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Variation in the Mating Behaviour of the Guppy, *Poecilia reticulata*

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Thesis submitted for the degree of doctor of philosophy
University of St Andrews

July 2005



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Abstract

This thesis focuses on variation in the mating behaviour of the guppy, *Poecilia reticulata*, a small poeciliid fish native to Trinidad. While there exists a wealth of literature on this topic few studies have drawn together the numerous, diverse influences on mating behaviour into a holistic analysis. Male guppies have two main forms of mating behaviour. They can undertake characteristic sigmoid displays, which facilitate female choice of males, or they can attempt sneak mating, which circumvents female choice. The expression of these behaviours is affected by numerous factors. Environmental factors such as predation regime; social factors like sex ratio; and morphological factors, for example colour pattern have all previously been shown to be influential. Moreover this variation is expressed at different levels. Population, shoal, family and individual variations in mating behaviour have all been recorded. Each chapter of this thesis investigates the influence of one or more factors at a different level of effect. Individual males are shown to have consistent levels of behaviour relative to other males that nonetheless vary with changes in sex ratio, which allows males to be described in terms of individual mating behaviour profiles. Phenotypic manipulation of male size reveals that females prefer larger males and that these larger males undertake more of both courtship displays and sneak mating attempts than their smaller counterparts, and this effect interacts with competitive environment. Variation in the water velocity profile is demonstrated to have similar effects on male mating behaviour in both field and laboratory situations with the frequency of displays decreasing in favour of sneaks with increasing water velocity. Population differences in male morphology are found to be apparent even in 10th generation laboratory reared fish, though differences in male mating behaviour and female mate choice are not as well defined as that seen in previous studies of natural populations. Male reproductive success with respect to these morphological and behavioural differences is elucidated. The final discussion integrates this behavioural variation with the different aspects of conflict inherent in the guppy mating system.

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Chapter 1

General Introduction

1.1) Introduction

The guppy, *Poecilia reticulata*, is a small poeciliid fish native to Trinidad and North-Eastern South America where population structures have provided a natural experiment (Haskins *et al* 1961). It has been studied extensively for over 80 years and is used as a model system for evolutionary, behavioural and ecological research. This thesis focuses on the reproductive behaviour of the guppy, exploring sources of variation in mating behaviour and examining the potential evolutionary consequences of these factors. A wealth of literature already exists. However, most studies concentrate on one source of variation or the effects in one small area, thus ignoring the numerous interrelating aspects of the guppy system. This thesis aims to draw together these disparate factors, highlighting interactions as well as the singular sources or areas of effect to create a more holistic image of the guppy mating system.

This introduction starts with an overview of the poeciliid family then spotlights the Trinidadian guppy. The mating system and reproductive biology of the guppy are then explored in more detail followed by an examination of mating behaviour. The theme of this thesis is variation and an examination of the sources of variation, both causal factors and areas of effect of these factors comes next. The introduction finishes by listing specific aims of this thesis.

1.2) Overview of the Poeciliidae

The Poeciliid family, of the order Cyprinodontiformes, includes around 200 species of small fish, mostly less than 5cm long, that inhabit temperate, subtropical and tropical latitudes of the Caribbean and the Americas (Meffe and Snellson 1989a). Poeciliids in general, including the genus poeciliidae, are a tolerant and highly adaptive group. They live in a wide range of habitats from desert to mesic regions; occupying streams, rivers, lakes, springs, fresh and brackish marshes, seacoasts and saline mangrove

swamps; typically inhabiting small shallow bodies of water or shallow marginal areas of larger bodies, and tolerate a range of temperatures and light regimes, as indicated by the latitudinal spread of species (Meffe and Snelson 1989b). They also exploit diverse food sources: the family includes piscivorous, herbivorous and omnivorous species, the latter being most common (Dussault and Kramer 1981). Even cannibalism has been recorded (e.g. Krumholz 1948, Thibault 1974). They are in turn exploited as prey by many species of fish, birds, reptiles and even invertebrates (Meffe and Snelson 1989b, references therein). Their adaptability, along with their varied mating systems, the ease with which they can be studied in the field and laboratory and their prolific breeding has made them ideal models for the study of cellular, organismal, population and evolutionary biology. Studies of poeciliids have contributed to subjects as diverse as physiology (e.g. Rosen and Bailey 1963); vicariance biogeography (e.g. Liley 1966, Carvalho *et al* 1991); parasitology (e.g. Lopez 1998); evolution of life history parameters (e.g. Reznick *et al* 1996, 2001); and natural and sexual selection (e.g. Bisazza 1993).

All poeciliids exhibit internal fertilization and viviparity (Wourms 1981). However, these traits encompass a wide range of reproductive strategies which can be divided into pre- and post-mating. Males may engage in forced copulation or court females, males of several species being able to exhibit both these behaviours. Furthermore they may attempt to interfere with conspecifics' copulation attempts. Females, too, show a range of behaviours from no apparent interest in sexual partner, to passive permittance of mating to actively choosing males, all examples of different pre-mating strategies. Variations in post-mating strategy include the distinction between ovoviviparous and viviparous poecilliines and species with superfetation (Thibault and Schultz 1978). In addition there are species complexes consisting of males and unisexual and bisexual females. These reproductive strategies are affected by many factors, such as predation, habitat and food availability, and in turn affect the evolution of species, specifically the connections between mating strategies and sexual selection. The mating behaviour of one member of this group, the guppy (*Poecilia reticulata*), is the subject of this thesis.

1.3) The Trinidadian Guppy

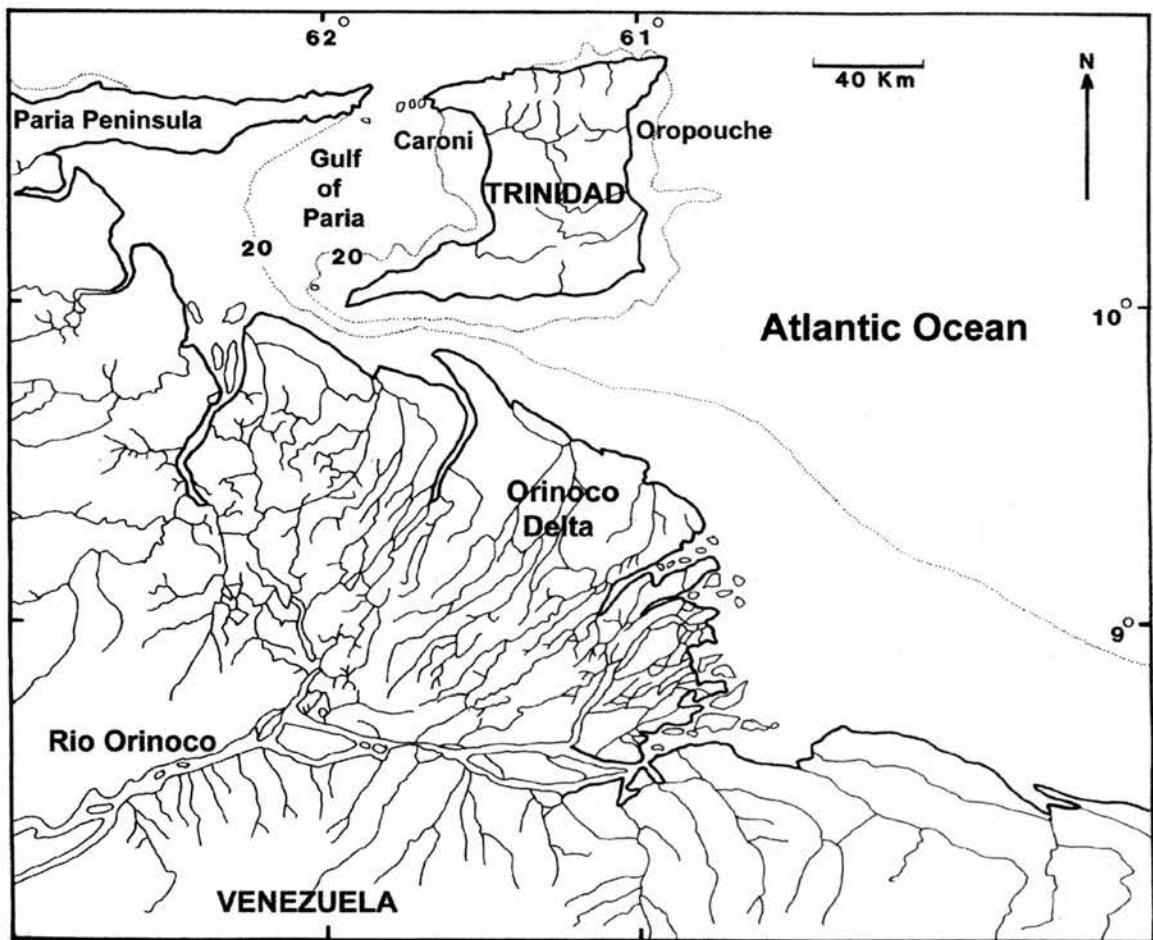
The guppy was first described by Wilhelm Peters in 1859 as *Poecilia reticulata* (Parenti and Rauchenberger 1989) and formally classified as *Girardinus guppyi* by Günther (British Museum) in 1866, the original name being restored by Rosen and Bailey (1963). They were first used in research in the early 20th century with Winge's (1922, 1937) observations of brood successions. Investigations of the behavioural ecology of the guppy began with examination of their sexual behaviour and reproductive biology (Clark and Aronson 1951) and Haskins and his co-workers described geographic variation in these traits in natural populations (Haskins and Haskins 1950, Haskins *et al* 1961). Baerends *et al* (1955) and Liley (1966) provided the first characterisation of the guppy's mating behaviour. This laid the foundations for the myriad studies in the burgeoning fields of ecological, behavioural and evolutionary research that have supplied so much useful background material for contemporary investigations (see Meffe and Snelson 1989a and Houde 1997 for reviews).

The guppy is a ubiquitous generalist, colonising almost any small or large; fresh, brackish or polluted; transient or permanent body of water. The natural range of the guppy extends from Venezuela, Surinam and Guyana in north eastern South America to Trinidad and Tobago and several of the Windward islands in the Caribbean (Rosen and Bailey 1963). Additionally, use as a mosquito control agent and release from domestic stocks has facilitated the existence of countless feral populations throughout the world (Courtney and Meffe 1989). While guppies in several different countries have been studied over the years (e.g. Australia: Brooks 1999; Cuba: Barus *et al* 1995; Japan: Karino and Haijima 2001, Karino and Matsunaga 2002, Karino and Shinjo 2004; South Africa: Brooks and Caithness 1995a, 1995b, 1999; Venezuela: Winemiller *et al* 1990, Alexander and Breden 2004), the vast majority of research has focused on populations in Trinidad: the archetypal Trinidadian guppy.

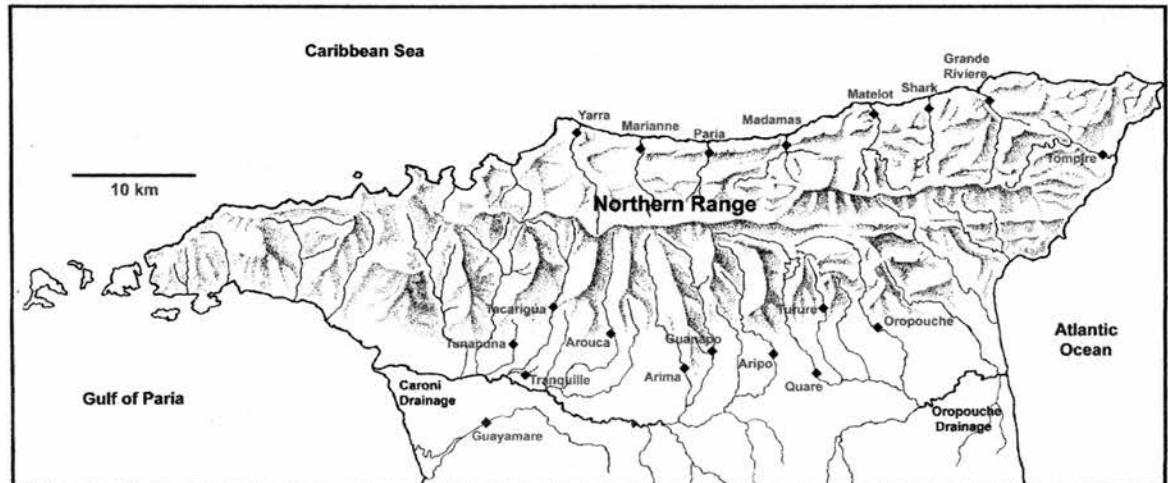
Guppies are found all over Trinidad (Magurran and Philip 2001) but are especially well studied in the many rivers and streams of Trinidad's Northern Range (Figure 1.1). Colonisation of Trinidad by guppies is postulated to have occurred in at least two waves reflecting historical climatic and geological events (Carvalho *et al* 1991, Shaw

Figure 1.1: Map of Trinidad a) geographical location, b) the Northern Range where most guppy research takes place

a)

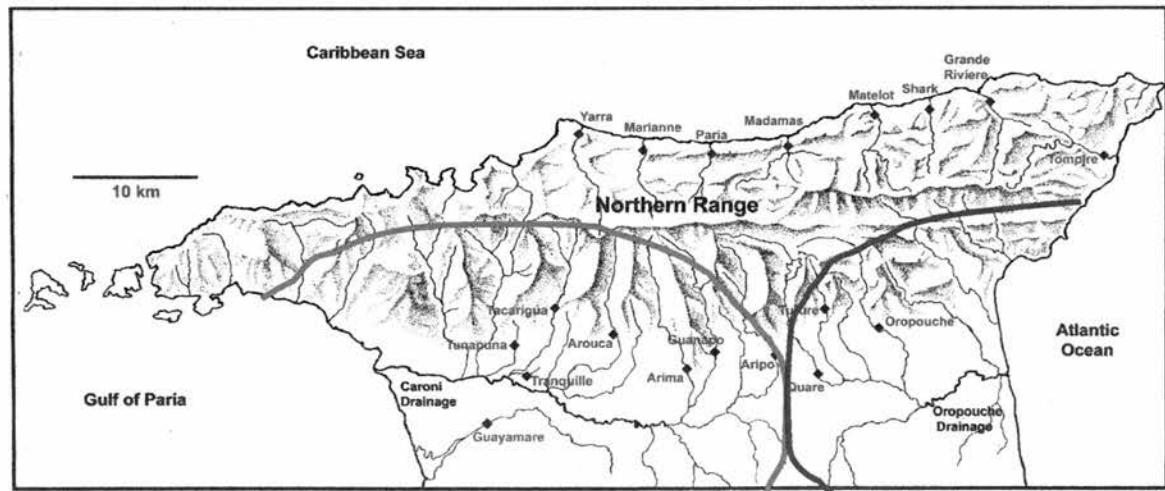


b)



et al 1991, Fajen and Breden 1992, Breden *et al* 1999). During the last glaciation in the Pleistocene (circa 10,000 years BP) sea level in the Caribbean was approximately 100m lower, thereby making Trinidad a peninsula of continental Venezuela and making differences in river drainage patterns likely. Guppies probably became initially established in Trinidad during this time (Carvalho *et al* 1991). Further colonisation events may have been facilitated by the guppy's high tolerance for diverse conditions. Poeciliids are secondary freshwater fish and can bear relatively high salinity (Briggs 1984). Periodic immigration from the Orinoco Delta, only 20-30km away, may also be a factor (Carvalho *et al* 1991). These waves of migration have resulted in contemporary genetic distinction between the two main drainages in the Northern Range: the Caroni drainage on the western side of the island and the Oropouche drainage in the east (Figure 1.2) (Carvalho *et al* 1991, Shaw *et al* 1991, Fajen and Breden 1992, Breden *et al* 1999). Ongoing population level differentiation most likely augments these differences.

Figure 1.2: Map of the Northern Range in Trinidad showing the Caroni Drainage (red) and the Oropouche Drainage (blue).



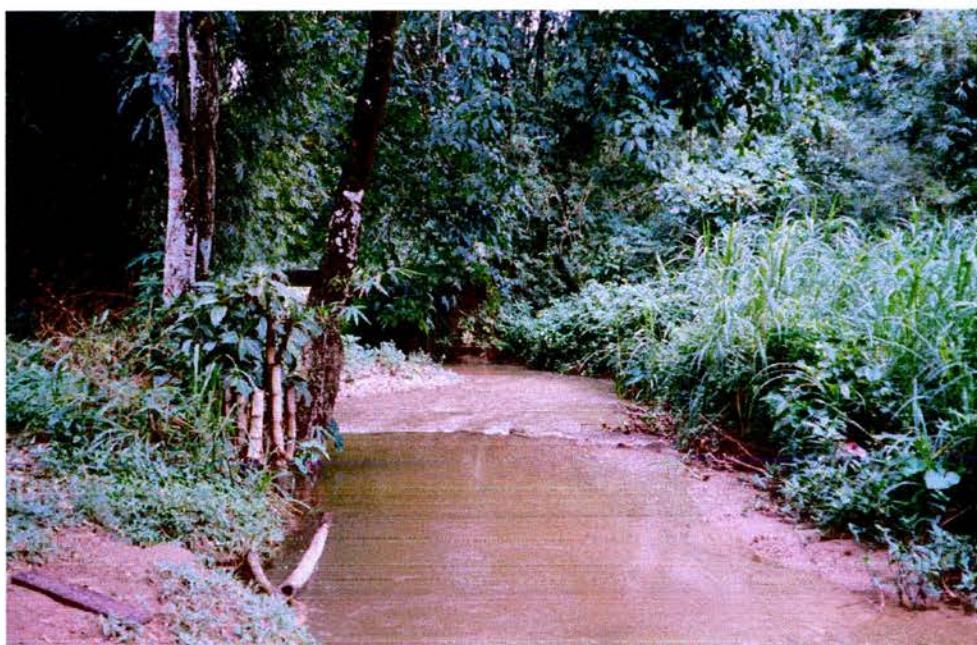
Guppies are part of an active trophic system (Magurran and Philip 2001). They are opportunistic omnivores being able to ingest, and remain healthy on a diet of, benthic invertebrates, zooplankton and vegetable matter (Dussault and Kramer 1981). While often considered to be grazers, they can also drift feed by maintaining position in the current and catching food particles that pass, snatch surface food, such as small insects, and actively kill small invertebrates (personal observation). They are in turn a food source for a variety of predators. Predation regimes are generally divided into two types, traditionally called high and low predation (Figure 1.3) (Haskins *et al* 1961). Low predation regimes are generally situated in the upper reaches of rivers and are separated from the downstream, high predation, regions by a barrier waterfall which keeps out other predators (Haskins *et al* 1961; Magurran and Philip 2001). Low predation regimes are alternatively named *Rivulus* habitats, after the killifish, *Rivulus hartii*, which are almost invariably found there (Seghers 1973, Magurran and Philip 2001). *Rivulus* is a gape limited predator so is regarded as presenting more of threat to juveniles (Seghers 1973). High predation regimes, in contrast, are much more species rich (Magurran and Philip 2001). In addition to *Rivulus hartii*, other species such as *Aequidens pulchar* and the pike cichlid, *Crenicichla alta* inhabit these areas (Magurran and Philip 2001). These are larger predators which are able to prey on adult guppies (Seghers 1973, Magurran and Philip 2001). The pike cichlid in particular is a voracious predator of guppies and high predation areas are also called *Crenicichla* habitats in recognition of this (Seghers 1973, Magurran and Philip 2001). These species assemblages, while most common in Trinidad are confined to the southern slopes of the Northern Range (Magurran and Philip 2001). On north facing slopes *Rivulus* is joined by freshwater prawns, *Macrobrachium sp.*, which may also be guppy predators (Endler 1983; Magurran and Seghers 1990; Rodd and Reznick 1991) most likely of juveniles (Endler 1995). Downstream *Crenicichla* is replaced by predatory gobies that exert a similar predation pressure on guppies (Reznick and Bryga 1996). Associated ecological variables and the evolutionary implications of this predation dichotomy are discussed later (sections 1.6 and 1.7).

Figure 1.3: Typical a) low and b) high predation habitats in Trinidad

a)



b)



1.4) Mating System and Reproductive Biology

Guppies are sexually dimorphic with larger dull coloured females (Figure 1.4a) and smaller brightly coloured males (Figure 1.4b). Guppies have a promiscuous mating system (Liley 1966; Farr 1989; Houde 1997) in which both males and females have several mates often resulting in broods with multiple paternity (Liley 1966; Farr 1989; Houde 1997). The benefits to males of multiple mating are intuitively obvious. In a species in which males do not participate in parental care, a male can best increase his reproductive success by achieving as many fertilizations as possible (Smuts and Smuts 1993; Rowe 1994; Parker and Partridge 1998; Gavrilets 2000; Magurran 2001). The benefits of polyandry are less evident though multiple mating of females has been documented in laboratory (Constantz 1984) and wild (Haskins *et al* 1961, Kelly *et al* 1999) populations. However females do gain clear advantages. Offspring of multiply mated females are larger (Evans and Magurran 2000, Ojanguren *et al* 2005) and produced in a shorter time (Evans and Magurran 2000). These offspring also have better developed schooling abilities and escape responses than offspring from singly mated females (Evans and Magurran 2000). Moreover, multiple mating in males and females has interesting evolutionary ramifications (see section 1.7).

Guppies are ovoviparous, giving birth to a brood of live, precocious young circa once every four to six weeks (Liley 1966; Farr 1989; Houde 1997). There is no superfetation, i.e. a situation where two or more broods at different stages of development occur in the same female (Wourms 1981), in this species; broods are produced sequentially (Constantz 1989). Ova are matured in paired ovaries before the birth of a brood, then a few days after parturition a mature batch of eggs is fertilized (Constantz 1989). Gestation lasts 25 to 35 days (Houde 1997) and is lecithotrophic, i.e. with no maternal contribution to eggs after fertilization (Wourms 1981; Constantz 1989; Reznick and Yang 1993). Fertilization is internal and females' ability to store sperm means females can produce up to eight broods after just one fertilization (Winge 1937; Liley 1966; Farr 1989; Houde 1997). Sperm are stored within folds of the ovary (Constantz 1984) where they are nourished by extracellular ovarian sugars (Gardiner 1978). Consequently females are the choosy sex, selecting males for mating when receptive which occurs when virgin (immediately upon sexual maturity) and for a few days after parturition (Liley 1966; Farr 1989; Houde 1997).

Figure 1.4: The guppy; a) larger, dull coloured female; b) smaller, colourful male

a)



b)



Males, on the other hand, are extremely ardent in pursuit of mating opportunities, devoting a large proportion of their energy budget to harassing females (Magurran and Seghers 1994b, Magurran 2001) (see section 1.5). To achieve a copulation, and therefore potentially fertilization, the male attempts to insert his gonopodium into a female's gonopore (genital opening). The gonopodium is a modified anal fin formed from stiffening and lengthening of the fourth and fifth anal rays (Rosen and Bailey 1963). The guppy's gonopodium is relatively short and bilaterally symmetrical (Constantz 1989, Rosen and Bailey 1963). When erect it forms a groove for sperm transport (Constantz 1989). It has a thin hood-like caecum extending beyond its tip which, with the aid of an extensive nervous plexus, helps direct gonopodial thrusts towards the female's genital pore (Constantz 1989). How males achieve the opportunity to copulate is the subject of the next section.

1.5) Mating Behaviour

In the context of this thesis the most relevant aspect of mating is pre-copulatory mating behaviour. Males have two methods of achieving a copulation. They may undertake a visual courtship display (Figure 1.5a), which involves stereotypical swimming motions and fin postures, often called a sigmoid display (Liley 1966; Farr 1989; Houde 1997). The male positions himself in front, or just to the side, of a female. He contorts his body into a characteristic sigmoid shape, extends his fins and quivers, displaying his attractive (at least to a female guppy) markings (Liley 1966; Farr 1989; Houde 1997). A display may last from part of a second to around 15 seconds (personal observation). The alternative behaviour is called gonopodial thrusts or sneaky mating (Figure 1.5b) (Liley 1966; Farr 1989; Houde 1997). The male orients himself behind the female, brings his gonopodium into a forward position, swims forward and attempts to insert the gonopodial tip into the female's gonopore (Liley 1966; Farr 1989; Houde 1997).

Correspondingly, females have differing roles in pre-copulatory mating behaviour. They may exercise choice, facilitated by sigmoid displays, which involves both cooperation and direct influence on which of many competing males will sire her offspring (Liley 1966; Farr 1989; Houde 1997) or they may be recipients of unsolicited gonopodial thrusts (Liley 1966; Farr 1989; Houde 1997). Females may

Figure 1.5: Male guppy mating behaviour; a) courtship display; b) sneak mating attempt

a)



b)



base their choice of males on several factors. For example, it has been widely demonstrated that female guppies prefer males with more orange colouration (Houde 1987, 1994; Houde and Endler 1990; Nicoletto 1993; Brooks and Caithness 1995; Kodric-Brown 1995; Houde and Hankes 1997; Jaroensutasinee 2000) though Kodric-Brown and Nicoletto (2001) found this preference decreases with age. There are other aspects to colour pattern variation. Females are more likely to mate with males having novel colour patterns than with males having a colour pattern with which they were familiar (Hughes *et al* 1999), an example of the ‘rare male effect’. Females also prefer males with larger bodies (Reynold and Gross 1992; Magellan *et al* 2005); symmetric colouration (Sheridan and Pomiankowski 1997); larger tails (Bischoff *et al* 1985) and, in some populations, longer gonopodium (Brooks and Caithness 1995).

Females may also elicit matings from preferred males, using an activity known as the glide response or simply glides (Liley 1966; Farr 1989; Houde 1997). A responsive female orients towards the favoured male and glides smoothly towards him with a rigid posture, moving only her fins (Liley 1966, Houde 1997). Sometimes the female’s body is arched laterally towards the male as she glides (Houde 1997). Most researchers regard this as a purely sexual response (e.g. Houde 1987, Reynolds and Gross 1992), but Liley (1966) suggests that the gliding motion may function to minimise the male’s tendency to react with fear to the approach of the usually larger female as the usual male-female interactions are aggressive with the female as the aggressor (Liley 1966).

The culmination of male and female sexual behaviour is copulation (Figure 1.6). Assuming the female’s response to his display is favourable, the male starts to circle around the female, still displaying. The female turns with the male and angles her body slightly so that her genital opening is nearer the male. The male swings his gonopodium forward and attempts to insert it from below and behind the female. If he is successful, the pair rotates rapidly around each other then pull forcefully apart. Sometimes insemination occurs immediately. On other occasions the male and female have to circle each other several times before insemination occurs and sometimes the pair separate with no successful insemination (Houde 1997). If insemination is successful, the male jerks his body quite rapidly immediately following copulation then at a diminishing rate for several minutes afterwards. He may follow the female

Figure 1.6: Consensual copulations in the guppy. The female tilts her body and curls her tail around to allow the male to attempt to insert his gonopodium and allow copulation; a) just prior to copulation, b) copulation attempt

a)



b)

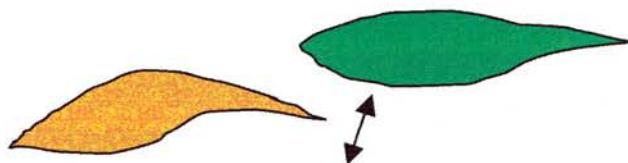


during this time (Houde 1997) but doesn't begin displaying again until after a refractory period that can last as long as an hour (Houde 1997).

