MORPHOLOGICAL VARIATION AND ITS TAXONOMIC IMPLICATIONS FOR INSULAR POPULATIONS OF *PSEUDOCRENILABRUS PHILANDER* (PISCES:CICHLIDAE)

THESIS
Submitted in Fulfilment of the
Requirements for the Degree of
MASTER OF SCIENCE
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by

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ALL NATURE IS BUT ART, UNKNOWN TO THEE;

ALL CHANCE, DIRECTION WHICH THOU CANST NOT SEE;

ALL DISCORD, HARMONY NOT UNDERSTOOD;

ALL PARTIAL EVIL, UNIVERSAL GOOD.

Alexander Pope: *An Essay on Man*

CONTENTS

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ABSTRACT

The cichlid fish *Pseudocrenilabrus philander* is widely distributed in southern Africa. Many of the populations occur in small, insular, geographically isolated water bodies, some of which are in arid areas. These small allopatric populations have been isolated for thousands of years and gene flow between them is non-existent or severely restricted. Populations were found to be different in terms of coloration, size of individuals, sexual dimorphism and behaviour. This thesis involved the determination of the taxonomic status of these isolated populations, from a morphological point of view. This has been part of a larger project, involving genetic and behavioural studies, to determine whether the different populations are geographic races of a single species, or whether they are species. Heritability of the observed differences was tested by breeding through three generations under uniform laboratory conditions.

The populations from which wild-caught individuals were drawn could be identified on the basis of colour. There were some differences in anatomy between populations, but none of these were entirely distinctive for a particular population. When bred under laboratory conditions, populations did not show a tendency towards uniformity, as would be expected if morphological differences were environmentally induced. However, there were slight changes in the oral and pharyngeal bone form which may be diet related. Although there are morphological differences

between populations and between wild-caught and laboratory-bred populations, it is suggested that the populations of *P. philander* are not fully differentiated and thus in the stages of incipient speciation.

Other studies on *P. philander* have introduced an element of uncertainty in that they show different results. Behavioural work suggests that different populations would behave as different species if they were to become sympatric. Karyological and mitochondrial studies showed that there were no differences between populations. Protein electrophoresis showed that populations were genetically unique. Since the various species and subspecies of *Pseudocrenilabrus* have been based on morphological criteria, the approach and conclusions in this study are based entirely on morphological criteria.

These studies have practical implications for conservation, as some of the small populations are threatened with extinction. If the differences between the populations were ecophenotypic (i.e. related to their environment), then threats to some populations would not affect the conservation status of the species as a whole. If, however, such differences were genetic (i.e. the populations have evolved separately), then the extinction of small, isolated populations could mean the loss of actual species. This study strongly suggests that populations are incipient species and thus should be conserved.

Chapter 1

INTRODUCTION

The genus *Pseudocrenilabrus* Fowler (1934), is a habitudinally eurytopic genus, occurring in rivers, lakes and swamps, and is distributed widely in Africa. *P. philander* (Weber) occurs in southern Africa, *P. ventralis* (Pellegrin) in central Africa and *P. multicolor* (Schoeller) in eastern Africa as far north as Egypt. There are reported overlaps in the distribution of *P. multicolor* and *P. philander*, with *P. ventralis* apparently confined to the upper reaches of the Zaire River System (Loiselle, 1982a). *P. philander* and *P. multicolor* may also be more complex taxonomically, as these species consist of distinctive, geographically separate populations (Trewavas, 1936; Loiselle, 1982 a & b; Greenwood, 1989) which may be incipient species or even a complex of distinct species.

The focus of this thesis is on *Pseudocrenilabrus philander* which is widely distributed in southern Africa and lives in a variety of geographically isolated waters. These allopatric populations have been isolated in sinkholes, springs, rivers and lakes, with little possibility of gene flow between them. Most populations have differentiated and exhibit unique coloration, differ from one another in terms of maximum adult size, in some morphological and behavioural aspects and in the degree of sexual dimorphism (Ribbink 1971). In this authors opinion, the degree of differentiation may be sufficient, in at least some cases, for

each to be regarded as distinct species. In other cases, interpopulation differences may suggest that speciation is incipient. Alternatively, the differences between the populations may be ecophenotypic. Ribbink (1975) suggested that clinal variation may also exist.

Some cichlid groups of the African Great Lakes have speciated greatly, are narrowly stenotopic and are often specialized (Fryer 1959; Fryer & Iles 1972; Ribbink 1987; Ribbink, Marsh, Marsh, Ribbink & Sharpe 1983; Witte 1984). However, cichlids which live in changing environments tend to be eurytopic and generalised (Fryer 1969) and might therefore be expected to differentiate slowly, having a poor representation of species numbers (Fryer 1960; Fryer 1977; Fryer & Iles 1969; Greenwood 1984a; Lowe-McConnell 1969). Ecological studies of *P. philander* show that it is eurytopic, occupying a wide variety of habitats. As it is predicted that eurytopic species differentiate more slowly than stenotopes, the rapid changes which might be anticipated had the *P. philander* populations followed the evolutionary trends of haplochromines of the African Great Lakes (Ribbink 1993), are not likely.

The taxonomic status of the various isolated populations of *P. philander* is unclear. In an endeavour to resolve the taxonomic issues, a multi-faceted study on the systematics of *P. philander*, in which populations were characterized genetically and phenotypically, was launched. The study involves behavioural analyses (Ribbink 1975), karyology (Oellermann, Ribbink & Twentyman-Jones, in prep.), mitochondrial DNA analyses (de

Villiers, Harley & Ribbink, 1992), protein electrophoresis (Grant 1990) and a morphological study. A thorough morphological study is essential to taxonomy. My study provides the morphological component of the project. The aims of the thesis are to document the differentiation of populations and the heritability of such differences, and so draw taxonomic conclusions. To test the ecophenotypic nature of the populations and thus heritability of the observed morphological differences, several generations were bred under controlled laboratory conditions.

The Recognition Concept (Paterson 1985) is followed in determining the taxonomic relationship of the allopatric populations of *P. philander*, as it is the most practical guide for evaluating the status of the populations. The reason for choosing the Recognition Concept, rather than any of the other species concepts, is justified in the discussion.

Behavioural studies (Ribbink 1975) suggest that the differences between *P. philander* populations may have reached a level indicative of the populations being incipient species. Based on morphological differences discussed in this thesis, it is my contention that all populations are conspecific, showing levels of divergence that are compatible with that of incipient speciation. Objective arguments in this thesis will debate the issue.

Population genetic studies have shown that small, insular populations are prone to extinction (Frankel & Soule 1981; Schonewald-Cox, Chambers, MacBryde & Thomas 1983). So this study also has practical implications for conservation as it

demonstrates that populations of *P. philander* are unique (and possibly incipient species) and thus worthy of protection (see Ribbink & Twentyman-Jones 1989).

NOMENCLATURAL HISTORY

Chromis philander was described by Weber in 1897, but was subsequently placed in the genus *Haplochromis* (see Boulenger 1915). In 1963, however, Wickler proposed a new genus, *Hemihaplochromis*, into which he placed *Haplochromis philander* and the Egyptian Mouthbrooder, *Haplochromis multicolor*. In 1973, however, Trewavas found that *Haplochromis philander* had been described as *Pseudocrenilabrus natalensis* by Fowler in 1934. This had been done in error, as Fowler had thought that the single specimen from Durban, on which he had based his description, was of the marine family Labridae. Under the International Rules of Zoological Nomenclature, the oldest published name is accepted as the valid one.

Pseudocrenilabrus philander has also been referred to as *Haplochromis moffatii* (Castelnau) 1861 and *Tilapia ovalis* (Steindachner) 1866, both trivial names considered by Boulenger (1915) to be synonyms of *philander*, *moffatii* being the senior synonym. However, Trewavas (1936, 1973) regarded *moffatii* as a *species dubia* (and possibly a *Tilapia*). Barnard (1978) disagreed with Trewavas's reasoning and used the name *moffatii*. Since the type specimen of *Chromis moffatii* Castelnau 1861 is lost, the most recent revisor of the genus (Greenwood 1989) has suggested that, at least for the moment and until a thorough revisionary

study of the taxon had been undertaken, Trewavas's views be adopted. For the nomenclatural history of *T. ovalis*, see Regan (1922).

In 1902, Hilgendorf described a fish from Otavifontein closely resembling *P. philander*, as *Paratilapia luebberti*. Trewavas, in 1936, considered that taxon to be only subspecifically distinct from *philander* and subdivided that species into three subspecies, namely: *Haplochromis philander philander* (Weber 1897), native to Natal and Mozambique; *Haplochromis philander dispersus* (Trewavas 1936), native to rivers and lakes of the Transvaal, Botswana, Zimbabwe, Katanga and Angola; *Haplochromis philander luebberti* (Hilgendorf 1902), only from Otavifontein in South West Africa.

Sterba's observations (1962) indicate that the taxonomy of this species is more complex than suggested by Trewavas (1936). Ribbink (1975) suggested that the isolated populations of *P. philander* which he studied are probably incipient species, and Loiselle (1982b) also differentiated the populations.

Subsequently Greenwood (1984) found that the diagnostic features for the subspecies were not at all trenchant and suggested that the recognition of these subspecies should be deferred until a thorough taxonomic and ethological revision of the various allopatric populations of the species had been undertaken.

Chapter 2

MATERIALS AND METHODS

1) COLLECTION

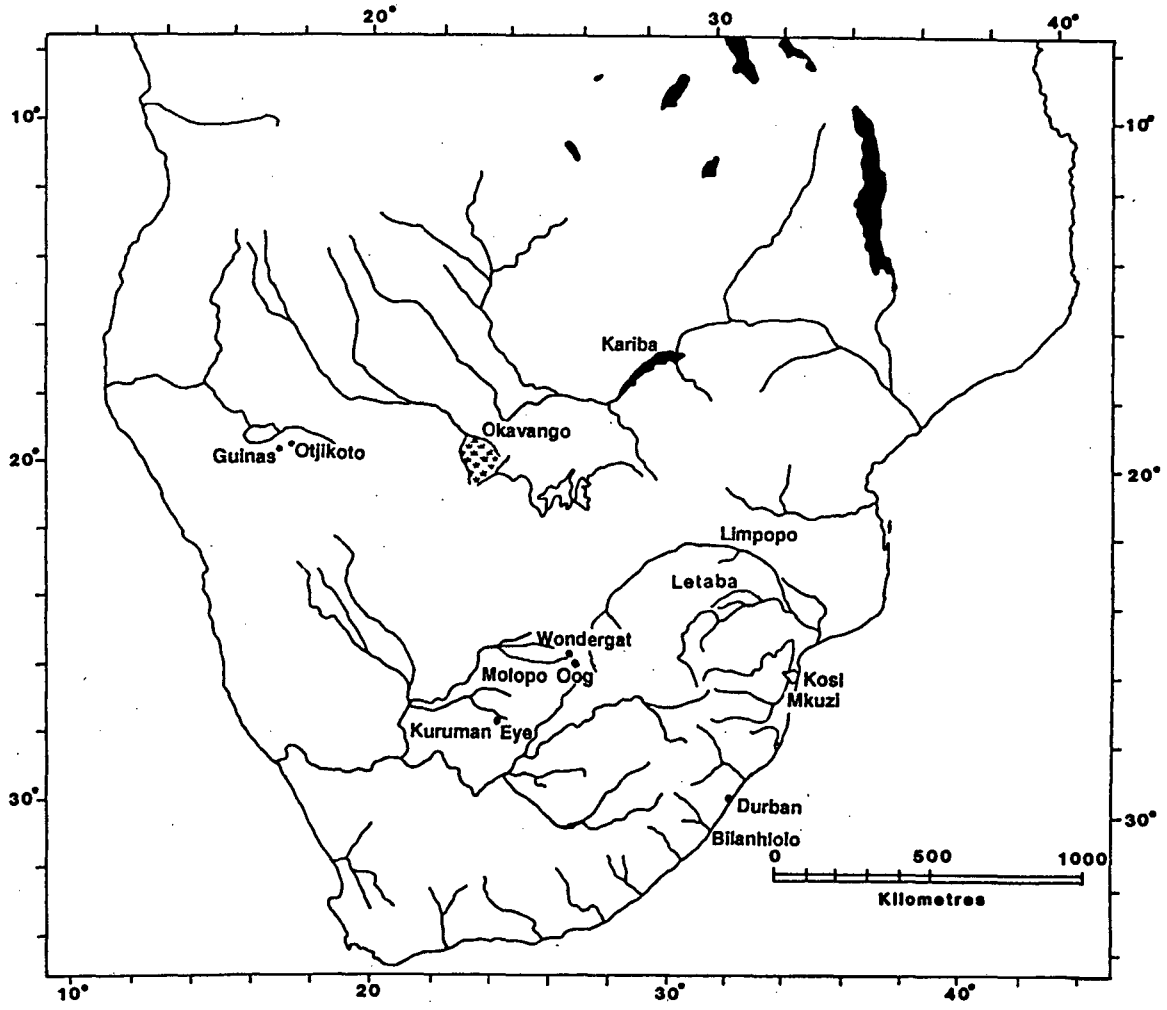

Figure 2.1: Map of southern Africa, showing collection sites of *P.philander* populations for this study.

P.philander were obtained from thirteen widespread localities within southern Africa (Fig. 2.1): Durban Bluff Nature Reserve (29° 56'S, 30° 59'E); Bilanhlolo (30° 50'S, 30° 19'E); Kosi (27°S, 32° 49'E); Muzi Pan (27° 1'S, 32° 19'E); Letaba River

(23° 50'S, 31°E); Lake Funduzi (22° 53'S, 30°18'E); Lake Kariba (16° 40'S, 28° 50'E); Wondergat (25° 52'S, 25° 53'E); Kuruman Eye (27° 27'S, 23° 27'E); Molopo Oog (25° 53' S, 26° 01'E); Ngquma Lagoon, Okavango (18° 55'S, 22° 25'E); Lake Otjikoto (19° 16'S, 17° 32'E); Lake Guinas (19° 12'S, 17° 20'E).

The following populations were studied in the field: Durban Bluff; Molopo Oog; Wondergat; Kuruman Eye; Lake Guinas; Lake Otjikoto. For the other populations, specimens were collected by colleagues, who also provided habitat descriptions.

Figure 2.2: A SCUBA diver taking notes in Lake Guinas.

Where possible, notes on the behaviour, distribution, habitat preferences and live colours of *P.philander* were made in the field (Fig. 2.2). Fish were collected by means of handnets in the shallows and by a curtain-net deployed by free or SCUBA divers in

deeper water. At Molopo Oog an electro-fisher was used to catch the fish which lived in dense vegetation or among the rocks in shallow water.

Fish were packed in oxygenated water in sealed plastic bags and insulated by polystyrene boxes for transport to the laboratories by road and air.

2) COLOUR DESCRIPTIONS

Colours and markings of live specimens were recorded for all wild populations either in the field, or as soon as possible after being brought to the laboratory. This was done by means of detailed notes on the coloration and markings of adult fish, using the notations of Barel, van Oijen, Witte & Witte-Maas (1977) (Fig. 2.3), and also with the use of a colour chart (Appendix 1).

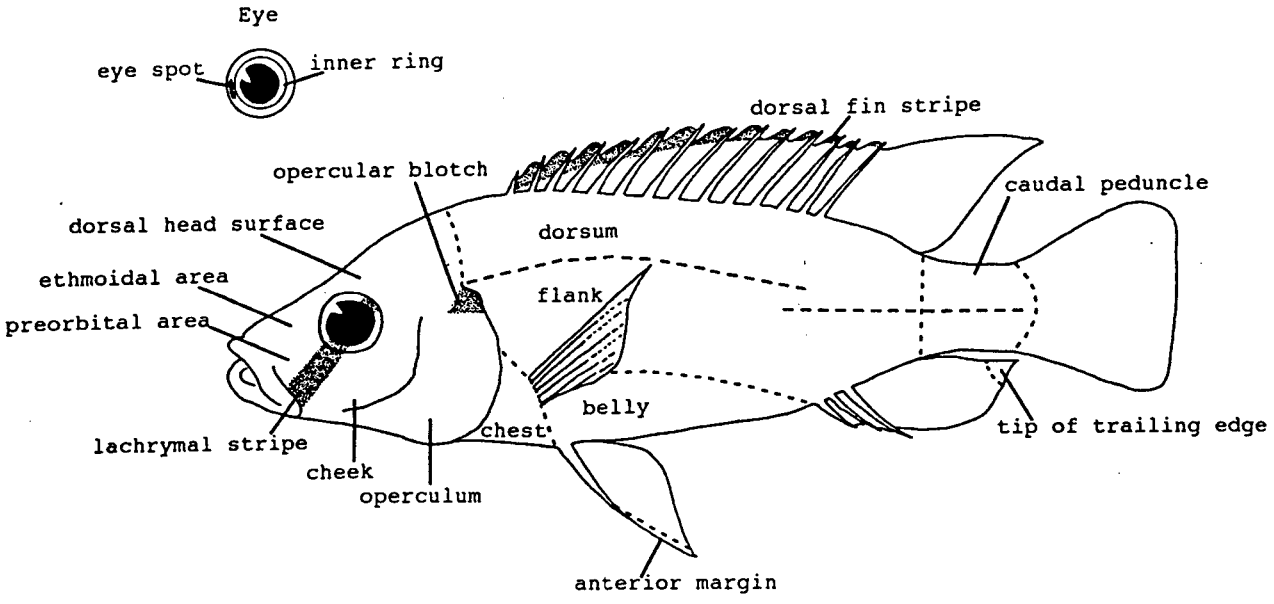

Figure 2.3: Illustration of *P.philander* showing marked areas used in the description of colours and markings.

Descriptions were supported by photographs, using a Nikon F2 camera, 105mm lens and Agfachrome CT 100 film. Later, diagnostic portraits of fish from each population were painted. These portraits were based on studies of live fish, on field notes and on colour photographs. (This thesis does not contain the full set of paintings as they have not been completed by the artist.)

3) MORPHOMETRICS AND ANATOMY

Morphological variation was determined by means of: (1) qualitative (descriptive) features; (2) morphometrics, using the conventional method (including absolute and proportional measurements) and the Truss method (Bookstein, Chernoff, Elder, Humphries, Smith & Strauss 1985); (3) meristic features (counts); (4) osteology; (5) dentition.

Due to difficulties in obtaining enough specimens of the rare populations and having to rely on colleagues to send us samples from remote areas, it was difficult to obtain adequate sample sizes in all cases. This problem was exacerbated by the need to share the samples with other workers for karyological and genetic studies. Although there was access to Okavango specimens (in the collection of the JLB Smith Institute of Ichthyology), the fish used here are restricted to a single known locality from which few live individuals were brought to the laboratory, hence the low sample size.

Fish from different populations were kept in separate tanks but under uniform conditions in the laboratory: all fish were fed the same diet (Tetramin flakes, grated ox-heart and *Daphnia* sp.);

temperature was maintained at 24-25 C; light regime was 12 hours light to dark.

Specimens of *P.philander* were killed by a high concentration of the anaesthetic, SANDOZ MS222. The fish were then pinned out, so that their fins were spread, and fixed in 10% formalin. After 10 days the specimens were rinsed in water and preserved in 70% Ethanol.

Morphometric measurements followed the procedures established by Greenwood (1956-1973; Greenwood & Gee 1969; Greenwood & Barel 1978) and further developed by Barel *et al.* (1977).

(i) All measurements were taken as the direct distance between the tips of the dividers placed on the defining points for the measurement involved.

(ii) All measurements were obtained from the left side of the fish unless damage or malformations prevented this, when the right side was used.

(iii) A dissecting microscope (with magnification of 8x-20x) was used when taking most measurements, and especially for those in the head region, where small distances were involved.

(iv) Distorted specimens were not included, as this would affect the accuracy of measurement.

(v) Specimens were kept wet while working on them, as slight shrinkage due to drying could significantly influence some of the measurements.

3.1) Definitions of Proportional Measurements based on Greenwood (Greenwood pers. comm.; Greenwood 1956-1973; Greenwood & Gee 1969; Greenwood & Barel 1978) and Barel *et al.* (1977) (Fig.2.4).

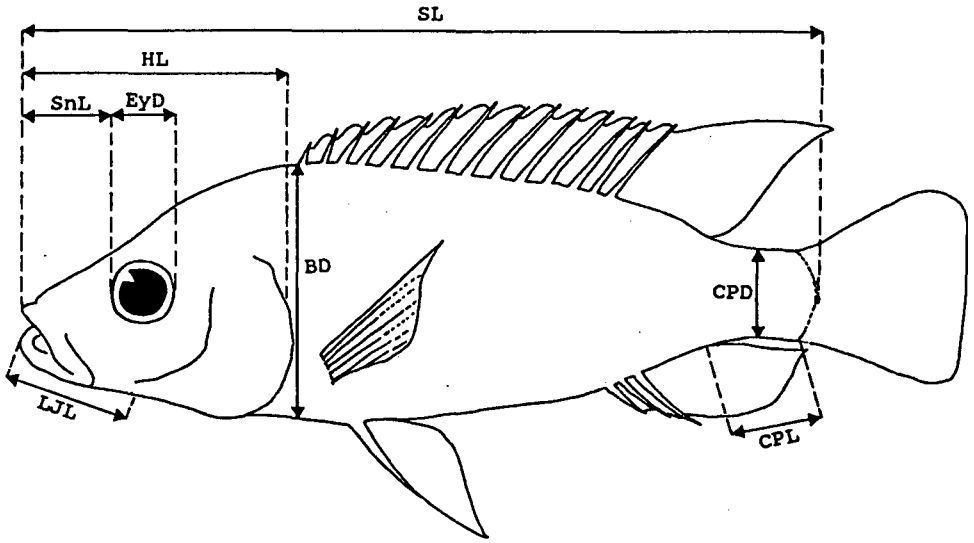

Figure 2.4: Some of the traditional morphometric measures used on *P.philander*.

STANDARD LENGTH (SL)

Definition: from the rostral tip of the upper jaw (symphysis between the premaxillae) to the origin of the caudal fin (caudal border of the hypurals). The measuring point for the latter is at the intersection of the vertical line through the hypurals and the line through the lower part of the lateral line. An external indication of the caudal border of the hypurals can be found by bending the caudal fin laterally to form a vertical skin fold. The mouth is kept closed and the premaxillae in a non-protruded position. The body is kept flat and the caudal peduncle horizontally aligned.

BODY DEPTH (BD)

Definition: the maximum vertical depth of the body.

Proportion: as percentage of standard length.

CAUDAL PEDUNCLE LENGTH (CPL)

Definition: the distance between the vertical line through the posterior point of the anal fin insertion and that through the caudal border of the hypurals.

Proportion: as percentage of standard length.

CAUDAL PEDUNCLE DEPTH (CPD)

Definition: the least vertical depth of the caudal peduncle.

Proportion: the ratio length/depth of the caudal peduncle. It indicates slenderness of the caudal peduncle.

CAUDAL FIN LENGTH (CFL)

Definition: the horizontal distance between the vertical line through the caudal border of the hypurals and the posterior margin of the caudal fin.

Proportion: as percentage of standard length.

HEAD LENGTH (HL)

Definition: from the rostral tip of the premaxillae to the most posterior point of the caudal margin of the bony operculum.

Proportion: as percentage of standard length.

HEAD WIDTH (HW)

Definition: the greatest width of the head when viewed dorsally. This width usually coincides with a point on the posterior part of the left and right preoperculum.

Proportion: as percentage of standard length.

INTERORBITAL WIDTH (IOW)

Definition: the least width between points on the dorsal bony margin of the left and right orbits.

Proportion: as percentage of the head length.

PREORBITAL DEPTH (POD)

Definition: from the rostral corner of the bony orbit to the rostral corner of the lachrymal.

Proportion: as percentage of the head length.

SNOUT LENGTH (SnL)

Definition: from the rostral point of the bony border of the orbit to the rostral tip of the premaxillae.

Proportion: as percentage of the head length.

SNOUT WIDTH (SnW)

Definition: the greatest width of the snout when viewed dorsally. This width is mostly found between a point on the left and right lachrymals.

Proportion: as the ratio length/width of the snout.

HORIZONTAL EYE DIAMETER (EyD)

Definition: the greatest horizontal distance between the anterior and posterior bony margins of the orbit.

Proportion: as percentage of the head length.

CHEEK DEPTH (ChD)

Definition: the greatest depth from the ventral point of the bony margin of the orbit to a point along the ventral border of

the adductor muscle.

Proportion: as percentage of the head length.

LOWER JAW LENGTH (LJL)

Definition: from the rostral tip of the lower jaw (symphysis of the dentary) to the posterior (caudal) tip of the retro-articular process.

Proportions: as percentage of the head length.

LOWER JAW WIDTH (LJW)

Definition: the greatest width, in ventral view (note: operculum is pressed closed).

Proportions: as the ratio length/width of the lower jaw.

ASCENDING PROCESS OF PREMAXILLA (AsPr)

Definition: from the symphysis of the upper jaw (premaxilla) to the tip of the ascending process, i.e. length of the ascending process.

Proportions: as percentage of the head length.

DENTIGEROUS ARM OF PREMAXILLA (DA)

Definition: the direct distance from the premaxillary symphysis to the caudal tip of the premaxilla.

Proportions: as the ratio ascending process length to dentigerous arm length.

LOWER PHARYNGEAL ELEMENT: LENGTH/WIDTH RATIO (LPL/LPW)

Definition: the width is the distance between the tips of the horns. The length is measured from the rostral point of the element perpendicularly to the line connecting the tips of the horns.

3.2) Truss measurements

Morphometric measurements of the body were based on the Truss method of Bookstein *et al.* (1985). Strauss and Bookstein (1982) pointed out several weaknesses of the traditional character sets used for morphometric analyses, the first being that most characters tend to be aligned with one of a very few axes (such as the "longitudinal"), with insufficient sampling of depth and breadth. Coverage of form, in terms of measurements, is very uneven. Some morphological landmarks are used repeatedly, so uncertainty in the position of a morphological feature would affect a series of measurements. Because the Truss configuration covers form more completely than "traditional" (conventional) character sets, it can considerably enhance discrimination between groups, when differences are not specific to a few structures (Strauss and Bookstein, 1982). Strauss and Bookstein (1982) also found another weakness in the traditional method, where points of measurement are often on soft-body parts. Such measurements could be affected by distortion of specimens due to preservation methods. By using anatomical landmarks which are bony points (and thus consistent features of morphology), such as those used by Truss, this can ensure consistency from form to form.

In this study, the Truss method was used as well as the conventional method of Greenwood (as set out in Barel *et al.* (1977)) to show up or enhance any differences between the populations of *P. philander*. Points were selected on the body so that measurements were in one plane (Fig. 2.5).

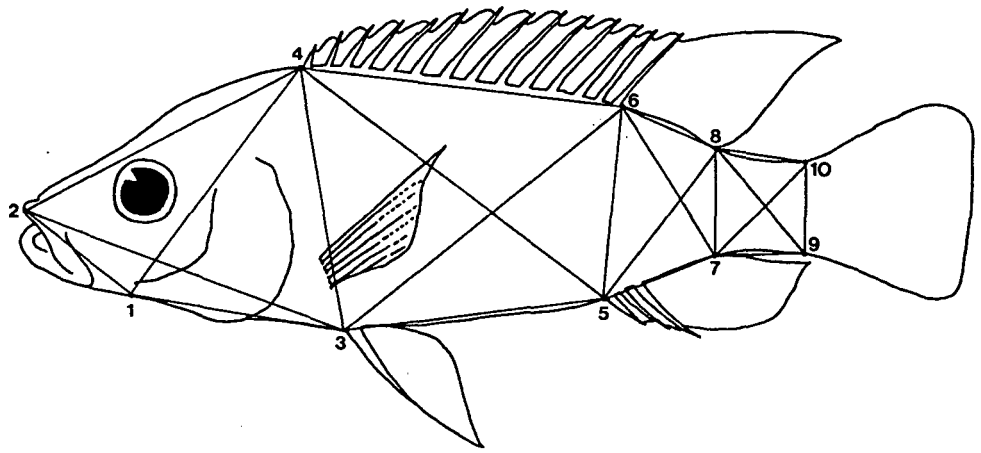

Figure 2.5: Illustration of *P.philander* showing points used for the Truss network of measurements.

These points are defined as follows:

1] Against the anterior margin of the pectoral girdle, where the left and right cleithra meet.

2] At the rostral tip of the upper jaw (premaxillary symphysis).

3] At the posterior face of the pelvic girdle, between the pelvic fins.

4] At the origin of the dorsal fin (anteriorly at the base of the first fin spine).

5] At the origin of the anal fin (anteriorly at the base of the first fin spine).

6] Posteriorly at the base of the last spinous ray of the dorsal fin.

7] Posteriorly at the base of the last branched ray of the anal fin.

8] Posteriorly at the base of the last branched ray of the dorsal fin.

9] At the base of the ventral caudal fin rays, i.e. the ventral end of the groove formed when the fin is bent laterally towards the observer. (See page 11 on Standard Length measurements.)

10] At the base of the dorsal caudal fin rays, opposite Point 9, i.e. the dorsal end of the groove formed when the fin is bent laterally towards the observer.

4) MERISTICS

4.1) Definitions of Counts (Meristics) and Angular Measurements (Barel *et al.* 1977).

SCALE COUNTS:

SCALES IN THE LATERAL LINE (ScLL)

Definition: the number of scales in the upper part of the lateral line plus the number of scales in the lower part which lie caudal to the last upper lateral line scale (Barel *et al.* 1977).

Note: the count is from the first pored scale to the end of the upper lateral line; where the lateral line divides, proceed down the row diagonally forwards, to the first scale in the horizontal series which continues the lateral line posteriorly, omit that scale and continue the count to the last large pored scale, excluding the smaller pored scales at the caudal fin origin. (Note: the first one or two scales in this line may not be pored.) Poreless and missing scales are included in the count.

A separate count of pored scales only, was also carried out.

SCALES BETWEEN LATERAL LINE AND DORSAL FIN ORIGIN (ScDL)

Definition: the number of scales in a (diagonal) series starting

at the origin of the dorsal fin and continuing to the scale immediately above the pored lateral line scales.

SCALES BETWEEN PECTORAL AND PELVIC FIN BASES (ScPP)

Definition: the number of scales from the ventral origin of the pectoral fin to the rostral origin of the pelvic fin.

SERIES OF SCALES ON THE CHEEK (ScCh)

Definition: the number of cheek scale rows in a line (not necessarily vertical), from the lowest point of the orbit to the ventral edge of the adductor muscle (the scales ventral to the eye are arranged in more or less horizontal rows).

SCALES AROUND THE CAUDAL PEDUNCLE (ScCP)

Definition: the count is from the first or second scale after the insertion of the anal fin, along the diagonal row (in the direction of the caudal fin) to the median scale, and continues on the other side of the body, back to the anal fin.

NUCHAL (PREDORSAL) SCALES (ScNuc)

Definition: the number of scales in a straight line from the dorsal fin origin to the first scale on the head.

FIN RAY COUNTS:

DORSAL FIN RAYS: SPINOUS (DFSp) AND BRANCHED (DFBr)

Definition: the number of spinous rays in the dorsal fin, and separately, the number of branched rays.

Note: The first spine is often small and depressed, so care must be taken to include it. When counting the last branched ray(s), careful examination is required, because the last two rays may

appear to be one deeply divided ray, but if the split continues to the fin base, then two rays must be counted (Barel *et al.* 1977).

ANAL FIN RAYS: SPINOUS (AFSp) AND BRANCHED

Definition: the same counts as for the dorsal fin.

Note: careful examination of the last branched ray, as for the last branched ray of the dorsal fin

GILL RAKERS (TOTAL)

Definition: gill raker counts are for the outer row on the first gill arch; the formula reads ceratobranchial, epibranchial-ceratobranchial junction, epibranchial.

Note: The gill raker in the angle between upper and lower elements of the arch is counted separately; for example, 9+1+2.

VERTEBRAE: TOTAL, ABDOMINAL, CAUDAL

Definition: total vertebral counts exclude the fused first preural (PU1) and first ural (U1) centra; abdominal vertebrae are those centra with pleural ribs; caudal vertebrae are those centra without the ribs.

5) OSTEOLOGY

5.1) Definitions of proportional skull measurements (Greenwood pers. comm.) (Fig. 2.6).

NEUROCRANIAL LENGTH (Ncl L)

Definition: from the rim of the basioccipital to the mid-point of the vomer.

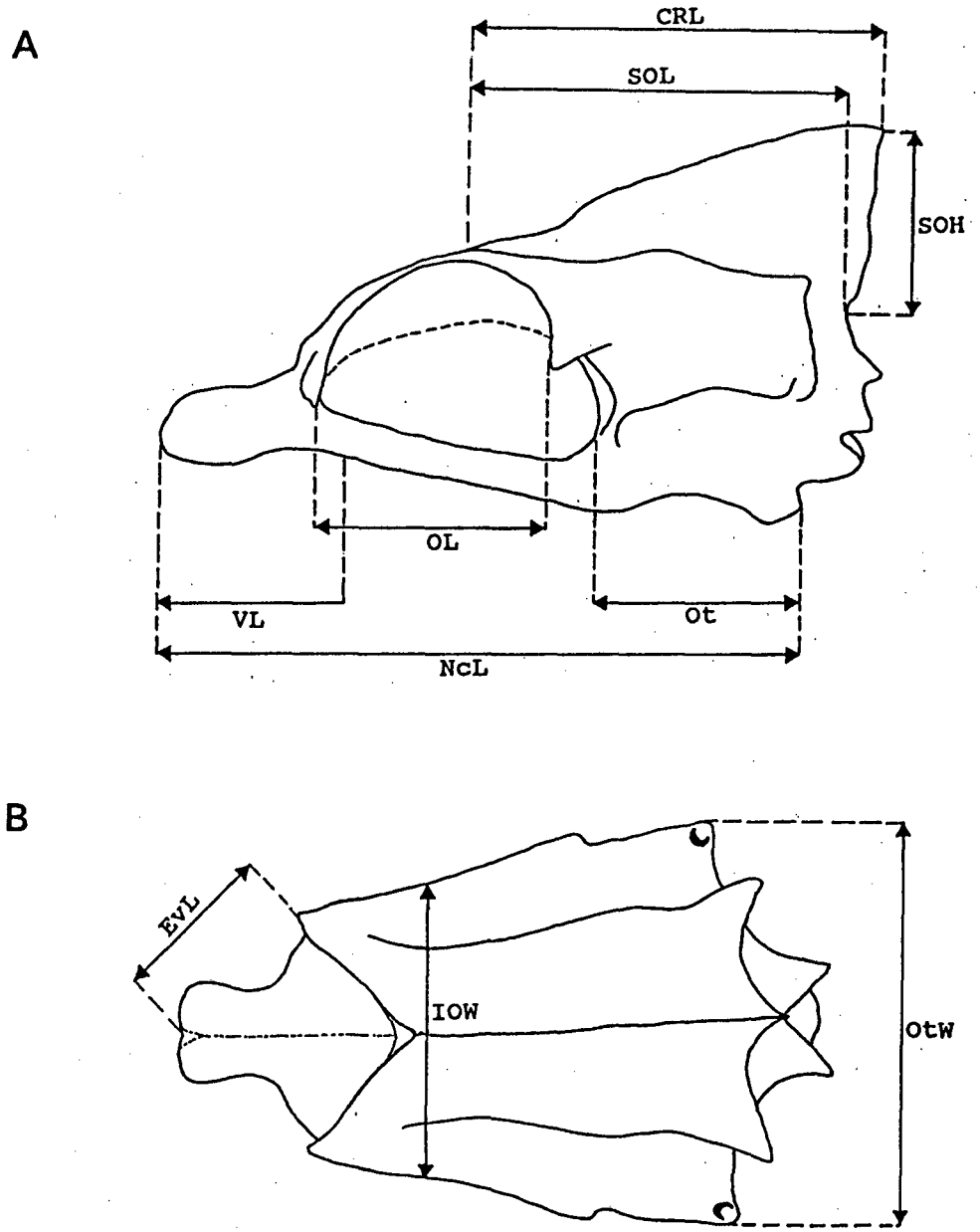

Figure 2.6: Lateral (A) and dorsal (B) views of the skull showing traditional morphometric measures.

