

C.1.
GEOGRAPHIC VARIATION IN COURTSHIP
BEHAVIOUR OF THE GUPPY,
POECILIA RETICULATA

by

PETER J. BALLIN

A.B., University of California, Berkeley, 1968

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the Department
of
Zoology

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

December, 1973

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study.

I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of ZOOLOGY

The University of British Columbia
Vancouver 8, Canada

Date 3 JAN 1974

ABSTRACT

This thesis attempts to elucidate some adaptive modifications of behaviour in response to environmental influences in geographically isolated populations of the guppy. Poecilia reticulata, Peters, I examined courtship behaviour of ♂ guppies from three Trinidad streams which differ in several physical and biotic parameters. Males of two headstream populations (the Paria and Upper Aripo Rivers) are larger and more brightly colored than ♂♂ of a lowland stream (the Guayamare River). Headstream ♂♂ are more conspicuous in their courtship than downstream ♂♂ in a one ♂-one ♀ encounter: they exhibit more display behaviour and move around the ♀ more. It was concluded that differences in behaviour are genetic.

I then investigated effects of ♂ interactions on courtship. Paria ♂♂ were much more aggressive than Upper Aripo or Guayamare ♂♂ upon encountering other ♂♂ of the same race. Increased aggressiveness in P ♂♂ occurred in the presence of ♀♀, suggesting that ♂♂ fight over ♀♀. Display behaviour was reduced (especially in P ♂♂, who fought) when two ♂♂ were introduced to one another and a ♀ after an isolation period. However, no decrease was noted without an isolation period. Display behaviours decreased with the addition of a third ♂ in the Paria and Upper Aripo races, but not in the Guayamare. Increasing the number of ♂♂ generally heightened the level of ♀-oriented activity and reduced the distance between ♂♂ and the ♀.

Preferences for fish of the same race occurred. When three ♂♂ , one of each race, were presented to a virgin ♀ simultaneously, Paria ♂♂ fought only in the presence of Paria ♀♀ , Upper Aripo displays were more successful in eliciting sexual responses, especially from Upper Aripo ♀♀, and Guayamare ♂♂ thrust more at Guayamare ♀♀ . Choice behaviour experiments revealed that virgin Paria and Guayamare ♀♀ respond selectively to ♂♂ of their own race. In another experiment, Upper Aripo ♀♀ responded much more readily to displays from Upper Aripo males. Females seem more likely to complete full sexual responses with ♂♂ of their own race.

It appears that relatively light predation and good visibility have resulted in the evolution of displaying and fighting as the primary mating strategies in headstreams. Heavy predation and poor visibility have resulted in selection for downstream ♂♂ which display less frequently and rely more heavily on tactile signals to insure insemination. A simple model is presented to suggest how behavioural differences evolved. The results are discussed in light of other studies on geographic variation in color and mating behaviour.

ACKNOWLEDGEMENTS

I thank Dr. N. R. Liley, my research supervisor, for introducing me to guppies, providing support and facilities, and critically reading the manuscript. I am also thankful to my research committee, Dr. I. E. Efford and Dr. J. D. McPhail, for comments and discussions.

I am especially grateful to Dr. M. Cullen, who as visiting professor at U.B.C., gave generously his advice, resourcefulness, and good cheer.

I express my appreciation to a number of people who kindly assisted me during this study:

To fellow students, especially Dr. D. L. Kramer and Dr. B. H. Seghers, for discussions and comradeship; Mr. W. P. Wishlow, for technical assistance and a perspective on sanity; Mr. C. Parkinson and Mr. A. Koppel for help with equipment; Ms. J. Meredith for critical reading of the manuscript; Mr. S. W. Borden, Ms. D. Lauriente, and Ms. R. Kardynal for the computer work; Ms. L. Duncan for measuring guppy photos; Ms. V. L. Lennox for drawing some figures and putting up with me most of the time; and Ms. P. Waldron for typing.

TABLE OF CONTENTS

v

	Page
Abstract.....	ii
Acknowledgements.....	iv
List of Tables.....	x
List of Figures.....	xii

Chapter

1.	Introduction.....	1
2.	The Environment and the Animals.....	4
	A. The environment.....	4
	1. Locations and isolation of the streams....	4
	2. Abiotic features.....	6
	3. Biotic features.....	6
	B. The fish.....	8
	1. Range.....	8
	2. Morphology.....	8
	3. Sex ratios.....	11
	4. Distribution of fish in the streams.....	13
	C. Summary.....	14
3.	Materials and Methods	15
	A. Maintenance.....	15
	B. Subjects.....	16
	C. Observational set-ups.....	17
	D. Recording and analyzing instruments.....	18
4.	A Comparison of Male Courtship Strategies.....	19

	Page
A. General introduction and behaviour patterns described.....	19
B. A descriptive analysis of the courtship of the three populations (Experiment 1).....	21
1. Introduction.....	21
2. Materials and Methods.....	21
3. Results.....	24
a. Orientation.....	24
b. Contact movements.....	29
c. Display.....	29
d. Correlations of behaviours.....	35
C. Threshold distances for courtship (Experiment 2)....	35
1. Introduction.....	35
2. Materials and Methods.....	36
3. Results.....	36
D. Competition in courtship I (Experiment 3).....	40
1. Introduction.....	40
2. Materials and Methods.....	40
3. Results.....	40
E. Pilot experiments on the role of male-male aggression during courtship (Experiments 4, 5, and 6).....	42
1. Introduction.....	42
2. Aggression and territory (Experiment 4).....	44
a. Introduction.....	44
b. Materials and Methods.....	44
c. Results.....	44

Chapter	Page
3. Aggression and females (Experiment 5).....	47
a. Introduction.....	47
b. Materials and Methods.....	47
c. Results.....	47
4. Aggression, females, and density (Experiment 6)...	49
a. Introduction.....	49
b. Materials and Methods.....	49
c. Results.....	49
F. Competition in courtship II (Experiment 7).....	50
1. Introduction.....	50
2. Materials and Methods.....	50
3. Results.....	52
G. Competition in courtship III (Experiment 8).....	59
1. Introduction.....	59
2. Materials and Methods.....	59
3. Results.....	60
a. Effects of the different numbers of males on the courtship performed.....	60
b. Contributions of different males to the total courtship.....	61
c. Interactions between the males.....	63
d. Comparison of the races.....	67
H. Summary of the male's courtship.....	70
1. The behaviour of single males.....	70
2. The behaviour of two or more males.....	70

Chapter	Page
5. Discussion of Male Courtship Strategies.....	74
6. The Consequences of Geographic Isolation.....	81
A. Introduction.....	81
B. Breeding between the races.....	81
C. Inter-population competition in courtship (Experiment 9).....	82
1. Introduction.....	82
2. Materials and Methods.....	83
3. Results.....	83
a. Male behaviours.....	83
b. Females as recipients of male behaviours....	87
c. Female behaviours.....	89
d. Male-female interaction.....	92
D. Female selectivity.....	94
E. Choice test 1 (Experiment 10).....	94
1. Introduction.....	96
2. Materials and Methods.....	96
3. Results.....	96
F. Choice test 2 (Experiment 11).....	99
1. Introduction.....	99
2. Materials and Methods.....	99
3. Results.....	101
G. Reactive distances of females (Experiment 12).....	101
1. Introduction.....	101
2. Materials and Methods.....	103

Chapter	Page
3. Results.....	103
H. Summary of the female's response.....	105
7. Discussion of Geographic Variation.....	106
A. Geographic variation in color.....	106
B. Geographic variation in mating behaviour.....	113
Literature Cited.....	118
Appendix	
1. Some Speculation and Findings about Black Markings of the Male Guppy.....	131
2. A Description of Guppy Behaviour Patterns Pertaining to Courtship.....	134

LIST OF TABLES

Table		Page
1a.	Temperature, pH, and DH variation; Trinidad, 1969.....	7
1b.	Stream dimensions, flow, and velocity; Trinidad, 1969....	7
1c.	Water turbidity and color, substrate composition, and amount of lighting; Trinidad.....	7
2.	Carotenoid pigment in wild fish collected in 1967.....	12
3.	Sex ratios in wild fish.....	12
4.	Standard lengths of fish observed in Experiment 1.....	23
5a.	Orientation distances.....	26
5b.	Occurrence of fish within one male fish-length (approx. 2 cm) of each other.....	26
6.	Angle of male long axis to long horizontal axis of female while orientating.....	27
7.	Display distances.....	32
8.	Differences in frequencies of courtship activities of individual males when tested alone with a female (Experiment 1) or in the presence of another male (Experiment 7).....	53
9.	Male charges in a competition situation of two males and one female.....	54
10.	Mean percentage of time in trial in which a male was within one male fish-length (approx 2 cm) of a female in one-male and two-male situations.....	55
11.	Occurrence of males within a 2 cm radius of a female in the two-male situation.....	56

Table		Page
12.	Correlation coefficients of courtship activities in a two-male, one-female situation.....	58
13.	Relative amount of display behaviour of first and second males on days 2 and 3.....	66
14.	Summary of morphological, behavioural, and environmental differences between the races.....	73
15.	Response indices (# glides/ # displays) for virgin females with males of different races in competition with one another.....	95

LIST OF FIGURES

Figure		Page
1.	Northern Trinidad.....	5
2.	Male guppies from the Paria, Upper Aripo, and Guayamare Rivers of Trinidad.....	9
3.	Sizes of wild-caught male guppies in Trinidad.....	10
4.	Three-way choice maze.....	17
5.	Relative positions recorded in Experiment 1.....	22
6.	Mean angles of orientation by males with respect to the horizontal plane of the female.....	28
7.	Occurrence of males in positions above, below, and in front of a female.....	30
8.	A comparison of mean number of bouts of sigmoid displays and mean percentage of time in the trial spent displaying by males of three races of guppies...	33
9.	Schematic representation of male displays in various positions relative to a female.....	34
10.	Aquarium set-up for Experiment 2, top view.....	37
11.	Mean times until first display and mean number of bouts of display at the shortest distance (0 cm) from the females.....	39
12.	Courtship of two males in the presence of one female, expressed as percentages of total courtship ($\sigma'1 + \sigma'2$) (a.) and time in trial (b.).....	41
13.	Courtship of two males with one female, expressed in seconds.....	43

Figure	Page
14. Number of charges by males in each of three observation periods in all-male aquaria.....	46
15. Mean number of male charges in: (1) 5-male groups, (2) the same 5-male groups into which 5 females were introduced, and (3) the same groups as in (2) from which 3 females were removed.....	48
16. Number of male charges in groups of 5 fish of one sex, and these same groups after 5 additional fish of either the same or opposite sex were introduced.....	51
17. Comparison of the courtship performed by different numbers of males with one female.....	62
18. Contributions of different males to total courtship on each day of the experiment.....	64
19. Comparison of the courtship performed by males of the three races on each of the days of the trials...	68
20. A simple model showing factors influencing male conspicuousness.....	75
21. Experimental set-up for Experiment 9.....	84
22. Comparison of the courtship performed by three males, one of each race, to single virgin females of all races.....	86
23. Comparison of the courtship performed by three males, one of each race, to virgin females of each race.....	88

Figure	Page
24. Relative positions of fish and thrusts received by virgin females from males of different races in competition with one another.....	90
25. Display behaviours received by virgin females of different races from males of different races in competition with one another.....	91
26. Responses of virgin females to males of different races.....	93
27. Experimental set-up for Experiment 10.....	97
28. Experimental set-up for Experiment 11.....	100
29. Preferences for males of different races by virgin and experienced females.....	102
30. Aquarium set-up for Experiment 12.....	104
31. Areas of the male guppy's lateral surface used to quantify black markings.....	133

Chapter 1. Introduction

Numerous studies of mating behaviour have been carried out at the species level, but little inquiry has been directed toward interpopulation differences. Indeed, very little effort has been expended to clarify the adaptive nature of animal variation in general (see Chapter 7). Since survival depends upon the ability of individuals to contribute to the subsequent generation, any given population must have evolved or be evolving the most efficient means to do so in its particular ecological situation. There are good reasons for believing that the local breeding population is the evolutionary unit of importance (e.g., Ehrlich and Raven, 1969), and therefore it would seem propitious to begin a search for behavioural differences at the population level.

If we find behavioural differences between populations of animals, can we then relate these differences to dissimilarities in the environment? If we can then ascertain that the selective agents in the environment influence genotypes associated with behavioural differences, we should gain some understanding of how behaviour evolves.

The guppy, Poecilia reticulata Peters, is an ideal animal with which to assess behavioural differences between natural populations. Few animals express such a high degree of morphological variability and occur in such a multitude of diverse environments. Especially striking are differences in color patterns of ♂♂ from different areas. These color patterns, not expressed in the larger, drably attired ♀♀, seem to be involved in intra-specific communication and appear to be important in mating (Baerends, Brower, and Waterbolk, 1955; Haskins et al., 1961; Liley, 1966). In

addition, ♂ guppies spend much of their day involved in courtship activities, and are easily observed. A complete qualitative and quantitative description of guppy mating behaviour by Liley (1966) is available as a baseline for comparison of natural populations.

The populations chosen for this study originate in three geographically isolated streams in Trinidad, where the guppy is native, and represent three of the most dissimilar demes with respect to morphology and ecology. Two of the populations originate in headstreams where the water is clear, predators relatively few, and ♂ guppies large and brightly colored. Physical factors remain fairly constant throughout the year in these two locations. However, color patterns of the ♂ guppies differ: those of the Paria River impress one as being orange-red and those of the Upper Aripo River appear bluish with iridescence and large ocellated black spots. The third population comes from a larger lowland river where the water is turbid, predators abundant, and ♂ guppies small and relatively dull in coloration. Here, in the Guayamare River, physical conditions such as turbidity, water flow, and temperature are more variable than in the headstream locations. A more extensive description of the fish and the environment is given in Chapter 2.

Haskins et al. (1961), in conjunction with an ecological study of guppies in Trinidad, found that lab-raised brighter (to humans) ♂♂ were more successful than "duller" ♂♂ in inseminating ♀♀. The study also indicated that the brighter guppies were more likely to suffer predation. The authors suggested that sexual selection was possibly pressuring for more conspicuous ♂ coloration, while predation was selecting for ♂

crypsis. In neither case was the behaviour of the fish taken into consideration. This is a major omission, as Seghers (1973) has shown with respect to antipredator behaviour and I hope to show with respect to courtship behaviour.

My first objective was to determine, through descriptive and experimental analyses, whether or not ♂ courtship behaviour does indeed differ from population to population. My second objective was to relate observed differences in courtship strategies to differences in the natural environment. Thirdly, I examined the success of different courtship strategies and the responsiveness of ♀♀ to various ♂♂ in an attempt to elucidate the effects of geographic isolation and sexual selection as mechanisms in the evolution of behaviour and ethological isolation.

Chapter 2. The Environment and the Animals

A. The environment

1. Locations and isolation of the streams

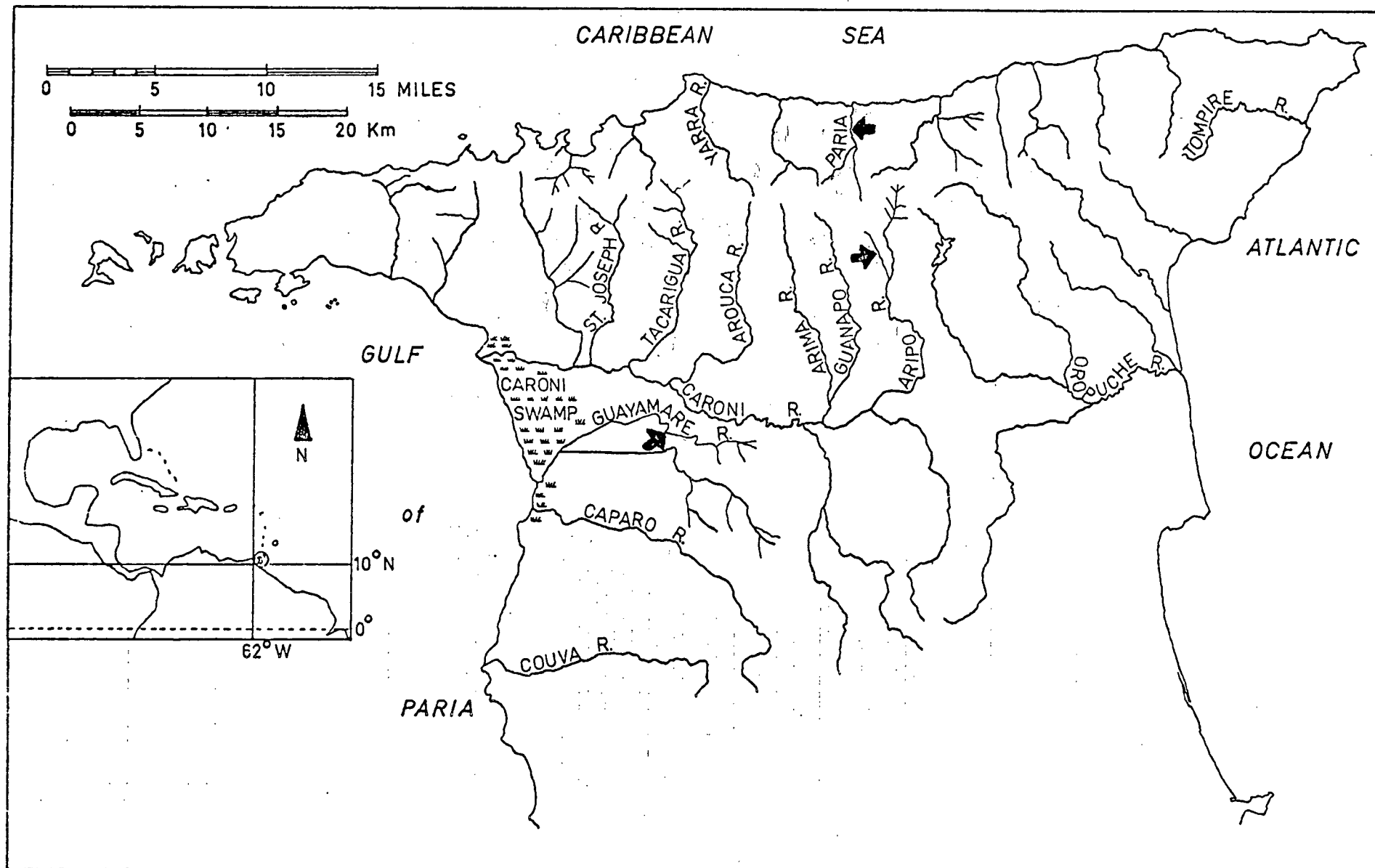
The three streams from which the fish originate--the Paria, Upper Aripo, and Guayamare Rivers--are shown on the accompanying map of north-west Trinidad (Fig. 1). The Paria and the Upper Aripo begin in the Northern Mountain range at altitudes of approximately 1000 m and course through relatively untractable jungle. The Guayamare is a lowland stream which flows through open country cultivated mostly with sugar cane.

A ten-meter waterfall at the mouth of the Paria and a five-meter waterfall between the Upper and Lower Aripo effectively separate these streams from any upstream movements of guppies. Thus, we can consider the guppy populations of these two streams to be in absolute geographic isolation. Guppies can be washed down stream, but the chance of any of the three populations under study mixing are slim. To reach the Caroni system, gene flow from the Paria would have to extend through many kilometers of ocean, where guppies are rarely, if ever, found. Gene flow from the Upper Aripo would involve about 20 kilometers of the Lower Aripo and the Caroni, a canal to the Guayamare, and then several kilometers downstream.¹

In addition, data from Haskins et al. (1961) indicate that guppies tend to adhere to localized groups, suggesting that a displaced fish might cease its wanderings upon encountering more guppies. Furthermore, Eaton

¹The existence of this canal was reported to Liley and Seghers but never observed by them.

Fig. 1. Northern Trinidad. Arrows indicate sources of populations studied. After Seghers (1973).



(1970) and Ehrlich and Raven (1969) suggest that reproduction by some migrants may not significantly affect the unique spectrum of genotypes in a population. Therefore, I believe that we may safely refer to the three populations as being geographically isolated.

2. Abiotic features

Some measurements of chemical and physical parameters of the three streams may be found in Tables 1a, b, and c. Note that the lowland river, the Guayamare, may be distinguished from the other two streams by its higher temperature, lack of shading, greater depth, larger volume of flow, and, perhaps most important to courtship, its turbidity.

3. Biotic features

Vegetation is absent except during flood waters in the Guayamare, when plants along its banks are submerged. The guppies seem to feed principally on invertebrate drift. Most important for this study is what feeds upon guppies.

The ubiquitous cyprinodontid Rivulus hartii has circumvented the waterfall barriers to the isolated headstreams, joining an otherwise impoverished fish fauna. This fish is moderately abundant in the Upper Aripo and relatively scarce in the Paria. As Seghers (1973) has demonstrated, Rivulus is an important predator mostly upon immature and young ♂ guppies. Potential aerial predators in the form of kingfishers (Chloroceryle spp.) have been sighted along the Paria. The predator situation in the Guayamare is quite different. Here, among an abundance of large fishes, the characid Hoplias malabaricus and the cichlid

Table 1a. Temperature, pH, and DH variation, Trinidad, 1969.

Stream	Dates	N	Temp. mean and range	pH mean and range	DH(ppm) mean and range
Guayamare	16/3-30/6	9	29.1 27-31	7.0 6.8-7.1	108.6 70-130
Upper Aripo	29/3-3/7	3	24.6 24.5-24.8	7.5 7.4-7.6	183.3 150-210
Paria	18/4-29/6	4	25.4 25-26.5	7.0 7.0-7.1	96.7 90-100

Table 1b. Stream dimensions, flow, and velocity; Trinidad, 1969.

Stream	Date	Width mean & range(m)	Depth mean & range(m)	Volume of flow (m ³ /sec)	Velocity (m/sec)
Guayamare	30/6	2.00	1.50	1,080	.400
Upper Aripo	no data.....similar to Paria				
Paria	18/4	3.50	.17	.0779	.168
	8/29/6	3-4	.14-.20	.0729-.0828	.163-.172

Table 1c. Water turbidity and color, substrate composition, and amount of lighting; Trinidad.

Stream	Turbidity	Color	Substrate	Lighting (Cover)
Guayamare	turbid-- always, but variable	brown	mud and silt	no cover to occasional sugar canes along shore
Upper Aripo	clear	white	rocky, silt in pools	75% cover * of low bush- es--medium to dense shade with only a few spots exposed
Paria	clear	lightly stained	rocky	50% cover of low bushes --medium shade

*% of cover indicates how much sky is obscured by vegetation,
after Seghers, 1973

Crenicichla alta are the most significant guppy predators (Seghers, 1973).

A more detailed treatment of guppy environments may be found in Seghers (1973). Haskins et al. (1961) give a general account of guppy ecology.

B. The fish

1. Range

The original distribution of Poecilia reticulata is fresh water of the island of Trinidad and the northeastern corner of South America, of which Trinidad is faunistically a part. Because of its remarkable adaptability, the guppy has been introduced into tropical waters all over the world to aid in mosquito control.

2. Morphology

♀ guppies from the three populations are virtually indistinguishable, although after practice, I can discern Paria ♀♀ from the other two types by their deeper bodies and darker shading. In marked contrast, the colorful, smaller ♂♂ differ greatly and are easy to assign to their home streams.

In the clear headwaters of the Paria and Upper Aripo, the ♂♂ are relatively large and conspicuously colored. Compare them with the smaller and much less conspicuous Guayamare ♂♂ (Figs. 2 and 3). Liley (unpubl.) has shown a genetic basis for these size differences. Note the presence of large areas of orange-red in the Paria fish. Seghers has compared the percentage of the body surface covered by carotenoid pigments in several races and has quantitatively confirmed this impression (Table 2; Liley and Seghers, unpubl). Furthermore, the quality of the carotenoids seems

Fig. 2. Male guppies from the Paria, Upper Aripo, and Guayamare Rivers of Trinidad. (Ektachrome: N.R. Liley).

Guaymare



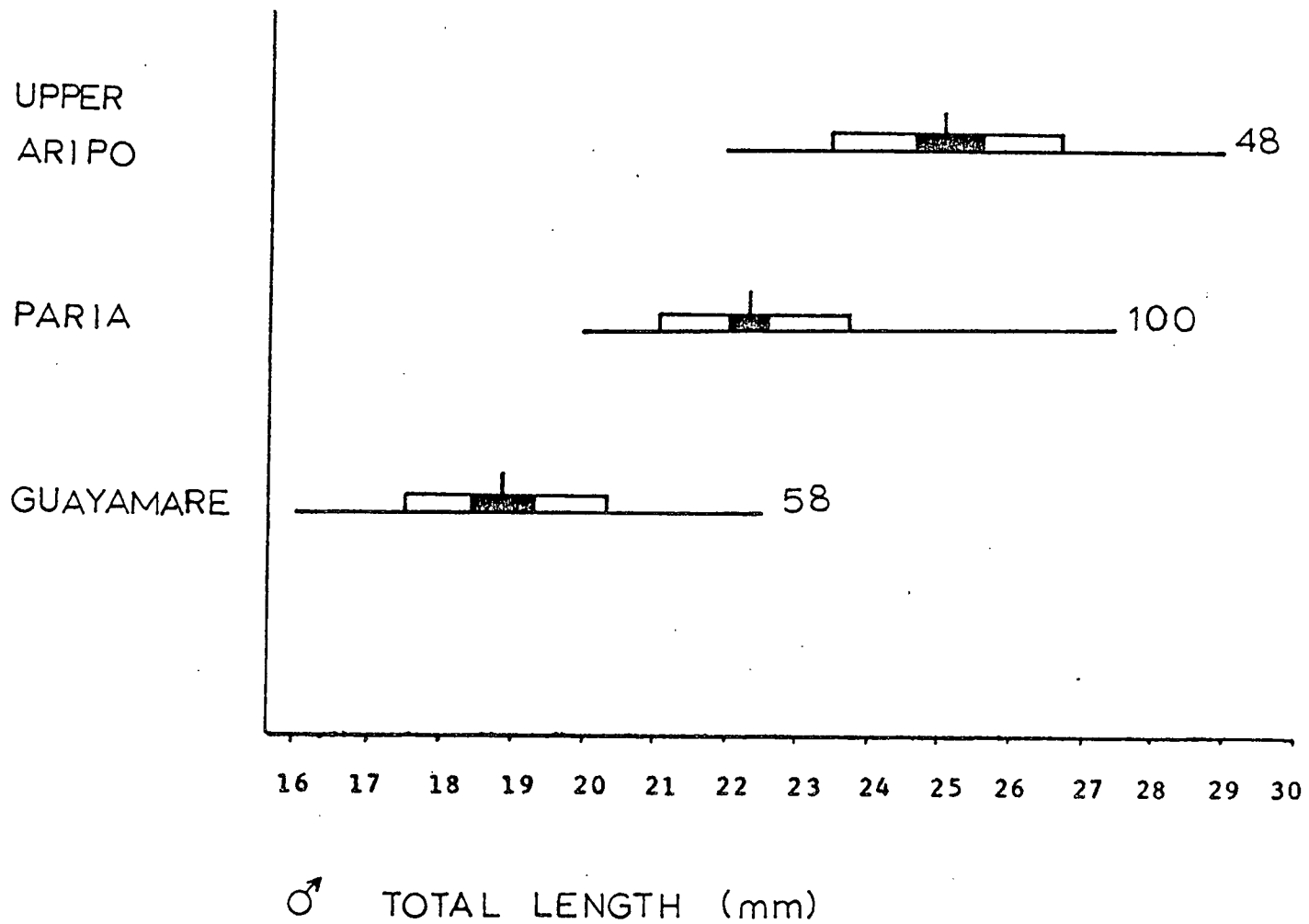
U. ARIPO (LAB)



Paria (Lab)



Fig. 3. Sizes of wild-caught ♂ guppies in Trinidad. The vertical line is the mean, the shaded bar ± 2 S.E., the empty bar ± 1 S.D., the horizontal line the range, and the number indicates the sample size. From Seghers, 1973.



different from those of the other races. Turning to other colors, Upper Aripo fish appear bluish, containing guanidine pigment, and exhibit iridescence and large black spots, often circled with iridescent white. The Guayamare fish express a great variety of colors, but they are subdued. Furthermore, they lack the markings on the body and tail which make the headwater fish conspicuous to the human and, presumably, to the piscine eye. An analysis of the amount of black markings and their possible significance was undertaken and is reported in Appendix I.

Two correlations should be considered: (1) the more conspicuous ♂♂ occur in clear water and the less conspicuous ♂ occurs in turbid water; (2) the larger ♂♂ occur with a small predator (Rivulus in the Upper Aripo and Paria) and the smaller ♂ occurs with large predators (Crenichthys and Hoplias in the Guayamare). Regarding the causal basis of the second correlation, Seghers (1973) has observed that large guppies give Rivulus handling difficulties and ♂♂ are eaten more readily than ♀♀; whereas small guppies appear to be more effective in evading large predators and ♀♀ are at no advantage.

3. Sex ratios

Seghers (1973) has found that unbalanced guppy sex ratios coincide with the abundance of Rivulus. Thus we find that ♀♀ outnumber ♂♂ by 2 to 1 in the Upper Aripo, while the other two streams have more nearly balanced sex ratios (Table 3). Laboratory reared fish from all populations exhibit a 1:1 sex ratio. Seghers presents convincing evidence that

Table 2. Carotenoid pigment in wild fish collected in 1967.
Expressed as the percentage of surface area covered
on the left side of the body. From Seghers, 1973.

<u>Stream</u>	<u>N</u>	<u>Avg</u>	<u>SD</u>	<u>Range</u>
Paria	33	28.9	11.3	10.4-50.7
Upper Aripo	40	12.4	7.7	1.4-33.8
Guayamare	50	9.4	4.1	1.4-20.8

Table 3. Sex ratios in wild fish. From Seghers, 1973.

<u>Stream</u>	<u>♂♂</u>	<u>♀♀</u>	<u>Immatures</u>	<u>N</u>	<u>♂/♀</u>
Paria	292	256	212	760	1.14
Upper Aripo	252	528	565	1345	.48
Guayamare	110	120	85	315	.92

Rivulus is indeed the cause of this imbalance. Male guppies were less adept at avoiding capture than ♀♀ and were at a disadvantage in size-selective predation.

4. Distribution of fish in the streams

Observations by Seghers indicate that guppies tend to inhabit pools and back-eddies where water velocities are nil or very close to it. Field and laboratory observations by Seghers show that Guayamare fish swim near the water surface and close to shore, while the headstream fish swim near the substrate and show no preference of position in relation to shore. Positions taken by guppies in the Guayamare seem to correspond to where the predators are not.

The degree of schooling behaviour shown by the guppies corresponds to the degree of predation upon them. Thus, we find schooling behaviour well developed in fish from the Guayamare, poorly developed in the Upper Aripo, and absent in the Paria (Seghers, 1973).

These differences in positional preferences and schooling behaviour persist in laboratory-reared stocks. Seghers and I therefore, believe that these differences reflect genetic differences which result from selection due to predation.

C. Summary

1. Three geographically isolated populations of guppies originate from three ecologically different streams in Trinidad.
2. The populations with larger, more conspicuous ♂♂ frequent clear headstreams of the Paria and Upper Aripo Rivers, which contain only a small guppy predator. The smaller, less conspicuous ♂ occurs in a turbid lowland stream, the Guayamare, in which several large predators occur.
3. The predator Rivulus is probably responsible for an unbalanced sex ratio (1♂:2 ♀♀) in the Upper Aripo River. Sex ratios are nearly balanced in the other two streams.
4. Differences in positions in streams seem to have resulted from selection by predators.
5. The degree of schooling expressed by guppies corresponds to the degree of predation upon them.
6. Difference in coloration, size, and behaviour persist in laboratory populations. This implies that the differences have a genetic basis.

Chapter 3. Materials and Methods

A. Maintenance

Fish from the Paria, Upper Aripo, and Guayamare Rivers (the fish populations will hereafter be referred to as P, UA, and G, respectively) were housed in physical and visual isolation from one another in 43 liter (51.5 cm x 27 cm x 31 cm deep) and 61 liter (62 cm x 32 cm x 31 cm deep) aquaria. Similar conditions prevailed in all the stock aquaria, as follows:

Photoperiod: 12L : 12D

Lighting: cool white 40 or 30-watt fluorescent bulbs suspended 15 cm above the water

Substrate: medium gravel

Vegetation: all possible combinations of Vallisneria, Ceratopteris, and Lemna.

Water: one half to one-third of each aquarium was changed regularly with Vancouver city water and local well water

pH: approx 6.0

Filtration: outside filters of charcoal and fiberglass wool. Some aquaria used sub-gravel filters, but these were replaced due to a tendency for the water to become acid.

Food: twice daily on Tetra-Min, trout chow, frozen brine shrimp, and chopped Tubifex worms

Temperature: during the first part of this study, fish were housed in four separate rooms. Temperature was controlled by heater-thermostats at about 25 C in three of the rooms: the fourth room was supposedly under thermostatic control, but in fact varied from 24 to 31 C. Fish were moved to new facilities in October, 1970, where the controlled temperature room kept the water at $25 \pm .5$ C.

Density: an attempt was made to keep aquaria at approximately equal densities by non-selectively removing immature fish to large troughs. G fish seemed to multiply more quickly than either of the other races.

In addition, aquaria with the most plants usually contained the most young. An "average" 43 l aquarium held about 50 fish, of which slightly more than half were immature.

B. Subjects

Fish were collected in Trinidad in 1967 (Liley and Seghers) and 1969 (Seghers). See Seghers (1973) for collecting techniques. Wild-caught to sixth generation laboratory-bred animals were observed in experiments. All fish observed, except virgin ♀♀ were from the stock aquaria. Care was exercised to select fish from as many aquaria as possible to minimize possible inbreeding and drift effects. With the exception of the seven-month period preceding Experiment 1, fish of the same race were occasionally interchanged between aquaria.

Virgin ♀♀ were obtained by allowing about a dozen pregnant ♀♀ to drop young in a well-planted 43 l aquarium. The mothers were removed when sufficient numbers of young appeared, usually in a month's time. Males were removed when their sex became apparent.