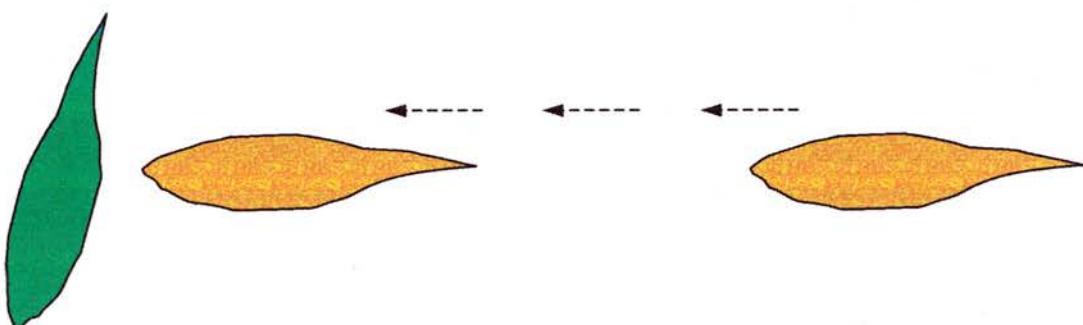
Following a female after copulation is just one example of a collection of associated behaviours that a male can undertake. Following, or attending, a female occurs not only after copulation but at any time a male attempts to monopolise a female (Houde 1997). Another type of mating behaviour is chasing females (e.g. Magellan *et al* 2005) which occurs when a female attempts to escape a male's persistent advances by swimming rapidly away. The male may then follow closely on the female's tail. This often happens when one male tries to interfere with the mating attempts of another male and can result in a female being chased by two or more ardent admirers (Houde 1997). Interfering with another male's mating attempts, that is endeavouring to deprive an individual of vital resources in the form of females, is just one of a suite of male-male aggressive interactions. Others include tail-beating (Magurran and Seghers 1991) (Figure 1.7a); biting (Magurran and Seghers 1991; Kodric-Brown 1993; Bruce and White 1995) (Figure 1.7b); rapid approach or lunging (Gorlick 1976) (Figure 1.7b) and fighting (Figure 1.7c) which involves stereotypical head-to-tail posture along with biting and tail-beating (Magurran and Seghers 1991). These behaviours often take place in the presence of a female in which context they can be seen as direct competition for matings (Farr 1980). They can also occur without a female's involvement in which case they serve as indirect competition (Farr 1980). Indeed, the beaten male will frequently withdraw from interactions with any individual so, temporarily at least, is not competing for females. Although Houde (1997) considers aggressive behaviour to be primarily an artefact of keeping fish in artificial laboratory conditions, several researchers have observed aggressive behaviour in field situations (Magurran and Seghers 1991, personal observation) and all the aggressive behaviours described here have been seen in both laboratory and field conditions (personal observation).

Figure 1.7: Plan diagrams of aggressive interactions between male guppies; a) Tail beating: the aggressor (orange) aligns himself so that his tail can contact the recipient (green) then beats his tail vigorously back and forth; b) lunging and/or nipping: the aggressor lunges towards the recipient and bites him, often around the gonopodium. Either action can occur alone; c) fighting: two males line up head to tail, often displaying towards each other and tail beat (large arrows) and nip (small arrows) each other.

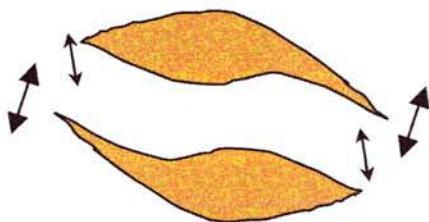
a)



b)



c)



1.6) Sources of Variation

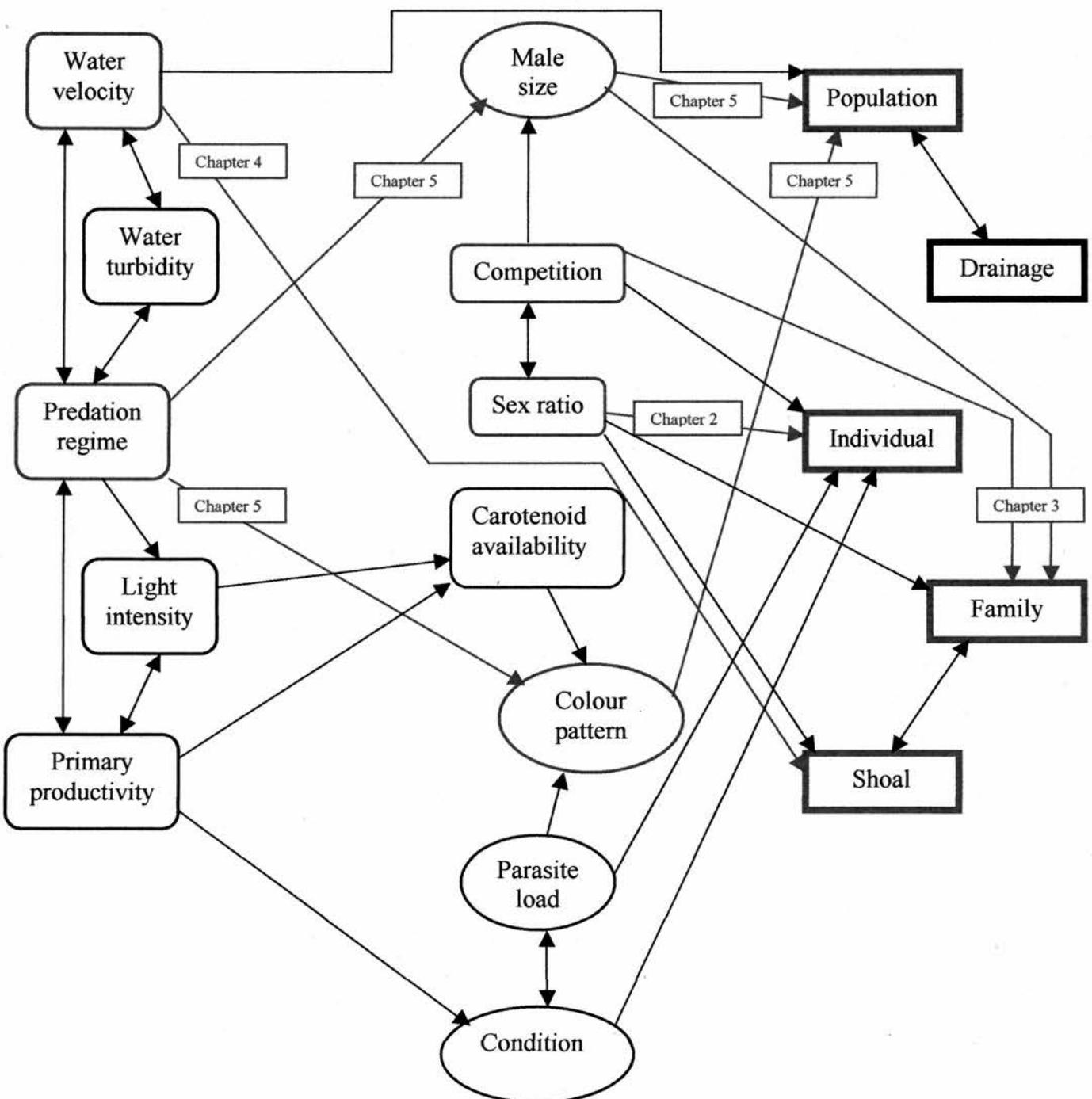
Variation in guppy mating behaviour is under the influence of numerous, diverse and potentially interacting factors (Figure 1.8). Similarly the effects of this variation can be expressed at several different levels, often simultaneously (Figure 1.8). These various causal factors and areas of effect are discussed separately below.

1.6.1) Causal Factors

Causes of variation in the guppy in general, and mating behaviour in particular, can be divided into several categories. Environmental factors, for example, are widespread and far-reaching and many have already been shown to affect mating behaviour in the guppy. For instance, light intensity (Endler 1987), water velocity (Nicoletto 1996) and water turbidity (Luyten and Liley 1985, 1991) have all been demonstrated to affect male mating behaviour. Another facet of environmental variation, in addition to the abiotic factors already referred to, is associated biota. This can include social environment, in the form of conspecifics; other taxa that inhabit the same environment but don't necessarily directly interact with guppies, such as associated plant and insect species; available prey species and perhaps the most widely recognised of the many environmental variants in the guppy system – predation regime (e.g. Reznick 1982; Reznick and Endler 1982; Endler 1987; Reznick *et al* 1996a; Reznick *et al* 2001; Houde 1997).

Guppy habitats, as mentioned above (section 1.3), can be divided into high and low predation regimes in reference to the available guppy predators. In high predation areas, males that show conspicuous displays attract the attention, not only of females, but also of predators (Endler 1987, Magurran and Nowak 1991). In these situations a switch to the less noticeable sneak mating is common (Endler 1987, Magurran and Nowak 1991). In low predation areas, where the small fish *Rivulus hartii* is the main guppy predator, males do not need to be so concerned with remaining unnoticed and consequently carry out relatively more displays than their high predation counterparts (Endler 1985, Magurran and Nowak 1991). High (or low) predation populations, wherever they are geographically, will often be subject to similar associated environmental variables. For example high predation habitats are characterised by

Figure 1.8: Synoptic diagram showing some of the myriad sources and levels of variation in guppy mating behaviour. Some of these have been investigated already. Those that are covered in this thesis are indicated in blue. The bold boxes on the right show the possible levels of variation. All other boxes show sources of variation; square boxes are environmental factors and oval boxes are those intrinsic to the individual. Arrows show interconnections between factors.



relatively high light intensity and reduced vegetation cover, lower water velocity and higher turbidity and primary productivity (Grether 2000; Magurran 2001; Reznick *et al* 2001). Low predation habitats, in contrast, are usually further upstream and so at higher altitude. These tend to have more canopy cover and therefore lower light intensity, higher water velocity and lower turbidity and primary productivity (Grether *et al* 1999; Magurran 2001; Reznick *et al* 2001). These various factors, individually and in combination, also affect mating behaviour (Endler 1987; Luyten and Liley 1985, 1991). For example, males court less and use visually conspicuous behavioural elements less often (Endler 1987) and court at closer distances to females (Long and Rosenqvist 1998) under high light level. However, maximum risk due to predation naturally occurs at highest light intensities so either light intensity or predator presence can be used as a cue for predator avoidance (Endler 1987). Similarly, in the presence or absence of predators males display more in clearer water (Luyten and Liley 1985, 1991) suggesting one or both of the factors are used as behavioural cues.

Another biotic influence on mating behaviour is the conspecific environment. While guppies are able to recognise other individuals (Griffiths and Magurran 1997, 1999) and prefer to shoal with known fish (Griffiths and Magurran 1997, 1999) guppy shoals are transient entities with fish moving around within and between groups (Reznick *et al* 1996b; Houde 1997; Croft *et al* 2003). Shoal demography therefore changes over varying timescales. One factor that affects mating behaviour is the density of fish (Jirotkul 1999a). More fish in a given area means more opportunity for competition and males accordingly decrease their courtship displays as density increases (Jirotkul 1999a). A related factor is local sex ratio, which again can change over a few minutes. At female biased sex ratios there is more opportunity for courtship and more choice over where to direct this courtship. At male biased sex ratios, increased competition from other males will influence mating behaviour. However, the results from previous studies on this subject are conflicting. Farr (1976) found that males increased the frequency of displays in the presence of competitors, while Jirotkul (1999b) found that the frequency of displays decreases at male biased sex ratios and sneak mating was most frequent at equal sex ratios.

Environmental variables in turn influence, and may be influenced by, other sources of variation. Conditional variation, that is variation due to individual state, is at least in

part affected by the environment and affects mating behaviour. Males that have developed resistance to parasites, for example, display more than those who are in worse condition due to parasite infection and females favour these resistant males (Lopez 1998). Although in Lopez's (1998) study colour pattern was unaffected by parasite load, Houde and Torio (1992) showed that males infected by parasites had reduced orange colour compared to their brothers who were not infected. Females accordingly preferred the non-affected siblings (Houde and Torio 1992). Orange colouration is condition dependent in a more general way. The intensity of orange is in part due to the level of carotenoid intake (Kodric-Brown 1989) and the main source of carotenoids is the algae that guppies feed on (Grether 2000). Fish in poorer condition will not forage as effectively so will not be able to express their full orange colour potential. Moreover, an interacting environmental variable is again apparent here. In areas that are more open, and therefore have higher light intensity, primary productivity is higher so carotenoid bearing algae are more abundant. Fish in these, usually high predation (Reznick *et al* 2001), sites will not have to work as hard to obtain sufficient carotenoids in their diet (Magurran 2001).

Phenotypic factors such as colour pattern are themselves connected with mating behaviour variation. Jirotkul (2000) showed that male guppies with 15% mean orange coverage interfered with other courting males more and engaged in courtship activities for longer than males with 10% mean coverage and Nicoletto (1993) found a correlation between area of orange covered, sustained swimming activity and display rate. Moreover, differences in appearance and behaviour in males give females criteria on which to base their choice of mates (reviewed in Houde 1997).

An interesting interaction between environment, a phenotypic factor and ontogenetic variation can be seen in size of fish. Female guppies have indeterminate growth but males cease growing upon reaching sexual maturity (Liley 1966). In high predation environments it is adaptive for males to grow quickly and therefore maturing at a smaller size while the opposite is true in low predation habitats (Reznick 1982; Reznick and Endler 1982; Endler 1987; Reznick *et al* 1996a). Body size effects on mating behaviour, both male mating behaviour and female mate choice, have been shown in several species (Table 1.1). In sailfin mollies, *Poecilia latipinna*, larger males exhibit higher rates of courtship and lower rates of gonopodial thrusting

compared to smaller males (Farr *et al* 1986). In addition, intermediate sized *Poecilia latipinna* males varied their behaviour according to the size of competitors, exhibiting intermediate rates of behaviour when with males of their own size, but ‘small male’ type behaviour when with mixed sized males, an example of the effect of competitors (Travis and Woodward 1989). In the mosquitofish, *Gambusia affinis*, larger males were more likely to court females while smaller males tended to chase non-receptive females and attempt to inseminate them forcibly (Hughes 1985). In this case the difference in body size and behaviour was correlated with female preference for larger males (Hughes 1985). This same phenomenon is seen in *Xiphophorus nigrensis* populations (Ryan *et al* 1990). In the guppy Reynolds and Gross (1992) found that females prefer males with larger bodies whereas Endler and Houde (1995), who examined female preference for a variety of male traits, including size, in 11 guppy populations that ranged across drainages and predation regimes, could not detect a clear trend. There was no preference with respect to male size in nine of Endler and Houde’s populations, one locality in which females preferred smaller males and a preference for larger males in another. Males also have female size preferences, tending to favour larger females (Ojanguren and Magurran 2004). A final effect of ontogeny concerns female choice of males. Kodric-Brown and Nicoletto (2001) showed that females’ preference is not absolute but changes as females age.

A final factor that is likely to influence mating behaviour variation is genotype. As yet, direct indication of a genetic effect on behaviour is limited though there is plenty of evidence for indirect influence. Luyten and Liley (1985) provide some evidence for the inheritance of male mating behaviour. Offspring of fish taken from four rivers in Trinidad and raised in identical laboratory conditions showed levels of courtship behaviour consistent with those of their wild fathers, suggesting a genetic basis to observed behavioural differences between populations (Luyten and Liley 1985). Farr (1976) postulates that male-male competition for females generates selection pressure for males to maximise display rate (Farr 1976). However, Rodd and Sokolowski (1995) found no evidence for a genetic basis for variation in courtship behaviour, though their findings did indicate a genetic component of sneaky behaviour (Rodd and Sokolowski 1995). There is more evidence for the inheritance of female choice. Female responsiveness to (Brooks and Endler 2001b) and choice of (Houde 1988b) males have been shown to have a genetic component. However, most of the evidence

for a genetic influence on mating behaviour is indirect. Many of the factors known to affect mating behaviour have themselves been shown to be inherited. Male colour pattern, for example, is inherited (Houde 1992, Brooks and Endler 2001a). In fact, prior to the widespread availability of genetic markers, colour pattern was used as a reliable predictor of paternity (Houde 1997).

Table 1.1: The expression of size effects on male mating behaviour and female mate choice in poeciliid fishes

Species	Effect	Reference
<i>Poecilia latipinna</i>	Larger males court more and sneak less than smaller males	Farr <i>et al</i> 1986
<i>Poecilia latipinna</i>	Mating behaviour varies with competitor size	Travis and Woodward 1989
<i>Gambusia affinis</i>	Larger males court Smaller males sneak	Hughes 1985
<i>Gambusia affinis</i>	Females prefer larger males	Hughes 1985
<i>Xiphophorus nigrensis</i>	Larger males court Smaller males sneak	Ryan <i>et al</i> 1990
<i>Xiphophorus nigrensis</i>	Females prefer larger males	Ryan <i>et al</i> 1990
<i>Poecilia reticulata</i>	Females prefer larger males	Reynolds and Gross 1992
<i>Poecilia reticulata</i>	Female preference varies across population	Endler and Houde 1995
<i>Poecilia reticulata</i>	Males prefer larger females	Ojanguren and Magurran 2004
<i>Poecilia reticulata</i>	Female preference changes with age	Kodric-Brown and Nicoletto 2001

1.6.2) Levels of Effect

The factors that influence mating behaviour in the guppy can be expressed at various different scales. Perhaps the most obvious division of effect is at the population level. Population effects can also be differentiated. First, there are clear differences between populations as elegantly demonstrated by Endler and Houde (1995) in their examination of female preferences for various male traits in 11 different Trinidadian populations. Only two of the traits tested (females preferred more orange and less blue-green) showed consistency in female preference between localities. For all other traits (areas of all other colours individually and in total; body size, tail size, total size and body height; and number of traits used as choice criteria) females were inconsistent between localities. Second, as discussed above, are the division of populations into high and low predation regimes. For example adult size (Reznick *et al* 1996); male colour pattern (Houde 1987); male behaviour (Rodd and Sokolowski 1995); number and size of offspring (Reznick *et al* 1996a) and gonopodium length (Kelly *et al* 2000) have all been shown to vary with predation regime. Finally there is the grouping of populations into those from different drainages. The Caroni and Oropuche drainages are the usual distinctions, but further drainages on the northern slope of the Northern Range are Paria and Yarra (Carvalho *et al* 1991) which are genetically included in the Caroni drainage (Carvalho *et al* 1991, Shaw *et al* 1991). Interestingly, the high and low predation distinctions found on the south slope of Trinidad's Northern Range are mirrored by those of the drainages on the north slope (Reznick *et al* 1996a,b).

The next level of effect is within population. Populations are made up of numerous, dynamic, mixed sex and age shoals that inhabit a geographically distinct area. Males and females are often affected differently by influential factors, as are individuals at different stages of development. This can be ably demonstrated with reference to the classic source of variation in guppy populations, predation pressure. Male guppies, being brightly coloured and undertaking distracting courtship displays to females are more conspicuous to predators than other shoal members and are therefore likely to be preferentially predated (Endler 1987). Similarly, predators such as *Rivulus*, a gape limited predator, will preferentially predate smaller individuals, usually juveniles or even smaller adults (Reznick and Endler 1982). Thus individuals within a shoal, and

more generally within a population, will be subject to different predation pressures. Other factors, such as light intensity which will highlight the brightly coloured males more than other individuals (Endler 1987), or available nutrients which may be more accessible to smaller or larger individuals (Dussault and Kramer 1981), will likewise have different effects on different subsets of the population. A connected level of variation is within family variation which, in addition to being subject to the above mentioned within population level factors, has the advantage for the researcher of partially removing some potential variation as family members are likely to be more similar than totally unrelated individuals.

Finally, there is the within individual variation. Both internal and external environments are changeable. Some factors, for example adult male size (Meffe and Snelson 1989a) cannot change with circumstances, but many others can. Even a genetically determined trait such as male colour pattern can change over time (Houde 1997). Males express different areas of colour more intensely when excited (Baerends et al 1955; Liley 1966; Houde 1997) and the expression of orange colouration, for instance, depends in part on the consumption of dietary carotenoids (Kodric-Brown 1989), which may vary over time. Mating behaviour certainly varies within a single individual. As stated above, females change their preferences for male type with age (Kodric-Brown and Nicoletto 2001) and will also practice mate copying, in that they will change the selected male after observing other females' choices (Dugatkin and Godin 1992, Brooks 1996). Moreover female choice may be condition dependent (Dugatkin and Godin 1998). A female in poor condition will be less interested in males than one in the peak of health (Dugatkin and Godin 1998). Finally female choice varies with internal state, specifically the current stage of their reproductive cycle; unless a female has recently given birth or is virgin, she will not respond to males at all (Liley 1966). Similarly individual male mating behaviour may vary with the presence or absence of predators (Magurran and Nowak 1991) or light intensity (Endler 1987, Long and Rosenqvist 1998); with nutritional state (Abrahams 1993) or parasite load (Lopez 1998); with immediate social environment (Jirotkul 1999a, b); with female size (Ojanguren and Magurran 2005) or reproductive state (Liley 1966, Crow and Liley 1979) among others.

1.7) Aims

This thesis aims to examine variation in mating behaviour using different combinations of causal factors and scales of effect in order to elucidate the importance of variation in an evolutionary context. The first data chapter (Chapter two) looks at variation in male mating behaviour at the scale of the individual using changes in sex ratio, a social factor. The next chapter continues the theme of the effects of competition on male mating behaviour with the additional developmental factor of male size. This time the scale of effect moves to the next level of variation between siblings. Another aspect of mating behaviour, female choice of males, is also introduced here. Chapter four again moves up in scale of effect to variation in male mating behaviour between unrelated individuals. This time the factor concerned is changes in water velocity, an abiotic environmental variable. A combination of laboratory and field experiments illustrates the applicability of laboratory studies to natural conditions. The final data chapter (Chapter five) brings together many of the influences shown in previous chapters in an examination of variation in male mating behaviour and female mate choice at the population level. This chapter also goes one step further in establishing the reproductive success of males from different populations who have the attributes associated with different predation regimes. Finally the impact of variation at these different scales and the evolutionary implications of this variation are discussed and potential future studies highlighted by the work presented here are put forward.

1.7.1) Specific Aims

- To examine the plasticity and consistency of male mating behaviour under varying sex ratios and to test the hypothesis that male guppies have intrinsic levels of mating behaviour relative to other males
- To investigate the applicability of phenotypic manipulation in relation to adult size in the guppy and hence test the hypothesis that female mate choice and male mating behaviour are influenced by male size using siblings that differ only in size

- To explore the effects of the water velocity profile on male mating behaviour, testing the hypothesis that this behaviour varies with varying water velocity and comparing these effects in field and laboratory situations
- To examine differences in male morphology and mating behaviour between populations that differ in predation regime origin, exploring female mate choice in relation to these differences with the prediction that male reproductive success will reflect these differences

Overall Hypothesis

- The magnitude of variation in male mating behaviour increases as the scale over which variation is examined increases. Specifically, the smallest amount of variation will be found in individual males; more variation will be found between siblings; an increased amount of variation will occur between unrelated individuals and males from different populations will show the most variation.

Chapter 2

Behavioural Profiles: Individual Consistency in Male Mating Behaviour Under Varying Sex Ratios

2.1 Abstract

Behavioural profiling reveals whether individuals express a behaviour consistently and whether this consistency is maintained relative to other individuals across contexts. In this study wild guppies were examined in equal, female- and male-biased sex ratios. Male display and sneak frequency was highly repeatable and males maintained a consistent level of behaviour relative to other males across sex ratios. Male guppies, therefore, have individual mating behaviour profiles. Functional significance and potential causal factors are discussed.

2.2 Introduction

Several studies have examined the individuality of behavioural patterns and used this as a basis for discussion of, for example, personality dimensions (e.g. Budaev 1997; Budaev and Zhuikov 1999; van Oers *et al* 2004) or behavioural syndromes (Sih *et al* 2004). The first step in the demonstration of any of these hypotheses is the establishment of individual consistency in the behaviours necessary to illustrate the phenomenon in question. This is usually assessed using repeatability (Boake 1989; Kolok 1999; Watkins 1997). A further requirement may be to show that this consistency is maintained across situations. This ‘behavioural profiling’ is itself of interest and the establishment of individual behavioural profiles in one specific behaviour type, mating behaviour, is the focus of this study; specifically, whether individual male guppies show consistent reproductive behaviour across contexts where the advantages of employing alternative mating tactics differ.

Operational sex ratio (OSR); defined as the number of sexually active males relative to the total number of sexually active adults of both sexes (Jirokul 1999), is known to affect mating behaviour (see Andersson 1994 for examples). At female biased sex ratios there is more opportunity for courtship and more choice over where to direct this courtship. At male biased sex ratios, increased competition from other males will influence mating decisions. Examples of the influence of sex ratio on alternative male mating behaviours can be found in numerous diverse taxa. In the giant kangaroo rat, for example, males increase competition for females in male biased sex ratios (Randall *et al* 2002) while in the blue crab a male biased sex ratio induces males to trade courtship for forced capture of females (Jivoff and Hines 1998). Among aquatic species, the effects of sex ratio on mating behaviour has been demonstrated in the frog, *Rana dalmatina*, in which males switch from calling to actively searching for females in a male biased sex ratio (Lode *et al* 2004) and in the sockeye salmon operational sex ratio influences both the level of aggression and courting carried out by males (Quinn *et al* 1996). In this study, individual male mating behaviour will be tested both within sex ratios, to assess repeatability, and between sex ratios, to measure consistency of behaviour across contexts.

The guppy, *Poecilia reticulata*, is an ideal model species with which to investigate mating behaviour profiles. Males have two alternative mating behaviours, courting (sigmoid displays) or sneaking (gonopodial thrusts), and all males can use either behaviour at any time (Liley 1966, Farr 1989). Guppies have been studied extensively and many factors have already been shown to influence the use of these alternative mating behaviours including predation regime (Endler 1987; Magurran and Seghers 1994b; Nicoletto and Kodric-Brown 1999; Rodd and Sokolowski 1995) and associated environmental variables (Reznick *et al* 2001), such as light intensity (Endler 1987) and water turbidity (Luyten and Liley 1985, 1991); colour pattern (Houde 1988a; Jirotkul 2000a; Kodric-Brown 1989; Nicoletto 1991) and gonopodium length (Reynolds *et al* 1993); water velocity (Magellan and Magurran: in press, Nicoletto 1996); male size (Magellan *et al* 2005, Rodd and Sokolowski 1995); male age (Houde 1997); population density (Jirotkul 1999b) and parasite load (Lopez 1998). In addition sex ratio varies in wild populations (Pettersson *et al* 2004, Seghers 1973) so males are likely to be adapted to deal with this. Indeed sex ratio has already been demonstrated to affect mating behaviour (Farr 1976, Jirotkul 1999a) though the exact relationship is as yet inconclusive. Farr (1976) found that males increased the frequency of displays in the presence of competitors while Jirotkul (1999b) found that the frequency of displays decreases at male biased OSRs and sneak mating was most frequent at equal OSRs (Jirotkul 1999b). Surprisingly, despite the widespread attention received by the guppy mating system, to date no studies have assessed the repeatability of male mating behaviour.

The aim of this study, therefore, is to describe individual male mating behaviour profiles in the guppy. Repeatability measures will be used to assess the consistency of mating behaviour within and across sex ratios and the relationship between total frequency and relative frequency of the two types of male mating behaviour will be illustrated. Specific predictions are that males will show individual consistency in the absolute frequency of sigmoid displays and sneak mating attempts and that this consistency will be maintained relative to other males across sex ratios. Further predictions are that the frequency of displays will increase in a female biased sex ratio where there is more opportunity for courtship while increased competition from other males will increase the incidence of interference with mating attempts suggesting sneaky mating is the more viable option in a male biased sex ratio.

