FEMALE MATING DECISIONS IN THE TRINIDADIAN GUPPY, *POECILIA RETICULATA*
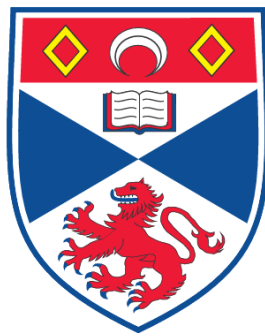
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**A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews**

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FEMALE MATING DECISIONS IN THE TRINIDADIAN GUPPY,

POECILIA RETICULATA

Thesis submitted by

Miguel Barbosa

For the requirements of the degree of Doctor of

Philosophy

School of Biology, St Andrews University

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DECLARATION

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To my son as an example of how work and fun can walk hand in hand

TABLE OF CONTENTS

Chapter three – The facultative adaptation of offspring sex ratio, number
and size under extreme population sex ratios

Chapter four - Female Trinidadian guppies actively promote polyandry

Chapter five - No mating benefits from multiple mating in guppies: a two-generation test

References 129

Appendix

LIST OF FIGURES

(4 x 60 n=240) to area C. The test female (n=60) was allocated to area B. The barriers that divide all areas were transparent with small holes that enabled both visual as well as chemical interactions between all individuals in the tank. Time spent near the group of individuals was only valid if the test female swam freely and stayed inside the area delimited by a grey line (less than one and half body length from the barrier).

Figure 4.2 – Percentage of total time spent by the test female near a single and four males (experiment) or near a single male and four females (control) during 3 days of observation. Filled bars represent time near the single individual and open bars represent time near the group of individuals. Means and 95% confidence intervals are represented.

Figure 4.3 – Daily variation in total time spent by the test female near a single and four males (a) or near a single male and four females (b) during 3 days of observation. Filled bars represent time near the single individual and open bars represent time near the group of individuals.

Figure 5.1 – Mean differences in the proportion of fitness components measured for F1 and F2 from two mating treatments, single (open) and multiple (filled) mated females. Error bars denote 95% confidence intervals. Significance * was based on Bonferroni corrected *p*-vales (*alpha* = 0.006).

Figure 5.2 – Percentage of mortality rate (a) and birth success (b) in parents and offspring allocated to a single or a multiple mated female treatment.

Figure 6.1 – Principal coordinate analysis (PCoA) showing the dispersion to centroid of offspring phenotype for multiple (green) and single (red) mating treatments. a - Fathers, b- sons and c - daughters. Open triangles - offspring from multiple mating treatment; open circles - offspring from single mating treatment.

LIST OF TABLES

ABSTRACT

Contrary to traditional belief, the decisions that females make before, during and after mating shape its outcome and ultimately fitness. The aim of this thesis was to examine how females modify and adjust their mating decisions in line with social and environmental variability and how these directly and indirectly affect mating benefits. To address this aim I have formulated four main questions that correspond to chapters 2 to 6 in this thesis.

Firstly I asked whether there was evidence for female choice being driven by mating benefits. More precisely, in chapter 2, I reviewed the literature in search of evidence for direct and indirect benefits in female choice among freshwater fish species. Direct mating benefits were defined as an increase in female's reproductive success (number of offspring). Conversely, increases in offspring reproductive success were considered to be indirect benefits. The results showed that despite the multiple suggestions and the great amount of information available, to date there is still no evidence for both direct (increase of F1) or indirect (increase in F2) mating benefits, nor their influence in female mating decisions if freshwater fishes species. Furthermore, although polyandry occurred in more than 60% of the species reviewed, I was unable to confirm that polyandry was maintained because of indirect benefits. These findings justified the need to experimentally investigate the drivers of female mating decisions in freshwater fish species and lead to the questions addressed on chapters 5 and 6. For the experimental chapters 3-6 I used the Trinidadian guppy, *Poecilia reticulata*, as a model species. Their unique and remarkable ecological and biological characteristics coupled with easy maintenance in laboratory conditions make guppies an ideal species for behavioural studies. But in particular, the fact that guppies live in a promiscuous mating system where females despite being constantly harassed by

males may be able to control paternity, makes guppies the ideal species to address my aims.

The second question was how much were females in control of their mating decisions, and how social environment could limit these decisions. In particular, in chapter 3 I looked at how females adapt and adjust their mating decisions in line with extreme differences in population sex ratio. In chapter 4, I examined to what extent male sexual harassment affects female reproductive behaviours. Results from these two chapters indicated that female guppies have a remarkable reproductive plasticity that enable them to control their mating decisions. When faced with extreme differences in population sex ratio, female mating decisions were made in an optimal way that maximized the ratio of female reproductive benefits per investment. This translated into producing bigger offspring when in a strong female biased environment, than when in a male biased environment. Further, chapter 4 illustrated that female guppies can, despite high levels of male sexual harassment, be in control of their mating decisions. These two chapters demonstrated and have reinforced previous findings of the remarkable reproductive adaptation of female guppies to differences in the social environment.

The third question I addressed was: do multiply mated females have greater direct or indirect benefits than single mated females? To answer this question I followed for the first time reproductive success of females over two generations. I measured fitness directly (number of F1 and F2) and took as well as multiple indirect measures of fitness components for two generations in search for evidence of direct and indirect mating benefits in explaining the maintenance of female multiple mating. The results of chapter 5 revealed that female guppies do not have a higher number of F1 and F2 from polyandry or either from mating with males possessing allegedly good quality

traits. I, therefore, stressed the idea that potential differences in sexual selection pressure between laboratory and wild populations may influence the expression and intensity of mating benefits between thus explaining the difficulty of finding mating benefits.

In my fourth and last question, I used a novel statistical approach based on the analysis of the dispersion in phenotypes, to look for potential alternative explanations for the prevalence of polyandry. The results of this analysis show offspring from multiple mated mothers were phenotipically more diverse than offspring from single mated mothers. Given the direct relationship between phenotypic diversity and potential fitness gains in stochastic systems, female guppies are likely to get greater benefits from mating with males with different phenotypes than with males with a particular sexual trait. This result provides an alternative explanation for the maintenance of polyandry in resource free systems.

Overall the results of this thesis reinforce previous suggestions that female guppies are active participants in the mating process, and not necessarily limited to post-copulatory mechanisms of selection of sperm. It also showed the remarkable ability of females to adjust their reproductive investment in line with changes in the social conditions. Interestingly, my results contradict the commonly accepted assertion that females' mating preference converges towards unique male sexual traits. This result stresses the need to look at alternative explanations to justify female mating decisions.

Chapter one

GENERAL INTRODUCTION

Understanding the mechanisms underlying female reproductive decisions, and deducing how these affect female and offspring fitness have been, and still are, central questions in evolutionary ecology (Fisher 1915; Wright 1930; Trivers 1972; Roughgarden 2004). Even though both male and female participate in the mating process, it has become increasingly evident that the reproductive decisions that females make before, during and after mating are particularly important in shaping the outcome of the reproductive process (Andersson & Simmons 2006). Furthermore, the introduction of new molecular and genomic tools in behavioural experiments has led to the realization that the role of females in the mating process is not restricted to pre mating choice driven by male-male competition (Parker 1970; Birkhead & Møller 1993). In fact, females can efficiently use post-copulatory selection of sperm, or cryptic choice, to overrun or reinforce mating choices (Birkhead & Pizzari 2002). Post-copulatory selection enables females to create fertilization bias that favours certain males (Eberhard 1996). All these facts signal the importance of female mating decisions and their potential to impose changes in the strength and direction of sexual selection on males. Therefore, examining the patterns of female mating decisions and their fitness consequences can give us invaluable insights into the process of sexual selection. The aim of this PhD was to ask how females modify and adjust their reproductive decisions in different contexts and how these ultimately affect fitness.

FEMALE MATING DECISIONS: DIRECT AND INDIRECT FITNESS BENEFITS

In spite of the Portuguese popular saying "os homens sao todos iguais" (males are all the same) the fact is that some males bring more mating benefits than others. For many species, male sexual traits such as bright colouration, exaggerated body parts, frequency of sexual behaviours, ornamentation, among others are reliable indicators of male quality and of potential reproductive success (Andersson 1994; Candolin 2003). It is has been assumed that male sexual traits are major drivers of female mating decisions, since they are the basis on which females assess how much they should invest in reproduction (Sheldon *et al.* 1997; Sheldon 2000). Thus, if a given male sexual trait is indicative of reproductive benefits, then there is a selective advantage for females to mate with males possessing such traits (Iwasa & Pomiankowski 1994; Wedell & Tregenza 1999; Ishikawa & Mori 2000). Females may obtain direct benefits in the form of increased paternal care, protection against predators, nuptial gifts during mating, etc when they chose to mate with a male with a particular trait (Trivers 1972). These direct benefits will ultimately increase female fecundity, and consequently fitness. On the other hand, if male sexual traits are linked to their genetic quality, then females not only get direct benefits but also indirect benefits expressed in their offspring (Kirkpatrick 1996). The argument behind indirect benefits is that offspring inherit the good qualities of their father and these good paternal genes enhance their viability (good genes) and/or attractiveness (sexy sons) (Fisher 1915, 1958; Weatherhead & Robertson 1979; Kirkpatrick 1996). In contrast to direct benefits, indirect benefits increase offspring fitness, but not necessarily mother's fitness directly. Interestingly, in spite of extensive research, underlying support for female mate choice based on mating benefits remains highly debatable

(Charmantier & Sheldon 2006). This is particularly evident in regards to the justification of female mating choice based on indirect benefits that will increase offspring fitness (Kokko *et al.* 2003; Hunt *et al.* 2004; Akcay & Roughgarden 2007; Kotiaho & Puurtinen 2007). This controversy justifies the need to review the current evidence for both direct and indirect benefits. Therefore, I begin this thesis by reviewing both theoretical and empirical evidence in support of female mating decisions based on both types of mating benefits in freshwater fishes. An increase in a female's (mother's) lifetime reproductive success (number of F1) was taken as support for direct benefits. On the other hand, a positive relationship between male sexual traits and an increase in offspring lifetime reproductive success (number of F2) was interpreted as support for indirect benefits. This classification was adopted because it is currently accepted as the most accurate way of categorizing mating benefits (Hunt *et al.* 2004; Kotiaho & Puurtinen 2007). In chapter 2 I reviewed the literature in search of evidence for direct and indirect benefits as drivers of female mating choice in freshwater fishes.

PLASTICITY AND ADJUSTMENT OF FEMALE MATING DECISIONS IN RESPONSE TO SOCIAL CONDITIONS

Females are expected to base their reproductive decisions predominantly on the potential benefits from mating with a particular male. However, these decisions and the scale of mating benefits obtained from them may be driven and constrained by external factors, rather than merely by male quality. For example, the social conditions experienced by females prior to and during mating dramatically affect the relationship between mating benefits and costs (Real 1990; Charnov 2001) and consequently a female's investment in reproduction (Mousseau & Fox 1998). It has

been theoretically and empirically demonstrated that the proportion of males and females in a population has a direct effect on levels of sexual harassment, competition and intra/inter-sexual conflict, which eventually influences female reproductive decisions (Charnov 2001; Rankin & Kokko 2007). Furthermore, differences in social conditions have a direct impact on the amount of resources available, and consequently on the total energy females can allocate to reproduction (Reed *et al.* 1996). All these factors highlight the important evolutionary and ecological role of social factors by illustrating how these can potentially act as sources of variation in female and offspring fitness. It is, then, essential to take into account the social environment when addressing questions regarding female mating decisions. The theoretical prediction is that female mating decisions should co-vary with the conditions experienced by them in ways that optimize fitness (Smith & Fretwell 1974; Charnov 1982; Sheldon 2000). In other words, reproductive success is optimized when females respond optimally to both environmental and social cues. One way in which females can optimize their reproductive investment is by adjusting the sex ratio of their brood. This is particularly relevant if the relative benefits of producing sons and daughters differ for a given set of social conditions (Trivers & Willard 1973; Frank 1990; Clutton-Brock & Vincent 1991). There are numerous examples of how females skew their sex ratio in response to social factors (Emlen 1997; Duchateau *et al.* 2004; Martins 2004; Reed *et al.* 2008). However, sex ratio adjustment is not universal, and in many species females lack the ability to determine or control the sex of their brood (Helle *et al.* 2008). This absence, however, does not limit females from using other reproductive strategies of optimization. Females can adjust their resource allocation to progeny instead (Smith & Fretwell 1974; Charnov 1982; Hunt & Simmons 2004). Sex ratio adjustment and differential allocation are two examples

that illustrate well the remarkable plasticity in female reproductive optimization. But more importantly, these emphasize the importance of examining the extent to which differences in social conditions experienced by females prior to mating affect their reproductive investment in studies of female mating decisions. This question was addressed in the third chapter. In particular, I looked at how females adjust the sex ratio of their brood (compensatory mechanisms) and/or invest differentially (brood and offspring sizes) in response to extreme social conditions. Chapter 3 aimed to develop a better understanding about the plasticity in female mating decisions, and particularly to shed light into how variability in social conditions affects reproductive investment and potentially mating benefits.

FEMALE MATING DECISIONS: WHY MATE POLYANDROUSLY?

Mating benefits are, for any individual, the main objective of reproduction. However, females and males have different reproductive costs, and hence different ways of achieving mating benefits. Males maximize the number of offspring by maximizing the number of sexual partners, whereas female reproductive success is limited by the progeny they can produce per mating event, and thus it is thought to be independent of the number of males with whom they mate (Bateman 1948; Ihara 2002). Females are, therefore, expected to show a conservative approach to mating, whereas males should mate promiscuously. This prediction is in line with evidence showing that the costs of mating are greater for females than for males, as a result of physical and also physiological and energetic costs (Chapman *et al.* 1995). Moreover, mating with several males has additional costs, including loss of time and energy in evaluating and rejecting potential mates, increase in predation risk, reduced longevity due to risk of infection and physical injury (e.g. Rowe 1994). However, in spite of the

potential costs females of many species mate with more than one male during the same reproductive season (Birkhead & Møller 1998). Even more puzzling is the fact that even in species that were previously thought to be monandrous, polyandry is widespread (Avise *et al.* 2002). Females clearly benefit when polyandry increases their access to limited resources, increases paternal care provided by males or increases female chances of obtaining future mates (Hoeck & Garner 2007). Direct benefits are, however, not enough to explain polyandry in species where males provide females with only their sperm. And this has led many to ask why females mate polyandrously when males provide only sperm to them? (Keller & Reeve 1995; Jennions & Petrie 2000; Zeh & Zeh 2003; Jennions *et al.* 2007). This question is currently one of the greatest paradoxes in behavioural ecology, and one for which there are still no consensus answers (Simmons 2005; Andersson & Simmons 2006; Charmantier & Sheldon 2006; Akcay & Roughgarden 2007; Kotiaho & Puurtinen 2007; Miller & Moore 2007; Dibattista *et al.* 2008; Huk & Winkel 2008; Kotiaho *et al.* 2008).

The main argument used to justify polyandry in these systems is that females can get indirect benefits by mating polyandrously, namely by enhancing the genetic quality of their offspring (Jennions & Petrie 2000). A result of polyandry is that sperm competition at the site of fertilization is promoted, resulting in the fittest sperm fertilizing the eggs (Simmons 2005). If this superiority is genetically linked to father's quality then females are likely to get indirect benefits for their offspring. Polyandry gives females the opportunity to bias their mating decisions towards high quality males. As a result of this bias, females may produce offspring of higher viability and mating potential, thus gaining indirect benefits (sexy and good sperm hypotheses - Weatherhead & Robertson 1979; Keller & Reeve 1995; Kirkpatrick 1996; Yasui

1998). Although, indirect benefits are the most parsimonious explanation for the prevalence of polyandry in the absence of direct benefits, there is little empirical evidence showing a relationship between female mate choice and an increase in offspring fitness. Furthermore, many argue today that evidence supporting the existence of indirect benefits is weak and based in conflicting assumptions of what is indirect benefits (Kirkpatrick & Barton 1997; Westneat & Stewart 2003; Gustafsson & Qvarnstrom 2006; Akcay & Roughgarden 2007). Consequently, female choice of polyandry for indirect benefits remains very debatable and more investigation is therefore warranted.

FEMALE MATING DECISIONS: ACTIVE CHOICE FOR POLYANDRY

Several points have been put forward to challenge indirect benefits as a driver of polyandry. Firstly, and foremost, polyandry may not be a female choice but rather a consequence of male sexual harassment. In fact, this idea has been getting increased support (Lee & Hays 2004; Arnqvist & Kirkpatrick 2005; Dibattista *et al.* 2008; Le Galliard *et al.* 2008). If so, there is no need to invoke any type of benefits to justify the prevalence of polyandry. Therefore, is imperative that female decisions are examined before making any assumptions about female driven mating choice for benefits (Kotiaho & Puurtinen 2007). Clarifying to what extent polyandry is in fact a female mating decision is a logical first step before attempting to justify its evolutionary advantages. In the fourth chapter I have experimentally examined female pre-mating choice. More specifically, I asked whether females, when given the choice, preferred to be associated with a single or a group of males and if this preference changed through time. This chapter laid the basis for a comprehensive

understanding of the strength of female mating choice of polyandry in systems where this choice may be masked by male sexual harassment.

POLYANDRY AND THE PARADOX OF INDIRECT BENEFITS

A second reason for the paucity of data supporting female choice of polyandry for indirect benefits is that to date few studies have tried to measure the benefits of polyandry directly (i.e. number of F1 and F2) (Veen *et al.* 2001; Head *et al.* 2005; Huk & Winkel 2008). Instead, most studies have used indirect measures of fitness such as offspring viability, survival rate, swimming speed, growth rate, among others, to examine indirect benefits (see Hunt *et al.* 2004, for examples). Indirect benefits are a measure of offspring fitness, which can depend, or not, on offspring survival, genetic heterogeneity, growth and fecundity. An unambiguous and more accurate test of the indirect benefits of offspring fitness is to measure them directly by looking at the number of grandchildren produced by polyandrous and monandrous females living in similar social/environmental conditions (Kotiaho *et al.* 2008). In the fifth chapter I used this approach to investigate if there was evidence of either type of mating benefits. More precisely I asked whether polyandrous females obtained an increase in the number of offspring (direct benefits) and/or whether polyandrous offspring had themselves more offspring (indirect benefits).

FEMALE MATING DECISIONS: POLYANDRY AND THE BENEFITS OF
DIVERSIFYING THE BROOD

Finally, the last explanation proposed for polyandry I investigated is whether females may be mating polyandrously in order to enhance genetic quality or the diversity of their brood (Yasui 1998; Foerster *et al.* 2003). This may give females some benefits since their offspring may be better adapted to environmental changes, but may not necessarily increase the fitness of their offspring under experimental conditions. In fact, it has been previously suggested that polyandry may have evolved and be maintained because of the indirect benefits obtained from producing offspring of greater heterozygosity (Keller & Reeve 1994; Cornell & Tregenza 2007; Rubenstein 2007). Using polyandry to increase genetic quality/diversity is thought to better explain the prevalence of polyandry in cases where females are unable to escape costly multiple mating attempts. It seems that polyandry may persist as a strategy of costs minimization (Hosken & Stockley 2003). In the fifth chapter I have used a novel statistical approach to test the idea that females may use polyandry to increase offspring diversity, instead of increasing offspring fitness directly. In particular, I looked at the multivariate dispersion in the phenotypes of offspring from two mating treatments: monandrous and polyandrous. Instead of comparing mean differences between the offspring of monandrous and polyandrous females, examining multivariate dispersions allows testing for differences in diversity in the offspring phenotypes. This provides a novel explanation for the prevalence and maintenance of polyandry in systems where females get no direct benefits from males.

In conclusion, the main aim of this thesis was to examine the role female mating decisions in the mating process, in particular how these decisions are modified

to accommodate environmental/social variability in order to maximize mating benefits. This aim was addressed using the Trinidadian guppy, *Poecilia reticulata* as model species. This small neotropical poeciliid fish has been, and still is one of the preferred species for sexual selection studies with a focus on female reproductive behaviour (Endler 1988; Houde 1988; Reznick & Miles 1989; Reynolds & Gross 1992; Nicoletto 1995; Magurran *et al.* 1996; Houde 1997; Rodd *et al.* 1997; Kodric-Brown & Nicoletto 2001; Magurran 2005; Pitcher *et al.* 2008). The reason for this huge effort lies in the unique biological and ecological characteristics of guppies. For my study guppies are particularly well suited for four reasons. The first of these is the mating system and the way it is controlled by female choice (Houde 1997). Secondly, in spite of the harsh environment they live in and being constantly harassed by males, female guppies have been shown to possess enough reproductive plasticity to overcome these adversities in their favour (Reznick & Yang 1993; Reznick 1996; Houde 1997; Rodd *et al.* 1997; Godin *et al.* 2005). The efficiency of reproductive plasticity is well demonstrated by how prolific guppies are, and how remarkably fast they can invade new systems. Thirdly, female guppies can use post-copulatory selection to replace sperm or to reinforce pre-mating decisions (Evans *et al.* 2003). This ability allows females to have an active decision-making role in the mating process, in spite of high levels of male sexual harassment. Finally, polyandry is extremely common, both in laboratory and wild conditions (Becher & Magurran 2004). And although mating benefits have been suggested to justify polyandry (Evans & Magurran 2000), the nature of these benefits remains unclear. In conclusion, guppies are a remarkably tractable species to work with and at the same time one for which there is a plethora of information available. These two factors combined make

guppies the ideal species to test many of the current female mating decisions themes that remain largely unresolved.

STUDY SPECIES – DISTRIBUTION, BIOLOGY AND ECOLOGY

The Trinidadian guppy, *Poecilia reticulata* is a small poeciliid fish (Fig. 1.1) native from North-eastern South America and adjacent islands, including Trinidad and Tobago. In fact the name Trinidadian guppy is believed to have originated from the fact that many natural studies with guppies are carried out in Trinidad and Tobago (Magurran 2001). Nowadays, however, the distribution of guppies is not restricted to the North-eastern South America. The bright colours, the easy maintenance and prolific reproductive success made guppies a favorite pet fish among aquarists worldwide. Additionally, guppies were introduced in many countries as a means of controlling the larvae of the mosquito responsible for malaria. Although the success of this action remains very debatable, this introduction allowed guppies to colonize new countries and aquatic systems. As a result of both the aquarium trade and malaria control, guppies can now be found in every continent in the world with the exception of Antarctica (Magurran 2005). Their widespread geographical distribution allied to their prolific ability to colonize new systems, make guppies an ideal species tool to examine the impact of invasive species on native fauna.

In terms of their reproductive biology, like all other Poeciliidae fish the Trinidadian guppy is a livebearer (Rosen & Bailey 1963) with females producing one brood every 3-4 weeks on average (Reznick *et al.* 1996). A single copulation is enough for complete fertilization for multiple broods, and females can also store

sperm for several months (Constantz 1989). Trinidadian guppies, however, display a typical way of maternal allocation, lecithotrophy (Wourms 1981). This means that there is no connection between the placenta and the young, thus all maternal provision is made prior to egg fertilization. Additionally, Trinidadian guppies also exhibit a variation in the mode of bearing in terms of developing broods carried by the mother. Trinidadian guppies lack superfetation meaning that all young in the brood are in the same development stage (Thibault & Schultz 1978; Reznick & Miles 1989). Finally, mixed paternity broods are typical among female guppies. On average each brood is sired by 3-4 fathers each (Becher & Magurran 2004). Multiple paternity of broods is a direct consequence of polyandry, which can be consensual or not and is extremely common among populations of Trinidadian guppies (Kelley *et al.* 1999; Neff *et al.* 2008).

Guppies live in a typical promiscuous mating system where females are constantly sexually harassed by males (Houde 1987, 1997). Male sexual harassment in guppies is one of the biggest recorded for aquatic fishes, with females being targeted at a rate of one sexual attempt every minute (Magurran & Seghers 1994a; Evans *et al.* 2003a). There is, however, no experimental evidence of a direct link between male sexual harassment and reduction of female reproductive success. Curiously, in spite of the high level of male sexual harassment, it is ultimately females that through their mating preferences and decisions determine paternity (Houde 1988, 1997). Female mating choice and its decisions are thought to be based primarily on the variation in male colour pigments and, to a smaller degree, on male body size and behaviour (Haskins & Haskins 1950; Reynolds & Gross 1992; Nicoletto 1995; Kirkpatrick 1996; Houde 1997; Jayasooriya *et al.* 2002; Pilastro *et al.* 2002; Evans *et al.* 2004a; Magurran 2005). Females are receptive to males either

when virgin or shortly after parturition (Liley 1966; Hughes *et al.* 1999). By contrast, males show a remarkable sexual zeal. Male guppies display two types of sexual behaviours: sigmoid displays used to solicit consensual matings, and gonopodial thrusting, employed in sneaky mating (Liley 1966). During a sigmoid display males court females directly, by swimming laterally, displaying their dorsal fin and twisting their body (Fig. 1.2). Females may respond positively or reject the male approach. If they respond positively, then the female positions herself in front of the male, followed by swimming side by side while the male inserts the gonopodium into the female's genital pore for internal fertilization (Baerends *et al.* 1955; Liley 1966; Farr 1977). In gonopodial thrusting males try to copulate with the female by sneaking from behind without prior display or receptivity from the female (Houde 1997). The same individual may use the two behaviours interchangeably (Matthews *et al.* 1997; Matthews & Magurran 2000). Although there is evidence that females prefer males that display sigmoid sexual behaviour over those that use sneaking (Karino and Koboyashy 2005), how the frequency of both sexual behaviours translates into paternity is not yet fully understood (Matthews & Magurran 2000; Evans *et al.* 2003a).