INTERORBITAL WIDTH (IOW)

Definition: the least width between points on the bony orbits.

Proportion: as a percentage of the neurocranial length.

ETHMO-VOMERINE LENGTH (EVL)

Definition: directly from the anterior margin of the lateral ethmoid to the mid-point of the vomer.

Proportion: as a percentage of the neurocranial length.

ORBITAL LENGTH (OL)

Definition: from the tip of the ethmoid (anteriorly) to the tip of the sphenotic (posteriorly).

Proportion: as a percentage of the neurocranial length.

OTIC REGION (Ot)

Definition: from the anterior point where the parasphenoid curves upwards to meet the prootic, to the posterior rim of the basioccipital.

Proportion: as a percentage of the neurocranial length.

OTIC WIDTH (OtW)

Definition: the maximum width across the otic region, on the pterotic bones.

Proportion: as a percentage of the neurocranial length.

VOMERINE LENGTH (VL)

Definition: length of the vomer from its mid-point to its suture with the parasphenoid.

Proportion: as a percentage of the neurocranial length.

SUPRAOCCIPITAL CREST HEIGHT (SOH)

Definition: from the tip of the supraoccipital bone (highest point) to the base directly below (lowest point, usually the suture between the supraoccipital and the exoccipital bones).

SUPRAOCCIPITAL LENGTH (SOL)

Definition: from the base of the supraoccipital crest (suture between the supraoccipital and exoccipital bones) to the suture between the supraoccipital and the frontal bone.

Proportion: as the ratio length/ height of the crest.

SUPRAOCCIPITAL CREST LENGTH (CRL)

Definition: length of the whole crest, from the dorsal tip of the supraoccipital to the margin of the crest anteriorly, including that part of the frontal bone which contributes to the crest.

Proportion: as a percentage of the neurocranial length.

5.2) Truss measurements on the skull

Points were selected to make up a Truss network, but these were not all in one plane (Fig. 2.7).

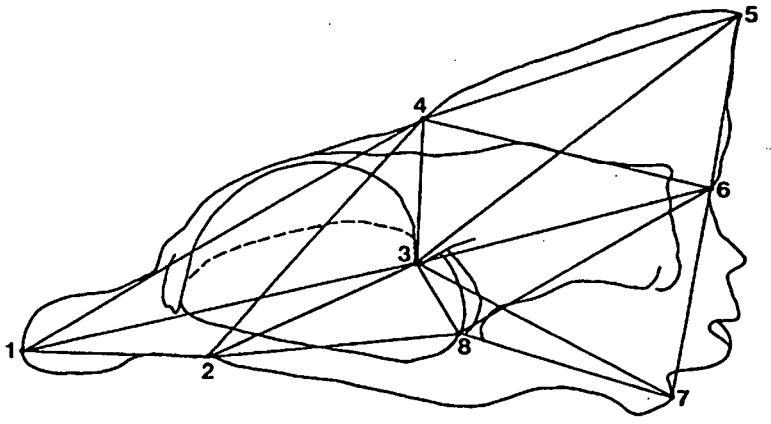

Figure 2.7: Lateral view of the skull showing points used for the Truss network of measurements.

These points are defined as follows:

1] At the mid-point of the vomer.

2] At a point on the suture between the vomer and the parasphenoid.

3] At the ventral orbital tip of the sphenotic.

4] At a point on the suture between the supraoccipital crest and the frontal bone.

5] At the tip (highest point) of the supraoccipital crest.

6] At the base (lowest point) of the supraoccipital crest (usually on the suture between the supraoccipital and the exoccipital bone).

7] At the rim of the basioccipital.

8] At a point on the suture between the parasphenoid and the anterior margin of the prootic.

6) DENTITION

JAW-TEETH

Jaw-teeth were studied using a Nikon microscope at a relatively high magnification (40x). Tooth counts were made, such as number of teeth in the outer rows of the premaxilla and dentary. A note was made of the number of inner rows anteriorly and anterolaterally. Tooth form, such as tooth size and number of cusps, was also described.

PHARYNGEAL BONES AND TEETH

Pharyngeal teeth were studied, using a microscope (at a magnification of 40x) and a Scanning Electron Microscope - JEOL SEM 100 (for greater magnification). Tooth rows (antero-posterior) were counted and tooth form described.

7) CLEARING AND STAINING

Clearing of tissues and staining of cartilage and bone was based on the techniques of Potthoff (1984).

8) X-RAYS

Fish specimens were X-rayed for vertebral counts and for examination of the axial and caudal fin skeletons. A Picker Soft X-ray unit, set at 34 kilovolts, 8 milliamperes and tube height of 45 centimeters.

9) STATISTICAL PROCEDURES

Statistical analysis was carried out using the BMDP Statistical Software Package (Dixon 1990) at Rhodes University. The data sets were subjected to Discriminant Function Analysis, a multivariate statistical procedure used to distinguish between two or more groups of cases. Data sets are made up of discriminating variables that measure characteristics on which the groups are expected to differ (Klecka 1975; Dixon 1990). The mathematical objective of Discriminant Analysis is to weight and linearly combine the discriminating variables in some fashion, forming discriminant function coefficients, so that the groups are as statistically distinct as possible (Klecka 1975). The BMDP produces standardised discriminant functions, canonical variables, for which the within-group variances are one and their overall mean is zero (Dixon 1990; Klecka 1975). The first canonical variable is the linear combination that best discriminates among the groups, the second canonical variable is the next best linear combination orthogonal to the first one, etc. (Dixon 1990). Results of a Discriminant Function Analysis are more easily visualised after plotting on a graph defined by the first two discriminant functions or, as in this case, the first two canonical variables.

The objectives of Discriminant Analysis are: *analysis* and *classification*.

Analysis allows for the interpretation of data. This technique can be used to study spatial relationships of groups and it can identify the variables which contribute most to differentiation of the groups. Statistical tests measure the success of the discriminating variables in distinguishing between groups (Klecka 1975).

Once a set of variables is found which discriminates groups with known memberships, new cases with unknown memberships can be *classified* (although this was not an objective of this study).

Another use of *classification* is in testing the adequacy of the derived canonical variables. By classifying the cases used to derive the functions (canonical variables) in the first place and comparing predicted group membership with actual group membership, one can empirically measure the success in discrimination by observing the proportion of correct classifications (Dixon 1990; Klecka 1975).

Multivariate statistical methods are used in morphological studies as they provide numerical and graphical techniques for examining data which consist of measurements on a number of individuals or cases. In this way, classical comparative morphology and anatomy can be described quantitatively (Albrecht 1980).

Data were subjected to Analysis of Variance (ANOVA) to test for significance of differences found between populations of wild-caught fish and between populations of laboratory-bred fish. ANOVA was also used to test for significant differences between

wild and laboratory-bred fish for each population. A Scheffe multiple range test was applied in each ANOVA test, to indicate which populations were significantly different.

Chapter 3

GEOGRAPHIC DISTRIBUTION AND HABITATS

GEOGRAPHIC DISTRIBUTION

Pseudocrenilabrus philander has a wide distribution in southern Africa, extending from the east to the west coasts, from Natal and the Orange River in the south, and northwards into central and tropical East Africa (including Malawi, Mozambique, Zaire, Zambia, Zimbabwe). Although *P.philander* occurs in the Lake Malawi Basin, it has not been found in the lake itself (Trewavas, 1936).

P.philander occurs in a variety of habitats, such as streams, rivers, shallow pans and "vleis" (marshes), and in lacustrine habitats, such as lakes, sinkholes and springs (Fig. 3.1). Many populations are totally isolated geographically, being separated from others by great distances. The most insular populations are those confined to sinkholes or springs in arid or semi-arid areas in the south western parts of its distribution. Palaeoclimatic evidence shows that in general, southern Africa was substantially wetter in the period 16 000 to 11 000 BP (Butzer, Fock, Stuckenrath & Zilch 1973; Deacon & Lancaster 1988). Thus it appears that *P. philander* dispersed after 16 000 BP, when a falling in lake and groundwater levels resulted in fragmentation of water bodies.

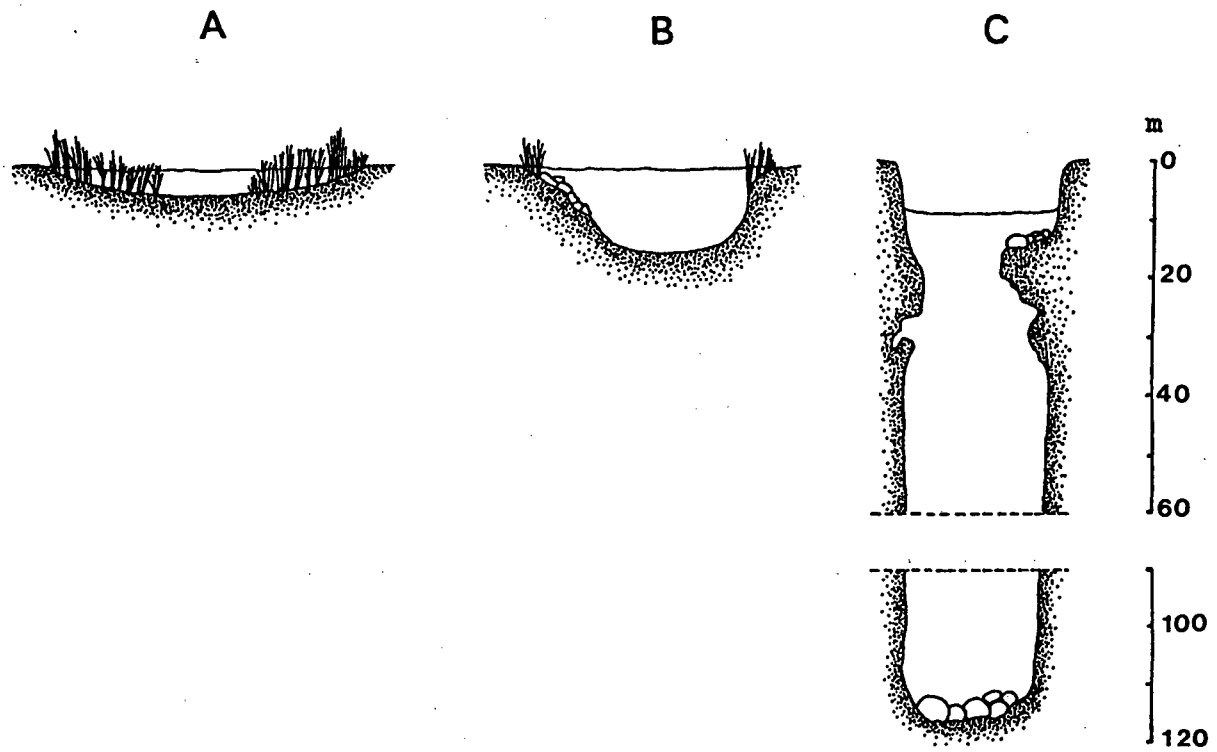

Figure 3.1: Illustration of the different types of habitats in which *P.philander* occurs:- A: shallow pan or "vlei"; B: stream or river; C: deep sinkhole lake.

The range of habitats occupied by *P. philander* include habitats with the following substrata: rocky, muddy, sandy and detritic. They may live in waters with vegetation (e.g. Molopo Oog, Kuruman Eye, many pans and vleis such as that in the Durban Bluff Nature Reserve) or without much vegetation (e.g. the sinkhole lakes), in shallow (e.g. Kuruman Eye, Molopo Oog) or deep water (e.g. Lakes Otjikoto and Guinas and Wondergat) and in water over wide temperature and pH ranges and hardness values (Jubb 1961; Loiselle 1982b). It can also tolerate salinities of up to 20 parts per thousand (Millard & Broekhuysen 1970; Whitfield, Blaber & Cyrus, 1981). Although it occurs in clear

waters, water clarity may be reduced by algal blooms in the warmer months (e.g. Lake Kariba; Otjikoto) or by disturbance of the muddy substrate in times of flood (e.g. Lake Kariba; rivers in Natal and Kwazulu, especially in cyclones). *P. philander* can survive temperatures as low as 16°C for prolonged periods (Loiselle, 1982), and can withstand brief drops in temperature (to 9.5°C) and increases in temperature (to 35°C) (Mike Coke *in lit.*). The optimal range, however, is 24°C to 29°C (Loiselle, 1982b).

HABITATS

i) Lakes

P. philander has colonized lacustrine habitats (Greenwood 1973a; Trewavas 1973; Loiselle 1982a, b), such as Lake Sibaya, lakes in Mozambique and Lake Kariba (an artificial lake). Lake Sibaya is a natural coastal lake, which is isolated and fresh (Hill 1969). The substrate is made up of both vegetated and open sandy areas, which may be covered with detritus. *P. philander* is found in the shallow, marginal areas, and offshore, down to depths of 40m (the deepest part of the lake). However, sheltered bays and inlets, characterized by steep slopes close inshore and dense submerged vegetation, are the preferred habitats (Bruton 1979). Males hold territories in water from 5-24m deep (Ribbink 1975). Mouthbrooding females and juveniles inhabit areas of submerged plants in deeper water (15-40m), while shoals of fry are found from 3-30m depth (Bruton 1979).

Lake Kariba is a large, man-made lake on the Zambezi River. The substrate is either sandy or muddy, with extensive aquatic

vegetation in most areas (Kit Hustler *in lit.*). Water clarity is variable, depending on wind and wave action and rainfall. Visibility was estimated as being usually about 2m by Kit Hustler (*in lit.*), although no information was given as to how the estimate was derived. Water temperature in the surface water ranges from 17-32°C (Welcomme 1972). Although the lake is deep, *P. philander* was found mainly in the shallows, along the gently sloping edges, particularly in areas of macrophyte growth (Hustler & Marshall 1990).

P.philander are the commonest fish along the sandy shores of many of the lakes in Zululand, Natal (Crass 1964).

ii) Rivers and streams

Figure 3.2: A shallow, slow flowing stream below the Malemane Eye (Western Transvaal), which *P.philander* inhabits.

P. philander occurs in small streams (Fig. 3.2) and large rivers (for example Limpopo, Okavango, Orange, Pongolo and Zambezi Rivers), although it tends to occur along the edges of the faster flowing rivers (Crass 1964; Loiselle 1982a) and in the shallows where flow rate has decreased and where submerged vegetation is more abundant (Bell-Cross & Minshull 1988).

In the Okavango, *P. philander* occupies the river, isolated pools and the flood-plain. *P. philander* is common in the littoral vegetated areas, but is not found in the deeper, open river channels. It does, however, occur in the weeded fringes of these open areas, while the open waters are inhabited by *Hydrocynus* and other fishes (Glenn Merron pers. comm.).

The type of substrate (as well as water clarity) in rivers and streams varies, depending on stream flow. Vegetation cover may be in the form of aquatic plants and reeds on the edges of the water. Temperature of the water may range from 14-25^{o}C in a small stream (Ben van der Waal pers. comm.). Fish feed on virtually any small aquatic organisms, such as midge larvae, insects and shrimps (Crass 1964; Bell-Cross & Minshull 1988).

iii) Pans and Lagoons

These are shallow waterbodies, with a very slow flow-through (Fig. 3.3). The 'vlei' in the Durban Bluff Nature Reserve is fringed with vegetation such as reeds and bulrushes and grass. The water is clear, and the fish are found in the shallow waters (less than 1m in depth) (pers. obs.).

Figure 3.3: Shallow 'vlei' in the Durban Bluff Nature reserve showing fringing vegetation.

In Ngquma Lagoon (in the Okavango), the substrate is sandy with fringing reeds and bulrushes. The water is clear and is about 1-1.5m deep; water temperature is on average 24-25°C. *P. philander* occurs in the littoral, vegetated areas of the lagoons in the Okavango (Glenn Merron pers. comm.).

The substrate of Muzi Pan (Mkuzi Swamps) is overlain with leaf detritus from overhanging fringing vegetation. The water is not very clear, with Secchi disc readings of about 0.6m (Skelton, Whitfield & James 1989) and ambient water temperature ranges from 24-34°C. (Nick James pers. comm.). Although *P.philander* was found to be more common in the open water lagoons than in the riverine habitats of the Mkuzi system, it showed its ecological versatility and tolerance by occurring in all the habitats

sampled by Skelton, *et al*. (1989), i.e. in the river and associated flood plain, in streams, in large open water lagoons, in the lower swamp and in isolated pans.

iv) Springs

The springs from which fish were collected for this study, were Molopo Oog and Kuruman Eye, situated in semi-arid regions (Fig. 3.4). The waterbodies are clean and clear, with a constant flow-through of water welling up from the "eyes" (22 million litres per day from Kuruman Eye (Jubb 1971) and 25 million litres per day from Molopo Oog (Ribbink & Twentyman-Jones 1989). Ambient water temperature is fairly constant for both springs (22°C) (Jubb 1971) but with seasonal fluctuations away from the subterranean sources.

Figure 3.4: Aerial view of Molopo Oog, showing the aridness of the surrounding countryside. The source is on the right-hand side and the weir on the left-hand side of the photograph.

Molopo Oog is about 1km long and about 200m wide at its widest part, near the source, the rest being a narrow channel dammed at the end by a weir. The water is nearly 3m deep at the source, becoming shallower (0.3m) near the weir. The substrate is sandy, but overlain with mud and detritus along the edges. The whole waterbody is fringed with reeds (*Phragmytes sp.*) (Fig. 3.5), forming dense cover for the *P. philander* adults and juveniles to hide from the introduced predatory bass, *Micropterus salmoides*. *P. philander* also make use of the cracks and crevices between the rocks of jetties for breeding sites and for protection (similar to the rock-dwelling, Mbuna species of Lake Malawi). Mouthbrooding females and fry occur in the extreme shallows.

Behavioural observations suggest that *P. philander* feed on epiphytes on the sandy and muddy substrates, and on the algae (Aufwuchs) on rocks.

Figure 3.5: Molopo Oog, with a dense reed fringe.

The spring of the Kuruman Eye was originally the source of the Kuruman River, but domestic and agricultural demands on the water are such that the river no longer flows (Jubb 1971). Today, the water flows into a "miniature lake" (Jubb 1971), which is a small (70x30m), shallow (0.5-1.5m deep) waterbody situated in the middle of the town of Kuruman (Fig. 3.6). The fishes in Kuruman Eye include an endemic population of *P.philander*, *Tilapia sparrmanii*, *Barbus* species and *Clarias gariepinnus*. In creating a park and beauty spot for the town, carp were introduced to the spring and most of the natural reeds were removed and replaced by water lilies (resulting in the removal of fish breeding sites).

Figure 3.6: Kuruman Eye, a spring in the centre of the town of Kuruman.

The substrate is sandy where the water enters the area, but is covered with mud and detritus further away from the inlet. The sandy areas are used by *P. philander* for breeding. Behavioural

observations suggest that adults and juveniles feed on epiphytes, and that adults also hunt for copepods in the sandy substrate. Small fry remain and feed in the shallows.

v) Sinkholes

The sinkhole lakes, Wondergat, Guinas and Otjikoto are situated in semi-arid regions, being totally separated from other waterbodies (Fig. 3.7, 3.8 and 3.9).

Sinkholes are formed when part of the roof of water-filled underground caves collapses, often leaving deep bodies of water. Wondergat is 60-75m deep, Lake Otjikoto 27-36m and Lake Guinas about 120m deep. The sinkholes have a fairly small surface area (approximately 3600m for Wondergat, 2800m for Guinas and 4200m for Otjikoto).

Figure 3.7: Wondergat, a sinkhole in the Western Transvaal. A shelf sloping down to a depth of about 20m can be seen in the foreground.

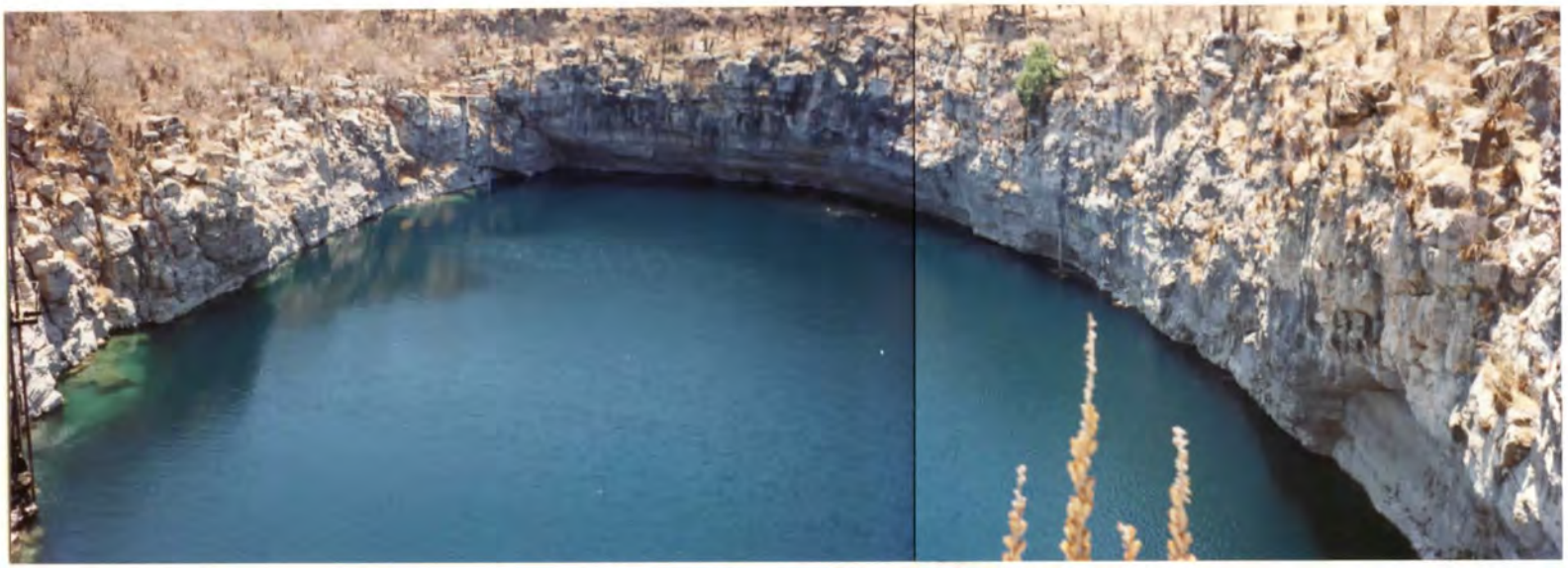

Figure 3.8: Lake Guinas in Namibia. Some of the submerged rocky shelves can be seen along the western wall of the sinkhole.

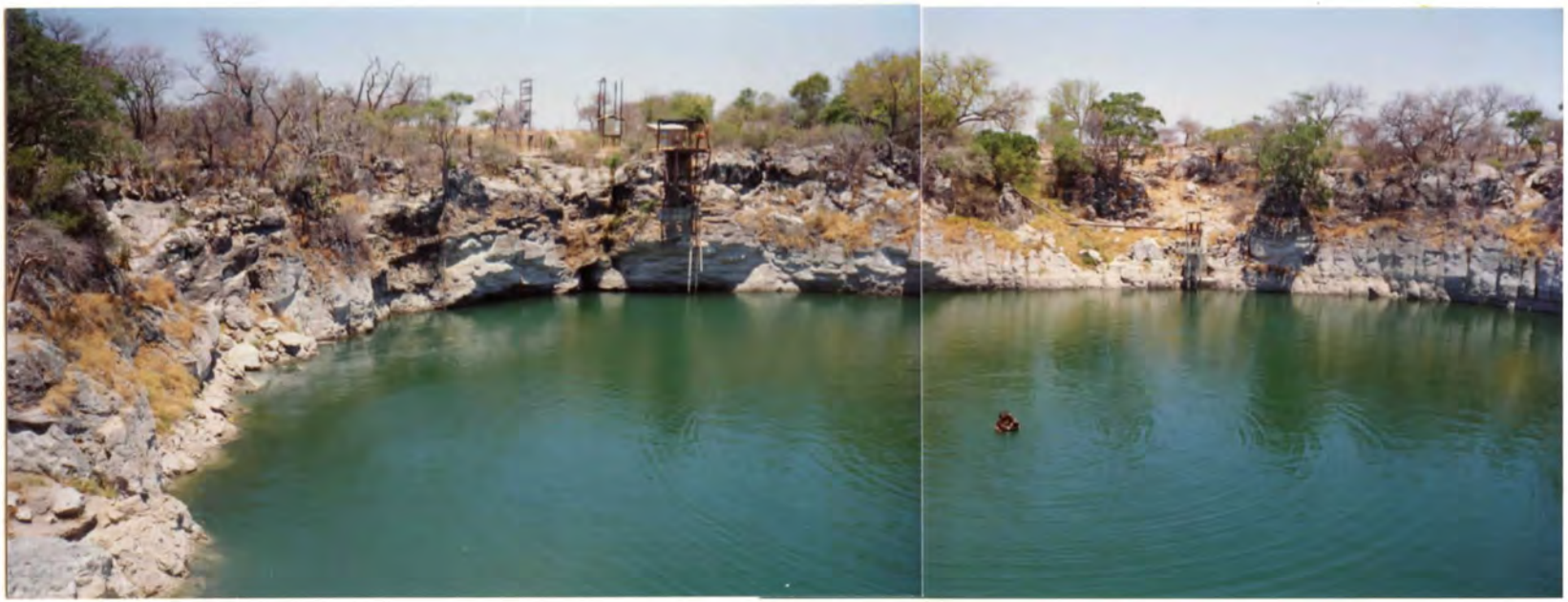

Figure 3.9: Lake Otjikoto (Namibia), with an algal bloom clouding the water.

Water clarity is generally very good, with underwater visibility of about 15-20m. This may decrease in the rainy season due to algal blooms (which cloud the visibility above the thermocline, such as that in Lake Otjikoto). Ambient water temperature is 21°C in Wondergat and about 24°C in Lakes Otjikoto and Guinas, with seasonal fluctuations.

Water level fluctuates seasonally, the extent of which depends on the effects of rainfall on the water table. Water flow is hardly detectable in Otjikoto (Penrith 1978), or Wondergat (Gomes 1985). Circulation in Guinas, however, is very slow, but was strong enough to affect the neutrally buoyant nets used by divers for catching fish. Vertically suspended rope transect lines were angled by an anticlockwise flow.

The vertical walls of the sinkholes are sheer, with very few ledges and crevices. Wondergat and Otjikoto have more ledges and platforms and also piles of rubble (broken rocks from the walls of the sinkholes), than Guinas. *P.philander* use the ledges for breeding and nesting sites; fry use the cracks and crevices in the vertical walls for protection from predators (either conspecifics or other fish). *P. philander* was rare in Guinas, but more common in Wondergat and Otjikoto, possibly because there are more breeding sites and more areas affording protection for fry in the latter two sinkholes.

Submerged macrophytes do not occur in sinkholes, so fish feed on surface insects and on the algae growing on the rocky walls ("Aufwuchs"). Fish feed mostly near the surface, where algal productivity is highest, due to greater light penetration in the water (Ribbink & Twentyman-Jones 1990). However, *P. philander*

were found to depths of about 36m in all three sinkholes (and may even occur at greater depths than the limits to which we dived). Penrith (1978) reported catching *P. philander* in traps at depths of 100m in Guinas.

Chapter 4

COLOUR VARIATION

Most members of each geographically isolated *Pseudocrenilabrus philander* population studied are characterised by their distinctive coloration. The fact that there is, at least modally, such a distinction suggests that populations are beginning to diverge in this particular characteristic. To determine the taxonomic significance of these interpopulation colour differences, the various colorations have to be described. Such descriptions are also necessary to determine whether or not any clinal gradations exist between populations.

GENERAL

Along the eastern region of their distribution (Sibaya, Durban, Kosi, Bilanhlolo), adult *P. philander* were found to have a golden-yellow ground coloration (Fig. 4.1). Those from the more western regions (Lake Kariba, Okavango, Wondergat) have a blue body coloration (Fig. 4.2). Fin colour and markings vary between populations. For example, males of the Kosi population have black pelvic fins and a conspicuous dorsal fin stripe, while those of Wondergat are not as dark, or there may be little or no black on the fins at all (such as the fish from Bilanhlolo). Colour and markings of the unpaired fins (i.e. dorsal, anal and caudal) were found to differ in intensity between populations.

Although the ground coloration of females is the same as that of males, their fin and body colours are duller than those of males.

A

B

Figure 4.1: Adult male *P.philander* of (A) Durban and (B) Kosi populations, showing golden-yellow ground coloration.

A

B

Figure 4.2: Adult male *P.philander* of (A) Okavango and (B) Wondergat populations, showing blue ground coloration.

LOCAL

(1) Durban Bluff Nature Reserve

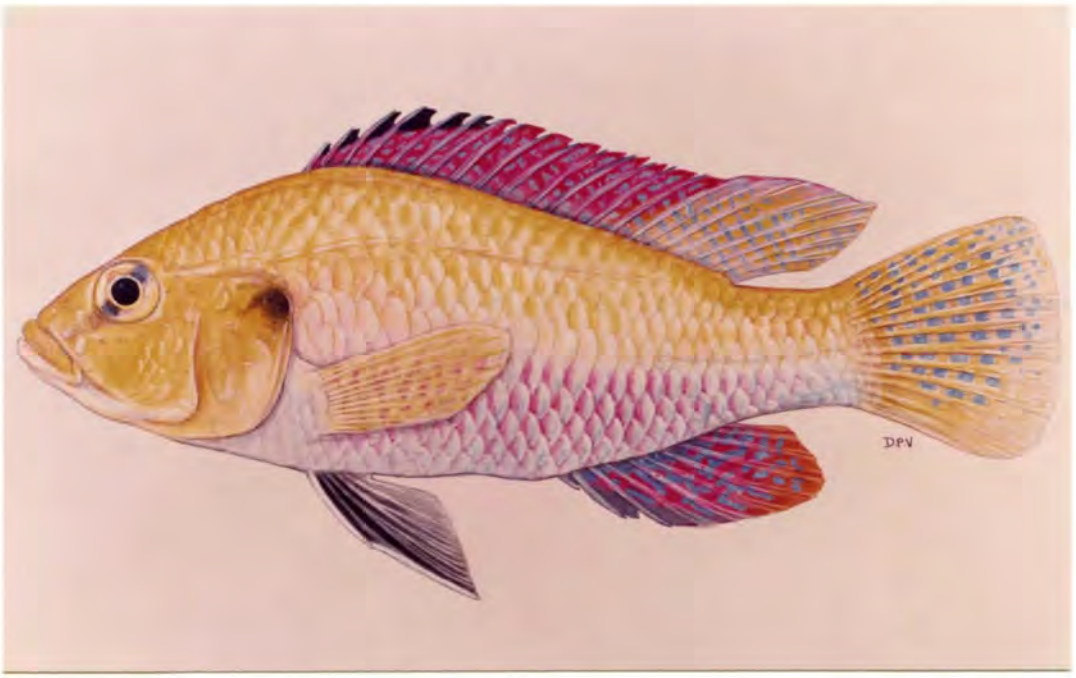

Figure 4.3: Adult male *P.philander* from Durban Bluff Nature Reserve.

Male coloration: background colour golden-yellow. Preorbital, ethmoidal area and dorsal head surface golden-yellow. Cheek and operculum dark golden-yellow. Opercular blotch conspicuous dark brown. Lips golden-yellow, almost orange. Broken line from lower lip (not on lip), along lower edge of cheek to operculum a very pale, fluorescent blue. Inner ring of the eye light golden coloured, iris black. Dark brown eye stripe (lachrymal stripe) not very conspicuous, extending to posterior end of lower lip. Eye spot pale red, but more obvious in some and not in others. Dorsum bright golden-yellow, extending onto caudal peduncle.

Flank golden-yellow with reddish colour on scales. Ventral flank silvery-blue with reddish tinge on scales. Chest and belly silvery-white. Dorsal fin magenta and blue checks anteriorly with yellow and blue checks posteriorly. Dorsal fin stripe black on lappets of anterior six fin spines. Pectoral fins bright golden-yellow. Pelvic fins black, becoming grey to transparent on trailing edge; white anterior margin. Anal fin magenta and blue checks; grey anterior margin, with orange (mandarin orange) trailing edge. Caudal fin golden-yellow and pale fluorescent blue checks.

Female coloration: background colour pale, silvery-grey. Preorbital, ethmoidal, dorsal head surface and operculum silvery-grey. Opercular blotch dark brown. Cheek silvery-grey with mauve tinge. Lips grey with fluorescent silver mark posterior to lower lip. Inner ring of eye pale orange, iris black. Eye spot pale red. Eye stripe very pale. Dorsum silvery-grey. Dorsal flank, caudal peduncle and ventral flank silvery-grey with blue tinge. Mid flank with gold tinge on scales. Chest and belly pale grey-white. Dorsal fin transparent with very pale blue checks; fin base and posterior of fin (on rays) with yellow tinge. Dorsal fin stripe grey. Pectoral and pelvic fins transparent. Anal fin yellow, but with grey anterior margin. Caudal fin transparent with very pale blue checks; base and ventral portion of fin yellow.

Sexual dimorphism: males have a golden-yellow body coloration, females are pale, silvery-grey; males have more conspicuous body markings, such as eye stripes and fin markings; the anal fin in females is yellow, but checkered with jet black anteriorly, in males.

Distinguishing features: males have a deep golden-yellow body; the dorsal fin has a magenta background; a large portion of the anal fin is black and there is no orange tip to the fin.

