DIFFERENTIAL SPERM EXPENDITURE BETWEEN SPECIES IN THE

SAILFIN MOLLY, POECILIA LATIPINNA

THESIS

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Master of SCIENCE

by

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CHAPTER I

INTRODUCTION

Sexual selection results from differences in mating success (Darwin 1871). This may take the form of intersexual selection, or mate choice, whereby the limiting sex selects mates from the limited sex, and intrasexual competition, in which members of the limited sex compete among themselves for access to the limiting sex. Male mating success should vary more than female mating success because females are often the limiting sex (Bateman 1948). Females are usually the limiting sex because: (1) The average proportion of fertilizable females to sexually active males in a population at a given time, or operational sex ratio (OSR), is often male biased and causes females to become a limiting resource (Emlen & Oring 1977); (2) Females often have both a greater initial investment in offspring and (3) greater parental investment (Trivers 1972, 1985).

Intersexual selection

Many hypotheses have been proposed to explain the mechanisms by which individuals select mates. Each of these models assumes that selection will favor individuals that choose mates such that they maximize the ratio of benefits to costs associated with

mating. Hypotheses based on non-genetic benefits propose that females select mates based on resources males provide to females, such as food and/or breeding territories that directly increase a female's fecundity. Hypotheses based in indirect, or genetic benefits, propose that females select males that provide heritable fitness advantages to their offspring (Andersson 1994).

Genetic benefits models of female choice are typically classified as either indicator or non-indicator hypotheses. Indicator hypotheses propose that females prefer heritable male traits correlated with viability fitness that increases the fitness of their offspring. The first such model, Fisher's runaway process (1930), which was formalized by Lande (1980, 1981) and Kirkpatrick (1982), suggests that a rare phenotypic trait could be initially favored by natural selection. If the viability advantage was heritable, then females that mated with the males with the trait would produce male offspring with both the viability advantage and a reproductive advantage. Such a mating advantage would subsequently drive the elaboration of the male trait beyond its natural selection optimum. Another indicator hypothesis (not-mutually exclusive to Fisherian processes) is the good genes hypothesis, which states that females choose males exhibiting heritable phenotypes that will benefit their offspring (Zahavi 1975). One extension of the good genes hypothesis, the handicap principle suggests that elaborate male traits lead to a viability disadvantage, so only those males fit enough to overcome selection pressures survive (Zahavi 1975). Another good genes hypothesis, parasite-mediated sexual selection, suggests that male phenotypes correlated to parasite resistance are the targets of female preference (Hamilton & Zuk 1982). Each of the indicator models assume that when

females select males on the basis of an indicator trait female preferences and male traits will coevolve as the alleles are passed on to offspring (Andersson 1994).

Non-indicator models of female choice suggest that a female preference may exist before the evolution of a male trait. Most non-indicator hypotheses are based on the transmission of male phenotype signals through the environment and female perception of these signals. The sensory drive hypothesis suggests that environmental factors direct selection of sensory systems which affect communication efficiency (Endler & McLellan 1988; Endler 1992). The sensory exploitation hypothesis similarly suggests that biases of the female sensory system (due to selection in other contexts, such as foraging) can modify existing male traits (Ryan 1990; Ryan & Rand 1990). An extension of sensory exploitation is the pre-existing bias hypothesis, which suggests that female preferences may bias evolution toward preferred novel male traits due to the evolutionary history of the female sensory system unrelated to mate choice (Endler 1992). Although these hypotheses do not assume an initial correlation between the male traits and female fitness, it is possible that future correlations could arise.

Species recognition

Mate choice involves two processes that may overlap: species recognition, such that conspecific individuals are identified and mate-quality recognition where high quality mates are chosen (Pfennig 1998). When species recognition and mate-quality reinforce

each other, selection should decrease variation in species recognition signals and increase the potential to identify high-quality mates by selecting for traits indicating mate-quality (Ryan & Keddy-Hector 1992; Pfennig 1998; Ptacek 2000). However, when species recognition and mate-quality signals are conflicting, for example when heterospecifics resemble high-quality conspecific mates, the outcomes of selection are less clear (Pfennig 1998). Because fitness benefits from mating with a heterospecific individual are almost always less than those gained from mating with a low-quality conspecific, mate-quality may be compromised for a conspecific mating (Pfennig 1998). Another conflict in mate choice may occur if signals used in species recognition are not the same signals used for mate-quality recognition because individuals may be unable to recognize both types of signals simultaneously, leading to heterospecific matings (Pfennig 1998).

The potential conflict in species versus mate-quality recognition is highest in sympatric populations of closely related species. In cases where closely related species are sympatric in only some areas, one might expect to find reproductive character displacement, which is the pattern of greater divergence of a reproductively isolating trait between closely related taxa in areas of sympatry than in areas of allopatry (Brown & Wilson 1956). Sympatry increases the chances of character displacement when related species exist together (Pfennig 2000; Sætre et al. 1997). While evolution of mate recognition systems may also occur in allopatry (Crapon de Crapona & Ryan 1990; Wymann & Whiting 2003), lack of selection against recognition of heterospecifics can lead to mating mistakes if encountering a heterospecific. In sailfin mollies, *Poecilia latipinna*, males from populations sympatric with the closely related species Amazon mollies, *P. formosa*, have a significantly higher strength of preference for conspecific females than males from populations allopatric with *P. formosa*, indicating reproductive character displacement in male preferences (Ryan et al. 1996; Gabor & Ryan 2001). However, it is not clear how such sympatric species respond when mate-quality cues conflict with species recognition cues.

Intrasexual selection

Intrasexual selection is the conflict between members of the same sex for access to mates and/or for access to the mates' gametes. An OSR that is male-biased usually results in male-male competition for females (Emlen & Oring 1977). Intrasexual selection often involves either direct aggressive interactions, such as using antlers for head to head combat in deer (Shuster & Wade 2003), or less overt mechanism, such as mate guarding (Andersson 1994), which can involve male parental care (Thornhill & Alcock 1983). Intrasexual selection is expected to favor elaboration of male traits involved in competition. In addition, it may lead to the formation of social hierarchies (Pfennig et al. 2000) and/or the evolution of alternative mating strategies (Shuster & Wade 2003).

In addition to the overt, often aggressive behavior by individual males prior to mating, males may also compete post-copulation via sperm competition. Sperm competition occurs as the rivalry between the sperm of two or more males for the fertilization of a given set of ova, and may select for a range of behavioral, morphological

and physiological tactics that maximize reproductive fitness (Parker 1970). Males may adopt strategies based on: (1) the strategies of other males in the population (raffle principle-Parker 1990, 1993); (2) social structure (OSR) that remains fixed for at least a short period of time (Evans & Magurran 1999); and/or (3) according to the male's quality (Pizzari et al. 2003). Such factors are likely interactive with one another, ultimately influencing final male sperm expenditure. Sperm expenditure is the amount of sperm that a male allocates while mating with a female. Sperm expenditure may increase as the *risk* of sperm competition, which is the likelihood that sperm will compete with a rival's sperm, increases (Parker et al. 1997; Pizzari et al. 2003), but as the *intensity* of sperm competition increases, which is the number of rival's ejaculates with which a male's sperm competes, less favored, lower quality males should decrease sperm expenditure due to the inability to compete with higher quality males (Pizzari et al. 2003). If males do not have enough sperm to compete effectively against other males' sperm, then mating with higher-quality females may be too costly and males may pursue matings with lower quality females.