Before all behavioural recordings, fish were given some time to become accustomed to the test situation. Fish which did not remain calm were left until they became so, or else finally rejected. "Calm" is defined as swimming about in a relaxed fashion, not remaining motionless with rapidly beating pectoral fins, and not swimming energetically at the sides of the aquarium. White-cloud Mountain fish, Tanichthys albonubes Lin, were utilized as "dither fish" (Barlow, 1968a) to calm guppies in several experiments.

Only mature fish were used in experiments. Fish were never fed prior to observation sessions on a given day.

At the conclusion of experiments 1, 3, 7, and 8 fish were anesthetized in MS 222 and were either photographed for later measurement or measured with calipers. Males not photographed were sketched in stenciled outlines to maintain a record of markings. All fish were returned to stock aquaria.

c. Observational set-ups

Fish were observed in three types of container. None contained vegetation. Food, water conditions, temperature, and photoperiod were the same as in the stock aquaria. Observation containers always included some water in which fish had lived.

1. Nine aquaria, hereafter referred to as the observation aquaria, were employed in several experiments. Each had a volume of 23 l and dimensions of 41 cm x 21.5 cm x 26 cm deep.
2. Another aquarium, hereafter referred to as the long aquarium, was used for several tests. The volume was 22-l and the dimensions were 61.5 cm x 17 cm x 21 cm deep.
3. A three-way choice maze was constructed by Mr. Colin Parkinson of the Department of Zoology workshop. Specifications:

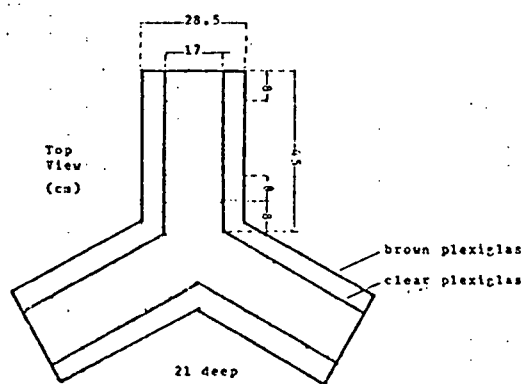


Fig. 4. Three-way Choice Maze

The maze was lighted by four 40-watt fluorescent bulbs 206 cm above the water and one 100-watt incandescent bulb 82 cm above the water

4. I observed fish in the observation aquaria and the long aquarium by sitting quietly between 40 and 70 cm in front of them. Fish in the maze were observed from a distance of approximately 130 cm above and to one side of the apparatus.

D. Recording and analyzing instruments

The major equipment employed is listed here and referred to in the experimental sections.

I photographed fish for measurement with a Pentax Spotmatic 35 mm camera and Kodak High Speed Ektachrome Type B film.

An Olympus 35 mm camera, especially outfitted for bulk-loading and timed half-frame exposures photographed fish on Kodak Tri-X Pan film in experiments 1 and 7.

A Vanguard Motion Analyzer was used to analyze film obtained in experiments 1 and 7. Measurements were punched directly onto perforator tape which was decoded in an IBM 1130 computer.

I built a keyboard which transferred my observations to a computerized data acquisition terminal (hereafter referred to as CDAT), built by Mr. Stephen Borden of the Bio-Science Data Centre. As I observed fish, I pressed buttons corresponding to their behaviour patterns and positions. The machine received this information and punched it on perforator tape, which was later decoded by the IBM 1130. Frequencies, durations, intervals, and sequences of activities were then available for print-outs and statistical analyses.

A four-channel Rustrak event recorder

A Phillips cassette tape recorder

Chapter 4. A Comparison of Male Courtship Strategies

A. General Introduction and Behaviour Patterns Described

My initial goal was to determine whether or not population differences in ♂ patterns and coloration have any significance in courtship. It soon became apparent that differences in behaviour were more important features to observe. Color patterns have probably evolved to enhance the signal value of behaviour patterns rather than the converse.

When one gazes into an aquarium of guppies, one cannot help but be impressed by the seemingly incessant attention given to ♀♀ by ♂♂. For convenience, we may divide the ♂'s courtship activities into three categories: orientation, contact movements, and display (Liley, 1966).

Orientation is simply maintaining visual and spatial bearings on the ♀, usually facing her. Contact movements: a ♂ may thrust his gonopodium at the genital pore of a ♀, but this rarely inseminates her. From time to time a ♂ displays to a ♀. When this sigmoid display (Baerends et al. 1955) is performed to a receptive ♀, it may elicit a sexual response resulting in copulation, the normal mode of sperm transfer. However, since a ♀ only becomes receptive at approximately three-week intervals (Liley, 1966: 43), most of the ♂'s displays just lead to more ♂ courtship. The display, which lasts about two seconds, is highly ritualized and is emphasized by changing colour and marking patterns, spreading median fins, quivering, and rocking to and fro. Its effectiveness as a signal is presumably thereby enhanced. Of all the ♂'s courtship activities, the

display most often leads to copulation.

A number of studies of guppy reproductive behaviour have been reported in the literature (see Liley, 1966: 26-28). Sexual communication appears to be largely visual, although Amouriq (1964, 1965a, b, and 1967) and Gandolfi (1969) report the presence of olfactory cues. Liley (1969) finds difficulty in attaching importance to a chemical sex attractant in guppies. For a detailed description and analysis of guppy courtship refer to Liley (1966), and Appendix 2).

The accompanying list of ♂ behaviour patterns recorded in my experiments follows Liley (1966) except where noted.

Orientating: includes Following, Watching, Circling, Retreating, and Weak sigmoids

Gonopodial swing: (Occasionally performed when the ♂ was not courting).

Sigmoid display: only the fully developed display was recorded. The adjectives frontal and general were dropped in favor of locating the displays by denoting them as "in front" if they were performed within a twenty-degree angle extending horizontally forward from a point between the ♀'s eyes, or "not in front". The objection to "frontal" is that the term is used to denote a type of display in many fishes and not its location.

Leap

Thrust

Copulation attempt and Copulation

Jerk

Snout contact with genital pore of ♀: this behaviour did not occur in the fish Liley studied, even though he looked for it. It did occur infrequently in the populations observed here.

Chase: a ♂ initiates a fleeing response by the ♀, usually by contact with her genital region, and pursues her.

Non-courtship behaviour associated with courtship occurring between ♂♂:

Sparring

Tail-beating

Charge: aggression which includes bites at another fish, attempted bites, and chasing culminating in attempted bites. Few, if any, bites actually made contact.

B. A descriptive analysis of the courtship of the three populations (Experiment 1)

1. Introduction

The purpose of this set of observations was to determine whether there are differences in courtship between ♂♂ of the Paria, Upper Aripo, and Guayamare Rivers, and to describe the nature of these differences.

2. Materials and Methods

A ♀ and a ♂ from one race which had been residing in separate aquaria were introduced, at about 0900 hours, into one side of an observation aquarium which had been divided in half (20 cm x 21.5 x 25 cm deep). At about 1700 hours a brown, opaque plexiglas partition was placed between the two fish, then removed at about 0900 hours the following morning. Observations commenced if both fish were calm. If the fish were not calm, they were left together until 1700 hours, when they were separated until the next morning. This procedure was continued until the fish met the behavioural criteria, which was usually in two or three days. A few fish never calmed sufficiently and were not used. Of 18 independent trials attempted for each race, 13 P, 16 UA, and 17 G were

completed. The standard lengths of the fish observed are found in Table 4.

Each observation period lasted 15 minutes and all trials were recorded between 0900 and 1300 hours (lights on at 0800 hrs.).

All of the courtship behaviour listed in Chapter 4.A., along with information on the relative position of the ♂ to the ♀ was recorded with CDAT. Relative positions recorded were as follows: A ♂ moved, above or below a ♀ from the level position.

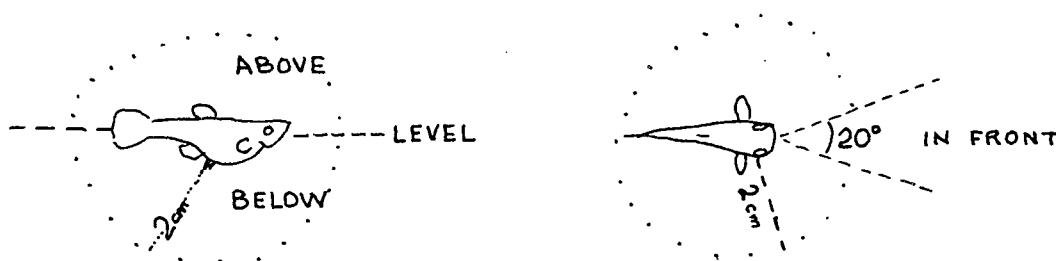


Fig. 5 Relative positions recorded in Experiment 1.

Occurrence of a ♂ within a 2 cm radius around the ♀ was also noted. Two cm represents approximately one ♂ fish length and was thus easily estimated. Every 15 seconds for the duration of a trial, the Olympus camera photographed the observation chamber and an overhead mirror, which allowed a view from the top.

Measurements of orientation and display distances and angles were made on the motion analyzer. Unfortunately the camera failed repeatedly so that only about one-half the trials were photographed.

The rationale for measuring spatial relationships and amount of ♂ movement to various positions relative to the ♀ is as follows. Males

Table 4. Standard lengths of fish observed in Experiment 1.

Race	♂♂ mean (mm)	Range	♀♀ mean (mm)	Range
P	16.40	14.50-17.75	24.90	23.00-27.00
UA	16.40	15.25-19.25	25.30	22.50-29.00
G	15.75	13.75-19.75	26.18	22.50-30.25

who changed position more often would presumably catch the eye of a predator and a ♀ more readily than ♂♂ who changed position less. Greater distances from the ♀ might place ♂♂ over deeper water where predators might lie in wait. Since the environments of the populations differ in both number of predators and visibility, I thought that the ♂♂ might exhibit adaptive differences in their utilization of space.

3. Results

Data was first analyzed parametrically (one-way analysis of variance), but Bartlett's test indicated that the variances of most of the behavioural measures were probably not homogeneous. The non-parametric Kruskal-Wallis one-way analysis of variance was therefore employed to compare the populations. On the photographic data, a parametric one-way analysis of variance was justified and used. Comparisons of pairs of races was done by Kruskal-Wallis simultaneous probabilities in the one case, and Scheffe's test for multiple comparisons with unequal sample size in the other case.

a. Orientation

1) Time spent orientating

The three races differed in percentage of time in the trial spent orientating and the length of each bout of orientation. Fewer displays by G ♂♂ (see part c) resulted in more and longer orientation periods. G ♂♂ oriented to the ♀♀ 93% of the total trial time, while P ♂♂ did so 82% and UA ♂♂, 85% of the time ($p = .04^2$). The mean bout lengths of orientation were: G, 51.1 seconds; P, 30.8 seconds, UA, 36.2 seconds

²All probability values arise from the Kruskal-Wallis one-way analysis of variance unless otherwise stated.

($p = .003$). Time and number of times not attending to the ♀ did not differ between the races.

2) Distance of ♂ from ♀ during orientation

Photographic analysis revealed that the mean distance of the ♂ from the ♀ during orientation was significantly less in the UA than in the P and G races ($p < .001$, parametric one-way analysis of variance; Table 5a). However, a different impression was gained from less precise data collected on the data acquisition system (though the source of this difference is not clear). Here the presence of a ♂ within one ♂ fish-length to a ♀ was scored. The results (Table 5b) show P ♂♂ came within a fish-length of a ♀ significantly less often than UA or G ♂♂ ($p = .04$) and spent less time there ($p = .001$). Once G ♂♂ moved within their length of a ♀, they remained there longer than P or UA ♂♂ ($p = .0002$).

3) Orientation angles of ♂ with respect to ♀

The angles at which ♂♂ oriented to ♀♀ were measured from the film. An uncanny consistency of ♂ long axis direction in relation to the long axis in the horizontal plane of the ♀ was recorded: 90° on either side of her in all races (Table 6).

Angles above and below the horizontal plane of the ♀ showed great variation (Fig. 6). P ♂♂ averaged slightly greater angles below the ♀♀ than did UA ♂♂ who slightly exceeded G ♂♂.

4) Changes in ♂ position with respect to ♀

As stated earlier, ♂♂ spent most of their time orientating, often changing position relative to the ♀. When movement of the ♂♂ to positions above and below the plane of the ♀♀ are examined, racial differences

Table 5a. Orientation distances .

Race	P	UA	G
Number of fish	7	9	5
Number of measurements	65	71	50
Mean distance (cm)	4.5	3.4	4.9
S.D. (cm)	2.6	1.9	2.3
Range (cm)	1.5-13.4	1.0-14.6	1.4-11.1

One-way analysis of variance, $p < .001$.

Table 5b. Occurrence of fish within one ♂ fish-length (approx. 2 cm) of each other.

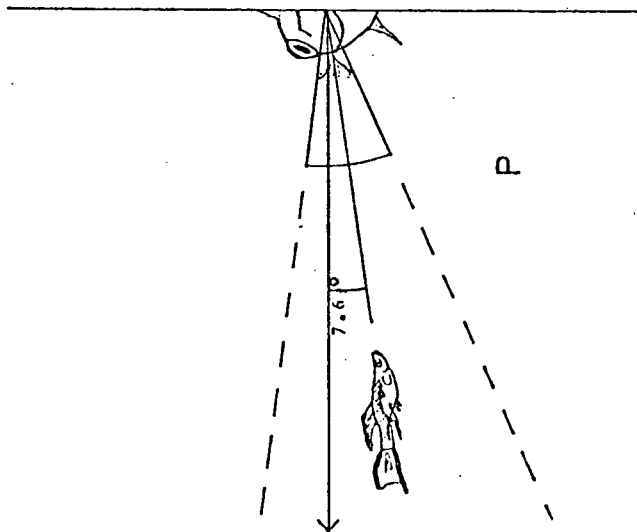
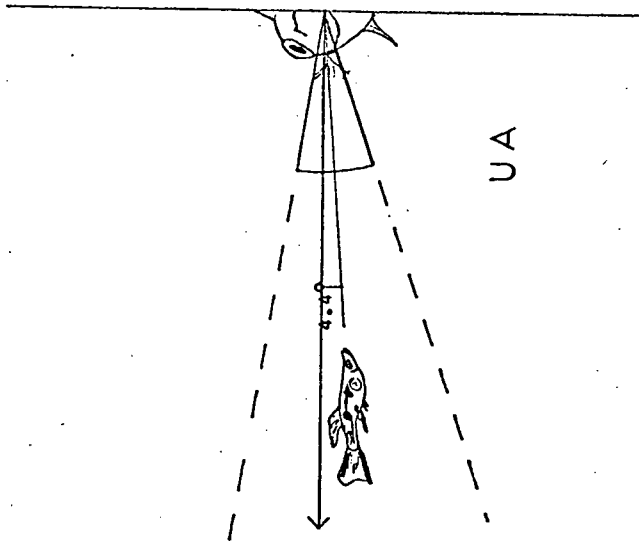
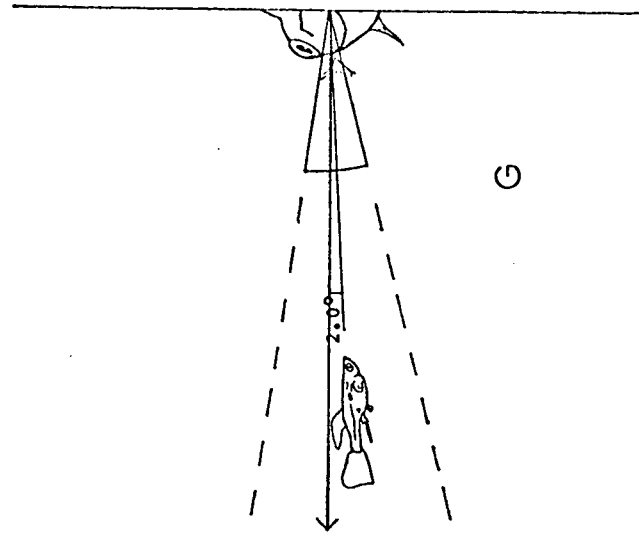
Race	P	UA	G	Kruskal-Wallis one-way analysis of variance
n	13	16	17	
Mean number of bouts	20.6	36.6	34.1	$p = .04$
Mean percentage of time	5.28	12.71	19.34	$p = .001$
Mean bout length (sec)	2.12	2.77	5.61	$p = .0002$

Table 6. Angle of ♂ long axis to long horizontal axis of ♀ while orientating.

Race	No. of fish	No. of Measures	Mean angle (right or left)	S.D.
P	7	65	91.0	10.1
UA	9	71	89.5	2.9
C	5	50	90.1	1.6

One-way analysis of variance, $p \gg .1$.

Fig. 6. Mean angles of orientation by $\sigma\sigma'$ with respect to the horizontal plane of the ♀. Angles were the same on both the left and right sides of the ♀♀. The wedges closest to the ♀ represent standard deviations around the mean. One-way analysis of variance indicated that $p > .1$ that the differences arose by chance.



become apparent: (Fig. 7a). G ♂♂ moved to positions below the ♀ less often ($p = .04$) and remained there for the smallest percentage of time ($p = .1$). P ♂♂ tended to spend a greater percentage of time both above and below the ♀ than other ♂♂ (Fig. 7b). Besides moving above the ♀♀ less often and for less time ($p = .01$), UA ♂♂' mean bout length in that position was also shorter ($p = .03$). Mean bout length below the ♀ did not differ between the races (Fig. 7c). The times above and below do not total 100% because ♂♂ were often level with the ♀; i.e., some part of the ♂'s body lay within two horizontal planes extending from the ♀'s most dorsal and most ventral body parts (Fig. 5).

The probabilities of differences between the races in being in a position within a 20° horizontal angle directly in front of the ♀ is slight (Fig. 7a, b, c).

Overall there was an indication that P and UA ♂♂ moved around the ♀ more than G ♂♂ while orientating (mean position changes in trial: P, 69.5; UA, 68.6; G, 63.1).

b. Contact movements

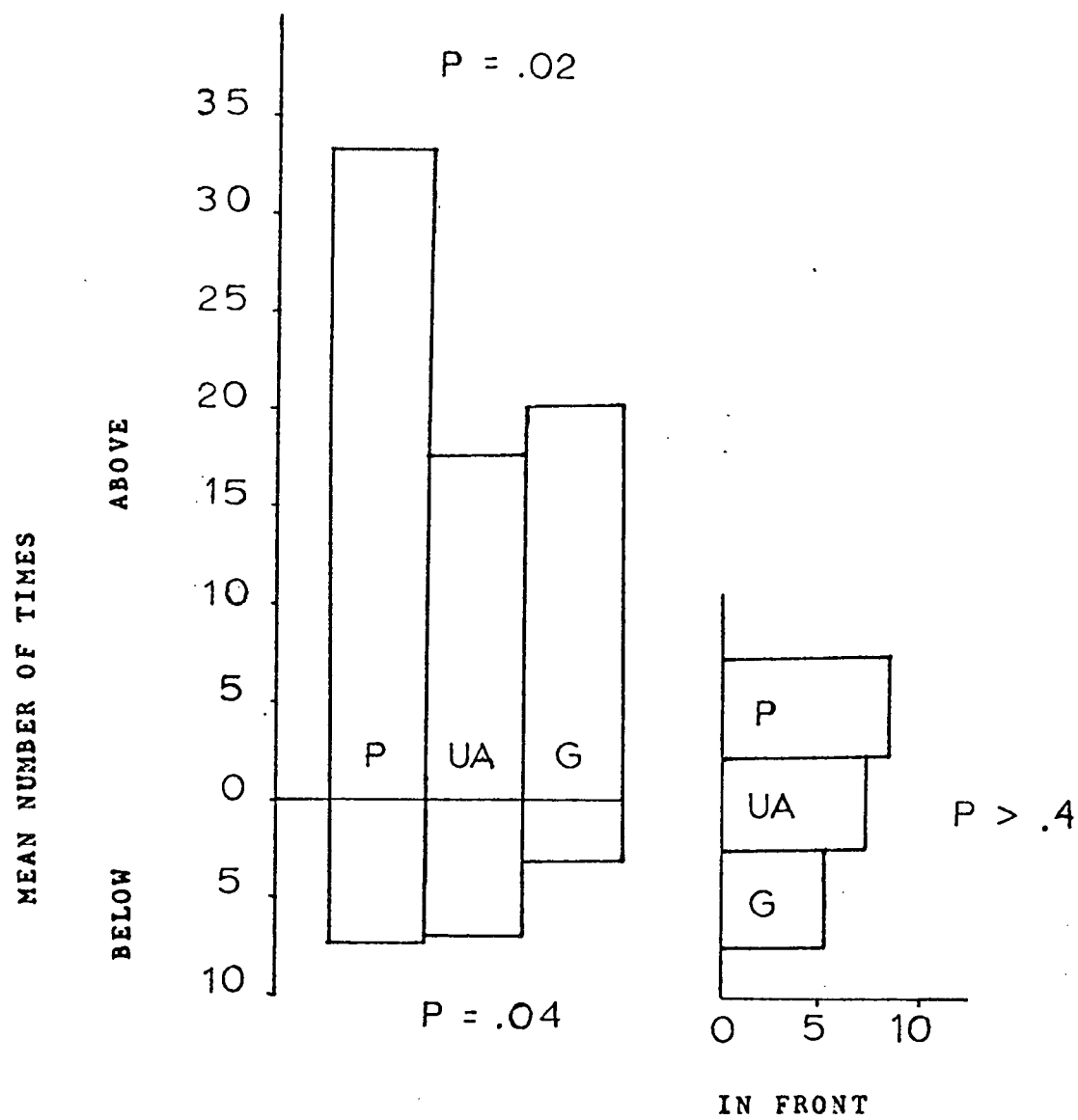
The frequency of thrusts did not differ significantly between races in this experimental situation ($P = 1.3$, $UA = .6$, $G = 1.0$; $p > .5$). Very few copulation attempts occurred because all but eight ♀♀ (3 P, 1 UA, 4 G) were unreceptive. There was a suggestion that snout contact with the ♀'s genital pore, though infrequent, occurred more often in UA ♂♂ than others ($P = 1.7$, $UA = 3.1$, $G = 2.2$; $p < .3$).

c. Display

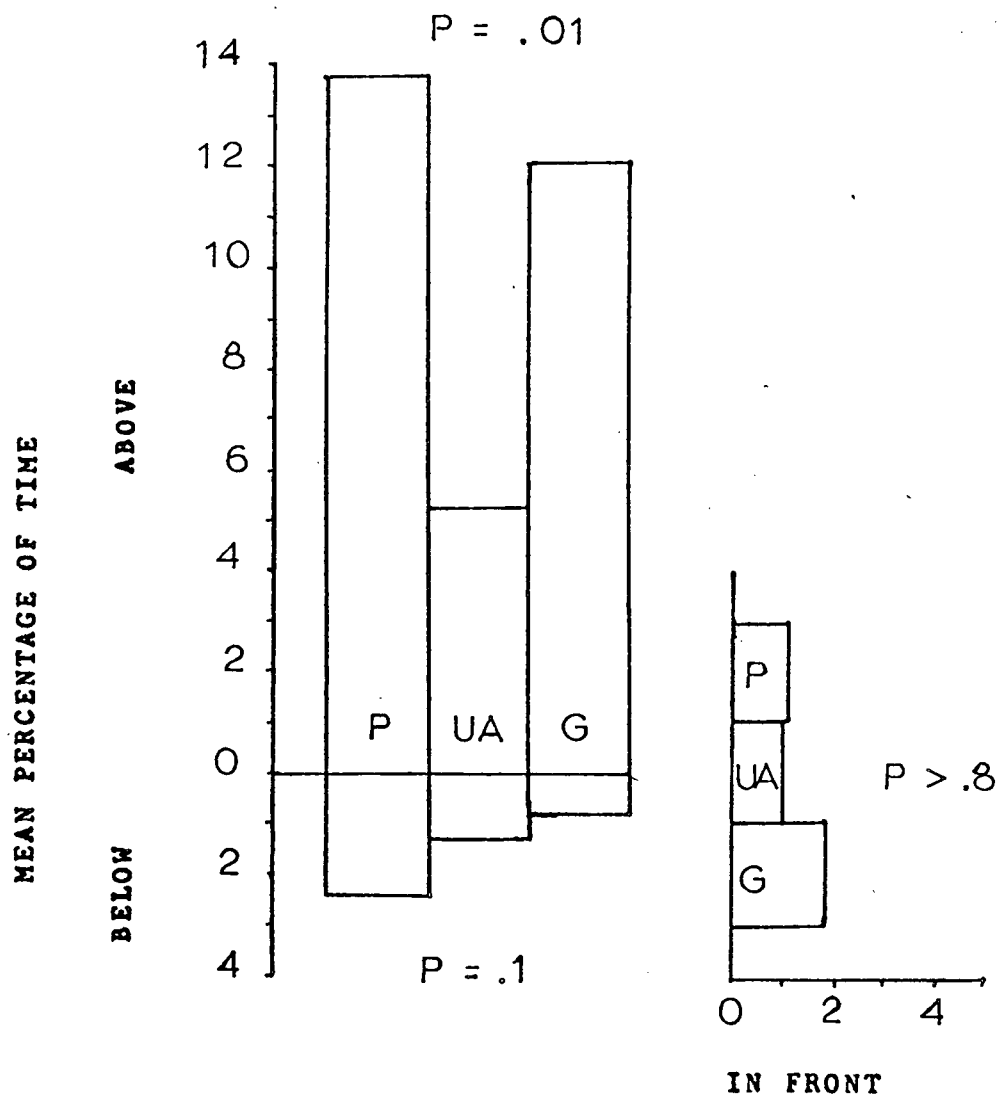
The distance from the ♀ at which the ♂♂ performed sigmoid displays was approximately five cm and did not differ between the races (Table 7).

Fig. 7. Occurrence of ♂♂ in positions above, below, and in front of a ♀. N = 13 for P, 16 for UA, and 17 for G. Probability values are derived from the Kruskal-Wallis one-way analysis of variance.

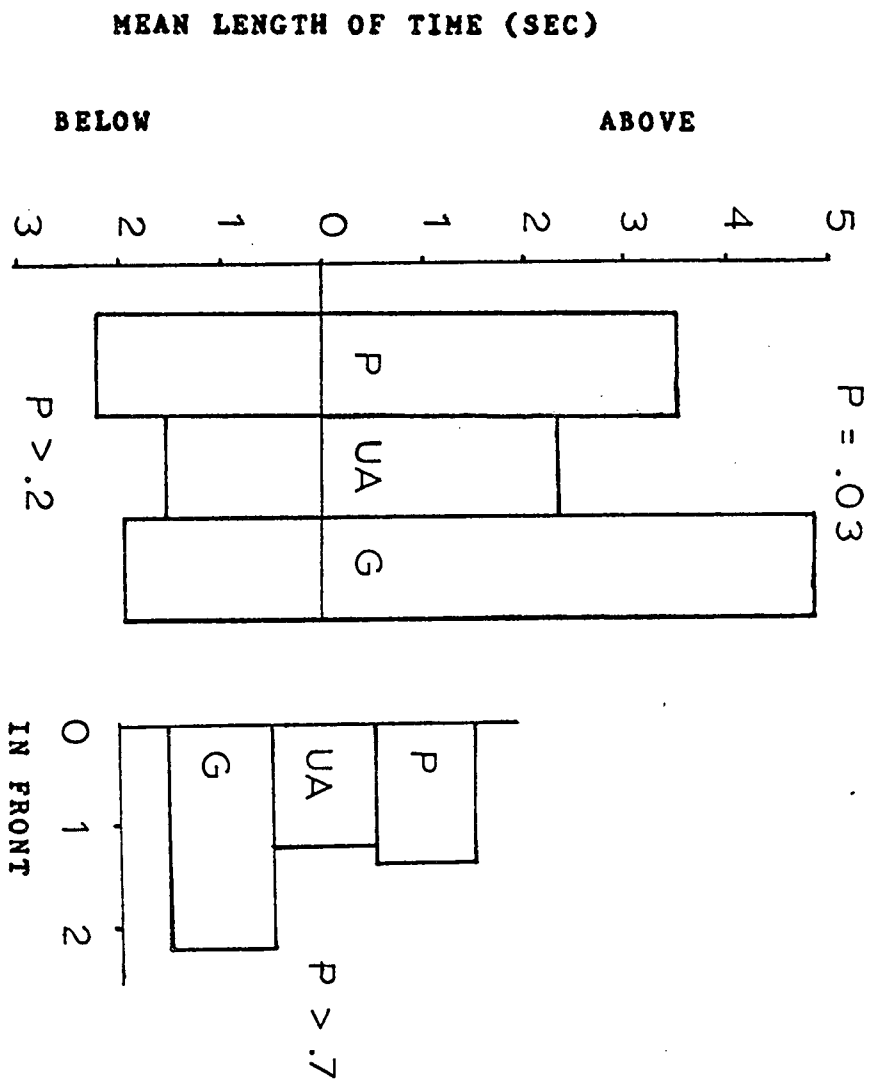
- a. Mean number of times ♂♂ moved into different positions.
- b. Mean percentage of time ♂♂ spent in different positions.
- c. Mean length of time ♂♂ spent in different positions. N = 11 for P, 13 for UA, and 11 for G in the position below the ♀.



a.



b.



C.

G ♂♂ displayed less frequently and for less time when all races are compared ($p = .02$ and $.04$ respectively, Fig. 8). Difference in position relative to ♀♀ during display occurred not just because of variation in total display activities between the races. For instance, P ♂♂ displayed above the ♀ longer and more often ($p = .0002$ and $.001$, respectively) than UA and G ♂♂, and UA ♂♂ displayed within a fish-length (approximately 2 cm) of the ♀ more often and for longer times ($p = .0009$ and $.0008$, respectively) than P and G ♂♂. Other differences between races in location of display more closely followed the overall differences in bouts and percentage time of display mentioned above and in Fig. 8 (Fig. 9a,b). The average number of changes in display position (above, below, level; inside or outside one fish-length) during a trial was 7.7 for G ♂♂, 28.2 for P ♂♂ and 27.9 for UA ♂♂, but the number of position changes per display did not differ (P, 2; UA, 1.8; G, 1.8). I consider the number of position changes in a time period the most ecologically relevant measure because the amount of motion complements racial differences in the very obvious display behaviour and thereby enhances racial differences in conspicuousness.

If we examine display times in various positions as a proportion of total display times for each race separately, we find G ♂♂ in front of the ♀ more often (32%) than P and UA ♂♂ (20% and 14%, respectively). P ♂♂ remained above most and UA ♂♂ remained within a fish-length most (Fig. 9c).

While relative positions of two fish necessarily depends upon both of their movements, I believe that the differences found here are due to differences in the ♂♂ rather than a reflection of ♀ response patterns.

Table 7. Display Distances

Race	n	distance (cm)	Range (cm)
P	8	4.5	1-13
UA	14	5.3	2-15
G	6	5.2	3-8

One display (the first clearly measurable on the film) was measured for each fish.

Fig. 8. A comparison of mean number of bouts of sigmoid displays and mean percentage of time in the trial spent displaying by ♂♂ of three races of guppies. Kruskal-Wallis one-way analysis of variance.