2.3 Methods

To establish male mating behaviour profiles, fish were collected from a 20-metre stretch of the Arima River in Trinidad's Northern Range. They were allowed to rest for several days before experimentation to get used to captivity, during which total length (TL) was measured under light anaesthesia in order to match the size of experimental males as closely as possible (male TL: 21 to 24 mm). Thirty-nine experimental males were identified by colour drawings and housed separately. All other fish (67 females and 68 males) were placed together in a stock tank.

Observations were carried out in 320x170mm tanks filled with 8l of river water treated with a water conditioner and an anti-fungal solution. Each tank had an air supply, a river gravel substratum and a leaf from a stream. The leaf provided a substrate for bacteria which process fish excretory products so aiding maintenance of water quality and also supplied a refuge. The fish were fed on flaked fish food at least once per day.

In order to carry out the large amount of observations required, six tanks were set up in series. Stock fish were added to all tanks in the evening. The following morning, one hour after being fed, five test males were identified, added to five of the experimental tanks at 15-minute intervals and allowed to settle for 1 to 2 hours. This set-up meant that once a male had been observed in one tank (and therefore with one group of stock fish), he could be moved to the next tank which did not contain an experimental male and allowed to settle for 1 to 2 hours while the other tanks were being observed. This also ensured that no two males were exposed to the same five sets of stock fish as, for example, male 1 would be observed in tanks A, B, C, D and E; male 2 in tanks B, C, D, E and F; male 3 in tanks C, D, E, F and A etc. As a further precaution, at the end of each day's observations all stock fish were replaced which also ensured that stock fish did not become over familiar with the tanks. The experiment was carried out in 2 parts. The first part was to establish 'base line' mating behaviour for each male. Each experimental male was observed for 10 minutes in each of 5 tanks containing an equal sex ratio (5 females, 4 stock males, 1 experimental male) recording the frequency of sneaky mating attempts and sigmoid displays. At the end of each observation, the experimental male was placed in the next available tank if observations still remained or in a holding tank if his observations

were complete. The second set of observations assessed how this 'base line' behaviour changed in different sex ratios. For this, tanks contained male-biased (3 females, 6 stock males, 1 experimental male) or female-biased (7 females, 2 stock males, 1 experimental male) sex ratios. These sex ratios were chosen based on a brief survey of the Arima River which revealed schools with an OSR ranging from 0.14 (i.e. female biased: n=11) to 0.77 (i.e. male biased: n=5). Only 1 out of 17 schools had an even sex ratio (OSR of 0.5). This compares to OSRs of 0.3, 0.5 and 0.7 in this experiment. Each experimental male was observed twice for 10 minutes in each biased sex ratio. When all observations had been completed, all the fish were returned to their site of capture.

To assess repeatability of male behaviour repeated measures analyses of variance were carried out for each sex ratio for both behaviours to obtain within- and between-subject mean squares. Repeatability was calculated following the method of Lessells and Boag (1987). Standard errors of repeatability were calculated following Becker (1992). Differences between sex ratios were analysed by taking the mean of behavioural frequency for each male in each sex ratio and performing further repeated measures analyses of variance. Post-hoc tests comparing all pairwise interactions (Tukey's) were carried out to discover where differences occurred. Repeated measures analysis of variance assumes normality, which was achieved through square root transformations on all data, and sphericity. None of the analyses violated this assumption (Greenhouse-Geisser epsilon: 0.835-1.000). Kendall's coefficient of concordance was then used on these means to assess whether the ranking of males was the same across sex ratios. Finally, to ascertain the relationship between total frequency and the relative frequency of mating behaviour the mean frequency of sneaks and displays were totalled for each sex ratio to give the total frequency of mating behaviour and the mean frequency of displays was divided by this total to give the proportion of displays (relative frequency). The data for mean total frequency of behaviour in each biased sex ratio was \log_{10} transformed to achieve normality. Pearson correlations were carried out for each OSR.

2.4 Results

As expected, in line with earlier work (Jirokul 1999, Magellan *et al* 2005) the frequency of both sneaks and displays varied significantly between sex ratios (Figure 1: displays: $F_{(2,76)}=15.59$, $p<0.001$, sneaks: $F_{(2,76)}=4.69$, $p<0.012$) There was a significant decrease in both variables from equal OSR (displays: 2.97 ± 0.932 , sneaks: 2.52 ± 0.817 (mean \pm sd)) to male biased OSR (displays: 2.15 ± 1.087 , sneaks: 2.06 ± 0.786) (Figure 1). There was also a (non-significant) decrease in the frequencies of both behaviours from equal to female biased OSR (Figure 1: displays: 2.90 ± 0.980 , sneaks: 2.45 ± 0.932). The frequencies of both sigmoid displays and sneak mating attempts by individual males show highly significant repeatabilities in all OSRs with values ranging from 0.62 to over 0.99 (Table 1); the difference between individuals is highly significant (Table 1: $p<0.001$ in all cases). Individual males have consistent levels of mating behaviour within sex ratios. Furthermore, individual male rankings were the same in all sex ratios (sneaks: $W=0.099$, $p=0.021$; displays: $W=0.249$, $p<0.001$; $N=39$; $df=2$; Figure 2). Thus the consistent levels of behaviour exhibited by males within sex ratios are also maintained, relative to other males, between sex ratios. There was no relationship between total frequency and the proportion of sneaks and displays in equal ($r =0.047$, $p=0.774$) or female biased ($r =0.134$, $p=0.414$) sex ratios. However, in the male biased sex ratio a significant positive correlation indicates that an increase in the total frequency of mating behaviour can be attributed mainly to an increase in display activity ($r =0.340$, $p=0.034$).

Table 1: Repeatabilities and F-ratios for frequency of displays and sneak mating attempts in each sex ratio

Condition	r \pm se	df	F	p
Displays-equal sex ratio	0.941 ± 0.0144	38,156	403.929	<0.001
Displays-female biased sex ratio	0.820 ± 0.0531	38,39	342.850	<0.001
Displays-male biased sex ratio	0.624 ± 0.0991	38,39	152.915	<0.001
Sneaks-equal sex ratio	0.965 ± 0.0087	38,156	370.133	<0.001
Sneaks-female biased sex ratio	0.993 ± 0.0023	38,39	275.336	<0.001
Sneaks-male biased sex ratio	0.833 ± 0.0497	38,39	266.631	<0.001

Figure 1: Mean display (black bars) and sneak (white bars) frequencies for all males between sex ratios. Error bars represent standard deviation.

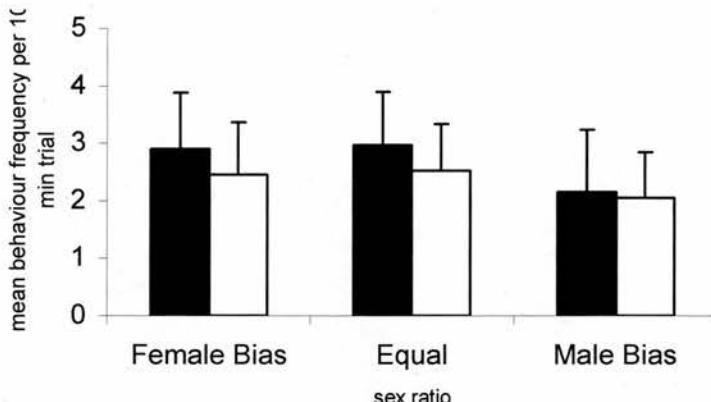
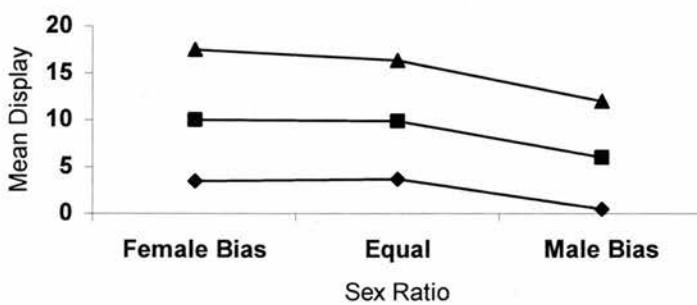
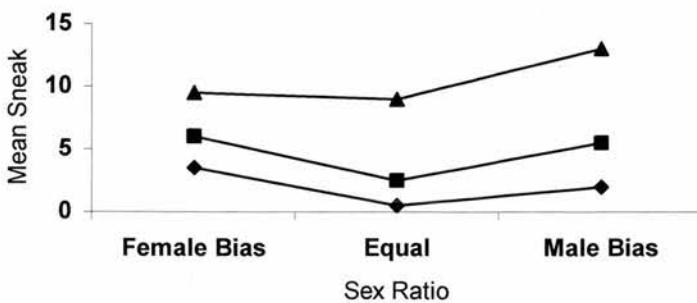


Figure 2: Representative samples showing consistency in male behaviour across sex ratio. a) displays: high display rate-male 33; medium -male 39; low -male 30. b) sneaks: high sneak rate-male 2; medium -male 37; low -male 28.

a)



b)



2.5 Discussion

Male guppies are clearly shown to have individual mating behaviour profiles. Males are extremely consistent in the frequencies of displays and sneak mating attempts. Moreover for displays, and to a lesser extent sneaks, a male with a high level of behaviour maintained that high level relative to other males in all sex ratios examined. There was no evidence to suggest that males that display more, sneak less, or vice versa and apart from a weakly significant result for male biased OSR there was no relationship between the relative frequency of behaviour types and the total frequency of behaviour. Males could, therefore, be profiled as 'high courters-medium sneakers' or 'low courters-high sneakers' and so on. These individual profiles indicate males are constrained in how they behave in different contexts, which has implications for modelling of mating behaviour.

A consistently high level of sexual activity seems, at least superficially, to be advantageous. Female poeciliids generally accept displays only as virgins or for a few days after parturition (Liley 1966, Farr 1989). Males with higher courtship display rates will encounter greater numbers of receptive females, so selection should maximise display rates (Farr 1989). Mathews and Magurran (2000) and Pilastro and Bisazza (1999) have demonstrated that sperm is transferred during sneaky mating attempts in guppies. It follows that males with higher sneaky mating rates will be able to inseminate more females than those with lower rates. Selection therefore should also favour higher sneaking rates (Farr 1989). So, overall there has been intense selection for males to maximise sexual activity (Farr 1989). However a proportion of the males observed had low levels of either displays or sneaks. It is likely that additional selective pressures counteract any advantages gained by maintaining high levels of sexual activity. For example, active males are likely to preferentially attract the attention of predators (Endler 1987; Farr 1975; Luyten and Liley 1985; Reynolds 1993; Reynolds *et al* 1993).

Increased levels of mating behaviour at male biased sex ratios would seem to be adaptive as increased competition for mating opportunities is likely to lead to more intense sexual selection (Jirokul 2000b). Previous studies have yielded conflicting results. Farr (1976) found that males increased the frequency of displays in the

presence of competitors, while Magellan *et al* (2005) found that the frequency of displays was unaffected by the presence or absence of competition but sneak mating attempts increased with competition. These results are most in agreement with Jirotkul (1999a) in that the frequency of displays decreases at male biased sex ratios and sneak mating was most frequent at equal sex ratios (Jirotkul 1999a).

There are several possible explanations for the consistency of male mating behaviour. Repeatability gives an upper limit to broad-sense heritability (Boake 1989, Lynch and Walsh 1998). Luyten and Liley (1985) provide some evidence for the inheritance of male mating behaviour. Offspring of fish from four Trinidadian rivers raised in identical laboratory conditions showed levels of courtship behaviour consistent with those of their wild fathers, suggesting a genetic basis to observed behavioural differences between populations (Luyten and Liley 1985). However, Rodd and Sokolowski (1995) found no evidence for a genetic basis for variation in courtship behaviour, though their findings did indicate a genetic component of sneaky behaviour (Rodd and Sokolowski 1995). Male mating behaviour is influenced by many variables most of which were impossible to quantify in the wild fish used in this study. Factors such as male size (Magellan *et al* 2005, Rodd and Sokolowski 1995), which due to males determinate growth (Houde 1997) does not change after sexual maturity, and male age (Houde 1997), which was constant over the timescale of this study, affect mating behaviour. It may be that an amalgamation of factors for each individual male combine to produce the consistent level of behaviour seen here. Clearly further work is needed to elucidate the factors governing the consistency of male mating behaviour in guppies. However, as this paper shows, the establishment of behavioural profiles is a constructive technique that can be applied across taxa to elucidate behavioural hypotheses.

Chapter 3

Quantifying male attractiveness and mating behaviour through phenotypic size manipulation in the Trinidadian guppy, *Poecilia reticulata* *

3.1 Abstract

Although many studies have examined the effects of male size on attractiveness and mating behaviour, few have taken genetic background into consideration. Phenotypic manipulation permits the experimental adjustment of morphological traits while keeping genetic background constant. Here, male guppies, *Poecilia reticulata*, an ideal model for this type of manipulation, were raised at different temperatures to produce sibling pairs that differ in size. These were then used to investigate male mating behaviour and male attractiveness, assessed through female mate choice, in relation to this size dimorphism. Further, male-male competition, which is intrinsic to male mating behaviour, is also likely to be affected by male size. Through the use of repeated measures analysis it is demonstrated that females significantly prefer larger males and that male size and competition significantly affect several aspects of male mating behaviour. Larger siblings perform more sneaky mating attempts and spend more time chasing females. The frequencies of both these behaviours increase with competition. While display frequency is unaffected by male size and competition, display duration and the amount of time spent attending females are reduced in the presence of competitors. This study highlights the use of phenotypic manipulation as a valuable tool for investigating behavioural interactions and confirms that both male size and competition are significant factors in the guppy mating system.

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3.2 Introduction

Male body size plays a central role in many aspects of sexual selection. Larger males may have direct advantages when competing with other males for mating opportunities (Andersson 1994), but their body size may also be used by females as a cue to assess their overall quality and attractiveness (Jennions and Petrie 1997, Candolin 2003). There is today evidence that females actively choose larger males in groups as diverse as insects (Iyengar *et al* 2002), lizards (Censky 1997, Olsson and Madsen 1998), mammals (Bro-Jørgensen 2002), and fish (Reynolds and Gross 1992; Quinn and Foote 1994). This female preference is often interpreted from a ‘good genes’ perspective (Møller and Alatalo 1999, Jennions *et al.* 2001), where size indicates general male vigour, but it can also evolve as a runaway process (Fisher 1958) or through sensory bias for exaggerated phenotypes (Ryan 1994). Males, on the other hand, also experience size effects. In particular, their relative body size commonly influences mating behaviour, for example, through the expression of conditional mating tactics (Gross 1996; Moczek and Emlen 2000; Brockmann 2002; Aubin-Horth and Dodson 2004), or as context-dependent, flexible mating behaviour (Farr *et al* 1986; Rodd and Sokolowski 1995; Shine *et al* 2003). Given the attention these questions and sexual selection in general has received during the last decade (e.g. Andersson 1994; Jennions and Petrie 1997; Candolin 2003), it is somewhat surprising that a central feature of size-related effects remains largely unexplored. Traditionally, size-related effects have been investigated using males of unknown relatedness, matched for coloration or other secondary sexual characters (e.g. Andersson 1994, Houde 1997). Unfortunately, this also means that effects attributed to male size *per se* are confounded with differences in genetic background and related covarying factors. However, this problem can be circumvented through phenotypic manipulation (Sinervo and Basolo 1996, Sinervo and Svensson 1998), a developmental approach recently applied to evolutionary biology. By experimentally generating relevant phenotypes rather than working with ones already available, phenotypic manipulation makes it possible to disentangle factors that would otherwise be beyond reach (Sinervo and Basolo 1996, Sinervo and Svensson 1998). Given that care is taken so that relevant treatments are used and unwanted correlated effects avoided (Rose *et al* 1996, Sinervo and Svensson 1998), the approach can be successfully used to address life-history effects (Sinervo and Huey 1990),

physiological performance (Pettersson and Brönmark 1999), growth strategies (Schmitt *et al* 1999) and the expression of secondary sexual characters (Ketterson and Nolan 1999). To use phenotypic manipulation to address the effect of male body size on female choice and male mating behaviour while controlling for genetic background, a system is needed where males have phenotypically plastic growth, female choice is prominent, and male mating behaviour is flexible. The guppy, *Poecilia reticulata*, provides exactly this (Houde 1997, Magurran 2001).

The guppy is a small poeciliid native to Trinidad, where it is widely distributed and found in virtually every freshwater habitat (Magurran and Phillip 2001, Magurran 2001). Guppies have a promiscuous mating system (Houde 1997) with a central feature being female choice for colourful males (Houde and Endler 1990, Houde 1997). In contrast, males switch between two different mating strategies, either courting females using sigmoid display behaviour (Houde 1997) or engaging in forced mating attempts (sneaking), thereby potentially overriding female choice (Magurran 1998, 2001). The relative success of the two types of behaviour may be influenced by male size (Endler 1995). Growth patterns in guppies are phenotypically plastic and can be affected by factors such as water temperature (Liley and Seghers 1975), social environment (Rodd *et al* 1997) and food abundance (Reznick *et al* 2001). While female guppies have indeterminate growth, males cease growing after maturation (Houde 1997). The fact that male guppies have determinate growth while growth itself is a plastic trait provides a simple means of experimentally producing adult males that differ in size (Sinervo and Basolo 1996). Water temperature during development is a suitable factor to manipulate (Atkinson 1994), since guppy populations experience considerable variation in water temperature in nature (Liley and Seghers 1975, Magurran and Phillip 2001), and that temperature manipulation of ectotherm development generally leads to consistent effects on phenotypes, with low temperatures inducing larger body size and higher temperature leading to a smaller body size (Atkinson 1994, Johnston 2001).

The main aim of this study is to use phenotypic manipulation of male guppies to address effects of male body size on two aspects of sexual selection: male attractiveness as judged by female choice, and male mating behaviour. The study uses sibling pairs raised at different temperatures in a split-brood design, generating full-

sib pairs of small and large individuals. This design allows for the study of the effect of male size separately while controlling for genetic differences. In line with Reynolds and Gross (1992), females are expected to prefer larger siblings. Further, male size is expected to influence the relative use of sigmoid display behaviour and forced mating attempts. In particular, larger males are expected to devote a larger proportion of time to display behaviour. Finally, the presence of male competitors is expected to increase the overall time that males devote to mating behaviour and induce a switch from display to sneaking behaviour.

3.3 Methods

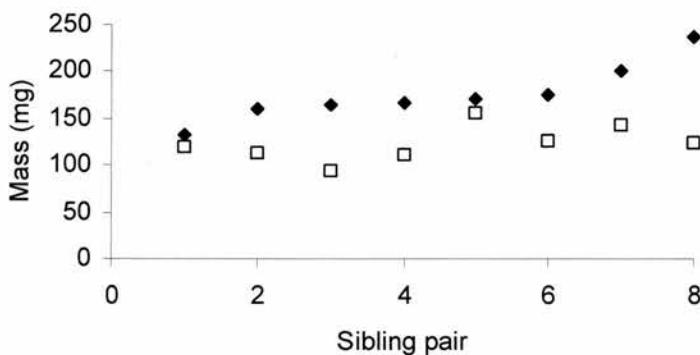
3.3.1 Experimental Animals

The experimental guppies were descendants of wild-caught fish from the upper Aripo River in Trinidad's Northern Range. This population occurs in a locality where there are few predators, and is conventionally termed a low predation site (Endler 1995). The goals were to quantify female preferences for male size (Experiment 1) and to examine size-dependent male mating behaviour in relation to male-male competition (Experiment 2). By taking advantage of temperature-dependent phenotypic plasticity (Atkinson 1994) eight size dimorphic full-sib pairs were produced. In many ectotherms, including guppies, a lowered rearing temperature induces a larger size at maturity (Atkinson 1994) and can be used generate particular phenotypes while controlling for genetic background and other confounding effects (Sinervo and Basolo 1996, Sinervo and Svensson 1998). The same eight sib-pairs were used in both experiments.

To standardize the effect of temperature on critical stages of early embryology (Takahashi 1975; Stearns and Kawecki 1994; West-Eberhard 2003), parental males and females of the full-sib broods were raised and mated at intermediate temperature (26° C). Pregnant females were kept at this temperature until giving birth, thereby standardizing embryological development. Broods were then split and siblings transferred to either low (23° C) or high (29° C) water temperature, in which they were raised singly. The median water temperature of guppy sites in Trinidad is 25.8° C, with 90% of the populations within the range of 23 – 29°C (Liley and Seghers 1975, Magurran and Phillip 2001). The guppies were kept in transparent 5-l PVC jars and had visual contact with other guppies. They were fed live brine shrimp and commercial flake food *ad lib* daily, and apart from water temperature, rearing conditions were identical. Fish in the lower temperature treatment matured more slowly and achieved a larger size than those raised at a higher temperature (paired t-test, $t=8.59$, $df=10$, $p<0.0001$) with large males being, on average, 15% bigger than their smaller siblings (mean standard length \pm SE: larger males = 22.3mm \pm 0.4mm, smaller males = 19.4mm \pm 0.6 mm) (Figure 3.1). One large and one small male sibling from each brood were chosen to maximise size difference while matching for

colour pattern. Their water temperature was adjusted to 25° C and the fish were then acclimatized to this temperature for a minimum of 3 months to avoid any short term temperature dependent effects.

Figure 3.1: Size difference between large and small siblings. Large, cold-reared siblings – black diamonds, small, warm-reared siblings – white squares.



Twenty-four virgin females, from the same population as the males (Upper Aripo), were allowed to choose between large and small siblings (Experiment 1). Virgin females are receptive to male courtship, and exhibit clear preferences for particular male phenotypes (Liley 1966, Houde 1997). Wild females are rarely responsive to courtship and are often pursued by several males (Magurran and Seghers 1994). Experiment 2 therefore used non-receptive females and examined the effects of male-male competition when assaying the behaviour of large and small siblings. This set of trials involved a further 15 males (standard length: 15-18 mm) and 15 females from a mixed stock of laboratory-bred descendants from Trinidadian populations. These guppies were housed in separate tanks to ensure absence of familiarity between the sexes (Kelley *et al* 1999). Throughout both experiments, fish were kept at 25° C with a 12-hour light/dark regime and were fed live brine shrimp *ad lib* daily. Although males were no longer completely naïve of females at the start of Experiment 2, they had had no opportunity to mate and were matched in experience.

3.3.2 Experiment 1

The aim of the first experiment was to quantify female preferences for male size. To do this, two 61×30 cm tanks, filled to a depth of 25 cm were marked with vertical lines 22 cm from each side. The central (17 cm wide) area was designated the ‘neutral zone’. Two perforated plastic bottles (34 cm tall×10 cm diameter) for males were placed against each side. A plastic, non-transparent 15×7 cm ‘start box’ with a 3×2 cm exit hole facing towards the back of the aquarium was suspended in the centre of the tank. The tank was illuminated from above using a standard 15-W, 45 cm fluorescent aquarium light. All other light sources were removed so that the observer, sitting 1 m away, was not likely to affect fish behaviour. Preliminary trials established that the fish did not react to the observer’s presence. Both tanks had two airstones each and the bottom was covered with gravel. Trials were initiated by placing a pair of male siblings in the tank, one in each bottle. A female was then placed in the start box and allowed to exit at will. If she had not left the start box after 15 minutes she was replaced with another female and re-tested at a later date. Each trial began when the female left the start box, and for 15 min the following measures were recorded: the time that the female spent in each male’s zone, and the time oriented towards each male whilst in its zone. During the trial, the female was able to see both males from anywhere in the tank and the males were able to see each other. At the end of the trial, all fish were removed. Each female was observed with one pair of males in 2 trials, with the males exchanging places for the second trial. Each male pair was placed with 3 different females (6 trials), giving 48 trials in total. The order of males and females was randomised, as were the tanks and bottles used.

3.3.3 Experiment 2

The aim of the second experiment was to quantify size-dependent male mating behaviour in relation to male-male competition. All males were tested individually in two conditions, giving 16 trials each with and without competition. The same 61×30 cm tanks as in the previous experiment were used, but plastic bottles and start boxes were removed. Five non-virgin stock females, taken at random from the holding tank, were added to the experimental tank. They were fed, then left overnight to acclimate. The next day, the experimental male, either alone (no competition) or with 5

randomly selected stock males (competition), was added to the tank, fed, then left to acclimate for 2 hours. During each 15 min trial the following variables were recorded for each focal male (identified by colour pattern): number of sneaky mating attempts; number of sigmoid displays; cumulative duration of sigmoids; total time spent chasing females; total time attending females. At the end of the observation, the males were removed. Females were left in the tank throughout one day's observations. If any offspring were found in a tank, all the trials from that tank for that day were excluded from the analysis and the 5 females therein were not used in any trials for 3 days. This avoided changes in male behaviour due to female receptivity, which is high just after parturition (Liley 1966). The order of experimental males and of trial conditions was randomised.

3.3.4 Statistical procedures

Female preference in Experiment 1 was quantified as time spent with each male and as time spent observing each male whilst being close to it. These two measures were also combined into a choice index which evaluated the relative amount of time spent with each male as follows:

$$\text{Choice index} = \frac{\text{time spent oriented towards male (s)}}{\text{time spent in that male's area (s)}}$$

As the time spent with one male affects the time available to spend with the other, it is important to take this effect into account in the analysis (Houde 1997, Wagner 1998). One alternative has been to use the difference in time spent with each male and to standardize this by the total amount of time spent near the males (e.g. Houde 1997). However, this approach does not take the absolute values into account. As the negative correlation between the time spent with the two males is itself potentially dependent on the experimental setup (i.e. decreasing dependency with increasing size of the no-choice zone), an alternative method was used in which the significance of the statistical dependency is directly quantified. This was done for all three measures of female preference. The three different measures were each evaluated with SAS Proc GLM (SAS Institute 1999) as a doubly repeated measures design, using family as a random factor (Littell *et al* 1996; Hosn 1999; Stevens 2002). The double pairwise

structure results from the fact that the first repeated factor (sibling size: large and small) is itself observed as a repeated factor (position in tank: left and right). As a consequence, the dependent measure, female preference, is a function of two repeated factors: size and position, each with two levels. By using a doubly repeated design, the full dataset (i.e. three replicates (females) per sibling pair, each encountering the males in the two alternate positions) can be used without either discarding information (Scheiner 2001) or inflating the degrees of freedom (Scheiner 2001, Stevens 2002). The female choice data was also used to investigate whether females were responding to large and small guppies in a consistent way or if differences that were detected could be explained as correlated responses to males having been raised under cold and warm conditions (Huey and Berrigan 1996, Rose *et al* 1996). To quantify this effect, female preferences were analysed as functions of male size as separate (i.e. large and small siblings as two groups) and pooled regressions (Zar 1999). If separate regressions described the preference functions significantly better than the pooled regression, this would indicate that cold-raised and warm-raised males were not judged the same way. Conversely, if the pooled regression described the data well, this indicated that there was no significant correlated effect of the experimental treatment.

Male mating behaviour in Experiment 2 was evaluated using SAS Proc GLM (SAS Institute 1999) with respect to male size and presence or absence of male-male competition. The analysis was carried out in a two-step process. First, the full data set on all five measured male behavioural variables was analysed to assess overall effects. This main model was a mixed model repeated measures design without replication, with one random factor (family), two factorial variables (fixed effects: type of mating behaviour; male size), and one repeated factor (fixed effect: presence and absence of male competitors). Analogous to model formulation in randomised block designs, the main model was reduced by omitting the interaction between the random factor (family) and male size (Zar 1999). This formulation meant that both fixed factors, their interaction, and the random factor were quantifiable. Having identified significant overall effects, each behavioural variable was analysed separately. Tukey HSD post-hoc tests were used to identify significant differences between experimental groups (SAS Institute 1999, Stevens 2002). Model assumptions were tested using graphical methods according to standard practise (Lynch and Walsh

1998, Zar 1999). When necessary to meet model assumptions, data were $\log(X)$ or $\log(X+1)$ transformed to improve variance homogeneity and normality (Zar 1999).