Cost of sperm production

Although the traditional view of sexual selection theory has focused on the relatively higher cost of egg production as compared to sperm production, spermatogenesis can be energetically costly and limit male reproductive success (Dewsbury 1982; Nakatsuru & Kramer 1982; Shapiro et al. 1994; review in Wedell et al. 2002). Physiological changes associated with sperm production when males are provided with stimuli from females are referred to as the priming response (Olsén & Liley 1993; Bozynski & Liley 2003), which

may be a mechanism by which males can conserve energy to produce more sperm (Liley & Kroon 1995). It is possible that in addition to female stimuli, other factors influence the amount of sperm males have available for transfer. For example, Evans & Magurran (1999) suggest that the amount of sperm males have available is indicative of their motivational state. Male guppies (*Poecilia reticulata*) from both male-biased and female-biased groups had more sperm available than males from equal sex-ratio groups. This result suggests that males from male-biased groups are able increase sperm production in response to the intensity of sperm competition (in male biased groups) or in response to expected matings (in female biased groups).

Male mate choice and sperm production

Male mate choice is not documented as well as female mate choice, but is expected when the OSR is female biased. Male mate choice is also expected when males provide paternal care, when males are more resource limited by sperm, territory, or food, when mating is costly to males, or when there is a large difference in mate-quality of available females (Andersson 1994). For example, choosy males may increase their number of offspring and thus their fitness by mating with females with higher fecundity. In many species of fish female fecundity will increase with size, and males exhibit preferences for large females (e.g., *P. latipinna*: Travis & Trexler 1987; Ptacek & Travis 1997; Gabor 1999; pipefish, *Syngnath us typhle*: Berglund et al. 1986; sockeye salmon, *Oncorhynchus nerka*: Foote 1988; three-spined sticklebacks, *Gasterosteus aculeatus*: Kraak & Bakker 1998). There are a number of measures of male mating preferences. In *P. latipinna*, male mate choice has been measured by examining association times (Gabor 1999), male mating attempts (Ptacek & Travis 1997), and male sperm production (Aspbury & Gabor 2004a, b). However, sperm production prior to mating is not always indicative of sperm transferred (Pilastro et al. 2002). In guppies, sperm primed did not correlate to fertilization success (Evans & Magurran 2001); however, Pilastro & Bisazza (1999) found a relationship between the amount of sperm stripped and the amount transferred to females, also in guppies. When there is high variation in female quality, males may conserve more sperm for future matings than when variation in female quality is low (Reinhold et al. 2002). Males with depleted sperm supplies may forgo mating with highquality females if there is a perceived risk of sperm competition and if favoring lowerquality females will increase the chance of fertilizing eggs (Galvani & Johnstone 1998). Because females prefer larger males, larger males may mate with several females and adjust sperm transfer accordingly so that no relationship exists between sperm primed and transferred (Aspbury & Gabor 2004a)

Sperm priming has been used to measure male mate choice in *P. latipinna*. Male *P. latipinna* prime more sperm for larger females and smaller males prime less sperm than larger males (Aspbury & Gabor 2004a). Smaller males primed more sperm with females present relative to the amount of sperm males had available when isolated from females, indicating that sperm may be more costly for smaller males. Sperm competition is a likely factor influencing sperm production and possibly sperm transfer in *P. latipinna* because both males and females mate multiply. Mate quality may also factor into the risk of sperm competition because larger, more fecund females have more multiply sired

broods than smaller females (Trexler et al. 1997) and may be the object of male-male competition (Gage 1998).

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Males also may exhibit mate choice by controlling sperm production and transfer if they belong to a species assemblage that consists of both unisexual gynogenetic species and bisexual species (Foran & Ryan 1994; Ryan et al. 1996; Gabor & Ryan 2001). Gynogenetic species consist of only females but require sperm from males of closely related bisexual species to initiate embryogenesis (Hubbs & Hubbs 1932; Balsano et al. 1989). Males that mate with these sexually parasitic females gain no offspring, and thus do not increase their fitness. Male *P. latipinna* are sexually parasitized by the gynogenetic species, Amazon molly, *Poecilia formosa*, and male *P. latipinna* prefer to mate with conspecific females over *P. formosa* (Gabor & Ryan 2001). Male *P. latipinna* also prime more sperm for conspecific females than *P. formosa* (Aspbury & Gabor 2004b).

Natural history of the study system

The sailfin molly, *Poecilia latipinna* (Family: Poeciliidae) is a livebearing fish native to brackish waters of southern Mexico near Rio Tuxpan along the Gulf of Mexico through North Carolina. *Poecilia latipinna* was also introduced to several sites outside of its native range, including the San Marcos and Comal rivers in central Texas (Brown 1953).

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Female *P. latipinna* are sexually mature around 30-32 mm; female size is indeterminate and is positively correlated with brood size within a population (Trexler et al. 1997). Interpopulation variation in maturity exists and females from some populations mature at 16 mm (Trexler et al. 1997). Females have an ovulatory cycle of 30 days and are sexually receptive as virgins and 1-2 days after dropping a brood (Liley 1966). They also mate multiply and are capable of storing sperm for several months (Baerends et al. 1955).

Males are mature when the anal fin fuses completely to form the gonopodium, the organ used for sperm transfer. At maturity males also exhibit enhanced coloration on the caudal fin and enlarged dorsal fin. There is a large size difference at maturity among males, which varies by age, and is determined by Y-linked alleles (reviewed in Travis 1994). Small males mature in 30 days while larger males may take up to 60 days to mature. Average male size also varies between populations (Trexler et al. 1997). Size differences within a population correlate to behavioral differences. There are three mating behaviors that males typically exhibit: 1) courtship displays that include erecting the dorsal fin and sigmoid curving, 2) nibbling the female's gonopore, which usually occurs before gonopodial thrusting (mating attempts) and may provide cues regarding female receptivity (Farr & Travis 1986), and 3) gonopodial thrusting whereby the male attempts to inseminate the female by attaching his gonopodium to the female's gonopore. Larger males court females more often than small males; small males rely primarily on gonopodial thrusting (Travis & Woodward 1989). Large males also are more aggressive toward small males and limit their access to females (Travis & Woodward 1989; Travis

et al. 1990). Intermediate-size males show variability in behavior relative to the size of other males (Travis & Woodward 1989).

The Amazon molly, *Poecilia formosa* (Family: Poeciliidae) is a livebearing fish native in rivers and streams along the Gulf Coast from southern Texas to Vera Cruz, Mexico. *Poecilia formosa* has mollies have also been introduced to sites outside of their native range in central Texas. This clonal, all female, gynogenetic species formed from a single hybridization event that occurred approximately 100,000 years ago (Avise et al. 1991; Schartl et al. 1995). Poecilia formosa was also the first parthenogenetic vertebrate described (Hubbs & Hubbs 1932). *Poecilia formosa* sexually parasitize males from parental species, P. latipinna and P. mexicana, for sperm to initiate gynogenesis (Hubbs & Hubbs 1946; Kallman 1962; Darnell et al. 1967). Laboratory experiments show other species in the genus *Poecilia* may also act as sperm donors (Schlupp et al. 2002) and field experiments show that P. latipunctata sometimes acts as a sperm donor outside of a laboratory setting (Niemeitz et al. 2002). Although other species may act as sperm donors, the parental species are the main sperm donors in the field. Range of P. formosa molly is therefore limited by the range of the potential sperm donors, although other factors such as marine currents may also limit the geographic distribution of P. formosa (Schlupp et al. 2002).

Due to the hybrid origin of the species, *P. formosa* possesses higher levels of genetic heterozygosity than similar bisexual species (Schartl et al. 1995). Although the species has high heterozygosity, there is little genetic variation among individuals

compared to similar bisexual species (Avise et al. 1991). *Poecilia latipinna* is the paternal species and genetic analysis indicates that *P. mexicana limantouri* is the maternal species of *P. formosa* (Schartl et al. 1995). Most DNA is derived from either parental species, although there are two alleles and one chromosome derived either from mutation or from specialized ancestry (Turner et al. 1980).