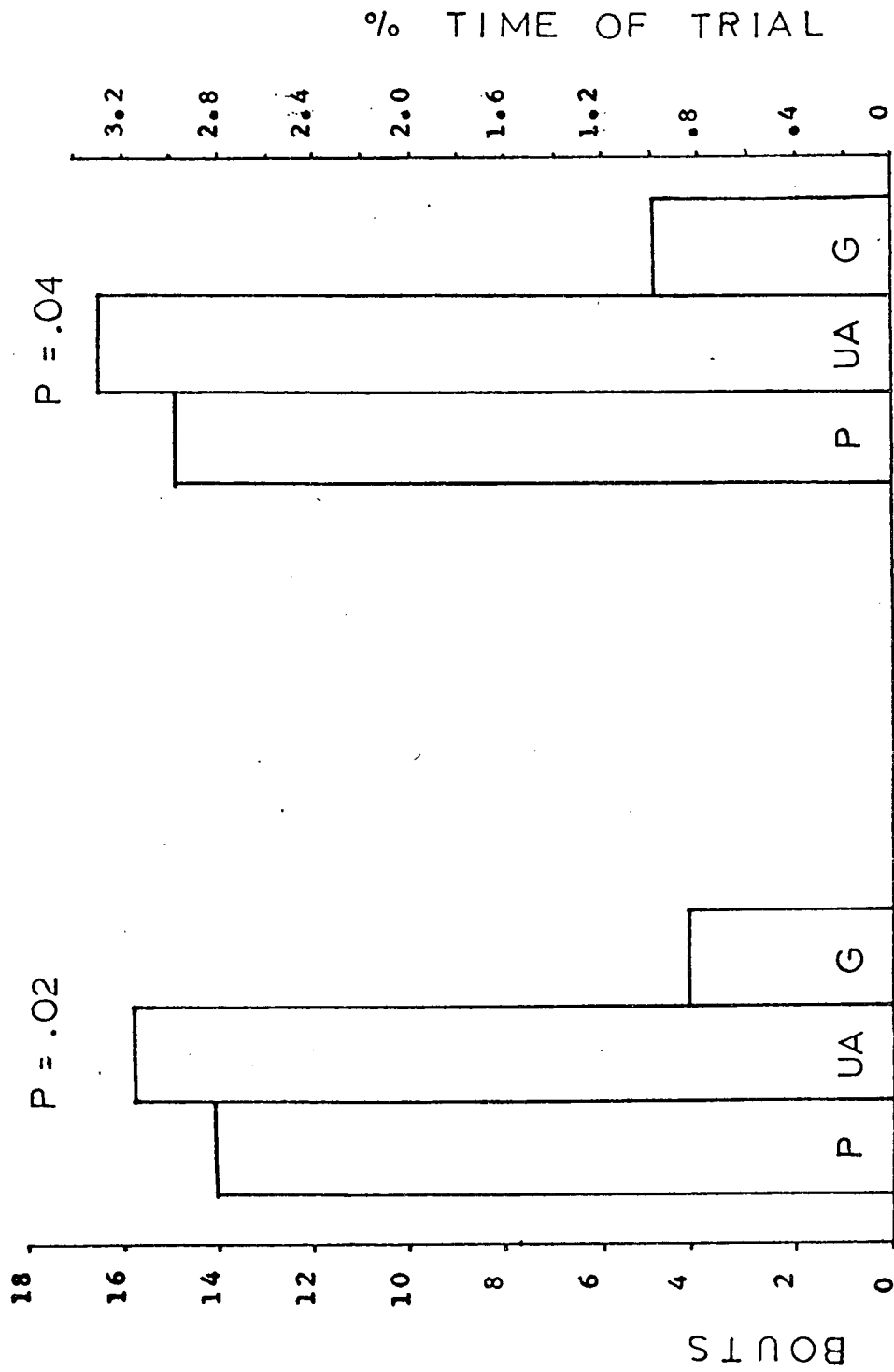
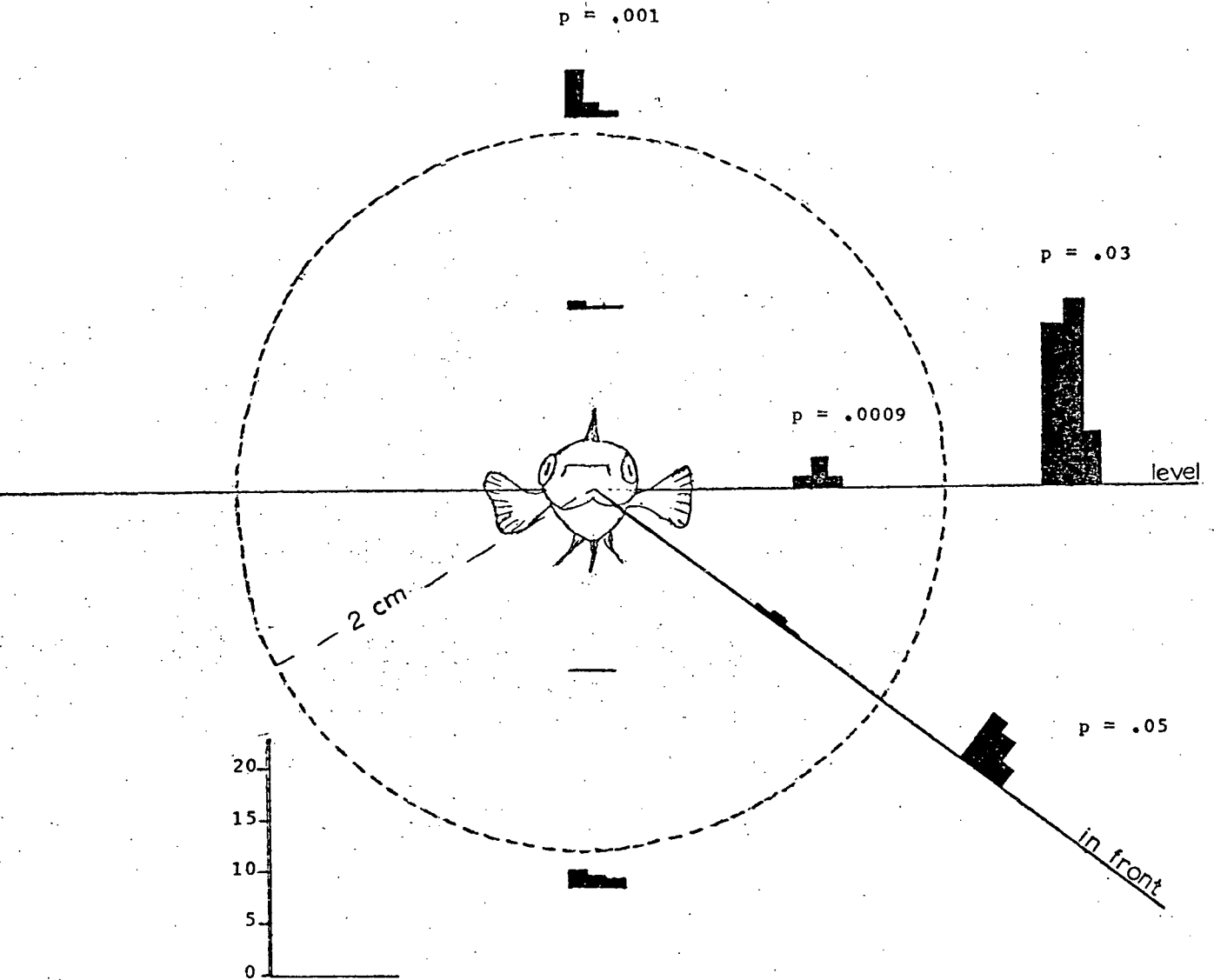
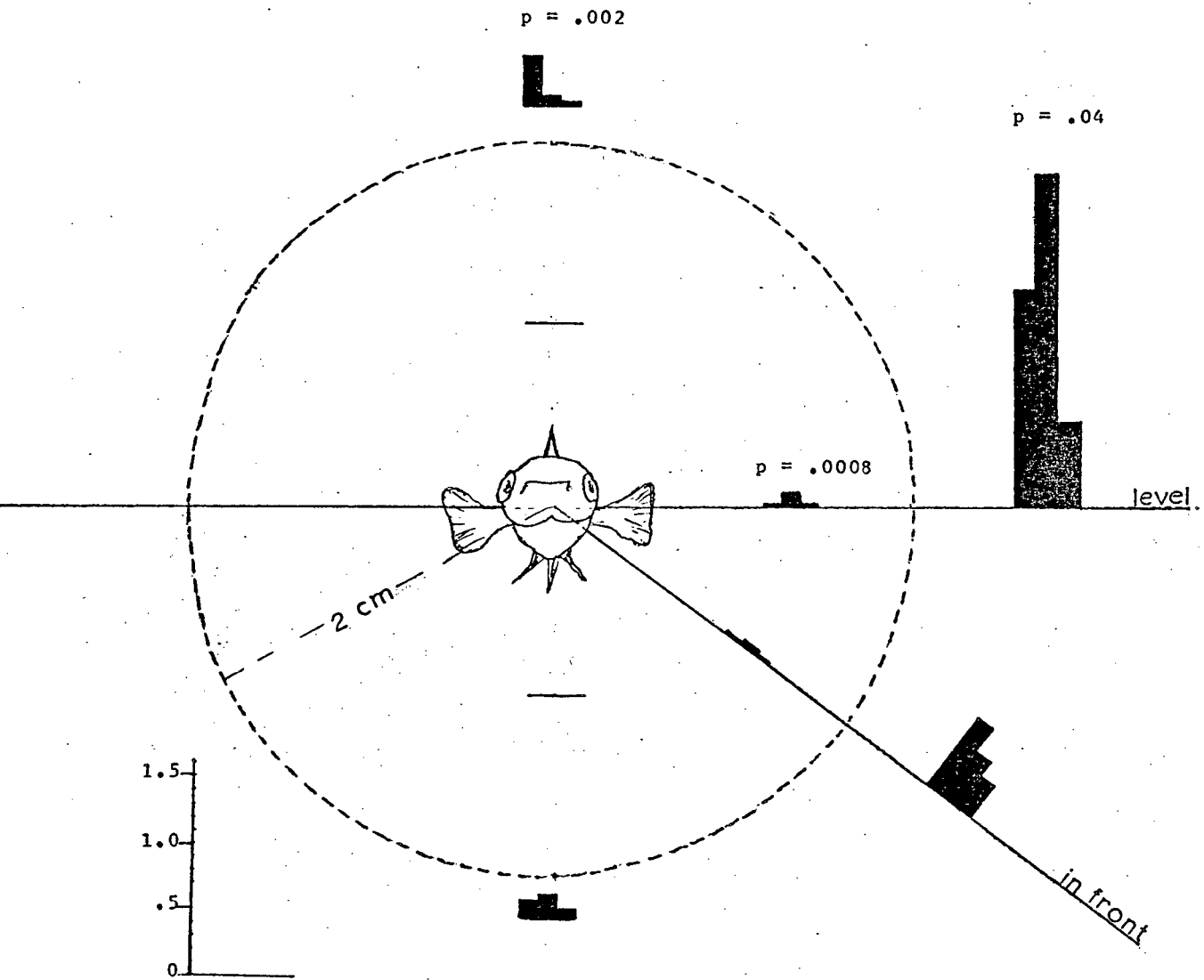


Fig. 9. Schematic representation of ♂ displays in various positions relative to a ♀. The fish represents a ♀, the dotted circle represents a 2 cm cylinder with its axis horizontal and coincident with the fish's long axis. Each group of histograms represents the occurrence of P, UA, and G ♂♂ (in that order) displaying in various positions relative to the ♀: above, below, level, in front, and inside or outside approximately 2 cm of the ♀. Level displays occurred on both sides of the ♀. Probability values from the Kruskal-Wallis one-way analysis of variance are inserted where a difference between races exists.

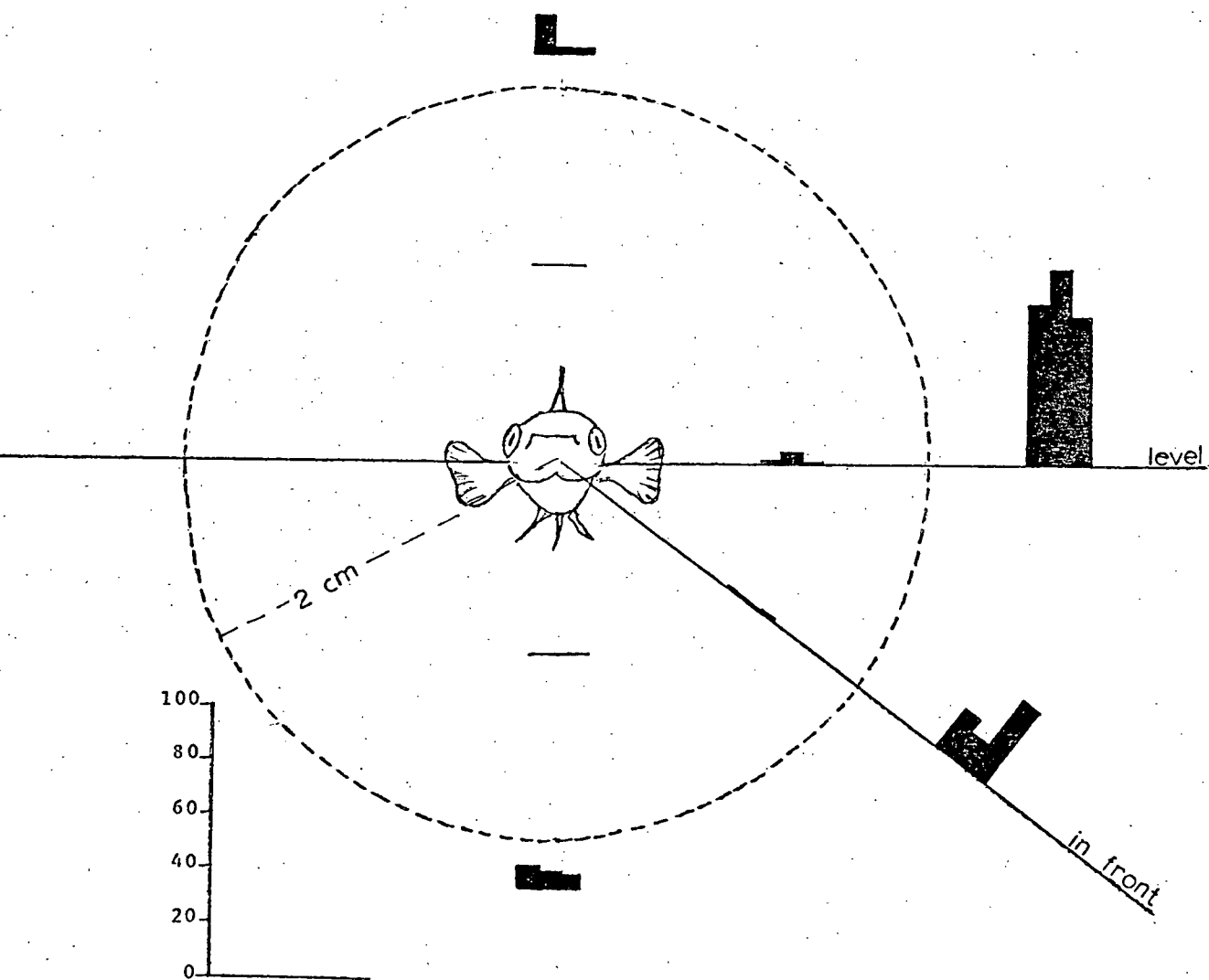
- a. Number of times ♂♂ were found displaying in various positions relative to a ♀.
- b. Percentage of time in trial ♂♂ were found displaying in various positions relative to a ♀.
- c. Percentage of time ♂♂ were found displaying in various positions relative to a ♀, expressed as percentages of total display time for each race (i.e., each race displayed 100%).



a.



b.



C.

Fish did not differ in mean number of gonopodial swings ($P = 4.6$ per trial, $UA = 4.2$, $G = 5.0$; $p > .8$), or leaps ($P = .8$, $UA = .8$, $G = .5$, $p > .8$).

d. Correlations of behaviours

Correlation coefficients were obtained between all recorded aspects of courtship, between ♀ length and all aspects of ♂ courtship and spatial relations, and between ♂ length and all aspects of his courtship and spatial relations. The only significant relation obtained was a strong positive correlation between numbers of displays and leaps in G ♂♂ ($R = .82$, $p < .0001$). I know from personal observations that leaps are always preceded by displays--P and UA ♂♂ did not leap enough to yield significant correlation coefficients. Apparently the size of either the ♂ or ♀ fish had no bearing on ♂ courtship activity in this situation.

C. Threshold distances for courtship (Experiment 2)

1. Introduction

This experiment was undertaken before the photographic analysis of Experiment 1 was completed. Because of the greater turbidity and higher degree of predation in the Guayamare River I hypothesized that P and UA ♂♂ would display at greater distances from the ♀ than G ♂♂. The experiment therefore was designed to compare the courtship of the ♂♂ of the three races when prevented from approaching nearer than certain distances to ♀♀. Furthermore, having shown the discrepancy in display frequencies between the races in the previous experiment, I hypothesized that

P and UA ♂♂ would also display more frequently and with shorter latencies than G ♂♂.

2. Materials and Methods

Three ♀♀ of the same size ($24.2 \pm .1$ mm), one of each race, were placed in the long aquarium as shown in Fig. 10. These ♀♀ were chosen for calmness. Eight ♂♂ of each race were individually isolated the same day. The following day, six ♂♂ of each race were tested three times each with three different minimum distances from the ♀♀ -- 0, 5, and 10 cm--determined by insertion of clear plexiglas partitions (Fig. 10). Males were observed in an order (P, G, UA) so that they had approximately equal isolation periods between trials. The order in which a ♂³⁷ was tested at the minimum distances was determined by chance. Dither fish swam in an aquarium behind the long aquarium (Fig. 10).

I recorded the following: time until first display, number of displays, display distances (+1 cm, distances marked on aquarium) and whether or not the ♂ ever attended to the ♀ during the test. Attentiveness was defined as ♂ orientation and approach to the ♀♀.

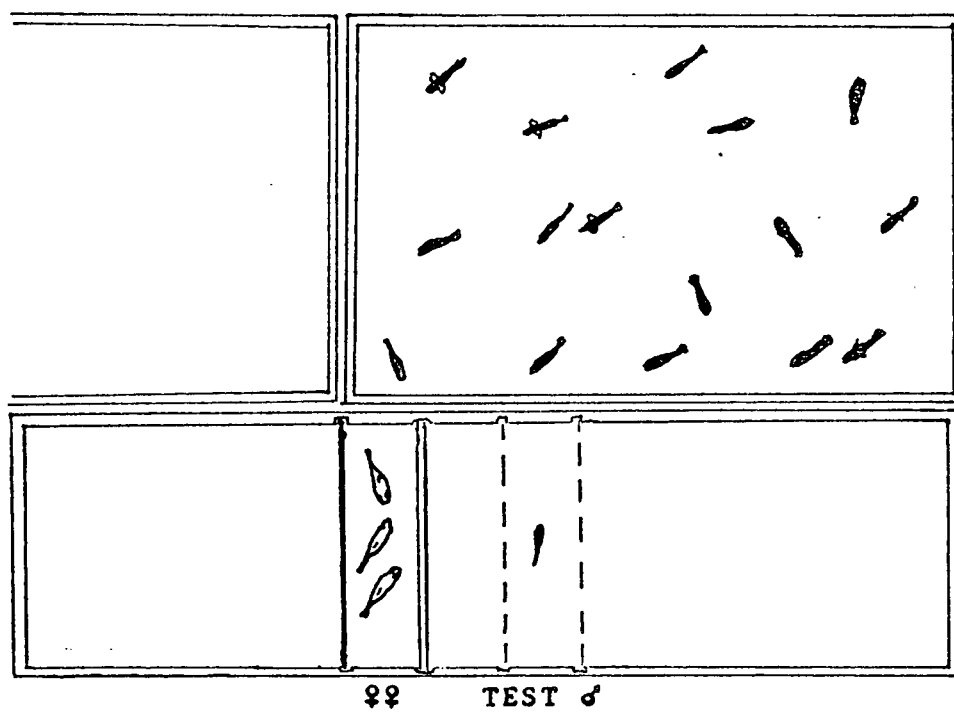
3. Results

When ♂♂ were placed in the long aquarium they generally began attending to the ♀♀ immediately if they were going to attend at all. One UA ♂ did not behave calmly enough on his first trial and was replaced.

Contrary to expectation, no fish, with the exception of one P ♂, displayed with the 5 and 10 cm partitions in place. This was not because the fish displayed only while very close to the ♀♀, because virtually

Fig. 10. Aquarium set-up for Experiment 2, top view. Three ♀♀ enclosed in a 5 cm wide section bounded by opaque plexiglas on one side and clear plexiglas on the side of the ♂. The ♂ could swim freely throughout most of the aquarium (approaching to within 2 mm of the ♀♀) but could be limited in his most proximal approach to the ♀♀ by a clear plexiglas partition placed at either 5 or 10 cm from the ♀♀'s partition (positions shown by dotted lines).

DITHER FISH



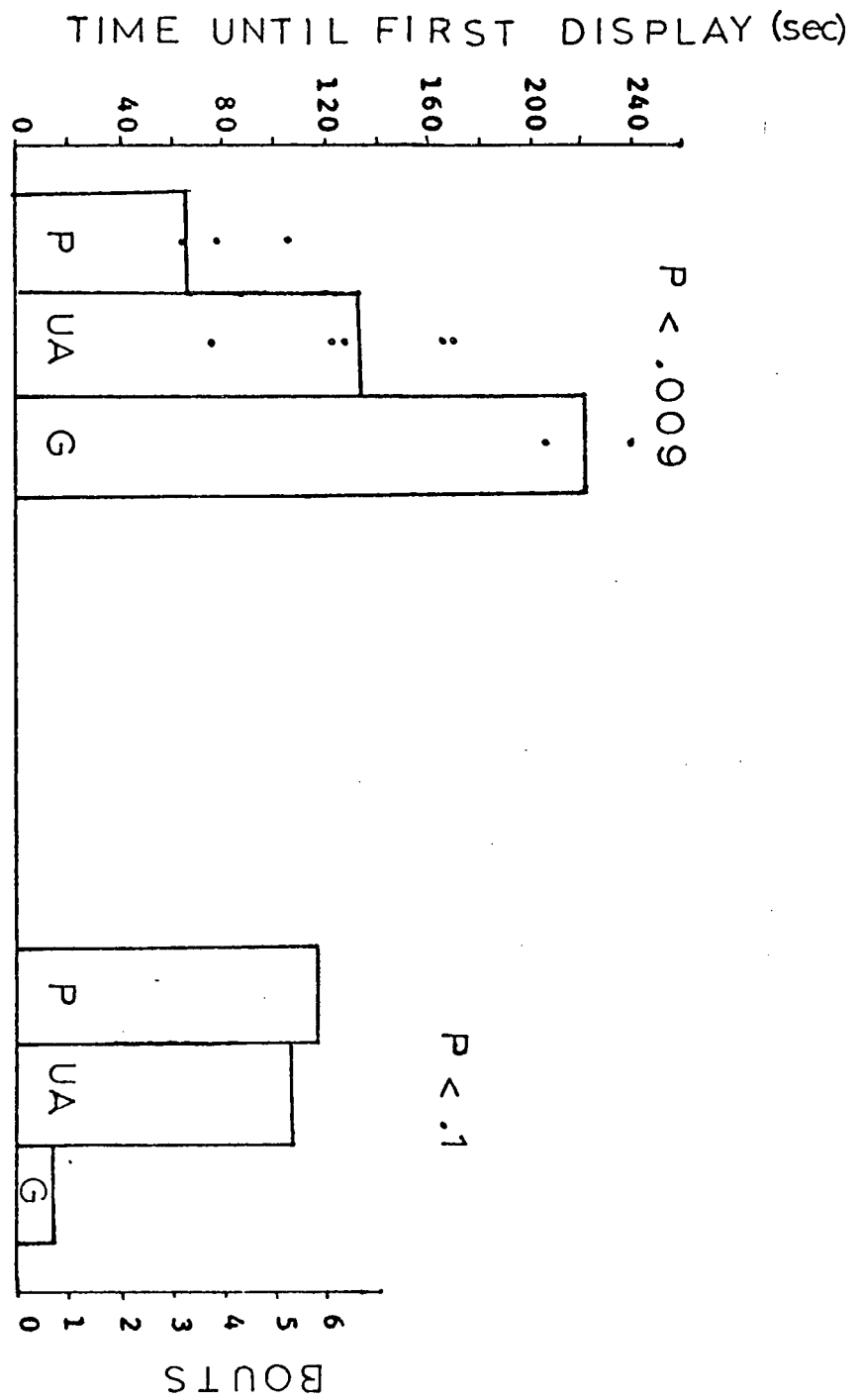
5 cm

all the fish displayed between 4 and 7 cm from them as in Experiment 1 (Table 7). The apparent reason for this behaviour is that the ♂ must first come very close to the ♀ before initiating the display. Almost all displays were preceded by the ♂ and ♀ being separated only by the thickness of the plexiglas partition (3 mm), with the ♂ backing off to perform.

Fig. 11 shows times until first displays and frequencies of displays. The Kruskal-Wallis one-way analysis of variance reflects the differences between all three races in time until the first display ($p < .009$). P and UA ♂♂ displayed most frequently (for both P-G and UA-G comparisons, $p = .004$, Mann-Whitney U test, one-tailed) and with a shorter latency to the first display (P-G, $p = .067$; UA-G, $p = .047$; Mann-Whitney U test, one-tailed). UA ♂♂ took longer to display than P ♂♂ ($p = .064$, Mann-Whitney U test, two-tailed).

At the two greater distances G ♂♂ paid less attention to the ♀♀ than did other ♂♂. In 1/12 cases with UA, 3/12 with P, and 7/12 with G, the ♂ did not attend to the ♀♀ at all during the five-minute trials with the partitions 5 or 10 cm from the ♀♀. Expected frequencies were too low in too many cells to compare the races with the χ^2 test, but a comparison of each pair of races with the Fisher exact probability test yielded a two-tailed probability value of .05 for the discrepancy between UA and G ♂♂ to occur by chance. My impression was that most of the G ♂♂ not attending did not notice the ♀♀.

Fig. 11. Mean times until first display and mean number of bouts of display at the shortest distance (0 cm) from the ♀♀. Probability values are from the Kruskal-Wallis one-way analysis of variance. The dots are the data points. See text for further statistical comparisons.



D. Competition in courtship I (Experiment 3)

1. Introduction

Because of the social nature of guppies, a ♂ probably would have little opportunity to court ♀♀ in the absence of other ♂♂. The following questions may be posed: Does the manner and amount of courtship change in the presence of another ♂ of the same race? Is there competition? Are the differences between the races retained in a competitive situation? This experiment was designed to begin to answer these questions.

2. Materials and Methods

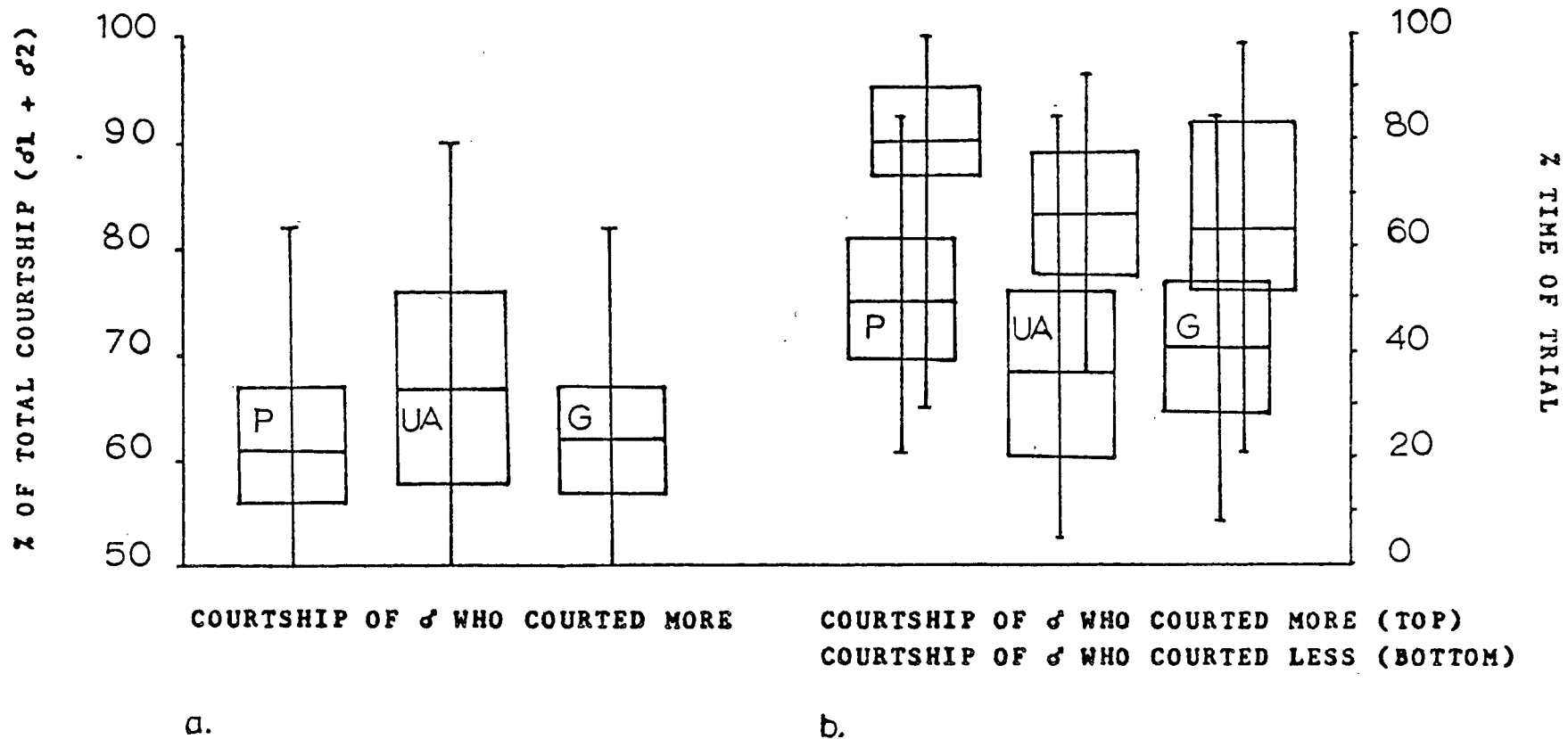
One ♀ and two ♂♂ of one race were placed in an observation aquarium between 1400 and 1800 hours. Observations took place the following morning, usually between 1000 and 1300 hours. Each trial lasted ten minutes. A Rustrak event recorder was employed to chart the duration of time each ♂ was courting.

Although 18 independent trials were attempted for each race, fish which did not behave calmly enough reduced the number of trials analyzed to 15 P, 13 UA, and 15 G.

3. Results

Although one of the pair of ♂♂ courted an average of nearly two-thirds of the time both ♂♂ courted, there is no reason to believe that the results reflect any differences in courtship time between ♂♂ within any of the races (one-way analysis of variance; Fig. 12a). However,

Fig. 12. Courtship of two ♂♂ in the presence of one ♀ , expressed as percentages of total courtship ($\sigma_1 + \sigma_2$) (a.) and time in the trial (b.). The horizontal lines represent the mean values for 15 P, 13 UA, and 15 G ♂♂. The rectangles are the 5% confidence limits and the vertical lines are the ranges.



racial differences appeared in the time spent in courtship by the ♂ who courted more---P ♂♂ courted most ($p = .05$; Fig. 12b). Total courtship time (♂#1 plus ♂#2) tended to be higher for P ♂♂ ($p = .08$). The time spent in simultaneous courtship did not differ between races (Fig. 13). There were no grounds for expecting these results on the basis of findings in Experiment 1.

Correlation coefficients were obtained for length of all fish with amount of courtship. Male courtship time was correlated with neither ♂ nor ♀ length. These findings do not necessarily contradict those of Baerends et al. (1955), who found that ♂♂ courted larger ♀♀ if given a choice. My results show that the length of ♀ in the size range offered (20-28 mm S.L.) did not effect the amount of courtship when the ♂♂ had no choice. This finding allows me to assume that ♀♀ of different sizes in the one-♀ experiments do not have different stimulatory properties.

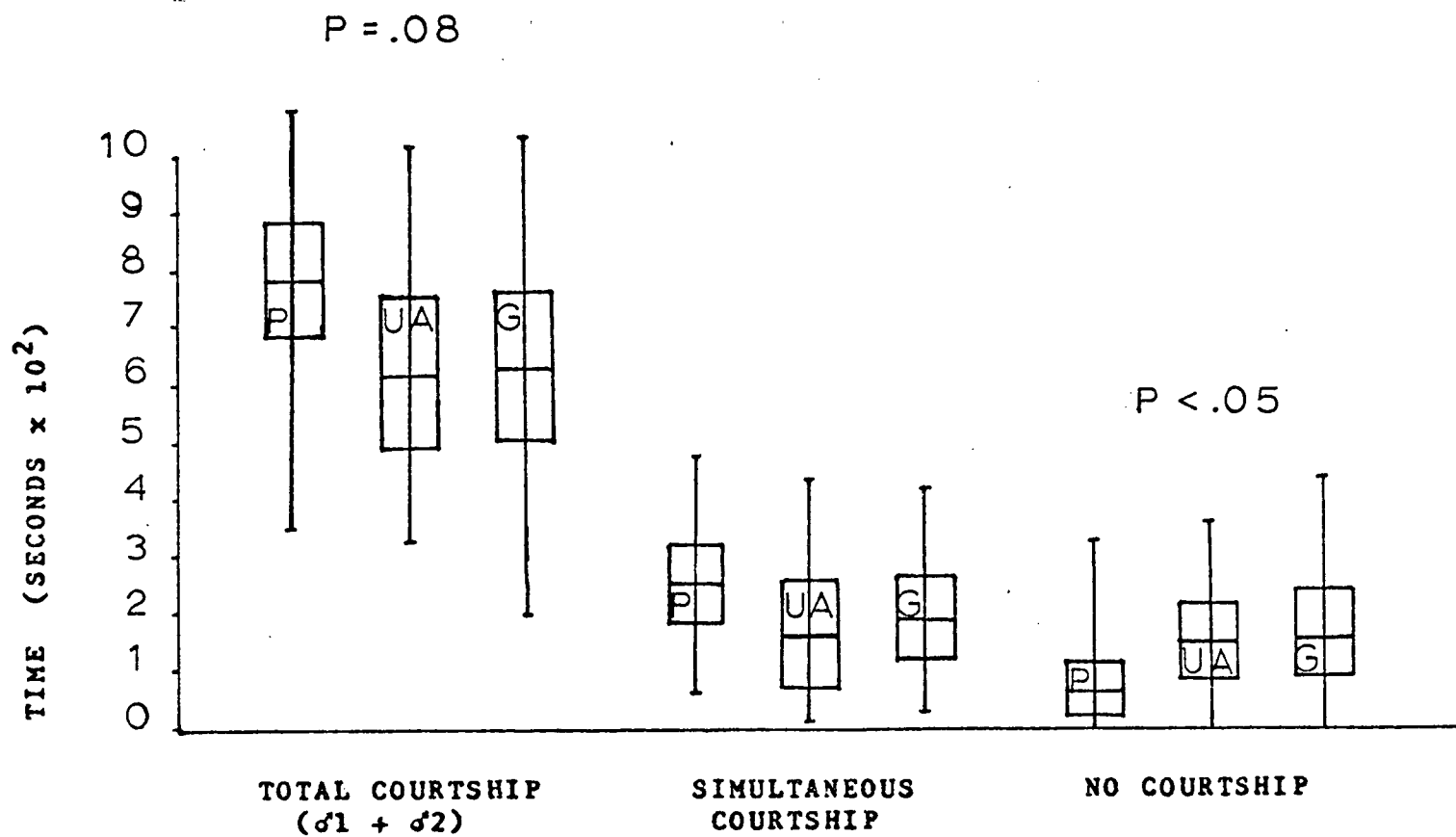
The results and impressions from these observations suggest that some mechanisms are in operation which determine which ♂♂ get the most opportunity to mate.

E. Pilot experiments on the role of ♂-♂ aggression during courtship (Experiments 4, 5, and 6)

1. Introduction

Observations in Experiment 3 and daily checks of the stock aquaria at the onset of this study suggested that Paria fish engaged in considerable aggressive activity, something which was noticeably absent in the

Fig. 13. Courtship of two♂♂ with one ♀, expressed in seconds. The horizontal lines represent mean values for 15 pairs of P, 13 pairs of UA, and 15 pairs of G ♂♂. The rectangles are the 5% confidence limits and the vertical lines are the ranges. One-way analysis of variance yielded the probability values.



other races. For a controlled check on differences in aggression and for some clues as to its functional significance and relation to courtship, the following experiments were performed.

