

BEHAVIOURAL ADAPTATIONS TO STREAM VELOCITY
IN THE GUPPY, POECILIA RETICULATA

by

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ABSTRACT

Populations of guppies living in different streams vary in their behaviour and morphology. Some populations live in fast-flowing streams while others occupy slow-flowing water. I hypothesized that behavioural differences between the populations may represent adaptations to stream velocity. I tested seven predictions based on this hypothesis in two experiments. In Experiment I, I examined the behaviour of laboratory reared guppies descendant from three natural populations. These guppies were examined at .03, .07, .10, and .20 m/sec stream velocities. Experiment II used wild-caught guppies from four populations. Their courtship behaviour was examined in still water (.00 m/sec) and at .08 m/sec velocity.

As predicted, in Experiment I guppies from slow-flowing water showed 1) greater cohesion, 2) less aggressive behaviour, and 3) a greater preference for the surface region of stream tanks than fast water fish. Also as predicted, slow water males performed fewer sigmoid displays but more thrusts and gonopodial swings than fast water guppies. The persistence of these behavioural differences in laboratory raised descendants of isolated populations demonstrates a genetic contribution to the differences. Therefore the differences are presumably the product of natural selection and represent evolutionary adaptations.

Experiment I also showed that stream velocity directly affects the performance of courtship behaviour by males. The frequency of sigmoid displays and gonopodial swings

decreased markedly as stream velocity increased. As stream velocity increased, fast water males maintained their frequency and duration of sigmoid displays better than slow water males, thus providing evidence for behavioural adaptation to stream velocity. The higher ratio of sigmoid displays : thrusts exhibited by fast water fish may also represent an adaptation to stream velocity.

Stream velocity had no direct effect on group cohesion and aggression in the guppy. However, it directly affected guppy stream depth preference. Slow water fish could not maintain position at the top of the stream in faster-flowing water, whereas fish bred from an intermediate stream velocity population were able to do so. Fast water fish always preferred the bottom of the stream bed.

In Experiment II wild-caught fish from fast and slow-flowing streams were tested together in two stream conditions. Again, as predicted, slow water fish performed fewer sigmoid displays but more thrusts and gonopodial swings than fast water fish. Because of the slow velocity used during the experiment, no effect of a fast stream velocity on courtship behaviour was obtained. During the experiment males generally preferred to court females from their own population. These preferences may represent partial isolating mechanisms.

I concluded that differences between guppy populations in courtship behaviour and stream depth preference represent adaptations to different stream velocities. Stream velocity is therefore presumed to have been one of several environmental factors that interacted to shape the guppy's present behaviour.

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CHAPTER I

INTRODUCTION

Within a species, individuals vary in their behaviour and ability to survive and reproduce. Those individuals best adapted to their particular environment contribute more of their genes to subsequent generations. Thus, natural selection may link specific behaviour patterns to certain ecological factors. In this thesis I examine whether individuals fish derived from different natural populations are adapted to a specific ecological factor, namely stream velocity.

To demonstrate the adaptive (or survival) value of a structure or behaviour, it is necessary to demonstrate that it contributes to reproductive success. Strictly speaking, this requires assessing reproductive success with and without the character. This is seldom done since 1) it is usually impossible to find two populations differing in only one character and 2) it is rarely possible to measure reproductive success under natural conditions. However, the combined use of comparative and experimental methods can determine the adaptive value of behaviour (see Hinde, 1975). In this approach, correlations are made between population differences and ecological variables in the environment. These correlations generate hypotheses on the observed relationships and the hypotheses may be tested by further comparisons or by experiments in the field and laboratory.

Natural populations of the guppy, Poecilia reticulata (Peters), display considerable variation in their morphology and behaviour. This fact, along with the guppy's short generation period and easy maintenance in the laboratory, makes it ideal for studying the adaptive value of behaviour. Different populations of the guppy were first studied in detail in their natural environment by (C.P.) Haskins, (E.F.) Haskins, McLaughlin, and Hewitt (1961). They found that populations of guppies occupying different streams varied genetically in their colour patterns. Since this study, considerable interest has been shown in these natural populations. Inter-population variation has been found in guppy size, sex ratio, schooling, antipredator behaviour, male courtship behaviour, and male aggression (Seghers, 1973, 1974a, 1974b; Ballin, 1973; Liley and Seghers, 1975; Farr, 1975; and Snyder, 1978). Studies on the variation in male colour patterns have also continued (Endler, 1978, 1980). In most of these studies the investigators suggest that morphological and behavioural differences between populations of guppies are adaptations to different predation pressures. Liley and Seghers (1975), however, suggest that many other features of the environment likely result in selection pressures which compete and interact with those due to predation.

Some populations of guppies occur in fast-flowing mountain streams while others occupy slow-flowing or stagnant water. This fact led me to hypothesize that different populations may be adapted to particular stream velocities and, consequently, that stream velocity may have acted in the past as a selection

pressure, partly moulding the species' present behaviour. To test this hypothesis, I examine the relationship between social behaviour and water velocity. Thus, unlike previous studies, I examine the effect of a physical factor on the behaviour of different guppy populations rather than the effect of predation.

Water velocity is closely correlated with turbidity: fast-flowing waters are almost invariably clear, slow-flowing waters are generally turbid (see Chapter III). During my experiments, turbidity was kept constant and stream velocity was manipulated. It is important to recognize, however, that the social behaviour observed is almost certainly coadapted to both prevailing flow and turbidity conditions (as well as biotic factors such as predation). Male courtship behaviour was chosen as a social behaviour on which to concentrate during this study since factors that affect courtship behaviour may also directly affect reproductive success.

The hypothesis that guppy populations are adapted to different stream velocity and turbidity conditions led to the following predictions:

- 1) Guppies derived from slow-flowing turbid water should show greater cohesion (schooling) than fish from fast-flowing clear streams.

- 2) Slow water guppies should be less aggressive than fast water guppies.

- 3) Guppies from slow-flowing turbid water should use the top of the water column (surface region), whereas fish from fast-flowing water should remain near the bottom of the stream.

4) Male guppies from turbid slow-flowing water should perform sigmoid displays less frequently but thrusts and gonopodial swings more frequently than males from fast-flowing streams. (For a description of these behaviour patterns see Chapter II.)

5) For all guppy populations the frequency of courtship behaviour (sigmoid displays, thrusts, and gonopodial swings) should decrease with increasing stream velocity and the frequency of sigmoid displays should decrease more than the frequency of thrusts.

6) Males from slow-flowing water should be less able to maintain their courtship behaviour and stream position at high velocities than fish from fast-flowing streams. Slow water fish should therefore show a greater decrease than fast water fish in their frequency of courtship behaviour as stream velocity increases.

7) At fast velocities the sigmoid displays performed by males from turbid slow-flowing water should be of shorter duration than the displays of males from fast-flowing water.

These predictions were tested in two experiments. The first experiment tested all of the predictions, using three stocks of guppies containing at least fifth generation descendants of wild-caught guppies. These stocks, each bred from a different natural stream population, were cultured under similar still water conditions before being tested in four stream velocities. Therefore, behavioural differences found between stocks should not be due to prior exposure to different environmental factors but instead represent genetic

differences between stocks. In the second experiment several populations of wild-caught guppies were used to test predictions 4, 5, and 6 under two stream conditions. In addition, this experiment examined male mate preferences for females from different populations.

The predictions were in part based on previous studies. I now give a more detailed rationale for each. Prediction 1 assumes that in turbid water one might expect fish to keep close together for communication or to provide protection from predators (as suggested by Seghers, 1973, 1974b). It follows that populations with a tendency to school should be less aggressive than those which are more spaced out (Prediction 2). Thus, stream velocity and turbidity should indirectly affect aggression in guppies by affecting schooling behaviour. The third prediction (that fast water fish should remain near the bottom of the stream) is based on the assumption that fast water fish may need to retain visual contact with the stream bed to hold their position and make use of the deceleration of water close to the substrate. On the other hand, by maintaining a position in the well-lit surface layer, slow water fish should be assisted in schooling and find each other more easily.

Males from clear fast-flowing water are predicted to sigmoid display more but thrust and gonopodial swing less than males from turbid slow-flowing water (Prediction 4). Ballin (1973) and Snyder (1978) found that populations of guppies demonstrated these differences in courtship when observed in still water. Ballin speculated that males from turbid waters

may display less frequently and thrust more frequently than those from fast-flowing clear streams partly as a response to stream turbidity. In his view, female guppies are less able to see the displays of males in turbid water. Therefore, in turbid water males should benefit less from displaying and more from attempting copulations by performing thrusts. (Thrusts represent insemination attempts without the cooperation of females, whereas displays occasionally elicit female cooperation and therefore result in successful insemination more often than thrusts (Kadow, 1954; Liley, 1966).)

As stream velocity increases, more energy should be required by fish to maintain position in the stream and to perform courtship. Sigmoid displays, which involve fin spreading by males and the maintenance of a position perpendicular to females (and thus to the direction of stream flow), should be particularly difficult to maintain as stream velocity increases (Prediction 5).

Predictions 6 and 7 are of particular importance to the hypothesis that guppy courtship behaviour is adapted to stream velocity. If fast water males are better able to maintain their courtship at high velocities than males from slow-flowing water it follows that they are better adapted to the fast-flowing water environment.

Before presenting the two experiments that test these predictions, in Chapter II I describe the courtship behaviour patterns studied during the experiments and provide background material on the natural environment of the guppy. Chapter III details the collection of stocks, field observations, and

general methods relevant to both experiments. Experiment I (Chapter IV) and Experiment II (Chapter V) are followed by a general discussion on the evolution of morphology and behaviour in the guppy (Chapter VI).

CHAPTER II

GUPPY COURTSHIP BEHAVIOUR AND NATURAL ENVIRONMENT

Reproductive Biology and Courtship Behaviour in the Guppy

The reproductive biology of the guppy is similar to that of other Poeciliid fishes (Breder and Rosen, 1966). Sexual dimorphism is quite marked, the male is considerably smaller and more colourful than the female. P. reticulata is ovoviviparous and prolific; it has a generation period of about three months, with females undergoing a well defined cycle of brood production.

Male courtship behaviour consists of orientation to the female, display behaviour and physical contacts. I measured male courtship by recording the frequency and duration of several courtship behaviour patterns. The following brief descriptions of these behaviour patterns are based on the detailed account in Liley (1966):

Gonopodial Swing: The gonopodium, a modified anal fin, is brought forward to one side of the mid-line. The organ then returns to the rest position, the entire action taking only one or two seconds. Gonopodial swinging may be performed at practically any stage during courtship and while the male is in any orientation with respect to the female. In this work I recorded all gonopodial swings in which the gonopodium was swung 45 degrees or more.

Sigmoid Display: The male takes up a position in front of or to the side of the female, twists his body into a sigmoid or S-shape in the horizontal plane, and vibrates the whole body for several seconds. During the display the caudal and dorsal fins may or may not be fully spread.

Leap: Following a sigmoid display the male may perform a leap in which he shoots away from the female along a straight or curved path.

Thrust: The male approaches the female from below and behind, swings the gonopodium forward, and directs it vigorously towards the female's genital pore. The gonopodium may or may not make momentary contact with the female's opening.

Copulation Attempt: The copulation attempt is distinguished from the thrust chiefly by the fact that it is directly preceded by a sigmoid display. In making a copulation attempt the male moves, still displaying, into a position parallel to one side and just below the female. From this position the male thrusts upwards and forwards as in thrusting.

Copulation: If, during a copulation attempt contact is made between the gonopodium and the female's genital pore then it is maintained for a fraction of a second. The male then jumps away and performs a series of jerks.

Jerks: Jerks may follow either thrusts or copulations and are short, sharp, forward and upward movements involving the whole body.

In P. reticulata insemination occurs almost entirely during the recognizable acts of copulation which are preceded by sigmoid displays. Very occasionally, however, the more commonly observed thrusts may also result in insemination (Kadow, 1954; Liley, 1966).

For most successful matings the female plays an active role which involves several receptive behaviour patterns. During my experiments female courtship behaviour was not recorded since females are only rarely observed during their receptive periods. Liley (1966) describes the cycle of receptivity in females and details their behaviour patterns.

Aggression or agonistic behaviour occasionally occurs between males during courtship and has been described in some detail by Liley (1966). Population differences in male

aggression were studied in Experiment I (Chapter IV). I recorded all inter-male attacks. An attack consists of a charge towards the flanks of another male. There is no evidence that biting or nibbling occurs and in many cases the male fails to make contact.

The Environment of the Guppy

Poecilia reticulata is restricted in its natural distribution to coastal areas of northeastern South America and a few of the Caribbean islands (Rosen and Bailey, 1963). Within this region, however, it is found in large numbers and in a wide range of stream environments. Among the many studies on this species only those of Haskins et al. (1961), Seghers (1973), Liley and Seghers (1975), Farr (1975), and Endler (1978) deal with the biology of the animal in its natural environment. All of these investigators studied the guppy on the island of Trinidad, as did I.

A mountainous chain extends along northern Trinidad. The north-facing slopes of these mountains are divided by several roughly parallel streams emptying individually into the Caribbean Sea with no fresh-water connections between them. The south-facing slopes contain approximately parallel streams that are connected in the lowlands by draining into the westward-flowing Caroni River. Most of these streams contain populations of guppies.

Since guppies rarely occur in the open sea, populations in the northward-flowing streams are virtually isolated from each other. Populations in the upper regions of

the southward-flowing streams are probably also genetically isolated since 1) the upper courses of these streams flow in deep valleys and are well separated at all seasons, 2) several of these streams contain waterfalls which likely act as effective barriers to upward migration of fish (Liley and Seghers, 1975), and 3) guppies remain fixed in their stream locations over periods of months or years (Haskins et al., 1961). In contrast to these populations, those in the lowland rivers are probably not as genetically isolated. I examine fish from the upper regions of northward and southward-flowing streams as well as from lowland rivers. The populations studied represent isolated populations from the full range of ecological conditions in which guppies live.

Seghers (1973) made quantitative ecological measures of Trinidad streams. Based on physical features, his streams were classified into four categories: springs, headstreams, midstreams, and lowland rivers. Going from springs to lowland rivers the streams become wider, deeper, warmer, and more turbid. Stream velocity generally increases from springs through headstreams and midstreams but decreases considerably in the lowland rivers (Table I). Additional information on the chemical composition of such waters is given in Haskins et al. (1961) and on stream substrates in Endler (1978).

In addition to differences in the physical features of Trinidad streams, there are also striking differences between the faunas of the streams. The springs and isolated headstreams commonly contain only P. reticulata and a small cyprinodontid, Rivulus hartii, a predator of the guppy. In contrast, the

Table I. Classification and physical features of streams in Trinidad.
Modified from Seghers (1973).

Stream type	Number of Streams Studied	Width (m)	Depth (m)	Velocity (m/sec)	Volume of Flow (m ³ /sec)	Temp. (°C)	Shade ^a	Turbidity ^b
Spring	4	0.50-1.0	0.05-0.15	0.11- 0.29	0.0085- 0.0128	24.3- 26.2	3-4	0
Headstream	7	1.20-5.0	0.06-0.15	0.32- 0.67	0.028- 0.267	24.6- 27.4	2-3	0-1
Midstream	5	3.0-8.0	0.13-0.20	0.42- 1.18	0.150- 1.129	24.3- 30.0	2	1
Lowland R.	3	2.0-25.0	1.5-3.0	0.33- 0.40	0.563- 22.50	26.9- 29.1	0-1	2

^a0 - no shade

1 - small amount of shade restricted mainly to streambank

2 - medium shade (50% cover)

3 - medium to dense (75% cover with few exposed parts)

4 - very dense cover with virtually complete shading

^b0 - always clear

1 - turbid only after heavy rains

2 - turbid throughout year

midstreams and lowland rivers have as many as sixteen species of fish, including several large guppy predators (eg. Hoplias malabaricus, Crenicichla alta, Aequidens pulcher, Astyanax bimaculatus and Hemibrycon sp.). For details see Boeseman (1960, 1964), Seghers (1973), and Liley and Seghers (1975).

Guppy populations in these different environments exhibit considerable variation in morphology and behaviour. As summarized by Liley and Seghers (1975), guppies from springs and isolated headstreams are generally larger, the males more brightly coloured, and the females outnumber males by as much as four to one. They show relatively poorly developed schooling behaviour and avoidance responses. These characteristics are associated with clear, fast-flowing water, relatively low temperatures, and a virtual absence of aquatic predators other than Rivulus. In contrast, in the lowland rivers, guppies are small, the males less brightly coloured and sex ratios are near one to one. These guppies show vigorous avoidance responses and often occur in small schools along the edge of a stream or river. Here, guppies occur in slow-flowing turbid water, high temperatures, an absence of shade, and the presence of numerous predators.

The evolutionary significance of correlations between these different physical and biotic factors and the observed variation in guppy morphology and behaviour will be considered at the end of this thesis (Chapter VI).

CHAPTER III

STOCK COLLECTING, FIELD OBSERVATIONS, AND GENERAL METHODS

Collecting Stocks

The stocks of fish were collected in Trinidad from five sites (see map, Fig. 1). Fish were collected from the northward-flowing Paria stream at a point above a waterfall barrier, 10-14 meters high; from two of the southward-flowing streams: the small Petite Curucaye stream, at a point close to its source, and from the Upper Aripo (= Naranjo) stream, above a series of falls 3-5 meters high. Fish were also collected from the Caparo and Guayamare rivers. These rivers lie in the sugar-cane planted lowland region. They are both relatively large streams and flow westward, emptying into the Gulf of Paria at the Caroni Swamp. Detailed information on each collection site can be found in Seghers (1973).

The stocks were collected during two different field expeditions and transported by air to the laboratory in Vancouver. Fish in Experiment I were bred from populations collected by N. R. Liley and myself in June, 1976. Experiment II used wild-caught fish collected by Liley and P. H. Luyten during May and June, 1978. The guppies were collected with fine-mesh circular dip nets. Lowland populations were collected by netting from small schools along the edges of rivers, while headstream and spring populations were collected from points across the stream beds.