Female mating choice varies remarkably with social and environmental conditions, namely with predation risk (Breden & Stoner 1987; Magurran & Seghers 1990). Under low levels of predation, females are more receptive to mating and prefer colourful males, whereas under high predation they become less receptive and their preference is for less colourful males (Gong & Gibson, 1996; Rodd & Reznick, 1997). Females are also likely to spend more time foraging, schooling and in anti-predator behaviours in areas of high predation risk (Magurran & Nowak 1991). Males use this change in female mating behaviour as a signal, switching from courtship

behaviour (low level of predation) to a coercive mating system (high level of predation) (Endler, 1987; Evans & Magurran, 1999a; Evans et al., 2002). This flexibility of female behaviour in line with social and environmental conditions and how it affects how females chose their mates, is thought to be responsible for the rainbow of behaviours and colourations seen in male guppies (Brooks & Cainthness 1995; Houde 1997; Brooks & Couldridge 1999; Brooks and Endler 2001a; Brooks 2002)

Because female guppies have been suggested to efficiently control paternity, a simple variation in female mating preference can have a profound effect in male life history and evolution. The relationship between female behavioural response to differences in social/environmental conditions and how these directly mediate male sexual behaviour can have profound effects on sexual selection. It is fascinating to study such system where a simple change in female behaviour and their perception of male preference can have a profound effect on every aspect of guppies life history. Additionally, guppies are easily maintained under laboratory conditions and easily amenable to behavioural experimentation. It is therefore not surprising that the guppy system has produced a voluminous literature in behavioral and evolutionary ecology over the past 60 years. This small-bodied fish species continues to allow us to expand our knowledge in evolutionary ecology

Figure 1.1 – Female and male of Trinidadian guppy *Poecilia reticulata*. Guppies exhibit a strong size and sexual dimorphism. Females are larger and plain, whereas males are smaller and display bright patterns of body colourations.
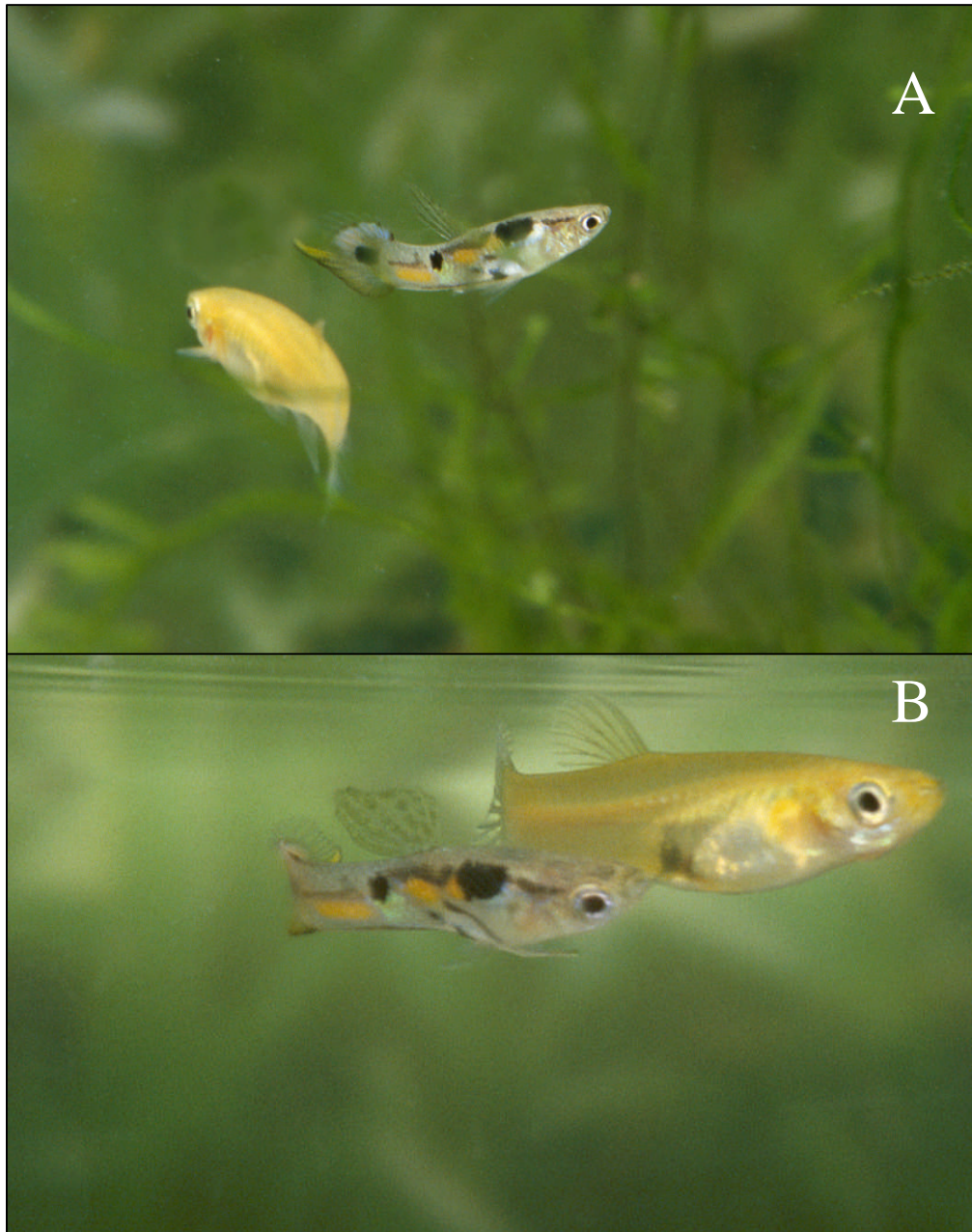
Figure 1.2 – Sequence of male sexual display. Males court females directly by swimming laterally, displaying their dorsal fin and twisting their body (a). Females show receptivity to mating by positioning themselves in front of the male and swimming side by side while the male inserts the gonopodium into the female's genital pore for internal fertilization (b).

Chapter two - Female mating decisions: maximizing fitness?[1]

ABSTRACT

Sexual selection theory assumes that maximizing fitness is the ultimate goal in every mating decision. Fitness can be maximized directly by increasing the number of offspring (direct benefits), or indirectly by maximizing offspring's lifetime reproductive success (indirect benefits). Whereas there is considerable evidence in the literature for the influence of mating decisions on direct benefits, indirect benefits have been more elusive. In chapter 2 I review the variables that influence mating decisions made by females of freshwater fish and how these affect their fitness directly, as well as indirectly. There is evidence that females enhance their fitness by matching their mating decisions to current environmental conditions, using pre and post copulation mechanisms that enable them to maximize benefits from mating. Male sexual traits and courtship displays are signals used by females as a way of assessing male quality in terms of both direct and indirect benefits. Polyandry is very common among freshwater fish species, and indirect benefits have been hypothesized as drivers of its predominance. Despite intensive theoretical work, and multiple suggestions of the effects of indirect benefits, to date no study has been able to demonstrate experimentally the existence of indirect benefits in freshwater fish species. Additionally, most studies of direct benefits measure short-term benefits of mating decisions. In both cases, lifetime reproductive success was not assessed. Therefore, I am led to conclude that evidence as to whether female mating decisions result in direct and/or indirect benefits in freshwater fish species is still lacking. These

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results should be considered in light of the ongoing debate about the significance of indirect benefits in female mating decision.

INTRODUCTION

Deciding who will be the father of their offspring is arguably the most important question any female faces. While both males and females contribute equal genetic material to their offspring, energetic investment is typically much higher in females (Trivers 1972; Clutton-Brock & Vincent 1991). It is ultimately the mating decisions that females make before, during and after mating that shape their fitness, as well as that of their offspring. The male sex has developed complex advertising strategies (ornamentation and elaborated courtship displays) to influence female mating decisions (Darwin 1871; Møller & Thornhill 1998). Hence, female mating decisions are under strong evolutionary pressure by sexual selection (Kirkpatrick 1996). Consequently, it is a central issue in evolutionary ecology to understand the variables that influence female mating decisions, and how these affect fitness.

Sexual selection theory suggests that female mating decisions are influenced by the changes in fitness (mating benefits) associated to each mating choice (Iwasa & Pomiankowski 1994). Two types of mating benefits have been proposed: 1) direct and 2) indirect benefits (Kirkpatrick 1982; Kirkpatrick & Ryan 1991). Direct benefits involve a direct pay back to the female from reproducing with a particular male. Examples of these benefits include nutritional gifts during copulation, parental care, enhanced fertilization rate, access to good resources and protection from male harassment (see Jennions & Petrie 2000 for examples). Direct benefits are thus defined by an increase in female's lifetime reproductive success. By contrast, indirect

benefits will be reflected in their offspring by increasing their lifetime reproductive success (Kokko 2001; Arnqvist & Kirkpatrick 2005). Indirect benefits are thought to be mediated through two mechanisms: good genes (Kirkpatrick 1996) and sexy sons (Weatherhead & Robertson 1979). Both of these mechanisms assume that offspring will inherit the qualities of their father and these qualities will enhance their viability (good genes) and/or their mating potential (sexy sons). However, separating indirect benefits according to these two mechanisms is often thought unnecessary since both are expressed in an increase in offspring fitness regardless of the evolutionary mechanism (Kirkpatrick 1985; Kokko 2001; Kokko *et al.* 2002, but see Cameron *et al.* 2003). Mating decisions that maximize the number of offspring are selectively advantageous, but so are mating decisions that maximize their offspring's fitness. These two facets of fitness may thus, at times, be conflicting (Nicoletto 1993).

Direct benefits are frequently easy to quantify; demonstrating indirect (genetic) benefits, however, poses a greater challenge because there are so many variables that might influence offspring fitness and as a result studies are often contradictory. A recent meta-analysis showed that only 43% of the studies, that claim to have demonstrated the influence of indirect benefits in female mating decisions in numerous organisms, were successful in demonstrating a correlation between male secondary sexual traits and offspring fitness (Alatalo *et al.* 1998). By contrast, recent theoretical work advocates that indirect benefits are "nonexistent" and that female mating decisions can be explained exclusively based on direct benefits (Roughgarden 2004; Akcay & Roughgarden 2007, but see Jennions & Petrie 2000). Reviewing female mating decisions and how these affect the two types of benefits is essential to solve the ongoing debate about direct / indirect benefits. Here, for the first time, mating decisions from a female's perspective are reviewed using fresh water fish as

model organisms. Additionally, possible links between these decisions, the type of benefit obtained (direct / indirect) and how these change in different environmental conditions are also explored in chapter two.

How do females make decisions about when and with whom to reproduce? The type of female mating decision and consequently the type of mating benefit depends largely on the costs associated with mating with a particular male. The differential allocation hypothesis proposes that it is advantageous for females to invest only the sufficient energy relative to the amount of benefits (direct or indirect) they will get from mating with a particular male (Alatalo *et al.* 1998; Sheldon 2000). Thus, the question of when and with whom to mate should be viewed in the context of a trade-off between future benefits (direct and indirect benefits) *vs.* current reproductive costs (predation, energy costs, loss of mating status). Predation risk, food availability, diseases, competition and habitat conditions are all environmental variables that have a direct effect on fecundity, fertility, growth and mortality (Charnov 2001). These variables shape the population structure and consequently affect the trade off between future benefits *vs.* current reproductive costs. The way these variables influence female and offspring fitness are thus important to understand the mating decisions made by the female and consequently the type of mating benefit.

Male-male competition and female choice are the two main forces driving sexual selection (Parker 1970; Birkhead & Møller 1993; Andersson 1994). Classical evolution theory suggests that there is a positive correlation between strength of sexual selection and number of non-mating individuals in a population (Bateman 1948). The number of reproductive partners each individual has defines its mating system. There are essentially six types of mating systems, which vary from one extreme of total promiscuity to the other of strict monogamy. Only the dichotomy

monandry/polyandry is directly related to female mating decision. Polyandry occurs when female mates with more than one male in a reproductive season, monandry occurs when the female only mates with a single male in one reproductive season. Male decisions also affect the balance between reproductive costs and benefits. For example, in promiscuous mating systems it has been found that females who mate with several males receive less sperm from males and consequently have lower rates of fertilization (Pizzari *et al.* 2003, but see Evans & Magurran 2000). However, this review addresses female mating choice, and hence I have focused mostly on monandry *vs.* polyandry.

If a given trait is indicative of the reproductive benefits associated with a particular male, then it is selectively advantageous for the female to mate with a male who has that trait (Andersson 1994; Wedell & Tregenza 1999). Additionally, if any of the male characteristics are linked to the genetic quality of the male, then females not only get direct benefits but also indirect benefits expressed in their offspring (Kirkpatrick 1996; Møller & Jennions 2001). Male quality signals are major drivers of female mating decisions, since they are the basis on which females assess how much they should invest in reproducing with any male. Using reliable male quality signals has, hence, high selective advantages.

Freshwater fish species have been used extensively in studies of reproductive behavioural ecology. The reason for this is that freshwater species show a great variety of reproductive strategies and behaviours both within and between species. These range from species with internal, external fertilization, gonochoric and hermaphrodite sexual patterns, with and without parental care, monandrous and polyandrous mating systems as well as pre and post mating sperm selection mechanisms. Additionally, freshwater fishes can be found in almost all aquatic

habitats living in highly variable environmental systems. The diversity of reproductive strategies allied with a wide geographical/environmental range illustrates the evolutionary adaptability existent in this group as well as the biological importance of how different expressions of reproductive strategies can coexist and thrive in different environments. For all these reasons freshwater fish are a good model to test the relationship between female mating decisions in terms of female and offspring fitness. Surprisingly, however, there are only a few studies addressing female mating decisions and how these vary in this group of fish species. Therefore, the aims of chapter two were to review the evidence for direct and indirect benefits and the influence of female mating decisions on the two types of benefits. And in a second degree I reviewed how female mating decisions vary under different environmental conditions (i.e. predation regimes, competition, sex ratio) and mating systems (i.e. polyandry, monandry). Finally, I have also explored how female perception of male quality based on signals affects their mating decisions and their mating benefits.

To conduct this review I compiled data on mating benefits and reproductive behaviour and ecology of freshwater fish species published in international peer-reviewed journals. Information was extracted directly from graphs and /or tables from the published papers. I made an extensive survey, and in order to ensure that most of the relevant papers were analyzed, a variety of different keywords (e.g. mating benefit*, reproduct* success, female fitness, offspring fitness, freshwater, etc) were used. Using this approach allow me to examine the main question on a much larger scale and at the same time reducing the number of missed data. Despite believing that the majority of work published in this area is likely to have been collected, I cannot discount the possibility that some studies might have been missed. In this review

freshwater fishes were considered to be those that spend at least their reproductive season in freshwater systems.

WHICH ENVIROMNENTAL VARIABLES INFLUENCE FEMALE MATING DECISIONS?

Predation risk, food availability, diseases, competition and habitat conditions are all environmental variables that have a direct effect on fecundity, fertility, growth and mortality (Charnov 2001). Females may enhance their fitness by matching their mating decisions to the current environmental conditions (Reznick & Yang 1993; Kodric-Brown 1995; Reznick *et al.* 2002). In freshwater fish species, plasticity in mating decisions is common (Godin *et al.* 2005).

Predation imposes high costs on mating and can have a large influence on female mating decisions (Lima & Dill 1990). Predator mediated female mating changes in behaviour have been described for several freshwater species, such as the three spined stickleback *Gasterosteus aculeatu*s. and the Trinidadian guppy *Poecilia reticulata* (Gong & Gibson 1996; Candolin 1997).

Females of the Trinidadian guppy adjust their preferences according to predation risk (Breden & Stoner 1987; Magurran & Seghers 1990). In low levels of predation, females are more receptive to mating and prefer colourful males whereas in high predation they become less receptive and their preference is for less colourful males (Gong & Gibson 1996; Rodd & Reznick 1997). Males use this change in female mating behaviour as a signal, switching from courtship behaviour (low level of predation) to a coercive mating system (high level of predation) (Endler 1987; Evans & Magurran 1999a; Evans *et al.* 2002). This flexibility in mating decision enables

both female and male guppies to achieve a high reproductive success (Evans *et al.*, 2002). Reznick and colleagues (1997) have demonstrated that the Trinidadian guppies adjust their size and reproductive strategy according to predation risk. In areas of high predation risk individuals mature early and females produce many small offspring, whereas individuals that live in low predation areas mature significantly latter and females produce fewer but bigger offspring. They demonstrated that populations that had been transplanted from high predation sites to low predation sites took as little as 4 years (rate of change was thought to be 10,000 to 10 millions times faster than average rate from fossil record) to adapt to their new environment and shift from a typical r-selection reproductive strategy to become more K-selected organisms. Females that are able to adapt their reproductive strategy as environmental variables change will get greater fitness benefits, than females that are unable, or take longer, to evolve. Females of Trinidadian guppy show a great plasticity in terms of reproductive strategies, by doing so they are maximizing the ratio of reproductive benefits per investment (Jennions & Telford 2002).

Food availability has an effect on both fecundity and fitness (Reznick & Yang 1993; Magurran & Seghers 1994b; Bryant & Grant 1995) and thus should also influence mating decisions. It is particularly relevant for species where males cannibalise juveniles, whenever food is scarce. In the cichlid *Xenotilapia tenuidentata* females prefer to mate with males in good nutritional state, to minimize the chances of cannibalism during the brooding period (Takahashi 2003). Cannibalism and egg predation are two factors that play an important role in female decisions. In fact, it has been suggested that in some species cannibalism may play a major role in the regulation of offspring population density (Thibault 1974; Dahlgren 1979). In the mottled sculpin *Cottus bairdii* large males are more successful at protecting eggs from

predation (Fiumera *et al.* 2002). However, larger males are also more likely to eat conspecifics. Thus, there is a trade-off between male size, female size and offspring survival. Females choose to spawn with a given male according to her size and his size (Downhower *et al.* 1983).

Susceptibility to diseases and parasites are key factors determining offspring fitness, and hence are large contributors to the indirect benefits component of fitness. Females of three-spined stickleback prefer to mate with colourful males (Bakker & Milinski 1990, 1991). Offspring sired by these colourful males are more resistant to infections by parasites and hence have higher survival rate (Barber *et al.* 2001). They, however, have a slower growth rate, which may have consequences in terms of future reproductive success of offspring (Ali & Wootton 2000). The fact that offspring sired by colourful males are more immune to parasites than offspring sired by dull males, suggests a mechanism for the maintenance of heritable variation in both parasite resistance and male colouration. This mechanism may explain the decisions made by the female in light of indirect benefits, as Barber and colleagues (2001) mention "our results provide a clear positive test of one of the key predictions of indirect models…". The fact that there is a male sexual character that is heritable and provides some advantage does not indicate or reflect necessarily that there is an increase in the lifetime reproductive success of the offspring, a critical assumption of the indirect benefits model (Kirkpatrick 1982, 1985; Andersson 1994; Kokko 2001; Kokko *et al.* 2002, but see Cameron *et al.*, 2003). To date, no information has been gathered to substantiate the claim that females of three spined stickleback choose their mate based on indirect benefits, or that indirect benefits are correlated with male colouration.

In conclusion, most freshwater fish species inhabit very dynamic systems, where environmental conditions are constantly changing. This uncertainty should be

reflected in plasticity on the type of mating decisions made by females. Predation risk and parasitism have been shown to influence female mating decisions. However, little is known about the effects of competition and habitat structure on mating decisions but see (Goddard & Mathis 1997).

POLYANDRY *vs.* MONANDRY: WHY DO SOME FEMALES MATE WITH MULTIPLE MALES?

Traditionally, males have been suggested to mate promiscuously whereas females were thought to be mostly monogamous. However, recent evidence suggests that polyandry occurs in most taxa, even in species previously thought to be strictly monogamous (Jennions & Polakow 2001; Avise *et al.* 2002). Consequently reproductive studies should shift to a perspective that accounts for polyandry (Zeh & Zeh 2003; Feldheim *et al.* 2004). As in other animal groups, polyandry is very common among freshwater fish species. 60% of the species examined in this review are thought to live in this type of mating system (Table 2.1).

Polyandry is associated with multiple direct reproductive benefits, for example increased fecundity and fertility (Arnqvist & Nilsson 2000), reception of nutrients during copulation (Kaitala & Wiklund 1994), or a combination of these (Hardling & Kaitala 2005) among others. However, direct benefits are not enough to explain the existence of polyandry in species where males provide females with only their sperm. In the absence of any apparent direct benefits, indirect benefits have been suggested to drive polyandry (Petrie 1994; Petrie *et al.* 2001, but see Gustafson & Qvarnstrom 2006). In a recent review, Jennions and Petrie (2000) reinforce the idea that polyandry is unlikely to have evolved based only on direct benefits, and that polyandry will lead invariably to indirect benefits.

Table 2.1 - Summary of freshwater fish species examined in chapter two according to their mating system, type of mating benefit and female choice mechanism

Family	Species	Mating system	Female mating benefits		Female choice mechanism		References
			Direct	Indirect	Pre	Post	
Adrianichthyidae	*Oryzias latipes*	Monandry					(Grant *et al.*, 1995a; Grant *et al.*, 1995b)
Anablepidae	*Jenynsia multidentata*	Polyandry					(Bisazza *et al.*, 2000)
Callichthyidae	*Corydoras aeneus*	Polyandry			no	no	(Kohda *et al.*, 2002)
Catostomidae	*Carpiodes carpio*	Polyandry	yes	no	no	no	(Vila-Gispert & Moreno-Amich, 2002)
Centrarchidae	*Lepomis macrochirus*	Polyandry	yes		yes		(Coleman *et al.*, 1985; Cote & Gross, 1993)
	Lepomis megalotis	Polyandry	yes		yes		(Goddard & Mathis, 2000)
	Micropterus dolomieui	Polyandry	yes		yes	no	(Wiegmann *et al.*, 1992; Wiegmann & Baylis, 1995)
	Micropterus salmoides	Polyandry	yes		yes		(Vila-Gispert & Moreno-Amich, 2002)
Cichlidae	*Aequidens coeruleopunctatus*	Monandry	yes				(Jennions & Polakow, 2001; Velez et al., 2002)

Table 2.1 - Continued

Family	Species	Mating system	Female mating benefits		Female choice mechanism		References
			Direct	Indirect	Pre	Post	
	Cichlasoma nigrofasciatum	Monandry	yes		yes		(Wisenden 1993)
	Lamprologus ocellatus	Monandry	yes		yes		(Walter & Trillmich 1994; Brandtmann *et al.* 1999)
	Oreochromis mossambicus	Monandry	yes		yes		(Nelson 1995)
	Sarotherodon galilaeus	Monandry	yes		yes		(Smith 1977)
	Xenotilapia tenuidentata	Monandry	yes				(Takahashi 2003)
Cottidae	*Cottus bairdii*	Monandry	yes		yes	no	(Downhower *et al.* 1983; Goto 1987; Fiumera *et al.* 2002)
	Cottus hangiongensis	Polyandry	yes		yes		(Goto 1987)
Cyprinidae	*Cyprinus carpio*	Polyandry	no		no	no	(Vila-Gispert & Moreno-Amich 2002)
	Rhodeus sericeus	Monandry	yes	yes	yes	no	(Candolin & Reynolds 2001; Smith *et al.* 2002; Reichard *et al.* 2004

Table 2.1 - Continued

Family	Species	Mating system	Female mating benefits		Female choice mechanism		References
			Direct	Indirect	Pre	Post	
Cyprinodontidae	*Cyprinodon pecosensis*	Polyandry	yes	yes	yes	no	(Kodric-Brown 1983; Kodric-Brown 1995)
Eleotridae	*Gobiomorphus breviceps*	Monandry	yes	no			(Hamilton *et al.* 1997; Hamilton & Poulin 1999;)
Gasterosteidae	*Gasterosteus aculeatus*	Polyandry	yes	yes	yes	?	(Bakker & Milinski 1990; 1991; Barber & Arnott 2000; Barber *et al.* 2001)
Gobiidae	*Neogobius melanostomus*	Polyandry	yes		yes		(MacInnis & Corkum 2000)
	Padogobius martensi	Monandry	yes		yes	no	(Bisazza *et al.* 1989)
Percidae	*Etheostoma blennioides*	Polyandry	yes		yes		(Dalton 1991)
Poeciliidae	*Gambusia affinis*	Polyandry			yes		(Evans *et al.* 2003c)
	Gambusia holbrooki	Polyandry	yes	yes	yes		(Bisazza *et al.* 2001)
	Heterandria formosa	Polyandry	yes		yes	yes	(Henrich 1988)
	Poecilia reticulata	Polyandry	yes	yes	yes	yes	(Endler 1980; Houde 1992; Reynolds & Gross 1992; Endler & Brooks 1995; Nicoletto 1995; Brooks 2000; Evans & Magurran 2000; Brooks & Endler 2001b, a; Brooks & Kemp 2001; Evans *et al.* 2003b)

Other authors suggest that there may be both direct and indirect benefits associated to female polyandry (Kirkpatrick 1996; Evans & Magurran 2000; Konior *et al.* 2001) (Table 2.1). The hypothesis that indirect benefits drive polygyny has, however, been refuted by a 28 year study on flycatchers. Gustafson and Qvarnström (2006) demonstrated experimentally that the offspring of females that mate with polygamous males are less fit than offspring from monogamous males. Furthermore, sons of polygamous males do not inherit their father's large forehead patch, a morphological trait preferred by females. In conclusion, this study suggests that the direct costs of polygyny are not compensated by indirect benefits.