2. Aggression and territory (Experiment 4)

a. Introduction

This experiment was performed to investigate the differences in aggression between Paria and the other two races, and the possible defence of territories or formation of dominance hierarchies. (Field data suggest the possibility of territories in the Paria stream-Seghers, pers. comm.)

b. Materials and Methods

Each of three observation aquaria were arranged with three rocks in a line parallel to the front of the aquarium, approximately midway between front and back, and equidistant from one another and the sides. The rocks were provided as reference points should a fish wish to establish a territory. Six ♂♂ from each of the three races were placed separately in each of three observation aquaria. Fish were individually recognizable. After six days fish were observed for ten-minute periods. Observations were repeated after seven and nine days. I sat quietly in front of the aquarium and tallied charges and noted sparring and tail-beating.

c. Results

Fig. 14 shows scores for each of the three days the fish were observed. Clearly, P ♂♂ were more aggressive than UA and G ♂♂. The total scores

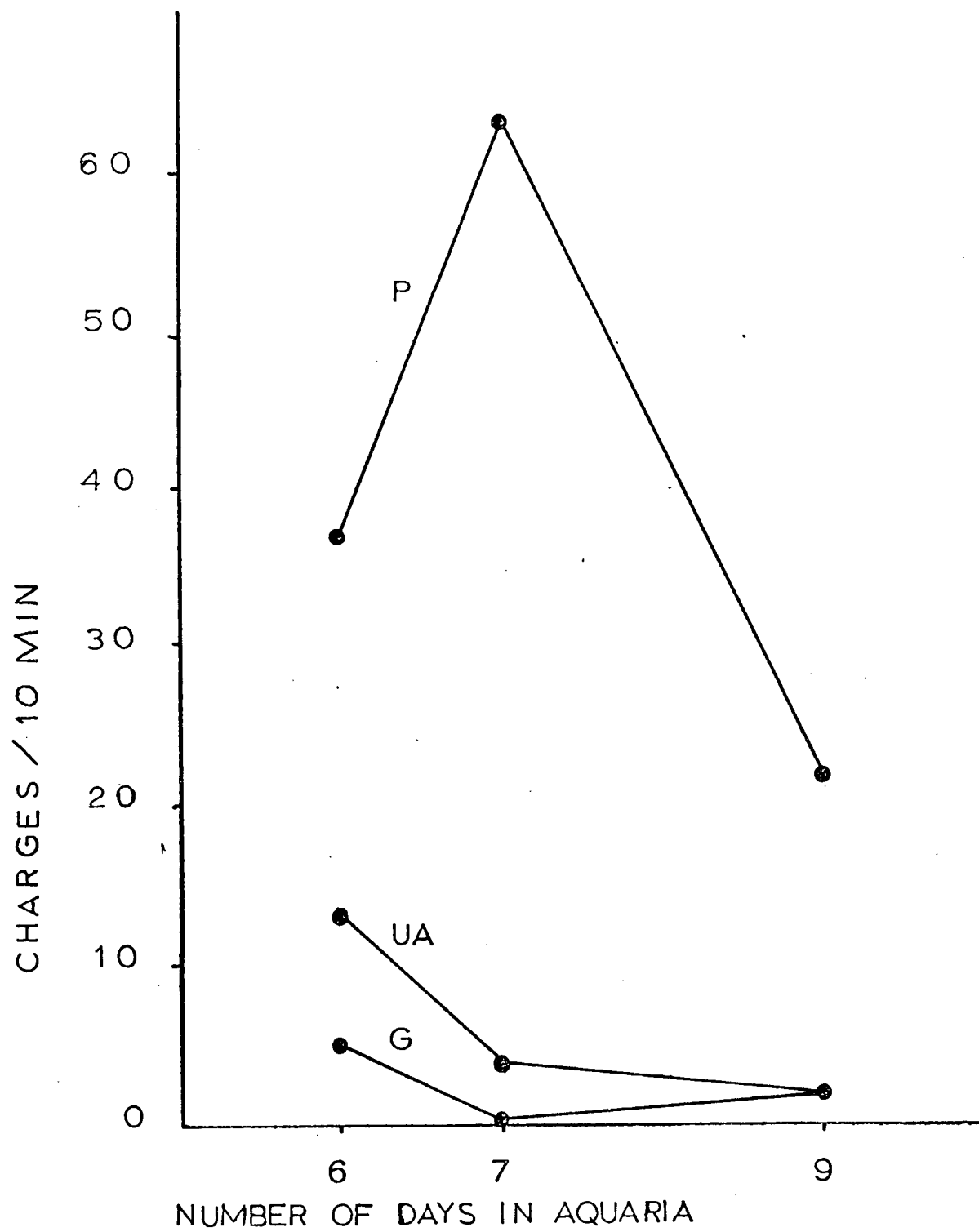
were not a result of the activity of all the fish, however. In most cases one or two fish did all the charging. I found no evidence of a dominance hierarchy.

The only territory formed was by a Paria fish--that is, he always was found in the same place and always returned to it after chasing another fish. This fish had a peculiar wobble, but was not obviously ill. Later observations have shown this association--wobble, site tenacity, and aggression--to occur occasionally in both P♂♂ and ♀♀. These fish did not die and usually resumed normal swimming when replaced into stock aquaria. I have also observed this wobble as an occasional impermanent condition in stock aquaria. I have no explanation of this behaviour and I did not pursue the problem because it did not seem to have bearing on courtship.

Observations were terminated due to an increasing confusion between aggressive and sexual behaviour. Initially, sparring and chasing were clearly aggressive. Sparring ♂♂ assumed positions parallel or anti-parallel to one another and chases were usually terminated by a bite attempt and no further pursuit by the aggressor. Later, chases persisted and attention was directed at the genital region of the other ♂. In addition, sigmoid displays were also performed. It is possible that the sigmoid display has some appeasement function, as the ♂ usually performs it to the much larger ♀, who occasionally charges (see also Appendix 1 regarding the possible functions of black coloration in ♂♂).

When ♀♀ were introduced to these ♂♂ after day 9, a few ♂♂ persisted in courting other ♂♂ and attempting copulation with them.

Fig. 14. Number of charges by ♂♂ in each of three observation periods in all-♂ aquaria.



I interpret this as abnormal behaviour resulting from the deprivation of a proper sex object in an animal with an extremely low response threshold for sexual activity.

3. Aggression and females (Experiment 5)

a. Introduction

The previous experiment and observations by Liley (♂♂ raised in isolation from ♀♀ tend to become homosexual; pers. comm.) suggested that ♀♀ are a necessary part of the environment for the development of normal ♂ responses. This experiment was designed to test the effects of ♀ presence on ♂ aggression, and the possibility that ♂ aggression serves as a mechanism of competition for ♀♀, as seen in two species of the genus Gambusia (Warburton, Hubbs, and Hagen, 1957; McAlister, 1958).

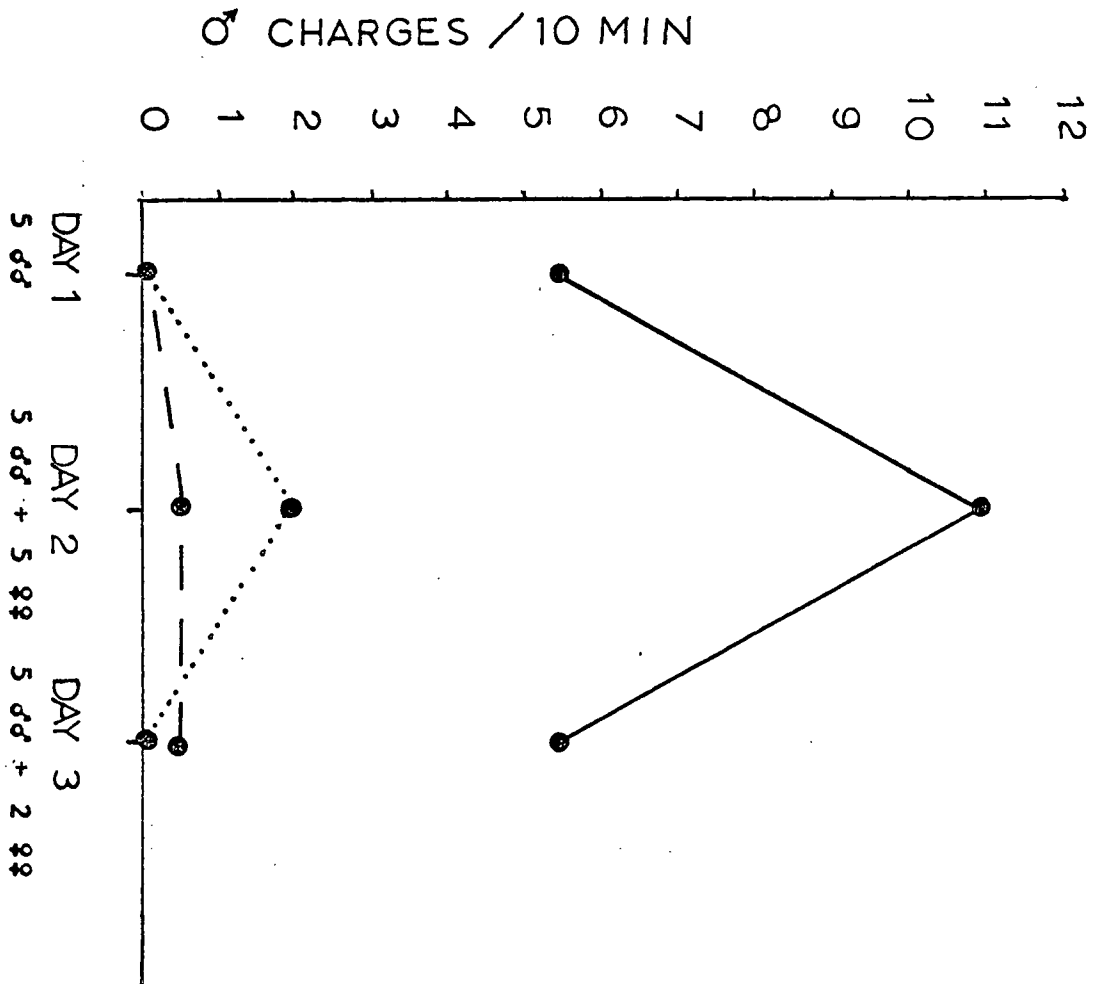
b. Materials and Methods

The observation aquaria were employed minus the rocks. Two aquaria were used for each race, and five ♂♂ from stock aquaria were placed in each. The next day they were scored for ten-minute periods for aggression as in the previous experiment. At the conclusion of observations, five ♀♀ were added to each aquarium, and scoring commenced on day 2. At the end of this series of tests, three of the ♀♀ were removed to stimulate a more highly competitive situation for the ♂♂, if indeed they were competing for the ♀♀.

c. Results

Fig. 15 shows that the addition of ♀♀ increased the total amount of aggression in all races, with the most aggression by far occurring in

Fig. 15. Mean number of ♂ charges in: (1) 5-♂ groups, (2) the same 5-♂ groups into which 5♀♀ were introduced, and (3) the same groups as in (2) from which 3♀♀ were removed. Solid line indicates P groups, dashed line, UA, and dotted line, G. $N = 2$ groups for each race.



Paria. In addition, one P ♀ defended a territory and charged other ♀♀ twelve times and a ♂ once on day 2, and charged ♂♂ four times on day 3. The reduction in aggression on day 3 implies one or more of the following: (1) fewer ♀♀ meant the ♂♂ were less stimulated to fight, (2) the ♂♂ had established more stable relationships, (3) the decrease in aggression was dependent upon the addition of fish regardless of sex.

4. Aggression, females, and density (Experiment 6)

a. Introduction

The previous experiment posed the problem of whether the increased ♂ aggression was a function of the presence of ♀♀ or just a higher density of fish. The present experiment was designed to get an indication of the effect of sex and density on aggression.

b. Materials and Methods

The manner of scoring and the length of scoring periods were as in the previous experiment. Three observation aquaria were used for each race. The day before the first test two of these aquaria received five ♂♂ each and one aquarium received five ♀♀. At the conclusion of the first scoring session, five additional ♂♂ were added to one of the ♂ aquaria, five ♀♀ were added to the other ♂ aquarium, and five ♀♀ were added to the ♀ aquarium. The all-♂ and the all-♀ trials were run as controls for density and ♀ roles in aggression.

c. Results

The only aggressive acts on day 1 were in all-♂ P aquaria. The only noteworthy increase in aggression on day 2 occurred where ♀♀ were added

to P ♂♂ (Fig. 16). These results suggest that ♀♀ fish may stimulate aggression among P ♂♂, and that increased density alone may not be the primary cause of aggressive interactions.

F. Competition in courtship II (Experiment 7)

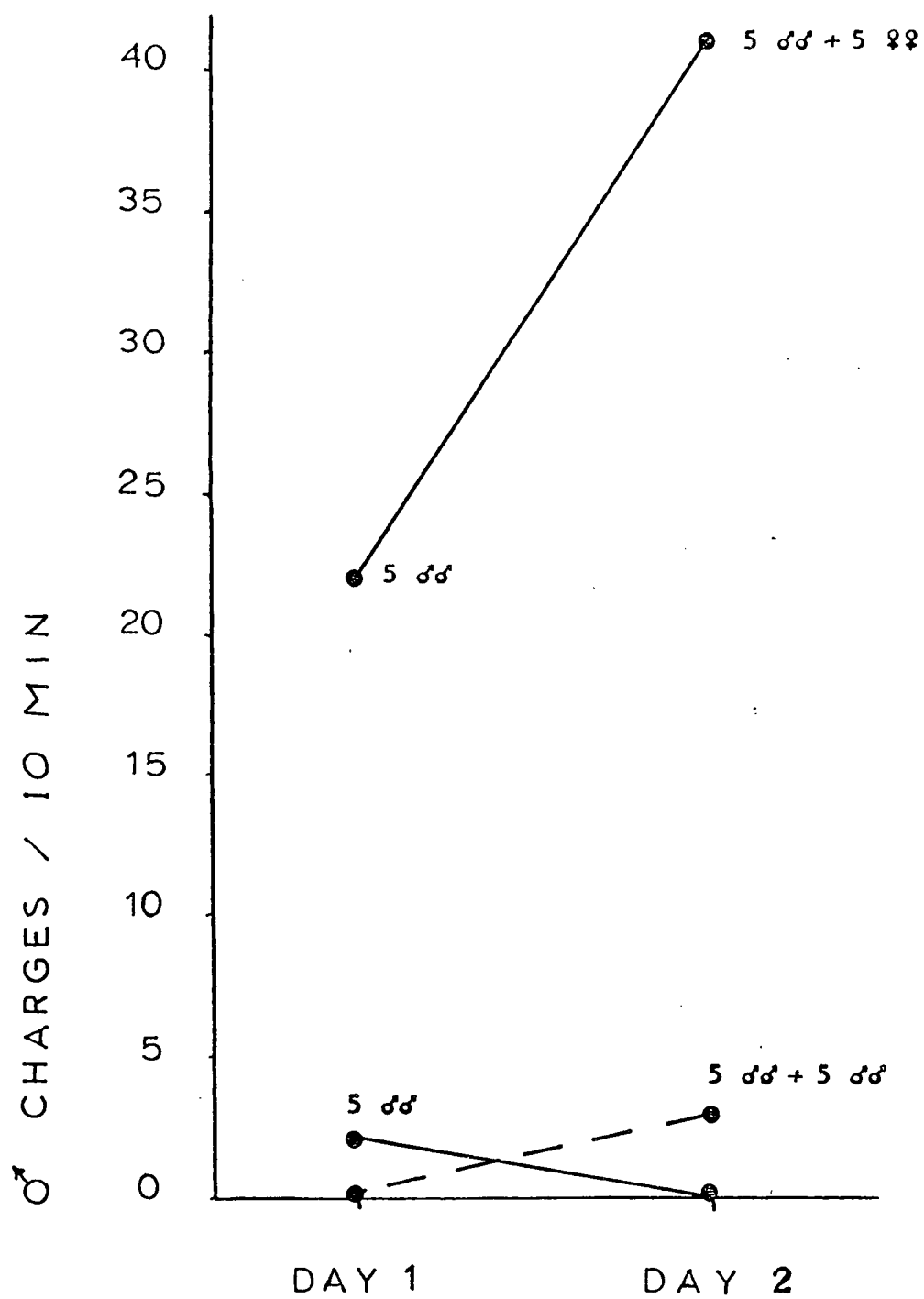
1. Introduction

To further investigate mechanisms of competition I undertook a detailed analysis of how a ♂ established himself as the dominant suitor, if he indeed did so. This analysis was much strengthened by using the ♂♂ of Experiment 1, who had some previous courtship recorded. An analysis of changes in courtship behaviour was thereby possible.

2. Materials and Methods

Fish were isolated in each observation aquarium after observation in Experiment 1, at about 1700 hours. Between 1250 and 1600 hours the following afternoon, the center partition was removed and both ♂♂ and one of the ♀♀ were allowed in the center section. Recording commenced immediately. Each trial lasted ten minutes. Nine independent trials were attempted for each race, and 9 P, 8 UA, and 8 G were completed. The following ♂ behaviours were recorded on the CDAT: display, leap, thrust, charge, charge by the ♀, chase, and amount of time each ♂ spent within 2 cm of the ♀. Sparring and any unusual aspects of a trial were noted. The Olympus camera shot a frame every 15 seconds, but due to its incessant malfunctioning, only 5 P, 4 UA, and 3 G trials yielded photographs suitable for analysis.

Fig. 16. Number of ♂ charges in groups of 5 fish of one sex, and these same groups after 5 additional fish of either the same or opposite sex were introduced. Only groups where charges occurred are shown. Solid lines indicate P groups; dashed line, UA.



3. Results.

Changes in courtship from the 1♂-1♀ Experiment 1 were quite evident within each race in this 2 ♂♂-1 ♀ situation (Table 8). Frequency of displays and leaps of single ♂♂ were reduced in all races. P ♂♂ decreased their frequency of thrusts greatly whereas UA and G ♂♂ increased theirs slightly. If we compare races we find the degree of changes much greater in P ♂♂ than in UA and G ♂♂ (displays: $p < .02$; leaps; $p < .001$; thrusts: $p < .02$). Furthermore, P ♂♂ seemed to rely on a different strategy than the other ♂♂. They fought (Table 9).

There was a tendency for pairs of UA ♂♂ to display more than pairs of P and G ♂♂ (means: UA = 10, P = 4, G = 2; $p < .1$)³. UA ♂♂ also tended to display within 2 cm more often than P or G ♂♂ (UA 5/8 trials, 13 times total; P 2/9, 4; G 2/8, 3; $p = .11$). No difference between the races in mean number of chases, leaps, or thrusts were recorded.

All ♂♂ decreased their distance from the ♀ when another ♂ was present. The percentage of time in a trial in which the ♂ was within a ♂ fish-length of a ♀ did not differ between the races in the competitive situation, although it did differ when the ♂ was alone with a ♀ (Table 10). Note that P ♂♂ increased their time very close to a ♀ more than five fold, UA ♂♂ by just under three fold, and G ♂♂ by just over two fold from the 1-♂ to the 2-♂ experiment. The data in Table 11 arose from sampling the positions of the ♂♂ every 15 seconds in the trial. One or

³But see Fig. 13 in Experiment 3. There, total courtship was measured. Because of the deprivation period prior to observation here, almost all of the fish were courting for the duration of the trial.

Table 8. Differences in frequencies of courtship activities of individual ♂♂ when tested alone with a ♀ (Experiment 1) or in the presence of another ♂ (Experiment 7). Difference expressed as mean % change in number of bouts performed for the same ♂♂ from Experiment 1 to Experiment 7.

	P	UA	G	
Displays	73.3 reduct.	27.4 reduct.	54.6 reduct	p < .02
Leaps	71.4 reduct.	19.0 reduct.	22.3 reduct.	p < .001
Thrusts	66.3 reduct.	9.3 increase	9.5 increase	p < .02

Kruskal-Wallis one-way analysis of variance, 2 df, corrected for ties.

Numbers for ranking were arrived at by the following formula:

$$\% \text{ change} = \# \text{ bouts in competition} / (.667) (\# \text{ bouts alone})$$

where .667 is a correction factor for the difference in the length of the trials. When both the numerator and the denominator were 0, the trial was discarded. When the numerator was 0, the number arrived at was 0. When the denominator was 0, the number arrived at was infinity.

Table 9. Male charges in a competition situation of two ♂♂ and one ♀.

	P	UA	G	
Mean number of charges	28	4	4	$p < .02$
Median number of charges	26	0	0	
Number of trials in which charges occurred	7/9	2/8	2/8	

Kruskal-Wallis one-way analysis of variance, 2 df.

Table 10. Mean percentage of time in trial in which a was within one σ fish-length (approx 2 cm) of a φ in one- σ (Experiment 1) and two- σ (Experiment 7) situations.

	P	UA	G	
1 σ -1 φ	5	12	19	p = .001
2 σ -1 φ	26	36	44	p < .3

Kruskal-Wallis one-way analysis of variance, 2 df.

N = 13 and 18 for P, 16 and 16 for UA, and 17 and 16 for G.

Table 11. Occurrence of $\sigma\sigma$ within a 2 cm radius of a φ in the two- σ situation. Numbers represent the percentage of photographs in which no $\sigma\sigma$, one σ , or two $\sigma\sigma$ appeared within the 2 cm radius.

	P	UA	G
N (pairs of $\sigma\sigma$)	5	6	3
% no $\sigma\sigma$	57	57	54
% 1 σ	30	32	38
% 2 $\sigma\sigma$	13	11	8

Each entry is the average of at least 35 frames and usually 40 frames measured for each pair of $\sigma\sigma$.

both of the ♂♂ were within 2 cm of a ♀ in almost half of the observations in all races (P, 43%, UA 43%; G, 46%).

Correlation coefficients were obtained between all measures taken. There was no indication that the larger ♂♂ were in the closer position relative to a ♀ in any of the races. Size was negatively correlated with both number of displays and number of thrusts in the G race only ($p < .008$ and $p < .05$, respectively). No other correlations between size and behaviour appeared. It follows that larger or smaller size confers no advantage to one ♂ over another in the frequency of performance of some courtship activities, with the above exception in the G race.

Certain behaviour patterns were correlated with one another, as well as with close proximity to a ♀. Many more significant positive correlations in display and contact behaviour were found in P ♂♂ (Table 12). This indicates that when a P ♂ performs one display or contact behaviour more frequently, he also does more of everything else. Since these correlations did not appear in the one ♂-one ♀ experiment, this may be a response to competition. P ♂♂ who were charged more tended to display less, and charged less when close to a ♀. On the other hand, more aggressive P ♂♂ were neither larger nor did they display more than less aggressive ♂♂. The high positive correlation between charges and displays and charges and leaps in UA ♂♂ results from the effectiveness of only two ♂♂, one of whom only charged once. Displays, leaps, and thrusts performed by one ♂ did not appear to influence the performance of those behaviours in the other ♂.

Table 12. Correlation coefficients of courtship activities is a two-♂, one-♀ situation.

Activities correlated	Race	R	Probability of R being 0
Number of displays and percentage of time of trial ♂ is within 2 cm of ♀	P	.16	> .1
	UA	.02	> .1
	G	-.14	> .1
Number of leaps and number of displays	P	.71	.0008
	UA	.90	< .0001
	G	.37	> .1
Number of thrusts and number of displays	P	.65	.003
	UA	-.23	> .1
	G	.61	.01
Number of charges and number of displays	P	-.35	> .1
	UA	.80	.0001
	G	-.13	> .1
Number of times charged and number of displays	P	-.41	.09
	UA	.05	> .1
	G	.08	> .1
Number of times within 2 cm of ♀ and number of leaps	P	.40	> .1
	UA	.05	> .1
	G	-.34	> .1
Number of times within 2 cm of ♀ and number of thrusts	P	.63	.005
	UA	.33	> .1
	G	.22	> .1
Number of times within 2 cm of ♀ and number of charges	P	-.44	.07
	UA	.11	> .1
	G	.21	> .1
Number of leaps and number of thrusts	P	.55	.02
	UA	-.22	> .1
	G	.21	> .1
Number of leaps and number of charges	P	-.03	> .1
	UA	.74	.0009
	G	-.13	> .1
Number of thrusts and number of charges	P	-.21	> .1
	UA	-.18	> .1
	G	-.20	> .1

Previous experience with a particular test ♀ had no effect on the ♂'s performance in competition. In addition, the frequency of courtship behaviours when a ♂ was alone with a ♀ in Experiment 1 had no bearing on his performance in this experiment. Thus, a very active courter in the single-♂ situation did not necessarily outperform a less active courter when they were placed in competition.

C. Competition in courtship III (Experiment 8)

1. Introduction

The results of Experiments 3-7 suggest further investigation of the roles of aggression, territory, and "possession" of a ♀ in relation to courtship. Braddock (1949) found that prior residence gave platyfish, Xiphophorus maculatus, a greater potential for dominance. Heuts (1968) gathered similar evidence for ♀ Xiphophorus hybrids. In Experiment 8 the experimental design allowed examination of the effects of different numbers of ♂♂ upon the courtship performed to one ♀, the contribution to courtship by each of three ♂♂ with different residency periods with the ♀, and the interactions between the ♂♂.

2. Materials and Methods

Females were selected for tameness and one ♀ was placed in each observation tank at about 1000 hours. In the afternoon a ♂ was added. Observation took place the following morning, for 10 minutes between 0945 and 1335 hours. Another ♂ of the same race was added that afternoon between 1600 and 1845 hours, and the three fish were observed the next morning. This procedure was repeated once more so that a total of three

♂♂ were observed with a ♀ over a three-day period. A control series was run wherein single ♂♂ were observed for three days in the same way as in the experimental trials. A Rustrak event recorder was employed to register displays and chasing, and charges and leaps were written down. No ♂♂ were used more than once. Of 15 trials attempted for each race, 15 P, 15 UA, and 13 G were completed.

3. Results

a. Effects of the different numbers of ♂♂ on the courtship performed.

I hypothesized that if no competition took place between the ♂♂, the total number of displays, chases, and leaps would on all days be a multiple of day 1 of the trial--when only one ♂ was present. Note that there is no deprivation period as in Experiment 7. The assumption that single ♂♂ courted much the same on each of three consecutive days was tested. Guppies whose parents came from both the Diego Martin and Petite Curacaye streams in Trinidad were used due to a shortage of other fish, but my impression is that these findings can be generalized. Sixteen replicates failed to disprove my assumption (Wilcoxon test between each pair of days⁴). In fact, display frequency rose on the third day of the trials (means: day 1, 1.63; day 2, 1.81; day 3, 2.88).

To test the hypothesis, that no competition took place between ♂♂ expected frequencies and durations were derived in the following way. The average frequency or duration of an activity by all ♂♂ on day 1, in

⁴ Hereafter, probability values followed by "Wilcoxon" will refer to the Wilcoxon matched-pairs signed-ranks test, two-tailed.

each race, was added to the frequency or duration of that activity of each individual of the race to give expected numbers for day 2. Similarly, twice the average frequency or duration was added to individual results of day 1 to yield expected numbers for day 3. Thus, if the presence of other $\sigma\sigma$ did not affect another σ 's performance, the expected and observed figures for each day would be equal. Trials in which a given behaviour never occurred were excluded from analysis of that behaviour.

The results are summarized in Fig. 17. All the probability values following are from the Wilcoxon test. Frequency and total duration of displays were significantly below expected on the third day in P and UA $\sigma\sigma$ ($p < .01$), and display duration was also low on the second day in UA $\sigma\sigma$ ($p < .01$). Thus, increasing numbers of $\sigma\sigma$ inhibited these behaviours. G $\sigma\sigma$ showed no evidence of change in displays (Fig. 17a), which means that each σ displayed as if no other $\sigma\sigma$ were present. Both P and UA $\sigma\sigma$ leaped less than expected on the third day (P: $p < .05$; UA: $p < .02$). G $\sigma\sigma$, in contrast, leaped more on the third day (Fig. 17b). Frequency and total time of chasing increased more than expected on each consecutive day in all races (day 2: $p < .01$, with the exception of UA frequency, $p < .05$; day 3: $p < .01$; Fig. 17c). Not surprisingly, increasing numbers of $\sigma\sigma$ enhanced chasing, which appears to be a σ 's attempt to remove a ♀ from the presence of other $\sigma\sigma$.

b. Contributions of different males to the total courtship

If we sum the amount of a given behaviour on one day of the trial and divide that total by the number of participants we arrive at an

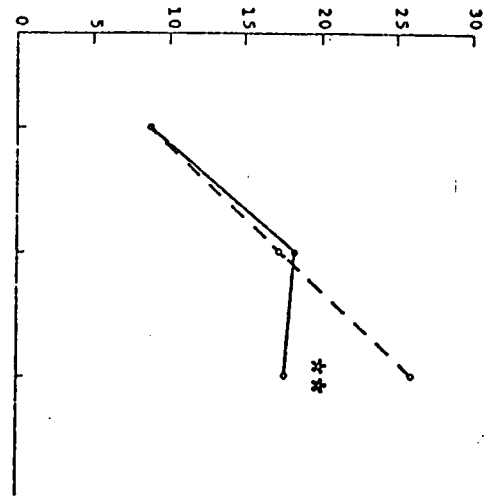
Fig. 17. Comparison of the courtship performed by different numbers of ♂♂ with one ♀. One ♂ was present on day 1, two on day 2, and three on day 3. The darkened circles and solid lines represent observed data and the open circles and dashed lines are expected values. The number of samples for each data point are shown under the race designations. ** signifies $p < .01$ and * signifies $p < .05$. Probability values are derived from the Wilcoxon matched-pairs signed-ranks test, two tailed.

- a. Comparison of mean frequency and duration of displays.
- b. Comparison of mean frequency of leaps.
- c. Comparison of mean frequency and duration of chases.

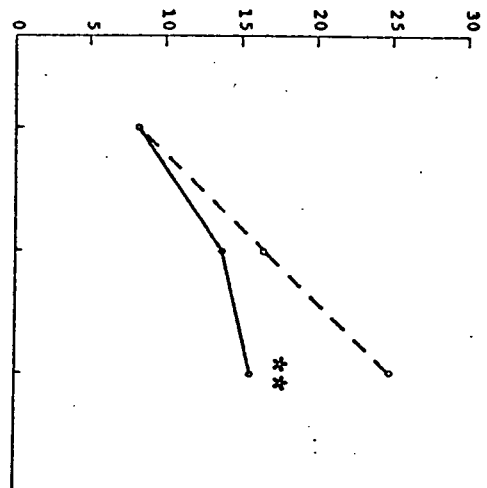
DISPLAYS

P
N = 14

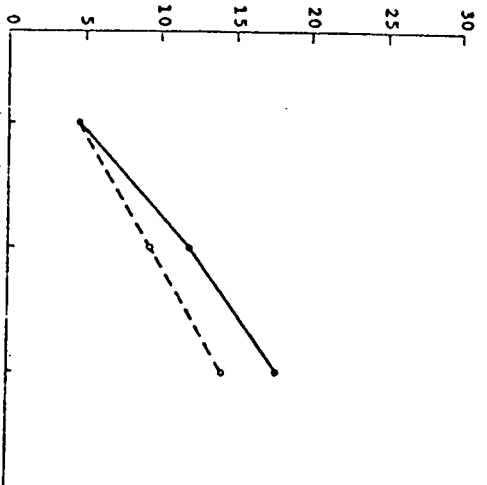
MEAN FREQUENCY



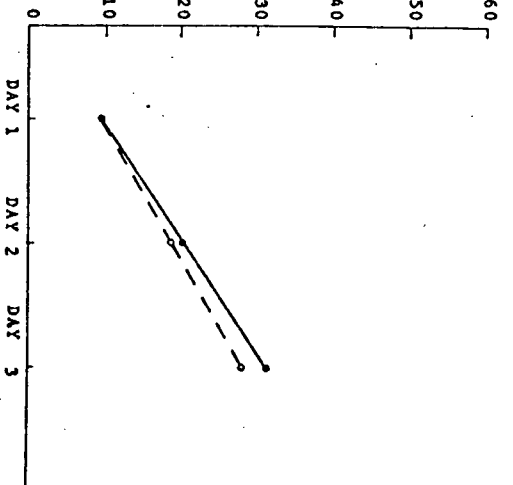
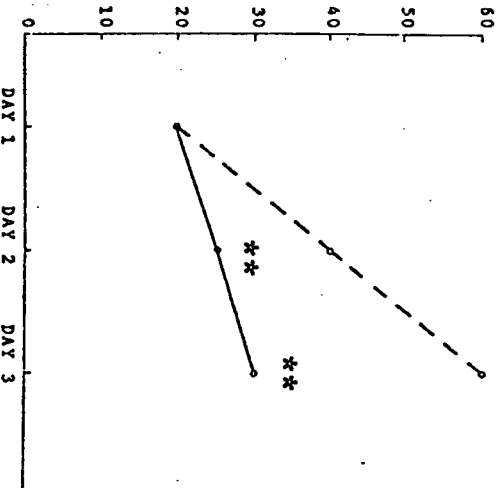
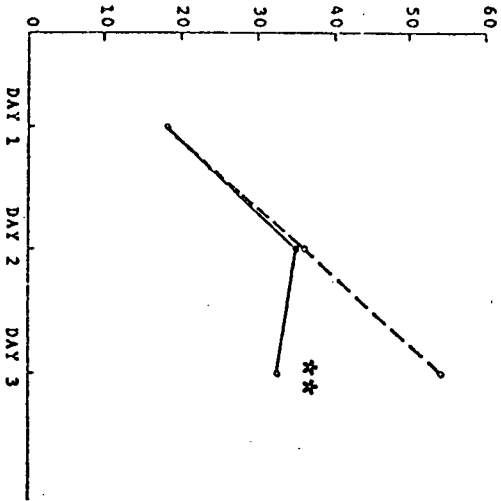
UA
N = 15



G
N = 13



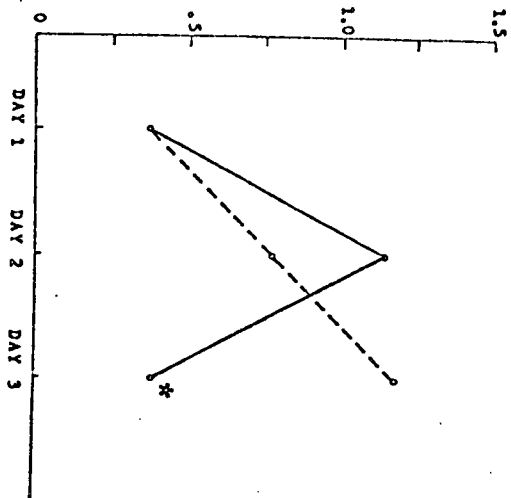
MEAN DURATION (SEC)