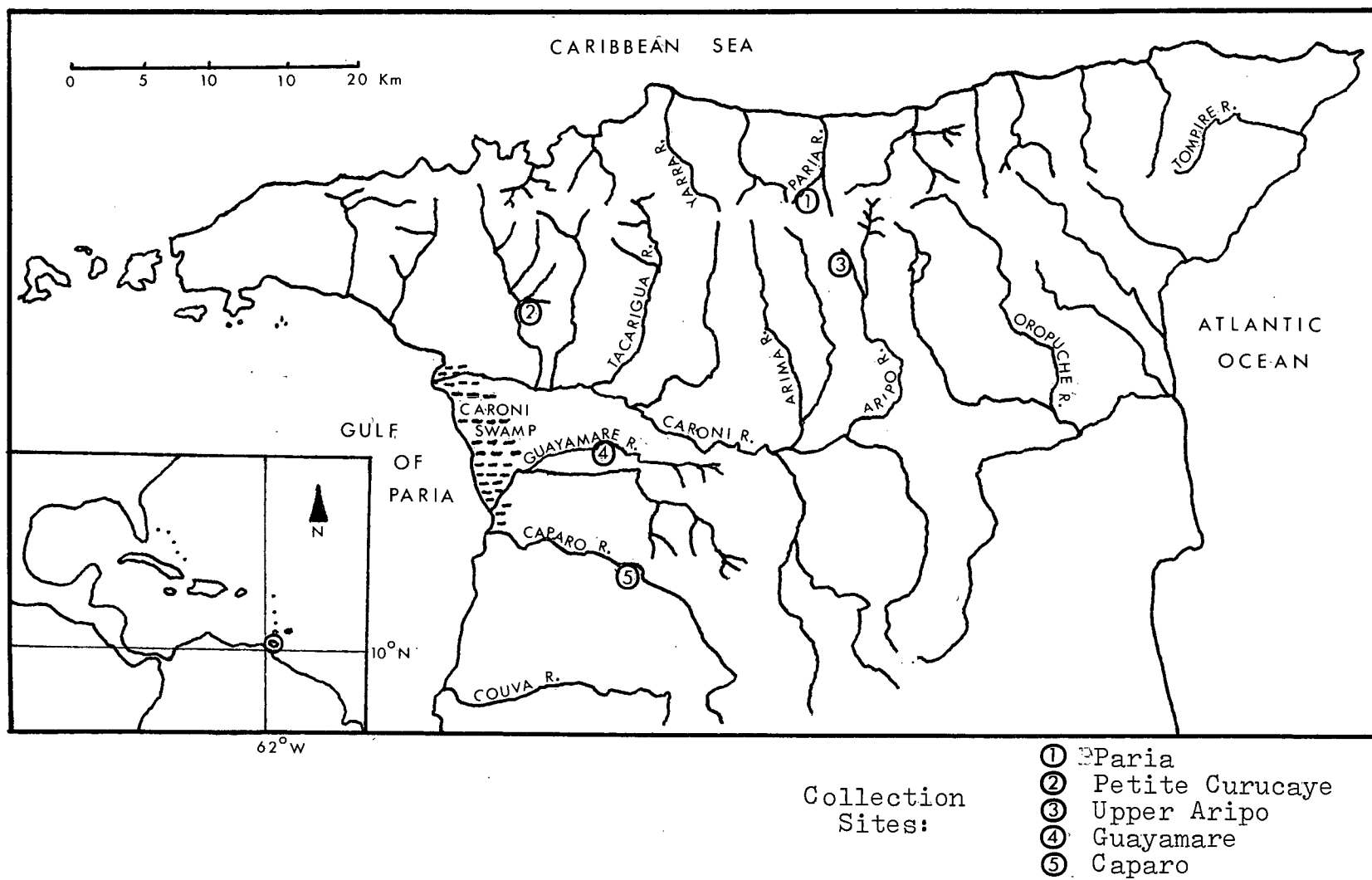


Figure 1. Map of the northern half of Trinidad, showing the major river systems. Inset map indicates the position of the island off the northeast coast of Venezuela.

Field Observations

In addition to collecting stocks in June, 1976, Liley and I observed the guppies in their natural streams, noting their distribution and measuring stream velocity. Stream velocity in the fastest section of each stream was found to be within the range given for the stream by Seghers (1973, and Table I), except for the lowland rivers where it was lower, since we did not measure it following a heavy rain as did Seghers.

The two headstreams, the Paria and Upper Aripo, consist mostly of riffles, although there are occasional smooth-flowing sections and a few pools. The density of guppies appeared slightly higher in the smooth-flowing and pool sections but guppies were dispersed across the entire stream bed, including some riffles (where the velocity ranged from 0.17 to 0.50 m/sec). Guppies were observed to remain near the substrate in these streams and to perform courtship behaviour in all river sections, including riffles.

In the two lowland rivers, the Guayamare and Caparo, the situation was entirely different. These rivers consist of long smooth-flowing sections. Here guppies occurred in schools near the water surface at the river edges. The rivers flowed at a velocity of 0.14-0.16 m/sec at the centre; however, the velocity at their edges was usually less. Throughout the lowland region, guppies were also found in drainage trenches and pools near the rivers, where there is almost no stream flow. Unlike the other streams, lowland rivers fluctuate widely in depth during the wet and dry seasons and often overflow their

banks during the rainy season, dispersing fish. Thus, for lowland populations, the stream velocity in which the guppies live and perform courtship is relatively low (often near 0 m/sec and usually less than 0.16 m/sec).

The mountain spring, Petite Curucaye, consists of numerous very shallow riffles which are frequently broken by smooth-flowing sections and pools. Here, stream velocity is fairly slow (less than 0.30 m/sec) and guppies were found dispersed over the entire stream bed.

Maintaining Stocks

Each laboratory stock was started with at least 50 males and 50 females. All stocks were maintained under similar controlled laboratory conditions. The fish were housed and reared in glass tanks of 43 and 61 litre capacity, located in three aquaria rooms. Each stock was housed in up to eight tanks which were spatially isolated from other stocks. Young were removed from the wild-caught fish and allowed to mature in separate tanks. These isolated fish were occasionally transferred between tanks of the same stock to equalize densities and reduce inbreeding.

All tanks had beds of medium or fine gravel and were filtered with external charcoal filters. The tanks were planted with Vallisneria, Ceratopteris, and Lemna. One-third of the water in each tank was changed twice monthly with fresh dechlorinated water.

The tanks were lit by 30 or 40-watt fluorescent tubes suspended 15 cm above the water on a 13L-11D photoperiod.

Temperature in the aquaria rooms was maintained at 23-26°C, eliminating the need for individual tank heaters.

The fish were fed twice daily on a diet of commercially prepared dried food, occasionally supplemented with frozen brine shrimp and chopped Tubifex worms.

The Stream Tanks

All tests were performed in two specially constructed stream tanks (Fig. 2) designed by Liley and myself. These were constructed in the U.B.C. Department of Zoology workshop from clear plexiglas and mounted on plywood bases. Various stream currents were created by using a submersible pump (Model 4SMD, Little Giant Pump Company) that forced water through two 2.5 cm plastic tubes. These plastic tubes were connected to two plexiglas tubes, one located at each end of the circular stream tank channel. The plexiglas tubes each had six 6 mm-holes spaced 4.5 cm apart. Water forced through these holes created a fairly even current across the width of the stream tank channel.

The stream velocity was controlled by altering a variable autotransformer (output voltage range 0-140 V; Powerstat, Type 3PN, Superior Electric Company) connected to the submersible pump. The stream velocity produced was measured using a floating vial and timing its movement over a 1 meter distance.

During all tests fish were restricted to the observation area of the stream tank channel. This area was 100 X 25 X 15 cm. The fish were restrained by screens (2.5 mm-mesh) located at each end of the area. A reference grid of 10 rectangles (7.5 X

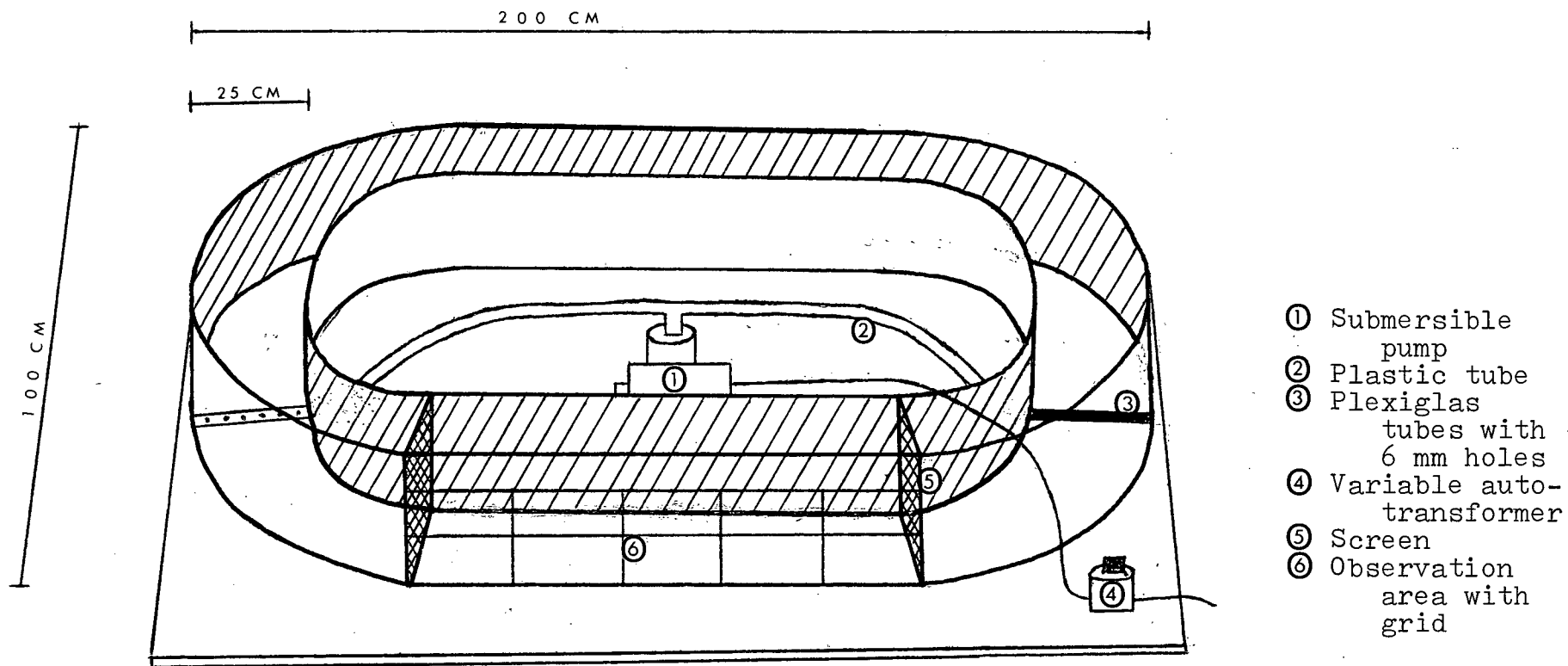


Figure 2. Schematic diagram of stream tank.

20 cm) was marked on the front of this observation area. A sheet of black plexiglas was placed at the back of the observation area during Experiment I, providing a background against which the fish were easily observed. During Experiment II a white plexiglas sheet was used, making the observation area slightly brighter.

The stream tanks were located in a separate laboratory room, kept under the same photoperiod and temperature as the aquaria rooms. The stream tanks were lit by eight 40-watt fluorescent tubes located 1.9 meters above the water.

Each stream tank held 500 liters of water, at least 10 liters of which was drawn from tanks in which the stocks had been housed. The tanks were filtered with a submersible charcoal filter and aerated with an air stone.

Recording of Observations

Observations were made while sitting approximately 1 meter from the front of the stream tanks. Behaviour patterns were recorded on a 4-channel Rustrak paper chart event recorder at a chart speed of 6 cm/min. From these recordings frequencies, sequences, or durations were calculated. Fish distribution was recorded by hand using check sheets containing models of the reference grid.

CHAPTER IV

EXPERIMENT I : THE SOCIAL BEHAVIOUR OF P. RETICULATA
POPULATIONS AT DIFFERENT WATER VELOCITIESIntroduction

In this experiment I examine the behaviour of three stocks of guppies, each one bred from either a headstream, lowland river, or spring population. The experiment tests the predictions that fish from the lowland stock should show 1) greater cohesion, 2) less aggressive behaviour, and 3) greater preference for the surface region of stream tanks than headstream fish. It also tests the prediction that lowland males perform fewer sigmoid displays but more thrusts and gonopodial swings than headstream males. The spring stock was bred from a population living in an intermediate stream velocity and was predicted to show a level of courtship intermediate between the lowland and headstream stocks.

The main purpose of this experiment was to examine the effect of stream velocity on each stock's courtship behaviour. The three stocks were therefore tested in each of four stream velocities. I predicted 1) that courtship behaviour should decrease for all stocks as stream velocity increases and 2) that fish from the headstream stock, descendant from fish that lived in fast-flowing water, should be better able to continue performing courtship in fast-flowing water than fish from the lowland stock.

To determine whether differences between populations are largely genetic, at least fifth generation descendants of wild-caught fish were used. Young were removed from the original wild-caught fish within a few days of birth and allowed to mature in isolation. All stocks had been maintained under similar (still water) conditions, eliminating any environmental effect of stream velocity, for several generations.

Materials and Methods

Adult guppies from Caparo (lowland), Paria (headstream), and Petite Curucaye (spring) stocks were tested. These stocks, containing fifth to eighth generation descendants of wild-caught fish, were cultured and maintained as outlined in the General Methods (Chapter III).

From each stock, four groups of 10 fish, 5 of each sex, were tested (a total of 20 males and 20 females per stock). Each stock's four groups were tested during different weeks, with each group tested in a different stream velocity on each of four consecutive days. The stream velocities in which each group was tested varied according to the following schedule:

	<u>Day 1</u>	<u>Day 2</u>	<u>Day 3</u>	<u>Day 4</u>
	Stream Velocity (m/sec)			
<u>Group</u>				
1	.07	.03	.10	.20
2	.10	.07	.20	.03
3	.03	.20	.07	.10
4	.20	.10	.03	.07

The stream velocities used lie well within the range of velocities for streams inhabited by guppies. Faster velocities were not used since the stream tanks did not contain areas of decreased velocity, where guppies could rest (eg. behind rocks).

The 5 males and 5 females for each group were drawn from the stock tanks four days before testing and placed, each sex separately, in two 12-liter holding tanks. On the morning of each consecutive test day these fish were introduced into the stream tank. (The stream velocity in the tank was set before introducing the fish.) At the end of each observation day the fish were returned to the two holding tanks. This procedure of isolating the sexes ensured the start of courtship behaviour soon after their introduction, eliminating the need for a long acclimation period.

Each of the group's five males was observed three times a day for ten minutes; approximately 1, 3, and 4 hours after introduction of the fish. In total, this procedure produced three 10-min recordings for 20 males from each of the three stocks in each of four stream velocities. The number and duration of sigmoid displays was recorded, as well as the number of gonopodial swings, thrusts, copulation attempts, and aggressive attacks, both initiated and received. Males were easily distinguished from one another by their distinct colour patterns, especially their black markings.

During each test the distribution of the ten fish (both males and females) was also recorded. The position of each fish was recorded using the grid of 10 rectangles marked on the

front of the stream tank. Recordings were made at 30 sec intervals for a 10 minute period (20 recordings). This series of recordings was repeated four times during each test day, beginning approximately 1, 3, 4, and 5 hours after introduction of the fish. Thus 80 recordings of distribution were made for each group in each stream velocity.

The distribution recordings were used to determine group cohesion and depth. An "index of cohesion" (designed by Seghers, 1973) was calculated. For each 30 sec observation, the maximum density for any of the 10 grid squares was recorded. The index is the mean maximum density for the 80 observations in each test. It has a theoretical minimum of 1 (one fish in each of the 10 squares) and a maximum of 10 (entire group in the area of one square).

Occasionally during this experiment a male died or became "sick". If so, it was replaced with another fish and eliminated from the data analysis. Following the experiment each group of fish was anaesthetized in MS222 (tricaine methyl sulphamate), measured with calipers, photographed and then housed in separate post-experiment breeding tanks.

Statistical Treatment of Data

When comparing the same stock under different stream conditions I used the Friedman two-way analysis of variance (Siegel, 1956). When comparing different stocks under the same stream conditions the Mann-Whitney U test was used. (Siegel, 1956). Correlations between the size of males and

courtship behaviour patterns (see Appendix) were measured using the Spearman rank correlation coefficient (Siegel, 1956).

Results and Discussion

Stream Depth Location

The depth of fish is given in relation to stream velocity for each stock in Figure 3. At the slowest flowing condition (0.03 m/sec) the Paria (headstream) fish were in the top half of the tank less than 20% of the time, whereas the Caparo (lowland) and Petite Curucaye (spring) fish were located in the top half of the tank more than 50% of the time.

To determine the effect of stream velocity on depth location, a comparison was made of the "group depths" for each stock at different velocities (Table II). No significant difference was found between velocities for the Paria and Petite Curucaye stocks (although fish were located less often in the top of the tank with increasing velocity, Fig. 3). In contrast, the lowland Caparo stock showed a significant variance in its group depths. This variance was clearly due to a decrease in the time spent in the top half of the stream tank as stream velocity increased (Fig. 3). The number of times fish were located in the top half of the stream tank decreased between the slowest and fastest velocities by 28% for the Paria stock, by 35% for the Petite Curucaye stock, and by 76% for the Caparo stock (Table II). Thus, of the two stocks that showed a preference for the top of the stream tank in slow-flowing water, the Petite Curucaye

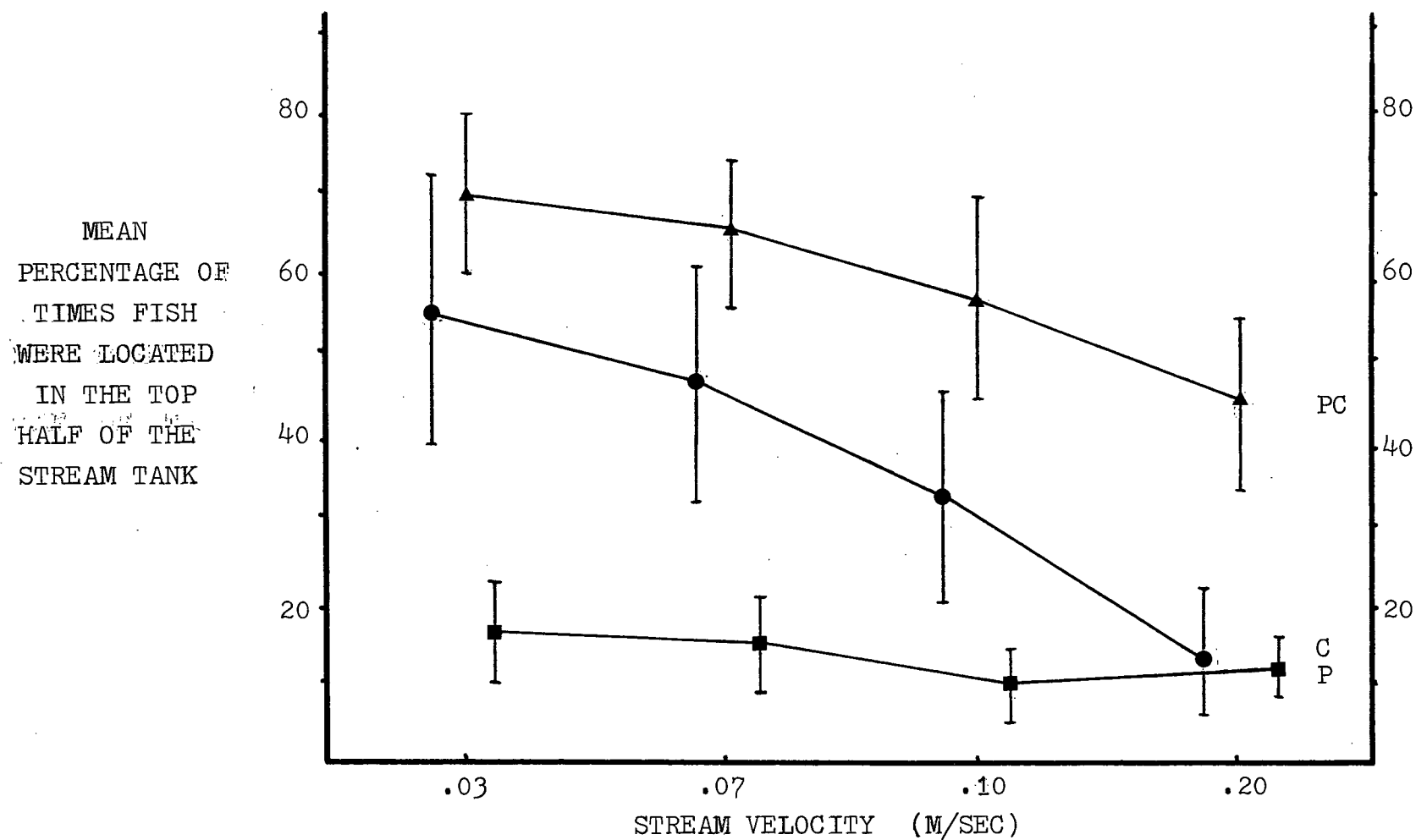


Figure 3. "Group depth" in relation to stream velocity.
Means \pm 95% confidence intervals.
C = Caparo, P = Paria, PC = Petite Curucaye (N = 16).
(Lowland) (Headstream) (Spring)

Table II. Comparison of "group depth" for each stock at different stream velocities.