The drivers of polyandry vary extensively, as is illustrated by the case of *Protomelas spilopterus* and the Malawi blue cichlid *Pseudotropheus zebra*. These two species of cichlids live in the same lake (Lake Malawi), and are both polyandrous (Parker & Kornfield 1996; Kellogg *et al.* 1998). However, the level of polyandry is different in the two species, *P. spilopterus* mates with 1-3 males maximum whereas the Malawi blue cichlid mates with 5-6 males (Parker & Kornfield 1996; Kellogg *et al.* 1998). The first species is quite rare, and there is low male density. *P. spilopterus* females, therefore, mate with any male they meet, because of the risk of being predated before meeting another male. The Malawi blue cichlid, on the other hand, is very abundant, and in this species polyandry seems to be a mechanism to avoid inbreeding. Maintenance of genetic diversity may also be one reason for polyandry in the Trinidadian guppy (Becher & Magurran 2004). The benefits of polyandry are well known, it is however still not clear whether those benefits arise from mating with multiple males (to enhance genetic diversity), or has something to do with the quality of the males that constitute that group. This can be experimentally investigated by manipulating social structure according to male quality and examining lifetime

reproductive success of offspring. In general, for species living in unpredictable

environments it is selectively advantageous to maximise genetic variability, as

insurance towards survival of the offspring in unknown future conditions (Ivy &

Sakaluk 2005, but see Arnqvist & Nilsson 2000).

Polyandry is not always entirely a female decision, particularly in systems

where females are constantly harassed by males (Lee & Hays 2004; Dibattista *et al.*

2008; Le Galliard *et al.* 2008). In some freshwater species females have rates of

harassment up to once every minute (Magurran & Seghers 1994a; Magurran *et al.*

1996). In mating systems where males only achieve mating through coercive mating

and females do not cooperate, the opportunity for female decisions to influence

mating was thought to be limited. This however is not always the case. The

mosquitofish *Gambusia holbrooki* lives in a coercive mating system, but still females

demonstrate some level of mating choice. After being deprived from mating for some

time females tend to prefer multiple large and dull males over smaller and brighter

ones (Bisazza *et al.* 2001). By moving closer to a particular male females increase the

probability of being inseminated by that male. By doing so females may not only be

selecting the best male but also be selecting the sneaking qualities that will be

essential in a system like this.

Female mating decision has been traditionally considered to be restricted to

pre-mating behavioural decisions driven by male-male competition and quality of a

resource (e.g. Parker, 1970). Females were then assumed to be merely passive

participants in the decision of who fathers their offspring (Birkhead & Møller 1993).

Additionally, there are cases where females are not able to mate with the preferred

male, either because of coercive mating, harassment or due to a lack of "high quality"

males in the population. This traditional perception of passivity in female

reproductive decision has, however, changed in the light of new findings. Females of some species are able to use post-mating physiological mechanisms in order to select the sperm that fertilizes their eggs (Evans *et al.* 2003b). Females can use both behavioural pre-mating and physiological post-mating mechanisms to select when and with whom to reproduce (Olsson *et al.* 1996; Birkhead & Pizzari 2002). Polyandric females, thus, can possess both direct and indirect post-mating mechanisms, respectively by using the sperm of the preferred male to fertilize their eggs in detriment of other males (e.g. sperm storage, abortion) or by promoting sperm competition (Zeh 1997) (Table 2.1). Females may therefore use post mating mechanisms, or cryptic mating choice, to permit direct selection of who sires their offspring through sperm selection. Females may also use post-mating mechanisms to overcome costs associated with polyandry (Hellriegel & Ward 1998). These post-mating choice mechanisms generally reinforce pre-mating decisions (Evans *et al.* 2003b).

There is evidence that polyandry can potentially have some negative effects on female fitness, such as reducing their longevity by increasing the risk of infection and physical injury, as well as by increasing the risk of predation due to an increase of time devoted to mating, among others see (Arnqvist & Nilsson 2000) for examples. In fact, it has been verified that increasing the number of sexual partners is only beneficial to a certain extent, after which the negative effects overrun any benefits (Hardling & Kaitala 2005). A study on fitness consequences of enforced monogamy in fruit flies showed that polyandric females have shorter lives, but reproduce more often than monandric females. However the life-long female fitness was similar in both mating systems (Martin & Hosken 2003). It remains to be shown if this is a

general trend among other organisms, and if so, it raises the question of offspring fitness being affected by the mating system (Wedell & Tregenza 1999).

Finally, monandry is favoured if females require only one successful mating to have their fitness maximized, and the number of offspring per female is unlikely to increase as a function of number of males but rather in function of number of eggs produce by the female (Bateman 1948; Ihara 2002). These conditions are, however, relatively rare in freshwater fish.

In conclusion, polyandry is more prevalent in freshwater fish than monandry. There are multiple theoretical selective advantages to mating with several males including both direct and indirect reproductive benefits (see Table 2.1). However, polyandry also carries costs, and ultimately, females should decide to mate with the number of males that maximizes this trade-off. Most previous studies use indirect measures of fitness (e.g. predation avoidance, growth). Future research should examine the effects of mating system on both female and offspring lifetime reproductive success.

HOW DO FEMALES ASSESS MALE QUALITY?

Variance in female reproductive lifetime success and offspring fitness depends on the reliability of the signals sent by the male (Nilsson *et al.* 2002; Candolin 2003). Females may use a particular male sexual trait(s) (coloration, fin size), courtship display and/or male resource quality (territory, nest) to assess male quality. The conspicuousness of male sexual traits and/or behaviours of these traits are thought to reveal some honest signal about male's quality that may enhance female as well as offspring fitness (Sumner *et al.* 1994; Day 2000, but see Møller & Jennions 2001).

The degree of asymmetry of a sexual trait has also been shown to be used by females to assess male quality (Watson & Thornhill 1994). Asymmetries of secondary sexual characters may express information about the phenotypic and genetic quality of the male (Møller & Pomiankowski 1993). Both social and environmental conditions change during a single breeding season or between breeding seasons, conditionally expressed traits that vary in intensity and symmetry and can be rapidly turned on and off, such us nuptial colouration, are likely to be used by females as indicators of male quality and honesty (Kodric-Brown 1995; Mazzi *et al.* 2003). In the Trinidadian guppy, male colouration and courtship display frequency are positively correlated with sperm ejaculation and fertilization rate (Matthews *et al.* 1997; Matthews & Magurran 2000; Evans & Magurran 2001; Pilastro *et al.* 2002) (Table 2.2). In this particular example, male phenotype is an honest signal of male quality and thus of female mating benefit (the back-up signal hypothesis - (Iwasa & Pomiankowski 1994). These signals, however, are not always reliable and may not be indicative of mating benefits. Some studies have shown that males may use particular sexually selected traits to deceive females in order to get reproductive benefits at expenses of females (Warner *et al.* 1995; Witte & Ryan 1998). Another problem is that despite the honesty of male signals, females may not be able to process that information because of the costs associated in searching (Reynolds & Gross 1990) or simply because they may misinterpret the male quality signals, and consequently do not mate with the "best quality" male.

In this review only one signal used by females to assess male quality, was not an honest indicator of male quality (Table 2.2). These results should, however, be analyzed with caution because of two factors that may lead to bias in the results.

Table 2.2 - Summary of freshwater fish species examined in chapter two according to type of male sexual traits selected by females and their honest value

Family	Species	Trait	Honest	References
Anablepidae	*Jenynsia multidentata*	Large males and sneaking qualities	yes	(Bisazza *et al.* 2000)
Callichthyidae	*Corydoras aeneus*	Courtship intensity	yes	(Kohda *et al.* 2002)
Centrarchidae	*Lepomis macrochirus*	Nest location (colonial)	yes	(Coleman *et al.* 1985; Cote & Gross 1993)
	Lepomis megalotis	Opercula flap length	yes	(Goddard & Mathis 2000)
	Micropterus dolomieui	Male size and nest quality	yes	(Wiegmann *et al.* 1992; Wiegmann & Baylis 1995)
Cichlidae	*Cichlasoma nigrofasciatum*	Nest quality and presence of predators	yes	(Wisenden 1993)
	Oreochromis mossambicus	Nest and male size		(Nelson 1995)
Cottidae	*Cottus bairdii*	Male size and number of embryos in the nest	yes	(Fiumera *et al.* 2002)
	Cottus hangiongensis	Male size	yes (size)	(Goto 1987)
Cyprinidae	*Rhodeus sericeus*	Initially male colouration and size and then mussel quality	yes/no	(Candolin & Reynolds 2001; Smith *et al.* 2002; Reichard *et al.* 2004; Smith *et al.* 2004)

Table 2.2 - Continued

Family	Species	Trait	Honest	References
Cyprinodontidae	*Cyprinodon pecosensis*	Male colouration	yes	(Kodric-Brown 1983; Kodric-Brown 1995)
Eleotridae	*Gobiomorphus breviceps*	Male colouration and amount of parasites in the body	yes	(Hamilton *et al.* 1997; Hamilton & Poulin 1999; 2001)
Cottidae	*Cottus bairdii*	Male size and number of embryos in the nest	yes	(Fiumera *et al.* 2002)
	Cottus hangiongensis	Male size	yes (size)	(Goto 1987)
Cyprinidae	*Rhodeus sericeus*	Initially male colouration and size and then mussel quality	yes/no	(Candolin & Reynolds 2001; Smith *et al.* 2002; Reichard *et al.* 2004; Smith *et al.*, 2004)
Cyprinodontidae	*Cyprinodon pecosensis*	Male colouration	yes	(Kodric-Brown 1983; Kodric-Brown 1995)
Eleotridae	*Gobiomorphus breviceps*	Male colouration and amount of parasites in the body	yes	(Hamilton *et al.* 1997; Hamilton & Poulin 1999; 2001)
Poeciliidae	*Gambusia affinis*	Male size,	yes	(Hughes 1985; Evans *et al.* 2003c)
	Gambusia holbrooki	Male size, size group and dull colouration	yes	(Bisazza *et al.* 2001)
	Poecilia latipinna	Male size	yes	(Witte & Ryan 1998)

39

Table 2.2 - Continued

Family	Species	Trait	Honest	References
	Poecilia reticulata	Male size, colouration and boldness	yes	(Endler, 1980; 1987; Houde, 1987; 1988; Houde & Endler, 1990; Endler & Brooks, 1995; Houde, 1997; Matthews *et al.*, 1997; Brooks & Couldridge, 1999; Brooks & Endler, 2001a; Pilastro *et al.*, 2002; Evans *et al.*, 2004a; Evans *et al.*, 2004b; Herdman *et al.*, 2004)
	Xiphophorus pygmaeus	Male size		(Hankison & Morris, 2002)

Firstly, the publication bias problem or "file drawer". Most of the published literature shows only the positive correlations and significant differences and negative and non-significant results are seldom reported. This may lead to a potential under representation of dishonest signals. Secondly, I considered male sexual signals to be honest if females got any type of benefit from mating with that particular male. I made, however, no distinction between signals that correspond to the condition of the male at a particular point in time (e.g. Andersson 1994) and heritable signals, which can be costly and disadvantageous for the male, as proposed by the handicap model (Zahavi 1975). There is a positive correlation between offspring survival, male colouration and mussel (nest) quality in the bitterling *Rhodeus sericeus*. Mussel quality is not, however, correlated with male quality (Smith *et al.* 2004). Females of bitterling may use multi-traits to assess male quality. Females base their initial decision on male colouration and behaviour (courtship) and secondly on nest inspection (Candolin & Reynolds 2001).

In chapter 2 I have also shown that, similarly to what has been described for some insect and bird species (e.g. Candolin 2005), females of some freshwater fish species use multiple signals to assess male quality. The same male signal in the same species living in different systems may send different information (the multiple message hypothesis - (Iwasa & Pomiankowski 1994). Hence, females use different signals to assess male quality according to the system in order to maximize their benefits. Females of upland bully *Gobiomorphus breviceps* living in different populations use different male sexual traits to assess male quality (Table 2.2). In some populations brighter males are selected whereas in others dull males are preferred. In some females, definition of male quality is based on the amount of parasites in the

body, the more parasites the better in some populations and the fewer parasites the better in others (Hamilton *et al.* 1997; Hamilton & Poulin 1999, 2001).

Few studies have looked at the effect of asymmetry in sexual traits in female preference in freshwater fish species. A recent study that looked at female preference in three spined stickleback according to pelvic spines size and asymmetry, has shown that females prefer smaller symmetrical pelvic ray fins than longer and asymmetric ones (Mazzi *et al.* 2003). Although their study used video screens in laboratory and therefore likely to underestimate real life mate choice decisions, it revealed that three spined stickleback females had the ability to discriminate between potential males based on small differences in pelvic spine symmetry. This suggests that pelvic spine symmetry is likely to be under enormous sexual selection through female choice. This discrimination increased significantly with female age. The older the female, the more selective she was in selecting the male. Another study with the same species has revealed that females are able to use male breeding coloration as a way of avoiding parasitized males, and hence choose males in better conditions for parental care (Bakker & Milinski 1990).

In conclusion, females use multiple traits to assess male quality, and signals vary between species and populations. Females in different populations may interpret the same signal differently. Females are also able to choose a male based on the symmetry of their sexual traits. In this review all signals but one sent by males seem to be honest indicators of male quality and thus represent female mating benefits (direct and/or indirect), these results should, however, be analyzed with prudence due to the file drawer problem and the differentiation between condition and heritable signals.

INDIRECT OR DIRECT BENEFITS?

Females may get direct benefits (increase their fitness), indirect benefits (increase in offspring fitness) or both if choosing to mate with a high quality male. Direct benefits are thought to be easily quantifiable, and hence usually accounted for. Indirect benefits are, however, more elusive. Because there are so many variables that might influence offspring fitness, indirect selection is extremely difficult to demonstrate.

In this chapter 16% of the species studied have been proposed to provide evidence of indirect benefits (Table 2.3). However, it has yet to be to successfully demonstrated experimentally, that there is a relationship between genetic heritability of a specific male sexual trait(s) and an increase in offspring lifetime reproductive success. Reynolds and Gross (1992) argued that, in the Trinidadian guppy offspring sired by large size males grew faster than offspring sired by other types of males. This faster growth would potentially lead to an increase in the fecundity of daughters, but not sons (Table 2.3). Their experiment, however, did not investigate the fecundity of female offspring through their life, thus falling short of demonstrating the existence of indirect benefits, neither does it exclude the possibility of variable female investment in offspring. Further, it was later proposed that female guppies were selecting colourful males and that this selective choice was amplifying the trait but not giving any fitness advantages to the offspring (Nicoletto 1995). Brooks (2000) demonstrated that female guppies get more attractive offspring by mating with colourful males but at the expense of reduced offspring survival and few numbers of sons reaching maturity, hence contradicting the assumptions of indirect benefits hypothesis. Other studies use relationship between a male sexual trait (performance) and female choice (Nicoletto 1993), male sexual trait and offspring quality (Evans *et al.* 2004b),

offspring predation avoidance ability (Evans & Magurran 2000), and offspring growth (Barber *et al.* 2001) as evidence of indirect benefits. Offspring fitness is a measure of lifetime reproductive success, which can depend on offspring survival, growth and fecundity. Studies need to demonstrate experimentally a co-variation of a male trait, and offspring lifetime reproductive success to be able to show that there are indirect benefits behind a particular female mating decision (Kokko 2001).

In terms of direct benefits, in spite of more than 80% of the species examined here are claimed to have some type direct benefits, again I found no experimental evidence to support this hypothesis (Table 2.3). As it happens with indirect benefits, direct benefits can only be accepted if a female's lifetime reproductive success is shown to have increased through mating with a high quality male. All studies examined in this review only look at the direct benefits at short term, failing to address lifetime consequences. It is quite possible that a female mating with high quality male has more offspring, of higher quality in that particular brood, but lower or equal fitness over her lifetime as whole.

In conclusion the literature includes numerous studies that demonstrate female mating decision leading to short-term direct and indirect benefits of various types. However, it is now necessary to assess how these short-term benefits affect female's fitness as a whole, as this is what sexual selection should maximize. Because of this I am led to conclude that is still premature to assume that both direct and indirect benefits are indeed responsible for the evolution of female mating decisions in freshwater fish species.

Table 2.3 - List of freshwater fish species examine in this chapter according to the type of mating benefit. Definition of indirect benefits used by authors for a given species is given

Family	Species	Female mating benefits		Type of Indirect benefits	References
		Direct	Indirect		
Catostomidae	*Carpiodes carpio*	yes	no		(Vila-Gispert & Moreno-Amich 2002)
Centrarchidae	*Lepomis macrochirus*	yes			(Coleman *et al.* 1985; Cote & Gross 1993)
	Lepomis megalotis	yes			(Goddard & Mathis 2000)
	Micropterus dolomieui	yes			(Wiegmann *et al.* 1992; Wiegmann & Baylis 1995)
	Micropterus salmonoides	yes			(Vila-Gispert & Moreno-Amich 2002)
Cichlidae	*Aequidens coeruleopunctatus*	yes			(Jennions & Polakow 2001)
	Cichlasoma nigrofasciatum	yes			(Wisenden 1993)
	Lamprologus ocellatus	yes			(Walter & Trillmich 1994; Brandtmann *et al.* 1999)
	Oreochromis mossambicus	yes			(Nelson 1995)

Table 2.3 - Continued

Species	Female mating benefits		Type of Indirect benefits	References
	Direct	Indirect		
Sarotherodon galilaeus	yes			(Smith 1977)
Xenotilapia tenuidentata	yes			(Takahashi 2003)
Cottus bairdii	yes			(Downhower *et al.* 1983; Fiumera *et al.* 2002)
Cottus hangiongensis	yes			(Goto 1987)
Cyprinus carpio	no			(Vila-Gispert & Moreno-Amich 2002)
Rhodeus sericeus	yes	yes	Male colouration may increase offspring fitness. Not tested.	(Candolin & Reynolds 2001; Smith *et al.* 2002; Reichard *et al.* 2004; Smith *et al.* 2004)
Cyprinodon pecosensis	yes	yes	Increase viability of offspring. But offspring fitness yet to be tested	(Kodric-Brown 1983; Kodric-Brown 1995)
Gobiomorphus breviceps	yes	no		(Hamilton *et al.* 1997; Hamilton & Poulin1999, 2001)
Economidichthys pygmaeus	yes			(Daoulas *et al.* 1993)

Table 2.3 - Continued

Species	Female mating benefits		Type of Indirect benefits	References
	Direct	Indirect		
Economidichthys trichonis	yes			(Daoulas *et al.* 1993)
Neogobius melanostomus	yes			(MacInnis & Corkum 2000)
Padogobius martensi	yes			(Bisazza *et al.* 1989)
Etheostoma blennioides	yes			(Dalton 1991)
Gambusia holbrooki		yes?	Sneaking characteristics that are likely to increase fitness. Yet to tested.	(Bisazza *et al.* 2001)
Heterandria formosa	yes			(Henrich 1988)
Poecilia formosa	no			(Woodhead & Armstrong 1985)
Poecilia reticulata	yes	yes	Predation avoidance, swimming abilities, increase growth. Offspring fitness yet to be tested.	(Endler 1980; Houde 1992; Reynolds & Gross 1992; Nicoletto 1993, 1995; Brooks 2000; Evans & Magurran 2000; Evans *et al.*, 2004b)

DISCUSSION

Data on female mating decisions for 36 species of freshwater fishes have been reviewed and analyzed in this chapter. More specifically, I have explored how females make their decisions and tried to identify possible links between these decisions and the type of mating benefit (direct/indirect). I have also to a lesser degree reviewed how females adjust and modify their mating decisions in line to environmental and social variables.

There are studies that reported that female mating decisions in freshwater fish are made according to current environmental conditions, revealing some level of plasticity. Environmental variables such as predation risk, food availability, diseases, competition and habitat conditions have strong effects on reproductive success (Martin 2001). Freshwater fish species inhabit very dynamic systems, where environmental conditions are likely to vary between reproductive seasons (Johnston & Leggetta 2002), so it is selectively advantageous to have female flexible mating decisions that can be adjusted to the environmental factors associated with reproductive success (Emlen & Oring 1977).

Females use male secondary sexual traits (signals) to assess their quality and the accuracy of their mating decisions, depending on the reliability of these signals sent by the male (Nilsson *et al.* 2002). Table 2.2 showed that only one signal used by females to assess male quality, was a dishonest indicator of male quality. This result is in harmony with other studies that show a positive correlation between female mating decisions and male sexual traits and behaviours (e.g. Soler *et al.* 1998). Additionally, as has been described for other organisms, freshwater females use multiple traits to assess male quality, and signals vary between species and

populations, and by doing so they allegedly maximize their benefits (but see Møller & Jennions 2001).

In more than half the species examined in chapter two, females mate polyandrously. This result concurs with other studies that found similar results for other organisms (Jennions & Petrie 2000; Avise *et al.* 2002; Akcay & Roughgarden 2007), and reinforces the need to change the classical view of females mating monogamously and males mating promiscuously. Curiously I could not find any strong evidence that indirect benefits are behind polyandry. This result raises the question: Why do females, which do not get any apparent direct benefits, mate polyandrously? There are at least three possible reasons. Firstly, females might not be able to escape un-wanted mating, and thus polyandry may be forced rather than chosen. Secondly, females may be mating with multiple partners in order to enhance genetic diversity. This is a particular case of indirect benefits, where the offspring fitness is enhanced as a whole, but not necessarily in each individual. Thirdly, females may be mating with multiple males and using post mating selection mechanisms to select the best sperm. In general females appear to be more in control of who sires their offspring than previously thought. In fact, even in coercive mating systems, where females never cooperate in mating, females show some type of reproductive strategies (post copulation mechanisms and/or staying closer to a given individual) that enable them to influence which males father their offspring (Bisazza *et al.* 2001; Evans *et al.* 2003b). This result is in line with the results described for bird species where it has been shown that mothers control offspring quality according to father quality (Cunningham & Russell 2000, 2001; but see Petrie *et al.* 2001).

Finally and foremost, no substantial evidence for both direct and indirect benefits arising from female mating decisions was found. It is thought that direct

benefits are easily demonstrated. However, none of the studies examined here were able to prove the existence of these benefits in female mating decisions in freshwater fish species. All studies fell short of demonstrating a relationship between male quality and female's lifetime reproductive success. The majority of studies describe a positive relationship between male quality and an increase in the number of offspring (e.g. Fiumera *et al.* 2002), better predation avoidance skills by offspring (e.g. Evans *et al.* 2004b) or an increased survival rate of offspring (e.g. Wiegmann & Baylis 1995), which at a short term will give some direct benefits to the female, but not necessarily increase their lifetime reproductive success. Future studies should focus on trying to follow female's fitness throughout life and then assess whether there is direct benefits from mating with a high quality male or if these benefits are only reflected in the short-term.