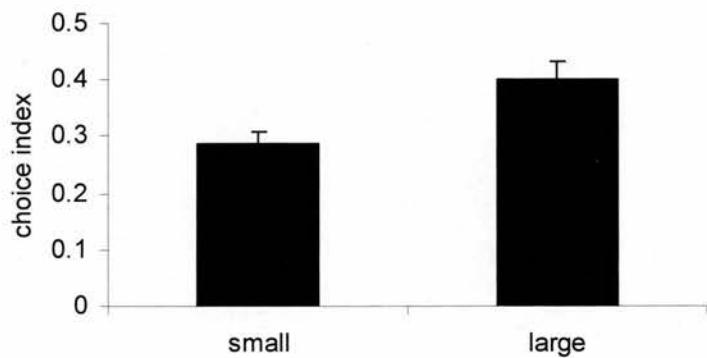
3.4 Results

3.4.1 Experiment 1

Females did not spend more time with either of the two male phenotypes (small males: 308 ± 26 s (mean \pm SE); large males: 379 ± 32 s; doubly repeated ANOVA, $F_{1,16}=3.40$, $p=0.084$). However, they did spend more time observing the larger male (small males: 89 ± 11 s; large males: 152 ± 22 s; doubly repeated ANOVA, $F_{1,16}=12.31$, $p=0.003$). Further, the choice index, i.e. the ratio of time a female spent oriented towards a male to the time she spent in that male's area, showed a strong female preference for larger males (Figure 3.2; doubly repeated ANOVA, $F_{1,16}=19.11$, $p=0.0005$). Position effects and interaction effects were non-significant for all three measures (doubly repeated ANOVAs, $F_{1,16}=0.20-1.59$, $F_{7,16}=0.47-2.26$, $p>0.084$). Separate family effects and their corresponding interactions were also non-significant for all three measures (doubly repeated ANOVA, $F_{7,16}=0.72-1.90$, $p>0.14$). There was no evidence of significant negative correlations in the pairwise choice tests, i.e. that the time spent with one male reduced the time available to spend with the other ($r_p=0.113-0.257$, $p=0.077-0.446$, $N=48$). All three relations were weakly positive. Male behaviour was not formally quantified during the experiment, but no consistent behavioural differences between the phenotypes were observed (personal observation).

The relation between male size and female preferences was similar for both groups of males. When each of the female choice criteria (time with male, time observing male, and the choice index) were regressed against male total length, females responded in the same way to males from the two different rearing conditions (separate vs. pooled regressions, $F_{2,13}=0.001-0.907$, $p=0.428-0.999$). The responses were stronger towards larger males, but regressions were not significantly different. Hence, as differences in male rearing temperature did not translate into significant influences on female preferences, the results suggest that the post-rearing acclimatisation period had been sufficiently long.

Figure 3.2: Mean choice index (+ SE), i.e. the ratio of time a guppy female spent oriented towards a male to the time she spent in that male's area. Small denotes smaller male sibling, Large denotes the larger male sibling.

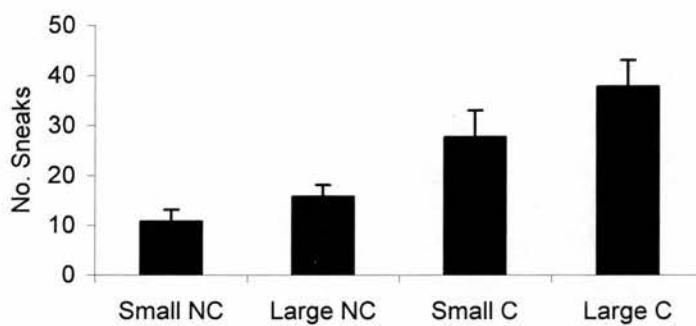


3.4.2 Experiment 2

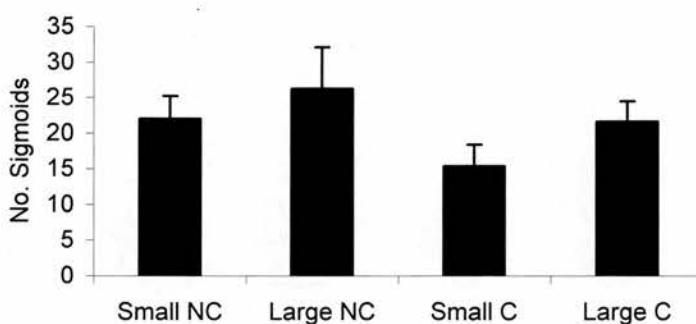
The analysis of the full set of all five measured male behavioural variables showed significant main effects and interactions (Table 1), while overall family effects were non-significant (results not shown). Subsequently, all five variables were analysed separately and four out of five proved to be significantly influenced by the experimental treatments (Figure 3.3, Table 2), while family effects remained non-significant (results not shown). Number of sneaky mating attempts was influenced by both male size and by the presence of competitors (Figure 3.3a, Table 2). Larger siblings performed more sneaky mating attempts (mixed model repeated ANOVA, size: $F_{1,7}=5.73$, $p=0.048$; Table 2), and individual sneaking frequency more than doubled when competitors were present (Figure 3.3a; mixed model repeated ANOVA, comp: $F_{1,7}=35.47$, $p=0.0006$; Table 2). The number of sigmoid displays were not affected by either male size or the presence of competitors (Figure 3.3b; mixed model repeated ANOVA, both main treatments: n.s.; Table 2), while cumulative amount of time spent performing such displays was significantly reduced when competitors were present (Figure 3.3c; mixed model repeated ANOVA, comp: $F_{1,7}=42.43$, $p=0.0003$; Table 2). There was no effect of male size on the cumulative amount of time spent performing sigmoids (Figure 3.3c; mixed model repeated ANOVA, size: n.s.; Table 2). The fourth variable, the total time spent attending females, showed no difference between large and small siblings (Figure 3.3d; mixed model repeated ANOVA, size: n.s.; Table 2), but that males significantly reduced the time attending females when competitors were present (Figure 3.3d; mixed model ANOVA, comp: $F_{1,7}=8.46$, $p=0.023$; Table 2). Finally, the total time spent chasing females was strongly influenced by both male size and by the presence of competitors (Figure 3.3e, Table 2). Larger siblings spent more time chasing females than small siblings did (Figure 3.3e; mixed model ANOVA, size: $F_{1,7}=9.54$, $p=0.018$; Table 2), and the presence of competitors increased the chasing of females even further (Figure 3.3e; mixed model ANOVA, comp: $F_{1,7}=84.56$, $p<0.0001$; Table 2). There was no interaction between male size and the presence of competitors for any of the behavioural variables (Table 2).

Figure 3.3: Behavioural variables during male guppy mating behaviour (mean+SE): a) the number of sneaky mating attempts; b) the number of sigmoid displays; c) the total (cumulative) time spent performing sigmoid displays; d) the total time spent attending females; e) the total time spent chasing females. In the No Competition (NC) treatment, the experimental male encountered a group of five females, whereas in the Competition (C) treatment, the experimental male encountered a group of five males and five females. Small denotes smaller male sibling, Large denotes the larger male sibling.

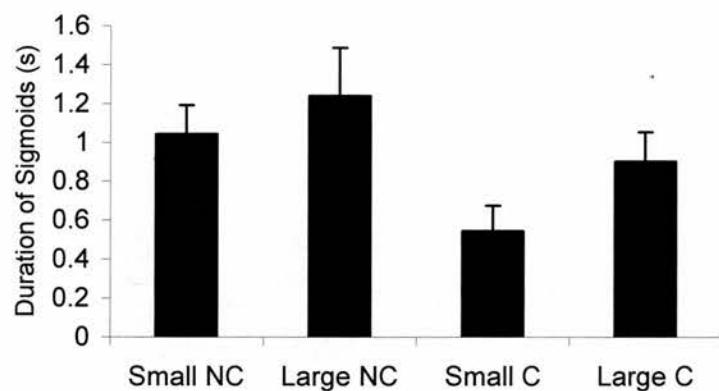
a)



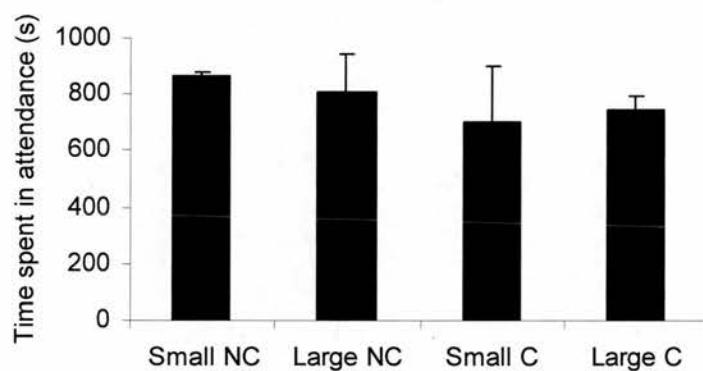
b)



c)



d)



e)

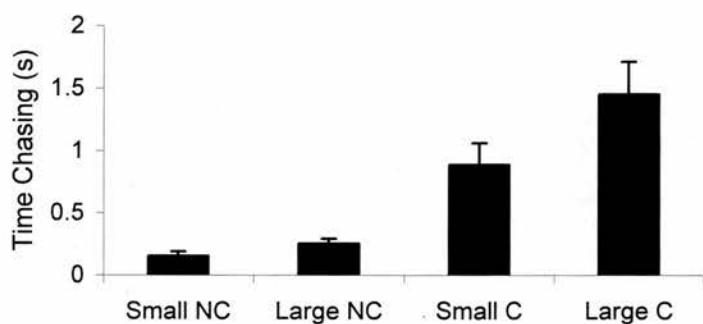


Table 1: Multivariate ANOVA of the combined set of five types of male mating behaviour (B, for details see Table 2) in relation to male size (S), and presence of male competitors (C). The model includes family effects (random) and full interactions, but these were all non-significant and are not shown here.

Source	Numerator	Denominator	F	p
	df	df		
Behaviour (B)	4	4	258.73	<0.0001
Size(S)	1	7	0.003	0.869
Competition(C)	1	7	11.04	0.013
B x S	4	4	6.64	0.047
B x C	4	4	41.18	0.002
S x C	1	7	1.41	0.274
B x S x C	4	4	1.29	0.406

Table 2: Separate ANOVAs of five types of male mating behaviour in relation to size (Size) and presence of male competitors (Comp). Sneaks denotes the number of sneaky mating attempts, sigmoid number the number of sigmoid displays, sigmoid duration (Sigmoid Dur) the total (cumulative) time spent performing sigmoid displays, attend the total time spent attending females, and chase the total time spent chasing females. Sneaking was log (X) transformed, and sigmoid duration and chase were log (X+1) transformed to meet model assumptions. The models include family effects (random) and interactions between family and Size, but these were all non-significant and are not shown here.

Behaviour	Source	df	F	p
Sneaks	Size	1,7	5.73	0.048
	Comp	1,7	35.47	0.0006
	Size x Comp	1,7	0.06	0.813
Sigmoid No	Size	1,7	1.33	0.287
	Comp	1,7	3.70	0.096
	Size x Comp	1,7	0.12	0.742
Sigmoid Dur	Size	1,7	0.43	0.531
	Comp	1,7	42.43	0.0003
	Size x Comp	1,7	0.33	0.586
Attend	Size	1,7	<0.01	1.000
	Comp	1,7	8.46	0.023
	Size x Comp	1,7	1.68	0.236
Chase	Size	1,7	9.54	0.018
	Comp	1,7	84.56	<0.0001
	Size x Comp	1,7	0.01	0.931

3.5 Discussion

These results demonstrate that experimentally generated male size variation has marked effects on male attractiveness as well as on male mating behaviour. By controlling for genetic background, the relative importance of a 15% difference in male body size was quantified and found to result in a significant female preference for the larger siblings in terms of time spent observing males, as well as in terms of the choice index. Further, the size difference had strong effects on the relative use and intensity of alternative mating behaviours. Larger siblings performed more sneaky mating attempts and spent significantly more time chasing females than smaller siblings. Interestingly, presence of male competitors made this pattern even more pronounced, indicating that the added competitors influenced mating intensity but not the relative use of alternative mating behaviours. Hence, this experimental design provided a precise quantification of how size variation between full siblings translates into male attractiveness and mating behaviour, adding a new way to investigate size-related effects on female choice and male mating behaviour in ectotherms (Andersson 1994; Gross 1996; Jennions and Petrie 1997). In particular, the technique is likely to be a valuable tool to evaluate selection pressures in systems such as the Trinidadian guppy, where population differentiation and local dynamics (e.g. Reznick *et al* 1996; Magurran 1998; Croft *et al* 2003; Pettersson *et al* 2004) generate size variation with potentially far-reaching effects on mating behaviour and sexual conflict (Magurran 1998; 2001).

Previous investigations of female preference for male size in guppies have yielded conflicting results. Although Reynolds and Gross (1992) and Karino and Matsunaga (2002) found that females preferred larger males, Endler and Houde (1995), who examined female preference for a variety of male traits (including size) in 11 guppy populations that ranged across drainages and predation regimes, could not detect a clear trend. There was no preference with respect to size in nine of Endler and Houde's populations, though females from one locality showed a preference for smaller males and there was a preference for larger males in another. This investigation (Experiment 1) identified a strong preference for larger males when male colour and genetic background is held constant. It would be interesting to determine whether this outcome is repeated in other populations.

Sneaky mating attempts more than doubled under competition as males devoted significantly more time to pursuing females (Experiment 2). This result is in line with Jirokul's (1999) finding that males sneak most at intermediate sex ratios and Farr's (1976) observation that sneaky mating tends to increase under competition. Unlike Farr (1976) however, no elevation in display rate was detected when males were competing and the total time spent displaying was lower when male competitors were present. Sneaking behaviours, while generally associated with smaller male poeciliids (Hughes 1985; Farr *et al* 1986; Travis and Woodward 1989; Ryan and Causey 1989) were not expected to show such a pattern in this case since it is relative size as opposed to absolute size of males that influences mating behaviour (Houde 1997). All males in this study, being individually reared, were large in comparison to stock males, so were not expected to be induced into undertaking 'small male type' behaviour. In fact it was larger siblings who invested most time in mating behaviour, including significantly more sneaky mating attempts, suggesting an additional size related factor at work. Finally, there was no interaction between male size and sneaking (or any other behaviour) indicating that brothers of different sizes respond in the same way to intrasexual competition.

This investigation shows that phenotypic manipulation can be successfully used to investigate mate preferences and mating behaviour. Rather than altering fully developed adult phenotypes (e.g. Andersson 1982, Karino and Matsunaga 2002), this approach benefits from involving the organism's developmental machinery and generally results in more thorough responses (Sinervo and Basolo 1996, Sinervo and Svensson 1998). As such, it adds to the growing number of study systems and topics investigated using phenotypic manipulation, e.g., life-history effects (Sinervo and Huey 1990); physiological performance (Pettersson and Brönmark 1999); growth strategies (Schmitt *et al* 1999); and secondary sexual characters (Ketterson and Nolan 1999). Of course, unless clones are used, genetic differences can never be completely controlled for, even among full siblings. However, the present approach drastically reduces these effects compared to studies with unrelated individuals. By further matching sibling pairs by colour pattern, confounding influences can be kept at a minimum.

Whenever phenotypic variation is generated by the experimenter, it is important to avoid correlated effects caused by the experimental treatment (Huey and Berrigan 1996, Rose *et al* 1996). In particular, early embryology may be sensitive to manipulations (Stearns and Kawecki 1994, West-Eberhard 2003). Pregnant females were therefore kept at an intermediate temperature until they gave birth, giving all juveniles a standardized temperature climate during embryological development (Takahashi 1975; Elphick and Shine 1998; Johnston 2001). At birth, juvenile guppies are developmentally advanced (Takahashi 1975, Veggetti *et al* 1993) but still retain a considerable flexibility in terms of muscle growth (Veggetti *et al* 1993). Subsequent manipulation of developmental temperature primarily affected muscle growth, with secondary sexual characters (e.g. male coloration, gonopodia) responding allometrically (Pettersson and Magurran pers. com.). To avoid behavioural effects of the difference in rearing temperature, siblings were transferred to an intermediate water temperature (25° C) after both males had ceased growing. They were kept at this temperature for a minimum of 3 months to allow for as complete acclimatisation as possible (Huey and Berrigan 1996). As the experimentally generated male size variation had similar influences on female choice in both cold-reared and warm-reared males, this indicated that acclimatisation had been sufficiently long and that correlated, confounding effects of the rearing regime played a negligible role in this system. It should be noted that males raised in the two different temperatures could potentially have experienced long-term effects on behaviour. However, this effect should typically have been the opposite of what we did observe in the experimental trials (e.g. Elphick and Shine 1998, O'Steen and Bennett 2003). A high temperature may induce persistent increases in ectotherm activity (Elphick and Shine 1998, O'Steen and Bennett 2003), but in the present experiment, the smaller males from the 29° C treatment were equally or less active than their larger siblings. Hence, by not disturbing critical stages of early development and by allowing for a thorough acclimatisation after experimental manipulation, this approach allowed the generation of phenotypic variation while keeping other factors controlled (Sinervo and Svensson 1998), a methodology which is likely to be a valuable tool for future studies of behavioural interactions among ectotherms.

This experiment suggests that, all other things being equal, being large is a win-win situation. Not only are large males more attractive to females; they also engage in

more mating activity. This latter observation may be a result of increased stamina in larger fish. In laboratory swimming trials, larger males can swim for longer at higher water velocities (personal observation) and can presumably also maintain strenuous mating activities for longer (see Blake 1983). Nicoletto (1993) found that male display rate was correlated with sustained swimming performance. Furthermore, as Reynolds and Gross (1992) showed, larger males sire offspring with higher growth rates which results in these larger sons being preferred by females and larger daughters having increased reproductive output (Reynolds and Gross 1992), suggesting that condition dependent selection may be an important factor in male size determination. Integrating condition dependence with genetic effects will be a major challenge for future studies.

Chapter 4

Habitat Use Mediates the Conflict of Interest Between the Sexes *

4.1 Abstract

Males of many species have alternative mating behaviours and must make decisions over which behaviours to use in order to maximize reproductive success. Guppies have two alternative mating behaviours, display behaviour which facilitates female choice and sneak mating, a form of sexual coercion, which circumvents female choice. The choice of which behaviour to use and the circumstances in which behaviours are employed can be potentially costly to both sexes. In this study the influence of variation in water velocity on male mating behaviour is examined in nature and in laboratory conditions in which male size can be controlled for. Questions posed are first whether males use alternative behaviour when subjected to changes in water velocity and second if females have the potential to ameliorate male harassment by moving to higher water velocities where males may be excluded due to their smaller size. The results show that females do preferentially use higher available water velocities than males and that mating activity decreases with increasing water velocity. However, at higher water velocities males switch from displays to sneak mating behaviour, the behaviour type which most opposes female interest. Females thus have the potential to reduce, but not entirely escape, the detrimental effects of this sexual conflict by moving to higher water velocities.

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4.2 Introduction

Decision making is a ubiquitous part of life. Individuals must decide whether to mate or to forage, to fight or to flee, to sleep or to play. Within these broad categories decisions must be made concerning, for example, which food is edible, whether fighting is too risky or safe places to sleep. Male mating is a good example of a behaviour type in which a range of decision making possibilities exist. Males are typically selected to produce as many offspring as possible (Rowe 1994; Parker and Partridge 1998; Gavrilets 2000) and males of many species have alternative methods of achieving this (Gross 1996). Taxa as diverse as primates (e.g. Smuts and Smuts 1993, Eberle and Kappeler 2004); pinnipeds (e.g. Van Parijs *et al* 2003, Lidgard *et al* 2004), ungulates (e.g. Bro-Jorgensen and Durant 2003); rodents (e.g. Randall *et al* 2002); birds (e.g. Pilastro *et al* 2003a; Pryke and Andersson 2003; Duckworth *et al* 2004), anurans (e.g. Leary *et al* 2004); reptiles (e.g. Orell and Jensson 2003); arachnids (e.g. Andrade 2003, Elgar *et al* 2003) and insects (e.g. Foitzik *et al* 2002) all exhibit alternative mating behaviours. Amongst fishes alone there are a wide variety of alternative mating strategies (see Taborsky 1994 and Henson and Warner 1997 for reviews).

Alternative mating behaviours can be expressed at various levels and are influenced by many different factors. In some species alternative mating behaviours are determined at an early stage of development. Most salmon species, for example, have two alternative reproductive phenotypes which have very different morphologies (e.g. coho salmon: Gross 1991, Atlantic salmon: Gage *et al* 1995). The anadromous males are larger and court females while smaller parr males gain fertilizations by sneaking in to ejaculate while anadromous males and females spawn (e.g. Gross 1991, Gage *et al* 1995). Likewise the bluegill sunfish (*Lepomis macrochirus*) has two alternative reproductive phenotypes, parental and cuckolder males (e.g. Leach and Montgomerie 2000). Other species show ontogenetically variable mating behaviours. The blue-headed wrasse (*Thalassoma bifasciatum*) is a protogynous hermaphrodite (Warner *et al* 1975). Fish are born as females and females choose the largest, brightest males for mating. When for some reason the large male is no longer available, the next largest

fish, which is female, becomes male (Warner *et al* 1975). Interestingly, when the wrasse population is large, some fish are born as males and adopt a sneaky mating behaviour (Warner *et al* 1975) similar to the satellite (salmon) and sneaker (sunfish) males mentioned above. Other species exhibit plasticity in the expression of alternative mating behaviours (Gross 1996; Taborsky 1994; Henson and Warner 1997), in that all males can carry out the alternative behaviours at any time. The influences on the expression of alternative mating behaviours are many and varied. Male size plays a role in many species (e.g. guppy: Rodd and Sokolowski 1995; topi antelope: Bro-Jorgensen and Durant 2003; anolis lizards: Orrell and Jensson 2003; salmon: Aubin-Horth and Dodson 2004). Competition from other males can also be important (e.g. guppy: Jirotkul 1999; giant kangaroo rat: Randall *et al* 2002; topi antelope: Bro-Jorgensen and Durant 2003; orb-web spiders: Elgar *et al* 2003) as can female quality (e.g. rock sparrows: Pilastro *et al* 2003a, guppy: Ojanguren and Magurran 2004) and male condition (e.g. guppy: Abrahams 1993; house finch: Duckworth *et al* 2004; toads: Leary *et al* 2004).

The guppy is one species in which males can choose between alternative mating behaviours (Liley 1966, Farr 1989) and that choice can be potentially costly for females as well as males. Males have two methods of achieving a copulation. They can undertake a courtship display in which the male extends his fins and forms his body into a characteristic sigmoid shape in front, or to the side, of a female (Liley 1966, Farr 1989) facilitating female mate choice. However, the alternative method, gonopodial thrusting or sneaky mating, is a form of sexual coercion that circumvents female choice (Magurran 1996, 2001). This involves the male swimming behind the female and attempting to insert his gonopodium into the female's genital opening by force (Liley 1966, Farr 1989). The decision over which mating behaviour to employ can be potentially costly for both sexes. Females are constantly harassed by males, receiving approximately one sneaky mating attempt per minute in high predation sites (Magurran and Seghers 1994a) which constrains their foraging behaviour leading to potential fitness costs (Magurran and Seghers 1994b) and may indirectly increase their risk of predation (Magurran and Seghers 1994a). Males must also bear these costs. Courting females is incompatible with foraging (Abrahams 1993) and there is

evidence that display behaviour makes males more susceptible to predation (Luyten and Liley 1985; Endler 1987; Reynolds 1993; Reynolds *et al* 1993). In addition male mating decisions may lead to sexual conflict scenarios due to the discrepancy between male and female interests (Smuts and Smuts 1993; Rowe 1994; Parker and Partridge 1998; Gavrilets 2000; Magurran 2001).

An important environmental feature for all stream dwelling organisms is water velocity and guppies are no exception. Guppies inhabit the many rivers of Trinidad's Northern Range where water velocity varies considerably (Magurran and Phillip 2001) so that individuals must be adapted to deal with large scale seasonal changes (Lytle and Poff 2004) and can experience smaller scale local variation (Moss 1998). Some aspects of the effects of flow rate on mating behaviour have been examined. Males raised in higher velocities spend more time undertaking courtship displays than low velocity males (Nicoletto 1996). Nicoletto and Kodric Brown (1999) took this one step further and found that wild males inhabiting sites with a higher flow regime swim significantly faster and have higher display rates. However, the influence of a fluctuating velocity environment on male mating decisions has yet to be examined.

Unlike more sedentary species fish are able to choose which flow regimes to inhabit and can switch between them as circumstances dictate. For example, Harding *et al* (1998) showed that rainbow darters, *Etheostoma caeruleum*, preferentially inhabited lower water velocity refugia. Other studies have found ontogenetic shifts in water velocity use in relation to flow rate. For example, young (age 0) bull trout, *Salvelinus confluentus*, are more frequent in low velocity water near stream margins (Polacek and James 2003) whereas in a New Zealand freshwater fish, the giant kokopu, *Galaxias argenteus*, small fish tend to occur in shallow backwaters adjacent to fast flowing water and larger individuals are most common in slower flowing pools (Whitehead *et al* 2002). In the guppy sexual dimorphism suggests that size related differences in the use of the water velocity profile allows larger females, who are constantly harassed by males seeking to mate with them (Magurran and Seghers 1994a), to use faster flowing water to escape the attention of smaller males. If this is the case males will face decisions over the optimal behaviours to employ at different

water velocities. Mating behaviour is likely to be a strenuous activity as suggested by the positive correlation between sustained swimming performance, which is a measure of general condition and vigour, and increased mating activity (Nicoletto 1993, 1996). It follows that displaying in higher water velocities is likely to mean increased energy expenditure. Additionally, sigmoid displays could be more energetically expensive than sneak mating as they involve spreading the body out making it more water resistant and displaying in front of females who often orientate themselves rheotactically. Therefore we hypothesized that males choose to decrease the overall intensity of mating behaviour with increasing water velocity, and specifically that the frequency of display behaviour decreases faster than the frequency of sneaks as water velocity increases. Conversely females, who are targets of male sexual coercion, may be able to escape this constant attention by moving into areas of higher water velocity where males may be excluded due to their smaller size, and anyway should be less able to harass females, leading to the hypothesis that females preferentially use higher water velocities than males.

We first examined these hypotheses in a field experiment. However, as with all field studies, we found it impossible to control all variables. Besides the many potentially confounding environmental variables, male size may affect male performance at different water velocities. Larger males are likely to be stronger so perhaps can endure higher water velocities than their smaller conspecifics; indeed it is larger males that tend to be found in faster flowing areas (personal observation). Male size could not be quantified in the field. Therefore a further laboratory based experiment in controlled conditions was undertaken in which the male decision making hypothesis could be further tested and which allowed the incorporation of male size.

