AN EXAMINATION OF THE ADAPTIVE SIGNIFICANCE OF INTERPOPULATION VARIATION IN THE BEHAVIOUR OF THE GUPPY *Poecilia reticulata*

by

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Populations of guppies (*Poecilia reticulata*) are found in rivers in Trinidad which differ in biotic and abiotic characteristics. Guppies from these rivers differ in a number of behavioural and morphological characteristics. Guppies in a turbid lowland river were found to be more cohesive and inhabit areas closer to shore than fish in clear headstream rivers. Males in the turbid lowland river exhibited a greater frequency of thrusts in their courtship than did males in the headstream rivers. Conversely, headstream males exhibited a greater frequency and duration of sigmoid displays in their courtship than did lowland males.

These differences in courtship behaviour between males in headstream and lowland rivers persisted in males bred in the laboratory under identical conditions. This strongly suggests that the differences in behaviour are at least in part genetically determined, and that therefore the behaviours are a product of natural selection.

Five experiments were designed in order to test the hypothesis that the identified differences in courtship behaviour exhibited by the populations are adaptations, in part, to differences in the turbidity of the rivers in which the populations naturally occur. If they are adaptations, they will contribute to the mating success of males in their particular environments. Males from turbid and clear rivers were placed in competition for mating with females under both clear and turbid water conditions. Mating success was
determined on the basis of number of inseminations and sperm contributed to females by males of the different populations. Sperm where identified using a radioactive labelling technique and standard autoradiographic methods.

In clear water, male guppies from a clear headstream river were more successful at mating with their own females and as successful at mating with lowland females as males from a turbid lowland river. In turbid water, males from the turbid lowland river were more successful at mating with their own females and equally successful at mating with headstream females as males from the clear headstream river. These results are consistent with the hypothesis that a high frequency of display and displays of long duration in male courtship are adaptations to clear water conditions. The results are also consistent with the hypothesis that high frequencies of thrusts in male courtship is an adaptation to turbid water conditions.

Males exhibiting similar courtship behaviours from two geographically isolated headstream rivers were significantly more successful at mating with females from their own populations. This suggests that other factors, such as colour or pheromones, may also be important in determining mating success.
Table of Contents

Abstract.................................................................................. ii
List of Tables........................................................................... vi
List of Figures.......................................................................... vii
Acknowledgements................................................................... viii

CHAPTER ONE
Introduction............................................................................ 1

CHAPTER TWO
Review of the Behaviour and Natural Environments
of the Guppy
I. Behaviour................................................................. 13
II. The Study Area..................................................... 19
   Physical Parameters of the Study Rivers
   A. Methods......................................................... 24
   B. Results........................................................ 26

CHAPTER THREE
Geographic Variation in the Courtship Behaviour of
Poecilia reticulata
I. Introduction........................................................................ 29
II. Behaviour in the Natural Environments
   Methods..................................................................... 31
   A. General behaviour............................................ 33
   B. Male courtship behaviour............................... 34
   Results and Discussion........................................ 35
III. Behaviour in Laboratory Raised Fish
   General Methods.................................................. 39
   Observation Procedures....................................... 40
   Results and Discussion........................................ 42
IV. Summary of Results.................................................... 46

CHAPTER FOUR
Mating Success in Clear Water Competition
I. Introduction............................................................... 49
II. Background and Preliminary Studies in
   Sperm Labelling..................................................... 51
III. Injection of Radioactive Tracer and
   Recovery of Labelled Sperm From Females... 53
IV. Stains and Autoradiographic Techniques.... 54
V. Experimental Design and Procedure.......... 56
VI. Assessment Procedures and Sources of
   Error................................................................. 66
VII. Results and Discussion............................................ 78
VIII. Summary of Results................................................. 90
CHAPTER FIVE
Mating Success in Turbid Water Competition
I. Introduction ................................................. 92
II. Experimental Design and Procedure ....................... 93
III. Results and Discussion ................................ 101
IV. Summary of Results ...................................... 105

CHAPTER SIX
General Discussion
The Adaptive Significance of Geographic Variation in the Behaviour and Morphology of Poecilia reticulata .................................................. 107

LITERATURE CITED ........................................... 122

APPENDICES
A. Mean numbers of males, females and juveniles in 2500 cm² areas in the four rivers .................. 130
B. Statistical values for Bartlett's, ANOVA and Scheffes' tests on data from the field observations of populations in the four rivers .. 131
C. Statistical values for Bartlett's, ANOVA and Scheffes' tests on data from the laboratory observations of populations from the four rivers 132
D. Mean sizes, and standard errors, of guppies used in this study ........................................ 133
E. Formulas for Stains ........................................ 134
**List of Tables**

TABLE ONE  
Comparison of the Physical Characteristics of the Naranjo, Paria, Lower Aripo and Guayamare Rivers... 28

TABLE TWO  
Means of the Behavioural and Physical Characteristics Observed and Recorded in the Naranjo, Paria, Lower Aripo and Guayamare Rivers... 36

TABLE THREE  
Comparisons of the Means of the Behavioural and Physical Characteristics Observed and Recorded in the Naranjo, Paria, Lower Aripo and Guayamare Rivers 37

TABLE FOUR  
Means of the Frequencies and Durations of the Behaviours Observed and Recorded in Laboratory Raised Males from the Naranjo, Paria, Lower Aripo and Guayamare Rivers 43

TABLE FIVE  
Comparison of the Means of the Frequencies and Durations of Behaviours Observed and Recorded in Laboratory Raised Males from the Naranjo, Paria, Lower Aripo and Guayamare Rivers 44

TABLE SIX  
Mating Success in Clear Water Competition - Experimental Outline (1 - 4) 61

TABLE SEVEN  
Proportions of Sperm Appearing as 'Labelled' in Background Radiation Control Smears 75

TABLE EIGHT  
Proportion of Labelled Sperm in Isotope Injected Males 76

TABLE NINE  
Summary of Results of Mating Success in Clear Water Competition, Experiments 1 - 4 81

TABLE TEN  
Mating Success in Turbid Water Competition - Experimental Outline (5) 98

TABLE ELEVEN  
Summary of Results of Mating Success in Turbid Water Competition, Experiment 5 104
List of Figures

FIGURE ONE
Illustration of Male Courtship Behaviours.............. 16

FIGURE TWO
Map and Location of the Island of Trinidad, West Indies Indicating Study Rivers................. 21

FIGURE THREE
Cages and Pond Used in Experiments 1 - 4.............. 59

FIGURE FOUR
Outline of Experimental Procedure...................... 65

FIGURE FIVE
Photomicrograph of Female Oviduct Smear Illustrating an Area of Heavy Sperm Concentration Not Suitable for Tallying................................. 69

FIGURE SIX
Photomicrograph of Female Oviduct Smear Illustrating a Low Level of Labelled Sperm............. 71

FIGURE SEVEN
Photomicrograph of Female Oviduct Smear Illustrating a High Level of Labelled Sperm.......... 73

FIGURE EIGHT
Results of Experiment 1................................. 83

FIGURE NINE
Results of Experiment 2................................. 85

FIGURE TEN
Results of Experiment 3................................. 87

FIGURE ELEVEN
Results of Experiment 4................................. 89

FIGURE TWELVE
Experimental Tank Used in Experiment 5................. 96

FIGURE THIRTEEN
Results of Experiment 5................................. 103

FIGURE FOURTEEN
The Mean Number of males (M), Females (F), and Juveniles (J) in 2500 cm² Areas in the Four Rivers................................. 130
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CHAPTER ONE

INTRODUCTION

A major thrust of ethological research has been aimed at elucidating the functions of the behaviour of organisms. The word function is used here not only in the sense of 'what is it for' but also in the stronger sense posed by Hinde (1975), "through what consequences does natural selection act to maintain the character?" These consequences, or selective pressures, elicit what Liley and Seghers (1975) refer to as a species' 'response', namely its morphological, physiological and behavioural characteristics. It is through analysing this response in terms of survival value, suggested Tinbergen (1965), that some suggestions can be formulated as to how the characteristic evolved.

The guppy, *Poecilia reticulata*, a live bearing teleost in the Family Poeciliidae, offers a unique opportunity to investigate the selective pressures affecting the morphological and behavioural characteristics of a species. The guppy exhibits marked intraspecific variation in behaviour and morphology and exists in a wide range of natural environments in the West Indies and Venezuela. Pioneering work by Winge (1922a, b; 1923, 1927, 1930, 1932, 1934), Winge and Ditlevson (1938, 1948) and Haskins (1951) concentrating on sex determination and colour inheritance in the species has also led to the establishment of a variety of laboratory strains identifiable by genetic markers. A short ovarian cycle (23 - 30
days), rapid maturation (2 - 3 months), hardiness and ease of collection and shipment combine to make the guppy an ideal organism to study from both the comparative and experimental approaches.

Studies attempting to shed light on microevolutionary trends in the species have concentrated on correlating observed variation in behaviour and morphology with biotic and abiotic differences in environments. These correlations have given rise to hypotheses which in turn have been tested experimentally. This study follows this approach.

Guppies are markedly sexually dimorphic. Males exhibit an elaborate and highly species-specific innate courtship pattern (Haskins et al., 1961). Females exhibit a number of receptive behavior patterns which are usually necessary for successful insemination (Liley, 1966). In size, the male is smaller than the female and while colouration in the male is pronounced, it is virtually absent in the female. Winge (1922a, b, 1923, 1927, 1930, 1932, 1934,) and Winge and Ditelevson (1938, 1948) have extensively studied the sex-determining system in Poecilia reticulata as well as modes of colour pattern inheritance. These authors have found sex-determination to have a genetic basis in the species, involving a pair of sex-chromosomes and a number of sex-genes on other chromosomes. The system is of the X-Y type with a large number of polymorphic colour patterns behaving genetically as though linked to the Y chromosome. Haskins and Haskins (1951) extended this work and found another large complex of the patterns acting as though linked to the X chromosome also. Haskins et al. (1961) mentioned that the
system is relatively unspecialized and that the sex chromosomes are at an early stage of evolutionary development as compared to *Drosophila*, for example.

As in all poeciliids, fertilization is internal. Rosen and Gorden (1953) described the functional morphology of the gonopodium, the modified anal fin in the male through which spermatophores are transferred to the female. Sperm is kept viable in the folds of the ovary (Winge, 1922a; Stolk, 1950) and can be stored up to ten months (Hogarth and Sursham, 1972). One insemination may be sufficient to fertilize a number of broods (Hildemann and Wagner, 1954; Baerends, Brouwer and Waterbolck, 1955). Winge (1937) suggested that new inseminations always compete successfully with sperm already in the ovary. Hildemann and Wagner (1954) found, however, that earlier inseminations could also contribute to the progeny of a given brood. The latter authors also cited evidence for multiple inseminations in laboratory stocks. Haskins, et al., (1961) contended that multiple inseminations are virtually non-existent in the wild. My research supports the findings of Hildemann and Wagner (see Chapter Four).

Fontaine (1945) and Trinkaus and Drake (1952) have shown that the guppy is actually ovoviviparous, the developing embryo receiving little or no nourishment from the female. Breder and Rosen (1966) reviewed the research on gestation in the guppy. Females exhibit an ovarian cycle of 23 to 30 days, ripe ova being present immediately after the release of a brood (Turner, 1937). Sexual maturity is reached in approximately 2 months, this can vary depending upon environmental conditions (Haskins
et al., 1961). Size of brood is considered by Turner (1937), Purser (1938) and Affleck (1960). Affleck suggested that brood size is in part a function of female length, with numbers varying from 2 to 100.

Isolated populations of guppies exist in headstream and lowland rivers on the island of Trinidad, West Indies. Headstream rivers are characterized by water which is clear, low in temperature and high in velocity. Predator species and numbers are low. Lowland rivers, on the other hand, are slow, turbid, high in temperature and contain diverse and numerous guppy predators (Seghers, 1973; Liley and Seghers 1975).

Correlated with headstream river conditions, Liley and Seghers (1975) have found populations of guppies in which individuals are large, males are brightly coloured and which exhibit a sex ratio of as much as four females to one male. Guppies in these rivers characteristically have poorly developed anti-predator and schooling behaviours. Individuals of lowland river populations are smaller, less brightly coloured, exhibit a 1:1 sex ratio and well developed anti-predator and schooling behaviours.

Of these relationships, the most extensively investigated to date is that between predation and its possible selective effects on guppy behaviour and morphology. Seghers (1970, 1973, 1974) studied the responses of natural populations to predation. He found that populations subject to high predation pressures (such as those in lowland rivers) were much more cohesive than those subject to predation by only a single species such as *Rivulus* (typical of headstream rivers). He
tested the hypothesis that schooling is a response to predator pressure by presenting wild and laboratory raised 'schoolers' (lowland river populations) and non-schoolers (headstream river populations) in equal numbers to large predators. He found the predators to take a greater number of 'non-schoolers' than 'schoolers'. Seghers identified differences between populations in reaction distance, alarm threshold and microhabitat selection in the anti-predator complex of adaptations in the species. The use of guppies raised under similar conditions in the laboratory provided evidence for Segher's contention that many of the observed behavioural differences between populations are, in part, due to genetic differences.

Liley and Seghers (1975) cite evidence to suggest that variation in size may also, in part, be a response to predation. A headstream river predator, *Rivulus*, was found to prey selectively on small fish while larger lowland river predators were unselective or took larger guppies. Their research was based on the observed correlation between high predator density and size and small guppies in lowland rivers and low predator density and size and large guppies in headstream rivers.

The suggestion that predation exerted a selective effect on the colour patterns of male guppies (Haskins, et al., 1961) has been tested by Endler (1978, 1980). Endler (1980) placed stocks collected from 11 locations in Trinidad randomly in tanks representing a variety of substrate and predator conditions. After five and fourteen months exposure to predation, censuses of the fish in the various tanks were
taken, although none was taken before the predators were added. The fish were scored for number, size and position of spots, colour and body size. He found guppies to be less conspicuous at high predator as compared to low predator intensities and found a good background matching in colour patterns, that is, course gravel matched large spots and fine gravel matched small spots. While Endler did not offer specific details on his scoring methods, he concluded from his results that natural selection acts differentially on different components of colour patterns. Furthermore he concluded that colour pattern variability reflects a selective balance between predation and another biotic feature, sexual selection.

Haskins et al., (1961) were the first to test the hypothesis that male color patterns which are brilliant to the human eye constitute a selective advantage in breeding. 'Bright' and 'dull' males of inbred laboratory stocks were allowed to compete for females. Since the stocks could be identified by genetic markers, the progeny of the mating could be assessed for parentage. The authors found a statistically greater number of offspring to be of descent of bright males. They did not however, investigate the mechanism of the breeding advantage, suggesting only that it might be through female choice, inter-male competition or associated physiology.

Endler (1980) suggested on the basis of his research that competition between males of different colour patterns was a prime determinant of reproductive success in low predator rivers.

Farr (1976) and Farr and Herrenkind (1974), on the other
hand, contend that behavioural differences, in terms of variation in display rates and aggression affecting intermale competition, were the prime determinants of reproductive success in the guppy. They suggest that colour acts only as a 'clue' in terms of kinship. Farr (1977) concluded in a later study that female choice based on both rare colour (rare-male phenomena) and behavioural characteristics was the central mechanism rather than inter-male aggression.

Mating a number of inbred laboratory stocks with various behavioural and morphological characteristics, Farr (1980) concluded from his results that high courtship display rate in males conferred a breeding advantage to males. Farr assessed the progeny of the matings and further concluded that males respond to the display rate of a competitor by increasing their display rates and that colour, first suggested by Haskins et al., (1961) to be the a prime determinant of reproductive success, only had effect if there were no overriding behavioural differences.