It has been suggested that indirect benefits may not be the driving force in female mating decisions (Møller & Alatalo 1999) and that we should change towards a view that assumes that female mating decision can be explained based only on direct benefits (Roughgarden 2004; Gustafsson & Qvarnstrom 2006; Akcay & Roughgarden 2007). My results are in partial agreement with these two studies. I could not find any experimental proof that indirect benefits are indeed behind female mating decision in freshwater fish species, despite several suggestions (e.g. Reynolds & Gross 1992; Nicoletto 1995; Barber *et al.* 2001). The fact that I was unable to review any information that lead to the support of indirect benefits does not necessary imply that they do not exist. Indirect benefits may be present but their effect may be small or negligible and thus easily ignored. Further, it has also been suggested that indirect benefits may be species specific (Møller & Alatalo 1999) or only be visible in the long term (Møller & Jennions 2001). Most studies look at a maximum of two

generations, which may be a short time to observe indirect benefits. Future experiments should try to clarify experimentally to what extent indirect benefits contribute to female mating decisions freshwater fish.

In conclusion, chapter two highlights the importance of understanding female mating decision in studies of evolutionary ecology. It also reinforces the active role of females in the mating process as well as in shaping its outcome. Several matters arisen from this chapter that are worthy of further research: firstly, there is strong evidence that females of freshwater fish adapt their mating decision according to current environmental conditions (e.g. predation levels, competition, sex ratio) showing mating plasticity. Secondly, polyandry seems to be prevalent among freshwater fishes. Despite the suggestion that indirect benefits are behind polyandry, I could not confirm this. Finally, and despite the potential, and great amount of information available, currently there is no evidence for the existence of both direct and indirect benefits, nor of their influence in female mating decision in freshwater fishes. Therefore, I conclude that it is still premature to make this prediction given the available data. It is only when we reveal the effects of direct /indirect benefits that we can truly understand the mechanisms of sexual selection. Further studies to elucidate this issue must ensure that female and offspring fitness is directly estimated, rather than assumed to be proportional to any life history traits.

Chapter three – The facultative adaptation of offspring sex ratio, number and size under extreme population sex ratios

ABSTRACT

In many species females adjust their reproductive decisions in response to environmental variables, in ways that optimize their fitness or the fitness of their offspring. In chapter three I tested for two possible mechanisms of female reproductive optimization, 1) compensatory adjustment of sex ratio and 2) differential allocation of resources, under experimentally controlled extreme sex-ratio scenarios. The Trinidadian guppy, *Poecilia reticulata*, is a neotropical fish species that inhabits extremely variable environments. This variability has, through natural selection, pressured females to develop plasticity in their reproductive decisions. Thus, when faced with extreme differences in social environment (sex ratio), I predicted that females should adapt their reproductive investment accordingly. However, this experiment provided no evidence of sex ratio compensation. Instead I observed an over production of daughters in both sex ratio treatments. Nevertheless, offspring size at birth was significantly different between the two sex ratio treatments. Sons produced by females in a female sex ratio were bigger than sons produced in a male biased sex ratio environment. Curiously, I did not see an expected complementary balance between number and size at birth of offspring between treatments. Potential differences in food intake between females allocated to sex ratio treatments may have caused this result. In conclusion, results suggest that 1) female guppies are unable to regulate the sex ratio of their progeny and 2) female guppies allocate differentially to

progeny in response to differences in social environment in a way that selects for increased reproductive potential.

INTRODUCTION

Female reproductive investment is predicted to co-vary with the environmental and social conditions experienced by them in ways that maximize fitness (Sheldon 2000). However, reproductive investment is usually constrained by resource availability. Therefore, natural selection favours an optimization of female reproductive allocation (Smith & Fretwell 1974; Charnov 1982). In other words females are expected to maximize the ratio of reproductive benefits per investment (Jennions & Telford 2002). Additionally, female reproductive optimization can in some cases be further constrained if sons and daughters have different fitness returns. As a result, females are expected to bias their reproductive investment towards the sex with the greater fitness potential in any given context (Frank 1990; Clutton-Brock & Vincent 1991). Females may therefore adjust the sex of their brood in response to these fitness differences between sexes (Trivers & Willard 1973).

Numerous sex ratio models have been proposed to explore the circumstances under which adjusting the sex ratio is favorable (e.g. Emlen 1997). In general, females should always adjust the sex ratio of their offspring in response to environmental/social factors if this adjustment results in a significant increase in fitness (Trivers & Willard 1973; Clutton-Brock *et al.* 1984). Interestingly, this prediction holds for all types of mechanisms of sex determination. Apparently, sex ratio adjustment is not constrained by the species mechanism of sex determination, but rather by the trade-off costs/benefits (West & Sheldon 2002). According to this study, patterns of sex ratio adjustment are expected to be more frequent in species in

which the benefits of adjusting the sex ratio are far greater than its costs. However, in spite of the theoretical predictions and the increasing amount of evidence showing species skewing their sex ratios, whether this adjustment is adaptive or not remains highly controversial (West & Sheldon 2002). This is especially true for vertebrates where adjustment of sex ratio in response to population sex ratios have seldom been tested (Zann & Runciman 2003), prompting a need for further studies.

But how do females optimize their investment if they are not able to determine or control the sex of their progeny before birth? This seems to be the rule among most vertebrate species (Helle *et al.* 2008). In this case, females are expected to adjust their resource allocation to progeny in response to environmental/social conditions (Hunt & Simmons 2004). Here, the trade off sons *vs.* daughters is replaced by a trade-off in terms of the total number *vs.* size of offspring (Smith & Fretwell 1974). In some contexts, fewer but bigger offspring are a better investment, whereas under other conditions many but smaller offspring are more favorable (Clutton-Brock & Vincent 1991). Females are thus expected to judge conditions and make an optimal investment to offspring accordingly (Smith & Fretwell 1974; Charnov 1976). This optimal investment should always be the maximum if both sexes are equally costly to produce (Kishi & Nishida 2008).

In both sex ratio adjustment and differential resource allocation, it is assumed that females are able to assess environmental/social conditions and predict future ones, and thus adjust their investment accordingly. This may not always be the case, especially in stochastic environments. Mixed and/or random female allocation strategies may arise in these environments (Hunt & Simmons 2004). A way to prevent this is by allowing females to evaluate the social environment characteristics before mating. I used this approach to examine how females living under extreme social

conditions vary their reproductive investment. More precisely, I used extreme differences in adult sex ratio as social factor to examine if females 1) can either compensate the sex ratio of their offspring and/or 2) differentially allocate resources to sons and daughters. The Trinidadian guppy, *Poecilia reticulata,* was used as a model species to examine these two of female reproductive strategies. This species is an ideal candidate to test these two ideas because of the extraordinary plasticity in terms of female reproductive decisions (Reznick & Yang 1993; Godin *et al.* 2005). Furthermore, the fact that most reproductive decisions are linked to social/environmental factors in a way that optimizes reproductive investment and fitness return of females (e.g. Reznick *et al.* 2002), is of particular relevance for this study. Finally, there is conflicting information regarding the question of whether guppies are able to compensate their sex ratio. Whereas, some experiments showed they can (Geodakyan *et al.* 1967; Geodakyan & Kosobutskii 1969), others have reached the opposite conclusion (Farr 1981; Brown 1982; Watt *et al.* 2001). This uncertainty warrants re-investigation. Furthermore, female guppies are likely to observe the benefits of adjusting the sex ratio of their brood since the mean time of gestation to sexual maturation of offspring is 8weeks (Houde 1997). Given that guppies live on average 15 weeks after gestation, by compensating the sex ratio of their offspring females will get direct benefits.

Sex ratios in guppies are extremely variable, even at birth, but female biased in adult populations (wild) (Rodd & Reznick 1997). This variation has been reported to influence some major components of guppy's life history traits. For example, sex ratio fluctuations have a direct impact on male sexual behaviour, sperm production, sexual harassment, as well as on female preference and reproductive investment (Evans & Magurran 1999a, b; Jirotkul 1999; Reznick *et al.* 2002; Field & Waite

2004). Furthermore, the fact that adult sex ratios are female biased is believed to influence the opportunity for sexual selection (Jirotkul 2000).

Extreme sex ratio conditions can also influence maternal resource allocation. For example, strongly male biased sex ratios can significantly reduce the amount of time females spend feeding (Magurran & Seghers 1994b). Food limitation has a direct effect on the resources individuals have available, and therefore maternal allocation to offspring size and growth (Reznick & Yang 1993; Bashey 2006). Female guppies seem to respond to environmental adversity by producing fewer but bigger offspring (Reznick & Yang 1993). Bigger offspring have generally greater fitness potential and higher survival rate than smaller ones (Reznick *et al.* 1996; Bashey 2008). In line with these facts, I predicted differences in reproductive investment between females allocated to different social environments.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN

200 individuals were reared from birth (100 females and 100 males). All individuals were reared in individual tanks until reaching three months old. After this, I randomly allocated each female to either a male biased sex ratio (n_{tank}=50) (MSR), or to a female (n_{tank}=50) (FSR) biased sex ratio treatment. Sex ratio proportions (male/female) were in the MSR 6/1 and in the FSR 1/6. In all treatments females were allowed to settled and assess the environmental conditions of the tank for 24 hours before a male was introduced. Because, the level of sexual harassment in the MSR could potentially affect results, all tanks were divided into two identical sized parts by transparent perspex. Stress conditions were, therefore, kept identical for both

treatments. All individuals were kept in these conditions until babies were born. After birth each baby was individually measured and allocated to an individual tank, where it stayed for 12 weeks. Only one brood per tank was allowed. This decision was based on previous evidence suggesting the first brood is the one that shows the maximum sex ratio compensation (Geodakyan *et al.* 1967; Geodakyan & Kosobutskii 1969). All individuals were sexed before the end of the experiment. All individuals were maintained in identical light cycle (12 hour) and water temperatures (20-24 c). Additionally, all individuals were fed once a day with live *Artemia*.

STATISTICAL ANALYSIS

Sex ratios were expressed as the ratio of sons per daughters, hereafter. Compensatory regulation of primary sex ratio within sex ratio treatments was examined by testing for significant departures (95 % confidence intervals) from a 1:1 sex ratio proportion using a binomial test (Sokal & Rohlf 1995). I proposed two alternative hypotheses. In a female biased sex ratio I expected to see a departure from equal sex ratio towards an over production of sons. By contrast, in a male biased sex ratio, an over production of daughters was predicted. Differences in sex ratio proportions within treatments were analyzed using unpaired t-tests. In order to assess if there was any effect of sex ratio on maternal allocation, I looked for mean differences between sex ratio treatments in terms of brood size and size at birth in offspring using a one-way analysis of variance. Bonferroni corrections were employed to prevent an inflation of type I error from multiple comparisons (Sokal & Rohlf 1995). Because both brood size and offspring size at birth can be influenced by female size, differences in female allocation (brood and offspring sizes) were analyzed using a one-way analysis of variance with values standardized for female

standard length (cm). This analysis would therefore help reveal the investment in terms of proportion of female size to brood and offspring sizes. Proportion data were arcsine transformed prior to analysis to achieve normality and homoscedasticity (Sokal & Rohlf 1995). All analyses were performed in R 2.7.2 (R development team 2008) and results were considered to be significant for $\alpha = 0.05$.

RESULTS

In both treatments, females produced on average more daughters than sons (Table 3.1). Therefore, there was no evidence of a compensatory effect in the experiment (Table 3.1, Fig 3.1). There was a significant departure from the expected alternative hypothesis in the females allocated to the female biased sex ratio treatment (Table 3.1, Fig 3.1). However, this was caused by the female bias in offspring observed in both treatments. Interestingly, sex ratios remained stable within treatments, (two tailed, MSR, $t_{63} = -1.25$, $p = 0.21$; FSR, $t_{84} = -1.13$, $p = 0.25$). Mortality rate was significantly higher in the female sex ratio treatment (G-test, $p < 0.005$). Finally there was no significant difference in brood size (two tailed $t_{96} = -0.01$, $p = 0.10$) and gestation time (two tailed $t_{91} = -0.36$, $p = 0.71$) between sex ratio treatments (Table 3.1).

Table 3.1 - Summary of sex ratio results. * Differs significantly from an expected probability of even sex ratios 0.5 (binomial test, *Alpha* = 0.006)

Treatment	Brood success (%)	N Sons	N Daughters	Sex ratio (m:f)	Mortality (%)	Mean brood size ($\pm$ SE)	Mean gestation Time ($\pm$ SE)
Female	86%	85	105	0.810	13.9%	4.53 ± 3.02	44.45 ± 25.9
Male	80%	79	100	0.790*	2.5%	4.40 ± 3.08	46.17 ± 19.53
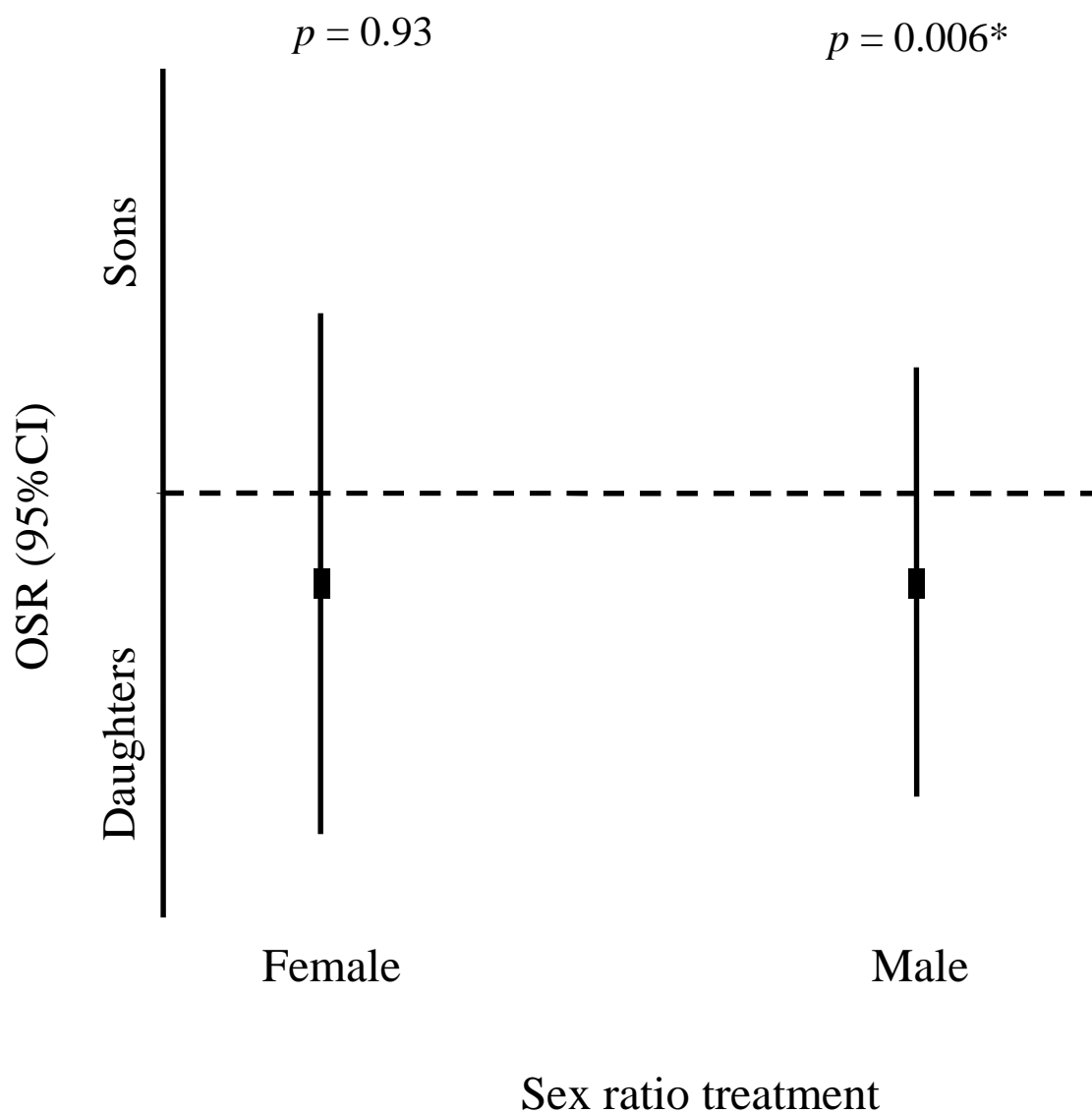
Fig 3.1 – Results from the binomial test on the operational sex ratio (sons/daughters) (95% CI)). Dotted line indicates a even sex ratio. *Alpha* levels are indicated (*significant for $p < 0.005$)

In terms of differential maternal resource allocation, a two-way ANOVA revealed that the interaction between sex ratio treatment and size at birth is not significant (GLM, $F_{1, 112} = 0.65$, $p = 0.423$). Interestingly, females allocated to a FSR treatment produced bigger offspring than females allocated to the MSR treatment (Table 3.2, Fig 3.2,). Sons produced in the female biased sex ratio were bigger than the other (Fig 3.2). Finally, whereas siblings in the female biased sex ratio had different sizes at birth, siblings in the male biased sex ratio had similar sizes (Table 3.2). After standardizing for female size, females allocated to the FSR treatment produced, proportionally to their size, bigger babies but similar sized broods (GLM, $F_{1, 71} = 6.21$, $p = 0.04$; $F_{1, 71} = 0.04$, $p = 0.83$) (Fig 3.3a, b). This result suggests that females allocated to the FSR treatment invest more resources into their progeny than females allocated to the MSR treatment.

Table 3.2 – One-way ANOVA to test the variation of size at birth between and within sex ratio treatments. FRS – female biased sex ratio and MSR – male biased sex ratio. *Significance was based on Bonferroni corrected *p*-vales (*alpha* = 0.008)

Comparison	df	MS (residual)	F-ratio	*Alpha*
FSR$_{daughters}$ x FSR$_{sons}$	178	0.0007	16.90	0.003*
MSR$_{daughters}$ x MSR$_{sons}$	168	0.0005	2.98	0.090
FSR$_{daughters}$ x MSR$_{daughters}$	184	0.0009	1.38	0.240
FSR$_{sons}$ x MSR$_{sons}$	162	0.0003	32.87	0.004*
FSR$_{daughters}$ x MSR$_{sons}$	182	0.0008	0.032	0.857
FSR$_{sons}$ x MSR$_{daughters}$	164	0.0004	44.02	0.0001*
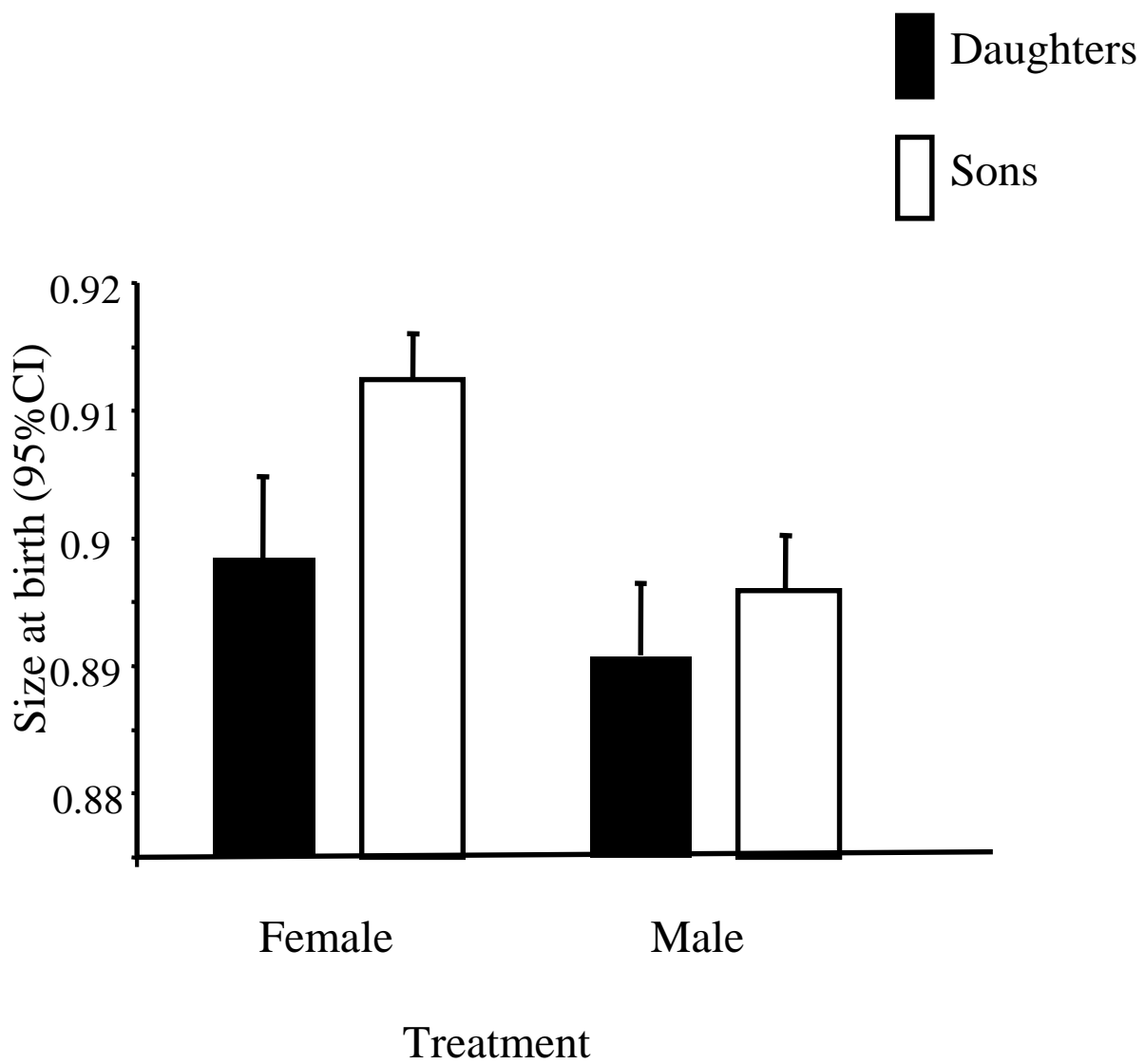
Fig 3.2 – Mean size at birth of sons and daughters produced by females allocated to a female or male biased sex ratio treatment. Error bars denote 95% confidence intervals
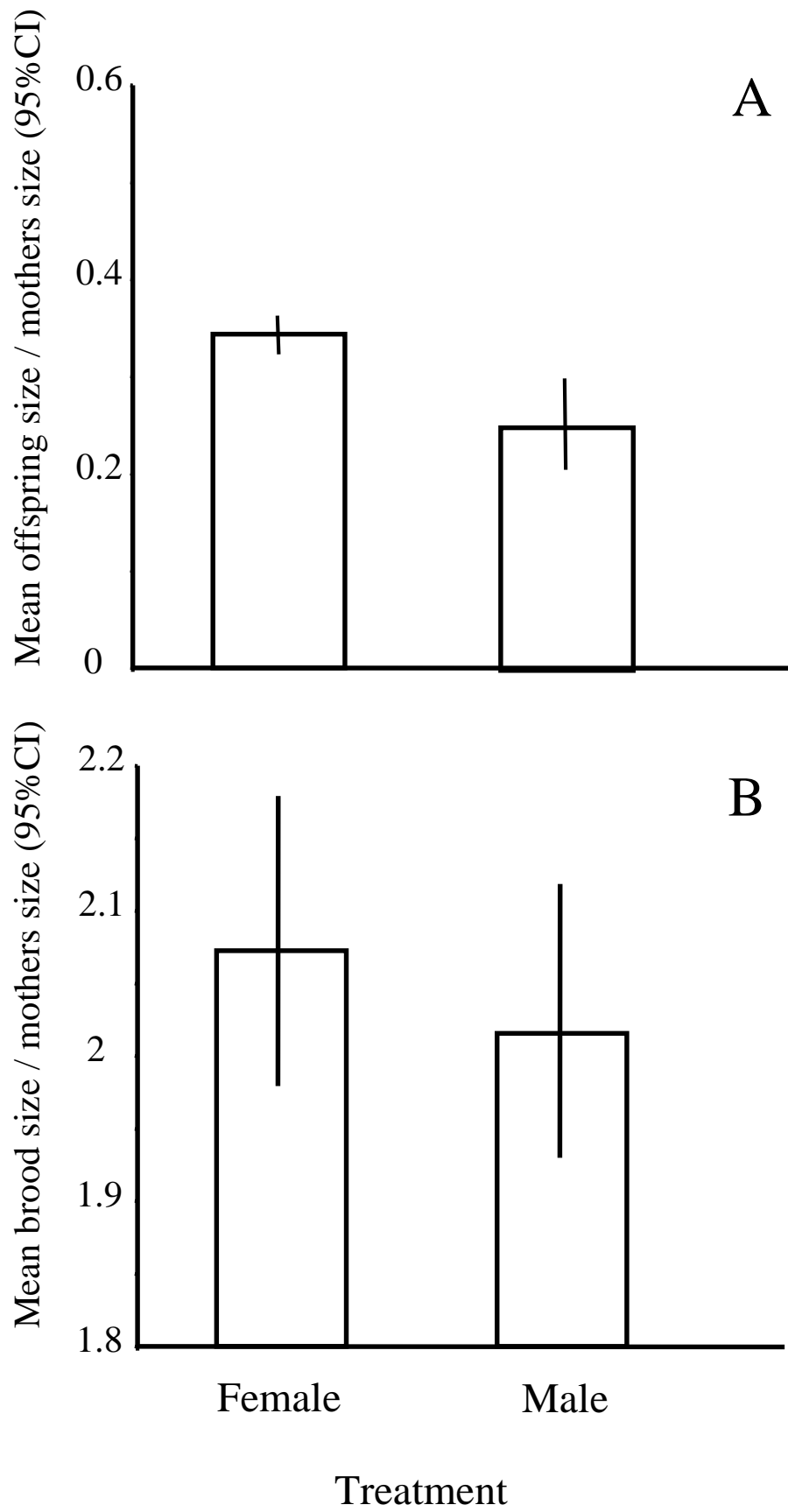
Fig 3.3 – Mean sizes at birth (a) and brood size (b) between sex ratio treatments. Values standardized by mother's sizes. Error bars indicate 95% confidence intervals

DISCUSSION

Environmental and social conditions experienced by females prior and during mating dramatically affect female's investment in reproduction (Mousseau & Fox 1998). Females adapt and optimize their reproductive investment in response to environmental and social cues to maximize their fitness and/or the fitness of their offspring (Sheldon 2000). In this chapter I looked at how a female's reproductive investment changes when faced with extreme differences in adult sex ratios. In particular, I asked whether female guppies were able to adjust the sex ratio of their brood (compensatory mechanism) and/or invest differentially (brood and offspring sizes) in response to an all female or all male sex ratios environments. I did not see any evidence for females, or for that matter males, modifying the sex ratio of their brood in compensatory adjustment. However, I found that there were differences in female reproductive allocation between the two sex ratio treatments. Broods were proportionally larger and babies were significantly bigger at birth in a female dominated environment than in a male biased sex ratio situation. These differences remain significant even after data on female size was taken into account. Therefore, females allocated a greater amount of resources to reproduction proportionally to their size in a female biased environment. Female guppies have been reported to adjust their reproductive allocation by varying the number, size and growth of offspring in response to changes in predation, competition and food scarcity (e.g. Bashey 2006). Here I showed that differences in sex ratio also cause female guppies to adjust their reproductive allocation.

