A COMPARATIVE STUDY OF THE LIFE HISTORIES OF THE SISTER SPECIES, *PSEUDOBARBUS AFER* AND *PSEUDOBARBUS ASPER*, IN THE GAMTOOS RIVER SYSTEM, SOUTH AFRICA

THESIS

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ABSTRACT

This thesis explores the biology, ecology, and life-history styles of two closely-related redfin minnows, *Pseudobarbus afer* and *P. asper* (Pisces; Cyprinidae), which both occur in the Gamtoos River system of South Africa.

Five of the seven species of flexible-rayed redfin minnows are in the South African Red Data Book - Fishes. This investigation was designed to provide the data which would enable conservation authorities to manage the remaining populations of the *Pseudobarbus* species.

A thorough understanding of the Gamtoos River system was necessary to properly interpret the findings of this study. The palaeo river systems and the changing climates since the break-up of Gondwanaland are discussed so that the present day environments could be considered as well as the past environmental changes.

P. afer and *P. asper* occur in the Gamtoos River system with no physical barrier separating the two species. *P. afer* only occurs in the clear mountain streams of the Cape Fold Mountain Belt whereas *P. asper* occurs in the highly saline and turbid Karoo section of the system.

P. afer were found to be the more precocial form of the sister species. They had bigger eggs, lower relative fecundity, shorter breeding season, lower gonadosomatic indices, larger first feeding larval fish, matured later and had a longer life-span than did *P. asper*, which had more altricial life-history attributes. They differ in their tradeoffs with *P. asper* devoting more resources earlier to reproduction and having a shorter lifespan. The improvement in the one aspect of fitness (early maturity) leads to the deterioration in another, namely lifespan. Both species undertake breeding migrations to riffle areas where they spawn in mid-channel immediately above a pool after an increase in water flow. *P. afer* and *P. asper* are non-guarders of their non-adhesive eggs and young, open substrate spawners on coarse substrates (rocks) and have photophobic free embryos. The breeding season is shorter for *P. afer* whereas *P. asper* can spawn as late as April and impoundment releases can induce them to spawn.

A study of comparative neuroecology revealed that of the four groups of fish analyzed (males and females of both species) male *P. afer* had the largest brains, especially the optic lobes and cerebellum. *P. asper* females had the smallest brains. No neural compensation in the external gustatory centre, the facial lobe, was found for *P. asper* inhabiting the turbid waters. *P. afer* also had significantly larger eyes and longer barbels. *P. afer* males were also found to have the highest density and largest nuptial tubercles as well as the most pronounced breeding colouration.

It was concluded that *P. asper* is the more derived of the sister species pair with regard to life-history attributes. It is further suggested that investment per offspring is important in determining the life-history trajectories. Paedomorphosis has occurred and by this mechanism variability has been restored to the redfin minnows in the Groot River which enables them to survive in the highly variable, intermittent Karoo stream. The more precocial *P. afer* do not require this variability in the more constant and predictable environment of the Wit River.

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CHAPTER - 1

INTRODUCTION

There are 54 *Barbus* and *Pseudobarbus* species in southern Africa of which 44 are less than 150 mm fork length. The genus *Pseudobarbus*, which was separated from *Barbus* by Skelton (1988), consists of seven species. The distribution of the *Pseudobarbus* species is confined to a geographically distinct region, the Cape Fold Mountain and associated Karoo drainages, except for *P. quathlambae* which is confined to the Lesotho highlands. This distribution is the result of climatic, geological and sea level changes over geological time scales and recently their distribution has been influenced by human impacts. The present day distribution of the *Pseudobarbus* species are, with one exception, entirely complementary and allopatric. *P. afer* and *P. asper* are sister species in the Gamtoos River system. *P. afer* is restricted to the stable, clear, acidic, perennial mountain streams, whereas *P. asper* occurs in the widely fluctuating, turbid, alkaline, seasonal rivers of Karoo origin (Figures 1, 2 & 3). The knowledge of the interrelationships of these two closely-related species (Skelton, 1980a) and their occurrence in two extreme environments without a physical barrier separating them makes them suitable for a comparative life-history study.

The common ancestor to the monophyletic *Pseudobarbus* probably evolved, or became established, during the late Eocene to Oligocene (45-25 X 10^6 y BP) (Skelton, 1980a). Deterioration of catchment areas by damming, water abstraction, poor land management, and the introduction of exotic predator fish such as *Micropterus* spp. are having a severe impact on *Pseudobarbus* populations (Cambray & Stuart, 1985; Skelton, 1987; Cambray & Cambray, 1988). Due to the ever increasing habitat degradation, five of the seven *Pseudobarbus* species are now in the Red Data Book (fishes) (Skelton, 1987). Because of their uniqueness, they are one of the most important groups of fishes to conserve in South Africa and are characterized by a suite of distinct characters. There have been very few studies on these small barbs.

In the New World cyprinids little more is known than the body size, habitat, and associated species. Consequently, it is unlikely that the significance of the fauna will be fully appreciated without an increased interest in and understanding of their biologies (Mayden, 1991). There is also very little known about many of the small African cyprinids (Cambray, 1982). Basic data such as phylogeny, age, growth, population dynamics, spawning seasons and habitat, behaviour, early ontogeny and diet are lacking for the majority of the smaller cyprinids in Africa. It is

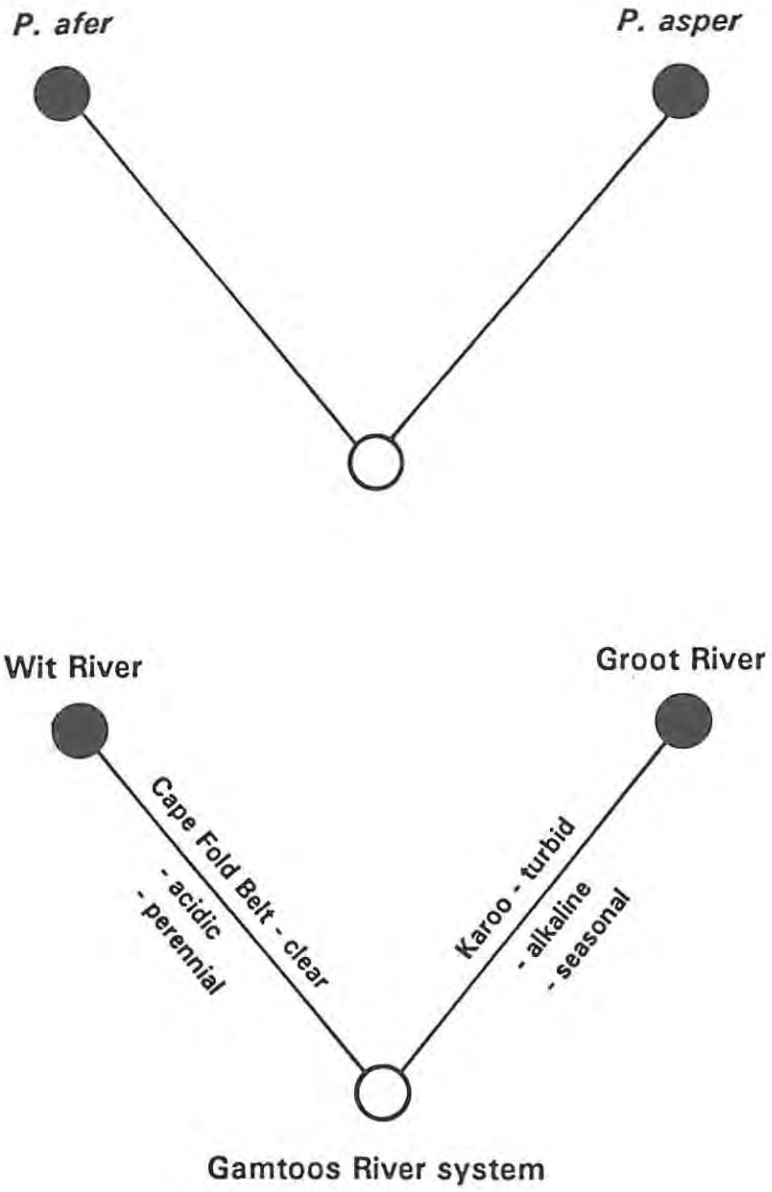
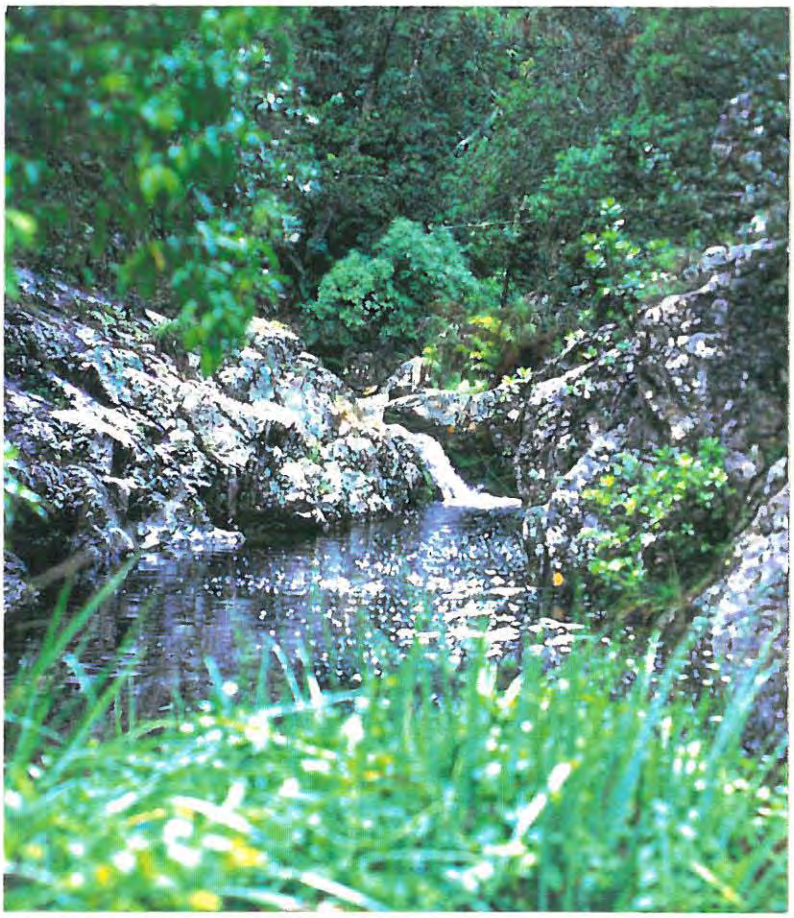


Figure 1. Sister species and "sister environments" of the Gamtoos River system.

Figure 2. Male *Pseudobarbus afer* (above) and *Pseudobarbus asper* (below).



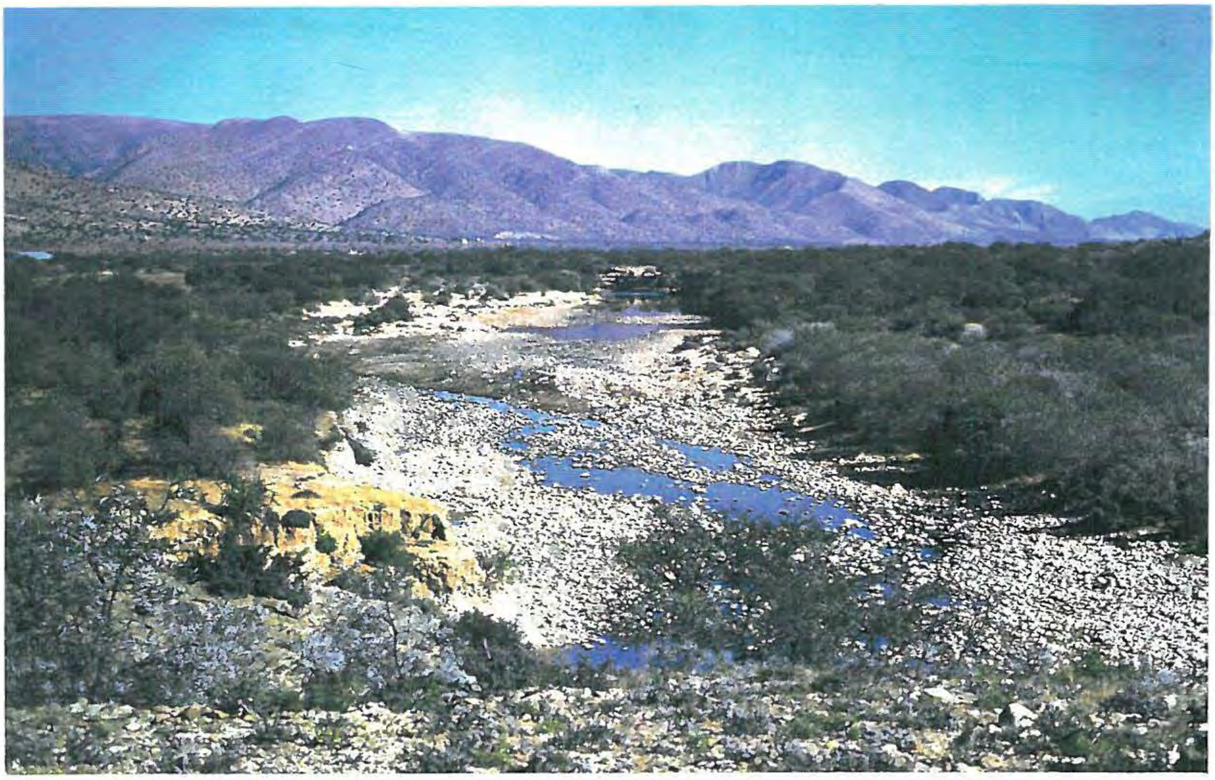


Figure 3. Wit River, a clear, perennial mountain stream (above) and Groot River a turbid, intermittent Karoo stream (below).

encouraging therefore that there have been several recent studies on the smaller species in Africa (eg. Cambray & Meyer, 1988; Skelton, 1988; Naran, 1992; Polling *et al.*, 1992a,b). Studies of the interrelationships and possible Gondwanic origins increase their significance (Skelton, 1980a, 1988). In North America the studies on the small cyprinids, such as shiners, minnows and chubs, have been done mainly because of their importance as food for piscivorous game fish, such as salmonids, pikes and bass. In Africa, bass and some salmonids have been introduced because in some rivers there were "only" minnows. Much of the past effort of conservation bodies has been centred on hatcheries and culturing of aliens while the indigenous fauna was mainly ignored except for several of the larger species.

There have been very few field studies on *Pseudobarbus*: Skelton (1980a) undertook a thorough cladistic analysis; Cambray & Stuart (1985) worked on the ecology of one species (*P. burchelli*); Rondorf (1975, 1976) completed several ecological studies on *P. quathlambae*; Cambray & Meyer (1988) described the early ontogeny of *P. quathlambae*; Cambray (1990a) described the impact of entrainment on a population of *P. asper*; Cambray (1990b) discussed *P. asper* migrations; Cambray (1991a) noted how water releases from a dam were sufficient to initiate spawning in *P. asper* and Cambray (1991b) compared the egg envelope ultrastructure of three species with those of several *Barbus* species. Jubb (1967) and others have made passing references to the biology and/or ecology of these seven species of *Pseudobarbus*. The present study adds to the existing data base for this distinct group of African minnows.

'Phenotype' pairs, such as *P. afer* and *P. asper*, which arise from dichotomies have been considered to be r- and K- strategists, generalists/specialists, altricial/precocial, maintenance and dispersal phenotypes etc. Insight on the mechanisms behind these dichotomies could be gained through studying *P. afer* and *P. asper.*

Balon (1990) has suggested that less attention should be given to arbitrary variables like age and size at maturity or fecundity as single and decisive values of what he terms "the elusive life-history theory." For an understanding of life history and evolution comparative patterns and mechanisms of reproductive styles and early ontogeny would be more rewarding. In this study a combination of both the life-history attributes (which are generally based on field studies in the multivariate environment) and ontogenetic studies which unfortunately at this stage remain laboratory bound (Cambray, 1982) has been completed.

One of the main questions asked in the present study is, "Why are there two redfin minnow species, *P. afer* and *P. asper*, in the Gamtoos River system since there is no physical barrier separating the populations?" In the other systems where *P. afer* occurs there is no influence from a Karoo river and there is only one redfin species present, *P. afer*. If *P. afer* and *P. asper* are indeed sister species (Skelton, 1980a) then they must have the same ancestor. It would therefore be essential to consider the two environments in which these species occur today. *P. afer* inhabits a clear, second order mountain stream, while *P. asper* inhabits a turbid, saline, fifth order stream. It would also be important to understand the past environments as much as possible and this information is reviewed.

Some authors have suggested that organisms "... keep open two or more life-history options, and allow the co-evolving environment to determine which one will be most successful" (Bruton, 1989: 506). Balon (1990) argued that the life-history trajectories which are established during development may become fixed by 'genetic assimilation', and the adoption of phenotypic options may be regarded as the first draft of evolution. Evidence to support or refute these ideas was sought in the Gamtoos River system *Pseudobarbus* species.

In North American minnows studied by Gotelli & Pyron (1991: 30) "... both historical factors such as phylogenetic relationships, and ecological factors, such as latitude, probably contributed to interspecific variation in life-history traits of minnows." The African minnows, *P. afer* and *P. asper*, occur at almost the same latitude but the importance of historical factors such as changing climatic patterns followed by water quality changes were considered in this study.

Since the two *Pseudobarbus* species are closely related it was of interest to assess the phylogenetic constraints and the trade-off constraints on the demographic variables of *P. afer* and *P. asper*.

An important point in the present study was that *P. asper* is considered to be the more derived form of the sister species pair (Skelton, 1980a). *P. afer* and *P. asper* spread through the palaeo river systems during river piracy events to the present day Gamtoos River. With changing river conditions *P. afer* were left as relicts in the more stable mountain streams. *P. asper* evolved in the Groot River while it was still part of the palaeo-Groot-Kariega-Sundays system and when the Groot River section was captured by the Gamtoos River system so were the *P. asper* populations (Skelton, 1980a). Since *P. asper* is more derived, and more generalised, the question can be posed whether it became so by heterochronic shifts during development and in this case paedomorphosis? Thus the question is whether the sister species pair is an example of an altricial/precocial pair, an example of phenotypic variability of one species (ecophenotypes) or

whether they are really sister species which were once altricial/precocial ecophenotypes? For the present study the sound morphological base established by Skelton (1980a, 1988) is accepted as the null hypothesis which states that *P. afer* and *P. asper* are closely-related sister species and that *P. asper* is the more derived of the two species.

'The adoption of alternative phenotypic states in nature is probably more widespread than is currently realised and many populations which are currently recognised as species may, in fact, be no more than ecophenotypes of one or another homeorhetic state' (Bruton, 1989: 544). *P. afer* and *P. asper* species may be species (the null hypothesis) or ecophenotypes. Skelton (1980a, 1988) noted that there were four different forms of *P. afer* / *P. asper* which could be separated by lateral line scale counts. One of the options he faced was to put all the forms into one polytypic species (*P. afer*), however this was not a suitable taxonomic solution, and he elected for two separate species, with some previous *P. asper* (they were *B. asper*) populations incorporated into *P. afer*. The phenotypic variability of this sister species pair was therefore clearly evident from Skelton's study.

These two mechanisms of adaptation, phenotypic variability and genetic selection, are not incompatible, but their time courses are different (Wootton, 1990). Phenotypic adaptation is more rapid and can occur within a generation whereas genetic adaptation occurs between generations. Wootton (1984; 1990) regarded phenotypic adaptation as a 'tactical' response by individuals to their environment while genetic adaptation is a 'strategic' response observable at the level of the population (gene pool), and is driven by the adaptive differences between individuals. Balon (1990) stressed the need for a realistic model of life history based on comparative studies of ontogenies. A comparison of the life histories of these two closely related *Pseudobarbus* species living in different aquatic environments would be of value to understanding phenotypic variability and genetic selection of African minnows.

Only a few studies on African minnows have considered life-history theory in their discussions (eg. Cambray, 1982; Kleynhans, 1987). The two redfin minnows provided material for research into life-history evolution. The relative importance of different selection forces operating on the life-history phenotypic options employed in different sections of the same river system by two closely related redfin minnows were examined. It was hoped that this approach would provide some insight into the way the life histories of these two redfin minnows evolved.

An organism should be studied over its entire ontogeny, from single cell at activation until death

(Balon, 1985). Many studies on southern African fish have only considered the definitive phenotypes (juvenile and adult fish) and have neglected the embryos and larval fish. This is restrictive and these studies do not consider the mechanisms through which the diversity formed. In the present study the early life-history stages were studied in relation to spawning and rearing sites.

Skelton (1980a) suggested that our understanding of the taxonomy of *P. afer* and *P. asper* could be furthered by investigating their habitat preferences. Field studies geared to answering systematic questions, concentrating on breeding behaviour, feeding regimes and ecological interactions in relation to the annual cycles of the environment were undertaken in the present study.

Heterochronic shifts in the duration or timing of ontogenetic events enable variability within individuals (Balon, 1990). If these shifts are persistent over time then an alternative life-history style may develop. It has been argued that heterochronic shifts due to environmental influences are important in the formation of an ecophenotype (Balon, 1985).

Hypothesis

It is hypothesized here that the environmental influences of the southern Karoo rivers caused heterochronic shifts during the early ontogeny of the ancestor of *P. asper*. This gave rise to an alternative life-history trajectory unlike that of *P. afer* in the mountain streams. *P. asper* would be the more altricial form compared to the more precocial form *P. afer*. The life-history styles and phenotypic options of these two species differ in the two distinct sections of the Gamtoos River system. The two different habitats impose distinct selection pressures on the life histories of the two species. Brundin (1986: 606) has noted that "...in pairs of sister groups, we often are able to observe a dual trend towards conservatism and change. The two alternatives have always functioned as a double insurance of survival." This question was investigated for the sister group, *P. afer* and *P. asper*.

CHAPTER - 2

STUDY SITES IN THE GAMTOOS RIVER SYSTEM

Introduction

The nature of aquatic organisms inhabiting a river is determined by physical and chemical characteristics of the system as well as the evolutionary history of the taxa. These characteristics originate from the interplay between land form and climate within the basin. An understanding of this background is essential for the interpretation of the present distribution and life-history styles of *P. afer* and *P. asper*. Information on geology, tectonic history and climatic patterns of the area is required to interpret present day drainage patterns of the Gamtoos River system. The Gamtoos River drains areas with considerable differences in physical and chemical characteristics which together with historical changes have influenced present freshwater fish distribution.

Gamtoos River system

The Gamtoos River system, with a catchment area of 34450 km^2 and a virgin mean annual runoff of 501 x 10^6 m^3 a^{-1}, is one of the major rivers in the southern Cape of South Africa (Anon, 1986), although it only contributes 1,1 % of the total mean annual runoff in South Africa (Noble & Hemens, 1978). The Gamtoos River system extends well inland with tributaries draining the southern side of the Great Escarpment (Figures 4 & 5). The main tributary, the Groot River, traverses the Karoo before penetrating through a series of strike-valley sections, interspersed with shorter transverse portions in the Cape Fold Mountains. At the confluence of the Groot and the Kouga Rivers it becomes the Gamtoos River which meanders in a south-easterly direction and then passes through a 7 km estuary before reaching the Indian Ocean (Figure 4).

The river system has two "headwater" catchment areas - in the mountains of the Great Escarpment and lower down in the Cape Fold Mountains respectively. The Gamtoos River system rises at *ca.* 1700 masl and is approximately 600 km in length, with an average gradient of 2,95 m km^{-1} (Figure 5). These parts of the system are of ancient pre-Gondwanaland break-up origin (see below). The Gamtoos River has been grouped with the southern Karoo rivers because it is usually very turbid with medium to very high dissolved salt levels (Noble & Hemens, 1978).

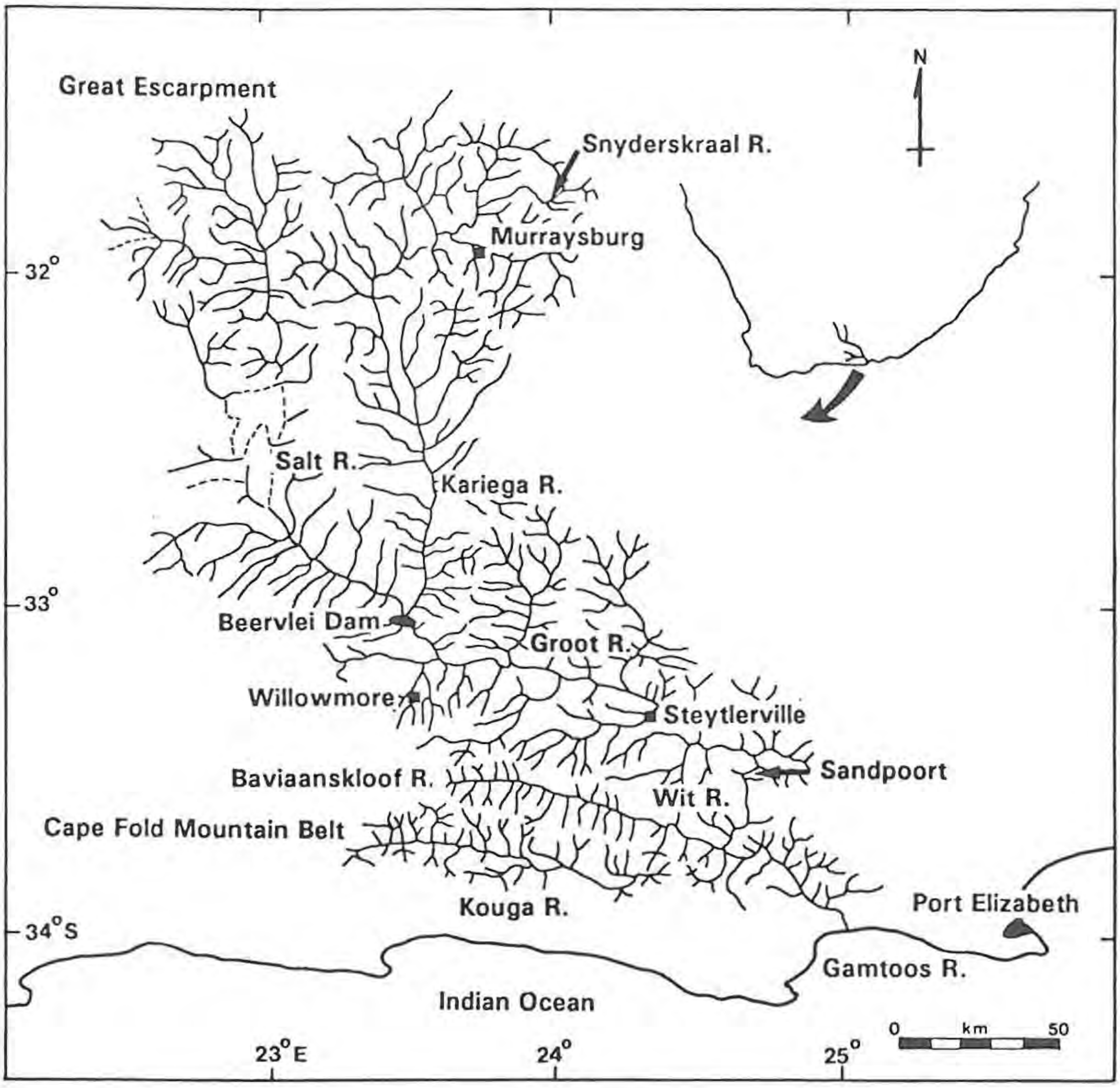


Figure 4. Map of the Gamtoos River system.

The Karoo part of the system is a sandbank river with intermittent flows due to low and sporadic rainfall, with the upper catchment experiencing extended periods of drought followed by extreme floods (Figures 6). The Groot River mainly flows through areas of livestock farms and occasional arable fields. Approximately 500 km from the source, the river cuts through the Cape Fold Mountains (Figure 5). Some of the inflowing Cape Fold Mountain Belt streams, such as the Wit River (Figure 7), are more typical of the "reservoir" type river with permanent flows.

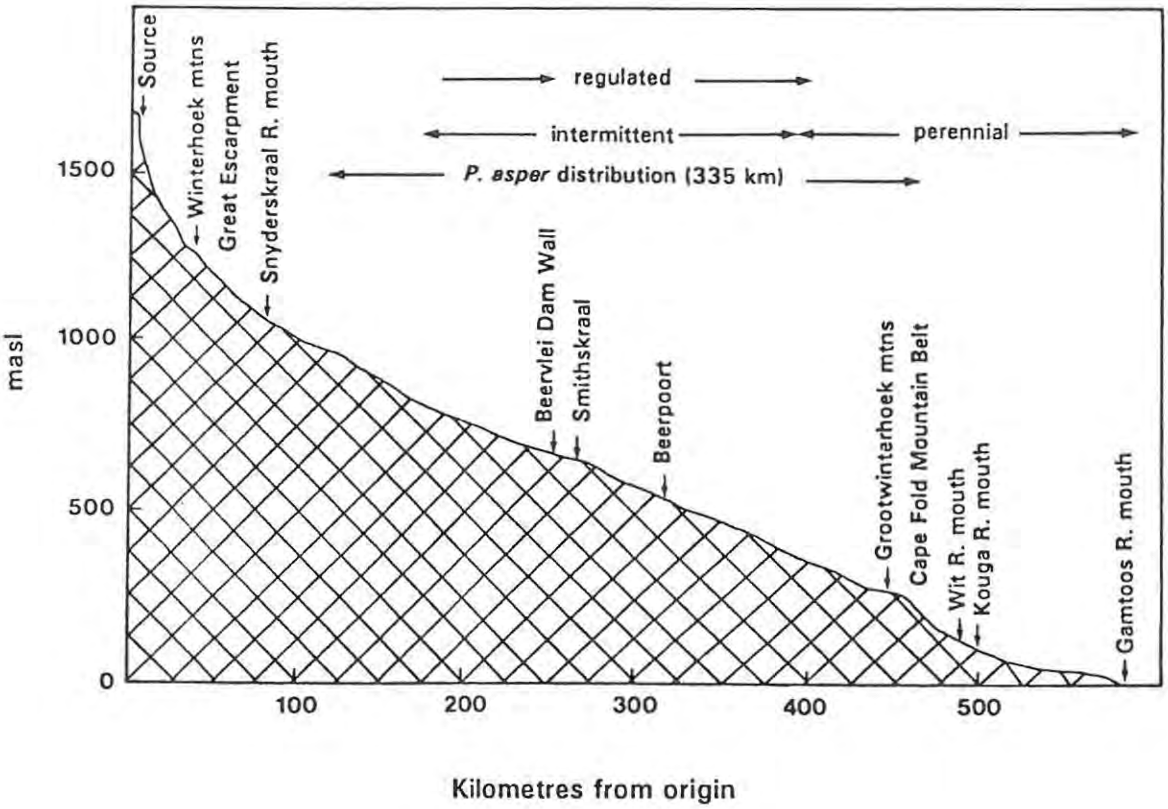


Figure 5. Vertical profile of the Gamtoos River system, taking Snyderskraal River as the source. Average gradient 2,95 m km^{-1}.

Geology

The two major tributaries of the Gamtoos River, the Sout and Kariega Rivers, flow from the Great Escarpment and penetrate through relatively soft geological formations. From the Willowmore area those rivers form the Groot River which meanders over soft Bokkeveld beds, then through fault-fractured quartzite, Bokkeveld shale and Enon conglomerate which all weather fairly easily. The mountainous areas consist of quartzites of the Ordovician-Silurian Table Mountain Group which is fairly resistant to erosion, except in areas where it is broken by joint planes or faults (Rust & Illenberger, 1989). In the lower estuarine reaches the river cuts through Uitenhage sediments bordered by cliffs of conglomerates (Haughton, 1969).

Sediment yields along the upper course of the Gamtoos River system range from 1000 t km^{-2} y^{-1} in the upper Kariega catchment (highest in South Africa [Rooseboom, 1978]), to 400 t km^{-2} y^{-1} along the Sout River to 150 t km^{-2} y^{-1} at the coast.

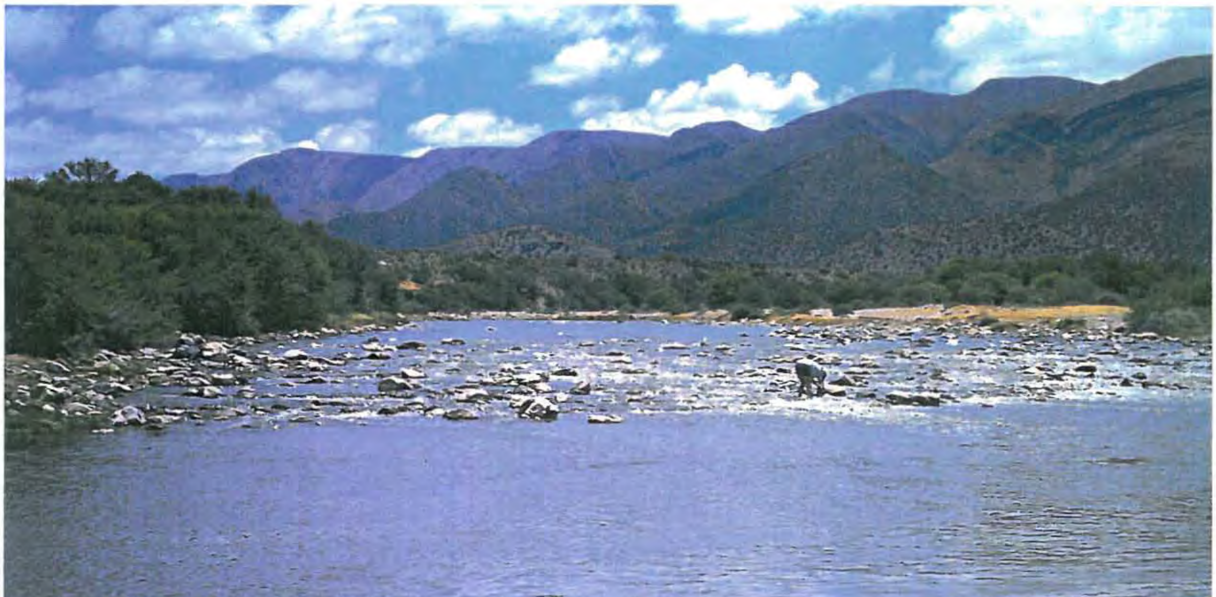


Figure 6. Groot River during a dry period (above) and in spate (below).

