>9.28 ± 0.37</td>
<td>4.81 ± 0.25</td>
</tr>
<tr>
<td>Novel</td>
<td>57.87 ± 1.30</td>
<td>73.13 ± 1.29</td>
<td>23.00 ± 0.39</td>
<td>11.37 ± 0.41</td>
<td>7.57 ± 0.36</td>
</tr>
<tr>
<td>Late</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>50.75 ± 0.68</td>
<td>63.95 ± 0.94</td>
<td>20.40 ± 0.48</td>
<td>9.42 ± 0.26</td>
<td>5.20 ± 0.31</td>
</tr>
<tr>
<td>Novel</td>
<td>58.24 ± 0.68</td>
<td>73.36 ± 0.73</td>
<td>23.16 ± 1.17</td>
<td>13.22 ± 1.47</td>
<td>8.57 ± 0.41</td>
</tr>
<tr>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>48.29 ± 0.63</td>
<td>61.41 ± 0.81</td>
<td>20.55 ± 0.46</td>
<td>9.45 ± 0.28</td>
<td>5.01 ± 0.24</td>
</tr>
<tr>
<td>Novel</td>
<td>56.28 ± 0.78</td>
<td>71.41 ± 0.91</td>
<td>23.73 ± 0.58</td>
<td>11.17 ± 0.28</td>
<td>10.94 ± 2.97</td>
</tr>
</tbody>
</table>
Table 3.4: Mean ± SD size measurements (mm) of partner and novel males provided to females. Males were size-matched within each replicate and among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>52.84 ± 0.88</td>
<td>66.79 ± 1.10</td>
<td>22.21 ± 0.64</td>
<td>9.55 ± 0.44</td>
<td>5.70 ± 0.38</td>
</tr>
<tr>
<td>Novel</td>
<td>66.24 ± 1.02</td>
<td>84.74 ± 1.15</td>
<td>27.68 ± 0.57</td>
<td>12.07 ± 0.32</td>
<td>11.20 ± 0.53</td>
</tr>
<tr>
<td>Mid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>52.19 ± 1.01</td>
<td>65.65 ± 1.21</td>
<td>21.79 ± 0.37</td>
<td>9.34 ± 0.25</td>
<td>5.30 ± 0.30</td>
</tr>
<tr>
<td>Novel</td>
<td>67.56 ± 1.87</td>
<td>86.06 ± 2.36</td>
<td>28.42 ± 0.81</td>
<td>12.55 ± 0.38</td>
<td>11.98 ± 1.04</td>
</tr>
<tr>
<td>Late</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>58.21 ± 0.97</td>
<td>73.20 ± 1.61</td>
<td>24.54 ± 0.67</td>
<td>11.00 ± 0.44</td>
<td>7.75 ± 0.47</td>
</tr>
<tr>
<td>Novel</td>
<td>68.06 ± 0.80</td>
<td>87.17 ± 1.09</td>
<td>28.39 ± 0.41</td>
<td>11.98 ± 0.26</td>
<td>12.03 ± 0.49</td>
</tr>
<tr>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner</td>
<td>53.22 ± 1.59</td>
<td>67.35 ± 1.84</td>
<td>22.73 ± 0.67</td>
<td>9.88 ± 0.45</td>
<td>5.79 ± 0.47</td>
</tr>
<tr>
<td>Novel</td>
<td>66.48 ± 1.13</td>
<td>85.21 ± 1.40</td>
<td>28.31 ± 0.70</td>
<td>12.27 ± 0.53</td>
<td>11.21 ± 0.64</td>
</tr>
</tbody>
</table>
Table 3.5: Results of a GLM repeated-measures ANOVA comparing main effects and interactions of factors on the percent of observations and number of visits of focal individuals to the three compartments in the choice aquarium.

<table>
<thead>
<tr>
<th>Effect</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Observations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment</td>
<td>5.210</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Focal sex</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Stage of pair bonding</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Compartment*Focal sex</td>
<td>6.075</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Compartment*Stage of pair bonding</td>
<td>0.350</td>
<td>0.90</td>
</tr>
<tr>
<td>Focal sex*Stage of pair bonding</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Compartment<em>Focal sex</em>Stage of pair bonding</td>
<td>0.645</td>
<td>0.69</td>
</tr>
<tr>
<td>Visits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment</td>
<td>24.752</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Focal sex</td>
<td>55.126</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Stage of pair bonding</td>
<td>1.242</td>
<td>0.30</td>
</tr>
<tr>
<td>Compartment*Focal sex</td>
<td>19.305</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Compartment*Stage of pair bonding</td>
<td>1.725</td>
<td>0.12</td>
</tr>
<tr>
<td>Focal sex*Stage of pair bonding</td>
<td>0.836</td>
<td>0.48</td>
</tr>
<tr>
<td>Compartment<em>Focal sex</em>Stage of pair bonding</td>
<td>1.177</td>
<td>0.32</td>
</tr>
</tbody>
</table>
Table 3.6: Mean ± SE number of aggression and courtship behaviors directed toward potential mates in male and female choice experiments.

<table>
<thead>
<tr>
<th>Stage of pair bonding</th>
<th>Early</th>
<th>Mid</th>
<th>Late</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression toward partner</td>
<td>2.20 ± 1.65</td>
<td>2.89 ± 2.24</td>
<td>0.50 ± 0.27</td>
<td>4.00 ± 2.40</td>
</tr>
<tr>
<td>Aggression toward novel</td>
<td>7.00 ± 3.44</td>
<td>13.67 ± 6.98</td>
<td>8.10 ± 7.34</td>
<td>13.00 ± 7.25</td>
</tr>
<tr>
<td>Courtship toward partner</td>
<td>0.70 ± 0.37</td>
<td>0.33 ± 0.17</td>
<td>0.30 ± 0.15</td>
<td>0.50 ± 0.34</td>
</tr>
<tr>
<td>Courtship toward novel</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression toward partner</td>
<td>0.00 ± 0.00</td>
<td>0.10 ± 0.10</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Aggression toward novel</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Courtship toward partner</td>
<td>0.20 ± 0.13</td>
<td>0.80 ± 0.61</td>
<td>0.20 ± 0.13</td>
<td>2.40 ± 1.32</td>
</tr>
<tr>
<td>Courtship toward novel</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.10 ± 0.10</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>
Table 3.7: Results of a general linear model repeated-measures ANOVA comparing main effects and interactions of factors on measures of aggression and courtship toward potential mates.