Sex in guppies is genetically determined by a typical *xy* system (Lindholm & Breden 2002). Despite being intuitively easy to think that genetic determination of sex

may limit the adaptive adjustment of sex ratio, recent evidence suggests otherwise. The mechanism of sex determination appears to play a minor role in sex ratio adjustment (West & Sheldon 2002). Instead potential benefits seem to be the limiting factor of sex ratio adjustment. In guppies the benefits from sex ratio adjustment are potentially high. It has been shown that fluctuations in population sex ratio in guppies dramatically affect male and female reproductive behaviour and investment (Jirotkul 1999). Additionally, the costs of producing sons or daughters are identical (Farr 1981) and are, therefore, not expected to act as a resource investment constraint. In summary there are clear benefits for sex ratio adjustment in guppies. However, this study found no evidence for sex ratio adjustment under extreme social conditions. Despite early studies reporting compensatory adjustment of sex ratio in guppies (Geodakyan *et al.* 1967; Geodakyan & Kosobutskii 1969), more recent investigations have been unable to replicate those results (Brown 1982; Watt *et al.* 2001). This study was in accordance with the latter. Thus, the costs and/or the physiological constraint of sex determination in guppies must prevent females (or males) from adjusting the sex of their offspring in response to environmental conditions.

In spite of the variation in sex ratio observed among different broods within treatments, which is typical in this species (see Pettersson *et al.* 2004), on average more daughters were produced in both treatments. This over production of daughters has been reported before (e.g. Watt *et al.* 2001). The interesting point here is the similarity in the ratio male/female between different studies. In this study the ratio was 0.797, whereas in two other studies was of 0.759 and 0.905 (Farr 1981; Brown 1982) respectively. This is more fascinating if we bear in mind the likely differences in stock type, age and life histories between individuals used in these studies. What can explain the typical and similar over production of daughters in laboratory

experiments? An explanation put forward is that female biased sex ratios are primarily caused by the rate of accumulation of mutations and deleterious alleles in the Y-chromosome, which leads to higher mortality of male offspring. This situation is common in populations with high levels of inbreeding (Farr 1981). Although more experimental, and especially genetic, evidence is needed to support this hypothesis, all fish used here descended from fish that have been in laboratory for several generations. It is then likely that a similar process of deterioration and mutation of Y-chromosome may have caused the result of over production of daughters.

There is considerable evidence that different environmental and social contexts affect female reproductive allocation in guppies e.g. (Reznick & Yang 1993; Reznick *et al.* 1996; Bashey 2006). Here I saw a significant effect of population sex ratio in offspring size at birth. Offspring produced in an all female sex ratio were bigger than offspring produced in an all male sex ratio. The difference was only significant between sons. Given the relationship between size at birth and potential fitness e.g. (Reznick & Yang 1993; Bashey 2008), it is selectively advantageous to produce bigger sons in an all female environment. The results from this study, therefore, suggest maximization of the ratio of reproductive benefits per investment.

The mystifying question here is why are females producing proportionally larger daughters? Guppies are lecithotrophic species, meaning that all resources are allocated to eggs prior to fertilization (Wourms 1981). Additionally they also have superfetation, and are therefore unable to have different stage embryos at the same time. As a result differential allocation of sex is very unlikely in guppies, which may explain why daughters are also bigger in the female biased sex ratio. An interesting result was the fact that in all treatments daughters were on average smaller than sons. Given the equal investment of the mother in the egg, this may indicate that sons are

more efficient than daughters at converting dietary protein into biomass. A similar mechanism has been proposed for other species (Redondo *et al.* 1992).

Life-history theory predicts a trade-off between size and number of offspring (Smith & Fretwell 1974). Although for some species this balance may be absent (Tejedo 1992; Gibbs *et al.* 2005), in guppies it has been documented in several empirical studies (e.g. Reznick & Yang 1993 but see Ojanguren & Magurran 2007). Furthermore there is evidence that this balance between size and number of offspring is genetically constrained by maternal investment (Shikano & Taniguchi 2005). It is therefore, puzzling that I did not see any evidence for this life history balance in this study. A major assumption of the size vs. number life history trade off theory is that food is a limiting resource (van Noordwijk & de Jong 1986). In this study food was not a limiting factor as it was in others (Reznick & Yang 1993; Reznick *et al.* 1996; Bashey 2006, 2008). Although all individuals were fed identical portions, differences in food intake may have occurred between treatments. In particular, females allocated to a male sex ratio environment may have had less opportunity to feed because of the negative effects of male harassment on female feeding time (Magurran & Seghers 1994b). Consequently, females in this treatment may have had fewer reserves to allocate to reproduction in comparison to females allocated to a female biased sex ratio. In contrast there is no evidence that intense levels of female-female competition (female biased sex ratio treatment) affect reproductive output (Borg *et al.* 2006). In environments where levels of stress are reduced and where the production costs of either sex is equal, females should allocate the maximum amount of resources to reproduction (Kishi & Nishida 2008). Results from my study lend support to this hypothesis. Alternatively, the absence of a trade-off between brood and offspring sizes may be attributed to the artificial conditions imposed on the fish. Other studies

with guppies in extreme artificial conditions have either failed to observe or had to adjust trade off predictions (Reznick 1983; Kodric-Brown & Nicoletto 2001; Ojanguren & Magurran 2007). The social environment in which individuals are housed and how it is artificially manipulated can influence their behaviour in unpredictable ways (e.g.Field & Waite 2004). Therefore, caution should be taken when interpreting results from this study in light of life history theory expectations.

In conclusion this study contributes to understanding how females vary their reproductive investment according to the social environment they live in a way to optimize fitness. In spite of the clear benefits of sex ratio adjustment this mechanism seems to be absent in guppies. Constraints at a physiological level must prevent this mechanism. Nevertheless, it is shown that females invest differentially in reproduction when facing different social conditions. The higher reproductive investment in a female biased environment may either be caused by females having more resources available, or be an adaptation to maximize investment in offspring with the most successful gender in such conditions. Either way, the result of this differential reproductive investment is that both the females and their offspring fitness are maximized in a female biased environment.

Chapter four – Female Trinidadian guppies actively promote polyandry

ABSTRACT

Polyandry is extremely common among a wide range of organisms. In promiscuous mating systems females are often sexually harassed by males, but at the same time obtain more mating benefits from multiple mating. It remains unclear whether polyandry is imposed by males or is instead a female mating decision. In this chapter I investigated this question by recording the time spent by female guppies near a single or a group of males with similar size and colour patterns over three consecutive days. I accounted for the effect of schooling by using a control treatment where a group of females replaces the group of males. Results showed that females promote polyandry, by spending significantly more time near the group of males, but not with the group of females. In the presence of a group of males, overall female courtship time did not change through the length of the study; but it shifted from the single male to the group of males. In the presence of a group of females, test females decreased their courtship time throughout the experiment, but did not seek the group of females. Additionally, because the proportion of time spent between the two male groups was independent of male size and coloration, female choice for mates may not be necessarily limited to these two male sexual traits. This study highlights the current view of the active role of females in the mating process.

INTRODUCTION

Females and males have different reproductive objectives. Males maximize the number of offspring by maximizing the number of sexual partners. By contrast, female reproductive success is limited by the progeny they can produce per mating event (Ihara 2002). These conflicting goals generate sexual conflict, which is enhanced in promiscuous systems. In these systems constant sexual harassment may limit or mask female mating decisions and consequently affect the strength and direction of sexual selection (Clutton-Brock & Parker 1995). However, females have the potential to reinforce or weaken males' contribution to mating through pre-mating decisions. Understanding female mating decisions in the context of male sexual harassment can help us better understand the forces at play in sexual selection.

The costs of mating are thought to be greater for females than for males, as a result of physical, physiological and energetic costs (Chapman *et al.* 1995). Mating with several males has additional costs, including loss of time and energy in evaluating and rejecting potential mates, increase in predation risk, reduced longevity due to risk of infection and physical injury (e.g. Rowe 1994). Furthermore, in some species, males adjust the level of sperm investment according to female promiscuity, by reducing it if a female has had many partners, thus encouraging monandry (Pizzari *et al.* 2003; Redpath *et al.* 2006). However and despite all the costs associated with polyandry, female multiple mating is widespread in most taxa (Birkhead & Møller 1998). In fishes, and in particular in freshwater species, this ubiquity is well documented with 60% of the species studied showing this reproductive pattern (Barbosa & Magurran 2006).

The prevalence of polyandry is linked to the reproductive benefits associated with this reproductive pattern (Arnqvist & Nilsson 2000). Two types of benefits have been proposed to explain the evolution of polyandry: 1) direct benefits that are reflected in an increase in female fitness. Direct benefits are though to translate into an increase in the number of F1 produced. The second type of mating benefits is indirect benefits. These benefits are believed to enhance the genetic quality or diversity of offspring and thus increasing their reproductive success (number of F2) (Jennions & Petrie 2000). Most of our knowledge about polyandry comes from studies that try to estimate female benefits that arise from multiple mating. However, the extent to which polyandry is promoted by females in promiscuous systems is yet to be addressed. It is particularly relevant for these systems because females are sexually harassed but also obtain benefits from multiple mating. Hence polyandry may be simply a consequence of male harassment rather than of female decision. Clarifying to what extent polyandry is in fact a female mating decision is a logical first step before attempting to justify its evolutionary advantages.

The guppy *Poecilia reticulata* is a freshwater fish species living in a promiscuous mating system where the frequency of male sexual harassment is high. Females exhibit preference for bright colourful males (Houde 1997), and are able to change the course of mating through post-copulatory mechanisms (Evans *et al.* 2003b). Female mating response to males does not change with the increase of male sexual coercion (Ojanguren & Magurran 2007). Additionally the fitness consequences for females of increased male density remain controversial (Head & Brooks 2006). Finally and foremost, polyandry is common in this species (Becher & Magurran 2004), and its benefits over single mating have been suggested (Evans & Magurran

2000). There is, therefore, enough information to predict that female guppies can promote polyandry.

However, the question remains: do female pre-mating decisions have the power to influence the outcome of mating? There is evidence that in some species male sexual harassment does not limit female mating choices (see Clutton-Brock & Parker 1995; for examples). In guppies in particular, it has been shown that females move to areas of high predation in order to avoid sexual harassment (Croft *et al.* 2004; Croft *et al.* 2006b; Darden & Croft 2008). Additionally, in two closely related species, females of sailfin molly (*Poecilia latipinna*) and of the mosquito fish (*Gambusia holbrooki*) chose to stay near a particular type of male in order to potentially reduce the levels of male harassment (Bisazza *et al.* 2001; Schlupp *et al.* 2001). This may be an adaptive behaviour that permits females to select which male mates and at the same time control the level of sexual harassment by other males, as is common in other species (Censky 1997). These examples illustrate how females are able to exert pre-mating decisions. In line with these previous studies, I predict that female guppies can, if given the choice, promote polyandry.

In chapter 4 I tested this prediction by asking three questions. First, I asked whether receptive female guppies prefer to associate with a single male or a group of males. Second, I asked whether female preference differs when the alternative to the single male is either a group of males or a group of females. Females may chose to associate with the group for reasons other than seeking additional mates. For example, females may prefer to be near the group for schooling reasons, rather than to pursue extra mating. In fact, females show strongest schooling preferences for other females (Griffiths & Magurran 1998). Thus, by comparing female behaviour in two contrasting situations (a choice of a group of males, or a choice of a group of females)

I will address the motivation for associating with groups of fish. Finally, I asked if this preference changes through time. Virgin female guppies are highly receptive to first mating (Hughes *et al.* 1999). However, this receptivity may decrease when copulation has been achieved, especially if males are of identical size and colouration. Accordingly, I predicted that the proportion of time allocated to be near males should decrease with time.

MATERIAL AND METHODS

STUDY SPECIES

All fish used in this experiment ($N = 360$) were laboratory-reared descendents of wild caught guppies from Lower Tacarigua, Trinidad. This population naturally experiences both high predation and intense male sexual harassment (Magurran & Seghers 1994b). As a result females spend more time schooling, preferentially with other females (Magurran *et al.* 1992; Griffiths & Magurran 1998) and engaging in anti-predator behaviours (Croft *et al.* 2003a; Croft *et al.* 2003b; Croft *et al.* 2006a), whereas males allocate considerable time to mating activity, particularly into sneaky mating (Evans *et al.* 2002). It has also been found that males in this area have smaller sperm reserves (Evans & Magurran 1999a). All these factors lead to the suggestion that sexual selection in this area is reduced (Endler 1995). The difference in time allocated to mating and schooling between males and females in this population can help tease apart the effects of associating with other individuals for schooling or mating. If females typically school with other females (Griffiths & Magurran 1998), show reduced interest in males (Gong 1997) and exhibit pronounced sexual segregation (Croft *et al.* 2006b; Darden & Croft 2008), then a voluntary association

between a female and given male(s) can be interpreted as a means to promote polyandry.

Virgin females and males were reared in individual tanks (30 x 20 x 16 cm) until sexual maturity. Because visual contact before mating may affect mating decisions (Breden *et al.* 1995) all individuals were raised in visual isolation. All fish were kept under a 12 hour light/night regime and fed daily with live artemia.

EXPERIMENTAL DESIGN

In order to investigate if females prefer to associate with multiple males or with a single male, I recorded the proportion of time spent by females (test individual) near a single or a group of males (experimental). This method has been widely used in studies investigating female guppy mating preference, as the amount of time a female spends near a male reflects her mating preference (Godin *et al.* 2005). Additionally, to test if this preference was caused by other factors such as group schooling, a control treatment was setup. Here, instead of a group of males I allocated a group of females. Finally, to examine if female sexual motivation changed through time I looked at the proportion of time allocated to the single male and the group of males during three consecutive days. The two treatments (experimental and control) allows three key comparisons: 1) whether females prefer to be associated with single or multiple males (within treatment), 2) if this is motivated by schooling or promoting mating (across experimental and control treatment and finally 3) if this preference is maintained through time (within treatment during three days).

All males (N=180; experimental single n=30 and group n=4 x 30; control single n=30) used in this study were matched for size (standard length, cm) ($X \pm$ SE,

single ♂ = 2.08 ± 1.27; group ♂ = 2.01 ± 1.62; control ♂ = 2.14 ± 1.30 one-way

ANOVA, $F_{(2, 177)}$ = 15.7, p = 0.23) and coloration (Table 4.1). All males were

photographed prior to the experiment and the proportion of black, orange, blue and

green pigments in the body recorded using Image j. Particular attention was paid in

selecting individuals with bright orange spots because they are favoured by females of

this population (Jayasooriya *et al.* 2002) regardless of any possible differences in

male motivation (Evans *et al.* 2004a). All females used in the control treatment

(n=150; test females n=30 and group n=4 x 30) were matched for size (test ♀ = 2.32 ±

1.81; group ♀ = 2.28 ± 3.10; t_{149} = 2.28, p = 0.13).

Table 4.1 – Results from a one-way ANOVA to compare the proportion of colour
pigments between males used in the experiment. Significance was based on
Bonferroni corrected *p*-vales (*alpha*=0.0125)

Pigments	MS residual	df	F-test	*Alpha*
Black	7.31	177	0.009	0.90
Orange	5.58	177	0.478	0.62
Blue	5.78	170	0.248	0.78
Green	12.62	175	0.902	0.40

60 tanks, 30 experimental and 30 control were setup (Fig. 4.1). Each tank was

comprised of three areas (A, B, C see Fig. 4.1) separated by two barriers of

transparent Plexiglas. I randomly assigned one male to area A (n=60) and either four

males or four females to area C (n♂=120, n♀=120). In area B I allocated the test

female (n=60) (Fig. 4.1). All individuals were allowed to settle for 24 hours before

observations started. After this settling period the barrier that divided areas A and B

was raised to allow the test female to sexually interact with the single male. There was another settling period of 30 minutes, where the female and single male were allowed to interact before observations started.

Observations consisted in recording for 30 minutes the time the test female spent near the single male or near the group individuals. When observations were finished for the day, the barrier that divided areas A and B was placed back and both test female and single male returned to their areas. This experimental proceeding was repeated for three consecutive days.

The proportion of time spent by the test female near the group of individuals over total time spent near both single and group (Time near group / (Time near single + Time near group)), was then calculated. This variable summarizes the time allocation to the single *vs.* group of males and avoids the problem of non-independence of variables. I only account for the time spent near the single male by the test female, when he was approached by the test female and not the other way around. Additionally, time spent near the group of individuals was only valid if the female swam freely and stayed within an area less than one and half body length from the barrier (see Fig. 4.1). By using this methodology I avoided recording situations where females could be near both the single and group of individuals at the same time.

STATISTICAL ANALYSIS

Mean differences in total time associated with the single male or the group of males/females was analyzed using a t-test for dependent variables. I analyzed the difference in the proportion of time spent between the group of males (experimental) and the group of females (control) during three days using a one-way within-subject repeated measures analysis of variance (RM). This analysis examined both if group *per se* had an effect in the decision of female (e.g. schooling) and if female decision was consistent through time. The design had 3 levels (day 1, 2 and 3), with "fish test" (test female) as within–subject and "fish treatment" (experimental *vs.* control) as between subject factors. The within-subject effect tested the relative shift in time spent with the group, whereas the between subject effect gave information about differences in the behaviour of the test female between male and female groups. Proportion data were arcsine transformed and each observation weighted by the denominator of the proportion prior to analysis to achieve normality and homoscedasticity (Sokal & Rohlf 1995). All the analyses were performed using STATISTICA® 7.0 software. Results were considered significant for $\alpha = 0.05$, and we report magnitude of effects as well as the results of hypothesis testing.

RESULTS

1) Did females prefer to be associated with multiple males, rather than with a single male? As Fig 4.2, shows, the test female spent 26 % more time associated with the group of males than with the single male. This difference is statistically significant ($T_{29} = 3.64$, $p < 0.05$). When the group was constituted by females rather than males

(control), the test female spent 66 % more time near the single male, a statistically significant difference (T_{29} = -14.1, $p < 0.05$) (Fig. 4.2).

2) Was female preference to be associated with the group sexually or socially motivated? There were no significant differences in total time allocated to the single or the group of individuals between experimental and control tanks (RM, $F_{(2, 116)}$ = 2.56, $p = 0.07$). What did change, however, was how females in the experimental and control tanks allocated their time between the two (single and group) (RM, $F_{(2, 57)}$ = 71.1, $p < 0.05$) (Fig. 4.2). Females allocated to the experimental tanks spent 12.8 % of their total time (three days of observation) near the group, whereas females in the control tanks only spent 5.92 % of their total time near the group (Fig. 4.2).

3) Was female preference maintained through time?