(2) Bilanhlolo

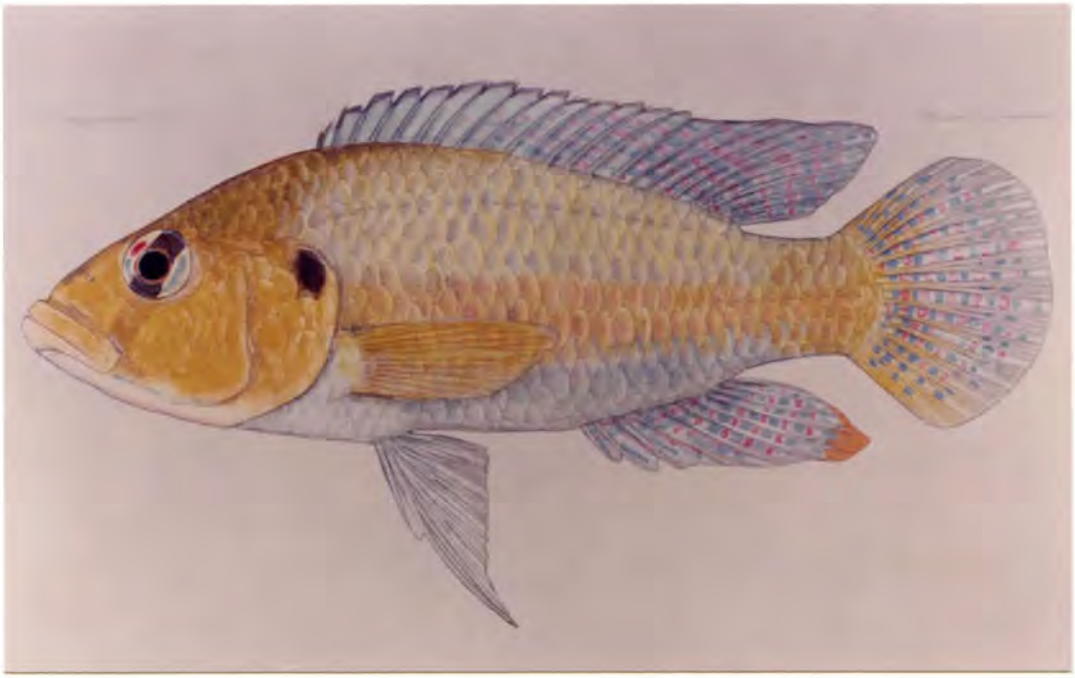

Figure 4.4: Adult male *P.philander* from the Bilanhlolo River.

Male coloration: background colour yellow-gold. Preorbital and ethmoidal areas yellow-gold; dorsal head surface with khaki tinge. Upper lip, cheek and operculum bright yellow-gold. Opercular blotch dark brown. Lower lip pale yellow, in some males almost white. Marking along lower edge of cheek to operculum fluorescent blue (almost silver and not very conspicuous). Inner ring of the eye copper-yellow; iris black. Eye stripe conspicuous dark brown, with lighter marking extending towards lower lip. Eye

spot conspicuous red; blue markings in the eye. Dorsum silvery-grey, but with khaki-gold tinge. Dorsal flank silvery-grey with very pale yellow tinge. Mid-line and caudal peduncle khaki with mauve tinge. Ventral flank and belly silvery-blue; chest paler, nearly white. Dorsal fin transparent but with pale blue and magenta checks. Dorsal fin stripe faint black. Pectoral fins yellow; pelvic fins transparent. Anal fin checkered with magenta and bright blue; pale carrot-orange on trailing edge. Caudal fin magenta and bright blue checks; yellow tinge at fin base and ventrally. Dorsal part of fin transparent at trailing edge.

Female coloration: background pale yellow-gold. Preorbital, ethmoidal and dorsal head surface khaki-gold. Lips, cheek and operculum yellow-gold with khaki tinge. Opercular blotch brown, but not very obvious (absent in some). Stripe along lower edge of cheek to operculum fluorescent silver. Inner ring of eye copper-gold; iris black. Eye spot conspicuous red; blue markings in the eye. Dorsum khaki-gold. Dorsal and ventral flank silvery-blue with pink tinge. Mid-line and anterior of flank with gold tinge. Chest and belly pale grey to white. Dorsal fin transparent, with pale blue checks posteriorly (on fin rays). Pectoral fin pale yellow-gold. Pelvic fin transparent, with yellow tinge and white anterior margin. Anal fin yellow-gold. Caudal fin transparent with yellow tinge at base and darker yellow on ventral part.

Sexual dimorphism: males and females are yellow-gold, but females are much paler; fin markings and coloration are also much paler in the female.

Distinguishing features: there is no black on the pelvic fins and a very narrow black dorsal fin stripe.

(3)Kosi

Male coloration: background colour orange-gold. Preorbital, ethmoidal and dorsal head surface dark gold (orange-gold). Lips, cheek and operculum yellow-gold. Dark brown opercular blotch laterally extended towards the eye. Stripe on lower lip pale fluorescent blue. Eye stripe conspicuous dark brown; eye spot faint red. Dorsum gold but with blue tinge. Dorsal flank orange-gold (reddish gold anteriorly), fading to yellow-gold on ventral flank. Chest and belly greyish white. Dorsal caudal peduncle has a blue tinge, medial portion gold and ventral portion with blue-grey tinge. Dorsal fin has a yellow background colour, but a magenta and pale blue checkered pattern on the branched rays. Dark black dorsal fin stripe and conspicuous red margin on the fin. Pectoral fins yellow-gold; pelvics very black, transparent posteriorly, with white anterior edge. Anal fin black on anterior edge, fading to grey and pale blue checks. Caudal fin yellow-gold with blue and magenta checks fading to a transparent trailing edge.

Female coloration: background colour silvery-grey with pink tinge. Preorbital, ethmoidal, dorsal head region and lips pink-grey. Cheek and operculum tinged with gold. Opercular blotch dark brown. Stripe below lower lip, extending below cheek towards the operculum, pale fluorescent blue. Eye stripe present, but not very conspicuous. Eye spot very pale red. Base of the dorsal fin yellow-gold. Dorsum and dorsal flank pink-grey, ventral flank blue tinged, becoming yellow-gold (pale) towards the belly. Belly yellow-gold, chest greyish-white. Dorsal fin pink-grey with pale blue checks on branched rays. Dorsal fin

stripe grey. Pectoral fins yellow, pelvics transparent with white anterior margin. Anal fin yellow. Caudal fin yellow, fading to transparent, with pale blue checks on the trailing edge.

Sexual dimorphism: males have a checkered anal fin, of which a large portion anteriorly is black.

Distinguishing features: conspicuous dark brown eye stripe extending anteriorly towards the mouth; orange-gold body colouring; large portion of anal fin is black (unlike the Lake Sibaya form which has the entire anal fin black (Ribbink 1975)).

(4) Muzi Pan (Mkuze)

Male coloration: background colour khaki. Preorbital, ethmoidal and dorsal head surface khaki. Cheek khaki (almost golden-yellow); operculum has pink sheen (tinge) and brown opercular blotch. Lower lip khaki; fluorescent blue marking (stripe) extending below cheek towards operculum. Inner ring of eye pale gold; iris black. Eye spot yellow-orange. Eye stripe conspicuous (black) and extended towards upper lip. Dorsum silver-blue with pink sheen. Mid-line and caudal peduncle golden-yellow, but red tinge on scales. Ventral flank has a blue sheen; chest and belly silver-white. Dorsal fin magenta and blue checks anteriorly (between fin spines), with pale magenta and blue checks posteriorly, and an overall yellow tinge (between branched rays). Pectoral fins yellow. Pelvics black anteriorly, but transparent trailing edge, and white margin on anterior edge. Anal fin with magenta and blue checks, blackish anterior edge and orange marking on trailing edge. Caudal fin pale magenta and blue checks, yellow tinge at fin base and ventral part of fin.

Female coloration: background silvery grey, but yellow tinge. Preorbital, ethmoidal and dorsal head surface khaki. Cheek silver; operculum and lower cheek have a pink sheen. Lips khaki; stripe along lower cheek fluorescent silver-blue. Inner ring of the eye gold; iris black. Eye spot red. Dorsum and flank silver with yellow tinge. Chest and belly silvery white. Dorsal and pectoral fins transparent with yellow tinge, dorsal fin has faint checkered pattern (magenta and blue). Pelvics transparent with white anterior edge. Anal fin yellow. Caudal fin transparent with yellow tinge and darker yellow at fin base.

Sexual dimorphism: dimorphism in intensity of body coloration, with males being darker in colour than females; anal fin of females yellow, males checkered with black anteriorly.

Distinguishing features: none.

(5) Letaba River (Limpopo)

Male coloration: background colour yellow-gold. Preorbital, ethmoidal, dorsal head surface, lips, cheek and operculum yellow-gold. Opercular blotch grey-brown. Lower lip fluorescent blue with stripe extending along lower cheek and as a very faint stripe over the operculum. Inner ring of the eye copper coloured; iris black. Eye stripe present, darker in some than in others. Eye spot red, but not very conspicuous. Dorsum silvery-blue. Flank and caudal peduncle yellow-gold with reddish on the scales. Chest and belly black. Dorsal fin with yellow ground colour and pale blue and magenta checks; conspicuous black margin (dorsal fin stripe) to the 6th/7th spiny ray. Pectoral fins yellow-gold. Pelvics black (but transparent), darker anteriorly and with a

white anterior margin. Anal fin magenta and blue checks with a yellow-gold ground colour, faint black margin anteriorly and pale orange tips on the trailing edge. Caudal fin with yellow-gold ground colour and magenta and blue checks.

Figure 4.5: Adult male *P.philander* from the Letaba River.

Female coloration: background colour silvery-grey. Preorbital, ethmoidal and dorsal head surface silvery-grey with yellow-gold tinge. Cheek and operculum silver-grey; grey-brown opercular blotch. Lower lip fluorescent blue, with pale stripe extending along lower edge of cheek, but not onto the operculum. Inner ring of the eye copper coloured; iris black. Eye stripe not very conspicuous and no red eye spot. Dorsum, dorsal flank and dorsal caudal peduncle dark grey-silver. Flank and ventral caudal peduncle lighter grey-silver. Chest and belly even lighter grey-

white. Dorsal fin yellow ground colour with pale blue and magenta checkered pattern. Pectoral fins pale yellow. Pelvics transparent with a white anterior margin. Anal fin pale yellow. Caudal fin yellow with a very faint checkered pattern.

Sexual dimorphism: dimorphism in intensity of body colour; fluorescent blue stripe along lower cheek does not continue onto the operculum of females as in the males; anal fin of females pale yellow, males have a checkered fin, black anteriorly and a carrot-orange tip to the trailing edge.

Distinguishing features: males have a black chest and belly (similar to that of the Lake Sibaya populations described by Ribbink (1975: Plate 4); the anterior portion of the anal fin is black while the rest of the fin is magenta with checks.

(6) Lake Funduzi

Male coloration: background colour golden-yellow, tending to khaki. Preorbital, ethmoidal, dorsal head surface, cheek and operculum golden-yellow. Opercular blotch dark brown-copper. Lower lip fluorescent blue, with blue line extending along lower cheek to operculum. Inner ring of the eye copper coloured. Eye spot just visible, and reddish brown. Eye stripe not very conspicuous, with a "shadow" of a stripe extending down towards the mouth. Dorsum khaki-yellow. Dorsal and ventral flank and caudal peduncle silvery-blue. Flank along mid-line golden yellow. Chest and belly silvery-white. Dorsal fin transparent, but with a yellow tinge and magenta and blue checks. Black dorsal stripe not very conspicuous, but all fin spines black-tipped. Pectoral fins pale yellow, but transparent. Pelvics have a white anterior

margin, and are black becoming dark grey and transparent posteriorly. Anal fin golden yellow with blue markings; trailing edge with a carrot-orange tip. Caudal fin yellow, but darker yellow on the ventral portion; magenta and blue checks very light.

Female coloration: background colour khaki-yellow. Preorbital, ethmoidal, dorsal head surface and upper lip khaki-yellow. Lower lip fluorescent blue with blue stripe extending along lower cheek. Opercular blotch dark brown. Inner ring of the eye copper coloured. Eye spot very pale and red-brown. Eye stripe not very conspicuous with a "shadow" extending across the cheek. Dorsum khaki-yellow. Dorsal and ventral flank and caudal peduncle silvery-blue. Mid-line of body and extending onto the caudal peduncle, golden-yellow. Chest and belly silvery-white. Dorsal fin transparent, but with a yellow tinge. Dorsal fin stripe black along fin spines; anteriorly tinged red with blue markings (checks). Pectoral fins transparent with yellow tinge at fin base. Pelvics transparent with white anterior margin. Anal fin golden-yellow. Caudal fin pale yellow, darker at fin base and ventral portion of fin, with pale blue and magenta checks.

Sexual dimorphism: general background colour of females more "muddy" than males; golden-yellow anal fins in both sexes, but males have blue spots and magenta anteriorly, and a carrot-orange tip to the trailing edge.

Distinguishing characters: the edge of the caudal fin (males) is magenta and the posterior tip of the dorsal fin is orange.

(7) Lake Kariba

Figure 4.6: Adult male *P.philander* from Lake Kariba.

Male coloration: background colour appears golden-yellow when the body is viewed from some angles, but appears blue from other angles. Preorbital, ethmoidal, dorsal head surface and cheek (brightest below eye) golden-yellow. Stripe on lower lip extending along lower edge of cheek to operculum fluorescent blue (upper lip also has fluorescent blue coloration). Inner ring of eye copper coloured. Eye stripe conspicuous black, extending towards mouth. No red eye spot. Dorsum golden-yellow. Dorsal flank (reddish tinge on scales) and dorsal caudal peduncle silvery blue. Ventral flank (reddish tinge on scales) and ventral caudal peduncle golden-yellow. Chest and belly silvery-white (red tinge on scales behind pectoral fins). Dorsal fin has conspicuous

black dorsal fin stripe on anterior portion (on first 6/7 fin spines), fading to grey on lappets of posterior portion of fin. Anteriorly, red below dorsal fin stripe; posteriorly, yellow with faint blue checks. Pectoral fins transparent yellow. Pelvics black with white anterior margin and grey-transparent trailing edge. Anal fin with dark grey-black anterior edge and very red, with blue checks; transparent with checks posteriorly. Chrome yellow marking on trailing edge. Caudal fin transparent golden-yellow with blue checks.

Female coloration: background colour very pale mustard-yellow. Preorbital, ethmoidal, dorsal head surface and upper lip mustard (dirty) yellow. Cheek and operculum silver with mauve tinge. Lower lip fluorescent blue, with a broken line extending along lower edge of cheek to operculum. Inner ring of the eye copper coloured. No red eye spot. Prominent black eye stripe, extending (as a much lighter marking) down across the cheek to the mouth. Dorsum mustard yellow. Dorsal flank silvery-blue with mauve tinge. Ventral flank pale yellow-gold with silvery-blue behind the pectoral fin. Caudal peduncle silvery-blue. Chest and belly silvery with yellow tinge. Dorsal fin transparent with pale yellow tinge; narrow, grey dorsal fin stripe. Pectoral fins transparent but yellow at base. Pelvics transparent with white anterior margin. Anal fin yellow anteriorly and at fin base with transparent trailing edge (may have a few very pale blue checks). Caudal fin pale transparent yellow, darker ventrally, with pale blue checks.

Sexual dimorphism: males have very dark, strong colours and markings while females are very pale; females have a yellow anal

fin, but there may be a few very pale blue checks; males have a magenta anal fin anteriorly, the remainder is checkered and the trailing edge has a carrot-orange tip.

Distinguishing features: blood-red on dorsal fin (anteriorly, below dorsal fin stripe) and on anal fin.

(8) Wondergat

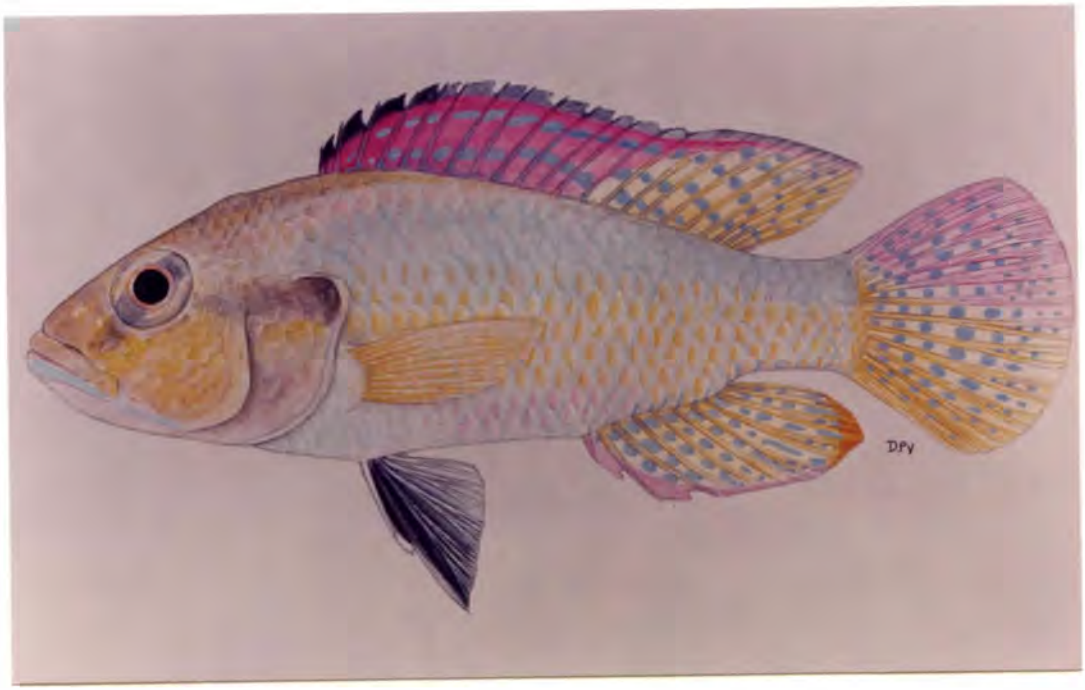

Figure 4.7: Adult male *P.philander* from Wondergat.

Male coloration: background colour grey-gold, but scales edged with blue. Preorbital, ethmoidal and dorsal head surface grey-gold. Cheek and operculum yellow-gold. Opercular blotch grey-brown, extending towards the eye. Lower lip fluorescent blue, with a faint blue stripe continuing along the lower edge of the cheek towards the operculum. Inner ring of the eye copper

coloured; iris black. Eye stripe faint. Dorsum grey-gold. Dorsal flank and dorsal caudal peduncle (above the lateral line) silvery-blue. Ventral flank and ventral caudal peduncle yellow-gold. Chest and belly white with silvery-blue tinge. Dorsal fin transparent grey with black dorsal fin stripe (darker and wider on the spiny rays) and magenta anteriorly. Checked blue and magenta more conspicuous on branched rays. Pectoral fins yellow. Pelvics black fading to transparent grey; white anterior margin. Anal fin light magenta anterior margin; yellow posteriorly with blue checks and carrot-orange trailing edge. Caudal fin transparent yellow but darker yellow ventrally. Checked pattern on fin: blue and magenta dorsally; blue and yellow ventrally.

Female coloration: background grey-pink but with silvery-blue tinge. Preorbital, ethmoidal, dorsal head surface, cheek and operculum grey-gold (khaki). Opercular blotch grey-brown. Lower lip pale fluorescent blue with broken blue stripe extending along lower edge of cheek. Inner ring of the eye copper coloured; iris black. Eye stripe conspicuous in some and virtually absent in others. Eye spot pale red. Dorsum darker grey-pink than the rest of the body, with gold tinge. Dorsal flank and dorsal caudal peduncle silvery-blue tinge, ventrally (flank and caudal peduncle) yellow-gold. Chest and belly grey-white. Dorsal fin grey with dark grey dorsal fin stripe. Pectoral fins yellow. Pelvics transparent with white anterior edge. Anal fin yellow and may have a few faint blue markings. Caudal fin transparent grey, and yellow-gold ventrally, with a very faint checkered pattern.

Sexual dimorphism: males more brightly coloured than females; anal fin of females yellow with some faint blue markings;

carrot-orange tip to the trailing edge of anal fin of males.

Distinguishing characters: females have a few faint blue markings on the yellow anal fin; distinct carrot-orange coloured trailing edge of anal fin.

(9) Kuruman Eye

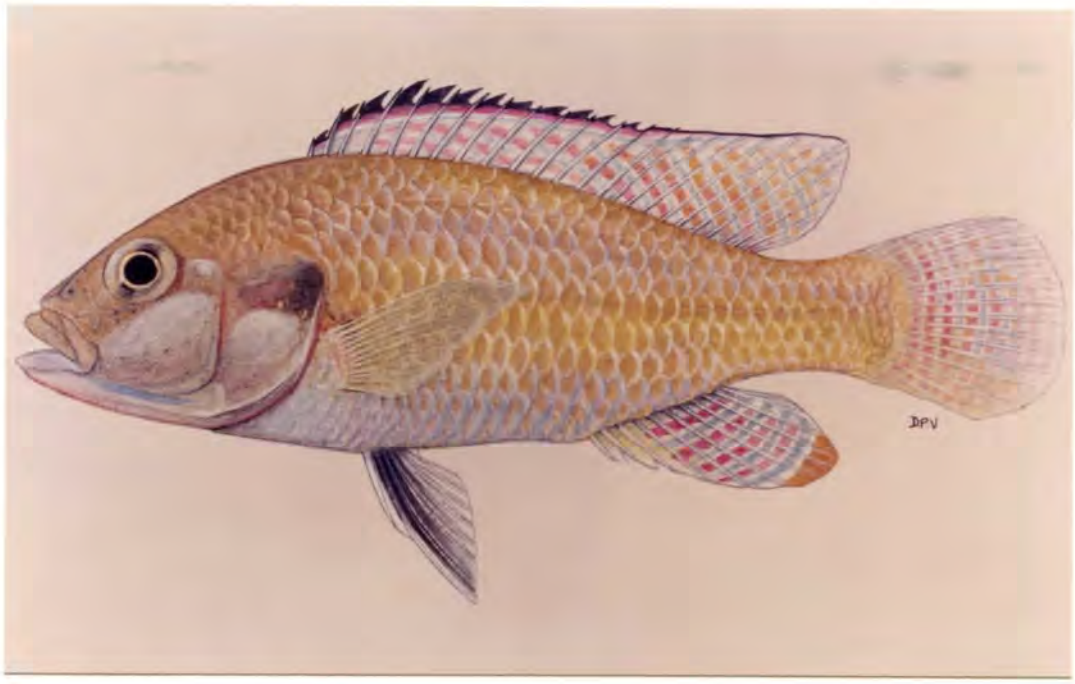

Figure 4.8: Adult male *P.philander* from Kuruman Eye.

Male coloration: background colour khaki. Preorbital, ethmoidal and dorsal head surface khaki. Cheek and operculum silvery with a pink (lilac) sheen. Lower lip has a fluorescent light blue stripe, extending along the lower cheek as a sky-blue stripe. Inner ring of the eye pale gold; iris black. No red eye spot; no eye stripe. Dorsum khaki. Dorsal flank and dorsal caudal peduncle silvery-pink with blue sheen. Ventral flank and ventral

caudal peduncle yellow-gold. Chest and belly silver-white. Dorsal fin transparent with blue and magenta checks. Dorsal fin stripe black along the whole length of the fin. Pectoral fins yellow. Pelvics black, fading to transparent grey posteriorly and white anterior margin. Anal fin blue and magenta checks with bright carrot-orange trailing edge. Caudal fin pale blue and magenta checks and yellow tinge on ventral part of fin.

Female coloration: background colour same as male, but paler. Preorbital, ethmoidal and dorsal head surface khaki. Cheek and operculum silver with pink sheen. Lower lip khaki, but with very pale blue tinge, extending as a more conspicuous stripe to the operculum. Inner ring of the eye pale gold; iris black. No red eye spot; no eye stripe. Dorsum and dorsal flank khaki with a gold tinge. Mid-flank yellow-gold; ventral flank silver-blue (very pale). Chest and belly white. Dorsal fin transparent with a yellow tinge and blue and magenta checks. Dorsal fin stripe narrow and light along the length of the fin. Pectoral fins pale yellow. Pelvics grey with white trailing edge. Anal fin transparent, with blue and magenta checks, with yellow anterior edge. Caudal fin magenta and blue checks, with yellow tinge on ventral part of fin.

Sexual dimorphism: males darker in colour than females.

Distinguishing characters: anal fin of females is transparent with blue and magenta checks, and yellow anteriorly; general pallid ground colour in both males and females.

(10) Molopo Oog

Figure 4.9: Adult male *P.philander* from Molopo Oog.

Male coloration: background colour silver-blue. Preorbital, ethmoidal and dorsal head surface mustard yellow (khaki). Cheek and operculum golden-yellow. Opercular blotch brown. Lower lip fluorescent blue, with line extending along lower cheek. Inner ring of the eye orange-red; black iris. No red eye spot. Dorsum, dorsal caudal peduncle and ventral flank silver-blue. Dorsal flank yellow-gold. Scales are reddish with blue or gold edges. Silver-white chest and belly. Dorsal fin magenta and blue checkered pattern, yellow tinge to posterior portion of fin. Dorsal fin stripe black along the whole fin. Pectoral fins yellow. Pelvics grey with black anterior edge and white margin. Anal fins yellow-orange with magenta and blue checks. Base of fin

magenta; very bright carrot-orange tip to trailing edge of fin. Caudal fin magenta and blue checks on yellow-orange background, which is darker ventrally on the fin; margin red (carmine) and narrow edge black.

Female coloration: background colour silver-blue with pink tinge. Preorbital, ethmoidal and dorsal head surface khaki. Cheek and operculum pale gold with pink tinge on cheek. No opercular blotch. Stripe (sometimes broken) from edge of lower lip along lower cheek to operculum fluorescent blue. Inner ring of the eye copper coloured; iris black. No red eye spot. No eye stripe. Dorsum yellow-gold at base of the dorsal fin. Dorsal flank silver-blue. Ventral flank golden-yellow. Chest and belly silver-white. Dorsal fin magenta and very light blue checks (darker magenta posteriorly). Narrow, light, black dorsal fin stripe. Pectoral fins yellow. Pelvics transparent grey with white anterior margin. Anal fin yellow anteriorly, with blue and magenta checks posteriorly. Caudal fin magenta and blue checks (some fluorescent blue) with a very narrow black margin.

Sexual dimorphism: males have a blue body coloration when viewed at a certain angle; males are very brightly coloured (e.g. very distinct carrot-orange marking on anal fin), while females are paler.

Distinguishing characters: males have a yellow-gold head, but dorsal head surface is khaki; anal fin of females has conspicuous blue and magenta checks on a dark yellow background.

(11) Okavango

Male coloration: background colour silver-blue. Preorbital,

ethmoidal and dorsal head surface khaki (with grey tinge). Cheek grey; operculum gold-tinged. Opercular blotch grey-brown. Lower lip fluorescent blue, with stripe extending along lower edge of cheek (not onto operculum). Inner ring of the eye coppery-red; iris black. Eye stripe conspicuous dark brown, extending over the iris and towards the upper lip. Eye spot red, but not conspicuous. Dorsum khaki-grey. Dorsal flank and dorsal caudal peduncle bright blue-silver. Mid flank yellow-gold. Ventral flank silver-blue extending onto ventral caudal peduncle. Chest and belly light blue-silver (nearly white). Dorsal fin yellow with pale blue and magenta (checkered pattern). Wide dorsal fin stripe conspicuous black, with the lappets black (to the 6th dorsal fin spine) for approximately one half the dorsal fin height, becoming narrower posteriorly. Pectoral fins yellow-gold. Pelvics black, with grey trailing edge and white margin on leading edge. Anal fin yellow-gold with pale blue and magenta (checkered pattern). Trailing edge has a very small carrot-orange marking on the tip. Margin on leading edge grey-black. Caudal fin yellow-orange, interradial membranes blue and magenta checks. Fin base yellow-orange (i.e. base of radials near the caudal peduncle).

Female coloration: background colour khaki-grey. Preorbital, ethmoidal and dorsal head surface khaki-grey. Cheek and operculum silver-grey with pink tinge on cheek and yellow-gold tinge on operculum. Opercular blotch dark brown. Lower lip fluorescent silver-blue, stripe (more silver than blue) extending along lower part of cheek to the operculum. Inner ring of the eye coppery-red; iris black. Light eye stripe; no red eye spot. Dorsum khaki-grey. Dorsal flank and dorsal caudal peduncle khaki. Mid-line

pink tinge, ventral flank blue-silver, with yellow-gold anteriorly (beneath pectoral fin). Chest and belly grey-white. Dorsal fin pale yellow with pale magenta and blue checks Faint black dorsal fin stripe with magenta stripe below. Pectoral fin yellow (paler than male). Pelvics transparent white. Anal fin pale yellow-gold with a few blue spots near the body and towards the trailing edge. Caudal fin with pale magenta and blue checks, pale yellow ground colour, darker towards the base of the fin (ventrally, but near the body).

Sexual dimorphism: males have a distinct blue body coloration, while females are khaki-grey. Although females also have blue spots on the anal fin, the spots are pale and few and they lack an orange tip on the trailing edge and the magenta anteriorly (which males have on the anal fin).

Distinguishing characters: males have a distinct blue body coloration and very black pelvic fins; both upper and lower lips of males are fluorescent blue.

(12) Lake Otjikoto

Male coloration: background colour khaki dorsally with a blue sheen on the flank. Preorbital, ethmoidal and dorsal head surface khaki. Cheek silvery with a pink sheen; operculum silver. Opercular blotch dark brown. Lower lip fluorescent blue, with blue stripe extending below cheek and trace of blue on operculum of some. Inner ring of the eye pale gold; iris black. Eye stripe black; no red eye spot. Dorsum ("dirty") khaki. Dorsal and ventral flank pink-silver with a blue tinge; same on caudal peduncle. Flank yellow-gold along mid-line and mid-caudal

peduncle. Chest and belly silver grey. Dorsal fin transparent with a yellow tinge and faint blue and magenta checks on the fin rays of some. Faint dorsal fin stripe (black) on the first few spines, and red tips to the rest of the rays and spines. Pectoral fins yellow. Pelvics transparent, but grey (darker anteriorly) with transparent posteriorly. Anal fin yellow with blue checks and red tinge anteriorly with a grey-black edge. Orange mark on trailing edge inconspicuous, but pale orange in some. Caudal fin yellow, darker at base and ventral part of fin; faint blue checks.

Female coloration: background colour khaki ("dirty"), with blue tinge. Preorbital, ethmoidal and dorsal head surface khaki. Cheek silvery pink; operculum silver. Opercular blotch dark brown. Lower lip khaki with trace of fluorescent blue, stripe extending below cheek (not onto operculum). Inner ring of the eye pale gold; iris black. Eye stripe black; no red eye spot. Dorsum "dirty" khaki. Dorsal and ventral flank silvery pink with blue sheen, extending onto caudal peduncle. Mid flank and mid caudal peduncle pale yellow-gold. Chest and belly silver-white. Dorsal fin transparent yellow with light black dorsal fin stripe and red band below this. Pectoral fins pale yellow. Pelvics transparent. Anal fin yellow. Caudal fin transparent, with yellow at base and ventral part with faint blue checks.

Sexual dimorphism: males are not very brightly coloured, but are still more colourful than females. Anal fin of females is yellow, while that of males is black anteriorly, has checks on a magenta background and is mauve on the trailing edge with a pale orange tip.

Distinguishing characters: coloration of both sexes very pale in comparison to other populations; magenta background of anal fin; trailing edge of anal fin of males has a mauve tinge.

(13) Lake Guinas

Male coloration: background colour "metallic" gold with a green-blue tinge on the flank. Preorbital, ethmoidal and dorsal head surface khaki-gold. Cheek and operculum gold. No opercular blotch. Lower lip fluorescent blue, with blue stripe extending below the cheek. Inner ring of the eye copper-gold; iris black; no red eye spot. Prominent eye stripe across the eye black and extending to the posterior part of the lower lip. Dorsum, dorsal and ventral flank silver-blue. Mid-flank gold with green-blue tinge. Chest black; belly silver-white. Dorsal fin yellow with a few very pale spots posteriorly (on the branched rays). Prominent dorsal fin stripe black and a slight red tinge below this. Pectoral fins yellow. Pelvics black with a white anterior margin. Anal fin dark grey anterior margin and powder-blue fin with a few fluorescent blue spots and an orange tip to the trailing edge. Caudal fin yellow, but blue tinge on the ventral edge.

Female coloration: background colour khaki ("dirty"). Preorbital, ethmoidal and dorsal head surface khaki. Cheek and operculum khaki-gold ("dirty" grey). Lower lip khaki, no blue, but broken line of blue on lower edge of cheek. Opercular blotch brown, but not very dark. Iris black; inner eye ring pale gold. No red eye spot; no eye stripe. Dorsum and flank "dirty" grey. Dorsal flank tinged blue. Dorsal fin yellow, faint black dorsal fin stripe. Pectorals yellow; pelvics transparent. Anal fin yellow; caudal fin yellow.

Sexual dimorphism: males brightly coloured, being almost "metallic" gold in colour, while females are dull and "dirty" coloured. Anal fin of males is black, and the remainder is powder blue-mauve.

Distinguishing characters: anterior part of the anal fin of males is black, and the rest is powder blue-mauve; "metallic" gold body colour.

SUMMARY

Males of the different populations of *P.philander* studied show differences in terms of their body coloration and fin markings, with differentiation of colour forms over the east-west distributional range. Females of all the populations are generally dull, grey or khaki coloured fish (following the basic body coloration of males), without the striking fin markings shown by the males. Although the coloration in some populations appears to have a higher level of differentiation than in other populations, there appears to be no clinal gradation of ground colour and fin markings and colour. In other words, there is no gradual change in ground colour or pattern over geographical distance.

Fish bred in the laboratory over three generations were found to have the same colours as those of the wild populations, indicating that colour is heritable for *P. philander*. Cross-breeding experiments on populations of *P. philander* carried out by Ribbink (1975) produced forms with intermediate coloration, demonstrating genotypic variation between groups. Ribbink's work

also showed that populations bred true under laboratory conditions after several generations.

Although the populations from which some individuals were derived may be recognised on the basis of the fishes coloration, in other individuals this was not an accurate means of trenchantly determining their origin. This situation suggests that populations are not differentiated on the basis of coloration, thus supporting the thesis of incipient speciation.

Chapter 5

MORPHOLOGICAL FEATURES OF INDIVIDUALS FROM WILD-CAUGHT POPULATIONS

In addition to differing in colour, some of the allopatric populations of *Pseudocrenilabrus philander* appeared to differ from one another with regard to anatomical features. To establish the extent of this apparent anatomical differentiation and to evaluate the significance of these differences careful measurements were made, using standard meristic techniques (Barel *et al.* 1977, which includes Greenwood's work : Greenwood, 1956-1973; Greenwood & Gee, 1969; Greenwood & Barel, 1978), as well as Truss measurements (Bookstein *et al.* 1985).