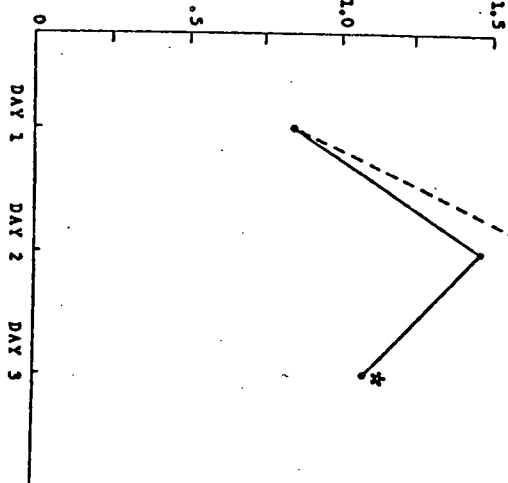
LEAPS

MEAN FREQUENCY

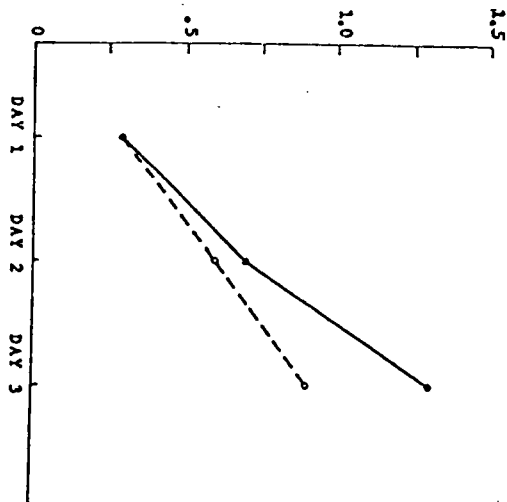
P
N = 13



UA
N = 13



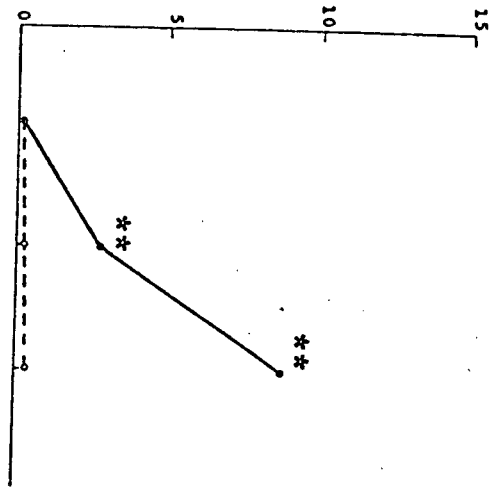
G
N = 10



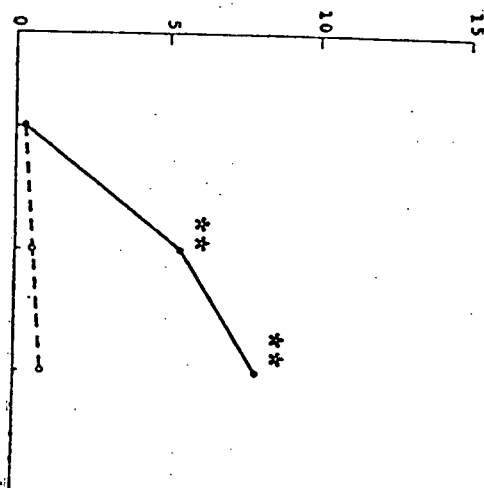
CHASES

P
N = 14

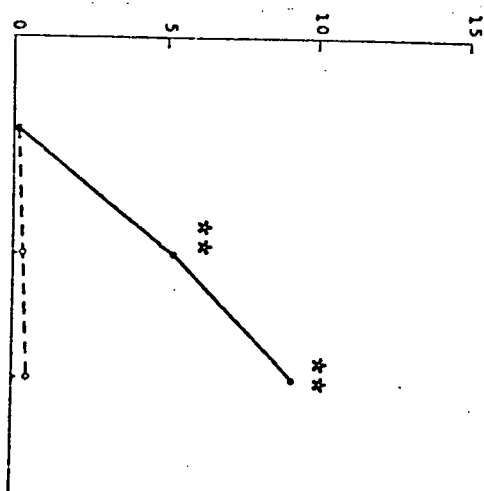
MEAN FREQUENCY



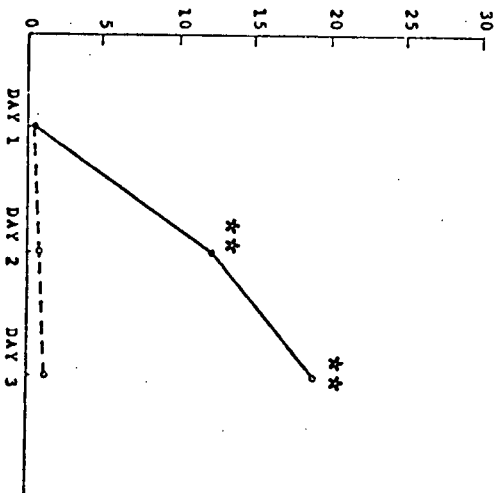
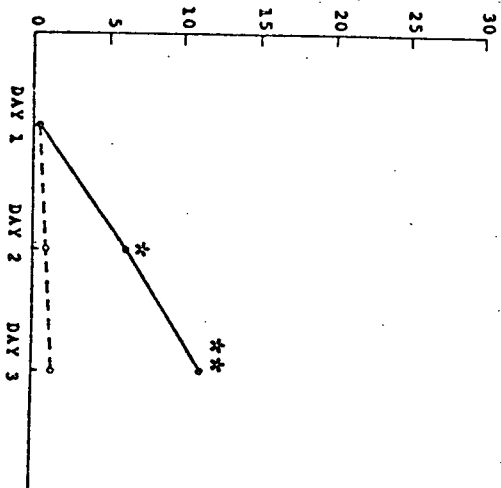
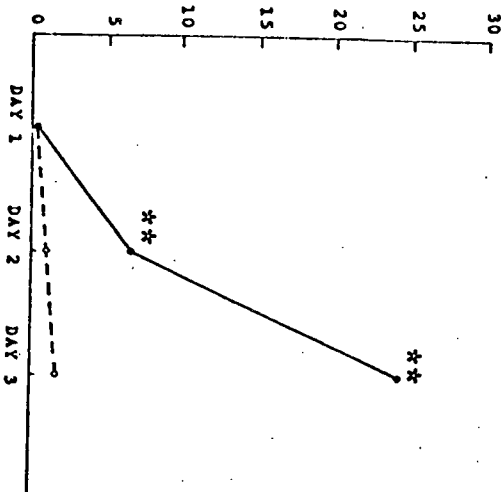
UA
N = 12



G
N = 12



MEAN DURATION (SEC)



expected level of performance for a ♂ if all ♂♂ were contributing equally. If prior residence confers any dominance to the first ♂ we would expect him to exceed the other ♂ or ♂♂ and the average amount of activity toward the ♀. Fig. 18 compares the performances of displays and leaps for all the ♂♂ on each of the three days of the trial. In no case is there a statistically significant indication that the first ♂ had an advantage over the others, or that any of the ♂♂ differed from one another on days 2 and 3. The low frequency of leaps precludes the seemingly large differences exhibited in Fig. 18 from reaching statistical significance. The decreased level of display behaviours observed with increased ♂ density in the P and UA races was therefore not dictated by a territorial or "possessive" ♂, but by some other interactions of the fish.

c. Interactions between the males

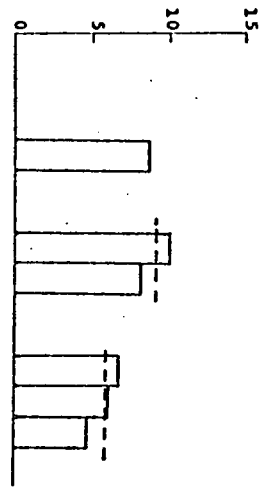
The mechanisms of interactions between ♂♂ were not always clear. I could see no effects of subtle body contacts during chasing and changes in body and fin posture to the ♀ on ♂ behaviour. Fighting was the only activity which obviously changed ♂ - ♂ behaviour. Here, their attentions were totally directed toward one another rather than toward the ♀.

Aggressive behaviour need only be considered in P ♂♂. Only four charges occurred in 750 fish-minutes (number of fish multiplied by observation times where at least two ♂♂ were present) in UA and only two charges occurred in 650 fish-minutes in G; whereas 247 charges occurred in 700 fish-minutes in P (in 11/14 trials). The amount of P aggression in this experiment when two ♂♂ were present (47.86 charges/100 fish-

Fig. 18. Contributions of different ♂♂ to total courtship on each day of the experiment. For each day, ♂ 1 is on the left, etc. Dashed lines represent the average values for all the ♂♂ and the expected value for each ♂. No statistically significant differences appeared. See text for discussion.

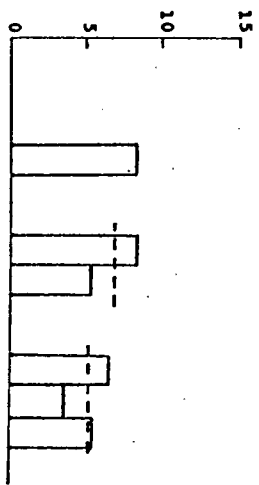
P

N = 14



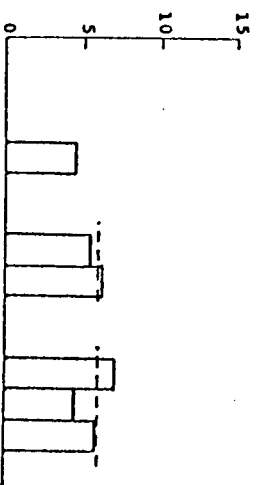
UA

N = 15



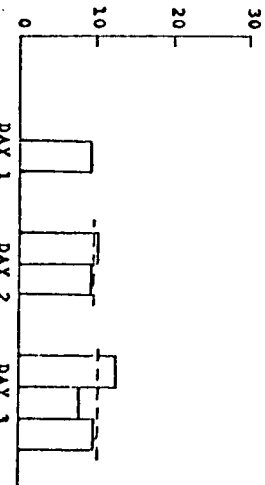
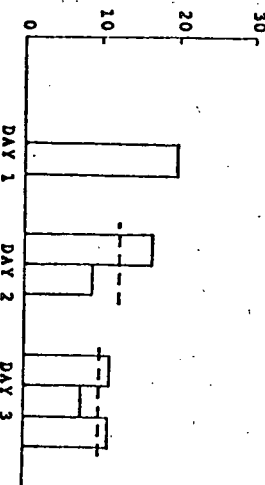
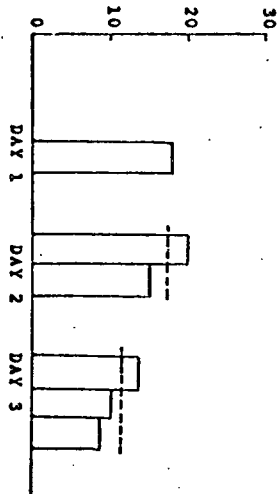
G

N = 13



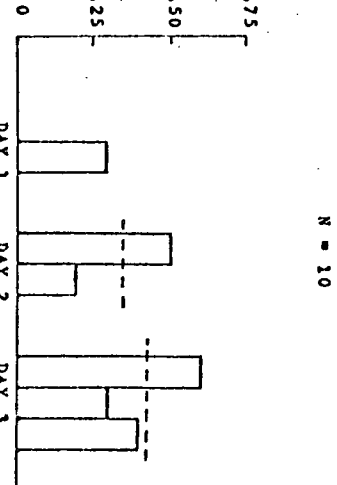
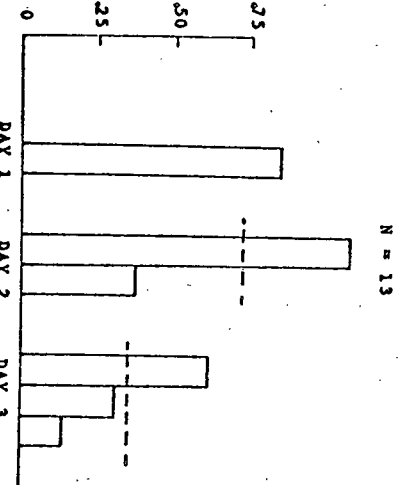
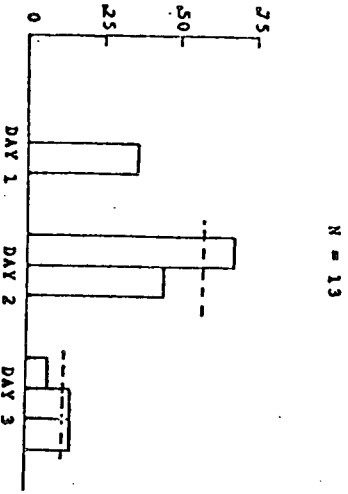
DISPLAYS

MEAN DURATION (SEC) MEAN FREQUENCY



LEAPS

MEAN FREQUENCY



minutes) was markedly lower than in Experiment 7 (140.00 charges/100 fish minutes; $p = .0344$, Mann-Whitney U test corrected for ties). Apparently familiarity with the other ♂ and the ♀ reduced the level of aggression. Friedman two-way analysis of variance shows no differences in the frequency of charges between P ♂♂. In the nine trials where charging occurred on day 2, first ♂♂ out-charged second ♂♂ by nearly three to one (92-32), but this difference is statistically insignificant (Wilcoxon) because one ♂ charged 49 times. On day 3 first and second ♂♂ charged equally (27 and 24 times), while third ♂♂ charged more (72 times). Again, differences were not statistically reliable. No consistency appeared in who charged whom between first and second ♂♂ on the two days they were observed together. In addition, charges did not bear any relation to displaying in any of the races.

However, one ♂ of the first two introduced performed more display behaviour than the other on both the second and third days of the experiment. The likelihood that this results by chance are slim. (Table 13). The results of experiments 1 and 7⁵ suggest that interactions are essential in determining which ♂ displays most. This experiment suggests that the results of these interactions are stable for at least a two-day period. Remember that fish were not separated from one another in this experiment.

The effects of size on ♂-♂ interactions appear to be negligible. Length (P, $\bar{X} = 17.2$ mm; UA, $\bar{X} = 18.8$ mm; G, $\bar{X} = 18.2$ mm) was significantly correlated with displaying in several instances, mainly in P and UA, but

⁵No relation existed between the amount of displaying of single ♂♂ in non-competitive and competitive situations.

Table 13. Relative amount of display behaviour of first and second ♂♂ on days 2 and 3. Probability values are derived from the binomial test.

Race	Total No. of Trials	No. of trials in which same ♂ displayed most both days	No. of trials in which same ♂ did not display most both days	
P	13	11	2	p = .011
UA	14	10	4	p = .090
G	12	9	3	p = .073

Race	No. of Trials in which leaps occurred	No. of trials in which ♂ who displayed most both days also leaped most	No. of trials in which ♂ who displayed most both days did not leap most	
P	11	9	2	p = .033
UA	5	5	0	p = .031
G	6	5	1	p = .109

did not correlate with the ♂ who displayed most on the third year. Leaps and charges bore no relation to length.

d. Comparison of the races

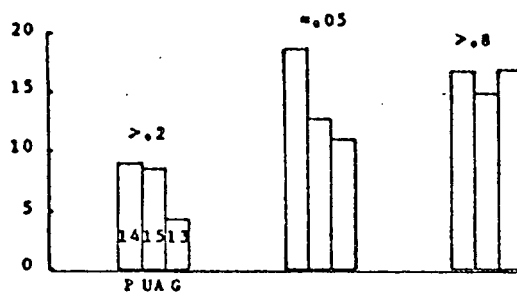
Kruskal-Wallis one-way analysis of variance was employed to compare the behaviour of ♂♂ of the three races over the three days. Fig. 19 summarizes the comparison of displays, leaps, and chases. On day 1, G ♂♂ displayed less than the other ♂♂, but not significantly less as in Experiment 1 (this was probably owing to the shorter observation time). UA ♂♂ performed more leaps than the other ♂♂ ($p < .05$), a difference not detected in the conditions of Experiment 1. All ♂♂ spent about the same amounts of time chasing ♀♀ ($p > .2$), but UA ♂♂ chased more often than the others ($p < .01$).

The total time displaying doubled in P and G ♂♂ on the second day, but increased only slightly over day 1 in UA ♂♂. P spent the most time displaying ($p < .05$). The number of displays did increase in UA ♂♂, but P ♂♂ doubled and G ♂♂ more than doubled their display frequencies. P ♂♂ displayed more frequently and G ♂♂ displayed less frequently ($p = .05$). The mean display bout length of first ♂♂ in P and G remained at about two seconds on each of the three days, but dropped from 2.8 seconds on day 1 to 1.9 and 1.8 seconds on days 2 and 3, respectively, in UA ($p < .05$, Friedman two-way analysis of variance). All races increased their frequency of leaps on day 2, P most and UA least, but G ♂♂ leaped less ($p < .05$). These results contrast with those of Experiment 7, where in a two-♂ situation frequencies of displays and leaps were reduced when

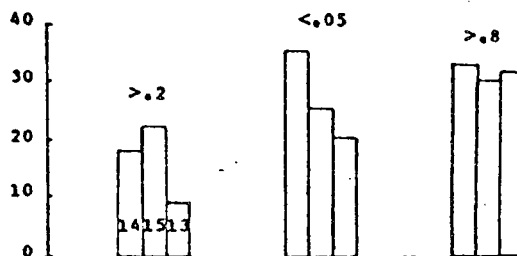
Fig. 19. Comparison of the courtship performed by ♂♂ of the three races on each of the days of the trials. Each group of three histograms represents P, UA, and G ♂♂, in that order. Kruskal-Wallis one-way analysis of variance probabilities appear above the histograms. Sample sizes are shown on the first group of histograms for a given behaviour.

DAY 1 DAY 2 DAY 3

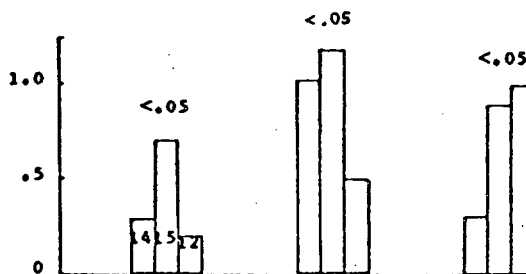
a. MEAN DISPLAY
FREQUENCY



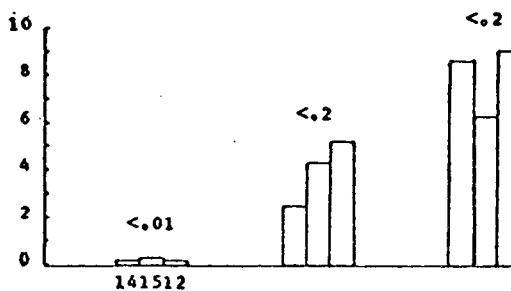
b. MEAN DISPLAY
DURATION (SEC)



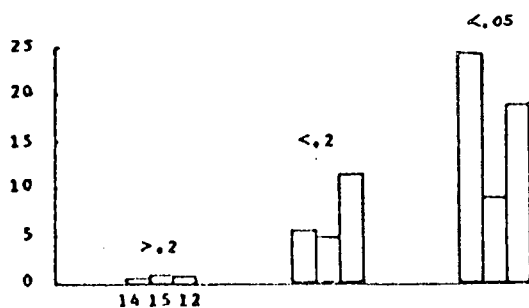
c. MEAN LEAP
FREQUENCY



d. MEAN CHASE
FREQUENCY



e. MEAN CHASE
DURATION (SEC)



compared to the one-♂ situation of Experiment 1. Furthermore, it was the P ♂♂ who reduced these behaviours the most. The differences are that in the present experiment no period of isolation was imposed upon the ♂♂, one ♂ was with the ♀ longer than the other, and the ♂♂ had the opportunity to learn about one another and the ♀. G ♂♂ tended to do more chasing ($p < .2$). No difference in chasing were found in Experiment 7.

By the third day differences in displaying disappeared ($p > .8$). P ♂♂ did not display more than on the second day, UA ♂♂ displayed only slightly more, and G ♂♂ displayed more, in proportion with the increase from day 1 to day 2. Leaps differed, but P ♂♂ leaped less than the other races ($p < .05$). The pattern of change of leaping differed quite strikingly. Two P ♂♂ together leaped much more frequently than one, but three P ♂♂ leaped with the same frequency as one. The amount of leaping of UA ♂♂ on the third day was mid-way between the scores of days 1 and 2. G ♂♂ simply doubled their average number of leaps on consecutive days. UA ♂♂ spent less time chasing on day 3 ($p < .05$) and tended to chase less often ($p < .2$). The addition of the third P ♂ induced a very sharp upward surge in chasing when compared to the previous days, and G ♂♂ chased more in direct proportion to their increase in numbers over day 2.

As noted in part c above, the presence of aggressive actions in P and the absence of it in UA and G are of an almost all or nothing nature.

Differences in behaviour of individual ♂♂ between the races are mentioned in parts a, b, and c of this experiment and generally follow the differences discussed above.

H. Summary of the male's courtship

1. The behaviour of single males

P and UA ♂♂, through behaviour and color patterns, are probably more obvious than G ♂♂ in courtship. P and UA ♂♂ displayed more than G ♂♂, but distance from the ♀ during display was the same and close proximity to the ♀ was mandatory to initiate display in all races. P ♂♂ orientated at greater distances and at greater angles relative to the ♀ and moved above her and displayed there more frequently. G ♂♂ positioned themselves at more acute angles above and below the ♀, and, while displaying, changed positions relative to the ♀ least often. UA ♂♂ came closer to a ♀ while displaying, chased her more often, and leaped more than the other races when there was a longer introductory period with a ♀. P ♂♂ had a longer and G ♂♂, a shorter, latency to display. G ♂♂ paid the least attention to ♀♀ at short enforced distances from them. Gonopodial swings and thrusts were similar for all races.

2. The behaviour of two or more males

Neither previous experience with a ♀, previous performance in a one♂-one♀ situation, prior residence, nor size correlated with a ♂'s performance in the presence of other ♂♂. However, a consistency existed in one ♂'s courtship behaviour in relation to another ♂.

P ♂♂ were much more aggressive than UA and G ♂♂ upon encountering other ♂♂. Increased aggressiveness occurred in the presence of ♀♀, suggesting that ♂♂ fight over ♀♀. P ♂♂ who were charged tended to display less when first encountering one another, but this relationship

did not hold after some time together, and the level of aggression dropped.

Pairs of P ♂♂ courted and displayed more than pairs of UA and G ♂♂ after an afternoon and night with a ♀. When only the top courters of each pair were considered, P ♂♂ courted more. Two ♂♂ introduced to one another and a ♀ after an isolation period displayed and leaped less than they did individually with a ♀. However, if two ♂♂ had time to interact prior to observation, no decrease was noted. The addition of a third ♂ with time for learning led to less displaying and leaping than expected in P and UA ♂♂ but not in G ♂♂.

My impression is that ♂♂ usually attempt to maximize the amount of attention they receive from the ♀ before displaying, and the presence of another ♂ or ♂♂ made this difficult. Upon being introduced to one another, all ♂♂ moved closer to the ♀ to the extent that interracial differences disappeared. One or both ♂♂ were within a fish-length of the ♀ for almost half the time of the trial in the two-♂ situation, and one, two, or three ♂♂ of different races remained close even longer. P ♂♂ decreased their frequency of thrusts greatly in the presence of a second ♂, whereas UA and G ♂♂ increased theirs slightly over the single ♂ situation. Smaller G ♂♂ displayed and thrust more than larger ones. UA ♂♂ tended to display more and more closely to the ♀. More ♂♂ meant more chasing in all races.

There were no interracial differences in display frequency when three ♂♂ were present (see Experiment 9, whether of the same race or not). In three-♂ situations P ♂♂ leaped less than other races, and G ♂♂ thrust more. G ♂♂ behaved almost as if no other ♂♂ were present.

Table 14 summarizes morphological, behavioural, and environmental differences between the races by ranking each race from 1 to 3 ($1>2>3$) for each parameter considered.

Table 14. Summary of morphological, behavioural, and environmental differences between the races. Rank 1>2>3. An asterisk after a race denotes a statistically valid difference between that race and the others. More than one asterisk means that the races marked differ significantly.

Rank	1	2	3
Parameter			
Size	UA*	P*	G*
Brightness	P and UA		G
Sex ratio	P*	G*	UA*
Water clarity	P and UA		G
Predators	G	UA	G
Schooling	G*	UA	P
% time close proximity to ♀	G*	UA*	P*
Angle of orientation	P	UA	G
Position changes during courtship	P	UA	G
Bouts of sigmoid display	UA	P	G*
Latency to display	P*	UA*	G*
Attentiveness to ♀ at a distance	UA*	P	G*
Charges between ♂♂	P*		UA and G
Reduction in display and contact behaviours in the presence of a second ♂ (after isolation)	P*	G	UA
Leaps with 3 ♂♂ present	G	UA	P*
Thrusts with 3 ♂♂ present	G*	P	UA

Chapter 5. Discussion of Male Courtship Strategies

It is tempting to assume that behaviour patterns have evolved in the same manner as other features of animal populations, by the selection of inheritable differences.

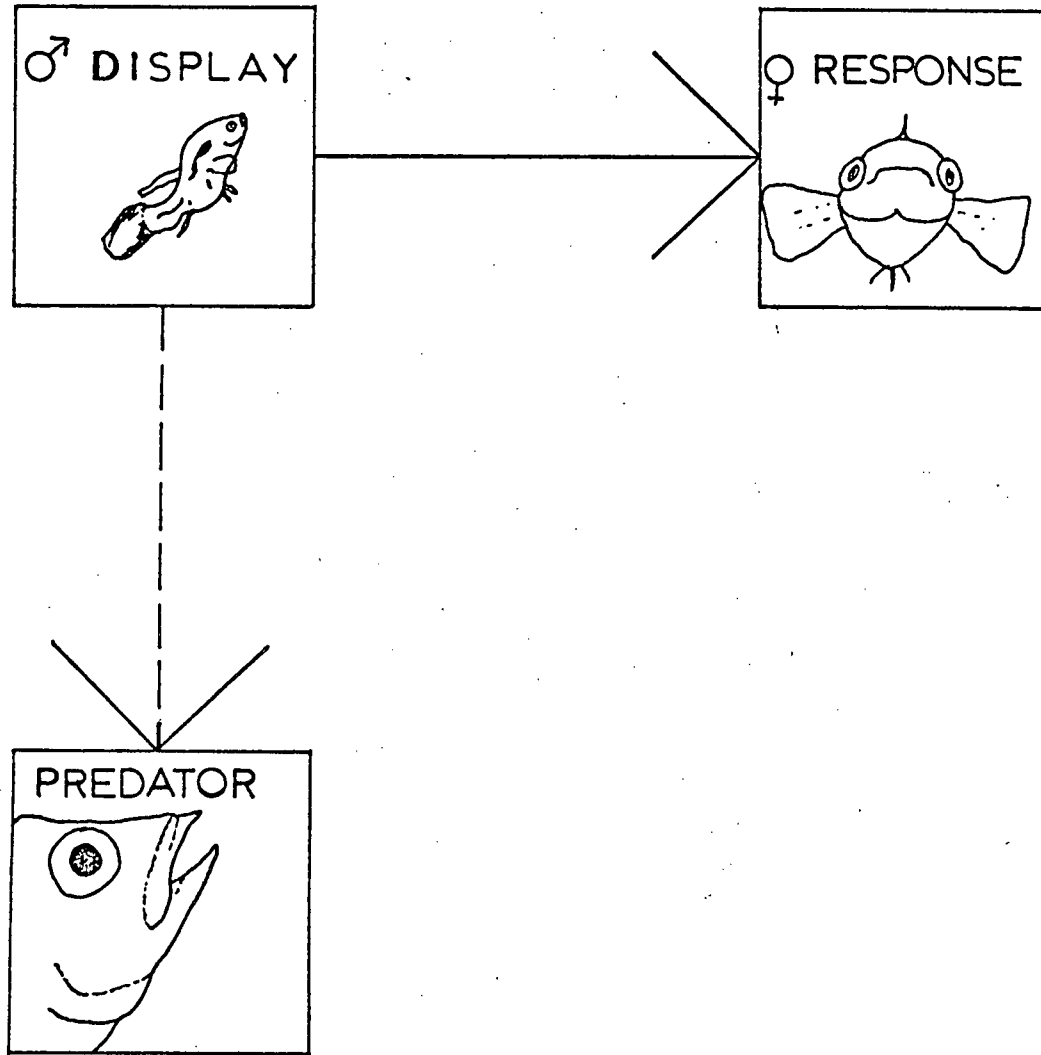
It is impossible to be certain, however strong the implication about the evolution of behaviour, unless one can demonstrate the existence of "genetic" variations in behaviour upon which selection could act.

Bastock (1956)

The field and experimental evidence gathered by Seghers (1973) provides strong arguments in favor of the adaptive nature of antipredator strategy, and it seems likely that the same forces have effected the evolution of courtship behaviour. First consider the behaviour of single ♂♂ in reference to the simple model presented in Fig. 20. It is safe to say that the ♂'s courtship behaviour is goal directed to copulation with a ♀. To achieve this goal maximum conspicuousness, with behaviours such as displays, is probably optimal. If, however, the environment includes the eyes of piscivores as well as those of guppy ♀♀, there must exist an evolutionary trade-off in conspicuousness. Quite simply, the greater the predator component of the model is, the less conspicuous can the behaviour of the ♂ guppy be. Conversely, reduced predation pressure allows for maximization of ♀-attracting activities.

The guppies studied fit the model well. G ♂♂ are not as quick to display and are less conspicuous in display behaviour, utilization of space, size, and color than are P and UA ♂♂. Seghers' findings suggest

Fig. 20. A simple model showing factors influencing conspicuousness.



that color is not important in prey selection by guppy predators. The relatively dull coloration of G ♂♂ probably is associated with the turbid waters which they inhabit (see Chpt. 7). These guppies also show a genetic preference for the shallow waters near shore. If the ♂♂ fixed their attention on ♀♀ at relatively large distances, not only might they lose sight of them, but they might find themselves over deeper waters where predators lurk. On the other hand, in the streams where predation pressure is relatively low, the ♂♂ exhibit elaborate display behaviour, display readily, utilize more space (which may also be a function of the clear water), and are larger and brighter.

The display itself and the distance from the ♀ at which it is performed seem conservative components in the ♂'s behaviour repertoire, apparently not as easily affected by selective pressures as most other courtship activities. Observations of display behaviour and distance in turbid water would be instructive.

The model fits well with the ideas of Giesel (1972), who suggests that the potential rate of evolution in species where ♂♂ suffer higher mortality than ♀♀ may be greater than expected from existing theory. He proposes that such species should have greater niche breadth, be better able to track short-term environmental changes genetically, and be more efficient colonizers.

It may be wise to emphasize that all three races of ♂♂ perform all the same behaviours, and that the major differences between them are quantitative. Similarly, Seghers finds antipredator adaptations to differ quantitatively. P fish hardly respond to predators while G fish are

highly responsive to them. UA guppies fall between these two extremes, as they did with many of the behavioural measures I employed.

Moving closer to the ♀ in the presence of other ♂♂ seems to be a way in which a ♂ gains the ♀'s attention and minimizes the attention she can get from other ♂♂. It is probably significant that G ♂♂ thrust more than the competitors in the presence of others. Thrusts may occasionally result in insemination and thus allow a ♂ to transmit his genes without endangering his survival as much as by displaying. In addition, the low visibility in the Guayamare might allow more of these thrusts to result in success than would the clear waters of the other two streams. Insemination through thrusts probably does not involve cooperation by the ♀ and a ♂ in turbid water would probably have a better chance of approaching a ♀ unnoticed. Also, smaller G ♂♂ thrust and displayed more than larger ones. Seghers (1973) has evidence that smaller ♂♂ are better at avoiding large predators. Perhaps smaller G ♂♂ can "afford" to be more obvious.

The flurry of activity when several ♂♂ encounter a ♀ is quite conspicuous, even though the total space occupied by all the fish at one moment may be less than that occupied when a single ♂ and ♀ are interacting. Perhaps the group of fish give a schooling effect, i.e., make it difficult for a predator to single out an individual for attack. It may also be that there are more eyes with which to spot a predator, but the ♀ seems to have the ♂♂'s undivided attention. Observations in the field could contribute much information to clarify this problem.

Other differences emerge in situations where more than one ♂ is present. For instance consider the aggressive behaviour of P ♂♂. Many

P ♂♂ even have a slight spike, outlined in black, on the ventral edge of the caudal fin, and exhibit a "backing" motion reminiscent of sword-tails, where the spike functions in aggression between ♂♂ (Hemens, 1966). Fighting is a strategy which has evolved here to further a ♂'s chance of successful insemination. It is also a behaviour which makes a fish very conspicuous, so we can understand why it is not often seen among G ♂♂. But why do P ♂♂ fight so much more than UA ♂♂? I believe that we can turn to Rivulus for the answer. Seghers found that Rivulus density in the Paria was very low, with few large individuals. This is probably the reason for sex ratio differences: P, 1.14, and UA, .48. The Paria had the highest ratio of ♂♂ to ♀♀ of 19 streams which Seghers checked, and a very dense guppy population. Could the low predator pressure and increased competition between ♂♂ have opened the gates for aggression? Has aggression emerged as a population control device in the absence of predation (cf. Breder and Coates, 1932)? The latter hypothesis can be tested.

Social status may greatly influence insemination success within a population. Using different genetic types, but controlling for the differences, Gandolfi (1971) finds that the dominant ♂ of a pair leaves far more offspring than the subordinate. Though there are no success statistics, dominant ♂♂ who exhibited their ranks by chasing other ♂♂ away from ♀♀ in both Gambusia heterochir (Warburton et al., 1957) and G. hurtadoi (McAlister, 1958) were more intensely colored. Perhaps competition is a clue to the color polymorphism in guppy populations (see Chapt 7 and Appendix 1). As with the guppies, size was not correlated with aggressiveness in the latter two studies. In Mollienesia (= Poecilia) latipinna

larger ♂♂ dominated smaller ones. Males were more aggressive when courting, but levels of aggression dropped when several ♂♂ were thrusting or attempting copulation (Parzefall, 1969). There seems to be a similar relationship between courtship and aggression in mollies and guppies (also see Baerends et al., 1955). The absence of obvious stable dominance hierarchies in guppies may be related to a comparatively low level of aggression (Collins et al., 1967).