Stock	Mean number of times per 200 that fish were in the top half of the stream tank				Overall Difference (Friedman 2-way ANOVA)
	Stream velocity (m/sec)				p
	.03	.07	.10	.20	
Caparo	109.9	93.9	65.3	26.9	<.01
Petite Curucaye	137.4	131.4	113.4	89.1	N.S.
Paria	32.0	28.6	19.8	23.0	N.S.

fish (derived from an intermediate stream velocity population) were better able to maintain their location at the top of the stream tank in fast-flowing water, whereas the Caparo fish were unable to do so.

Comparing stream location between the three stocks (Table III), in the slowest velocity there was a significant difference between Caparo and Paria stocks but no significant difference between Caparo and Petite Curucaye stocks. In the highest velocity this situation was reversed, with Caparo and Paria stocks not significantly different and Caparo and Petite Curucaye stocks now significantly different from each other. This is clearly due to the change in the Caparo stock's "group depth" (Fig. 3).

Each stock exhibited a similar location in the stream tank to the location their parent population was observed in the wild (see Chapter III). The persistence of these differences in laboratory raised fish suggests that there is likely a genetic basis to stream depth location and that these stocks vary in this trait. The fact that Petite Curucaye fish are better able to maintain position at the top of the stream in faster-flowing water, whereas Caparo fish are unable to do so, may represent an adaptation by the Petite Curucaye fish to living in faster-flowing water. The Paria stock, whose parent population lived in the fastest stream velocity, as predicted, exhibited a consistent preference for the bottom of the stream tank at all velocities. I speculate that Paria fish have been selected to prefer a position near the stream bed because maintaining a position at the surface is difficult in fast water.

Table III. Comparison of "group depth" between stocks at different stream velocities.

Stream velocity (m/sec)	"Group depth" Comparison between stocks*		
	C > P	C \neq PC p	P \neq PC
.03	<.001	N.S.	<.002
.07	<.001	N.S.	<.002
.10	<.01	<.02	<.002
.20	N.S.	<.002	<.002

*Mann-Whitney U test.

C = Caparo, P = Paria, PC = Petite Curucaye.

Index of Cohesion

Comparing each stock separately (Fig. 4), none of the stocks showed significant variances in cohesion under the four velocities ($p < .05$, Friedman test).

Comparing cohesion between stocks (Table IV), I found that Caparo had a significantly higher index than Petite Curucaye at all stream velocities. The Caparo stock indices were also significantly higher than those of Paria in the slowest and fastest velocities. The Paria and Petite Curucaye indices were not significantly different in any of the stream velocities.

These results support the hypothesis that stocks vary in their tendency to school and that these tendencies are under genetic control. The prediction that the lowland (Caparo) stock would have a greater tendency to school than the headstream (Paria) stock was confirmed. The Petite Curucaye stock was similar to the headstream stock in its schooling behaviour. In Trinidad, lowland fish were found in schools while headstream and spring populations were dispersed over the stream bed (see Chapter III). Again, each stock exhibited behaviour similar to the wild population from which it was descended.

The results indicate that differences existing between stocks in their index of cohesion, or degree of schooling, are not affected by stream velocity, at least within the range of velocities tested. Perhaps differences in cohesion are due to different predation pressures (as suggested by Seghers, 1973) or to other factors, such as stream turbidity, or to a combination of factors.

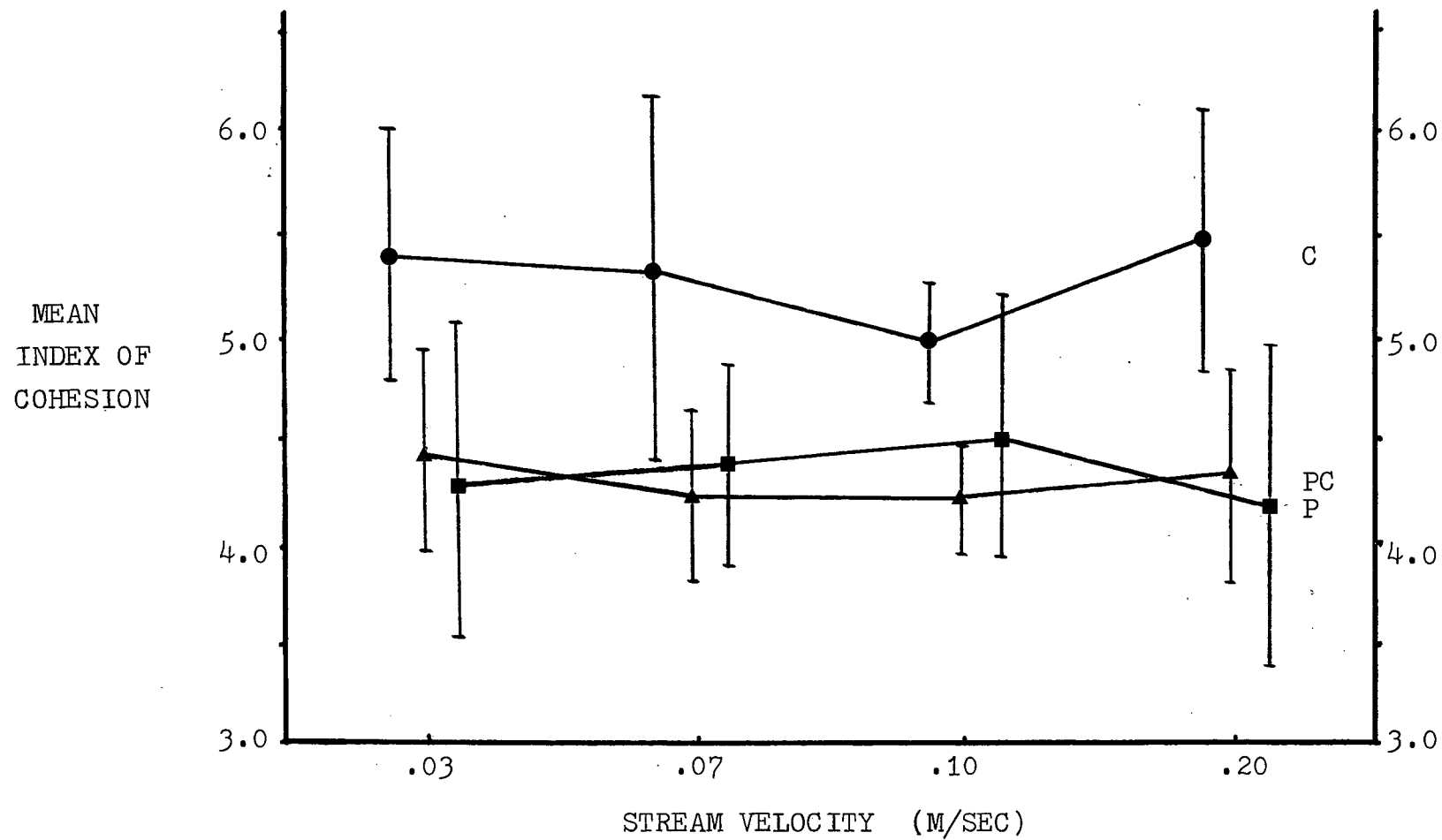


Figure 4. Index of Cohesion in relation to stream velocity.
Means \pm 95% confidence intervals.
C = Caparo (Lowland), P = Paria (Headstream),
PC = Petite Curucaye (Spring). (N = 16).

Table IV. Comparison of "Index of Cohesion" between stocks at different stream velocities.

Stream velocity (m/sec)	Index of Cohesion Comparison between stocks*		
	C > P	C > PC	P < PC
.03	<.01	<.03	.03 N.S.
.07	N.S. (\approx .06)	<.01	N.S.
.10	N.S. (\approx .08)	<.01	N.S.
.20	<.01	<.01	N.S.

*Mann-Whitney U test (Predictions based on Seghers, 1973).
C = Caparo, P = Paria, PC = Petite Curucaye.

Male Aggression

For each stock male aggression in relation to stream velocity is given in Figure 5. The number of male attacks did not differ significantly between stream velocities for any of the stocks ($p < .05$, Friedman test), although it did decrease somewhat for the Paria stock.

Although aggression varied widely among individual Paria males (Fig. 5), they were significantly more aggressive than Caparo and Petite Curucaye males at most stream velocities (Table V).

Ballin (1973) found Paria males to be more aggressive than another lowland population of males (Guayamare). The results of this experiment confirm my prediction that Paria (headstream) males should be more aggressive than lowland males (Caparo) and also indicate that the spring population (Petite Curucaye, which had not previously been studied) is relatively non-aggressive. Once again, these behavioural differences between populations likely have a genetic basis.

Stream velocity does not appear to affect aggression among males directly, although there may be a slight decrease in aggression with increasing velocity.

MEAN
NUMBER OF
ATTACKS
PER MALE
PER 30 MIN

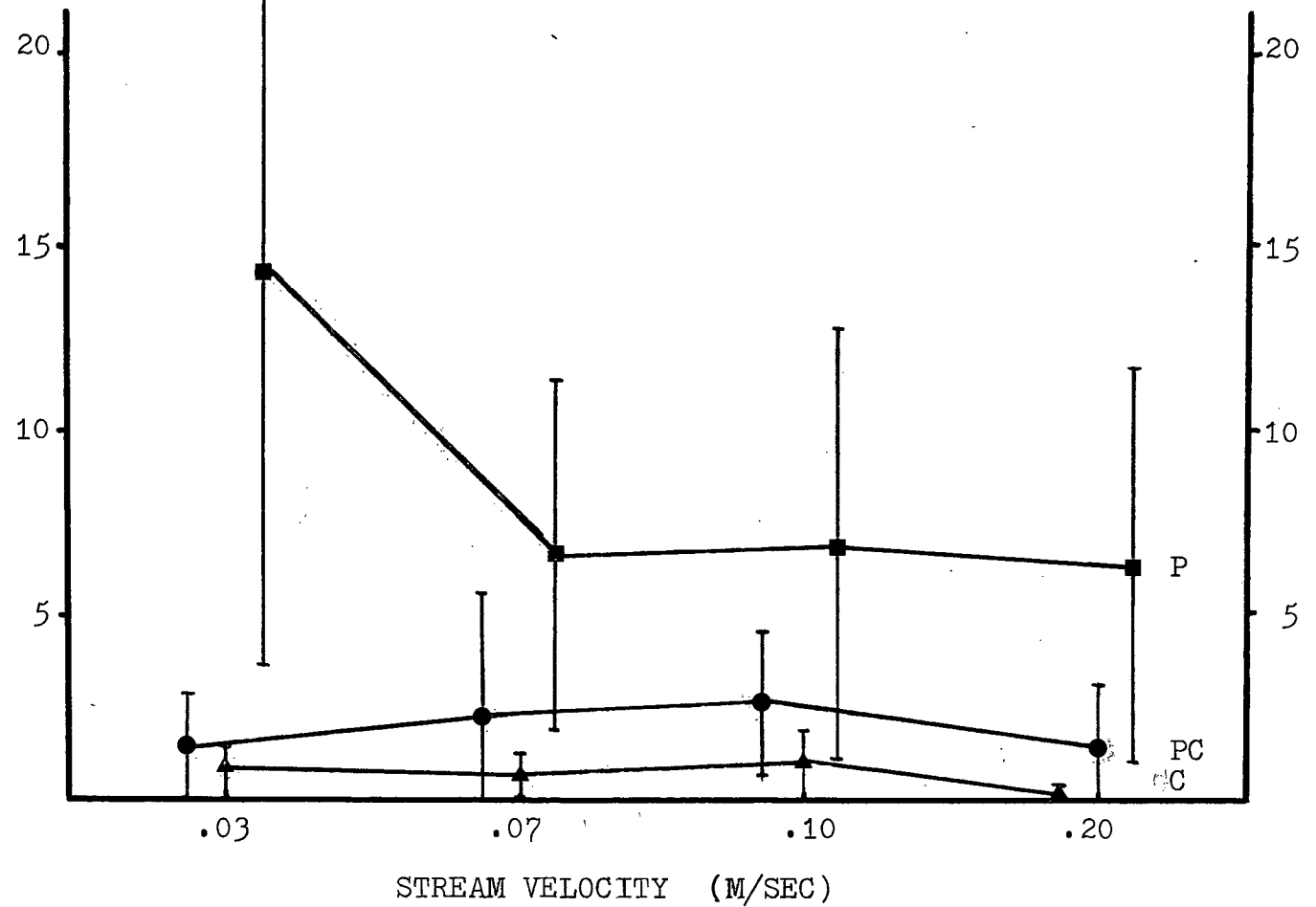


Figure 5. Male aggression in relation to stream velocity.
Means \pm 95% confidence intervals.
C = Caparo (Lowland; N = 19), P = Paria (Headstream; N = 19),
PC = Petite Curucaye (Spring; N = 17).

Table V. Comparison of Male Aggression at different stream velocities.

Stream velocity (m/sec)	Male Attacks Comparison between stocks*		
	C < P	C \neq PC	P \neq PC
.03	<.001	N.S.	<.002
.07	<.025	N.S.	N.S. (\approx .05)
.10	<.05	N.S.	<.02
.20	<.01	N.S.	N.S.

*Mann-Whitney U test, 1-tailed for C < P, 2-tailed for C \neq PC, P \neq PC.

C = Caparo, P = Paria, PC = Petite Curucaye.

Courtship Behaviour Patterns

Thrusts and Sigmoid Displays

The relationship between the mean number of thrusts performed by males and stream velocity is shown in Figure 6. Comparing each stock separately, there was no significant variance in the number of thrusts performed at the four velocities (Table VI).⁴

As predicted, Caparo males consistently exhibited more thrusts at all velocities than Petite Curucaye males. Petite Curucaye males in turn consistently performed more thrusts than Paria males. These differences were significant between Caparo and Paria males at the two fastest velocities (Table VII).

For each stock there was a significant variance in the number of sigmoid displays performed at the different stream velocities. This variance was due to a decrease in the number of sigmoid displays performed by males as stream velocity increased (Fig. 7).

Also as predicted, Paria males performed more sigmoid displays at all stream velocities than Petite Curucaye males, and Petite Curucaye males in turn always displayed more frequently than Caparo males. The differences between Paria and Petite Curucaye and between Paria and Caparo were significant at the two slowest velocities (Table VII).

Each population's ratio of sigmoid displays : thrusts at the four stream velocities is given in Figure 8. All three stocks showed a significant variance in their ratios between the four stream velocities. The variance was due to a

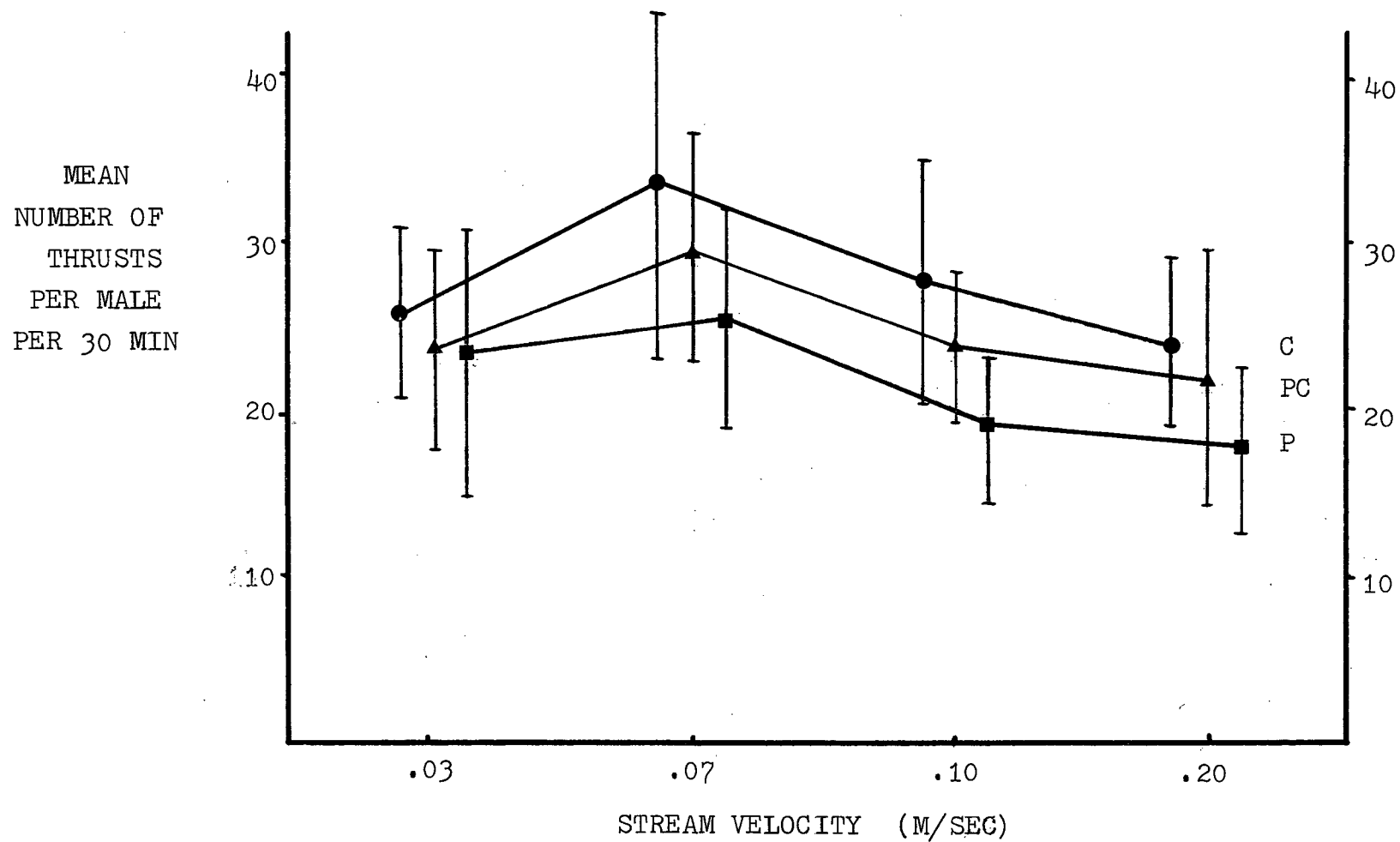


Figure 6. Number of thrusts in relation to stream velocity.
Means \pm 95% confidence intervals.
C = Caparo (Lowland; N = 19), P = Paria (Headstream; N = 19),
PC = Petite Curucaye (Spring; N = 17).

Table VI. Comparison of number of Thrusts, Sigmoid Displays, and the ratio of Sigmoid Displays : Thrusts for each stock at different stream velocities.

Stock	Stream velocity (m/sec)				Overall Difference (Friedman 2-way ANOVA) p
	.03	.07	.10	.20	
	Mean number of Thrusts per male per 30 mins				
Caparo	25.9	34.0	28.2	24.5	N.S.
Petite Curucaye	23.5	30.1	24.1	22.5	N.S.
Paria	23.5	25.9	19.4	18.1	N.S.
	Mean number of Sigmoid Displays per male per 30 mins				
Caparo	33.9	29.3	22.4	11.1	<.001
Petite Curucaye	34.5	36.6	33.5	20.6	<.01
Paria	49.4	48.1	34.1	24.2	<.001
	Ratio of Sigmoid Displays : Thrusts				
Caparo	1.31	0.86	0.79	0.46	<.01
Petite Curucaye	1.47	1.22	1.39	0.92	<.01
Paria	2.10	1.85	1.76	1.34	<.01

Table VII. Comparison of number of Thrusts, Sigmoid Displays and the ratio of Sigmoid Displays : Thrusts between stocks at different velocities.