The proportion of time spent near the group changed significantly throughout the three days of observation (RM, $F_{(2, 116)}$ = 79.1, $p<0.05$), and this shift was influenced by the sex of the group of individuals (RM, $F_{(1, 58)}$ = 177.3, $p < 0.05$) (Fig. 4.3a, b). The test female decreased the time she spent near the single male throughout the experiment by 69% in the experimental treatment and by 34.5% in the control treatment (Fig. 4.3a, b). The major difference between the two was that in the experimental treatment this decrease was accompanied by an increase of 58.8% in time spent near the group of males (Tukey HSD, df = 144.84; day 1 - day 2, $p < 0.05$; day 2 - day 3, $p =0.07$). Whereas, in the control treatment time spent by the test female near the group of females remained constant throughout (increase of 5 %) (Tukey HSD, df = 144.84; day 1 – day 2, $p = 0.65$; day 2 – day 3 $p = 0.052$) (Fig. 4.3a, b).
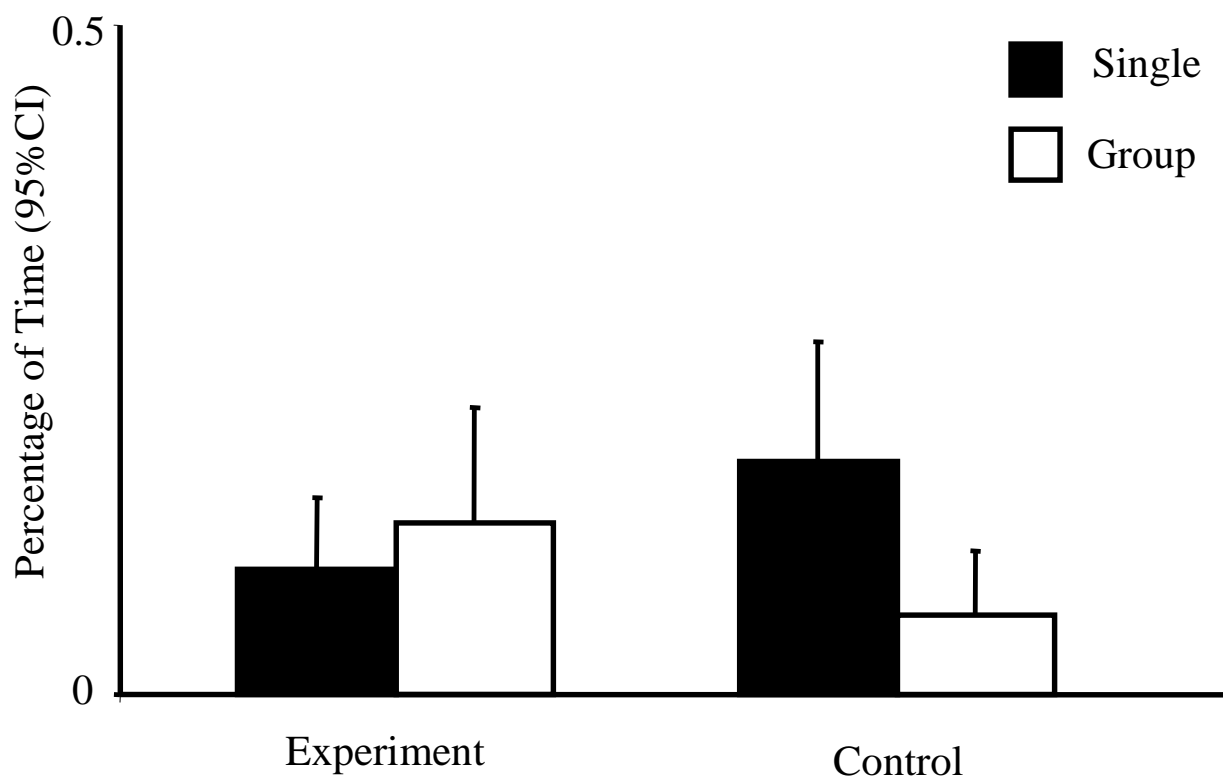
Figure 4.2 – Percentage of total time spent by the test female near a single and four males (experiment) or near a single male and four females (control) during 3 days of observation. Filled bars represent time near the single individual and open bars represent time near the group of individuals. Means and 95% confidence intervals are represented
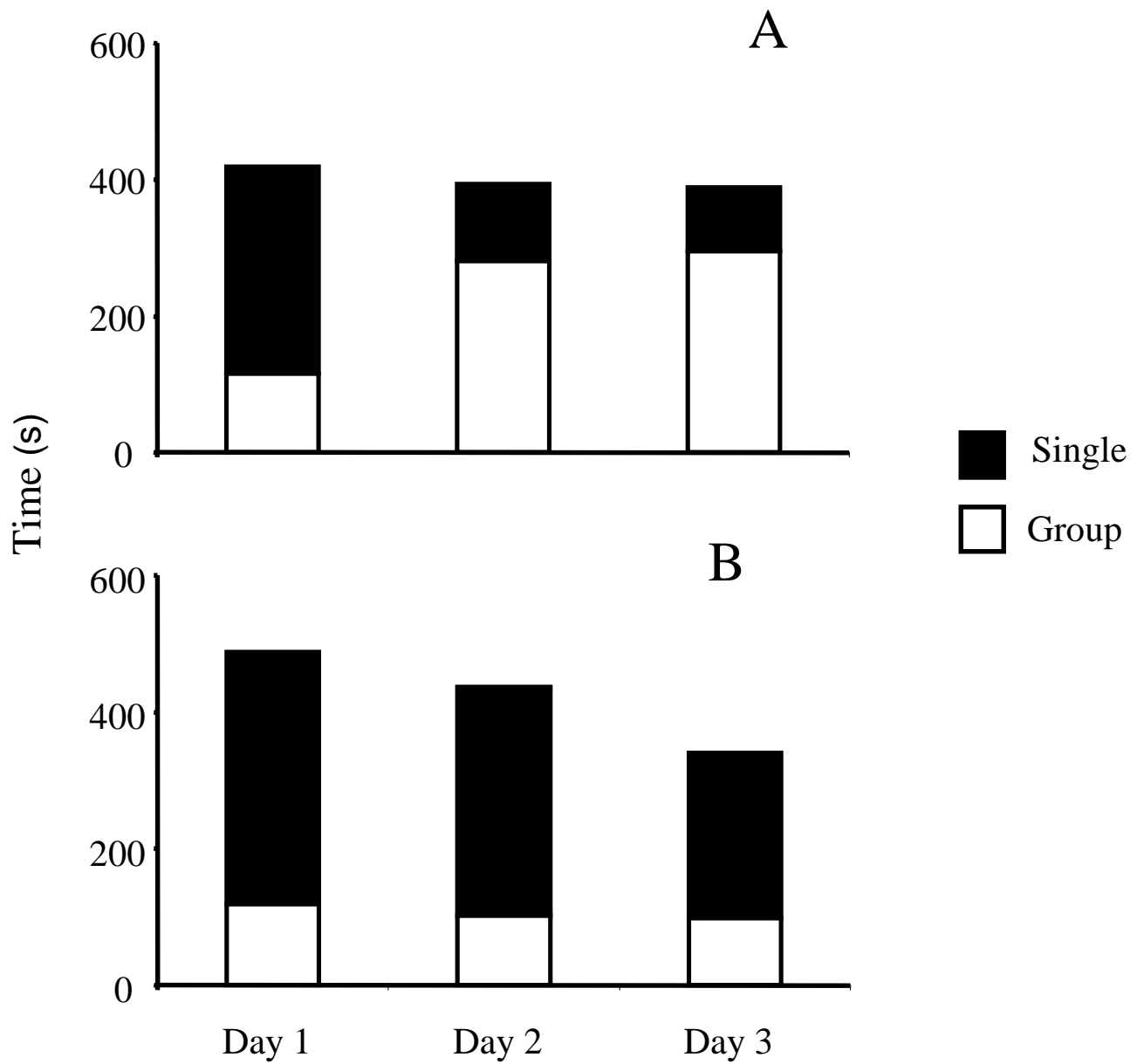
Figure 4.3 – Daily variation in total time spent by the test female near a single and four males (a) or near a single male and four females (b) during 3 days of observation. Filled bars represent time near the single individual and open bars represent time near the group of individuals

DISCUSSION

I predicted that females should be keener to be associated with the group of males rather than with the single male. This prediction was based on the assumption that female guppies get more mating benefits from mating with multiple males than by mating with a single male (Evans & Magurran 2000). The results from this study are in line with the prediction. I observed a shift in the proportion of time spent by test females near the two different male groups as the trial progressed. Female guppies spent the first day near the single male. This type of indiscriminate behaviour is typical of virgin females (Houde 1997). After presumably having assured copulation, females significantly increased the proportion of time near the group of multiple males in the remaining two days of observations. The time spent near a given male or males is an accurate indicator of female's sexual motivation (Godin *et al.* 2005). Female guppies are able to exercise some level of male choice, although the high levels of sexual harassment may undermine this (Magurran 2001). In a system characterized by intense male sexual harassment such as this one, the decision by a female to move close to a given male increases her probability of being mated. Thus, the outcome of associating with a group of males is that females are mated by multiple males. Additionally, I did not observe the same pattern of time allocated to the group of individuals when this was constituted by females rather than males. Here, females, spent most of their time near the single male, suggesting that the association with the group of males is driven by sexual motivation rather than to ensure group schooling/protection. Thus, results from this study show that, when given the opportunity and under controlled stress levels, female guppy behaviour can promote polyandry.

The interaction between the evolution of female choice and male sexual harassment has been well studied in a range of different species (reviewed in (Clutton-Brock & Parker 1995). Although intense male sexual harassment is usually more costly to females than males, some females can use sexual harassment to maximize mating benefits and at the same time decrease the level of sexual harassment (Clutton-Brock *et al.* 1993). It has been proposed that 1) if there is a correlation between male traits and mating benefits (Clutton-Brock *et al.* 1991) and 2) if selective pressure to avoid un-wanted mating has pressured females to grow bigger than males and thus minimize male coercion (Smuts & Smuts 1993), then females are likely to control sexual harassment for their own benefit (Censky 1997). Among male guppies colouration is thought to be a reliable proxy for female preference and mating benefits (Endler & Houde 1995; Evans *et al.* 2003b). Additionally, females are larger than males (Magurran 2005). Therefore, female guppies meet the characteristics that suggest that they are able to avoid sexual harassment (Croft *et al.* 2004; Darden & Croft 2008), and actively drive their pre-mating decisions. This study shows that indeed they do.

Female association with multiple males may be linked to other reasons such as schooling and sexual conflict rather than re-mating. It has been shown that female guppies are more prone to school than males (Croft *et al.* 2003a), and preferentially with same sex-conspecifics (Griffiths & Magurran 1998). The comparison between the experimental and control tank helped tease apart the effects of schooling from sexual effects. If schooling was the sole responsible for the female behaviour observed here, I would expect to see similar trends in both the experimental and control tanks, with females preferring to associate with multiple individuals regardless of their sex. However, I saw the opposite: in the experimental tanks females increased

their time near the group of males, when the group was constituted by females rather than males (control tanks) the test female showed little interest in the group.

Could sexual conflict provide an alternative explanation to the results? When males can force females to copulate with them, conflicts of interest between the sexes are likely to occur (Clutton-Brock & Parker 1995). In guppies this is well illustrated, and male sexual harassment is an important factor in sexual segregation and conflict (Magurran 2001; Croft *et al.* 2006a; Croft *et al.* 2006b; Darden & Croft 2008). Recent studies have added support to the idea of sexual conflict as the main factor behind polyandry (Lee & Hays 2004; Hardling & Kaitala 2005; Dibattista *et al.* 2008; Le Galliard *et al.* 2008). I tried to control and separate the sexual motivation of the female from the potential intra and inter-gender conflict by analyzing results within and across experimental and control tank.

Nevertheless, there may be alternative explanations for my results. Firstly, females may prefer to associate with a single male rather than a group of females, because of female-female competition. In a study of male choice, (Herdman *et al.* 2004) demonstrated that female competition is negligible. In their study, the operational sex ratio (OSR) was 0.5 (two males and two females), whereas here the OSR in the control tank was 0.16 (one male and 5 females). Because female competition increases as OSR becomes more female biased, I may have had a significant effect of female-female competition. The greater interest in the single male showed by test females in the presence of other females suggests this played a part in the patterns observed. This reinforces the assumption that female guppies are in control of their mating decisions.

Yet another alternative explanation is that the test female may have been associated with the group of males in the experimental tanks because of male-male

competition and the effect of males paying less attention to the female. Male-male competition and aggression play a limited role in female guppy choice (Houde 1997). Additionally, in male biased environments females receive more sneaking attempts (Jirotkul 1999). Therefore, it is unlikely that male-male competition can explain the results observed here.

Is female preference maintained through time regardless of male size and colouration? Results confirm previous claims that virgin female guppies do not discriminate between males (Houde 1997). This initial indiscriminate behaviour has been suggested to be an adaptation of females to ensure fertilization (Sheldon 2000). The novel contribution of this study is that females increased time near the group of males in the second and third days. Female guppies have previously been shown to pursue second mates, when these were more attractive (Pitcher *et al.* 2003). Here I showed that this happens also for equally attractive males. I observed a shift in the proportion of time spent near each male group, but not in total time spent near males. Because males used in this study were matched for size and colouration, results suggests that female choice of mates is probably not restricted to size and colouration. Others have been intrigued by the evolutionary reasons for the exuberant sexual behaviours and colour morphs in male guppies, in particular their evolutionary advantages in areas where visual cues are likely to play a minor role in paternity success (Magurran 2005). As seen for other fish species (Landry *et al.* 2001), it is likely that female guppies use other cues, rather than only male's size and colouration, to select their mates.

In conclusion this study underpins the importance of understanding the effects of female pre-copulatory decisions in actively shaping the outcome of the mating process. Females actively moved near new males by shifting their time to them after

being preferentially associating with the single male in the first day of observation. This result was reinforced by the difference seen in the proportion of total time spent near the groups between the experiment and control. Finally, this study also hints on the possibility that female guppies may base their male mating preferences on other factors (diversity) rather that only size and colouration as a proxy of mating benefits.

Chapter five – No mating benefits from multiple mating in guppies: a two-generation test

ABSTRACT

The prevalence of female multiple mating or polyandry in many species has been attributed to the gains of indirect benefits. These benefits are believed to be the primary reason for the evolutionary maintenance of polyandry in species where females get nothing but sperm from males. However, a crucial assumption of indirect benefits is that these will increase the viability and/or attractiveness of offspring, which consequently will increase its offspring's reproductive success. Interestingly, despite intensive theoretical work, and multiple references to the effects of indirect benefits defined as an increase in offspring fitness, have yet to be experimentally demonstrated. Unless a direct relationship between multiple mating and an increase in offspring reproductive success is shown, arguments for the persistence of polyandry due to indirect benefits may be overstated. In this chapter I compared the reproductive success of offspring of multiple and single mated mothers using direct measures of fitness (net fitness). Results showed that it is unlikely that the maintenance of polyandry depends exclusively on indirect benefits. The fitness of offspring from polyandrous females was no greater than those from monandrous females. In spite of lack of indirect benefits, the results showed that polyandrous females produced proportionality more babies than monandrous females. This result indicates that when number of F1, mortality rate and birth success are taken into account together and analyzed over multiple broods, a significant direct benefit may arise from polyandry.

As a result I propose that the potential long term contribution of both direct benefits and male sexual harassment as the most parsimonious explanation for the prevalence of polyandry in guppies.

INTRODUCTION

Why is female multiple mating or polyandry so common across many different species? This question, which has intrigued many behavioural ecologists over the years, stemmed from the need to account for the observed discrepancy between sexual selection theory and empirical data from female mating choice studies (Keller & Reeve 1995; Jennions & Petrie 2000; Simmons 2005). The Bateman principle suggests that females will maximize fitness by mating with a single or few mates (Bateman 1948). Additionally, given the fact that mating involves costs to females such as an increase in the transmission of diseases, predation and physical harm from males (Chapman *et al.* 1995), females are expected to have a more reserved approach to mating than males. However, empirical evidence has shown the opposite. In fact, females actively seek and mate with more than one male during the same reproductive season, with this being the rule rather than the exception (Birkhead & Møller 1998; Simmons 2005). Additionally, studies have shown that females are also able to bias paternity through post-copulatory selection (Eberhard 1996; Birkhead & Pizzari 2002). Trying to explain the inconsistency between theory and empirical observations in female mating choice studies is currently a major unsolved question in evolutionary biology.

Returning to the initial question, why is polyandry so prevalent? An adaptive explanation is that its benefits outweigh its costs (Simmons 2003, 2005). Two types of benefits have been put forward to explain the prevalence and evolution of polyandry. 1) Direct benefits, which give a direct fitness advantage to females (Møller & Jennions 2001), and 2) indirect benefits, which are reflected in the offspring's fitness (Jennions & Petrie 2000). Indirect genetic benefits provide a more robust explanation for the maintenance of polyandry in systems where males provide females with no material benefits (Weatherhead & Robertson 1979; Jennions & Petrie 2000). The main argument behind indirect benefits is that by mating polyandrously, females increase the probability of having their eggs fertilized by a genetically superior male. And if male quality is transmitted to offspring, then females obtain indirect benefits by producing offspring of greater mating potential and/or viability, the sexy and good sperm mechanisms (Keller & Reeve 1995; Kirkpatrick 1996; Yasui 1998). Alternatively, indirect benefits may arise from the combination of a given set of parental alleles, which will result in females producing more attractive and viable offspring, as predicted by the genetic compatibility hypothesis (Zeh & Zeh 1996). Indirect benefits are commonly used to justify the prevalence of polyandry in absence of direct benefits (Jennions & Petrie 2000). However, evidence supporting indirect benefits has been criticized for being empirically inaccurate and based on weak evidence (Kirkpatrick & Barton 1997; Westneat & Stewart 2003; Gustafsson & Qvarnstrom 2006).

There is, therefore, an ongoing debate on the evolutionary reasons for the maintenance of polyandry in the absence of direct benefits (see Simmons 2005). Much of the debate is fuelled by conflicting results from empirical tests of indirect benefits. Whereas some describe their existence (Foerster *et al.* 2003; Head *et al.*

2005), others argue they are nonexistent (Arnqvist & Kirkpatrick 2005; Jennions *et al.* 2007). This, lack of consensus occurs not only across different species, but also within the same species (Barbosa & Magurran 2006). Interestingly, despite the lack of consensus, it is unanimously agreed that the current available data suffers from one vital drawback: offspring fitness is not measured directly (Veen *et al.* 2001; Head *et al.* 2005; Huk & Winkel 2008). An unambiguous test of the hypothesis that polyandrous females obtain indirect benefits is to compare the number of grandchildren between them and monandrous females in similar conditions of habitat and mating frequency (Hunt *et al.* 2004; Kotiaho & Puurtinen 2007).

In chapter five I examined the existence of mating benefits from polyandry in guppies, a species living in a resource free mating system and for which mating benefits have been previously suggested (Evans & Magurran 2000). In particular I asked whether polyandrous females obtain an increase in the number of offspring (direct benefits) and/or the offspring of polyandrous females have greater net fitness (indirect benefits). Additionally, because differences in fitness may be small and reflected in more than one component of fitness (Hunt *et al*. 2004), I also looked at differences between monandrous and polyandrous females using a set of multiple components of fitness. I have also incorporated information on the costs (mortality and birth success) of all participants of the mating process. Finally, I have examined the contribution of paternal phenotype, size and sexual behaviour to offspring survival and viability (sexy and good sperm hypotheses). The main goal of this study was to provide a direct analysis of the benefits of polyandry and to discuss the results in light of the current evolutionary explanatory hypothesis of polyandry. In particular, the aim of chapter five was to obtain crucial information for the significance of polyandry in a resource free mating system species.

MATERIAL AND METHODS

STUDY SPECIES

The Trinidadian Guppy, *Poecilia reticulata* is a neotropical fish species that lives in a resource free mating system, where males only contribution to mating is their sperm (Houde 1997). Female multiple mating is extremely common (Becher & Magurran 2004). Although the evolutionary reasons for the maintencae of polyandry in guppies are still unknown, female guppies have been suggested to get more mating benefits from multiple mating (Evans & Magurran 2000). In fact, fitness predictor measures hint that female guppies can gain both direct and indirect benefits from polyandry. These include: short gestation period and large broods (Evans & Magurran 2000), faster growing babies (Reynolds & Gross 1992), offspring with better schooling and predator avoidance behaviors (Evans & Magurran 2000) as well as producing bigger offspring (Ojanguren *et al.* 2005).

EXPERIMENTAL DESIGN

All fish used in this experiment were laboratory-reared descendents of wild caught guppies from Lower Tacarigua, Trinidad. Females from this population prefer males with high proportion of orange pigmentation (Jayasooriya *et al.* 2002; Evans *et al.* 2004a). Furthermore, the proportion of pigmentation is highly heritable (Brooks & Endler 2001a, b) and is a reliable indicator of male quality (van Oosterhout *et al.* 2003). Finally, sperm load and quality is correlated to male phenotype (Pitcher &

Evans 2001; Skinner & Watt 2007), with preferred phenotypes being favoured in post-copulatory selection (Evans *et al.* 2003b). Thus, to control for potential differences in sperm load and female post-copulatory selection, I used males with similar sizes and identical colour patterns. By doing so, I have tried to minimize possible confounding effects arising from female/male mating preferences.

A protocol similar to that of Tregenza & Wedell (1998) was used, in which the number of matings was kept constant whereas the number of mates varied. This methodology helps to circumvent the potential confounding effects of maternal and paternal investment (Simmons 2005; Huk & Winkel 2008). Because visual contact before mating may affect female mating decisions, all individuals were raised separately and in visual isolation insuring that all were virgins (Breden *et al.* 1995). After 12 weeks (by then females and males were fully mature), each female was allocated to either a single or a multiple mating treatment tank. Females were allowed to settle for 24 hours before mating trials begun. I then introduced a male to each single treatment tank for four successive days. In the multiple mating treatment, a new male was introduced to the tank on each one of the four days. Males in both treatments were introduced at 7.00 am and removed at 5.00pm. The level of female stress in all tanks was therefore identical. All males were removed at the end of the fourth day, and females were left to produce babies.

When first generation babies (F1) were born, I allocated each baby to individual tanks, where they stayed for 12 weeks. After 12 weeks, according to their sex, a male or female were introduced a to each tank and kept together until babies were born (F2). When F2 were born, I again allocated each one to individual tanks where they stayed for 12 weeks. A total of 80 females (40 each treatment) were used,

that produced 291 F1, which then produced 641 F2 (N=1283 fish, mother, father, F1 + partner, F2). All experimental individuals were kept under a 12 hour light/night regime and fed daily with baby fish food.

DIRECT, INDIRECT AND PERFORMANCE MEASURES OF FITNESS

The definition of direct and indirect benefits used in this study follows the criteria used by Birkhead and Pizzari (2002). Direct benefits are all types of reproductive advantages obtained by females from mating with a particular male(s) and which will increase only her fitness. Indirect benefits on the other hand are any advantages that are passed on to F1 by parents that ultimately increase F1 fitness, but not necessarily parent's fitness. Following this description, at each generation net fitness was measured as the number of offspring (F1 and F2). Multiple performance measures of fitness were also recorded for both F1 and F2. Measures recorded for F1's were considered to be direct benefits, as these reflect a direct pay back to the female's fitness. On the other hand, when recorded for F2 these were considered to be indirect benefits. The performance measurements of fitness were: gestation period, size at birth, growth rate, sexual maturation, schooling behaviour, predator avoidance behaviour, sexual behaviour. Each individual was measured immediately after birth and growth rate was measured for each individual on a weekly basis for 12 consecutive weeks. Individuals were placed on a petri dish and their picture taken. I measured total and standard length using ImageJ 1.37V software (National Institute of Health, USA, http://rsb.info.nih.gov.ij/java). Sexual maturation was only recorded for males, since the timing of maturation is difficult to determine precisely for females without sacrificing them. Typically, males are considered sexually mature when the apical hood of the gonopodium extends beyond the tip of

the first ray of the anal fin (Houde 1997). Both schooling and predator avoidance behaviors were recorded within less than 12 hours after babies were born, using a protocol used extensively in this species (see Evans & Magurran 2000; for further details). Briefly, schooling behaviour was assessed in pairs of newly born siblings. Pairs of siblings were randomly selected and allowed to settle for 10 minutes in a circular tank (diameter 44 cm) before observation. I then recorded the length of time they swan together during five minutes. Schooling was only measured when the fish were <less than 3 cm apart, and when they swam synchronously. This was repeated for all pairs of siblings within the same brood. In case of odd number of siblings I randomly selected a previous offspring from the same brood and repeat the observation with the last offspring. Predator avoidance behaviour followed Evans and Magurran's (2000) protocol using the definition of predator escape used by (Birkhead *et al.* 1998). Predator avoidance behaviour was tested by recording the time it took to capture an individual with a small net in a circular tank. Each newborn sibling was allowed to settle for 10 minutes before the test. Each catching was repeated twice and the mean time to capture was calculated. Finally, in terms of sexual behaviour, male guppies display two types of sexual behaviours: sigmoid displays, used to solicit consensual mating, and gonopodial thrusting, employed in sneaky mating. Studies have shown that females prefer to mate with males that display sigmoids (Karino & Kobayashi 2005). The preference associated to sigmoiding is likely to translate in a greater number of babies sired by the male (Evans & Magurran 2001). Sexual behaviour was measured on all 12 week-old males. Each male was placed in an observation tank alone with four experienced females. Total number of sigmoids and gonopodial thrusts were recorded for 10 minutes.

MORTALITY / BIRTH SUCCESS

I recorded mortality rate and birth success, separately, at parental and F1 stages. Mortality rate was calculated as the proportion of individuals that did not survive to the next generation. Birth success was calculated as number of pairs that produced babies / total number of pairs.

STATISTICAL ANALYSIS

Mating benefits were tested using univariate analysis of variance. All fitness measures were selected as dependent factors and treatment (single/multiple) as independent factor. Mother's size was entered as covariate. Separate analyses were performed for each of the 8 measures of fitness to explore how each measure varied between treatments. Bonferroni correction tests were employed to prevent type I error from multiple single comparisons (Sokal & Rohlf 1995). In order to achieve normality of residuals and homoscedasticity of variance variables were transformed *a priori*. Since the dataset had many zeros, which is typical of biological datasets, I used Log_e (x+0.5) to overcome this problem. The results of ANOVA can change dramatically depending on the constant value used. I have opted for a constant value of 0.5 because this value gives a more accurate result over others (e.g.1, 2), especially when running ANOVA on data of unknown distribution (Yamamura 1999), which was our case. To determine whether the mortality rate and birth success were independent between single and multiple mating treatments, both in F1 and F2 generation, a goodness of fit G-test was performed. This test was used to compare the observed frequency distribution with the expected frequencies for the null hypothesis

of a homogeneous distribution between treatments. In order to examine whether any of the father's proxies of quality contribute significantly to F1 viability (birth success) and survival (mortality rate), a multi-regression analysis with a binominal distribution with a Logit link function (GLZ) was used. The binomial response variable varied between 0 (dead/no birth) and 1 (survival/birth). The ratio of the deviance statistic to the respective degrees of freedom was used to evaluate the goodness of fit of the model. Values close to 1 indicate that there is no over-dispersion and that the dependent variable is appropriately scaled. All the analyses were performed using STATISTICA® 7.0 software. Results were considered significant for $\alpha = 0.05$, and I report magnitude of effects as well as the results of hypothesis testing.