<table>
<thead>
<tr>
<th>Effect</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aggression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential mate (partner/novel)</td>
<td>5.816</td>
<td>0.02</td>
</tr>
<tr>
<td>Focal sex</td>
<td>15.271</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stage of pair bonding</td>
<td>0.490</td>
<td>0.69</td>
</tr>
<tr>
<td>Potential mate*Focal sex</td>
<td>5.889</td>
<td>0.02</td>
</tr>
<tr>
<td>Potential mate*Stage of pair bonding</td>
<td>0.139</td>
<td>0.94</td>
</tr>
<tr>
<td>Focal sex*Stage of pair bonding</td>
<td>0.479</td>
<td>0.70</td>
</tr>
<tr>
<td>Potential mate<em>Focal sex</em>Stage of pair bonding</td>
<td>0.147</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Courtship</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potential mate (partner/novel)</td>
<td>11.256</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Focal sex</td>
<td>1.384</td>
<td>0.24</td>
</tr>
<tr>
<td>Stage of pair bonding</td>
<td>1.722</td>
<td>0.17</td>
</tr>
<tr>
<td>Potential mate*Focal sex</td>
<td>1.099</td>
<td>0.30</td>
</tr>
<tr>
<td>Potential mate*Stage of pair bonding</td>
<td>1.900</td>
<td>0.14</td>
</tr>
<tr>
<td>Focal sex*Stage of pair bonding</td>
<td>1.722</td>
<td>0.17</td>
</tr>
<tr>
<td>Potential mate<em>Focal sex</em>Stage of pair bonding</td>
<td>1.831</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Figure 3.1: Mean (± SE) strength of preference of focal individuals for a partner at various stages after pair bond formation. Negative values indicate a time-based preference for the novel individual.
Figure 3.2: Mean (+SE) number of visits to compartments containing the partner (solid) and novel (open) individuals by males (A) and females (B) at four stages of pair bonding. Note difference in scales.
Figure 3.3: Mean percent observations (+ SE) in compartments containing either the partner (solid) or novel individual (open) averaged over the 3 day experimental period for male choice (A) and female choice (B) experiments.
Female mate preference is independent of male hormone-mediated courtship behavior

Abstract

For many monogamous species, the display of specific types of courtship behavior during pair bond formation have been hypothesized to be more important for informing mate choice decisions than morphological signals. Across a variety of vertebrate taxa, courtship behaviors have been linked to steroid hormone levels. Here, the relationship between proximate hormonal mechanisms (specifically androgens) and courtship behavior was explored in male convict cichlids, *Amatitlania nigrofasciata*, as well as their influence on female mate preference. In one experiment, 11-ketotestosterone (KT), or the anti-androgenic endocrine disrupter flutamide, was administered to males that were then provided with a choice of potential mates. Males given KT did not differ in courtship behavior from controls. Males administered flutamide showed similar preference for females as controls, but demonstrated less overall courtship behavior. The influence of these treatments on female mate preference was investigated in a second experiment. Females showed no preference between males administered KT and untreated males. Females given a choice between untreated and flutamide-treated males, however, also showed no overall preference. These data suggest that while androgens may function to regulate levels of courtship behavior in males, female preference remains independent of these behaviors.
Introduction

In order to understand the ethological significance of sexually selected characters, it is necessary to explore the process of mate choice and the traits that influence mate choice decisions. A variety of characteristics have been implicated to influence these decisions that differ from species to species. Often, these are morphological signals, but can be behavioral as well (Kirkpatrick & Ryan 1991; Candolin 2003). For instance, in the jumping spider, *Maevia inclemens*, male movement patterns have a greater influence on female mate choice than physical phenotypic differences between two discrete male morphs (Clark & Uetz 1992). The role of behavioral signals in mate choice has been hypothesized to be especially true in monogamous systems, where displays of courtship behavior occur throughout pair bond formation (Wachtmeister & Enquist 2000; Wachtmeister 2001). These behaviors associated with the formation and maintenance of a pair bond have long been implicated in coordinating parental behavior within a pair and synchronizing reproductive physiology, but their contribution to informing mate choice decisions is poorly understood.

A strong link between steroid hormones secreted by the gonads and courtship behavior has been documented across a wide range of vertebrate taxa. In addition to organizational roles these hormones play in shaping the physiology and neural circuitry of developing animals, these endocrine signals often activate specific mating behaviors later in life. Specifically, androgens and their aromatized metabolites influence male reproductive behavior in birds (Adkins & Adler 1972; Silver et al. 1979; Adkins-Regan 1996; reviewed in Ball & Balthazart 2002), reptiles (Crews 1991; reviewed in Godwin &
Crews 2002), mammals (Young et al. 1964; Goy 1966; Phoenix 1973; reviewed in Hull et al. 2002) and fish (Borg & Mayer 1995; Páll et al. 2002a; 2002b; Stacey & Sorensen 2002; Desjardins et al. 2008). The specific effects of androgens, however, differ among these taxa and are influenced by phylogenetic, social, and environmental factors (Crews & Moore 1986; Wingfield et al. 1990).

Among monogamous species, males vary in levels of circulating androgens depending on reproductive status (Adkins-Regan 1981; Wingfield 1984; Wingfield et al. 1990; Oliveira et al. 2001). Levels are generally higher in the breeding season and decrease during the non-breeding season, which may include periods of parental care. These observations come mainly from avian systems (Wingfield & Farner 1978; Wingfield 1984), but similar patterns have been observed in non-avian vertebrates as well (Hirschenhauser & Oliveira 2006). In an interspecific analysis of fish, Oliveira et al. (2001) found general support for monogamous males showing higher levels of circulating androgens when courting and decreased levels when caring for young. Van Breukelen (2008) confirmed this pattern in the convict cichlid where unpaired, courting males in both the field and the laboratory have significantly higher circulating levels of androgens than parental males.