Contrary to my results and those of Henderson (unpubl.), Farr (1972) finds an increase in display behaviour from the one ♂-one ♀ situation to two ♂♂ -one ♀ to two ♂♂ -two ♀♀ . In higher densities up to 15 pairs, displays per individual remained constant. Henderson found a decrease in reproductive activity at higher densities. I believe these differences emphasize the need to examine and compare populations originating from different ecological circumstances.

Results of the competition experiments suggest that G ♂♂ change their behaviour relatively little in the presence of other ♂♂ . Perhaps much of the evolution of the brighter colors in P and UA ♂♂ can be explained by inter-♂ competition (see Chapt. 6 & 7). In addition, results in ~~Chapter 4~~ and Experiment 9 in the next chapter suggest that ♂♂ of each race might have a dominant mating strategy. G ♂♂ thrust, P ♂♂ fight, and UA ♂♂ have particularly effective displays.

Variability within populations must be mentioned along with variation between populations (see also Barlow, 1961). For most behaviours, measurements from individuals from all the races overlap almost completely. To quote Lindsey (1962): "Perhaps, within a species, each population can carry in its pool of built-in variability the potential to alter rapidly

so as to resemble any other population, given only the appropriate selective pressures acting on alleles already present". The ideal way in which to ascertain this would be to take a Trinidad stream devoid of fishes, screen off sections, and introduce various guppy populations and various predator populations in the appropriate combinations, with controls. There are such streams available. Behavioural and morphological sampling could take place periodically. If Trinidad could not be used, predator complements could be added to large aquaria (see Seghers, 1973:176) and observations could be made on which guppies were most readily preyed upon.

Chapter 6. The Consequences of Geographic Isolation

A. Introduction

Like most researchers working with geographically isolated populations, I was interested in the possibility of occurrence of reproductive isolation between races. Reproductive isolation is first, presumedly, a by-product of the accumulation of genetic changes between diverging races. When effective reproductive isolation occurs, races become species (Dobzhansky, 1970: Chpt. 11). Many borderline cases between "races" and species have been examined by Mayr (1963). Examples are the descriptive and experimental studies of reproductive isolation between "incipient species" or "semispecies" of fruit flies, undertaken by Dobzhansky and his students (see Chapter 7). In Chapter 6 I sought to determine to what extent divergence in courtship behaviour of the guppy has evolved by involving fish of all three races in experiments together.

B. Breeding between the races

All possible crosses between the races (4 wild-caught ♂♂ placed with 4 virgin ♀♀) produced viable, fertile offspring. The range of variation within each race would have made many replications necessary to establish heritability coefficients of behaviours for the "hybrid" offspring. Lack of space (for 6 "hybrid" groups plus 3 non-hybrid control groups) precluded such a behavioural analysis. Examination of ♂ color patterns suggested that most of the offspring were sired by one ♂. Generally, the ♂♂ of the more brightly colored races left more colorful ♂ offspring. In addition to the predominance of Y-linked color patterns (see Winge, 1922a, b, 1927), the P ♂♂ seemed to contribute a lot of color to their ♂ young

(e.g., G♂ x P ♀ Fl ♂♂ were as bright as P ♂♂). Atz (1962) found that reciprocal crosses of several Xiphophorus species did not always produce similarly pigmented offspring (see also Kallman, 1970b).

I attempted to determine comparative reproductive success of by comparing color patterns of potential fathers and ♂ offspring. Six ♂♂ of each race were placed with six virgin ♀♀ of a single race for two weeks. The ♀♀ were then removed to individual quarters in 4-liter jars, where they dropped young. Unfortunately, space was insufficient and not enough young were raised to permit conclusions to be drawn. However, the method of following ♂ parentage by color phenotype seems reasonably accurate. Individual ♀♀ delivered young from more than one ♂, but results suggested that one ♂ was responsible for most of the offspring of one ♀, (see Hildemann and Wagner, 1954). Further experiments in this area could be important in elucidating some factors of population structure as well as interracial competition.

C. Inter-population competition in courtship (Experiment 9)

1. Introduction

The major question asked in this experiment was: does one race of ♂♂ with its particular combination of morphology and behaviour stand a greater chance of reproductive success than another race when competing for the same ♀? The results are presented in two parts: one concerning the effects of interracial competition on the ♂♂'s behaviour, and the other concerning the responses of the ♀♀ to the ♂♂'s behaviour.

2. Materials and Methods

For each trial one ♂ of each race, after an isolation period of about two days, was introduced into the long aquarium, arranged as illustrated in Fig. 21. After the ♂♂ had about ten minutes of exploration time, a virgin ♀ was introduced to them. Seven ♀♀ of each race were observed for a total of 21 independent trials. A trial lasted 30 minutes or until copulation took place. The CDAT was employed to record frequency and duration of behaviours and the identity of the actor in the case of the ♂♂ and response behaviour and the identity of its elicitor in the ♀. Male behaviours recorded were display, leap, thrust, copulation and which fish was closest to the ♀. In addition; aggression, jerks, and other noteworthy events were spoken into a tape recorder.

3. Results

a. Male behaviours

Very intense ♀-oriented activity commenced when the highly responsive virgin ♀ was presented with previously isolated ♂♂. Only one copulation occurred (G♂ and UA ♀), however, despite the ♀♀'s receptivity. The reason was obvious: as soon as one ♂ displayed to a ♀ and elicited a response, another ♂ closer to her attempted copulation--the displaying ♂ then rushed toward the ♀ and disrupted the attempt of the other ♂, with no copulation ensuing. This sequence of events was repeated many times in each trial.

It appeared that the ♂♂ showed no racial discrimination toward one another--they behaved as they would have to members of the same race. The comparison of races, lumping all the ♀♀, closely resembles that of the

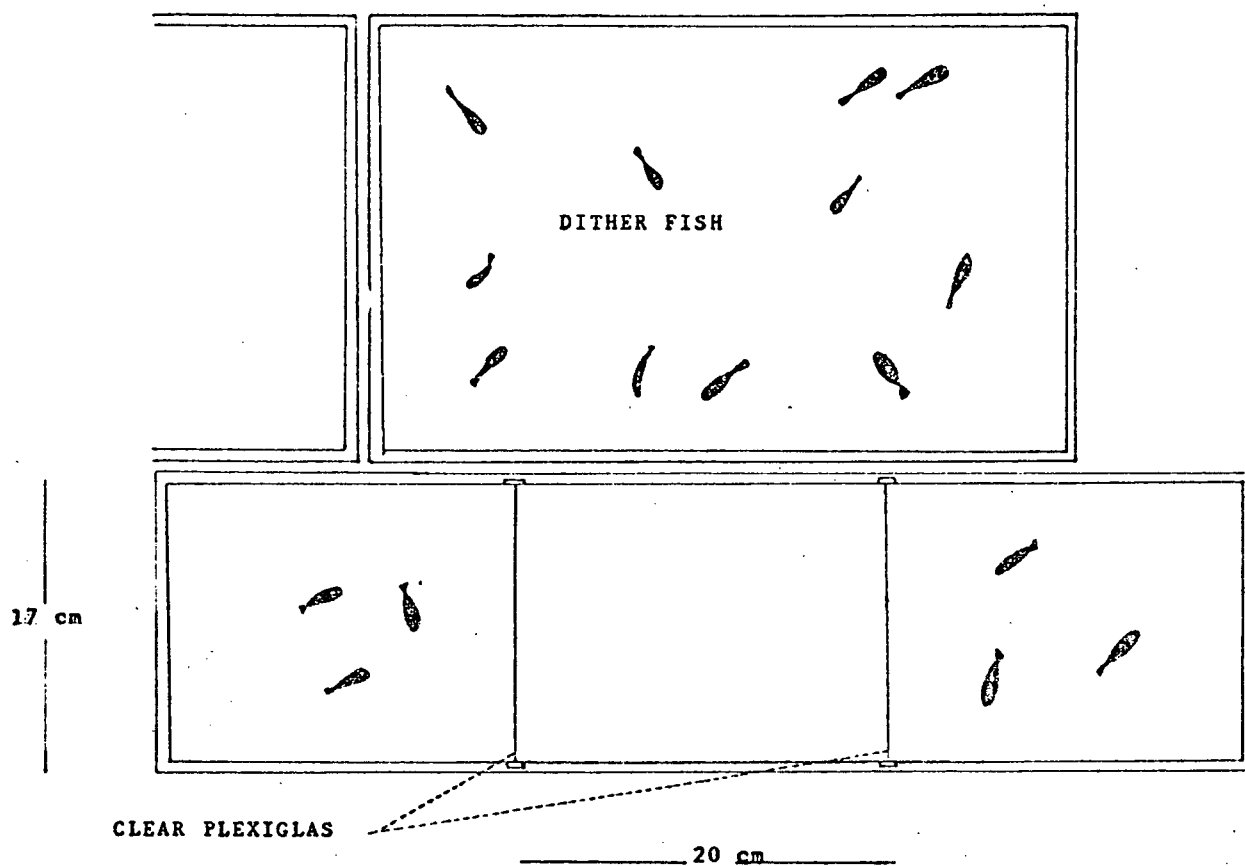


Fig. 21. Experimental set-up for experiment 9. Three ♂♂, one of each race, were placed in the empty compartment along with a virgin ♀. Dither fish were visible to the experimental fish on all sides but the observer's.

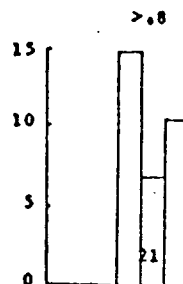
three-♂ situation of Experiment 8: the frequency and duration of displays did not differ and P♂♂ tended to leap less than other ♂♂ (Fig. 22a, b, and e). Bouts of display tended to be longer in UA ♂♂, however (Fig. 22c). The relatively low frequency of displays (7-15 in 30 minutes for all ♂♂ as opposed to 4-16 in 15 minutes for all single ♂♂ in Experiment 1) probably arose from the vigor of the ♂♂'s pursuits, which kept the ♀♀ moving rather quickly and thereby made it difficult for the ♂♂ to position themselves for display. The relatively low display frequency of UA ♂♂ may be related to their performance of jerks in six trials (G,2; P,0). Although jerks are commonly associated with successful copulation, no UA ♂♂ copulated successfully. The jerk may be a "consummatory" sort of activity and have a diminishing effect on further courtship. G ♂♂ thrust more often than other ♂♂ ($p < .01$, Friedman two-way analysis of variance;⁶ Fig. 22d). Males of any one race were not consistently closer to the ♀♀.

The relative infrequency of aggression on the part of P ♂♂ was initially puzzling. Charges occurred in only four of the 21 trials. Closer examination of the data revealed that all four of these trials involved P ♀♀. Furthermore, P ♂♂ engaged in another aggressive behaviour in four trials, two of these with P ♀♀ (no overlap with trials in which charges occurred). These fish pushed their tails in another ♂'s face, an action which sometimes precedes tail-beating or an attempted bite, and usually results in the other fish moving away. Thus, we find that some

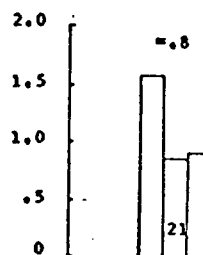
⁶Probability values throughout this experiment arose from the Friedman two-way analysis of variance, 2df.

Fig. 22. Comparison of the courtship performed by three ♂♂ one of each race, to single virgin ♀♀ of all races. Each group of histograms represents the behaviour of ♂♂ from the P, UA, and G races, in that order. Probability values derived from the Friedman two-way analysis of variance appear above the histograms. Sample sizes appear on the histograms.

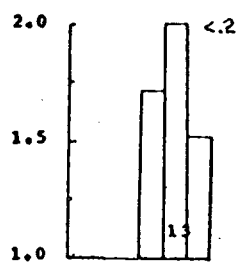
a. MEAN DISPLAY FREQUENCY



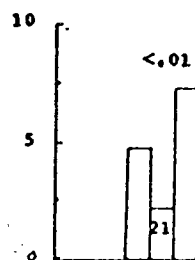
b. MEAN DISPLAY DURATION
(% TIME OF TRIAL)



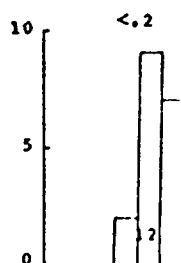
c. MEAN DISPLAY BOUT
LENGTH (SEC)



d. MEAN THRUST FREQUENCY



e. NUMBER OF TRIALS IN
WHICH LEAPS OCCURRED



form of recognizable P ♂ aggression took place in six of the seven P ♀ trials and two of the fourteen trials with other ♀♀ . The only other aggression was from a UA ♂ in the presence of a UA ♀ .

The possibility of some selectivity taking place between ♂♂ and ♀♀ prompted the examination of the ♂♂'s behaviour to ♀♀ of each race separately. P ♂♂ displayed to their own ♀♀ more than the other ♂♂ in five of seven trials ($p < .1$). Males tended to exhibit higher frequencies, durations, and average bout lengths of display than other ♂♂ to ♀♀ of their own race, with the exception of G ♂♂ (Fig. 23a, b, and c). G ♂♂ thrust more than other ♂♂ to their own ♀♀ ($p = .02$; Fig. 23d). Few leaps occurred, but P ♂♂ leaped only with P ♀♀ . P ♂♂ tended to spend the most time as the closest ♂ to P ♀♀ (Fig. 23a). My impression is that (1) there was an affinity between P fish, and (2) if UA and G ♂♂ did not show a preference for their own ♀♀ , they showed an avoidance of P ♀♀ (Fig. 23e).

No comparative score of success (insemination) could be obtained in this experiment because the intense competition between the ♂♂ prevented copulation. However, it seems reasonable to suggest that the ♂ exhibiting the greatest amount of courtship behaviour and remaining closest to a ♀ has the highest probability of success.

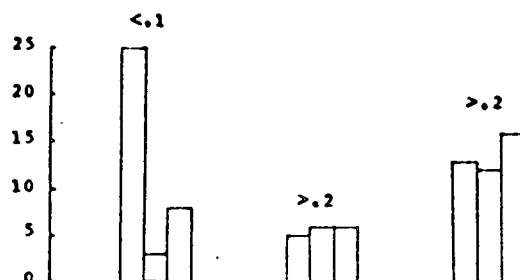
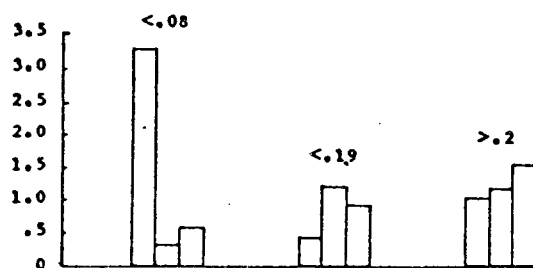
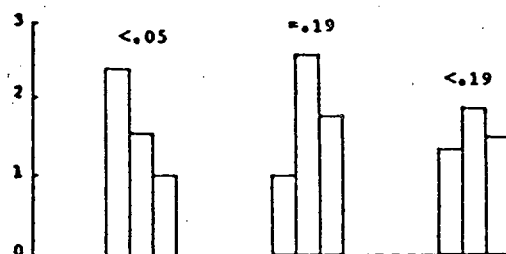
b. Females as recipients of ♂ behaviours

It seems likely that ♂♂ receive some cues from ♀♀ which increase their propensity to court them. Certainly ♂♂ are much more responsive to virgin ♀♀ than non-virgin ♀♀ , even if no apparent overt sexual response on the ♀♀'s part is involved. Compare the following results, where

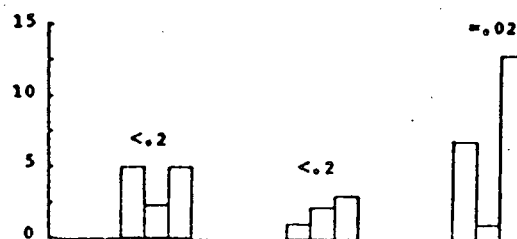
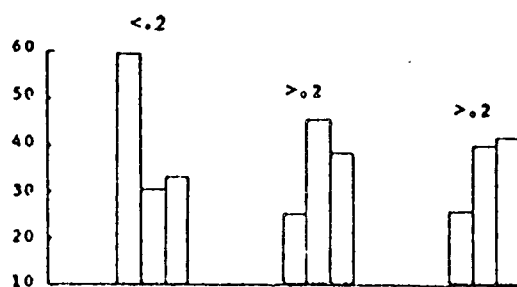
Fig. 23. Comparison of the courtship performed by three ♂♂, one of each race, to virgin ♀♀ of each race. Each group of histograms represents the behaviour of ♂♂ from the P, UA, and G races, in that order. Probability values derived from the Friedman two-way analysis of variance appear above the histograms. N = 7 for each race.

TO P
♀♀TO UA
♀♀TO G
♀♀

a. MEAN DISPLAY FREQUENCY

b. MEAN DISPLAY DURATION
(% TIME OF TRIAL)c. MEAN DISPLAY BOUT
LENGTH (SEC)

d. MEAN THRUST FREQUENCY

e. MEAN TIME CLOSEST TO ♀
(% TIME OF TRIAL)

applicable, with the ♂ competition in Fig. 23.

P ♀♀ were closest to P ♂♂ for a greater percentage of time and greater average lengths of time than UA and G ♀♀ ($p = .06$ and $.05$, respectively; Fig. 24a and b). Although the other ♀♀ were closest to their own ♂♂ slightly more of the time, differences were insignificant. Both P and G ♀♀ were approached most closely more often by P and G ♂♂ than were UA ♀♀ ($p = .07$ and $.06$, respectively; Fig. 24c).

Females received more thrusts from ♂♂ of their own race ($p < .02$, χ^2). However, these results arise largely because G ♀♀ were thrust at more by their own ♂♂ than others ($p = .02$). UA ♀♀ tended to receive fewer thrusts from P ♂♂ than did the other ♀♀ ($p = .09$; Fig. 24d). G ♂♂ thrust more to all ♀♀ and G ♀♀ were thrust at more by all ♂♂ ($p < .01$, Friedman, and $< .05$, Kruskal-Wallis, respectively; Fig. 24e).

Bouts of displays were received more from members of the same race than from others ($p < .01$, χ^2), but close associations between ♂ and ♀ P guppies and ♂ and ♀ G guppies seemed to account for this. P ♀♀ received longer displays from P ♂♂ than other ♀♀ ($p < .02$), UA ♀♀ received shorter displays from G ♂♂ than did UA ♀♀ ($p < .02$; Fig. 25a). P ♀♀ also got a greater percentage of display time from P ♂♂ ($p = .03$), in contrast to UA ♀♀ who tended to get less display time from P ♂♂ than other ♂♂ ($p = .19$; Fig. 25b). Display frequencies followed the same pattern (Fig. 25c). P ♀♀ received leaps only from P ♂♂ ($p = .12$) and UA ♀♀ received fewer leaps from P ♂♂ ($p = .13$; Fig. 25d).

c. Female behaviours

Female guppies exhibit a rather stereotyped sexual response, as

Fig. 24. Relative positions of fish and thrusts received by virgin ♀♀ from ♂♂ of different races in competition with one another. Each set of histograms represent activities of P, UA, and G ♂♂ , in that order. Kruskal-Wallis one-way analysis of variance. The first set of histograms in e. represent ♂♂ , and Friedman two-way analysis of variance is used.

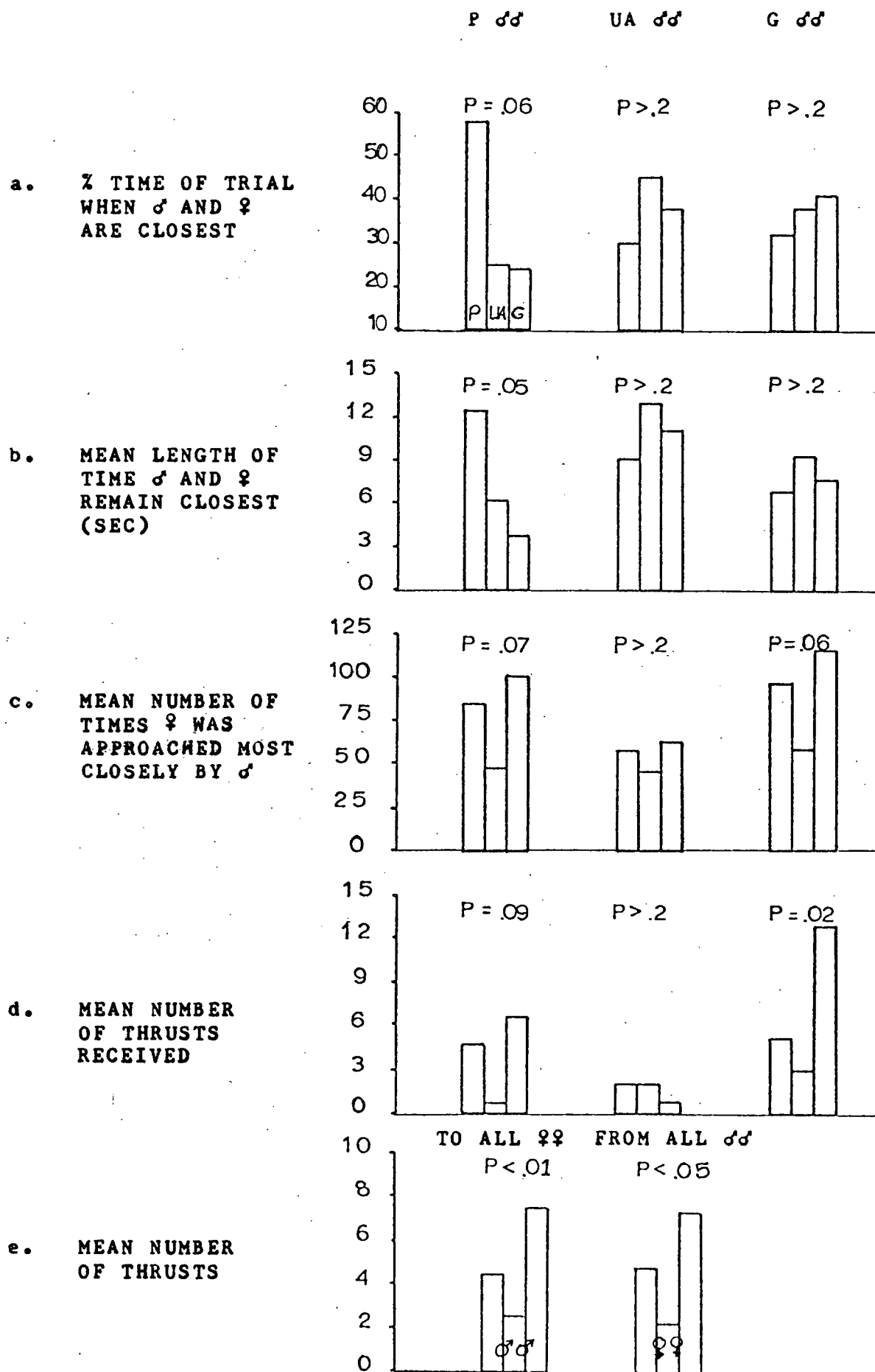
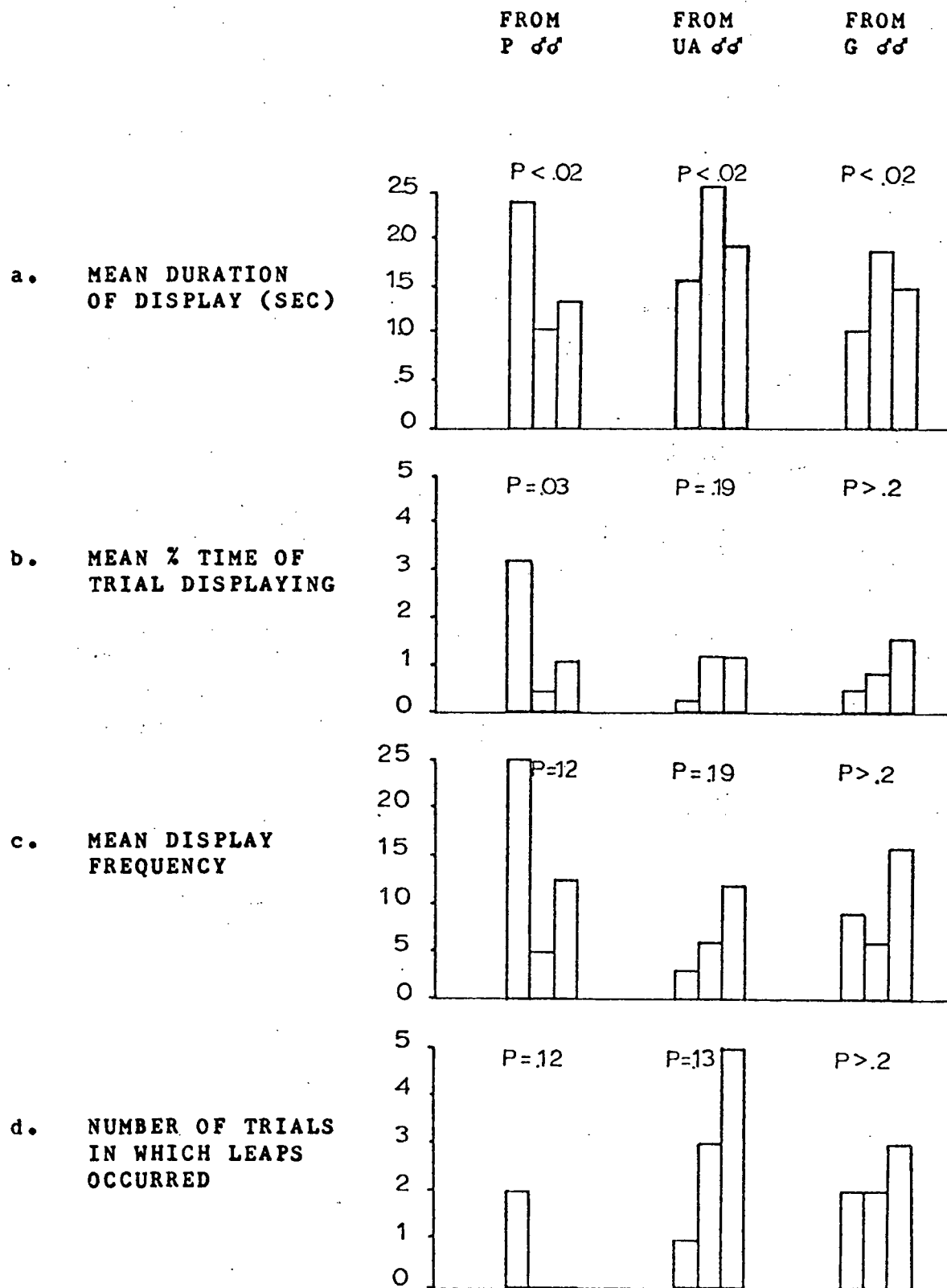


Fig. 25. Display behaviours received by virgin ♀♀ of different races from ♂♂ of different races in competition with one another. Each set of histograms represent P, UA, and G ♀♀ , in that order. Kruskal-Wallis one-way analysis of variance.



described by Liley (1966). See Appendix 2. The occurrence of the response depends upon a combination of the physiological readiness of the ♀♀ and the motivating capacities of the ♂, and appears to be identical in all three races.

The glide is the first response of a receptive ♀ to a displaying ♂. Females tended to glide to their own ♂♂ in more trials than to others ($p < .1$, χ^2). Upon closer analysis, P ♀♀ tended to glide more to P ♂♂ ($p = .17^7$) and less to UA and G ♂♂ ($p = .10$ and $.09$, respectively) than UA and G ♀♀ (Fig. 26a). P ♀♀ tended toward longer bouts of gliding to P ♂♂ ($p = .12$) and G ♀♀ had longer bouts of gliding to G ♂♂ ($p < .05$) than did the other ♀♀ (Fig. 26b). There were tendencies for P ♀♀ to glide for a greater total time to P ♂♂ ($p = .09$), UA ♀♀ to glide more to UA ♂♂ ($p = .14$), and G ♀♀ to glide less to P ♂♂ ($p = .07$) than did the other ♀♀ (Fig. 26c).

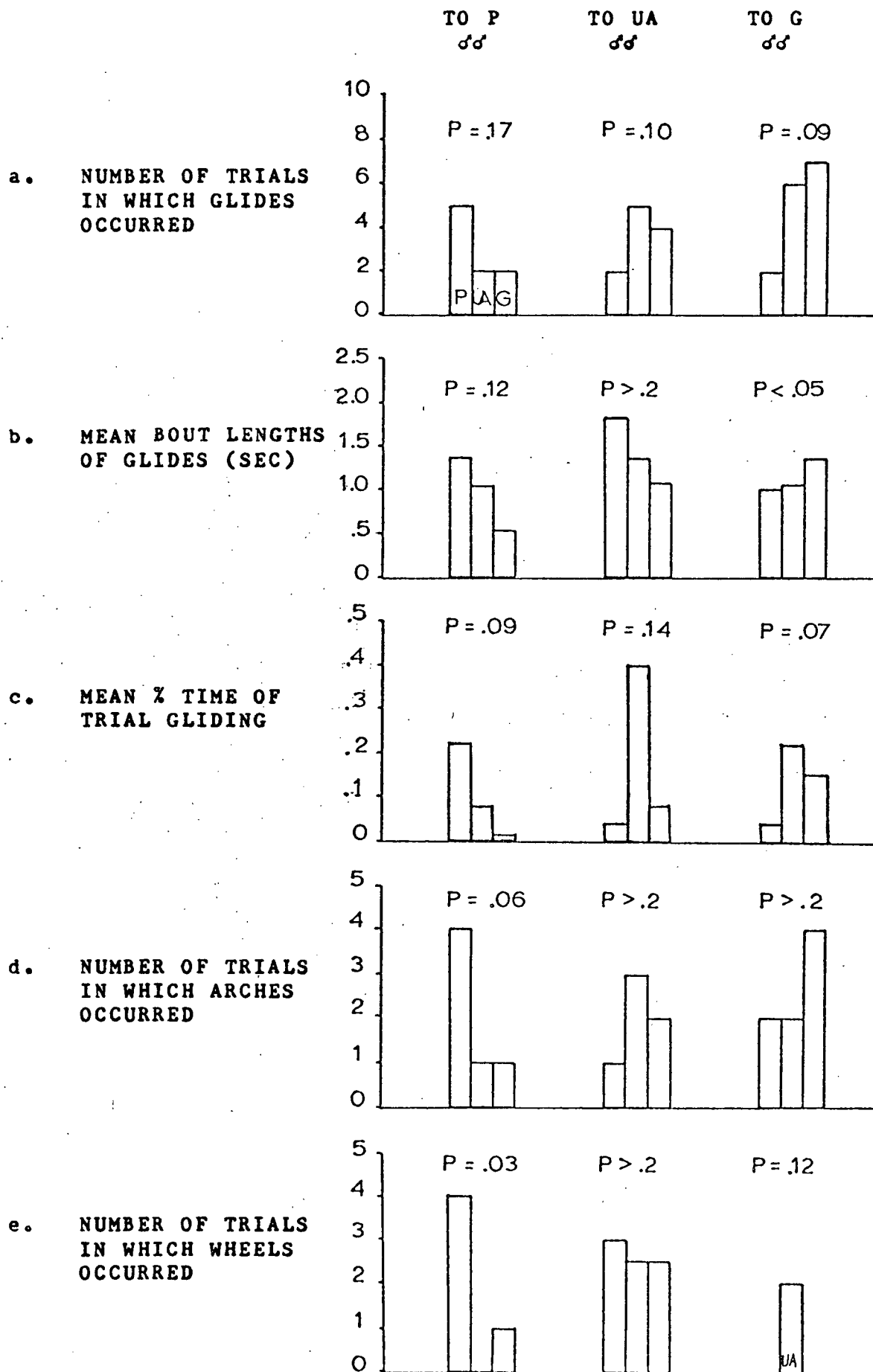
The subsequent ♀ responses, arching and wheeling, were infrequent. Nevertheless, the number of trials in which arches occurred was highest with fish of the same race ($p < .05$, χ^2). P ♀♀ performed arches and wheels more for P ♂♂ than did the other ♀♀ ($p = .06$ and $.03$, respectively; Fig. 26d and e).

d. Male-female interaction

The ♂ who elicited the first ♀ response in a trial was of the same race as the ♀ ($p < .01$, χ^2). An index of response was formulated by

⁷Unless otherwise mentioned, probability values are derived from the Kruskal-Wallis one-way analysis of variance.

Fig. 26. Responses of virgin ♀♀ to ♂♂ of different races. Each set of histograms represent activities of P, UA, and G ♀♀, in that order. Kruskal-Wallis one-way analysis of variance.



dividing the number of glides by the number of ♂ displays. It seems clear that UA ♂♂ have very attractive displays. They were able to elicit more responses from all ♀♀ combined than the other ♂♂ ($p < .01$), but this was coupled with the greater responsivity of UA ♀♀ to all ♂♂ ($p < .05$; Table 15a). UA ♂♂ elicited more responses from UA and G ♀♀ than did other ♂♂, and P ♂♂ elicited fewer ($p = .194$ and $< .005$, respectively; Friedman two-way analysis). G ♂♂ evoked fewer responses from P ♀♀ than did P and UA ♂♂ ($p < .039$, Friedman two-way analysis). UA and G ♂♂ evoked more responses from UA ♀♀ than other ♀♀ ($p < .01$ and $< .02$, respectively). UA ♂♂ and ♀♀ were much more successful in responding to one another than ♂♂ and ♀♀ of other races ($p < .001$; Table 15b).

D. Female selectivity

The purpose of the following experiments is to examine the selectivity of the ♀♀ to ♂♂ of different races suggested in the previous experiment. Two major problems appear in attempting to assess the responses of the ♀ : (1) experienced ♀♀ rarely respond to ♂♂, and (2) virgin ♀♀ respond so readily to ♂♂ that anything but ♂-ness is probably not noticed.⁸ Despite these difficulties, I thought that ♀♀ might discriminate between if given a choice, even if their selections were not sexually motivated (e.g. schooling).

E. Choice Test 1 (Experiment 10)

⁸I once accidentally bent a ♀ while transferring her to an aquarium of virgins. Almost immediately another ♀ began a sexual response to this apparently displaying fish. Females do not seem to respond to models, however (Liley, pers. comm.).