Research directions

Because males contributing sperm to Amazon mollies do not receive the fitness benefits of paternal offspring, the unisexual-bisexual species complex would be expected to be unstable. However, *P. formosa* has persisted for 100,000 years, suggesting stability (Avise et al. 1991; Schartl et al. 1995). There are several possibilities that may explain the persistence of *P. formosa*. The hybrid origin of unisexual females may cause males of the parental species to recognize unisexual females as potential mates (Dries 2003). Second, because *P. formosa* only produce female offspring, they can produce twice as many female offspring as female P. latipinna, resulting in a higher reproductive potential. Male P. latipinna may also experience a conflict in mate quality and species recognition when P. formosa are larger than conspecific females (Gumm & Gabor 2005). Although male P. latipinna do not gain paternal inheritance by mating with P. formosa, female P. latipinna copy the mate choice of P. formosa, which may favor the persistence of sexual parasitism by P. formosa (Schlupp et al. 1994). Finally, male Atlantic mollies, Poecilia *mexicana*, expended less sperm to conspecific females than to Amazon mollies (Schlupp & Plath 2005). Male sailfin mollies may also expend less sperm to Amazon mollies than

to female sailfin mollies. This thesis investigates some of the strategies male sailfin mollies employ to reduce the costs of mating with female Amazon mollies.

In Chapter II, I examined sperm availability in male sailfin mollies after mating with female sailfin mollies and Amazon mollies. In experiment 1, I examined whether sperm availability after mating reflects sperm expenditure. I expected males to have less sperm available after mating with female sailfin mollies than after mating with Amazon mollies. Surprisingly, I found that male sailfin mollies had more sperm available after mating with female sailfin mollies than after mating with Amazon mollies. I suggest that ready sperm may increase as a result of mating with conspecific females and as a result of rapid spermiation. In experiment 2, I investigated whether spermiation may rapidly occur during mating in the sailfin molly. Male sailfin mollies were mated with female sailfin mollies or Amazon mollies for different trial lengths. Sperm availability was measured as the difference between sperm left after mating and a baseline measure of sperm primed. Rapid spermiation during mating trials may increase sperm availability for transfer. I found that sperm availability increased as trial time increased with female sailfin mollies, but found no relationship for trials with Amazon mollies. My results indicate that male sailfin mollies rapidly produce sperm during mating trials with sailfin mollies. Rapid spermiation for conspecific females may provide at least three benefits to males: (1) males reduce physiological costs associated with spermatogenesis, (2) males will have more sperm available and higher quality sperm available for sperm competition, and (3) males may avoid expending as much sperm when mating with heterospecific females.

In Chapter III, I examined seasonal variation in reproduction in the sailfin molly. The sailfin molly occupies habitats in parts of the southern temperate zone in North America, but little is known about seasonal patterns in reproductive parameters such as female egg production and male sperm production. This study sought to: (1) examine the seasonal patterns in egg availability; (2) examine the seasonal patterns in male sperm availability; (3) determine whether variation in sperm availability follows the same pattern of variation observed in female fecundity; and (4) examine the relationship between female size and fecundity. Because both egg and sperm production can be energetically costly, individuals may reduce costs associated with reproduction by reducing the availability of ready gametes during the time of year when mating is infrequent. Male sailfin mollies were collected at the beginning of each month for a year. Ready sperm was extracted from males immediately upon collection. Female sailfin mollies were also collected, throughout the year, and the total number of eggs in the females was counted. These data suggest that males exhibit seasonal variation in sperm availability and that this variation follows a pattern similar to the variation observed in female fecundity. While the number of fecund females varied among seasons, season did not affect the number of eggs carried in fecund females. The pattern of seasonal variation in egg production and sperm availability indicates that males reduce costs associated with sperm production by synchronizing with female reproduction.

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CHAPTER II

DIFFERENTIAL SPERM TRANSFER BY MALE SAILFIN MOLLIES IN A UNISEXUAL-BISEXUAL SPECIES COMPLEX: RAPID SPERMIATION DURING MATING?

It is increasingly evident that sperm production is costly to males (Dewsbury 1982; Nakatsuru & Kramer 1982; Shapiro et al. 1994; reviewed in Wedell et al., 2002; Aspbury & Gabor, 2004). Spermatogenesis and spermiation, the last stage of spermatogenesis when sperm detach from the Sertoli cells (Grier 1973), are both regulated by male hormones (reviewed in Weltzein et al., 2004; Walker & Cheng, 2005). Female hormones may also stimulate spermatogenesis and spermiation (e.g., Miura et al. 1999; reviewed in Akingbemi, 2005). The physiological changes associated with sperm production when males are provided with stimuli from females are referred to as the priming response (Olsén & Liley 1993; Bozynski & Liley, 2003). The priming response allows males to budget energy associated with sperm production and conserve energy in the absence of females (Liley & Kroon 1995).

In addition to reducing physiological costs associated with spermatogenesis, differential sperm production for desirable females may increase male mating success

and can indicate male mate choice (Aspbury & Gabor 2004 a, b). For example, courtship and sexual behavior are positively correlated with sperm production in some species (e.g., guppies, *Poecilia reticulata*, Matthews et al. 1997; Evans et al. 2002, Amarillo fish, Girardinichthys multiradiatus, Macias-Garcias & Saborio 2004). Male mate choice may be expected when the operational sex ratio is female biased, when males provide paternal care, when males are resource limited, when mating is costly to males, when sperm production is costly, or when there is a large difference in the quality of available females as mates (Andersson 1994; e.g., refs. Gabor & Ryan 2001; Wong et al. 2005). For example, choosy males may increase their reproductive success by mating with females with higher fecundity. In many species, female fecundity increases with size, and males exhibit preferences for large females (Andersson 1994; e.g., sailfin mollies, P. latipinna, Travis & Trexler 1987; Ptacek & Travis 1997; Gabor 1999; Japanese beetle, Popillia japonica newman, Saeki et al. 2005; salamanders, Desmognathus santeelah, Verrell 1995, zebra finches, Taeniopygia guttata, Jones et al. 2001). Thus, male mate preference may translate into males producing more sperm for larger females.

One scenario where control of sperm production and sperm expenditure should be strongly favored is within a species assemblage that consists of a bisexual species and a closely related unisexual gynogenetic species (Gabor & Ryan 2001; Aspbury & Gabor 2004b). Gynogenetic species consist of only females but require sperm from males of closely related bisexual species to initiate embryogenesis (Hubbs & Hubbs 1932; Balsano et al. 1989). The Amazon molly, *Poecilia formosa*, is a gynogenetic species of hybrid origin that requires sperm from males of either parental species, *P. latupinna* or *P*. *mexicana*, to initiate embryogenesis Hubbs & Hubbs 1932; Kallman 1962; Darnell et al. 1967). Males cannot directly increase their fitness by mating with Amazon mollies because the male genes are not incorporated into offspring, though males may indirectly increase their fitness when female sailfin mollies copy the mate choice of Amazon mollies (Schlupp et al. 1994). Male sailfin mollies prefer to mate with female sailfin mollies over Amazon mollies if given a choice, and more so in populations sympatric with Amazon mollies (Hubbs 1964; Ryan et al. 1996; Gabor & Ryan 2001). Males also show a stronger association preference for female sailfin mollies over Amazon mollies (Schlupp et al. 1994; Gumm et al. 2006). Male sailfin mollies produce more sperm when in the presence of female sailfin mollies than when in the presence of Amazon mollies (Aspbury & Gabor 2004b). The following experiments examine sperm availability after mating in the sailfin molly and how it may be affected by sperm expenditure and rapid spermiation during mating.