Despite extensive studies exploring the relationship between steroid hormones and courtship behaviors, few attempts have been made to understand the link between these behaviors and their effect on mate choice decisions. Here, the relationship between endocrine-mediated courtship behavior and mate choice was explored in the monogamous convict cichlid. Convict cichlids are serially monogamous and form pair
bonds that last through parental care of young to independence (Keenleyside et al. 1990; Keenleyside 1991; Wisenden 1995). The two primary goals of this study were to first establish if androgen manipulation alters courtship behavior in male convict cichlids and secondly, determine if female mate choice decisions are influenced by these behaviors. It was hypothesized that increasing androgen levels would both increase courtship and cause males to be more preferred by females and conversely, that decreasing androgen effects would reduce male courtship and decrease female preference for males.
Methods

Experimental replicates were conducted using laboratory-raised animals bred from a combination of captive and wild-caught individuals. Stock populations were housed in 473 L, single-sex stock tanks kept on a 14L:10D photoperiod and fed commercial trout pellets daily. All aquaria were maintained at 20°C (±2°C). At the conclusion of each replicate, subjects were placed into separate stock aquaria to avoid pseudoreplication. Each individual used in experimental replicates was measured in 4 separate morphological dimensions prior to testing: standard length (SL), height (H), width (W) and mass (M).

Preference tests were conducted in 76 L aquaria divided into 3 compartments with opaque dividers made from corrugated plastic (Figure 4.1). This design is similar to that used in previous studies (Santangelo & Itzkowitz 2004; Santangelo 2005; Santangelo & Itzkowitz 2006; Gagliardi-Seeley et al. 2008) and allows potential mates to physically interact, but controls for any influence of intra-sexual competition on the mate search process. The central compartment (approximately 20 cm in length) was slightly smaller than the 2 outer compartments, which were equal in size (approximately 28 cm in length). Each divider contained a single opening to allow a focal individual to move freely throughout the choice tank. Openings were offset to prevent individuals in the outer compartments from visually interacting. Outer compartments contained a single terracotta flower pot (5 cm diameter) as a nest site.

At the start of both male and female choice experiments, potential mates were placed in the outer compartments between 0900h and 1100h. As in many vertebrates,
body size influences mate choice decisions in convict cichlids. Both males and females prefer the larger of two potential mates in a variety of contexts (Nuttal & Keenleyside 1993; Trienfenbach & Itzkowitz 1998; Beeching & Hopp 1999; Gagliardi-Seeley et al. 2008). Additionally, in any given pair males are generally larger than their female partner and females resist mating with males smaller than themselves (Leese et al. 2010). In both experiments, males were selected to be on average 10 - 15 mm larger than females to reflect the natural situation (Wisenden 1995) and potential mates in outer compartments were size-matched (± 5% total body length, see Tables 4.2 & 4.4). Focal individuals (for sizes, see Tables 4.1 & 4.3) were added to the tank approximately 24 h after choice individuals and introduced into the neutral compartment to control for the influence of sequential mate assessment similar to that observed in sticklebacks (Bakker & Milinski 1991). The entire tank was video recorded for 20 min daily on 3 consecutive days after introduction of the focal individual. Santangelo & Itzkowitz (2004) and Gagliardi-Seeley et al. (2008) demonstrated that 3 days is sufficient for both sexes to form a preference and furthermore, this preference is indicative of eventual mate choice if replicates are allowed to continue to spawning. In males, this preference can be inferred from a greater number of courtship behaviors directed toward one female and in females, by the amount of time spent with one male compared to another (Santangelo & Itzkowitz 2004). Recordings were taken between 1600h and 1700h each day to control for possible variation in circadian rhythms. Recordings were later analyzed using JWatcher behavioral event recorder freeware offered through UCLA and Macquarie University (copyright 2000-2010 D.T. Blumstein, J.C. Daniel & C.S. Evans).
Behavioral Observations

Cichlid fishes show a suite of stereotypic behaviors described previously in various ethograms (Baerends 1986; Enquist et al. 1990; Wisenden 1994a; 1994b; Wisenden 1995; Santangelo & Itzkowitz 2004; Oldfield & Hofmann 2011). These include, but are not limited to: bites, chases, frontal and lateral displays, brushes, tailbeats and mouth-wrestling. Difficulties arise in classifying these behaviors as ‘courtship’ or ‘aggression’ because similar types of behavior are often shown in both intra- and inter-sexual interactions (Baerends 1986; pers. obs.). Applying these labels is especially difficult during the early stages of pair bond formation in which males often behave aggressively toward females, and females tend to evade their advances (Baerends 1986). Oldfield & Hofmann (2011) attempted to further delineate behavioral function based on the response of the individual toward whom the behavior was directed, for example discriminating between a ‘bite’ and an ‘affiliative bite’ based on the receiver’s reaction. Given that convict cichlids demonstrate mutual mate choice, this approach, however, can lead to erroneous conclusions based on the receptivity of the individual receiving a signal rather than the intention of the individual displaying a given behavior. A male may actively and aggressively court a female, but if the female is unreceptive, a pair bond is unlikely to form. For this reason, in the male choice experiment, the simplifying assumption was made that behaviors directed toward a member of the opposite sex indicated motivation to mate and/or courtship, even though the individual to whom the behavior was directed may have responded adversely. This assumption is likely untrue of either sex during the parental phase or after already being in a pair bond (see Chapters 2
& 3), and thus these trials were kept to a brief period of interaction (3 days) as pairs
sometimes form and spawn within 7 days of being introduced (Bockelman & Itzkowitz
2008). For the male choice experiment, all interactions with females over the 3 days were
summed and used as an overall measure of courtship. In addition to the behavioral
measures taken, the amount of time each focal individual spent with a potential mate was
recorded. For the female choice experiment, only the time budget analysis is presented, as
time spent with a male indicates eventual mate choice in (Santangelo & Itzkowitz 2004;
Santangelo 2005; Gagliardi-Seeley et al. 2008).