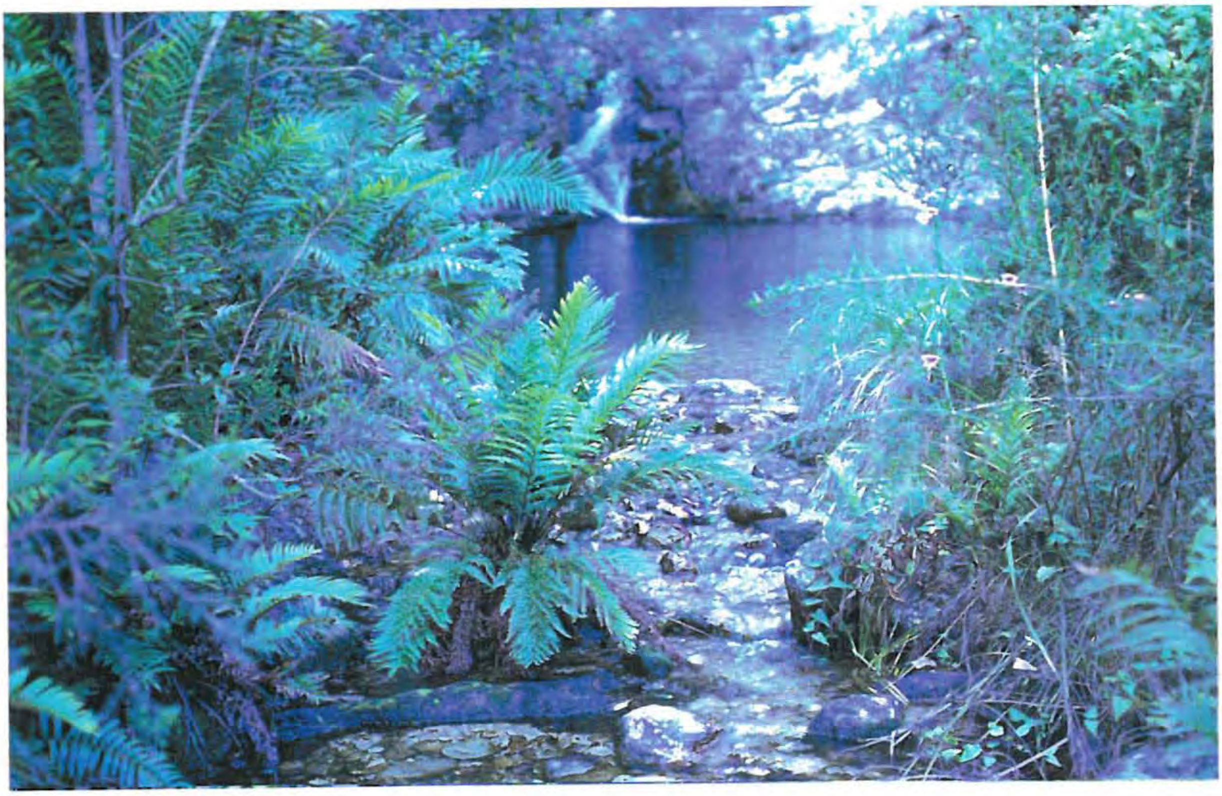


Figure 7. Typical pool and vegetation along the Wit River.

Geomorphology of the Gamtoos River system
(Palaeogeology and Palaeogeography)

Two important features to the Gamtoos River system are the Great Escarpment and the Cape Fold Mountains (Figure 5) which indicate the presence of resistant geological elements (thick quartzites, sandstones and dolerite sills) and a history of successive local rejuvenations along the larger rivers (Partridge & Maud, 1987). The Great Escarpment separates the elevated interior from the coastal margins and was formed following continental rifting. That it is still present as a major topographic feature is due to the high elevations and central position of southern Africa in Gondwanaland prior to rifting (Dingle *et al.*, 1983; Partridge & Maud, 1987). The Great Escarpment, the single most important geomorphic feature of the subcontinent, "... neatly defines much of the limnology of the subcontinent" (Allanson *et al.*, 1990: 9). There is evidence that a major part of the erosion after the fragmentation of Gondwanaland occurred in the period following rifting and that by the mid-Cretaceous the Great Escarpment had receded some 100 km from the coast and by the end of the Cretaceous 120 km, close to its present position

(Matthews, 1978; Partridge & Maud, 1987). This continuous scarp retreat since the break-up of Gondwana would mean that the Orange River and its associated fauna would have extended further coastwards in the east, and southwards to the fold belt (Wellington, 1958; Partridge & Maud, 1987, their Figure 16).

Palaeo-drainage features confirm the continuity of surfaces, such as defunct channel systems, dismembered drainage systems, pans and the gravel lags of old channels (Partridge & Maud, 1987). The drainage pattern established on the African surface exerted an important influence on later geomorphic evolution of the subcontinent (Partridge & Maud, 1987).

During the evolution of the Gamtoos River system there have been expansions and exchanges with adjacent rivers which are important in understanding the present distribution of freshwater fish. During the early Cretaceous the present headwaters of the Gamtoos River system flowed into the Orange River and entered the Atlantic Ocean through the Olifants River system (Dingle & Hendey, 1984; Partridge & Maud, 1987)(Figure 8). By the mid-Tertiary the Groot River and its tributaries were part of the Gamtoos River system (Partridge & Maud, 1987). The present distribution of the *Pseudobarbus* includes members in both the Olifants (*P. phlegethon*), the Orange (*P. quathlambae*) and the Gamtoos River systems (*P. afer* and *P. asper*) which confirms that these rivers have had a history of expansions, contractions and river piracies.

The ancestral equivalents of the Gamtoos River complex were the palaeo-Groot-Kariega-lower Sundays in the north, to the south was the palaeo-Baviaanskloof-Gamtoos and parallel to these two flowed the palaeo-Kouga-Krom (Figure 9; Rust & Illenberger, 1989).

Five of the seven redfin minnows, not including *P. asper* and *P. quathlambae*, only occur in rivers draining the Cape Fold Mountain ranges which can be dated to either an early Permian orogeny (Halbich *et al.*, 1983) or the early and middle Triassic (Partridge & Maud, 1987). At the break-up of Gondwanaland, some 140×10^6 years ago, the Cape Fold Mountain belt already existed and its east-west trend was the result of a much older episode of crustal folding and thrust faulting (Rust & Illenberger, 1989). The initial drainage lines in this area would have been mainly east-west within the strike valleys (Du Toit, 1926; Barnard, 1943; Maske, 1957)(Figure 9). The palaeo-Baviaanskloof valley was formed by a tensional fault during the break-up of Gondwanaland into which Enon gravels were deposited at least 140 million years ago (Rust & Illenberger, 1989).

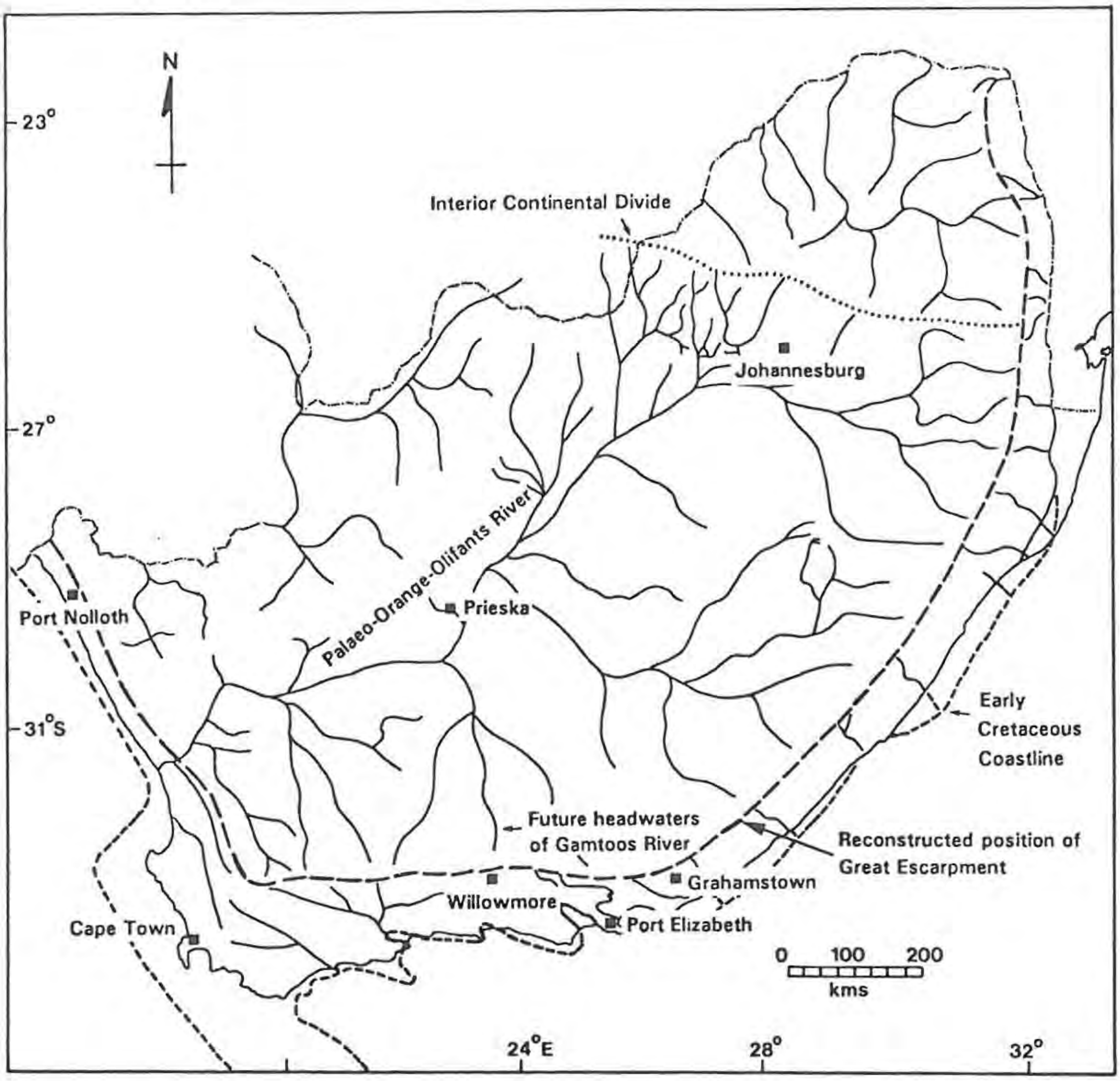


Figure 8. Reconstruction of the principal elements of early Cretaceous drainage (after Partridge & Maud, 1987).

River piracy is common in the Cape Fold Mountain belt (Rust & Illenberger, 1989). One of the best examples occurs in the Gamtoos River system where the Kouga River flows through a strike valley and has two major 90° bends which is usually a good indication of river piracy (Figure 9; Rust & Illenberger, 1989). The development of joints in the Table Mountain quartzite provided areas for headward erosion and when the joints were in a north-south direction watersheds could be breached through the weakened quartzite, as had happened with the Kouga River (Figure 9). These tributaries have steep gradients with a high capacity to erode. Should the neighbouring drainage basin have been breached there would have been a substantial increase in the discharge. In this way large "poorts" (ravines) are excavated through the mountain chains by headward erosion of small tributaries (Rust & Illenberger, 1989). The area is characterised by deep, narrow V-shaped incised valleys such as the Wit River (collecting sites for the present study).

A southward flowing tributary of the palaeo-Baviaanskloof-Gamtoos pirated the upper reaches of the palaeo-Kouga-Krom about 60-100 X 10^6 y BP (Illenberger pers. comm.). Another tributary of the palaeo-Baviaanskloof-Gamtoos penetrated northwards through the main range of the Baviaanskloof-Elandsberge mountains and captured the upper catchment of the easterly flowing palaeo-Groot-Kariega-lower Sundays (Figure 9; Haughton *et al.*, 1937; Rust & Illenberger, 1989). Dating this piracy to roughly 60 x 10^6 y BP (Illenberger, pers. comm.) is important as Skelton (1980b) suggested that *P. asper* were already in the Groot River when it was captured (Chapter 3).

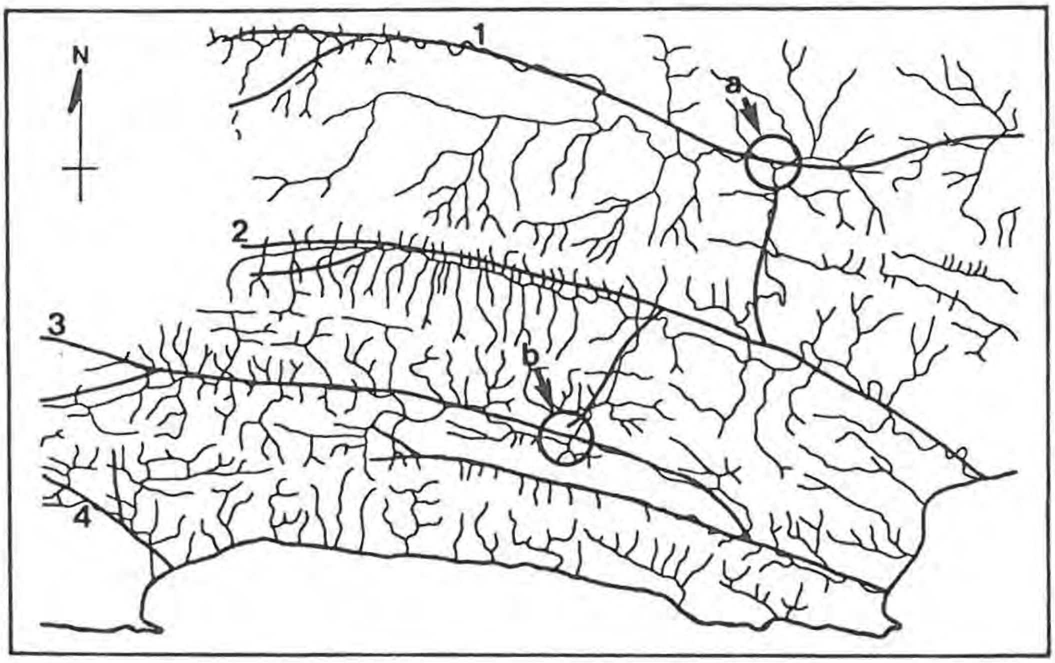


Figure 9. Present drainage (thin lines) compared to palaeo-drainage systems (thick lines) shortly after the break-up of Gondwanaland, with three main systems flowing eastwards down the major structural valleys. Areas of impending river piracy by headward erosion of tributaries along cross-joints are circled: (a) - Groot River captured; (b) - Kouga River captured. The palaeo-systems are: (1) - palaeo-Groot-Kariega-lower Sundays; (2) - palaeo-Baviaanskloof-Gamtoos; (3) - palaeo-Kouga-Diep-Krom; (4) - palaeo-Keurbooms (modified from Rust & Illenberger, 1989).

Sea level transgressions and regressions

The coastal platform of the southern Cape belongs to the Post-African I cycle which was initiated by the Miocene uplift (Partridge & Maud, 1987). The Terminal Miocene resulted in a world-wide sea-level drop of over 100m (Brain, 1985). There was a major glacio-eustatic drop in sea level during the Pliocene (De Swart & Bennett, 1974; Corbett, 1979). Climatic deterioration during the late Pliocene increased the global ice volume and caused eustatic reductions in sea level, followed by warmer periods where the sea level was higher than at present. Regressions of sea levels extended river courses, increased their gradients and may also have provided an opportunity for inter-connections of systems and exchanges of fauna. The present distribution of *P. afer* in small coastal streams probably indicates fish movement between systems during regressions of the sea levels (Skelton, 1980b).

Vegetation types

Most of the Gamtoos River system falls within the eastern Cape which forms a major climatic, topographical, geological and pedological transition zone marked by environmental heterogeneity over short distances. This region is regarded as a tension zone and one of floral transition with a diverse mosaic of vegetation types (Lubke & van Wijk, 1988). The flora is estimated at 3 600 - 4 000 species and lies within 21 of the 70 Acocks's (1975) veld types (Gibbs Russell & Robinson, 1981).

The vegetation map of Lubke *et al.* (1986) differs from Acocks (1975) and uses a biome type hierarchy. The Groot River passes through Karoo or Subdesert vegetation characterized by dwarf subdesert shrublands and succulent Karroid vegetation types. When the Groot River enters the Cape Fold Mountain Belt it passes through Valley Bushveld. Afromontane elements of forest vegetation survive in some well watered ravines, such as the Wit River.

The Wit River has a long, narrow stand of forest along the river. The area had the second highest number of species in the eight forest sites in the Baviaanskloof/Kouga complex and the second highest number of species in the 23 forest sites in the inland mountains of the southern Cape studied by Geldenhuys (1989). The vegetation is typical of the Forest Biome (Rutherford & Westfall, 1986). The patterns in species richness and composition in the forests were found to be related to the geomorphological history of the Gamtoos and Gouritz river systems. These rivers connect the coastal forests with the inland mountains (Geldenhuys, 1989). Vegetation along the Wit River is characteristic of small riverine areas where there are micro-climates created in the ravines (Vlok, 1989). It is clearly evident at the Wit River site that the mountain range allows this forest to persist in an area with a totally different climate. The dry slopes surrounding the Wit River make it reasonable to assume that here it is mainly the rainfall pattern which limits the forest.

Possible dispersal corridors for forest taxa from the coast to the inland mountains were the Zunga River, the Gamtoos River through the Groot River Poort and Baviaanskloof, the Keurbooms River and the Gouritz Poort. The Zunga and the Gamtoos Rivers were effective corridors for stepping stone dispersal. There are many species of forest taxa shared between Groendal, Groot River Poort, Wit River and the Baviaanskloof (Geldenhuys, 1989). It should be noted that these areas all have isolated populations of *P. afer* (see Chapter 3 Figure 18).

It is interesting to make a comparison with the plant species. Aridity and drought stress in areas such as the eastern Cape midlands and lowlands (Zucchini & Adamson, 1984) were said to have pressurized taxa to evolve a low stature, shorter life cycle, drier and smaller propagules, spinescence, deciduousness and storage organs (Geldenhuys, 1989). *P. asper* also has some of these life-history attributes such as shorter life span and smaller eggs to cope with the "drier" area in which it evolved.

The wide Sundays River valley (where a population of *P. afer* occurs) stretches from the coast to the Great Escarpment and has separated the southern Cape forests from forests to the east since at least the Late Pliocene (ca 2,5 X 10^6 y BP) (Geldenhuys, 1989). Populations of *P. afer* were probably also fragmented at that time and some populations became extinct as forests contracted. The separation of the southern Cape forests from those to the east since the Pliocene implies long isolation and stability of the forest species. This stability, possibly indicating developmental constraints, may also be important in the interpretation of the present life-history style of *P. afer*.

There has been a general increasing aridity in the southern Cape region since the Miocene (Deacon, 1983) which suggested to Geldenhuys (1989) that over time the inland forest patches became increasingly isolated. Geldenhuys (1989) proposed that the widespread inland species of moist sites represent relicts of the temperate austral forests or high-altitude forests of tropical latitudes which covered the southern tip of Africa during the Palaeocene. Also included at inland sites such as the Wit River are elements of the subtropical forest which replaced the temperate forest after the Oligocene-Miocene (Axelrod & Raven, 1978; Deacon, 1983). Areas such as the Wit River valley have provided suitable refuge areas for certain forest species as well as *P. afer*.

During the Holocene (10 000 y to present) karroid vegetation replaced the retreating forest during the warmer and drier periods (Geldenhuys, 1989). Similarly the isolation and fragmentation of *P. afer* populations possibly occurred, and the Groot River became drier, more turbid and saline and *P. asper* evolved.

Water chemistry

The rivers of the Cape Fold montane limnological region (Allanson *et al.*, 1990) can be divided into two groups (Harrison & Agnew, 1962). Those which are strongly acidic (pH 5,0-5,9), unbuffered, low in TDS and peat stained and those, like the Wit River, with a pH of 6,0-6,9 and clear water. This division of the rivers is further supported by their invertebrate faunas. The characteristics of the Wit River are dominated by the geology of the Cape Fold Mountains which consist of poorly buffered Table Mountain Sandstone. The water is typically clear with a low conductivity, flowing through fynbos vegetation in the upper catchment. The common soil of the Fynbos biome is the undifferentiated rock and lithosol group. The silicaceous soils generally have a low nutrient status (Rutherford & Westfall, 1986). These typically oligotrophic rivers have a unique fauna with many endemics (Allanson *et al.*, 1990). Water chemistry details clearly indicate the differences between the Karoo section and the Cape Fold Mountain section of the Gamtoos River system (Table 1). Allanson *et al.* (1990) noted that rivers such as the Wit River contrast strongly with the generally alkaline, turbid, sediment-laden rivers of the rest of southern Africa, and these latter characteristics describe the Groot River. It was these differences that influenced the choice of this research project. The physical and chemical parameters of the Wit and Groot Rivers differed dramatically during the survey period (Table 1). The pH readings for the Wit River were slightly acidic to neutral whereas the pH of the Groot River was as high as 8,5 (Table 1). The conductivity in the Groot River reached 92 times the highest recorded for the Wit River (Table 1). The Karoo section had a consistently higher and more variable conductivity which is in sharp contrast to the Wit River (Figure 10). The Gamtoos River system has no major inputs of either industrial effluents or raw sewage. Agricultural runoff from the arable fields along the Groot River may carry inorganic fertilisers and pesticides but this aspect has not been investigated. In comparison the Wit River is located mostly in a Wilderness area.

Table 1. Water analysis of the Wit and Groot Rivers giving the extreme ranges of the monthly readings during the period 22 February 1987 to 23 April 1989.

	Wit River	Groot River	Difference between highest reading, taking Wit River as one
pH	6,6 - 7,2	8,0 - 8,5	-
Electrical conductivity (mSm^{-1})	7,7 - 10,94	118,0 - 1006,0	92
Total dissolved solids (mgl^{-1})	44,0 - 159,0	763,0 - 5063,0	32
Turbidity (ntu)	0,2 -3,0	1,7 - 36,0	12
Secchi disk (cm)	500 plus*	8 - 129	-
Anions			
Chloride (mgl^{-1})	12,408 - 23,47	255,9 - 3172,8	135
Alkalinity as $CaCO_3$ (mgl^{-1})	2,5 - 30,0	10,0 - 245,0	8
Cations			
Calcium (mgl^{-1})	0,5 - 2,0	29,0 - 235,0	118
Magnesium (mgl^{-1})	1,4 - 2,9	31,0 - 313,0	108
Sodium (mgl^{-1})	10,0 - 16,0	50,0 - 1500,0	94
Potassium (mgl^{-1})	0,4 - 0,9	0,5 - 27,8	31

* Secchi disk was always visible to bottom of deepest pool.

Photoperiod, water temperature and rainfall

The mean number of daylight hours per month is shown in Figure 11a. The longest days occur in the months of December and January and the shortest in June and July. The highest and lowest water temperatures follow the photoperiod oscillations Figures 11b & d.

South Africa's climate is influenced by the movement of the southern hemispheric sub-tropical high pressure belt, the circumpolar belt of westerlies, the warm Agulhas ocean current to the east and the cold Benguela current to the west. Altitude, mountain orientation and distance from the Indian Ocean are some of the physical configurations and variables of the country which act on the prevailing atmospheric conditions in the eastern Cape (Stone, 1988). In southern Africa there are temporal as well as spatial variations in the rainfall patterns (Tyson *et al.*, 1975). Rainfall in South Africa exhibits a pseudo-regular pattern with time and has distinct regional patterns (Abbott & Dyer, 1976). The upper Gamtoos (Groot River) falls into a region with an oscillation of 10 years. In general the upper Groot River is in an autumn rainfall zone and the Cape Fold Mountain area is an all year round rainfall zone. Dyer (1976) showed that the coherence between rainfall and sunspots in the area of the upper Gamtoos River is 0,85 at a frequency of 0,1 cycles per year (period of ten years). In this way he was able to correctly estimate future periods of wet and dry years including the serious drought for the 1980s which has caused severe hardship to many people in this region. The present study was conducted when southern Africa was experiencing the crippling drought predicted by Dyer.

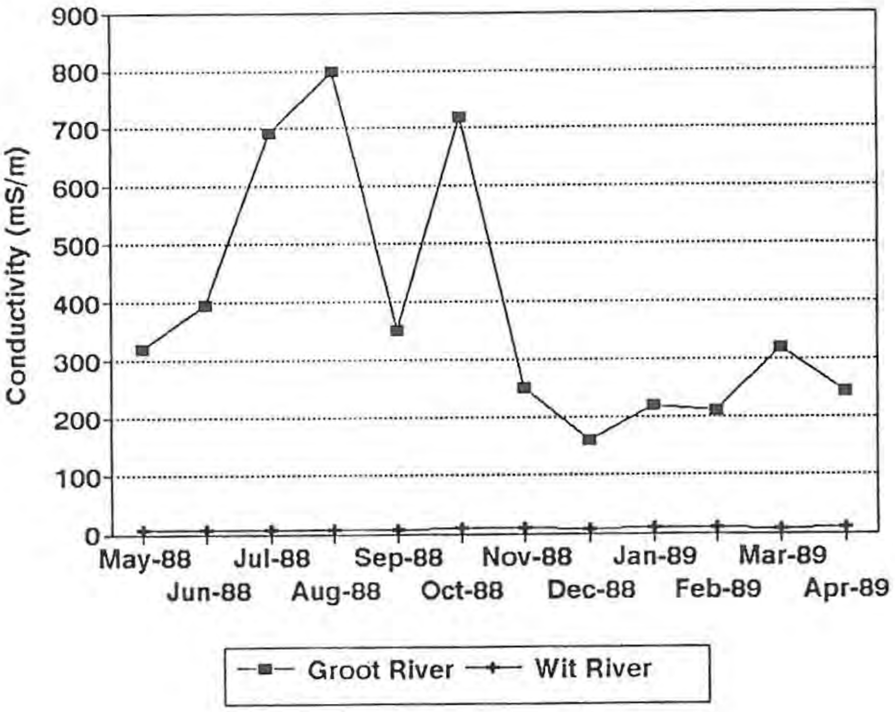


Figure 10. Fluctuations in the conductivity readings for the Groot River and the Wit River between May 1988 and April 1989.

According to the modified Koppen System, there are seven climatic belts in the eastern Cape (Schulze, 1947). From the coast to approximately 50 km inland the Gamtoos River is in the subtropical belt with all months between 10-22,2 °C and having at least 60 mm of rain. From near the junction of the Groot River with the Kouga River to near Beervlei Dam the area is classified as having a steppe climate with less than 8 months over 10 °C and with hottest months below 22,2 °C. Beyond this area is the desert belt with 8 months of over 10 °C and with maximal rainfall in autumn.

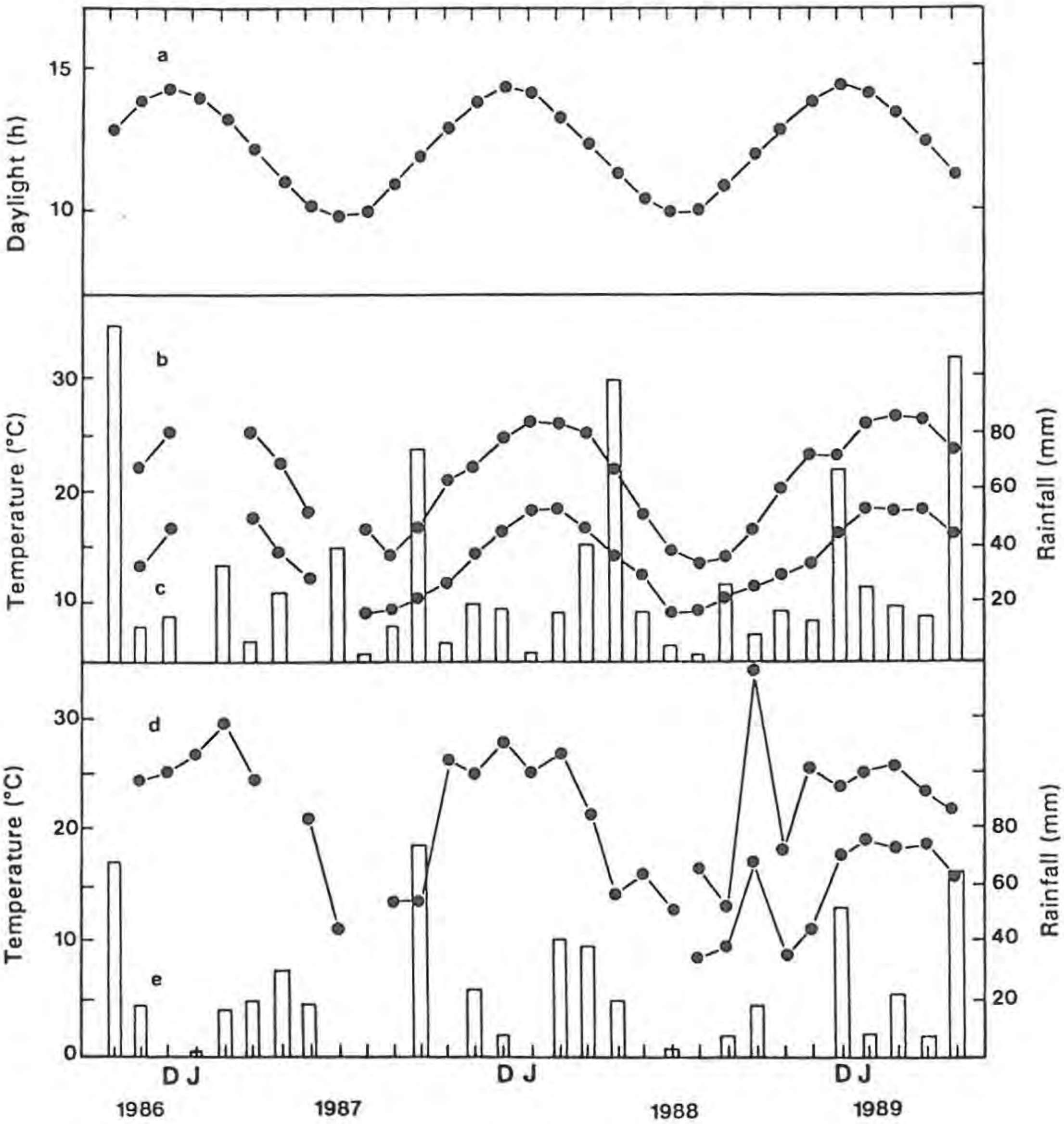


Figure 11. (a) Mean number of daylight hours; (b) maximum and minimum water temperatures, Wit River; (c) monthly rainfall Wit River; (d) maximum and minimum water temperatures, Groot River; (e) monthly rainfall, Groot River at Fullarton.

Marker (1988) recorded that the mean annual rainfall in the catchment area of the Gamtoos

River ranges from 500-1500 mm whereas on the rainfall map in Pitman *et al.* (1981) the mean annual precipitation ranges from 200 to 500 mm. The precipitation in the upper Kariega catchment is over 300 mm y^{-1}. Thereafter the river flows into a drier, flatter area with a mean annual rainfall of between 200-300 mm which rises to 400-500 mm in the Cape Fold Mountain Belt. Köpke's (1988) analysis of long periods of data showed that the Gamtoos River from the mouth to Steytlerville is in a spring rainfall area and that the catchment above this is in an autumn rainfall zone. The eastern Cape as a whole is in a transitional zone of climatic types and the seasonality of rainfall is less pronounced than in other parts of the country (Stone, 1988).

The nearest rainfall recording station for the Wit River was 10 km downstream on the farm 'The Havens' owned by Mr O. Edmeades, located within the Wit River catchment but in the more exposed area and not within the micro-climate of the Wit River or on the same slope. Over a 23 year period (1965-1988) the average yearly precipitation was 433 mm with a range of 175 (1984) to 858 mm (1981). The annual rainfall to support an Afromontane Forest (such as exists along the Wit River) is between 600-800 mm y^{-1} on low fertility sandstone soils (Vlok, 1989). The only true limit to potential establishment and growth of forest in the southern Cape is an annual rainfall below 500 mm (Geldenhuys, 1989). The upper Wit River catchment occurs in the Fynbos Biome where rainfall varies between 210 to greater than 3000 mm y^{-1} (Rutherford & Westfall, 1986). Although rain can occur throughout the year the months of March (51 mm) and October (46 mm) had the highest rainfall for the 23 year period covered by the Edmeades data. The annual rainfall pattern for the survey period in the Wit River catchment was below average, 1986 - 303 mm, 1987 - 247 mm and 1988 - 325 mm. Peak rainfall months were October 1986 (120 mm), September 1987 (76 mm), April 1988 (101 mm), December 1988 (68 mm) and April 1989 (113 mm) (Figure 11). The percentage deviation from the mean annual rainfall can be as high as 30 - 40 % in the catchment area.

The mean annual evaporation rates along the Gamtoos River system ranged between 1400 mm at the coast to 2300 mm on the edge of the Great Escarpment (Pitman *et al.*, 1981). The mean annual evaporation rates in the upper catchment of the Kariega and the Sout Rivers are 2000-2100 mm and 2100-2200 mm respectively. The river then passes through areas with high evaporative rates up to 2300 mm y^{-1}. Thereafter a trend to lower rates follows, with 1500-1600 mm in the Cape Fold Mountain Belt and between 1400-1500 mm y^{-1} in the estuarine area. The low rainfall coupled with high evaporative rates result in a low runoff except during thunderstorms.