4.3 Methods

4.3.1 Field Study

The Arima River flows approximately North-South through Trinidad's Northern Range. River width varies considerably from approximately 1 to 10m while depth varies from a few centimetres to over one meter. A survey was first carried out to find sites inhabited by guppies and where observations were possible. This identified seventy-two sites along approximately 2km (Trinidad 1: 25,000 Topographic Maps 1971: Grid references: 686396 to 686837) of river such that sites were no closer than 5m to avoid the possibility of observing fish more than once. At each site virtual quadrats were formed by planting thirty-six plastic rods, approximately 50cm long, in a grid pattern so that a square of 1.25m per side was created. Each side was made up of 6 rods placed 25cm apart forming a virtual grid 5x5 grid of 25cm² squares with a rod at each junction. This set up allowed fish to behave as normal while being able to record where behaviour occurred. The location of grids varied across the width of the river from marginal to central. In narrow areas the grid took up the entire width of the river. Areas of riverbank and fallen obstacles were incorporated into the grid area. Water depth within grids varied from zero to 80cm. In the following text 'site' signifies area of river and 'grid' represents the entire experimental set up made up of 25 'squares'.

Sites were observed randomly to avoid any systematic time or location effects. A 15 minute wait followed placement of a grid to allow fish to recolonise the site and for normal behaviour to resume. Of the fish available in each grid focal watches of up to 5 males, identified primarily by colour pattern and to a lesser extent by size and position; and 3 females, identified primarily by size with contributions from position and colour, were carried out for 2 minutes or until the fish left the grid or could no longer be identified (mean ± se: 49 ± 1.67 s). Care was taken to ensure selected males did not interact with each other though they did interact with females. Recorded variables were time spent in each square within the grid for both sexes with the number and position in the grid of sigmoid displays and sneak mating attempts also

recorded for males. After observations water velocity was measured in the centre of each grid square between 5 and 10cm from the substrate, or midway between surface and substrate for depths less than 10cm. Two readings, each averaged over 10 seconds to an accuracy of 0.01 ms^{-1} were taken using a Höntzsch flow measuring instrument $\mu\text{P-TAD}$. These were combined into one measure per grid square by taking the mean of the two readings giving 25 equidistant water velocity measures per grid.

To quantify sex differences in water velocity use, a single water velocity score for males and for females in each grid was obtained. Only those grids in which both male and females were found were included. For each sex in each grid, water velocity was multiplied by the time spent in that water velocity. This was totalled and divided by the total time for that sex in that grid. Normality was achieved with square root transformations and variances were equal so analysis was by paired t-test with water velocity as the dependent variable and sex as the factor. To assess differences in male behaviour in different water velocities a single water velocity score was obtained for each individual male for each type of mating behaviour (displays and sneak mating attempts). Individual males rather than grids could be used as the experimental unit in this analysis as males did not interact with, and therefore did not influence, each other. This score was achieved by multiplying the frequency of behaviour by the water velocity in which that behaviour occurred, totalling this for each individual then dividing this total by the total frequency of that behaviour for that individual. These scores were rounded to the nearest 0.5 cms^{-1} to allow frequency of occurrence of each behaviour at the different water velocities to be calculated. Separate Spearman rank correlations were carried out for each behaviour, with water velocity forming the independent variable and the frequency of behaviour as the dependent variable.

4.3.2 Laboratory Study

The fish used for this study were descendants of wild caught Lower Tacarigua fish that had been maintained in the lab for several generations. A 1 liter Blazka-type flume (Peake and McKinley 1998) was used for this study. As fish were unable to leave the test chamber, so could not move between water velocities, only male

behaviour was concentrated on in this experiment. Prior to the experiment males and females were maintained in separate tanks for 2 weeks so that males were more motivated to carry out mating behaviour. Males were divided between two tanks to ensure no familiarity between males that were in the flume together. Five water velocities were chosen, 0, 3, 7, 11, and 15 cm^{-2} , which cover the range found in field observations of where fish occurred and could carry out mating behaviour. Ten males were observed in each water velocity giving fifty trials in total. For each swimming trial 3 females taken from the pool of 30 and 2 males, one from each male tank, were placed in the flume test chamber. This sex ratio was chosen to mimic field conditions, to ensure that there was always a female available for the focal male to interact with and because guppies tend to be more settled with a few individuals. Males were used only once so that there was no familiarity with the equipment. The order of water velocities was randomised. The fish were allowed to settle for 10 minutes during which water flow rate was gradually increased to the pre-determined rate for that trial. In the case of 0 cm^{-2} the power was switched on to allow the Archimedes screw to turn slowly so that these fish were subject to as similar noise conditions as possible to those swimming at faster flow rates. One male was selected as the focal individual and observed for 10 minutes. As before, the frequency of sneak mating attempts and sigmoid displays were recorded. As displays may be of any duration, an additional measure, the total duration of displays, was also included as this could be affected by changes in water velocity. After the trial, females were returned to the pool and the stock male to a holding tank. The experimental male was placed in a petrie dish containing just enough water to cover him and photographed using a Nikon Coolpix 990 digital camera. Male length was measured from these images using the image analysis software ImageJ (ImageJ 1.27z, National Institutes of Health, Bethesda, MD, U.S.A.). Lengths were taken 3 times to ensure no systematic measuring error and the mean of these measurements was used for analysis. A mean display duration was obtained by dividing the total duration by the frequency of displays. The variation in frequency of sneaks, frequency of displays and mean duration of displays with different water velocities were tested separately with one-way analyses of covariance including male length as a covariate. Normality was achieved for frequency of displays and frequency of sneaks by performing ($\log + 1$) transformations. Variances

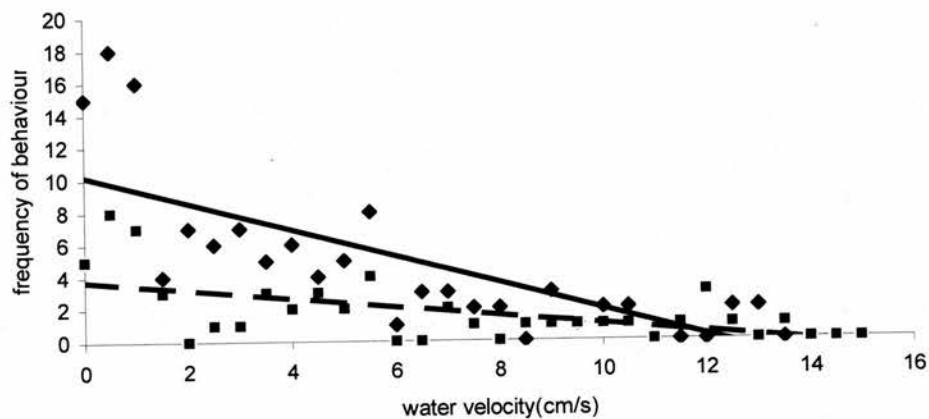
were equal. Further analyses of covariance assessed variations in total behaviour and proportions of behaviour with water velocity.

4.4 Results

4.4.1 Field Study

The water velocities in the study areas ranged from 0 to 49cms^{-1} , this also being the widest range of velocities found in a single grid. Of the 284 males observed 124 carried out displays and 53 attempted sneak mating. Only 29 males carried out both behaviour types during the observation period. The maximum velocity at which sneak mating occurred (16cms^{-1}) was higher than that at which displays were carried out (13cms^{-1}) while the minimum was the same for both behaviours (0cms^{-1}). One hundred and eighty eight females were observed. The maximum water velocity at which females were found (25cms^{-1}) was slightly higher than that at which males were found (24cms^{-1}) while the minimum water velocity was the same for both sexes. The difference between male and female water velocity use at each site was highly significant (t-test: $t_{1,69}=-3.08$, $p<0.003$) with females using the higher water velocities of those available (females: $4.63 \pm 0.438 \text{ cms}^{-1}$, males: $4.04 \pm 0.400 \text{ cms}^{-1}$ (mean \pm se)). The effect of water velocity on male mating behaviour was equally notable. The frequency of displays per unit time decreased as water velocity increased, with water velocity accounting for over 60% of the variation in display rate ($r_s=0.794$, $n=30$, $p<0.001$, $r^2=0.630$) (figure 4.1). The frequency of sneak mating attempts likewise decreased significantly with increasing water velocity ($r_s=0.617$, $n=30$, $p<0.001$, $r^2=0.380$) (figure 4.1).

Figure 4.1: The frequency of water velocities in which display behavior (diamonds) and sneak mating attempts (squares) occurred from the field study. Each point represents a single score for each male. Regression lines: displays-solid line, sneaks-dashed line.

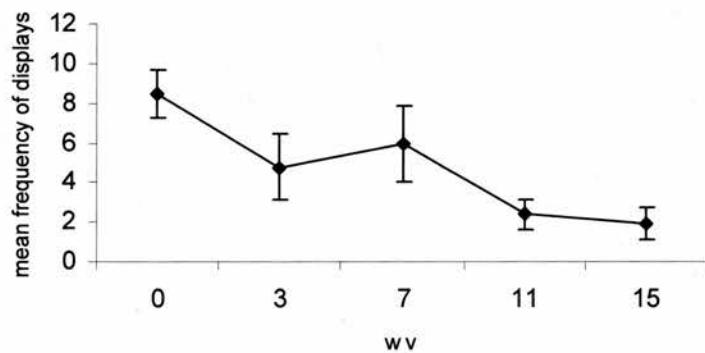


4.4.2 Lab Study

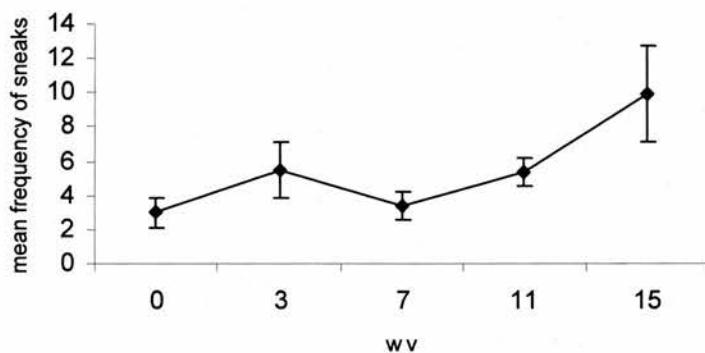
When the frequency of behaviour rather than frequency of water velocity in which behaviour occurred (as in the field study) was examined the results are similar to those of the field study though with an interesting addition. Both the frequency of displays (ANCOVA: $F_{4,44}=3.61$, $p=0.012$) (figure 2a) and duration of displays (ANCOVA: $F_{4,44}=6.32$, $p=0.016$) (figure 2b) decrease significantly with increasing water velocity. However, in contrast to the field results, in the controlled conditions the frequency of sneak mating attempts actually increases with increasing water velocity (ANCOVA: $F_{4,44}=2.84$, $p=0.035$) (figure 2c). In fact, the total frequency of mating behaviour remains approximately the same in all conditions (ANCOVA: $F_{4,44}=0.65$, $p=0.630$) but the proportion of sneak mating attempts increases (and the proportion of displays decreases) with increasing water velocity (ANCOVA: $F_{4,44}=6.06$, $p=0.001$). Male length is a significant covariate for only the duration of displays (ANCOVA: $F_{1,44}=6.46$, $p<0.001$). For both the frequency of sneaks (ANCOVA: $F_{1,44}=3.24$, $p<0.079$) and the frequency of displays (ANCOVA: $F_{1,44}=2.53$, $p<0.119$) length is not a significant covariate at $\alpha=0.05$.

Figure 4.2: Behaviours from the lab study: a) frequency of displays, b) frequency of sneak mating attempts, c) mean duration of displays. Each point represents the mean of the ten males examined at that water velocity (cms^{-1}). Errors bars represent se of mean.

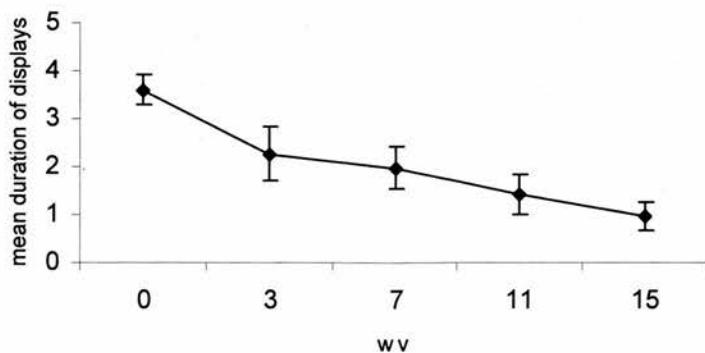
a)



b)



c)

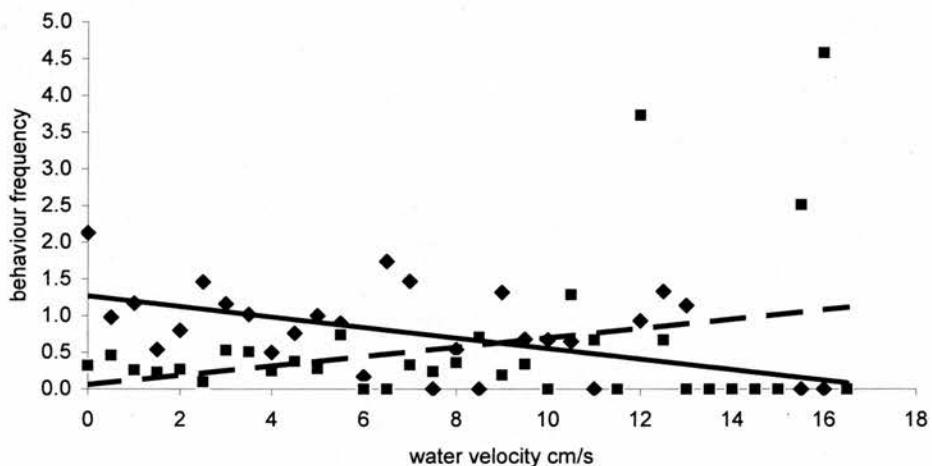


4.5 Discussion

As predicted, water velocity influences male mating behaviour. In the controlled lab study, the intensity of mating behaviour remained approximately the same with increasing water velocity with the significant decrease in the frequency of displays being compensated for by the significant increase in the frequency of sneak mating attempts. In contrast, and in agreement with the hypothesis, the field study shows both behaviour types decrease with increasing water velocity with display behaviour decreasing at a faster rate. These apparently contradictory results can be accounted for by the different conditions in the two studies. In field conditions, where males (and females) are not restricted to only one part of the water velocity profile, males are able to wait until displaying becomes more feasible. In laboratory conditions, fish were unable to move between water velocities so males switch to a behaviour type that is probably less energetically demanding. In fact, when the male behaviour results from the field are amalgamated across all sites a similar pattern to the lab study is evident, with display behaviour decreasing as water velocity increases while the frequency of sneak mating attempts remains approximately constant (figure 3). Unfortunately however, the problem of maintaining independence of data means no statistical analysis was possible for this effect; individual males would have to be included in more than one (and in some cases up to ten) data categories leading to pseudoreplication (Hurlbert 1984). In addition the presence of predators in the wild is likely to affect both male and female behaviour (Seghers 1973).

Male guppies continue to carry out mating behaviour in faster flowing areas of the water velocity profile. The ability to maintain even a small amount of courtship behaviour in higher water velocities may have indirect fitness benefits. Guppies from faster flowing site populations (Nicoletto and Kodric-Brown 1999) or raised in faster flows (Nicoletto 1996) can swim faster and have higher display rates. Females prefer males with higher display rates (Farr 1980; Bischoff *et al* 1985; Nicoletto 1993, 1996) and higher sustained swimming speeds (Nicoletto 1993, 1996) indicating that males that display in higher velocities may be demonstrating their superior condition, as has been seen in other species. In the stream goby, *Rhinogobius brunneus*, for example,

Figure 4.3: The frequency of behaviors in the field study. The results for all males at all water velocities are shown, each point representing the frequency of displays (diamonds) or sneak mating attempts (squares) for each males in each water velocity. Regression lines: displays-solid line, sneaks-dashed line.



the maximum speed of the current in which a male courts should be indicative of his quality and of the subsequent survival of eggs under his care. Females use male courtship display in the water current as an honest indicator of parental quality (Takahashi and Kohda 2004). For guppies, it could be expected that larger, stronger males benefit most from this female preference as males with increased muscle development have longer prolonged swimming speeds and more intense displays (Nicoletto 1996) and it was larger males that were most often seen in higher velocity areas (personal observation). Therefore it would seem that selection favours larger, stronger males. However, there are costs associated with higher water velocities. Predators favour higher water velocities (Seghers 1973) and males, being brightly coloured, are likely to attract the attention of predators more than the dull coloured females, particularly larger (Reynolds 1993, Reynolds *et al* 1993) and displaying (Luyten and Liley 1985; Endler 1987; Reynolds 1993; Reynolds *et al* 1993) males, so mating behaviour in faster areas of the velocity profile can be a risky decision.

Two further potential reasons for the decrease in display activity found in both studies involve the energetic cost of higher water velocities. Display behaviour is likely to become more energetically expensive as water velocity increases as suggested by the positive correlation between sustained swimming performance, which is a measure of general condition and vigour, and increased display intensity (Nicoletto 1993, 1996). A male will have to devote more of his energy budget to swim against the current at higher velocities leaving less available for displaying, in a similar manner to that of foraging juvenile Atlantic salmon, for example, who reduce reaction and attack distances in response to increasing current velocity (Godin and Rangeley 1989). Furthermore, females' rheotactic positioning in faster flows (personal observation) means that in order for a male to display in front of a female he would have to position himself perpendicular to the direction of water flow. This, combined with the mechanics of courtship displays with fins extended and body contorted into a sigmoid shape, will greatly increase the male's surface area and therefore his water resistance potentially further increasing energy expenditure and making it more likely for the male to be swept downstream away from the female he was courting. Male guppies,

however, are enthusiastic in their pursuit of females so at higher water velocities that make displaying more difficult a switch to their alternative mating behaviour is expected, as is shown in the lab study. Conversely, it may be that females swimming in faster currents are less able to avoid sneak matings and males may just exploit this. This interesting result has potential implications for sexual conflict.

Sexual conflict can occur whenever characteristics that augment the reproductive success of one sex are detrimental to the fitness of the other (Smuts and Smuts 1993; Rowe 1994; Parker and Partridge 1998; Gavrilets 2000; Magurran 2001). It arises because one sex, typically males, is selected to produce as many offspring as possible while the other sex, usually females, invest their reproductive effort into producing fewer high quality offspring. Sexual conflict can occur before, during and after mating. In the context of this study, for the guppy pre-mating sexual coercion in the form of sneak mating attempts circumvents female choice leading to potential reproductive costs. The functional consequences to females are not just reproductive. Harassment by males can leave females more vulnerable to predation (Shine *et al* 2000, Arnqvist and Rowe 2002) and reduce foraging efficiency (Pilastro *et al* 2003b). Coercion from males seeking to copulate can be physiologically stressful to females (e.g. Shine *et al* 2004) and in some cases extremely aggressive, sometimes resulting in injury or even death to females (e.g. non-human primates: Smuts and Smuts 1993). In the guppy potential fitness costs are incurred through sexual harassment constraining female foraging behaviour (Magurran and Seghers 1994b), which may also indirectly increase their risk of predation (Magurran and Seghers 1994a). However females are not totally helpless in the face of this male aggression. Female counterstrategies have evolved which allow females to avoid harassment or ameliorate the effects of sexual coercion. Avoidance techniques can involve gaining protection from other individuals (Pilastro *et al* 2003b) or simply moving away from males.

Thus in order to provide evidence for sexual conflict, in addition to the effects on males shown above, it is also necessary to show a corresponding effect of water velocity on female use of the water velocity profile. As predicted, females did indeed use a higher mean water velocity of those available within sites than males, presenting

indirect support for the hypothesis that females use areas of higher water velocity to escape the constant attention of males. Sexual dimorphism in size and microhabitat use has been demonstrated in other taxa, for example the pygmy grasshopper, *Tettix subulata* (Forsman and Appelqvist 1999). In several species of blennioid fishes there is sexually dimorphic and ontogenetic variation in microhabitat use, with smaller juveniles and females residing in the open while males prefer shelter (Hastings 2002). In the guppy, this sexual dimorphism in the use of the water velocity profile is just one of a wide range of behavioural differences between the sexes (for a review see Magurran and Macías-Garcia 2000).

The maximum water velocities used in the wild were similar for each sex indicating that males are capable of swimming in higher water velocities. However, it did tend to be larger males who were found in these areas (personal observation). There are three potential compatible explanations for this. First, males are so eager to pursue females that they will follow females even when there is only a small chance to mate with them. Second, it may be that females are also constrained by higher water velocities making them less able to avoid males who take advantage of this, or there is a further reason for male and female movement into higher flows. One possibility is that guppies, though mainly considered to be benthic feeders (Dussault and Kramer 1981) can also undertake drift feeding and higher flows increase the likelihood of encountering food items (Moss 1998). In fact, observations of guppies in higher water velocities in their natural environment suggest that this is exactly what they are doing (personal observation).

For aquatic species water velocity is likely to be of primary importance and numerous studies have addressed this topic. However, the influence of flow rate on mating behaviour has received surprisingly little attention. Many studies focus on the physiological differences (e.g. Jennions and Kelly 2002, Odell *et al* 2003), while of those that do examine behavioural aspects of water velocity variation the vast majority focus on swimming performance (e.g. Peake *et al* 1997; Ward *et al* 2003; Pedersen and Malte 2004). One study in which mating behaviour is examined looks at the river blenny, *Salaria fluviatilis*, which builds nests with entrances that are

progressively closer to the direction of flow as water speed increases (Vinyoles *et al* 2002) and in the stream goby, *Rhinogobius brunneus*, females prefer males who court in faster currents probably because such males are demonstrating their superior physical condition (Takahashi and Kohda 2004). Similarly in the guppy, *Poecilia reticulata*, the most studied aspect of the influence of water velocity on behaviour has been swimming performance (Nicoletto 1991, 1993, 1996; Nicoletto and Kodric Brown 1999). As this study has demonstrated, water velocity influences far more than just swimming ability.

In conclusion, by moving to higher water velocities where males are excluded due to their smaller size, females can ameliorate the effects of male sexual coercion. However, this is just one side of the story. The maximum water velocities used were similar for each sex so at least larger males are capable of swimming in higher water velocities. Furthermore, in higher water velocities males have the option of switching to sneak mating, which is anyway the behaviour that is conflicting most with female interests as it is irreconcilable with female choice. While females have the potential to use the variation in the water velocity profile to avoid some male harassment, they cannot escape from the battle of the sexes. Males still make the decision to approach females in higher water velocities, despite the potential energy and predation costs (to which females will also be vulnerable). It seems then that the fitness benefits to males of carrying out mating behaviour in varying flow regimes outweigh any associated costs.

Chapter 5

Mate Choice, Sexual Coercion and Gene Flow in Guppy Populations

5.1 Abstract

Several mechanisms can regulate gene flow between populations. Female choice, male and female mating behaviour and post-copulatory mechanisms, such as sperm competition and cryptic female choice, are all potential influences. Guppy, *Poecilia reticulata*, populations in Trinidad are subject to differing environmental conditions, particularly differences in predation level, which produce differences in morphology and behaviour. Moreover, pre-copulatory female choice, varied male mating behaviour and sperm storage provide arenas for gene flow mediating mechanisms. Here, the potential for pre-copulatory mediation of gene flow is assessed via female mate choice and male mating behaviour trials using fish from pairs of populations that differ in predation regime. Population differences in male morphology, upon which female mate choice is partially based, are explored. Finally, the reproductive success of males from different populations is examined, via microsatellite DNA analysis, to ascertain prospective post-copulatory gene flow regulating mechanisms. In one population pair, females from the high predation population are shown to prefer larger, more colourful males from the corresponding low predation population. Males from this high predation population carry out more sneak mating attempts, the mating behaviour that is most in conflict with female interests. Despite this female preference, high predation males achieve greater reproductive success than their low predation counterparts. The other population pairing shows a different pattern with no female preference in relation to population, no distinction between males in their mating behaviour, minor differences in male morphology and no difference in mating success between males from the two populations. The evolutionary implications in terms of population differences, sexual conflict and gene flow are discussed.

5.2 Introduction

A population is defined as a group of individuals of a species in one area (Begon *et al* 1996). Geographically distinct populations often have a different morphology and/or behaviour, reflecting adaptations to different environmental conditions (Krebs and Davies 1993). Gene flow nonetheless occurs and populations remain part of the same species (Begon *et al* 1996). Thus species are composed of local populations connected to each other by a recurrent exchange of migrants which link local populations genetically (Roughgarden 1979): a view encapsulated in the metapopulation concept (Begon *et al* 1996). Arguably the most obvious influence on this gene flow is the motility of individuals within a species. However, assuming individuals can meet, various other factors may also play a part. Mechanisms that affect gene flow may occur before, during or after copulation and may be male or female mediated. A pre-copulatory mechanism is mate choice, particularly female mate choice. Females of a given population may, in general or individually, prefer certain phenotypes (Jennions and Petrie 1997, Ptacek 2000), the corresponding genotypes of which would be preferentially passed on to the next generation. Mating behaviour is another potential gene flow determining pre-copulatory mechanism, particularly in species with alternative mating behaviours. Depending on the prevailing environment different behaviours may have more chance of resulting in fertilization of gametes (Andersson 1994, Henson and Warner 1997). During or just after copulation other processes can occur. From a male perspective, sperm competition can have a substantial influence on gene flow (Birkhead and Möller 1992) while from a female standpoint cryptic female choice can be equally significant (Eberhard 1996). Finally, for those species with parental care, parents may be able to exhibit post-copulatory control on gene flow through preferential treatment of some offspring (Clutton-Brock 1991).

The guppy, *Poecilia reticulata*, is an ideal model species with which to investigate gene flow mechanisms. Guppies inhabit the many rivers of Trinidad's Northern Range so various geographically isolated, but potentially capable of interbreeding, populations exist in the different rivers (Magurran and Phillip 2001). Within these broad boundaries further populations are differentiated within rivers, usually separated by a barrier such as a waterfall, traditionally designated high predation and low predation populations in reference to the prevailing predation regime (Haskins *et*

al 1961). These populations differ in morphology (e.g. Houde 1988b; Nicoletto and Kodric-Brown 1999; Reznick *et al* 2001) and behaviour (e.g. Luyten and Liley 1985; Endler 1987; Magurran and Seghers 1990; Evans and Magurran 1999). There are two compatible reasons for this behavioural divide. High predation populations are subject to higher, and more varied, predation risk. Displaying advertises male presence not only to females but also to predators, so a switch to the less conspicuous sneaking behaviour is prudent (Luyten and Liley 1985; Endler 1987; Magurran and Seghers 1990; Evans and Magurran 1999). Moreover, high predation habitats are characterised by higher turbidity (Reznick *et al* 2001) in which visual components of courtship are less apparent so, again, sneak mating is the more beneficial tactic in these environments (Luyten and Liley 1985).

Migration in the guppy is known to occur (Haskins *et al* 1961; Endler 1977; Reznick *et al* 1996; Houde 1997; Croft *et al* 2003). Haskins *et al* (1961) showed that an introduced marker phenotype travelled 9.8km within 16 months suggesting a migration rate of 0.75km per generation (Endler 1977) so over relatively few generations encounters between populations are likely. Indeed one, human facilitated, migration event has been well documented. Genetic material from fish introduced by Caryl Haskins in 1957 into the Turure River, above a barrier waterfall, has been shown by allozyme (Shaw *et al* 1992) and mitochondrial DNA (Becher and Magurran 2000) studies to be present in fish below this barrier. Guppies, moreover, are potentially subject to a range of gene flow regulating mechanisms. Females exhibit pre-copulatory mate choice and both population (Endler and Houde 1995, Godin and Briggs 1996) and individual (Godin and Dugatkin 1995, Brooks and Endler 2001) preference functions have been described. Males have two alternative mating behaviours (Liley 1966, Farr 1989), sigmoid displays which facilitate female choice (Liley 1966, Farr 1989) and sneak mating which circumvents female choice (Liley 1966, Farr 1989). Additionally, females' ability to store sperm for several months (Houde 1997) and evidence that sperm from more recent matings preferentially fertilizes ova over stored sperm (Evans and Magurran 2001) provides the background for scenarios of sperm competition and/or cryptic female choice and recent work reflects this (e.g. Pilastro *et al* 2002; Evans *et al* 2003; Pilastro *et al* 2004).