Table 15. Response indices (# glides/ # displays) for virgin ♀♀ with ♂♂ of different races in competition with one another.

a.

	P ♀♀	UA ♀♀	G ♀♀	
All ♂♂	.134	.437	.112	$p < .05$

	P ♂♂	UA ♂♂	G ♂♂	
All ♀♀	.126	.306	.222	$p < .01$

b.

	P ♀♀	UA ♀♀	G ♀♀	
P ♂♂	.185	.130	.052	$p < .2$
UA ♂♂	.138	.652	.280	$p < .01$
G ♂♂	.079	.459	.089	$p < .02$
	$p < .039$	$p = .194$	$p < .005$	$p < .001$

1. Introduction

I gave virgin and experienced ♀♀ deprived of recent experience with ♂♂ a choice of swimming to one of three ♂♂ of different races. The experimental hypothesis was that ♀♀ of one race would make consistent choices of ♂♂ of one race, not necessarily their own (recall Haskins et al., 1961; ♀♀ select brighter ♂♂).

2. Materials and Methods

First, one ♂ of each race was introduced into the three-way choice maze, then one ♀ was placed behind clear plexiglas partitions, as illustrated in Fig. 27 (see also Fig. 4). After five minutes, I raised the partitions by a pulley system, and the ♀ was free to proceed to a ♂. The ♀ completed her trial when she touched a partition with a ♂ behind it. I recorded her choice of ♂ and the length of time she took to reach him. A ♀ had five minutes to complete her trial before I terminated it. Six White Cloud Mountain minnows served as dithers in each of the three side compartments of the maze.

All trials were independent. Position effects were controlled by rotating ♂♂ to different arms of the maze for each trial, so that ♀♀ saw ♂♂ of a given race in each of the three arms approximately equally. Twenty-four G and 23 P virgins were tested (no UA were available at the time). Twelve experienced ♀♀ of each race, isolated from ♂♂ (but not from one another) for 28-36 days, were tested.

3. Results

General observations of differences in behaviour between virgins and experienced ♀♀ in the maze must first be noted. When a virgin was

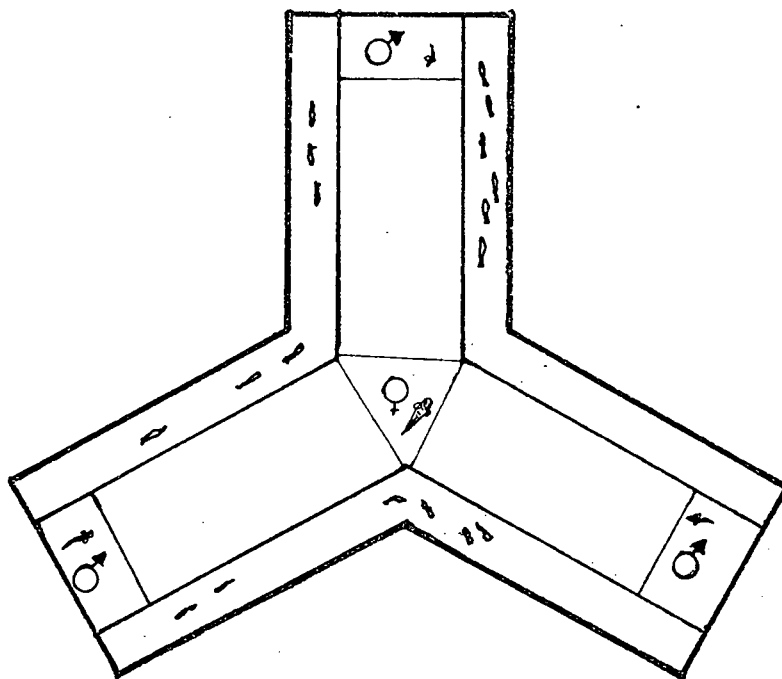


Fig. 27. Experimental set-up for Experiment 10. The clear partitions surrounding the ♀ were removed by a pulley system. All lines but the outer ones represent clear plexiglas.

placed in the maze, she generally swam around until she apparently noticed a ♂---then swam immediately to him. In contrast, the experienced ♀♀ seemed to take no more notice of the ♂♂ than of the dither fish. Five of the experienced ♀♀ never reached a ♂, and those that did took an average of 82.9 seconds, compared to 43.6 seconds for the virgins ($p = .00006$, Mann-Whitney U test, two-tailed).

Females exhibited no preferences of ♂♂. Neither did they show a bias in their selection of arms of the maze. The behaviour of the ♂♂ in this experiment and the next was quite consistent: almost all of them swam at the clear partition, orientating to the ♀♀.

Experienced ♀♀ of all races demonstrated no differences in the length of time taken to reach any race of ♂♂. P virgins also did not differ, but G virgins tended to take less time to reach G ♂♂ ($p < .1$, Kruskal-Wallis⁹). When the data for experienced ♀♀ were lumped, a trend appeared in the direction of less time taken to reach the G ♂♂ ($p < .1$).

P ♀♀ (both virgins and non-virgins) took longer to reach a ♂ than other ♀♀ (virgins: $p < .011$, Mann-Whitney U test; non-virgins: $p < .05$, Kruskal-Wallis). I attribute this difference to the tendency of P ♀♀ to freeze near the substrate in unfamiliar surroundings (see Seghers, 1973); whereas UA and G fish tend to swim about rapidly.

⁹"Kruskal-Wallis" hereafter refers to the Kruskal-Wallis one-way analysis of variance.

F. Choice test 2 (Experiment 11)

1. Introduction

This choice test was designed to measure relative length of time spent near ♂♂ of the three races. It seemed that the highly exploratory ♀♀ could then make choices based on comparison. The experimental hypothesis for the experienced ♀♀ was that a race of ♀ would spend different amounts of time close to ♂♂ of different races. For the virgins, two possibilities were advanced: (1) ♀♀ would prefer ♂♂ of their own race, and (2) ♀♀ would prefer the races which appear brighter to the human eye (P and UA) over the duller one (G).

2. Materials and Methods

The maze was arranged as illustrated in Fig. 28. Dither fish were employed. Males were introduced and their positions changed between trials as in the previous experiment. One ♀ was introduced into the center of the maze and a stopwatch began when she began moving. Each trial, thus begun, lasted ten minutes. The time the ♀ spent near each ♂ began when she made contact with the partition behind which he swam, and ended when she swam out of his area (see Fig. 28). In this way, total time spent with each ♂ and number of approaches to each ♂ were recorded.

The same experienced ♀♀ were used as in the previous experiment, now isolated from ♂♂ for 37-41 days, with the exception of that experiment. Some of the virgins tested previously participated in this experiment, with the addition of new UA virgins. Twelve virgins and 12 non-virgins of each race were used.

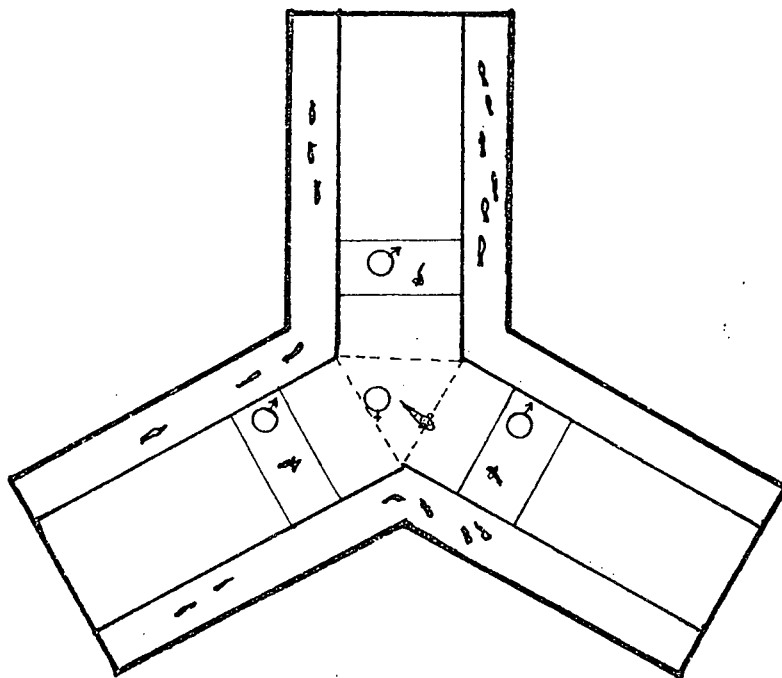


Fig. 28. Experimental set-up for Experiment 11. The dashed lines delimit a ♂'s "area". All lines but the outer ones represent clear plexiglas.

3. Results

Most fish swam up to each ♂ before choosing one a second time. As in Experiment 10, the virgins were markedly more responsive to the ♂♂. They made more approaches to the ♂♂ and spent more time in their areas than the non-virgins ($p = .008$ and $.034$, respectively; Mann-Whitney U test). Females showed no positional biases.

No virgin ♀♀ showed differences in activity, measured by number of approaches, but the experienced ♀♀ did ($p < .05$, Kruskal-Wallis), with P ♀♀ being least active (P-UA: $p = .142$; P-G: $p = .007$; Mann-Whitney U test).

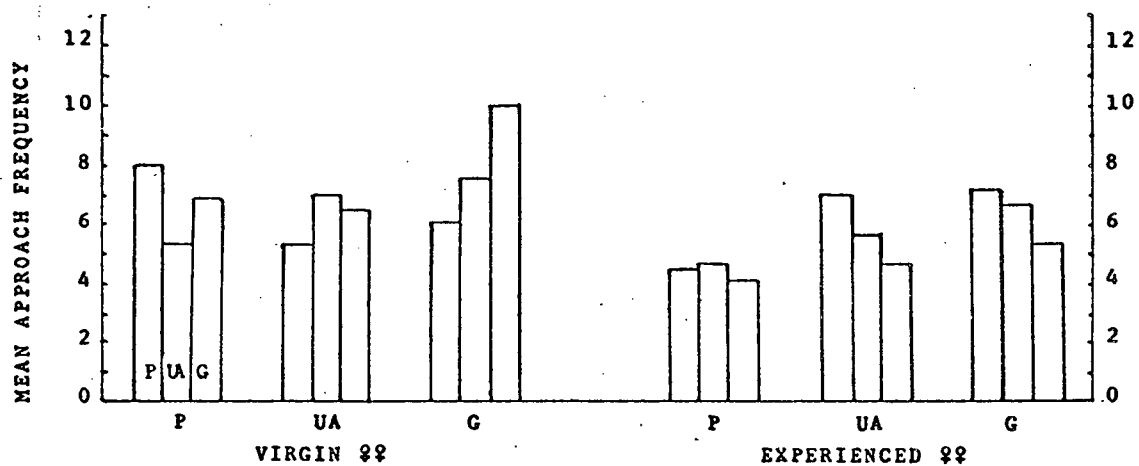
Fig. 29 reveals that experienced ♀♀ showed no preferences for ♂♂ of any race, with one exception. Pairs comparison was permissible for P ♀♀ ($p < .01$, Friedman two-way analysis of variance), where they spent longer average times with UA ♂♂ than their own ($p < .02$, Wilcoxon). Virgin ♀♀, on the other hand, exhibited distinct preferences ($p < .01$, χ^2 , 1 df) as indicated by the number of trials in which ♀♀ approached most and spent most time with ♂♂ of their own race. While P and G ♀♀ selected their own ♂♂ in pairs comparisons, UA ♀♀ did not. No ♀♀ showed preferences when the pairs comparison did not include a ♂ of their race. No evidence was found to support the hypothesis that ♀♀ prefer the ♂♂ brighter to humans.

G. Reactive instances of ♀♀ (Experiment 12)

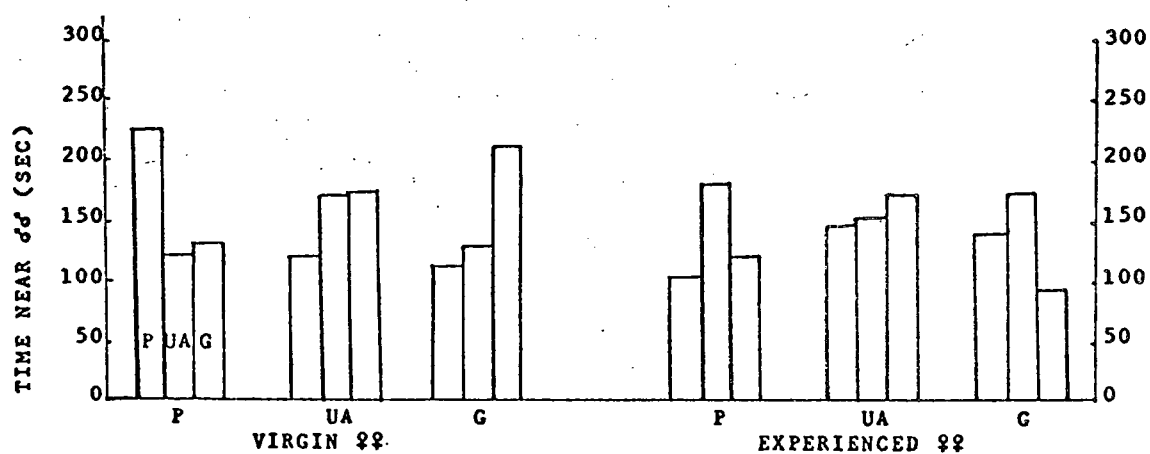
1. Introduction

This experiment was designed with the following in mind: (1) a more conspicuously colored ♂ should be more noticeable to a ♀ at a

Fig. 29. Preferences for ♂♂ of different races by virgin and experienced ♀♀. Each set of three histograms represents responses of ♀♀ of one race to P, UA, and G ♂♂, in that order. The boxes below the figures show the probabilities of virgin ♀♀ choosing ♂♂ of races other than their own by chance (Wilcoxon matched-pairs signed-ranks test, one-tailed). Under each column the comparison is made between ♂♂ of that race and the ♀'s own race. N = 12 for each group of ♀♀.



	P♂♂	UA♂♂	G♂♂
VIRGINS P	---	< .01	N.S.
UA	N.S.	---	N.S.
G	.005	< .02	---



	P♂♂	UA♂♂	G♂♂
VIRGINS P	---	.05	< .025
UA	N.S.	---	N.S.
G	.025	< .025	---

distance, (2) in association with the turbid water of their natural habitat, G ♀♀ might take longer to notice ♂♂ at relatively great distances (see experiment 2), and (3) ♀♀ might perceive their own race of ♂ at a greater distance.

2. Materials and Methods

To ensure that ♀♀ were responsive, virgins were used (25 P, 22 G, but only 4 UA were available at the time). Dither fish were employed. A ♂ was introduced behind a clear partition in the long tank, followed by a ♀, as illustrated in Fig. 30. After the ♀ calmed, the opaque partition blocking her view of the ♂ was removed. One stopwatch recorded the time from removal of the partition until the ♀ began swimming in a straight line towards the ♂, and another stopwatch recorded the length of time taken by a ♀ to reach the ♂'s partition once she began swimming toward him. A different ♂ was used for each trial and ♂♂ of the three races were presented in a regular sequence. Each ♀ participated in three trials, one with each race of ♂. Observer bias was avoided by placing ♀♀ individually in jars inconspicuously labeled and having someone mix the jars up. Thus, I did not know the race of the ♀ I was testing. Males were consistent in swimming at the partition between them and the ♀, usually orientating to her.

3. Results

It was generally clear when a ♀ noticed a ♂. She would cease swimming about or sitting on the bottom and swim rapidly straight toward him. No differences appeared in the distances at which the ♀♀ reacted

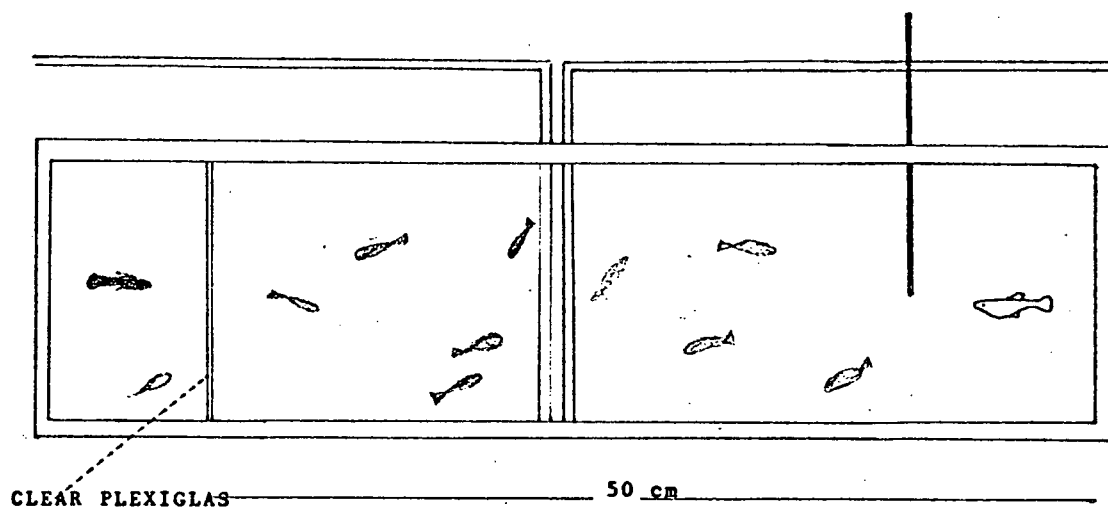


Fig. 30. Aquarium set-up for Experiment 12. The ♂ was confined behind a clear plexiglas partition on the left, and the ♀ was released from behind an opaque partition on the right. Dither fish swim behind the experimental aquarium.

to the ♂♂, either between ♀♀ of different races, or between ♂♂ of different races with ♀♀ of one race (average = 34.6 cm for 79 runs on P ♀♀, 8 on UA, and 64 on G, each ♀ being used a maximum of three times). Females of one race did not vary either in the time taken to start toward the ♂ or in the time taken to swim to ♂♂ of the different populations.

It is evident from the last three experiments that virgin ♀♀ of the P and G races discriminate in favor of their own race of ♂♂, but only when given opportunity for close observation and comparison.

H. Summary of the female's response

Virgin ♀♀ of the P and G races discriminated in favor of ♂♂ of their own races if they were given opportunity for close observation and comparison of all three races of ♂♂. If given opportunity to interact with all three races of ♂♂ at once, ♀♀ tended to respond more to ♂♂ of their own race. This was accompanied by the tendency of ♂♂ to direct more display and contact behaviour toward their own ♀♀. First responses by ♀♀ were directed to their own ♂♂ when ♂♂ of each race were present. Response indices indicated that UA ♀♀ responded much more readily to UA ♂♂ displays than did ♀♀ of other races to their ♂♂'s displays. Thus, whether by initial innate recognition (P and G ♀♀), or by increased responsiveness to displays (UA ♀♀), ♀♀ of each race seemed more likely to complete full sexual responses with ♂♂ of their own race.

Chapter 7. Discussion of Geographic Variation

Geographic variation has generally been regarded as the manifestation of adaptation at the population level. Geographic barriers maintain differences between populations in different ecological situations: whereas lack of physical barriers allow less distinct and more gradual differences between populations (clinal variation). Numerous accounts of geographic variation in color and considerably fewer of variation in mating behaviour have appeared in the literature. However, few studies suggest the selective agents involved, or the adaptive significance of these variations. I can do little more than add to the list of observations and speculations on color variation. However, information about variation in mating behaviour in the guppy, as discussed in Chapter 5, adds to the knowledge about the evolution of races and species.

A. Geographic variation in color

Subspecies of field mice from environmentally similar islands differ in coat color, at least partly due to a founder effect (Berry, 1970). The presence of few ground predators may have hastened the development of these differences (Delany, 1970). With the guppies we have dissimilar environments, but a parallel can be drawn between the oceanic islands of terrestrial animals and the land-bounded "islands" of the guppies. I suspect that lowland river guppies were ancestral to the upstream populations. Aerial predators and tornadoes could have been dispersal agents. Seghers and I both have the impression that the Guayamare race harbors considerably more variation than the Paria race, and not in color alone. In our support, Bromley (unpubl.) has demonstrated a polymorphism for

malate dehydrogenase in G fish while UA and P are monomorphic (cf. Northcote, et al., 1970; color and meristic differences of rainbow trout did not match lactate dehydrogenase differences in similar environments above and below falls).

Despite the large color and color-pattern differences between the guppy races, occasional males from the Guayamare River look very much like Upper Aripo or Paria males. Even rarer are UA males which resemble G males, and P males seem never to appear like members of the UA and G races. Remember that I was working with three of the morphologically most dissimilar races. All the races in Trinidad are polymorphic, no two are the same, and the extent of polymorphism seems to vary. Kallman and Atz (1966) find that species or subspecies of Xiphophorus with extensive geographic ranges are more polymorphic than those with restricted ranges, but remark: "There is as yet no answer to the question why certain patterns are widespread while others occur only in single species or are absent from certain populations of others" (p. 128).

HersHKovitz (1968: he regards differences in color or color-pattern in marmoset races non-adaptive) theorizes that when certain mammals become relatively free of predation, color loses its concealment functions and becomes either more attractive or neutral, which I interpret as selection for intraspecific communication or no selection at all. Contrast this with the drab plumage of island birds which suffer less predation than related species, often colorful, on the mainland (Grant, 1965). Superficially it appears that with marmosets and mice we have a situation similar to the guppies--relax predation and the colors emerge. However, there is no good evidence to suggest that guppy color has a direct link

with vulnerability to predation (Seghers, 1973; Chpt. 4, suggests careful re-examination of Haskins et al., 1961). Furthermore, the relative importance of visual communication to different species must be considered. While vision appears to be of primary importance to guppies, I expect that olfaction, audition, and taction are more important to mice. If colors were very important to intraspecific communication they would exist in the presence of predation also (cf. Selander, 1965).

Many species of animals exhibit color or pattern variation between allopatric populations with no apparent correlation with the severity of ecological barriers or distance between them (e.g., butterflies; Dowdeswell and Ford, 1953; frogs; Volpe, 1961; cyprinodont fishes: Gordon, 1947; Gordon and Gordon, 1957; Rosen and Kallman, 1969; Scheel, 1970; birds: Moreau, 1957; Moreau and Southern, 1958; Selander, 1964; Sibley and Short, 1964; Hall et al., 1966; Vuilleumier, 1971). How much color variation is due to random genetic drift? How much is due to adaptive differences in sexual-aggressive communication in different geographic areas? How specific are responses between animals in the same population compared with animals from different populations?

Other cases of color variation exist where the investigators were able to get some correlation with environment or even an indication of selective value: porpoises--predatory behaviour and more striking counter-shading in clearer waters (Perrin, 1969; pers. comm.); birds--vegetation and substrate color (Davis, 1951; Buchanan, 1964; Chaniot, 1970; Barlow and Williams, 1971; Johnson and Brush, 1972); quantitative aspects of territorial display and possibly predation (Collias and Collias, 1971);

absence of related or similar species (Sibley, 1961; Mayr, 1963: 318-319; cf. Cody, 1969); lizards--mate discrimination (McKinney, 1971; but contrast some birds: Selander, 1964; Short, 1965); frogs--amount of light on the forest floor (Savage and Emerson, 1970); fishes--a larval escape response and a predator (McPhail, 1969); potential predators (Fryer, 1959; 237-281; McKenzie and Keenleyside, 1970); butterflies--activity periods (Hovanitz, 1953); spiders--heat absorption and/or camouflage (Muniz, in Levins, 1968); snails--predation (Wolda, 1963; 1965; 1969; Currey et al., 1964; Owen, 1965; Parkin, 1971; cf. Komai and Emura, 1955; Clarke, 1968).

The incidence of gray morphs of the poeciliid Brachyrhaphis episcopi is higher in the silty water above a waterfall than below it (Dressler, 1971). The colors are not sex-limited, and males do not discriminate in aggressive or courtship behaviour. The paper gives no information about predators. It is tempting to speculate that underwater visibility has been a factor in the evolution of the Brachyrhaphis and guppy morphs. Species of temperate fresh-water fishes tend to be duller in turbid environments, but I could find no evidence that this is genetic. Though probably not important in camouflage, the brightness of male guppies correlates positively with water clarity.

Why have bright colors when they cannot be seen? This question immediately suggests the converse: why have bright colors when they can be seen? Pleiotropism is not a satisfying answer. An adaptive, functional solution is required. For example, consider Bartnik's (1972) study of two allopatric subspecies on longnose dace. The males of the eastern subspecies (Rhinichthys cataractae cataractae) have bright crimson nuptial

coloration, which is absent in males of the western subspecies (R. c. dulcis). R. c. cataractae males use the red coloration for sex discrimination, while R. c. dulcis males appear to rely mostly on tactile stimuli. Furthermore, the colored subspecies spawns mostly during daylight, and the uncolored subspecies spawns at night, possibly in response to a predator, which is absent in the East.

Although naive guppy females of at least the P and G races discriminate in favor of males of their own race visually, I cannot rule out factors other than color in their choices (in contrast to some birds--Mayr, 1963: 318-319). Haskins et al. (1961: 387) have the impression that intra-male competition is more important in making more conspicuous males more attractive to females. Although I have found that brilliance of male color has little to do with aggression or attractiveness to females, I have the impression that coloration influences intra-male behaviour more than male-female behaviour, within populations. Consider, in comparison, that the tail-spike of the male swordtail, Xiphophorus helleri, apparently functions as a signal to other males only (Hemens, 1968; Franck and Hendricks, 1973). Higher ranking males have more color in the related Gambusia heterochir (Warburton et al., 1957) and G. hurtadoi (McAlister, 1958). Noble and Curtis (1935) and Haskins and Haskins (1949) consider the male's bright colors to be intimidating devices. Breder and Coates (1935) could not avoid concluding that there was no sex recognition in guppies, but this is now impossible to accept. In support of the male-intimidation hypothesis, Haskins et al. (1961) suggest that most mating is performed by young males who are just beginning to show color. (I would be cautious in extending this statement to all guppy populations,

however). Furthermore, it seems that the brighter P and UA males attend to one another far more than G males.

A few cases have been studied where hybrid zones and introgression occur between differently colored forms with no obvious environmental correlates (e.g., birds: Short, 1965; Hubbard, 1969; lizards: Zweifel, 1962; McKinney, 1971; salamanders: Stebbins, 1949). An intermediate hybrid may be adapted to an intermediate ecological situation (e.g., Hagen, 1967). Opportunities for exploring these kinds of differences exist within the larger streams of Trinidad. Guppies tend to form sub-populations with relatively little gene flow between pools (Haskins et al., 1961; Seghers, 1973).

Clines in morphological types of a species can be more or less steep, indicating relatively different amounts of gene flow, and thereby relative degrees of discreteness of populations. Differences in color are usually responses to local conditions, and represent antipredator adaptations (e.g., elephant shrews: Corbet, 1970; birds: Pitelka, 1951; Sibley and West, 1959; Johnston and Selander, 1964; Johnston, 1966; frogs: Schaaf and Smith, 1970). Other cases exist where the concomitants of clinal color variation are more obscure (e.g., birds: Miller, 1941; Mayr and Stresemann, 1950; Dickinson, 1952; Galbraith, 1956, 1969; Vaurie, 1957; Banks, 1964; Berry and Davis, 1970; fish: Hubbs and Miller, 1965).

Variation in color is generally linked to crypsis. Some researchers have considered intraspecific signaling. A possibility which almost all investigators have neglected is mimicry. Behaviour differences associated with geographical color differences have been found only by McPhail (1969),

Collias and Collias (1971), McKinney (1971), and Bartnik (1972).

Streams and Pimentel (1961) have stated, "Selection seldom acts on one character without affecting other characters". The current state of knowledge leads us to the conclusion that unexplained variations in color are due largely to pleiotropisms (Dobzhansky, Scudder, pers. comms.). I believe this to be a premature conclusion because it explains nothing. Explanations for color variations have been sought mainly as adaptive mechanisms for a given animal's association with other species, especially predator avoidance and prey capture. The importance of colors in intra-specific communication, emphasized by an animal's behaviour, must be given attention.

As I mentioned earlier, I initially intended to study the signal value of guppy coloration, but found that polymorphism in addition to geographic variation made the problem too complicated. The correlations of black markings and behaviour, discussed in Appendix 1, suggest further work. Perhaps certain color patterns are linked quantitatively with behaviours. Certainly enough is known about guppy color inheritance to begin (Winge, 1922a, b, 1923a, 1927, 1930; Winge and Ditlevsen, 1948; Haskins and Haskins, 1951, 1954; Haskins et al., 1961; Haskins et al., 1970), but the work would appear to require much space and many hours. Nevertheless, the guppy seems to me a leading candidate for behaviour-genetic analyses, hopefully using colors as marker genes. Some related work on behaviour-genetics and, separately, color, has begun on species of the related genus Xiphophorus (Kallman and Atz, 1966; Kallman, 1970a, b; Franck, 1970).

B. Geographic variation in mating behaviour

The probable consequence of geographic variation in mating behaviour is sexual isolation and speciation. Therefore, most investigators who have looked at racial differences in mating have been searching for speciation in action, and not so much for the adaptive significance of the differences. Obviously, reinforced sexual isolation is adaptive, but the evolution of different mating patterns or mate choices in isolated populations where no secondary contact has occurred is puzzling. Pleiotropism may be a reason for many of the cases on record, but, as mentioned earlier, this is not a satisfactory explanation to me.

The report of McKenzie and Keenleyside (1970) is the only study which I came across that outlined direct environmental correlates to geographic differences in mating behaviour, and here the significance of the courtship differences was not clear. Intraspecific variation in social organization in ungulates (Estes, 1966; Klopfer, 1972), rodents (Anderson, 1961), and primates (Gartlan and Brian, 1968; Crook, 1970) is likely to result from the physical and social environment, and is not necessarily genetic. Similarly, geographical variation in some birdsongs (Marler and Tamura, 1964; Thielcke, 1965, 1969) and nesting sites and nest structure (Walkinshaw and Zimmerman, 1961) show no indication of a genetic basis. In other studies mate preferences (Godfrey, 1959) and courtship (Ferguson, 1970; McKinney, 1971) differ, but environmental correlates are not evident.

In general, members of one subspecies or race of Drosophila succeed much more frequently in mating with their own rather than another subspecies or race (Stalker, 1942; Dobzhansky, 1944; Dobzhansky and Mayr, 1944; Dobzhansky and Streisinger, 1944; Bateman, 1949; Spieth, 1951; Dobzhansky

and Spassky, 1959; Dobzhansky and Mather, 1961; Ehrman, 1961, 1964, 1965; Dobzhansky et al., 1964; Anderson and Ehrman, 1969; Baimai, 1970). This generality extends even to strains within races (Carmody et al., 1962). Laboratory studies have demonstrated possibilities of how ethological isolation evolves (Knight et al., 1956; Santibanez and Waddington, 1958; Dobzhansky and Pavlovsky, 1971; also see Powell, 1971). All in all, either Drosophila are unusual animals or investigators have examined a preponderance of cases where races differed in mating. Why should a mating system be so subject to variation? Are other characteristics of fruit flies as pliable? Is pleiotropism so often expressed in mating behaviour?

Many studies showing mating differences and preferential mate selection between closely related species have been reported (e.g. crickets: Hoy and Paul, 1973; Drosophila: Tan, 1946; Koopman, 1950, Merrell, 1954; fishes: Clarke, Aronson, and Gordon, 1954; Heinrich, 1967; Nelson, 1968; Franck, 1969, 1970; Parzefall, 1969; Gerald, 1971; Rubinoff and Rubinoff, 1971; frogs: Littlejohn and Loftus-Hills, 1968; Licht, 1969; lizards: Gorman, 1969; birds: Ramsay, 1961; Crook, 1964; Sharpe and Johnsgaard, 1966; Smith, 1966; Davies, 1970; mammals: Blair, 1953; Fisler, 1965). In many cases ethological isolating mechanisms may have evolved due to reinforcing selection. In other instances the differences have evolved in allopatric species and the selective agents are not apparent.

Usually no suggestions are given by investigators regarding the significance of differences between populations. When concomitant variation in environmental parameters are given, they are only correlations and not causal relationships, as Mayr (1963) suggests. If we pursue

neo-Darwinian thought in an effort to substantiate the adaptive significance of the phenotypic variations we see, we have the following statement of Mayr with which to contend: "Geographic variation as a whole is adaptive. It adapts each population to the locality it occupies. However, not all the phenotypic manifestations of this genotypic adaptation are necessarily adaptive" (e.g., Mayr, 1963; Dobzhansky, 1970).

Dobzhansky stated that the origin of isolation is a process different from the origin of other species differences: "Race formation is essentially the development of genetic patterns which are adapted to a definite environment. Speciation is a process resulting in fixation of these patterns through the development of physiological isolating mechanisms" (Dobzhansky, 1940). This latter sentence seems fine in the case of reinforcement, but if two populations are isolated we must look to either teleology or pleiotropism if selection is not working directly on mating behaviour or physiology.

There are no environmental factors obvious to me which should influence guppy female selectivity of males. The opportunity for reinforcing selection seems non-existent in the absence of secondary contact. I could understand choices biased toward males with more attractive colors or displays, if females had a similar response threshold retained from ancestral populations (cf. Selander, 1969; Oortmerssen, 1970). While this type of sexual selection may take place within populations, the choice of G females for their more dully colored, less elaborately performing males refutes this hypothesis of Haskins et al. (1961). There is good evidence to support the hypothesis that male guppy courtship differences are the results of differences in selection pressure operating in different

environments. (see Chpt. 5). The males do not appear to discriminate between females of different populations--they respond to females which respond to them.

Could it be that an ancestral population, believed to be similar to the G race, contained females which varied genetically in response threshold to different males? Could the evolution of male conspicuousness in isolated populations have preceded female selectivity? Could the females more responsive to the type of male now more prevalent in the population have left more offspring, until most of the females possessed an innate "template" of males of their race? Or would the genetic preferences of females prove inconsequential with experience? This can be tested.