The catchment of the Groot River covers a large area of Karoo. Rainfall records were taken from data collected at Beervlei Dam (33° 04' 30" S and 23° 29' 30" E) which is 704 m asl and c. 60 km above one of the sampling sites (Figure 5). For a 21 year period (1959-1980) the average yearly precipitation was 195 mm with a range of 68 mm (1969/1970) to 351 mm (1973/1974). This is well below the 500 mm average annual rainfall for South Africa (Allanson *et al.*, 1990). As with the Wit River rain can occur throughout the year with the peak months being February (31 mm), March (33 mm) and April (23 mm) for the 21 year period. In the present study peak rainfall months were October 1986 (72,6 mm), September 1988 (76,8 mm) and April 1989 (65,2) (Figure 11e). The annual rainfall for the survey period was above average during 1987 (202,7 mm) and below average in 1988 (182,0 mm).

The major characteristic of southern African hydrology is variability (Alexander, 1985; Braune, 1985) for which the Groot River is a good example (Figure 12). The Groot River is a dynamic system which can fluctuate from extended periods of no flow to short periods of flash-flooding with resultant irregular perturbations in the riverine habitats (Cambray, 1990b). Open exposed land, such as occurs along the Groot River catchment, results in high evaporative rates (Anon, 1986) which exacerbate the runoff variability. In South Africa when rainfall is converted to runoff a figure of 8,6% is realised compared to 65,7% for Canada and 9,8% for Australia for similar yearly annual rainfalls (Braune, 1985).

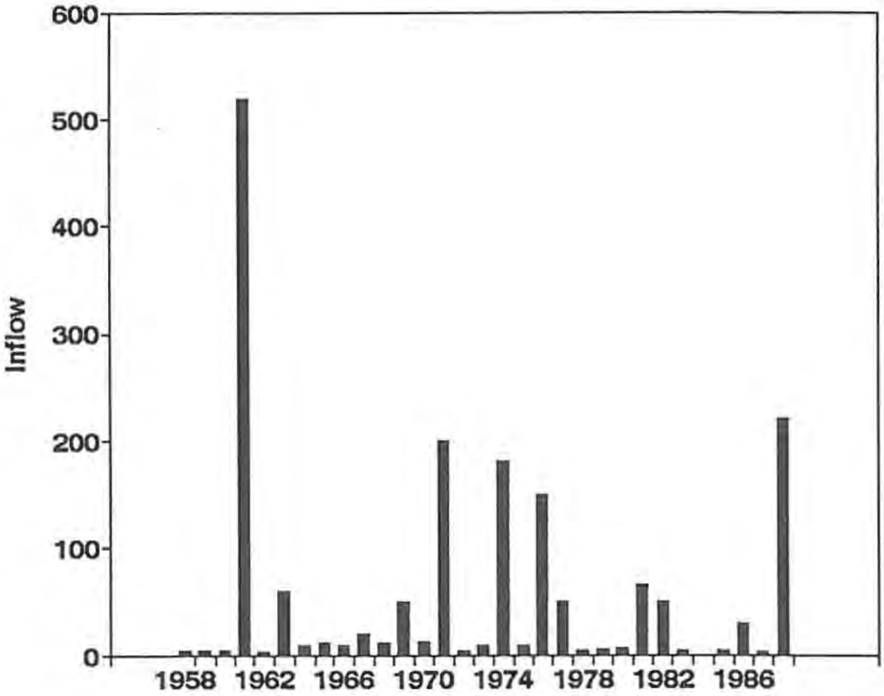


Figure 12. The yearly inflow (10^6 m^3) into Beervlei Dam over the period 1958 - 1989 (from Cambray, 1991a).

Palaeoclimate

It is important to have an understanding of the palaeoclimates when trying to explain present day faunal distribution patterns. During the past 100 million years southern Africa experienced warmer, damper climates than at present (Rust & Illenberger, 1989). As the continent drifted in a northerly direction after the breakup of Gondwanaland climatic conditions changed as various ocean currents formed along the coasts. There is thought to have been considerable climatic fluctuation during the late Tertiary and the Quaternary (Livingstone, 1975; van Zinderen-Bakker, 1978). The increasing aridity since the Cretaceous-Palaeocene has contributed to the narrowing and fragmentation of the forest belt. These climatic-ecological perturbations fragmented the ranges of species into isolated forest refugia.

In the western and south-western Cape pollen core analysis by Coetzee (1978) has shown that there was a tropical climate in the early Tertiary (Palaeocene and early Eocene), which was followed by a lowering of temperature to sub-tropical and temperate climes during the Eocene (60-40 X 10^6 y BP). The temperature oscillated during the Oligocene (40-25 X 10^6 y BP) from warm, humid climates of the early Miocene, followed by cooling in the mid-Miocene and a return to warmer climes during the late Miocene. The Pliocene was colder and drier and the Quaternary climates fluctuated between cooler-wetter climates to warmer-drier climates than at present.

There has been a progressive decline in temperature from the Palaeocene (60 X 10^6 y BP) to the end of the Miocene (ca 6 x 10^6 y BP) after which there has been a series of oscillations. The global cooling during the Miocene was mediated in part by the development of the circum-Antarctic current which started to flow once the northward drift of Australia relative to Antarctica opened a seaway around Antarctica which resulted in the Terminal Miocene Event. There was a temperature plunge between 6,5 - 5 million years ago, rapid accumulation of the Antarctic ice sheet and a dramatic sea-level drop of over 100 m worldwide (Brain, 1985). During the Pliocene 5-4 million years ago the temperatures rose and then plunged again 2,6-2,5 million years ago when the major ice cap developed in the northern hemisphere. Since then the earth's temperature has oscillated between glacial and interglacial conditions on at least 17 occasions. During the Pleistocene there has been an intensification of the shift towards increasing aridity that began in Tertiary times (Partridge & Maud, 1987).

Global temperature changes over the past 50 million years have broken the African land-mass into a series of discontinuous habitats between which the biological thresholds for certain species have been exceeded. The alternating cycles of higher and lower temperature in southern Africa have caused expansions and contractions of temperature zones and the biota within these zones (Brain, 1985). This is the same pattern noted for the southern Cape forests (Geldenhuys, 1989).

There is evidence for periglacial conditions during the Pleistocene in the higher mountain areas of the Eastern Escarpment including those in the Drakensberg which suggest that there were temperatures 9 °C lower than at present (Harper, 1969). Butzer & Helgren (1972) discussed the late Cenozoic evolution of the Cape Coast between Knysna and Cape St Francis, and concluded that there were repeated and drastic changes in the vegetation and the climate in what is today the most mesic environment in southern Africa. Brain (1981) suggested that African habitats have been repeatedly affected by low-temperature episodes during the last few million years and that minimum winter temperatures were depressed by 5 - 10 °C on each occasion. He then showed the greatly increased area of southern Africa which would have a mean minimum temperature during the coldest month of below 0 °C (and this area includes most of the area at present occupied by *Pseudobarbus* species). Brain (*op cit.*) argued that these periods would profoundly have influenced the distributions of frost-sensitive plants and animals.

Southern Africa is now in a warm interglacial period (Brain, 1981).

River classification

Although rivers are receiving renewed attention (J.M. King & J.H. O'Keeffe pers. comm.) Harrison's (1959) attempt at river classification in South Africa remains the most detailed to date. The hydrobiological regions defined within South Africa were based on the existing knowledge of geology, climate, physiography and hydrobiology. Allanson *et al.* (1990) reviewed river classification systems for southern African rivers and have suggested that more work is required. The Gamtoos River occurs in hydrobiological Regions A (The Cape System Region) grouped together with rivers such as the Keurbooms, lower Gouritz, Breede, Palmiet, Great Berg and part of the Olifants. The upper catchment area of the Gamtoos lies in Region C (The Central Arid Region) which is grouped together with the upper catchment of the Gouritz, part of the Olifants and the Orange River (Harrison, 1959). The Cape System Region was split into four main types because of geological variation. The lower Gamtoos River receives tributaries, such as the Wit River, which flow over Table Mountain Sandstone, as noted above, and have water low in total dissolved solids (TDS), unbuffered, acidic and of the colourless type. These types of rivers often contain many specialized endemics. A large part of the upper catchment of the Gamtoos River rises in the Central Arid Region C. Rivers in this region are temporary, alkaline and often saline. The lower main channel sections of the Gamtoos River is influenced by the occasional flood waters received from Region C rivers which are high in TDS.

A less detailed river classification scheme was suggested by Noble & Hemens (1978), using similar criteria such as geology, water chemistry and biota. The Gouritz, Gamtoos, Sundays and Great Fish Rivers were grouped together as Southern Karoo rivers whilst the contribution of Harrison's (1959) Region A rivers for the Gouritz and Gamtoos Rivers is ignored. Both these rivers are transitional in their lower reaches and influenced by Table Mountain Sandstone and by Karoo sediments.

Stream Order

The branching pattern of the river channels in drainage basins permits the classification of streams by the stream order method (Horton, 1945; Strahler, 1957). Streams are categorized according to the order of the streams in a hierarchy eg. first order streams have no tributaries; second order are formed by the junction of the first order streams etc. In this study the modified method of Strahler (1957) is used. There can be sudden changes in faunal abundance below the confluence of streams due to differences in flow, sediment load and other hydrological factors. These differences lead to a shift in ecological factors which in turn favours one species group over another (Welcomme, 1983).

The Wit River is a second order stream whereas the Groot River in the vicinity of the sampling sites is a fifth order stream (data from 1: 250 000 maps).

Hydrology

Rate of flow is one of the most important factors determining the distribution of aquatic organisms in a river system. The flow rate in turn influences a number of physical and chemical factors which act directly on fish. Flow regime of a stream depends on the area of the drainage basin of the stream in question. The area of the drainage basins of the Groot and Wit vary considerably. However, both can have series of spates during the rainfall months. Both the

Groot and the Wit Rivers have seasonal variations in flow produced by the annual rainfall pattern as well as inter-annual flow variations which are more marked in the larger Groot River.

In rivers which have a succession of high and low waters there are changes in types of habitats available to fish. There may be no distinction between pool and riffle during flood. In the Groot River as the water falls the riffles dry out and the main channel breaks down into a series of isolated pools. During these times the spawning beds of *P. asper* are dessicated.

Vertical profiles

The vertical profiles of the Wit and Groot River (Gamtoos River system) are compared in Figures 5 & 13. Overall the vertical profiles of both rivers are concave consisting of a steep upper portion ("rhithron"; Illies, 1961) near the source followed by reaches of progressively less gradient closer to the mouth ("potamon"; Illies, 1961).

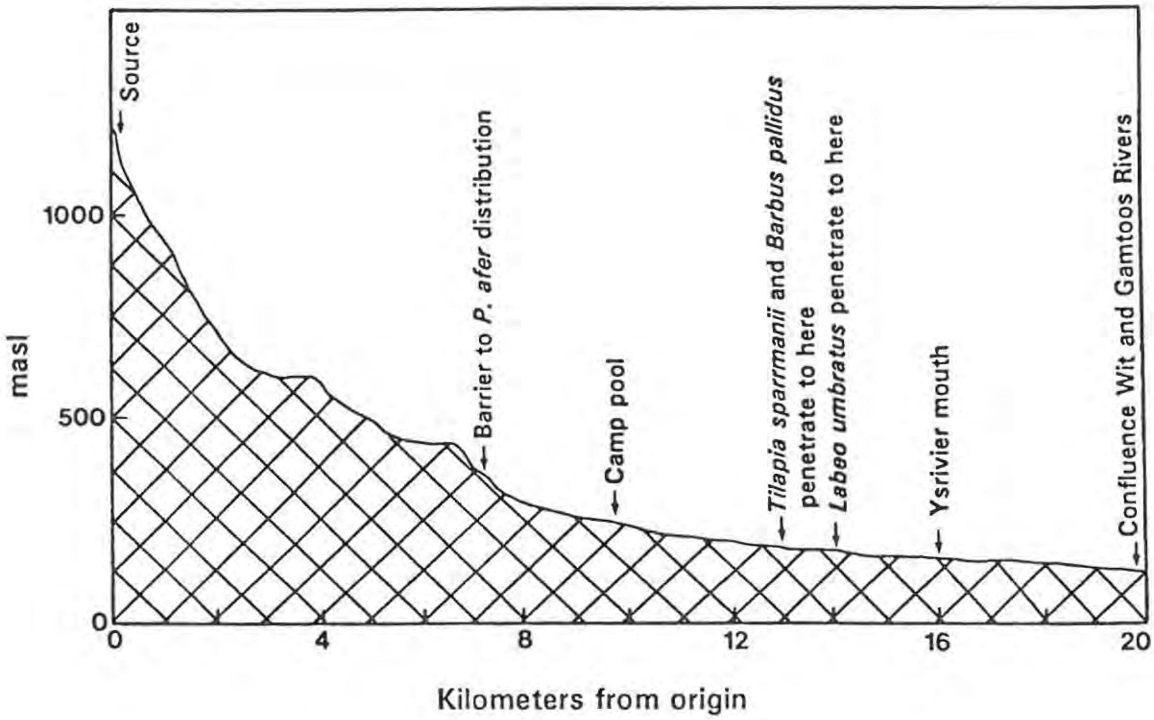


Figure 13. Vertical profile of the Wit River, a mountain tributary of the Groot River system with an average gradient of 60,95m km^{-1}.

The upper section of the Wit River is a typical rhithron-like zone. There are alterations between steep, narrow, shallow riffles and flatter, wider, deeper reaches (pools). During the rainy season the riffles have high, turbulent flows over coarse bottoms of boulders and rocks with very little attached vegetation. The pools have deeper, flatter bottoms with finer material and some rooted vegetation. This is the habitat of *P. afer*. As a result of the severity of the habitat diversity there are few resident fish species.

In contrast *P. asper* inhabits the potamon reaches of the Groot River. These consist of wide, flat meandering channels, mud bottoms and, in the summer months, considerable rooted vegetation.

Cross sections of the study sites

The cross sections of the two sites show the open, wide valley of the Groot River with exposed river channel in sharp contrast to the narrow, precipitous Wit River which is shaded by the steepness of the valley as well as by a riparian forest (Figure 14a).

STUDY SITES

Wit River, Gamtoos River system

The Wit River is 17 km long and occurs in the 1700 km^2 Kouga-Baviaanskloof Wilderness area. It is a perennial, second-order, headwater stream which flows through a deeply incised gorge in the Baviaanskloof Mountains (Figures 13 & 14a). The river cuts through quartzite rocks along a joint in a northerly direction which is probably related to the underlying rock structure (Rust & Illenberger, 1989). The lower reaches of the river have been farmed and water is abstracted for irrigating citrus orchards and crops. The vegetation of the area is described above.

Beerpoort and Smithskraal, Groot River, Gamtoos River system

The two study sites on the Groot River were on farms, Beerpoort owned by Mr J. Erasmus and Smithskraal owned by Mr D. Hume (Figure 5).

The river is wide, with a boulder and silt bed (Figure 6). The banks are steep and lined with

Acacia karroo trees. In both areas stock farming and lucerne fields occurred along the river. Weirs and causeways are also common in this area as well as water abstraction directly from the river (Cambray, 1990a). The vegetation of the area is described above.

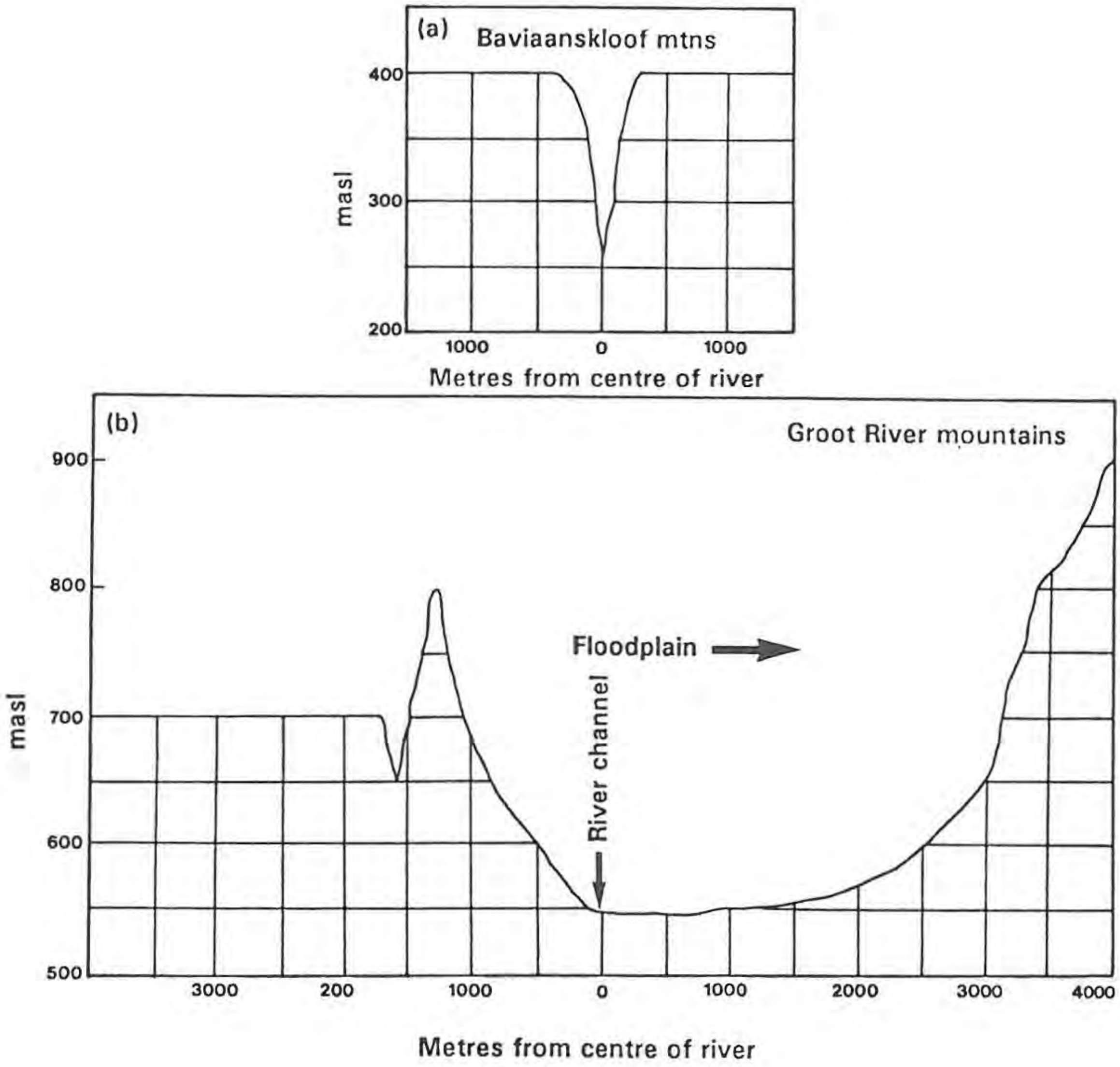


Figure 14. Cross sections of (a) Wit River collecting site for *P.afer* and (b) a Groot River collecting site at Netley Weir for *P.asper*.

Water flow into Beervlei Dam on the Groot River

The unstable and erratic flow in the Groot River forced the Department of Water Affairs in

1957 to construct a flood control dam, Beervlei Dam, (33° 04' 30" S and 23° 29' 30" E) near Willowmore to minimise the impact of flash-flooding to farm lands (Figures 4 & 5). After the dam wall was raised in 1967 it had a storage capacity at full supply level of 92,580 X 10^6 m^3 (Department of Water Affairs, Pretoria, pers. comm.). The full supply capacity has since been reduced, due to silt retention, to 90,828 X 10^6 m^3 (Department of Water Affairs, Pretoria, pers. comm.). The area under irrigation from the reservoir is 2660 ha.

The Beervlei Dam wall is a complete barrier to all migrating fish species with the possible exception of elvers of *Anguilla* species (Cambray, 1990b).

The yearly inflow pattern into the reservoir over the period 1958-1989 has been very irregular (Figure 12) varying from no inflow (1983 -1985) to 524.2 X 10^6 m^3 (1960/61). The inflow pattern on a monthly basis for the period 1980 - 1989 also highlights the sporadic nature of the riverine flow varying from no inflow for long periods to 205 X 10^6 m^3 in March 1988 (Figure 15). Prior to March 1988 Beervlei Dam had last spilled in 1981. During the floods of February/March 1988 Beervlei Dam rapidly went from 0% to overflowing in less than 12 days. The dam reached 145% on March 10, 1988 and continued to spill until May 1988. Subsequently the Department of Water Affairs, together with the local farming community below the Beervlei Dam wall, planned a series of eight water releases during the summers of 1988/89 and 1989/90 to flush out the pools of brack water between the Beervlei Dam wall and Steytlerville to improve the water quality for irrigation. The releases were in decreasing volumes of water over a 4-5 day period commencing with a strong flushing flow of between 56,64 and 65,35 m^3 sec^{-1}. The varying release flows were a result of experimentation by the Advisory Committee of the water scheme in order to reach an acceptable release pattern to satisfy the needs of all irrigators along the system (Cambray, 1990a).

Beerpoort causeway

Beerpoort causeway is approximately 2 km upstream of Netley Weir. The river is 150 m wide here and upstream of the causeway is a riverine pool, which when full is 150 m wide, 300 m long and 4 m deep. In the deepest section there is an irrigation pipe for water extraction. The substrate is deep, soft silt, with some patches of exposed boulders. During summer an annual aquatic grass occurs in the more sheltered areas of the pool. Below the causeway there is a boulder run, with small pools. The banks have either no vegetation or *Acacia karroo*.

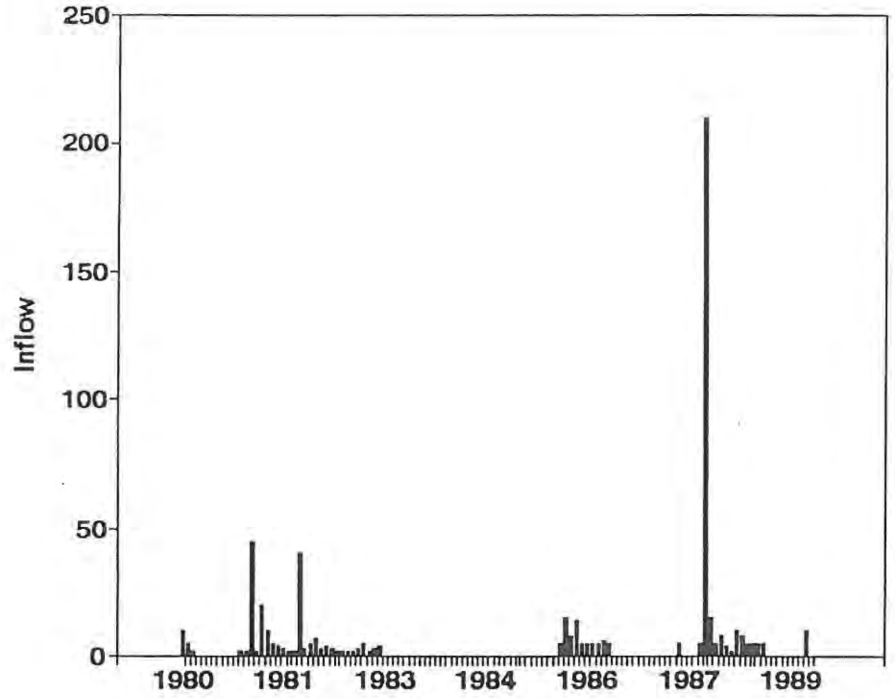


Figure 15. The monthly inflow (10^6 m^3) pattern into Beervlei impoundment over the period 1980 - 1989 (from Cambray, 1991a).

Netley Weir

The 162 m wide concrete Netley weir is on the Groot River approximately 70 km downstream of Beervlei Dam and 2 km below the Beerpoort causeway. The weir consists of two steps and there is a one metre wide sluice gate at one end of the weir (Cambray, 1990b, his Figure 2). The pool above the weir is silted, and the substrate is very soft silt (Figure 16). When full the pool is 162 m wide, over 500 m long and up to 6 m deep. There is a bare rock face on one side of the pool and the other side has a stand of *Acacia karroo* trees.

Mean annual rainfall in the area of the Netley weir is 195 mm annum^{-1}. A river flow gauging weir at Sandpoort (33° 27' 30" S, 24° 41' 30" E), just above the entry of the Groot River into the Grootrivierpoort, recorded very little flow before the March 1988 floods (Figure 15). During the March floods the flood waters connected the more permanent section, which occurs in the Cape Fold Mountain Belt, of the Gamtoos River system with the previously dry Karoo tributary. Fish then underwent a migration up the river from the large permanent pools in the mountains (Cambray, 1991a).

Figure 16. Demonstration of the soft silt behind Netley Weir, Groot River.

Summary

The summary of this chapter is presented overleaf in tabular format (Table 2).

Table 2. Summary of the general characteristics of the Wit and Groot Rivers of the Gamtoos River system. For references and explanations see text.

Characteristic	Wit River (*P. afer*)	Groot River (*P. asper*)
Length (km)	17	500
Source (masl)	1219	1740
Gradient (m km^{-1})	60,95	2,95
Geology	Resistant quartzites and Table Mountain Sandstones	Soft Bokkeveld beds
Sediment yields (t km^{-2} y^{-1})	150 - 200	400 - 1000
Important geomorphological feature	Cape Fold Mountain Belt	Great Escarpment
Orogenesis	Early and middle Triassic	
Ancestral equivalent 1	Palaeo-Baviaanskloof-Gamtoos	Palaeo-upper Orange-Groot-Olifants
Ancestral equivalent 2	Palaeo-Baviaanskloof-Gamtoos	Palaeo-Groot-Kariega-lower Sundays
Flow	Perennial	Intermittent
Stream order	Second	Fifth
Phytochrological region	Tongaland-Pondoland and Afromontane	Karoo-Namib
Biome	Similar to Forest	Nama-Karoo
Vegetation types	Valley Bushveld with Afromontane elements	Karoo and Subdesert
pH	Acidic-neutral	Alkaline
Clarity	Clear	Turbid
Conductivity	Low	High
Nutrient status	Oligotrophic	Eutrophic
Mean annual rainfall (mm)	400 - 500	200 - 300
Peak rainfall months	March and October	February, March and April
Mean annual evaporative rates (mm)	1400 - 1500	2000 - 2300
River type	Reservoir	Sandbank
River classification	Cape system region	Central arid region
River regulation	Not regulated	Regulated by a number of in channel weirs and the Beervlei Dam

CHAPTER - 3

REVIEW OF THE BIOGEOGRAPHY AND SYSTEMATICS OF THE REDFIN MINNOWS IN THE GENUS *PSEUDOBARBUS*.

Biogeography

African cyprinids

Redfin minnows belong to the Cyprinidae which is the most speciose of all freshwater teleost fish families with 1700 valid species in at least 220 genera. The family has a wide geographical distribution and utilizes most types of freshwater habitats and some species are highly tolerant to salinity (Howes, 1991).

The cyprinids are a major component of the fish fauna of Africa and have a pan-African distribution. There are 477 species of African cyprinids in 24 genera in three subfamilies, the cyprinines, the bariliines, and the leuciscines (Skelton *et al.*, 1991).

The fossil record at present does not entirely help to explain present day distribution of African cyprinids. Fossil records are few with the earliest African cyprinid fossils dating from the Miocene (van Couvering, 1977). The absence of Tertiary fish fossils from southern Africa has made dating the fauna difficult (Skelton, 1986).

Lowe-McConnell (1987) summarized and categorized African freshwater fish distribution into five groups: widely distributed remnants of archaic elements; endemic families; Gondwanic elements shared with South America; elements shared with the Orient (some of possible Gondwanic origin) and marine derivatives.

Earlier studies emanating from southern Africa suggested that the ancestors of the present ichthyofauna, with the exception of *Galaxias zebratus*, dispersed southwards in a series of invasions through former drainage connections between river systems (Barnard, 1943; Farquharson, 1962; Gabie, 1965; Jubb, 1964, 1967; Jubb & Farquharson, 1965; Gaigher & Pott, 1973 and Bowmaker *et al.*, 1978). The earliest invasion in the mid-Pliocene was thought to have provided the ancestors of fishes now found in the southern Cape rivers, such as the flexible-rayed redfins (Jubb & Farquharson, 1965). Following this, further invasions resulted in competitive displacements of some of the earlier species and climatic changes eliminated species from certain areas.

Distributional patterns of the Cyprinidae have been explained by dispersal, vicariance or panbiography (Howes, 1991). The dispersal hypothesis presented various difficulties and has been challenged by proponents of vicariance biogeographical theory (Howes, 1991). Fragmentation (vicariance) of populations has occurred through both hydrographic and climatic changes (Beadle, 1981). Panbiographers see tracks which mark the distributional boundaries of cyprinid groups as indicative of past large-scale 'form-making' processes. Thus there are conflicting views on palaeogeography and a lack of understanding of the phylogenetic relationships within the Cyprinidae (Howes, 1980, 1981). Higher-level ostariophysian interrelationships need to be resolved before any acceptable hypotheses can be put forward to explain the African-Asian fish distribution 'track' (Skelton *et al.*, 1991).

The ten African ichthyological provinces (Roberts, 1975) are not strict biogeographical units (Greenwood, 1983). These provinces are a useful tool for discussing regional patterns of African fish distribution (Skelton *et al.*, 1991).

Cyprinids increasingly dominate the Cape ichthyofaunal province as one moves southward (Bowmaker *et al.*, 1978). Cyprinids in this province can be divided into the Karroid and the Cape groups (Skelton, 1986). Endemic Cape fishes are mainly confined to the Cape Fold Mountain drainages and include the only African galaxiid, *Galaxias zebratus*, the only two species in the anabantid genus *Sandelia*, and at least three distinctive groups of barbine cyprinids (Skelton, 1986; Skelton *et al.*, 1991). The Cape ichthyofaunal province therefore contains elements that suggest its relationships lie closer to areas outside Africa (Reid, 1978, 1982; Howes, 1985, 1987). Howes (1984), Travers (1984) and Skelton (1986) suggested a "Gondwanoid" origin and history for the African freshwater fishes. The absence of small *Barbus* species from Arabia may be because African species are not necessarily monophyletic with Asian species (Banister & Clarke, 1977).

Palaeo-distribution of Pseudobarbus species

The group of cyprinid minnows known as "redfins" is only found in rivers of the Cape Fold Mountains in South Africa and the Drakensberg and Maluti mountains of Lesotho (Figure 17). Distribution of the species is entirely complementary and allopatric with the exception of *P.*

tenuis which is found sympatrically with *P. asper* in the Gourits River and with *P. afer* in the Keurbooms River (Skelton, 1980a; Albany Museum records). An area cladogram demonstrated the relationship of the present distribution ranges of the redfin minnows in relation to their phylogenetic relationships (Figure 17; Skelton, 1986). The redfin minnow lineage is distributed in a vicariant pattern consistent with the hydrographic history of the region (Skelton, 1986, 1988).

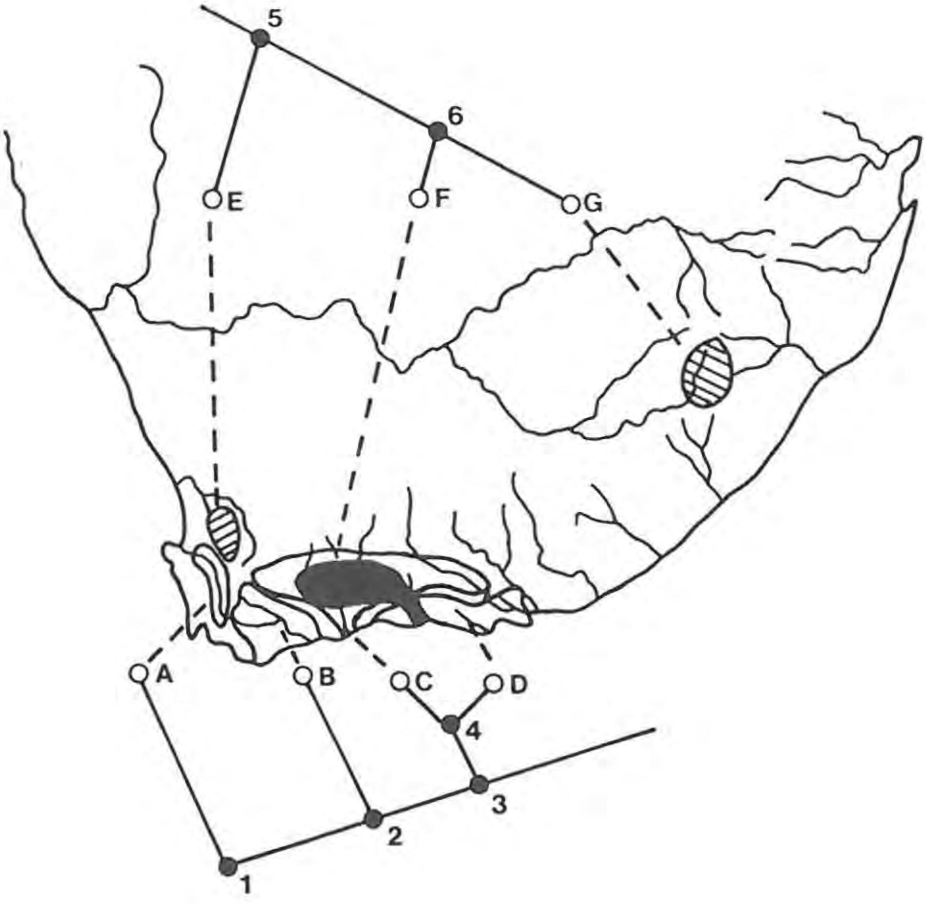


Figure 17. Area cladogram of the *Pseudobarbus* species (from Skelton, 1986).

A. *P. burgi* : Eerste River, Berg River and Verlorevlei River;

B. *P. burchelli* : Breede and adjacent river systems;

C. *P. asper* : Gourits and Groot River (Gamtoos);

D. *P. afer* : Coastal rivers from Mossel Bay to Sundays River (Algoa Bay);

E. *P. phlegethon* : Olifants River system;

F. *P. tenuis* : Gourits and Keurbooms River systems;

G. *P. quathlambae* : Headwater tributaries of the Orange River.

The common ancestor of the *Pseudobarbus* probably achieved a widespread distribution in the proto-upper Orange River and rivers of the Fold Mountain Belt region (Skelton, 1980a). Skelton (1980a) explained the allopatric distribution of the *Pseudobarbus* as due to vicariance of a widespread ancestral species following the isolation of each of the major drainage basins. *Pseudobarbus* species are linked to the Cape Fold Mountain Belt - montane archipelago distribution track and therefore the common ancestor to this lineage evolved or became established during a time when temperate conditions were widespread over southern Africa. Skelton (1980a) estimated that this may have been during late Eocene to Oligocene times (45-25 X 10^6 y BP) when climates were known to have been cooler before the continent reached its present latitudes. Another possible period was during the late Miocene to Pliocene when it was also cool. This accords with the suggested entry of cyprinids into Africa during the early Miocene (van Couvering, 1977).