RESULTS

There were no significant differences between treatments in terms of mother's sizes ($X \pm$ SE, monandrous $\female$ = 2.66 ± 0.37 cm; polyandrous $\female$ = 2.65 ± 0.31 cm; t_{78} = -0.13, p = 0.90) and fathers' sizes (monandrous $\male$ = 2.20 ± 0.19 cm; polyandrous $\male$ = 2.18 ± 0.21 cm; t_{78} = -0.37, p = 0.71). The proportion of black, orange, blue and green pigmentation in fathers did not differ between treatments (black t_{78} = -1.04, p = 0.31; orange t_{78} = 0.99, p = 0.32; blue t_{78} = -0.73, p = 0.42; green t_{78} = -1.74, p = 0.09). There were also no differences in time spent near males and time to first mate between treatments (t_{78} = -1.86, p = 0.06 and t_{78} = -1.02, p = 0.34) respectively. However, males in the single mating treatment displayed significantly more often than males in the multiple treatment (t_{78} = -2.67, p = 0.01). Finally, birth success in both treatments was statistically indistinguishable. In the single treatment the birth

success was of 43 % (40/93) whereas in the multiple was of 51.2 % (40/78) (*G*-test goodness-of-fit, $p > 0.05$).

DIRECT BENEFITS

Number of F1 (net direct benefits) did not differ significantly between the two treatments (Table 5.1). However, polyandrous females had on average 8.2 % more F1 than monandrous ones (Fig 1). Mother's size could not predict brood size ($R_{s'} = 0.003$, $p = 0.60$), nor gestation time ($R_{s'} = 0.01$, $p = 0.22$). In terms of the multiple components of fitness measured, none of the components measured indicate that F1 from polyandrous females achieve higher fitness than F1 from monandrous females. In fact, in terms of predator avoidance behaviour the pattern was the opposite, with monandrous F1 taking significantly longer to be captured by a simulated predator than F1 from polyandrous females (Table 5.1, Fig 5.1). Differences in size at birth could not explain this ($R_{s'} = 0.02$ $p = 0.72$). Finally, the frequency of sexual behaviour in F1 sons was not correlated with of their fathers ($R_{s'} = 0.01$, $p = 0.34$).

INDIRECT BENEFITS

There was also no significant difference in the number of F2 (net indirect benefits) between monandrous and polyandrous females (F2) (Table 5.1). The F1 from monandrous females produced on average 7.5% more F2 than F1 from polyandrous females (Fig 5.1). In terms of the other components of fitness examined on F2, it was only in predation avoidance behaviour that the F2 from polyandrous

treatment did significantly better than F2 from the monandrous treatment (Table 5.1, Fig 5.1). Interestingly, there was significant but weak negative correlation between size at birth and time to evade predator ($R_{s'}$ = 0.01, p = 0.001). F2 from the monandrous treatment were significantly bigger and grew faster than their counterparts (Table 5.1). Parent sizes were not, however, significantly different (F1 monandrous father = 2.10 ± 0.19 cm; F1 polyandrous father = 1.80 ± 0.27 cm; t_{298} = 1.45, p = 0.07; F1 single mother = 2.18 ± 0.18 cm; F1 multiple mother = 2.03 ± 0.35 cm; t_{329} = -0.18, p = 0.56). As well as growing faster, the F2 from the monandrous treatment also matured significantly sooner than polyandrous F2 ($p < 0.05$) (Table 5.1). Individuals that grew faster reached sexual maturation sooner than slower growing individuals ($R_{s'}$ = 0.24, $p < 0.001$).

Table 5.1 – Mean values for mating benefits for the two generations (F1 and F2) and respective test of significance from the comparison between monandrous and polyandrous females. ns - values considered significant (s) after bonferroni correction for *alpha* < 0.006. m denotes monadrous whereas p for polyandrous mating treatments

Category	Fitness component	N	Mean (SE)	*Alpha*	Bonferroni correction	Treatment
First generation (F1)	F1	80	3.47 (0.23)	0.03	ns	
	Gestation period	80	42.7 (1.44)	0.71	ns	
	Size at birth	290	0.84 (0.004)	0.33	ns	
	Growth rate	289	0.12 (0.008)	0.18	ns	
	Sexual maturation	171	49.5 (1.07)	0.27	ns	
	Schooling	64	0.55 (0.18)	0.53	ns	
	Pred. avoidance	253	217.3 (7.40)	0.0001	s	m > p
	Sexual behaviour	164	9.67 (0.55)	0.01	ns	

Table 5.1 - Continued

Category	Fitness component	N	Mean (SE)	*Alpha*	Bonferroni correction	Treatment
	F2	80	8.06 (0.86)	0.77	ns	
Second generation (F2)	Gestation period	239	105.5 (15.2)	0.02	ns	
	Size at birth	629	0.86 (0.002)	0.0001	s	m>p
	Growth rate	641	0.12 (0.001)	0.0005	s	m>p
	Sexual maturation	262	42.9 (0.07)	0.0003	s	m>p
	Schooling	137	0.22 (0.01)	0.34	ns	
	Pred. avoidance	571	261.2 (6.7)	0.0001	s	p>m
	Sexual behaviour	189	10.9 (0.42)	0.05	ns	
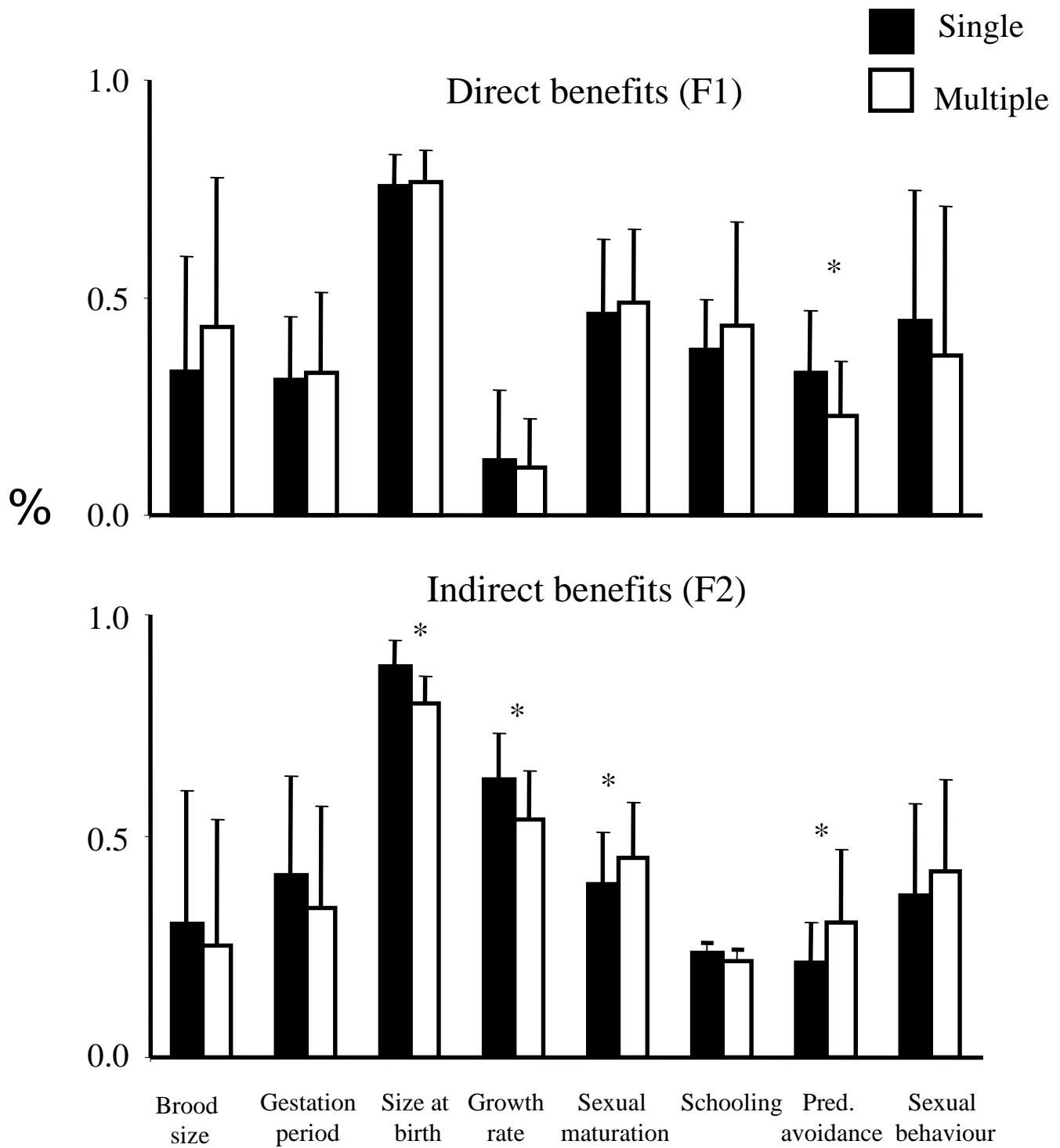
Figure 5.1 - Mean differences in the proportion of fitness components measured for F1 and F2 from two mating treatments, single (open) and multiple (filled) mated females. Error bars denotes 95% confidence intervals. Significance * was based on Bonferroni corrected *p*-vales (*alpha* = 0.006)

MORTALITY / BIRTH SUCCESS

Polyandrous females exhibited lower mortality rates and higher birth success than monandrous females (Fig 5.2a, b). This pattern remained through the F1 generation. However, *G*-test goodness of fit test revealed these differences were not statistically significant ($p > 0.05$) in both paternal and F1 generations. Polyandrous females had 3.1% less mortality than monandrous females, whereas among their offspring this difference was of 3.5% (Fig 5.2a). In terms of birth success, polyandrous females had 8.6 % higher birth success than monandrous females (Fig 5.2b). At F1 generation this difference was smaller, with polyandrous F1 having 2 % greater birth success than monandrous F1 (Fig 5.2b). Finally, fathers size, sexual behaviour, time spent near female and different proportion of colour pigments were not good predictors of both mortality rate and birth success of F1 (Table 5.2).

Table 5.2 – Results for the maximum likelihood estimates of the model parameters for the response variable F1 birth success and F1 mortality rate. Distribution : binomial, link function: logit. * Results considered significant for a p level of 0.05

Response variable	Parameter	df	Estimate	SE	Wald- stat	*Alpha*
Birth success	Intercept	1	-2.38	2.38	0.99	0.31
	Father size	1	-0.19	1.28	0.02	0.88
	Sexual behaviour	1	0.01	0.09	0.03	0.85
	Time spent near female	1	-0.03	0.90	0.001	0.97
	% black	1	14.3	8.44	2.90	0.08
	% orange	1	7.10	7.32	0.93	0.33
	% blue	1	-5.74	9.54	0.36	0.54
	% green	1	4.17	4.72	0.78	0.37

Table 5.2 – Continued

Response variable	Parameter	df	Estimate	SE	Wald- stat	*Alpha*
	Intercept	1	1.35	2.21	0.37	0.53
	Father size	1	-1.92	1.25	2.38	0.12
	Sexual behaviour	1	-0.15	0.14	1.16	0.27
Mortality rate	Time spent near female	1	-0.15	0.94	0.02	0.86
	% black	1	14.9	7.47	3.99	0.55
	% orange	1	0.11	6.98	0.0002	0.98
	% blue	1	6.36	8.59	0.54	0.45
	% green	1	1.47	4.54	0.10	0.74
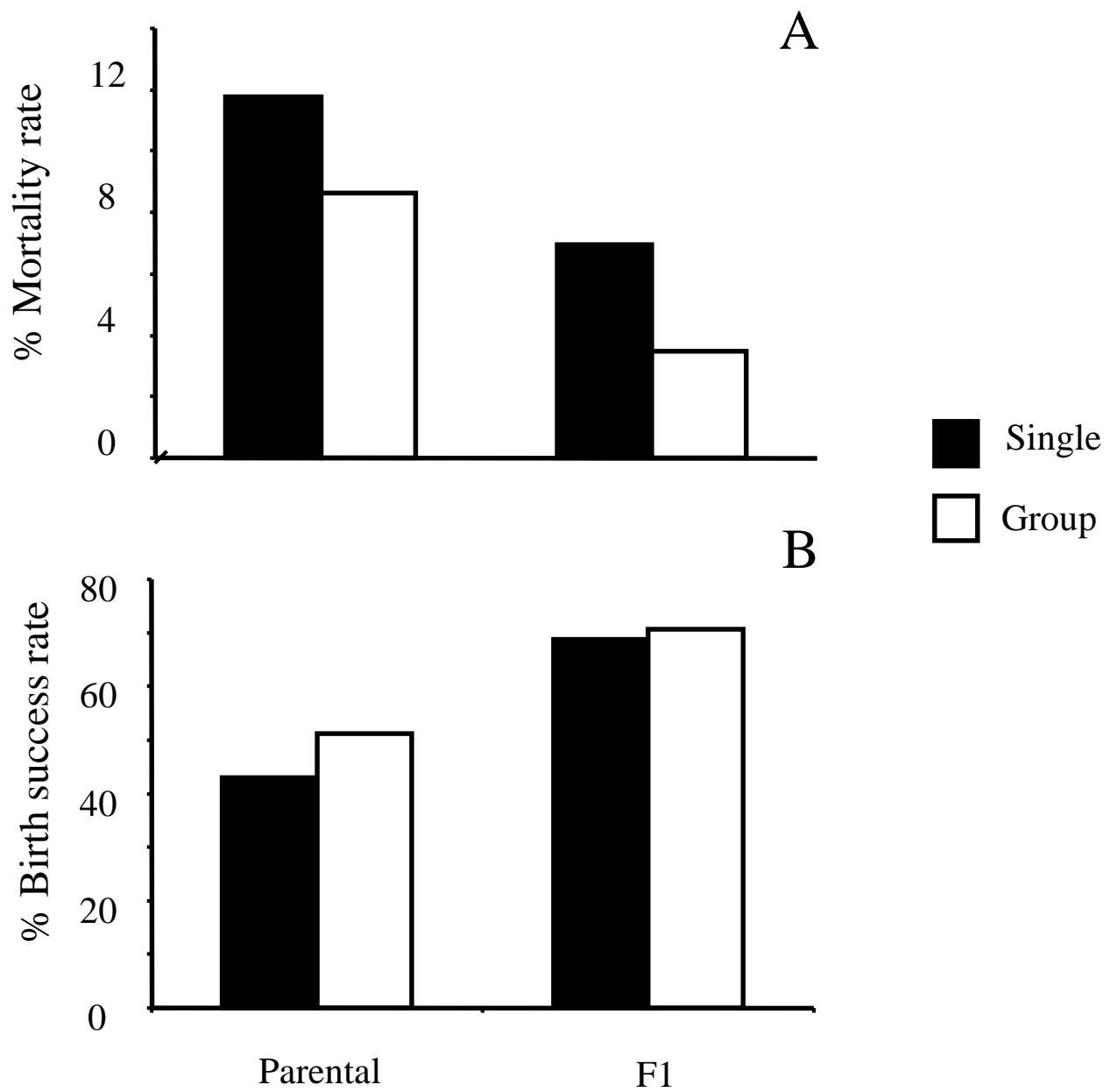
Figure 5.2 - Percentage of mortality rate (a) and birth success (b) in parents and offspring allocated to a single or a multiple mated female treatment

DISCUSSION

There is currently an intense debate on whether females accrue any mating benefits from polyandry (Simmons 2005). This debate is maintained mainly because of the contradictory results studies have reported. Here I examined the potential mating benefits in guppies, a species for which mating benefits have been previously suggested (Evans & Magurran 2000). In this chapter I used direct measures of fitness as well as multiple measures of fitness components to have an accurate estimate of the potential benefits of polyandry. The results from this study reveal that mating benefits from polyandry are small and not statistically significant.

Polyandrous female guppies have previously been shown to produce larger broods, bigger and faster growing babies than monandrous ones (Reynolds & Gross 1992; Evans & Magurran 2000; Ojanguren *et al.* 2005). Such patterns, however, were not observed here. Offspring from polyandrous females were of identical size at birth and grew at a slightly slower pace than offspring from monandrous females. Gestation time was also identical and non-significant between treatments. It is, however, important to state that in spite of lack of statistical significance, polyandrous females produced on average 8.2 % more F1 than monandrous females. The maintenance of sexual behaviour in a population is governed by a cost-benefit ratio. For some species, fecundity and fertility benefits are thought to be more important than any other benefit (Hoeck & Garner 2007). And as pointed out by Hardling & Kaitala (2005), even if direct benefits of polyandry are small and non significant, if these outweigh the costs, expressed in terms of mortality rate, then theoretically there is enough evolutionary force to maintain polyandry based exclusively on direct benefits. In guppies, in particular, survival is a component of life-history trade offs that has a major influence

on individual fitness (Reznick *et al.* 2006). In this study, polyandrous females not only produce 8.2 % more babies, they also suffer 3.1 % lower mortality rate and 8.6% higher birth success. Female guppies produce multiple broods throughout their lives (Houde 1997; Magurran 2005). Therefore, benefits may become statistically significant when assessed in terms of overall fitness for all broods a female produces in her life rather than for a single one. Future research should focus on assessing whether these three components when added together and analyzed over multiple broods lead to significant benefits in terms of overall fitness.

In this study I found no evidence for indirect benefits, in terms of net indirect fitness (number of F2) or in the measures of performance recorded. The results show that polyandrous females have no greater net indirect benefits than monandrous ones. In fact, I saw the opposite, monandrous females produced a proportionately greater number of F2. Indirect benefits from polyandry have been previously suggested for guppies (Houde 1992; Reynolds & Gross 1992; Nicoletto 1995; Brooks 2000; Evans *et al.* 2004b). In this study I have used the definition of mating benefits as an increase in the number of F1 (direct) or F2 (indirect) (Birkhead & Pizzari 2002). Additionally, I also used several indirect indicators of fitness components (e.g. growth rate, size at birth, schooling, etc). There were therefore, fundamental differences in how mating benefits were perceived between studies. This must account for the differences in results. However, here I have shown experimentally and using direct measures of fitness for two generations that polyandrous females have no greater net indirect benefits than monandrous females. Thus, the persistence of polyandry in guppies cannot be explained exclusively by an increase in the number of grandchildren.

Guppies have all the biological characteristics, which theoretically predict that indirect selection will act. These are: sufficient variation and heritability in a male sexual trait, that this trait is correlated with male quality, and finally that the costs of searching for a mate are low (Bjorklund 2006). So the question arises: are the benefits of polyandry expressed in terms of offspring quality rather than in number? Polyandrous females may produce offspring with better survival rate and/or better viability (Keller & Reeve 1995; Yasui 1998; Andersson & Simmons 2006). This higher survival and viability rate may give polyandrous offspring a greater net fitness (increase number of F2) (Fisher *et al.* 2006). Results from this study confirm this prediction; polyandrous F1 had higher survival rate and better birth success than monandrous F1. This difference, however, did not translate into polyandrous F1's achieving greater net fitness (number of F2). Furthermore, none of the proxies of paternal quality (colouration, size, sexual behaviour) were able to account for differences in F1 mortality and birth success. A counter argument would be that I failed to see any differences in F1 fitness because males allocated to the multiple treatment were less preferred by females. All initial males used in this experiment were of identical size and colouration (proxies of male quality, (Houde 1997; Evans *et al.* 2004b)). Furthermore, male sexual display was not statistically different between males in the two mating treatments. It is then unlikely that female preference in male traits could be responsible for the lack of differences. In conclusion, since I did not find any correlation between paternal quality and offspring survival and mating potential, the mechanisms of sexy and good sperm for the maintenance of polyandry in guppies must be rejected.

An alternative explanation is that polyandry is maintained because of intense male sexual harassment (Simmons 2003, 2005; Lee & Hays 2004; Dibattista *et al.*

2008). In fact this hypothesis is considered by many as the only possible explanation for the prevalence of multiple mating in systems where males provide no direct material benefits to females (Westneat & Stewart 2003; Akcay & Roughgarden 2007). Although, male sexual harassment is very intense among guppies, female guppies display strong male choice (Houde 1997). Additionally, the fitness consequences of male sexual harassment in guppies are still debated (Head & Brooks 2006; but see Ojanguren & Magurran 2007). The experimental design used here did not test the effect of sexual harassment on female fitness, nor was this the aim of this study. However, in identical stress regimes, polyandrous females produce more F1's than monandrous. It is unlikely that in a system such as the one that the guppy inhabits, in which male sexual harassment is intense, females would be able to choose all her mates. Male sexual harassment must always be taken into account as a major factor influencing the choice of mates but not necessarily the frequency.

In conclusion the main goal of this study was to examine the mating benefits of polyandry in guppies. This study showed that mating benefits from polyandry are small and not statistically significant. There was no evidence that polyandrous females obtain greater net indirect fitness than monandrous ones, both in number of F2 produced as well as in terms any other component of fitness recorded. The idea that the evolution and prevalence of polyandry in resource-free mating species can only be explained by indirect benefits is thus rejected. The lack of any mating benefits could, however, be a consequence of the artificial conditions our fish are allocated to. Comparisons and extrapolations of these results with results from natural population, where both sexual and natural selections act in different ways, must be done cautiously. Results from this study however, hint that mortality and birth success when analyzed together over several generations may increase the magnitude of

benefits. Thus, there is potential for direct benefits to act in favour of polyandry.

Finally, the typical male sexual harassment environment female guppies live is likely

but not necessarily exclusive explanation for why polyandry is so common among

guppies.

Chapter six – Offspring phenotypic dispersion and polyandry

ABSTRACT

The prevalence of female multiple mating in systems where females get no direct benefits remains enigmatic. The main argument used to justify polyandry in these systems is based on the idea that females can get indirect benefits from polyandry, namely by enhancing the genetic quality of their offspring. Two hypotheses have been proposed: the trade-up hypothesis, which proposes females bias paternity towards males with a preferred phenotype; and the genetic diversity hypothesis, which suggests that the advantages of polyandry are linked to enhanced variability among offspring. To test which of these two hypotheses best explains the high levels of polyandry in a resource free mating species I studied the offspring of Trinidadian guppies under experimentally induced monandry and polyandry. In chapter six I compared the dispersion in the phenotypes of offspring produced by monandrous and polyandrous females. The dispersion of phenotypes was calculated as measure of variation of several phenotypic variables in each offspring. Results showed that polyandrous broods were phenotypically more diverse than monandrous broods. However, this was only statistically significant for sons but not for daughters. Interestingly, polyandrous fathers were phenotypically less diverse than monandrous fathers. Accordingly, results suggest that brood diversification was generated by females biasing paternity rather than just a consequence of inherited differences in the paternal phenotypes. Results from this study support the genetic diversity hypothesis as a possible driving force of polyandry in guppies.

INTRODUCTION

The evolution and maintenance of female polyandry remains enigmatic and has stimulated an intense debate among evolutionary biologists (Simmons 2005). In systems where males provide females with direct mating benefits, such as paternal care, male nuptial gifts or protection against predators, the advantages of polyandry are easily understood. However, when male's only contribution to mating is their sperm, understanding the prevalence and maintenance of polyandry is more puzzling. This is particularly so when females accrue direct costs from repeated mating. However, in spite of the associated costs, in many of these resource-free mating systems, polyandry seems to be selected by females (Birkhead & Møller 1998).

One hypothesis put forward to account for the adaptive significance of polyandry in these systems is that females may use polyandry as a way to swamp/replace the sperm of a previous un-wanted mating event. In these resource-free mating systems, males often sexually coerce females, which may restrict female mating choice. Sperm storage and cryptic selection may help females circumvent this constraint (Birkhead & Pizzari 2002). The trade-up hypothesis proposes that females should replace previous sperm if they encounter a better quality male, particularly if there is a male phenotype that genetically increases the fitness of offspring (Halliday 1983). Polyandrous females may, therefore, obtain indirect genetic benefits from polyandry by biasing paternity towards a particular male phenotype that will maximize the genetic quality of their offspring.

The trade-up hypothesis falls short of explaining polyandry when there is variation in what constitutes an ideal phenotypic male. In this case, it is thought that

polyandry may persist as a strategy of costs minimization (Hosken & Stockley 2003). However, there is an alternative hypothesis: if phenotypic/genetic variation is reproductively advantageous, then females may use polyandry as a way to increase the diversity of their offspring. This is known as the genetic diversity hypothesis (Yasui 1998). In fact, it has been suggested that polyandry may have evolved and be maintained because of the indirect benefits obtained from producing offspring of greater heterozygosity (Cornell & Tregenza 2007; Rubenstein 2007). Genetic/phenotypic diversity is likely to play a vital role in determining the short and long-term success (e.g. mortality, reproductive) of an individual (Kussel & Leibler 2005). The potential benefits of producing diverse offspring are enhanced in stochastic systems, where changes occur at small and unpredictable scales.