As Liley and Seghers (1975) have stated, it may be misleading to search for the biological function of a piece of behaviour or morphology. Similarly, it is misleading to search for the prime determinant of reproductive success. It is more likely that the interaction of a great number of biotic and abiotic features determine the survival value of a character and its contribution to mating success.

While predation has been most intensively studied in terms of its selective effects on guppy behaviour and morphology, there has been a recent effort to expand research to include a
number of abiotic features, including temperature, water velocity, and, in this study, turbidity.

Liley and Seghers (1975) found temperature to have an effect on the size of adult male guppies. Males of both headstream and lowland rivers grew larger under a 23 °C regime than those under a 28 °C regime, although under both regimes headstream males grew larger than lowland males. There were no significant differences in the effects on females of the stocks used. With evidence suggesting the size differences in males are at least in part due to genetic differences, the authors identified another selective factor which has likely played a part in the evolution of the species.

Stream velocity has been found to have a direct effect on the courtship behaviour of males (Crow, 1981). Increasing water velocity causes a corresponding decrease in courtship display frequency, but males from headstream rivers (high velocity) are better able to maintain frequency and duration of display than males from lowland rivers (low velocity). Using both fish shipped directly from the rivers and laboratory stocks of the same populations raised under similar conditions, Crow (1981) concluded that the observed variation in ability to maintain display has a genetic component and is an adaptation, in part, to stream velocity.

Ballin (1973) provided initial evidence that not only does male courtship differ in frequency and duration between different environments but he also suggested that two distinct behavioural strategies exist. In particular Ballin proposed that there are two routes to insemination in males, one through
courtship display and active participation by the female, the other involving simply a gonopodial thrust by the male made whether the female is receptive or not.

On the basis of the observed differences in guppy morphology and the associated differences in physical and biotic conditions discussed above, and particularly differences in turbidity, I predicted that males in clear headstream rivers would rely heavily upon the visual components (displays) in their courtship. On the other hand, I suggested that in fish from turbid lowland rivers, the non-visual components would play a more prominent role in courtship (thrusting). As the two insemination strategies are present in both types of populations, differences were expected to be quantitative rather than qualitative.

In headstream rivers sexual selection has been thought to play a role in the evolution of large size and bright colouration (Liley and Seghers, 1975), and is predicted to result in more vigorous or persistent courtship displays, all features emphasizing the visual component.

In lowland rivers, predation is more intense and the water is turbid. These factors would probably result in selection against a vigorous, conspicuous display and favour a mating strategy not as heavily reliant on visual aspects.

This study was organized into two stages. In the first stage the behaviour of guppies from four populations, two from clear headstream rivers, one from a turbid lowland river and one from an intermediate midstream river, was observed in the field and the laboratory in order to:
a) confirm the existence of differences in the behaviour of males from different populations and determine whether or not they are in the predicted direction, that is, a higher display frequency and duration in headstream as compared to lowland males, and a higher frequency of thrusting in lowland as compared to headstream males. It was also predicted that males from a midstream river would exhibit characteristics intermediate between these two extremes.

b) determine whether these differences are, in part, due to genetic differences. Offspring of fish from the four populations were bred and raised under identical conditions in the laboratory and the males' courtship behaviour observed. If the differences identified in the field persisted in the laboratory raised fish, this would provide strong evidence (but not proof) that the differences have a genetic basis.

In the second stage of the study I attempted to test the hypothesis that the observed differences in behaviour are to some extent adaptations to the different physical conditions, in particular to differences in turbidity, in which the populations naturally occur. If the display strategy in male courtship behaviour is an adaptation to clear water conditions, one would expect males exhibiting a high frequency and duration of display to have greater mating success in clear water than males exhibiting lower frequencies and durations of display. Conversely, if the thrust strategy is an adaptation to turbid water conditions, one would expect males exhibiting high frequencies of thrusts to have greater mating success in turbid water than males exhibiting lower frequencies of thrusts.
The hypothesis gave rise to the following predictions:

(c) that headstream males (high frequency and duration of display in courtship) would have greater mating success than lowland and midstream males (lower frequencies and durations of display) when placed in competition for females of all populations in clear water. This prediction was tested in experiments 1 and 2.

(d) that lowland males (high frequency of thrusts in courtship) would have greater mating success than headstream males (lower frequencies of thrusts) when placed in competition for females of both populations in turbid water. This prediction was tested in experiment 5.

Furthermore, it would be expected that males from different populations, but exhibiting similar courtship strategies, would be equally successful at mating with females in clear water conditions. This led to two further predictions:

(e) that there would be no difference in the mating success of lowland and midstream males when placed in competition for females of both populations in clear water. This prediction was tested in experiment 3.

(f) that there would be no difference in the mating success of males from two isolated headstream rivers when placed in competition for females of both populations in clear water. This prediction was tested in experiment 4.

As there was no reason to expect qualitative differences in the courtship of the males of the two different populations there was no reason to expect females to be selectively responsive to any type of courtship and therefore it was
predicted that both headstream and lowland river females would mate freely with headstream males in clear water and lowland males in turbid water.
CHAPTER TWO

REVIEW OF THE COURTSHIP BEHAVIOUR AND NATURAL ENVIRONMENTS OF THE GUPPY

I. Behaviour

The courtship behaviour of *Poecilia reticulata* has been extensively studied and described. Clark and Aronson (1951) offered the first detailed description of courtship patterns and presented preliminary results on the effectiveness of these patterns in bringing about insemination. The male pattern is the most conspicuous, the male orients in front of the female, then arches its body in a sigmoid display with fully open or closed caudal fin. The male then turns and attempts to insert the gonopodium into the female's genital pore.

While Breder and Coates (1935) suggested sexual discrimination in the guppy to be poor, other authors including Noble and Curtis (1935) and Haskins and Haskins (1949, 1950) concluded from their studies that sexual discrimination of males for conspecific females was well developed. Breder and Coates (1935) also suggested that the females played no active role in courtship. This contention was supported by Haskins and Haskins (1949). Later investigations, however, supported Stepanek's (1928) report in which he suggested that females are actively involved. Latham (1949) elaborated on this suggestion and Clark and Aronson (1951) finally identified and described a distinct, stereotyped female response behaviour. Kadow (1954)
and Liley (1966) offer detailed descriptions of the response which consists essentially of the female swimming in a tight circle with the genital pore exposed and thereby cooperating with the male in the mating attempt.

Ballin (1973), Farr (1978) and Crow (1981) have identified significant variations in the courtship pattern of males from a number of different natural populations. I have predicted that males from clear headstream rivers will exhibit higher display frequencies (emphasizing the visual component) and males from turbid lowland rivers will exhibit higher thrust frequencies (emphasizing the non-visual component). The following elements of the male's courtship pattern were observed and recorded. These behaviours are illustrated in Figure 1.

(A) Sigmoid Display. The sigmoid display begins up to 15 cm infront of the female; the male quivers with its body curved into and arch or an S-like shape while moving from infront of to beside the female. The male's caudal fin is either fully extended or tightly closed during the display.

(B) Gonopodial Thrust. The male swims alongside and slightly below the female, brings the gonopodium and ipsilateral fin forward and attempts to insert it into the female's genital pore with a quick jabbing motion. I specifically define the gonopodial thrust as a copulation attempt by the male without prior display and without prior female response.

(C) Copulation Attempt. If the male's gonopodium touches the female's genital pore for a fraction of a second, after either a sigmoid display or a thrust, and this is
FIGURE 1

Illustrations of males courtship behaviours.

1. Three variants of the sigmoid display.
2. Gonopodial thrust.
3. Copulation Attempt.
5. Aggressive encounter (chase).

Adapted from Baerends, Brouwer and Waterbolk, 1955. See text for further details.
1. 

2. 

3. 

4. 

5.
followed by post-copulatory jerking in the male (a number of jerky jumps away from the female) the behaviour is recorded as a copulation attempt. It would be less accurate to record this behaviour as an actual copulation since it is difficult to establish whether spermatophores have actually been transferred during any one attempt. Evidence does exist, however, to suggest that these criteria are indicative of successful inseminations. Baerends, Brouwer and Waterbolk (1955), using the oviduct smear technique, found 15 of 16 females to be inseminated after copulation attempts as described above.

(D) Gonopodial Swing. The gonopodial swing is defined as the sideward and forward movement of the gonopodium by the male when there is no female near or no attempt at insemination. Clark and Aronson (1951) and Liley (1966) found this behaviour to increase in frequency with increased sexual activity. Baerends, Brouwer and Waterbolk (1955) suggested that it may be a displacement activity.

(E) Aggressive Encounter. During an aggressive encounter a male gives chase or nips at another male. If the attacked male nips or chases in return, this is also considered as an aggressive encounter.

As discussed in the Introduction, two behavioural sequences are believed to result in insemination. In one strategy, males perform sigmoid displays to females and attempt to inseminate with the female's cooperation (Clark and Aronson, 1951; Kadow, 1954; Baerends, Bouwer and Waterbolk, 1955; Liley, 1966; Farr 1975, 1980). In this strategy, which I term the display strategy, the male typically orients in front of the
female, exhibits a sigmoid display and attempts to copulate with the female. Successful copulation involves the female's cooperation, expressed as a set of stereotyped receptive behaviour patterns (Liley, 1966).

The second strategy involves simply the male's orientation to the female, a gonopodial thrust and a copulation attempt (Kadow, 1954; Liley, 1966; Farr, 1980). The thrust strategy does not appear to involve female cooperation or acceptance. It must be noted that gonopodial thrusts, like displays, rarely result in a successful insemination and often result in only a small amount of sperm being transferred to the female (Clark and Aronson, 1951; Baerends, Brouwer and Waterbolk, 1955; Liley, 1966). Farr (1980), however, contends that the frequency of gonopodial thrusts resulting in inseminations as compared to displays may be underestimated. Most research to date has used virgin females, most of which immediately respond to courtship display. This biases the results towards insemination through display. Secondly, female guppies are receptive to male displays only during a 3-5 day period after the birth of a brood when ripe ova are present. During the remainder of the 30 day ovarian cycle females are unresponsive to male courtship and inseminations can only occur through thrusting. In wild populations, while the display strategy could result in more inseminations during the female's receptive period, gonopodial thrusting and sperm storage during the non-receptive period could result in successful fertilization.
II. The Study Area

The natural distribution of the guppy ranges from the coastal regions of Venezuela and British Guiana, through the islands of Trinidad and Tobago and into both the Windward and Leeward Islands of the Lesser Antilles (Rosen and Bailey, 1963). Populations exist in swamps, ponds, streams and rivers in these areas, including some brackish environments.

The island of Trinidad, the general research area of this study, lies off the coast of Venezuela opposite the mouth of the Orinoco River. The Northern Range, a chain of tree covered mountains, part of the coastal cordillera of Venezuela, extends along the northern margin of the island. The average elevation of the range is approximately 600-700 m (Liley and Seghers, 1975) and the peaks of El Tucuche and El Aripo extend above some 1000 m. A series of short (less than 16 km), narrow, roughly parallel rivers dissect the northern slopes of the Range, emptying into the Caribbean Ocean. The southern drainage of the Range is composed of a number of similar but longer rivers which form the tributaries of the larger Caroni and to a lesser extent, the Guayamare Rivers. These latter flow through the lowlands and empty into the Gulf of Paria (see Figure 2). While areas of low salinity exist along the northern coast and that of the Gulf of Paria, it is highly probable that _P. reticulata_ does not exist in the open sea. Populations in the northern slope rivers are thus effectively isolated from each other and from those in southern slope and lowland rivers. The
FIGURE 2

Map and location of the island of Trinidad, West Indies indicating study rivers

1. Paria River.
2. Naranjo River.
3. Lower Aripo River.

Adapted from Seghers, 1973.
upper ends of the southern rivers are as effectively isolated as those in the north but in their lower reaches they are connected by floodwaters and their common outflow (Haskins et al., 1961; Liley and Seghers, 1975).

Four rivers, one on the northern slope, two on the southern slope and one in the lowlands were selected for this study. Differences in physical and biotic characteristics determined the choices. The northward flowing Paria (P) (see Figure 2) is approximately 13 km in length and is blocked at its mouth by a 12 m waterfall. The Naranjo River (also referred to as the Upper Aripo by Liley and Seghers, 1975) is a tributary of the southern flowing Aripo River. The Aripo River is punctuated at its midpoint by a series of 3 m high falls. The major fish predators are isolated below the falls in the Lower Aripo. The only fish predator of the guppy existing in the Paria and Naranjo Rivers is *Rivulus hartii* (Cyprinodontidae).

Endler (1978) contended that the freshwater prawn *Macrobrachium crenulatum*, which exists in at least one of these rivers, the Paria, is a serious predator of the guppy. Endler states that the cutoff point for the eyes of arthropods in terms of sensitivity is at the orange or red wavelengths or longer. His discussion implied that this may be responsible for a shift in colour patterns in male guppies toward predominantly red patches where *M. crenulatum* is present. My own observations of the prawn in the Paria, based upon some 20 individuals in the company of guppies and many more incidental encounters, have not revealed an approach or capture by the prawn.
Observations that *M. crenulatum* is mainly nocturnal places some question on Endler's conclusions.

The third river, the Lower Aripo, contains a number of serious fish predators including *Aequidens pulcher* and *Crenicichla alta* (Cichlidae), *Astyanax bimaculatus*, *Hemibrycon* sp., *Hoplias malabaricus*, and *Hypostomus robinii* (Loricariidae) (Seghers, 1973; Liley and Seghers, 1975; pers. obs.). Seghers (1973) identified the Lower Aripo as a midstream river, intermediate in physical characteristics between headstream and lowland rivers.

The fourth study river, the Guayamare, flows through low lying agricultural land and empties into the Gulf of Paria via the Caroni Swamp. The Guayamare drains to some extent into the Caroni River, the major drainage for the southern slopes, via an artificial canal located some 16 km east of the Caroni Swamp. The predator species in the Guayamare River are much the same as the Lower Aripo with the addition of two further species of characids. Seghers (1973) and Liley and Seghers (1975) provide further and more specific details on the predator fauna of these and other Trinidad rivers.

These authors also presented data on various abiotic characteristics of the rivers. The data suggested the following trends from headstream rivers (the Naranjo and Paria) through midstream (the Lower Aripo) to lowland Rivers (the Guayamare); increasing width, depth, volume of flow, temperature and turbidity and decreasing velocity and shade. Since my predictions concerning differences in behaviour of populations of guppies in these rivers were based on these trends,
especially in turbidity, it was essential to examine whether the trends remained consistent over time. The following section presents my analysis of the physical characteristics of the four rivers utilized in this study.

Methods

One location on each of the four rivers, the Naranjo, the Paria, the Lower Aripo and the Guayamare was selected and identified as representative of that river. Using topographical maps, a number of locations were visually examined (where vegetation allowed) and a general area was chosen which was accessible, contained populations of guppies and was representative of the uniform conditions in each river. A grid reference map was made of each location on which was indicated the physical appearance of the river at that location. Specific points were identified at each location using a tape measure and a random number table. These points were identified on the grid reference map in centimeters from permanent landmarks. Recordings were always made at the specific points indicated on the map.