Experiment 1: sperm availability after mating with two different species

The objective of this study was to determine how the previously demonstrated greater strength of male mating preferences for female sailfin mollies than Amazon mollies (measured as association time, mating attempts, and sperm production) relates to male sperm availability. I chose to examine sperm availability over sperm transfer because sperm transferred to females may indicate insemination success while sperm availability may be a more accurate measure of male investment. Sperm loss may occur during sperm transfer, and therefore sperm recovered from females after sperm transfer does not

necessarily reflect male sperm expenditure. Sperm loss affects sperm availability for future matings, and thus affects a male's future reproductive success. Because males expend sperm during mating, sperm availability after mating likely reflects potential sperm allocation. For example, high sperm availability after mating may indicate that little sperm was expended during mating. If sperm expenditure is a variable that contributes to male mating preferences for conspecific females, then I expect that sperm expenditure by male sailfin mollies will exhibit the same pattern as observed previously in this species: male sailfin mollies are expected to expend more sperm when mating with female sailfin mollies than when mating with Amazon mollies. When males of the other parental species of Amazon mollies, P. mexicana, mated with female P. mexicana and Amazon mollies in a choice experiment, more sperm was left in the reproductive tract of female P. mexicana than of Amazon mollies (Schlupp & Plath 2005). This may indicate higher sperm expenditure by male *P. mexicana* to conspecific females. I expect that male sailfin mollies will have more sperm available after mating with Amazon mollies than after mating with female sailfin mollies.

Methods

The sailfin molly, *Poecilia latipinna*, is a livebearing fish native to brackish waters of southern Mexico and the southern United States near Rio Tuxpan along the Gulf of Mexico through North Carolina. The Amazon molly, *P. formosa*, is a livebearing fish native to rivers and streams along the Gulf Coast from Vera Cruz, Mexico to southern Texas. This clonal, all female, gynogenetic species formed from a single

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hybridization event that occurred as far as 100,000 years ago, based on genetic evidence (Avise et al. 1991; Schartl et al. 1995; but see Dries 2000, 2003). Amazon mollies require sperm to initiate embryogenesis, and sexually parasitize males from both of their parental species, *P. latipinna* and *P. mexicana* (Hubbs & Hubbs 1946; Kallman 1962; Darnell et al. 1967).

In poeciliid fishes, spermiation occurs when sperm detach from the Sertoli cells (Grier 1973), and can be influenced over short time periods by environmental factors and by the availability of receptive females (Evans & Magurran 1999; Constantz 1989). Spermatogenesis (the formation of the male gametes) depends more on long term environmental variation (Constantz 1989). In poeciliids, sperm cells are transferred to females via the gonopodium, in bundles (spermatozeugmata) that contain 4000 to 5500 sperm cells (see Kallman 1975 for review). Female poeciliids store sperm and may have up to eight broods from stored sperm (Constantz 1989).

In the following experiment I used male and female sailfin mollies originating from a population sympatric with Amazon mollies in Tamaulipas, Mexico (2003), and Amazon mollies originating from another population sympatric with sailfin mollies also in Tamaulipas, Mexico (1989; 1998). All fish were maintained at Texas State University in 38 l (54 x 29 x 33 cm) and 53 l aquaria (76 x 32 x 32 cm). Fish were maintained on a 14-h light:10-h dark cycle using UV lighting to simulate daylight, and fed Ocean Star International Inc. Spirulina Flake mixed with Ocean Star International Inc. Freshwater Flake food twice daily until satiation and supplemented daily with live brine shrimp.
Females have a 30 d ovarian cycle (Liley 1966) and thus were isolated for a minimum of 30 d in single sex tanks to control for receptivity. As the study progressed, some females were isolated for more than 30 d. Females were at least 30 mm standard length to ensure maturity. Males were identified as mature by the fusion of the anal fin into the gonopodium. Males were isolated for at least 7 d in single sex tanks.

Mating trials were conducted from August-October 2004 between 0800-1700 h. On day zero of the experiment, male standard length (SL) was recorded and sperm was extracted following established protocols (Aspbury & Gabor 2004 a, b). Removing sperm on day zero allowed males to only have sperm available that was primed for the stimulus females. Following sperm extraction, males (mean SL \pm SE= 30.0 \pm 1.8 mm, range = 21.1-49.7 mm) were placed in separate 18 l aquaria. One female conspecific and one female heterospecific (size matched \pm 2 mm SL; mean SL sailfin molly, 35.2 \pm 1.0 mm, range = 32.0-51.0 mm; mean SL Amazon molly, 35.8 \pm 1.1 mm, range = 32.2-52.5 mm) were haphazardly paired with each male. Aquaria were divided in half with a clear Plexiglas divider with the male on one side and the females on the other.

On day three of the experiment, one female, picked randomly, was removed from the tank. Three days has been shown to be a sufficient amount of time for males to build sperm stores back up to their baseline level (Aspbury & Gabor 2004a). After removing the tank divider the male was allowed to physically interact with the remaining female for 60 min; timing started with the first mating attempt (gonopodial thrust). If the male did not attempt to mate with the female within 60 min, the trial ended. During the first 10 min of the mating trial, the number of gonopodial thrusts directed at the female was recorded. Most observed mating attempts occurred during the first 5 min of the trial. Sperm was extracted from the male immediately following the mating trial. Following sperm extraction, all fish were returned to the test tank for another three days. The second mating trial was repeated after three days with the female that was not mated in the first trial. Following completion of both mating trails, all fish were returned to the test tank for another three days. On the third day after the completion of both of the mating trials (day 9), I extracted sperm from the male for the measure of sperm primed for the stimulus females. This measure of sperm production is more accurate than using the day 0 measure of sperm production, since males vary sperm production based on female species and size (Aspbury & Gabor 2004 a, b). Sperm samples were coded so that species identity of the female mated during the trial could not be identified when counting.

To determine if male sperm production decreases across days, I tested males (N=10) in the same conditions as outlined above in May 2005. Sperm was stripped from each male on the same days as the males in the experimental groups, but these males never participated in mating trials. I compared the amount of sperm stripped from males across days three, six and nine using a repeated measures ANOVA.

To analyze sperm availability, I subtracted the day 9 count by the sperm remaining in males after each mating trial. Positive values were possible when the amount of sperm remaining after mating is higher than the day 9 sperm count. Mating trials were included for analysis if males attempted to mate with one or both females. All statistical analyses were performed using S-Plus 6.1 (Lucent Technologies, Inc.) and Statview 5.0 (SAS Institute, Inc.). All data met assumptions of parametric analyses. Alpha was set at 0.05 and all tests were two-tailed.

Results

Male sailfin mollies were more likely to direct mating attempts at sailfin mollies than Amazon mollies (Fisher's exact test on gonopodial thrusts: P=0.002, N=24/24 male mating attempts with the sailfin molly and N=15/24 male mating attempts with the Amazon molly).

The day 9 measure of sperm primed was positively dependent on both male SL and average female SL for both species. Larger males had a higher sperm count, and males in the presence of larger females had a higher sperm count (day 9 sperm count = $-4.0 \times 10^7 + 6.1 \times 10^5$ (average female SL) + 8.0×10^5 (male SL); $F_{2,21}=24.40$, P<0.0001, $r^2=0.69$). In the control experiment, I found no significant increase or decrease in sperm stripped from non-mated males during the full length of the experiment (ANOVA: $F_{3,27}=0.577$, P=0.635).

Males had significantly more sperm available after mating with sailfin mollies than after mating with Amazon mollies (Fig. 2.1a; paired *t* test: t_{23} =2.834, *P*=0.009). When the sperm available was subtracted by the baseline measure of sperm primed, this difference was also significantly different when mating with Amazon mollies than when mating with sailfin mollies (Fig. 2.1b; paired *t* test: t_{23} =2.834, *P*=0.009).

Discussion

In this experiment, male sailfin mollies had more sperm remaining after mating with female sailfin mollies than after mating with Amazon mollies. There are many hypotheses that may explain these results that I will discuss. First I will list these, then I will discuss each in detail. Male sailfin mollies may expend more sperm when mating with Amazon mollies because: (1) Males perceive benefits of heterospecific mate choice copying; (2) males perceive a risk of sperm competition when mating with female sailfin mollies or reserve sperm for future matings; (3) males expend more sperm to Amazons from a foreign population; (4) choosy female sailfin mollies prevent/limit sperm transfer; (5) it is easier to expend more sperm to Amazon mollies; (6) male sailfin mollies may rapidly produce sperm for conspecific females during mating.