Initial dichotomies of the redfin lineage aligns the species within adjacent major drainage basins of the Cape Fold Mountains (Figure 17; Skelton, 1986) with *P. burgi* in the Berg and other west coast rivers, *P. burchelli* in the Breede and adjacent streams, *P. asper* in the Gourits and Gamtoos and lastly *P. afer* in rivers east of the Gourits. There has been intercommunication between drainage basins by river piracy (Barnard, 1936; Taljaard, 1951). These drainage changes provide a feasible theoretical explanation for the widespread distribution of this group of *Pseudobarbus* species. The distribution of three other redfins (*P. phlegethon*, *P. tenuis* and *P. quathlambae*) provided a more complex problem in relation to modern drainage lines (Skelton, 1986). The pattern of distribution of the fishes of the karroid category coincides with these three redfin minnows. *Barbus anoplus*, *B. motebensis* and *B. gurneyi*, members of a karroid group of fishes, are thought to be the sister group to the redfin minnows (Skelton, 1980a). The distribution of the *Pseudobarbus* and karroid groups are to a large extent allopatric and complementary with one overlap.

The general pattern of freshwater fish distribution can be explained by the geomorphic and hydrographic evolution of the subcontinent. Skelton (1986) considered the evolution of the Orange River basin to be of central significance to the distribution patterns of temperate southern African freshwater fishes. The Upper Orange River once flowed through part of the present Olifants River and switched to the present outlet at the beginning of the Miocene (see Chapter 2). This explains the distribution of redfins in the Orange and Olifants Rivers and dates this vicariant event in the redfin minnow lineage. *P. tenuis* and *P. quathlambae* possibly vicariated within the Orange River when progressive dissection of the upland peripheries isolated ancestral stocks (Skelton, 1986). Skelton's hypothesis was that *P. tenuis* was later translocated by catchment transfer to the Gourits River system and eliminated from the Orange River. *P. quathlambae* in the Lesotho Highlands remained as an isolate compared with south coastal species or species groups.

An explanation for the presence and distribution of *P. afer* and *P. asper* in the Gamtoos River system has been problematical for some time. The form of *P. asper* in the mountain tributaries was not typical of the form which occurred in the Groot River (Barnard, 1943). Jubb (1965) found difficulty in separating *P. asper* from *P. afer* collected from certain coastal tributaries and suggested that they were closest to *P. afer*. The widespread distribution of *P. afer* made it difficult for Skelton (1980a) to pinpoint the dichotomy between *P. afer* and *P. asper*. The coastal rivers and larger inland drainages of the Gourits and Gamtoos River systems suggested a dichotomy. The distribution of *P. afer* into many coastal rivers was said to be via interconnections during periods of lower sea-levels. The regions between the Gourits and Gamtoos Rivers were once sand dunes and as such were poorly drained with meandering rivers (Dingle & Rogers, 1972; Birch *et al.*, 1978) which Skelton (1980a) considered to have facilitated transverse drainage patterns and interconnection of rivers. *P. asper* was probably present in the Groot River before river capture and appears to have been prevented from dispersing beyond the confines of the parent river (Skelton, 1980a). He further suggested that ecophysiological and competitive factors may have been involved in the maintenance of this distribution pattern. This was partly based on evidence of salinity tolerances (Hofmeyr, 1966). *P. asper* were found to be more tolerant to chlorides than *P. afer* which fully corresponds to the limits of their present day distribution pattern. Recent work has indicated that the palaeo-Baviaanskloof-Gamtoos River captured the upper catchment of the easterly flowing palaeo-Groot-Kariega-lower Sundays about 60 X 10^6 y BP (Figure 9; Rust & Illenberger, 1989; Illenberger, pers. comm.). If *P. asper* was already present in this section, as proposed by Skelton (1980a), then this date would indicate an earlier origin for the *Pseudobarbus*.

At present there are low divides between the Groot River of the Gamtoos River system and the Gourits River. It is quite feasible that sheet flooding during periods of high flow in the past connected these two river systems, allowing the dispersal of *P. asper* from the Groot River to the Gourits River system.

Cape Fold Belt Distribution track

Distribution of redfin species is coincident with a distinctive pattern of several other organisms in the Cape Fold Belt - eastern highlands area (Skelton, 1980a) such as diverse palaeogenic invertebrate groups (Stuckenberg, 1962). Other works on the invertebrates of the Cape Fold Belt have also added important information (Harrison & Agnew, 1962; Harrison, 1965; Endrödy-Younga, 1978). Harrison (1965) divided riverine invertebrates into two groups, an "old element" which was distributed approximately like Stuckenberg's (1962) pattern and a more recent group that had higher temperature tolerances. Amphibians and reptiles (eg. FitzSimons, 1962; Poynton, 1964; Greig & Burdett, 1976; Poynton & Broadley, 1978), a small endemic "Cape" avifauna (Roberts, 1978; Winterbottom, 1978), some mammal species (Davis, 1962; Meester, 1962; Bigalke, 1978) follow the Cape Fold Belt-eastern highland distribution pattern. The Capensis flora (Taylor, 1978) has a characteristic mainly mountainous distribution pattern which is similar to the centres in Stuckenberg's palaeoendemic distribution track. The montane forest vegetation (White, 1978; Geldenhuys, 1989) also conforms to the pattern of the distribution track which White (1978) termed the Afromontane archipelago.

This distribution track includes both palaeogenic and post-Gondwanoid elements which have been "welded" together by the geological and geographical history of the region (Skelton, 1980a). Older Gondwana, as well as post-Gondwana elements are present in the Capensis flora (Axelrod & Raven, 1978; Taylor, 1978), the Amphibia, the freshwater invertebrates (Harrison, 1965) and the Coleoptera (Endrödy-Younga, 1978). *Galaxias zebratus* is the only freshwater fish likely to be a Gondwanoid form (Skelton, 1980a).

Past and present floral patterns within this distribution track are very informative. The Capensis flora dating from when the mega-continent broke up some 120-140 X 10^6 y BP is known as Gondwanoid flora. With the continent over 15° further south than at present the southern half of the flora was adapted to temperate conditions. As the continent drifted northwards warmer and wetter climates led to the Capensis flora only surviving in the higher lying regions. The oscillations since the Miocene (van Zinderen-Bakker, 1976; Coetzee, 1978) allowed the vegetation to expand and then contract leaving relicts in the cooler moister areas. The Afromontane flora had a similar history (Geldenhuys, 1989; Wild, 1968). The present distribution of the flora represents relicts from a former continuous distribution.

Present day distribution of Pseudobarbus *species with special reference to* P. afer *and* P. asper

P. afer and *P. asper* occur only in rivers draining Cape Fold Mountain ranges in the southern Cape Province (Figure 18). *P. afer* has been collected from coastal rivers east of the Gourits River system to the Sundays River system. *P. afer* occurs in deeply entrenched river systems of Tertiary origin along the Outeniqua-Tsitsikamma coastal plain (Albany Museum records). This study in the Gamtoos River system has increased the known range of the species in both the Kouga and Baviaanskloof Rivers.

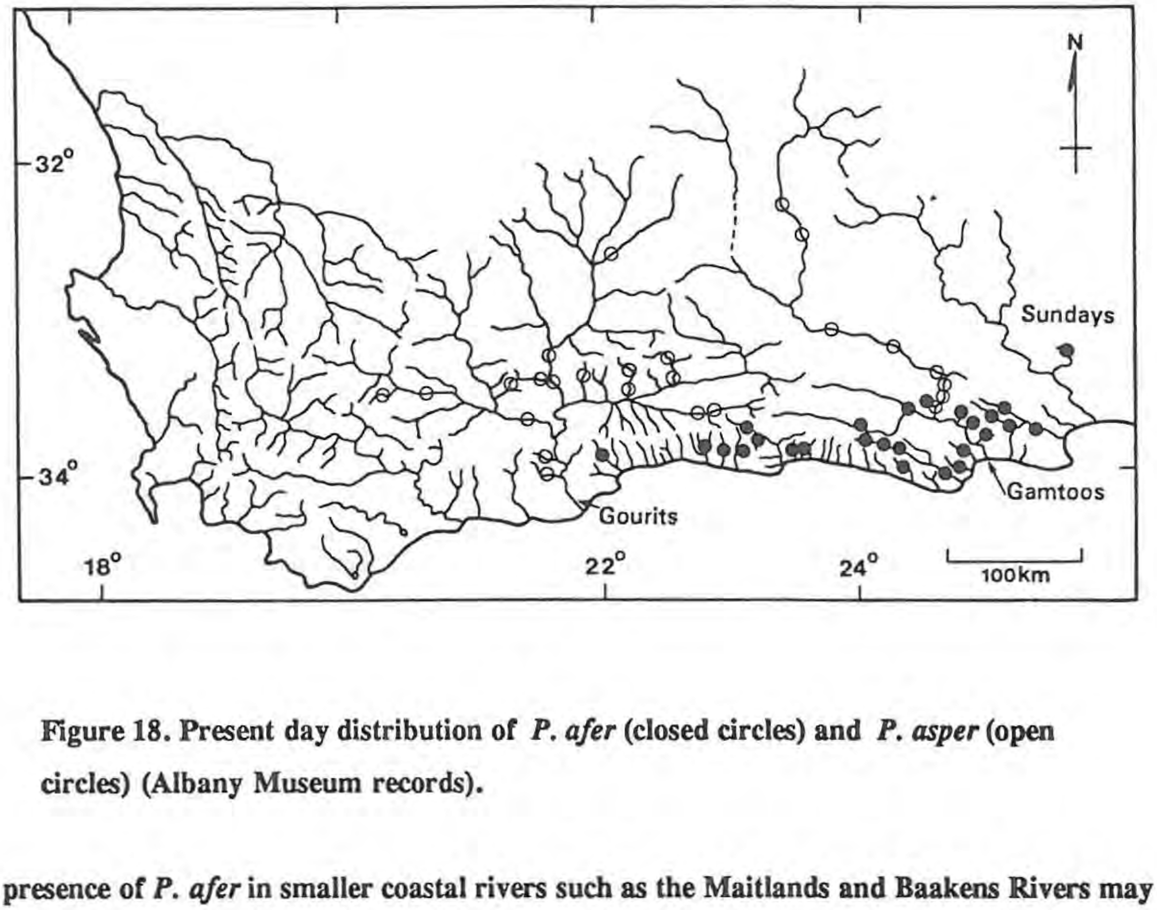


Figure 18. Present day distribution of *P. afer* (closed circles) and *P. asper* (open circles) (Albany Museum records).

The presence of *P. afer* in smaller coastal rivers such as the Maitlands and Baakens Rivers may possibly be explained by interconnections of these systems with larger systems such as the Gamtoos or Swartkops during former sea-level regressions. Skelton (1980b) illustrated this by examining the depth contours in Algoa Bay and extending the possible courses of the rivers during a hypothetical 90 m regression. Freshwater fish could change systems during these regressions. The sea level may have dropped by as much as 180 m below the present level with small rivers along the eastern Cape coast of today having once been headwater tributaries of major river systems which cut deep gorges across the continental shelf (Marker, 1988).

A single isolated population of *P. afer* occurs in the Witte River (Sundays River system), a small Suurberg stream which according to Forbes & Allanson (1970) is the only permanent tributary of the system which does not have highly mineralized water. Skelton (1980b) considered this to be a relict population which reached this stream during more favourable conditions possibly when the Sundays River system was linked to other systems draining the Fold Mountains and suggested the Swartkops River as an example. This would have been during a pluvial period with runoff from the Fold Mountains sufficient to improve water quality of the mainstream. Partridge & Maud (1987) and Rust & Illenberger (1989) have noted past connections of the present day Gamtoos system with the Orange and Sundays Rivers. Possibly, the present isolated population of *P. afer* in the Witte River is from the palaeo-Groot-Kariega-lower Sundays connection. Part of the *Pseudobarbus* population later moved into the present day Gamtoos River system when the palaeo-Baviaanskloof-Gamtoos captured the Groot River (Figure 19).

Palaeo-Orange-Olifants River
↓
Pseudobarbus quathlambae
ancestor remained in Orange River
↓
Ancestors of other six
Pseudobarbus species
moved into lower Orange
or came from the
lower Orange (= Olifants)
↓
↓
Ancestor of
Pseudobarbus afer - Pseudobarbus asper
moved into the
palaeo-Groot-Kariega-lower Sundays
P. afer and *P. asper* evolved here,
one population of *P. afer* remained
rest moved into
↓
↓
palaeo-Baviaanskloof-Gamtoos
here *P. afer* succeeded in mountain streams
and *P. asper* inhabited the
now more saline and turbid Groot River
↓
during this time lower sea levels
allowed *P. afer* to migrate into what are
now small river systems along the coast
↓
while inland *P. asper* migrated into the Gouritz system

Figure 19. Possible movement and evolution of *Pseudobarbus* species in palaeo-river systems.

P. asper only occurs in two river systems, the Groot River of the Gamtoos River system and in the tributaries and mainstream of the Gourits River system (Albany Museum Records, Figure 18). Survey trips undertaken during the course of the present study have extended the known range of this species to the upriver side of Beervlei Dam (Figure 20) within the Gamtoos River system. The largest populations of this species in the Gamtoos River system occur in the Groot River between Steytlerville and Beervlei Dam.

Systematics of the *Pseudobarbus* species

There has been considerable confusion in the past between a clear separation of *P. afer* and *P. asper*. Skelton (1980a) recognized four forms. They were then '*B. afer*' (*typical*), '*B. afer*' (Gamtoos), '*B. asper*' (typical) and '*B. asper*' (variant) (Figure 21). Until recently all redfin minnows were placed in the genus *Barbus* including *P. afer* and *P. asper* (Skelton, 1988). Boulenger (1911) described *Barbus asper*. The taxonomic history of *P. afer* is somewhat more involved (see Skelton, 1988).

For his study on the *Pseudobarbus* species Skelton (1988) used cladistic analysis which involved the identification of homologies and evolutionary direction of character change from a plesiomorphic (primitive) character state to an apomorphic (derived) character state. He established phylogenetic relationships based on shared derived character states (synapomorphies).

As redfins are distinctive species, finding an outgroup for them proved problematical (Skelton, 1980a). In his analysis Skelton (1980a, 1988) established that, of all *Barbus* in southern Africa, *B. anoplus*, *B. amatolicus*, *B. motebensis* and *B. gurneyi* are most similar to the flexible-rayed redfins. Their close relationship was supported by several synapomorphies, such as development of large tubercles (see Chapter 7), aspects of squamation, reduction of ossification of epineurals, reduction in size of supraneurals and shape of the metapterygoid (Skelton, 1980a, 1988).

Using a broad-based character analysis Skelton (1980a, 1988) revealed that there were two monophyletic lineages within the redfin minnows and that the flexible-rayed redfin minnows were sufficiently unique and morphologically distinct, relative to other *Barbus* species, to justify separate generic status. This distinctive group of African cyprinid fish was placed in the genus *Pseudobarbus* which at present includes seven extant redfin minnow species. *Pseudobarbus* was

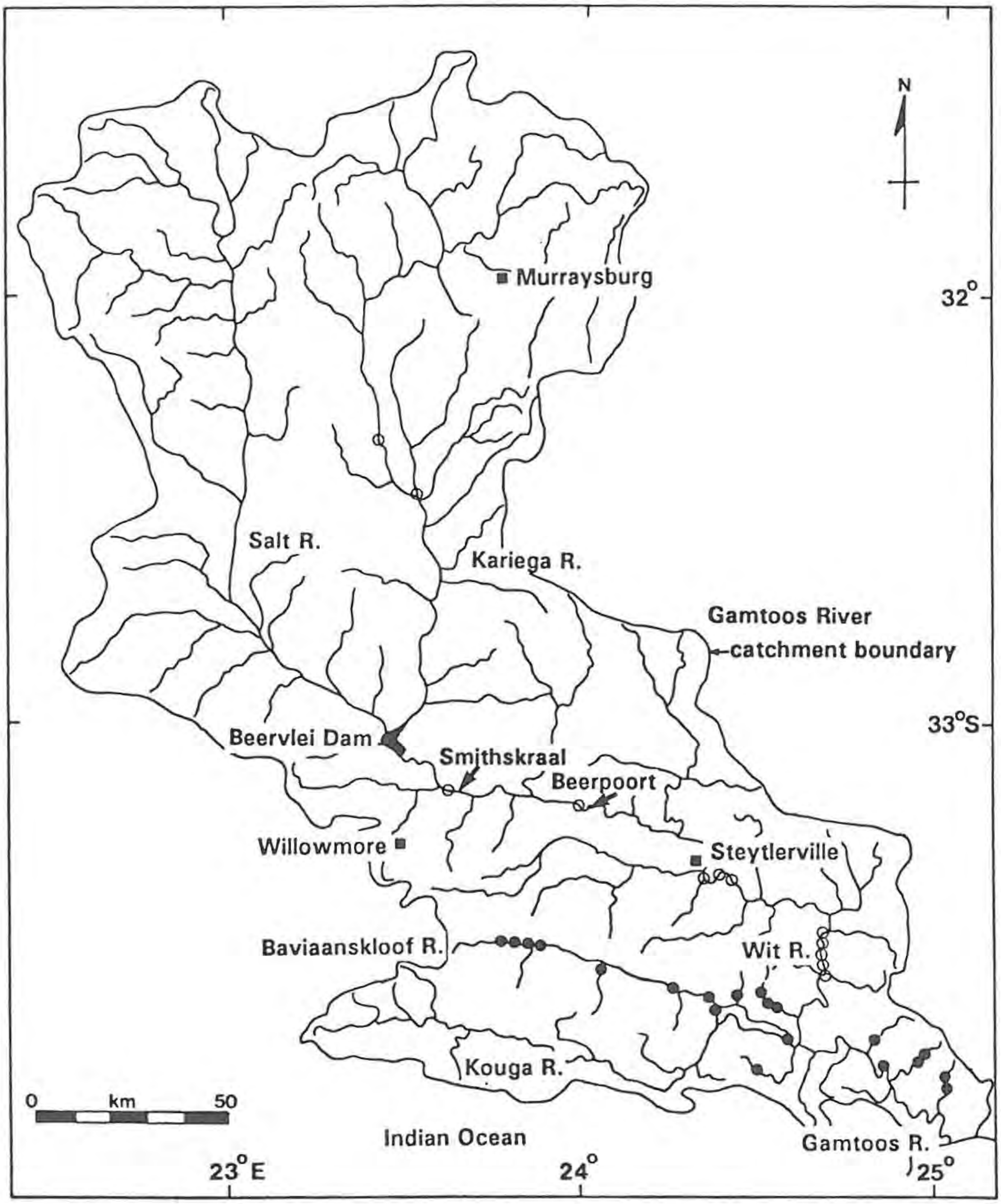


Figure 20. Localized distribution records of *P. afer* (closed circles) and *P. asper* (open circles) in the Gamtoos River system showing catchment boundaries (Albany Museum records).

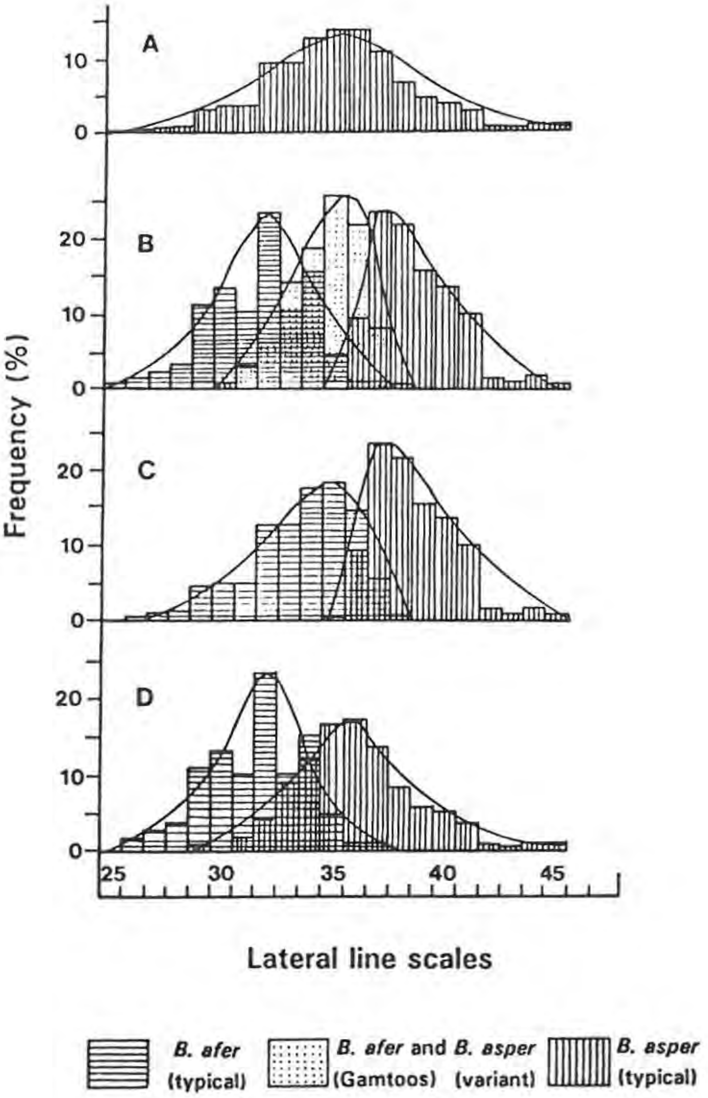


Figure 21. Frequency distribution (%) of lateral line scales of *B. afer* and *B. asper* populations (now *P. afer* and *P. asper* see text). (A) All samples of both species combined. (B) *B. afer* plus *B. afer* (Gamtoos) plus *B. asper* (variant). (C) *B. afer* plus *B. afer* (Gamtoos) plus *B. asper* (variant) and *B. asper*. (D) *B. afer*, *B. asper* plus *B. afer* (Gamtoos) plus *B. asper* (variant), (from Skelton, 1980a and 1988.

raised by Smith (1849) as a sub-genus of *Barbus* and included two species, *P. burchelli* and '*B. pallidus*'. Jordan (1919) recorded the type species of *Pseudobarbus* as *P. burchelli*. Skelton (1980a, 1988) noted that the original definition of *Pseudobarbus* by Smith (1849) was inadequate and therefore included a new designation. Barnard (1943) and Jubb (1965, 1967) had considered redfin minnows, including both the flexible and serrated dorsal spine groups, to be a natural group due to the bright red colour on the fins of all the species. The seven minnow-sized species in the monophyletic group of flexible-rayed redfin minnows (Figure 22) are now characterized by a suite of unique characters. These characters include bright red fins, prominent nuptial tubercles on head, scales and fins, distinctive pharyngeal teeth and alimentary tract and various skeletal characters (Skelton, 1980a, 1988). Besides the synapomorphous characters there was a great similarity of form between the flexible-rayed redfins which suggested monophyly (Skelton, 1980a).

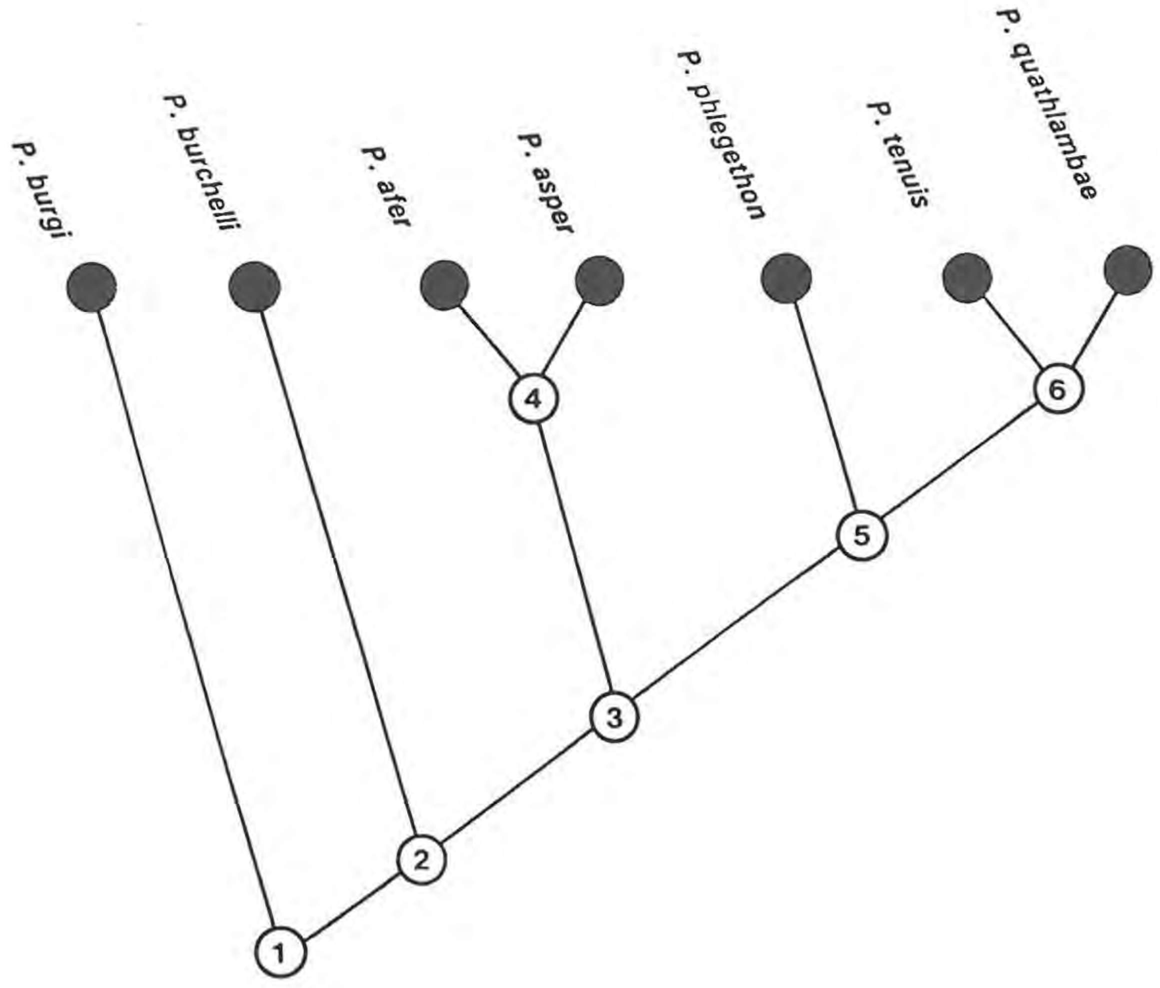


Figure 22. Phylogeny of the flexible-rayed redfin *Pseudobarbus* species (from Skelton, 1980a).

There are several synapomorphies such as the loss of the anterior pair of barbels, the loss of the mandibular sensory canal, a slight increase in the number of scale radii, reduction in the number of head tubercles, and the development of a more pointed flange on the exoccipital, which show a monophyly for *P. afer*, *P. asper*, *P. phlegethon*, *P. tenuis* and *P. quathlambae* (Skelton, 1980a). Of these five characters probably the most reliable are loss of mandibular sensory canal and decrease in number (but increase in size) of head tubercles. The major feature of the flexible-rayed redfin tubercle pattern was the large size, the regularity and form of the pattern, and the development of regular rows on the borders of the scales. Skelton (1980a) considered this character a synapomorphy.

The majority of redfin minnows were found to have a similar scale size (approx. 30-38 in lateral line; 12-16 around the caudal peduncle) (Skelton, 1980a). *P. asper* and *P. quathlambae*, by comparison, had relatively small scales which Skelton considered apomorphic. Skelton (1980a, 1988) established that the sister species of *P. tenuis* was *P. quathlambae* and that the small scale size of *P. quathlambae* and *P. asper* was independently derived.

The long and involuted intestines of flexible-rayed redfins was considered synapomorphic, as most small *Barbus* species have shorter intestines (Skelton, 1980a). There was a wide degree of intraspecific differences in the length of the gut of *P. afer* and *P. asper* and Skelton (1980a) suggested that the evidence was equivocal as to whether these differences are phenotypic or genotypic.

The high vertebral count (36-38) of flexible-rayed redfins is in accordance with Jordon's Rule (Lindsey, 1975) and redfins are endemic to higher latitudes than other small *Barbus* species (Skelton, 1980a). High vertebral number was therefore an adaptational specialisation of redfins to a temperate environment, and the high vertebral number was considered to be synapomorphous for the group.

In cladism, species are classified by their position in the phylogenetic hierarchy and not by their phenetic similarity (Ridley, 1986). Cladists represent all relationships as sister groupings. Several character transformations within the flexible-rayed redfins permitted reconstruction of the phylogeny of the species. Skelton (1988) resolved the interspecific boundaries between *P. afer* and *P. asper* and considered them sister species (which is a pair of species, or group of species, that share a more recent common ancestor with each other than with any third species). *P. afer* and *P. asper* are morphologically extremely similar but Skelton (1980a) could not find a clear synapomorphy which would confirm their close relationship. Skelton (1980a) noted three differences between the two species - size of scales, length of intestine and pattern of pigmentation (Figure 23). In these three characters *P. asper* is more unusual with smaller scales, a longer intestine and different pigmentation. These are often phenotypic characters and may indicate that the two 'species' are ecophenotypes. The caudal peduncle scale count was the main distinction that Skelton (1980a) could find. *P. asper* also inhabits turbid, saline waters which are distinct from the remainder of the habitats occupied by redfins (see Chapter 2). *P. asper* is probably the most derived morphologically and physiologically of the sister species pair (Skelton, 1980a).

Skelton (1980a, 1988) expanded the known *P. afer* to include what he termed '*B. asper*' (variant). *P. afer*, as now known, is a variable and widespread species occurring in perennial coastal rivers from the Sundays to the Outeniqua coastal plain east of the Gourits River system.

The problems of the relationship of *P. afer* and *P. asper* would be better understood if there was experimental evidence of the effect of physico-chemical parameters on characters such as scale size (Skelton, 1980a). There could be several reasons for reduced scale size. Small scale size can reduce friction in strong current environments of fast swimming oceanic fishes and in some cases the fish have lost them entirely. Small scales increase flexibility therefore eels (anguilliforms) and benthic fish have small scales on the ventral (contact) surface. The reduced breast scales of the *Pseudobarbus* group correlate with the inferior mouth and suggested benthic feeding habits for the species (Skelton, 1980a). Reduction of the pelvic axillary scales correlates with fish species which live near or at the bottom (Norman, 1975) . The small scales of *P. asper* may be a direct result of salinity of the Groot River because salinity tends to retard embryological development which can lead to an increase in meristic characters (Barlow, 1961; Fowler, 1970). The smaller scales of *P. asper* may also be a physiological response to an environment which has a higher conductivity than other *Pseudobarbus* environments (Skelton, 1980a). However, *P. quathlambae* inhabit mountain streams with low conductivity and they have the smallest scales of any of the *Pseudobarbus* species. It may be that this character is highly variable within the *Pseudobarbus*.

The redfin minnows have a higher variability of taxonomic characters than do many African small *Barbus*-like species (Skelton, 1988). Fish which live at higher latitudes usually have a greater range of variability and that character variability in fishes is inversely proportional to the diversity of the fauna (Nikolsky, 1976). It was therefore suggested that in the higher latitudes in which the redfin minnows occur, together with the lower faunal diversity at these latitudes

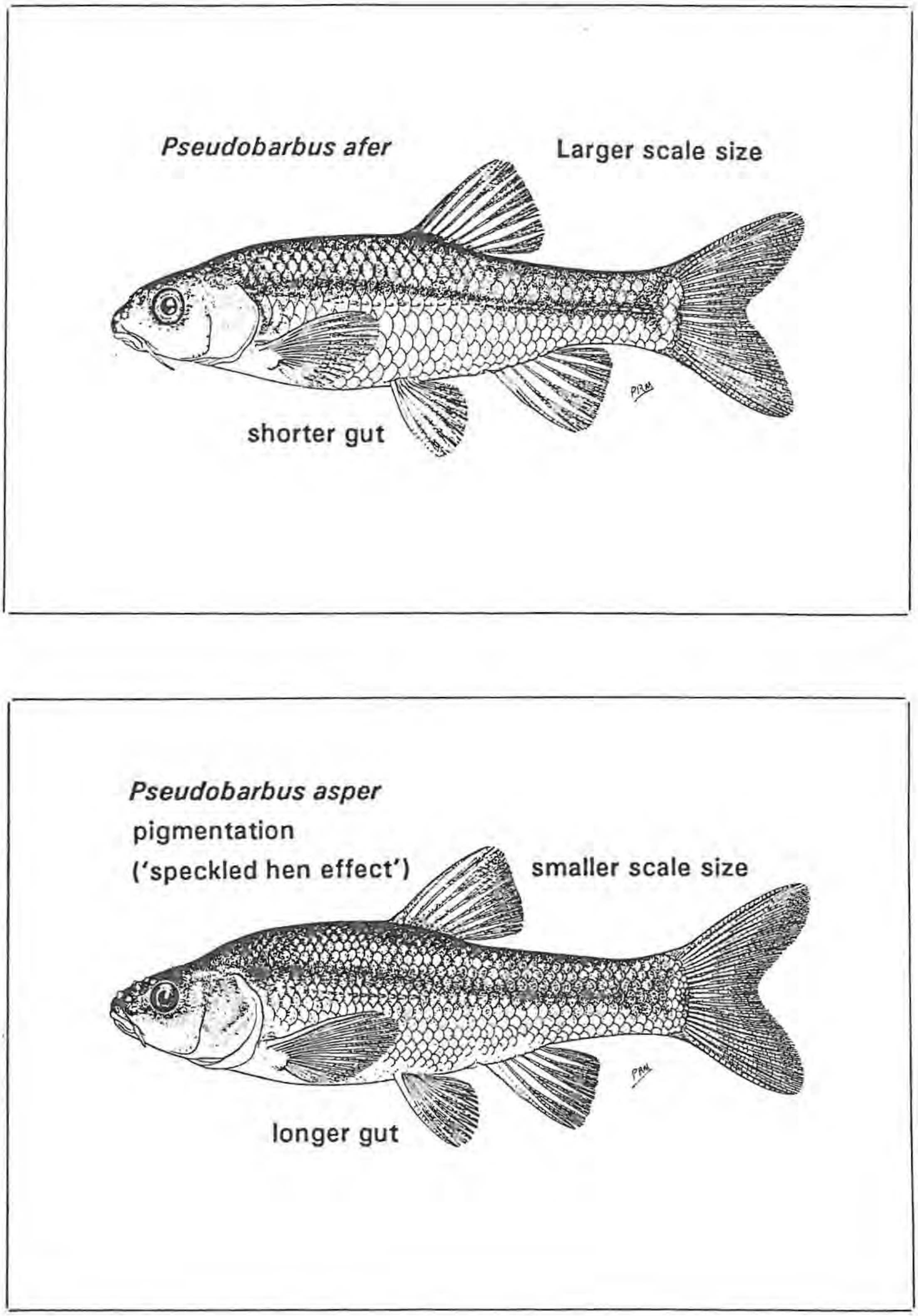


Figure 23. Meristic and morphological differences between *Pseudobarbus afer* and *P. asper* (Drawings by P. Meakin).

a greater variability of characters relative to other African minnows may be favoured (Skelton, 1988). In addition the polyploidy of the tetraploid *Pseudobarbus* species may be a factor (see below). The Cape Fold Mountain Belt streams do not offer a diversity of physical environments, yet the redfin minnow group now consists of seven recognised species. *P. asper* is the only species which occurs in a distinctly different environment, but it is morphologically close to *P. afer*.