The locations were each visited on 8 days during the period May to November, 1978 for the express purpose of collecting physical data. All the rivers were visited on any one day and the visits were spaced 20 to 30 days apart. The order in which the rivers were visited on any one day was rotated. This approach was used in order to incorporate any seasonal effects on the rivers during the study period and at the same time control for short term weather changes.
The following physical characteristics were measured:

1. Depth: in cm at 3 points in each of the Naranjo (N), Paria (P), and Lower Aripo (LA) and at 2 points in the Guayamare (G).
2. Temperature: in °C at the same points in each of N, P, LA, and G.
3. Stream velocity: using a meter stick, a stopwatch and a small buoyant flask, the time it took the flask to travel 1 m was recorded. Three replicates were taken at the same points in the N, P, LA, and G. Data was recorded in m/sec.
4. Turbidity: a) using a sechi disk, the depth at which the disk disappeared from sight was recorded in cm. Recordings were made at 2 of the 3 points in the N, P, and LA, and the 2 points in the G.

   b) using a turbidity meter. The meter was composed of a 30 cm grey polyethylene cylinder 7.5 cm in diameter and sealed at one end with a disk of clear polyethylene. This formed the container for a column of water. This cylinder was placed, once filled with water, atop a black polyethylene box with one open side. In the top of the box was a 2 cm aperture lined on the inside with black foam rubber. The light receptor of a Lunasix 3 Light Meter fitted into this aperture and was shielded from all light save that coming through the column of water. A light was then placed over the top of the cylinder (powered by a water protected and fully recharged 5 volt
battery). This device was designed by N.R. Liley to measure relative rather than absolute values of turbidity. The Lunasix 3 Light Meter was set at din 16 and readings were taken on a scale 0 - 30, 0 being the lowest light level and therefore reflecting the highest turbidity. Control readings were taken with the cylinder empty. Since these read in all cases 10.3 on the scale, the actual scale readings were used as data.

One column of water was measured at each of 2 points in the N, P, LA, and G.

5. Light Striking the Surface: was measured with a Lunasix 3 Light Meter held at the water's surface and set at din 19. The scale on the meter offered a relative measure (15.0 = approx. 2800 lux, 22.0= approx. 350000 lux). Three replicate measures were taken at 10 m intervals at both the middle and edge of the N, P, LA, and G locations.

Results

The means and standard errors of the five physical characteristics measured in the four rivers are summarized in Table 1. Depth and light reaching the surface were found to increase from the headstream rivers to the lowland river. Water velocity was found to decrease from headstreams to lowlands with the exception of the Paria, which was relatively slow in comparison to the Naranjo but still faster than the lowland Guayamare. Temperature increased from headstream to lowland
although the trend was not found to be as distinct as the others. Turbidity decreased markedly from lowland to headstream.

The trends identified in the physical characteristics of the four rivers in 1978 are generally consistent with those identified by Seghers (1973) and Liley and Seghers (1975). On the basis of these results I predicted that male *P. reticulata* in the clear Naranjo and Paria rivers would exhibit a higher frequency of display strategy than would males from the turbid Guayamare River. I also made the prediction that Guayamare males would rely more heavily on the non-visual courtship strategy (thrusting) than would headstream males and that males from the Lower Aripo would occupy a midpoint between the two extremes.
TABLE 1. Comparison of the physical characteristics of the Naranjo, Paria, Lower Aripo and Guayamare Rivers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NARANJO</th>
<th>PARIA</th>
<th>LOWER ARIPO</th>
<th>GUAYAMARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Depth (cm)</td>
<td>30.71 (2.863)</td>
<td>30.71 (2.863)</td>
<td>44.31 (1.897)</td>
<td>114.24 (1.430)</td>
</tr>
<tr>
<td>Mean Temperature (°C)</td>
<td>25.48 (0.103)</td>
<td>24.21 (0.099)</td>
<td>25.89 (0.160)</td>
<td>25.69 (0.159)</td>
</tr>
<tr>
<td>Mean Water Velocity (m/sec)</td>
<td>0.646 (0.738)</td>
<td>0.143 (0.007)</td>
<td>0.162 (0.009)</td>
<td>0.047 (0.004)</td>
</tr>
<tr>
<td>Mean Turbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sechi Disk (cm)</td>
<td>100.0 (0.000)</td>
<td>100.0 (0.000)</td>
<td>31.27 (3.840)</td>
<td>5.400 (0.465)</td>
</tr>
<tr>
<td>Turbidity meter&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.91 (0.149)</td>
<td>10.03 (0.033)</td>
<td>8.03 (0.328)</td>
<td>5.04 (0.124)</td>
</tr>
<tr>
<td>Mean Light Reaching&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.62 (0.026)</td>
<td>16.56 (0.424)</td>
<td>18.51 (0.308)</td>
<td>20.78 (0.491)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Standard error in brackets.

<sup>b</sup> Relative measure based on 0 (turbid) - 10.3 (clear) scale. See text for details.

<sup>c</sup> Relative measure based on 0 (low) - 30 (high) scale. See text for details.
CHAPTER THREE

GEOGRAPHIC VARIATION IN THE COURTSHIP BEHAVIOUR OF MALE *Poecilia reticulata*

I. Introduction

The observations of populations of guppies in their natural environments reported in this chapter were carried out for two reasons. The first was to confirm the report by Seghers (1978), based on laboratory observations, that guppies from predator dense, turbid lowland rivers would exhibit a higher degree of cohesion and live closer to shore than guppies in clear, low predator headstream rivers. Seghers (1978) interpreted this increased cohesion and proximity to shore in lowland rivers as responses to predation pressure. Greater cohesion in lowland rivers can also be predicted on the basis of the greater turbidity of these rivers. Location of mates in the narrow, clear headstream rivers would appear to be much less a problem than in the wide, turbid lowland rivers. Therefore I predicted a higher level of cohesion in the Guayamare River (G) than in the headstream Naranjo (N) and Paria (P) Rivers, with the Lower Aripo River (LA) reflecting its midpoint turbidity (see Chapter 2) between the two extremes. I also predicted that G and LA fish would occupy a position in the river closer to shore than those in the N and P.

The second aim of the observations of natural populations
in the field was to test the prediction that male guppies in
turbid lowland rivers would more frequently exhibit the thrust
strategy in their courtship whereas those in headstreams would
more frequently exhibit the visual oriented display strategy.

It was predicted that N and P males would exhibit (1) a
higher frequency of sigmoid displays, (2) a greater duration in
sigmoid displays and (3) a lower frequency of gonopodial
thrusts than males from the LA or G. No differences were
predicted in the number of copulation attempts.

If differences as described above were found, the question
arose whether the differences in behaviour were a result of
experiential influences during development or whether they had
a genetic basis. To distinguish between the two possibilities,
fish from the four populations were raised under identical
conditions in the laboratory. As the fish matured, their
behaviour was examined, again under standard laboratory
conditions. If the behavioural differences between populations
persisted under these conditions it would provide strong
support for the claim (but not proof) that the differences have
a genetic basis.

Investigations of the populations in their natural
environments is the subject of the first section of this
chapter. The analysis of behaviour in laboratory raised fish is
the subject of the second section.
II. Behaviour in the Natural Environments

Methods

The studies discussed below were carried out during the period May to November, 1978. Preliminary observations of the Naranjo, Paria and Lower Aripo Rivers up to 1 km upstream and downstream from the locations identified in Chapter Two showed that guppies occurred in a number of distinct microhabitats within each river. In the N and P Rivers these microhabitats were of three types. The glide was defined as an area of relatively shallow water (approximately 1 - 15 cm deep) flowing at a relatively high velocity. Glides were found at both the edges and in the middle of the two rivers. A shallow was an area of deeper (approximately 15 - 40 cm) and slower water. Shallows were found only along the edges of the rivers. Pools were relatively slow moving bodies of water (at times no recordable velocity) with a minimum depth of 40 cm and averaging 100 cm in the N and 81.5 cm in the P.

In the LA guppies were present in both shallows and pools but were not seen in glides.

The Guayamare River presented a somewhat different situation. Guppies were found to be thinly distributed within 1 km of the location described in Chapter 2. Since a number of other locations on the G were easily accessible, it was decided to observe guppies in two further locations in order to provide a comparable sample. The G does not contain glides, shallows or pools as described above. There are two discernible
microhabitats in which guppies occur, one along the edge of the relatively uniform main body of the river, the other in large, slow moving pools. While depth does not vary appreciably between the two microhabitats, water velocity is noticeably reduced in the pools (0.08 m/sec in the main body of the river against 0.005 m/sec in the pools).

In order to obtain measures of behaviour representative of guppies occurring in the entire rivers, groups of guppies were observed according to the following schedule;

Naranjo: 8 glides, 8 shallows, 4 pools = 20 locations
Paria: 8 glides, 8 shallows, 5 pools = 21 locations
Lower Aripo: 8 shallows, 8 pools = 16 locations
Guayamare: 8 main body, 3 pools = 11 locations

I had hoped to observe fish in each of 8 microhabitats in each river but the relative infrequency of some and the restricted access to others brought this number down.

I refer to my observations taking place on groups of fish rather than schools in order to avoid any confusion with respect to the connotations associated with the latter. Schools defined as close aggregates of fish with some measure of integrity were found only to exist in the main body of the G and to some extent in the shallows and pools of the LA. Guppies in the N and P, while not being distributed randomly throughout the rivers, could not be said to exist in identifiable aggregates.

The following physical and behavioural characteristics were observed and recorded in each of the 68 locations.
A. General behavioural and physical parameters.

1. Number of guppies per 2500 cm²: A 2500 cm² grid with markings along the edges at every cm was gently lowered into the water and placed on the bottom substrate of glides and shallows in the N, P, and LA Rivers; 5 minutes were allowed before any observations were carried out. The number of guppies in the grid were counted every minute for 3 minutes noting number of adult males, females, and juveniles. In the G river and the pools of the N, P, and LA turbidity and/or depth obscured the grid so in these locations it was held approximately 30 cm above the water surface and numbers per minute for 3 minutes were recorded on a tape recorder and later transcribed into a field notebook.

2. Distance between fish: Using the grid and the recording methods described above, the distance between two fish at the edge of the grid was recorded in cm. Starting in the bottom right corner of the grid, 15 distances between separate pairs of fish were recorded, moving in a clockwise direction around the grid. A mean was then calculated for each location.

3. Depth: The depth of the water at each location was measured in cm by lowering a tape measure in the center of the grid.

4. Distance to shore: the distance of a group of fish to shore was measured using a tape measure, distance to shore being defined here as the distance between the
edge of the river at the waterline and the nearest
guppy. Five measurements were taken at each location,
spaced 1 minute apart, and a mean calculated.

5. Velocity of water: After all the observations had
been completed, including those one male courtship
described below, water velocity was measured using
the method described in Chapter 2. A mean was
calculated over three replicates.

It should be pointed out that, as mentioned earlier, fish
in the LA and to a greater extent the G tend to be in small
schools, with the rest of the rivers being devoid of guppies.
The measure of number of guppies per 2500 cm² in these rivers
therefore does not accurately represent fish density since it
measures only the numbers associated with schools. The measure
more accurately represents the cohesion of fish in areas where
they are found in the rivers.

B. Male Courtship Behaviours.

The following procedures were used in recording the
courtship behaviours of males. A male was identified in the
general grid area (holding the grid was dispensed with at this
point) and visually followed for three minutes. Observations of
males which disappeared from sight within three minutes were
disregarded. For each of five males at each location the
following behaviours were recorded:

1. Number of sigmoid displays per 3 minutes.
2. Number of gonopodial thrusts per 3 minutes.
3. Number of copulation attempts per 3 minutes.

Durations of sigmoid displays were also recorded using a
stopwatch after the above had been completed. Ten durations were usually recorded per location, this was reduced to five in some due to the infrequency of display and/or low density of fish. A maximum of 2 durations were recorded for any one male.

**Statistical Analysis**

All data were transformed to log(x+1) in order to ensure normality and homogeneity of variance (Quenouille, 1950). All statistical analyses were carried out on the transformed data. Adjusted means were then obtained using the adjustment recommended by Elliot (1973); 1.15 times the variance of the transformed counts was added to the mean of the transformed counts and the antilog of this adjusted mean was then taken and 1 subtracted in order to obtain the adjusted mean.

Homogeneity of variance was confirmed using Bartlett's Test (Snedecor and Cochran, 1967) at the 0.01 level of significance (see Appendix B). The data were then subjected to analysis of variance. Those comparisons in which the ANOVA identified significant differences were then subjected to Scheffé's (1959) Test for specific comparisons between rivers. The results of the ANOVAs and Scheffé Tests are summarized in Table 3. The F values, degrees of freedom and levels of significance for the ANOVAs and the Scheffé F statistics, contrast values and levels of significance are included in Appendix B.

**Results**

Table 2 lists the means of the behavioural and physical characteristics observed and recorded in the four rivers. Table 3 indicates a number of significant differences between these
TABLE 2. Means of the behavioural and physical characteristics observed and recorded in the Naranjo, Paria, Lower Aripo and Guayamare Rivers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NARANJO</th>
<th>PARIA</th>
<th>LOWER ARIPO</th>
<th>GUAYAMARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of fish per 2500 cm²</td>
<td>2.81</td>
<td>5.63</td>
<td>6.95</td>
<td>12.35</td>
</tr>
<tr>
<td>Distance between fish (cm)</td>
<td>27.99</td>
<td>18.61</td>
<td>7.73</td>
<td>4.12</td>
</tr>
<tr>
<td>Depth of water (cm)</td>
<td>35.31</td>
<td>32.10</td>
<td>34.34</td>
<td>83.15</td>
</tr>
<tr>
<td>Distance to shore (cm)</td>
<td>49.26</td>
<td>71.04</td>
<td>30.36</td>
<td>16.86</td>
</tr>
<tr>
<td>Water velocity (m/sec)</td>
<td>0.477</td>
<td>0.142</td>
<td>0.059</td>
<td>0.070</td>
</tr>
<tr>
<td>Number of sigmoid displays/3 min</td>
<td>3.62</td>
<td>5.40</td>
<td>1.43</td>
<td>1.84</td>
</tr>
<tr>
<td>Number of gonopodial thrusts/3 min</td>
<td>0.47</td>
<td>0.76</td>
<td>1.22</td>
<td>2.93</td>
</tr>
<tr>
<td>Number of copulation attempts/3 min</td>
<td>0.12</td>
<td>0.10</td>
<td>0.25</td>
<td>0.15</td>
</tr>
<tr>
<td>Duration of sigmoid display (sec)</td>
<td>4.13</td>
<td>4.08</td>
<td>2.73</td>
<td>2.87</td>
</tr>
</tbody>
</table>
TABLE 3. Comparisons of the means of the behavioural and physical characteristics observed and recorded in the Naranjo, Paria, Lower Aripo and Guayamare Rivers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ANOVA</th>
<th>Scheffé Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G-LA</td>
<td>G-N</td>
</tr>
<tr>
<td>Number of fish per 2500 cm²</td>
<td>&lt; 0.0001</td>
<td>G&gt;LA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Distance between fish (cm)</td>
<td>&lt; 0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Depth of water (cm)</td>
<td>0.0007</td>
<td>G&gt;LA</td>
</tr>
<tr>
<td>Distance to shore (cm)</td>
<td>0.0071</td>
<td>-</td>
</tr>
<tr>
<td>Water velocity (m/sec)</td>
<td>0.0002</td>
<td>-</td>
</tr>
<tr>
<td>Number of sigmoid displays/3 min</td>
<td>&lt; 0.0001</td>
<td>-</td>
</tr>
<tr>
<td>Number of gonopodial thrusts/3 min</td>
<td>&lt; 0.0001</td>
<td>G&gt;LA</td>
</tr>
<tr>
<td>Number of copulation attempts/3 min.</td>
<td>0.4733&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Duration of sigmoid display (sec)</td>
<td>&lt; 0.0001</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Scheffé comparisons significant at the 0.01 level of significance.

<sup>b</sup> Scheffé comparisons not significant at the 0.01 level of significance.

<sup>c</sup> ANOVA not significant at the 0.01 level of significance. Scheffé not performed.
characteristics. Keeping in mind that number of fish per 2500 cm² is not a true measure of density across the entire LA and G rivers, the measure does indicate than G guppies are significantly more cohesive in specific areas than guppies in the N, P, or LA. Both LA and P fish exhibit greater numbers per area than those in the N. The distance between fish was also significantly lower in both the G and LA than it was in the N and P. These results tend to support the prediction, based on similar findings by Crow (1981) and Seghers (1974) in laboratory observations, that guppies in lowland rivers live in more cohesive units and in greater proximity to each other than do those in headstream rivers.