If sperm availability after mating reflects sperm allocation, then male sailfin mollies expend more sperm when mating with Amazon mollies. There are at least two potential explanations why male sailfin mollies may expend more sperm to Amazon mollies. First, males may perceive benefits of heterospecific mate choice copying in this experiment (Schlupp et al. 1994) because males primed sperm in the presence of females of both species. Heterospecific mate choice copying occurs when a female sailfin molly observes the mate choice of an Amazon molly and as a result prefers the same male as

the Amazon molly preferred (Schlupp et al. 1994). Males may then indirectly increase fitness by mating with Amazon mollies. If males perceive benefits of mate choice copying then males should mate with Amazon mollies but reduce costs associated with mating by reducing sperm transfer to Amazon mollies (Schlupp & Plath 2005). Alternatively, males may expend less sperm when paired with sailfin mollies in expectation of potential sperm competition or more matings in the near future. It remains unclear how males assess the risk of sperm competition without the presence of competitors (but see Parker et al. 1997). Perhaps some aspect of male mating strategy may promote higher sperm expenditure to Amazon mollies when males cannot simultaneously choose between both species of female. In green tree frogs, Hyla cinerea and H. gratiosa, females respond to calls of heterospecific males during no choice tests even though they prefer the call of conspecific males (Gerhardt 1974; Oldham & Gerhardt 1975). More work needs to be done to assess how male preferences affect sperm allocation strategies in choice, no choice, and sequential choice experimental designs.

Another alternative explanation for my finding is that male sailfin mollies may not expend as much sperm when mating with Amazon mollies native to the same population as the male sailfin mollies. I used Amazon mollies that were foreign to the population of sailfin mollies due to a lack of Amazons from the male population at the time of this study. Males from the population I studied have not coevolved with Amazon mollies from the populations, and thus may not have as strong avoidance for these Amazon mollies as they would for Amazon mollies from their own population. Given that Gabor & Ryan (2001) examined male mating preference with sailfin molly females that were from either a native or a foreign population and found no significant difference between the two treatments, I did not expect a different response to non-native Amazons. Amazon mollies do not show much genetic variation among individuals compared to similar bisexual species (Avise et al. 1991), decreasing the likelihood that the results were affected by using a foreign population of Amazon mollies. Another possibility is that choosy female sailfin mollies avoid and prevent males from transferring sperm. This possibility seems unlikely because female avoidance of males was observed in both species of females at similar frequencies (D. Robinson personal observation). Alternatively, it may be easier for male sailfin mollies to expend sperm to Amazon mollies than to female sailfin molly. This also seems unlikely because male *P. mexicana* transferred more sperm to conspecific females than to Amazon mollies.

The favored alternative hypothesis is that the positive measure of sperm availability may indicate rapid sperm production during the mating trials due to stimulation from physical interactions with females. Ready sperm for mating can be influenced over short time periods by environmental factors and by the availability of receptive females (Constantz 1989; Evans & Magurran 1999). In my study some males had positive sperm expenditure (that is they had more sperm left than their baseline level of sperm primed) after mating with sailfin mollies more often than after mating with Amazon mollies. Male sailfin mollies prime more sperm for female sailfin mollies than for Amazon mollies over seven days (Aspbury & Gabor 2004b), and this pattern may 35

also occur with rapid spermiation during mating. In the following experiment I test this hypothesis.

Experiment 2: rapid spermiation for conspecific females

While many studies have examined costs of sperm production through examining sperm priming (e.g., Olsén & Liley 1993; Liley & Kroon 1995; Evans & Magurran 1999; Bozynski & Liley 2003) and other studies have examined sperm expenditure and sperm transfer (e.g., Evans et al., 2003; Schlupp & Plath 2005), no studies, to our knowledge, have examined the potential for rapid spermiation between species. Rapid spermiation may provide a mechanism by which choosy males can increase mating success by restricting spermiation to times when a desirable female or conspecific female is accessible for mating.

The following experiment seeks to determine whether rapid spermiation is occurring during mating trials for conspecific or heterospecific females. Rapid spermiation during mating should increase the amount of sperm that males have available for transfer. If rapid spermiation is occurring, then there should be a positive slope in sperm availability over time as the rate of spermiation increases and expenditure decreases (Fig. 2.2a). Males should also have more sperm available after mating as trial length increases. If rapid spermiation does not occur, then there should be a negative slope in sperm availability over time as more sperm is expended (Fig. 2.2b). If the rate of rapid spermiation is equal to the rate of sperm loss, then there may not be a change in sperm availability as mating time increases (Fig. 2.2c). There may also be no change in sperm availability if rapid spermiation does not occur and males only expend sperm in the beginning of the mating (Fig. 2.2d). Because male sailfin mollies prefer female sailfin mollies over Amazon mollies, I expect that rapid spermiation during mating will occur for female sailfin mollies but not for Amazon mollies.

Methods

Sailfin mollies were collected from Spring Lake, TX (the headwaters of the San Marcos River) and *P. formosa* were collected from the San Marcos River near Martindale, TX. Both populations were introduced into the San Marcos River in the 1940s (Brown 1953).

Fish maintenance is detailed in experiment 1. Mating trials were conducted from March – June 2006 and started between 0830-1030 h. On day zero of the experiment, I extracted sperm from the males so that the only sperm available for mating was primed during the trial. Following sperm extraction, one female was placed opposite the male in a tank divided in half with a clear Plexiglas divider. Male sailfin mollies were placed haphazardly with a female of one species. Male and female pairs were assigned to one of five treatments (N=16 per treatment, N=80 per female species) differing in the amount of time for mating: (1) 10 min; (2) 60 min; (3) 120 min; (4) 240 min; and (5) 480 min.

On day three the divider was removed and the male could physically interact and potentially mate with the female for 10 - 480 min depending on the treatment; time started with the first mating attempt (gonopodial thrusting). Immediately following trials, sperm was extracted from the male. Then male and female were once again divided by a Plexiglas divider. On day six, thus three days after the trial, sperm was extracted from the male again to obtain the baseline amount of sperm that the male primed in the presence of a female. Afterwards, the male and female were measured for SL. Males were not used for more than one trial. Females were isolated again upon completion of the trial for 30-60 days. Some females were reused but were used maximally three times for trials.

I used standard sperm extraction and counting procedures (Aspbury & Gabor 2004a, b). Sperm available after mating (day 3) was subtracted by the baseline measure of sperm primed (day 6), on the assumption that sperm priming up to day 3 of the trial was the same as priming between days 3 and 6. As with experiment 1, positive numbers indicate that more sperm was available after mating than baseline measure of sperm primed.

Male SL is positively correlated with sperm production. To remove male size effects from the analysis, I expressed sperm expenditure per SL. A cube root transformation on scaled sperm expenditure met the assumptions of homoscedasticity and normality for ANOVA while maintaining the positive and negative relationships in the dataset. I determined the relationship between trial time and sperm availability using a separate linear regression for each species. A *t*-test determined the difference in slopes between the regressions. Statistical analyses were performed using S-plus 7.0 and Statview 5.0.

Results

There was a slightly positive significant relationship between mating trial time and sperm availability for female sailfin mollies (Fig. 2.3a; linear regression: $r^2=0.059$, $F_{1,78}=4.909$, P=0.030). There was no significant relationship between time and sperm availability for Amazon mollies (Fig. 2.3b; $F_{1,78}=0.297$, P=0.587). Including female SL as an independent variable did not change these relationships. The slopes of the regressions for each species were not significantly different from each other (*t*-test: $t_{79}=-$ 1.1247, P=0.262).