When Skelton (1980a: 347) speculated on the evolution of the redfins he had to be cautious because of the lack of information on the environment in which the species occurred as "almost nothing is known about redfin biology and ecology." In addition only the endpoints of the evolutionary process are known as no fossil records have been recorded. The hypothesized ancestor to the present day *Pseudobarbus* species was probably of moderate size, fusiform shape, sexually dimorphic, had a barbelled inferior mouth and an elongated involuted gut. There was a tendency towards a reduction in the supraneural bones, with the anterior intramuscular bones being established early in the history of the group. Skelton (1980a) hypothesized that the ancestral species was probably similar to *P. burgi*, *P. burchelli* and *P. afer*. These three species exist in the Cape Fold Mountain Belt streams, which are ancient and the geology of the region has probably had the same influence on the physical and chemical nature of the rivers since the Mio-Pliocene (5,1 x 10^6 y BP), which is the postulated time of the origin of the redfin minnows (Skelton, 1980a).

The biogeographical hypothesis which Skelton (1980a) proposed was that speciation of the group was due to geographical vicariance. Many of the species have been isolated for a long time and probably this factor, rather than environmental change, has led to evolutionary change.

Polyploidy in the tetraploid Pseudobarbus *species*

In a review of the karyotypes of cyprinids 70% of the 315 species had a diploid chromosome number of about 50 (Oellermann, 1988). A smaller number of cyprinids had about 100 chromosomes and a third cluster of fewer species had about 150 chromosomes. In southern African *Barbus*, *Pseudobarbus* and *Varicorhinus* there were at least three levels of ploidy: diploid, tetraploid and hexaploid (Oellermann, 1988).

It has been proposed that the cyprinid family consisted of species with diploid and tetraploid origins (Ohno *et al.*, 1967). Species now in the tetraploid group experienced a polyploidic event

(Mayr *et al.*, 1986) sometime during their evolution which has doubled their chromosome numbers (2n = 96). The polyploid cyprinids may be related phylogenetically which has been suggested partly on the grounds of the polyploidization (Arai, 1982). Some of the cyprinid polyploidizations may represent derived, single-step transitions (apomorphies) which could unite several species, or species groups into putatively monophyletic lineages (Buth *et al.*, 1991). Buth *et al.* (1991) pointed out that 'although chromosome numbers differ among both tetraploid and hexaploid forms, the same chromosome numbers typically occur in species belonging to the same genus or related species group.' Among the examples of cyprinid species in the tetraploid group are species in the genera *Acrossocheilus*, *Aulopyge*, *Barbodes*, *Barbus*, *Carassioides*, *Carassius*, *Cyprinus*, *Neolissocheilus*, *Percocypris*, *Pseudobarbus*, *Schizothoraichthys*, *Sinocyclocheilus*, *Spinibarbus* and *Tor* (Mayr *et al.*, 1986; Oellermann, 1988).

The chromosome number of 96 of *P. afer* and *P. burgi* may have been derived from a diploid ancestral species with 48 chromosomes, or one with 50 chromosomes (Oellermann, 1988). Further work on the karyology of *Pseudobarbus* species has established that five of the species examined had 2n = 96-100 chromosome numbers which supported the hypothesis that the lineage is monophyletic (Naran, 1992). It had previously been suggested that the outgroup of the *Pseudobarbus* lineage was probably a group of small *Barbus* species (*B. anoplus*, *B. motebensis* and *B. gurneyi*) (Skelton, 1980a). *B. anoplus* have a diploid complement of 48 chromosomes (Oellermann, 1988). Four Robertsonian fusions could then give rise to 96 chromosomes of the redfin minnows if the tetraploid population started off with 100 chromosomes. It is of interest to note that *B. anoplus* co-occurs with *P. asper* in the Groot River but is not present in the *P. afer* habitats.

Polyploidy provides redundant gene loci (Becak *et al.*, 1966) which are more likely to escape natural selection pressures than conserve active loci, and in this way can more easily accumulate mutations (Wolf *et al.*, 1969). Therefore polyploidy in the tetraploid *Pseudobarbus* species may have played an important role in their evolution. Uyeno & Smith (1972) suggested that the advantages of polyploidy for cyprinids was a larger size, a longer life, faster growth and a greater ecological adaptability than many of the diploid cyprinids. The above-mentioned advantages of polyploidy fit the hexaploid yellowfish species (eg. *Barbus kimberleyensis*) and other southern African hexaploids (eg. *Barbus marequensis*) which are relatively large, long-lived and ecologically flexible species (Oellermann & Skelton, 1990). However, these advantages do not appear to be appropriate for the smaller, shorter-lived tetraploid *Pseudobarbus* species. For the southern African hexaploids it has been suggested that hexaploidy is the basis of the characteristic life-history attributes of these species (Oellermann & Skelton, 1990).

CHAPTER - 4

COMPARATIVE REPRODUCTIVE STYLES

Introduction

Reproduction is "the axis about which the biology of species revolve" (Meien, 1939 in Wootton, 1990). Fishes have evolved diverse sets of reproductive styles (for reviews see Breder & Rosen, 1966; Balon, 1975a, 1981a). The fitness of each reproductive style is sensitive to energy allocation parameters and mortality of the juvenile and adult phases of the life cycle. To establish which reproductive style is most adaptive requires knowledge of life-history characteristics of the population and what the effect of environmental factors are on the species (Ware, 1984).

When classifying ecological groupings of fishes Kryzhanovsky (1948, 1949 and Kryzhanovsky *et al.*, 1951 in Balon, 1990) combined selected spawning features, environmental factors and ontogenetic factors. Later Balon (1975a,b; 1981) expanded these ideas into an evolutionary classification of reproductive guilds in which there are three ethological sections (nonguarders, guarders and bearers) each of which is divided into ecological groups (such as open substrate egg scatterers) which are then further divided into guilds (such as rock and gravel spawners with benthic larvae). Before placing a species into a guild the spawning substrate and style of reproduction must be established as well as associated characters of eggs, embryos and larvae.

A combination of morphological, developmental, behaviourial and ecological criteria were evaluated on a comparative basis in order to place *P. afer* and *P. asper* in a particular guild. The framework of the classification of reproductive styles of Balon (1975a and 1981) provided useful information on evolutionary trajectories. Balon (1988 a,b) suggested that the main trends of reproductive guilds are shaped by epigenetic bifurcations and heterochronies during development. Since there are very few differences between *P. afer* and *P. asper* (Chapter 3) they might be phenotypic options or what Balon (1990) has termed the 'first draft of evolution'.

In their study of the life-history phenotypic options of the sculpin, *Cottus gobio*, Mann *et al.* (1984: 179-181) posed the question, "Do the different reproductive strategies in this species arise from genetic (ultimate) differences or from environmental (proximate) influences?" They were able to do a reciprocal transfer experiment which gave support to the thesis that there was a strong environmental influence upon life-history phenotypic options. Their results suggested that genetically determined life-history phenotypic options are overshadowed by the effects of productivity and temperature. The two redfin minnows, *P. afer* and *P. asper*, may be geographic variations (ecophenotypes) or there may be a genetic explanation for the few morphological and meristic differences. The variability of the reproductive life-history attributes, egg size, fecundity, age at maturity and seasonal and lifetime reproductive effort between *P. afer* and *P. asper* may or may not be within the range of a single species.

The hypothesis tested in this chapter was that in the more stable mountain stream the *Pseudobarbus* species would have more precocial life-history phenotypic options including larger larval size at first exogenous feeding than the species in the highly variable Karoo stream which would be more altricial.

Methods

P. afer and *P. asper* were collected monthly by means of minnow seines and scoop nets as described by Cambray (1982). The reproductive cycle was determined by measuring changes in gonadal condition over a 31 month period, October 1986 - April 1989. The following data were recorded: fork length (FL) (mm), standard length (SL) (mm), total mass (g), gonad mass (g), gonadal maturation stages, monthly development of ova, nuptial tubercle formation, egg collections from the rivers and the appearance of newly hatched free embryos. The raw data and a selection of *P. afer* and *P. asper* used in this study were lodged at the Albany Museum, Grahamstown.

Fish were blotted dry and their mass was measured to an accuracy of 10^{-3} g. Gonads of both sexes were dissected out, blotted dry and their mass was measured to an accuracy of 10^{-4} g to assess seasonal changes in sizes of ovaries which reflected the growth of the developing oocytes as they accumulated yolk.

Condition factor

The relative condition factor (K_n) was defined as:

$$K_n = Mo/Mp$$

where *Mo* was the observed mass of the fish and *Mp* was the predicted mass from the length-mass relationship (LeCren, 1951). K_n measures the deviation from the mass predicted for a fish of a given length for that population. Since K_n would be specific to a population it is useful to quantify changes in condition within a population but not for comparing the condition of fish from two or more populations (Wootton, 1990). In this study K_n was used to follow differences in total relative condition and somatic relative condition of the minnows on a monthly basis (Cambray & Bruton, 1984) and not for comparing relative condition of *P. afer* to *P. asper*.

The relationship between length and mass for *P. afer* (n = 770) and *P. asper* (n = 460) was analyzed by measuring the mass and lengths of a sample of fish collected during the winter period when somatic and total mass readings are closest. The relationship can be expressed as;

$$M = aL^b$$

Where M = mass in grams

L = SL (mm)

a and b are constants estimated by regression analysis

These curvilinear relationships for *P. afer* and *P. asper* were fitted as recommended by Sokal & Rohlf (1973).

Significance of differences between mean monthly total and somatic condition at the 95% confidence level for all length groups for both sexes of both species was tested using the Student's *t*-test. A *t*-test was used because the data set was unbalanced. The level of significance for each *t*-test was reduced by dividing the nominal level of significance by the number of individual *t*-tests performed to ensure that the overall level of significance was not higher than 5% (Miller, 1981). The data for October 1986, October 1988 and October 1989 were subjected to a one-way analysis of variance to establish whether there were differences in the prespawning condition of *P. afer* and *P. asper* females. When there were significant differences the source of variation between months was analyzed by the Scheffe multiple range test (Zar, 1984).

Gonadosomatic index (GSI)

The gonadosomatic index (GSI) was calculated for each specimen using the equation:

$$GSI = (gonad\ mass \times 100) / total\ mass\ of\ fish.$$

This index was used to describe the gonadal maturation cycle of *P. afer* and *P. asper* and if there were significant differences in the mean GSI values between months the data were subjected to a one-way analysis of variance for both males and females of each species. The source of variation between months was analyzed by the Scheffe multiple range test (Zar, 1984).

Maturation stages

The dissected gonads were assigned to stages of maturation after viewing the gonads at a magnification of 10-40 X through a stereoscopic microscope. The stages used were those of Nikolsky (1963) except that an additional stage was added for females called "partially spent" (stage 6), followed by spent (stage 7) (Table 3; Cambray & Bruton, 1984).

Median size at maturity

Median size at maturity was the length at which 50% of the catch was mature.

Seasonality of ova size

Preserved ovaries were examined from each of the ten largest female *P. asper* and *P. afer* to follow changes in mean ova size. The ovaries were put into petri dishes and the ova near the posterior end of the ovary were separated and scanned with an eyepiece micrometer in a stereoscopic microscope. The ten largest ova were selected and their diameter measured (accuracy 0,05 mm) for each female giving a total of 100 ova per month.

Table 3. Developmental stages of the gonads of *Pseudobarbus afer* and *Pseudobarbus asper* (modified from Cambray & Bruton, 1984).

Stage	Description
Stage 1 (juveniles)	Gonads small, ovary thin, translucent, without visible oocytes (X40 magnification). Testes very thin, thinner than ovaries and almost transparent.
Stage 2 (inactive)	Includes immature virgins and recovering spent fish. Ovaries small to moderate, oocytes (<0,2 mm) and distinguishable at X10 magnification. Testes thin, white and strap-like.
Stage 3 (maturing)	Ovaries noticeably enlarged and almost fill the width of the abdominal cavity. Oocytes are now visible to the naked eye. The largest (>0,6 mm) are yellowish, some have the nucleus obscured by yolk deposition, and the smallest (<0,2 mm) are still translucent. Testes are swollen and whiter.
Stage 4 (late maturing)	Ovaries almost fill the entire body cavity. There are noticeably more mature oocytes scattered throughout the ovary than at stage 3. Ovaries are a distinct yellow colour. Testes whiter and more enlarged occupying more than half the width of the body cavity. Sexual products are not extruded when light pressure is applied.
Stage 5 (ripe-running)	Gonads are at their maximum size. Ovaries now distend the body cavity. Oocytes large, translucent yellow, and when handled usually shed a few eggs with only slight pressure on the abdomen. Testes are brilliant white, width similar to stage 4, and milt is easily extruded under slight abdominal pressure.
Stage 6a (females - partially spent)	Ovary noticeably smaller than a stage 5 ovary with a smaller number of mature oocytes present. Mature ova are still scattered throughout the ovary. Recruitment oocytes, that is smaller yolked oocytes are more dominant than in previous stage. Difficult to interpret as similar to stage 4.
Stage 6b (spent males)	In males the partially spent condition could not be distinguished from stage 5. As in *B. anoplus* the freshly collected testes were slightly pink, but in preserved material only the reduced size could be noticed in some specimens. Spent males had thin testes, which were flaccid and translucent.
Stage 7 (fully spent)	An additional stage was added for the females of both species since ovaries could be classified as partially spent (stage 6) or fully spent. Ovaries small and flaccid and some retained only a few large oocytes which were granular in structure and a pale yellow (resorbing) and irregularly arranged in the ovary.

Fecundity terminology

Definitions of fecundity that are acceptable in all circumstances have not been devised (Bagenal, 1978). The number of eggs that a female spawns over a defined time period depends on the number of eggs per spawning and the number of spawnings (Bagenal, 1978; Wootton, 1979). If possible fecundity should be measured as the number of eggs produced per spawning (batch spawning) which is a function of body size and ideally should be defined as the number of viable eggs actually spawned (Foucher & Beamish 1980), which would account for incomplete spawning or interrupted maturation of cells during the trophoplasmic growth phase followed by resorption (Mason, 1985). These methods are usually not practical under field conditions, so fecundity is often measured as the number of eggs present in the ovary immediately before spawning on the assumption that few mature eggs are retained. Thus the fecundity of temperate-zone fishes is usually determined from the annual production of maturing/mature oocytes which are destined for potential release (Mason 1985).

Fecundity should be recorded as the product of the number of spawnings and the mean number of eggs per spawning in species, such as *B. anoplus*, which spawn several times within a breeding season (Cambray, 1982). The term *multiple-spawning* as used here refers to repeated reproduction within a season or year (Hubbs, 1985; Burt *et al.*, 1988). Ideally the fish should be collected immediately before spawning commences, providing that all the oocytes destined for later spawnings are mature enough to be counted. Bagenal (1968) recommended that all fish to be used for a fecundity study should be collected on the same day. The main problem in studies on fecundity of multiple-spawning fish is to distinguish between reserve and the developing oocytes (Bagenal, 1978). A common criterion has been presence or absence of yolk, with a count of yolked eggs giving the fecundity for the season.

Absolute fecundity is sometimes defined as the number of mature yolked ova in the ovary just prior to spawning (Bagenal, 1973). Cambray (1982) defined absolute fecundity of *B. anoplus* as the number of all yolked ova (mature and immature) in the ovary just prior to spawning. In the present study yolked ova in different size classes were counted as outlined below.

Fecundity methodology

Fish fecundity has traditionally been estimated by: direct count of eggs in ovaries; counts or estimates made when females are stripped of their eggs; counting the eggs in a given mass or volume of the ovary, determining the total mass or volume of the ovary and then estimating the total number of eggs present by proportion (Carlander, 1950).

In most studies on North American minnows direct counts of eggs rather than estimates have been made with minute and yolkless ova usually excluded. Phillips (1969) compared the accuracy of volumetric and gravimetric methods to direct counts of eggs for a minnow species and concluded that the best approach for small fish was to decide which ova are mature and then determine their numbers by actual counts.

There were relatively few eggs in each *Pseudobarbus* female so the direct count approach was used to determine the absolute fecundity of *P. afer* and *P. asper*. In addition closer examination could be made of different egg sizes.

Fish of different lengths were selected from data sheets whilst the mass and gonad mass section of the data sheet was covered. Only those fish were used for this study. In many studies only the females with fully extended abdomens are chosen for fecundity studies (e.g. Heins *et al.*, 1980). Cambray (1982) suggested that this method for selecting females might be misleading as only the most fecund fishes in the collection are studied.

A range in sizes of adult females for both species (*P. afer* n = 14; *P. asper* n = 14) was selected from the October collection. The ovaries were dissected out, measured to an accuracy of 10^{-4} grams and placed in vials containing 5% formalin. After the eggs had hardened, the vials were shaken until the eggs had separated. The contents were then transferred to a grooved perspex counting tray (Cambray, 1982). All counting of ova was done at 20X. All the oocytes with yolked nuclei or fully yolked ova ($\geq$ 0,2 mm), were measured with a calibrated eyepiece micrometer in a stereoscopic microscope (accuracy 0,05 mm). After measuring, the ova were grouped into seven 0,2 mm egg size categories (from $\geq$ 0,2 mm to $\geq$ 1,4 mm).

Regressions for each ova size category (where applicable) related to length and mass for both species were calculated. The following formulae were used:

$$F_{batch} = aL^b$$

where F_{batch} is batch fecundity

L is standard length

a and b are constants

Similarly the relationship between ova number (F_{batch}) and fish mass (M) can be described by the function :

$$F_{batch} = aM^b.$$

Curvilinear regression lines expressing the relationship between standard length or mass were fitted as recommended by Sokal & Rohlf (1973). A test for equality of the regression lines for both length and mass of both species was done for each egg size group (Zar, 1984).

Early life-history methodology

Adult *P. afer* were collected from the Wit River and adult *P. asper* were collected from the Groot River. Fish of both species were collected during their breeding season so that the mature eggs were nourished in the field and not under artificial conditions and diets. At the laboratory they were injected with carp pituitary at 18h00 and left overnight in covered aquaria. The parental groups consisted of 5 to 7 males and 10 to 15 females. Ova were stripped into petri plates and the testes were mashed in a separate container and immediately added to the eggs. A saline solution was not used for sperm because it might have influenced hardening and final size of egg envelopes of especially *P. afer* which come from an area of low salinity. The eggs were then put into nylon meshed hatching trays and incubated at 23°C ± 1°C in aquaria with a continuous water current passing over the eggs and an airstone under the hatching trays. The eggs were from multiparent spawnings so that there was heterogeneity. The photoperiod was controlled by an automatic timer at 13 light: 11 dark hours during incubation. Until the embryos hatched aquaria were covered with a black plastic sheet to simulate the natural spawning habitats when the eggs would be in dark areas between and under boulders. When the embryos started to hatch half of each aquaria was uncovered. Once the fish showed positive phototaxis the entire aquaria were exposed to the 13:11h photoperiod which would approximate spawning habitat photoperiods. Larval fish were fed a laboratory culture of rotifers and finely ground tropical fish food.

Sampling was continuous for the first hour after activation. Thereafter sampling times gradually decreased as fish grew and developmental events slowed. For the purpose of this study behaviour of free-embryos at hatching, age at first swim-up, and age and size at first feeding of *P. afer* and *P. asper* were seen as important comparative life-history events, and these are reported here.

The detailed comparative early ontogeny of these two species will be published separately.

Life-history terminology

Use of the terms *tactic*, *trait* and *strategy* have been criticised (eg. Balon, 1990). Strategies and tactics imply rational planning which is inappropriate in the context of evolutionary biology (Wootton, 1984). The term *style* is used here instead of *strategy*. The term *attribute* as used here is synonymous with *trait* and the term *phenotypic option* is synonymous with *tactic*.

Within a *life-history style* there are a number of *life-history attributes*, such as length at maturity. In different environments an individual may have different *phenotypic options* such as early or delayed maturity. The range of the expression of these phenotypic options is under genetic control. The variability of expression of a life-history attribute is here called *phenotypic variability* which is synonymous with the term *phenotypic plasticity* which in today's world has a throw-away connotation. *Phenotypic variability* is a term used to describe a range of *phenotypic options* available to an individual such as changes in egg size, growth rate, age at maturity etc. which are used to confront a particular environment. Reciprocal transfer experiments conducted by Mann *et al.* (1984) are a good example of a freshwater fish species demonstrating *phenotypic variability*.

Variable phenotypic trajectory is synonymous with *plastic trajectory* as used by Stearns & Crandall (1984). This *trajectory* merely reflects the *phenotypic options* which an individual has in its genome. In the models of Stearns & Crandall (1984) two life-history attributes (age and size at maturity) were considered and they established that organisms matured along a trajectory of age and size which depended on demographic conditions.

Balon (1990) presented his arguments for the use of the terms *altricial* and *precocial*, which he adopted from the accepted terminology for birds (eg. Ricklefs, 1979). The terms of *altricial* and *precocial* are used here, as parental investment per progeny was seen as an important difference between *P. afer* and *P. asper* which influences their life-history trajectories. The main attributes of the altricial form are a relatively smaller and incompletely developed young compared to larger, better developed young in the precocial forms. Balon (1990) noted that the differences between altricial and precocial forms in ontogeny are small, with the generalist a little more inclined towards attributes of altriciality and the specialist more inclined towards the attributes of precociality. It has been recommended that it should be made clear whether these terms are used to refer to a intraspecific life-history dichotomy or used in an interspecific sense (Balon, 1990; see his Figure 20). Following from Chapter 3 which outlined Skelton's (1980a, 1988) work these terms will be used in an interspecific sense when referring to *P. afer* and *P. asper*. Balon (1990) has also suggested that these terms reflect the effect behind the mechanism of epigenetic bifurcation and the importance of the interplay between environment and the genome.

Alprehost is short for "Altricial ⇌ Precocial Homeorhetic States" which Balon (1990: 31) has suggested "... is the cause of evolutionary patterns" and the ontogeny of each taxon is created in a sequence of these alternative altricial to precocial states. *Alprehost* is best described in Balon (1990; his Figure 20).

Reproductive style terminology

Breder & Rosen (1966) and Balon (1975a, 1981) have classified modes of reproduction of teleost fishes. Breder & Rosen (1966) divided fish taxa into seven main divisions based upon number of anatomical, developmental and behaviour characters. An ecological classification of 32 guilds which are descriptive categories was proposed by Balon (1975a, 1981). Potts (1984) noted that these classifications are helpful in relating species to their environment, but there are problems when explaining behaviourial change or intraspecific variability.

The classification of the reproductive styles of the two redfin minnows follows the comprehensive classification of Balon (1975a, 1981). This classification is based on spawning site, adaptations of eggs and embryos to the site and degree of parental care as outlined in the introduction to this chapter.

Results

Condition factor

The condition factor for *P. afer* collected in winter was described by the equation:

$$M = -11{,}1866L^{3{,}13683}$$

$$(n = 772, r = 0{,}9977)$$

and for *P. asper* by:

$$M = -11{,}0504L^{3{,}08024}$$

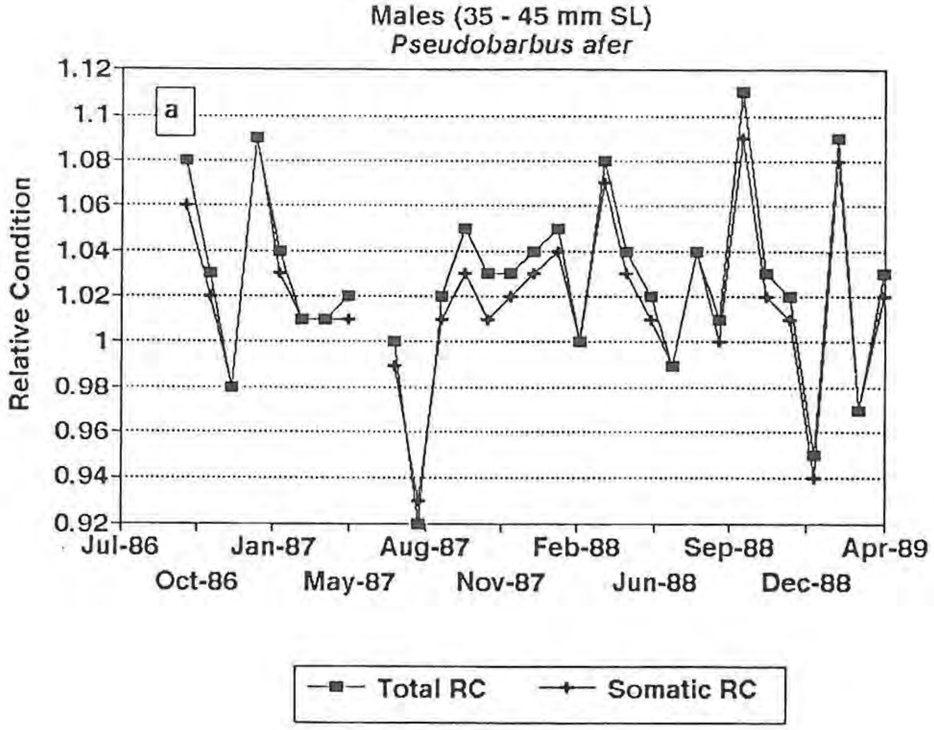
$$(n = 460, r = 0{,}9919)$$

The total and somatic relative condition factors during the study period for the length groups 35-45 mm SL and >45 mm SL are shown in Figures 24 - 27.

In male *P. afer* (35-45 mm SL) there was no significant difference between the somatic and total condition (Figure 24a). A similar seasonal fluctuating pattern occurred in the small female *P. afer* with no significant differences between somatic and total condition for the entire period (Figure 24b). The changes in condition factor of the small *P. asper* males followed the same seasonal pattern with winter lows and early summer highs and no significant difference between the total and somatic condition (Figure 25a). In contrast the small female *P. asper* showed significant differences between the somatic and relative condition between September and December 1987 but remained relatively stable in the winter months (Figure 25b and Table 4).

In the larger male *P. afer* (> 45 mm SL) there were no significant differences between somatic and total condition during the study period but a very similar seasonal trend as was seen in the smaller males was apparent with the low August 1987 and high October 1988 condition factors (compare Figures 24a and 26a). In both years there was a decrease in condition during December (post breeding period) followed by an increase in January. There was also a seasonality in the condition of the large male *P. asper* with the highest condition during November followed by a decrease during the breeding season of both somatic and total condition (Figure 27a). There were no significant differences between somatic and relative conditions during the study period.

In the female *P. afer* (> 45 mm SL) there was a seasonal trend in condition and significant differences between somatic and relative condition indices during October to November 1986, September to October 1987 and October 1989 (Figure 26b; Table 5). These periods were



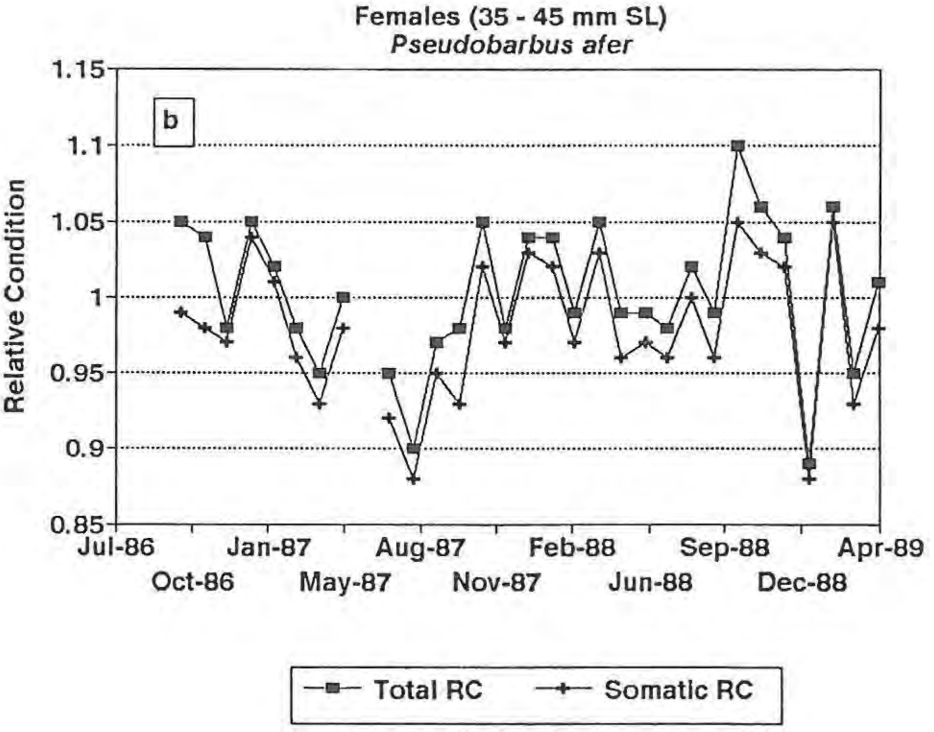


Figure 24. Monthly changes in the mean total condition compared to the mean somatic condition of (a) *P. afer* males and (b) *P. afer* females of 35-45mm SL, (for statistical tests see text).

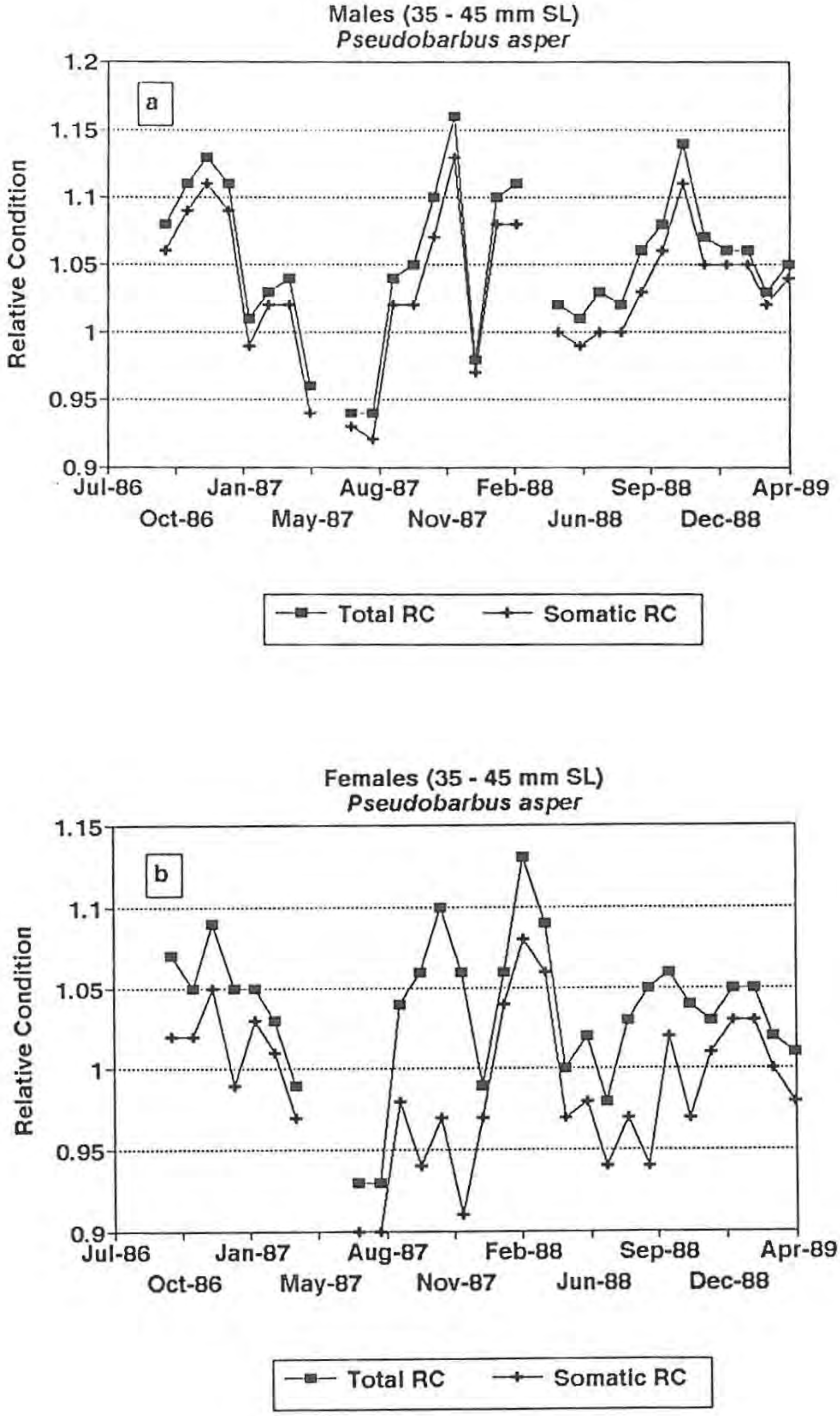


Figure 25. Monthly changes in the mean total condition compared to the mean somatic condition of (a) *P. asper* males and (b) *P. asper* females of 35-45mm SL, (for statistical tests see text).