Stream velocity (m/sec)	Number of Thrusts Comparison between stocks*			Number of Sigmoid Displays Comparison between stocks*			Ratio of Displays:Thrusts Comparison between stocks*		
	C > P	C > PC	P < PC	C < P	C < PC	P > PC	C < P	C < PC	P > PC
.03	N.S.	N.S.	N.S.	<.05	N.S.	<.05	<.01	N.S.	<.025
.07	N.S.	N.S.	N.S.	<.01	N.S.	N.S. (\approx .05)	<.01	N.S.	=.025
.10	<.025	N.S.	<.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
.20	<.05	N.S.	N.S.	N.S. (\approx .05)	N.S.	N.S.	<.025	N.S.	N.S.

*Mann-Whitney U test.

C = Caparo, P = Paria, PC = Petite Curucaye.

MEAN
NUMBER OF
SIGMOID
DISPLAYS
PER MALE
PER 30 MIN

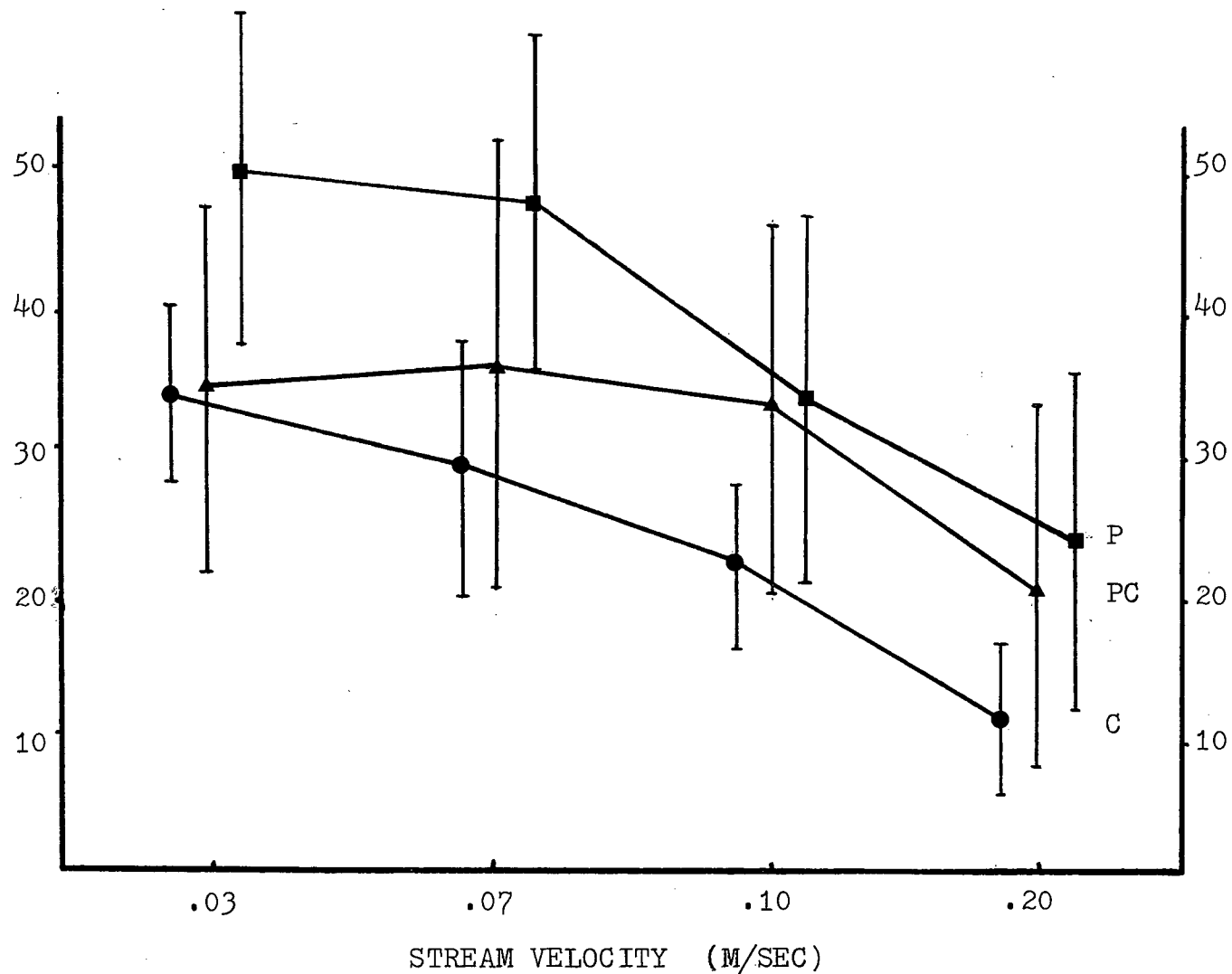


Figure 7. Number of sigmoid displays in relation to stream velocity.
Means \pm 95% confidence intervals.
C = Caparo (Lowland; N = 19), P = Paria (Headstream; N = 19),
PC = Petite Curucaye (Spring; N = 17).

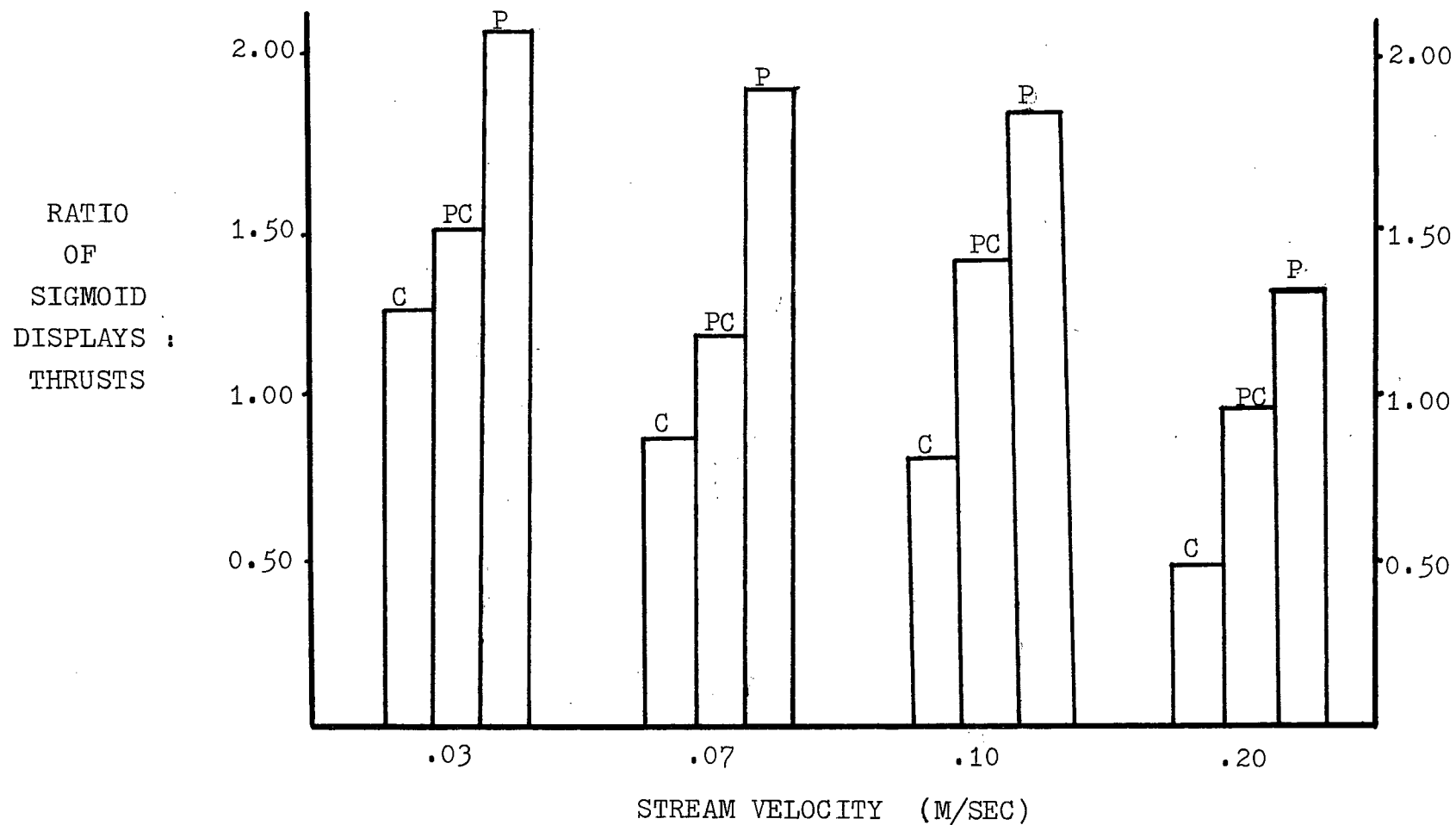


Figure 8. Ratio of sigmoid displays : thrusts in relation to stream velocity.
 C = Caparo (Lowland; N = 19), P = Paria (Headstream; N = 19),
 PC = Petite Curucaye (Spring; N = 17).

decrease in the ratio as stream velocity increased (Fig. 8). This means that, as predicted, the frequency of sigmoid displays decreased more than the frequency of thrusts as stream velocity increased.

At all velocities Paria had the highest ratio of sigmoid displays : thrusts and Petite Curucaye in turn had a higher ratio than Caparo. These differences in courtship were significant between the Paria and Caparo stocks at three of the velocities and between Paria and Petite Curucaye at two of the velocities (Table VII).

Differences in the frequencies of thrusts and sigmoid displays between the Caparo and Paria stocks are consistent with results for similar stocks tested in still water (Ballin, 1973, Snyder, 1978). As predicted, the headstream population used more displays while lowland fish used more thrusts. The Petite Curucaye stock, bred from an intermediate stream velocity population, used an intermediate courtship strategy. These differences between stocks again presumably reflect genetic differences.

The prediction that each population differs in its ability to perform courtship in increasing stream velocity was supported. Specifically, in faster-flowing water headstream fish were better able to maintain their frequency of displays than lowland fish. In slow-flowing water the Caparo stock displayed more than it thrusts, however, it was unable to maintain this ratio in faster velocities. In fact, in the fastest velocity it displayed less than half as frequently as it thrusts (Fig. 8, Table VI). The Paria stock performed

more than twice as many sigmoid displays as thrust in slow-flowing water, and at the faster velocities it was always able to maintain a higher frequency of displays than thrusts.

The ratio of sigmoid displays : thrusts decreased between the slowest and fastest velocities by 65% for the Caparo stock and by only 36% for the Paria stock. Thus Paria (headstream) fish appear better adapted to displaying in faster-flowing water than Caparo (lowland) fish. (The Petite Curucaye stock had an intermediate ratio and were able to maintain this ratio to about the same degree as the Paria stock - the ratio decreased by 37% between the fastest and slowest stream velocities.)

The ratio of sigmoid displays : thrusts for Caparo in slow-flowing water (1.31) was similar to the ratio for Paria at the fastest velocity (1.34) and to Petite Curucaye's ratio at the second fastest velocity (1.39). Although this could be coincidental it is interesting that in the wild these populations perform courtship at these relative velocities and thus may be achieving roughly similar ratios. Perhaps males perform an optimal ratio of sigmoid displays : thrusts for successful mating.

If an optimal ratio of sigmoid displays : thrusts does exist, then the previously observed differences between populations in their ratio of sigmoid displays : thrusts could represent adaptations to different stream velocities. Since sigmoid displays decrease more than thrusts with increasing stream velocity, males that live in fast-flowing water may have evolved a tendency to perform a higher ratio of sigmoid displays : thrusts (as observed in slow water) in order to still

be performing at the optimal ratio when in fast-flowing water. As noted, when headstream fish were observed in fast-flowing water their ratio was close to that of lowland fish when observed in slow-flowing water. Thus, apparent differences in male courtship observed between populations in still water may not actually exist in the wild. This point is important when considering the adaptive significance of variation in guppy courtship behaviour. Previously observed courtship differences may represent adaptive responses to increasing stream velocity rather than adaptations only to turbidity and predation as suggested by Ballin (1973).

The mean number and duration of sigmoid displays, mean number of sigmoid displays longer than 1.5 secs, and percentage of sigmoid displays longer than 1.5 secs are given in Table VIII for each stock at the four stream velocities. In general the duration of displays and percentage of displays longer than 1.5 secs decreased as stream velocity increased.

At all stream velocities Petite Curucaye males have the longest sigmoid displays and the greatest percentage of displays longer than 1.5 secs. At slower velocities Caparo males have slightly longer displays and more displays longer than 1.5 secs than Paria males. However, as predicted, at the faster stream velocities the opposite is the case, with Paria males having slightly longer displays than Caparo males and more sigmoid displays longer than 1.5 secs.

As expected, increasing stream velocity does decrease the duration of sigmoid displays for fish from all three stocks. However, there was not always a direct relationship

Table VIII. Mean number and duration of Sigmoid Displays at four stream velocities.

Stream velocity	Mean number of Sigmoid Displays /male /30 mins			Mean duration of Sigmoid Displays (secs)			Mean number of Sigmoid Displays >1.5 secs			Percentage of Sigmoid Displays >1.5 secs		
	C	PC	P	C	PC	P	C	PC	P	C	PC	P
.03	33.9	34.5	49.4	1.4	1.8	1.2	13.1	18.8	18.2	38.4	54.5	36.7
.07	29.3	36.6	48.1	1.3	1.5	1.1	10.5	16.8	14.9	35.7	45.8	31.0
.10	22.4	33.5	34.1	1.0	1.3	1.0	4.2	14.0	8.7	18.8	41.8	25.6
.20	11.1	20.6	24.2	0.9	1.5	1.0	1.8	8.9	5.9	16.5	43.4	24.6

between a stock's ability to perform longer sigmoid displays and its natural stream velocity. Fish derived from populations living in faster-flowing water (Paria and Petite Curucaye) did, as predicted, perform longer displays in the faster velocities than the lowland (Caparo) fish. However, the males from the intermediate stream velocity (Petite Curucaye), performed displays of the longest mean duration at all stream velocities, which was not predicted.

Gonopodial Swings

The mean number of gonopodial swings performed by each stock in relation to stream velocity is shown in Figure 9. All stocks exhibited a significant variance in the number of gonopodial swings performed at different velocities (Table IX). This variance was due to a decrease in gonopodial swings as stream velocity increased (Fig. 9). Clearly the performance of gonopodial swings is affected by stream velocity.

Under all stream velocity conditions both the Caparo and Petite Curucaye stocks performed significantly more gonopodial swings than the Paria stock (Table X). The Caparo and Petite Curucaye stocks were not significantly different at any of the velocities.

The difference between the Caparo and Paria stocks confirms my prediction that lowland males should perform more gonopodial swings than headstream males. This prediction was based on the differences found between similar headstream and lowland populations by Ballin (1973) and Snyder (1978). The previously unstudied Petite Curucaye stock's performance of

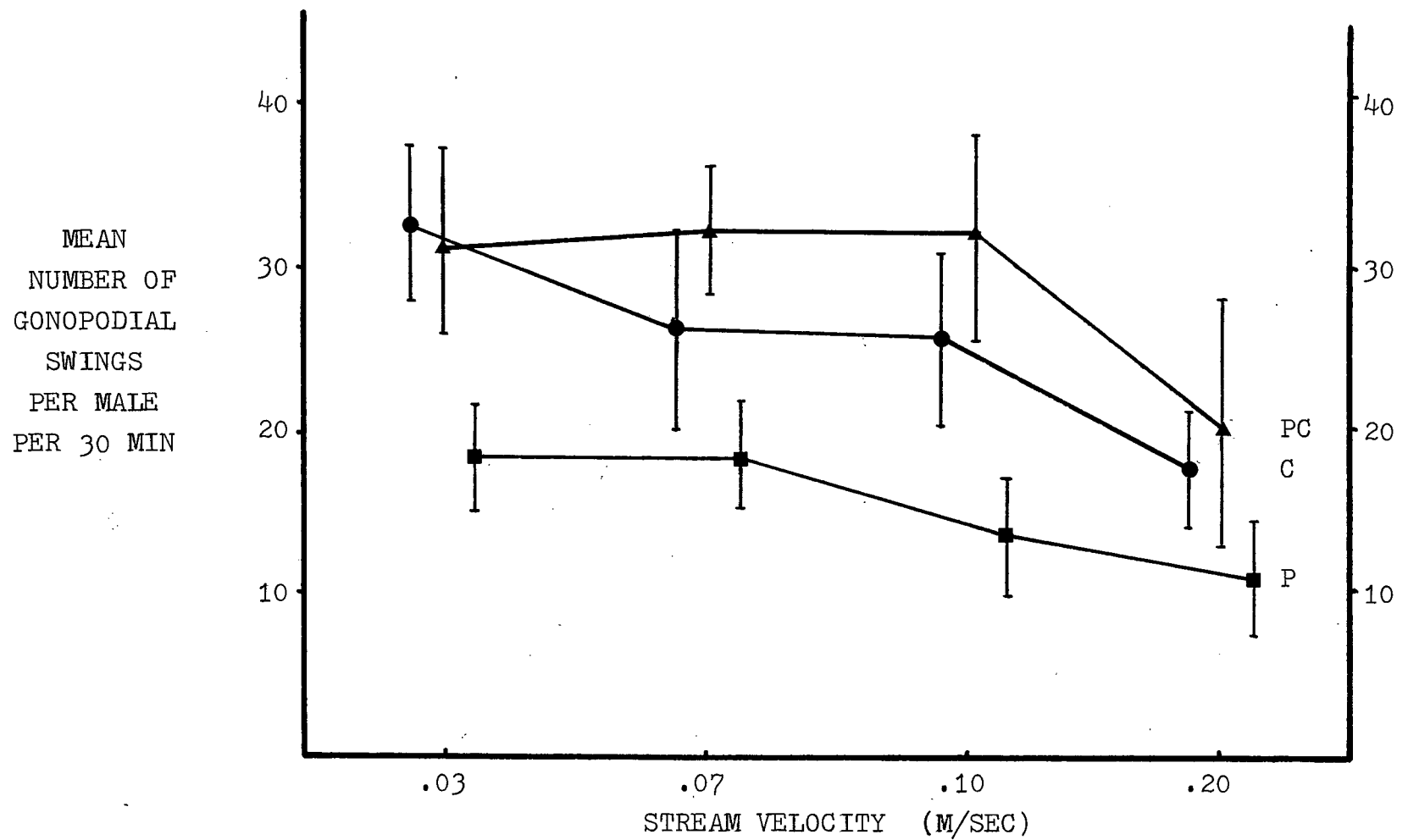


Figure 9. Number of gonopodial swings in relation to stream velocity. Means \pm 95% confidence intervals. C = Caparo (Lowland; N = 19), P = Paria (Headstream; N = 19), PC = Petite Curucaye (Spring; N = 17).

Table IX. Comparison of number of Gonopodial Swings performed at different stream velocities for each stock.

Site	Mean number of Gonopodial Swings				Overall Difference (Friedman 2-way ANOVA) p
	Stream velocity (m/sec)				
	.03	.07	.10	.20	
Caparo	32.8	26.5	25.5	18.3	<.001
Petite Curucaye	31.9	32.6	32.1	21.0	<.01
Paria	18.7	18.8	13.8	11.5	<.001

Table X. Comparison of number of Gonopodial Swings
between stocks at different stream velocities.

Stream velocity (m/sec)	Number of Gonopodial Swings Comparison between stocks*		
	C > P	C > PC P	P < PC
.03	<.001	N.S.	<.001
.07	<.025	N.S.	<.001
.10	<.001	N.S.	<.001
.20	<.001	N.S.	<.025

*Mann-Whitney U test.