Discussion

In experiment 1, male sailfin mollies had significantly more sperm available after mating with female sailfin mollies than after mating with Amazon mollies. Furthermore, this average was positive for female sailfin mollies, indicating that more sperm was left after mating with female sailfin mollies than was primed in the presence of both species. However, this positive value did not exclude zero. Experiment 2 separated the possibility that the amount of sperm remaining after mating reflects sperm expenditure by measuring sperm availability over mating trial time. If males mating with conspecific females produced more sperm during the trial than males mating with heterospecific females, I expected sperm availability to increase with time for trials involving conspecific than heterospecific females. The results presented here demonstrate that sperm availability did in fact increase as trial time increased when males mated with female sailfin mollies, indicating that the spermiation rate outpaced the sperm loss rate, most likely because of enhanced spermiation when male sailfin mollies mated with conspecific females.

By contrast, no significant trend in sperm availability over time was observed for matings with Amazon females. This may occur if the rate of sperm production equaled the rate of sperm loss. Schlupp & Plath (2005) determined that male Atlantic mollies (P *mexicana*), the other parental species of Amazon mollies, transferred less sperm when mating with Amazon mollies than mating with female Atlantic mollies. Because male sailfin mollies prefer to mate with female sailfin mollies over Amazon mollies (Hubbs 1964; Ryan et al. 1996; Gabor & Ryan 2001), I would expect the same pattern of sperm expenditure with male sailfin mollies. The amount of sperm available may not change over time if males lose little sperm to Amazon mollies and if this rate of low sperm expenditure is compensated for by low rates of sperm production. However, this pattern could also occur due to high sperm loss and high sperm production, although this seems less likely since males prefer to mate with female sailfin mollies. Alternatively, males may expend most sperm during the first few minutes of the mating trial, which would also produce no trend in sperm availability over time. Currently, I can not definitively differentiate between these results with out further study.

Interestingly, I did not find that cumulative sperm availability decreased over time with either species. Such decreases would be expected if no additional sperm was produced during the mating. Additionally, the slopes of the regressions for female sailfin mollies and Amazon mollies were not significantly different. Both of these results suggest that some spermiation may occur when male sailfin mollies mate with Amazon mollies. Because it seems likely that rapid spermiation for conspecific females occurs, future studies should not use sperm availability after mating to determine the proportion of sperm expended during mating, as such estimates may overestimate sperm available prior to mating and underestimate the proportion expended. The regressions were not affected by female SL, although a prior study indicated that sperm primed increased with female SL (Aspbury & Gabor 2004a). The effect of female SL may be controlled in this experiment because males primed sperm for the same females with which they mated.

It appears unlikely that sperm available after mating accurately reflects male sperm expenditure. Ready sperm for mating can be influenced over short time periods by environmental factors and by the availability of receptive females (Constantz 1989; Evans & Magurran 1999). In experiment 1, some males had positive sperm availability (that is they had more sperm left than their baseline level of sperm primed) after mating with sailfin mollies more often than after mating with Amazon mollies. Male sailfin mollies prime more sperm for female sailfin mollies than for Amazon mollies over seven days (Aspbury & Gabor 2004b), and this pattern may also occur with rapid sperm production during mating. Rapid sperm production for conspecific females has at least three possible benefits to males: (1) males reduce physiological costs associated with spermatogenesis; (2) males will have more sperm available and higher quality sperm (newer sperm) available for sperm competition (Roche et al., 1968); and (3) males may expend less sperm when mating with heterospecific females. I would expect selection to favor male strategies that reduce costs associated with spermatogenesis and increase male mating success (Liley & Kroon 1995).

Little work has been done on rapid spermiation during mating (but see Olsén & Liley 1993) and little is known about how common rapid spermiation may be for conspecific females. In other species systems where hybridization can occur, rapid spermiation for conspecific females may reduce gene flow between species. Systems where priming responses have been found (e.g. Stacey & Sorensen 1991; Bozynski & Liley, 2003) may be good candidates to examine rapid spermiation.

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Figure legends

Figure 2.1. Amount of sperm (a) left after mating, and (b) available after mating with female sailfin mollies and Amazon mollies. (a) Male sailfin mollies, *Poecilia latipinna*, had more sperm remaining after mating with sailfin mollies (N=24; mean sperm cells \pm SE= (6.9 \pm 2.0) x 10⁶ cells) than after mating with Amazon mollies (N=24; (3.2 \pm 1.1) x 10⁶ cells). (b) Males showed greater sperm availability for sailfin mollies, *P. latipinna*, (N=24; (2.7 \pm 1.0) x 10⁶ cells) than for female sailfin mollies (N=24; (-1.1 \pm 1.1) x 10⁶ cells). Sperm availability equals the difference between sperm extracted after mating and the day 9 measure of sperm production.

Figure 2.2. Possible rates of sperm loss and spermiation over time. Expected rates of sperm loss and spermiation for (a) female sailfin mollies and (b) Amazon mollies. Thin lines indicate the rate of sperm loss. Thick lines indicate rate of spermiation. (c) Alternatively the rate of sperm loss and spermiation may be equal. Only one line is visible and represents the rate of sperm loss and spermiation. (d) Sperm loss may also only occur during the beginning of the mating.

Figure 2.3. Sperm availability over time in the sailfin molly, *Poecilia latipinna*. Sperm availability was measured as the difference between the baseline measure of sperm expenditure and the amount of sperm remaining in males after mating (*N*=16 per treatment). Males were paired with sailfin mollies (a) or Amazon mollies (b) for 10, 60, 120, 240, or 480 minutes.





Sperm cells/minute



minutes

(c)

Sperm cells/minute



minutes



(a)

CHAPTER III

SEASONAL SPERM AVAILABILITY AND FEMALE FECUNDITY IN THE SAILFIN MOLLY

Introduction

Seasonal variation in animal reproduction may occur due to a range of environmental factors. In marine organisms, reproduction may coincide with tidal cycles (e.g., Morgan & Christy, 1994; Yamahira, 2001) and lunar cycles (reviewed in Takemura *et al.*, 2004). Freshwater and terrestrial animals in tropical climates may exhibit seasonal variation that coincides with peaks and dips in precipitation, high food availability, and low predation risk (reviewed in Brown & Shine, 2006). Similar to other taxa in tropical climates, *Poeciliidae*, livebearing fishes, exhibit seasonal variation (e.g. Reznick, 1989; Morris & Ryan, 1992; Winemiller, 1993).

In temperate climates, reproduction often coincides with day length and temperature (e.g., Winemiller & Rose, 1992). Day length and temperature also often correspond to high food availability in temperate climates (e.g., Love *et al.*, 1991; Sarma *et al.*, 2005). Poeciliid fishes from temperate latitudes often show seasonal variation in reproduction triggered by day length and temperature (reviewed in Constantz, 1989).

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Most research examining seasonal variation in reproduction at temperate latitudes investigates seasonal variation in fecundity of females (e.g., Machado et al., 2002; reviewed in Constantz, 1989; but see Grier, 1981; Burns, 1985). Sailfin mollies (Poecilia *latipinna*) exhibit little reproduction during the winter months, though the exact months may vary by population (e.g. Snelson, 1984; Farr & Travis, 1986; Snelson et al., 1986). Size at maturity also varies by population, and is affected by salinity and temperature (Trexler et al., 1990). In addition, male sexual behaviour has been observed year round, although male sexual behaviour increased when females were receptive (Farr & Travis, 1986). However, patterns of sperm production across the seasons and how these patterns correlate with female fecundity are not well understood. Male sailfin mollies may still gain paternity during the non-mating season because female poeciliids, including the sailfin molly, store sperm (Constantz, 1989). However, some authors have suggested that there is second male sperm precedence when females are given the choice between two males in *Poecilia reticulata* (Evans & Magurran, 2001; Pitcher et al., 2003). In addition, males mating with females that are not receptive are less likely to gain paternity than males that mate with females right after parturition, when female are most receptive (e.g., sailfin mollies, Poecilia latipinna, Farr & Travis, 1986; reviewed in Constantz, 1989). Consequently, we may expect that the patterns of seasonal variation in sperm production would mirror those patterns observed in female fecundity. Therefore, the first objective of this study was to determine the seasonal patterns of female fecundity and male sperm reserves, and their relationship to each other in a population of sailfin mollies. This particular population was introduced into a constant temperature spring in the southern

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part of temperate North America. Seasonal variation in this population may be correlated to variation in day length.