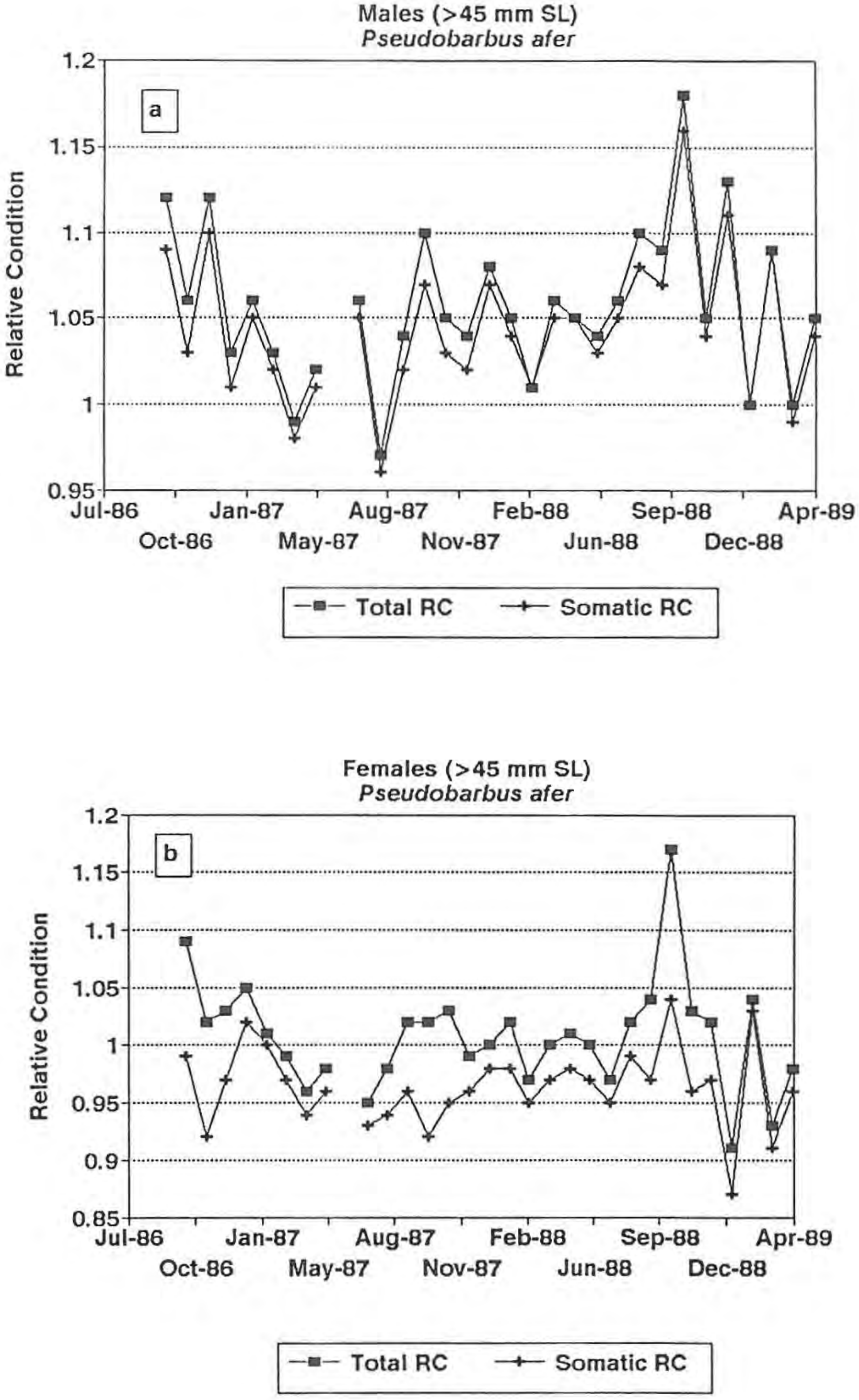


Figure 26. Monthly changes in the mean total condition compared to the mean somatic condition of (a) *P. afer* males and (b) *P. afer* females of greater than 45mm SL, (for statistical tests see text).

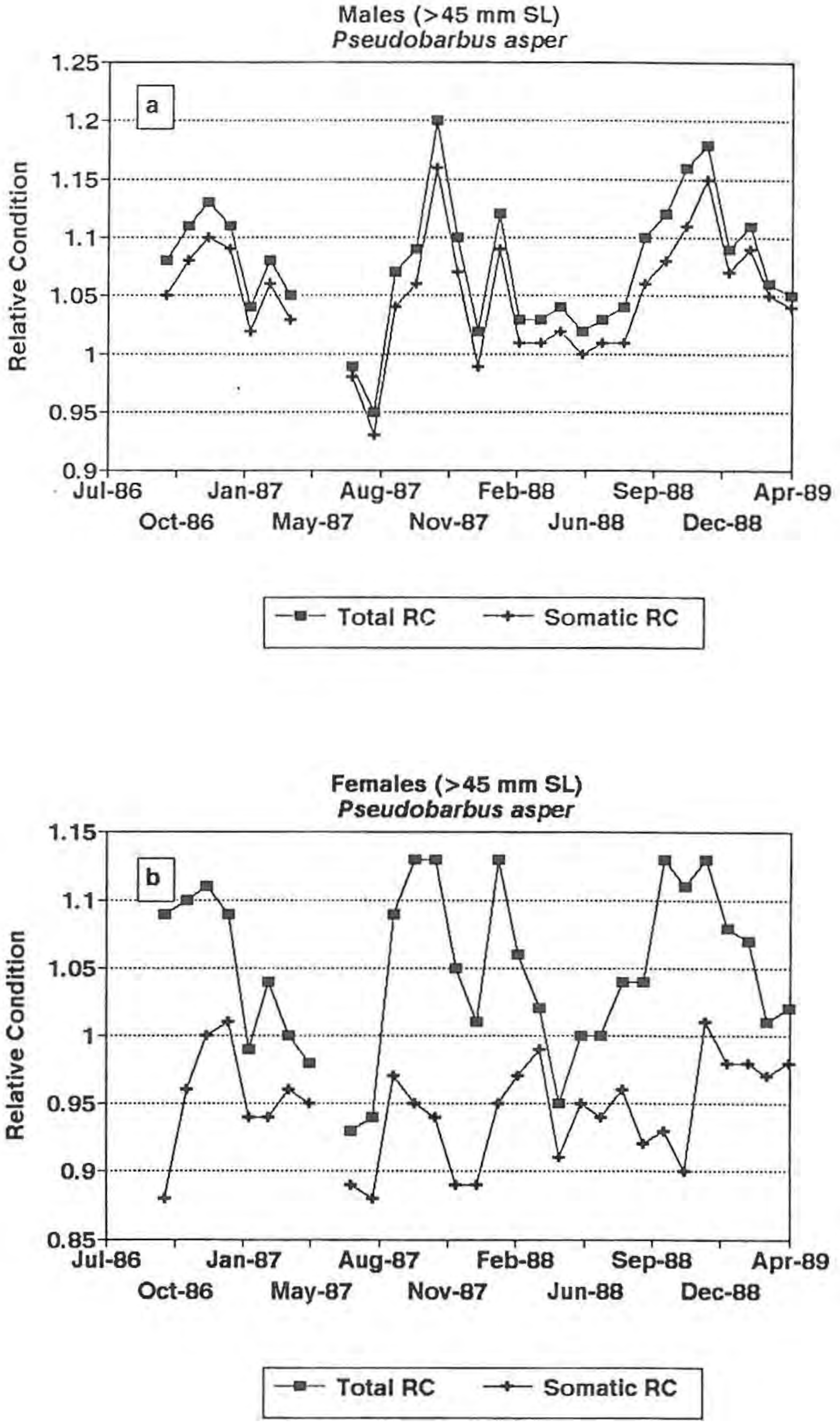


Figure 27. Monthly changes in the mean total condition compared to the mean somatic condition of (a) *P. asper* males and (b) *P. asper* females of greater than 45mm SL, (for statistical tests see text).

Table 4. Analysis of the difference between the mean monthly somatic relative condition compared to the mean monthly total relative condition of *P. asper* females 35 - 45 mm SL (95% confidence interval, significance level adjusted, Miller [1981]).

Date	DF	*t*	Significance 5% level
Oct. 1986	4	2,3207	n.s.
Nov.	54	1,9913	n.s.
Dec.	24	2,3931	n.s.
Jan. 1987	6	0,9187	n.s.
Feb.	12	2,4519	n.s.
Mar.	40	1,0843	n.s.
Apr.	84	2,1075	n.s.
July	16	1,315	n.s.
Aug.	56	2,2555	n.s.
Sept.	112	3,7581	*
Oct.	86	9,0815	*
Nov.	32	6,0926	*
Dec.	14	4,4874	*
Jan. 1988	16	0,8865	n.s.
Feb.	74	1,4922	n.s.
Mar.	2	1,5358	n.s.
Apr.	8	1,35	n.s.
May	30	1,9781	n.s.
June	16	1,5258	n.s.
July	34	2,0655	n.s.
Aug.	38	2,2736	n.s.
Sept.	12	2,871	n.s.
Oct.	20	1,4022	n.s.
Nov.	8	0,435	n.s.
Dec.	162	2,0	n.s.
Jan. 1989	182	2,5352	n.s.
Feb.	70	1,7811	n.s.
Mar.	116	1,818	n.s.
Apr.	66	1,937	n.s.

Table 5. Analysis of the difference between the mean monthly somatic relative condition compared to the mean monthly total relative condition of *P. afer* females greater than 45 mm SL (95% confidence interval; significance level adjusted, Miller [1981]).

Date	DF	*t*	Significance 5% level = *
Oct. 1986	58	5,8874	*
Nov.	40	6,1197	*
Dec.	34	2,7446	n.s.
Jan. 1987	12	1,7724	n.s.
Feb.	42	0,9174	n.s.
Mar.	22	1,3684	n.s.
Apr.	32	1,4272	n.s.
May	24	1,2583	n.s.
July	10	1,4881	n.s.
Aug.	78	2,1812	n.s.
Sept.	62	4,1076	*
Oct.	70	7,6493	*
Nov.	42	3,2502	n.s.
Dec.	50	0,9923	n.s.
Jan. 1988	10	0,6112	n.s.
Feb.	62	2,8318	n.s.
Mar.	28	0,9706	n.s.
Apr.	58	1,7906	n.s.
May	32	1,274	n.s.
June	44	1,7935	n.s.
July	74	2,5703	n.s.
Aug.	36	1,3441	n.s.
Sept.	30	3,1868	n.s.
Oct.	28	4,5865	*
Nov.	26	2,8203	n.s.
Dec.	18	1,362	n.s.
Jan. 1989	36	2,6955	n.s.
Feb.	28	0,8099	n.s.
Mar.	52	1,2323	n.s.
Apr.	28	0,9762	n.s.

followed by a loss of both somatic and total condition followed by increases in either January (1987) or February (1989). As with both length groups of male *P. afer* the highest total relative condition occurred during October 1988 which was highly significantly different from October 1987.

In contrast the large female *P. asper* had longer periods of high total condition followed by post spawning lows February 1986, December - January 1987 and November 1988 (Figure 27b). There were significant differences for 20 months between the somatic and total conditions (Table 6). There were no significant differences between the prespawning high total condition indices for the three years for female *P. asper* (> 45 mm SL) unlike the significant differences between October 1987 and October 1988 for the total condition of the *P. afer* females (Table 7).

Maturation stages

Maturation stages for *P. afer* and *P. asper* for the study period are shown in Figures 28 -31. Seasonality of both species was clearly apparent and *P. asper* had a longer breeding season. *P. afer* could breed from October to February whereas *P. asper* could breed from October to as late as April.

Gonadosomatic index (GSI)

In both sexes of both species there was a seasonal pattern of gonadal activity. The proportion of ripe fish was greatest from October to November for *P. afer* and from October to February for *P. asper* (Figures 32 & 33).

In both species the mean GSI values for females were significantly higher during the breeding season than at other times of the year (Tables 8 - 9). In the males and females of *P. afer* and *P. asper* there were no significant differences between the three October periods in GSI values (Tables 8,10 & 11).

An analysis of the differences between the mean monthly GSI values of female *P. afer* and *P. asper* showed that there were significant differences for most months (Table 12). The difference was not as pronounced but still evident in males with significant differences for 15 months

Table 6. Analysis of the difference between the mean monthly somatic relative condition compared to the mean monthly total relative condition of *P. asper* females greater than 45 mm SL (95% confidence interval; significance level adjusted, Miller, 1981).

Date	DF	*t*	Significance 5% level = *
Oct. 1986	28	7,3863	*
Nov.	28	5,4884	*
Dec.	104	6,7777	*
Jan. 1987	18	3,05	n.s.
Feb.	38	2,4255	n.s.
Mar.	12	2,4519	n.s.
Apr.	6	0,7626	n.s.
July	24	2,3956	n.s.
Aug.	46	4,0163	*
Sept.	34	6,1677	*
Oct.	14	5,5362	*
Nov.	64	10,0512	*
Dec.	176	14,4234	*
Jan. 1988	72	8,0551	*
Feb.	92	13,5711	*
Mar.	28	3,7428	*
Apr.	74	1,6614	n.s.
May	78	2,0785	*
June	98	3,9372	*
July	50	4,0438	*
Aug.	54	4,1581	*
Sept.	44	4,2193	*
Oct.	22	5,4733	*
Nov.	64	13,4078	*
Dec.	90	7,3331	*
Jan. 1989	14	3,1159	n.s.
Feb.	36	4,5097	*
Mar.	68	2,4484	n.s.
Apr.	48	0,9331	n.s.

(Table 13). *P. asper* males and females therefore allocate significantly more resources to their gonads than do *P. afer* males and females.

Table 7. Test of significance (95% confidence level) between the prespawning period (October 1986, October 1987 and October 1988) total relative condition of *P. afer* and *P. asper* females greater than 45 mm SL (ANOVA and Scheffe methods; * = significant).

Date	n	Significance
P. afer		
October 1986/ October 1987	38	n.s.
October 1986/ October 1988	20	n.s.
October 1987/ October 1988	32	*
P. asper		
October 1986/ October 1987	23	n.s.
October 1986/ October 1988	48	n.s.
October 1987/ October 1988	41	n.s.

The changes in the GSI values for female *P. asper* and *P. afer* are shown in Figures 32a & b. The highest GSI recorded was for a *P. asper* female (69,67 mm SL) collected during October 1986 (GSI = 28,13). In contrast the highest GSI for a *P. afer* female (57,78 mm SL) was 15,46 and this specimen was collected during October 1987. The area under the GSI curve for *P. asper* (Figure 32) between September 1987 and April 1988 is 2,87 times that of the area under the *P. afer* GSI curve for the same period. In the following season, September 1988 to April 1989, this figure was 2,32 indicating higher reproductive effort in *P. asper*. The pattern in the reproductive seasonality of female *P. asper* showed an increase in gonad mass which commenced in May and reached a peak in the October - November period during which spawning occurred. In the summer of 1987/88 the protracted spawning period was from October to April. The possibility of a spawning as late as April was confirmed in April 1989 when an artificial release of water from Beervlei Dam was sufficient to trigger spawning (Cambray, 1991a). *P. asper* was therefore able to spawn over a 6-7 month period. In contrast the *P. afer* population in the Wit River had

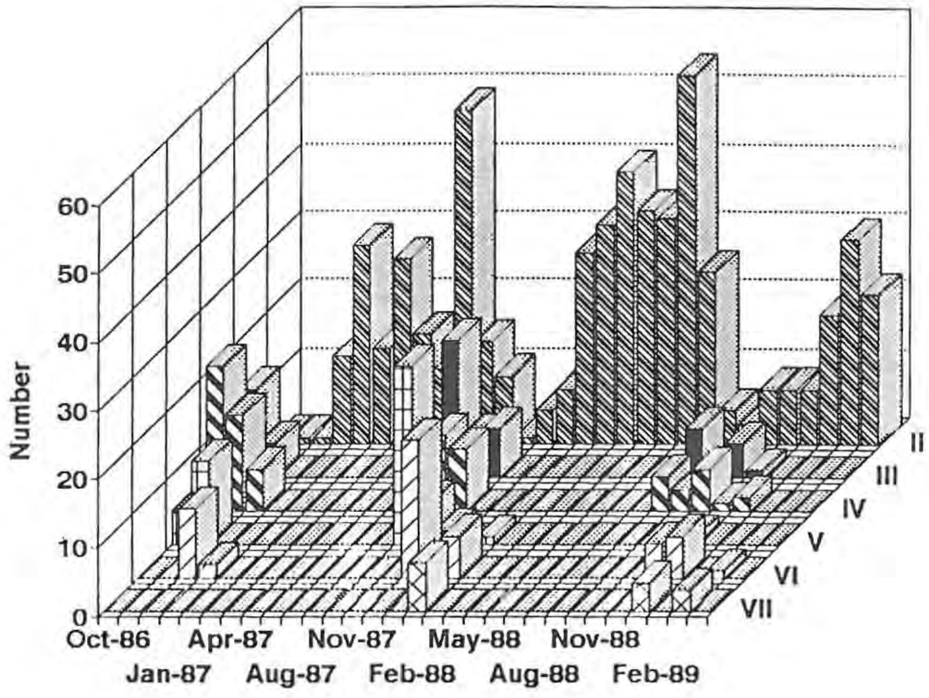


Figure 28. Number of ***Pseudobarbus afer*** females at each developmental stage (II-VII as described in Table 3), caught per month. Maturity stage I (immatures) are not included here as they are uninformative for seasonal maturity trends.

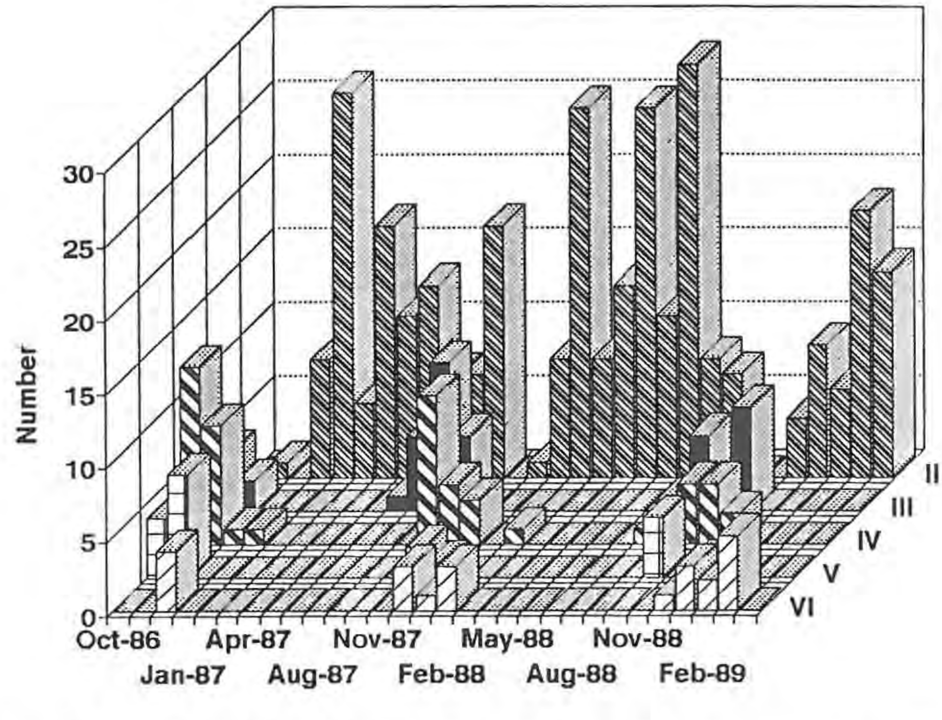


Figure 29. Number of ***Pseudobarbus afer*** males at each developmental stage (II-VI as described in Table 3), caught per month. Maturity stage I (immatures) are not included here as they are uninformative for seasonal maturity trends.

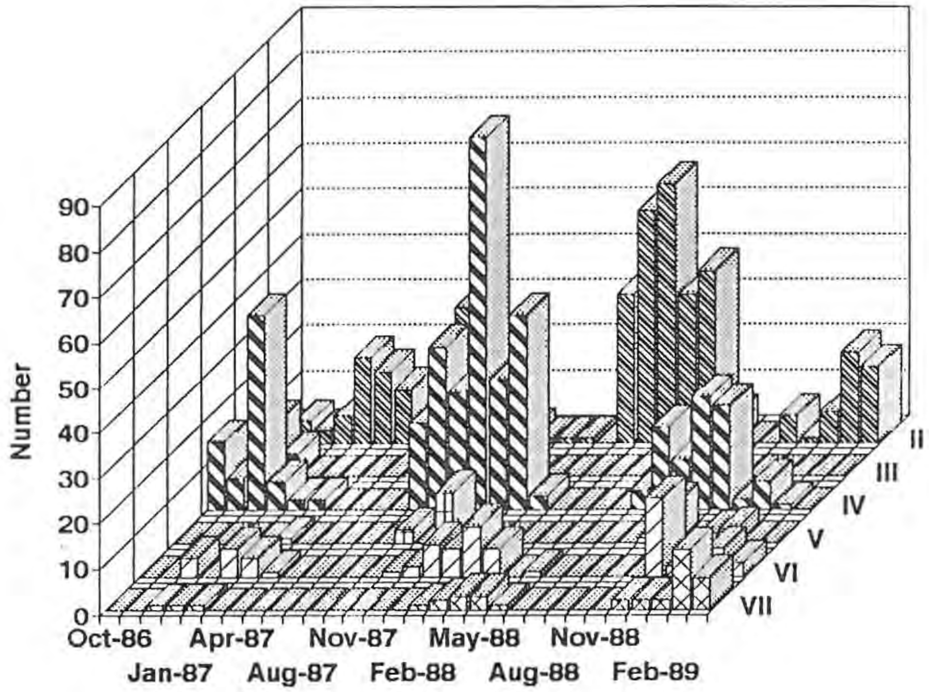


Figure 30. Number of *Pseudobarbus asper* females at each developmental stage (II-VII as described in Table 3), caught per month. Maturity stage I (immatures) are not included here as they are uninformative for seasonal maturity trends.

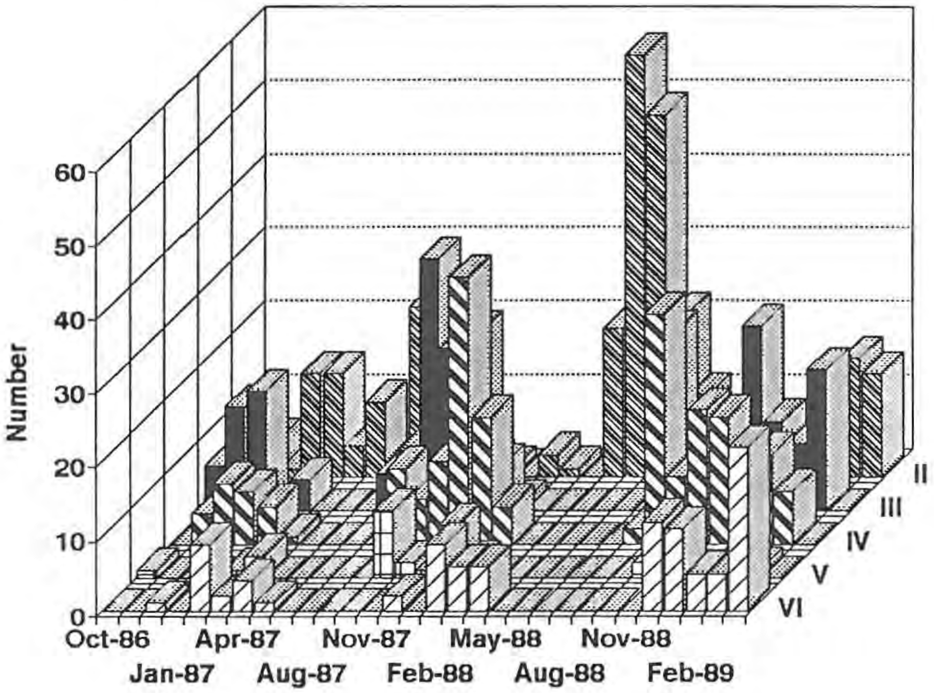


Figure 31. Number of *Pseudobarbus asper* males at each developmental stage (II-VI as described in Table 3), caught per month. Maturity stage I (immatures) are not included here as they are uninformative for seasonal maturity trends.

a briefer reproductive season (Figure 32b) during each of the three seasons investigated. The population had usually finished breeding by February (1987, 1989), however, some individuals were still capable of spawning in February 1988. The reproductive season was therefore limited to 4-5 months with the major spawning during October-November.

Table 8. Analysis of the differences between the mean monthly gonadosomatic indices of female *P. asper* over 45 mm SL (95% confidence interval, ANOVA and Scheffe methods).

Date	n	P
Oct.1986/Nov.1986	30	n.s.
Oct.1986/Dec.1986	68	P<0,01
Oct.1986/Jan.1987	25	P<0,01
Oct.1986/Feb.1987	35	P<0,01
Oct.1986/Mar.1987	22	n.s.
Oct.1986/Apr.1987	19	P<0,01
Oct.1986/Jul.1987	28	P<0,01
Oct.1986/Aug.1987	39	P<0,01
Oct.1986/Sep.1987	33	P<0,05
Oct.1986/Oct.1987	23	n.s.
Oct.1987/Nov.1987	40	n.s.
Oct.1987/Dec.1987	97	n.s.
Oct.1987/Jan.1988	45	n.s.
Oct.1987/Feb.1988	55	n.s.
Oct.1987/Mar.1988	23	n.s.
Oct.1987/Apr.1988	46	P<0,01
Oct.1987/May 1988	48	P<0,01
Oct.1987/Jun.1988	58	P<0,01
Oct.1987/Jul.1988	34	P<0,01
Oct.1987/Aug.1988	36	n.s.
Oct.1987/Sep.1988	31	n.s.
Oct.1987/Oct.1988	19	n.s.
Oct.1988/Nov.1988	44	n.s.
Oct.1988/Dec.1988	57	P<0,05
Oct.1988/Jan.1989	19	n.s.
Oct.1988/Feb.1989	30	P<0,01
Oct.1988/Mar.1989	46	P<0,01
Oct.1988/Apr.1989	36	P<0,01
Oct.1986/Oct.1988	26	n.s.

Table 9. Analysis of the differences between the mean monthly gonadosomatic indices of female *P. afer* over 45 mm SL (95% confidence interval, ANOVA and Scheffe methods).

Date	n	P
Oct.1986/Nov.1986	51	n.s.
Oct.1986/Dec.1986	48	n.s.
Oct.1986/Jan.1987	37	$P<0{,}01$
Oct.1986/Feb.1987	52	$P<0{,}01$
Oct.1986/Mar.1987	42	$P<0{,}01$
Oct.1986/Apr.1987	47	$P<0{,}01$
Oct.1986/May 1987	43	$P<0{,}01$
Oct.1986/Jul.1987	36	$P<0{,}05$
Oct.1986/Aug.1987	70	$P<0{,}01$
Oct.1986/Sep.1987	62	n.s.
Oct.1986/Oct.1987	66	n.s.
Oct.1987/Nov.1987	58	$P<0{,}05$
Oct.1987/Dec.1987	62	$P<0{,}01$
Oct.1987/Jan.1988	43	$P<0{,}01$
Oct.1987/Feb.1988	68	$P<0{,}01$
Oct.1987/Mar.1988	51	$P<0{,}01$
Oct.1987/Apr.1988	66	$P<0{,}01$
Oct.1987/May 1988	53	$P<0{,}01$
Oct.1987/Jun.1988	59	$P<0{,}01$
Oct.1987/Jul.1988	74	$P<0{,}01$
Oct.1987/Aug.1988	55	$P<0{,}01$
Oct.1987/Sep.1988	52	$P<0{,}01$
Oct.1987/Oct.1988	51	n.s.
Oct.1988/Nov.1988	29	n.s.
Oct.1988/Dec.1988	25	$P<0{,}01$
Oct.1988/Jan.1989	34	$P<0{,}01$
Oct.1988/Feb.1989	30	$P<0{,}01$
Oct.1988/Mar.1989	42	$P<0{,}01$
Oct.1988/Apr.1989	30	$P<0{,}01$
Oct.1986/Oct.1988	45	n.s.

Table 10. Analysis of the differences between the mean monthly gonadosomatic indices of male *P. asper* over 45 mm SL (95% confidence interval, ANOVA and Scheffe methods).

Date	n	P
Oct.1986/Nov.1986	12	n.s.
Oct.1986/Dec.1986	28	n.s.
Oct.1986/Jan.1987	15	n.s.
Oct.1986/Feb.1987	28	n.s.
Oct.1986/Mar.1987	11	n.s.
Oct.1986/May 1987	9	n.s.
Oct.1986/Jul.1987	27	n.s.
Oct.1986/Aug.1987	25	n.s.
Oct.1986/Sep.1987	16	n.s.
Oct.1986/Oct.1987	12	n.s.
Oct.1987/Nov.1987	28	n.s.
Oct.1987/Dec.1987	49	n.s.
Oct.1987/Jan.1988	32	n.s.
Oct.1987/Feb.1988	19	n.s.
Oct.1987/Mar.1988	13	n.s.
Oct.1987/Apr.1988	33	n.s.
Oct.1987/May 1988	49	n.s.
Oct.1987/Jun.1988	40	n.s.
Oct.1987/Jul.1988	24	n.s.
Oct.1987/Aug.1988	28	n.s.
Oct.1987/Sep.1988	38	n.s.
Oct.1987/Oct.1988	19	n.s.
Oct.1988/Nov.1988	31	n.s.
Oct.1988/Dec.1988	40	n.s.
Oct.1988/Jan.1989	30	n.s.
Oct.1988/Feb.1989	31	$P<0,05$
Oct.1988/Mar.1989	40	$P<0,01$
Oct.1988/Apr.1989	36	$P<0,01$
Oct.1986/Oct.1988	17	n.s.

Table 11. Analysis of the differences between the mean monthly gonadosomatic indices of male *P. afer* over 45 mm SL (95% confidence interval, ANOVA and Scheffe methods).

Date	n	P
Oct.1986/Nov.1986	31	n.s.
Oct.1986/Dec.1986	20	n.s.
Oct.1986/Jan.1987	18	n.s.
Oct.1986/Feb.1987	33	$P<0,01$
Oct.1986/Mar.1987	18	n.s.
Oct.1986/Apr.1987	21	$P<0,05$
Oct.1986/Jul.1987	29	$P<0,01$
Oct.1986/Aug.1987	22	$P<0,05$
Oct.1986/Sep.1987	25	n.s.
Oct.1986/Oct.1987	40	n.s.
Oct.1987/Nov.1987	34	n.s.
Oct.1987/Dec.1987	33	$P<0,05$
Oct.1987/Jan.1988	33	$P<0,01$
Oct.1987/Feb.1988	42	$P<0,01$
Oct.1987/Mar.1988	30	$P<0,01$
Oct.1987/Apr.1988	35	$P<0,01$
Oct.1987/May 1988	37	$P<0,01$
Oct.1987/Jun.1988	32	$P<0,01$
Oct.1987/Jul.1988	41	$P<0,01$
Oct.1987/Aug.1988	31	n.s.
Oct.1987/Sep.1988	33	n.s.
Oct.1987/Oct.1988	32	n.s.
Oct.1988/Nov.1988	18	n.s.
Oct.1988/Dec.1988	10	n.s.
Oct.1988/Jan.1989	16	n.s.
Oct.1988/Feb.1989	13	n.s.
Oct.1988/Mar.1989	27	n.s.
Oct.1988/Apr.1989	16	n.s.
Oct.1986/Oct.1988	22	n.s.