Fish in the high predator G stay closer to shore and exist in areas of greater depth than do those in the low predator N and P. This supports Segher's (1973) contention that remaining close to shore is, in part, a response to increased predation. Both LA and N fish remained closer to shore than P fish.

The analysis of male courtship behaviour revealed a number of significant differences between populations, all in the predicted directions. Males from the headstream N and P rivers exhibited significantly more sigmoid displays and displays were of significantly longer duration than those of males in the LA and G. Conversely, males in the G were found to exhibit a significantly higher rate of gonopodial thrusting than those in the other three rivers. The low number of copulations observed in the four rivers did not differ significantly.

The differences in courtship identified were consistent with those found in the same stocks raised in the laboratory.
(Ballin, 1973; Snyder, 1978). Crow (1981), studying the Paria and Caparo (lowland river) populations under laboratory conditions obtained similar findings. He found Paria males to exhibit significantly more displays than Caparo males while the latter exhibited significantly more thrusts than the former.

The three predictions; that N and P males would exhibit (1) a higher frequency of sigmoid displays, (2) a greater duration in sigmoid displays and (3) a lower frequency of thrusts than males from the LA and G, were supported. Also as predicted, fish from the G exhibited a higher level of cohesion than those of the N and P. The midstream LA population was found to occupy an intermediate position for this characteristic. The number of observed copulation attempts were low and no significant differences were identified. These findings support the hypothesis that while all populations exhibit both thrusts and displays, males from clear headstream populations rely more on the visually oriented display strategy in their courtship while males from turbid lowland populations rely more on the thrust strategy.

III. Behaviour in Laboratory Raised Fish

Methods

Stocks were collected from Naranjo, Paria, Lower Aripo and Guayamare Rivers and kept in separate glass tanks of 21 and 38 l capacity at the Asa Wright Nature Centre (Simla), Trinidad. These fish will henceforth be referred to as the parental generation. The stock tanks were filled and regularly changed
with tap water, the source of which was a natural stream. They were maintained in natural light and temperature conditions in a large open air room at the Centre.

Young of all four stocks were collected as they appeared and were placed in separate tanks, and are referred to as the first generation. The fish in these tanks were allowed to mature and were used as subjects of the behavioural analysis, which was begun 2 months after the establishment of the first generation. Food for all stocks consisted of the commercially prepared dried food Tetra-min occasionally supplemented with locally collected mosquito larvae.

Behavioural observations were carried out in a separate room in the Centre between 800 and 1700 hours. One tank of 21 l capacity was utilized as the observation tank. The tank was shielded on three sides and illuminated by a fluorescent light suspended 2 m above the tank.

Observation Procedure

Ten mature first generation females of a given stock were taken from their tank and placed in the observation tank. Ten first generation males of the same population were then taken from the tank and placed in a 10 l capacity tank. A drawing was made of each male indicating its colour pattern to be used for subsequent identification during the observations. The males were then placed with females in the observation tank and left for a 30 minute acclimatization period. Each male was then observed for 10 minutes during which behaviours were recorded using a Rustrak recorder connected to a four key board.
The following behaviours were recorded for each male:

1. Number of sigmoid displays per 10 minutes.
2. Number of gonopodial thrusts per 10 minutes.
3. Number of copulation attempts per 10 minutes.
4. Durations of sigmoid displays (by keeping the key in (1.) depressed for the duration of the display).
5. Number of gonopodial swings per 10 minutes.
6. Number of aggressive encounters per 10 minutes (due to the infrequency of copulation attempts and aggressive encounters, the same key was used for these behaviours, one stroke indicating the former, two rapid strokes, the latter.

Descriptions and definitions of these behaviours are presented in Chapter 2. Observing and recording these behaviours in 10 males for 10 minutes per male constituted one trial. Three trials were carried out for each of the four populations. After each trial the fish were anaesthetised in 1:600 MS 222 (Tricane methane sulphonate - Sandoz) to distilled water. The mean sizes of all fish used in observations and experiments are recorded in Appendix D.

The data was subjected to the same statistical analysis as that used and described in the previous section. Homogeneity of variance was confirmed using Bartlett's Test (Snedecor and Cochran, 1967) at the 0.01 level of significance. F values, degrees of freedom and levels of significance for the ANOVAs and the Scheffé F statistics, contrast values and levels of significance are included in Appendix C.
**Results**

The means of the frequencies and durations of the behaviours observed and recorded are shown in Table 4. Table 5 summarizes the results of the statistical analyses. Laboratory raised Naranjo and Paria males displayed at higher frequencies and for longer durations than Guayamare males. G males in turn performed gonopodial thrusts at higher frequencies than both N and P males. These results are consistent with those observed in the field, namely that headstream males exhibit a higher frequency of the display strategy and lowland males a higher frequency of the thrust strategy in their courtship. Since the first generation fish were raised under identical laboratory conditions away from natural selective agents, the findings provide strong evidence that there is a genetic contribution to the observed differences.

Differences between the field (Table 3) and laboratory (Table 5) results are almost confined to the midstream Lower Aripo River. The significantly greater frequency of thrusting exhibited by G males as opposed to LA males identified in the field was not confirmed in the laboratory raised fish. Laboratory raised LA males were found, however, to exhibit a statistically greater number of displays than G males, a difference not found in the field. Similarly, LA males exhibited a higher frequency of thrusting than P males in the laboratory. Whether these differences between field and laboratory are a result of experiential factors during growth, differences between the observation tank and the natural streams or differences in my methods of recording observations
TABLE 4. Means of the frequencies and durations of the behaviours observed and recorded in laboratory raised males from the Naranjo, Paria, Lower Aripo and Guayamare Rivers.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>NARANJO</th>
<th>PARIA</th>
<th>LOWER ARIPO</th>
<th>GUAYAMARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sigmoid displays/10 min</td>
<td>14.20</td>
<td>11.07</td>
<td>4.71</td>
<td>2.57</td>
</tr>
<tr>
<td>Number of gonopodial thrusts/10 min</td>
<td>3.57</td>
<td>2.60</td>
<td>5.81</td>
<td>9.03</td>
</tr>
<tr>
<td>Number of copulation attempts/10 min</td>
<td>0.23</td>
<td>0.13</td>
<td>0.10</td>
<td>0.13</td>
</tr>
<tr>
<td>Duration of sigmoid display (sec)</td>
<td>2.63</td>
<td>2.09</td>
<td>1.64</td>
<td>1.26</td>
</tr>
<tr>
<td>Number of gonopodial swings/10 min</td>
<td>16.21</td>
<td>8.71</td>
<td>8.70</td>
<td>10.27</td>
</tr>
<tr>
<td>Number of aggressive encounters/10 min</td>
<td>1.42</td>
<td>1.70</td>
<td>0.03</td>
<td>1.87</td>
</tr>
</tbody>
</table>
TABLE 5. Comparison of the means of the frequencies and durations of behaviours observed and recorded in laboratory raised males from the Naranjo, Paria, Lower Aripo and Guayamare Rivers.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>ANOVA</th>
<th>Scheffe comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G-LA G-N G-P LA-N LA-P N-P</td>
<td></td>
</tr>
<tr>
<td>Number of sigmoid displays/10 min</td>
<td>&lt;0.0001</td>
<td>LA&gt;\text{a} N&gt;G P&gt;G N&gt;LA P&gt;LA -</td>
</tr>
<tr>
<td>Number of gonopodial thrusts/10 min</td>
<td>&lt;0.0001</td>
<td>- G&gt;N G&gt;P - LA&gt;P -</td>
</tr>
<tr>
<td>Number of copulation attempts/10 min</td>
<td>0.5355\text{c}</td>
<td>- - - - - - -</td>
</tr>
<tr>
<td>Duration of sigmoid display (sec)</td>
<td>&lt;0.0001</td>
<td>LA&gt;\text{G} N&gt;G P&gt;G N&gt;LA P&gt;LA N&gt;P</td>
</tr>
<tr>
<td>Number of gonopodial swings/10 min</td>
<td>&lt;0.0001</td>
<td>- N&gt;G - N&gt;LA - N&gt;P</td>
</tr>
<tr>
<td>Number of aggressive encounters/10 min</td>
<td>1.0715\text{c}</td>
<td>- - - - - - -</td>
</tr>
</tbody>
</table>

\text{a} Scheffe' comparisons significant at the 0.01 level of significance.

\text{b} Scheffe' comparisons not significant at the 0.01 level of significance.

\text{c} ANOVA not significant at the 0.01 level of significance. Scheffe' not performed.
is difficult to establish. It is interesting to note, however, that all these differences involve the LA stock and generally support its status as an intermediate population between the headstreams and lowland.

As in the field, the number of copulation attempts observed was low and there were no significant differences between the populations.

Two behaviours were measured in the laboratory and not in the field: gonopodial swings because they were difficult to distinguish in the field situation and aggressive encounters because they were non-existant in the field. While two other laboratory studies found P males to be significantly more aggressive than the lowland G (Ballin, 1973) and Caparo (Crow, 1981) males, no significant differences were found in this study. Farr (1975) also found aggression to be virtually non-existent in the field and found it to decrease reproductive success in laboratory strains of guppies (Farr, 1980). These findings shed some doubt on Ballin's (1973) suggestion that aggression is functional in inter-male competition and Gandolfi's (1971) and Gorlick's (1976) contentions that it is important in establishing status and social hierarchies in wild populations.

N males were found to exhibit significantly more gonopodial swings than males of the other populations. The significance of gonopodial swings in terms of male courtship has yet to be determined. Clark and Aronson (1951) and Liley (1966) have found this behaviour to increase with increased sexual activity, and Crow (1981) speculated that it may
function as an 'intention movement' before display. Ballin (1973) and Snyder (1978) found gonopodial swings to be in greater frequency in lowland populations as opposed to headstreams. Crow (1981) has suggested that these observations may reflect non-courtship gonopodial swings which represent displacement activities as first suggested by Baerends, Brouwer and Waterbolk (1955).

IV. Summary of Results

Four populations of *Poecilia reticulata*, two from clear headstream rivers (Naranjo and Paria), one from a turbid lowland river (Guayamare) and another from an intermediate midstream river (Lower Aripo) were observed in their natural environments and in the laboratory and a number of behaviours recorded. A number of predictions were tested through observation.

A. Field Observations.

1. The lowland population was found to be more cohesive than the headstream populations.

2. The lowland population was found to inhabit areas closer to shore than the headstream populations.

3. Males of the lowland population were found to exhibit a higher frequency of gonopodial thrusts during courtship than headstream males.

4. Males of the headstream populations where found to exhibit a higher frequency and duration of sigmoid displays during their courtship than lowland males.
5. No differences were found in the frequency of copulation attempts exhibited by males of the four populations.

6. Behaviours performed by fish from the midstream LA population were generally found to be intermediate between the headstreams and lowland.

B. Laboratory observations.

1. With fish from the four populations born raised under identical conditions in the laboratory, males of the lowland population were found to exhibit a higher frequency of gonopodial thrusts during courtship than headstream males.

2. Males of the headstream populations were found to exhibit a higher frequency and duration of sigmoid displays during their courtship than lowland males.

3. Males from one headstream river (N) were found to perform a higher frequency of gonopodial swings than males from the other populations.

4. No differences were found in the frequency of copulation attempts or aggressive encounters between males of the four populations.

5. Differences between field and laboratory results tended to support the intermediate character of the midstream (LA) population.

The results support the prediction that males from clear headstream rivers rely more heavily on the visual display strategy in their courtship, while males from the turbid lowland river rely more heavily on the non-visual thrust strategy in their courtship. Since the differences were found persist in stocks bred and raised under identical conditions in
the laboratory, strong evidence was found to support the prediction that the differences are, at least in part, genetically determined.
CHAPTER FOUR

MATING SUCCESS OF MALES IN COMPETITION
FOR FEMALES IN CLEAR WATER

I. Introduction

Observations of guppies in four rivers in Trinidad have established that significant differences exist between populations in levels of cohesion and male courtship behaviours. Strong evidence was found to suggest that the differences have, in part, a genetic basis. This implies that the different behaviours are the product of natural selection and thus have some adaptive significance for the fish in the different environments. In order to be adaptive, the behavioural patterns must make the organism better able to survive and reproduce as compared to other members of the same species (Wilson, 1975). They must, in other words, confer some measure of reproductive success on the organisms.

Experiments described in this chapter and chapter 5 were designed to test the hypothesis that differences in cohesion and mating strategy exhibited by the populations are adaptations, in part, to differences in the turbidity of the rivers in which the populations naturally occur. Males from the four populations were placed in competition for mating with females under clear (this chapter) and turbid (Chapter 5) water conditions. Mating success was determined on the basis of number of inseminations and sperm contributed to females by
males of the different populations. Sperm were identified using a radioisotope labelling technique and standard autoradiographic methods.

The hypothesis led to the following predictions for mating success in clear water:

1. That males from the clear headstream populations (Naranjo and Paria) would be more successful at mating, than males from the turbid midstream (Lower Aripo) and lowland (Guayamare) rivers, when placed in competition for females of all populations. Assuming that colouration and display evolved under epigamic sexual selection in both types of environment, the pressure of this type of selection would be expected to be more intense and progressed further in clear water. If the vigorous and conspicuous display strategy in headstream males is a response to this pressure, it should be more likely to attract and stimulate all females. This prediction was tested in two experiments:

Experiment 1: Headstream (Naranjo) males in competition for mating with lowland males (Guayamare) for females of both populations.

Experiment 2: Headstream (N) males in competition for mating with midstream males for females of both populations.

2. That there would be no significant differences in mating success between males of the midstream (LA) and lowland (G) rivers when placed in competition for females of both populations. While LA males exhibited behaviours intermediate between those exhibited by headstream and lowland males, the
courtship of LA males tended to be more similar to that of lowland males. Also, while the water of the headstream rivers was clear, the lowland and midstream rivers differed only in the degree of turbidity. I therefore predicted that there would be no differences in mating success between males of the lowland and midstream populations. This prediction was tested in one experiment:

Experiment 3: Midstream (LA) males placed in competition for mating with lowland males (G) for females of both populations.

3. That there would be no significant differences in mating success between males of the two headstream populations (N and P) when placed in competition for females of both populations. Since no major differences in courtship behaviour exist between the two populations and the environmental conditions in which they exist are similar, no differences in mating success were expected between males of these populations. This prediction was tested in one experiment:

Experiment 4: Headstream (N) males placed in competition for mating with headstream males (P) for females of both populations.

II. Background and Preliminary Studies in Sperm Labelling.

The radioisotopes thymidine - 3H and thymidine 14C are exclusively incorporated into the DNA of cells (Reichard and Esteborn, 1951). These isotopes have been used extensively in studying DNA synthesis and the cell cycle in man (eg. Bell, et
al., 1967; Cole and Mckalen, 1963), the rat (Saski, 1965), mice (Layde and Baserga, 1964), Drosophila (Plaut, et al., 1966) and a number of other organisms and cell cultures (see Baserga and Malamud, 1969, for an extensive review).

The possibility of using these isotopes in labelling the sperm of Poecilia reticulata was first suggested to me by N.R. Liley. Later we became aware of studies by Billard (1966) and Crowley (1968) documenting successful incorporation of thymidine - $^3$H into guppy sperm using standard autoradiographic methods.

I carried out a number of pilot studies in 1977 - 1978 in order to determine if the isotopes could be used with success. The preliminary studies, using standard autoradiographic procedures (see Gross, et al., 1951; Jofites, 1963; Rogers, 1967; and Baserga and Malamud, 1969, for comprehensive reviews), revealed that from 70 - 80% of sperm taken from males 21-28 days after an injection of the isotopes could be labelled. A spermazoa was defined as labelled if it had incorporated the isotope into its DNA. This incorporation was identified by a black grain in the emulsion over the cell evident upon microscopic examination (see Figures 5 - 7).