Anatomical differences between geographically isolated populations could be heritable indications of evolutionary divergence and could therefore be of considerable taxonomic value. Alternatively, they might be ecophenotypic responses to different conditions (Greenwood 1965a; Mayr 1988).

This chapter presents the differences which exist between wild-caught populations. In the next chapter, the heritability of characters is examined and the modifications to the phenotypes kept under laboratory conditions are described to determine the respective contributions to these differences of heritable and ecophenotypic effects.

Ideally, one should have a reasonably large sample size over a large size range for anatomical measurements (such as those done here), but because many of the populations studied were rare or

endangered, samples were kept small; or, as in the case of the Lake Guinas population, fish were so rare under natural conditions that an adequate sample was impossible to collect. We also had to rely on samples sent from remote areas by colleagues who did not provide sufficient material for morphological, behavioural and genetic studies (the other aspects of the project). The limitations of such small numbers which are imposed on a study of this nature are fully recognised and are borne in mind in an evaluation of the results.

Figure 5.1: A fixed, preserved specimen of *P.philander* as used for the morphometric measurements and meristic counts

DESCRIPTIONS

Durban

Description is based on 10 specimens, 40-95.5mm standard length (SL).

Supraoccipital crest is prominent, being 19.8-31.8% (x=27.5% SD=3.8) of the neurocranial length. Neurocranial width ranges from 46.4-55.8% (x=52.6% SD=2.9) of neurocranial length.

Infraorbital series has 5 or 6 bones (with the second and third or third and fourth canal-bearing bones fused); each lachrymal commonly has 5 pores opening into the laterosensory canal, but two specimens only have 4.

The height of the ascending process of the premaxilla as a percentage of head length, ranges from 21.9-34.8% (x=27.8% SD=4.1); in general, for this population, the ascending process is slightly longer than the dentigerous arm. Lower jaw length as a percentage of the head length ranges from 39.7-52.4% (x=43.9% SD=3.5).

There are 41-60 teeth in the outer tooth-row of the premaxilla; these are bicuspid anteriorly and unicuspid or tricuspid (or a mixture of both types) posteriorly. Inner-row teeth are bicuspid or tricuspid (or of both types). 31-50 outer row teeth on the dentary; these are bicuspid anteriorly and either bi- or unicuspid posteriorly; inner-row teeth tricuspid, but there may be a mixture of uni-, bi- and tricuspids.

Gill-raker counts are 8 or 9 + 1 + 2 or 3.

Total counts for scales in both lateral-line series range from 28-30. Upper lateral-line is separated from the dorsal fin origin

by 5 scales; the cheek is covered by 3 or 4 rows of scales; 4-6 scales between the pectoral and pelvic fin insertions.

Dorsal fin has 23 or 24 rays, comprising 14 or 15 spinous and 9 or 10 branched rays; anal fin has 10-12 (mode=11) rays, comprising 3 spinous and 7-9 (mode=8) branched rays.

Lower pharyngeal bone usually (f.9) longer than it is wide; 22-25 tooth rows; teeth bicuspid, the more robust teeth situated centrally and along the posterior row.

Total vertebral count (excluding the fused first pre-ural [PU1] and first ural [U1] centra) is 26 (f.6), 27 (f.2) or 28 (f.2), comprising 13 (f.10) abdominal and 13 (f.6), 14 (f.2) or 15 (f.2) caudal vertebrae.

Note: frequencies are indicated by "f."

Kuruman

Description is based on 10 specimens, 38.5-48mm SL.

Supraoccipital crest height is 21.5-26.9% (x=24.2% SD=1.7) of the neurocranial length. Neurocranial width ranges from 49.6-57.3% (x=53.4% SD=2.4) of neurocranial length.

Infraorbital series has 5 bones (with the second and third or fourth and fifth bones fused) (6 in one specimen); in eight specimens the lachrymal has 4 pores opening into the laterosensory canal, but two specimens have 5.

The height of the ascending process of the premaxilla as a percentage of the head length ranges from 18.8-28.6% (x=25.1% SD=7.5); in general, the ascending process is only slightly longer than the dentigerous arm. Lower jaw length as a percentage of the head length ranges from 35.0-41.2% (x=37.5% SD=1.8).

There are 34-44 teeth in the outer tooth-row of the premaxilla; these are bicuspid anteriorly and unicuspid or tricuspid posteriorly. Inner-row teeth may be tricuspid, uni- or bicuspid, or a combination of uni-, bi- and tricuspid. 35-44 outer row teeth on the dentary; these are bicuspid anteriorly and either bi- or unicuspid posteriorly; inner row teeth are tricuspid.

Gill-raker counts are 8 or 9 + 1 + 2 or 3.

Total counts for scales in both lateral-line series range from 24-29. Upper lateral-line is separated from the dorsal fin origin by 4 or 5 scales; the cheek is covered by 3 or 4 rows of scales; 4 or 5 scales between the pectoral and pelvic fin insertions.

Dorsal fin has 23-25 rays (mode=24), comprising 14 spinous and 9-11 (mode=10) branched rays; anal fin has 11 or 12 rays, comprising 3 spinous and 8 or 9 branched rays.

Lower pharyngeal bone usually (f.7) longer than it is wide, but in some fishes (f.3), it is wider than long; 22-25 tooth rows; teeth bicuspid, the more robust teeth centrally situated and along the posterior row.

Total vertebral count 27 (f.10), comprising 13 (f.10) abdominal and 14 (f.10) caudal vertebrae.

Otjikoto

Description is based on 10 specimens, 38-62mm SL.

Supraoccipital crest height is 16.0-27.8% (x=22.9% SD=3.2) of the neurocranial length. Neurocranial width ranges from 49.6-56.4% (x=53.3% SD=2.1) of neurocranial length.

Infraorbital series has 5 or 6 bones (with second and third or

third and fourth bones fused); in all but two specimens the lachrymal has 4 pores opening to the laterosensory canal, the exceptional specimens have 5 on the left and 4 on the right.

The height of the ascending process of the premaxilla as a percentage of the head length ranges from 20.3-26.6% (x=23.7% SD=1.7); in general, for this population, the ascending process is slightly shorter than the dentigerous arm. Lower jaw length as a percentage of the head length ranges from 32.2-41.1% (x=38.1% SD=2.4).

There are 37-66 teeth in the outer tooth-row of the premaxilla; these are bicuspid anteriorly and unicuspid posteriorly. Inner-row teeth may be a combination of uni- and bicuspid, or uni-, bi- and tricuspid, or may be all tricuspid. 34-39 outer row teeth on the dentary; these are bicuspid anteriorly and either bi- or unicuspid posteriorly; inner-row teeth uni-, bi- or tricuspid, or combinations of these.

Gill-raker counts are 8-10 + 1 + 2.

Total counts for scales in both lateral-line series range from 23-29. Upper lateral-line is separated from the dorsal fin origin by 4 or 5 scales; the cheek is covered by 3 rows of scales; 3-6 scales between the pectoral and pelvic fin insertions.

Dorsal fin has 23 or 24 rays, comprising 13 or 14 spinous and 9-11 (mode=10) branched rays; anal fin has 10 or 11 rays, comprising 3 spinous and 7 or 8 branched rays.

Lower pharyngeal bone may be longer than it is wide (f.5), or wider than long (f.5); 20-26 tooth rows; teeth bicuspid, more robust teeth centrally situated and along the posterior row.

Total vertebral count 24 (f.1), 25 (f.1) or 26 (f.8), with 13

(f.10) abdominal and 11 (f.1), 12 (f.1) or 13 (f.8) caudal vertebrae.

Wondergat

Description is based on 10 specimens, 37.6-68.5mm SL.

Supraoccipital crest is low, ranging from 14.2 to 23.5% (x=19.3% SD=2.8) of the neurocranial length. Neurocranial width ranges from 44.7-54.9% (x=50.98% SD=2.6) of neurocranial length.

Infraorbital series has 4-6 bones: where the second and third bones may be fused; third and fourth bones may be fused; the sixth bone (dermosphenotic) may be missing. The lachrymal has 5 pores opening into the laterosensory canal, but two specimens have 5 on the left and 4 on the right, two have 4 on the left and 5 on the right.

The height of the ascending process of the premaxilla as a percentage of the head length ranges from 24.4-27.5% (x=26.6% SD=1.0); in general, for this population, the ascending process is only slightly longer than the dentigerous arm. Lower jaw length as a percentage of the head length ranges from 35.9-41.5% (x=39.5% SD=1.7).

There are 32-57 teeth in the outer tooth-row of the premaxilla; these are bicuspid anteriorly and unicuspid posteriorly. Inner-row teeth may be tricuspid, a combination of bi- and tricuspid, or a combination of uni-, bi- and tricuspid. 29-58 outer row teeth on the dentary; these are bicuspid anteriorly and either bi- or unicuspid posteriorly; inner rows bi- and tricuspid.

Gill-raker counts are 8-10 + 1 + 2 or 3.

Total counts for scales in both lateral-line series range from 23-29. Upper lateral-line is separated from the dorsal fin origin by 4 or 5 scales; the cheek is covered by 3 or 4 rows of scales; 3-5 scales between the pectoral and pelvic fin insertions.

Dorsal fin has 23 or 24 rays, comprising 14 spinous and 9 or 10 branched rays; anal fin has 10-12 (mode=11) rays, comprising 3 spinous and 7-9 (mode=8) branched rays.

Lower pharyngeal bone usually (f.7) longer than it is wide. 23-26 tooth rows; teeth bicuspid, the more robust teeth centrally situated and along the posterior row.

Total vertebral count 26 (f.6) or 27 (f.4), comprising 13 (f.10) abdominal and 13 (f.6) or 14 (f.4) caudal vertebrae.

Molopo Oog

Description is based on 10 specimens, 37.3-87.7mm SL.

Supraoccipital crest ranges from 22.1-30.3% (x=24.9% SD=2.6) of the neurocranial length. Neurocranial width ranges from 52.6-57.9% (x=55.3% SD=1.7) of neurocranial length.

Infraorbital series has 4-6 bones (no fixed pattern of missing bones in the series); the lachrymal has 4 pores opening into the laterosensory canal, but one specimen has 5 on each side, and one has 5 on the left and 4 on the right.

The height of the ascending process of the premaxilla as a percentage of the head length ranges from 21.6-26.9% (x=24.4% SD=1.8); in general, for this population, the ascending arm is longer than the dentigerous arm. Lower jaw length as a percentage of the head length ranges from 32.9-41.9% (x=38.4% SD=2.7).

There are 31-52 teeth in the outer tooth-row of the

premaxilla; these are bicuspid anteriorly and unicuspid posteriorly. Inner-row teeth may be tricuspid, a combination of bi- and tricuspid, or a combination of uni-, bi- and tricuspid. 32-48 outer row teeth on the dentary; these are bicuspid anteriorly and either bi- or unicuspid posteriorly; inner rows a combination of bi- and tricuspid.

Gill-raker counts are 9 or 10 + 1 + 2 or 3. Total counts for scales in both lateral-line series range from 27-31. Upper lateral-line is separated from the dorsal fin origin by 5 or 6 scales; the cheek is covered by 3 or 4 rows of scales; 4-6 scales between the pectoral and pelvic fin insertions.

Dorsal fin has 23-25 (mode=24) rays, comprising 14 or 15 spinous and 9 or 10 branched rays; anal fin has 11 rays, comprising 3 spinous and 8 branched rays.

Lower pharyngeal bone usually longer (f.8) than it is wide. 20-25 tooth rows; teeth bicuspid, the more robust teeth centrally situated and along the posterior row.

Total vertebral count 26 (f.10), comprising 13 (f.10) abdominal and 13 (f.10) caudal vertebrae.

Lake Kariba

Description is based on 10 specimens, 33.9-56.9mm SL.

Supraoccipital crest height ranges from 19.4-30.9% (x=24.5% SD=3.4) of the neurocranial length. Neurocranial width ranges from 53.6-66.9% (x=56.9% SD=3.9) of neurocranial length.

Infraorbital series has 4-6 bones ; the lachrymal has 4 (f.6) pores opening into the laterosensory canal, but one specimen has 5 on each side, and three have 5 on the left and 4 on the right.

The height of the ascending process of the premaxilla as a percentage of the head length ranges from 20.5-29.8% (x=25.0% SD=1.8); in general, for this population, the ascending process is slightly longer than the dentigerous arm. Lower jaw length as a percentage of the head length ranges from 35.5-45.9% (x=37.9% SD=2.9).

There are 27-51 teeth in the outer tooth-row of the premaxilla; these are bicuspid anteriorly and unicuspid posteriorly. Inner-row teeth may be tricuspid, a combination of bi- and tricuspid, or a combination of uni-, bi- and tricuspid. 26-46 outer row teeth on the dentary; these are bicuspid anteriorly and either bi- or unicuspid posteriorly; inner rows a combination of uni-, bi- and tricuspid.

Gill-raker counts are 8-10 + 1 + 2 or 3.

Total counts for scales in both lateral-line series range from 25-30. Upper lateral-line is separated from the dorsal fin origin by 4 or 5 scales; the cheek is covered by 2 or 3 rows of scales; 3-5 scales between the pectoral and pelvic fin insertions.

Dorsal fin has 23-25 rays (mode=25), comprising 14-16 (mode=14) spinous and 9-11 (mode=10) branched rays; anal fin has 11 or 12 rays, comprising 3 spinous and 8 or 9 branched rays.

Lower pharyngeal bone usually (f.6) slightly longer than it is wide. 22-28 tooth rows; teeth bicuspid, the more robust teeth centrally situated and along the posterior row.

Total vertebral count 27 (f.10), comprising 13 (f.10) abdominal and 14 (f.10) caudal vertebrae.

Lake Guinas

Description is based on 4 specimens, 29-37.8mm SL.

Supraoccipital crest is low, its height ranging from 15.8-20.7% (x=18.1% SD=1.9) of the neurocranial length. Neurocranial width ranges from 50.0-54.7% (x=52.0% SD=1.7) of neurocranial length.

Infraorbital series has 4-6 bones; in two specimens each lachrymal has 5 pores opening into the laterosensory canal, in one there are 4 on each side, and in another there are 4 on the left and 5 on the right.

The height of the ascending process of the premaxilla as a percentage of the head length ranges from 23.8-26.4% (x=25.4% SD=1.1); in general, for this population, the ascending process is slightly longer than the dentigerous arm. Lower jaw length as a percentage of the head length ranges from 37.7-40.7% (x=39.4% SD=1.1)

There are 30-46 teeth in the outer tooth-row of the premaxilla; these are bicuspid anteriorly, with some unicuspid teeth posteriorly in some cases. Inner-row teeth may be unicuspid, or a combination of uni-, bi- and tricuspid. 28-45 outer row teeth on the dentary; these are bicuspid anteriorly and posteriorly; inner rows a combination of uni-, bi- and tricuspid.

Gill-raker counts are 11 or 12 + 1 + 2 or 3.

Total counts for scales in both lateral-line series range from 23-26. Upper lateral-line is separated from the dorsal fin origin by 4 or 5 scales; the cheek is covered by 2 or 3 rows of scales; 4-6 scales between the pectoral and pelvic fin insertions.

Dorsal fin has 22-24 (mode=22) rays, comprising 12 or 14 spinous and 10 or 11 branched rays; anal fin has 12 rays, comprising 3 spinous and 9 branched rays.

Lower pharyngeal bone as long as it is wide (f.2), or the measured length of the bone may be longer (f.1) or shorter (f.1) than the width. 25-27 tooth rows; teeth bicuspid, the more robust teeth centrally situated and along the posterior row.

Total vertebral count 26 (f.4), comprising 12 (f.4) abdominal and 14 (f.4) caudal vertebrae.

Okavango

Description is based on 4 specimens, 31.5-54.6mm SL.

Supraoccipital crest is quite prominent in height, ranging from 22.4-29.7% (x=25.8% SD=3.0) of the neurocranial length. Neurocranial width ranges from 55.2-56.5% (x=55.9% SD=0.5) of neurocranial length.

Infraorbital series has 4-6 bones; the lachrymal has 4 pores opening into the laterosensory canal on each side, but one specimen had one large pore on the left and right sides.

The height of the ascending process of the premaxilla as a percentage of the head length varies from 22.9-36.7% (x=27.5% SD=5.5); in general, for this population, the ascending process is slightly longer than the dentigerous arm. Lower jaw length as a percentage of the head length ranges from 38.1-54.2% (x=42.6% SD=6.7).

There are 24-52 teeth in the outer tooth-row of the premaxilla; these are bicuspid anteriorly and unicuspid posteriorly. Inner-row teeth may be tricuspid, a combination of

bi- and tricuspid, or a combination of uni-, bi- and tricuspid. 20-25 outer row teeth on the dentary; these are bi- and tricuspid, or bicuspid anteriorly and bi- or unicuspid posteriorly; inner rows uni-, bi- and tricuspid.

Gill-raker counts are 9 or 10 + 1 + 2 or 3.

Total counts for scales in both lateral-line series range from 27-29. Upper lateral-line is separated from the dorsal fin origin by 4 scales; the cheek is covered by 3 or 4 rows of scales; 4-6 scales between the pectoral and pelvic fin insertions.

Dorsal fin has 24 or 25 rays, comprising 14 or 15 spinous and 10 or 11 branched rays; anal fin has 10 or 12 rays, comprising 3 spinous and 7 or 9 branched rays.

Lower pharyngeal bone usually (f.4) longer than it is wide. 20-27 tooth rows; teeth bicuspid, the more robust teeth centrally situated and along the posterior row.

Total vertebral count 26 (f.4), comprising 13 (f.4) abdominal and 13 (f.4) caudal vertebrae.

SEXUAL DIMORPHISM

There was marked sexual dimorphism in the *P. philander* of Wondergat where adult males were on average 93.6mm SL (± 6.0; 82-110; n=38) and females 42.9mm SL (±2.7; 38-51; n=66). However in the other populations studied, there was no large difference in size between the males and females.

OSTEOLOGY

Neurocranium: The skull form of *Pseudocrenilabrus* is of the generalized haplochromine type, being similar to that of

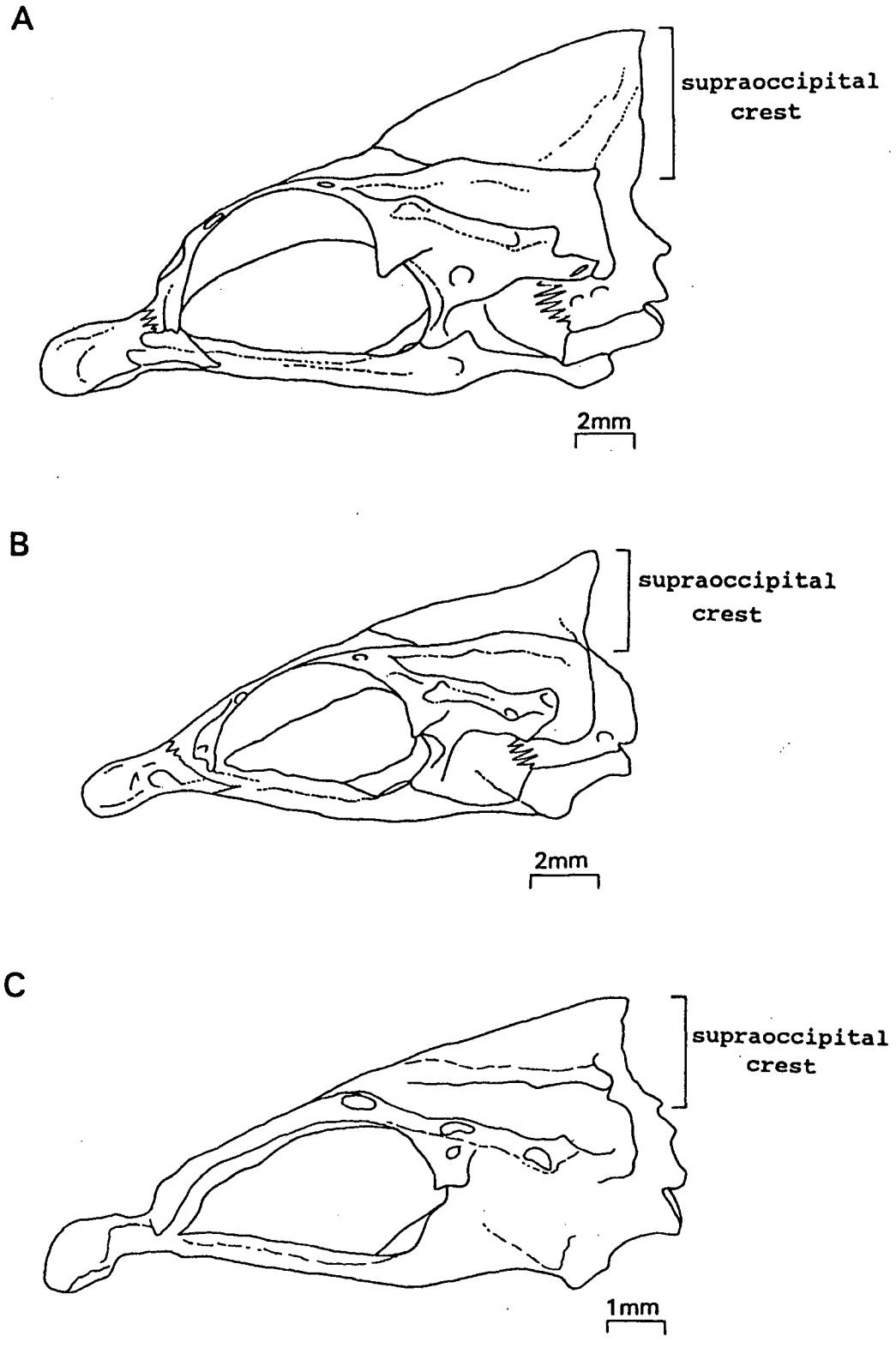

Figure 5.2: Neurocrania (left lateral view) of *P. philander* specimens from A: Durban, B: Wondergat and C: Guinas populations, showing variation of supraoccipital crests.

Astatotilapia (Barel *et al.* 1976; Greenwood 1979; 1989), but shows marked intraspecific variation (both inter- and intrapopulation variability) in height and shape of the supraoccipital crest (e.g. Fig. 5.2). The Durban population had tall crests and differed significantly (P=0) from Wondergat and Guinas fish, which had the lowest crests (Table 5.1).

Table 5.1: Mean height of supraoccipital crest (as percentage of neurocranial length) for wild-caught *P.philander* from each population (S.D.=standard deviation).

POPULATION	MEAN	S.D.	MINIMA & MAXIMA
Durban	27.5	± 3.8	19.8 - 31.8
Otijikoto	22.9	± 3.2	16.0 - 27.8
Wondergat	19.3	± 2.8	14.2 - 23.5
Kuruman	24.2	± 1.7	21.5 - 26.9
Kariba	24.5	± 3.4	19.4 - 30.9
Molopo	24.9	± 2.6	22.1 - 30.3
Okavango	25.8	± 3.0	22.4 - 29.7
Guinas	18.1	± 1.9	15.8 - 20.7

Neurocranial (otic) width (maximum width of the skull across the pterotics), expressed as a percentage of the neurocranial length, was also found to vary both within and between populations. Kariba, Okavango and Molopo Oog fish were found to have the widest skulls, while Guinas and Wondergat had the narrowest (Table 5.2); Kariba fish were significantly different (P=0.001) from Wondergat and Kuruman fish (Table 5.8).

Table 5.2: Mean neurocranial width (as percentage of neurocranial length) for wild-caught *P.philander* for each population (S.D.=standard deviation).

POPULATION	MEAN	S.D.	MINIMA & MAXIMA
Durban	52.6	± 2.9	46.4 - 55.8
Otijikoto	53.3	± 2.1	49.6 - 56.4
Wondergat	50.9	± 2.6	44.7 - 54.9
Kuruman	53.4	± 2.2	49.6 - 57.3
Kariba	56.9	± 3.9	53.6 - 66.9
Molopo	55.3	± 1.7	52.6 - 57.9
Okavango	55.9	± 0.5	55.2 - 56.5
Guinas	52.0	± 1.7	50.0 - 54.8

Infraorbital bones: Bones in the infraorbital series of *P. philander* show a high degree of variability in shape, size, number and arrangement (Figs. 5.3 & 5.4). The basic condition in *P.philander* is that of a lachrymal and four or five canal bones, but bones may be fused (often fusion of the second and third bones) or lost (the second and the last, the "dermosphenotic", are often absent). An interrupted and often reduced infraorbital series is a generic character of *Pseudocrenilabrus* (Greenwood 1989). There is no consistency in the pattern of fusion of the infraorbital bones between the populations, and the number of infraorbital bones may not be bilaterally symmetrical in an individual. However, the only exception is that members of the Molopo Oog population always have a reduction in number of bones in the series, but not always the same bones are missing or fused.

The lachrymal bone itself (the first bone in the series) shows considerable variability in terms of the number of pores opening

to the laterosensory canal. The range for *P.philander* is 4 or 5 pores (Table 5.3), and number of pores may also vary on right and left lachrymals of one individual. Greenwood (1989) found the same phenomenon in *P.multicolor* and *P.nicholsi*. In this study on *P.philander*, members of the Lake Kariba, Molopo Oog and Otjikoto populations have 4 pores on either side, whereas fish from Durban and Wondergat have 5 pores on each side. Kuruman individuals have 4 pores on the left and 5 on the right.

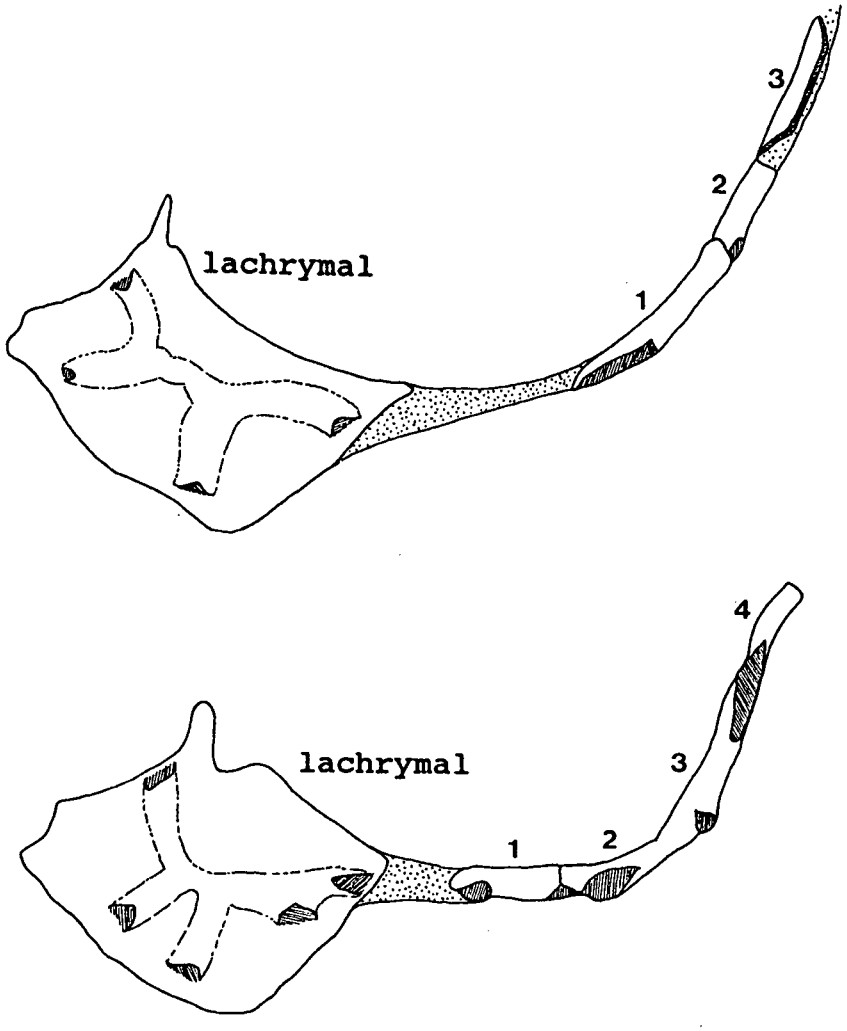

Figure 5.3: Semi-schematic diagrams to show some of the variation in the number and pattern of the bones in the infraorbital series.

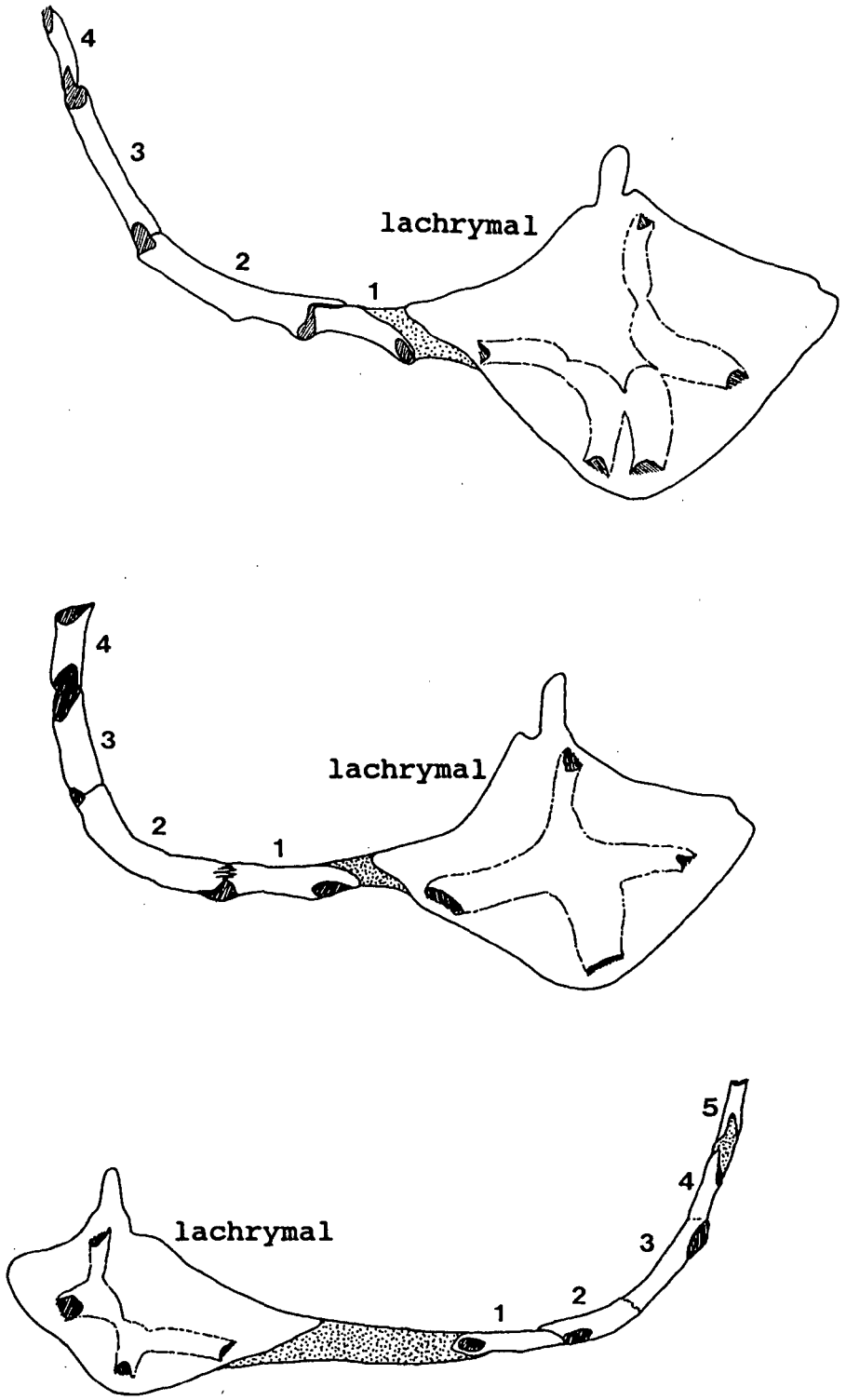

Figure 5.4: Semi-schematic diagrams to show some of the variation in the number and pattern of the bones in the infraorbital series.

Table 5.3: The number of specimens of wild *P.philander* having different numbers of sensory pores on left and right lachrymal bones (in the order left/right).

	4/4	4/5	5/4	5/5
Durban		2		8
Otijikoto	6	1	2	1
Wondergat		2	2	6
Kuruman	8			2
Kariba	6		3	1
Molopo	8		1	1
Okavango	3			
Guinas	1	1		2

Jaws: The ascending process of the premaxilla is longer than the dentigerous arm, for all the populations studied, except for that of Otjikoto but with no significant differences between the populations (Table 5.4).

Table 5.4: Ratio of the ascending process of the premaxilla to dentigerous arm length of the premaxilla of *P.philander* populations (S.D.=standard deviation).

POPULATION	MEAN	S.D.	MINIMA & MAXIMA
Durban	1.06	± 0.1	0.88 - 1.14
Otijikoto	0.91	± 0.1	0.74 - 0.98
Wondergat	1.04	± 0.1	0.88 - 1.28
Kuruman	1.02	± 0.1	0.96 - 1.14
Kariba	1.07	± 0.2	0.97 - 1.05
Molopo	1.11	± 0.1	1.03 - 1.23
Okavango	1.04	± 0.1	0.96 - 1.07
Guinas	1.08	± 0.1	0.94 - 1.24

Axial and caudal fin skeletons: For all populations the total vertebral counts (excluding the fused PU1 and U1 centra) vary

from 24-28 (comprising 12 or 13 abdominal and 11-15 caudal elements). The low vertebral count and the numbers of abdominal and caudal centra are plesiomorphic for the cichlidae (Greenwood 1989).

HEAD MUSCULATURE

Jaw muscle dissections confirmed the findings of Greenwood (1989), showing that *P.philander* is similar to the modal condition of cichlids seen in *Astatotilapia* (Anker 1978; Stiassny 1982; Greenwood 1985; 1989). There is considerable variation in cichlids in the degree of subdivision of the *pharyngocleithralis externus* muscle and its association with the ventral gill-arch musculature and other branchial structures (Stiassny 1982). Stiassny (1982) found that for *Astatotilapia*, *Aequidens* and *Chaetobranchus*, there are two sections to the *pharyngocleithralis externus* muscle: the A and B divisions. Greenwood (1989) found that for two of the specimens of *P. philander* that he worked on there was no B division in the *pharyngocleithralis externus* muscle. So the aponeurosis of the *pharyngohyoideus* muscle is connected with the large, single muscle, the A division of the *pharyngocleithralis externus* (Stiassny 1982). However, in this study all specimens had both divisions in the *pharyngocleithralis externus* muscle. As is the typical condition for cichlids (Anker 1978; Stiassny 1982; Greenwood 1985, 1989), the *pharyngocleithralis externus*, and *pharyngohyoideus* are joined to the lower pharyngeal bone by a tendon from their aponeurosis to the fourth ceratobranchial.