In addition to describing patterns of female fecundity and male sperm reserves across a season, this study also sought to examine the relationships between body sizes and fecundity and sperm reserves in the sailfin molly. Because females are often the limiting sex, male mating success should vary more than for females (Bateman, 1948). However, male mate choice may occur when there is a large difference in mate-quality of available females (Andersson, 1994). Sperm production can be energetically costly and limit male reproductive success (Dewsbury, 1982; Nakatsuru & Kramer, 1982; Shapiro et al., 1994; review in Wedell et al., 2002), and discriminating males may increase their reproductive success by mating with high-quality females. One mate-quality cue that varies within populations is female size. In many species of fish, female fecundity increases with size (reviewed in Andersson, 1994), and males exhibit preferences for large females (e.g., sailfin mollies: Travis & Trexler, 1987; Ptacek & Travis, 1997; Gabor, 1999; Aspbury & Gabor, 2004; pipefish, Syngnathus typhle; Berglund et al., 1986; sockeye salmon, Oncorhynchus nerka: Foote, 1988; three-spined sticklebacks, Gasterosteus aculeatus: Kraak & Bakker, 1998). The size of female sailfin mollies is indeterminate and is positively correlated with brood size within a population (Trexler et al., 1997). Thus male sailfin mollies that mate with larger females may have greater reproductive success.

The research presented here will: (1) examine the relationship between female size and fecundity; (2) examine the seasonal patterns in egg availability; (3) examine the seasonal patterns in male sperm availability; and (4) determine whether variation in sperm availability follows the same pattern of variation observed in female fecundity.

Materials and Methods

The sailfin molly is native to brackish waters of southern Mexico near Rio Tuxpan along the Gulf of Mexico through North Carolina. They have also been introduced into a few freshwater streams such as the San Marcos River and Comal River in central Texas, USA. The population in the headwaters of San Marcos River, Spring Lake, was introduced during the 1940's and originated from populations in Florida and Louisiana (Brown, 1953). The study fish were collected from Spring Lake, TX where year round there is little variation in the amount of water flow, and the clear water has stable nutrient availability and chemical properties, and a constant temperature of 21-23°C (Groeger *et al.*, 1997).

Sailfin molly life cycles are likely to vary somewhat from population to population, however some continuity is expected among populations. Female poeciliid fishes in general, have an ovulatory cycle of 30 days and are receptive as virgins and 1-2 days after parturition (Liley, 1966). Both sexes mate multiply and females are capable of storing sperm for several months (Baerends *et al.*, 1955). Previous studies concluded that female sailfin mollies are usually fecund from March-September (Travis, 1994) but in the lab this extends through October (personal observation). Female sailfin mollies from most populations are mature at 30 mm, and are identified by the presence of a brood spot, but this relationship may vary among populations (Travis, 1994). Males can be identified as mature by the fusion of the anal fin into the gonopodium. According to Travis & Woodward (1989) males from a Florida population are mature as small as 20 mm. In Spring Lake males are mature as small as 18 mm (personal observation). The data from the Spring Lake population will help determine the similarities to and differences from these other populations for some of these reproductive variables.

In this experiment, males were collected at the beginning of each month from July 2005 to June 2006. Female sailfin mollies were collected in September 2005, November 2005, December 2005, February 2006, April 2006, and May 2006. Differences in fecundity were analyzed across seasons. Females of all standard lengths, L_S , were selected to determine the relationship between female L_S and female fecundity, as well as to determine at what L_S females become mature. Upon collection, sperm was removed from all males (see Aspbury & Gabor, 2004 for sperm collection methods) to examine sperm availability across seasons.

Only females collected during the mating season were used to determine L_S at maturity because the presence of eggs or neonates could be used as a positive sign of maturity. Size at maturity was analyzed using a logistic regression to compare if different size categories of females were more or less likely to have eggs.⁻ Female L_S and egg number are positively correlated in sailfin mollies (reviewed in Travis, 1994), thus egg number was divided by female L_S Many females did not have eggs or neonates,

causing a violation of the assumption of normality that could not be corrected by a transformation so a Kruskal-Wallis ANOVA was performed to determine the difference in egg number among months. Many males also did not have sperm reserves, causing a violation of the assumption of normality that could not be corrected by transformation. Male $L_{\rm S}$ and sperm number are positively correlated in sailfin mollies (Aspbury & Gabor, 2004), thus sperm number was divided by male $L_{\rm S}$. A Kruskal-Wallis ANOVA was performed to determine the difference in sperm number among months. The least significant difference (LSD) in mean ranks was used to determine significance between pairs of months (Sokal & Rohlf, 1995). When comparing proportion of fecund females and males with sperm among seasons, months were grouped into season based on observations of mating seasons in other populations (Travis, 1994) and personal observations in the lab: early mating (March-June), late mating (July-October) and nonmating (November-February) seasons. A X^2 GOF test determined whether proportions of fecund females and proportions of males with sperm varied among seasons. A Fisher's exact test was used when at least one category had fewer than five values.

Results

Size at maturity

During the mating season females as small as 21.4 mm contained eggs. However, finding fecund females of this L_S was rare. Most females of 27 mm or greater were fecund, and the number of fecund females from 27-30 mm did not significantly differ from the number of fecund females larger than 30 mm (logistic regression: X^2 =0.462, d.f.=1, *P*=0.497). The proportion of fecund females smaller than 27 mm differed

significantly from the proportion of fecund females greater than 30 mm (logistic regression: X^2 =30.502, d.f.=1, P<0.001). Therefore, females smaller than 27 mm were excluded from further analyses.

Fecundity of mature females

Female L_8 was positively correlated to egg number (Spearman rank correlation: r_s =0.518, Z=6.053, P<0.001). Differences in egg counts among months were significant (Kruskal-Wallis ANOVA: H=57.524, P<0.001, Fig. 3.1, Table I). It should be noted that the few fecund females collected during the non-mating season were from the February collection, late in the season, and some eggs extracted at that time had characteristics indicating they were maturing embryos (criteria according to Reznick, 1981). February egg counts were also significantly higher than November egg counts (Fig 3.1, Table I).

Sperm availability in males

Differences in sperm counts among months were significant (Kruskal-Wallıs ANOVA: H=101.078, P<0.001, Table II). Median sperm count peaked in May (Fig. 3.2). The frequency of males with sperm differed significantly from the frequency of mature females with eggs during the early and late mating seasons: early in the season, 80% of the males had sperm and 98% of the females had eggs (P=0.003, Fisher's exact test) and late in the season 21% of the males had sperm and 60% of the females had eggs (X^2 =12.217, d.f.=1, P<0.001). The frequency of males with sperm did not differ significantly from the frequency of females with eggs in the non-mating season (X^2 =1.62, d.f.=1, P=0.203).

Discussion

The present study sought to clarify variation in reproduction with fish size and season in a population of sailfin mollies from a constant temperature spring and temperate climate. The relationship found between $L_{\rm S}$ and female fecundity was similar to that found in previous studies (Travis & Trexler, 1987; Travis *et al.*, 1990). The strong relationship between fecundity and $L_{\rm S}$ indicates that $L_{\rm S}$ is an honest indicator of mate quality. Larger, more fecund females have more multiply sired broods than smaller females (Travis et al., 1990; Trexler et al., 1997) and are the object of male-male competition (reviewed in Travis, 1994). Even though males mating with larger females have the potential to sire more offspring, males may not always gain a fitness advantage in terms of offspring number from females who have mated multiply but this remains to be tested. Males may still benefit from mating with larger females, such as an increased likelihood that the brood will not be aborted (Trexler, 1997). Additionally, in some species there is evidence of multiple matings causing increased brood size and may be evidenced by superfetation, which is the presence of embryos at different stages of development (reviewed in Reznick & Miles, 1989). Superfetation has not been subsequently found in sailfin mollies (Travis et al., 1990). The present study also did not find evidence of superfetation. Even though larger female sailfin mollies are more fecund, it is still unclear whether males benefit from mating with larger females.