In chapter six I tested which of these two hypotheses better explains the adaptive significance of the high levels of polyandry in a resource free mating system. To do this I looked at the multivariate dispersion in the phenotypes of offspring from two mating treatments: monandrous and polyandrous. If females use polyandry as a way to bias paternity towards a preferred male phenotype that confers genetic benefits to offspring, then I expected to see a smaller variation in the phenotypes of polyandrous broods in comparison to monandrous broods. Conversely, if polyandry can be used as a female strategy to promote brood diversification, then polyandrous broods should show higher levels of phenotypic variability than monandrous broods. Finally, if females are unable to or unwilling to bias paternity, then I expected to see offspring phenotypic dispersion mimic parent dispersion. Therefore the main aim of this study was to evaluate how these two hypotheses provide with a better explanation for female reproductive behaviour under high levels of polyandry.

To address this aim I used the Trinidadian guppy (*Poecilia reticulata*) a fish species that lives in highly dynamic, promiscuous mating systems where the male's only contribution to mating is their sperm (Houde 1997). Male sexual harassment is a common feature among guppies, with females being targeted at a rate of one sexual attempt every minute (Magurran & Seghers 1994a). Polyandry in guppies may, therefore, be imposed by males rather than promoted by females. Nevertheless, females have been shown to obtain indirect benefits from mating polyandrously (Evans & Magurran 2000). The question about the nature of these indirect benefits remains, however, disputable. Female guppies show preference for males with large proportions of areas of orange colouration (Houde 1997), which is an indicator of male quality (van Oosterhout *et al.* 2003), known to be highly heritable (Brooks & Endler 2001a) and to affect offspring performance (Evans *et al.* 2004b). Therefore, the trade-up hypothesis has some support in this system, as shown recently by Pitcher and colleagues (2003). However, studies have also revealed that the benefits females gain from mating with males with larger proportions of orange colouration are limited (Evans & Rutstein 2008) and that female mate choice is rather variable, with different females finding different and novel male phenotypes more attractive than others (Brooks & Endler 2001a). It has been suggested that the remarkable variation in terms of color morphs, behaviour, life-history and reproductive behaviours, is what enabled guppies to thrive and evolve under different environmental and ecological gradients (Houde 1997; Magurran 2005). Promoting phenotypic diversity should, therefore, be advantageous in guppies. In conclusion, there is enough information in support of both hypotheses (trade up/phenotypic diversity) as possible explanations for the maintenance of polyandry in guppies. Here I tested which one provided a better explanation for female reproductive behaviour of polyandry in guppies.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN

I raised 80 females and 400 males in isolation until sexual maturation. During this period size at birth and individual growth rate were recorded on a weekly basis for 12 consecutive weeks for all individuals. I have also recorded, for each individual, the time to escape potential predator. Additionally, I measured male age at sexual maturation and frequency of sexual behaviour. Number of sigmoid displays and gonopodial thurstings were recorded for 10 minutes for each male in a single tank with four identical sized females (these females were not included in the statistical test). Finally, male colouration was also recorded. After 12 weeks each male was placed in a petri dish and both sides photographed. I then measured the proportion of black, orange, green and blue pigments using Image J software.

Females were randomly allocated to either a monandrous (n=40) or a polyandrous (n=40) mating treatment. I adopted a similar experimental design to that used by (Tregenza & Wedell 1998), in which the mating frequency remained constant between mating treatments whereas the number of mates varied. Accordingly, in the monandrous treatment each female was allowed to mate with the same male for four consecutive days, whereas in the polyandrous treatment a new male was introduced to the female each day, for four consecutive days. In both mating treatments males were introduced to females at 7.00 am and removed at 5.00 pm. The level of disturbance was therefore identical for both mating treatments. At the end of the fourth day, all males were removed and females kept individually in their home tank until broods were produced.

After birth each offspring had its size recorded and growth rate measured individually on a weekly basis for 12 consecutive weeks. I also recorded for all offspring's time to evade a potential predator, following the methodology used by Evans and Magurran (2000). Additionally, time to sexual maturation, frequency of sexual behaviour and proportion of black, orange, blue and green pigments on both sides of the body were recorded, for sons only (see above for details).

STATISTICAL ANALYSIS

Because the phenotypic traits studied here are assumed to be inherited, the variability among the offspring of the two treatments must be considered in the context of the variability of their parents. Mean differences in size at birth and growth rate between females were examined using a t-test. I also tested for the dispersion of variances using the Levene test. For fathers, the multivariate dispersion of their phenotypes was calculated as distances to each group centroid in a multivariate space (Anderson 2006). All analyses were performed using the Vegan package (Oksanen *et al.* 2008) in R 2.7.2 (R development team 2008). Size at birth, growth rate, proportion of pigments and frequency of sexual behaviour and time to escape predator were used to compute a distance matrices. I selected the Gower similarity index to compute the similarity matrices because of its efficiency when dealing with variables of different nature, as is our case. Next, based on the generated similarity matrices, I calculated the multivariate dispersions of distances within mating treatments using the Vegan function betadisper. This function calculates the multivariate dispersion by measuring the distance of each male to its group centroid. To test if the dispersions in the phenotypes in one treatment were statistically more variable than in the other, parametric and a non-parametric test was run. The dispersion of phenotypes within

mating treatments was first compared by performing an ANOVA on the distances of offspring within treatments to its centroid. In the second test, I used a randomization test with 900 permutations, using the function permutest.betadisper. The residuals were used to generate a permutation distribution of F under the null hypothesis of no difference in dispersion of phenotypes within mating treatments. This analysis was then repeated, but this time for sons and daughters. Differences in the dispersions of phenotypes in the sons and daughters from the two mating treatments were analyzed graphically by constructing a principal coordinate analysis (PCoA). All variables were standardized to between 0 and 1 by dividing by the range for each variable. Results were considered significant for $\alpha = 0.05$.

RESULTS

Females allocated to the monandrous and polyandrous treatments were on average of identical size ($X \pm$ SE, monandrous ♀ $= 2.66 \pm 0.37$ cm; polyandrous ♀ $= 2.65 \pm 0.31$ cm; $t_{78} = $ -0.137, $p = 0.890$). The Levene test for homogeneity of variances revealed that females of both treatments were equally variable ($p<0.08$). However, males in the monandrous mating treatments were phenotypically more diverse than males allocated to the polyandrous treatment (Fig. 6.1a). Both the ANOVA and the permutation test revealed that differences were statistically significant (Table 6.1).

In terms of phenotypic diversity among sons and daughters, results from our analysis differed for the two genders. Polyandrous sons were phenotypically more diverse than monandrous sons (Fig. 6.1b, c), but daughters did not differ in phenotypic dispersion between the two treatments. Results from both the ANOVA

and the permutation test reveal that this difference is statistically significant for sons but not daughters (Table 6.1).

Table 6.1 – Results for the ANOVA and Permutation tests for the homogeneity of multivariate dispersions in the phenotypes of fathers, sons and daughters from two mating treatments, m – multiple and s – single mated females. Number of permutations 999. *Significant values considered for $p < 0.05$

	Mean distance to centroid	df	MS (residual)	F ratio	*Alpha*	Permutation
Father	m - 0.11 s - 0.15	140	0.002	31.9	0.0001*	0.001*
Sons	m - 0.14 s - 0.12	143	0.001	7.63	0.006*	0.006*
Daughters	m - 0.10 s - 0.09	125	0.006	0.57	0.44	0.44
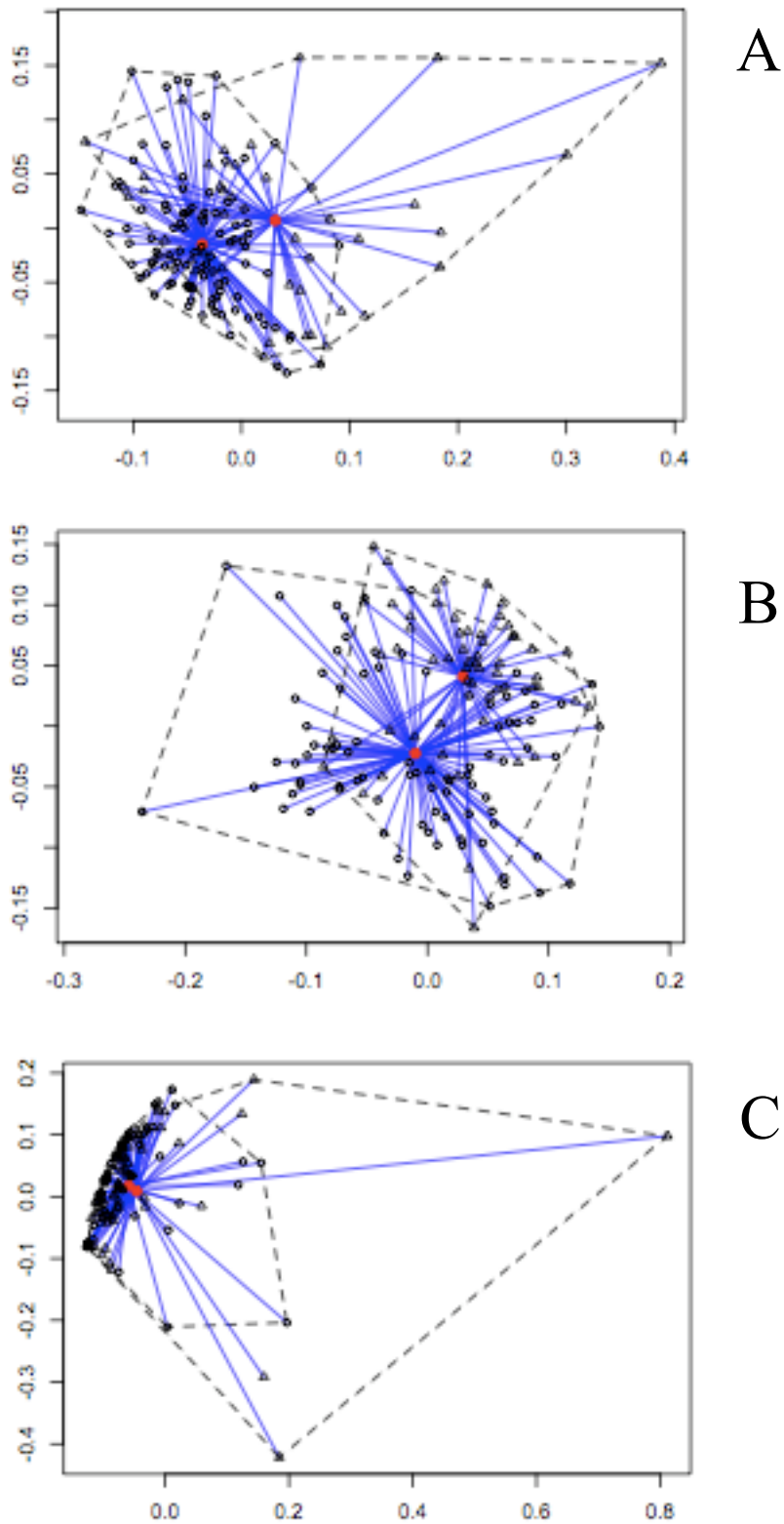
Figure 6.1 - Principal coordinate analysis (PCoA) showing the dispersion to centroid of offspring phenotype for multiple (green) and single (red) mating treatments. a - Fathers, b- sons and c - daughters. Open triangles - offspring from multiple mating treatment; open circles - offspring from single mating treatment

118

DISCUSSION

There is currently intense interest in the adaptive significance of polyandry in resource free mating systems (Simmons 2005; Akcay & Roughgarden 2007). The main argument used is based on the idea that, in the absence of direct benefits, polyandrous females can obtain indirect benefits by enhancing the genetic quality of their offspring (Jennions & Petrie 2000). Both the trade-up (Halliday 1983) and the genetic diversity (Yasui 1998) hypotheses are frequently employed in support of indirect genetic benefits (Foerster *et al.* 2003; Pitcher *et al.* 2003). However, the nature of benefits associated to each of the hypotheses is linked to different female reproductive strategies (Hosken & Stockley 2003). In the trade up hypothesis, polyandrous females are predicted to bias paternity towards males that possess a particular phenotypic trait that enhances the success of offspring. Consequently, I expected to see convergence in offspring phenotypes. By contrast, according to the diversity hypothesis, indirect benefits are directly attributed to offspring diversity. Accordingly, polyandrous females may use the contribution of multiple male phenotypes to produce broods of greater heterogeneity. Both hypotheses have been previously suggested as possible advantages of polyandry in guppies (Pitcher *et al.* 2003; Eakley & Houde 2004). This study showed that polyandrous guppies produced broods of greater phenotypic variability than monandrous guppies, despite the greater phenotypic variability among monandrous fathers in this study. Therefore, it seems that female guppies were not selecting a particular male phenotype, but rather promoting phenotypic diversity among their sons. If polyandrous females were trading up sperm for a particular male phenotypic trait, I would expect to see less variation in the phenotypes of males of polyandrous broods than among monandrous broods. Results, here, are consistent with those from Eakley and Houde (2004) that

showed that female guppies are more likely to re-mate with males of with novel colour patterns than with similar males to previous mates.

Phenotypic variability was greater among polyandrous sons, but not among polyandrous daughters. Typically, in promiscuous mating systems a male's reproductive success is more dependent on a particular phenotypic trait than female's reproductive success. Among guppies, paternity is mostly determined by female mating preference (Houde 1997). Males with preferred phenotypes are likely to be favoured in female pre and post copulatory selection (Evans *et al.* 2003b). On the other hand, as with most fish species, size is what determines female fecundity. Among guppies, males prefer large females to smaller ones (Dosen & Montgomerie 2004). Finally the fact that male traits bear more genetic variation than female, upon which sexual selection can act (Reznick et al 1997). In conclusion, because phenotype plays a greater role in determining male reproductive success than in females, that may explain why there were no significant differences in the variation of phenotypes in daughters from monandrous and polyandrous broods. Nevertheless, I should have seen a difference in the size at birth and/or growth rate in daughters between mating treatments, which I did not. It could be argued that other external factors such as sex ratio, size and density of the rearing environment must overrun any paternal and maternal contribution to the growth of individuals.

It has been previously suggested that female guppies trade up sperm when better quality males are introduced to the arena (Pitcher *et al.* 2003). Although the experimental setup used here was fundamentally different from this previous study, results here suggest that females may use polyandry to increase the diversity of their brood, rather than to bias for a particular male phenotype. This idea is reinforced by the fact that several of the underlying factors used in support of the trade up

hypothesis are rarely met in guppies. Firstly, according to the trade up hypothesis, polyandry should be common but not ubiquitous and mixed paternity broods rare (Lee & Hays 2004). Consequently, females are expected to bias paternity to males with a particular phenotype. However, in guppies both mixed paternity broods and polyandry are prevalent, both in laboratory and in wild conditions (Becher & Magurran 2004; Neff *et al.* 2008). The fact that female guppies may be able to control paternity through post copulatory mechanisms (Evans *et al.* 2003b) indicates that mixed paternity cannot be solely justified as a consequence of male sexual coercion. A final prediction of the trade up hypothesis suggests that females should be less discriminative in first mating to ensure fertilization, but then select the next male based on its quality (Halliday 1983). This prediction assumes that 1) females promote second mates and, thus polyandry and 2) some particular male phenotype produces offspring of higher fitness (good genes/sperm -indirect benefits) (Keller & Reeve 1995). Both assumptions are still debatable in guppies (Brooks 2000; Evans & Rutstein 2008). A hypothesis that is more consistent with the observed reproductive behaviour in female guppies is that polyandry, regardless of whether or not it is consensual, diversifies the phenotypes of their brood. As shown by Pitcher and colleagues (2003), although females bias paternity towards a specific male trait, all broods in their study were of mixed paternity. It can be argued, therefore, that females were indeed promoting phenotypic diversity of broods. It remains also unclear whether in the same brood offspring with the inherited paternal phenotype trait were fitter than their half siblings. This will eventually shed some light into which process (s) of benefits is behind female behaviour.

It is known that genetic and phenotypic diversity play a major role in determining the survival and reproductive success of a species (Kussell & Leibler

2005; Marshall *et al.* 2008). Polyandry enables females to diversify their broods, in particular if females are able to select paternity through post-copulatory mechanisms. In fact, polyandry in many species may have evolved because of the indirect benefits obtained from producing offspring of greater heterozygosity (Cornell & Tregenza 2007; Rubenstein 2007). The indirect benefits of brood diversification are enhanced in stochastic systems, like the one guppies inhabit, where regular changes in environmental (e.g. temperature) and ecological (e.g. predation, mate choice preferences) variables, occur on small temporal scales (Houde 1997; Magurran 2005). If there is variability in female mating choices, then there are advantages to not putting all of one's eggs in the same basket. That is, if there is not one best phenotype, and/or the performance of phenotypes is variable, then there is no pressure for stabilizing selection, but rather for diversifying selection. It has been found that in guppies males with uncommon and unfamiliar phenotypic patterns can achieve greater mating success (Farr 1977; Hughes *et al.* 1999). Furthermore, there is evidence that broods produced by familiar individuals are significantly smaller than broods produced by unfamiliar ones (Pitcher *et al.* 2008). Thus, diversity in brood phenotypes will potentially increase offspring fitness. Therefore, the gains of indirect benefits of producing broods of greater phenotypic diversity may provide an alternative explanation for the maintenance and adaptive significance of polyandry in guppies.

In conclusion, here I used a novel approach to compare hypotheses that aim to explain the adaptive significance of the high levels of polyandry in guppies. The results from this study showed that differences among polyandrous and monandrous broods are gender specific. For males, polyandrous broods were phenotypically more diverse than monandrous broods. By contrast, there were no differences among the

females of both treatments. Moreover, differences among treatments are greater in terms of variability rather than treatment averages. Thus, the results highlight the importance of examining variance in the data. The potential benefits of phenotypic diversity are particularly enhanced in a mating system where paternity is driven by a variable female mate preference, like the one that characterizes guppies.

Chapter seven

GENERAL DISCUSSION

This thesis aimed to examine how females modify their mating decisions in line with social/environmental variability and how these decisions ultimately affected fitness. I explored this theme by running experiments that tested for possible links between female mating decisions, different social conditions and their effect on mother and offspring fitness, direct and indirect mating benefits respectively. There is currently an intense debate on whether or not female mating decisions are being driven by mating benefits that arise from mating with a particular male (Kotiaho *et al.* 2008). In the second chapter I dealt with this question. In particular, I reviewed the empirical evidence for the relationship between female mating decisions, male type, environmentally variability and mating benefits. Data collected from 36 freshwater fish species indicated that to date there was still no experimental evidence in support for either direct or indirect benefits of female mating decisions (Barbosa & Magurran 2006). My conclusion was based on the assumption that mating benefits would translate into an increase in female (mother) or in offspring lifetime reproductive success. This result is consistent with recent reviews in other organisms (Avise *et al.* 2002; Akcay & Roughgarden 2007). Therefore, the first conclusion of this thesis is that there is insufficient evidence to support that in freshwater fish species females actively select their mates based only on the potential increase in either their lifetime reproductive success or on their offspring's (Barbosa & Magurran 2006).

This result raised the question of whether or not females have control of the mating process. In particular, if female guppies have the plasticity in mating decisions

and strategies to actively bias the mating process in their favour. Natural selection should lead females have evolved different reproductive strategies to overcome harsh and unpredictable conditions (Holand & Rice 1998). Guppies live in systems where social and ecological conditions change in a small and unpredictable scale (Houde 1997; Magurran 2005). Additionally, male sexual harassment among guppies is one of the highest recorded for aquatic systems (Magurran & Seghers 1994a; Matthews & Magurran 2000). Both factors could, therefore, impose limitations on female pre-mating decisions, which can justify the observed absence of female choice of males for mating benefits. In chapter 3 I showed that when faced with extreme social conditions female guppies were able to optimize their reproductive investment. In chapter 4 I observed that female guppies, when given the chance, showed strong pre-mating behaviour for promoting multiple mating. Using artificial insemination Evans and colleagues demonstrated that female guppies can efficiently use post-copulatory mechanisms to reinforce pre-mating preferences (Evans *et al.* 2003b), which can circumvent the limitation of pre-mating choice imposed by male sexual harassment. In chapter 4 I showed that male sexual harassment is unlikely to limit females from making their pre-mating decisions. Results from chapters 3 and 4 reinforce previous findings that described the remarkable plasticity and active role of female guppies in the mating process (Reznick & Yang 1993; Reznick 1996; Houde 1997; Rodd *et al.* 1997; Godin *et al.* 2005).

The results from these two previous chapters, however, posed the following question: if female guppies possess both the plasticity and the mechanisms to circumvent environmental and social constraints why were mating benefits not observed? But more importantly the conclusions from chapters 3 and 4 resurrect the debate on the prevalence of polyandry in guppies in the absence of benefits. There are

three possible explanations. Firstly, polyandry may be imposed by males rather than selected by females, as demonstrated for other species (Lee & Hays 2004; Dibattista *et al.* 2008; Le Galliard *et al.* 2008). Results from chapter 4, however, showed that female guppies when given the choice preferred to be associated with multiple males rather than with a single male. It is, therefore, unlikely, that polyandry is entirely caused because of male sexual harassment. Secondly, mating benefits may have been missed because they are not translated into an increase in female and/or offspring lifetime reproductive success (F1 and F2). Or thirdly, the magnitude of benefits may be small and easily negligible, specially if only one generation is analyzed (Møller & Alatalo 1999). In chapters 5 and 6 I addressed these two later possibilities. Results from chapter 5 showed that females accrued no significant mating benefits from mating polyandrously. There was no evidence that polyandrous females obtain greater net fitness than monandrous ones, both in number of F1 and F2 produced as well as in terms of any other components of fitness recorded). The last potential explanation for the lack of mating benefits may simple be due to laboratory artificial conditions. It is reasonable to advocate that laboratory fish are under smaller sexual and natural selection in laboratory than in those living under natural conditions. Because mating benefits evolve in response to differences in the pressure of sexual selection, one could expect to see some variation in the degree of mating benefits from laboratory and wild populations.

Interestingly, using a novel statistical approach in chapter 6 I found that polyandrous females produced offspring of greater phenotypic variability. Given the link between offspring phenotypic variability and their potential fitness advantages in constantly changing environments (Roughgarden 2004; Kussell & Leibler 2005; Marshall *et al.* 2008), polyandrous guppies may, therefore, be indirectly getting

mating benefits by producing more variable offspring. Consequently of maximizing the chances of having offspring well adapted to the constantly changing social and environmental surroundings typical of the guppy system (Houde 1997; Magurran 2005). The idea of female guppies using polyandry as a way to bias paternity towards males with a preferred phenotype was, therefore, rejected. Instead, I propose that female guppies may use polyandry as a way to enhance the variability of their offspring.

In conclusion, this thesis underpins the vital and active role of female guppies in all stages of the mating process. In particular, it reinforces the remarkable plasticity of female mating decisions in response to social variability experienced before mating in an optimal way. Several key points arise from this work that are relevant to be further emphasized. Firstly, the current scenario of females selecting mates based only on a particular set of sexual traits in order to obtain mating benefits has to be re-examined to accommodate random female mate preferences. The inability to observe mating benefits, specifically indirect, may be partially because we assume that female mating preference within populations are uniform and selective for the same set of male sexual traits. Accordingly, we should shift the way of examining female mating benefits towards one that looks at variation, rather than mean differences as a source of benefits. It is then time to implement the novel idea that females may make their mating decisions based on mate variability rather than on a particular male sexual trait. I therefore, propose that female guppies make their mating decisions as a way to increase the diversity rather than the genetic quality of their offspring.

FUTURE DIRECTIONS

With the conclusion of this thesis new ideas and gaps emerged that are worthwhile pursuing in the future. Currently there is growing interest in understanding the evolutionary reasons for polyandry, especially in systems where females get no direct benefits (e.g. guppies). The majority of studies compare the fitness of monandrous *vs.* polyandrous offspring. A novel way would be to group offspring by fathers and compare their fitness. This method would allow us to test if 1) offspring from a given father are consistently fitter than others or 2) instead it is the combination of mixed brood paternity that is responsible for the benefits. This test would ultimately allow us to have better understanding of the benefits and reasons for polyandry. A second idea for future studies would be to examine female potential lifetime reproductive success. Control populations could be setup in mesocosms and followed. By doing so we would have an exact measure of number of babies, their quality (standard measures, size at birth, predator escape, etc) per mother and father. This would allow us to have a full schedule of the potential mating benefits in guppies. Finally a last avenue of research worthwhile pursuing is the examination of sound and chemical cues in female-male interactions. Because of the high levels of male harassment, a female that moves near male to inspect him is very likely to end up mated by that male. On the other hand, the visibility of many of the rivers where guppies live is low, therefore, visual assessment of mates may be limited. Studies have shown that females use sound produced by males to base their mating decisions in other fishes (Landry *et al.* 2001). Future studies should try to assess whether or not guppies are able to produce sound, and if this can be used by females to base their mating decisions.

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