DENTITION

Outer row teeth are bicuspid anteriorly on the premaxilla and unicuspid posteriorly for all the populations except Durban and Kuruman, where there are tricuspid and unicuspid posterior teeth. However, in all populations, some of the bicuspid teeth may have the smaller cusp reduced, forming a "shoulder" on the tooth. As in the other species in the genus (Greenwood 1989), generally, there is a positive correlation in number of outer row premaxillary teeth with length of the individual.

Teeth on the dentary are a mixture of uni-, bi- or tricuspid teeth. All teeth may be bicuspid, or there may be bicuspid teeth anteriorly and unicuspid teeth laterally and posteriorly on the jaw. Greenwood (1989) suggests that the presence of these needle-like unicuspid teeth in adult *P.philander* is a result of the retention of the "juvenile" tooth form. If tricuspid teeth are present, these are found posteriorly.

Inner row teeth of both upper and lower jaws are small and fine, and are uni-, bi- or tricuspid (sometimes a mixture of all three), arranged in two rows anteriorly, becoming one row posteriorly.

The triangular-shaped lower pharyngeal bone carries rows of bicuspid teeth. The pharyngeal teeth are fine and slender, with those which are centrally (medially) situated and those in the posterior row being slightly thicker and more robust than the rest.

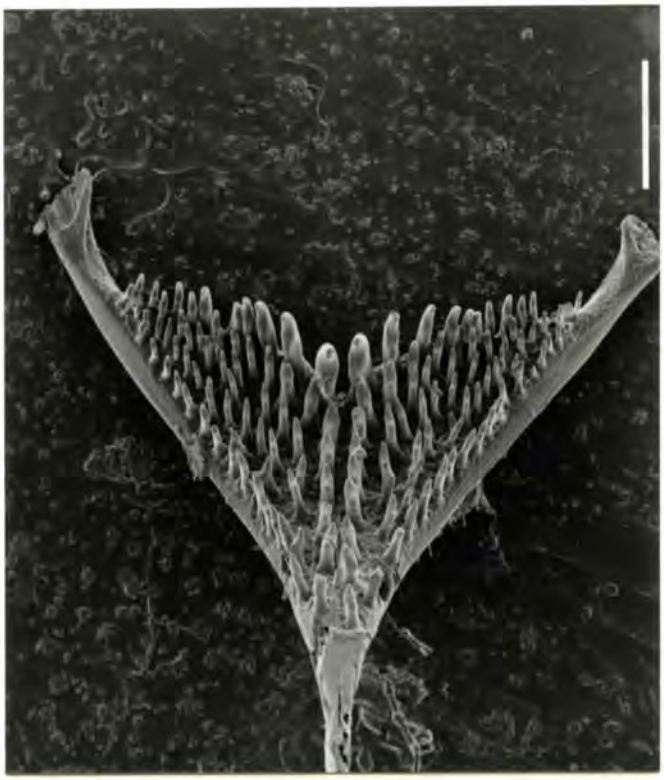

Figure 5.5: Scanning Electron Micrograph of the lower pharyngeal bone of a *P.philander specimen* from the Wondergat population (Mag. 16x).

ANALYSIS OF VARIANCE

Using One-way and Multifactor Analysis of Variance techniques, this study focuses on describing variation between samples from different populations of wild-caught *P. philander*. Proportional body (Table 5.5) and skull (Table 5.7) measurements were subjected to Analysis of Variance. Results showed significant differences among some of the populations for some of the variables. A Scheffe multiple range test separated character means into overlapping subsets (homogeneous groups), indicating which populations were significantly different.

Table 5.5: Body proportions of wild-caught *P. philander* populations* (BD, CPL and CPD as ratios to standard length; the remainder as ratios to head length). See MATERIALS & METHODS for abbreviations.

	DBN		OTJ		WON	
SL	63.9 ± 17.8	(40.0 - 95.5)	49.6 ± 6.4	(38.0 - 62.0)	45.8 ± 8.9	(37.6 - 68.5)
BD:SL	34.7 ± 0.9	(33.3 - 36.4)	35.0 ± 1.7	(32.1 - 37.6)	31.9 ± 1.4	(29.1 - 33.8)
CPL:SL	15.3 ± 1.2	(12.7 - 17.0)	12.4 ± 0.9	(10.4 - 13.2)	13.2 ± 0.9	(11.8 - 14.6)
CPD:SL	13.9 ± 0.3	(13.4 - 14.6)	12.3 ± 0.5	(11.5 - 13.2)	11.7 ± 1.0	(10.4 - 13.3)
HL:SL	35.5 ± 2.7	(28.2 - 38.5)	38.5 ± 3.3	(35.5 - 47.6)	38.6 ± 1.0	(36.6 - 39.6)
HW:HL	52.9 ± 4.7	(45.5 - 59.5)	49.3 ± 3.0	(42.4 - 52.3)	46.4 ± 3.6	(37.0 - 49.1)
IOW:HL	22.4 ± 3.0	(16.8 - 28.6)	20.8 ± 2.9	(15.3 - 27.3)	20.0 ± 2.6	(14.1 - 23.1)
POD:HL	19.8 ± 4.5	(15.9 - 31.8)	17.5 ± 0.9	(16.4 - 18.9)	17.0 ± 1.0	(15.7 - 18.8)
SNL:HL	33.3 ± 6.0	(27.5 - 49.6)	28.7 ± 1.9	(26.0 - 32.8)	29.1 ± 1.8	(26.8 - 31.9)
SNW:HL	32.7 ± 3.7	(27.5 - 39.1)	31.2 ± 2.3	(27.9 - 35.9)	31.2 ± 3.4	(27.3 - 37.0)
EYD:HL	25.4 ± 1.7	(21.9 - 28.6)	26.6 ± 2.6	(20.3 - 29.6)	28.7 ± 2.2	(23.9 - 31.1)
CHD:HL	22.4 ± 1.9	(19.8 - 26.2)	20.3 ± 0.9	(18.6 - 21.5)	19.2 ± 2.0	(16.2 - 22.4)
LJL:HL	43.9 ± 3.5	(39.7 - 52.4)	38.1 ± 2.4	(32.2 - 41.0)	39.5 ± 1.7	(35.9 - 41.5)
LJW:HL	20.1 ± 4.7	(14.5 - 27.0)	15.9 ± 1.9	(13.3 - 20.3)	20.5 ± 4.2	(13.6 - 26.8)
ASPR:HL	27.8 ± 4.1	(21.9 - 34.8)	23.7 ± 1.7	(20.3 - 26.6)	26.6 ± 1.0	(24.4 - 28.0)

	KUR		KAR		MOL	
SL	43.5 ± 3.1	(38.5 - 48.0)	44.4 ± 7.7	(33.9 - 56.9)	47.0 ± 15.1	(37.3 - 87.7)
BD:SL	33.7 ± 1.1	(31.7 - 34.8)	35.6 ± 2.4	(30.2 - 39.2)	35.1 ± 1.9	(32.3 - 37.6)
CPL:SL	14.3 ± 1.1	(12.4 - 16.1)	12.9 ± 1.5	(10.4 - 14.9)	12.6 ± 0.8	(11.5 - 14.5)
CPD:SL	12.9 ± 0.8	(11.5 - 14.3)	13.7 ± 1.0	(11.8 - 15.0)	13.2 ± 0.5	(12.3 - 14.1)
HL:SL	35.7 ± 0.9	(34.4 - 37.1)	34.5 ± 2.4	(28.6 - 37.2)	37.3 ± 1.6	(34.9 - 39.7)
HW:HL	51.1 ± 1.8	(46.9 - 53.6)	55.4 ± 2.9	(52.1 - 61.7)	52.7 ± 1.8	(49.7 - 55.4)
IOW:HL	22.4 ± 1.4	(21.0 - 25.8)	23.7 ± 1.2	(22.1 - 25.9)	21.2 ± 2.0	(16.2 - 23.1)
POD:HL	16.8 ± 1.9	(14.7 - 22.1)	16.7 ± 1.2	(15.1 - 19.4)	15.9 ± 1.6	(13.7 - 20.0)
SNL:HL	28.7 ± 2.2	(25.8 - 33.7)	27.8 ± 2.5	(23.6 - 34.1)	27.9 ± 2.8	(23.6 - 33.8)
SNW:HL	30.9 ± 1.6	(28.4 - 32.6)	32.7 ± 4.2	(22.8 - 37.1)	31.7 ± 3.4	(26.1 - 38.7)
EYD:HL	30.3 ± 1.3	(28.4 - 32.4)	29.5 ± 3.2	(23.9 - 36.7)	29.4 ± 2.2	(26.2 - 32.3)
CHD:HL	20.1 ± 1.9	(17.6 - 22.7)	21.1 ± 2.6	(16.7 - 26.3)	20.8 ± 2.9	(17.4 - 27.7)
LJL:HL	37.5 ± 1.8	(35.0 - 41.2)	37.9 ± 2.9	(35.4 - 45.9)	38.3 ± 2.7	(32.9 - 41.9)
LJW:HL	21.5 ± 3.0	(14.3 - 25.2)	21.1 ± 4.3	(16.1 - 29.1)	18.7 ± 2.7	(14.2 - 23.1)
ASPR:HL	25.1 ± 2.7	(18.8 - 28.6)	25.0 ± 3.0	(20.5 - 29.8)	24.4 ± 1.8	(21.6 - 27.0)

*Means±standard deviations, minima and maxima in parenthesis.

Table 5.6: Analysis of Variance on proportional body measurements of wild-caught *P. philander*. See MATERIALS & METHODS for abbreviations. (Dbn=Durban; Otj=Otjikoto; Won=Wondergat; Kur=Kuruman; Kar=Kariba; Mol=Molopo Oog.)

VARIABLES	F	P	POPULATIONS DIFFERING AT P<0.01
BD:SL	5.50	0.001	Kar > Won
CPL:SL	4.78	0.003	Dbn > Otj, Mol
CPD:SL	9.31	0.000	Won < Dbn, Kar, Mol Otj < Kar
HL:SL	4.04	0.006	Homogenous
HW:SL	5.45	0.001	Kar > Dbn, Won
IOW:SL	3.45	0.014	N.S.
POD:SL	4.22	0.005	Dbn > Kur, Mol
SNL:SL	3.62	0.111	N.S.
SNW:SL	3.12	0.022	N.S.
EYD:SL	5.97	0.001	Kur > Dbn, Otj
CHD:SL	4.13	0.006	Homogenous
LJL:SL	4.28	0.005	Dbn > Otj, Kur
LJW:SL	2.81	0.034	N.S.
ASPR:SL	1.24	0.315	N.S.

All four of the ratios based on standard length showed significant differences between populations. Five of the ten ratios based on head length showed significant differences between populations. Interorbital width (IOW), snout length (SnL), snout width (SnW), lower jaw width (LJW) and the ascending process of the premaxilla (AsPr) showed no significant differences between populations (Table 5.6).

Durban fish were characterised by the following features: greater pre-orbital depth (than Kuruman and Molopo Oog fish); longer lower jaw (than Otjikoto and Kuruman fish); longer caudal peduncle (than Otjikoto and Molopo Oog fish) (Table 5.6).

Otjikoto fish were characterised by: a deeper caudal peduncle

(than Lake Kariba fish) (Table 5.6).

Wondergat fish were characterised by: a deeper caudal peduncle (than Durban, Kariba and Molopo Oog fish) (Table 5.6).

Kuruman fish were characterised by: a greater eye diameter (than Durban and Otjikoto fish) (Table 5.6).

Lake Kariba fish were characterised by: greater body depth (than Wondergat fish); broader head (than Durban and Wondergat fish) (Table 5.6).

Table 5.7: Skull proportions of *P. philander* populations* (Ncl as a ratio to standard length; the remainder as ratios to neurocranial length). See MATERIALS & METHODS for abbreviations.

	DBN		OTJ		WON	
SL	63.9 ± 17.8	(40.0 - 95.5)	49.6 ± 6.4	(38.0 - 62.0)	45.8 ± 8.9	(37.6 - 68.5)
NCL:SL	26.1 ± 1.4	(24.4 - 29.0)	28.3 ± 2.4	(23.1 - 32.4)	29.7 ± 1.7	(25.3 - 31.6)
IOW:NCL	30.4 ± 1.7	(28.8 - 33.9)	25.5 ± 1.8	(23.1 - 28.6)	26.4 ± 2.0	(22.9 - 29.2)
EVL:NCL	28.2 ± 2.5	(23.3 - 31.4)	26.6 ± 1.0	(24.3 - 27.8)	26.5 ± 3.8	(21.7 - 34.3)
OL:NCL	36.5 ± 2.1	(33.7 - 40.5)	40.0 ± 3.0	(36.0 - 47.0)	42.2 ± 2.7	(37.6 - 47.0)
OT:NCL	33.2 ± 4.0	(29.2 - 40.1)	34.5 ± 3.5	(29.3 - 38.6)	31.5 ± 3.3	(27.5 - 39.1)
OTW:NCL	52.5 ± 2.9	(46.4 - 55.8)	53.3 ± 2.1	(49.6 - 56.4)	51.0 ± 2.6	(44.7 - 54.9)
VL:NCL	30.1 ± 4.2	(23.3 - 40.4)	26.8 ± 1.4	(24.4 - 28.6)	26.9 ± 1.1	(25.0 - 28.5)
SOH:NCL	27.5 ± 3.8	(19.8 - 31.8)	22.9 ± 3.2	(16.0 - 27.8)	19.3 ± 2.8	(14.2 - 23.5)
SOL:NCL	49.0 ± 5.7	(40.5 - 60.7)	47.9 ± 3.9	(39.8 - 54.8)	41.9 ± 5.4	(35.3 - 52.9)
CRL:NCL	55.4 ± 9.2	(38.8 - 67.3)	60.7 ± 2.4	(56.9 - 65.1)	50.3 ± 9.1	(33.3 - 63.6)

	KUR		KAR		MOL	
SL	43.5 ± 3.1	(38.5 - 48.0)	33.9 ± 7.7	(33.9 - 56.9)	47.0 ± 15.1	(37.3 - 87.7)
NCL:SL	27.0 ± 0.8	(25.6 - 28.0)	26.7 ± 2.0	(22.2 - 29.5)	27.9 ± 1.6	(25.5 - 30.1)
IOW:NCL	28.9 ± 2.1	(25.4 - 31.8)	29.7 ± 1.7	(26.0 - 31.6)	29.2 ± 1.6	(26.5 - 30.7)
EVL:NCL	23.4 ± 3.4	(17.2 - 27.7)	26.9 ± 1.8	(24.0 - 29.2)	24.6 ± 3.7	(17.2 - 28.3)
OL:NCL	43.8 ± 3.3	(38.1 - 48.0)	40.0 ± 3.4	(35.5 - 46.3)	44.1 ± 4.7	(36.6 - 49.1)
OT:NCL	32.5 ± 4.1	(27.3 - 38.8)	31.7 ± 2.0	(29.0 - 36.4)	32.4 ± 3.3	(28.6 - 39.3)
OTW:NCL	53.4 ± 2.2	(49.6 - 57.3)	57.0 ± 3.9	(53.5 - 67.0)	55.3 ± 1.7	(52.6 - 57.9)
VL:NCL	26.9 ± 1.4	(24.4 - 29.2)	24.8 ± 3.3	(17.7 - 29.0)	26.5 ± 1.8	(24.1 - 30.1)
SOH:NCL	24.2 ± 1.7	(21.5 - 26.9)	24.5 ± 3.4	(19.4 - 30.9)	24.9 ± 2.6	(22.1 - 30.3)
SOL:NCL	49.8 ± 3.7	(43.8 - 56.0)	50.0 ± 3.7	(45.5 - 57.5)	57.3 ± 6.5	(52.4 - 68.3)
CRL:NCL	52.1 ± 5.5	(42.7 - 60.0)	53.9 ± 7.4	(40.0 - 63.1)	61.5 ± 4.7	(53.5 - 66.7)

*Means±standard deviations, minima and maxima in parenthesis.

Table 5.8: Analysis of Variance on proportional skull measurements of wild-caught *P. philander*. See MATERIALS & METHODS for abbreviations. (Dbn=Durban; Otj=Otjikoto; Won=Wondergat; Kur=Kuruman; Kar=Kariba; Mol=Molopo Oog.)

VARIABLES	F	P	POPULATIONS DIFFERING AT P<0.01
NCL:SL	4.78	0.003	Won > Dbn
IOW:NCL	7.69	0.000	Dbn, Kar > Otj, Won
EVL:NCL	3.81	0.009	Dbn > Kur
OL:NCL	5.73	0.001	Mol > Won
OT:NCL	0.47	0.798	N.S.
OTW:NCL	5.53	0.001	Kar > Won, Kur
VL:NCL	4.23	0.005	Dbn > Otj, Kar
SOH:NCL	7.86	0.000	Won < Dbn, Kur
SOL:NCL	4.41	0.004	Dbn < Kur, Mol
CRL:NCL	2.11	0.092	N.S.

Seven of the nine ratios based on neurocranial length showed significant differences between populations. Neurocranial length (NcL), as a percentage of standard length, showed a significant difference between Wondergat and Durban populations. Otic length (Ot) and crest length (CrestL) were non-significant between populations (Table 5.8).

Durban fish were characterised by the following features: greater interorbital width (than Otjikoto and Wondergat fish); greater ethmovomerine length (than Kuruman fish); greater vomerine length (than Otjikoto and Lake Kariba fish); shorter supraoccipital crest length (than Kuruman and Molopo Oog fish) (Table 5.8).

Wondergat fish were characterised by: greater neurocranial length (than Durban fish); lower supraoccipital crest height (than Durban and Kuruman fish) (Table 5.8).

Lake Kariba fish were characterised by: greater otic width (than Wondergat and Kuruman Fish); greater interorbital width (than Otjikoto and Wondergat fish) (Table 5.8).

Molopo Oog fish were characterised by: greater orbital length (than Wondergat fish) (Table 5.8).

DISCRIMINANT FUNCTION ANALYSIS

The purpose of a Discriminant Function Analysis in this study was to statistically distinguish between populations of wild-caught *P. philander*, based on morphological variation. A Discriminant Function Analysis using morphometric data (both conventional and Truss measurements) from the wild-caught specimens was conducted. Using all measured variables (see MATERIALS and METHODS) as discriminating variables, specimens were classified into population groups.

I. Conventional body measurements of wild-caught fish

Table 5.9: The percentage of correct predictions for body measurements of wild-caught fish.

ACTUAL GROUP	PERCENT CORRECT	PREDICTED GROUP							
		DBN	OTJ	WON	KUR	KAR	MOL	OKA	GNS
DBN	90.0	9	1	0	0	0	0	0	0
OTJ	100.0	0	10	0	0	0	0	0	0
WON	100.0	0	0	10	0	0	0	0	0
KUR	90.0	0	1	0	9	0	0	0	0
KAR	80.0	1	0	0	0	8	1	0	0
MOL	80.0	0	0	0	1	1	8	0	0
OKA	75.0	0	0	0	0	1	0	3	0
GNS	100.0	0	0	0	0	0	0	0	4

Where: DBN=Durban; OTJ=Otjikoto; WON=Wondergat; KUR=Kuruman; KAR=Kariba; MOL=Molopo; OKA=Okavango; GNS=Guinas.

It was found that wild-caught Otjikoto, Guinas and Wondergat fish were 100% distinguishable (Table 5.9). The remainder had some of the specimens incorrectly classified; these were placed in other groups they were more similar to. For example, the Durban group had one specimen classified as belonging to Otjikoto, although all of the Otjikoto specimens were correctly classified. Overall, 89.7% of cases were correctly classified.

The first three variables ranked as important discriminating variables were: length of the ascending process of the premaxilla; caudal peduncle depth; diameter of the eye.

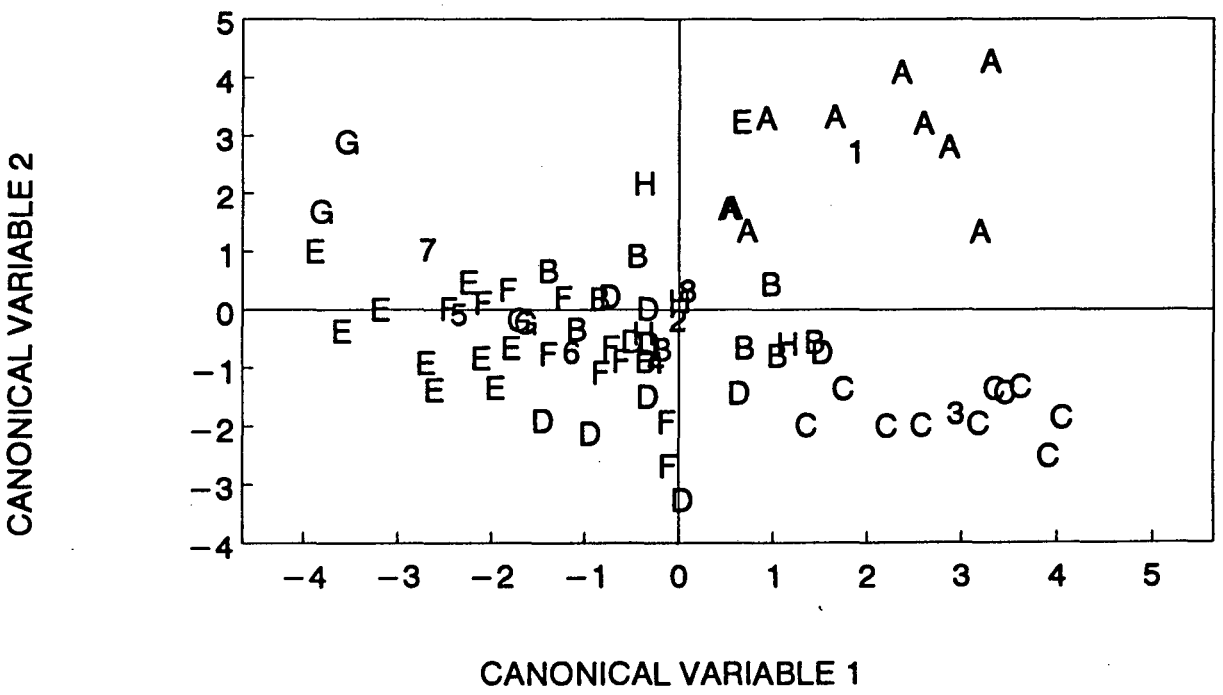

Figure 5.6: A discriminant function plot based on body measurements of wild-caught fish (A=Durban; B=Otjikoto; C=Wondergat; D=Kuruman; E=Kariba; F=Molopo; G=Okavango; H=Guinas).

A plot of the discriminant values illustrates the overlap of some groups and the separation of others (Fig. 5.6). Note the

well-defined Durban and Wondergat groups which were distinguished from the other groups (although the Durban group had slight overlap) along the first canonical variable and separated from each other along the second canonical variable.

II. Truss measurements on the body of wild-caught fish.

Table 5.10: Percentage correct predictions for the Truss body measurements of wild-caught fish.

ACTUAL GROUP	PERCENT CORRECT	PREDICTED GROUP DBN	OTJ	WON	KUR	KAR	MOL	OKA	GNS
DBN	83.3	5	0	0	0	0	1	0	0
OTJ	80.0	0	8	1	0	1	0	0	0
WON	100.0	0	0	10	0	0	0	0	0
KUR	90.0	0	0	0	9	1	0	0	0
KAR	90.0	0	0	0	1	9	0	0	0
MOL	100.0	0	0	0	0	0	10	0	0
OKA	75.0	0	0	0	1	0	0	3	0
GNS	100.0	0	0	0	0	0	0	0	4

Where: DBN=Durban; OTJ=Otjikoto; WON=Wondergat; KUR=Kuruman; KAR=Kariba; MOL=Molopo; OKA=Okavango; GNS=Guinas.

The overall percentage of correct predictions for the Truss method was similar (90.6% overall) to that for the conventional body measurements (89.7%) for wild-caught fish, and again, Wondergat and Guinas had 100% correct classification. Molopo Oog fish were also 100% correctly classified (Table 5.10).

The plot of discriminant function values showed fairly good separation of Otjikoto, Wondergat and Guinas populations, while the other groups showed considerable overlap (Fig. 5.7).

Wondergat and Guinas groups were distinguished from the other

populations along the first canonical variable. The Otjikoto group was distinguished from the other populations and Wondergat from Guinas along the second canonical variable.

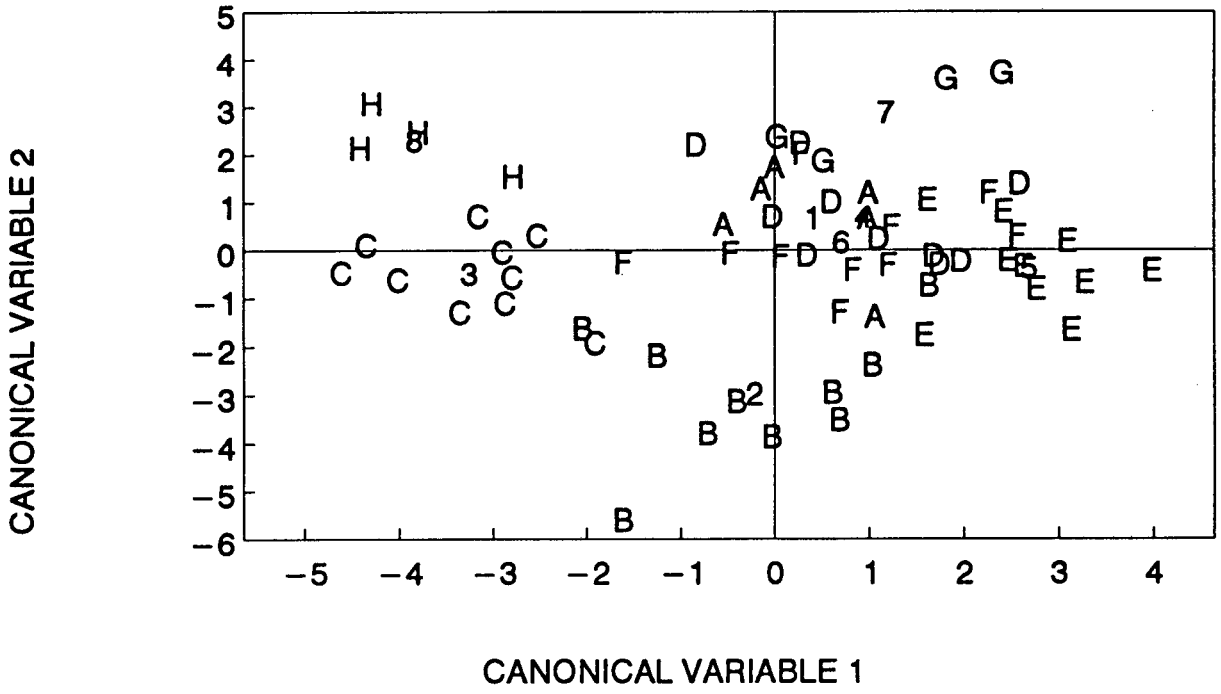

Figure 5.7: A discriminant function plot based on Truss body measurements of wild-caught fish (A=Durban; B=Otjikoto; C=Wondergat; D=Kuruman; E=Kariba; F=Molopo; G=Okavango; H=Guinas).

III. Conventional skull measurements on wild-caught fish

The overall percentage of correct classification of groups was only 79.7%, with Guinas being the only group having 100% correctly classified cases (Table 5.11).

Variables ranked as important discriminating variables were: interorbital width and length of the supraoccipital crest.

Table 5.11: Percentage of correct predictions into groups, based on conventional measurements on the skulls of wild-caught fish.

ACTUAL GROUP	PERCENT CORRECT	PREDICTED GROUP DBN	OTJ	WON	KUR	KAR	MOL	OKA	GNS
DBN	90.0	9	0	0	0	1	0	0	0
OTJ	90.0	0	9	0	0	0	1	0	0
WON	90.0	0	1	9	0	0	0	0	0
KUR	80.0	0	0	0	8	0	1	1	0
KAR	55.6	0	0	1	2	5	0	1	0
MOL	57.1	0	0	0	0	1	4	2	0
OKA	75.0	0	0	0	0	0	1	3	0
GNS	100.0	0	0	0	0	0	0	0	4

Where: DBN=Durban; OTJ=Otjikoto; WON=Wondergat; KUR=Kuruman; KAR=Kariba; MOL=Molopo; OKA=Okavango; GNS=Guinas.

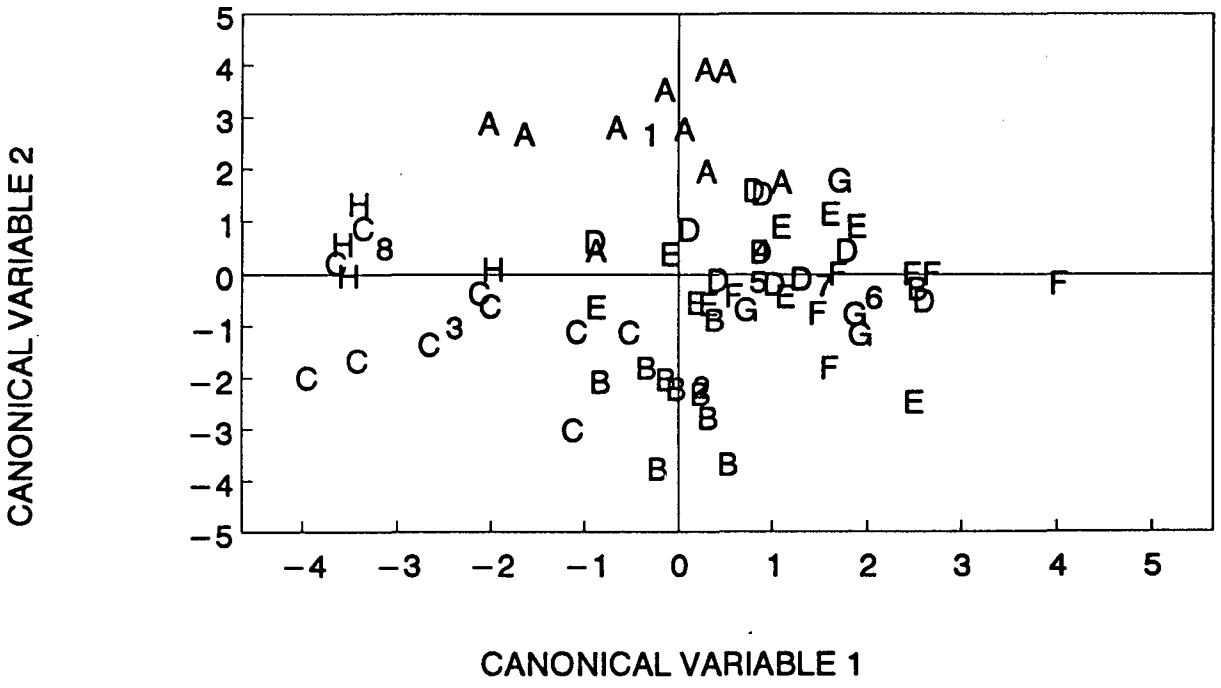

Figure 5.8: A discriminant function plot based on skull measurements of wild-caught fish (A=Durban; B=Otjikoto; C=Wondergat; D=Kuruman; E=Kariba; F=Molopo; G=Okavango; H=Guinas).

A plot of the discriminant function values showed considerable overlap of all populations, with some differentiation of the Durban, Otjikoto and Wondergat populations (Fig. 5.8). Although the Wondergat and Guinas groups were overlapped, they were distinguished from the other groups along the first canonical variable. The Durban group was separated from the other populations and the Otjikoto group also fairly well separated from the other populations along the second canonical variable.

IV. Truss measurements of the skull of wild-caught fish

Table 5.12: The percentage of correctly predicted cases of groups, based on skull Truss measurements of wild-caught fish.

ACTUAL GROUP	PERCENT CORRECT	PREDICTED GROUP							
		DBN	OTJ	WON	KUR	KAR	MOL	OKA	GNS
DBN	70.0	7	1	0	1	1	0	0	0
OTJ	70.0	0	7	1	0	1	1	0	0
WON	80.0	0	1	8	1	0	0	0	0
KUR	90.0	0	0	0	9	1	0	0	0
KAR	77.8	0	0	0	1	7	1	0	0
MOL	57.1	0	0	0	1	1	4	1	0
OKA	75.0	0	1	0	0	0	0	3	0
GNS	100.0	0	0	0	0	0	0	0	3

Where: DBN=Durban; OTJ=Otjikoto; WON=Wondergat; KUR=Kuruman; KAR=Kariba; MOL=Molopo; OKA=Okavango; GNS=Guinas.

Overall, the percentage of correctly classified cases was lower (76.2%) than those based on conventional measurements (79.7%). Guinas was the only group with 100% correct classification of cases (Table 5.12).

A plot of the discriminant function values showed overlap of

all populations, except Guinas (Fig. 5.9) which was distinguished from the other populations along the first canonical variable and from Wondergat along the second canonical variable. The Wondergat group showed some separation from the other groups along both canonical variables.

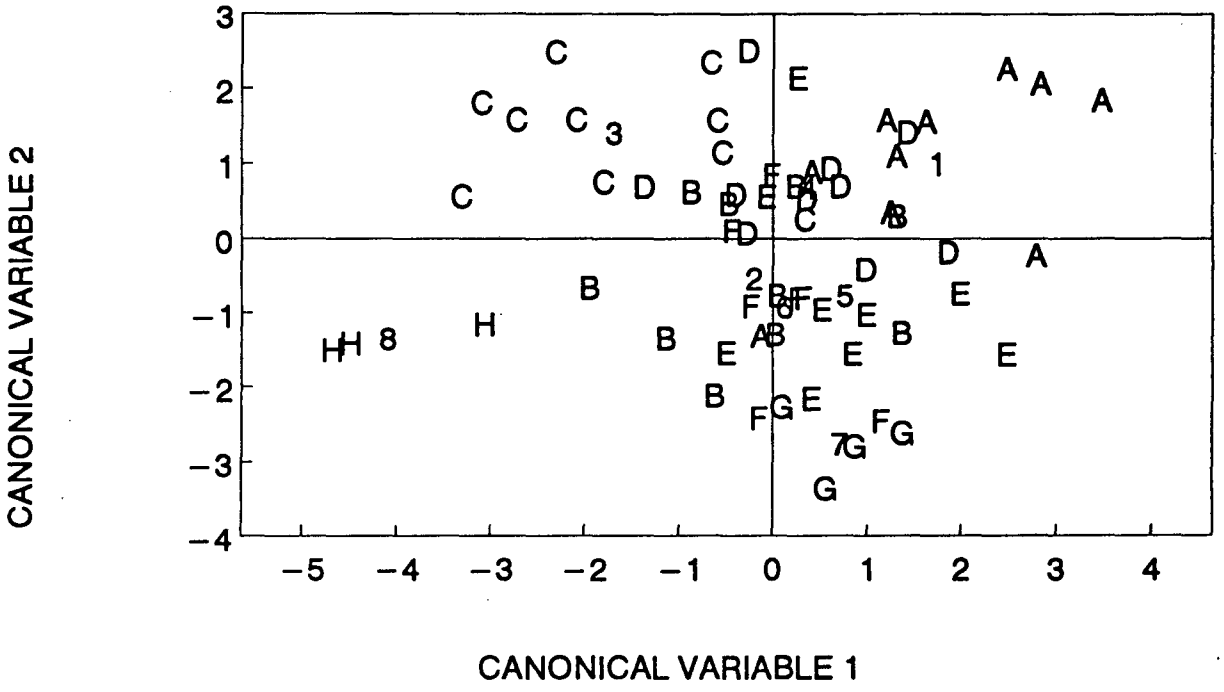

Figure 5.9: A discriminant function plot based on Truss skull measurements of wild-caught fish (A=Durban; B=Otjikoto; C=Wondergat; D=Kuruman; E=Kariba; F=Molopo; G=Okavango; H=Guinas).