Could the males transmit the genes for female bias toward their type? If the ancestral race were like the Guayamare, perhaps the rarer, more conspicuous males in the population inseminated a higher proportion of females than expected due to an advantage in intra-male competition (see Parsons, 1967; Ehrman and Petit, 1968; Ehrman, 1969, 1970). I have the impression that as many as 10% of G males fall within the range of "brightness" variation of UA males. The male offspring would possibly be at a disadvantage in predator-infested waters, but the females would not. A balanced polymorphism could result. In an isolated population with reduced predation, more conspicuous males would increase in frequency, and more females would carry their genes. This hypothesis is testable, since all crosses are possible. Remember that selection by females is just that: a choice must be presented. Choices within a population would be along

a continuum, and genotype changes would evolve ~~statistically~~. Now in a population with conspicuous males, less conspicuous ones could be maintained by a balanced polymorphism similarly to the reverse situation. Both mate selection and predator selection can promote diversity (Ford, 1965).

LITERATURE CITED

- Amouriq, L. 1964. L'activite et le phenomene social chez Lebistes reticulatus (Poeciliidae, Cyprinodontiformes). Compt. Rend. 259: 2701-2702.
- Amouriq, L. 1965a. L'activite et la phenomene social chez Lebistes reticulatus (Poeciliidae, Cyprinodontiformes). Ann. Sci. Nat. Zool. Biol. Animals 7: 151-172.
- Amouriq, L. 1965b. Origine de la substance dynamogene emise par Lebistes reticulatus femelle (Poisson Poeciliidae, Cyprinodontiformes). Compt. Rend. 260: 2334-2335.
- Amouriq, L. 1967. L'optimum de sensibilite de Lebistes reticulatus (Poisson Poeciliidae, Cyprinodontiformes) a l'hormone synthetique femelle. Rev. Comp. Animal 3: 57-60.
- Anderson, P. K. 1961. Density, social structure and non-social environment in house-mouse populations and the implications for regulation of numbers. Trans. N.Y. Acad. Sci. Ser. II 23: 447-451.
- Anderson, W. W., and Ehrman, L. 1969. Mating choice in crosses between geographic populations of Drosophila pseudoobscura. Amer. Midl. Nat. 81: 47-53.
- Atz, J. W. 1962. Effects of hybridization on pigmentation in fishes of the genus Xiphophorus. Zoologica 47: 153-181.
- Baerends, G. P., and Blokzijl, G. J. 1963. Gedanken Uber das Entstehen von Formdivergenzen zwischen homologen Signalhandlungen verwandter Arten. Z. Tierpsychol. 20: 517-528.
- Baerends, G. P., Brouwer, R., and Waterbolk, H. T. 1955. Ethological studies on Lebistes reticulatus (Peters). I. An analysis of the male courtship pattern. Behaviour 8: 249-334.
- Baimai, V. 1970. Additional evidence on sexual isolation within Drosophila birchii. Evolution 24: 149-155.
- Banks, R. C. 1964. Geographic variation in the white-crowned sparrow Zonotrichia leucophrys. U. C. Publ. Zool. 70: 1-123.
- Barlow, J. C., and Williams, N. 1971. Colorimetric analysis of the dorsal plumage of the red-eyed vireo (Aves: Vireonidae) in Canada. Can. J. Zool. 49: 417-419.
- Barlow, G. W., 1961. Causes and significance of morphological variation in fishes. Syst. Zool. 10: 105-117.

- Barlow, G. W. 1968. Dither--a way to reduce undesirable fright behavior in ethological studies. *Z. Tierpsychol.* 25: 315-318.
- Bartnik, V. G. 1972. Comparison of the breeding habits of two subspecies of longnose dace, Rhinichthys cataractae. *Can. J. Zool.* 50: 83-86.
- Bastock, M. 1956. A gene mutation which changes a behavior pattern. *Evolution* 10: 421-439.
- Bateman, A. J. 1949. Analysis of data on sexual isolation. *Evolution* 3: 174-177.
- Berry, R. J. 1970. Covert and overt variation, as exemplified by British mouse populations. *Symp. Zool. Soc. Lond.* 26: 3-26.
- Berry, R. J., and Davis, P. E. 1970. Polymorphism and behaviour in the Arctic skua (Stercorarius parasiticus (L.)). *Proc. Roy. Soc. Lond. B.* 175: 255-267.
- Blair, W. F. 1953. Experimental evidence of species discrimination in the sympatric species, Peromyscus truei and P. nasutus. *Amer. Natur.* 87: 103-105.
- Braddock, J. C. 1949. The effect of prior residence upon dominance in the fish Platypharodon maculatus. *Physiol. Zool.* 22: 161-169.
- Breder, C. M. Jr., and Coates, C. W. 1932. A preliminary study of population stability and sex ratio of Lebistes. *Copeia* 1932: 147-155.
- Breder, C. M. Jr., and Coates, C. W. 1935. Sex recognition in the guppy, Lebistes reticulatus Peters. *Zoologica* 19: 187-207.
- Buchanan, D. M. 1964. The Mexican races of the least pigmy owl. *Condor* 66: 103-112.
- Carmody, G., DiazCollazo, A., Dobzhansky, T., Ehrman, L., Jaffrey, I.S., Kimball, S., Obrebski, S., Silagi, S., Tidwell, T., and Ullrich, R. 1962. Mating preferences and sexual isolation within and between the incipient species of Drosophila paulistorum. *Amer. Midl. Natur.* 68: 67-82.
- Chaniot, G. E. 1970. Notes on color variation in downy Caspian terns. *Condor* 72: 460-465.

- Clark, E., Aronson, L. R., and Gordon, M. 1954. Mating behavior patterns in two sympatric species of xiphophorin fishes: their inheritance and significance in sexual isolation. *Bull. Amer. Mus. Nat. Hist.* 103: 135-226.
- Clarke, B. C. 1968. Balanced polymorphism and regional differentiation in land snails, p. 351-368. In Drake, E. T. (ed.) *Evolution and environment*. Yale Univ. Press, New Haven.
- Cody, M. L. 1969. Convergent characteristics in sympatric species: a possible relation to inter-specific competition and aggression. *Condor* 71: 222-239.
- Collias, N. E., and Collias, E. C. 1971. Comparative behaviour of West African and South African subspecies of Plocéus cucullatus. *Ostrich*, Suppl. 9: 41-52.
- Collins, J. M., Cramer, J., and Wechkin, S. 1967. Nip frequency and dominance in male swordtails and platies. *Am. Zool.* 7: 806. (Abstr.)
- Corbet, G. B. 1970. Patterns of subspecific variation. *Symp. Zool. Soc. Lond.* 26: 105-116.
- Crook, J. H. 1964. The evolution and social organisation and visual communication in the weaver birds (Ploceinae). *Behaviour*, Suppl. 10: 1-178.
- Crook, J. H. 1970. The socio-ecology of primates, p. 103-166. In Crook, J. H. (ed.) *Social behaviour in birds and mammals*. Academic Press, London.
- Currey, J. D., Arnold, H. W., and Carter, M. A. 1964. Further examples of variation of populations of Capaea nemoralis with habitat. *Evolution* 18: 111-117.
- Davies, S. J. J. F. 1970. Patterns of inheritance in the bowing display and associated behaviour of some hybrid Streptopelia doves. *Behaviour* 36: 187-214.
- Davis, J. 1951. Distribution and variation of the brown towhees. *U. C. Publ. Zool.* 52: 1-119.
- Delany, M. J. 1970. Variation and ecology of island populations of the long-tailed field mouse (Apodemus sylvaticus (L.)). *Symp. Zool. Soc. Lond.* 26: 283-295.
- Dickinson, J. C. 1952. Geographic variation in the red-eyed towhee of the eastern United States. *Bull. Mus. Comp. Zool.* 107: 271-352.

- Dobzhansky, T. 1940. Speciation as a stage of evolutionary divergence. *Amer. Natur.* 74: 312-321.
- Dobzhansky, T. 1944. Experiments on sexual isolation in Drosophila. III. Geographic strains of Drosophila sturtevantii. *Proc. Nat. Acad. Sci.* 30: 335-339.
- Dobzhansky, T. 1970. Genetics of the evolutionary process. Columbia Univ. Press, New York. 505 p.
- Dobzhansky, T., Ehrman, L., Pavlovsky, O., and Spassky, B. 1964. The superspecies Drosophila paulistorum. *Proc. Nat. Acad. Sci.* 51: 3-9.
- Dobzhansky, T., and Mather, W. B. 1961. The evolutionary status of Drosophila serrata. *Evolution* 15: 461-467.
- Dobzhansky, T., and Mayr, E. 1944. Experiments on sexual isolation in Drosophila. I. Geographic strains of Drosophila willistoni. *Proc. Nat. Acad. Sci.* 30: 238-244.
- Dobzhansky, T., and Pavlovsky, O. 1971. Experimentally created incipient species of Drosophila. *Nature* 230: 289-292.
- Dobzhansky, T., and Spassky, B. 1959. Drosophila paulistorum, a cluster of species in statu nascendi. *Proc. Nat. Acad. Sci.* 45: 419-428.
- Dobzhansky, T., and Streisinger, G. 1944. Experiments on sexual isolation in Drosophila. II. Geographic strains of Drosophila prosaltans. *Proc. Nat. Acad. Sci.* 30: 340-345.
- Dowdeswell, W. H., and Ford, E. B. 1953. The influence of isolation on variability in the butterfly Manicola jurtina L. *Symp. Soc. Exp. Biol.* 7: 254-273.
- Dressler, R. L. 1971. Local polymorphism in Brachyrhaphis episcopi (Poeciliidae). *Copeia* 1971: 170-171.
- Eaton, T. H. Jr. 1970. Evolution. W. W. Norton and Company, New York. 270 p.
- Ehrlich, P. R., and Raven, P. H. 1969. Differentiation of populations. *Science* 165: 1228-1232.
- Ehrman, L. 1961. The genetics of sexual isolation in Drosophila paulistorum. *Genetics* 46: 1025-1038.

- Ehrman, L. 1964. Courtship and mating behavior as a reproductive isolating mechanism in Drosophila. Am. Zool. 4: 147-153.
- Ehrman, L. 1965. Direct observation of sexual isolation between allopatric and between sympatric strains of the different Drosophila paulistorum races. Evolution 19: 459-464.
- Ehrman, L. 1969. The sensory basis of mate selection in Drosophila. Evolution 23: 59-64.
- Ehrman, L. 1970. Simulation of the mating advantage in mating of rare Drosophila males. Science 167, 905-906.
- Ehrman, L., and Petit, C. 1968. Genotype frequency and mating success in the willistoni species group of Drosophila. Evolution 22: 649-658.
- Estes, R. D. 1966. Behaviour and life history of the wildebeest (Connochaetes taurinus, Burchell). Nature 212: 999-1000.
- Farr, J. A. 1972. A quantitative analysis of social behavior of the guppy, Poecilia reticulata, in relation to population density. Am. Zool. 12: 651 (Abstr.)
- Ferguson, G. W. 1971. Variation and evolution of the push-up displays of the side-blotched lizard genus Uta (Iguanidae). Syst. Zool. 19: 79-101.
- Fisler, G. F. 1965. Adaptation and speciation in harvest mice of the marshes of San Francisco Bay. U. C. Publ. Zool. 77: 1-108.
- Ford, E. B. 1965. Genetic polymorphism. Faber and Faber, London. 101 p.
- Franck, D. 1969. Genetische Grundlagen der Evolution tierischer Verhaltensweisen. Zool. Anz. 183: 31-46.
- Franck, D. 1970. Verhaltensgenetische Untersuchungen an Artbastarden der Gattung Xiphophorus (Pisces). Z. Tierpsychol. 27: 1-34.
- Franck, D., and Hendricks, R. 1973. Zur Frage der biologischen Bedeutung des Schwertfortsatzes von Xiphophorus helleri. Behaviour 44: 167-185.
- Fryer, G. 1959. The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with especial reference to the fishes, and a discussion of the evolution of a group of rock-frequenting Cichlidae. Proc. Zool. Soc. Lond. 132: 153-281.

- Galbraith, I. C. J. 1956. Variation, relationships and evolution in the Pachycephala pectoralis superspecies (Aves, Muscicapidae). Bull. Brit. Mus. Zool. 4: 131-222.
- Galbraith, I. C. J. 1969. The Papuan and little cuckoo-shrikes, Coracina papuensis and robusta, as races of a single species. Emu 69: 9-29.
- Gandolfi, G. 1969. A chemical sex attractant in the guppy Poecilia reticulata Peters (Pisces, Poeciliidae). Monitore Zool. Ital. (N. S.) 3: 89-98.
- Gandolfi, G. 1971. Sexual selection in relation to the social status of males in Poecilia reticulata (Teleostei: Poeciliidae). Boll. Zool. 38: 35-48.
- Gartlan, J. S., and Brian, C. K. 1968. Ecology and social variability in Cercopithecus aethiops and C. mitis, p. 253-292. In Jay, P. (ed.) Primates: studies in adaptation and variability, Holt, Rinehart, and Winston, New York.
- Gerald, J. W. 1971. Sound production during courtship in six species of sunfish (Centrarchidae). Evolution 25: 75-87.
- Giesel, J. T. 1972. Sex ratio, rate of evolution, and environmental heterogeneity. Amer. Natur. 106: 380-387.
- Godfrey, J. 1959. The origin of sexual isolation between bank voles (Clethrionomys). Proc. XVth Inst. Cong. Zool.: 203-204.
- Gordon, H., and Gordon, M. 1957. Maintenance of polymorphism by potentially injurious genes in eight natural populations of the platyfish, Xiphophorus maculatus. J. Genet. 55: 1-44.
- Gordon, M. 1947. Speciation in fishes: distribution in time and space of seven dominant multiple alleles in Platypoecilus maculatus. Advan. Genet. 1: 95-132.
- Gorman, G. C. 1969. Intermediate territorial display of a hybrid Anolis lizard (Sauria: Iguanidae). Z. Tierpsychol. 26: 390-393.
- Grant, P. R. 1965. Plumage and the evolution of birds on islands. Syst. Zool. 14: 47-52.
- Hagen, D. W. 1967. Isolating mechanisms in threespine sticklebacks (Gasterosteus). J. Fish. Res. Bd. Canada 24: 1637-1692.
- Hall, B. P., Moreau, R. E., and Galbraith, I. C. J. 1966. Polymorphism and parallelism in the African bush-shrikes of the genus Malaconotus (including Chlorophoneus). Ibis 108: 161-182.

- Haskins, C. P., and Haskins, E. F. 1949. The role of sexual selection as an isolating mechanism in three species of poeciliid fishes. *Evolution* 3: 160-169.
- Haskins, C. P., and Haskins, E. F. 1951. The inheritance of certain color patterns in wild populations of Lebistes reticulatus in Trinidad. *Evolution* 5: 216-225.
- Haskins, C. P., and Haskins, E. F. 1954. Note on a "permanent" genetic constitution in a natural population. *Proc. Nat. Acad. Sci.* 40: 627-635.
- Haskins, C. P., Haskins, E. F., McLaughlin, J. J. A., and Hewitt, R. E. 1961. Polymorphism and population structure in Lebistes reticulatus, an ecological study, p. 320-395. In Blair, W. F. (ed.) *Vertebrate speciation*. Univ. Texas Press, Austin.
- Haskins, C. P., Young, P., Hewitt, R. E., and Haskins, E. F. 1970. Stabilised heterozygosis of supergenes mediating certain Y-linked colour patterns in populations of Lebistes reticulatus. *Heredity* 25: 575-589.
- Heinrich, W. 1967. Untersuchungen zum Sexualverhalten in der Gattung Tilapia (Cichlidae, Teleostei) und bei Artsbastarden. *Z. Tierpsychol.* 24: 684-754.
- Hemens, J. 1966. The ethological significance of the sword-tail in Xiphophorus helleri (Haekel). *Behaviour* 27: 290-315.
- HersHKovitz, P. 1968. Metachromism or the principle of evolutionary change in mammalian tegumentary colors. *Evolution* 22: 556-312.
- Heuts, B. A. 1968. A presumed case of territoriality in poeciliids (Pisces--Cyprinodontiformes). *Rev. Can. Biol.* 27: 297-312.
- Hildemann, W. H., and Wagner, E. D. 1954. Intraspecific sperm competition in Lebistes reticulatus. *Amer. Natur.* 88: 87-91.
- Hovanitz, W. 1953. Polymorphism and evolution. *Symp. Soc. Exp. Biol.* 7: 238-253.
- Hoy, R. R., and Paul, R. C. 1973. Genetic control of song specificity in crickets. *Science* 180: 82-83.
- Hubbard, J. P. 1969. The relationships and evolution of the Dendroica coronata complex. *Auk* 86: 393-432.

- Hubbs, C. L., and Miller, R. R. 1965. Studies of Cyprinodont fishes. XXII. Variation in Lucania parva, Its establishment in western United States, and description of a new species from an interior basin in Coahuila, Mexico. Misc. Publ. Mus. Zool. Univ. Mich. 127: 1-104.
- Johnson, N. K., and Brush, A. H. 1972. Analysis of polymorphism in the sooty-capped bush tanager. Syst. Zool. 21: 245-262.
- Johnston, R. F. 1966. The adaptive basis of geographic variation in color of the purple martin. Condor 68: 219-228.
- Johnston, R. F. and Selander, R. K. 1964. House sparrows: rapid evolution of races in North America. Science 144: 548-550.
- Kallman, K. D. 1970a. Sex determination and the restriction of sex-linked pigment patterns to the X and Y chromosomes in populations of a poeciliid fish, Xiphophorus maculatus, from the Belize and Sibun Rivers of British Honduras. Zoologica 55: 1-16.
- Kallman, K. D. 1970b. Different genetic basis of identical pigment patterns in two populations of platyfish, Xiphophorus maculatus. Copeia 1970: 472-487.
- Kallman, K. D., and Atz, J. W. 1966. Gene and chromosome homology in fishes of the genus Xiphophorus. Zoologica 51: 107-135.
- Klopfer, P. H. 1972. Patterns of maternal care in lemurs. II. Effects of group size and early separation. Z. Tierpsychol. 30: 277-296.
- Knight, G. R., Robertson, A., and Waddington, C. H. 1956. Selection for sexual isolation within a species. Evolution 10: 14-22.
- Komai, T., and Emura, S. 1955. A study of population genetics on the polymorphic land snail Bradybaena similaris. Evolution 9: 400-418.
- Koopman, K. F. 1950. Natural selection for reproductive isolation between Drosophila pseudoobscura and Drosophila persimilis. Evolution 4: 135-148.
- Levins, R. 1968. Evolution in changing environments. Princeton Univ. Press, Princeton. 120 p.
- Licht, L. E. 1969. Comparative breeding behavior of the red-legged frog (Rana aurora aurora) and the western spotted frog (Rana pretiosa pretiosa) in southwestern British Columbia. Can. J. Zool. 47: 1287-1299.

- Liley, N. R. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour*, Suppl. 13: 1-197.
- Liley, N. R. 1969. Hormones and reproductive behavior in fishes, p. 73-116. In Hoar, W. S., and Randall, D. J. (eds.) *Fish physiology*, vol. 3, Academic Press, New York.
- Lindsey, C. C. 1962. Experimental study of meristic variation in a population of threespine sticklebacks, Gasterosteus aculeatus. *Can. J. Zool.* 40: 271-312.
- Littlejohn, M. J., and Loftus-Hills, J. J. 1968. An experimental evaluation of premating isolation in the Hyla ewingi complex (Anura: Hylidae). *Evolution* 22: 659-663.
- Marler, P., and Tamura, M. 1964. Culturally transmitted patterns of vocal behavior in sparrows. *Science* 146: 1483-1486.
- Mayr, E. 1963. *Animal species and evolution*. Harvard Univ. Press, Cambridge. 797 p.
- Mayr, E., and Stresemann, E. 1950. Polymorphism in the chat genus Oenanthe (Aves). *Evolution* 4: 291-300.
- McAlister, W. H. 1958. The correlation of coloration with social rank in Gambusia hurtadoi. *Ecology* 39: 477-482.
- McKenzie, J. A., and Keenleyside, M. H. A. 1970. Reproductive behavior of ninespine sticklebacks (Pungitius pungitius (L.)) in South Bay, Manitoulin Island, Ontario. *Can. J. Zool.* 48: 55-61.
- McKinney, C. O. 1971. An analysis of zones of intergradation in the side-blotched lizard, Uta stansburiana (Sauria: Iguanidae). *Copeia* 1971: 596-613.
- McPhail, J. D. 1969. Predation and the evolution of a stickleback (Gasterosteus). *J. Fish. Res. Bd. Canada* 26: 3183-3208.
- Merrell, D. J. 1954. Sexual isolation between Drosophila persimilis and Drosophila pseudoobscura. *Amer. Natur.* 88: 93-99.
- Miller, A. H. 1941. Speciation in the avian genus Junco. *U. C. Publ. Zool.* 44: 173-434.
- Moreau, R. E. 1957. Variation in the western Zosteropidae (Aves). *Bull. Brit. Mus. Zool.* 4: 309-433.
- Moreau, R. E., and Southern, H. N. 1958. Geographical variation and polymorphism in Chlorophoneus shrikes. *Proc. Zool. Soc. Lond.* 130: 301-328.

- Nelson, J. S. 1968. Hybridization and isolating mechanisms between Catostomus commersonii and C. macrocheilus (Pisces: Catostomidae). J. Fish. Res. Bd. Canada 25: 101-150.
- Nelson, K. 1965. The temporal patterning of courtship behaviour in the glandulocaudine fishes. Behaviour 24: 90-146.
- Noble, G. K., and Curtis, B. 1935. Sexual selection in fishes. Anat. Rec. 64, Suppl. 1: 84-85. (Abstr.)
- Northcote, T. G., Willisroft, S. N., and Tsuyuki, H. 1970. Meristic and lactate dehydrogenase genotype differences in stream populations of rainbow trout below and above a waterfall. J. Fish. Res. Bd. Canada 27: 1987-1995.
- Oortmerssen, G. A. van. 1970. Biological significance, genetics and evolutionary origin of variability in behaviour within and between inbred strains of mice (Mus musculus). Behaviour 38: 1-92.
- Owen, D. F. 1965. Density effects in polymorphic land snails. Heredity 20: 312-315.
- Parkin, D. T. 1971. Visual selection in the land snail Arianta arbustorum. Heredity 26: 35-47.
- Parsons, P. A. 1967. Behaviour and random mating. Experientia 23: 585-586.
- Parzefall, J. 1969. Zur vergleichenden Ethologie verschiedener Mollienesia-arten einschliesslich einer Höhlenform von M. sphenops. Behaviour 33: 1-37.
- Perrin, W. F. 1969. Color pattern of the eastern Pacific spotted porpoise Stenella graffmani Lonnberg (Cetacea, Delphinidae). Zoologica 54: 135-150.
- Pitelka, F. A. 1951. Speciation and ecologic distribution in American jays of the genus Aphelocoma. U. C. Publ. Zool. 50: 195-463.
- Powell, J. R. 1971. Genetic polymorphisms in varied environments. Science 174: 1035-1036.
- Ramsay, A. O. 1961. Behaviour of some hybrids in the mallard group. Anim. Behav. 9: 104-105.
- Rosen, D. E., and Kallman, K. D. 1969. A new fish of the genus Xiphophorus from Guatemala, with remarks on the taxonomy of endemic forms. Amer. Mus. Novitates 2379: 1-29.

- Rubinoﬀ, R. W., and Rubinoﬀ, I. 1971. Geographic and reproductive isolation in Atlantic and Pacific populations of Panamanian Bathygobius. *Evolution* 25: 88-97.
- Santibañez, S. K., and Waddington, C. H. 1958. The origin of sexual isolation between different lines within a species. *Evolution* 12: 485-493.
- Savage, J. M., and Emerson, S. B. 1970. Central America frogs allied to Eleutherodactylus bransfordii (Cope): a problem of polymorphism. *Copeia* 1970: 623-644.
- Schaaf, R. T. Jr., and Smith, P. W. 1970. Geographic variation in the pickerel frog. *Herpetologica* 240-254.
- Scheel, J. J. 1970. The genus Procatopus. *Tropical Fish Hobbyist* 168: 4-14.
- Seghers, B. H. 1973. An analysis of geographic variation in the anti-predator adaptations of the guppy, Poecilia reticulata. Ph.D. Thesis. Univ. British Columbia. 273 p.
- Selander, R. K. 1964. Speciation in wrens of the genus Campylorhynchus. *U. C. Publ. Zool.* 74: 1-259.
- Selander, R. K. 1965. Hybridization of rufous-naped wrens in Chiapas, Mexico. *Auk* 82: 206-214.
- Sharpe, R. S., and Johnsgard, P. A. 1966. Inheritance of behavioural characters in F₂ mallard x pintail (Anas platyrhynchos L. x Anas acuta L.) hybrids. *Behaviour* 27: 259-272.
- Short, L. L. Jr. 1965. Hybridization in the flickers (Colaptes) of North America. *Bull. Amer. Mus. Nat. Hist.* 129: 307-420.
- Sibley, C. G. 1961. Hybridization and isolating mechanisms, p. 69-87. In Blair, W. F. (ed.) *Vertebrate speciation*. Univ. Texas Press, Austin.
- Sibley, C. G., and Short, L. L. Jr. 1964. Hybridization in the orioles of the Great Plains. *Condor* 66: 130-150.
- Sibley, C. G., and West, D. A. 1959. Hybridization in the rufous-sided towhees of the Great Plains. *Auk* 79: 326-338.
- Smith, N. G. 1966. Evolution of some Arctic gulls (Larus): an experimental study of isolating mechanisms. *Ornith. Monogr.* 4: 1-99.
- Spieth, H. T. 1951. Mating behaviour and sexual isolation in the Drosophila virilis species group. *Behaviour* 31: 105-145.

- Stalker, H. D. 1942. Sexual isolation studies in the species complex Drosophila virilis. *Genetics* 27: 238-257.
- Stebbins, R. C. 1949. Speciation in salamanders of the plethodontid genus Ensatina. *U. C. Publ. Zool.* 48: 377-526.
- Streams, F. A., and Pimentel, D. 1961. Effects of immigration on the evolution of populations. *Amer. Natur.* 95: 201-210.
- Tan, C. C. 1946. Genetics of sexual isolation between Drosophila pseudoobscura and Drosophila persimilis. *Genetics* 31: 558-573.
- Thielcke, G. 1965. Gesangsgeographische Variation des Gartenbäumlaufers (Gerthia brachydactyla) im Hinblick auf das Artbildungsproblem. *Z. Tierpsychol.* 22: 542-566.
- Thielcke, G. 1969. Geographic variation in bird vocalizations, p. 311-339. In Hinde, R. A. (ed.) *Bird vocalizations*. Univ. Press, Cambridge.
- Vaurie, C. 1957. Systematic notes on palearctic birds. No. 30. The *Certhiidae*. *Amer. Mus. Novitates* 1855: 1-14.
- Volpe, E. P. 1961. Polymorphism in anuran populations, p. 221-234. In Blair, W. F. (ed.) *Vertebrate speciation*. Univ. Texas Press, Austin.
- Vuilleumier, F. 1971. Generic relationships and speciation patterns in Ochthoeca, Myiotheretes, Xolmis, Neoxolmis, Agriornis and Muscisaxicola. *Bull. Mus. Comp. Zool.* 141: 181-232.
- Walkinshaw, L. H., and Zimmerman, D. A. 1961. Range expansion of the Brewer blackbird in eastern North America. *Condor* 63: 162-177.
- Warburton, B., Hubbs, C., and Hagen, D. W. 1957. Reproductive behavior of Gambusia heterochir. *Copeia* 1957: 299-300.
- Winge, Ø. 1922a. A peculiar mode of inheritance and its cytological explanation. *J. Genet.* 12: 137-144.
- Winge, Ø. 1922b. One-sided masculine and sex-linked inheritance in Lebistes reticulatus. *J. Genet.* 12: 145-162.
- Winge, Ø. 1923. Crossing-over between the X and the Y chromosome in Lebistes. *J. Genet.* 13: 201-217.
- Winge, Ø. 1927. The location of eighteen genes in Lebistes reticulatus. *J. Genet.* 18: 1-42.

- Winge, Ø. 1930. On the occurrence of XX males in Lebistes. J. Genet. 23: 69-76.
- Winge, Ø., and Ditlevsen, E. 1948. Colour inheritance and sex determination in Lebistes. Compt. Rend. d. Lab. Carlsberg, Ser. Physiol. 24: 227-248.
- Wolda, H. 1963. Natural populations of the polymorphic landsnail Cepaea nemoralis (L.). Factors affecting their size and their genetic constitution. Arch. Neerl. Zool. 15: 381-471.
- Wolda, H. 1965. Some preliminary observations on the distribution of the various morphs within natural populations of the polymorphic landsnail Cepaea nemoralis (L.). Arch. Neerl. Zool. 16: 280-292.
- Wolda, H. 1969. Stability of a steep cline in morph frequencies of the snail Cepaea nemoralis (L.). J. Anim. Ecol. 38: 623-633.
- Zweifel, R. G. 1962. Analysis of hybridization between two subspecies of the desert whiptail lizard, Cnemidophorus tigris. Copeia. 1962: 749-766.

Appendix 1. Some Speculation and Findings About Black Markings of the Male Guppy

A. Introduction

As courtship proceeds, the black markings of the ♂ guppy become more and more prominent, although no special "aggressive" or "sexual" melanophore systems were noted (cf: Baerends et al., 1955). Because the black seems to be an arousal signal, I hypothesized that ♂♂ of the race which courted most should have the most black; i.e., Paria and Upper Aripo should have more black than Guayamare. In addition it is possible that the black markings also have some appeasement function (see Barlow, 1970, on the appeasement and arousal hypothesis of Tinbergen). When ♂♂ are engaged in aggressive activities, virtually all the black on the body blanches. Furthermore, when the ♂ displays in front of the ♀, he is in a position to be charged. Males do occasionally display to other ♂♂, and the possibility remains that this behaviour is not just misguided sexuality, but has a component of appeasement. Since P fish are much more aggressive than either of the other races, I predicted that P♂♂ should have the most black. The hypothesized order for the most to the least amount of black pigment, then, was P--UA--G.

B. Method of analysis

Fortunately, the anesthetic MS 222 expands melanophores so that an anesthetized ♂ has much the same appearance as a highly motivated courting ♂. The sketches of 32 P, 32 UA, and 30 G ♂♂ associated with Experiment 3 were employed to score black markings by estimating the amount

of black in each of eleven sections of the guppy's lateral surface (Fig. 31). 0 - 25% of the area black gave a score of 1, 26 - 50% = 2, 51 - 75% = 3, and 76 - 100% = 4). The use of stenciled outlines of the same size automatically corrected scores for size discrepancies between the races. An independent analysis was performed in addition to my own because of the subjectivity involved in the scoring.

C. Results

The two analyses yielded virtually identical results. The differences in the amount of black were significant as predicted (P had an average score of 1.25 per body section, UA = 1.08, and G = .93; P to G comparison, $p < .00023$; UA-G, $p < .0054$; P-UA, $p < .0329$; Mann-Whitney U test). No correlations existed between amount of black and the $\sigma\sigma$ who courted most within a race, however. In comparison, see Warburton et al., (1957), where dominant $\sigma\sigma$ of Gambusia heterochir may be recognized by increased intensity of black coloration. These results are perhaps not as objective as is desirable, but they do suggest further experiments on the value of black as a signal in courtship and aggression.

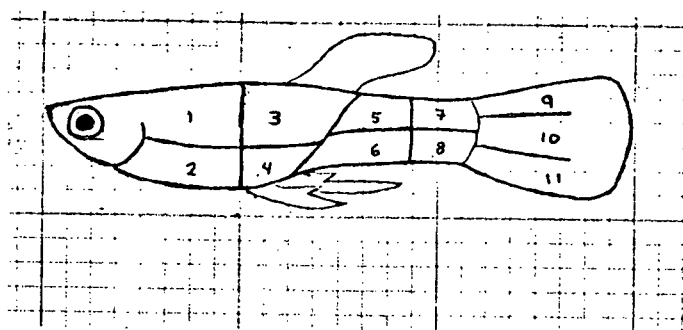


Fig. 31. Areas of the ♂ guppy's lateral surface used to quantify black markings.

Appendix 2. A Description of Guppy Behaviour Patterns Pertaining to Courtship

The following descriptions follow Liley (1966).

A. Male courtship

Orientating. The ♂ attends to the ♀, usually watching her.

He generally follows her when she moves, and attempts to maintain an optimal position of observation. He may orientate from positions above, below, or level with the ♀.

Gonopodial swinging. The gonopodium is moved forward on one side of the midline, accompanied by an arching in the vertical plane and often a sigmoid bending in the horizontal plane. This may occur at practically any time during courtship.

Sigmoid display. The ♂ bends his body into an S shape and quivers, with the pectoral fins working rapidly. Often the ♂ rocks back and forth. This posture is usually held for a few seconds.

Leap. As if spring-loaded, the ♂ shoots away from the ♀ along a straight or curved path for 10 cm or more. He then often resumes orientating, sometimes after a short period of immobility. Leaps may follow sigmoid displays, and are always preceded by them.

Thrust. The ♂ approaches the ♀ from behind, below, and slightly to one side, and brings his gonopodium forward and thrusts it at the ♀'s genital opening with an upwards and forward movement.

Jerk. A ♂ may make a series of short, sharp, forward and upward movements with his whole body following a gonopodial contact. His body moves no more than a few mm and he usually continues orientating.

Copulation and Copulation Attempt. While displaying, a ♂ moves into a position parallel to one side and just below the ♀. He then stops displaying, swims to line himself up with her, then directs his gonopodium as in thrusting. If he makes contact with the ♀'s genital pore, he rolls over on his side. The pair may swim in one or two small circles depending on the difficulty of gonopodial insertion. Contact is maintained for only a fraction of a second, after which the ♂ jumps away and performs a series of jerks. The only difference between copulation and copulation attempt is whether or not insemination has taken place.

B. Male agonistic behaviour

Sparring. Males line up parallel or anti-parallel, .5 to 1 cm apart, with straight, quivering bodies and median fins spread.

Tail-beating. From the sparring position, a ♂ lashes his tail toward the other ♂.

C. Female courtship

Glide. A receptive ♀ may glide in response to a display or Leap. The gliding ♀ seems rigid in motion, using her trunk little and relying mainly on her pectoral and caudal fins. Her attention is "fixed" on the ♂.

Arch. Following gliding the ♀ may stop and raise her tail and head slightly, often moving the tail slightly to one side away from the ♂. She thus makes her genital pore quite accessible to the ♂, who usually attempts copulation.

Wheel. The ♀ moves in a tight circle, assisting the ♂ in a copulation attempt.