It was also found that labelled sperm could be successfully recovered from females mated with injected males for a period of seven days. Neither the injection nor the isotope were found to have a significant effect on the courtship behaviours of isotope injected males as compared to saline injected and non-injected males.
III. Injection of Radioactive Tracer and Recovery of Labelled Sperm From Females.

Injection Techniques - Males

Males were taken from parental stock tanks and anaesthetized one at a time in a petri dish containing 1:600 MS 222 (Tricane methane sulphonate - Sandoz) to distilled water. The anaesthetized male was placed on a glass slide, the gonopodium layed forward and the abdominal region stroked with a blunt probe. This effectively stripped the male of sperm.

The male was then placed under a dissecting microscope on a wet cloth soaked in saline solution. Two µl of thymidine (methyl - 14C) - of 1.0 cu/l concentration was drawn into a 50µl capacity Hamilton syringe fitted with a #30 needle. The needle was slid anteriorly into the abdominal region of the male, just dorsal to the gonopodium. The isotope was injected into the fish. As the needle was withdrawn, the wound was held closed with a pair of forceps in order to ensure minimal loss of isotope. Where saline injected males were used, the stripping and injecting procedures were the same, except that a different syringe was used and 2.0 µl of saline (0.6% NaCl) solution injected. After the injection a fish was allowed to recover in a petri dish containing distilled water. Fish were then isolated from females and other experimental groups in tanks containing tap water (from a local freshwater stream) with 0.5 gm/l methylene blue dissolved.
Sampling Techniques - Females

Females were taken from the experimental cages (see Experimental Design, this chapter), anaesthetized in MS 222, and placed under a dissecting microscope on a wet cloth soaked in saline solution. Micropipettes fashioned by flaming and pulling 2.5 mm diameter glass tubing were used to take oviduct samples from the females. The micropipette was first partially filled with distilled water and then inserted into the female’s genital opening. The distilled water was flushed 4 times from pipette to oviduct to pipette. The micropipette was then withdrawn and the contents flushed onto a sterilized glass slide. The procedure was repeated three times for each female. The sample was spread over approximately two-thirds of the slide using the 'blood smear technique'. The smears were then allowed to air dry. A new micropipette was used for each female.

IV. Stains and Autoradiographic Techniques.

Staining

The slides with dry smears, each representing an oviduct sample from a female or a sperm sample from a male, were placed in the removable trays of staining dishes and stained as follows;

1. Soaked in 3 changes of distilled water, 10 minutes each.

2. Stained in Mayer's hematoxylin for 3 minutes (see Appendix E for formula).
3. Rinsed in distilled water.
4. Blued in 1.0% sodium acetate, 5 minutes.
5. Rinsed in distilled water.
6. Stained with aqueous eosin for 1 minute (see Appendix E for formula).
7. Rinsed in distilled water and allowed to dry.
8. One passage 50% ethanol for 5 minutes, then 2 passes 95% ethanol, 5 minutes each and allowed to dry.

All reagents were maintained at 18 °C in a cold water bath during the staining procedure and the hematoxylin replaced regularly.

**Autoradiographic Techniques**

The method of coating the smears with liquid emulsion is outlined in Baserga and Malamud (1969). The entire procedure subsequently to be discussed was carried out in a dark room under a light fitted with a Kodak Wratten Series I (red) filter. A 120 ml bottle of Kodak NTB - 3 Nuclear Emulsion was taken from refrigerated storage and the emulsion melted in the bottle in a water bath at 43.0 ± 0.5 °C. Sixty ml of the emulsion was diluted with 60 ml distilled water in a beaker and stirred slowly with a glass rod. All bubbles were removed from the diluted emulsion.

Each slide was dipped in the emulsion for 2 seconds, ensuring that the entire slide was coated. The slides were then placed vertically on the edge of paper towelling and allowed to dry. The filtered light was turned off during the drying period.
Once dry, the slides were placed in slide boxes taped with Scotch Brand black electrical tape. Each box contained a small amount of anhydrous CaSO₄ (Drierite) wrapped in gauze. The boxes were sealed with black tape and stored at room temperature for 14 days.

After the 14 day exposure period slides were removed from their boxes and processed as follows:

1. Kodak D19 developer for 3 minutes.
2. Distilled water for 1 minute.
3. Kodak Fixer for 10 minutes.
4. 1:15 dilution of Edwal Hypoeliminator to distilled water for 1 minute and allowed to dry.

V. Experimental Design and Procedure

All fish used in the competitive mating experiments were collected from the four rivers, the Naranjo (N), Paria (P), Lower Aripo (LA) and Guayamare (G) and held in the parental stock tanks described in Chapter 3. Only fish collected directly from the rivers were used in these experiments.

All experiments were conducted in an outdoor concrete pool at the Asa Wright Nature Centre (Simla), Trinidad. The pool measured 270 cm X 200 cm X 43 cm (Figure 3). A continuous flow of clear water was fed into the pool from a local natural stream. The water level in the pool was maintained at 24 cm depth. Each of the two replicates per experiment (see below) were conducted in one of the 6 cages placed in the pool. The cages were constructed by Liley and myself out of wood and
plastic mesh which did not allow the escape of fish, including newborn (Figure 3). A lid which fitted securely on the top of the cage allowed access.

Water temperature over the experimental period, August to October, 1978, averaged 26.3 °C at 1000 hours and 26.2 °C at 1600 hours. Water was maintained in clear condition and debris collecting on or around the cages was removed regularly. Fish were fed on the commercially prepared dried food Tetra-Min occasionally supplemented with locally collected mosquito larvae.

Four competitive mating experiments were conducted in clear water. In each experiment, males of two of the four populations, N, P, LA and G, were placed in competition for females of the same populations. Details of the four experiments are summarized in Table 6. Two replicates were conducted within each experiment with the isotope injected population of males reversed. This was done to cancel out any effects the isotope might have on the males. It also compensated for the fact that not all sperm in isotope injected males could be expected to be labelled (see Section VI, this chapter).

In experiment 1, replicate 1, Naranjo males injected with isotope were placed in competition with Guayamare males injected with saline solution. In replicate 2, Guayamare males injected with isotope were placed in competition for mating with Naranjo males injected with saline solution. The data from these two replicates was later combined for analysis. Experiment 2 included two replicates of LA and N males placed
FIGURE 3

Cages and pool used in Experiments 1 - 4.

A. Diagramatic organization of experimental cages in pond.
   1. Drain
   2. Water inflow

B. Proportions of an experimental cage.
   3. Removable lid
   4. Fine gauge plastic mesh
   5. Water level maintained in pond
in competition for mating, experiment 3, two replicates of LA and G males and experiment 4, two replicates of N and P males (see Table 6).

The following is a description of the experimental method used in all the replicates for all the experiments. For the sake of brevity and clarity, the first replicate of experiment 1 is used as an example. The method is illustrated in Figure 4.

Twenty five males were removed from the N stock tank and were stripped and injected with 2.0 µl of thymidine - 14C as described in section III. Thirty males from the G stock were then stripped and injected with 2.0 µl saline solution. The males from the two populations were then placed in separate holding tanks. This constituted day 0 of replicate 1, experiment 1 (on the same day replicate 2 was begun, with the injection reversed).

On day 5 of the experiment this entire stripping and injection procedure was repeated.

On day 10, 35 N females and 35 G females were removed from their respective stock tanks and clipped for later identification. This involved clipping off the 2 posterior rays (6,7) of the anal fin of N females and the 2 anterior rays (1,2) of G females. While the rays were found to regenerate, the clipping procedure was sufficient to positively identify the females over the experimental period. At this time the females' oviducts were flushed using a micropipette and saline solution in order to remove any sperm which might be present. The females were then placed together, isolated from the males, in outdoor cage 1 (Figure 3). The same procedure was carried
TABLE 6. Mating Success in Clear Water Competition

Experimental Outline (1 - 4)

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>POPULATION TYPE</th>
<th>COMPETING POPULATIONS</th>
<th>REPLICATE</th>
<th>FEMALE POPULATION</th>
<th>FEMALES MATED</th>
<th>ANAL RAYS CLIPPED</th>
<th>MALE POPULATION</th>
<th>MALES MATED</th>
<th>CAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Head-stream - Lowland</td>
<td>Naranjo - Guayamare</td>
<td>1</td>
<td>N</td>
<td>30</td>
<td>6,7/1,2</td>
<td>N*</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Mid-stream - Head-stream</td>
<td>Lower Aripo - Naranjo</td>
<td>2</td>
<td>G</td>
<td>30</td>
<td>1,2/6,7</td>
<td>G*</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Mid-stream - Mid-stream</td>
<td>Lower Aripo - Guayamare</td>
<td>1</td>
<td>N</td>
<td>30</td>
<td>6,7/1,2</td>
<td>N*</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Lowland - Head-stream</td>
<td>Naranjo - Paria</td>
<td>2</td>
<td>G</td>
<td>30</td>
<td>1,2/6,7</td>
<td>N*</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

* indicates male population injected with isotope.
out for females for replicate 2, except the rays clipped were reversed and the females placed in cage 2.

The cages were checked regularly for young and these removed. On day 24 females were removed from the outdoor cages and the cages cleared of any debris and young. Thirty females from each stock were identified under the dissecting microscope and replaced in their original cages. The remaining females, included in case of mortalities, were discarded in another outdoor pond not used in this study.

On day 25, 20 N males and 20 G males were added to the 30 females from each stock already present in cage 1. This constituted the beginning of the mating period. The remaining 5 isotope injected N males were held in the holding tank. Sperm samples from these males taken on day 28 and 5 other N males taken from the cage at the end of the experiment were used to obtain an accurate assessment of the proportion of sperm labelled at the middle and end of the mating period (Figure 4, c). Smears taken from these males will henceforth be referred to as label level control smears.

The remaining 10 saline injected G males were held in their holding tank for stripping on day 28 (Figure 4, B). Sperm smears from these males served as controls in order to measure background radiation (naturally occurring radiation) and emulsion contamination by light. Both of these factors cause black grains to appear in the emulsion. If the grains occur over spermatazoa, they cannot be distinguished from grains caused by isotope in the DNA. Smears taken from these males will henceforth be referred to as background radiation control
smears.

After these males were stripped and smears prepared, they were measured using calipers, then killed in a high concentration of MS 222, and preserved in formaldehyde.

On day 32 the mating period of the experiment was terminated (Figure 4 A). The females were sampled and oviduct smears prepared as described in section III, 5 N males stripped and label level control smears prepared, all fish measured using calipers, killed in a high concentration of MS 222, and preserved in formaldehyde. As each smear was made, it was identified using a diamond etching pen. The smears were then stained and fixed as described in section VI and then stored in slide boxes with ten background radiation control smears distributed amongst them. All smears where later subjected to further autoradiographic processing as described in section IV.

Procedures for the other three experiments, LA-N, LA-G and N-P, and the replicates therein were identical to those described above. Each replicate was carried out in a separate cage, the two replicates of any one experiment being started on the same day. The experiments themselves were staggered due to available work space and the amount of work involved in any one processing day.

The setting of the mating period at 7 days was a tradeoff between two factors, the period over which high levels of labelled sperm could be expected to be present in isotope injected males and the period of time after insemination over which sperm would remain accessible by oviduct flushing. My pilot data suggested that the maximum sustained period for
FIGURE 4

Outline of Experimental Procedure.

A. Main experiment (represents one of two replicates).

Day 0  Males stripped, injected and isolated,
* = population injected with isotope.
Day 5  Males stripped, injected and isolated.
Day 10 Females' oviducts flushed, anal rays
clipped for identification and isolated
in experimental cages.
Day 25 Males placed in experimental cage with
females.
Day 32 Experiment terminated, females' oviducts
sampled for sperm, smears prepared.

B. Label level control males.

Day 0  Males stripped, injected and isolated.
Day 5  Males stripped, injected and isolated.
Day 28 Males sampled for sperm, smears prepared.
Day 32 Males from main experiment sampled for
sperm, smears prepared.

C. Background radiation control males.

Day 0  Males stripped, injected with saline
solution and isolated.
Day 28 Males sampled for sperm, smears prepared.

NOTE: This represents one replicate of two in each
experiment. In the second replicate the isotope
injected population of males is reversed as are
the label level control and background radiation
control males. The anal rays clipped in females
of the two populations are also reversed.
which high levels of labelled sperm could be expected to be present in injected males after the first of two injections was from day 22 to 32 post injection. This is due to the production of new and disintegration of old sperm in the testes (Crowley, 1968).

On the other hand, Liley (1966) and my pilot results suggested that sperm is accessible in the oviduct for a period of approximately 7 days after which it is incorporated into the female's ovarian folds. On the basis of these factors, the mating period was set at 7 days, from day 25 to day 32 of the experiment.

VI. Assessment Procedures and Sources of Error

Assessment Procedures

Smears were examined under a light microscope with a micrometer grid using light-field illumination. A smear was initially examined for sperm at a magnification of 400X. Upon identifying sperm, magnification was shifted to 1000X and the smear scanned until individual sperm could be identified. In some areas the density of sperm was too heavy to allow this identification (see Figure 5). A sperm was considered labelled if one or more silver grains in the emulsion overlying it was activated (ie. black grain). Beginning at the left side of the micrometer grid, labelled and unlabelled sperm were tallied using hand counters until a total of 100 sperm had been assessed, moving the grid to the right as many times as necessary. The smear was then again scanned until another area
in which individual sperm could be identified was found. Another 100 sperm were assessed, labelled and unlabelled, as described above. The smear was then scanned a third time, and a final 100 sperm assessed for label. Tallies for each smear in which sperm was present were recorded as number of labelled and unlabelled sperm per 300. The same assessment procedure was used for oviduct smears, label level control smears and background radiation control smears. Figure 6 illustrates a smear section with a low proportion of labelled to unlabelled sperm, Figure 7 illustrates a smear section with a high ratio of labelled to unlabelled sperm.

Measures of Mating Success

Two criteria were established in order to determine the mating success of males placed in competition for females in the four experiments.

A. Number of Attributable Inseminations.

In a number of cases it was possible to attribute the labelled or unlabelled sperm present in a smear to a male(s) of only one of the two populations. The criteria for attributing an insemination(s) to one of the populations were as follows: if a smear contained over 245/300 labelled sperm it was attributed to a male(s) from the isotope injected population. Similarly, if the smear contained over 284/300 unlabelled sperm it was attributed to a male(s) from the saline injected population.

These criteria referred to above are based on corrections for background radiation and sperm label level in isotope
FIGURE 5

Photomicrograph of female oviduct smear illustrating an area of heavy sperm concentration not suitable for tallying. 1000X magnification.
FIGURE 6

Photomicrograph of female oviduct smear illustrating a low level of labelled sperm. 1000X magnification.
FIGURE 7

Photomicrograph of female oviduct smear illustrating a high level of labelled sperm. 1000X magnification.
injected males. Smears from males injected with saline in the four experiments (background radiation controls) should contain no labelled sperm. If activated grains over sperm are found in these smears, the activation can be attributed to either background radiation or light contamination. Table 7 lists mean number of sperm with activated silver halide grains over them tallied for the background radiation control smears for each replicate of the four experiments. A grand mean has also been calculated. The total percent of sperm tallied as 'labelled' which may be due to background radiation is 5.45%.

The mean number of labelled sperm in isotope injected males was also calculated for each replicate. These means for males label level control smears are listed in Table 8. The percentage of labelled sperm for males over the four experiments, corrected for background radiation was 76.20%. This translates into 245/300 labelled sperm, the lower limit for an oviduct smear, and consequently an insemination, to be attributed to the isotope injected male(s).