SUMMARY OF RESULTS

It was interesting to find a clear-cut sexual dimorphism in adult size for the Wondergat *P. philander*. It is suggested that there may be selection for larger size because of competition between males for breeding areas, as space is limited in the sinkhole. However, this would not hold true for Lake Guinas where adults of both sexes were found to be very small (for

example, a male of 37mm SL was observed in full breeding colour displaying to a female). In this case, it is suggested that because of lack of space for breeding sites, the fish mature at a much smaller size (and possibly a younger age, although no data are available for this) than in Wondergat, in that way, reducing their generation time.

Greenwood (1989) stressed the importance of the reduced infraorbital series as a generic character for *Pseudocrenilabrus* and suggested that it was a derived feature which could be used to indicate how *Pseudocrenilabrus* evolved. This reductional trend was also found in some African cichlid genera as well as in some of the Neotropical genera (Greenwood 1989). Although there was considerable variation in the infraorbital series of wild-caught *P. philander* in this study, there was no pattern in the variation for any particular population. So this feature was not useful or reliable in distinguishing between populations of *P. philander*.

Greenwood (1989) found that when comparing *Pseudocrenilabrus* with *Astatotilapia*, the pattern of the infraorbital series in adult *Pseudocrenilabrus* was similar to that of much smaller individuals of *Astatotilapia*. He also found that there was variation in the number of lachrymal pores for *Pseudocrenilabrus* (as in this study where there were 4 or 5 pores, with the modal number being 4 pores on each side). However, in *Astatotilapia* there were almost constantly 5 pores on each side. Greenwood (1989) concluded that the condition of the infraorbital series in adult *Pseudocrenilabrus* (compared with *Astatotilapia* and other

African genera) was due to ontogenetic retardation.

Another apparent juvenile condition found in all populations of *P.philander*, was that of unicuspid teeth in both the premaxilla and dentary. Greenwood (1989) found that in specimens of *Astatotilapia*, of the same size as the *Pseudocrenilabrus* of his study, the juvenile lateral and posterior unicuspid teeth were replaced with bicuspid teeth.

Head musculature of *P. philander* was also similar to that of *Astatotilapia* (Anker 1978; Stiassny 1982; Greenwood 1985) and is thus a plesiomorphic form (Greenwood 1989).

After applying One-way Analysis of Variance to the data, results showed that there were some significant differences between the populations of wild-caught *P. philander*, in terms of anatomy and morphology, but none of these were entirely distinctive for any particular population.

Discrimination (or characterisation) is reliant on the inter-relationships of many measurements and requires a multivariate function analysis, based on a large number of separate measurements (Turner, Pitcher & Grimm 1989). The Discriminant Function Analysis technique was able to distinguish some of the populations from the others. In summary, based on discriminant function plots the following was found:

1) Conventional body measurements - Durban and Wondergat fish were well-defined and distinguished from other populations
2) Truss body measurements - Otjikoto, Wondergat and Guinas fish were fairly well distinguished from the other populations
3) Conventional skull measurements - overlap of all populations,

but Durban, Otjikoto, Wondergat and Guinas had the least overlap with the other populations

4) Truss skull measurements - overlap of all populations except Guinas; although Wondergat overlapped with the other populations, it was also fairly well distinguished.

Discriminant function plots showed that the Durban and Wondergat populations were fairly well differentiated from the other populations, while Otjikoto and Guinas showed some separation of populations. It is thus becoming increasingly apparent that some of the populations are distinct from the others, but is this a product of environmental conditions?

In the following chapter, laboratory-bred populations are examined to determine whether the variation between wild *P.philander* populations is heritable or the result of ecophenotypic interactions.

Chapter 6:

MORPHOLOGICAL FEATURES OF INDIVIDUALS FROM LABORATORY-BRED POPULATIONS

By breeding *P. philander* in the laboratory one can test whether the differences which exist between wild populations are ecophenotypic or heritable characteristics. If, under uniform conditions, the populations were to lose these differences and become uniform, then the *P.philander* populations would be exhibiting ecophenotypically induced variation. If, however, populations retained the characteristics of their wild parents, this would mean that the isolates had diverged genetically. The heritability of the observed differences in the wild populations was examined by breeding sequentially, three generations under uniform laboratory conditions.

The sample size of each laboratory population was small as instead of selecting and covering a few populations in greater detail (in other words a larger sample size), it was decided to examine all populations with a view to identifying those which might require follow-up studies.

SIZE RANGE

Size ranges of specimens derived from the different populations (for morphological study) were as follows: Durban 51.5-55.4 mm SL (n=6); Otjikoto 37-52 mm SL (n=6); Wondergat 53-60.8 mm SL (n=6); Kuruman 42.7-55 mm SL (n=6); Lake Kariba 45.5-54 mm SL (n=6); Molopo Oog 37.5-49 mm SL (n=9); Okavango 40.7-50

mm SL (n=6); Kosi 48.6-64.5 mm SL (n=6); Mkuze 44.5-51 mm SL (n=6); Letaba 40.3-47.8 mm SL (n=6).

COLOUR

If colour was influenced entirely by local environmental conditions, then fishes kept under uniform conditions would be expected to develop the same colours. At the termination of the study after three generations, individuals of the *P. philander* populations retained the body colour and fin markings characteristic of the population from which they were derived. However, in all cases, colour was noticeably less intense than that of newly-caught, wild specimens. When these fishes were placed in outside pools they reaquired the colour intensity of wild-caught fish. This was attributed to a more varied diet, including surface insects and an abundance of carotene-rich algae which were not available in indoor aquaria. The possibility of natural light (as opposed to artificial laboratory lighting) affecting colour intensity should also be considered, as illumination produces increased pigmentation (Odiorne 1957). Although these fish were kept under uniform conditions and were not affected by varied diets and lighting conditions, it is interesting to note how readily environmental conditions affect colour.

It is concluded that the ground colours and basic markings which characterize populations are heritable, but intensity of colour expression is influenced by both environmental and motivational states (Barel *et al.* 1977; Blouw & Hagen 1990).

Colour descriptions apply only to live, adult, sexually mature fish (with emphasis on the males in this study).

ANATOMICAL FEATURES

Jaws: Greenwood (1965a), Witte (1984) and Meyer (1987) demonstrated in their studies on wild and tank specimens that the oral jaw apparatus and pharyngeal jaw apparatus of haplochromines have a high degree of plasticity, and that morphological changes can be environmentally induced. Laboratory-bred *P.philander* were compared with wild-caught fish to determine whether aquarium conditions affected the feeding apparatus.

Results showed that there appeared to be no outstanding changes in the lower jaw elements from those of the wild specimens (Table 6.11). Slight changes in the jaw occurred in the Wondergat fish, with a significant increase (P=0.005) in lower jaw length of laboratory-bred fish from the wild condition, and a significant decrease (P=0.003) in the lower jaw width for laboratory-bred Durban fish.

It was found that there were changes in the length of the ascending premaxillary process in laboratory-bred fish when compared with the bone in wild populations. To determine whether these changes were not artifactual (in other words, whether apparent changes in length of the ascending process were due to relative changes in other skeletal elements), the length of the ascending process was measured relative to dentigerous arm length of the premaxilla, as well as a percentage of head length, and a percentage of neurocranial length. Neurocranial length and width were then measured relative to standard length.

In wild-caught specimens of the Otjikoto population, the length of the ascending process was found to be slightly shorter than that of the dentigerous arm, whereas in laboratory-bred fish a similar condition was found in the Otjikoto as well as in the Wondergat, Kuruman, Kariba and Okavango populations (Table 6.1). However, there was no significant difference between wild-caught and laboratory-bred fish (for length of the ascending process of the premaxilla relative to dentigerous arm length), except for the Okavango population (which showed a significant decrease, P=0.005) (Table 6.1).

Table 6.1: Mean ratios of the ascending process of the premaxilla to dentigerous arm length for each population for both wild and laboratory-bred *P.philander* (P<0.01).

	DBN	OTJ	WON	KUR	KAR	MOL	OKA
Wild-caught	1.06	0.91	1.04	1.02	1.07	1.11	1.04
Laboratory-bred	1.06	0.96	0.99	0.96	0.95	1.09	0.90
P-value	0.936	0.520	0.143	0.190	0.536	0.762	0.005

In relation to head length, the ascending process in laboratory-bred populations was not significantly different from that of the wild-caught populations (Table 6.2). Likewise, there were no significant differences between the wild-caught populations (Table 6.3). Within the laboratory-bred populations, Kariba fish had a significantly shorter ascending process of the premaxillae than in Durban and Wondergat fish (P=0.001); the ascending processes in Otjikoto fish were also significantly shorter than those in Durban fish (P=0.001) (Table 6.3).

Table 6.2: Ratios of: ascending process to head length; ascending process to neurocranial length; neurocranial length to standard length; neurocranial width to standard length (expressed as means for each population), for both wild and laboratory-bred *P.philander* (P<0.01).

	DURBAN	OTJIKOTO	WONDERGAT	KURUMAN	KARIBA	MOLOPO	OKAVANGO
ASCENDING PROCESS: HEAD LENGTH							
Wild-caught	27.9	23.7	26.6	25.1	25.0	24.4	27.5
Laboratory-bred	30.2	26.3	28.7	27.4	24.8	27.7	24.3
P-value	0.050	0.088	0.074	0.010	0.954	0.017	0.253
ASCENDING PROCESS: NEUROCRANIAL LENGTH							
Wild-caught	37.6	24.0	34.5	33.1	29.2	33.1	33.0
Laboratory-bred	41.7	34.7	38.3	36.8	32.4	35.9	32.6
P-value	0.000	0.004	0.007	0.017	0.284	0.116	0.645
NEUROCRANIAL LENGTH: STANDARD LENGTH							
Wild-caught	26.1	28.3	29.7	27.1	26.7	27.9	27.1
Laboratory-bred	28.0	28.3	30.2	27.2	28.0	28.0	27.7
P-value	0.002	0.952	0.793	0.793	0.086	0.976	0.407
NEUROCRANIAL WIDTH: STANDARD LENGTH							
Wild-caught	13.8	15.1	15.1	14.5	15.2	15.4	15.2
Laboratory-bred	15.1	15.8	15.8	15.7	15.5	16.3	15.5
P-value	0.001	0.023	0.079	0.002	0.962	0.124	0.335

In relation to neurocranial length, there was a significant increase in length of the ascending process between wild-caught

and laboratory-bred fish of the Durban (P=0), Otjikoto (P=0.004) and Wondergat (P=0.007) populations (Table 6.2).

In the wild-caught populations, Otjikoto fish were significantly different (P=0.001), with a shorter ascending process of the premaxillae than in Durban, Wondergat and Molopo Oog fish (Table 6.3). In the laboratory-bred populations, Kariba fish had significantly shorter (P=0) ascending processes than Durban and Wondergat fish; also, the ascending process of the premaxillae of Otjikoto and Molopo fish was significantly shorter than in Durban fish (Table 6.3).

Table 6.3: Relative skull measurements for wild-caught and laboratory-bred populations of *P.philander*, showing significant differences between populations (ANOVA). See MATERIALS & METHODS for abbreviations. (Dbn=Durban; Otj=Otjikoto; Won=Wondergat; Kur=Kuruman; Kar=Kariba; Mol=Molopo Oog.)

VARIABLES		F	P	POPULATIONS DIFFERING AT P<0.01
ASPR:HL	WILD	1.24	0.315	N.S.
	LAB	6.04	0.001	Kar < Dbn, Won Dbn > Otj
ASPR:NCL	WILD	5.72	0.001	Otj < Dbn, Won, Mol
	LAB	9.38	0.000	Kar < Dbn, Won Dbn > Otj, Mol
NCL:SL	WILD	4.78	0.003	Won > Dbn
	LAB	5.03	0.002	Won > Kur, Mol
NCW:SL	WILD	5.32	0.001	Dbn < Kar,Mol
	LAB	2.17	0.085	N.S.
NCW:NCL	WILD	4.27	0.005	Kar > Won
	LAB	5.44	0.001	Mol > Won
PHARL:NCL	WILD	2.22	0.078	N.S.
	LAB	2.19	0.081	N.S.
PHARW:NCL	WILD	3.53	0.013	N.S.
	LAB	5.43	0.001	Mol > Dbn, Otj, Kar
PHARW:NCW	WILD	1.92	0.121	N.S.
	LAB	2.19	0.081	N.S.
SOH:NCL	WILD	8.18	0.000	Dbn > Otj, Won
	LAB	3.12	0.022	N.S.

In the wild-caught populations, Durban fish had significantly shorter neurocrania (as a ratio of standard length) than Wondergat fish (P=0.003) (Table 6.3) and in the laboratory-bred populations Kuruman and Molopo Oog fish had significantly shorter neurocrania than Wondergat fish (P=0.002) (Table 6.3).

The neurocranial length (relative to standard length) (Table 6.2) of laboratory-bred fish remained very similar to that of the wild populations, with the exception of the Durban population, where there was a significant increase (P=0.002) from wild-caught to laboratory-bred fish.

Since both length of the ascending process of the premaxilla

and neurocranial length increased significantly in the laboratory-bred Durban fish, it is suggested that relative to the neurocranial length the ascending process has increased to a greater extent than the neurocranial length.

In summary: the neurocranial length to standard length ratio of laboratory-bred fish showed no change from the wild condition (with the exception of Durban fish); the ascending process of the premaxilla, in relation to neurocranial length, did change under uniform laboratory conditions; ratio of the ascending process to dentigerous arm length of the premaxilla did not change significantly between laboratory-bred and wild-caught populations (so length of the dentigerous arm must have increased along with the ascending process of the premaxilla). Thus changes in the length of the ascending process of the premaxilla were real and are suggested to be due to environmental changes. Laboratory-bred *P. philander* did not undergo such marked changes to the jaws as those which occurred in laboratory populations of *Haplochromis squamipinnis* (Witte 1984), and *Cichlasoma managuense* (Meyer 1987), or which occurred in the pharyngeal jaws of laboratory and some wild populations of *Astatoreochromis alluaudi* (Greenwood 1965a).

Table 6.4: Ratios of: lower pharyngeal bone length to neurocranial length; pharyngeal bone width to neurocranial width; pharyngeal bone width to neurocranial length, for both wild-caught and laboratory-bred populations (expressed as a mean for each population) (P<0.01).

	DURBAN	OTJIKOTO	WONDERGAT	KURUMAN	KARIBA	MOLOPO	OKAVANGO
PHARYNGEAL LENGTH: NEUROCRANIAL LENGTH							
Wild-caught	41.3	40.0	39.0	40.0	43.2	40.7	40.5
Laboratory-bred	39.6	37.4	42.2	43.4	43.3	43.8	46.3
P-value	0.158	0.202	0.008	0.052	0.840	0.032	0.001
PHARYNGEAL WIDTH: NEUROCRANIAL WIDTH							
Wild-caught	73.0	74.2	70.8	70.0	71.1	66.3	66.7
Laboratory-bred	68.5	64.0	72.7	67.5	66.4	71.8	68.5
P-value	0.208	0.051	0.881	0.240	0.360	0.081	0.363
PHARYNGEAL WIDTH: NEUROCRANIAL LENGTH							
Wild-caught	38.6	39.6	36.0	37.3	41.2	36.5	37.4
Laboratory-bred	37.3	35.9	37.9	39.1	36.6	42.3	38.3
P-value	0.369	0.204	0.293	0.013	0.006	0.007	0.421

To determine whether length and width of the lower pharyngeal bone was affected by laboratory conditions, measurements were expressed relative to other bones in the skull. Pharyngeal bone length (in relation to neurocranial length) increased in all laboratory populations except in Durban and Otjikoto fish in which it decreased, but only the Wondergat (P=0.008) and Okavango (P=0.001) populations showed a significant increase (Table 6.4).

There was no significant difference between wild-caught populations (Table 6.3), but in laboratory-bred populations, pharyngeal bone length in Otjikoto fish was significantly shorter (P=0.005) than in Molopo Oog fish (Table 6.3).

As neurocranial length (in relation to standard length) did not increase in the laboratory-bred populations except for the Durban fish (P=0.002) (Table 6.2), the change in pharyngeal bone length is real.

The width of the bone (in relation to neurocranial width) showed no significant difference within (Table 6.3) or between (Table 6.4) wild-caught and laboratory-bred fish.

Neurocranial width (in relation to standard length) of laboratory populations was very similar to that of the wild fish but with significant increases in fishes of the Durban (p=0.001) and Kuruman (P=0.002) populations (Table 6.2). In the wild-caught populations, Durban fish had significantly narrower neurocrania than Kariba and Molopo Oog fish (P=0.001) (Table 6.3). There were no significant differences between laboratory-bred populations (Table 6.3), suggesting that neurocranial width tended towards uniformity under laboratory conditions.

In relation to neurocranial length, laboratory-bred populations of Molopo Oog fishes showed a significant increase (P=0.001) in pharyngeal bone width, whereas Kariba fishes showed a significant decrease (P=0.006) between wild and laboratory-bred fish (Table 6.4). There were no significant differences between the wild-caught populations (Table 6.3). In the laboratory-bred

populations, Otjikoto, Kariba and Durban fishes had significantly narrower pharyngeal bones than did those from Molopo Oog (P=0.001) (Table 6.3).

To summarise, slight changes in the oral and pharyngeal jaws occurred under laboratory conditions. These involved significant increase in length of the ascending process of the premaxilla in relation to neurocranial length (in Durban, Otjikoto and Wondergat populations), as well as an increase in length and width of the pharyngeal bone (in Wondergat, Okavango and Molopo populations) and a decrease in length and width of the pharyngeal bone (in Durban, Otjikoto and Kariba populations).

Length and width of the neurocranium in laboratory-bred fishes remained very similar to that of the wild fish, although there was a significant increase in length (relative to standard length) between wild and laboratory-bred Durban fish, and a significant increase in width between wild and laboratory-bred Durban and Kuruman fish, so the proportional increases in the studied parameters of the oral and pharyngeal jaws are real.

Neurocranium: In all populations, except Kariba, the mean neurocranial width for each population was greater for laboratory-bred fish than for the wild-caught fish (Table 6.5), but not significantly greater. In the wild-caught fish, Wondergat had significantly narrower (P=0.001) neurocrania than Kariba (Table 6.3), while in the laboratory-bred fish Wondergat had significantly narrower (P=0.001) neurocrania than Molopo Oog (Table 6.3).

Table 6.5: Mean neurocranial width (as a percentage of neurocranial length) for each population for both wild and laboratory-bred populations (P<0.01).

	DBN	OTJ	WON	KUR	KAR	MOL	OKA
Wild-caught	52.5	53.3	50.9	53.9	56.9	55.3	56.0
Laboratory-bred	53.9	56.4	52.3	58.0	55.5	58.3	56.0
P-value	0.584	0.046	0.267	0.028	0.028	0.243	1.000

Table 6.6: Mean height of supraoccipital crest (as percentage of neurocranial length) for each population, for both wild and laboratory-bred populations (P<0.01).

	DBN	OTJ	WON	KUR	KAR	MOL	OKA
Wild-caught	27.5	22.9	19.3	24.2	24.5	24.9	25.8
Laboratory-bred	23.6	23.1	22.9	26.9	23.5	27.3	22.9
P-value	0.000	0.560	0.030	0.372	0.502	0.204	0.109

In the Durban, Kariba and Okavango laboratory-bred populations, the height of the supraoccipital crest was not as great as in the wild-caught fish (Table 6.6), but only the Durban fish had significantly lower crests. Within the wild populations, fish from Wondergat and Otjikoto had significantly lower supraoccipital crests (relative to neurocranial length)

than Durban fish (Table 6.3). However, in laboratory-bred populations there were no significant differences between the populations (Table 6.3).

Infraorbital bones: The number of lateral-line sensory pores on the lachrymal bones of the laboratory-bred populations was found to be reduced and varied (Table 6.7) relative to the wild-caught fish (Table 5.3). Members of the Mkuzi, Okavango, Kuruman and Molopo Oog populations had none, one, two or three lachrymal pores, whereas in the wild populations there were four or five pores on each lachrymal. The laboratory-bred Durban population is similar to that of the wild stock (Table 5.3; 6.7). The apparent loss of pores in the lachrymal suggests that their number can be environmentally modified, thus also suggesting that number of pores is not a useful nor a reliable character for use at lower taxonomic levels.

As in the wild-caught fish, there is great variation in the shape, size, number and arrangement of the bones in the infraorbital series of laboratory-bred fish. Again there is no consistent pattern in the variation in the infraorbital series or in the lachrymal bone of the series for any particular population, so this character is of no use in differentiating between populations of *P. philander*.

Table 6.7: Frequency distribution of the numbers of laboratory-bred *P.philander* populations showing different numbers of sensory pores on left and right lachrymal bones.

	0/0	0/1	1/0	1/1	1/2	2/1	2/2	2/3	3/2	3/3	4/4	4/5	5/4	5/5
Durban											1			5
Otjikoto											3			1
Wondergat													2	4
Kuruman	2		2	1	1									
Kariba											5			
Molopo Oog	3	1			2						3	1		
Okavango				1			1			1	3			
Letaba											4			
Mkuzi		1		1	1	1		1	1					
Kosi														5

ANALYSIS OF VARIANCE

By means of One-way Analysis of Variance, variation between populations was described. Some of the populations of the laboratory-bred fish were found to differ significantly from others on the basis of morphometric data (Table 6.8; 6.10). A Scheffe multiple range test was applied so as to indicate which populations were significantly different.

Table 6.8: Body proportions of laboratory-bred *P. philander* populations* (BD, CPL and CPD as ratios to standard length; remainder as ratios to head length). See MATERIALS & METHODS for abbreviations.

	DBN		OTJ		WON	
SL	53.8 ± 1.5	(51.5 - 55.4)	40.8 ± 5.1	(37.0 - 52.0)	56.9 ± 2.8	(53.0 - 60.8)
BD:SL	33.6 ± 1.0	(31.5 - 34.6)	36.2 ± 0.7	(35.1 - 37.2)	35.6 ± 1.1	(34.0 - 37.2)
CPL:SL	16.7 ± 1.0	(15.2 - 18.0)	14.3 ± 0.6	(13.5 - 15.0)	16.5 ± 1.0	(15.1 - 18.1)
CPD:SL	13.7 ± 0.5	(12.6 - 14.2)	13.8 ± 0.2	(13.6 - 14.0)	12.6 ± 0.4	(12.1 - 13.2)
HL:SL	38.6 ± 0.5	(37.9 - 39.3)	37.4 ± 0.9	(35.6 - 38.5)	40.4 ± 0.8	(39.3 - 41.4)
HW:HL	51.1 ± 0.9	(49.7 - 52.4)	52.2 ± 1.4	(51.0 - 55.2)	51.7 ± 1.2	(50.0 - 53.1)
IOW:HL	22.4 ± 0.9	(20.9 - 23.8)	22.6 ± 0.5	(22.1 - 23.2)	20.0 ± 1.0	(18.4 - 21.5)
POD:HL	18.8 ± 0.6	(17.9 - 19.6)	20.2 ± 1.4	(17.8 - 21.4)	21.0 ± 1.1	(19.9 - 22.9)
SNL:HL	32.3 ± 1.1	(30.8 - 34.1)	31.1 ± 2.4	(28.3 - 35.7)	34.8 ± 1.4	(33.2 - 37.5)
SNW:HL	29.6 ± 0.8	(28.3 - 30.7)	31.0 ± 1.0	(29.9 - 32.2)	32.4 ± 0.9	(30.6 - 33.3)
EYD:HL	26.8 ± 0.6	(25.6 - 27.3)	25.3 ± 1.1	(24.3 - 27.6)	27.3 ± 0.6	(26.5 - 28.0)
CHD:HL	21.0 ± 1.1	(19.1 - 22.7)	24.2 ± 2.4	(21.4 - 29.3)	23.4 ± 1.2	(21.5 - 25.0)
LJL:HL	41.0 ± 0.5	(40.5 - 42.1)	39.2 ± 0.2	(38.8 - 39.5)	43.7 ± 2.0	(41.7 - 47.9)
LJW:HL	15.3 ± 1.4	(13.2 - 17.2)	18.2 ± 1.5	(16.4 - 20.7)	17.6 ± 1.4	(15.4 - 19.2)
ASPR:HL	30.2 ± 1.4	(27.5 - 31.9)	26.3 ± 1.9	(23.6 - 29.3)	28.7 ± 1.7	(25.0 - 30.4)

	KUR		KAR		MOL	
SL	49.7 ± 4.2	(42.7 - 55.0)	49.4 ± 3.2	(45.5 - 54.0)	42.5 ± 3.9	(37.5 - 49.0)
BD:SL	35.9 ± 1.7	(33.7 - 37.8)	34.4 ± 0.4	(33.9 - 41.0)	33.8 ± 3.0	(25.6 - 36.3)
CPL:SL	15.8 ± 0.9	(14.3 - 17.0)	15.3 ± 0.3	(14.6 - 15.0)	15.9 ± 1.5	(13.6 - 18.5)
CPD:SL	13.3 ± 0.4	(12.8 - 13.9)	13.9 ± 0.2	(13.6 - 14.9)	14.5 ± 0.6	(13.7 - 15.4)
HL:SL	36.4 ± 1.0	(35.2 - 38.3)	36.8 ± 0.5	(35.9 - 40.5)	36.2 ± 1.6	(33.6 - 38.7)
HW:HL	55.7 ± 1.3	(54.2 - 58.3)	54.6 ± 1.5	(52.6 - 55.6)	57.8 ± 4.3	(54.2 - 69.1)
IOW:HL	24.7 ± 1.1	(22.8 - 26.3)	24.2 ± 0.7	(23.5 - 24.0)	23.0 ± 2.7	(20.0 - 29.4)
POD:HL	21.1 ± 1.6	(19.3 - 23.9)	18.2 ± 1.0	(17.5 - 19.8)	16.0 ± 1.7	(13.1 - 17.6)
SNL:HL	33.9 ± 1.5	(31.2 - 35.6)	32.2 ± 1.3	(30.0 - 39.2)	29.2 ± 1.8	(25.7 - 32.1)
SNW:HL	34.3 ± 1.2	(32.0 - 35.5)	31.5 ± 1.2	(30.0 - 34.2)	31.0 ± 3.8	(22.1 - 37.3)
EYD:HL	29.8 ± 1.0	(27.8 - 31.2)	27.6 ± 1.0	(26.3 - 28.9)	30.3 ± 2.4	(27.7 - 35.3)
CHD:HL	25.7 ± 2.2	(23.5 - 29.4)	24.8 ± 1.3	(22.6 - 25.1)	23.8 ± 4.6	(13.8 - 29.4)
LJL:HL	39.7 ± 2.1	(37.1 - 43.2)	38.1 ± 1.4	(35.9 - 42.7)	37.5 ± 5.4	(26.1 - 47.8)
LJW:HL	21.7 ± 3.5	(17.2 - 27.1)	16.1 ± 1.8	(13.7 - 26.3)	20.8 ± 3.7	(15.7 - 26.1)
ASPR:HL	27.4 ± 0.8	(26.1 - 28.1)	24.8 ± 1.3	(23.7 - 28.4)	27.7 ± 2.4	(24.1 - 31.6)

*Means±standard deviations, minima and maxima in parenthesis.

Table 6.9: Analysis of Variance on proportional body measurements for laboratory-bred populations of *P. philander*. See MATERIALS & METHODS for abbreviations. (Dbn=Durban; Otj=Otjikoto; Won=Wondergat; Kur=Kuruman; Kar=Kariba; Mol=Molopo Oog.)

VARIABLES	F	P	POPULATIONS DIFFERING AT P<0.01
BD:SL	2.29	0.071	N.S.
CPL:SL	4.89	0.002	Otj < Dbn, Won, Mol
CPD:SL	10.70	0.000	Won < Dbn, Otj, Kar, Mol Mol > Kur
HL:SL	16.59	0.000	Dbn, Won > Kur, Mol Won > Otj, Won
HW:HL	6.69	0.000	Mol > Dbn, Otj, Won
IOW:HL	6.33	0.000	Won < Kur, Kar
POD:HL	14.53	0.000	Mol < Dbn, Otj, Won, Kur Kur > Kar
SNL:HL	10.90	0.000	Mol < Dbn, Won, Kur, Kar Won > Otj
SNW:HL	2.99	0.026	N.S.
EYD:HL	10.12	0.000	Mol > Dbn, Otj, Won Otj < Kur, Mol
CHD:HL	3.27	0.018	N.S.
LJL:HL	2.77	0.036	N.S.
LJW:HL	4.75	0.003	Kur > Dbn
ASPR:HL	6.04	0.001	Kar < Dbn, Won

Three of the four ratios based on standard length showed significant differences between laboratory-bred populations, while body depth was not significant between populations. Seven of the ten ratios based on head length showed significant differences between populations. Snout width (SnW), cheek depth (ChD) and lower jaw length (LJL) were non-significant between populations (Table 6.9).

Durban fish were characterised by the following features: greater head length (than Kuruman and Molopo Oog fish); longer ascending process of the premaxilla (than Otjikoto) (Table 6.9).

Otjikoto fish were characterised by: smaller eye diameter (than Kuruman and Molopo Oog fish); shorter caudal peduncle length (than Durban, Wondergat and Molopo Oog fish) (Table 6.9). Wondergat fish were characterised by: greater head length (than Kuruman, Molopo Oog, Otjikoto and Lake Kariba fish); smaller interorbital width (than Kuruman and Lake Kariba fish); longer snout (than Otjikoto fish); lower caudal peduncle depth (than Durban, Otjikoto, Lake Kariba and Molopo Oog fish) (Table 6.9).

Kuruman fish were characterised by: greater preorbital depth (than Lake Kariba fish); wider lower jaw (than Durban fish) (Table 6.9).

Lake Kariba fish were characterised by: shorter ascending process of the premaxilla (than Durban and Wondergat fish) (Table 6.9).

Molopo Oog fish were characterised by: greater head width (than Durban, Otjikoto and Wondergat fish); smaller preorbital depth (than Durban, Otjikoto, Wondergat and Kuruman fish); shorter snout (than Durban, Wondergat, Kuruman and Lake Kariba fish); greater eye diameter (than Durban, Otjikoto and Kuruman fish); deeper caudal peduncle depth (than Kuruman fish) (Table 6.9).

Table 6.10: Skull proportions of laboratory-bred *P.philander* populations* (Ncl as a ratio to standard length; the remainder as proportions to neurocranial length). See MATERIALS & METHODS for abbreviations.

	DBN		OTJ		WON	
SL	53.8 ± 1.5	(51.5 - 55.4)	40.8 ± 5.1	(37.0 - 52.0)	56.9 ± 2.8	(53.0 - 60.8)
NCL:SL	28.0 ± 0.8	(26.7 - 28.8)	28.3 ± 1.1	(26.0 - 29.1)	30.2 ± 0.9	(29.4 - 31.9)
IOW:NCL	29.8 ± 1.0	(28.7 - 31.2)	27.5 ± 1.2	(26.1 - 29.6)	25.5 ± 1.1	(24.4 - 27.6)
EVL:NCL	28.0 ± 1.2	(25.7 - 29.4)	25.1 ± 2.2	(23.0 - 29.6)	29.0 ± 1.0	(27.6 - 30.6)
OL:NCL	40.8 ± 2.6	(38.2 - 45.7)	45.6 ± 3.4	(42.2 - 52.2)	40.4 ± 1.9	(38.2 - 42.9)
OT:NCL	31.0 ± 0.3	(30.6 - 31.3)	32.9 ± 3.2	(30.4 - 39.8)	31.3 ± 0.8	(30.6 - 32.6)
OTW:NCL	54.0 ± 1.4	(52.0 - 55.9)	56.3 ± 2.3	(53.1 - 60.0)	52.3 ± 1.1	(50.8 - 54.3)
VL:NCL	30.1 ± 1.2	(28.6 - 31.8)	29.1 ± 2.8	(24.8 - 33.6)	28.1 ± 1.6	(26.3 - 30.6)
SOH:NCL	23.6 ± 1.8	(21.0 - 26.4)	23.1 ± 3.3	(18.7 - 29.6)	22.9 ± 1.0	(21.5 - 24.4)
SOL:NCL	49.5 ± 3.8	(42.7 - 54.1)	48.4 ± 5.5	(40.0 - 55.6)	52.0 ± 2.8	(47.2 - 55.2)
CRL:NCL	42.6 ± 5.4	(36.0 - 49.7)	44.7 ± 4.3	(38.9 - 51.3)	51.0 ± 4.7	(42.8 - 57.1)

	KUR		KAR		MOL	
SL	49.7 ± 4.2	(42.7 - 55.0)	49.4 ± 3.2	(45.5 - 54.0)	42.5 ± 3.9	(37.5 - 49.0)
NCL:SL	27.2 ± 1.3	(24.7 - 29.1)	28.0 ± 0.6	(27.2 - 29.2)	28.0 ± 1.4	(26.1 - 30.4)
IOW:NCL	31.0 ± 2.0	(29.1 - 34.6)	30.7 ± 1.3	(28.5 - 32.3)	28.3 ± 5.1	(17.5 - 37.4)
EVL:NCL	29.5 ± 2.4	(26.7 - 32.9)	27.1 ± 1.1	(25.9 - 29.3)	26.7 ± 2.6	(21.7 - 29.8)
OL:NCL	46.4 ± 2.6	(43.2 - 50.0)	40.0 ± 2.4	(38.5 - 45.1)	44.5 ± 3.7	(41.2 - 53.2)
OT:NCL	33.5 ± 1.2	(32.2 - 36.0)	32.4 ± 0.9	(30.8 - 33.3)	32.9 ± 2.1	(29.8 - 36.3)
OTW:NCL	58.0 ± 3.9	(53.8 - 64.0)	55.4 ± 1.4	(52.6 - 56.6)	58.3 ± 3.8	(52.6 - 64.3)
VL:NCL	31.5 ± 2.0	(28.2 - 33.8)	28.0 ± 2.0	(25.2 - 30.8)	23.8 ± 8.6	(0.1 - 30.1)
SOH:NCL	27.0 ± 4.0	(21.4 - 33.1)	23.5 ± 1.7	(21.8 - 26.3)	27.3 ± 2.0	(24.3 - 31.3)
SOL:NCL	50.3 ± 3.8	(44.4 - 54.4)	48.2 ± 3.1	(42.1 - 51.7)	51.8 ± 3.9	(43.5 - 55.7)
CRL:NCL	44.5 ± 3.6	(40.0 - 48.9)	41.1 ± 4.1	(34.7 - 46.9)	50.4 ± 5.3	(38.3 - 57.3)

*Means±standard deviations, minima and maxima in parenthesis.