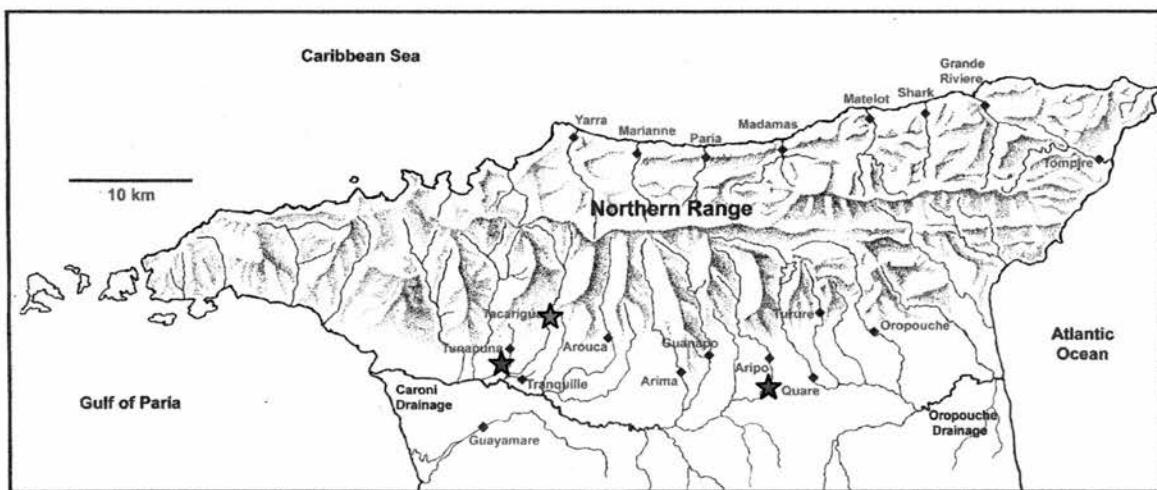
This study examines possible mechanisms for the regulation or facilitation of gene flow that may exist between related pairs of high and low predation guppy populations. Movement of guppies is predominantly male (Croft *et al* 2003) and upstream movement is limited by barrier waterfalls (Houde 1997). Therefore the effects of migration of low predation (upstream) males into high predation (downstream) populations are considered. First, female mate choice is investigated, testing the hypothesis that females prefer larger (Reznick *et al* 2001), more colourful (e.g. Houde 1997), low predation males, all traits demonstrated to be favoured by females (Endler and Houde 1995; Houde 1987, 1988, 1997; Farr 1980; Reynolds and Gross 1992; Magellan *et al* 2005). Next male and female mating behaviours are assessed: for males the hypothesis here, in agreement with earlier research (Luyten and Liley 1985; Endler 1987; Magurran and Seghers 1990; Evans and Magurran 1999), being that low predation males perform more sigmoid displays and high predation males undertake more sneak mating attempts, while for females, that their behaviour again favours the low predation males. Then male morphology is examined to confirm that these males do exhibit the morphological differences on which female choice is expected to be based. For female morphology the predictions are, in line with previous work (e.g. Houde 1997) that female length and weight will be correlated and that one, or both, of these variables can be used as a predictor for brood size. Finally, as low predation males undertake more display behaviour, the behaviour type shown to allow higher sperm transfer during copulation (Pilastro and Bisazza 1999) and also to be preferred by females (Houde 1997), paternity analysis is carried out to test the hypothesis that low predation males achieve a higher reproductive success than their high predation counterparts.

5.3 Methods

5.3.1 Experimental animals

All fish used in this study were pure lines descended from wild stock. Two pairs of populations were used: Upper Aripo (UA) and Lower Aripo (LA); and Tunapuna (Tun) and Lower Tacarigua (LT) (low predation and high predation populations respectively) (Figure 5.1). Males from all four populations and virgin females from Lower Aripo and Lower Tacarigua populations were maintained in separate tanks. All fish were fed artemia nauplii or commercial flake food once per day. Males were identified using coloured drawings. Fish were used first in female mate choice trials followed by assessment of male and female mating behaviour. Morphology for both sexes was gauged and finally those fish that produced offspring were subject to paternity analysis (Figure 5.2). The same families of fish (one female, two males, plus offspring) were kept together and used in all experiments.

Figure 5.1: Map showing the location of Lower Tacarigua (red star), Tunapuna (green star), Aripo (blue star) populations.



5.3.2 Female mate choice

Female choice of males was assessed following the method of Magellan *et al* (2005). Each of 50 Lower Tacarigua and 30 Lower Aripo virgin females was tested twice using one male from her own population and one from her paired population with males exchanging places for the second trial to ensure there were no side effects. The position of males in the first trial of a pair was randomised. The time spent in each male's zone was recorded as the measure of female preference for that male. Following completion of mate choice trials all three fish were transferred to a tank for the next phase of the experiment.

5.3.3 Male and female mating behaviour

To assess male and female mating behaviour fifteen 30x25x20cm tanks containing gravel, a filter and some java weed for cover were used. Immediately after fish were placed in the tank, observations commenced. Both males and the female were observed simultaneously. Recorded variables were: for males the frequency of sigmoid displays and the frequency of sneak mating attempts; for females frequency of glides and for males and females the frequency of consensual copulations. As male and female mating behaviour is likely to change with female receptivity (Liley 1966) and females are likely to become pregnant, and therefore non-receptive in around three days (Liley 1966) fish were observed over this length of time. Two opposing factors were also taken into account. First, females had just been involved in choice trials in which they may have selected a favoured male, and were likely to remember which male was selected (Magurran *et al* 1994; Griffiths 2003; Bhat and Magurran: in press) so immediate observation of behaviour was necessary. However, fish may react with caution to a new environment so a short amount of time to allow for acclimation was also necessary. For these reasons observations lasted for one hour divided into four 15 minute sections on day one, two 15 minute sections on day two and one 15 minute section on day three. Between observations fish remained in the tank together. Due to several reasons not all fish were examined on all three days; on day one 50 Lower Tacarigua and 30 Lower Aripo pairings were observed and by day three this had reduced to 46 Lower Tacarigua and 27 Lower Aripo pairings. Missing values were excluded from analyses. Following completion of mating behaviour trials males

were euthanased by immersion in benzocaine solution (Laird and Oswald 1975) ready for morphological examination while females were maintained in 2 litre plastic bottles containing gravel, a filtered air supply and some java weed until they produced a brood. All females and any broods produced were then euthanised by immersion in benzocaine solution ready for morphological examination.

5.3.4 Morphology

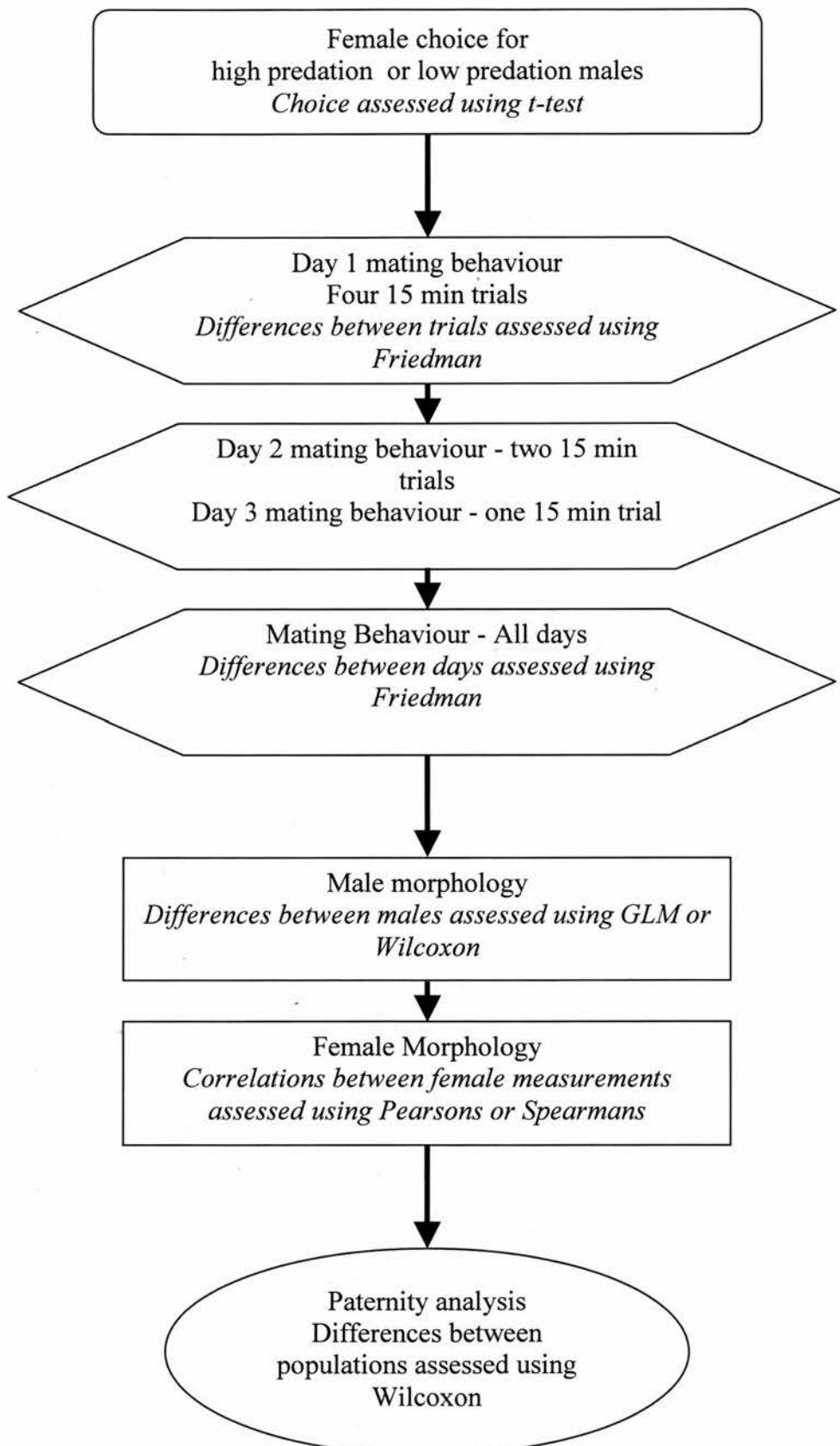
After euthanasia, both males and females were individually placed on a slide with a scale bar added to it and photographed using a Nikon 990 Coolpix digital camera. They were then weighed and placed individually into tubes containing 70% ethanol solution until ready for genetic analysis. Babies were euthanised, counted and placed in tubes with their mothers. Measurements were taken using image analysis software (ImageJ 1.27z, National Institutes of Health, Bethesda, MD, U.S.A.). For males, recorded variables were standard length (SL: from snout to caudal peduncle), gonopodium length and the size and number of orange and melanistic black spots. For females standard length was recorded.

5.3.5 Paternity Analysis

Paternity was determined via three polymorphic microsatellite DNA markers specifically designed for poeciliids. The first two loci were isolated by Parker *et al* (1998). Pooc-G49, (Genbank accession number AF026459) had a (GT)₆, GC, (GT)₄, GC, (GT)₇ repeat with primer sequences 5'-CAT AGA TTC TGC AGG CAG TG-3' and 5'-CTC AGT GAC TAT AAG GCC AAC-3'. Pooc-G10 (accession number AF026454) had a (GT)₁₀ repeat with primer sequences 5'-CTC ACT CGG TCT TCT AGC-3' and 5'-CAC TTG ACT GTG CCT GAC-3'. The third locus, isolated and characterised by J. S. Taylor and F. Breden (unpublished; accession number AF164205), had a core (TAA)_n repeat, with primer sequences 5'-GTG ACC GAA CGA AAG GAT A-3' and 5'-CCC CAA AGG AAC ACT GTA-3'. Genomic DNA was extracted from whole body tissue of offspring using a rapid one-tube extraction method (Gloor and Engels 1992, Estoup *et al* 1996), while adult DNA was extracted from tail and peduncle tissue and followed the cell lysis method (Sambrook 1989).

Polymerase chain reaction (PCR) amplifications were performed on a PTC-100 thermal cycler (MJ research Inc.). Reaction volumes of 15 μ l for the first, G49, locus consisted of 1 μ l DNA template, 0.4 μ l of each 10mM dNTP, 1.5 μ l 10x Taq buffer (16mM (NH₄)SO₄), 0.45 μ l 50mM MgCl₂, 0.05 μ l of each primer pair (50 pmol μ l⁻¹) and 0.06 μ l Taq polymerase (Bioline). Thermal cycles consisted of 3 min at 92°C followed by 31 cycles of 92°C for 30s, annealing at 63.1°C for 30s then 72°C for 30s. A terminal extension step of 5 min at 72°C followed by a 1 min cooling step to 10°C completed the program. The protocol for G10 was identical with the exception of the annealing temperature, which was 55°C and TAA had 26 cycles and an annealing temperature of 52°C. PCR products were resolved on 6% polyacrylamide gels (Sambrook 1989) and paternity was assigned following sequential assessment of allele sharing (G49, then G10, then TAA) between potential sires, mother and offspring.

Figure 5.2: Flow chart showing the sequence of steps for experimental procedures for males and females. Statistical analyses are shown in italics.



5.3.6 Statistical Methods

Analyses were carried out in three stages. First the entire data set for both population pairs was examined together then each population pair was examined individually. Figure 5.2 shows the sequence of analyses. Missing values were excluded from analyses.

In the analysis of dichotomous female choice, as time spent with one male affects the time available to spend with the other it is important to take this effect into account (Houde 1997, Wagner 1998). One approach is to directly quantify the significance of the statistical dependency by correlating the time spent with male A with the time spent with male B which will highlight any negative covariance (Magellan *et al* 2005). However, in this case negative covariance was evident in at least one set of dichotomous choice test results ($r_p=-0.503$; $df=28$; $p=0.005$) so an alternative approach was used in which the difference in time spent with each male was taken and standardized by the total amount of time spent near the males (e.g. Houde 1997, Stoner and Breden 1988). This was done for each data set (i.e. set 1 with male A on side A, set 2 with male A on side B). As there was no difference between these two sets ($t=-1.206$ to 1.762 , $p=0.084$ - 0.689 , df -see table 5.1) the means of the respective pairs of data were taken and used in one sample t tests compared to a mean of zero.

To assess differences in the frequency of displays and sneak mating attempts for males first the four 15-minute results for day 1 were examined using Friedman tests to check that behaviour was consistent over time. Consistent values were then combined as mean frequencies of behaviour over 15 minutes. Day 2 results were divided by 2 to give mean frequencies of behaviour over 15 minutes, then the results for all three days were compared. Again consistent results were combined as a mean and these means were used in Wilcoxon signed rank tests to establish differences in behaviour between populations. For the frequency of glides the majority of glides occurred on day one, presumably due to females' being at their most receptive at this time, so only these results were used in analyses. Again, the four results for day one were assessed for consistency using Friedman tests and appropriate means formed for use in Wilcoxon signed rank tests. The frequency of copulations was too low to allow any meaningful analysis.

To assess male morphology for those variables with normal distribution and equal variances a general linear model was used with both predation background and population pair as independent variables. For non-parametric variables Wilcoxon signed rank tests were used. Correlations between female length and mass were carried out using Pearson's correlations while correlations between brood size and female length, mass and gestation time used Spearman rank correlations.

Finally, paternity was analysed by converting absolute number of offspring for each male in each family into proportions of offspring fathered by that male and comparing these data in Wilcoxon signed rank tests.

5.4 Results

5.4.1 Female mate choice

When examining the standardized results for the entire data set for dichotomous female choice, females significantly preferred Upper Aripo males over their Lower Aripo counterparts at $\alpha=0.05$ (Table 5.1, Figure 5.3). For the whole data set and for the LT pairings females showed no discernible preference (Table 1, Figure 5.3).

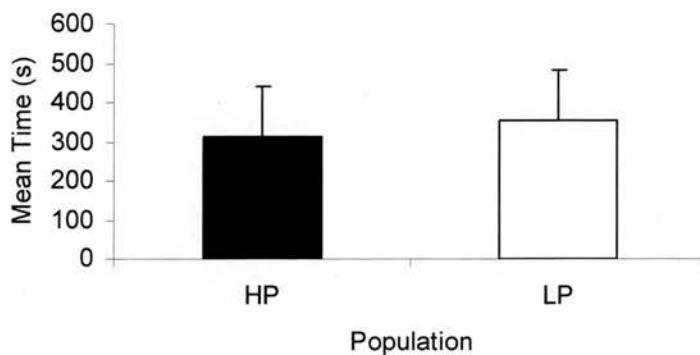
Table 5.1: Female choice results. Significant results are indicated by bold font.

Experimental Group	df	t	p
All fish	79	-1.587	0.119
Lower Tacarigua	49	-0.324	0.748
Lower Aripo	29	-2.042	0.050

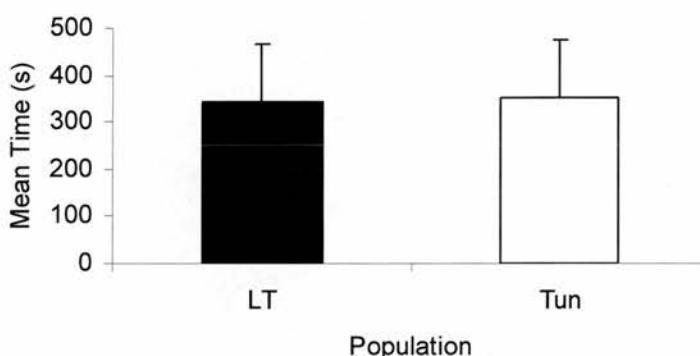
Figure 5.3: Mean time spent by females with males of each population a) HP- all high predation males; LP – all low predation males; b) LT – Lower Tacarigua males; Tun – Tunapuna males; c) LA – Lower Aripo males; UA – Upper Aripo males. High

predation populations are shown in black, low predation populations in white. Error bars show standard error of mean.

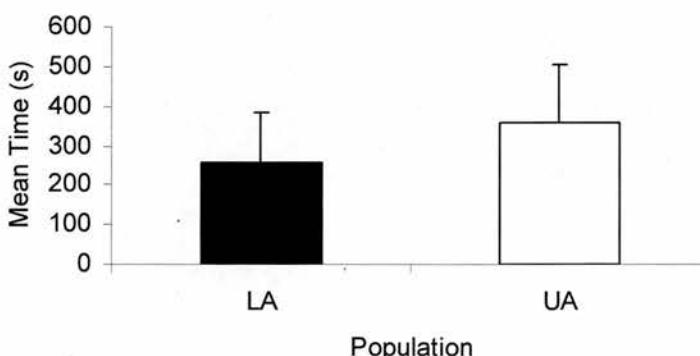
a)



b)



c)



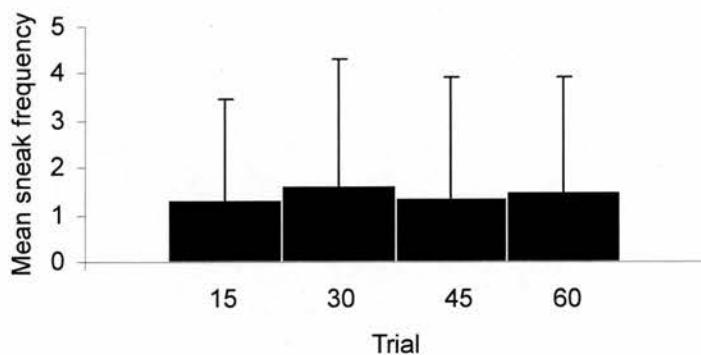
5.4.2 Mating Behaviour

The frequencies of both displays (Figure 5.4b) and glides (Figure 5.4c) showed a significant difference between the four 15-minute day one segments in all data sets examined (Friedman: N=60-162; df=3; displays: p=0.001-0.003; glides: p=0.003-0.018). Examination of means showed that in all cases this was due to a much lower frequency of displays and glides during the first 15-minute segment presumably because fish were adjusting to their new surroundings. These data were removed. Segments two, three and four frequencies subsequently showed no difference (Friedman: N=60-162; df=2; displays: p=0.520-0.923; glides: p=0.118-0.714) so were combined into means for day one. For the frequencies of sneak mating attempts there was no significant difference over day 1 for any data set (Friedman: N=60-162; df=3; p=0.179-0.829) (Figure 5.4a), accordingly all four segments were combined into means. Over the three days of observations there was no significant difference for frequencies of sneaks or displays in any of the six data sets (Friedman tests: N=24-146; df=2; sneaks-p=0.109-0.369 (Figure 5.4d), displays-p=0.764-0.923 (Figure 5.4e)) so these were combined as means for analyses (Table 5.2). The frequency of glides showed a highly significant difference between days (Friedman tests: N=24-146; df=2; p<0.001 in all cases). Examination of means showed that most glides were carried out on day one with the frequency decreasing from day one to two and day two to three (Figure 5.4f). Therefore only the data for day one were used for subsequent analyses.

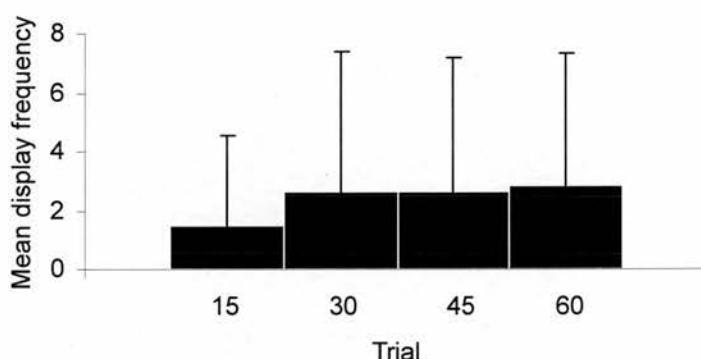
Figure 5.4: Differences between trials on day one (a, b, c) and days (d, e, f) for sneak mating attempts (a and d), displays (b and e) and glides (c and f). Error bars show standard error of mean. As the results for all fish together, Lower Tacarigua parings

and Lower Aripo pairings showed the same patterns only the results for all fish are shown.

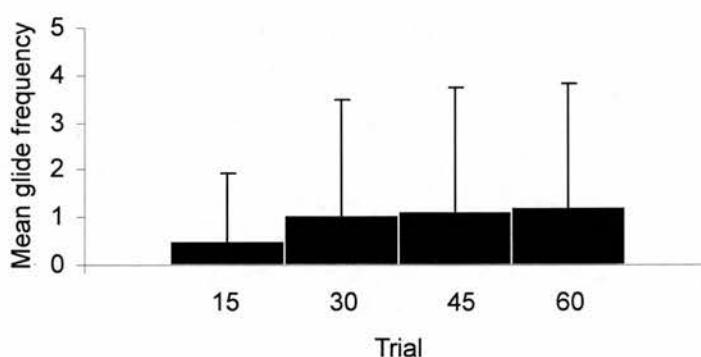
a)



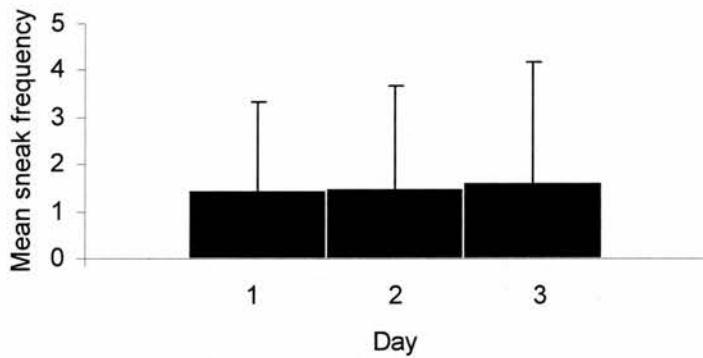
b)



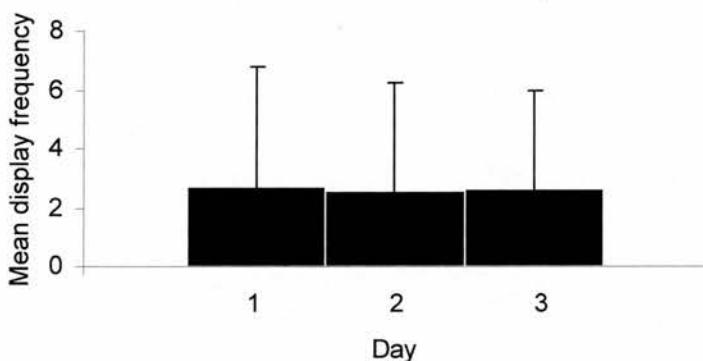
c)



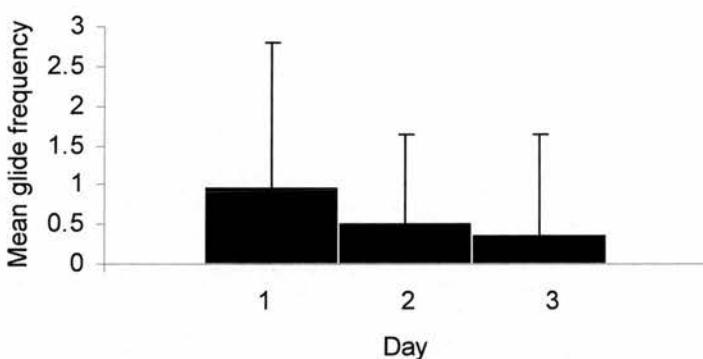
d)



e)



f)



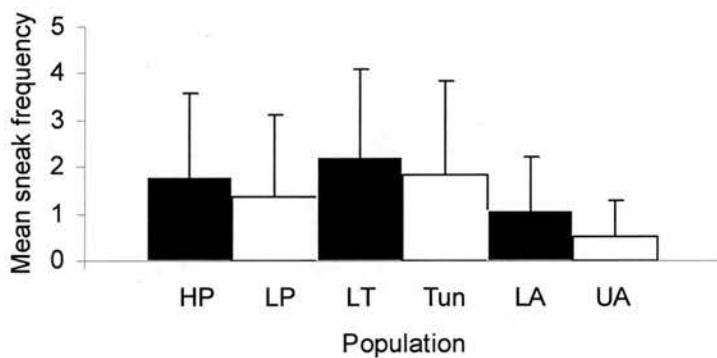
High predation males performed significantly more sneak matings than low predation males when all fish are examined together (Figure 5.5a, Table 5.2) and for Lower Aripo male pairs alone (Figure 5.5c, Table 5.2). Lower Tacarigua males did not show this difference (Figure 5.5b, Table 5.2). Unfortunately too few copulations were observed to allow statistical analysis.

Table 5.2: Mating behaviour results. Significant results are indicated by bold font. HP – high predation; LP – low predation.