Hormone Manipulation

In both experiments, males received one of 3 experimental manipulations. A
Silastic tubing capsule (inner diameter, 0.5 mm; outer diameter, 1.0 mm; Dow Corning,
Midland, MI) approximately 5.0 mm in length that contained powdered forms of either
11-ketotestosterone (KT) or flutamide was inserted into the abdominal cavity via a small
(~ 1.5 mm) incision just caudal to the pectoral fin. Control males were inserted with
empty capsules. Capsules of similar size release hyper-physiological hormone levels for
periods up to 7 d (van Breukelen 2008). Capsules were capped with silicone sealant on
both ends and inserted with an attached piece of string to allow for later removal. Visual
inspection of the capsules after removal insured that some KT or flutamide remained and
had been released for the duration of the experimental period. Incisions healed
completely in < 7 days after capsule removal without sutures or adhesive. There was no
infection or mortality during implantation or removal. All surgical techniques were
performed in compliance with IACUC protocol #86 through Lehigh University and in compliance with NIH guidelines for animal care and use in research.

Experiment 1: Male Choice

The male choice experiment examined the effect of androgen manipulation on male courtship behavior. Size-matched, untreated females were tethered to terra cotta pots in each outer compartment using monofilament line to prevent them from moving between compartments. Tethering was accomplished by placing a microfilament loop through the epaxial musculature just dorsal of the lateral line and securing the other end to the nest site. Tethers allowed for unhindered movement throughout the entire compartment and did not restrict the females’ general behavior (Santangelo & Itzkowitz 2004; pers. obs.). Focal males were implanted with blank (n = 12), KT (n = 12), or flutamide (n = 12) capsules and placed in the neutral compartment 24 h after the females. Males were allowed to visit freely both potential female mates throughout the 3 d experimental period. Video recordings were taken as described above and behaviors recorded for later analysis.

Experiment II: Female Choice

The female choice experiment examined the effects of androgen manipulation of males on female preference. Size-matched males were surgically implanted with silastic capsules as described above. In each replicate, one outer compartment contained a control male inserted with a blank capsule and the other compartment contained a male administered either KT (n = 10) or flutamide (n = 9). It was not necessary to tether the males in this experiment as their larger size prevented them from passing through the
openings in the dividers (30 mm diameter) while the smaller focal female could freely move. Focal females were placed in the neutral compartment 24 h after the treated males. As with the male choice experiment, the amount of time the focal female spent with each male was recorded.

Statistical Analysis

For comparisons of behavioral measures of focal individuals, males in KT and Flutamide treatment groups were independently compared to controls. The overall time spent with stimulus females as well as behaviors directed toward both females were compared with student t-tests. The number of behaviors demonstrated by males failed Levene’s test for equal variances and Kolmogorov-Smirnov test for normality (p < 0.05), thus parametric tests were deemed inappropriate. Square root and log transformation could not normalize the data, and behaviors were compared between treatments with non-parametric Mann-Whitney U tests. Further analysis within each treatment group was conducted to explore the preference for a given female over another. Females were designated ‘preferred’ or ‘non-preferred’ post hoc based on the time males spent with each female, the preferred being the female that the male spent the most time with. Paired t-tests were conducted comparing the time spent with each female within each treatment group and its non-parametric counterpart, the Wilcoxon Signed Ranked test, was used to compare behaviors toward each female.

In the female choice experiment, time spent with a given male was analyzed within each experimental condition with a paired t-test. This analysis was repeated with a post hoc designation of males as either preferred or non-preferred based on the time
females spent with a given male. Due to multiple analyses, a Bonferroni correction was applied to these results and alpha was set at $p \leq 0.025$. A measure of female responsiveness to males was obtained by summing the total time spent with both males in each replicate and dividing by the total observation time. These values were compared between the two choice conditions with a student t-test.

Statistical analyses were conducted with SPSS. All tests were two-tailed and, except where noted, alpha was set at 0.05.
Results

Experiment 1: Male choice

When compared to controls, treatment with KT had no significant effect on the mean time males spent with stimulus females ($t = -0.43$, $p = 0.67$) or the number of behaviors directed toward females ($Z = -0.26$, $p = 0.79$) (Figure 4.2). Similar results were observed when males were treated with flutamide. Flutamide treatment had no significant effect on the time males spent with females ($t = 0.09$, $p = 0.93$) or on numbers of behavior ($Z = -1.19$, $p = 0.24$), despite the mean number of behaviors among flutamide-treated males being approximately half that of the control group (24.67 ± 7.09 and 46.58 ± 14.06, respectively) (Figure 4.2B).

The lack of statistical significance in differences between control and flutamide-treated males appears to be the result of a basement effect on the behaviors in question. A high level of variation was observed in levels of male behavior directed toward females; when individual males were ranked by the number of behaviors directed toward females within each treatment group, a similar distribution can be easily observed (Figure 4.3). When viewed in this manner, males of similar relative rank in the flutamide treatment group consistently showed a lower number of behaviors toward females than their control counterparts. This pattern held true for each paired comparison by rank; however, many males showed such low levels of behavior that the effect of flutamide treatment appears to be masked. Half of the males in each group showed < 15 total behaviors during the entire observation period. When males showing > 20 behaviors were separated from these males, flutamide treatment resulted in a significant decrease in behavior toward
females when compared to controls (Z = -1.93, p = 0.05) (Figure 4.4). Notice the similar pattern, but difference in scales, between Figure 4.2B and Figure 4.4. In both analyses, mean levels of behaviors for flutamide-treated males are approximately half that of controls, but the variability within each group is drastically reduced when males showing low levels of behavior are excluded.