Conversely, oviduct samples contributed by the saline injected males should exhibit more than 284 unlabelled sperm. Up to 16 sperm associated with granules in the emulsion (5.45%) can be attributed to background radiation.

The number of attributable inseminations by males in each replicate were then combined and compared using the $X^2$ Test ($\alpha = 0.05$, Snedecor and Cochran, 1967).

B. Mean Number of Sperm.

The measure of mating success discussed above does not incorporate the data from oviduct smears which appear to be the
TABLE 7

Proportion of sperm appearing as 'labelled' in background radiation control smears.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male population injected with saline</td>
<td>N G</td>
<td>LA N</td>
<td>LA G</td>
<td>N P</td>
</tr>
<tr>
<td>Number of males</td>
<td>10 10</td>
<td>10 10</td>
<td>10 10</td>
<td>10 10</td>
</tr>
<tr>
<td>Total sperm tallied per smear</td>
<td>300 300</td>
<td>300 300</td>
<td>300 300</td>
<td>300 300</td>
</tr>
<tr>
<td>Mean 'label' due to background radiation</td>
<td>17.7 20.1 16.2 16.8 16.2 14.7 13.5 15.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 'label' due to background radiation, all experiments</td>
<td>16.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% 'label' due to background radiation, all experiments</td>
<td>5.45</td>
<td></td>
<td></td>
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</tbody>
</table>
### TABLE 8

Proportion of labelled sperm in isotope injected males.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male population injected with saline</td>
<td>N G</td>
<td>LA N</td>
<td>LA G</td>
<td>N P</td>
</tr>
<tr>
<td>Number of males</td>
<td>10 10</td>
<td>10 10</td>
<td>10 10</td>
<td>10 10</td>
</tr>
<tr>
<td>Total sperm tallied per smear</td>
<td>300 300</td>
<td>300 300</td>
<td>300 300</td>
<td>300 300</td>
</tr>
<tr>
<td>Mean number of labelled sperm</td>
<td>238.2 231.2 229.1 230.6</td>
<td>261.7 255.6</td>
<td>254.4 258.9</td>
<td></td>
</tr>
</tbody>
</table>

Mean number labelled sperm for all experiments corrected for background radiation: 228.60

% of labelled sperm for all experiments corrected for background radiation: 76.20
result of multiple inseminations, that is, those smears which contain less than 245 labelled sperm and those which contain less than 284 unlabelled sperm. These types of oviduct smears represent 64.4% of the oviduct smears found to contain sperm. In order to incorporate the data in these mixed oviduct smears, the total number of sperm contributed by males of the competing populations to females was compared using the Mann Whitney U test ($\alpha = 0.05$, Snedecor and Cochran, 1967).

The two sources of error discussed above could have a marked effect on the actual number of sperm contributed by males to females exhibiting mixed samples in the experiments. Background radiation could cause an overestimation of sperm contributed by isotope injected males and an underestimation of sperm contributed by saline injected males. Labelled sperm levels in isotope injected males below 100% could conversely result in underestimating the isotope injected males' contribution and overestimate the saline injected males' contribution. In an attempt to overcome these possible sources of error, I ran two replicates of each experiment with the injected population of males reversed. This should effectively cancel out the effects of the two sources of error, unless the errors were significantly different between replicates.

I therefore tested for differences in background radiation and male label level between replicates for each experiment using Mann Whitney U Tests ($\alpha = 0.05$, Snedecor and Cochran, 1967). None of the comparisons were found to be significantly different. It was therefore decided to use the raw scores and combine the replicates in comparing the mean number of sperm
contributed by males competing for females in the four experiments.

VII. Results and Discussion

Naranjo males were found to have inseminated more N females (p < 0.01) and contributed more sperm to N females (p < 0.001) than did Guayamare males (Figure 8A). Naranjo males were also found to have inseminated more N females (p < 0.05) and contributed more sperm to N females (p < 0.01) than Lower Aripo males (Figure 9B). These results are consistent with the prediction that headstream males utilizing the display strategy in their courtship would outcompete males utilizing the thrust strategy in clear water in competition for headstream females.

A similar prediction for midstream and lowland females was not supported, however. No significant differences were found in the number of inseminations or the amount of sperm contributed to G females by N and G males (Figure 8B). Neither were there any significant differences in the number of inseminations and sperm contributed by N and LA males to LA females (Figure 9A). These results suggest that while the display strategy (high display/low thrust) results in greater mating success for headstream males with their own females, the thrust strategy (low display/high thrust) employed by midstream/lowland males is equally successful in ensuring mating with midstream/lowland females. These results strongly suggest that other factors as well as male display strategy may be involved in determining mating success with midstream and
lowland females. These will be discussed below.

No significant differences were found in the number of inseminations and sperm contributed to females by males of the midstream LA and lowland G rivers. This result applies to both LA (Figure 10A) and G (Figure 10B) females. This supports my second prediction, that males from populations exhibiting similar courtship strategies from rivers with similar physical conditions would not differ significantly in their mating success with females of either population.

Males from similar headstream rivers with similar courtship strategies did, however, mate assortatively. Naranjo females where inseminated significantly more \((p < 0.005)\) and had significantly more sperm contributed to them \((p < 0.001)\) by Naranjo males than Paria males (Figure 11A). Conversely, P females exhibited the opposite trend, being inseminated more frequently \((p < 0.05)\) and carrying more sperm \((p < 0.001)\) from P males than N males (Figure 11B).

The results from the four experiments suggest that headstream populations are capable of some degree of interpopulation discrimination, exercised by the female, male, or both. That this discrimination is based only on the female in terms of preference for male courtship behaviour is unlikely since the only significant difference identified between the courtship behaviours of N and P males was the number of gonopodial swings, which are thought to be a displacement activities (Baerends, Brouwer and Waterbolk, 1955) or possibly intention movements (Crow, 1981). It is also possible that N and P females are capable of distinguishing males of their own
population on the basis of colour. Farr (1980) concluded from experiments with inbred stocks of guppies that male colouration could have some effect on male reproductive success, but only in the absence of behavioural differences. Haskins et al., (1961) also found females to be capable of discriminating between males of different colour patterns but they did not control for behaviour.

A third possibility exists, and that is that males of the two headstream populations are capable of discriminating for females of their own populations. Ballin (1973) suggested and Crow (1981) directly observed significant preferences in N males (Upper Aripo in Crow) for N females and P males for P females in experiments where the populations were observed together. These preferences were expressed as a higher number of displays and thrusts directed by males at females of their own population. The mechanism for this discrimination by males may be a population specific pheromone released by the female (Crow and Liley, 1979).

A high degree of interpopulation discrimination does not seem evident between the LA or G populations on the basis of the results illustrated in Figure 10. Colour differences between males of the LA and G populations are not as distinct as those between the N and P and consequently females of the G and LA populations may be less able to distinguish males of their own populations. Crow (1981) also found that while N and P males exhibited a significant preference in their courtship toward their own females, G males were less discriminating.

As mentioned earlier, no significant differences were
### TABLE 9

Summary of Results of Mating Success in Clear Water Competition, Experiments 1 - 4.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Population Sampled</td>
<td>N</td>
<td>G</td>
<td>LA</td>
<td>N</td>
</tr>
<tr>
<td>Number of Females Recovered</td>
<td>60</td>
<td>59</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Number of Female Samples With Sperm</td>
<td>28</td>
<td>26</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>Number of Samples Attributed to Male Population</td>
<td>11</td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
FIGURE 8

Results of Experiment 1

A. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Naranjo (N) and Guayamare (G) males to Naranjo females in clear water.

B. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Naranjo (N) and Guayamare (G) males to Guayamare females in clear water.

a. Comparison of numbers of sperm; Mann Whitney U Test.

b. Comparison of attributed samples, x^2 Test.

N.S. = not significant at 0.05 level of significance
A

NUMBER OF ATTRIBUTED SAMPLES

MEAN NUMBER SPERM PER 300

p < 0.001

NARANJO FEMALES

B

NUMBER OF ATTRIBUTED SAMPLES

MEAN NUMBER SPERM PER 300

N.S.

GUAYAMARE FEMALES

N.S.
FIGURE 9

Results of Experiment 2

A. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Lower Aripo (LA) and Naranjo (N) males to Lower Aripo females in clear water.

B. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Lower Aripo (LA) and Naranjo (N) males to Naranjo females in clear water.

a. Comparison of numbers of sperm; Mann Whitney U Test.

b. Comparison of attributed samples, $X^2$ Test.

N.S. = not significant at 0.05 level of significance
A

MEAN NUMBER SPERM PER 300

LOWER ARIPO FEMALES

B

MEAN NUMBER SPERM PER 300

NARANJO FEMALES

N.S. \(^a\)

p < 0.01 \(^a\)

N.S. \(^b\)

p < 0.05 \(^b\)
FIGURE 10

Results of Experiment 3

A. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Lower Aripo (LA) and Guayamare (G) males to Lower Aripo females in clear water.

B. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Lower Aripo (LA) and Guayamare (G) males to Guayamare females in clear water.

a. Comparison of numbers of sperm; Mann Whitney U Test.

b. Comparison of attributed samples, $X^2$ Test.

N.S. = not significant at 0.05 level of significance
A

NUMBER OF ATTRIBUTED SAMPLES
MEAN NUMBER SPERM PER 300

LOWER ARIPO FEMALES

B

NUMBER OF ATTRIBUTED SAMPLES
MEAN NUMBER SPERM PER 300

GUAYAMARE FEMALES
FIGURE 11

Results of Experiment 4

A. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Naranjo (N) and Paria (P) males to Naranjo females in clear water.

B. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Naranjo (N) and Paria (P) males to Paria females in clear water.

a. Comparison of numbers of sperm; Mann Whitney U Test.

b. Comparison of attributed samples, X^2 Test.

N.S. = not significant at 0.05 level of significance
A

MEAN NUMBER SPERM PER 300

p < 0.001\(^a\)

NARANJO FEMALES

B

MEAN NUMBER SPERM PER 300

p < 0.001\(^a\)

PARIA FEMALES

p < 0.05\(^b\)
found between the mating success of N males and G males with G females nor were any found between the mating success of N males and LA males with LA females. These results may be due to the relatively high levels of cohesiveness observed in the G and LA populations. G and LA females could be expected to encounter males of their own population more frequently than those of the N population. This may have counterbalanced any female preference for higher displaying males.

VIII. Summary of Results

Males of four populations of *Poecilia reticulata*, two from clear headstream rivers (Naranjo and Paria), one from a turbid lowland river (Guayamare) and another from an intermediate midstream river (Lower Aripo) were placed in competition for mating with females of these populations. Four experiments were designed to test the hypothesis that differences in cohesion and mating strategies between these populations are adaptions, in part, to differences in the turbidity of the rivers in which these populations naturally occur.

1. The prediction that males from clear headstream populations (N and P) would be more successful at mating than males from the turbid midstream and lowland populations (G and LA) when placed in competition for headstream females in clear water was supported.

2. The prediction that males from headstream populations would be more successful at mating than males from midstream and lowland populations when placed in competition for midstream
and lowland females in clear water was not supported. It was suggested that greater cohesion in midstream and lowland populations could have counteracted a preference of females of these populations for higher display frequency and duration exhibited by headstream males.

3. The prediction that there would be no significant differences in mating success between males of turbid midstream (LA) and turbid lowland (G) rivers when placed in competition for females of both populations in clear water was supported.

4. The prediction that there would be no significant differences between the mating success of two clear water headstream populations (N and P) when placed in competition for females of both populations in clear water was not supported. The results indicated interpopulation discrimination by both populations. It was suggested that this discrimination could be exercised on the basis of female preference for colour, or male preference on the basis of a pheromone or both.

The results suggest that increased display frequency and duration in headstream males are, in part, adaptations to the clear water conditions evident in their natural environment.
CHAPTER FIVE

MATING SUCCESS OF MALES IN COMPETITION
FOR FEMALES IN TURBID WATER

I. Introduction

Results in Chapter 5 show that headstream males exhibiting high frequencies and durations of display in their courtship are more successful at inseminating females of their own populations than are males exhibiting low levels of display and high frequencies of thrusts, in clear water. Headstream males employing the display strategy are also equally successful at inseminating females from midstream and lowland populations as are thrust strategy males from those populations, in clear water. These results argue strongly in favour of the hypothesis that high frequency and duration of display in male courtship are adaptations to the clear water conditions in which these males exist. Epigamic sexual selection is seen as a strong selective factor in these clear water rivers.

Turbidity and predation are both pressures existing in lowland rivers which presumably counteract sexual selection resulting in bright colour, large size and high frequency and duration of display. One would therefore expect fish in lowland populations to be smaller, less brightly coloured, more cohesive and exhibit courtship behaviour relying less on the visual component, that is, the display strategy. Observations of behaviour in lowland and midstream populations in the field
and the laboratory support these predictions (see Chapter 3). The experiment described in this chapter was designed to test the hypothesis that cohesiveness and a high frequency of thrusts in male courtship behaviour are adaptations, in part, to the turbid water conditions experienced by fish in lowland rivers. Turbidity, as determined by the two measures described in Chapter 2, was found to be significantly higher in the lowland Guayamare River than in the headstream Naranjo River (Mann Whitney U Test, Snedecor and Cochran, 1967; Sechi, $p < 0.01$; meter, $p < 0.01$). On the basis of the hypothesis I predicted that:

1. Males from the turbid lowland Guayamare river would be more successful at mating than males from the clear headstream Naranjo river when placed in competition for females of both populations in turbid water conditions. This prediction was tested in Experiment 5, using the sperm labelling and assessment methods described in Chapter 4.

II. Experimental Design and Procedure

I collected guppies from five rivers in Trinidad, West Indies, including the Naranjo and Guayamare, and shipped these to the University of British Columbia, Vancouver.

The stocks were housed, by Liley and Crow, in separate tanks in three controlled laboratories. The tanks in which they were held were of 43 and 61 l capacity with gravel substrates and planted with Vallisneria and Lemna. All tanks were maintained under 30 or 40 watt fluorescent tubes and filtered
by subgravel, and internal and external charcoal filters. One third of the water in each tank was regularly changed with dechlorinated tap water.

All three laboratories were on a 13 h light - 11 h dark photoperiod and maintained at 23 - 26 °C. Young were regularly removed from the wild caught tanks. All fish were fed on a commercially prepared dried fish food, Tetra-min, occasionally supplemented with brine shrimp or Tubifex worms.

This experiment was carried out at the University of British Columbia in two tanks designed by Liley and Crow (Figure 12). The tanks were constructed out of plexiglass and had a plywood base. Each tank held approximately 500 l of water and was aerated using air stones.

Water flow could be controlled through the use of a submersible pump (Model 4SMD, Little Giant Pump Company) which was regulated by a variable transformer (Powerstat, Type 3PN, Superior Electrical Company). Crow (1981) provides a detailed description of this control system.

Several alternatives were tried in attempting to make the water in the tanks turbid, the most successful being the particulate in water collected from streams along the Capilano Mountain Road, North Vancouver. Substrate from these streams was collected in plastic buckets and dechlorinated tap water added. The substrate was then stirred and allowed to settle for 1 hour. The water with the suspended particulate was then added to the tank water. A slight current established in each tank helped to keep the particulate suspended.

A white disk similar to the one used in measuring
FIGURE 12

Experimental tank used in Experiment 5.

1. Submersible pump.
2. Plastic tube.
3. Plexiglass tube with 6 mm holes.

Adapted from Crow, 1981.
turbidity in Trinidad streams and a plastic metric ruler were used to measure turbidity. Turbidity was checked twice a day during the experimental period and maintained at 6 ± 2 cm. This was done by removing water from the experimental tanks and adding turbid dechlorinated water.

Water temperature was maintained at 22 - 24 °C. The laboratory was on a 13 h light - 11 h dark photoperiod and each tank was lit by 8 40 watt fluorescent tubes suspended 2 m above the tank.