C = Caparo, P = Paria, PC = Petite Curucaye.

the highest frequency of gonopodial swings at all stream velocities was not predicted. The significance of this finding, however, is difficult to interpret, especially since gonopodial swings in fact occur during both courtship and non-courtship and their function remains unknown. (In the Appendix, which deals with the effect of stream velocity on correlations between courtship behaviour patterns, I consider in more detail the function of gonopodial swings.) At present the significance of the differences found between stocks in the performance of gonopodial swings remains unknown.

Copulation Attempts

The mean number of copulation attempts performed by males of each stock at the four stream velocities and the percentages of sigmoid displays that were followed by copulation attempts are listed in Table XI. No actual copulations were observed during the experiment.

Because copulation attempts occurred so infrequently (less than one per male per 30 mins), I did not analyze them statistically. The number of copulation attempts decreased with increasing stream velocity, except for the Paria stock. Since copulation attempts are always preceded by sigmoid displays, this decrease may reflect the decreasing number of sigmoid displays performed by males as stream velocity increased (Fig. 7).

Liley (1966) noted that copulation attempts are dependent on females maintaining their position during and immediately following a sigmoid display. Copulation attempts are

Table XI. Mean number of Copulation Attempts and percentage of Sigmoid Displays followed by Copulation Attempts.

Stream velocity (m/sec)	Mean number of Copulation Attempts /male /30 mins			Percentage of Sigmoid Displays followed by Copulation Attempts		
	C	PC	P	C	PC	P
.03	1.1	0.7	0.3	3.3	2.0	0.6
.07	0.8	0.5	0.7	2.7	1.3	1.4
.10	0.4	0.5	0.3	1.6	1.5	0.8
.20	0.2	0.1	0.5	1.9	0.6	2.2

C = Caparo, PC = Petite Curucaye, P = Paria.

unsuccessful because females are seldom receptive, and when unreceptive they rarely maintain their position near a displaying male. Thus a lack of female receptivity reduces the number of copulation attempts performed by males because females move away from displaying males. However, when males perform more copulation attempts this does not mean that females were necessarily more receptive. This is because many copulation attempts are performed to unreceptive females who have (inadvertently?) temporarily maintained their position during a male's display. Thus copulation attempts reflect female position immediately following a display and not receptivity. (When females are receptive, they continue maintaining their position following sigmoid displays, resulting in successful copulations. Thus copulations reflect female receptivity.) The fluctuation in the percentage of sigmoid displays that were followed by copulation attempts at different stream velocities (Table XI) likely simply reflects variations in female position during sigmoid displays.

Summary of Results

Guppies bred from populations living in slow-flowing (Caparo) and fast-flowing (Paria) streams differed in the following ways:

- 1) Caparo guppies were located in the top half of the stream tank significantly more often than Paria guppies (except at the fastest stream velocity, when they were unable to maintain their position).

- 2) Caparo guppies were significantly more cohesive than

Paria guppies at both the slowest and fastest velocities.

3) Caparo males were significantly less aggressive than Paria males at all stream velocities.

4) Caparo males performed significantly fewer sigmoid displays and more thrusts and gonopodial swings than Paria males in slow-flowing water..

5) For both guppy populations the frequency of sigmoid displays, thrusts, and gonopodial swings decreased as stream velocity increased. The frequency of sigmoid displays decreased more than the frequency of thrusts as stream velocity increased.

6) Caparo males were less able to maintain their courtship behaviour and stream position at high velocities than Paria males. Caparo males showed a greater decrease in the ratio of sigmoid displays : thrusts than Paria males as stream velocity increased.

7) In faster-flowing water the sigmoid displays performed by Caparo males were of shorter duration than those of Paria males.

Thus, all seven original predictions regarding these populations were in large part confirmed. In addition, as predicted, most of the courtship behaviour patterns of the intermediate stream velocity population (Petite Curucaye) were intermediate between the headstream and lowland populations.

The persistence of these differences in behaviour in laboratory raised descendants demonstrates that there is a genetic contribution to the differences. The differences therefore presumably represent the product of natural selection.

Stream velocity was found to have a direct effect on guppy depth location. However, no direct effect of stream velocity on cohesion and male aggression was found. The performance of courtship behaviour was clearly affected by stream velocity, with both sigmoid displays and gonopodial swings being markedly decreased by increasing velocity.

Support was gained for the hypothesis that population differences, especially in male courtship behaviour, represent adaptive responses to stream velocity.

CHAPTER V

EXPERIMENT II : MALE P. RETICULATA COURTSHIP BEHAVIOUR AND
MATE PREFERENCE IN STILL AND FLOWING WATER CONDITIONSIntroduction

In this experiment I study the courtship behaviour of wild-caught guppies from two pairs of headstream and lowland populations. The four populations were tested in still water and in one flowing water condition. Unlike Experiment I, the fish were maintained in the stream condition for a full week. However, I again tested the predictions that 1) lowland males perform fewer sigmoid displays but more thrusts and gonopodial swings than headstream males, 2) courtship behaviour should decrease for all stocks in the flowing water, and 3) fish from the headstream populations should be better able to maintain courtship in flowing water than lowland fish.

Striking behavioural differences between these wild-caught populations might be expected, due to their prior experience in different stream velocities and the lack of any laboratory selection occurring in still water tanks. Two pairs of populations were studied since parallel differences between them, reflecting differences found between stocks in Experiment I, would provide additional support for generality of the results.

To examine courtship behaviour in more detail I determined the number of "courtship bouts" performed by each population of males. (A courtship bout was defined as a series of

consecutive sigmoid displays and/or thrusts performed by a male to the same female.) Since, in Experiment I, both sigmoid displays and thrusts were found to decrease with increasing stream velocity, I predict that headstream males should perform more courtship bouts (observed in slow-flowing water) in order to maintain their level of courtship in fast-flowing water. The number of behaviour patterns performed during courtship bouts was also examined. This was done to determine whether all populations perform, on average, the same number of behaviour patterns to individual females once they begin courting them.

Another objective of this experiment was to determine male mate preference for females from different populations. I examined mate preference to test the prediction that males should court females from their own population more than females from another population. This prediction was based on Ballin's (1973) study, which found a degree of selectivity by different populations of males for females of their own population. Ballin's results, however, were inconclusive.

During this experiment male and female mate preferences were actually tested simultaneously. I determined male mate preference directly by observing courtship behaviour and recording which females males courted. Female choice and male mating success were determined at the same time but indirectly. This was done by Liley and Luyten, using an autoradiographic technique, measuring labelled and unlabelled sperm collected from the females' oviducts at the end of the experiment. The autoradiographic technique was used to

determine whether one stock of males had greater potential reproductive success (ie. inseminated more females) and whether this reproductive success was affected by stream velocity condition. Unfortunately, the results from this technique have not yet been analyzed. (Details of the technique used and the results will be reported elsewhere by Liley and Luyten.)

Materials and Methods

The procedure used in this experiment was designed in conjunction with Liley and Luyten.

Wild-caught adult guppies from four Trinidad populations were tested - Guayamare (lowland), Upper Aripo (headstream), Caparo (lowland), and Paria (headstream). Following their transport to Vancouver these populations were maintained under similar conditions (see General Methods, Chapter III).

Guayamare and Upper Aripo fish were tested together in groups containing 20 fish, 5 of each sex from both populations. In each group, males from one of the two populations had a radioactive label incorporated in their sperm. Each group of males was tested in both still and flowing water conditions but with a different group of females in each condition. The groups were as follows:

<u>Groups of Males</u>		<u>Females in Still water (A)</u>	<u>Females in 0.08 m/sec (B)</u>
1	5 UA* 5 GG	5 UA 5 G	5 UA 5 G
2	5 UA 5 G*	5 UA 5 G	5 UA 5 G

Groups of Males		Females in Still Water (A)	Females in 0.08 m/sec (B)
3	5 UA*	5 UA	5 UA
	5 G	5 G	5 G
4	5 UA	5 UA	5 UA
	5 G*	5 G	5 G

*Males injected with radioactive label.

UA = Upper Aripo, G = Guayamarea

Males from Groups 1 and 2 were tested first in still water (with Group 1A and 2A females) and then in a stream velocity of 0.08 m/sec (with Group 1B and 2B females). In contrast, males from Groups 3 and 4 were tested first in flowing water and then in still water. Following each test the females were killed and sampled for sperm. The same 20 males from each population were tested in both still and flowing water, whereas, 40 females from each population were tested but half in still water only and half in flowing water only.

The 0.08 m/sec velocity was chosen, assuming it to represent a fairly fast velocity considering that fish were to be maintained in it for a full week. (The second slowest velocity in Experiment I (0.07 m/sec) had only a slight effect on courtship behaviour, however, fish were maintained in it for less than a during the experiment.)

Males for each group were drawn from population tanks 25 days before testing and placed in isolated holding tanks with domestic stimulus females. These males were injected with thymidine-(methyl-¹⁴C) 25, 20, and 10 days before testing. Control males were injected with saline. The stimulus females were removed from the holding tanks five days before testing,

ensuring the start of courtship behaviour by the males soon after introduction to the stream tanks. At the end of the first test the males were returned to isolated holding tanks. Later they were introduced to a second stream tank under the alternate stream condition, with the second group of females. After this second test the males were killed.

Females for each group were drawn from the population tanks approximately 25 days before testing and placed directly in the stream tanks. Five to eight days before testing, the females were marked with a small dot on the top or bottom of their tails to distinguish them by population. The dot was made by intramuscular injection with indelible Trypan blue ink. The females were then returned to the stream tank which was set at the velocity to be used during the test. The test began when the females recovered from the marking procedure (5 to 8 days). At the end of each test females were killed and oviduct samples for sperm were taken.

Each test lasted one week, during which time the group of 20 fish (eg. Group 1A) was housed in the stream tank. The velocity was kept constant, at either 0.08 m/sec or with no current, for the entire seven days. The second test for each group of males (eg. Group 1B, at the second velocity) occurred 5 to 8 days after the end of the first test.

On days 1, 3, 5, and 7 of each test, two 10-min recordings were made for each of the group's 10 males. On each of these days one recording was made in the morning and one in the afternoon. Thus a total of eight 10-min recordings was made for each male. Males were distinguished from one another by

their individual colour patterns. Each recording contained the number of sigmoid displays, gonopodial swings, side-switches, leaps, and copulation attempts performed by the male. (Side-switches have not previously been described. They occur during sigmoid displays when the male, while displaying, switches the side of his body which faces the female. This is brought about by a rapid turn in the horizontal plane, head to tail, following which the sigmoid display continues.) For each behaviour pattern performed by a male the population to which the female he courted belonged was also recorded. Mate preference of males and the number and length of courtship bouts could therefore be determined.

This experimental procedure was repeated using Caparo and Paria fish, however, the males were not injected with radioactive label. Thus, in total, the procedure produced eight 10-min recordings for 20 males from each of the four populations in each of two stream velocity conditions.

Occasionally during the experiment a fish died or became "sick". If so, it was replaced with another fish and eliminated from the data analysis. Following the experiment each group of males was anaesthetized, measured with calipers, photographed and had a sperm smear taken.

Statistical Treatment of the Data

When comparing the same stock under the two stream conditions and when determining male mate preference for females, I used the Wilcoxon matched-pairs signed-ranks test (Siegel, 1956). When comparing different stocks under the

same stream conditions, the Mann-Whitney U-test was used (Siegel, 1956). Correlations between the size of males and various courtship behaviour patterns (see Appendix) were measured using the Spearman rank correlation coefficient (Siegel, 1956).

Results and Discussion

Thrusts and Sigmoid Displays

The mean numbers of thrusts performed by males in the two stream conditions are given in Figure 10. All four stocks performed more thrusts in the 0.08 m/sec velocity compared to still water. The increases, however, were not significant ($p < .05$, Wilcoxon test).

Comparing each pair of lowland and headstream stocks (Caparo with Paria; Guayamare with Upper Aripo), lowland fish usually performed more thrusts than headstream fish and in still water the difference between Guayamare and Upper Aripo males was significant (Fig. 10).

The mean numbers of sigmoid displays performed by each stock in the two stream conditions are given in Figure 11. The stocks performed either approximately the same or a decreased number of sigmoid displays in flowing water. The decrease was significant only for the Paria stock ($p < .05$, Wilcoxon test).

As predicted, under both stream conditions lowland males performed significantly fewer sigmoid displays than headstream males (Fig. 11).

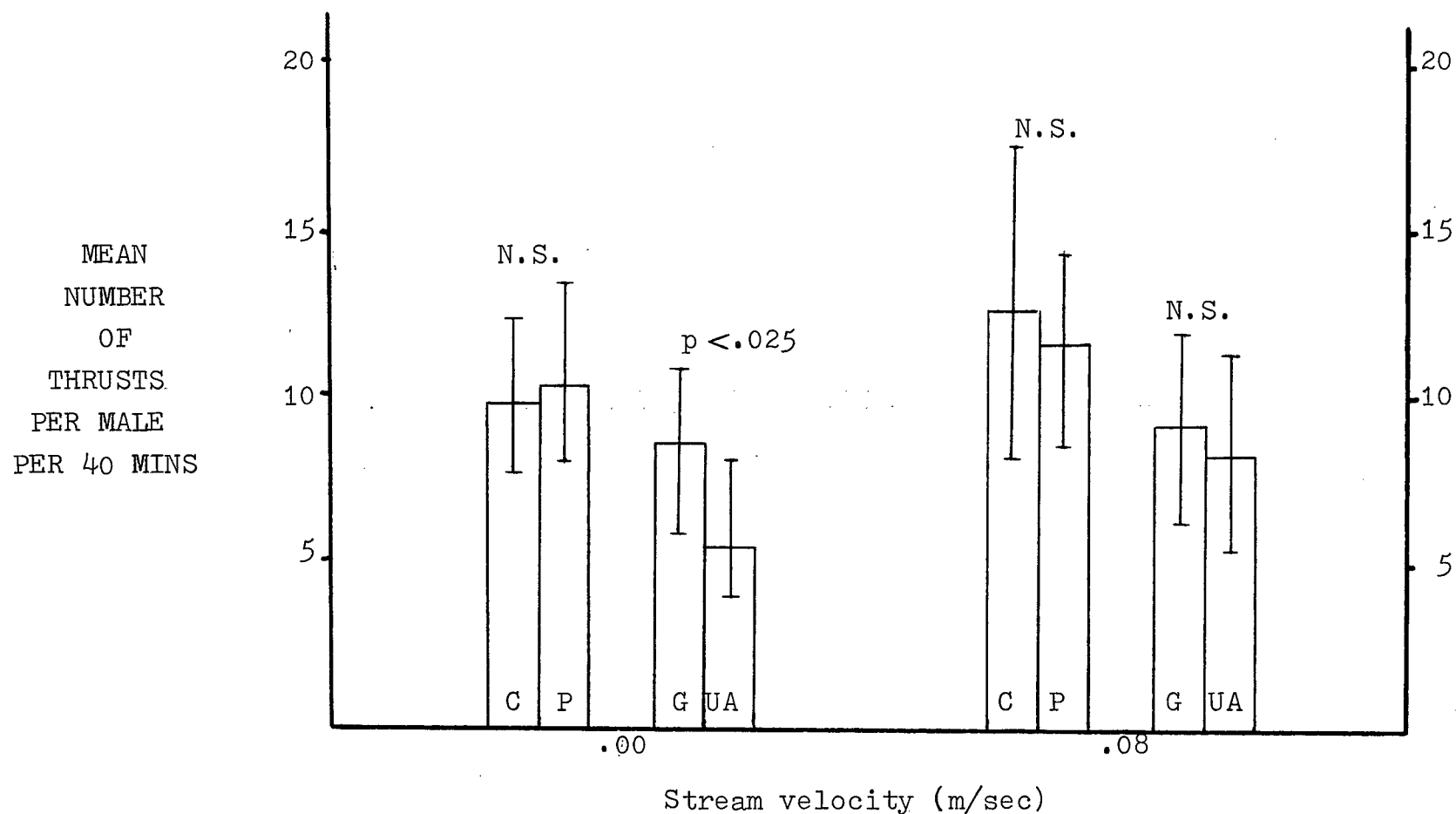


Figure 10. Number of thrusts in relation to stream velocity. Means \pm 95% confidence intervals. Probability values are from the Mann-Whitney U-test, 1-tailed for C > P, G > UA. C = Caparo (N(.00) = 19; N(.08) = 17), P = Paria (N(.00) = 20; N(.08) = 19), G = Guayamare (N(.00) = 17; N(.08) = 17), UA = Upper Aripo (N(.00) = 19; N(.08) = 17).

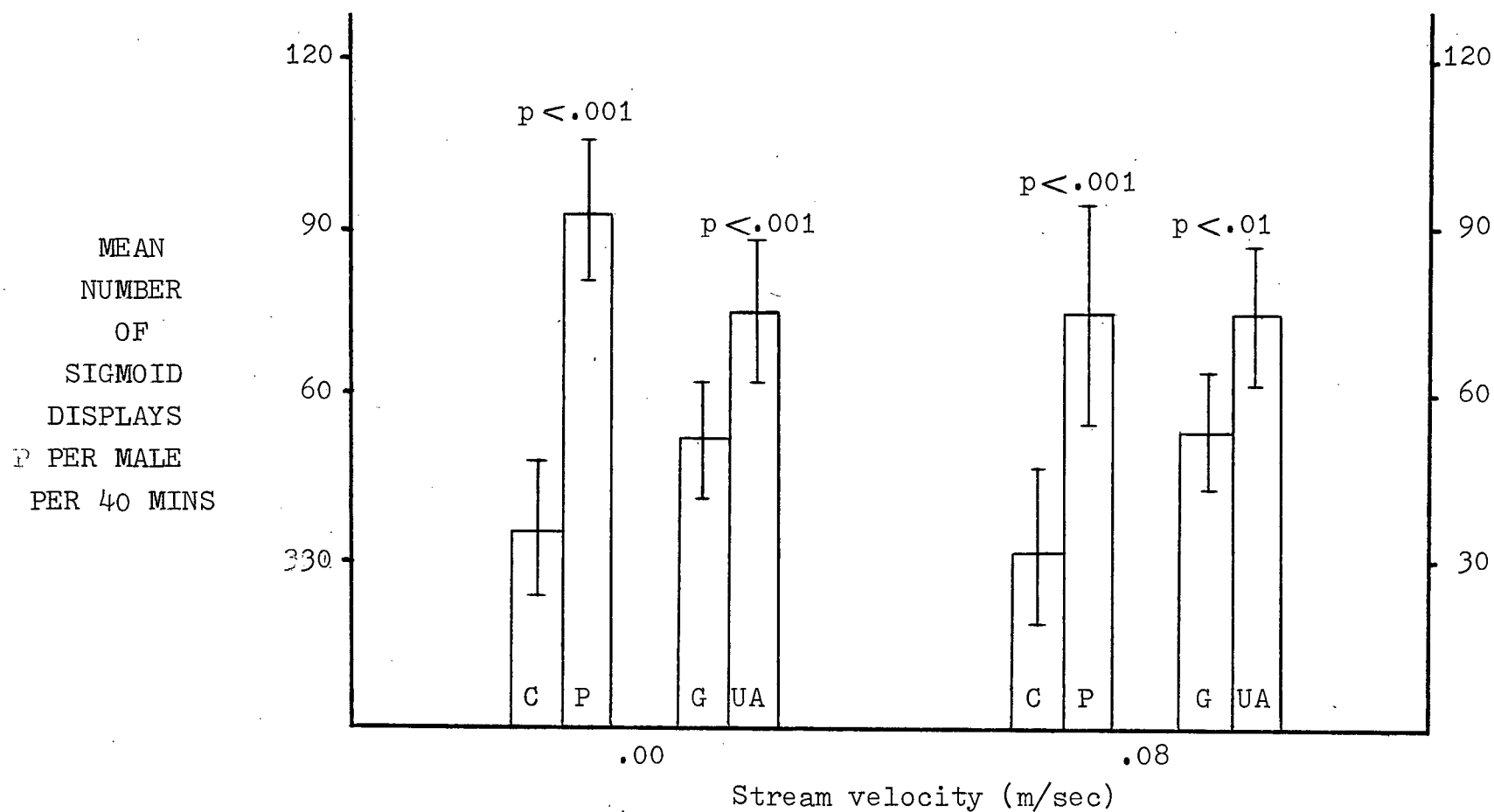


Figure 11. Number of sigmoid displays in relation to stream velocity. Means \pm 95% confidence intervals. Probability values are from the Mann-Whitney U-test, 1-tailed for $P > C$, $UA > G$. C = Caparo, P = Paria, G = Guayamare, UA = Upper Aripo.