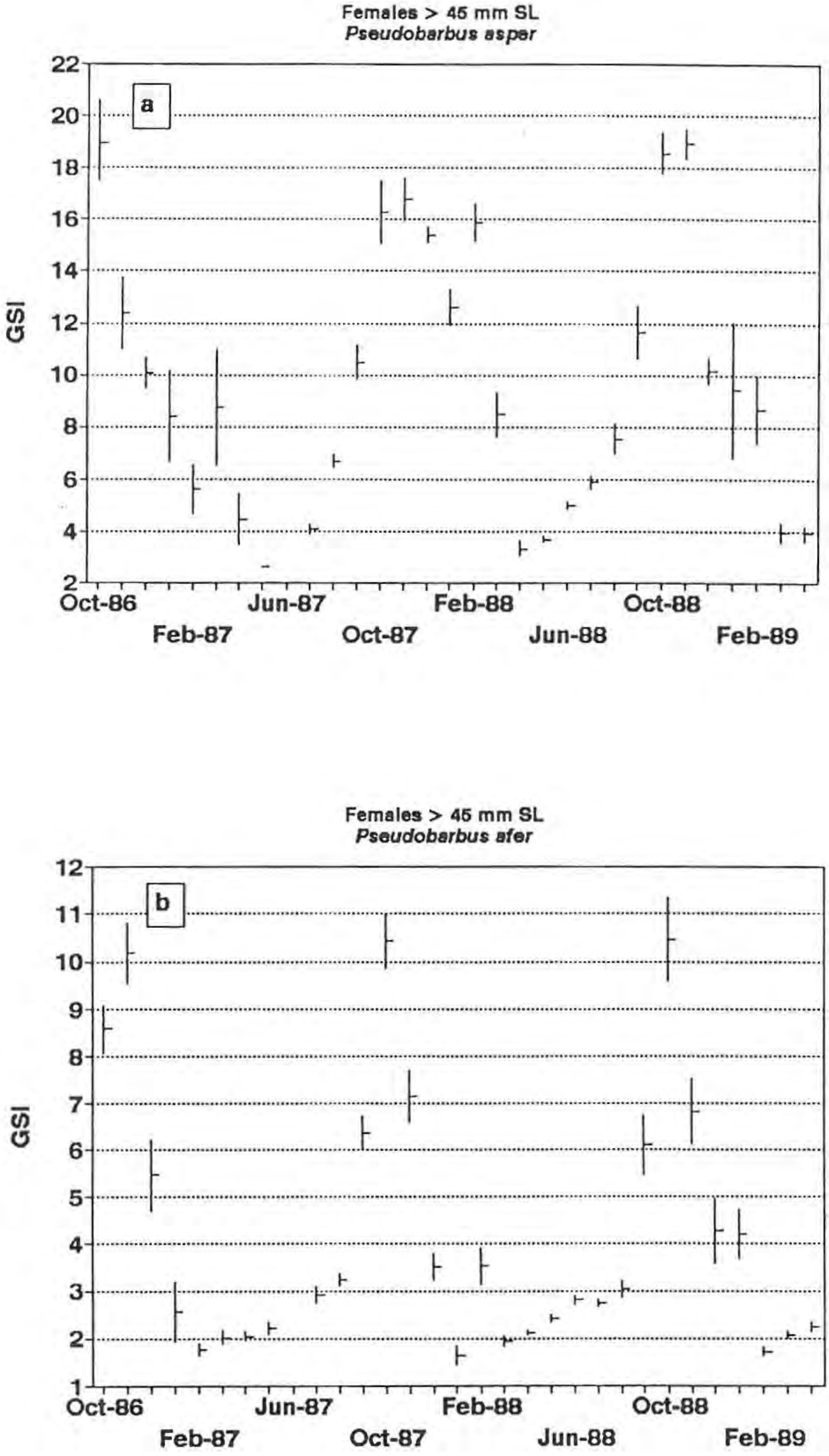
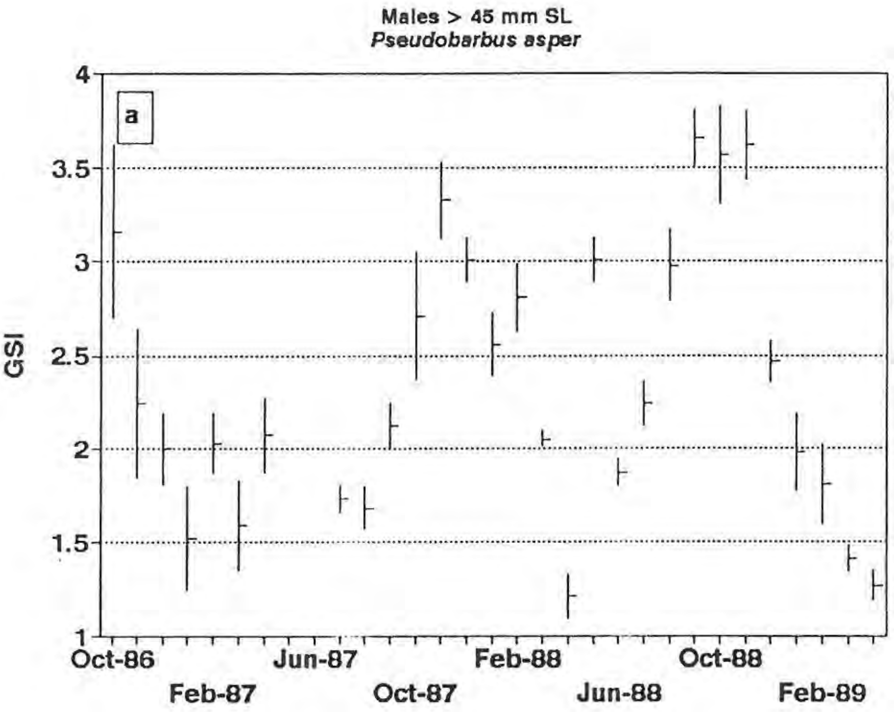


Figure 32. Monthly mean gonadosomatic indices for mature female (a) *P. asper* (n=790) collected in the Groot River and (b) *P. afer* (n=615) collected in the Wit River October 1986 to April 1989. Mean and ± one standard error.



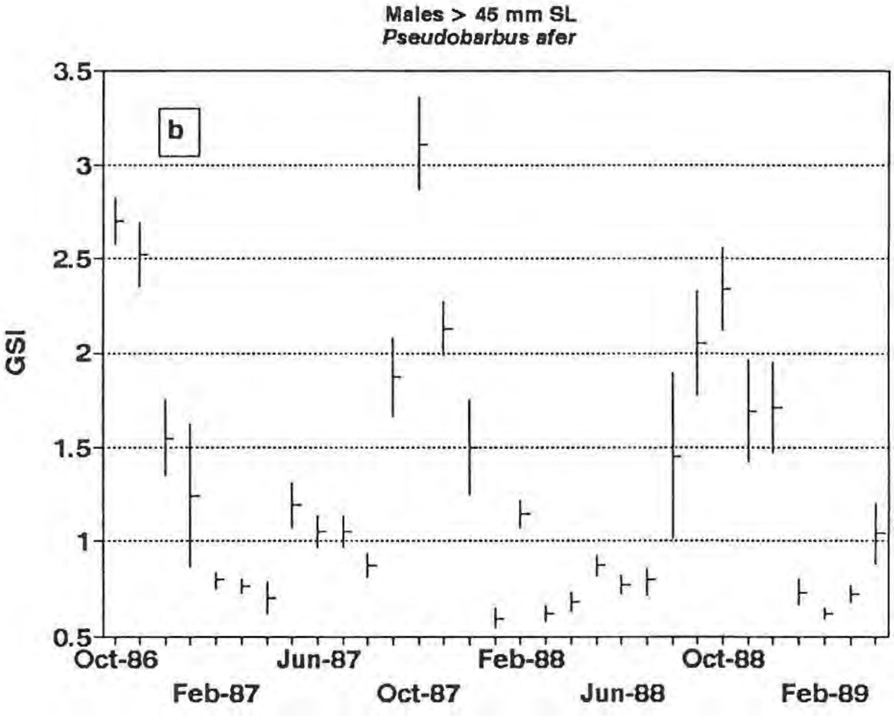


Figure 33. Monthly mean gonadosomatic indices for mature male (a) *P. asper* (n=563) collected in the Groot River and (b) *P. afer* (n=299) collected in the Wit River October 1986 to April 1989. Mean and ± one standard error.

Table 12. Analysis of the differences between the mean monthly gonadosomatic indices of female *P. afer* and *P. asper* over 45 mm SL (95% confidence interval, significance level adjusted Miller [1981]).

Date	DF	*t*	Significance 5% level = *
Oct.1986	43	7,5135	*
Nov.	34	1,561	n.s.
Dec.	69	4,2798	*
Jan.1987	15	2,5251	n.s.
Feb.	40	4,1377	*
Mar.	17	3,6926	n.s.
Apr.	19	4,438	*
July	17	3,6985	n.s.
Aug.	62	14,0502	*
Sept.	48	6,0436	*
Oct.	42	4,2109	*
Nov.	53	8,5645	*
Dec.	113	20,1122	*
Jan.1988	41	6,3247	*
Feb.	77	13,3537	*
Mar.	28	7,475	*
Apr.	66	3,9552	*
May	55	6,8115	*
June	71	10,5209	*
July	62	13,8621	*
Aug.	45	6,3529	*
Sept.	37	4,0974	*
Oct.	24	6,2884	*
Nov.	45	12,4292	*
Dec.	54	5,2061	*
Jan.1989	25	2,8995	n.s.
Feb.	32	4,6059	*
Mar.	60	4,3781	*
Apr.	38	4,5446	*

The seasonal pattern in GSI values for male *P. asper* and *P. afer* are shown in Figures 33a & b. There was sexual dimorphism in gonad sizes of both redfin minnow species, which is common for fish, and less energy was put into the maturation of male gonads. The highest GSI recorded for a *P. asper* male (44,39 mm SL) collected during November 1987 was 5,58. The highest GSI for a male *P. afer* (70,84 mm SL) collected in October 1987 was 5,1. The higher GSI for *P. asper* females was not apparent in the *P. asper* compared to *P. afer* males. However, the area under the GSI curves (Figure 33) for *P. asper* males between September 1987 to April 1988 was 1,85 times that of *P. afer* for the same time period. In the following season, September 1988 to April 1989 the figure was 1,83.

Table 13. Analysis of the differences between the mean monthly gonadosomatic indices of male *P. afer* and *P. asper* over 45 mm SL (95% confidence interval, significance level adjusted Miller [1981]).

Date	**DF**	***t***	**Significance 5% level = ***
Nov.	21	0,7232	n.s.
Dec.	26	1,0156	n.s.
Feb.1987	39	6,4934	*
Mar.	7	2,1575	n.s.
Apr.	8	6,4647	*
July	34	5,6664	*
Aug.	25	3,9435	*
Sept.	19	0,996	n.s.
Oct.	30	0,7974	n.s.
Nov.	28	3,6111	*
Dec.	48	6,4138	*
Feb.1988	27	9,1516	*
Mar.	9	2,9683	n.s.
Apr.	34	2,6091	n.s.
May	52	5,2201	*
June	73	3,744	*
July	31	10,2413	*
Aug.	25	3,368	*
Sept.	37	4,9673	*
Oct.	17	3,0899	n.s.
Nov.	27	5,9974	*
Dec.	29	2,199	n.s.
Feb.1989	23	3,1251	*
March	46	7,1501	*
April	30	1,3784	n.s.

In a review of the gonadosomatic indices for African minnows *P. afer* had the lowest (15,5%) and *P. burchelli* had the highest (33%) (Table 14). These high indices occurred in females during either October or November, that is, at the start of the reproductive season.

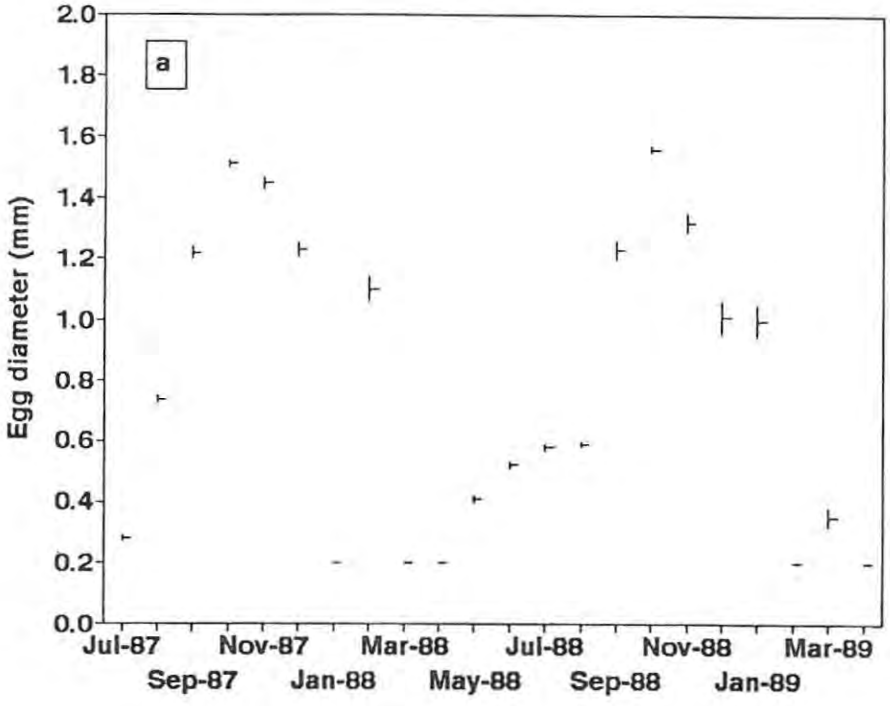
Table 14. Review of some of the recorded gonadosomatic indices of male and female African minnows in the genera *Barbus* and *Pseudobarbus*. Highest value recorded as well as mean value for month with highest indices (in brackets). These readings are approximate as they are taken from the graphs presented unless the GSI had been written out in the text.

Species	♂♂ GSI	♀♀ GSI	Reference
B. anoplus	4,5 (3)	22,6 (13)	Cambray & Bruton (1984)
B. trevelyani	(2,4)	(28)	Gaigher (1975)
P. afer	5,1 (3,1)	15,5 (10,5)	This study
P. asper	5,6 (3,7)	28,1 (19)	This study
P. burchelli	6	33	Cambray & Stewart (1985)

Seasonality of ova sizes

The seasonal changes in size of the largest ova of the two species is shown in Figures 34a & b. *P. afer* had consistently larger ova with the largest diameter up to 1,8 mm whereas the largest ova for *P. asper* was 1,3 mm. There was a highly significant difference between the egg diameters of *P. asper* and *P. afer* during both October 1987 ($t = 20,684$, $P < 0,001$, $DF = 198$) and October 1988 ($t = 28,283$; $P < 0,001$; $DF = 198$).

The major proportion of mature ova for *P. afer* in October 1987 had a mean egg diameter of 1,51 mm (n = 100; SE = 0,01) in diameter and in October 1988 1,56 mm (n = 100; SE = 0,01), and for *P. asper* 1,12 mm (n = 100; SE = 0,001) in October 1987 and 1,14 mm (n = 100; SE = 0,01) in October 1988. Assuming that the egg was spherical a 1,5 mm diameter egg would have a volume of 1,77 mm^3 whereas that of a 1,1 mm ovum of *P. asper* would be 0,7 mm^3. A *P. afer* ovum of 1,5 mm in diameter is 2,53 X the volume of a 1,1 mm *P. asper* ovum. The largest mean ovum volume for the 10 largest ova from the 10 largest *P. afer* occurred in October



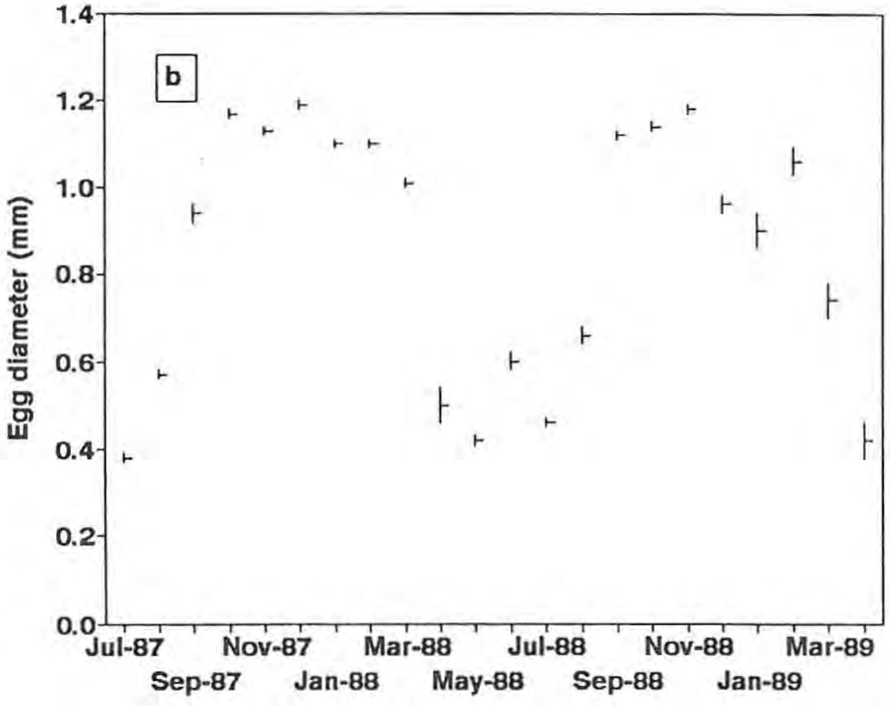


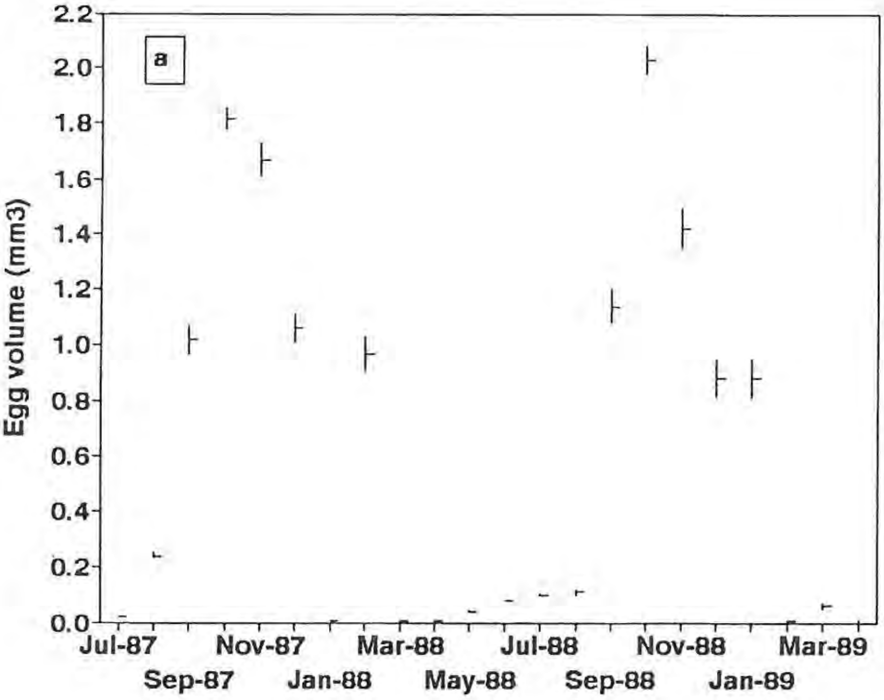
Figure 34. Seasonal changes in diameter of the largest ova in (a) *P. asper* (n=219 fish; n=2190 ova) and (b) *P. afer* (n=207 fish; n=2050 ova) females over the time period 12 July 1987 to 23 April 1989. Mean and ± one standard error.

1987 (1,82 mm^3) and in October 1988 (2,03 mm^3) (Figure 35b). For *P. asper* the highest mean ovum volume occurred in December 1987 (0,89 mm^3) and in November 1988 (0,86 mm^3) (Figure 35a). For October 1987 the difference in mean egg volume was a ratio of 2:1 (*P. afer* : *P. asper*) and for 1988 this ratio was 2,4:1. There were highly significant differences in egg volume between the two species in October 1987 (t = 20,777; P < 0,001; DF = 198) and October 1988 (t = 23,722; P < 0,001; DF = 198). In the extreme range the largest *P. afer* ovum had a volume of 3,05 mm^3 whereas the same figure for *P. asper* was 1,15 mm^3 (Figure 35) which was a ratio of 2,7:1 similar to the mean ovum volume ratios mentioned above.

Comparative ova size frequency differences between ripe female *P. afer* and *P. asper* collected during October 1988 demonstrated the smaller ova size of *P. asper* as compared to that of *P. afer* (Figure 36). In each species there was a high percentage of oocytes with yolked nuclei which would have had the potential to become mature ova. A similar comparison of ova size frequency differences for fish collected during February 1989 indicated that there was a higher percentage of oocytes with yolked nuclei in *P. afer* than for *P. asper* (Figure 37).

Length - fecundity relationship

The regression statistics for body length and number of ova in various size classes indicated a highly significant relationship in all groups especially those greater than 0,4 mm for both *P. afer* and *P. asper* (Tables 15 & 16). When all the yolked nuclei ≥ 0,2 mm in diameter were counted the highest absolute fecundity recorded was 4771 in a 63,7 mm SL *P. asper* collected on 28 October 1989. The highest absolute fecundity of a *P. afer* (73,1 mm SL) was 3922 of which only 783 (20%) were ≥ 0,8mm. For the above-mentioned *P. asper* this figure was 1662 (35%). As indicated in the previous section many of the recruitment ova with yolked nuclei of *P. afer* may go unutilized. It may therefore be more meaningful to compare only fully yolked 'mature' ova counts (≥ 0.8 mm) between the two species rather than counts including recruitment ova (Table 15). The main problem here was that *P. afer* has larger mature ova and therefore one was including all the ova from 0,8 to 1,5 mm compared to *P. asper* where only the ova from 0,8 to 1,1 mm were included. There was therefore a tendency to include eggs in more size classes for *P. afer* than *P. asper* although overall *P. asper* still had more eggs.



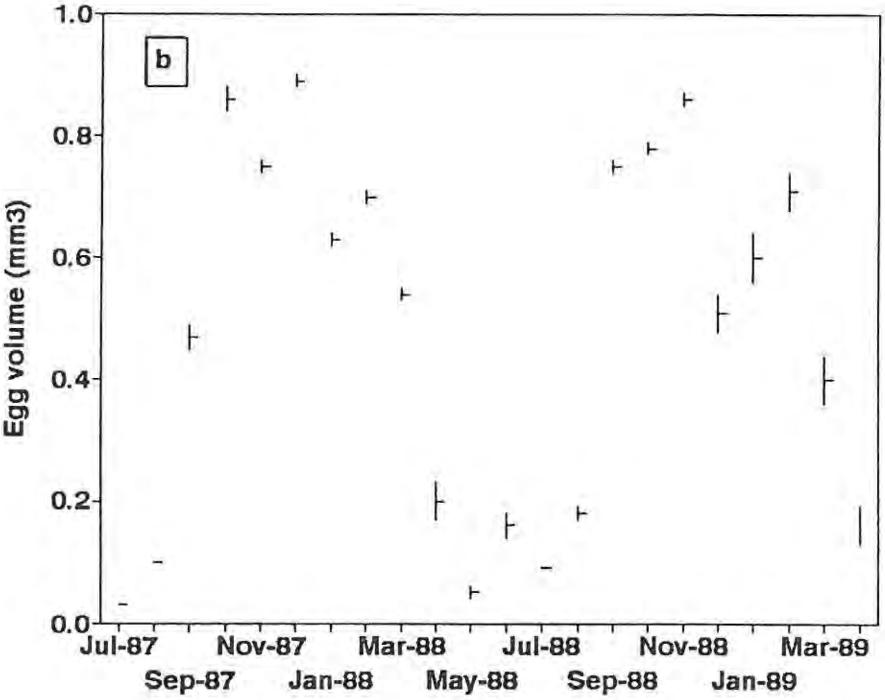


Figure 35. Seasonal changes in volume of the largest ova in (a) *P. asper* (n=219 fish; n=2190 ova) and (b) *P. afer* (n=203 fish; n=2030 ova) females over the period 12 July 1987 to 23 April 1989. Mean and ± one standard error.

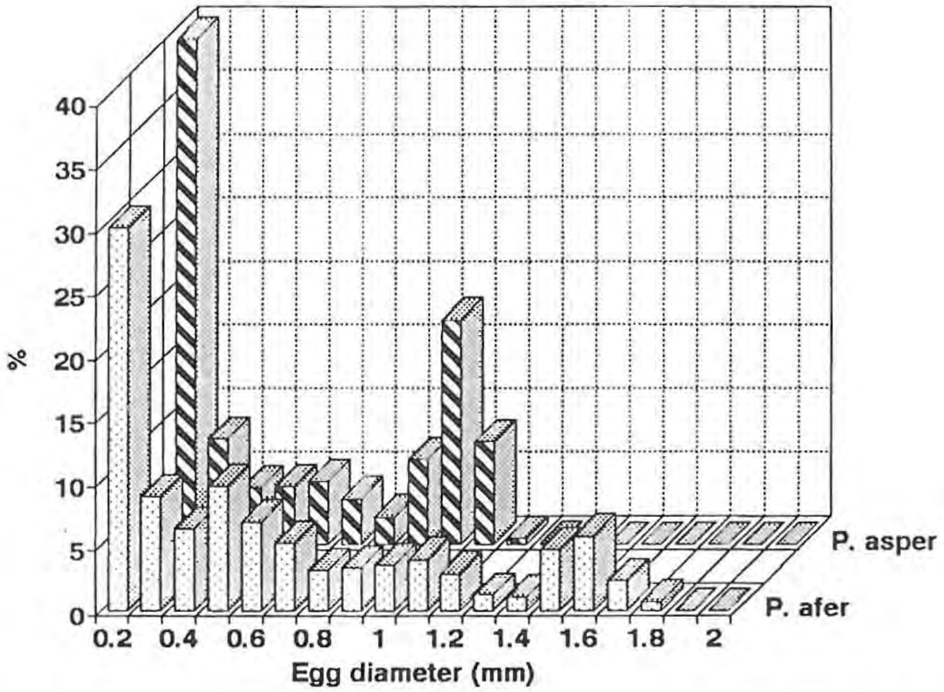


Figure 36. Comparative ova size frequency differences between *P. asper* (63,7mm SL)(n=2272 ova) collected on 28 October 1988 in the Groot River and *P. afer* (n=1368ova)(61,1mm SL) collected on 26 October 1988 in the Wit River.

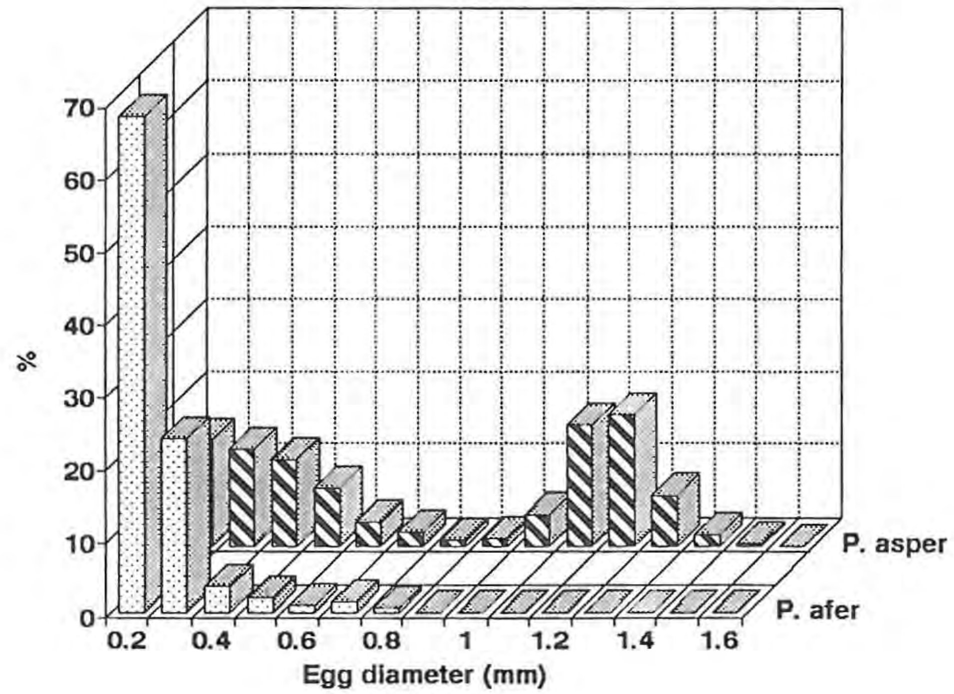


Figure 37. Comparative ova size frequency differences between *P. asper* (76,5mm SL)(n=3058 ova) collected on 18 February 1989 in the Groot River and *P. afer* (64,3mm SL)(n=239 ova) collected on 19 February 1989 in the Wit River.

Mass - fecundity relationship

At the 95% confidence level there was a highly significant relationship in body mass and fecundity in both *P. afer* and *P. asper* (Table 15 & 16) for all ova size groups.

Table 15. Regression statistics of the relationship between fecundity (F) including different ova sizes and standard length and mass in *Pseudobarbus afer* (n = 14).

$$F = ax^b.$$

(s.e. = standard error; r = correlation coefficient; *** P< 0,001)

Pseudobarbus afer				**Predictor Length**		
			Egg size (mm)			
	≥ 0,2	**≥ 0,4**	**≥ 0,6**	**≥ 0,8**	**≥ 1,0**	**≥ 1,2**
a	**-5,755**	**-7,05**	**-7,716**	**-7,875**	**-8,166**	**-10,928**
s.e. of a	**1,98**	**1,149**	**1,207**	**1,126**	**1,07**	**2,107**
b	**3,217**	**3,347**	**3,43**	**3,393**	**3,384**	**3,994**
s.e. of b	**0,504**	**0,292**	**0,307**	**0,286**	**0,272**	**0,536**
r	**0,88*****	**0,957*****	**0,955*****	**0,968*****	**0,963*****	**0,907*****

Pseudobarbus afer				**Predictor Mass**		
			Egg size (mm)			
	≥ 0,2	**≥ 0,4**	**≥ 0,6**	**≥ 0,8**	**≥ 1,0**	**≥ 1,2**
a	**5,754**	**4,907**	**4,529**	**4,238**	**3,914**	**3,325**
s.e. of a	**0,217**	**0,113**	**0,109**	**0,099**	**0,09**	**0,206**
b	**0,98**	**1,035**	**1,068**	**1,056**	**1,054**	**1,247**
s.e. of b	**0,159**	**0,083**	**0,08**	**0,073**	**0,066**	**0,152**
r	**0,872*****	**0,963*****	**0,968*****	**0,972*****	**0,977*****	**0,922*****

Test for equality of* P. afer *and* P. asper *fecundity regression lines

For the five egg size categories for both body length, body mass and fecundity for both species the regression lines were not equal (Table 17). The least significant difference was seen in the

ova size group ≥ 0,2 mm.

Table 16. Regression statistics of the relationship between fecundity (F) including different ova sizes and standard length and mass in *Pseudobarbus asper* (n = 14),

$$F = ax^b.$$

(s.e. = standard error; r = correlation coefficient; *** P< 0,001)

Pseudobarbus asper			Predictor - Length		
			Egg size (mm)		
	≥ 0,2	≥ 0,4	≥ 0,6	≥ 0,8	≥ 1,0
a	-1,286	-1,879	-4,479	-6,437	-7,372
s.e. of a	1,364	1,279	1,355	1,424	1,51
b	2,261	2,297	2,878	3,299	3,48
s.e. of b	0,335	0,314	0,333	0,35	0,371
r	0,89***	0,904***	0,928***	0,939***	0,938***

Pseudobarbus asper			Predictor - Mass		
			Egg size (mm)		
	≥ 0,2	≥ 0,4	≥ 0,6	≥ 0,8	≥ 1,0
a	6,746	6,291	5,76	5,299	5,01
s.e. of a	0,16	0,154	0,162	0,166	0,179
b	0,722	0,727	0,908	1,043	1,098
s.e. of b	0,095	0,092	0,096	0,098	0,106
r	0,91***	0,917***	0,939***	0,951***	0,948***

Spawning sites and reproductive guild

The redfin minnows are gonochoristic, oviparous, the eggs are not tended and they both (especially *P. asper*) have quantity not quality of progeny. *P. asper* spawned after an increased river flow either caused by rainfall or by water released from a dam (Cambray, 1991a) and *P. afer* spawned after an increase in water flow during rains. The eggs were located under boulders in mid-channel for both species and there was no observed parental guarding. The fish bred just above riverine pools (Figure 38). The late free embryos and early larval fish left the riffles and drifted into pools where they fed in the pelagic zone.

The reproductive guild of both species is lithophilic (A.1.3) (Balon 1975a, 1981). This is a primitive style with no parental care of the zygotes. Within the non-guarders Balon (1975a, 1981) recognized two main types, the egg scatterers (many cyprinids) and the egg hiders (some cyprinids, salmonids). It is typical of the non-guarders to produce a large number of small eggs, and the egg-hider group to produce fewer but larger eggs. It is possible that the *Pseudobarbus* species are crevice spawners but this has as yet not been verified and therefore they are classified here as egg scatterers.

Table 17. Test for equality of *P. afer* and *P. asper* regression lines (Tables 15 & 16) for number of ova in different size classes to length and mass (95% confidence levels).

Pseudobarbus afer and *Pseudobarbus asper* Length		
Eggsize (mm)	F	P
≥ 0,2	11,42	< 0,001
≥ 0,4	61,572	< 0,001
≥ 0,6	62,248	< 0,001
≥ 0,8	71,892	< 0,001
≥ 1,0	90,669	< 0,001
Pseudobarbus afer and *Pseudobarbus asper* Mass		
≥ 0,2	10,534	0,001 < P < 0,002
≥ 0,4	68,306	< 0,001
≥ 0,6	70,145	< 0,001
≥ 0,8	86,832	< 0,001
≥ 1,0	98,32	< 0,001

Figure 38. Spawning sites of *P. afer* in the Wit River (above; red marker) October 1987 and *P. asper* in the Groot River (below; yellow marker) March 1988.

Early life-history

At hatching the free embryos of both *P. afer* and *P. asper* exhibited negative phototaxis and during the swim-up period exhibited positive phototaxis after 5 days for *P. afer* and 4,25 days for *P. asper*. There was almost three days difference in the time to first feeding between *P. afer* (10,21 days) and *P. asper* (7,25 days). The *P. afer* larval fish were also larger at first feeding (8,99 mm TL) than *P. asper* (6,6 mm TL) (Table 18).

Table 18. Summary of several important early life-history events of *P. afer* and *P. asper*.

	P. afer	*P. asper*
Egg size (mm)	1,5	1,1
Egg volume (mm^3)	1,77	0,7
First cleavage (mins)	66	50
First heart-beat (days)	1,85	1,6
Negative phototaxis (days)	2,42	2,25
Positive phototaxis (days)	4,96	4,25
Swim-up (days)	4,96	4,25
First gulping movements (days)	6,54	5,0
Swimbladder (days) inflated	9,54	6,67
First feeding (days)	10,21 (8,99mm TL)	7,25 (6,6mm TL)

Size at sexual maturity

P. afer males and females matured at 39-40 mm SL (Figure 39). *P. asper* males matured at 41-42 mm SL whereas *P. asper* females matured at 43 mm SL (Figure 40). Between 39-43 mm SL appeared to be the threshold size at which 50% of the population of both sister species became sexually mature. *P. afer* were in their third year (2^+) when they matured and *P. asper* in their second year (1^+) (Chapter 9).