Table 6.11: Analysis of Variance on proportional skull measurements for laboratory-bred populations of *P. philander*. See MATERIALS & METHODS for abbreviations. (Dbn=Durban; Otj=Otjikoto; Won=Wondergat; Kur=Kuruman; Kar=Kariba; Mol=Molopo Oog.)

VARIABLES	F	P	POPULATIONS DIFFERING AT P<0.01
NCL:SL	5.03	0.002	Won > Kur, Mol
IOW:NCL	4.95	0.002	Won < Kur, Kar
EVL:NCL	3.63	0.011	N.S.
OL:NCL	1.85	0.132	N.S.
OT:NCL	0.64	0.674	N.S.
OTW:NCL	5.39	0.001	Mol > Won
VL:NCL	3.45	0.014	Won > Mol
SOH:NCL	3.09	0.023	N.S.
SOL:NCL	5.06	0.002	Homogeneous
CRL:NCL	3.63	0.011	N.S.

Only four of the nine ratios based on neurocranial length of laboratory-bred fish showed significant differences between populations. Ethmovomerine length (EvL), orbital length (OL), otic length (Ot), supraoccipital crest height (SOH) and crest length (CRL) were non-significant between populations. Neurocranial length (NcL), as a standard length ratio, showed a significant difference between Wondergat and Kuruman and Molopo Oog populations (Table 6.11).

Wondergat fish were characterised by the following features: greater neurocranial length (than Kuruman and Molopo Oog fish); smaller interorbital width (than kuruman and Lake Kariba fish); greater vomerine length (than Molopo Oog fish) (Table 6.11).

Molopo Oog fish were characterised by: greater otic width (than Wondergat fish) (Table 6.11).

Table 6.12: Variation between wild-caught and laboratory-bred populations.

VARIABLES	DURBAN	OTJIKOTO	WONDERGAT	KURUMAN	KARIBA	MOLOPO	OKAVANGO
BD	0.041	0.266	0.002	0.030	0.035	0.451	0.957
CPL	0.123	0.005 W<L	0.000 W<L	0.012	0.009 W<L	0.000 W<L	0.566
CPD	0.516	0.000 W<L	0.082	0.080	0.416	0.020	0.045
HL	0.005 W<L	0.371	0.015	0.071	0.073	0.259	0.188
HW	0.296	0.048	0.011	0.000 W<L	0.985	0.052	0.555
IOW	0.305	0.042	0.999	0.001 W<L	0.483	0.246	0.749
POD	0.286	0.005 W<L	0.000 W<L	0.000 W<L	0.089	0.991	0.018
SNL	0.398	0.052	0.000 W<L	0.000 W<L	0.022	0.320	0.113
SNW	0.018	0.719	0.697	0.005 W<L	0.000 W>L	0.702	0.271
EYD	0.076	0.665	0.049	0.139	0.682	0.362	0.279
CHD	0.175	0.005 W<L	0.003 W<L	0.000 W<L	0.058	0.014	0.675
LJL	0.124	0.154	0.005 W<L	0.107	0.786	0.984	0.454
LJW	0.003 W>L	0.167	0.284	0.821	0.016	0.255	0.732
ASPR	0.050	0.088	0.074	0.010	0.954	0.017	0.636
NCL	0.002 W<L	0.952	0.793	0.793	0.086	0.974	0.407
IOW	0.633	0.077	0.981	0.012	0.257	0.720	0.770
EVL	0.488	0.371	0.057	0.004 W<L	0.978	0.835	0.842
OL	0.938	0.880	0.001 W>L	0.475	0.225	0.061	0.546
OT	0.261	0.382	0.746	0.811	0.790	0.735	0.873
OTW	0.393	0.048	0.165	0.008 W<L	0.246	0.053	0.985
VL	0.458	0.101	0.324	0.003 W<L	0.188	0.838	0.327
SOH	0.001 W<L	0.561	0.012	0.354	0.506	0.208	0.115
SOL	0.004 W>L	0.032	0.133	0.213	0.677	0.511	0.005 W>L
CRL	0.003 W<L	0.000 W<L	0.765	0.010 W>L	0.000 W>L	0.007 W>L	0.001 W>L

One-way analysis of Variance was also used to determine the variation of the laboratory-bred populations from the wild-caught populations. Results showed some significant differences in both body and skull measurements (Table 6.12).

In the Durban fish, head length (and also neurocranial length) increased significantly under laboratory conditions, while width of the lower jaw decreased. Height and length of the supraoccipital crest (length of the supraoccipital crest includes portion contributed by the frontal bone) decreased under laboratory conditions, whereas length of the supraoccipital bone increased slightly (but significantly).

In the Otjikoto fish, pre-orbital depth, cheek depth, caudal peduncle length and depth and length of the supraoccipital bone increased significantly under laboratory conditions.

In the Wondergat fish, pre-orbital depth, snout length, cheek depth, lower jaw length, caudal peduncle length, and orbital length increased significantly under laboratory conditions.

In the Kuruman fish, head width, interorbital width, pre-orbital depth, snout length and width, cheek depth, ethmovomerine length, otic width and vomerine length increased under laboratory conditions, while length of the supraoccipital bone decreased.

In the Kariba fish, caudal peduncle length increased while snout width and length of the supraoccipital bone decreased under laboratory conditions.

In the Molopo Oog fish, caudal peduncle length increased while length of the supraoccipital crest decreased under laboratory

conditions.

In summary, there were significant differences (mostly increases in the measurements) found in the laboratory-bred as compared with the wild-caught populations. However, no particular character was found to differ significantly throughout all populations, although length of the supraoccipital bone was significantly different between wild-caught and laboratory-bred fish for all the populations except Wondergat fish. So, even under uniform conditions, the different populations did not undergo uniform morphological changes.

DISCRIMINANT FUNCTION ANALYSIS

A Discriminant Function Analysis was applied to the morphometric data obtained from the laboratory-bred populations of *P. philander*, with the aim of distinguishing between populations.

I. Conventional body measurements of laboratory-reared fish

Table 6.13: Percentage of correct predictions based on body measurements of laboratory-reared fish.

ACTUAL GROUP	PERCENT CORRECT	PREDICTED GROUP						
		DBN	OTJ	WON	KUR	KAR	MOL	OKA
DBN	100.0	6	0	0	0	0	0	0
OTJ	100.0	0	6	0	0	0	0	0
WON	100.0	0	0	6	0	0	0	0
KUR	100.0	0	0	0	6	0	0	0
KAR	100.0	0	0	0	0	6	0	0
MOL	100.0	0	0	0	0	0	9	0
OKA	100.0	0	0	0	0	0	0	6

Where: DBN=Durban; OTJ=Otjikoto; WON=Wondergat; KUR=Kuruman; KAR=Kariba; MOL=Molopo; OKA=Okavango.

All specimens were correctly classified, i.e. 100% correct classification overall (Table 6.13).

In this case, the most important discriminating variables were: length of the ascending process of the premaxilla; preorbital depth; caudal peduncle depth.

A plot of the discriminant function values for each group (Fig. 6.1) showed better differentiation than for the wild-caught fish. Again, Durban and Wondergat populations were well separated

(as in the wild-caught fish) from the other populations along the first canonical variable. The Kuruman and Molopo Oog populations were also fairly well-differentiated from the other populations and were separated from each other along the second canonical variable. Otjikoto, Kariba and Okavango populations were closely grouped, with considerable overlap.

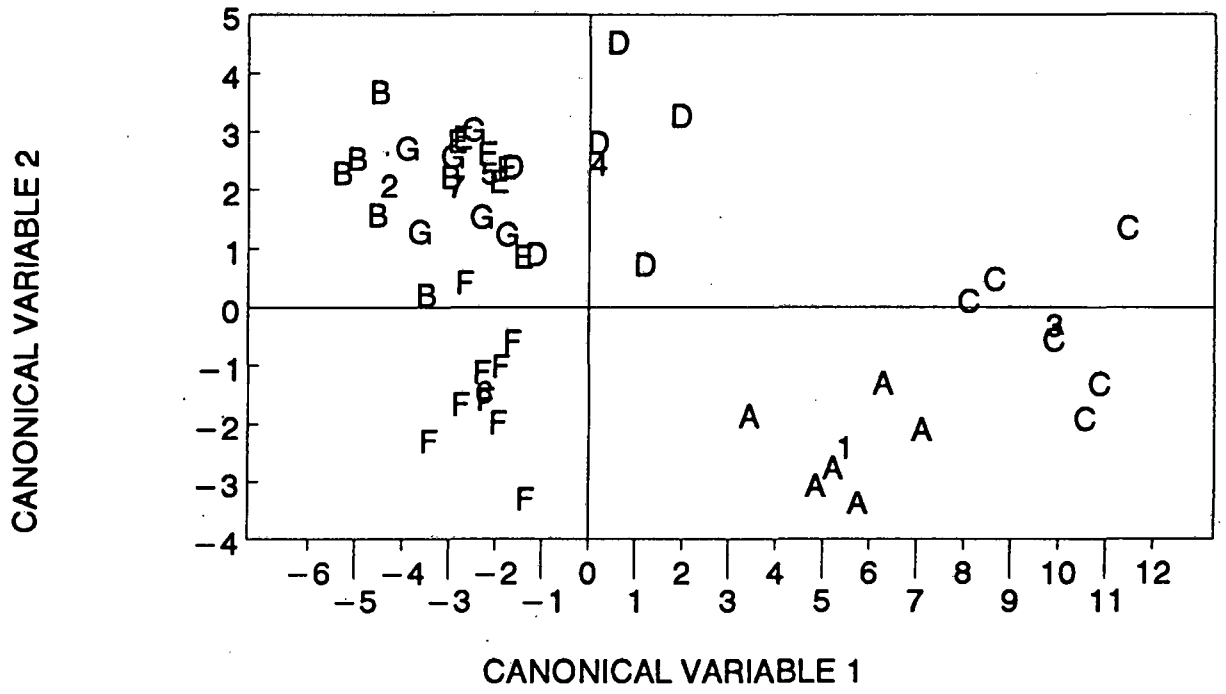

Figure 6.1: A discriminant function plot based on body measurements of laboratory-bred populations (A=Durban; B=Otjikoto; C=Wondergat; D=Kuruman; E=Kariba; F=Molopo; G=Okavango).

II. Truss measurements on the body of laboratory-reared fish.

Table 6.14: Percentage of correct predictions based on Truss measurements of laboratory-reared fish.

ACTUAL GROUP	PERCENT CORRECT	PREDICTED GROUP DBN	OTJ	WON	KUR	KAR	MOL	OKA
DBN	100.0	6	0	0	0	0	0	0
OTJ	100.0	0	6	0	0	0	0	0
WON	100.0	0	0	6	0	0	0	0
KUR	83.3	0	0	0	5	1	0	0
KAR	100.0	0	0	0	0	6	0	0
MOL	100.0	0	0	0	0	0	9	0
OKA	100.0	0	0	0	0	0	0	6

Where: DBN=Durban; OTJ=Otjikoto; WON=Wondergat; KUR=Kuruman; KAR=Kariba; MOL=Molopo; OKA=Okavango.

The percentage of correct predictions by Truss measurements was slightly lower (97.7% overall) than those based on conventional body measurements of laboratory-reared fish (Table 6.14).

The plot of discriminant function values (Figure 6.2) showed good differentiation of the Durban, Otjikoto and Wondergat populations from the other populations, along canonical variable one. The Otjikoto group was also separated from the other populations, but along canonical variable two. In the wild-caught fish, the Durban group was not separated from the other populations, as were the Wondergat and Otjikoto groups.

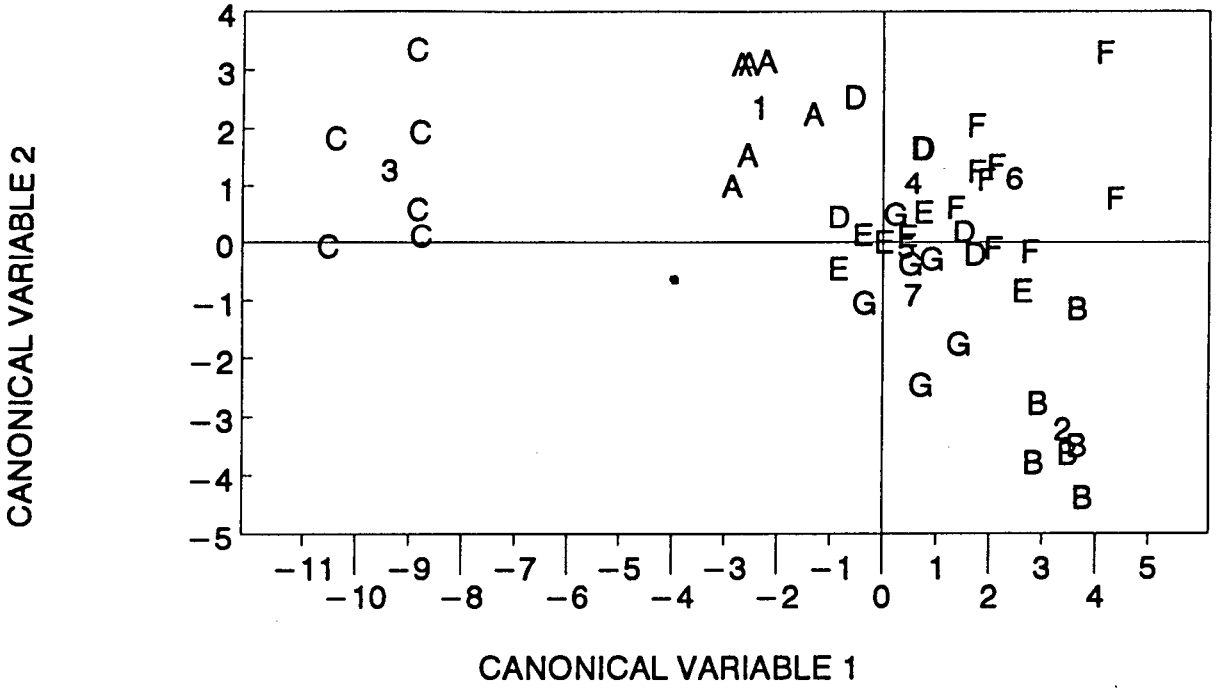

Figure 6.3: A discriminant function plot based on Truss body measurements of laboratory-bred populations (A=Durban; B=Otjikoto; C=Wondergat; D=Kuruman; E=Kariba; F=Molopo; G=Okavango).

III. Conventional skull measurements on laboratory-reared fish

Table 6.15: Percentage of correct predictions for groups, based on conventional skull measurements of laboratory-reared fish.

ACTUAL GROUP	PERCENT CORRECT	PREDICTED GROUP						
		DBN	OTJ	WON	KUR	KAR	MOL	OKA
DBN	66.6	4	0	0	1	1	0	0
OTJ	83.3	0	5	0	1	0	0	0
WON	100.0	0	0	6	0	0	0	0
KUR	83.3	0	1	0	5	0	0	0
KAR	83.3	0	0	0	0	5	0	1
MOL	77.8	0	1	0	0	0	7	1
OKA	83.3	0	0	0	0	1	0	5

Where: DBN=Durban; OTJ=Otjikoto; WON=Wondergat; KUR=Kuruman; KAR=Kariba; MOL=Molopo; OKA=Okavango.

Overall, the percentage of correctly predicted cases was 82.2%. Wondergat was the only group with 100% correctly classified cases and Durban had only 66.6% correct classification (Table 6.15).

The most important discriminating variables selected were: interorbital width and height of the supraoccipital crest.

A plot of discriminant function values showed considerable overlap of the population groups, with the Durban group showing some differentiation (as in the wild-caught fish (Fig. 5.8) and Wondergat being the only discretely differentiated group (Fig. 6.4). Wondergat and Durban groups were separated from the other populations along the first canonical variable, and from each other along the second canonical variable.

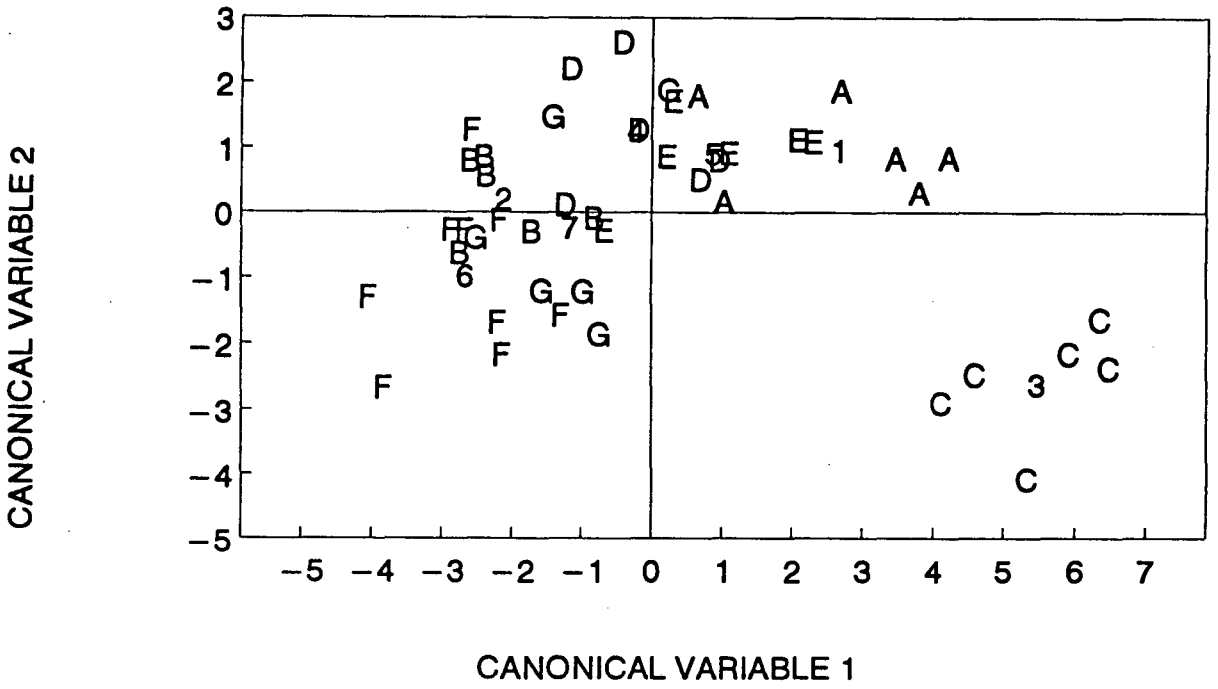

Figure 6.4: A discriminant function plot based on skull measurements of laboratory-bred populations (A=Durban; B=Otjikoto; C=Wondergat; D=Kuruman; E=Kariba; F=Molopo; G=Okavango).

IV. Truss measurements of the skull of laboratory-reared fish

Table 6.16: Percentage of correct classification of groups, based on skull Truss measurements of laboratory-reared fish.

ACTUAL GROUP	PERCENT CORRECT	PREDICTED GROUP DBN	OTJ	WON	KUR	KAR	MOL	OKA
DBN	100.0	6	0	0	0	0	0	0
OTJ	83.3	0	5	0	0	0	1	0
WON	100.0	0	0	6	0	0	0	0
KUR	83.3	0	0	0	5	0	0	1
KAR	100.0	0	0	0	0	6	0	0
MOL	77.8	0	1	0	0	0	7	1
OKA	83.3	0	0	0	0	1	0	5

Where: DBN=Durban; OTJ=Otjikoto; WON=Wondergat; KUR=Kuruman; KAR=Kariba; MOL=Molopo; OKA=Okavango.

The percentage of correct predictions, overall, was higher (88.8%) than that based on conventional skull measurements (82.2%). Durban, Wondergat and Kariba populations showed 100% correct classification (Table 6.16).

A plot of the discriminant function values (Fig.6.5) showed that the Durban and Wondergat populations were separated from the other populations along the first canonical variable. These groups were also separated from each other along the second canonical variable. However, in the wild-caught fish (Figure 5.9) Durban and Wondergat groups were separated from each other along the first canonical variable, but overlapped with all the other populations.

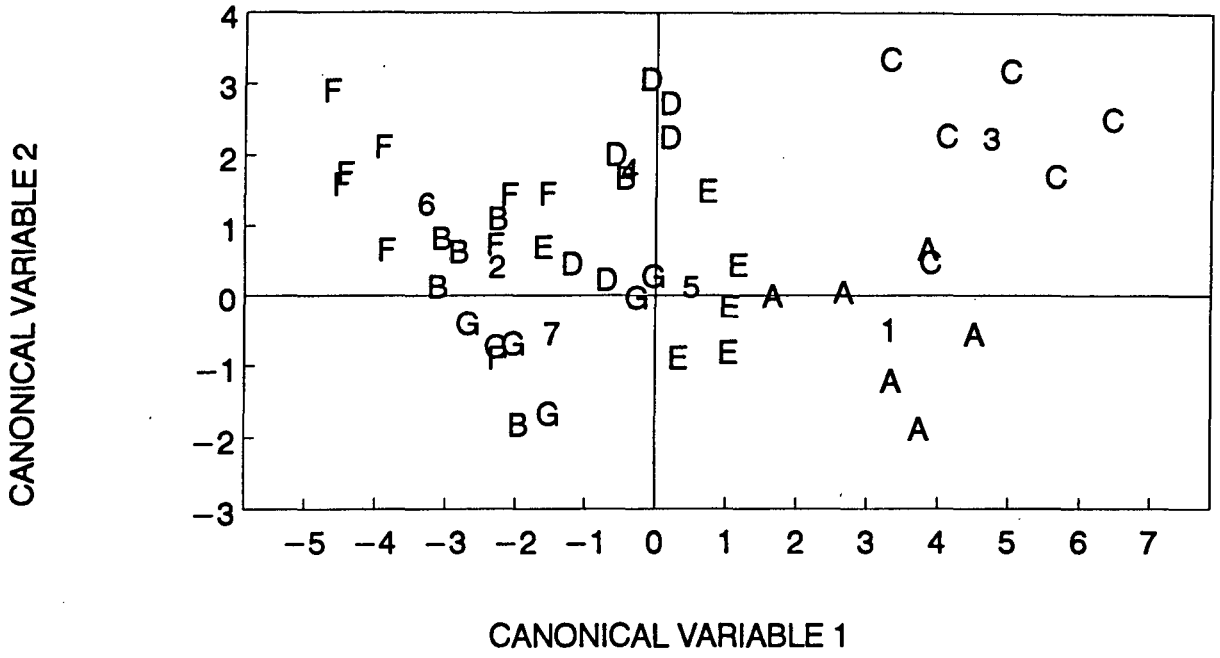

Figure 6.5: A discriminant function plot based on skull measurements of laboratory-bred populations (A=Durban; B=Otjikoto; C=Wondergat; D=Kuruman; E=Kariba; F=Molopo; G=Okavango).

SUMMARY OF RESULTS

After breeding in the laboratory three generations from the wild populations of *P.philander*, it was found that coloration of mature adult males which was characteristic of each population was retained. This strongly suggests that colour is heritable.

Slight changes in the bones of the jaw in laboratory-bred relative to wild-caught fish (such as the increase in the length of the ascending process of the premaxilla and an increase in the length and width of the pharyngeal bone), could be diet related (Witte 1984), as food composition differed greatly between natural and aquarium environments.

Of interest, was the remarkable reduction in the number of

lateral-line sensory pores in the lachrymal bones in the laboratory-bred populations (Table 6.7). It is suggested that the number of pores is environmentally modified, but the reasons for this are not known. The reductional trends of bones in the infraorbital series are a derived feature characterising *Pseudocrenilabrus* (Greenwood 1989). Greenwood (1989) also suggested that the condition of the infraorbital series was due to ontogenetic retardation.

Although sample size for each of the laboratory-bred populations was too small to provide definitive answers, some significant differences between populations were found, using ANOVA.

Application of Discriminant Analysis to the data (of laboratory-bred fish) and based on discriminant function plots, the results are summarised as follows:

1) Conventional body measurements - Durban and Wondergat fish were well differentiated, Kuruman and Molopo Oog fish less well differentiated and Otjikoto, Kariba and Okavango fairly closely grouped with overlap
2) Truss body measurements - Durban and Wondergat fish were well differentiated and Otjikoto also had very little overlap with other populations
3) Conventional skull measurements - overlap of all the populations with Durban showing some differentiation and Wondergat being the only discretely differentiated group
4) Truss skull measurements - although Durban and Wondergat

were separated from the other populations, there was some overlap between them.

As in the wild-caught populations, Durban and Wondergat fish were consistently differentiated from the other populations. It was interesting to note that a discriminant function plot based on conventional body measurements of laboratory-bred fish showed better differentiation of the groups than in the wild-caught fish and, in general, laboratory-bred fish showed clearer separation of the discriminated groups than did the wild-caught fish. This strongly points to the fact that these laboratory-bred populations of *P.philander* did not tend towards uniformity, showing that at least some of the populations were not differentiated on the basis of different environmental conditions. Otjikoto, Kuruman an Molopo Oog fish also showed some separation from the other populations. In a genetic study on the populations of *P. philander*, using protein electrophoresis, Grant (1990) suggested that based on allozyme frequencies, Durban and Wondergat fish were genetically similar. Grant (1990) found that Kuruman and Molopo Oog fish were also genetically similar.

Comparison of laboratory-bred and wild-caught fish showed that there were some obvious, but not necessarily statistically significant, differences within the populations, particularly in the number of sensory pores of the lachrymal (see pg. 11). However, when bred and kept under uniform laboratory conditions, populations of *P. philander* did not show a tendency towards uniformity as would be expected if the observed morphological differences were the result of environmental influence.

DEFORMITIES IN LABORATORY-BRED POPULATIONS

There was a high proportion of deformities in the laboratory-bred F1 generations of the Molopo Oog population (out of a total of 65 fish, 20 were deformed) and to a lesser extent in the Okavango population (of a total of 100 fish, 22 were deformed). Deformities of the jaws and head region were apparent, while vertebral counts and anatomical dissections showed that the remainder of the body was unaffected. Flank and belly scales on deformed specimens were irregularly arranged and did not show the usual pattern of size gradations both dorso-ventrally and antero-posteriorly. Pored lateral-line scales were not linearly arranged or were absent.

Foreshortening of the head was due to deformation of the upper and lower jaws. One badly deformed specimen from Molopo Oog had an upper jaw (maxilla and premaxilla) that was severely reduced in size relative to that in its siblings, giving it a "pug-nose" appearance. Its lower jaw (dentary and anguloarticular bones) was orientated sharply upwards, "overshooting" the upper jaw (Fig. 6.6). A more common deformity was that of the lower jaw. Changes in the relative proportions of the suspensorium and palato-quadrate arches resulted in the left and right articulatory points of the lower jaw being rotated laterally and anteriorly giving the head a foreshortened appearance (Fig. 6.7). When viewed from its medial aspect, the palato-quadrate arch, instead of appearing as a flat plate, is distinctly "convex" in the deformed specimens.

A

B

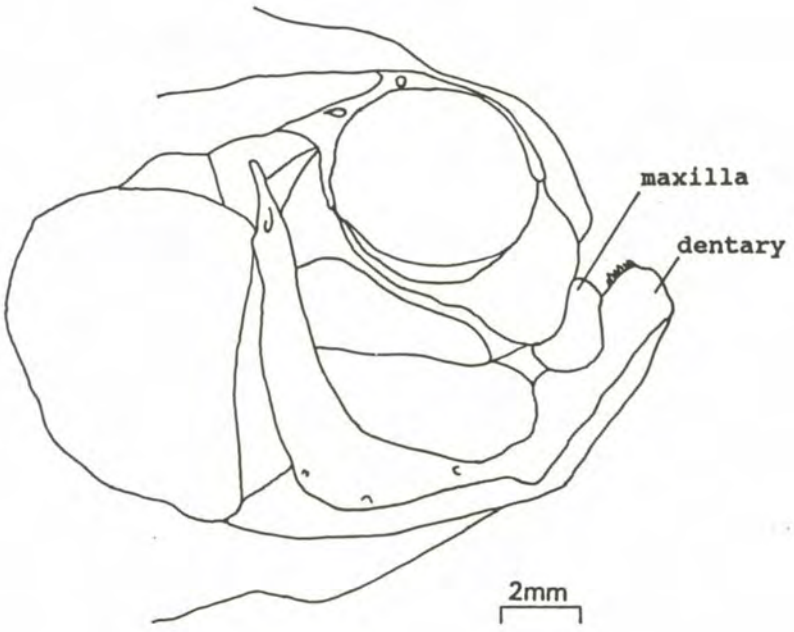

Figure 6.6: A: Deformed *P.philander* from the laboratory-bred F1 Okavango population with a "pug-nose"; B: Diagram of the head of the same fish showing deformation of the bones.

A

B

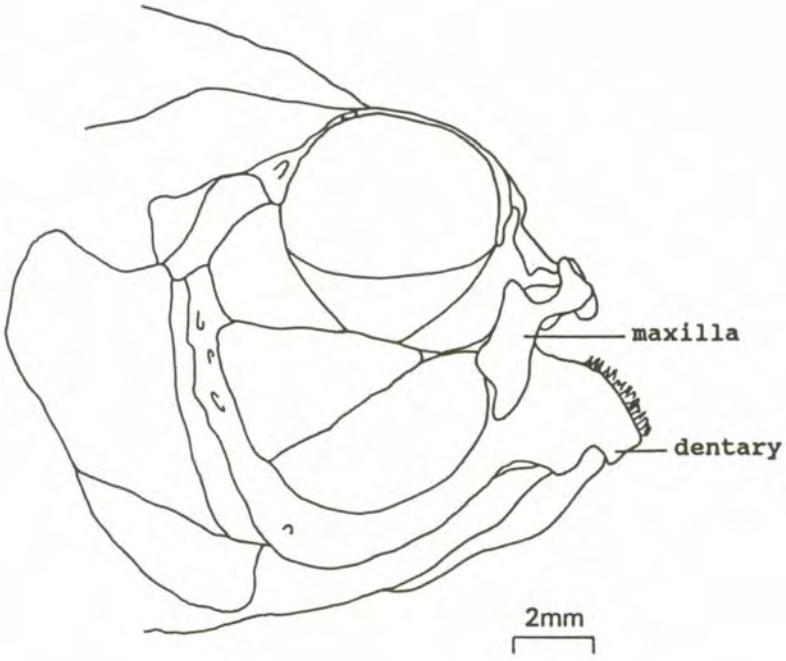

Figure 6.7: A: Deformed *P.philander* from the laboratory-bred F1 Okavango population with a foreshortened head; B: Diagram of the head of the same fish showing deformation of the bones.

A distortion of the palato-quadrate on one side of the head, results in a skewing of the lower jaw to one side and a consequent failure of the lower jaws to occlude correctly. Yet another malformation involves a shortening of the premaxillary dentigerous arms either uni- or bilaterally. A unilateral shortening resulted in either a lateral shift of the upper jaw in relation to the lower jaw, whereas a bi-lateral shortening of the dentigerous arms resulted in an overall shortening of the upper jaw relative to the lower jaw. Associated with this deformation, the lachrymal bones of the infraorbital series were also deformed in size and concavity of the inner aspect was greatly accentuated.

The deformed individuals did not breed. However, their normal siblings bred and produced fewer deformed young (out of a total of 71 Molopo Oog fish, 3 were deformed; out of 75 Okavango fish, 2 were deformed) than those of the other populations, suggesting that the high percentage of deformities in the F1 generation of laboratory-bred fish was not an effect of inbreeding but rather an environmentally induced effect on the parent fish (see DISCUSSION). Both populations were caught in areas where toxic chemicals have been used and both were drawn from bottleneck populations, so abnormalities could be ontogenetic or genetic (see page 11 of DISCUSSION).

The seemingly high proportion of deformities in the laboratory could be artifactual, since it is not always possible to sample the whole brood in the wild. Also, survival rate of deformed individuals in the wild is likely to be lower, thus reducing the number of deformed adults in the population.

Chapter 7:

DISCUSSION

This study has, as its primary objective, addressed the question of differentiation of the allopatric populations of *P. philander*: are the different populations geographic races or separate species?

This question is difficult to answer, as the morphological study, which is ultimately the most relevant and which is the focus of this thesis, is in conflict with the genetic and behavioural studies undertaken on *P. philander*.

Behavioural analyses of some of the *P.philander* populations (Durban, Lake Sibaya, Lake Kariba and Kuruman) were carried out by Ribbink 1975. It was found, in most cases, that the response of experimental fishes to individuals of their own population (and their own colour form) was greater in terms of aggression, courtship and other behaviour. The overall results suggest that the various populations recognise their own population characteristics; in other words, that distinctive behavioural differences have developed between the populations, indicating that assortative mating was likely to occur. So, if these allopatric populations were to become sympatric, it is probable that, given a free choice, they would be likely to behave as closed gene pools (in other words, behave as different species).

A karyological study (Oellermann, Ribbink and Twentyman-Jones, in prep.) showed that there were no differences between the populations of *P. philander* as they all had a diploid chromosome

number of 44 (comprising 10 metacentric, 10 submetacentric and 24 acrocentric chromosomes). However, morphological, anatomical and behavioural studies showed that there were some differences between populations. It is thus suggested that divergence of the taxa has occurred independently of gross chromosomal changes. This is also supported by the fact that African cichlids have a very narrow range in chromosome number, despite their wide geographic distribution, their varied phylogenies, their extensive adaptive radiation and explosive speciation. Interestingly, *P. philander* was found to differ from other cichlids studied, in that it was found to have heteromorphic sex chromosomes (X and Y chromosomes); a feature which appears to be rare in fishes and which has not previously been reported for African cichlids (Oellermann, Ribbink & Twentyman-Jones, in prep.). The reason for having heteromorphic rather than homomorphic sex chromosomes is unknown.