When exploring male preference for one female over another, males in each treatment group spent significantly more time with the preferred female than the non-preferred (control: t = 6.05, p < 0.001; flutamide: t = 6.33, p < 0.001; KT: t = 6.54, p < 0.001) (Figure 4.5A). Males also directed more courtship behavior toward the preferred female in control (Z = -2.41, p = 0.02) and KT (Z = -1.97, p = 0.05) treatment groups, but not in the flutamide group (Z = -1.73, p = 0.08), although there was a clear trend (Figure 4.5B). The lack of significant difference in behaviors directed toward preferred and non-preferred females among flutamide-treated males seems to be the result of an overall decrease in the number of behaviors performed as described above.

*Experiment 2: Female choice*

There was no difference in the overall responsiveness of females to male stimuli between the two choice conditions. Females spent an average of 67% of their time with a male in both treatment groups (t = 0.02, p = 0.98). Females also showed no preference for males based on experimental manipulation. This was true of females given the choice of a male treated with flutamide and a control (t = 0.24, p = 0.82) as well as a male administered KT and control (t = 0.05, p = 0.96; Figure 4.6A). When a similar analysis was conducted with males given a post hoc designation of either preferred or non-
preferred, females spent significantly more time with the preferred male (flutamide: $t = 4.44, p < 0.01$; 11KT: $t = 5.11, p < 0.01$; Figure 4.6B).
Discussion

These results suggest that androgens may facilitate courtship behavior in the convict cichlid, in that treatment with the androgen receptor blocker flutamide reduced courtship behavior toward preferred females when compared to controls. Unexpectedly, increasing the levels of KT had no effect on male courtship. This could be the result of a ceiling effect caused by non-natural laboratory housing conditions. Van Breukelen (2008) examined the androgenic profile of male convict cichlids from blood samples obtained in both the laboratory and the field. In the field, single, non-courting males were found to have relatively low levels of KT (0.34 ± 0.10 ng/ml) similar to parental males (0.39 ± 0.10 ng/ml), while actively courting males showed elevated levels (2.61 ± 0.85 ng/ml). When compared to males in the laboratory, parental males showed similar levels of KT (0.44 ± 0.10 ng/ml) to their parental counterparts in the field, as did courting males (2.53 ± 0.77 ng/ml). Unpaired males housed in single sex laboratory stock tanks, however, showed an average level of KT slightly higher than even courting males (3.29 ± 0.82 ng/ml). In holding with the challenge hypothesis (Wingfield et al. 1990), these results suggest that males housed in single sex stock aquaria may maintain elevated levels of KT because of the social context and thus artificially increasing levels with implants for this study may have had no measurable behavioral effect. Treatment with flutamide, however, did reduce male courtship so that these males performed approximately half as many behaviors toward preferred females as did controls.

The data do not suggest, however, that flutamide-treated males were avoiding females. In all three treatment groups, males spent the majority of their time in
compartments containing a female stimulus, and furthermore, males in each group showed a time-based preference for one female and spent significantly more time with the preferred female. The disagreement with the behavioral observations seems to suggest that while flutamide-treated males spent time with females, their motivation to actively court or form a pair bond appeared to be diminished. Separate mechanisms may be involved in the formation of partner preference and the performance of courtship behaviors. A related mechanistic pattern has been observed in monogamous male prairie voles, *Microtus ochrogaster*, where both affiliative partner preference and selective aggression toward unfamiliar females are associated with the maintenance of a pair bond, but each behavior appears to be mediated by unique neural mechanisms (Aragona et al. 2006). This implies an interesting consideration for mate choice studies in general in that time spent with a potential mate should be considered only as a single measure of mate preference in non-natural conditions. The display of other types of behaviors may be more indicative of an animal’s actual motivational state to mate.

Given the variation observed in the number of behaviors males performed toward females in the first experiment, it became difficult to predict a pattern for the preference of females for males in specific treatments. The lack of differences in behavior between males administered KT and controls suggests that females would not show a preference between males in these treatment groups, which is precisely what was observed. Females were expected, however, to prefer control males over males treated with flutamide as control males should have demonstrated more courtship behavior. Instead, while females
clearly formed preferences when given the choice of two males, these preferences were independent of treatment.

A general caveat must be considered when interpreting these data. While female choice was clearly independent of male treatment, it can only be inferred that the treatment reflects the changes in behavioral patterns seen in the male choice experiment. The levels of male behavior cannot be directly compared from observations in the female choice experiment because males could only interact with a focal female when she was present in their compartments. Thus by creating an experimental design focusing exclusively on the female, the ability to analyze the differences in male behavior within replicates was limited. Despite this drawback, results from the male choice experiment suggest that, on the whole, males treated with flutamide show a reduction in courtship behavior; thus, the independence of female choice from male hormone treatment can be extended to its independence from male courtship behavior in general.

This conclusion supports that of Santangelo & Itzkowitz (2004) who demonstrated that neither male nor female convict cichlids prefer partners that court at higher rates, suggesting that these behaviors are not used in mate choice decisions. This contradicts the assumption that courtship behaviors increase relative fitness through increased mating success (Wachmeister & Enquist 2000). Rather, the data support the idea that courtship in this monogamous species serves an ultimate function other than indicating mate quality.

Review of mate choice literature surprisingly shows that these results corroborate with the findings of many previous studies. Female guppies, *Poecilia reticulata*, show
preferences for male coloration patterns, but choose mates independent of display rate (Houde 1987). Zuk et al. (1990) found that female red jungle fowl, *Gallus gallus*, base their mate choice decisions almost exclusively on morphological traits like comb length and color, even though males actively court by crowing and wing flapping. Gibson et al. (1991) found little support for female mate choice for male vocal displays in a lekking species, but rather that females choose males based on site fidelity and the choices of other females. In instances like these, ‘courtship’ behavior may facilitate aspects of reproduction that are independent of mate choice. Thus, rather than reflecting the effects of sexual selection via the ability to attract and secure mates, the expression of these behaviors may reflect differences in the way natural selection acts between the sexes to increase fecundity.