All four stocks had a lower ratio of sigmoid displays : thrusts in flowing water than in still water (Fig. 12). This difference in ratio was significant for the Paria and Upper Aripo stocks ($p < .05$, Wilcoxon test).

Comparisons between each pair of stocks showed that, as predicted, the headstream stocks had significantly higher ratios of sigmoid displays : thrusts than the lowland stocks in both stream conditions (Fig. 12). These differences are also consistent with those found in Experiment I.

In summary, differences between these wild-caught populations in the frequencies of both thrusts and sigmoid displays and the ratio of sigmoid displays : thrusts are consistent with differences found between Caparo and Paria stocks in Experiment I. In both experiments lowland fish performed fewer sigmoid displays and more thrusts than the headstream fish. However, the marked effects of increasing stream velocity found in Experiment I were not found between the two stream conditions here.

There was no support for the prediction that headstream fish are better able to maintain displays in faster-flowing water than lowland fish. Comparing the ratio of sigmoid displays : thrusts for each stock in still water and at 0.08 m/sec, the ratio decreased between the two conditions by 30% for Caparo, 29% for Paria, 3% for Guayamare, and 30% for Upper Aripo (Fig. 12). Thus the lowland populations' ratios decreased by the same amount or less than the headstream ratios. It should be noted, however, that headstream males still used displays significantly more frequently than lowland

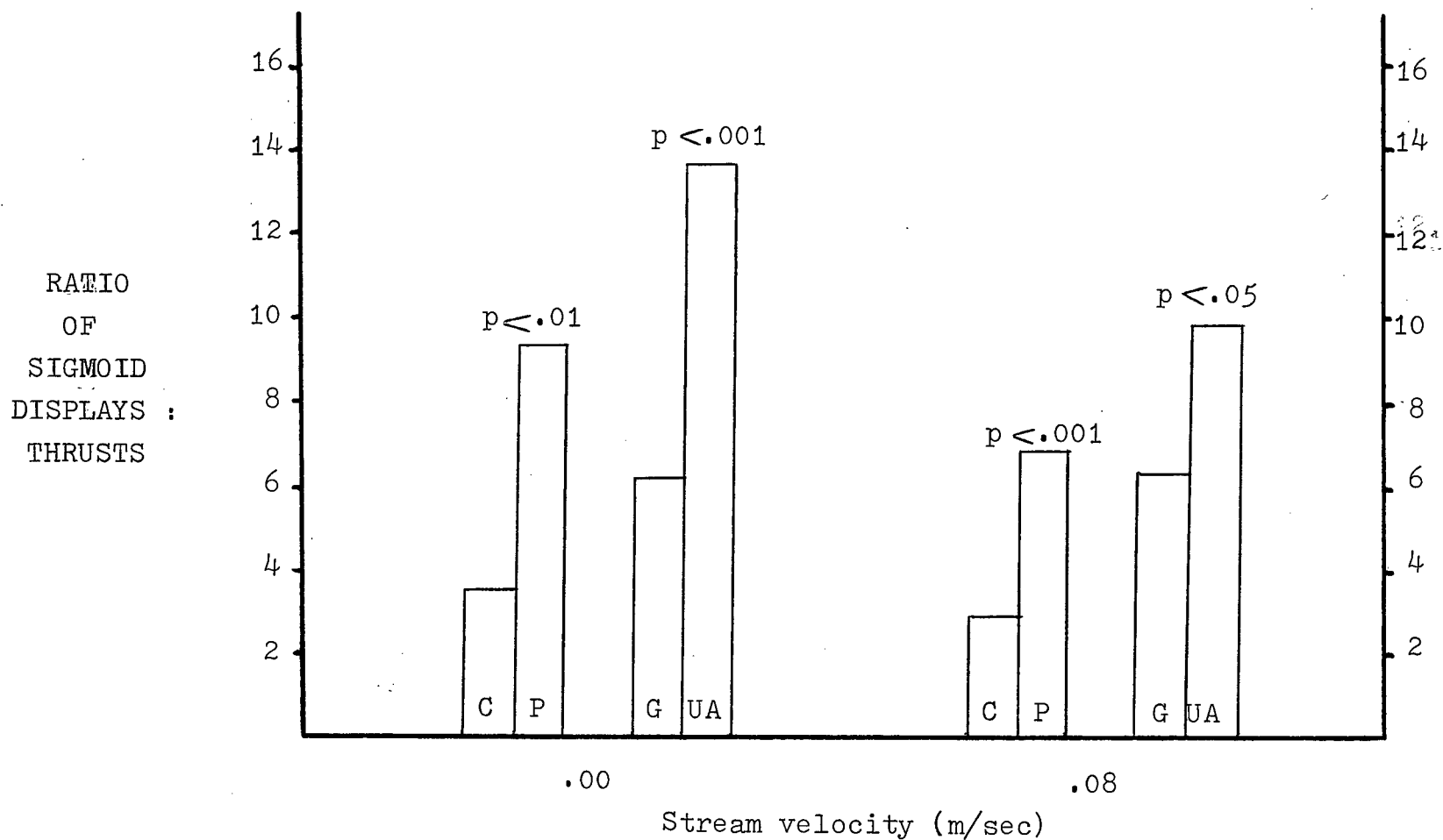


Figure 12. Ratio of sigmoid displays : thrusts in relation to stream velocity. Probability values are from the Mann-Whitney U test, 1-tailed for $C < P$, $G < UA$.
C = Caparo, P = Paria, G = Guayamare, UA = Upper Aripo.

males and the headstream ratios of sigmoid displays : thrusts were significantly higher in both stream conditions.

The prediction that headstream fish are better able to maintain courtship in fast-flowing water than lowland fish was not supported, likely because of the relatively slow stream velocity used during the experiment. Unlike Experiment I, the effect of a fast velocity was not actually tested. Differences in the frequencies of thrusts and sigmoid displays in Experiment I between 0.03 m/sec and 0.07 m/sec (the two slowest velocities; Figs. 6 and 7) are actually consistent with the results obtained in this experiment between still water (0.00 m/sec) and 0.08 m/sec. In both experiments thrusts increased slightly and sigmoid displays usually did not change significantly. Only at the higher velocities in Experiment I were the effects of increasing stream velocity evident. The results of the two experiments, however, cannot be compared directly because they tested fish for different periods of time. In addition, Experiment II examined fish in still water (0.00 m/sec) which represents a condition very different from that of flowing water, even of a slow velocity, since fish do not orientate upstream and males may show higher than normal rates of courtship. In still water males may orientate to females and court them from any direction.

As previously noted, I chose the 0.08 m/sec velocity for this experiment believing it to be a reasonably fast velocity since fish were to be maintained in it for a full week.

I had assumed that a slower velocity than the faster velocities

of Experiment I should be used, since in Experiment I fish were maintained in the velocity for only one day. The results obtained here contradict this assumption, since the effects of a fast stream velocity were not obtained. This was also perhaps due to the use of wild-caught guppies during this experiment. These guppies had previous experience in streams and would therefore likely be little affected by a slow velocity.

Gonopodial Swings

The mean numbers of gonopodial swings performed by each stock in the two stream conditions are given in Figure 13. All four stocks performed slightly fewer gonopodial swings in the flowing water condition, this difference was significant only for the Caparo stock ($p < .01$, Wilcoxon test).

Comparing stocks, lowland males generally performed more gonopodial swings than headstream males and the differences were significant between Guayamare and Upper Aripo males in both stream conditions (Fig. 13). These differences are consistent with the differences between Caparo and Paria stocks in Experiment I.

(Correlations between male size and sigmoid displays, thrusts and gonopodial swings are presented for this experiment in the Appendix.)

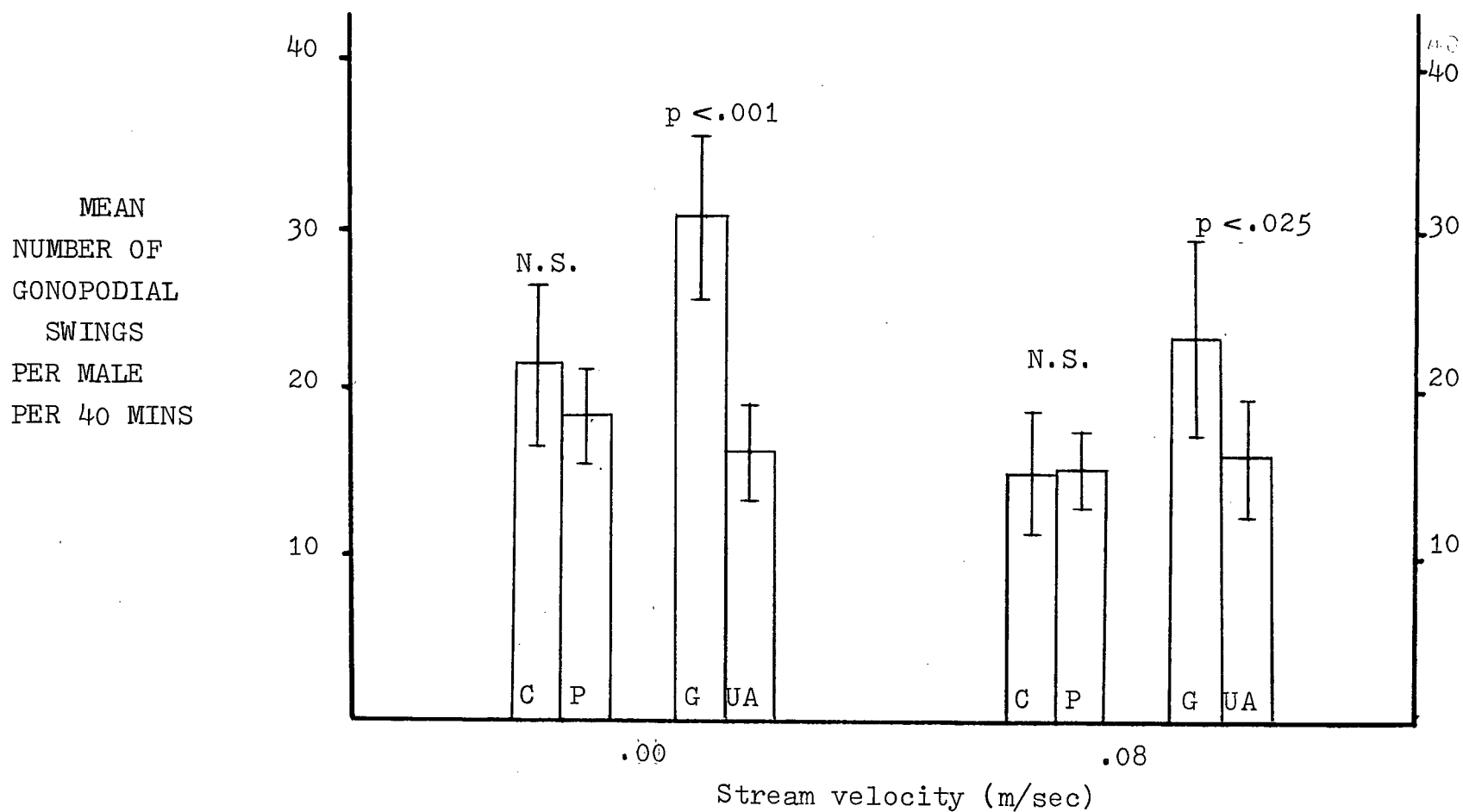


Figure 13. Number of gonopodial swings in relation to stream velocity. Means \pm 95% confidence intervals. Probability values are from the Mann-Whitney U-test, 1-tailed for $C > P$, $G > UA$. C = Caparo, P = Paria, G = Guayamare, UA = Upper Aripo.

Side-switches, Leaps, Copulation Attempts, and Copulations

Table XII gives the mean numbers of sigmoid display, side-switches, leaps, and copulation attempts performed by males of each stock in the two stream conditions. It also gives the percentages of sigmoid displays during which side-switches were performed and the percentages of sigmoid displays followed by leaps and copulation attempts.

These behaviour patterns occurred infrequently and were therefore not analyzed statistically. A decrease was generally seen in the frequencies of leaps and copulation attempts performed in flowing water relative to their frequency in still water.

Only six copulations were observed during the experiment. While in still water three Upper Aripo females were receptive: two to Upper Aripo males and one to a Guayamare male, one Paria female was receptive to a Caparo male, and one Guayamare female was receptive to an Upper Aripo male. In the 0.08 m/sec velocity one copulation was observed and it was between a Paria male and female. This small number of copulations at least indicates that females will mate with males from both lowland and headstream populations regardless of their own population of origin. Direct observations of copulations however were too infrequent to draw further conclusions regarding sexual selection by females.

Table XII. Mean number and percentage of Sigmoid Displays with or followed by Side-switches, Leaps, and Copulation Attempts for males from four stocks in two stream conditions.

	Mean number of Sigmoid Display Side-switches / male / 40 mins		Percentage of Sigmoid Displays with Side-switches	
	Stream velocity (m/sec)		Stream velocity (m/sec)	
	.00	.08	.00	.08
Caparo	1.4	0.3	4.0	0.9
Paria	2.0	1.3	2.1	1.7
Guayamare	1.8	2.0	3.4	3.8
Upper Aripo	0.8	1.2	1.1	1.6
	Mean number of Leaps / male / 40 mins		Percentage of Displays Followed by Leaps	
	Stream velocity (m/sec)		Stream velocity (m/sec)	
	.00	.08	.00	.08
Caparo	0.9	0.6	2.6	2.0
Paria	1.0	0.4	1.1	0.5
Guayamare	2.2	1.5	4.2	2.8
Upper Aripo	3.1	2.2	4.0	2.9
	Mean number of Copulation Attempts / male / 40 mins		Percentage of Sigmoid Displays followed by Copulation Attempts	
	Stream velocity (m/sec)		Stream velocity (m/sec)	
	.00	.08	.00	.08
Caparo	0.1	0.0	0.3	0.0
Paria	0.3	0.2	0.3	0.2
Guayamare	0.4	0.2	0.8	0.4
Upper Aripo	0.3	0.3	0.3	0.4

Courtship Bouts and the Number of Behaviour Patterns per Courtship Bout

Table XIII gives the mean number of courtship bouts performed by males of each population in the two stream conditions. As predicted, males from the headstream stocks performed more courtship bouts than lowland males. The differences were significant between Paria and Caparo in both stream conditions. Headstream males may have evolved a tendency to perform more courtship bouts in order to maintain an optimal number of courtship bouts in fast-flowing water.

Comparing each population with itself in the two stream conditions, no significant differences were found in the number of courtship bouts performed (Table XIII; Wilcoxon test). Therefore, in contrast to expectation, an increase in stream velocity did not decrease the number of courtship bouts performed. Either my hypothesis was wrong or the stream velocity used was not fast enough to detect the effect of stream velocity on the frequency of courtship bouts.

The proportion of courtship bouts with one, two, and three or more sigmoid displays and thrusts is shown in Figure 14. Headstream males did not differ from lowland males in the number of behaviour patterns performed to females during individual courtship bouts. In fact, both classes of fish performed remarkably similar proportions of bouts comprised of one, two, and three or more courtship behaviour patterns. This result leads me to speculate that, once a male begins courting a female, there is an optimal number of sigmoid displays and thrusts for which it is advantageous for the

Table XIII. Mean number of courtship bouts per male for each stock in two stream conditions.

Mean number of courtship bouts* per male per 40 mins				
	Stream velocity (m/sec) .00	Difference** between stocks p	Stream velocity (m/sec) .08	Difference** between stocks p
Caparo	32.9	<.025	32.5	<.025
Paria	78.0		64.7	
Guayamare	46.7	N.S.	49.1	N.S.
Upper Aripo	54.9		52.5	

*A courtship bout is defined as a series of consecutive sigmoid displays and/or thrusts performed by a male to the same female.

**Mann-Whitney U-test, 1-tailed for $P > C$, $U_A > G$.

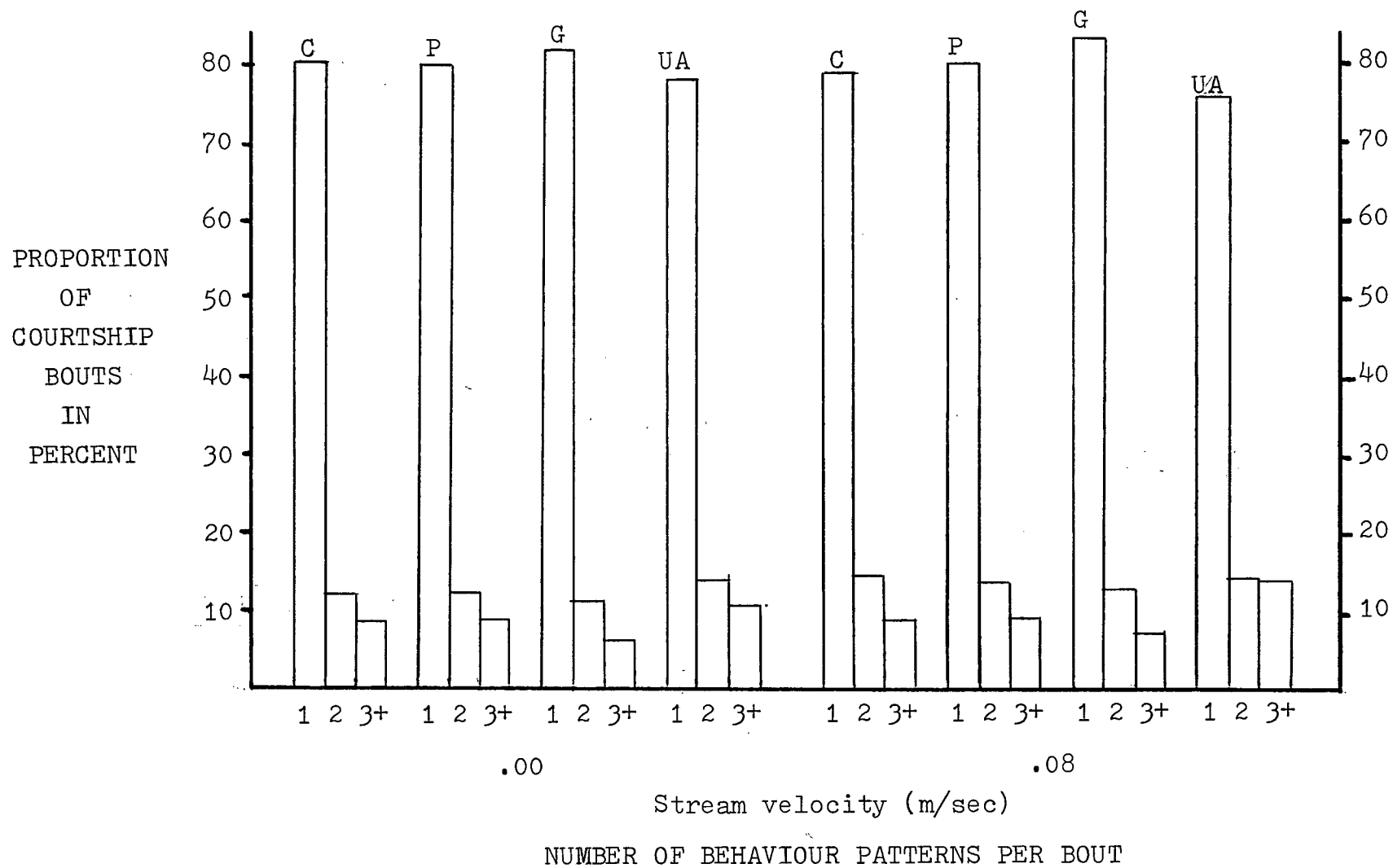


Figure 14. Proportion of courtship bouts with one, two, and three or more behaviour patterns per courtship bout.
C = Caparo, P = Paria, G = Guayamare, UA = Upper Aripo.

male to perform. Perhaps males do not increase their chance of mating success by performing more behaviour patterns to the same female during a courtship bout. It may be of greater advantage to leave the female after one or two displays or thrusts and seek out another female.