The number of eggs in mature females differed significantly by season. Most months within the early and late mating seasons were not significantly different from each other. February was also not significantly different from the mating season months but was significantly from November. The mating season may begin earlier in Spring Lake than in other populations of sailfin mollies. Populations of sailfin mollies from brackish marshes in Florida showed two peaks in reproduction, measured by the proportion of immature males present, one in May-June and another in August-September (Snelson, 1984). In the present study, neither the early May, 2006, nor the September, 2005 collections represent an extreme with respect to any measure of fecundity. In Spring Lake, where the water temperature is constant throughout the year (Groeger *et al.*, 1997), similar peaks in reproduction may not occur within the mating season. However, this study measured egg availability while Snelson (1984) measured the number of juveniles, thus peaks in reproductive output may not be as evident.

Males had the most sperm available during the early mating season, with no difference in sperm availability in the late and non-mating season. Unlike in this study, previous work with *P. mexicana* showed that males had the highest sperm reserves during the winter months (Monaco *et al.*, 1981). The opposite result was found in the present study. Male sailfin mollies had the highest amount of ready sperm during the early mating season, and males did not have high sperm reserves during the non-mating months. However, *P. mexicana* are found in tropical climates with less variation in day length. Reproduction in *P. mexicana* continues throughout the winter, which may explain why males continue to produce sperm during the winter months.

Poeciliids in temperature climates show seasonal variation due to temperature and day length (Constantz, 1989). In Spring Lake, where the water temperature is constant throughout the year (Groeger *et al.*, 1997), an increase in day length may explain the initial increase in sperm and egg number. However, an increase in day length and atmospheric temperature may increase food availability. Sailfin mollies feed primarily on vascular plants, but also consume mosquito larvae (Harrington & Harrington, 1961). Food availability may influence sperm and egg number throughout the year, though the effects of food availability and day length may only be separated through an empirical study.

The proportion of males with sperm and the proportion of females with eggs did not differ in the non-mating season. During the early and late mating season, more females had eggs than males had sperm, but some fecund females also had maturing embryos (Reznick, 1981). Although sperm and egg availability differed in proportion during the early and late mating season, male sperm number demonstrated seasonal variation similar to the variation in female fecundity and peaked during the early mating season. By synchronising sperm availability to the time of year when females are most fecund, males may reduce costs associated with spermatogenesis. One hypothesis is that males may become less discriminating and more willing to expend sperm during female encounters as the mating season progresses and the likelihood of fertilising a female's eggs decreases. For example, male amphipods, Gammarus aequicauda, and scorpionflies, *Panorpa cognata*, become less choosy as they age and mating opportunities decrease (Thomas et al., 1998; Engqvist & Sauer, 2002). While the number of males with sperm and number of fecund females was lower during the late mating season, the reduction in male sperm availability may reflect increased sperm expenditure.

In addition, sperm availability may be lower in the late mating season due to a lower rate of spermiation. Spermiation may occur during the early mating season and to a lesser extend during the late mating season. This would increase the amount of sperm males have available for transfer when a large number of receptive females are available. These variables remain to be examined.

Spermiation has been found to vary according to season in other fishes (e.g., Jackson & Sullivan, 1995; Kara *et al.*, 1995; Fauvel *et al.*, 1999; Caputo *et al.*, 2001). In the goby, *Aphia minuta*, spermiation synchronizes to oocyte production (Caputo *et al*, 2001). While male sailfin mollies exhibit year round sexual behaviour (Farr & Travis, 1986), male sailfin mollies may reduce spermiation and spermatogenesis during the nonmating season. Thus males can reduce costs associated with spermatogenesis during the non-mating season even though they continue sexual behaviours.

In conclusion, these data provide evidence for seasonal variation in the proportion of males with sperm available and the proportion of fecund females. Although the present study lasted only a year, the patterns of female reproduction were similar to those observed in other studies (Travis, 1994). Reduction in spermatogenesis and spermiation is likely correlated to seasonal influences as well as the availability of fecund females. Further research is necessary to explore peaks in reproduction as well as how sperm limitation in females may vary by season.

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	Feb	Apr	May	Sep	Nov	Dec	
Feb	X	34.8	22.2	1.1	38.6*	38.6	
Apr	Х	Х	12.6	33.6	73.4*	73.4*	
May	Х	Х	Х	21.1	60.8*	60.8*	
Sep	Х	Х	Х	Х	39.7*	39.7	
Nov	Х	Х	Х	Х	Х	0.0	
Dec	X	X	Х	Х	Х	Х	

Table I. LSD between mean ranks of months for egg availability in female sailfin mollies.

LSD, least significant difference **P*<0.05

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	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jan	Х	49.0	81.1*	68.8	111.8*	44.9	25.1	29.2	28.5	27.2	34.2	27.6
Feb	Х	Х	32.1	19.8	62.8	4.1	74.1	78.2	77.5	76.2	83.2	76.6
Mar	Х	Х	Х	12.3	30.7	36.2	110.3*	110.3*	109.6*	108.3*	115.3*	108.7*
Apr	Х	Х	Х	Х	43.0	23.9	97.3*	98.0*	97.3*	96.0*	103.0*	96.4*
May	Х	X	Х	Х	Х	66.9	139.0*	141.0*	140.3*	139.0*	146.0*	139.4*
Jun	X	Х	Х	Х	Х	Х	79.1	74.1	73.4	72.1	79.1*	72.5
Jul	Х	X	Х	Х	Х	Х	Х	4.1	3.4	2.1	9.1	2.5
Aug	Х	Х	Х	Х	Х	Х	Х	Х	0.7	1.6	5.0	1.6
Sep	Х	Х	X	X	Х	Х	Х	Х	Х	0.9	5.7	0.9
Oct	Х	Х	Х	X	Х	Х	Х	X	Х	X	7.0	0.4
Nov	Х	Х	Х	Х	Х	Х	Х	Х	X	X	X	6.6
Dec	X	X	X	X	X	X	Х	Х	X	X	X	X

Table II. LSD between mean ranks of months for sperm availability in male sailfin mollies.

LSD, least significant difference **P*<0.05

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FIG 3.1. Boxplots of variation in egg availability each month in female sailfin mollies from Spring Lake, TX from July 2005 to June 2006. Egg number was divided by female L_S Numbers above the boxplots represent the number of females counted each month. The horizontal line inside the box indicates the median egg number. The lower and upper edges of the box represent the 25% and 75% of the distribution, respectively. The whiskers represent the outer 0% and 100% of the distribution.

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FIG 3.2. Boxplots of variation in sperm availability each month in male sailfin mollies from Spring Lake, TX from July 2005 to June 2006. Sperm number was divided by male L_S Numbers above the boxplots represent the number of males counted each month. The line inside the box represents the median sperm number. The lower and upper edges of the box represent the 25% and 75% of the distribution, respectively. The whiskers represent the outer 0% and 100% of the distribution.

VITA

Donelle M. Robinson was born in Miami, Florida on October 10, 1981, the daughter of Joanne Marie Robinson and Nathaniel David Robinson, Jr. She graduated from Brentwood High School in Brentwood, Tennessee, in May 2000. At the University of Richmond in Virginia, she received the degree of Bachelor of Science in Biology in 2003. She began the Master of Science program in Biology at Texas State University-San Marcos in August 2004. While at Texas State University, Donelle was employed as a research assistant and taught labs in Organismal biology.

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