Mitochondrial DNA analyses showed that there was a very close relationship between all the populations, with a maximum interpopulation sequence divergence of only 0.0096 (less than 1%) (de Villiers, Harley & Ribbink 1992). These results suggest a recent origin for the various populations from a common ancestor and that their origins occurred almost at the same time. As in the chromosome analyses, the mitochondrial analyses also showed that the populations were conspecific despite the interpopulational differences in morphology and behaviour.

Analysis of genetic variation by means of protein electrophoresis was carried out by Grant (1990). The results showed a relatively large amount of interpopulation variation in

P. philander. All populations were found to be genetically unique. Grant (1990) suggests, on the basis of large allozyme frequency differences, that these apparently conspecific populations have diverged sufficiently from each other to be considered species or subspecies.

Differences between populations as shown up by the morphological and allozyme frequency analyses could reflect a more rapid fixation of these features in small isolated populations either through genetic drift or through selection by local environmental conditions. De Villiers, Harley and Ribbink (1992) attribute this to a single base change in a coding region of a gene which could result in a significant morphological change, but which would have hardly any effect on overall mtDNA sequence divergence (hence mtDNA analyses showing up all populations as conspecifics). The lack of consistency between the restriction enzyme mitochondrial DNA studies and the allozyme frequencies suggests opportunities for further research. Techniques using polymerase chain reaction and nucleotide sequencing could be used in more detailed studies of mtDNA variation (Grant 1990).

A final answer for the taxonomic categorisation of the *P. philander* populations is required. Clearly populations are diverging, but is there sufficient differentiation in their heritable characteristics for them to be categorised as distinct species? If the same criteria as those used to distinguish between members of species flocks of the African Great Lakes (Greenwood 1974; Barel *et al*. 1977; van Oijen *et al*. 1981; Lewis

1982a; Ribbink *et al.* 1983) are applied to *P. philander*, then several of these populations might also be considered new species. Anatomical and colour differences in Great Lakes cichlids are used as diagnostic features, and these are even more marked in the different *P. philander* populations than in several Great Lakes sibling species complexes.

Before being able to assess the taxonomic status of the allopatric populations of *P. philander*, it is necessary to clarify what is meant here by a "species".

Decisions regarding taxonomic placement of groups must be guided by a species concept that can be easily used. The **recognition concept** of Paterson (1985), which regards a species as "that most inclusive population of biparental organisms which share a common fertilization system", appears to be the most appropriate for this study. This concept focuses on mechanisms which facilitate reproduction (Paterson 1985). These mechanisms constitute the Specific-Mate Recognition System (SMRS), where "recognition" is a specific response of an individual to a signal from a conspecific (Paterson 1978; 1985). Using the recognition concept, systematists are able to evaluate a population with respect to the number of components of the SMRS which it has in common with other allopatric populations (Ribbink 1985). If populations have sufficient shared features in common to interbreed, should they become sympatric under natural conditions, and have a free choice of partners, then they are conspecific (Vrba 1980; 1984). This is hypothetical, as no natural experiment should be carried out and simulated natural

environments are too artificial to find a true answer to the question. Essentially, for allopatric populations to be conspecific they should have the same display patterns (Fryer 1977; Greenwood 1974), coloration (Barel *et al.* 1977; Fryer 1969; Fryer & Iles 1972; Marsh, Ribbink & Marsh 1981; Ribbink *et al.* 1983), habitat preferences, trophic adaptations and breeding seasonality, which all contribute to co-adaptation (Paterson 1978; 1982). Although it has not been shown affirmatively that these features are used by the species themselves in mate recognition, they are species attributes which can be used by systematists for the categorization of taxa (Ribbink 1985).

In studies of cichlids, differences in live coloration between closely related populations are frequently amongst the most important distinguishing features used by systematists (e.g. Greenwood 1965b; Barel *et al.* 1977; van Oijen *et al.* 1981; Lewis 1981, 1982b; Ribbink *et al.* 1983). However, where sibling species occur sympatrically, as is the situation at many locations within the African Great Lakes, studies of habitat segregation, assortative mating and differences in adult male coloration are required to enable taxonomic designations to be made (Holzberg 1978; Marsh *et al.* 1981; Ribbink *et al.* 1983). However, many species are highly habitat-specific, occupying clearly defined microhabitats, and are defined as being stenotopic. Such habitudinally allopatric populations with absolutely different habitat requirements are unlikely to be conspecific (Ribbink *et al.* 1983). Alternatively, if habitat specificity, behavioural use of that habitat and coloration are similar for allopatric populations, then the chances are that

they are conspecific. If some features or components of the SMRS's of allopatric populations are shared and others not, then value judgements would be necessary (Ribbink *et al.* 1983).

Another difficulty of taxonomic studies on cichlids is that of great intraspecific variation for most characters, as found in the Lake Victoria haplochromine species (Greenwood 1974; Barel *et al.* 1977). A quote from Greenwood (1974) emphasises this: "Within a group the morphological characters separating species are mainly slight proportional differences, squamation patterns, differences in male breeding coloration. In most of what may be termed 'specific characters', except male coloration, there is a high level of individual variability."

Greenwood (1965b; 1974) emphasised the role of male colour in the speciation of several cichlid species in Lake Nabugabo. Lake Nabugabo is a small lake on the western shore of Lake Victoria which was cut off from the main lake by sand bars approximately 4000 years ago (Greenwood 1965b). Although anatomically similar to the presumed parent species, the Lake Nabugabo species were markedly different in terms of coloration. However, it has not been shown experimentally whether the differences in male coloration are sufficient as an isolating mechanism between species.

Therefore, coloration may be an important component of a specific mate recognition system (Paterson's Recognition Concept), but without knowing the specific mate recognition signals for a species it is not possible to determine whether

particular differences in coloration between populations would be sufficient to inhibit or promote mating between such populations (Lewis 1982a).

Differentiation of the yellow and blue colour forms of *P. philander* over the east-west distributional range, appears to follow the dispersion and zoogeographic distribution of the ancestral populations, suggested by Jubb and Farquharson (1965). Such migration would have taken place during much wetter periods. It is unclear when isolation of *P.philander* populations occurred. Partridge (in. lit.) suggests that sinkhole formation occurred 75-65 million years ago, with their isolation from about 65-50 million years ago due to aridification of the western part of the subcontinent and thus disruption of drainage systems. But it is the colonisation of the isolated water bodies by fish, *P. philander* in particular, which is of evolutionary importance to this study. It is unlikely that the fish would have survived the glacial or dry periods, so based on palaeoclimatic and paleoecological evidence which show that in general warmer, wetter conditions occurred around 16 000 - 11 000 BP (Butzer, Fock, Stuckenrath and Zilch 1973; Deacon & Lancaster 1988), it is suggested that colonisation was likely to have occurred during this period. It is possible that flash floods would have aided in the distribution of fish. Subsequent drier conditions resulted in fragmentation of water bodies and thus of fish populations. Reduction in gene flow between such geographically isolated populations would lead to divergence of the populations from one another, over time (Mayr 1988; Greenwood 1991). Already some

differentiation may have occurred, as there is inter-population variation, although accurate identification of fish populations is not easy.

Observed colour differences of mature adult *P. philander* are heritable (genetic) differences between the populations. Ribbink (1975) showed that: cross-breeding produced intermediate colour forms; colours for each population remained true after several generations bred in the laboratory; colour differences were not due to changes in colour intensity related to reproductive phases of the fish, nor to stress (or other "emotions"), nor to changes associated with feeding or any other unstable environmental conditions.

In this study, each population of *P. philander* from the wild was characterized in terms of morphology and anatomy. Body colour, fin colour and markings of sexually mature adult males were found to show marked variation between populations, although the coloration shown in some populations appeared to show a higher level of differentiation than in other populations. Populations were bred under uniform laboratory conditions, through three generations, to test whether characteristics were heritable or ecophenotypic. They bred true, maintaining colours of the wild populations. So variation in colour appears to be an inherited and not an ecophenotypic characteristic. It is thus suggested that there has been some differentiation of *P. philander* populations, so the colour differences between these allopatric populations may represent early stages in the process of speciation.

In terms of external morphology, there is variation in body shape and size between the *P. philander* populations. Data sets for conventional morphometrics and Truss measurements were examined by means of Discriminant Function Analysis which classified and grouped the populations, using all measured variables as discriminating variables.

Although there was some overlap, the Durban, Wondergat and Otjikoto populations were consistently distinguished from others anatomically, based on both conventional and Truss data sets, in both wild and laboratory reared populations, again suggesting a genetic base for the distinguishing characteristics.

Using One-way Analysis of Variance (ANOVA), it was found that there were some significant anatomical differences between laboratory-bred and wild-caught fish. A Scheffe multiple range test was used to separate character sets into overlapping subsets (homogeneous groups). Modifications were mostly in the head region, and could be attributed to phenotypic plasticity (possibly diet related, see: Greenwood 1965a; Witte 1984; Meyer 1987).

One-way ANOVA showed that although there were some significant differences in variables between wild-caught and laboratory-bred fish for the measurements carried out, this is not consistent for any particular population.

Within the wild-caught and the laboratory-bred populations, there are significant differences between populations. The multiple range test separated homogeneous groups (populations); those with no overlap showed differences that were significant.

1. For example, height of the supraoccipital crest of wild-caught Wondergat fish was significantly different from that of Durban and Kuruman fish.
2. Another example: width of the skull (otic width) of wild-caught Wondergat and Kuruman fish was significantly different from Kariba fish. But in the laboratory-reared fish, Wondergat is significantly different from Molopo Oog.
3. It is interesting to see that for the measurement of body depth in the wild-caught fish Wondergat is significantly different from Kariba, but in laboratory-reared fish there is no significant difference between populations. It is also well-known that fish do get "fatter" under laboratory conditions with good feeding, so the true value of this as a discriminating variable has to be assessed very carefully.

Greenwood (1965a) found evidence for environmental influence on the shape of skeletal elements in the haplochromine, *Astatoreochromis alluaudi* both under natural conditions and in aquaria. It was suggested that this was either a result of different food, such as thin-shelled as opposed to thick-shelled snails (a physical factor), or due to a reduction in calcium intake (a physiological factor). Witte (1984), who compared wild-caught and tank-bred *Haplochromis squamipinnis*, was also able to show that oral jaw apparatus and pharyngeal jaw apparatus have a high degree of plasticity and that morphological changes can also be caused by environmental factors. Based on these

studies, the changes in the relative length of the ascending process of the premaxilla and the length and width of the pharyngeal bone in some of the laboratory-bred populations of *P. philander* (from the wild-caught fish) again demonstrate the plasticity of haplochromine jaw morphology to changes of environment (Greenwood 1965a; Witte 1984; Meyer 1987). Under natural conditions, the diet of *P. philander* consists of insects, midge larvae, shrimps, small fish and most other small aquatic organisms (Jackson 1961; Crass 1964; Bell-Cross & Minshull 1988), whereas in the laboratory, fishes were fed minced ox-heart, TETRAMIN flake food and *Daphnia* sp. Changes in the form of the premaxilla could possibly be due to a different way of "food-handling" in the laboratory (Witte 1984).

Skeletal anomalies are commonly observed in fish (Sindermann 1979; 1990). Such anomalies may have an hereditary basis, or there may be evidence of environmental effects, such as temperature, salinity, dissolved oxygen, radiation, dietary deficiencies, and toxic chemicals. In this study, it is not known whether the high percentage of observed deformities (in the Molopo Oog and Okavango populations) are a manifestation of inbreeding depression or an environmentally induced phenomenon. Such deformed fish would have a reduced ability to mouthbrood and to feed, and would probably not survive under natural conditions, so a comparative study with that of the wild situation would be artifactual.

The Molopo Oog population was severely reduced after the introduction of predatory bass into the spring, and was possibly

in a severe population bottleneck when specimens were collected. The Okavango fish were collected in an isolated pool, so founders of the laboratory population may all have been sibs, resulting in inbreeding. Other populations bred in the laboratory which were not under such serious bottlenecks in the wild, did not show as many deformities.

Another possibility considered, was that of the effect of toxic chemicals. Depending on concentrations, pollutants may result in mortality or in morphological or physiological abnormalities that can affect any life history stage (Sindermann 1990). The Molopo Oog water body and surrounding reeds had been sprayed to kill birds (pests to the local wheat farmers), and the Okavango Delta is continually sprayed to eradicate the Tsetse fly (which causes sleeping sickness in man and nagana in domestic cattle). Such chemicals would also prove harmful to the fish populations, by causing possible malformations. Increased occurrence of skeletal deformities and anomalies have been reported for several fish species from contaminated areas in southern California, the British Isles and Japan, as being pollution-associated (Sindermann 1990).

P. philander has a wide distribution in southern Africa and is able to live in a variety of different habitats. For example, *P. philander* is found to depths of approximately 40m in sinkholes (pers. obs.) and in Lake Sibaya (Bruton 1979). In sinkholes it has to feed on "Aufwuchs" (filamentous algae and microfauna in the algal mat on the walls of the sinkhole). Other populations are equally 'at home' in shallow "vleis" where there is a greater

abundance of food, particularly insects. *P. philander* also occurs in streams, pans, dams, rivers, lakes and springs, in vegetated and un-vegetated habitats, in clear or turbid waters and on rocky or sandy substrata. It is able to live in water with wide salinity ranges (Millard & Broekhuysen 1970; Whitfield, Blaber & Cyrus 1981), and broad temperature and pH ranges and hardness values (Loiselle 1982b). *P. philander* is thus a eurytopic fish which is able to live in many different, and often harsh, habitats. Despite this variation in habitats and despite its broad geographic range, the allopatric populations of *P. philander* have differentiated remarkably little when compared with, for example, the stenotopic Mbuna of Lake Malawi (Ribbink *et al.* 1983). To explain why differentiation of cichlids in sinkholes is slower than that of the Mbuna, it is suggested (Ribbink in press) that the fishes are at two different stages on a time scale of evolutionary specialization. The Mbuna may have also passed through a period of slow divergence in their evolutionary history before the species became so specialised that rates of differentiation increased, resulting in the present Mbuna flock of over 200 species. Although the eurytopic populations of *P. philander* have differentiated to some extent, they are still sufficiently plastic in their behaviour, habitat and feeding requirements to be relatively unaffected by environmental change and are thus slower to respond in an evolutionary sense, and hence their rate of differentiation is slower than that of stenotopes (Ribbink in press).

CONCLUSION

Although the Discriminant Function Analysis showed some differentiation of the Durban, Kuruman and Wondergat populations, after a consideration of the colour differences, morphology and behavioural patterns of the *P. philander* populations studied, it is concluded that populations are incipient species.

REFERENCES

ALBRECHT, G.H. 1980. Multivariate analysis and the study of form, with special reference to canonical variate analysis. *American Zoologist* 20: 679-693.

ANKER, G. Ch. 1978. The morphology of the head-muscles of a generalized *Haplochromis* species: *H.elegans* Trewavas 1933 (Pisces, Cichlidae). *Netherlands Journal of Zoology* 28: 234-271.

BAREL, C.D.N., F. WITTE & M.J.P. VAN OIJEN. 1976. The shape of the skeletal elements in the head of a generalized *Haplochromis* species: *H.elegans* Trewavas 1933 (Pisces, Cichlidae). *Netherlands Journal of Zoology* 26: 163-265.

BAREL, C.D.N., M.J.P. VAN OIJEN, F. WITTE & E.L.M. WITTE-MAAS. 1977. An introduction to the taxonomy and morphology of the haplochromine Cichlidae from Lake Victoria. *Netherlands Journal of Zoology* 27: 333-389.

BARNARD, K.H. 1978. Report on a collection of fishes from the Okavango river, with notes on Zambezi fishes. *Annals of the South African Museum* 36(5): 407-458.

BELL-CROSS, G. & J.L. MINSHULL. 1988. *The Fishes of Zimbabwe*. National Museums and Monuments of Zimbabwe, Harare, Zimbabwe. 294pp.

BLOUW, D.M. & D.W. HAGEN. 1990. Breeding ecology and evidence of reproductive isolation of a widespread stickleback fish (Gasterosteidae) in Nova Scotia, Canada. *Biological Journal of the Linnean Society* 39:195-217.

BOOKSTEIN, F., B. CHERNOFF, R. ELDER, J. HUMPHRIES, G. SMITH & R. STRAUSS. 1985. *Morphometrics in Evolutionary Biology*. Special Publication 15, The Academy of Natural Sciences of Philadelphia.

BOULENGER, G.A. 1915. *Catalogue of the Freshwater Fishes of Africa,* Vol 3. London, British Museum (Natural History).

BRUTON, M.N. 1979. The Fishes of Lake Sibaya. pp.162-245. In: *Lake Sibaya,* (ed.) B.R. Allanson. Monographiae Biologicae, Volume 36. Junk Publishers, The Hague.

BUTZER, K.W., G.J. FOCK, R. STUCKENRATH & A. ZILCH. 1973. Palaeohydrology of late Pleistocene Lake, Alexandersfontein, Kimberley, South Africa. *Nature* 243:328.

CASTELNAU, F. (de) 1861. *Memoir sur les poissons de l' Afrique Australe*. Paris. 78pp.

CRASS, R.S. 1964. *Freshwater Fishes of Natal*. Shuter & Shooter, Pietermaritzburg, South Africa. 167pp.

DEACON J. & N. LANCASTER. 1988. *Late Quarternary Palaeo-environments of Southern Africa*. Oxford University Press, New York. 225pp.

DE VILLIERS, D.L., E.H. HARLEY & A.J. RIBBINK. 1992. Mitochondrial DNA restriction enzyme variation in allopatric populations of *Pseudocrenilabrus philander* (Pisces: Cichlidae): a problem of incipient speciation. *South African Journal of Science* 88:96-99.

DIXON, W.J. 1990. *BMDP Statistical Software Manual, Volume 1*. University of California Press, Berkley. 629pp.

FOWLER, H.W. 1934. Fishes obtained by Mr H.W. Bell-Marley chiefly in Natal and Zululand in 1929-32. *Proceedings of the Academy of Natural Sciences of Philadelphia* 86: 405-514.

FRANKEL, O.H. & M.E. SOULE. 1981. *Conservation and Evolution*, Cambridge University Press, London. 327pp.

FRYER, G. 1959. The trophic interrelationship 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. *Proceedings of the Zoological Society of London* 132(2): 153-281.

FRYER, G. 1960. Some controversial aspects of speciation of African cichlid fishes. *Proceedings of the Zoological Society of London* 135(4): 569-578.

FRYER, G. 1969. Speciation and adaptive radiation in African lakes. *Verhandlungen International Vereiniging Limnologie* 17:303-322.

FRYER, G. 1977. Evolution of species of cichlid fishes in African Lakes. *Zeitschrift für Zoologische Systematik und Evolutionforschung* 15: 141-165.

FRYER, G. & T. D. Iles 1969. Alternative routes to evolutionary success as exhibited by African cichlid fishes of the genus *Tilapia* and the species flocks of the African Great Lakes. *Evolution* 23: 359-369.

FRYER, G. & T.D. ILES. 1972. *The Cichlid Fishes of the Great Lakes of Africa*. Oliver & Boyd, Edinburgh. 641 pp.

GOMES, N.A. DE N.C. 1985. Modern stromatolites in a karst structure from the Malmani subgroup, Transvaal sequence, South Africa. *Transactions of the Geological Society of South Africa* 88: 1-9.

GRANT, W.S. 1990. Population genetics of cichlids. *Final Report: National Programme for Ecosystem Research*. Unpublished.

GREENWOOD, P.H. 1956a. The monotypic genera of cichlid fishes in Lake Victoria. *Bulletin of the British Museum (Natural History) Zoology* 3: 295-333.

GREENWOOD, P.H. 1956b. A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae), Part I. *Bulletin of the British Museum (Natural History) Zoology* 4: 223-244.

GREENWOOD, P.H. 1957. A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae), Part II. *Bulletin of the British Museum (Natural History) Zoology* 5: 73-97.

GREENWOOD, P.H. 1959a. The monotypic genera of the cichlid fishes of Lake Victoria, Part II. *Bulletin of the British Museum (Natural History) Zoology* 5: 163-177.

GREENWOOD, P.H. 1959b. A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae), Part III. *Bulletin of the British Museum (Natural History) Zoology* 5: 179-218.

GREENWOOD, P.H. 1960. A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae), Part IV. *Bulletin of the British Museum (Natural History) Zoology* 6: 227-281.

GREENWOOD, P.H. 1962. A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae), Part V. *Bulletin of the British Museum (Natural History) Zoology* 9: 139-214.

GREENWOOD, P.H. 1965a. Environmental effects on the pharyngeal mill of a cichlid fish, *Astatoreochromis alluaudi*, and their taxonomic implications. *Proceedings of the Linnean Society of London*. 176 : 1-10.

GREENWOOD, P.H. 1965b. The cichlid fishes of Lake Nabugabo, Uganda. *Bulletin of the British Museum (Natural History) Zoology* 12: 315-357.

GREENWOOD, P.H. 1966. Two new species of *Haplochromis* (Pisces, Cichlidae) from Lake Victoria. *Annals and Magazine of Natural History* (13) 8: 303-318.

GREENWOOD, P.H. 1967. A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae), Part VI. *Bulletin of the British Museum (Natural History) Zoology* 15: 29-119.

GREENWOOD, P.H. 1973a. A revision of the *Haplochromis* and related species (Pisces, Cichlidae) from Lake George, Uganda. *Bulletin of the British Museum (Natural History) Zoology* 25: 139-242.

GREENWOOD, P.H. 1973b. Morphology, endemism and speciation in African cichlid fishes. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 1973: 115-124.

GREENWOOD, P.H., 1974. Cichlid fishes of Lake Victoria, East Africa: the biology and evolution of a species flock. *Bulletin of the British Museum (Natural History) Zoology,* Supplement 6: 1-134.

GREENWOOD, P.H. 1979. Towards a phyletic classification of the 'genus' *Haplochromis* (Pisces: Cichlidae) and related taxa. *Bulletin of the British Museum (Natural History) Zoology* 35 (4): 265-322.

GREENWOOD, P.H. 1984a. African cichlids and evolutionary theories. pp. 141-154. In: *Evolution of Fish Species Flocks,* (eds.) A.A. Echelle & I. Kornfield. Orono Press, Orono, Maine. 257pp.

GREENWOOD, P.H. 1984b. The haplochromine species (Teleostei, Cichlidae) of the Cunene and certain other Angolan rivers. *Bulletin of the British Museum (Natural History) Zoology* 47(4): 187-239.

GREENWOOD, P.H. 1985. Notes on the anatomy and phyletic relationships of *Hemichromis* Peters 1858. *Bulletin of the British Museum (Natural History) Zoology* 48(3): 131-171.

GREENWOOD, P.H. 1989. The taxonomic status and phylogenetic relationships of *Pseudocrenilabrus* Fowler (Teleostei, Cichlidae). *Ichthyological Bulletin of the J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa.* 54: 1-16.

GREENWOOD, P.H. 1991. Speciation. pp. 86-102. In: *Cichlid Fishes: Behaviour, Ecology and Evolution,* (ed.) M.H.A. Keenleyside. Chapman and Hall, London.

GREENWOOD, P.H. & J.M. GEE. 1969. A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae), Part VII. *Bulletin of the British Museum (Natural History) Zoology* 18: 1-65.

GREENWOOD, P.H. & C.D.N. BAREL. 1978. A revision of the Lake Victoria *Haplochromis* species (Pisces, Cichlidae) Pt VIII. *Bulletin of the British Museum (Natural History) Zoology* 33(2): 141-192.

HILGENDORF, F. 1902. Nueue Chromiden-Art aus Deutsch Südwest-afrika. *Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin* 1902: 141-143.

HILL, B.J. 1969. The bathymetry and possible origin of Lakes Sibayi, Nhlange and Sifungwe in Zululand (Natal). *Transactions of the Royal Society of South Africa* 38 (3): 205-216.

HOLZBERG, S. 1978. A field study of the behaviour and ecology of *Pseudotropheus zebra* (Boulenger), an endemic cichlid of Lake Malawi (Pisces: Cichlidae). *Zeitschrift für Zoologische Systematik und Evolutionforschung* 16: 171-187.

HUSTLER, K. & B.E. MARSHALL. 1990. Population dynamics of two cichlid fish species in a tropical man-made lake (Lake Kariba). *Hydrobiologia* 190: 253-262.

JACKSON, P.B.N. 1961. *The Fishes of Northern Rhodesia - A Check List of Indigenous Species*. The Government Printer, Lusaka. 140pp.

JUBB, R.A. 1961. *An Illustrated Guide to the Freshwater Fishes of the Zambezi River, Lake Kariba, Pungwe, Sabi, Lundi and Limpopo Rivers*. Stuart Manning, Bulawayo. 171pp.

JUBB, R.A. 1971. Cave and sinkhole fishes of southern Africa. Part 3. *African Aquarist* 4(7): 6-8.

JUBB, R.A. & F.L. FARQUHARSON. 1965. The freshwater fishes of the Orange River drainage basin. *South African Journal of Science* 61: 118.

KLECKA, W.R. 1975. Discriminant Analysis. pp.434-467. In: *Statistical Package for the Social Sciences*, (eds.) N.H. Nie, C.H. Hull, J.G. Jenkins, K. Steinbrenner & D.H. Bent. McGraw-Hill, New York.

LEWIS, D.S.C. 1981. Preliminary comparisons between the ecology of the haplochromine cichlid fishes of Lake Victoria and Lake Malawi. *Netherlands Journal of Zoology* 31: 746-761.

LEWIS, D.S.C. 1982a. Problems of species definition in Lake Malawi cichlid fishes (Pisces: Cichlidae). *J.L.B. Smith Institute of Ichthyology: Special Publication*. No. 23: 1-5.

LEWIS, D.S. 1982b. A revision of the genus *Labidochromis* (Teleostei: Cichlidae) from Lake Malawi. *Zoological Journal of the Linnean Society* 75: 189-265.

LOISELLE, P.V. 1982a. *Pseudocrenilabrus* The Dwarf African Mouthbrooders Part one: The *Pseudocrenilabrus multicolor* complex. *Freshwater and Marine Aquarium* 5(1): 30-35; 59-63.

LOISELLE, P.V. 1982b. *Pseudocrenilabrus* The Dwarf African Mouthbrooders Part two: The *Pseudocrenilabrus ventralis* and *Pseudocrenilabrus philander* complex. *Freshwater and Marine Aquarium* 5(2): 30-34; 66-71; 75.

LOWE-McCONNELL, R.H. 1969. Speciation in tropical freshwater fishes. pp. 51-75. In: *Speciation in Tropical Environments*, (ed.) R.H. Lowe-McConnell. Academic Press, London. 246pp.

MARSH, A.C., A.J. RIBBINK & B.A. MARSH. 1981. Sibling species complexes in sympatric populations of *Petrotilapia* Trewavas (Cichlidae, Lake Malawi). *Zoological Journal of the Linnean Society* 71: 253-264.

MAYR, E. 1988. *Towards a New Philosophy of Biology: Observations of an Evolutionist*. Belknap Press, Cambridge. 564pp.

MEYER, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* 41(6) : 1357-1369.

MILLARD, N.A.H. & G.J. BROEKHUYSEN. 1970. The ecology of South African Estuaries. Part X. St Lucia: a second report. *Zoologica Africana* 5(2): 277-307.

ODIORNE, J.M. 1957. Color changes. pp. 387-401. In: *The Physiology of Fishes*, (ed.) M.E. Brown. Academic Press Inc., New York. 526pp.

OELLERMANN, L.K., A.J. RIBBINK & V. TWENTYMAN-JONES. 1993. The karyology of allopatric populations of *Pseudocrenilabrus philander* (Pisces: Cichlidae) and the presence of dimorphic sex chromosomes. *Biological Journal of the Linnean Society*. In preparation.

PATERSON, H.E.H. 1978. More evidence against speciation by reinforcement. *South African Journal of Science* 74:369-371.

PATERSON, H.E.H. 1982. Perspective on speciation by reinforcement. *South African Journal of Science* 78:53-57.

PATERSON, H.E.H. 1985. The recognition concept of species. pp. 21-29. In: *Species and Speciation,* (ed.) E. Vrba. Transvaal Museum Monograph 4, Pretoria, South Africa. 176pp.

PENRITH, M.J. 1978. Otjikoto Lake. *S.W.A. Annual* 1978: 138-139.

POTTHOFF, T. 1984. Clearing and staining techniques. pp. 35-37. In: *Ontogeny and Systematics of Fishes - Ahlstrom Symposium,* (eds.) H.G. Moser, W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall & S.L. Richardson. American Society of Ichthyologists and Herpetologists, Special Publication No. 1.

REGAN, C.T. 1922. The classification of the fishes of the family Cichlidae. II. On African and Syrian genera not restricted to the Great Lakes. *Annals and Magazine of Natural History* (9) 10 (57): 249-264.

RIBBINK, A.J. 1971. The behaviour of *Hemihaplochromis philander*, a South African cichlid fish. *Zoologica Africana* 6:263-288.

RIBBINK, A.J. 1975. *A Contribution to the Understanding of the Ethology of the Cichlids of Southern Africa*. PhD. Thesis, Rhodes University, Grahamstown. 211pp.

RIBBINK, A.J. 1985. Species concept: problems of categorising closely related cichlids. Fifth Congress of European Ichthyologists. August 1985. Stockholm. Unpublished Conference Paper.

RIBBINK, A.J. 1987. African lakes and their fishes : conservation scenarios and suggestions. *Environmental Biology of Fishes* 19(1): 3-26.

RIBBINK, A.J. 1993. Biodiversity and speciation of freshwater fishes with particular reference to African cichlids. *Annual Symposium of the British Ecological Society and American Society of Limnology and Oceanography. Aquatic Ecology: Scale Pattern and Process. April 1992. University College, Cork, Ireland.* Blackwell Scientific Press. In Press.

RIBBINK, A.J., B.A. MARSH, A.C. MARSH, A.C. RIBBINK & B.J. SHARP. 1983. A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. *South African Journal of Zoology* 18: 149-310.

RIBBINK, A.J. & V. TWENTYMAN-JONES. 1989. Captive Propagation as a conservation tool. *Musee Royal de l'Afrique Centrale, Tervuren Belgique Annales Sciences Zoologiques* 257: 145-150.

RIBBINK, A.J. & V. TWENTYMAN-JONES. 1990. Colour morphs and breeding of *Tilapia guinasana. Journal of the American Cichlid Association: Buntbarsche Bulletin* 138: 2-8.

SCHOELLER, C.H. 1903. Ein neuer *Chromis. Blatter für Aquarien- und Terrarienkunde* 14: 185.

SCHONEWALD-COX, C.M., S.M. CHAMBERS, B. MACBRYDE & L. THOMAS (eds.) 1983. *Genetics and Conservation : a Reference for Managing Wild Animal and Plant Populations.* Benjamin/Cummings Publ., Menlo Park. 722 pp.

SINDERMANN, C.J. 1979. Pollution-associated disease and abnormalities of fish and shellfish: a review. *Fishery Bulletin* 76(4): 717-749.

SINDERMANN, C.J. 1990. *Principal Diseases of Fish and Shellfish.* Volume 1: Diseases of Marine Fish. Academic Press, San Diego. 521pp.

SKELTON, P.H., A.K. WHITFIELD & N.P.E JAMES. 1989. Distribution and diversity of Mkuze swamp fishes during a summer flood. *South African Journal of Aquatic Science* 15(1): 50-66.

STEINDACHNER, F. 1866. Ichthyologische Mittheilungen. 9.1. Ueber einige neue Süsswasser-fische von Angola. *Verhandlungen der Zoologisch-botanischen Gesellschaft in Wien* 16: 761-771.

STERBA, G. 1962. *Freshwater Fishes of the World.* Vista Books, London. 878 pp.

STIASSNY, M.L. 1982. The relationships of the neotropical genus *Cichla* (Perciformes, Cichlidae): a phyletic analysis including some functional considerations. *Journal of Zoology, London.* 197:427-453.

STRAUSS, R.E. & F.L. BOOKSTEIN. 1982. The truss: body form reconstructions in morphometrics. *Systematic Zoology* 31(2): 113-135.

TREWAVAS, E., 1936. Dr Karl Jordan's expedition to South-West Africa and Angola: the freshwater fishes. *Novitates Zoologicae* 40: 30.

TREWAVAS, E. 1973. I. On the cichlid fishes of the genus *Pelmatochromis* with proposal of a new genus for *P. congicus*; on the relationship between *Pelmatochromis* and *Tilapia* and the recognition of *Sarotherodon* as a distinct genus and II. A new species of cichlid fishes of rivers Quanza and Bengo, Angola, with a list of the known cichlidae of these rivers and a note on *Pseudocrenilabrus natalensis* Fowler. *Bulletin of the British Museum (Natural History) Zoology* 25(1): 1-37.

TURNER, G.F., T.J. PITCHER & A.S. GRIMM. 1989. Identification of the Lake Malawi *Oreochromis* (*Nyasalapia*) spp. using multivariate morphometric techniques. *Journal of Fish Biology* 35: 799-812.

VAN OIJEN, M.J.P., F. WITTE & E.L.M. WITTE-MAAS, 1981. An introduction to ecological and taxonomic investigations on the haplochromine cichlids from Mwanza Gulf of Lake Victoria. *Netherlands Journal of Zoology* 31: 149-174.

VRBA, E.S. 1980. Evolution, species and fossils: how does life evolve? *South African Journal of Science* 76: 61-84.

VRBA, E.S. 1984. Patterns in the fossil record and evolutionary processes. pp. 115-142. In: *Beyond Neodarwinism*, (eds.) M.W. Ho & P.S. Saunders. Academic Press, London.

WEBER, M. 1897. Beitrage zur Kenntniss der Fauna von Süd-Afrika. I. Zur Kentniss der Süsswasser-Fauna von Süd-Afrika. *Zoologische Jahrbücher für Systematik* 10:135-199.

WELCOMME, R.L. 1972. *The Inland Waters of Africa*. CIFA Technical Paper No. 1. FAO, Rome.

WHITFIELD, A.K., S.J.M. BLABER & D.P. CYRUS. 1981. Salinity ranges of some southern African fish species ocurring in estuaries. *South African Journal of Zoology* 16:151-155.

WICKLER, W. 1963. Zur Klassifikation der Cichlidae, am Beispiel der Gattungen *Tropheus, Petrochromis, Haplochromis* und *Hemihaplochromis* n.gen (Pisces Pisciformes) *Senckenbergia Biologica* 44(2): 83-86.

WITTE, F. 1984. Consistency and functional significance of morphological differences between wild-caught and domestic *Haplochromis squamipinnis* (Pisces, Cichlidae). *Netherlands Journal of Zoology* 34(4): 596-614.

APPENDIX 1

Colour chart used for the colour descriptions of the *P.philander* populations.