In summary, individuals are expected to be choosy when potential mates vary in quality. This prediction applies to monogamous species as well as those with other mating systems, and is generally supported. Most studies of mate choice show that animals do not mate randomly. The role of courtship behaviors in this process, however, may be overestimated. This might be especially true when courtship behaviors are mediated by steroid hormones that govern diverse suites of behavioral and physiological traits.
Table 4.1: Mean ± SE size measurements (mm) of focal males in male choice experiment. Males were size matched among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67.68 ± 1.70</td>
<td>86.63 ± 2.17</td>
<td>26.12 ± 1.58</td>
<td>13.11 ± 1.24</td>
<td>11.62 ± 0.99</td>
</tr>
<tr>
<td>Flutamide</td>
<td>67.18 ± 1.89</td>
<td>87.26 ± 2.55</td>
<td>28.03 ± 0.83</td>
<td>11.78 ± 0.46</td>
<td>11.93 ± 0.96</td>
</tr>
<tr>
<td>11KT</td>
<td>68.22 ± 2.02</td>
<td>86.18 ± 1.78</td>
<td>28.37 ± 0.88</td>
<td>12.40 ± 0.46</td>
<td>12.87 ± 1.10</td>
</tr>
</tbody>
</table>
Table 4.2: Mean ± SE size measurements (mm) of stimulus females provided to males. Females were size-matched within each replicate and among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female 1</td>
<td>52.18 ± 1.59</td>
<td>66.20 ± 1.94</td>
<td>21.52 ± 0.66</td>
<td>10.63 ± 1.08</td>
<td>5.82 ± 0.63</td>
</tr>
<tr>
<td>Female 2</td>
<td>51.94 ± 1.54</td>
<td>66.70 ± 1.97</td>
<td>21.73 ± 0.68</td>
<td>9.34 ± 0.35</td>
<td>5.50 ± 0.53</td>
</tr>
<tr>
<td><strong>Flutamide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female 1</td>
<td>53.68 ± 1.05</td>
<td>67.84 ± 1.43</td>
<td>22.02 ± 0.53</td>
<td>10.54 ± 0.31</td>
<td>6.29 ± 0.37</td>
</tr>
<tr>
<td>Female 2</td>
<td>54.89 ± 1.05</td>
<td>68.69 ± 1.34</td>
<td>22.28 ± 0.63</td>
<td>10.53 ± 0.34</td>
<td>6.44 ± 0.43</td>
</tr>
<tr>
<td><strong>11KT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female 1</td>
<td>53.52 ± 1.36</td>
<td>67.23 ± 1.76</td>
<td>22.59 ± 0.62</td>
<td>10.87 ± 0.49</td>
<td>6.01 ± 0.49</td>
</tr>
<tr>
<td>Female 2</td>
<td>52.96 ± 1.48</td>
<td>67.30 ± 1.93</td>
<td>22.43 ± 0.65</td>
<td>10.84 ± 0.43</td>
<td>5.98 ± 0.48</td>
</tr>
</tbody>
</table>
Table 4.3: Mean ± SE size measurements (mm) of focal females in female choice experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL</th>
<th>TL</th>
<th>H</th>
<th>W</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flutamide</td>
<td>53.32 ± 0.70</td>
<td>68.22 ± 1.69</td>
<td>21.53 ± 0.33</td>
<td>9.72 ± 0.31</td>
<td>5.88 ± 0.27</td>
</tr>
<tr>
<td>11KT</td>
<td>50.89 ± 0.81</td>
<td>64.83 ± 3.03</td>
<td>20.48 ± 0.48</td>
<td>9.56 ± 0.22</td>
<td>5.45 ± 0.32</td>
</tr>
</tbody>
</table>
Table 4.4: Mean ± SE size measurements (mm) of manipulated males provided to females. Males were size-matched within each replicate and among treatment groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SL (mm)</th>
<th>TL (mm)</th>
<th>H (mm)</th>
<th>W (mm)</th>
<th>M (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flutamide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>67.17 ± 1.68</td>
<td>86.78 ± 1.81</td>
<td>28.51 ± 0.60</td>
<td>12.06 ± 0.39</td>
<td>11.91 ± 0.75</td>
</tr>
<tr>
<td>Control</td>
<td>68.37 ± 1.25</td>
<td>87.43 ± 1.37</td>
<td>28.97 ± 0.66</td>
<td>12.29 ± 0.66</td>
<td>12.64 ± 0.66</td>
</tr>
<tr>
<td><strong>11KT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>65.96 ± 1.20</td>
<td>84.54 ± 1.53</td>
<td>28.85 ± 1.09</td>
<td>12.31 ± 0.39</td>
<td>11.80 ± 0.79</td>
</tr>
<tr>
<td>Control</td>
<td>65.04 ± 1.03</td>
<td>84.20 ± 1.39</td>
<td>28.02 ± 0.69</td>
<td>12.08 ± 0.34</td>
<td>11.40 ± 0.63</td>
</tr>
</tbody>
</table>
Figure 4.1: Preference tanks were divided into 3 compartments with opaque dividers. Focal individuals were placed in the neutral compartment but allowed to freely move throughout the entire tank.
Figure 4.2: Mean ± SE behavioral measures of focal males toward stimulus females. Males showed no difference in overall time spent with females based on treatment (A). Males administered KT showed no differences from control males in behaviors directed toward females, while flutamide treated males showed a non significant pattern of decreasing behavioral response toward females when compared to controls (B).
Figure 4.3: Number of behaviors directed toward females by individual males ranked from high to low within each treatment group (control: solid; KT: open, flutamide: hatched). Half of the males showed < 15 total behaviors toward females over the 3 day observation period.
Figure 4.4: Mean ± SE total number of behaviors directed toward females during 3 day observation period in males showing > 20 total behaviors. Males administered flutamide showed significantly fewer behaviors than control males. There was no difference between control and KT.
Figure 4.5: (A) Mean + SE time spent with each female in choice aquaria. All males showed a preference for one female over another independent of treatment. (B) Mean + SE behaviors toward each female. Control and KT treated males performed more behaviors toward one female, while flutamide treated males showed no difference. (Preferred female: solid; non-preferred female: open)
Figure 4.6: (A) Mean + SE time spent with each male in choice aquaria. Female preference for a given male was independent of experimental manipulation in both flutamide (control, solid; flutamide, hatched) and KT conditions (control, solid; KT, open). (B) Mean + SE time spent with each male in choice aquaria. Females spent significantly more time with one male (preferred, solid bars) over another (non-preferred, open bars) in both choice conditions.
General Discussion: Sexual Selection and Monogamy

The purpose of this dissertation was to explore and provide experimental evidence of the operation of sexual selection in a monogamous species. Recent advances in sexual selection theory suggest that monogamous species should be competitive and selective when choosing mates, but how this process transpires in nature continues to be poorly understood for most animal species (Kokko & Johnstone 2002; Clutton-Brock 2007). The studies here support the notion that both sexes of monogamous species are selective in mates and test several key hypotheses about the operation of sexual selection in a monogamous system.