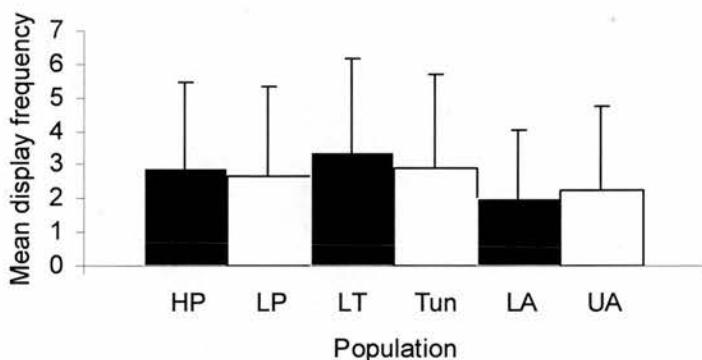
Experimental Group	Behaviour	n	Z	p	HP mean ±sd	LP mean ±sd
All fish	Displays	73	-0.918	0.359	2.88±2.575	2.65±2.681
	Sneaks	73	-2.344	0.019	1.80±1.782	1.36±1.762
	Glides	81	-0.175	0.861	1.14±2.032	1.14±2.300
Lower Tacarigua	Displays	46	-1.269	0.204	3.38±2.760	2.89±2.779
	Sneaks	46	-1.374	0.169	2.23±1.881	1.84±2.000
	Glides	51	-0.957	0.338	1.37±2.232	1.09±2.443
Lower Aripo	Displays	27	-0.312	0.755	2.03±1.999	2.26±2.506
	Sneaks	27	-2.044	0.041	1.08±1.130	0.53±0.747
	Glides	30	-1.035	0.301	0.74±1.594	1.23±2.072

Figure 5.5: Differences between populations in mating behaviour: a) frequency of sneak mating attempts; b) frequency of displays; c) frequency of glides. Error bars show standard error of mean. HP - all high predation males; LP – all low predation males; LT – Lower Tacarigua males; Tun – Tunapuna males; LA – Lower Aripo males; UA – Upper Aripo males. HP – black bars, LP – white bars

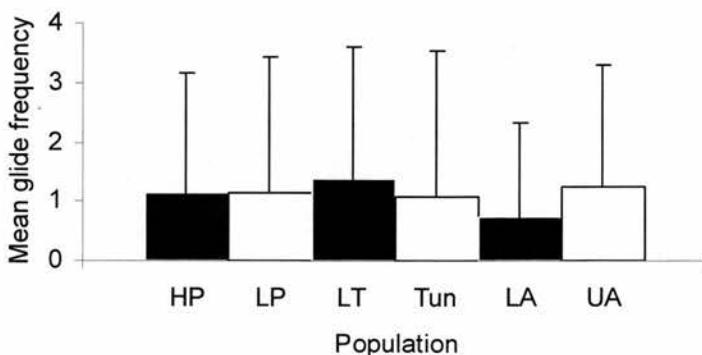
a)



b)



c)



5.4.3 Male Morphology

Overall, low predation males are larger (Figure 5.6a, Table 5.3) and have more (Figure 5.6c, Table 5.4), and a larger area of (Figure 5.6d, Table 5.4), black spots. In addition, Upper Aripo males have more (Figure 5.6e, Table 5.4), and a larger area of (Table 5.4), orange spots than their Lower Aripo counterparts. An interesting result comes from the LT population pair in which high predation males have significantly longer gonopodia, while the Lower Aripo pairing shows the opposite, non-significant trend (Figure 5.6b; Table 5.3). This is presumably due to the low predation males being larger so having allometrically larger gonopodia, and indeed when male length is included as a covariate this trend disappears (length: $F_{1,51}=13.788$, $p=0.001$; predation regime: $F_{1,51}=0.002$, $p=0.966$; HP male: 3.575 ± 0.06 mm, LP male: 3.571 ± 0.06 mm). However, when length is included as a covariate for the LT population pair, high predation males still have significantly longer gonopodia (length: $F_{1,89}=35.136$, $p<0.001$; predation regime: $F_{1,89}=5.603$, $p=0.020$; HP male: 3.690 ± 0.039 mm, LP male: 3.558 ± 0.039 mm).

Table 5.3: Male morphology (parametric). Significant results are indicated by bold font. All – all fish; HP – high predation; LP – low predation; LT – Lower Tacarigua population pairs; LA – Lower Aripo population pairs. '*' indicates significance at $\alpha=0.05$, '**' indicates significance at $\alpha=0.01$, after Bonferroni corrections.

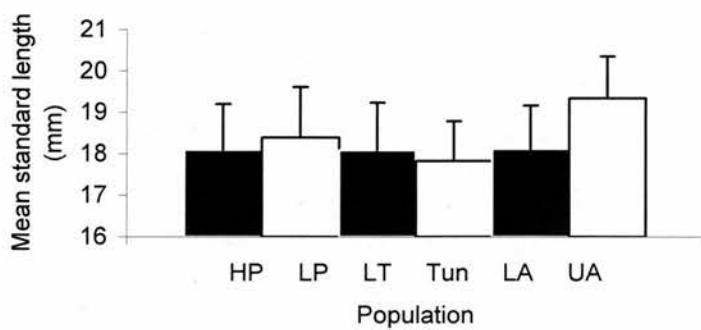
Exp group	Morphological variable	df	F	p	HP mean \pm sd	LP mean \pm sd
All	Length	1,142	7.014	0.009*	18.10 ± 1.100	18.39 ± 1.219
	Gonopodium	1,142	<0.001	0.990	3.63 ± 0.333	3.58 ± 0.317
LT	Length	1,90	1.486	0.226	18.09 ± 1.145	17.83 ± 0.959
	Gonopodium	1,90	7.186	0.009*	3.71 ± 0.300	3.54 ± 0.322
LA	Length	1,52	19.000	<0.001**	18.12 ± 1.040	19.34 ± 1.012
	Gonopodium	1,52	3.863	0.055	3.49 ± 0.344	3.66 ± 0.299

Table 5.4: Male morphology (non-parametric). Significant results are indicated by bold font. All – all fish; HP – high predation; LP – low predation; LT – Lower Tacarigua population pairs; LA – Lower Aripo population pairs.

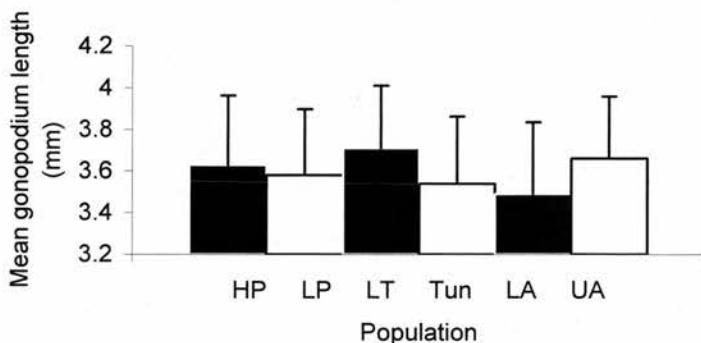
Exp group	Morphological variable	N	Z	p	HP mean	LP mean
All fish	No. black spots	146	-4.961	<0.001	3.05±1.393	4.58±2.108
	Total black area	146	-5.506	<0.001	4.35±2.198	6.99±3.205
	Mean black area	146	-1.157	0.247	1.50±0.688	1.61±0.658
	No. orange spots	146	-1.440	0.150	2.03±1.027	2.33±1.015
	Total orange area	146	-1.465	0.143	5.14±3.474	5.73±2.867
	Mean orange area	146	-1.501	0.133	2.38±1.529	2.53±1.034
LT fish	No. black spots	92	-3.675	<0.001	2.61±1.145	3.76±1.385
	Total black area	92	-3.884	<0.001	3.84±1.724	6.06±3.191
	Mean black area	92	-0.606	0.544	1.55±0.718	1.61±0.660
	No. orange spots	92	-0.232	0.817	2.15±1.074	2.15±0.942
	Total orange area	92	-0.213	0.831	5.24±3.679	5.55±3.165
	Mean orange area	92	-1.797	0.072	2.26±1.293	2.59±0.975
LA fish	No. black spots	27	-3.356	0.001	3.81±1.469	5.96±2.410
	Total black area	27	-3.892	<0.001	5.23±2.640	8.58±2.581
	Mean black area	27	-1.105	0.269	1.42±0.639	1.60±0.668
	No. orange spots	27	-2.537	0.011	1.81±0.921	2.63±1.079
	Total orange area	27	-2.114	0.034	4.67±3.104	6.05±2.295
	Mean orange area	27	-0.120	0.904	2.59±1.875	2.43±1.140

Figure 5.6: Differences between populations in male morphological variables (only the more significant results are shown): a) Mean standard length; b) Mean gonopodium length; c) Mean number of black spots; d) Mean total black area; e) Mean number of orange spots. Error bars show standard error of mean. HP- all high predation males; LP – all low predation males; LT – Lower Tacarigua males; Tun – Tunapuna males; LA – Lower Aripo males; UA – Upper Aripo males. HP-black bars, LP-white bars.

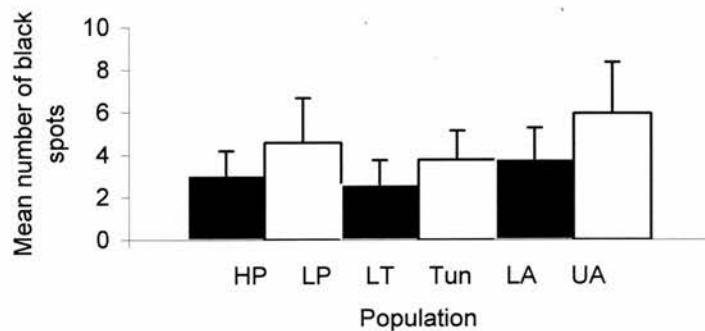
a)



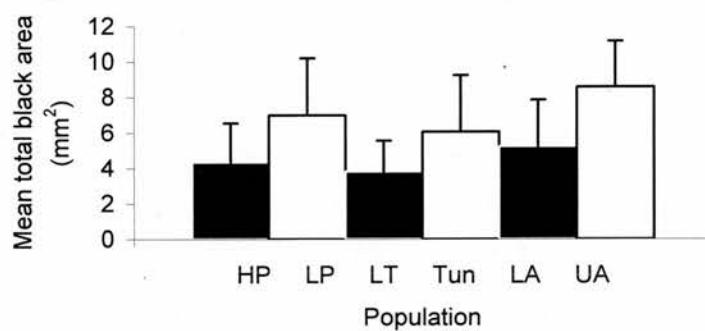
b)



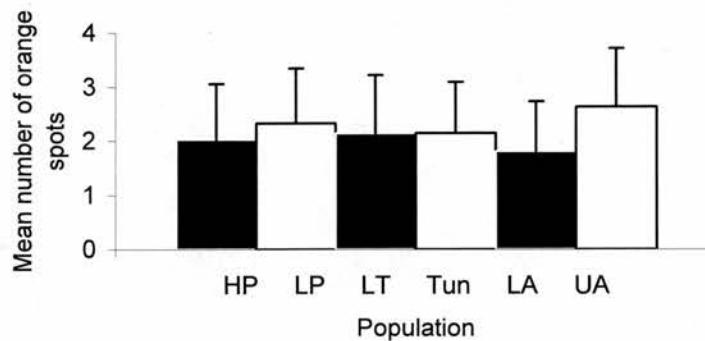
c)



d)



e)



5.4.4 Female Morphology

As expected female length and mass show highly significant positive correlations in all analyses (Figure 5.7; Table 5.5). In addition, length (and mass) is a significant predictor for brood size both when all fish are examined together and for the LT females only (Figure 5.8; Table 5.5). Lower Aripo females do not show this trend. Likewise there is no correlation between gestation time and brood size in any of the population groupings.

Table 5.5: Female morphology. Significant results are indicated by bold font. All – all fish; HP – high predation; LP – low predation; LT – Lower Tacarigua population pairs; LA – Lower Aripo population pairs.

Exp Group	Variables	N	r	p
All females	Length-mass	34	0.928	<0.001
	Gest-brood	34	0.001	0.997
	Length-brood	34	0.541	0.007
	Mass-brood	34	0.449	0.008
LT females	Length-mass	21	0.933	<0.001
	Gest-brood	21	0.048	0.837
	Length-brood	21	0.660	0.001
	Mass-brood	21	0.614	0.003
LA females	Length-mass	13	0.938	<0.001
	Gest-brood	13	-0.159	0.604
	Length-brood	13	0.167	0.586
	Mass-brood	13	0.198	0.518

Figure 5.7: Correlation between female standard length and mass. As all results were similar, for clarity only the trendline for all fish together is shown.

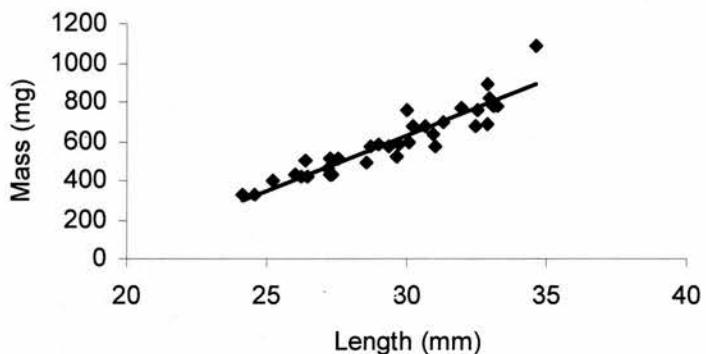
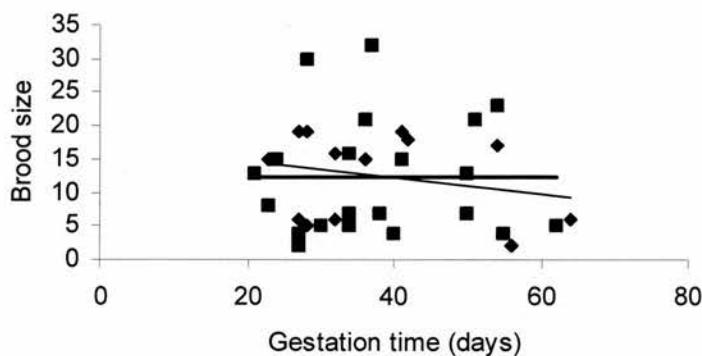
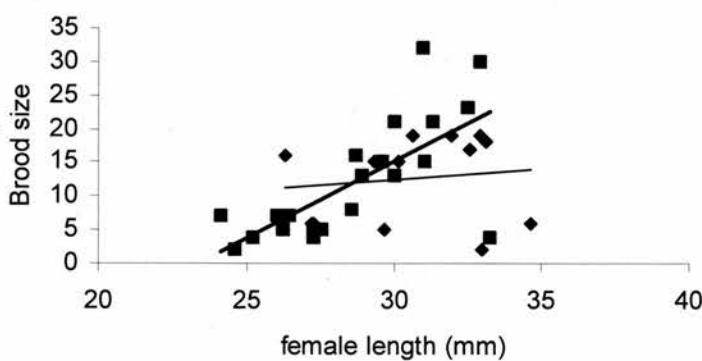


Figure 5.8: Correlations between brood size and a) gestation time; b) female length; c) female mass. As the results for all fish together and very similar to those for the Lower Tacarigua pairings, for clarity only data for Lower Tacarigua pairings (squares, heavy line) and Lower Aripo pairings (diamonds, light line) are shown.

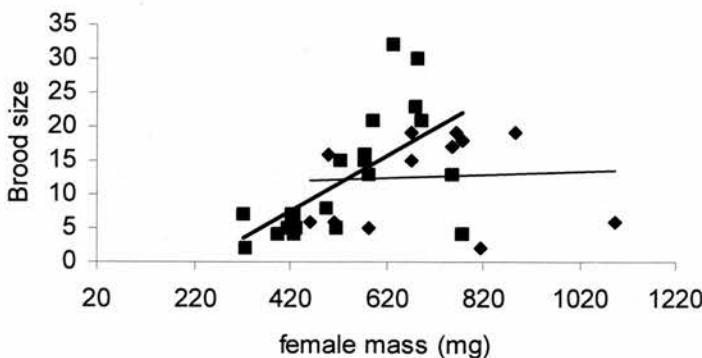
a)



b)



c)



5.4.5 Paternity Analysis

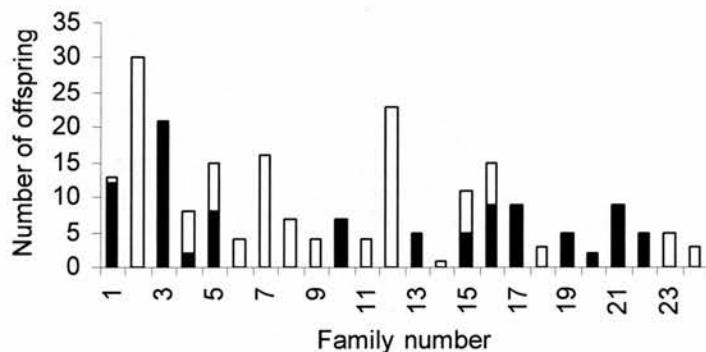
For all families together and for the LT families there is no significant difference in the proportion of offspring fathered by the high and low predation males (Figure 5.9a, Table 5.6). However, despite female preferences (Table 5.1) Lower Aripo males gain significantly higher paternity than their Upper Aripo counterparts (Figure 5.9b, Table 5.6).

Table 5.6: Paternity results. Significant results are indicated by bold font. HP – high predation; LP – low predation; LT – Lower Tacarigua population pairs; LA – Lower Aripo population pairs.

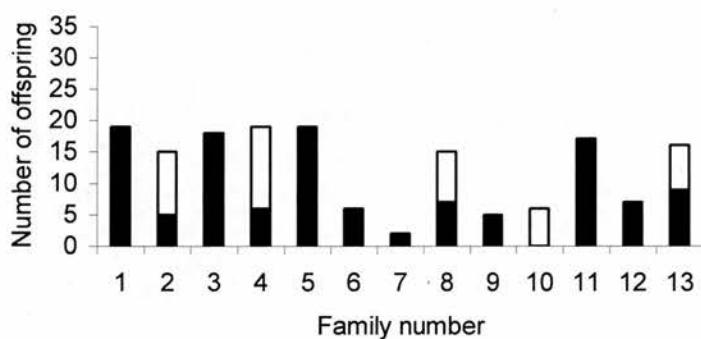
Exp group	N	Z	P	HP mean±sd	LP mean±sd
All families	37	-0.701	0.483	0.55±0.448	0.45±0.448
LT families	24	-6.38	0.523	0.45±0.463	0.55±0.463
LA families	13	-2.069	0.039	0.74±0.358	0.26±0.358

Figure 5.9: Number of offspring fathered by high predation (black bars) and low predation (white bars) males in a) Lower Tacarigua pairings and b) Lower Aripo pairings.

a)



b)



5.5 Discussion

5.5.1 Female Mate Choice

In agreement with the hypothesis, Lower Aripo females preferred the larger, more brightly coloured, low predation, males over those from their own high predation population, thus providing a means to facilitate gene flow between these populations. However, when all females were examined together and for Lower Tacarigua females alone there was no significant effect. Likewise, the frequency of female glide response was not affected by male source population. As Endler and Houde (1995) showed in their examination of mating preferences in 11 localities in Trinidad (not including the River Aripo), populations vary in the criteria used in female choice. While female choice is normally based primarily on colour pattern (Houde 1987, 1988, 1997, Endler and Houde 1995) and all low predation fish here showed significantly more black, multiple cues are likely to be used in mate choice (Brooks and Caithness 1995, Candolin 2003). The Aripo population pair showed a wider array of differences than either the Tacarigua pairings or all fish together, low predation males having more orange colouration and being larger than their high predation counterparts. Lower Tacarigua pairings did not show a difference in orange colouration between the high and low population males and this is indicative of the general result when all families are tested together. Females of most, if not all, populations share a preference only for orange colouration (Endler and Houde 1995) so this lack of differentiation in orange colour between Lower Tacarigua and Tunapuna males may at least in part explain the lack of female preference in this population. Moreover high predation females tend to be less consistent and show less intensity of preference than low predation females (Endler and Houde 1995, but see Evans *et al* 2004) suggesting a further reason for the lack of female choice shown here.

5.5.2 Mating Behaviour

Mating behaviour trials on day one consisted of four consecutive 15-minute observations. For both male displays and female glides the frequency during the first 15-minute period was lower than the rest of the observations. This presumably

reflects the effects of fish settling into their new environment and suggests a 15-minute acclimation period should be incorporated into experimental design for this species. Interestingly, the frequency of sneak mating attempts appears unaffected by adjustment to a new environment. One possible reason for this is that males are taking advantage of female distraction to carry out the mating behaviour that does not require female cooperation. When the frequencies of mating behaviour over the three days of observations are compared, male mating behaviour shows no effect of time. Female glide responses, however, reduce drastically after the first day, so much so that only day one observations were used in analyses. This is likely to be due to changes in female receptivity as females are only receptive for two or three days after parturition and when virgin (Liley 1966, Farr 1989) and the glide response only occurs when females are responsive to males. In this case it appears female receptivity begins to diminish after only one day and decreases rapidly thereafter.

When population differences in behaviour were investigated, high predation males did undertake significantly more sneak matings when examining all fish together and just the Lower Aripo pairings. Again, Lower Tacarigua fish did not show the same pattern. Contrary to predictions, low predation males did not perform more displays. Population differences in behaviour are expected to occur because in high predation areas, males that show conspicuous displays attract the attention of females, but they will also attract the attention of predators (Endler 1985, Magurran and Nowak 1991). High predation males are therefore likely to carry out a higher frequency of the less noticeable sneak mating behaviour (Endler 1985, Magurran and Nowak 1991). In low predation areas, where the small fish *Rivulus hartii* is the main guppy predator, males do not need to be so concerned with remaining unnoticed and consequently carry out relatively more displays than their high predation counterparts (Endler 1985, Magurran and Nowak 1991). It may be that the reason this expected difference in mating behaviour did not materialise is connected to the maintenance of these populations. All the fish used in this study were descended from wild caught individuals. However, the large number of generations (circa 10) spent in a laboratory environment, without the presence of predators to stimulate population differences in behaviour may have caused the erosion of these differences over time.

The combination of Lower Aripo females preferring Upper Aripo males and Lower Aripo males performing more sneak mating attempts suggests a role for sexual conflict via sneak mating circumventing female choice (Magurran 2001). Female efforts to select the most desirable males to father their offspring are being undermined by males from their own population apparently through sneak mating. Sexual conflict varies geographically within this species (Magurran and Seghers 1994a; Magurran 1996; Magurran 2001). Low predation populations tend to be more female biased (Seghers 1973, Magurran *et al* 1995) while high predation populations lean towards equal or male bias (Magurran 2001). More males mean greater potential for sexual coercion and therefore greater conflict between the sexes, which may be why the Lower Aripo fish in this study were apparently better able to circumvent female choice.

5.5.3 Morphology

Male colour pattern varies with predation regime in the same way the male behaviour does. Male guppies, being brightly coloured are likely to be preferentially predated (Endler 1987). Consequently males from populations that exist with numerous predators will be selected for less conspicuous colouration whereas those from low predation sites can afford brighter, female attracting colours (Houde 1987, 1997). This study confirms this effect. Low predation males were larger when all fish were considered together and for the Lower Aripo pairings. All low predation males had a higher number and total area of black spots and the Upper Aripo males, in addition, had higher number and total area of orange. This is reflected in the female choice results (see above).

An interesting finding from the Tacarigua pairing is that Lower Tacarigua males have longer gonopodia than do Tunapuna males. Male gonopodium length is a feature that shows between (Rosen and Bailey 1963) and within species polymorphism (Constantz 1975, Kelly *et al* 2000). This may have interesting consequences. Males that have short gonopodia may tend to display to elicit matings from the females, and females therefore are able to choose their mates (Chambers 1987). The shortness of the gonopodium means males are unable to line up the gonopodium by sight, so a degree of female cooperation may be essential. Conversely, males of species with long

gonopodia may be able to sight along the length of the gonopodium, so facilitating sneaky mating (Chambers 1987). Kelly *et al* (2000) documented geographical variation in gonopodium length in the guppy, finding that males from populations evolving under high predation intensity have longer gonopodia and postulate that longer gonopodia are advantageous in sneak mating, the behaviour type preferred by high predation males. High predation males did undertake significantly more sneak matings when examining all fish together and just the LA pairings. However, this argument is weakened by the absence of the same pattern in LT fish.

While male reproductive success is limited by the number of females they can fertilize, female reproductive success is limited by the number and size of broods they can produce (Houde 1997). In general, larger females produce more offspring (Liley 1966, Houde 1997) and the females in this study are no exception. Both measures of female size, length and mass, which are anyway strongly positively correlated, are positively correlated with brood size. A further advantage to females of being larger is that males tend to prefer larger females (Houde 1997, Ojanguren and Magurran 2004).

5.5.4 Paternity Analysis

Contrary to predictions it was high predation males that achieved a greater proportion of paternity, at least for the Lower Aripo population pairs. The Lower Tacarigua pairs and all fish taken together again did not show a significant result.

Despite the female preference for Upper Aripo males, it was Lower Aripo males, who performed more sneak mating attempts that achieved a higher reproductive success suggesting that in this system at least, cryptic female choice is not apparent and sperm competition provides a post-copulatory gene flow mediating mechanism. Sperm competition is widespread in nature (Birkhead and Møller 1992, Andersson 1994) and can take many forms. Common tactics to reduce the risk of rivals fertilizing a female include mate guarding, displacement of rival sperm and production of abundant sperm to out compete those of rivals (Birkhead and Møller 1992, Andersson 1994). Mate guarding occurs to some extent in the guppy (e.g. Magellan *et al* 2005) but certainly not for the duration of a females' receptive period, suggesting this is unlikely to be a method of sperm competition. Likewise, the structure of the male gonopodium (Rosen

and Bailey 1963) is such that removal of a previous male's ejaculate is possible. However, there is much more, and varied, evidence for copious sperm production as a competitive tactic. In one study, Evans *et al* (2003) found that relatively small individuals were advantaged in sperm competition, suggesting a possible trade-off between sperm competitive ability and body growth which would favour the smaller Lower Aripo males. However, in artificial insemination experiments, in which females are not able to exert control over sperm transfer, when equal numbers of sperm from two males compete for fertilization, relatively colourful individuals were found to achieve greater parentage than their less ornamented counterparts (Evans *et al* 2003). Additionally, during solicited copulations, male guppies with preferred phenotypes transfer more sperm to females than their less ornamented counterparts but this relationship breaks down during coercive copulations (Pilastro *et al* 2002). Sperm is transferred during sneak mating (Pilastro and Bisazza 1999, Matthews and Magurran 2000), though the absolute amount delivered is around one third of that transferred after courtship (Pilastro and Bisazza 1999) although this is very variable, all of which appears to indicate that Upper Aripo males would be at an advantage. The intensity of sperm competition may vary geographically in the guppy (Kelly *et al* 1999). Males from low risk sites have higher sperm reserves (Evans and Magurran 1999). However, sperm counts are broadly similar in high and low predation males kept for a number of generations in a constant laboratory environment (Matthews *et al* 1997) as these males were. Whereas the evidence for the exact nature of sperm competition in the guppy is conflicting, pilot work using more widely geographically separated populations than used in this study has suggested that sperm from a guppy females' own population is at an advantage in sperm competition (Matthews 1998) and this study provides additional evidence that this might be the case.

5.5.5 Conclusion

It has been shown here that high predation and low predation populations differ in female mate choice, male mating success, male morphology and male reproductive success although the results in several cases were not as strong as expected. As discussed above a possible reason for this is the erosion of population differences brought about by long habituation to laboratory conditions that do not show the same ecological variation as seen in wild populations. A notable finding here is the

difference between the two population pairs. Most differences occurred between the Lower and Upper Aripo populations, while the Lower Tacarigua and Tunapuna pairings show less apparent differentiation. Population differences in mechanisms of gene flow regulation is an interesting topic that would benefit from further research.