For each stock the proportions of courtship bouts with one, two, and three or more behaviour patterns were very similar under both conditions (Fig. 14). Stream velocity did not affect the number of behaviour patterns performed during courtship bouts.

Mate Preference of Males

Males from both headstream populations exhibited a preference for females of their own stock, consistently performing more sigmoid displays and thrusts to headstream females in both stream conditions (Table XIV). Males from the two lowland stocks, however, varied in their mate preference. Caparo males exhibited a significant preference for females of their own stock when observed in flowing water, whereas Guayamare males courted headstream females more than females from their own stock. Mate preferences were generally similar in both still and flowing water conditions.

It has often been assumed that male guppies are very promiscuous, courting all females regardless of stock, and that sexual selection is exercised only by females (Haskins et al., 1961; Farr, 1976, 1977; and Endler, 1980). In this experiment three of the four populations exhibited a preference for females of their own population, as predicted. They did not

Table XIV. Percentage of sigmoid displays and thrusts performed by males to females of their own or another stock.

Males	Females Courtied	Percentage of total sigmoid displays		Percentage of total thrusts	
		Stream velocity (m/sec)		Stream velocity (m/sec)	
		.00	.08	.00	.08
Caparo	Caparo	48.8	65.0**	49.7	68.9**
	Paria	51.2	35.0	50.3	31.1
Paria	Caparo	37.8	48.1	30.1	40.7
	Paria	62.2**	51.9	69.9**	59.3*
Guayamare	Guayamare	41.0	42.1	49.0	39.0
	U. Aripo	59.0	57.9	51.0	61.0
U. Aripo	Guayamare	41.6	43.0	46.0	34.3
	U. Aripo	58.4	57.0	54.0	65.7*

* $p < .025$ Wilcoxon test, one-tailed for a preference
 ** $p < .005$ for females from the same population as
 males (based on raw data and not the
 percentages shown).

simply court females randomly.

The reasons for the mate preferences exhibited by the populations of males are not entirely clear. Male preference for females may, at least in part, be influenced by female responses to male courtship. If this is the case, then male mate preferences may actually reflect female selection of males. Whether this is the case or not, the results indicate some inter-population discrimination and this may restrict inter-population breeding. The preferences may therefore represent partial isolating mechanisms between populations.

The fact that males exercise a choice in their courtship does not necessarily alter the role that females play in sexual selection, especially since female cooperation is critical for successful copulation (Liley, 1966). During the experiment, even when males exhibited a preference for one stock of females they still directed at least 30% of their courtship towards females of the alternate stock. Therefore sexual selection could still be exercised by females. The results of this experiment do, however, indicate that sexual selection in P. reticulata is not necessarily entirely controlled by female choice and inter-male competition. Future studies on inter-population sexual selection in the guppy should examine both male and female mate preferences.

Summary of Results

Population differences in the frequencies of courtship behaviour patterns between wild-caught headstream and lowland fish were consistent with differences found between the Caparo (lowland) and Paria (headstream) stocks tested in Experiment I. Lowland males were again found to use fewer sigmoid displays and more thrusts and gonopodial swings than headstream males. Since both pairs of populations showed results consistent with those obtained previously it seems reasonable to suggest that the differences observed are representative of headstream and lowland populations in general.

The effect of stream velocity on male courtship behaviour clearly demonstrated in Experiment I was much less pronounced in this experiment. Although the frequencies of sigmoid displays and gonopodial swings generally decreased slightly in flowing water compared to still water, in most cases the decreases were not significant. Lowland males did not show the predicted greater decrease in their courtship behaviour than headstream males. This is believed to be due to the slow stream velocity used during this experiment and the fact that wild-caught guppies were tested.

As predicted, headstream males performed more courtship bouts than lowland males and this may represent an adaptation to stream velocity. All populations performed roughly the same number of behaviour patterns during courtship bouts, suggesting that males may perform an optimal number of sigmoid displays and thrusts to individual females before leaving the

female and courting another one.

Three of the four populations exhibited a mate preference for females of their own population. These mate preferences may represent partial isolating mechanisms between populations.

CHAPTER VI

GENERAL DISCUSSION

The Evolution of Behaviour and Morphology in *P. reticulata*

In this discussion I first briefly review the results of my experiments and note that they provide evidence of behavioural adaptation to stream velocity. Then I review the studies done on natural populations of the guppy, combining my findings with those of others, to develop an overview of the factors which have likely acted as selection pressures to mold the guppy's present morphology and behaviour. Finally, I consider my results on male mate preferences and the possible formation of behavioural isolating mechanisms.

Studies of the ecological factors that are important in the evolution and maintenance of behaviour in a population are becoming increasingly common (see Brown, 1975; Krebs and Davies, 1978; and Keenleyside, 1979). Many of the studies, however, do not go beyond noting correlations between behavioural differences and environmental factors. In this thesis, however, I went beyond noting such correlations. My laboratory experiments on natural populations of guppies tested seven predictions based on the hypothesis that guppy social behaviour, and specifically guppy courtship behaviour, is adapted to stream velocity conditions.

In Experiment I, lowland and headstream guppy populations showed all of the predicted behavioural differences. Fish bred from a lowland population showed greater cohesion,

less aggressive behaviour, and a greater preference for the surface region of stream tanks than headstream fish. Lowland males performed fewer sigmoid displays but more thrusts and gonopodial swings than headstream males. Similar population differences in male courtship behaviour were again found in Experiment II. Thus, these behavioural differences are very likely representative of lowland and headstream populations in general.

The persistence of the behavioural differences in laboratory raised descendants of isolated populations proves a genetic contribution to the differences (Ehrman and Parsons, 1976). Such genetic differences are presumably the product of natural selection and represent evolutionary adaptations.

The direct effect of stream velocity on male courtship behaviour was clearly demonstrated in Experiment I. For all stocks of guppies the frequency of sigmoid displays and gonopodial swings decreased markedly as stream velocity increased. The best evidence of behavioural adaptation to stream velocity was obtained when headstream males were found to be better able to maintain their ratio of sigmoid displays : thrusts than lowland males as stream velocity increased. In addition, as predicted, in faster-flowing water the sigmoid displays of headstream males were of longer duration than those of lowland males. Thus, headstream males appear better adapted to displaying in fast-flowing water.

The higher ratio of sigmoid displays : thrusts exhibited by headstream males may itself represent an adaptation to stream velocity. Since sigmoid displays decrease more than

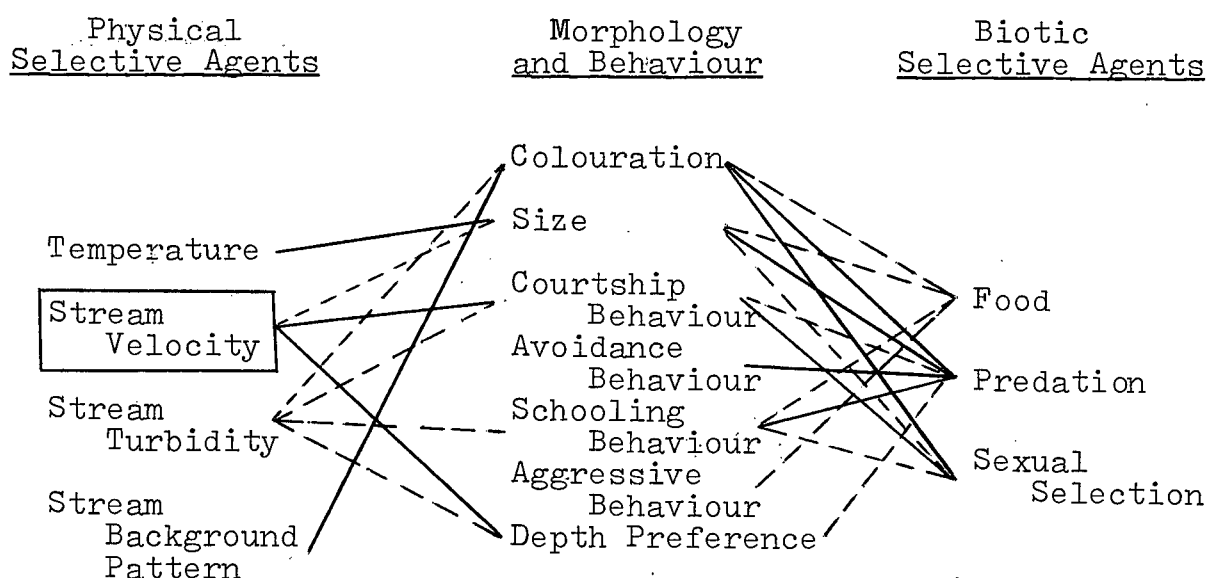
thrusts with increasing stream velocity, males living in fast-flowing water may have evolved the tendency to perform a higher ratio of sigmoid displays : thrusts (when observed in slow water) in order to still be performing at an optimal ratio when in fast-flowing water.

Although differences in the frequency of performing courtship behaviour patterns between populations were the same in both experiments, the direct effect of stream velocity on male courtship behaviour was less pronounced in Experiment II. This was likely due to the slow stream velocity used during this experiment.

Stream velocity was found to directly affect guppy depth location in Experiment I. Increasing velocity produced a decrease in the time spent at the top of the stream tank by lowland fish. Fish from the intermediate velocity spring population, however, were better able to maintain their position at the top of the stream tank than lowland fish. This result likely indicates another adaptation to stream velocity. In addition, headstream fish showed a preference for the bottom of the stream in all velocities and I suggest they evolved this preference in order to hold their position in fast-flowing water and make use of the deceleration of water close to the substrate.

Differences between populations of guppies in both male courtship and depth location appear to reflect adaptations to different stream velocities. This, however, does not mean that other environmental factors did not also play a role in shaping these behaviours. In fact, it is often wrong to search

for a single biological function or adaptation of a piece of behaviour or morphology. I agree with Liley and Seghers (1975) that a full assessment of the evolutionary significance of natural variation in the guppy requires consideration of many features of the environment. Both physical and biotic selection pressures have likely affected the morphology and behaviour of guppies. The studies dealing with guppies in Trinidad (Haskins et al., 1961; Seghers, 1973, 1974a, 1974b; Ballin, 1973; Liley and Seghers, 1975; Farr, 1975; Endler, 1978, 1980; and this study) suggest to me that the following selection pressures may have interacted to produce the observed differences between guppy populations:



(Solid lines indicate demonstrated evidence for selective influences. Broken lines indicate possible selective influences.)

For each character of morphology and behaviour listed I will briefly review the evidence for specific factors acting as selection pressures.

Male Colouration. The extreme polymorphism of male colour pattern in P. reticulata has long been noted and the genetics of colour inheritance has been studied in considerable detail (Winge, 1922, 1927; Fisher, 1930; Winge and Ditlevsen, 1947; Haskins and Druzba, 1938; Goodrich, Josephson, Trinkhaus, and Slate, 1944; Haskins and Haskins, 1951, 1954; Haskins, Young, Hewitt, and Haskins, 1970; and Yamamoto, 1975).

Natural populations of the guppy were first studied by Haskins et al. (1961) to gain an understanding for the colour polymorphism exhibited in the wild. They suggested that colour patterns in a given natural population may represent a balance between sexual selection, making the patterns brighter and more visible, and predation, making the pattern less conspicuous. Endler (1978) found that the colour patterns of populations of guppies in the wild show a marked change with predation intensity. Male colour patterns were less conspicuous at high, compared to low, predation intensities. In another study, Endler (1980) provided a direct test of the hypothesis that male colour pattern is subject to natural selection. In long term laboratory and field experiments he demonstrated that predation intensity and stream background colour patterns have predicted effects on the colour patterns of male guppies. Guppies became less conspicuous at higher compared to lower predation intensity. In addition, sexual selection by females appeared to produce males as different from their stream background as possible.

In laboratory choice experiments using natural guppy populations, Ballin (1973) found that bright colouration of

males did not influence female choice of mates. Using laboratory strains of guppies, Farr (1980a) found that females prefer conspicuous males only if all males exhibit equal courtship display rates. He suggests that male colouration does not influence female choice of males but rather that females prefer males with higher display rates. (During his experiments male reproductive success was measured directly by determining the parentage of offspring.) Farr (1977, 1980a) also found that within a group of competing males, females selected males with less frequent or rare colour patterns. It thus appears that females are more receptive to males whose colouration is different from either 1) their stream background (Endler, 1980) or 2) other males within a group (Farr, 1977, 1980a). Both predation and sexual selection have been demonstrated to act as selection pressures producing variability in male colouration.

Male Size. Experiments in which guppies from two different natural populations were raised at different temperatures have indicated that inter-population differences in the size of adult guppies, particularly males, are partly determined by genetic differences and are partly a phenotypic response to environmental temperature conditions (Liley and Seghers, 1975). Evidence that predation is size-selective has also been provided by experiments in which guppies were exposed to a variety of natural predators (Seghers, 1973; Liley and Seghers, 1975). Thus size selective predation may also be a factor involved in the evolution of inter-population differences in male size.

Liley and Seghers (1975) suggest that body size may be adapted to stream velocity and perhaps also represent some form of mating advantage. Large body size might allow fish to better manoeuvre in fast-flowing streams, and thus gain them an advantage in intra-specific behaviour and escape from predation. (Hubbs (1941) has noted that fish living in faster stream velocities are generally larger than those in slower-flowing water.) Sexual selection should favor larger guppies if females choose larger (and presumably fitter) males. Neither of these possibilities have as yet been tested experimentally. Only correlations between guppy size and stream velocity have been noted.

The effect of sexual selection and stream velocity on fish size could be tested by maintaining guppies under different stream velocities for several generations. Such experiments could determine whether larger males are more successful than small males in mating with females at different velocities.

Avoidance Behaviour. Models of aerial predators have been found to elicit different responses from two populations of guppies and their laboratory-reared progeny (Seghers, 1973, 1974a). Headstream males moved into deeper water and remained quiescent while lowland males did so to a much lesser degree. Seghers suggests that this genetic variation in behaviour reflects differences in selection pressures exerted by aquatic versus aerial predators.

Cohesion. Group cohesion or schooling may have evolved as an anti-predator strategy (Shaw, 1970). In laboratory

experiments, Seghers (1973, 1974b) demonstrated that populations of guppies having a greater tendency to school were relatively less vulnerable to predation. He suggested that genetic differences between populations in schooling behaviour, along with reaction distance to predators, alarm threshold, and microhabitat selection, are components of a coadapted complex of antipredator adaptations. In a field study, Farr (1975) found that populations of guppies living in streams containing Rivulus hartii (and not larger predators) were dispersed sparsely across the entire stream bed (and displayed low rates of sexual activity). Populations living in streams with large predators, however, lived in dense aggregates along the edges of the streams and males exhibited higher rates of sigmoid displays and thrusts. He speculated that the observed differences in schooling and courtship behaviour were entirely due to the presence or absence of Rivulus. He did not, however, test this hypothesis.

In addition to predation, stream turbidity may have acted as a selective agent in producing population differences in schooling behaviour. As noted earlier, in turbid water one might expect fish to keep close together for the purpose of communication. Because of the relationship between stream velocity and turbidity (slow-flowing lowland rivers are turbid while fast-flowing headstreams are clear), if turbidity affects schooling, stream velocity would indirectly affect schooling behaviour. I examined group cohesion in several stream velocities and found no direct effect of stream velocity on schooling behaviour, at least within the velocities tested.

Aggressive Behaviour. Aggression in the guppy may simply be coadapted with schooling behaviour since it is logical that fish living in schools should be less aggressive than if distributed over the stream bed. After finding that Guayamare lowland river guppies are less aggressive than Paria headstream guppies, Ballin (1973) suggested that aggression in headstream populations may be due to the absence of predation and presence of inter-male competition for females. Gorlick (1976) suggested that aggression may be an important factor in determining male dominance among domestic guppies. However, Farr (1980a), using laboratory strains of guppies, found that more aggressive males did not increase their reproductive success, in fact they decreased it. At present, there is no clear reason as to why headstream males are aggressive.

My experiments found no direct effect of stream velocity on aggression in males. Perhaps competition for resources, such as food, has produced inter-male aggression in headstream males. There is, however, no evidence for this possibility at present.

Depth Preference. The presumed genetic differences in depth preference of different guppy populations may represent adaptations to stream velocity. I outlined earlier the evidence from my experiment for this possibility. Stream turbidity may also have selected for differences in depth preference. Fish in turbid lowland waters may have evolved a preference for the surface region of streams since they would likely be assisted in schooling and find each other more easily in the well-lit surface region of rivers. The effect

of stream turbidity on depth preference (as well as on other behaviour) is presently being studied (Luyten, personal communication).

Courtship Behaviour. The reproductive biology and courtship behaviour of *P. reticulata* has been studied in detail (eg. Breder and Coates, 1935; Clark and Aronson, 1951; Rosen and Gordon, 1953; Hildeman and Wagner, 1954; Kadow, 1954; Baerends, Brouwer, and Waterbolk, 1955; Rosen and Tucker, 1961; Breder and Rosen, 1966; Liley, 1966; and Liley and Wishlow, 1974).

Farr (1980b) studied the role of sigmoid displays and thrusts in a laboratory strain of guppies. He found that males exhibited the same mean total number of displays regardless of their prior sexual experience or the receptivity of females they courted. However, he found that males thrust more frequently when with unreceptive females than with receptive ones. Farr therefore suggested that males utilize thrusts, which only rarely result in successful inseminations (Clark and Aronson, 1951; Baerends et al., 1955; and Liley, 1966), in order to "rape" unreceptive females. Unfortunately, his study does not deal with natural populations of guppies that vary genetically in their frequency and ratio of performing sigmoid displays : thrusts.

Working with natural populations of guppies, Ballin (1973) speculated that the lowland male guppies display less frequently than headstream males because of increased predation in lowland rivers, assuming males that display more frequently increase their risk of being eaten by predators. He also suggested that, due to poor visibility, males in turbid water

would benefit less from displaying and more from attempting copulations by performing thrusts. However, Ballin did not test these speculations. Farr (1975) previously noted correlations between guppy courtship behaviour and the presence of predators. He suggested that differences in courtship behaviour reflect a balance between adaptation to predation and sexual selection by females. Recently, Farr (1980a) showed that among laboratory strains of guppies, males which displayed at higher rates were preferred by females and demonstrated greater reproductive success than males who displayed less frequently. Thus he demonstrated that sexual selection by females favours a high rate of displaying in males. The effect of predation on display rate, however, remains to be tested.

My first experiment demonstrated that differences in courtship behaviour between natural populations have a genetic component. It showed that courtship differences between populations may represent adaptations to different stream velocities. The higher rates of performing sigmoid displays exhibited by headstream males and their high ratio of sigmoid displays : thrusts may represent adaptations to faster-flowing water, allowing them to maintain an optimal ratio of sigmoid displays : thrusts. Headstream males were also found to be better able to maintain their courtship in faster-flowing water than lowland males. Thus differences in courtship behaviour between populations may not simply represent adaptation to the biotic selection pressures of predation and sexual selection.