In Chapter 2, I addressed the hypothesis that the formation of a pair bond serves the ultimate function of facilitating mate choice decisions by acting as a prolonged period of mate assessment. I tested this in both males and females and showed that after forming a pair, both sexes will continue to visit novel potential mates, but this behavior does not appear to reflect active mate searching. The sexes differed significantly in the way in which they interacted with potential mates and these sex differences closely resemble the division of roles observed during periods of parental care. This suggests that after pair bond formation occurs, individuals are likely to remain with their given partner until spawning.

In the next set of experiments, I tested whether differences in mate quality influences the function of pair-bond formation by providing individuals of both sexes with a choice of their current pair-bonded partner and a novel potential mate of higher quality. I demonstrated in Chapter 3 that preference for a high quality (larger) mate
disappears when an individual has already formed a pair bond. I concluded that this lack of preference, however, is potentially due to separate mechanisms in each sex. For males, large novel females were met with aggression and appeared to be treated as territorial intruders. For females, however, some focal individuals appeared to switch their preference away from their partner to a high quality male. Together with data from Chapter 2, these results suggest that the role of pair bonding may differ between the sexes and that females may utilize it as a prolonged period of mate assessment while males do not.

Finally, in Chapter 4 I addressed the function of male courtship behavior in mate choice decisions for both male and female convict cichlids. I found that male courtship behavior could be reduced by experimentally blocking androgen receptors, but that this reduction in courtship behavior had no effect on female mate preference. Females were shown to form a preference for one male over another when given a choice, but this remained independent of male behavior. This observation has been made previously, but only by correlation (Santangelo 2004; Santangelo & Itzkowitz 2005). The experiments here not only confirmed this previous finding, but also provided a link between proximate mechanisms of endocrine-mediated behaviors and the process of pair bond formation and mate choice in this system.

These experiments align themselves well with earlier research in this model system and elucidate some of the ambiguities concerning the mate choice process previously observed. In particular, the work of Bockelman & Itzkowitz (2008) showed that females can respond quickly to mate loss and spawn with a new partner while males
take significantly longer to form a pair and reproduce with a new female after losing a mate. Each study here provides partial explanation for that seemingly paradoxical observation. First is that both sexes show a tendency to remain with their partner after the formation of a pair bond. Divorce and mate desertion have been observed in this system, but these results suggest that this is unlikely to occur due solely to active choice. In nature, intra-sexual competition, predation and other extrinsic factors may supersede individual preference for a pair-bonded partner and cause mate desertion and divorce.

Secondly, males appear to undergo a physiological transition that begins immediately after forming a pair bond. Van Breukelen (2008) showed that levels of circulating androgens in males are high when courting and lower when paired. While the studies here do not provide a detailed time-course, they support the idea that androgens decrease soon after formation of a pair bond and that a link exists between displays of courtship behavior and levels of androgens. I propose that after the formation of a pair bond, males essentially undergo a hormone-mediated behavioral switch that takes them from a courtship phase to parental care. They begin treating novel females as intruders and as such cannot actively pursue a new mate. This explains why pair-bonded males that lose a partner take significantly longer to form a new pair than unpaired males. There may be a recovery period for males to transition back from acting parental to actively courting again.

Females, on the other hand, do not appear to be constrained by the transition from courtship to parental care in terms of continuously assessing potential mates. The formation of a pair bond does appear to decrease the likelihood of females choosing a
novel individual of higher quality, but this suggests a potential trade-off between choosing the best of available mates and remaining with a pair-bonded partner. This also allows for the observation in Chapter 4 that females do not choose males based on levels of courtship to be integrated into this model. If females are free to switch to a new male partner even after forming a pair bond, but male courtship levels are tightly linked to their fluctuating androgen profile, females would benefit from using cues other than courtship in informing mate choice decisions. A simplistic model illustrating some of these factors is presented in Figure 5.1.

It is always with great care that results like these here can be extrapolated to other animal systems. On a fundamental level, these data support that sexual selection is operating within monogamous species and that both sexes demonstrate some degree of mate choice. It also reiterates that mate choice is a complex process and unlikely to be stripped down to one or even a few key components as it has been in some non-monogamous mating systems. The significance of this dissertation is that it demonstrates that the operation of an evolutionary process, like sexual selection, in any given system is dependent on the ecological niche unique to that organism. The term monogamy is applied to a wide range of animals based on a few key distinctions, but no behavioral repertoire can be applied to each and every one of those animals. Convict cichlids have been known to demonstrate a high level of faithful monogamy even in natural conditions, but this behavior may be facilitated in a completely different manner than other systems. Here, it appears that the extreme selection pressure on males to engage in parental care has constrained their ability to switch mates or take advantage of extra-pair fertilizations.
Future studies on the operation of sexual selection among other monogamous systems will help to further illustrate the subtleties and complexities that may be unique to a wider range of animal systems.
Figure 5.1: A schematic showing some of the sexually dimorphic physiologic and behavioral changes occurring during pair bond formation in the convict cichlid that influence mate choice and thus sexual selection.