The prevailing view of sexual conflict is that female advantage in this battle of the sexes promotes reproductive isolation whereas male success encourages gene flow (Parker and Partridge 1998; Magurran 2001; Tregenza *et al* 2000). Here, however, this situation is less clear. Female choice favours gene flow between populations while male characteristics, in the form of sperm competition, apparently prevents gene flow. However, cryptic female choice cannot be ruled out on the basis of this study. Nonetheless, incipient speciation is unlikely in these populations. Although one recent study has postulated incipient speciation in a Venezuelan guppy population (Alexander and Breden 2004), in this study Upper Aripo males did achieve some reproductive success, in one case being the sole father of a brood, and as numerous studies have shown (e.g. Godin and Briggs 1996; Magurran 2001; Evans *et al* 2002; Evans *et al* 2003; Pilastro *et al* 2002; Pilastro *et al* 2004) many diverse influences are involved in the guppy mating system. Indeed this study illustrates the diverse ways in which male-female interactions can effect gene flow.

Chapter 6

Discussion and Future Directions

This chapter brings together the conclusions of the previous chapters in terms of the sources of variation in guppy mating behaviour and the levels of effect of this variation. Evolutionary contexts are discussed and future directions suggested by the studies included in this thesis are highlighted.

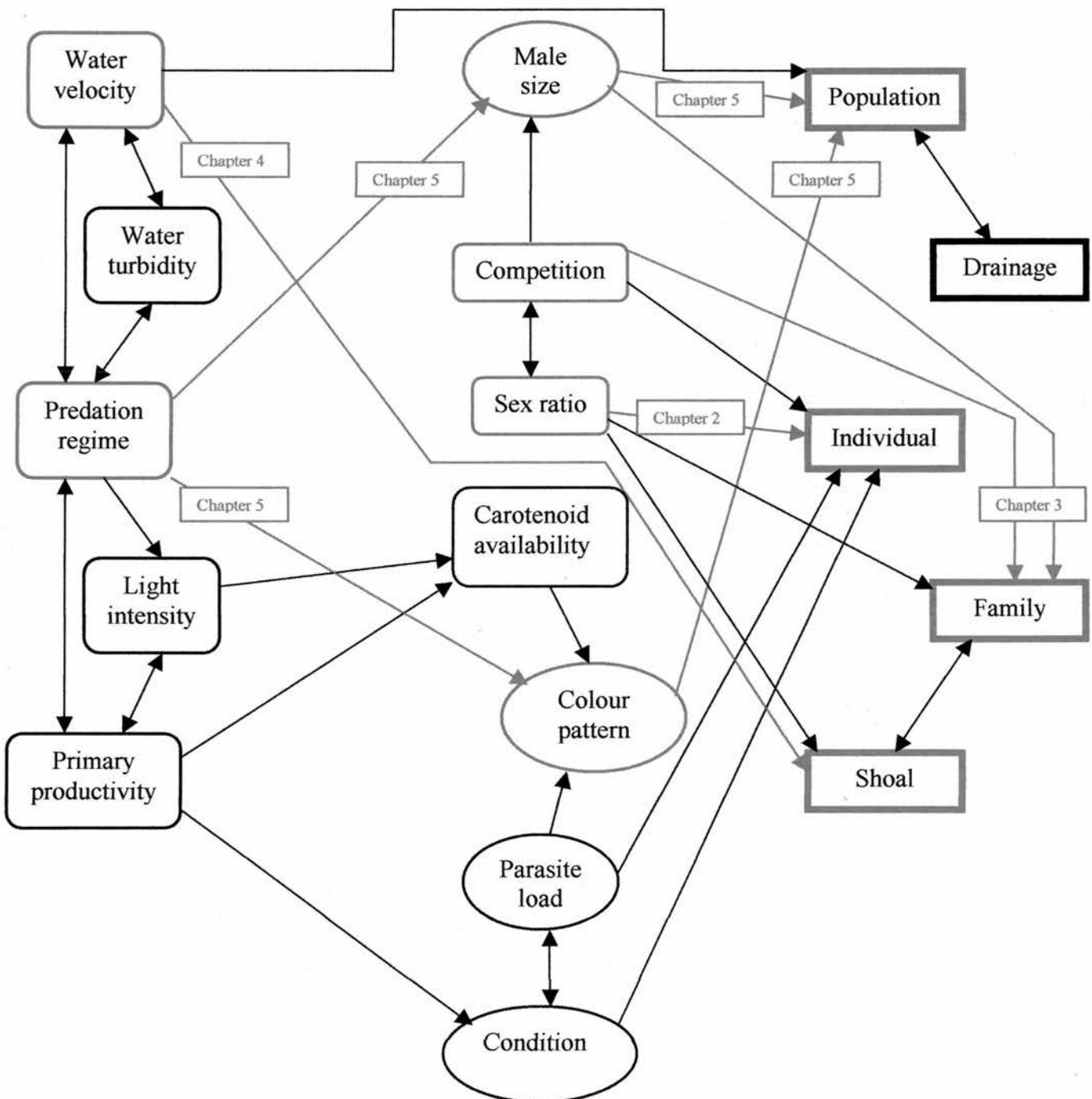
6.1) Sources of Variation

Overall individual consistency, rather than variation, in male mating behaviour emerged as perhaps the most striking result in this thesis (Chapter 2) providing great potential for further research (see section 6.3). From the perspective of variation in behaviour, variations between siblings (Chapter 3) in the context of size and competition were evident and differences between individuals from the same population, in relation to water velocity (Chapter 4) and sex ratio (chapter 2) were clearly shown. Variations between populations, hypothesised as showing the most obvious differences in behaviour as these males are most widely separated geographically, were the least apparent (Chapter 5). Figure 6.1 provides an overview of these effects.

6.1.1) Abiotic Environmental Variation

As expected, male guppy mating behaviour is influenced by numerous diverse factors. Both abiotic (water velocity) and biotic (sex ratio and competition) environmental factors are here demonstrated to have an effect. Male size, a developmental factor and, to a lesser extent, male population, to which male size can often be related, are also influential. Water velocity is a ubiquitous environmental feature for all stream dwelling organisms and male mating behaviour varies approximately linearly with changes in water velocity (Chapter 4). In both the laboratory and field studies increasing water velocity induces a reduction in sigmoid display behaviour and an increase in sneak mating behaviour suggesting that males switch behaviour types in

Figure 6.1: Figure 1.8 revisited. Synoptic diagram of the sources and levels of variation in guppy mating behaviour. The bold boxes on the right show the possible levels of variation. All other boxes show sources of variation; square boxes being environmental factors and oval boxes are those intrinsic to the individual. Arrows show interconnections between factors. Red markings show the connections demonstrated in this thesis



response to changes in an environmental variable. A recent study by Kodric-Brown and Nicoletto (2005) parallels, and adds a new dimension to, the results presented here. They contrasted courtship behaviour of guppies in high and low predation environments in Trinidad. In the low predation sites, in the Quare River, they found a positive correlation between display rate and water velocity whereas in the high predation, Oropuche River, sites there was no correlation (Kodric-Brown and Nicoletto 2005). Both the studies presented here used high predation fish; the field study was carried out in the Arima River, a high predation site, while the laboratory study used fish descended from Lower Tacarigua stock, another high predation locality, so both studies would be expected to agree more with the findings of Kodric-Brown and Nicoletto's (2005) high predation sites. In fact, these findings contrasted with both sets of Kodric-Brown and Nicoletto's (2005) data as both the lab and field studies showed a negative correlation between displays and water velocity. One possibility for this discrepancy may be that this study examined absolute frequencies of behaviour rather than rates as used in the Kodric-Brown and Nicoletto's (2005) study, but this is unlikely to account for the relatively large difference in results. Whatever the reason, this highlights once again the population differences that are so prevalent in the Trinidadian guppy system.

6.1.2 Biotic Environmental Variation

The two biotic environmental variables, sex ratio (Chapter 2) and competition (Chapter 3) are obviously related, an extremely female biased sex ratio being equivalent to no male competition. Male mating behaviour was shown to vary with sex ratio in both of these studies. However, the results are inconclusive. For displays there was a trend to decrease in frequency from equal sex ratio to a female sex ratio (Chapter 2) and a similar decrease from the equal sex ratio to no competition situations (Chapter 3). For sneak mating attempts there is a different pattern. The decreasing trend in sneaks from equal to female biased sex ratio seen in Chapter two is contrasted with an increase in sneaks from equal sex ratio to no competition in Chapter three. It appears that although both types of mating behaviour can be used by males at any time, they are under the influence of different variables or, looking at it

in another way, respond differently to the same influence. Another factor is the source of experimental animals. While the fish used in Chapter two were wild individuals that were tested in as close to natural conditions as the experimental design would allow, the fish in Chapter 3 were inexperienced, individually reared, laboratory stock, and were subject to an additional experimental condition, that of size manipulation. This again emphasizes the many diverse influences that must be considered in examination of the guppy mating system.

6.1.3) Individual Factors – Body Size

Male size, as mentioned above, was also examined in Chapter three and was shown to influence male mating behaviour with larger males undertaking relatively more sneak mating attempts and spending more time chasing females. This experiment used pairs of siblings and highlights the use of phenotypic manipulation as an experimental technique, in this case to produce males of different sizes. Body size effects have been shown in several poeciliid species. In sailfin mollies, *Poecilia latipinna*, larger males exhibit higher rates of courtship and lower rates of gonopodial thrusting compared to smaller males (Farr *et al* 1986). In addition, intermediate sized *Poecilia latipinna* males varied their behaviour according to the size of competitors, exhibiting intermediate rates of behaviour when with males of their own size, but ‘small male’ type behaviour when with mixed sized males, another example of the effect of competitors (Travis and Woodward 1989). In the mosquitofish, *Gambusia affinis*, larger males were more likely to court females while smaller males tended to chase non-receptive females and attempt to inseminate them forcibly (Hughes 1985). In this case the difference in body size and behaviour was correlated with female preference for larger males (Hughes 1985). This same phenomenon is seen in *Xiphophorus nigrensis* populations (Ryan *et al* 1990).

In wild situations, size differences are important both within and between populations. Within populations larger males may be preferred by females (e.g. Hughes 1985; Ryan *et al* 1990; Reynolds and Gross 1992) and may have the stamina to undertake more mating behaviour and at higher water velocities as shown in chapter four. Perhaps more significant, however, is between population size variation, in particular in relation to predation regime. In high predation populations it is adaptive for males

to mature as quickly as possible to avoid predation as juveniles (Reznick 1982; Endler and Reznick 1982; Reznick 1990). This results in males maturing at a smaller size (Reznick 1982; Endler and Reznick 1982; Reznick 1990). This situation is mirrored in low predation populations where males are not so constrained by predation and therefore mature slower and achieve a larger size at maturity (Reznick 1982; Reznick and Endler 1982; Reznick 1990). Chapter five demonstrates this phenomenon, with both Tunapuna and Upper Aripo (low predation) males being larger than their high predation (Lower Tagarigua and Lower Aripo respectively) counterparts. However in this case associated differences in behaviour, while present to a certain extent, are not as apparent as that found in previous studies (Endler 1995; Magurran *et al* 1995; Houde 1997). While the smaller, high predation, males did undertake more sneaky behaviour, there was no effect with respect to displays. High predation males, being in the presence of several predators, tend to opt for the less conspicuous sneak mating behaviour whereas low predation males, not being so constrained undertake more showy displays to attract females (Endler 1995; Magurran *et al* 1995; Houde 1997). It is possible that these circa 10th generation laboratory reared fish have to some extent lost this predator mediated adaptation so don't show the expected behavioural differences.

6.1.4 Female Mate Choice

Female mate choice, likewise, is under various influences, some of which have been demonstrated in this thesis. As already stated, females of many species prefer larger males (Hughes 1985; Ryan *et al* 1990; Reynolds and Gross 1992) and this is shown in chapter three and suggested in chapter five. Larger size may be associated with differences in behaviour (e.g. Farr *et al* 1986, Travis and Woodward 1989), for which females may also show a preference (e.g. Rosenthal *et al* 1996; Ryan and Causey 1989; Ryan and Wagner 1987) although this was not shown here. Moreover, a correlation between behaviour and colour pattern is often apparent. Jirokul (2000) found that male guppies with 15% mean orange coverage interfered with other courting males more and engaged in courtship activities for longer than males with 10% mean coverage and Nicoletto (1993) found a correlation between area of orange covered, sustained swimming activity and display rate. Likewise it has been widely demonstrated that female guppies prefer males with more orange colouration (Houde

1987, 1994; Houde and Endler 1990; Nicoletto 1993; Brooks and Caithness 1995a; Kodric-Brown 1995; Houde and Hankes 1997; Jaroensutasinee 2000) and, again, this may be apparent in chapter five. There are other aspects to colour pattern variation, for example, female *P. reticulata* are more likely to mate with males having novel colour patterns than with males having a colour pattern with which they were familiar (Hughes *et al* 1999), an example of the ‘rare male effect’. Further examples of female preference criteria in guppies include symmetric colouration (Sheridan and Pomiankowski 1997) larger tails (Bischoff *et al* 1985) and in some populations, longer gonopodium (Brooks and Caithness 1995b).

6.2) Evolutionary Contexts

The main evolutionary theme of the guppy mating system appears to be conflict. There are conflicts between male and female interests, between natural and sexual selection and intrasexual conflict in the form of male-male competition.

6.2.1) Sexual Conflict

Sexual conflict can occur whenever characteristics that augment the reproductive success of one sex are detrimental to the fitness of the other (Smuts and Smuts 1993; Rowe 1994; Parker and Partridge 1998; Gavrilets 2000; Magurran 2001). It arises because one sex, typically males, is selected to produce as many offspring as possible while the other sex, usually females, invest their reproductive effort into producing fewer high quality offspring. Sexual conflict can occur before, during and after mating and can be expressed in various ways. Male coercion of females to gain matings can be found in a number of species (e.g. spiders: Maklakov and Lubin 2004; garter snakes: Shine *et al* 2004; non-human primates: Smuts and Smuts 1993) often resulting in females receiving matings from males that are not preferred (Blanckenhorn *et al* 2002; Magurran 2001; Shine *et al* 2004). Male guppies have an obvious method of sexual coercion: sneak mating (Magurran 2001). Farr (1975) found that guppies perform up to 3 gonopodial thrusts in 5 minutes in natural populations and Magurran and Seghers (1994a) showed that females in high predation sites receive approximately one sneaky mating attempt per minute. All the studies presented here show sneak mating attempts are regularly carried out. Moreover males are able to

circumvent female choice of mates as indicated by the high sneak mating rate of the non-preferred Lower Aripo males in chapter five.

The functional consequences to females are not just reproductive. Harassment by males can leave females more vulnerable to predation (Arnqvist and Rowe 2002, Shine *et al* 2000) and reduce foraging efficiency (Magurran and Seghers 1994b, Pilastro *et al* 2003b). Coercion from males seeking to copulate can be physiologically stressful to females (e.g. Shine *et al* 2004) and in some cases extremely aggressive, sometimes resulting in injury or even death to females (e.g. non-human primates: Smuts and Smuts 1993). However females are not totally helpless in the face of this male aggression. Female counterstrategies have evolved which allow females to avoid harassment or ameliorate the effects of sexual coercion. Avoidance techniques can involve gaining protection from other individuals (Pilastro *et al* 2003b), simply moving away from males and many forms of cryptic female choice exist by which females can select the sperm used to fertilize their eggs (Eberhard 1996). An example of avoidance technique is shown in chapter four, where larger females use areas of higher water velocity which may be an attempt to avoid male harassment by moving into areas where smaller males may be excluded.

6.2.2 Natural Selection versus Sexual Selection

The second arena of conflict occurs between natural selection for predator avoidance and therefore crypsis and sexual selection for achieving mating opportunities. Male guppies have evolved bright colours and conspicuous displays to attract the attention of females (Liley 1966). However, this will also attract the attention of predators. In predator rich environments male guppies mature at a smaller size (Reznick 1982; Endler and Reznick 1982; Reznick 1990) and are less brightly coloured (Endler 1987) than their low predation counterparts. This situation is evident in the fish from chapter five in which low predation males are larger and have more, and a larger area of, black spots. Further the Upper Aripo fish have more, and a larger area of, orange spots. Females also base their choice of males on male size (Reynolds and Gross 1992) which was confirmed in both chapters three and five. Moreover, as chapter four showed, larger males are able to maintain mating behaviour across more situations (higher water velocities) than their smaller conspecifics. Thus, larger males seem to

be at an advantage and sexual selection should favour larger individuals. However, larger males are more conspicuous to predators so natural selection will favour smaller males (Reynolds 1993, Reynolds *et al* 1993).

6.2.3) Male-Male Competition

The third area of conflict involves male-male competition which can occur before, during or even after mating. Pre-mating indirect competition occurs whereby males are more colourful (Chapter 5), larger (Chapters 3 and 5) or more active (all chapters) than other males. As well as increasing their own rate of sexual behaviour, males may attempt to reduce the quality or quantity of sexual activity of other males through aggression (Farr 1989) engaging in direct competition for females (Jirokul 1999a, b) as seen in Chapter three where males were shown to interfere with the mating attempts of other males, an example of competition occurring during mating. Post-mating intrasexual conflict has also been demonstrated here in the form of sperm competition (Chapter 5). Sperm competition is widespread in nature (Birkhead and Møller 1992, Andersson 1994). Production of abundant sperm to out compete those of rivals is a common tactic (Birkhead and Møller 1992, Andersson 1994) and some variation of this appears to be transpiring here where the less preferred Lower Aripo males achieve a higher proportion of paternity than their low predation rivals. As discussed in Chapter five, this is an expanding area of research with is the focus of much recent interest (e.g. Evans and Magurran 1999; Kelly *et al* 1999; Pilastro and Bisazza 1999; Pilastro *et al* 2002; Evans *et al* 2003).

6.3) Future Directions

The results from this thesis have highlighted several areas that would benefit from future research. Behavioural profiling (Chapter 2), along with personality dimensions (e.g. Budaev 1997; Budaev and Zhuikov 1999; van Oers *et al* 2004) and behavioural syndromes (Sih *et al* 2004), is a relatively new area of interest to which the guppy system is ideally suited. Here, individual male mating behaviour profiles in relation to varying sex ratios have been illustrated. This could be expanded to encompass mating behaviour profiles in relation to other factors, such as age; other male mating behaviours including aggressive interactions; female mating behaviours, for example

individual variation in the intensity of the glide response; or non-mating behaviours like feeding patterns or schooling tendencies.

A related topic is the inheritance of behaviour. Repeatabilities, necessary for the establishment of behavioural profiles, provide an upper limit to broad-sense heritability (Boake 1989, Lynch and Walsh 1998). In the context of mating behaviour, the repeatability of the production of male mating signals gives information about whether males differ sufficiently for selection to act and whether the differences could be appreciably heritable (Boake 1989). Repeatability is not only useful in assessing heritabilities. It also provides a gauge of individual variation, the incorporation of which into experimental design can reveal interesting and biologically relevant relationships that may be masked by the traditional use of group means (Kolok 1999) and the measurement of inter-individual differences is an important first step in elucidating the functional causes and the ecological consequences of variation (Watkins 1997). Investigation into the inheritance of behaviour would be an interesting extension of these ideas allowing the elucidation of previous evolutionary influences (Boake 1989) and supplying clarification of the role of another potential influence on guppy behaviour.

Following on from the inheritance of behaviour, is the influence of developmental or social factors in behaviour, epitomised in the nature versus nurture debate. It is generally understood that characteristics depend on the intricate interactions between nature and nurture but in most cases little is known about the relative contribution of these two factors. Aspects of mate choice and mating behaviour, in particular, lend themselves well to addressing fundamental questions about inherited or learned behaviour. Natural and sexual selection operate on inherited phenotypes which, of course, are genetically mediated. However, the phenotype expressed depends also on environmental influence. For example, in guppies the inheritance of colour pattern is well established (e.g. Endler 1980). Yet an individual's colour pattern also depends on environmental influences. In guppies, for example, the potential for carotenoid consumption is also important (Kodric-Brown 1989). In much the same way the expression of behavioural traits is also likely to have a genetic component. However, in established social groups, such as fish shoals, social influence may contribute and this is particularly likely with interactive behaviours, such as mating behaviour. So far

most effort on teasing apart the influences of inheritance and social environment has focused on fruit flies, *Drosophila sp.* (e.g. Greenspan 1995). However, other taxa, including many fish species and in the context of this thesis particularly the guppy, also lend themselves to examination of nature/nurture questions.

Chapter five provides an illustration of the diverse mechanisms of gene flow regulation that can potentially influence the guppy system. These results are not decisive presenting a promising area of future research. Testing of further populations would supply additional insight into this relatively recent area of guppy research. While guppies could be considered to be undergoing evolution without speciation (e.g. Magurran 1998) the results presented here show that the potential exists. The numerous geographically isolated populations both on a local scale in Trinidad and on a global scale with the numerous natural and feral populations available provide an experimental arena in which theories of gene flow could be tested.

Guppy mating behaviour, and behaviour in general, is shown here to be affected by many factors. The huge amount of research on the guppy system has provided a wealth of information which is often conflicting. Relevant examples are the influences of sex ratio or male size on male mating behaviour. Even in the few studies presented here, the conclusions are by no means conclusive. Added to this is the related information for the Poeciliid family as a whole. Clarification of the exact role of influential factors and the elucidation of the factors that contribute to these dissimilar results could be aided with the use of meta-analytic techniques (e.g. Wachter 1988, Wachter and Straf 1990) and would provide a useful addition to the poeciliid literature.

6.4) Conclusions

The guppy is shown here once again to be an ideal model species with which to address evolutionary and behavioural questions. The nature of the guppy mating system makes it particularly suitable for studies of mate choice, mating behaviour variation and sexual selection. Guppies inhabit fluctuating environments so must be able to accommodate and react to variation and several aspects of this phenomenon have been presented here. No one aspect of the guppy system can be considered in

isolation. Conclusions can only be drawn with reference to the vast amount of existing literature on this subject. Despite this wealth of previous study, research into the guppy system is ongoing and as well as adding to the existent information this thesis provides potentially interesting new directions for this research to take.

This thesis has shown that guppy mating behaviour is under numerous diverse influences. Population differences in behaviour and morphology have been revisited but this time in the context of gene flow which can be seen to provide a fruitful area of future research. The influence of male size on mating behaviour has also been well studied but never before using related individuals which reduces the potential for confounding variables and, in phenotypic manipulation, provides a useful new tool for guppy research. Despite the plethora of previously demonstrated factors that affect guppy mating behaviour, water velocity, a ubiquitous influence for stream dwelling organisms, has not really been explored, and is here shown to have a considerable effect on male and female mating strategies. Sex ratio, likewise, is shown to be influential. The level of effects of these various factors is itself of interest and population, shoal, family and individual levels of effect have been elucidated. Finally, and for me the most interesting finding, is that although the focus of this thesis, and much of the ingoing research, has been variation in behaviour, guppies are shown to be much more consistent in their behaviour than has generally been thought. The incorporation of this consistency into research into the plasticity of mating behaviour provides an interesting new dimension for guppy research.

Appendix 1

This appendix contains the results of analyses for the extra variables that for clarity were not included in Chapter 2. These variables were subject to the same analyses as those in chapter two with the exception of ‘time attending’ in which normality was not achievable, so repeated measures ANOVA was unfeasible. Repeatabilities were therefore impossible to calculate for this variable and Friedman’s test was used to assess differences between sex ratios in place of repeated measures ANOVA used for the remaining variables (Table A2). These variables are: frequency of chases - when a male chases a female who is attempting to escape; frequency of interferences - when a male attempts to sneak mate with a female that is already being attended by another male; mean duration of sigmoid displays- the total duration of sigmoid displays divided by the frequency of displays (from Chapter 2); mean duration of chases - the total duration of chases divided by the frequency of chases; and time spent attending females - when a male follows close to a female and attempts to keep other males away.

All variables show highly significant repeatabilities (Table A1). In addition, for all variables except duration of displays differences between sex ratios are significant (Table A2) and individual males retain the same rankings in each sex ratio (Table A3). Duration of displays shows the highest level of repeatability suggesting that individual males have a characteristic ‘style’ of displaying which is not affected by changes in sex ratio. The number and duration of chases and the number of interferences show the same pattern of variation from a highest level in male biased sex ratios, through equal sex ratio to a lowest level in female biased sex ratios. These behaviours are perhaps most affected by the presence of other males as all can involve competitive interactions. Interfering with another male obviously requires another male to compete with and in the case of chases, often these are initiated by an attempt by one male to intrude between another male and a female. The duration of displays and the time spent attending females are less variable and vary in the opposite direction (duration of displays: Table A1, Time attending: equal OSR = 497.5 ± 15.0 s; male-biased OSR = 464.1 ± 17.0 s; female-biased OSR = 543.2 ± 11.3 s (mean \pm se)), with a high in female biased sex ratios and a low in male biased sex ratios.

Table A1: Repeatabilities and descriptive statistics for the frequency of chases (No Chase); the frequency of interfering with another male's mating attempts (No Interfere); the mean duration of sigmoid displays (Duration Displays) and the mean duration of chases (Duration Chases). The transformation used to achieve normality is included. Means and standard errors for both durations are in seconds.

Variable	Sex	Transform- ation	df	R	SE of R	F	p	mean±se
	Ratio							
No Chases	Equal	Square	38,156	0.852	1.12×10^{-3}	250.179	<0.001	2.24±0.208
		root						
	Male	Square	38,39	0.949	2.60×10^{-4}	175.351	<0.001	3.23±0.375
	Bias	root						
	Female	Square	38,39	0.525	0.0138	54.476	<0.001	0.82±0.144
	Bias	root						
	Equal	Square	38,156	0.846	1.99×10^{-3}	246.868	<0.001	2.02±0.161
		root						
	Male	Square	38,39	0.976	5.92×10^{-5}	178.785	<0.001	4.74±0.670
	Bias	root						
No Interfere	Female	Square	38,39	0.528	0.0137	50.193	<0.001	0.89±0.216
	Bias	root						
	Equal	Inverse	38,156	0.989	7.82×10^{-6}	701.861	<0.001	1.34±0.058
	Male	Inverse	38,39	0.970	9.19×10^{-5}	171.114	<0.001	1.18±0.084
	Bias							
	Female	None	38,39	0.756	4.83×10^{-3}	463.830	<0.001	1.34±0.062
	Bias							
	Equal	Inverse	38,156	0.910	0.0469	290.016	<0.001	0.90±0.062
Duration Chases	Male	Inverse	38,39	0.935	4.22×10^{-4}	206.131	<0.001	0.98±0.077
	Bias							
	Female	Inverse	38,39	0.951	2.41×10^{-4}	54.066	<0.001	0.47±0.060
	Bias							

Table A2: Differences between sex ratios for the mean frequency of chases (No Chase); the mean frequency of interfering with another male's mating attempts (No Interfere); the mean duration of sigmoid displays (Duration Displays); the mean duration of chases (Duration Chases) and the mean time spent attending females (Time Attending). The test statistic is F for all repeated measures analyses of variance (RM ANOVA).

Variable	Transformation	Statistical test	df	Test statistic	p
No Chase	Square root	RM ANOVA	2,114	31.417	<0.001
No Interfere	Square root	RM ANOVA	2,114	35.205	<0.001
Duration Display	None	RM ANOVA	2,114	1.806	0.169
Duration Chase	Log (X+1)	RM ANOVA	2,114	19.944	<0.001
Time Attending	Non-parametric	Freidman's	2	-	<0.001

Table A3: Examination of whether individual males rank at the same level relative to other males in each sex ratio assessed using Kendall's Coefficient of Concordance. Means of each variable in each condition are used and transformations are the same as those set out in Table A2 above.

Variable	N	df	W	p
No Chase	39	2	0.415	<0.001
No Interfere	39	2	0.635	<0.001
Duration Display	39	2	0.049	0.146
Duration Chase	39	2	0.268	<0.001
Time Attending	39	2	0.226	<0.001

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