My finding that different populations of a species vary genetically in their rates of performing behaviour patterns is not new. Within species genetic variation in courtship behaviour has previously been found to be mainly quantitative and likely polygenetic (see reviews by Bastock, 1967; and Broadhurst, Fulker, and Wilcock, 1974). How genes act to produce different levels of courtship in different populations remains unsolved. However, Bastock (1967) suggests that such differences are due to different threshold levels in the mechanisms controlling behaviour.

The above review shows that numerous factors have played a role in shaping each character of the guppy's present morphology and behaviour. My results, in particular, have shown that it is incorrect to concentrate on only biotic selection pressures when examining population differences in courtship behaviour. Interpopulation variability originally attributed to predation may in fact also be due to a combination of physical features such as stream velocity and turbidity.

To understand the nature of micro-evolutionary changes more fully we need eventually to assess the relative contributions of different selective pressures in shaping behaviour and morphology. This should be possible by continuing to study natural populations of the guppy. By performing long term experiments, and systematically varying stream velocity, turbidity, and predator conditions it may be possible to evaluate these selective agents' relative effects in shaping guppy morphology and behaviour.

In addition, using competition experiments similar to those in Experiment II, males from different populations with different characteristics could be tested to determine whether specific characteristics gain any mating advantages for individuals. Thus, the guppy should prove very useful in studying sexual selection not only using laboratory strains (as Farr (1980a) has done), but also using natural populations. The relative role of male size, colouration, aggressiveness, and rate of courtship display should be assessed, not only under one condition but under several different conditions. It is by examining natural populations under different conditions that the adaptive significance of various characteristics becomes more clear. Eventually a detailed understanding should be gained of the interaction of selection factors in shaping morphology and behaviour and of the adaptive significance of specific population characteristics. So far, my study, along with others, have indicated that specific characteristics of guppy morphology and behaviour are adapted to specific environmental factors. It remains now to test their relative importance under different conditions.

Adaptation of different populations to specific environments may eventually result in the formation of different species. This occurs when individuals from different populations no longer interbreed (Mayr, 1970). Although the populations of guppies I studied remain capable of interbreeding, it is interesting to note that some inter-population discrimination was demonstrated during my second experiment. The preferences of males for females from

their own population may represent a partial barrier to gene flow between populations. Sexual selection exercised by males (and/or females) may be maintaining differences between populations. Haskins and Haskins (1950) found a courtship preference by laboratory strains of males for females from their own strain and by wild-caught males for wild-caught females. My results, using different wild-caught populations, support their finding of inter-population discrimination.

One possible explanation for the preferential courtship exhibited by males during my experiment is that females may release different and identifiable pheromones.

Female guppies have been shown to release a sexual pheromone, especially during the time of their receptivity (Crow, 1976; Crow and Liley, 1979). The responses of males to sexual pheromones produced by headstream and lowland females have been studied by Snyder (1978). She found that males from each stock displayed to control females more when water which had previously held females from their own stock was added to their tank than when water from another stock was added. Thus, some form of olfactory discrimination may differentiate populations of females, and this may account for the observed mate preferences of males.

Continued study of the guppy may provide further insight into how isolating mechanisms develop. Differences in courtship behaviour between the guppy and three closely related species have been shown to function as ethological isolating

mechanisms (Haskins and Haskins, 1949; Liley, 1966). In this study, differences in courtship behaviour were found between populations of guppies and a degree of inter-population discrimination existed between the populations. It is adaptation to different environments, perhaps similar to those found in this study, that may eventually bring about behavioural isolation between populations.

In closing, I would like to stress that predictions about the roles of different selective factors and their effects on guppy behaviour should eventually be tested in field experiments. My study has made apparent the importance of examining fish in different stream velocity conditions. When populations are adapted to different stream velocities, unless they are examined in flowing water, the adaptive significance of their behaviour may be misinterpreted. My study showed that differences between guppy populations in courtship behaviour and stream depth preference likely represent adaptations to stream velocity. Until this study, these population differences had been considered only as adaptations to biotic selection pressures, such as predation.

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APPENDIX

Correlations between Male Size and Courtship Behaviour PatternsExperiment I

The mean total length for each population of males tested in Experiment I is given in Figure 15. Petite Curucaye males were slightly larger on average than Paria males and significantly ($p < .05$) larger than Caparo males (Mann-Whitney U-test, one tailed for $C < P$, $C < PC$, and $P < PC$). These differences are consistent with those found between wild-caught guppies collected from similar streams (see Seghers, 1973).

Seghers (1973) and Liley and Seghers (1975) showed that differences in the size of adult male guppies are partly genetic and partly a phenotypic response to environmental temperature. They suggest that size-selective predation may be involved in the evolution of inter-population differences in the size of mature fish. The data on male size presented here support the finding that size differences are partly genetic, since the differences were obtained between laboratory stocks raised under the same conditions.

To examine whether there is a relationship between male size and the performance of various courtship behaviour patterns I looked for correlations between male size and the frequency of gonopodial swings, thrusts, and sigmoid displays. This was done to determine whether, for example, larger

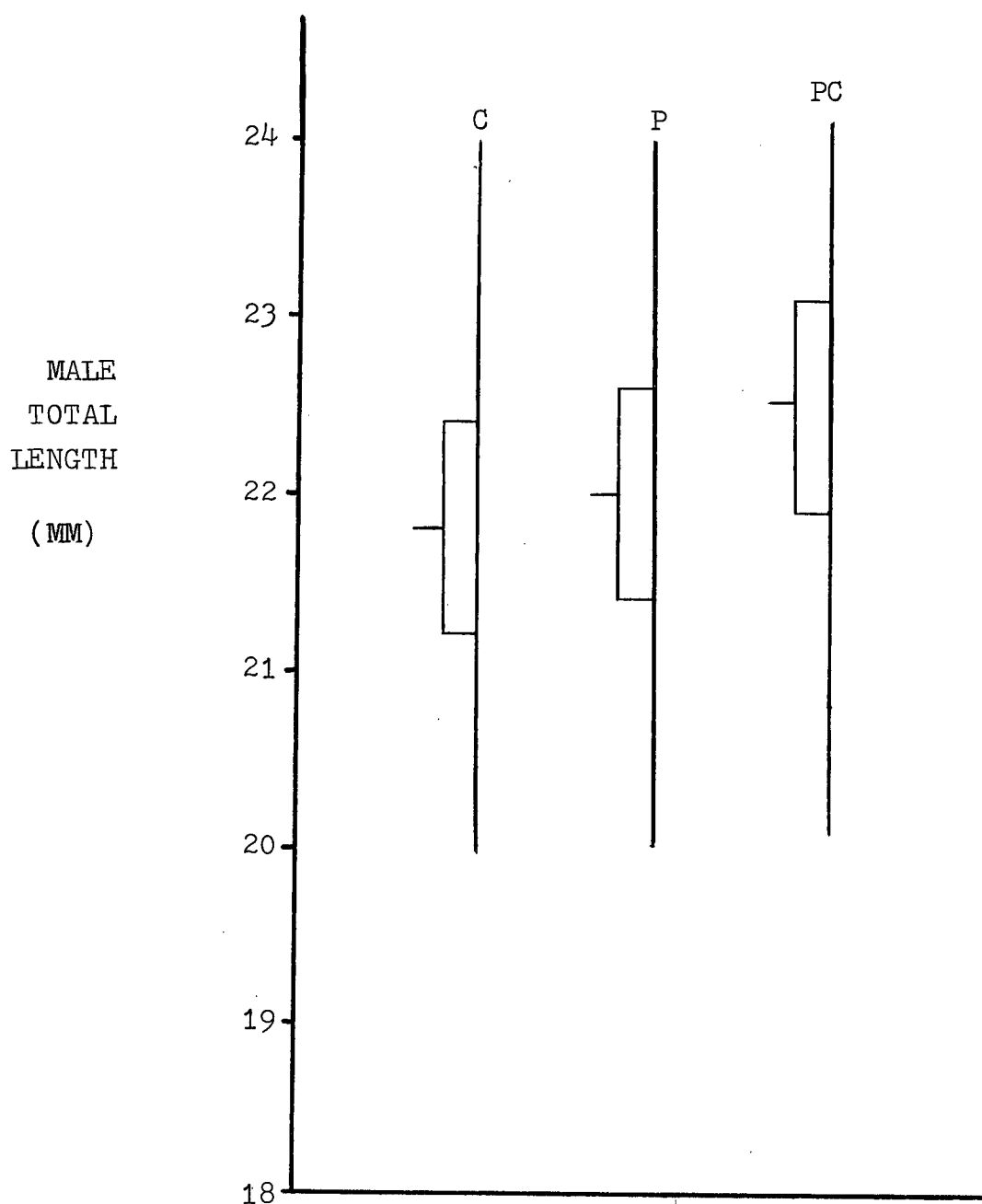


Figure 15. Total length of males tested in Experiment I. Horizontal lines are means, bars are 95% confidence intervals, and vertical lines are ranges.
C = Caparo (N = 19), P = Paria (N = 19),
PC = Petite Curucaye (N = 17).

males within a population perform more gonopodial swings, thrusts, and sigmoid displays. In addition, by comparing the correlations between these variables at the four stream velocities, I hoped to detect any effect of stream velocity on the correlations.

There were only two significant correlations between male size and the frequency of various behaviour patterns (Table XV). Two significant correlations in thirty-six comparisons would be expected by chance alone and I therefore attach no meaning to them. In addition, no effect of stream velocity on the correlations was detected.

Ballin (1973) also found no significant correlations between male size and courtship behaviour patterns. The size of individual males within a population does not appear to have a bearing on their courtship activity. Although the Paria and Caparo stocks did not vary significantly in male size, they differed significantly in their courtship behaviour (see Chapter IV). Thus, males of approximately the same size but from different populations can vary greatly in their courtship activity. The size of individual males

Experiment II

In Experiment II, wild-caught Upper Aripo males were significantly ($p < .01$) larger than Paria males, which in turn were significantly ($p < .001$) larger than both Caparo and Guayamare males (Fig. 16). The lowland stocks did not differ

Table XV. Spearman rank correlation coefficients between male size and courtship behaviour patterns and between various courtship behaviour patterns.
Experiment I.

Stream velocity (m/sec)												
.03			.07			.10			.20			
G.S.	S.D.	Th	G.S.	S.D.	Th	G.S.	S.D.	Th	G.S.	S.D.	Th	
Caparo												
Size	.228	.221	.156	.203	.167	-.081	.247	.202	-.018	-.017	.063	.111
Th	.050	.310		.395*	.054		.113	-.302		.206	.322	
S.D.	.281			.399*			.518*			.758*		
Petite Curucaye												
Size	-.223	-.296	.154	-.449	-.346	.362	-.125	.226	.654*	-.264	-.310	.139
Th	-.083	.332		.017	-.324		.059	.276		.278	.429*	
S.D.	.332			.312			.474*			.886*		
Paria												
Size	-.093	-.010	.224	.180	.064	.027	.061	.071	.578*	.043	.039	.079
Th	.345	.418*		.529*	.056		.518*	.352		.720*	.534*	
S.D.	.182			.316			.804*			.624*		

Size = Male Size, Th = Thrusts, S.D. = Sigmoid Displays, G.S. = Gonopodial Swings

*p < .05 Spearman rank correlation coefficients,
*p < .01 One-tailed test for positive correlations.

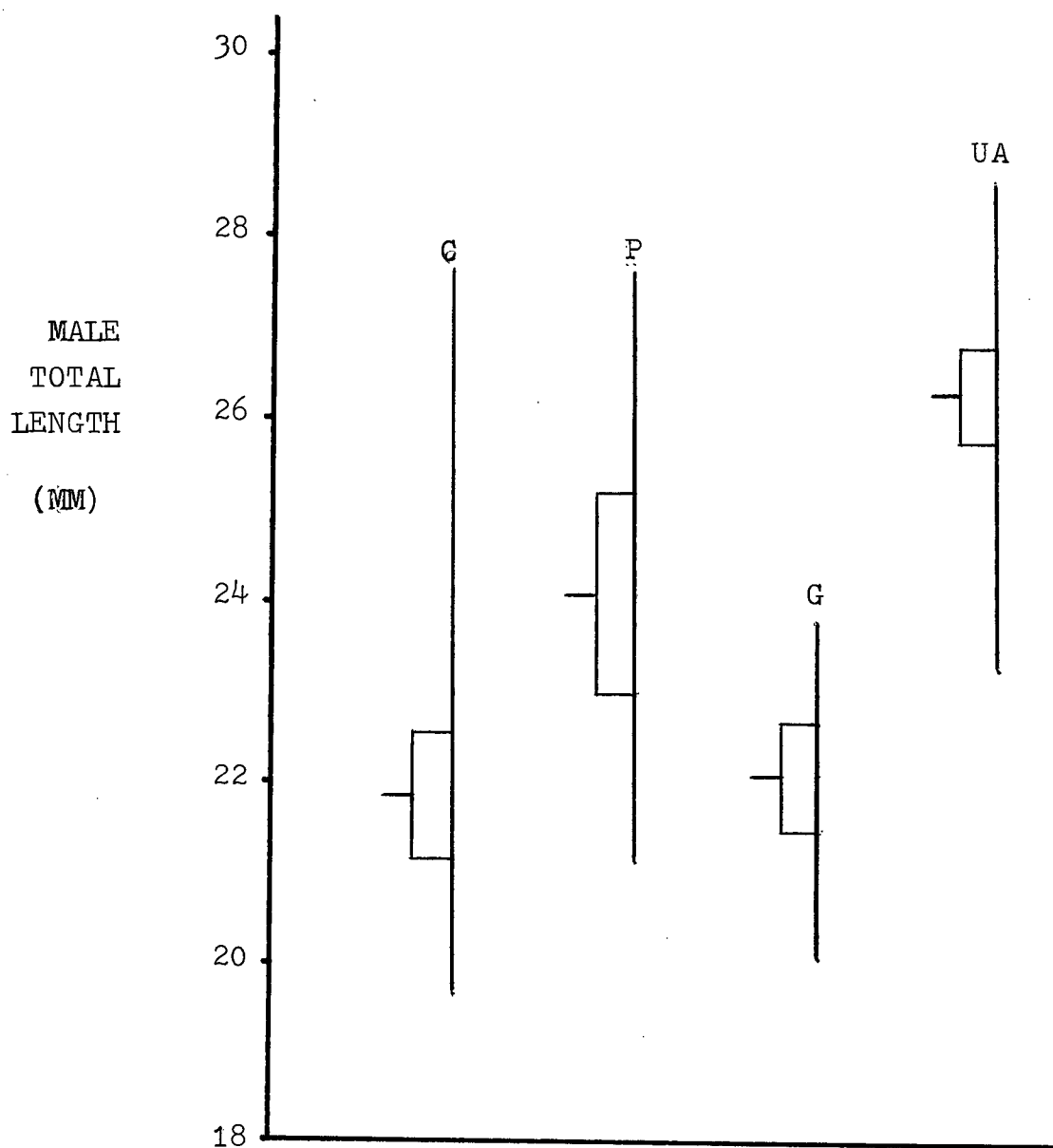


Figure 16. Total length of males tested for each stock. Horizontal lines are means, bars are 95% confidence intervals, and vertical lines are ranges.
 C = Caparo (N = 19), P = Paria (N = 20),
 G = Guayamare (N = 17), UA = Upper Aripo (N = 19).

significantly. (Mann-Whitney U-test, one-tailed for $U_A > P > G, C$; two-tailed for $G \neq C$). These differences in male size are consistent with differences previously found in wild-caught fish (Seghers, 1973).

There were no consistently significant correlations between male size and the frequency of gonopodial swings, thrusts, or sigmoid displays (Table XVI). (There was a significant correlation between male size and the frequency of thrusts performed by *Pañia* males in both stream conditions, however, this was not found in the other populations.) A lack of significant correlations between male size and male courtship behaviour patterns is consistent with the results obtained in Experiment I. The size of individual males does not appear to have a bearing on their courtship activity.

Correlations between Courtship Behaviour Patterns

Experiment I

To examine whether there is a relationship between various courtship behaviour patterns performed by males I looked for correlations between the frequencies of gonopodial swings, thrusts, and sigmoid displays performed by individual males. Table XV lists the correlation coefficients for each stock at the four stream velocities in which they were tested.

There were significant correlations between the frequencies of gonopodial swings and sigmoid displays for all three stocks in the two fastest stream velocities. There was also a consistently significant correlation between gonopodial swings

Table XVI. Spearman rank correlation coefficients between male size and courtship behaviour patterns and between various courtship behaviour patterns. Experiment II.

	Stream velocity (m/sec)					
	.00			.08		
	Gonopod Swings	Sigmoid Display	Thrusts	Gonopod Swings	Sigmoid Display	Thrusts
Caparo						
Male Size	.004	.525*	-.255	.064	.219	.188
Thrusts	-.025	-.554		.513*	.318	
Sigmoid Displays	.508*			.748*		
Paria						
Male Size	.050	.398*	-.442	.096	.433*	.103
Thrusts	-.461	-.335		.152	.396*	
Sigmoid Displays	.006			.596*		
Guayamare						
Male Size	.459*	-.314	-.396	.257	-.010	.106
Thrusts	-.002	.315	.315	.034	.365	
Sigmoid Displays	-.069			.626*		
Upper Aripo						
Male Size	.319	.340	-.223	.1188	.021	.000
Thrusts	.006	-.277		-.398	.026	
Sigmoid Displays	.180			.093		

Spearman rank correlation coefficients,
One-tailed test for positive correlations.

*p < .05, *p < .01

and thrusts performed by *Paria* males.

It appears that the relationship between gonopodial swings and sigmoid displays is affected by stream velocity. Although the correlations were not significant in the slower stream velocities (they were nearly significant at 0.07 m/sec), they generally became higher as stream velocity increased and were significant in the faster velocities.

I believe that these changes in the correlations between gonopodial swings and sigmoid displays in different stream velocities help to clarify the unknown function of gonopodial swings. I speculate that gonopodial swinging has a dual function and that each of these functions is seen to a greater extent depending on the fish's environment. In faster-flowing water, males that display frequently also gonopodial swing frequently and those that display infrequently rarely gonopodial swing. These results support the view that gonopodial swinging is related to sexual motivation, as suggested by Clark and Aronson (1951) and Liley (1966). Thus, gonopodial swinging may function as an "intention movement" or preparatory movement occurring before displaying. (For the *Paria* males it may also be similarly related to thrusting.) It is in still water that a second function becomes more apparent - that of being a type of "displacement activity". I found that gonopodial swinging was performed during non-courtship at a higher frequency when fish were in slow-flowing water. This "out-of-context" behaviour may represent a displacement activity, performed by males when they are slowly swimming about a tank. Males perhaps have less time or energy

to perform such displacement activities in fast-flowing water and the relationship between gonopodial swings and sexual motivation would become more evident in fast-flowing water. Admittedly these suggestions are speculative, however, they could explain the interesting affect of stream velocity on the correlations between gonopodial swings and sigmoid displays. They also could explain why Ballin (1973) did not find a correlation between gonopodial swings and sigmoid displays when he observed fish only in still water. In any case, stream velocity does appear to affect correlations between the two behaviour pattern.

Experiment II

In Experiment II, males from three of the four stocks showed a significant correlation between sigmoid displays and gonopodial swings in flowing water, as did Caparo males in still water (Table XVI). These results therefor support those of Experiment I. Stream velocity affects the correlations between these two behaviour patterns and perhaps this may be due to gonopodial swings exhibiting, to a greater and lesser degree, two different functions in each stream condition.