- **Male**: 
  - **11KT**
  - **Courtship**
  - **Territorial aggression/Parental defense**

- **Female**: 
  - **Ova mature**
  - **Time near nest**
  - **Continuous mate assessment**

`'Courtship' → Pair bond formation → Spawning → Parental Care`
References


Appendix A

Ethogram of observed cichlid behaviors. (Note: not an exhaustive listing)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Courtship</td>
<td>Brush</td>
<td>Mutual behavior where males and females pass by each other slowly, simultaneously extending paired fins and touching. Occurs with lateral orientation between male and female when one individual administers quick lashes of its tail toward the receiver. Signaler generally tilts body toward the substrate (approximately 45° angle) and vibrates its body at a high frequency.</td>
</tr>
<tr>
<td>Aggression</td>
<td>Bite</td>
<td>Individual opens mouth and advances toward receiver rapidly resulting in a bite. Receiver usually reacts adversely and tries to evade advances. Individual swims rapidly behind another, often includes biting behavior. A single chase begins with the increase velocity in swimming motion and ends when normal swimming speed resumes. Individuals extend paired and/or unpaired fins and gill opercula. Can be performed between individuals aligned head to head or laterally.</td>
</tr>
</tbody>
</table>
JOSEPH M. LEENE

Curriculum Vitae

Contact Information

Work Address
111 Research Drive
Bethlehem, PA 18015

Personal Address
4 Duh Dr., Apt 214
Bethlehem, PA 18015

Work Phone
610-758-5429

Personal Phone
717-521-3226

Work E-mail
jml206@lehigh.edu

Personal E-mail
Joseph.Leese@gmail.com

Education

Lehigh University
Ph.D. Integrative Biology
Advisor: Dr. Murray Itzkowitz
Dissertation title: Sex differences in mate assessment throughout pair bond formation in a monogamous species, *Amatitlania nigrofasciata*

DeSales University
B.S. Biology

Professional Employment

Lehigh University
Graduate Research Assistant
Sept. 2009 – Aug. 2010

Thorne Fellow
Sept. 2010 – Dec. 2010

Teaching Assistant

Office of David S. Glosser, Sc.D.
Neuropsychology Technician

Hymed Group, Ltd.
Research Assistant
June 2005 – Aug. 2006

DeSales University
Laboratory Assistant

Research and Field Experience

Bellairs Research Institute
May 2010 – June 2010
Holetown, Barbados

- Conducted field studies of interspecific territorial aggression in two species of damselfish (genus *Stegastes*).
➢ Explored sex differences in parental care behavior after brood loss in a monogamous species.

Discovery Bay Marine Lab  May – June 2007; 2008  Discovery Bay, Jamaica
➢ Investigated tradeoffs in behavior driven by natural and sexual selection pressures and tested for the presence of a behavioral syndrome in the beaugregory damselfish (*Stegastes leucostictus*).

Texas Parks and Wildlife  June – Aug. 2007; 2008  Diamond Y Springs, TX
➢ Monitored mating and social behavior of natural populations of several species of endangered pupfishes (genus *Cyprinodon*).

DeSales University  Feb. 2005 – May 2006  Center Valley, PA
➢ Investigated potential therapeutic effects of synthetic polyamine analogs on breast cancer cell growth.

Milton S. Hershey Medical Center  June 2004 – Aug. 2004  Hershey, PA
➢ Conducted research in the department of cellular and molecular physiology.

**Publications**


Publications in Review and Preparation

- J.M. Leese & E.K. Becker “Female mate preference is independent of male hormone-mediated courtship behavior in the monogamous convict cichlid.” In review.
- J.M. Leese “Sex differences in the function of pair bonding in the monogamous convict cichlid.” In review.

Poster Presentations

Invited Seminars


Contributed Oral Presentations


† denotes presentation by co-author

Teaching Experience

Lehigh University

Teaching Assistant


- BioS010 Bioscience in the 21st Century, Supervisor: Dr. Vassie Ware
- BioS382 Endocrinology of Behavior, Supervisor: Dr. Jill Schneider
- BioS120 Biology Core III: Integrative and Comparative, Supervisor: Dr John Nyby
- BioS336 Animal Behavior Laboratory, Supervisor: Dr. Murray Itzkowitz
- BioS115 Biology Core II: Genetics, Supervisor: Dr. Kathy Iovine
- BioS116 Genetics Laboratory, Supervisor: Dr. Meg Kenna
- BioS42 Cellular and Molecular Laboratory, Supervisor: Dr. Meg Kenna
- BioS41 Biology Core I: Cellular and Molecular, Supervisor: Dr. Matthias Falk

Mentor to Undergraduate Research Students

Jan. 2010 – May 2011

- Emily Becker ‘11 (Honors Thesis)
- Valerie Estella ‘11
- Monica Reibelak ‘10
- Caitlin Katrinic ‘10 (Honors Thesis)
- Gabrielle Navon ‘10

Guest Lecturer

Honors and Awards

- Guy Jordan Research Fund Award, American Cichlid Association (2011)
- Gordon C. Thorne Fellowship, Department of Biological Sciences, Lehigh University (2010)
- College of Arts and Sciences Summer Fellowship, Lehigh University (2009)
- 2nd Place Graduate Student Oral Presentation, Lehigh Valley Evolution and Ecology Symposium (2009)
- Animal Behavior Society/NSF Travel Grant (2009)
- 1st Place Graduate Student Oral Presentation, Lehigh Valley Evolution and Ecology Symposium (2008)
- Academic Excellence in Biology Award, DeSales University (2006)
- DeSales University Presidential Academic Scholarship (2002-2006)

Professional Affiliations and Service

- American Association for the Advancement of Science (2007 – Present)
- Sigma Xi, The Scientific Research Society (2009 – Present)
- New York Academy of Sciences (2011 – Present)
- National Park Service Bio-Blitz Team Leader, Delaware Water Gap National Recreation Area (Sept. 2010)
- Learning for Life Participant (March 2010 – Present)
- Biological Organization of Graduate Students (2006 – Present; Secretary ’08 - ‘09; Education Liaison ’10 - ’11)
- Ad hoc reviewer for Acta Ethologica & Behaviour