The autoradiographic methods used in this experiment was the same as those used in the four clear water experiments conducted in Trinidad (Chapter 3, sections II - IV). One competitive mating experiment, designated '5', was conducted in turbid water. Wild caught males from the Naranjo and Guayamare populations were placed in competition for mating with females of these same populations. Details of the design are listed in Table 10. Two replicates were run in the experiment, reversing the isotope injected population of males and the identification mark on females. These replicates were run simultaneously in two separate experimental tanks and the data later combined for analysis.

On day 0 of the experiment 25 Naranjo males were removed from the stock tank, were stripped and injected with 2.0 µl of thymidine - (methyl-3H)- at a concentration of 1.0 cu/l. 30 Guayamare males were stripped and injected with 2.0 µl saline (0.6% NaCl) solution. These males were then isolated in separate holding tanks. A further 30 Naranjo males and Guayamare males were removed from the stock tanks and processed
### TABLE 10. Mating Success in Turbid Water Competition.

Experimental Outline (5)

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPULATION TYPE</td>
<td>Headstream - Lowland River</td>
</tr>
<tr>
<td>COMPETING POPULATIONS</td>
<td>Naranjo - Guayamare</td>
</tr>
<tr>
<td>REPLICATE</td>
<td>1</td>
</tr>
<tr>
<td>FEMALE POPULATION</td>
<td>N</td>
</tr>
<tr>
<td>FEMALES MATED</td>
<td>30</td>
</tr>
<tr>
<td>PEDUNCLE MARK</td>
<td>top</td>
</tr>
<tr>
<td>MALE POPULATION</td>
<td>N*&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MALES MATED</td>
<td>20</td>
</tr>
<tr>
<td>EXPERIMENTAL TANK</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> indicates male population injected with isotope.
as above, except with the injections reversed, for replicate 2. On day 5 of the experiment the stripping and injection procedure was repeated for all males. On day 10 a third injection was given to all males in an attempt to lengthen the period over which high levels of labelled sperm would be present.

On day 10, 35 females from each of the populations were removed from the stock tanks and marked for later identification. The marking procedure involved injecting a small amount of Trypan blue into the musculature of the caudal peduncle of the female. Naranjo females were marked with a dot at the top of the peduncle, Guayamare females with a dot at the bottom. A further 35 females of each population were removed from the stock tanks and marked (reversed) for replicate 2. The oviducts of all females were flushed using a micropipette and saline solution. The females were then isolated in separate tanks and these tanks checked regularly for young, which if found, were removed.

On day 22 turbid water was added to the experimental tanks and turbidity brought to 6 cm.

On day 25, 30 of the females and 20 males of each population designated for each of the replicates were placed in the respective experimental tank (Table 10). The ten remaining saline injected males for each replicate and the five remaining isotope injected males for each replicate were stripped on day 28 in order to prepare background radiation control smears and label level control smears respectively (see Chapter 5, section V).
On day 32 the competitive mating experiment was terminated, all fish measured, oviduct smears prepared from females, and a further five isotope injected males stripped in order to prepare label level control smears. All smears were then processed, assessed as described in Chapter 5, section VI, and the fish killed in a high concentration of MS 222.

The essential elements of the experimental procedure are described in Chapter 3 and illustrated in Figure 4 with the exceptions discussed above.

Assessment procedures used for determining mating success in experiment 5 were the same as those for experiments 1-4 described in Chapter 3, section VI. The percentage of sperm counted as labelled attributable to background radiation was 5.47% and the level of labelled sperm in isotope injected males, corrected for background radiation was 73.9%. Thus, in estimating inseminations attributable to males of competing populations, smears containing over 238/300 labelled sperm were attributed to matings involving only males of the labelled population, while those exhibiting over 283/300 unlabelled sperm were attributed to matings involving only the unlabelled population. The number of attributable inseminations by males of each population in the replicates was then combined and compared using the X2 Test ($\alpha = 0.05$, Snedacor and Cochran, 1967).

In order to incorporate the data from oviduct smears which indicated multiple inseminations (51.06% of the oviduct smears) the total number of sperm contributed by males of the competing populations to females was compared using the Mann Whitney U
Test ($\alpha = 0.05$, Snedecor and Cochran, 1967). Since no significant differences were found in background radiation and male label level between the replicates ($p > 0.05$, Mann Whitney U Test, Snedecor and Cochran, 1967), it was assumed that combining the replicates would cancel any error in tallying due to these factors. It was therefore decided to run the tests on the combined raw tallies.

III. Results and Discussion

Guayamare males were significantly more successful at mating with Guayamare females in turbid water in terms of both inseminations attributable to one population ($p < 0.005$) and total number of sperm ($p < 0.001$) than were Naranjo males (Figure 13B). There was no significant difference in the number of attributable inseminations by G and N males with N females. Naranjo males did, however, contribute significantly more sperm to N females than did G males ($p < 0.01$, Figure 13A).

These results confirm the prediction that G males would be more successful at mating with G females in turbid water conditions. While N males were still found to be more successful with their own females in terms of total number of sperm contributed, comparing results in clear water (Figure 8A) and those for turbid water (Figure 13A) suggest that G males have increased their mating success considerably in turbid water. When comparing the number of samples attributable to one population of males between clear water (Table 9, experiment 1) and turbid (Table 11), the number in N females
FIGURE 13

Results of Experiment 5

A. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Naranjo (N) and Guayamare (G) males to Naranjo females in turbid water.

B. Mean number of sperm per 300 and number of oviduct smears attributed to males of one population (crosshatched) contributed by Naranjo (N) and Guayamare (G) males to Guayamare females in turbid water.

a. Comparison of numbers of sperm; Mann Whitney U Test.

b. Comparison of attributed samples, x² Test.

N.S. = not significant at 0.05 level of significance
A

NARANJO FEMALES

MEAN NUMBER SPERM PER 300

N.

G

p < 0.01

B

GUAYAMARE FEMALES

MEAN NUMBER SPERM PER 300

N.

G

p < 0.001

p < 0.005
<table>
<thead>
<tr>
<th>Experiment</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Population Sampled</td>
<td>NARANJO</td>
</tr>
<tr>
<td>Number of Females Recovered</td>
<td>59</td>
</tr>
<tr>
<td>Number of Female Samples With Sperm</td>
<td>25</td>
</tr>
<tr>
<td>Male Population</td>
<td>N</td>
</tr>
<tr>
<td>Number of Samples Attributed to Male Population</td>
<td>3</td>
</tr>
</tbody>
</table>
attributable to N males has dropped from 11 to 3 while the number attributable to G males increased from 2 to 5. This also suggests that G males are more successful in turbid water at mating with females from both populations than in clear water. This may be due to an increased success in insemination by thrusting with both N and G females. The greater cohesion of G fish would also increase the success of G males in mating with G females.

The results support the hypothesis that a high frequency of gonopodial thrusts in the courtship of lowland males and greater cohesion in lowland populations are, in part, adaptations to the turbid water conditions in which the populations exist.

IV. Summary of Results

Males of two populations of *Poecilia reticulata*, one from a clear headstream river (Naranjo) and one from a turbid lowland river (Guayamare) were placed in competition for mating with females of both populations. An experiment was designed to test the hypothesis that differences in cohesion and mating strategies between these populations are adaptations, in part, to differences in the turbidity of the rivers in which these populations naturally occur.

1. The prediction that males from the turbid lowland population (G) would be more successful at mating than males from the clear headstream population (N) when placed in competition for lowland females in turbid water was supported.
2. The prediction that males from the lowland population would be more successful at mating than males from the headstream population when placed in competition for headstream females in turbid water was not supported. Headstream males contributed significantly more sperm to headstream females, although not as much as in clear water. Also, the number of attributable matings by males of one population to headstream females was not significantly greater for headstream males as compared to lowland males.

3. In comparing the mating success of headstream males and lowland males in clear and turbid water, it was clear that overall mating success of headstream males decreased from clear to turbid while overall mating success for lowland males increased from clear to turbid water.

4. These results suggest that high display frequency and duration are adaptations, in part, to clear water conditions and that cohesion and high thrust frequency are adaptations to turbid water conditions.
CHAPTER SIX

GENERAL DISCUSSION

THE ADAPTIVE SIGNIFICANCE OF GEOGRAPHIC VARIATION
IN THE BEHAVIOUR AND MORPHOLOGY
OF *Poecilia reticulata*

Studies investigating the behaviour and morphology of *Poecilia reticulata* have been concerned to a large degree with correlating variation in these characteristics with differences in the biotic and abiotic conditions in which natural populations exist. The aim of these studies is to identify possible selective agents in the environments of the guppy which may, in an evolutionary sense, be responsible for the observed variation.

This chapter serves first as a summary of the results of this study. Secondly, it incorporates these results into the larger framework of research investigating the adaptive significance of both behavioural and morphological characteristics in this species. Finally, it outlines potential future directions for research aimed at gaining a more comprehensive understanding of the 'functions' of behaviour in *Poecilia reticulata*.

Differences were found to exist in the abiotic characteristics of four rivers in Trinidad in which guppies exist, namely, two geographically isolated headstream rivers, the Naranjo and Paria; a midstream river, the Lower Aripo; and a lowland river, the Guayamare. Turbidity in particular was found to increase markedly from the headstream to the lowland
Male guppies can attempt to inseminate females through two courtship strategies, the first is through display, the other through thrusting. The display strategy involves active female participation, the thrust strategy does not. In laboratory observations, Ballin (1973) first identified differences in the courtship behaviour of male guppies from clear headstream rivers and males from turbid lowland rivers. He found headstream males to exhibit displays at higher frequencies and for longer durations than lowland males while the latter exhibited thrusts at higher frequencies than headstream males. Ballin speculated that the lower display rate and higher thrust rate observed in lowland males relative to that observed in headstream males was a response to heavy predation in lowland rivers. I suggested that these differences could also be responses to differences in turbidity between the rivers, with males in the clear headstreams relying more heavily on the visual display strategy in their courtship and males from turbid rivers relying more on the less visually oriented thrust strategy.

Seghers (1973) observed in laboratory observations that fish from lowland rivers are more cohesive and inhabit areas closer to shore than those in headstream rivers. He interpreted these behaviours in lowland fish as also being anti-predator adaptations. High cohesiveness can also be interpreted as a response to turbidity, as it would facilitate the location of mates during courtship under turbid water conditions.

The first objective of this study was to confirm that the
differences in behaviour identified by Ballin (1973) and Seghers (1973) are present in fish in the natural environment. Guppies were observed in the low predator, clear Naranjo and Paria headstreams rivers, the high predator, turbid lowland Guayamare River and the intermediate Lower Aripo River.

Differences in the behaviour of populations of guppies observed in the four rivers were in the predicted direction. Fish in the headstream rivers were less cohesive and inhabited areas further from shore than did lowland fish. In their courtship behaviour, headstream males performed sigmoid displays at a higher frequency and for longer durations than did midstream or lowland males. Conversely, the lowland males were found to exhibit a higher frequency of gonopodial thrusts than did males of the other three rivers.

I also attempted to determine if the observed differences in behaviour were, in part, due to genetic differences. Guppies from the populations observed in the field were bred and raised under identical conditions in the laboratory and courtship behaviour observed in males. I predicted that the differences observed in the field would persist in the laboratory raised fish.

All of the behavioural differences observed in the field, save one, were found to persist in the laboratory raised fish. The difference in the frequency of thrusts exhibited by Guayamare males (higher) as compared to Lower Aripo males observed in the field was the only comparison not found to be statistically significant in the laboratory raised stocks. These results strongly support the suggestion that the
differences have a genetic basis.

This being the case, one would expect the different behaviours to be the product of natural selection and thus have some adaptive significance to the fish in their environments. In order to be adaptive, the behavioural patterns must make the organism better able to survive and reproduce as compared to other members of the same species (Wilson, 1975). The second objective of this study was to attempt to test the hypothesis that the observed differences in behaviour are, in part, adaptations to the differences in turbidity of the streams in which the populations naturally occur.

On the basis of this hypothesis I predicted that males from a clear headstream river would be more successful at mating than males from the the turbid midstream and lowland rivers when placed in competition for females in clear water. This prediction was partially supported. Headstream males were significantly more successful at mating with headstream females in clear water than were midstream and lowland males. This strongly suggests that headstream females choose males with high display frequency and duration. No significant differences were found, however, in the mating success of headstream males and midstream males when competing for midstream females, nor in the mating success of headstream males and lowland males competing for lowland females. I suggested that the high level of cohesion identified in lowland and midstream populations may have resulted in greater success in mating for lowland and midstream males through either display or thrusting.

A second prediction based on the hypothesis that the
observed behavioural differences between populations were, in part, adaptations to turbidity, was that males from the turbid lowland river would be more successful in mating with females when placed in competition with clearwater headstream males in turbid water. Lowland males were significantly more successful in mating with lowland females than were headstream males. This strongly suggests that males exhibiting a high frequency of thrusts in their courtship have an advantage in mating in turbid water. No differences were found in the number of inseminations attributable to the two populations in headstream females. Headstream males were found, however, to have been more successful in terms of total sperm contributed to headstream females. These results are consistent with the suggestion that female preference for males on the basis of colour and/or male preference for females of the basis of a pheromone contribute to mating success in headstream populations.

In comparing the results of the clear water and turbid water experiments, the overall mating success of headstream males increased in clear water as compared to turbid water. Conversely, the overall mating success of lowland males increased in turbid water as compared to clear water.

The prediction that males from two turbid water populations exhibiting similar courtship strategies would not exhibit differences in mating success when placed in competition for females of those populations was supported.

A similar prediction for behaviourally similar males from two clear headstream rivers was not supported. In this
experiment, males of each headstream population were significantly more successful at mating with females of their own populations. These results suggest that the responsiveness of females to the high display frequency and duration in males, while being an important component of mate choice in headstream populations, is at the same time not the only factor. High success rates of behaviourally similar males with females from their own populations suggests that either females may be choosing males on the basis of a non-behavioural characteristic such as colour (Haskins, et al., 1961; Endler, 1978, 1980) or possibly size (Liley and Seghers, 1975) or that males are actively choosing females on the basis of a population specific pheromone (Crow and Liley, 1979; Crow, 1981).

The results of this study clearly illustrate that altering turbidity has a direct effect on the mating success of guppies. Under clear water conditions, males from clear water rivers are more successful at mating with females from clear water rivers than are males from turbid rivers. Under turbid water conditions, on the other hand, males from turbid rivers are more successful than males from clear water rivers at mating with females from turbid rivers. Observations of guppies in the field and in the laboratory have confirmed that males from the two types of rivers differ in their courtship behaviour and that these differences are in part genetically determined. These results are consistent with the hypothesis that high frequencies and durations of display in the courtship of males from clear water headstreams are adaptations to clear water. The results are also consistent with the hypothesis that the
high frequencies of thrusts in the courtship behaviour of males from turbid lowland rivers are adaptations to turbid water.

My results also suggest that male courtship behaviour is not the only factor operative in insuring mating success in these populations. Some evidence has been found to suggest that female preference for males on the basis of colour or size may be, in part, an adaptation to clear water conditions.

Grant (1963) stated that an organism must be adapted to its total environment. This implies that the observed behavioural and morphological characteristics of an organism are 'responses' to a number of selection pressures, both biotic and abiotic. It is therefore unlikely that turbidity alone can totally explain the observed variability in the behaviour of the guppy. I turn now to consider the evidence for other selection pressures which have been suggested as influencing the behavioural and morphological characteristics of this species.

**Sexual Selection.**

Brown (1975) defined sexual selection as the differential production of progeny by different genotypes as a result of competitive mating. Darwin (1871) himself identified the two mechanisms through which sexual selection can operate, male competition or female choice. Both these alternatives, which are not mutually exclusive, have been suggested as having influenced the morphology and behaviour of the guppy.

Noble and Curtis (1935), Breder and Coates (1935) and Haskins and Haskins (1949; 1950) have all suggested that male colouration serves as a warning signal to other males, rather
than as a mechanism for female choice. Both Gandolfi (1971) and Gorlick (1976) concluded from their results that dominance hierarchies exist in laboratory populations of guppies and that the dominant males in these hierarchies were responsible for most of the progeny in these populations. Gorlick suggested that size, sex and aggression played an important part in influencing these hierarchies. Gandolfi on the other hand suggested that female choice on the basis of colour is the mechanism through which dominant males ensure mating success.

While both Ballin (1973) and Crow (1981) observed aggression in natural populations in the laboratory, observations of populations in their natural environments by both Farr (1975) and myself suggest that male aggression toward other males or females is almost non-existent in the wild. In competitive mating experiments with inbred stocks of guppies, Farr (1980) found aggression to decrease reproductive success in males. These results leave in question the role intermale competition plays in sexual selection in the guppy.

Endler (1980) concluded from a series of experiments in which natural populations varying in colour patterns were subjected to varying intensities of predation in a number of experimental environments that guppy colour patterns are affected by a balance between sexual selection and selection for crypsis by predators in a particular environment. In the absence of predation, Endler found colour patterns in male guppies to mismatch the background substrate in terms of brightness, patch size and colour frequencies. He suggested that inter-male competition and frequency-dependant mating were
the mechanisms selecting for this mismatch.

Mating a number of inbred stocks varying in behavioural and morphological characteristics, Farr (1980) concluded that high display rate in males was the prime determinant of reproductive success. Farr assessed the broods from these matings and further concluded that males respond to the display rate of a competitor and that colour only affected reproductive success if there were no overriding behavioural differences.

My results agree with those of Farr (1980) in that those males from natural populations exhibiting high frequencies and durations of display have greater mating success with females of the same population in clear water than do males exhibiting lower frequencies and durations of display. These results support the hypothesis that in clear water headstreams sexual selection in the form of female choice may select for the high frequencies and durations of display. In the absence of behavioural difference I also found evidence that colour patterns or size may effect female choice in clear water.

**Predation**

Predation has been correlated with behavioural and morphological characteristics in a variety of species of fish (see Worthington, 1937; Hoogland, et al., 1956; Lowe-McConnell, 1959; 1975; Fryer, 1959; Jackson, 1961; Gross, 1978). In the guppy, the suggestion that male colour patterns represent a balance between sexual selection (favouring bright) and predation (favouring dull) was first presented by Haskins et al., (1961). Endler (1978) predicted that the colour patterns of male guppies at a particular place are inconspicuous or
cryptic if they approximate the background in colour patch size, brightness and colour distribution. He suggested that colour patterns could be predicted based on the intensity of predation in any given river. He tested this prediction in his (1980) study mentioned above. Placing guppies in a variety of environments modeling natural situations in terms of substrate background and predator pressure, he tested the effects of predation on colour by censusing a number of generations of fish for number, size and position of spots, colour and body size. He found guppies to be less conspicuous at high predator as opposed to low predator intensities and found a good background matching to colour patterns, that is, course gravel matched to large spots and fine gravel matching small spots in those environments in which predation was most intense.

Farr (1978) observed male courtship behaviour in a number of populations of guppies in rivers in Trinidad and found that high display rate was characteristic of populations living in rivers with large or no predators while low display rate was characteristic of populations living in rivers in which *Rivulus hartii* was the only predator. My observations of guppies in the N and P rivers (*R. hartii* present) and the LA and G rivers (large predators present) furnished results which were opposite to those of Farr. The reasons for this discrepancy are unknown and difficult to trace since little is stated in the paper concerning his study sites. He observed 50 males in each river for unspecified periods of time and does not indicate whether the data reflect behaviour in one location or in a variety of microhabitats in each river. Other laboratory studies dealing
with populations from rivers containing large predators and other populations from rivers containing only *R. hartii* (Ballin, 1973, Crow, 1981) found results similar to mine. I suggest that predation is a major factor, along with turbidity, balancing sexual selection in the predator rich, turbid lowland rivers. Courtship behaviors which make the male less obvious to predators would likely be favoured in the lowland rivers, namely low display frequency and duration and the thrust strategy.

Liley and Seghers (1975) found *Rivulus*, which as mentioned earlier, exists in headwater streams, to prey selectively on smaller guppies while the large lowland predators *Crenicichla* and *Hoplias* were unselective or preyed on larger guppies. They suggested that size selective predation may be one of the factors involved in determining interpopulation variation in size. The fish used in this study from rivers in which *Crenicichla* and *Hoplias* were present (G and LA) were generally smaller than those from rivers in which only *Rivulus* was present (N and P, see Appendix D).

Seghers (1970; 1973; 1974) investigated the behavioral 'responses' of natural populations to predation. He found that populations subject to high predation pressures were more cohesive than those in which only *Rivulus* was a predator. These observations are substantiated by my estimates of cohesion in the field. He presented wild and laboratory raised 'schoolers' (Lower Aripo and Guayamare) and 'non-schoolers' (Naranjo, Paria) in equal numbers to large predators and found them to take significantly more non-schoolers than schoolers. He
related these findings to a large anti-predator complex of adaptations including reaction distance, alarm threshold and microhabitat selection. The responses in terms of 'schooling' might also have been influenced by the effects of turbidity on courtship behaviour.

**Stream Velocity**

The effects of stream velocity on behaviour has been investigated by Crow (1981). As in this study, guppies from lowland rivers were found to exhibit greater cohesion, a greater frequency of thrusts and a lower frequency of displays than headstream populations. He obtained similar results in wild and laboratory raised fish. Lowland guppies were found to be less aggressive and more adherent to the surface area of stream tanks than those from headstreams.

Stream velocity was also found to have a direct effect on courtship behaviour of males, decreasing the frequency of both sigmoid displays and gonopodial swings as velocity increased. Males from headstream rivers were found better able to maintain the frequency and duration of display in fast water than were lowland males. Crow (1981) concluded that courtship behaviour was therefore also, in part, adapted to stream velocity.

Crow found males from two isolated rivers to display selectively to females of their own populations. Results of my study, using the same populations, indicate that assortative mating takes place between these populations. The discrimination may be based on the male preference identified by Crow (1981) and Ballin (1973) (possibly on the basis of a species specific pheromone) or female preference based on
colour pattern.

Temperature

Liley and Seghers (1975) found temperature to have an effect on the size of adult male guppies. Males of both headstream and lowland rivers grew larger under a 23 °C regime than those under a 28 °C regime, although in both temperatures headstream Naranjo males grew larger than lowland Guayamare males. There were no significant differences found in the effects on females of these stocks. The authors concluded that size differences were in part due to genetic differences.

These studies and my own research lead me to conclude that a number of both biotic and abiotic selection pressures have contributed to the observed variation in the behaviour and morphology of guppies in the wild. Sexual selection in the form of female choice has likely favoured the evolution of large, brightly coloured males exhibiting vigorous courtship displays in clear water headstream rivers. Selective predation by *Rivulus* on smaller guppies is likely to have contributed to the large size of fish in these rivers as has low temperature, the latter especially in males. Stream velocity has presumably interacted to set an upper limit on the frequency and duration of courtship display. Predation is seen as a major selective factor responsible the less conspicuous colour patterns observed in male guppies in lowland rivers. Predation and turbidity may also have interacted to select for a less visual courtship strategy in males, one relying less on the conspicuous display strategy and more on the non-visual thrust strategy. Schooling, or cohesiveness, and preference for the
surface and edges of rivers would also be favoured in these lowland rivers, both as anti-predator strategies and to ensure mating under turbid water conditions. The smaller size of lowland fish may have been influenced by both predator preference and temperature.

The review also illustrates that most of the studies conducted to date have concentrated on one selective agent and few of either behavioural or morphological characteristics. Consequently, results are obtained in isolation from other factors and characteristics, and integration becomes difficult and sometimes contradictory. Also, studies using inbred lines of guppies leave some doubt as to their application to the natural situation. A further complication has involved distinguishing between phenotypic and genetic effects.

While it will remain impossible to assess all the possible selective factors affecting the guppy and determining their exact contributions to behaviour and morphology, it should be possible to design long term experiments such as those conducted by Endler (1980) in which a variety of biotic and abiotic conditions were simulated in experimental environments. Using natural populations, the actual selective effects of the conditions could then be assessed directly, by observing for changes in a number of morphological and behavioural characteristics over several generations. Direct and interaction effects could then be determined through the use of a multivariate scheme.

Ideally, these types of experiments could be carried out in the natural environments. Stocks could be collected and
placed in cages similar to the ones used in this study. Populations from headstreams could, for example, be placed in the Guayamare and the lowland fish in the Naranjo, both with and without representative predators in the cages. The stocks could then be allowed to breed and their behavioural and morphological characteristics assessed over a number of generations. The only serious limitation to conducting studies in the natural environment is a practical one, human intervention.
LITERATURE CITED


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Winge, 0. 1922b. One-sided masculine and sex linked inheritance in Lebistes reticulatus. J. Genetics 12: 145-162.


Winge, O. 1934. The experimental alteration of sex chromosomes into autosomes and visa versa, as illustrated by Lebistes. C. R. Lab. Carlsberg, ser. physiol. 21, 1.


APPENDICES
APPENDIX A

FIGURE 14

The mean number of males (M), females (F), and juveniles (J) in 2500 cm² areas in the four rivers. 95% confidence limits of the means are indicated. The data was obtained as described in Chapter 3.
**APPENDIX B**

Statistical values for Bartletts', ANOVA and Scheffés' Tests on the field observations of populations in the four rivers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BARTLETT'S</th>
<th>ANOVA</th>
<th>SCHEFFE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SCHEFFE COMPARISONS&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X^2$</td>
<td>$F$</td>
<td>df</td>
<td>$G$-LA</td>
</tr>
<tr>
<td>Number of fish per 2500 cm$^2$</td>
<td>5.183</td>
<td>0.159</td>
<td>44.531</td>
<td>3.200</td>
</tr>
<tr>
<td>Distance between fish (cm)</td>
<td>9.637</td>
<td>0.022</td>
<td>41.099</td>
<td>3.64</td>
</tr>
<tr>
<td>Depth of water (cm)</td>
<td>3.962</td>
<td>0.266</td>
<td>6.443</td>
<td>3.64</td>
</tr>
<tr>
<td>Distance to shore (cm)</td>
<td>0.199</td>
<td>0.978</td>
<td>4.480</td>
<td>3.64</td>
</tr>
<tr>
<td>Water velocity (m/sec)</td>
<td>10.992</td>
<td>0.012</td>
<td>7.754</td>
<td>3.64</td>
</tr>
<tr>
<td>Number of sigmoid displays/3 min</td>
<td>4.814</td>
<td>0.186</td>
<td>13.964</td>
<td>3.336</td>
</tr>
<tr>
<td>Number of gonopodial thrusts/3 min</td>
<td>4.199</td>
<td>0.241</td>
<td>18.224</td>
<td>3.336</td>
</tr>
<tr>
<td>Number of copulation attempts/3 min</td>
<td>4.785</td>
<td>0.197</td>
<td>0.839</td>
<td>3.336</td>
</tr>
<tr>
<td>Duration of sigmoid display (sec)</td>
<td>2.273</td>
<td>0.482</td>
<td>13.201</td>
<td>3.536</td>
</tr>
</tbody>
</table>

<sup>a</sup> Scheffe F calculated at 0.01 level of significance.

<sup>b</sup> Any comparison value, and thereby the contrast it represents, that is greater than the Scheffe F value is significant at the 0.01 level of significance (underlined).

<sup>c</sup> The ANOVA for this comparison was found to be not significant at the 0.01 level of significance, therefore no Scheffe was performed on the data.
### APPENDIX C

Statistical values for Bartlett's, ANOVA and Scheffé's Tests on the laboratory observations of populations from the four rivers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BARTLETT'S</th>
<th>ANOVA</th>
<th>SCHEFFE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SCHEFFE COMPARISONS&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$x^2$</td>
<td>$F$</td>
<td>df</td>
<td>$F$</td>
</tr>
<tr>
<td>Number a sigmoid displays/10 min.</td>
<td>5.208</td>
<td>0.157</td>
<td>36.627</td>
<td>3.116</td>
</tr>
<tr>
<td>Number of gonopodial thrusts/10 min.</td>
<td>11.272</td>
<td>0.011</td>
<td>11.170</td>
<td>3.116</td>
</tr>
<tr>
<td>Number of copulation attempts/10 min.</td>
<td>1.963</td>
<td>0.580</td>
<td>0.731</td>
<td>3.116</td>
</tr>
<tr>
<td>Duration of sigmoid display (sec)</td>
<td>8.857</td>
<td>0.052</td>
<td>80.439</td>
<td>3.962</td>
</tr>
<tr>
<td>Number of gonopodial swings/10 min.</td>
<td>5.927</td>
<td>0.115</td>
<td>19.278</td>
<td>3.116</td>
</tr>
<tr>
<td>Number of aggressive encounters/10 min.</td>
<td>8.443</td>
<td>0.038</td>
<td>1.072</td>
<td>3.116</td>
</tr>
</tbody>
</table>

<sup>a</sup> Scheffé $F$ calculated at 0.01 level of significance.

<sup>b</sup> Any comparison value, and thereby the contrast it represents, that is greater than the Scheffé $F$ value is significant at the 0.01 level of significance (underlined).

<sup>c</sup> The ANOVA for this comparison was found to be not significant at the 0.01 level of significance, therefore no Scheffé was performed on the data.
**APPENDIX D**

**MEAN TOTAL LENGTH OF GUPPIES OF ALL POPULATIONS USED IN OBSERVATIONS AND EXPERIMENTS. STANDARD ERRORS IN BRACKETS.**

A. Behavioural Observations (Chapter 3).

<table>
<thead>
<tr>
<th>Population</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guayamare</td>
<td>14.513 (0.165)</td>
<td>17.371 (0.597)</td>
</tr>
<tr>
<td>Lower Aripo</td>
<td>15.240 (0.132)</td>
<td>19.367 (0.278)</td>
</tr>
<tr>
<td>Naranjo</td>
<td>17.890 (0.234)</td>
<td>20.486 (0.414)</td>
</tr>
<tr>
<td>Paria</td>
<td>15.927 (1.952)</td>
<td>19.633 (0.451)</td>
</tr>
</tbody>
</table>

B. Competitive Mating in Clear Water (Chapter 4).

<table>
<thead>
<tr>
<th>Population</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guayamare</td>
<td>14.540 (0.719)</td>
<td>18.399 (0.211)</td>
</tr>
<tr>
<td>Lower Aripo</td>
<td>14.669 (0.144)</td>
<td>19.182 (0.288)</td>
</tr>
<tr>
<td>Naranjo</td>
<td>17.878 (0.258)</td>
<td>20.551 (0.317)</td>
</tr>
<tr>
<td>Paria</td>
<td>17.613 (0.212)</td>
<td>19.606 (0.361)</td>
</tr>
</tbody>
</table>

C. Competitive Mating in Turbid Water (Chapter 5).

<table>
<thead>
<tr>
<th>Population</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guayamare</td>
<td>14.708 (0.127)</td>
<td>18.636 (0.284)</td>
</tr>
<tr>
<td>Naranjo</td>
<td>18.118 (0.177)</td>
<td>20.849 (0.535)</td>
</tr>
</tbody>
</table>
APPENDIX E

FORMULAS FOR STAINS

AQUEOUS EOSIN Y

Dissolve 5 gm of Eosin Y in 1000 ml distilled water.

MAYER'S HEMATOXYLIN

Dissolve 1 gm of Hematoxylin in 1000 ml distilled water. Then add 0.2 gm sodium iodate and 50 gm aluminum sulfate. Stir.