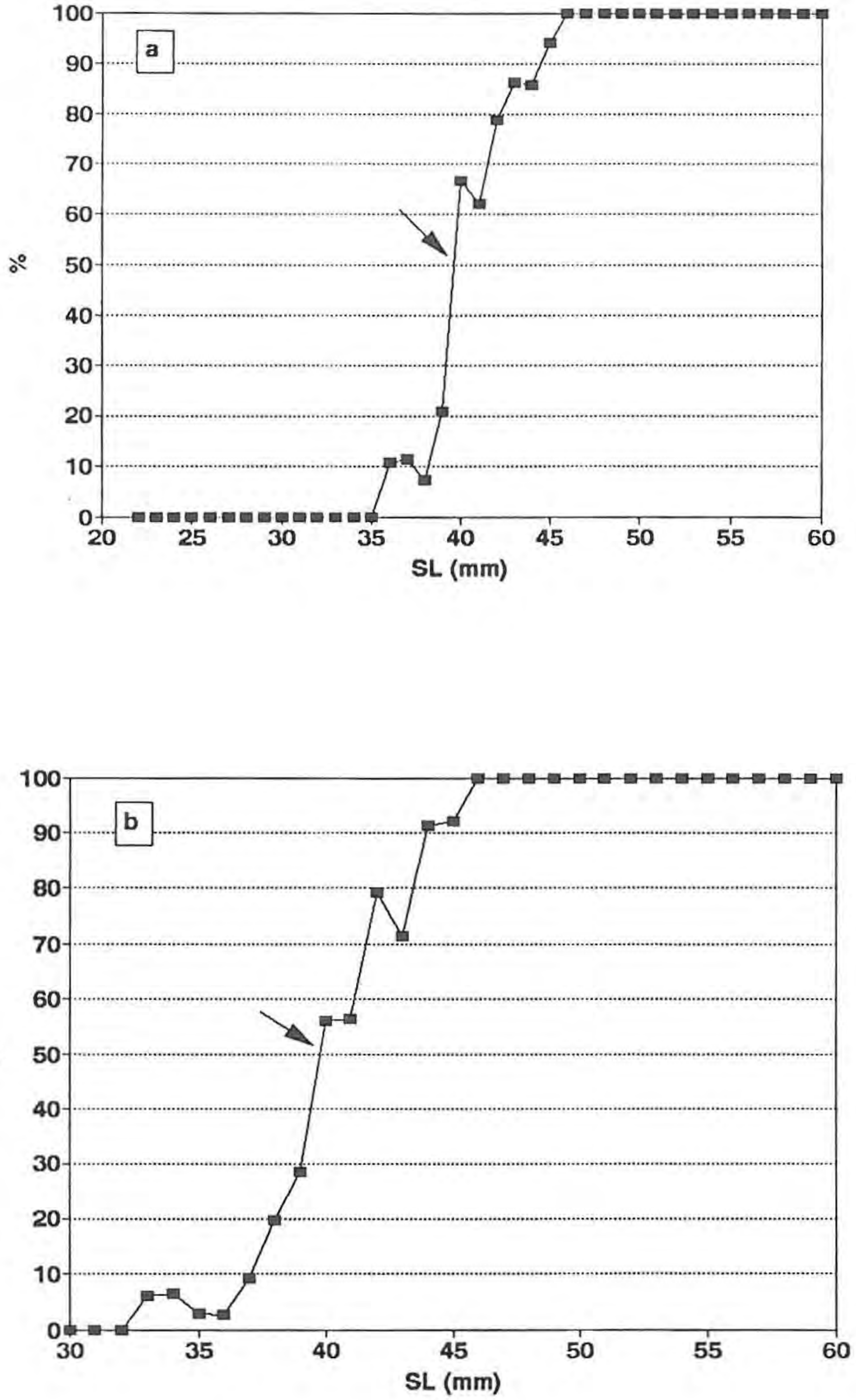
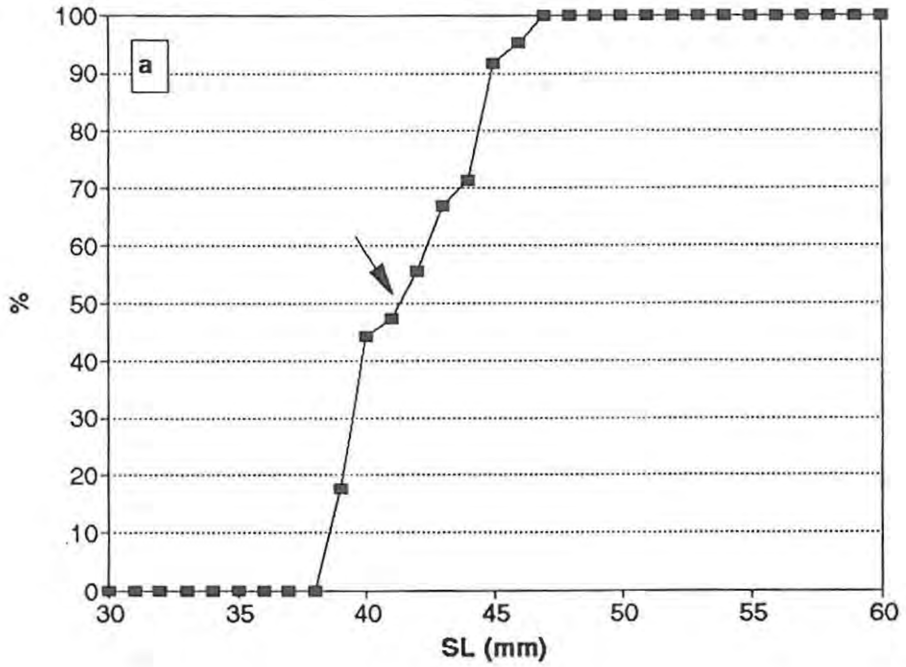


Figure 39. Size at maturity for (a) ♂♂ *P. afer* (n=568) and (b) ♀♀ *P. afer* (n=1002).



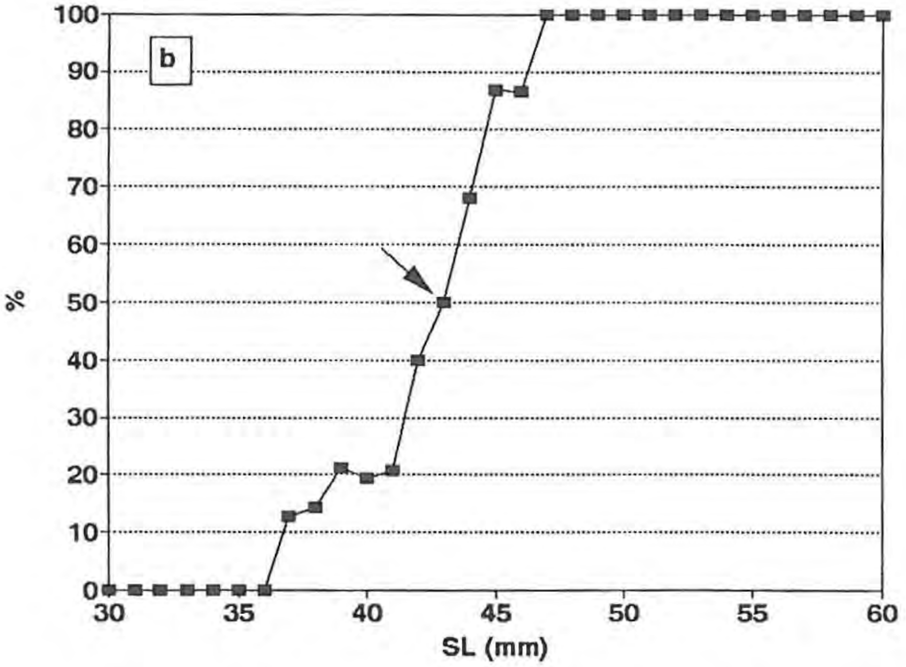


Figure 40. Size at maturity for (a) ♂♂ *P. asper* (n=1385) and (b) ♀♀ *P. afer* (n=1230).

Discussion

Wootton (1990: preface) believes that "... the key to understanding the ecology of any group of animals is an understanding of how individual animals react to environmental conditions by altering their allocations of time and resources to their different activities, and the consequences of these alterations for the reproductive success of individuals." Fisher (1958: 47) noted that "It would be instructive to know not only by what physiological mechanism a just apportionment is made between the nutriment devoted to the gonads and that devoted to the rest of the parental organism, but also what circumstances in the life-history and environment would render profitable the diversion of a greater or lesser share of the available resources towards reproduction." It is critical that at some stage in the life of a fish it must allocate resources to reproduction so that it can be represented genetically in the next generation (Silby & Calow, 1986). Age when this switch is made, amount and way the resource is allocated and time period over which it is allocated are all important phenotypic options in the life-history repertoire of a fish. Reproductive effort models conclude that organisms are expected to maximize their Fisherian reproductive value at each age by an allocation of resources to growth, maintenance, and reproduction (Partridge & Harvey, 1988). Resources have to be allocated not only to gamete production but also to the development of sexual characteristics (eg. tubercles and breeding colours) and reproductive behaviour. Individual species of fish have life-history characteristics which are a result of their inherited attributes and their present environment (Mann, 1989). Within these species individual fish have a suite of reproductive attributes which are determined by genotype and this in turn has been determined by the evolutionary history of the gene pool of which the individual is a member (Wootton, 1990). The reproductive style of an individual fish is a combination of the reproductive attributes characteristic of individuals which belong to the same gene pool.

The different allocations of time and resources to reproductive activities may be the key difference between *P. afer* and *P. asper*. An individual divides the energy it has available between its own maintenance and reproduction. Allocation patterns of time and energy over periods of environmental variability (which affect survival and reproduction) determine the lifetime reproductive success of an individual (=fitness). The following discussion clarifies what these different allocations mean to the survival of individual *P. afer* or *P. asper* living in their distinctly different environments, what the selection pressures are and how these may have led to the evolution of their observed reproductive styles.

Causal factors are the ultimate factors which are responsible for selection of a certain reproductive styles. Individuals of *P. afer* and *P. asper* have attributes with phenotypic ranges that may be expressed but which are defined by the genotype. If the attribute, such as egg size, is variable then an individual can exhibit a wide range in their expression with the environment experienced by the fish determining this expression. The phenotypic options used by an individual in response to environmental changes are variations in the expression of the attribute. An understanding of the variability of phenotypic options requires the identification of those environmental factors that cause variations within the lifetime of the individual (the proximate factors - cues). Proximate control factors are: light cycles, temperature, lunar cycles, meteorological cycles, hydrological cycles and food and energy supplies to the parents. This study has enabled these environmental factors to be identified to some extent.

The sister species, *P. afer* and *P. asper*, occur in the same river system with no physical barrier separating them. They have very few morphological differences (Chapter 3) but the 'sister' environments which they inhabit are distinctly different (Figures 6 & 7; Table 2). The Groot River is subject to physico-chemical extremes whereas the Wit River is relatively stable over time. The selection pressures which exist in these two distinct sections of the same river system have led to the evolution of the observed reproductive styles of these two redfin minnows. They are recognised as two distinct species because the attributes of individuals of each species may not be variable enough to exhibit wide enough ranges so that they could be expressed in each of the two environments. Alternatively physiological differences such as salinity tolerance (Hofmeyr, 1966) may prohibit the individuals of one species entering the environment of the other. This in turn would not allow full potential expression of an attribute, even though the individual fish had the potential phenotypic variability.

The most important ultimate and proximate factors that shape the reproductive ecology of fishes include: harshness and variability of the abiotic environment; availability of food for the parental fish and their offspring and the nature of the habitat of the parental fish (Wootton, 1990). If food is in short supply an individual may delay puberty, shorten the period of reproduction, reduce the number of gametes or may even forego breeding for a season in order to conserve energy for survival until more favourable times. It has been shown that *P. asper* inhabits a harsher and more variable abiotic environment than does *P. afer*. Food was more available for the parental *P. asper* fish and their offspring in the Groot River than for *P. afer* in the oligotrophic Wit River. The habitat of mature *P. asper* was large riverine pools whereas *P. afer* adults inhabited small pools. From these differences it would be expected that the reproductive ecology of *P. afer* and *P. asper* have been shaped by different ultimate and proximate factors.

Size and age at maturity

An individual has to attain either a threshold size or age before it is capable of initiating full gametogenesis (Munro, 1990a). At the onset of sexual maturity the allocation of resources in *P. afer* and *P. asper* changed from a two-way (growth and survival) to a three-way conflict (then including gamete production and the cost of sexual behaviour). To resolve the conflict, resources were allocated between the three demands in a way which would maximize the number of offspring produced in the lifetime of an individual under the environmental conditions which prevailed.

If we assume that redfin minnows would allocate resources in the same way whether they were in the Wit River or Groot River then their size at maturity should have been and was similar (*P. afer* ♂♂ and ♀♀ 39-40 mm SL and *P. asper* ♂♂ 41-42 mm SL, ♀♀ 43 mm SL) but their age at maturity differed (Chapter 9).

In another African minnow Cambray (1982) found that for male *B. anoplus* the median length at sexual maturity was 40-41 mm FL in the first year of the study and 38-39 mm FL in the second year. For female *B. anoplus* these figures were 39-40 and 38-39 mm FL. This length was obtained in six months if the fish were spawned early in the breeding season.

Stearns & Crandall (1984) regarded variability for age and size at sexual maturity as a life-history response to unavoidable stress. Their model suggested that organisms mature along a trajectory of age and size that depends on demographic conditions. The model also suggested that age and size at maturity are determined both by the genome and the environment, and in a way that separates the two influences. The manner in which an organism alters age and size at maturity under stress can be predicted. As stress increases the age and size at maturity may follow a variable phenotypic trajectory (Stearns, 1983). The attribute under selection is the entire trajectory, not age or size at maturity taken separately. They suggested that there is a need to understand the general properties of variable phenotypic trajectories for life-history attributes. The model assumed that maturity may be delayed because it resulted in a gain in fecundity or because it resulted in a reduced juvenile mortality, or both. It was assumed that the primary impact of environmental stress was on growth rate and that changes in growth rate impose unavoidable changes in size at given ages which have particular consequences for fecundity and for the juvenile mortality rates of the offspring produced. This model of variability for age- and size-at-maturity suggested that when growth is rapid (eg. *P. asper*) then changes in growth produce large changes in age-at-maturity but small changes in size-at-maturity which would indicate that the organisms always mature at a fixed size. When growth rates are intermediate, changes in growth produce large changes in size-at-maturity but small changes in age-at-maturity. When growth rates are slow, changes in growth produce large changes in age-at-maturity but small changes in size-at-maturity (eg. *P. afer*). The variability of the age/size at maturity trajectory can be seen in the two *Pseudobarbus* species. *P. asper* grow faster and attain maturity earlier than *P. afer* but both attain maturity at a similar size (see Chapter 9).

The Stearns & Crandall (1984) model indicates that selection operates on the shape of the age-size maturation trajectory and suggests that neither age nor size should be considered the attribute under selection. Sexual maturity will be discussed here but since age and growth are important in this discussion the section of the thesis (Chapter 9) which deals with age and growth will be referred to. There are at least four types of trajectories. An organism should mature earlier at smaller sizes if a change in the environment increases adult mortality without altering juvenile mortality. In this case the adult is worth less therefore it should mature earlier and convert its biomass into offspring which have a higher chance of survival. *P. asper* mature earlier but at a similar size to the slower growing *P. afer*. *P. asper* makes an earlier switch to allocating resources to reproduction (that is sexual maturity) away from growth and survival than do *P. afer*. This earlier switch maximizes the number of offspring produced in a lifetime for *P. asper* under the prevailing environmental conditions of the Groot River. The trade-off is a shorter lifespan for *P. asper* compared to that of *P. afer* (Chapter 9). Particular trajectories of age and size at maturity have evolved in each population of *P. afer* and *P. asper* and probably represent the genetically fixed and adaptive component of the species response. Whether *P. afer* has the ability to take a *P. asper* like age-size trajectory is not known. However, since the two redfin minnow species are closely-related there is a possibility that they both have the same variable phenotypic trajectories in their life-history repertoire which are under environmental control.

Food availability can alter the timing and onset of maturity as well as the number of eggs produced by the individual. It can also alter size at maturity (Pianka, 1970; Balon, 1981). In a number of studies it has been demonstrated that as food supply increased the fish grew faster with the age at maturity decreasing in rainbow trout, brown trout and sticklebacks (Scott, 1962;

Bagenal, 1969; Wootton, 1973). Experimental evidence has shown that fish which received higher food rations became sexually mature earlier, as was shown for the Pacific herring, *Clupea harengus pallasi*, the brown trout and threespine sticklebacks (Bagenal, 1969; Wootton, 1973; Hay *et al.*, 1988). Wootton (1990) noted that a common response to a change which increased the growth rate of a fish was the size at which a fish matured. The Wit River is oligotrophic whereas the Groot River is enriched and as would be expected *P. asper* occurring in the Groot River grew faster (Chapter 9). Early maturity is therefore a common response to increased growth rate (Wootton, 1990), and this would explain the difference in age at sexual maturity between *P. afer* and *P. asper*. Age at maturity is therefore more variable whereas there may a phylogenetic constraint on size at maturity of the two *Pseudobarbus* species.

Cambray & Bruton (1984) reviewed the length of the breeding season, fecundity, egg size and spawning habitats of some small African *Barbus* species. Their table is included and updated (Table 19).

Condition factor

Differences in the value of condition factor between fish populations can yield insight into the circumstances of their lives, especially with regard to food supply, timing and duration of breeding cycle, etc. (Weatherley & Rogers, 1978). Condition factor was used to define seasonal changes in condition of the redfin minnows in relation to size (age) and sex of the fish and also differences between seasonality and condition of the two redfin minnows.

There is a seasonal variation in the condition factor in both fish species. In immature/maturing *P. afer* and immature/maturing *P. asper* the highest condition factors were observed during summer months. Mature males and females of *P. afer* had a maximal condition factor in October. The peak condition for *P. asper* males occurred in either November (1987) or December (1986, 1988) and for mature females in December 1986, October and November 1987 and again in February 1988 (same season) and then in October, November and December 1988. The similarity in maximum total condition between the three years for *P. asper* females may have indicated that food was abundant enough that they could allocate maximum amounts to reproduction each year. In comparison *P. afer* females allocated significantly different amounts each year which was reflected in the condition values.

Table 19. The length of breeding season, fecundity, egg size and spawning habitats of some small African *Barbus* and *Pseudobarbus* species.

Species	Locality	Breeding season	No. of months	Fecundity	Mature egg size (mm)	Spawning habitat and spawning habits	Reference
B. anoplus	Natal	September to March	7	-	-	-	Crass (1964)
B. anoplus	Lake le Roux	November to March-April	5-6	63FL-3250	1,0	migration not necessary but some migrate - eggs adhere	Cambray (1982)
B. apleurogramma	Lake Victoria (stream)	ripe fish present October to December and March to June	-	35SL-818 58SL-1635	n.s.	migrates upstream	Welcomme (1969)
B. kerstenii	Lake Victoria (stream)	ripe fish present September to December and February to June	-	40-44SL-1170 70-74SL-2696	1,0	migrates upstream -eggs adhere	Welcomme (1969)
B. liberiensis	Sierra Leone (stream)	June-July single discrete season	n.s.	110TL-6-7000	1,0	migrates upstream -eggs adhere	Payne (1975)
B. paludinosus	Natal	mainly early summer	-	74FL-2200	1,0	-	Crass (1964)
B. paludinosus	Lake Victoria (stream)	only ripe fish in rainy seasons	-	86SL-6100 112SL-11450	0,8-1,0	migrates upstream	Welcomme (1969)
B. paludinosus	Lake Sibaya	October-February	5	-	-	-	Bruton (1979)
B. paludinosus	Lake Chilwa	extended throughout rainy season (November - April)	6	50-60TL-255-801 112TL-2513	-	possibly a spawning migration	Furse (1979)
B. toppini	Kruger Park	late summer	-	-	-	possibly a spawning migration	Pienaar (1978)
B. treurensis	Blyde River	October - November	2	108FL-2040	2,08	-	Kleynhans (1987)
B. trevelyani	Tyume River	end of September to February - March	6-7	65-69FL-900 >100FL-4000-5000	1,0-1,3	possibly spawns midstream, migration possibly not necessary	Gaigher (1975)
B. trimaculatus	Kruger Park	-	-	-	-	migrates upstream	Pienaar (1978)

Species	**Locality**	**Breeding season**	**No. of months**	**Fecundity**	**Mature egg size (mm)**	**Spawning habitat and spawning habits**	**Reference**
B. trimaculatus	Natal	spring and summer	-	117FL-8000	0,9	-	Crass (1964)
B. unitaeniatus	Kruger Park	late summer	-	-	-	-	Pienaar (1978)
B. viviparus	Natal	throughout summer	-	53FL-8000	0,9	-	Crass (1964)
B. viviparus	Lake Sibaya	September - February	6	-	-	-	Bruton (1979)
P. afer	Wit River	October - January or February	4-5	73,12SL-3922[1] (783)[2]	1,5	mid-channel riffles	This study
P. asper	Groot River	October - April	7	63,72SL-4771[1] (1662)[2]	1,1	mid-channel riffles	This study
P. burchelli	Breede River	October - November	6	123FL-10678	1,5	-	Cambray & Stuart (1985)

1 - All yolked ova ≥0,2mm
2 - mature ova ≥0,8mm

Meili (1987) established that condition factor did not merely reflect the trophic condition of the fish, but that it was to a large extent governed by other variables, such as gonad development and growth rate. The development of the gonads and the accumulation of storage products influences the seasonal changes in mass and therefore has a major influence on the gross condition factor.

Duration of breeding season

In cyprinids the factors which may determine spawning success are water level, water quality, nutrients, temperature, breeding substrate, and vegetation (Hontela & Stacey, 1990). All of these factors may fluctuate on a seasonal basis. Many species with extended spawning seasons are multiple spawners with individual females producing several clutches of eggs. In many oviparous teleosts multiple clutches of eggs are spawned periodically in a reproductive season and ova undergo group synchronous development (Wallace & Selman, 1981; Heins & Rabito, 1986). The ovaries of these species cycle through maturing, mature and ripe stages as noted for *Notropis leedsi* (Heins & Rabito, 1986) and suggested for *B. anoplus* (Cambray, 1982) and *P. asper* (Cambray, 1991a). During each cycle maturing oocyctes (smaller, heterogeneous, vitellogenic oocytes) are recruited and undergo yolk-loading which produces a separate, synchronous group of larger mature oocytes (Heins & Rabito, 1986). This process of yolk-loading decreases when the oocyctes reach their full mass and ripen (Selman & Wallace, 1986; Heins & Baker, 1988). Ripe ova appear for a few hours of the cycle whereas mature ova in some species are present during each inter-spawning period, therefore random field surveys are unlikely to include females with ripe eggs (Heins & Baker, 1988).

More limited and smaller spawning sites along the Wit River may indicate competition for spawning sites. The low male to female ratio (1♂♂:1,68♀♀ Chapter 6) may also indicate limited spawning areas. In small *Barbus* species in Sri Lanka one of the factors influencing spawning seasonality was access to (including competition for) breeding sites (Schut *et al.*, 1984).

Like most species which occur in environments which are seasonal, *P. afer* and *P. asper* have adaptive cyclic changes in their biology. In the reproductive seasonality of the two species there was a cyclical pattern of demand for energy and materials to fuel the "reproductive machinery" (sensu Miller, 1984) which was correlated to environmental seasonality (Figures 11 & 32). Food availability may have played an important role in reproductive seasonality. A shorter reproductive season for *P. afer* may have been the result of scarcer food resources when compared to those of *P. asper*. A shortage of food supply may delay sexual maturity, shorten the period of reproduction, reduce the number of gametes or prevent breeding entirely for a season so that energy can be conserved for survival (Sumpter, 1990). Gaigher (1984) has shown that an African *Labeo* (*L. umbratus*) may skip breeding seasons if conditions are not favourable. *P. afer* delayed maturity, have a shorter breeding season and fewer gametes than did *P. asper*.

The two critical environmental cues which ensure that fish are in breeding condition at the right time of the year are thought to be temperature and day length (Wootton, 1990). The temperatures of the Wit River and the Groot River differ, but because the rivers are situated near each other the photoperiods are similar (Figure 11). Local measurements of light would reveal the effect of the forested narrow valley and high mountains through which the Wit River traverses (Figures 7 & 14a). The light regime would therefore be different from the open Groot River *P. asper* localities (Figures 6 & 14b). These light regimes may have influenced the differences in the length of the breeding season of these two species.

The length of the breeding season of many small African *Barbus* species has been reported as between five to six months (Cambray, 1982). *P. asper* can breed from October to early April, a period of six to seven months, and *P. afer* breeds from October to January - February, a four to five month period. Therefore redfin minnows in the harsher environment of a Karoo river have a longer breeding season. A shorter breeding season of *P. afer* appears to be correlated with larger eggs. The *B. treurensis* in the Blyde River in the Transvaal have a fairly discrete breeding season, October-November, and large eggs (Kleynhans, 1987). However, *B. liberiensis* in Sierra Leone, have both a short spawning season and small (1,0 mm) mature ova (Payne, 1975).

Timing of reproduction

Endogenous and exogenous physiological mechanisms are both important for the timing of breeding to facilitate progeny survival. The annual reproductive cycle is under the control of the endogenous rhythm whereas the exogenous rhythm is driven by external environmental cues (Sumpter, 1990). In the larger ostariophysans fecundity/spawning is increased by postponing maturation in favour of somatic growth for several seasons, and potential recruitment is maximized in seasons when the timing of spawning is right. The smaller shorter-lived species usually do not delay maturation. In general small ostariophysan species have more prolonged breeding seasons than those of large ostariophysans (Cambray & Bruton, 1984; Munro, 1990b). Since their small body size limits their absolute fecundity, small species are often partial or small-brood spawners with each individual breeding several times in a season in order to maximize individual female fecundity. In this way an individual can maximize the chance that at least one spawning of the several per season occurs at an optimal or near-optimal time. In contrast to other small *Barbus* species, *B. treurensis* in the Blyde River have a discrete spawning period, which may be one of the reasons why this fish has not adapted to man-induced changes and is now a Red Data book species (Kleynhans, 1987). Both *P. afer* and *P. asper* have a similar photoperiodic history and as they occur in a temperate area they are 'long-day species'. This means that with the lengthening of daylight hours in spring the fish mature sexually. Photoperiod is therefore one of the proximate factors, and can be used as a predictive cue, which regulates gonadal growth in the minnows so that the fish are ready to spawn at the most favourable time of year. *P. afer* and *P. asper* initiate gonadal recrudescence under both increasing photoperiods and temperatures during spring. In an experimental approach it was established that there may be synergism between a long photoperiod and warm temperature for the shiner, *Notemigonus crysoleucas* (de Vlaming, 1975). If the requirements for the exogenous mechanism are not met then a species may produce a weak year class (Tomasson *et al.*, 1984) or resorb their gametes (Gaigher, 1984).

The way in which an individual can maximize its lifetime production of offspring is to spawn at a time of year that would tend to maximize the survival of the vulnerable early life-history stages. In the eastern Cape the weather is extremely variable both temporally and spatially (Chapter 2). The Groot River is in an autumn rainfall region, and this may help to explain the late spawning of *P. asper,* whereas the *P. afer* population is in a spring to year-round rainfall region. *P. asper* also seems to be an opportunistic breeder and reproduces whenever there is a possibility of success (Cambray, 1991a). That some of the *P. asper* population spawned as late as April would indicate reduced chances for the adults to survive the winter but a better chance that some of the offspring would survive. Since the larval fish must have appropriate food, protection from predators and benign abiotic conditions for survival, we can assume that these conditions are possibly present in the Groot River in April but not in the Wit River. It appears then that an April spawning in the Groot River is probably marginal. Wootton (1990) calls it the common-sense hypothesis that eggs are laid and larval fish hatched at times that maximize their survival. Possibly an individual *P. asper* female maximizes survival through its progeny at this time of year as the adult has a short lifespan.

In the laboratory studies it was determined that at 7,25 days at 23°C *P. asper* changed from endogenous to exogenous feeding. The relatively poor locomotory capacities of the larval fish would be decreasing because of the cooler water during April. Their choice of prey may be reduced to even less evasive prey than earlier spawned larval fish could capture which may have further reduced their chance of survival. The slower growth at that time of year would also make them more vulnerable to predation by other fish and invertebrates over a longer time period.

The optimum time for spawning in a river is unpredictable due to year-to-year variations in timing, pattern and extent of floods. Of the two rivers in this study, this statement is particularly true for the Groot River (Figures 11e, 12 & 15). Those members of a population which spawn too early risk losing their progeny either because the flood was transient with rapidly receding ("false start") waters or because rising waters after spawning meant that they developed in suboptimal (too deep or too fast-flowing) habitats. Unpredictability of flow in the Groot River means that if an individual *P. asper* postponed spawning it may have missed the optimum time to spawn whereas too late a spawning means the length of the first growth period is reduced for the larval fish. Similar to *P. asper* the small cyprinid, *Barbus tetrazona*, spawned at irregular intervals, associated with rainfall and rising water levels (Kortmulder & Feldbrugge, 1975 in Munro 1990b).

P. afer and *P. asper* are similar to the summer spawners of the Northern Hemisphere, such as the European minnow and several other cyprinids (Wootton, 1979) and *Notropis* spp. (Heins & Baker, 1988), where spawning takes place from spring and throughout the summer. In many species with a summer spawning season pattern, the female can produce several batches of eggs during a single spawning season. Both *P. afer* and *P. asper* are multiple spawners.

B. anoplus was found to have two discrete major spawnings, one in the early spring and one in late summer (Cambray & Bruton, 1984). Female *B. anoplus* cycle between partially spent (stage 6), back to stage 4 (late maturing) then to stage 5 (ripe - running) when environmental conditions are suitable with rainfall but not water flow being necessary to trigger spawning. *P. asper* was found to breed after water releases from Beervlei Dam at an interval of six to eight weeks (Cambray, 1991a). No rainfall was required and only a period of riverine flooding was sufficient to trigger spawning.

There would be a chance of natural hybridisation of *P. afer* X *P. asper* since there is an overlap in the spawning times of several months. Another factor such as water quality (chemical / physiological barrier) may separate the two species in the Gamtoos River system.

Control of final maturation

It is not known what factors regulate the final maturation in *P. afer* and *P. asper*. In the Nile the spawning of *Barbus bynni* occurs during flooding, although a rise in water level, inundation of shallow areas, increases in water velocity and turbidity might all be responsible for inducing the fish to spawn (Rahman & Moghraby, 1984). In addition, ionic changes and nutrient load might also influence reproductive success. It is difficult to establish what the factors are under laboratory conditions because of the possibility of under- or overestimation of the importance of the experimental variables as the modifying influences of the environment cannot be provided (Bye, 1984).

There are three factors considered to be relevant for the occurrence of spontaneous ovulation in goldfish (Hontela & Stacey, 1990). These are water temperature, presence of aquatic vegetation, and presence of males. Increasing water temperature may indicate that there is sufficient food supply for the fry.

Mueller (1984) and Cambray (1991a) have suggested that scouring of the substrate on the spawning grounds during flooding may be an important stimulus for spawning. Mueller (1984) worked on the speckled dace, *Rhinichthys osculus*, in the southern U.S. and Cambray (1991a) worked on the redfin minnow, *P. asper*. *P. asper* were induced to spawn after controlled releases of water from an impoundment. The water release cycles each commenced with a flushing/scouring flow that would have "cleaned" the spawning grounds of silt. The scouring of the mid-channel riffle spawning habitat may act as a synchronising cue for spawning of the *Pseudobarbus* species. There was probably a rapid ovulatory response to exogenous cues such as increased water flow and scoured spawning beds which in turn increased the chance that eggs would be deposited in an optimal site for development and hatching. This response was similar to goldfish (Hontela & Stacey, 1990) except that increased water flow through riffles and not vegetation was the exogenous cue for *P. afer* and *P. asper*.

Since fish are poikilotherms, temperature probably plays an important role during final maturation. Temperature changes are potential synchronizing cues for species which spawn at the start of floods. Temperature may be useful as a source of information about the arrival of suitable spawning conditions. Floods once regulated the seasonality of the spawning of two large *Barbus* species in the Orange River (South Africa). After the building of the Hendrik Verwoerd Dam wall, which dampened a number of the minor floods, fish spawned when a critical temperature was reached and not at every release of water from an upstream hydro-electric power station (Tomasson *et al.*, 1984). This indicated that water flow alone during the summer months was not sufficient to trigger spawning and that the temperature regime played a significant role. In the upper Orange River in Lesotho *P. quathlambae* spawned after a hail storm which lowered the river water temperature to 15 °C (Cambray & Meyer, 1988). Therefore not all fish in southern Africa require warm water conditions before they initiate spawning.

Temperature is a very important ultimate factor as it can influence fertilization success, survival of the larvae, and growth of juvenile fish (Hontela & Stacey, 1990). Therefore spawning at an appropriate temperature is highly advantageous as it greatly influences the survival of the young.

Sightings of spawnings are rare in many African cyprinids. In the present study spawning sites were located for both *P. afer* and *P. asper* which were similar to the spawning habitat of *P. quathlambae* in Lesotho (Cambray & Meyer, 1988).

Seasonality in relation to ultimate control factors

As with most populations which breed during floods *P. afer* and *P. asper* must spawn at a time and place where ultimate factors, such water quality, low predation pressure and suitable food availability, are optimal. However, there may be trade-offs between minimizing exposure to predators and maximizing oxygen and/or food availability for the offspring.

Munro (1990b) suggested that some large fish species only breed once a season in the absence of superabundant food. They trade present reproduction off against growth in order to maximize fecundity in subsequent years. *P. afer* in the Wit River was closer to that scenario than *P. asper* which allocated more resources to current reproduction, even late in the spawning season, than did *P. afer*.

Rainfall may serve as one of a suite of synchronizing cues (ie changes in conductivity, water level etc.). Hydrological factors and in particular chemical changes, are cues which may be used by fish to determine the onset of flood conditions for spawning. During periods of rising water there are a host of potential cues which depend on the rainfall pattern (eg. flash flood), the topography, geology of the catchment area and on stream order. As compared to the second order Wit River the water level in the higher order Groot River, below Beervlei Dam, may reflect the rainfall over a large and relatively distant catchment area. There are a host of chemical changes in the Groot River after flooding such as an increase in ion content and conductivity (Cambray, 1991a). Dry sunbaked soils may accumulate various chemicals. The rising waters may flush out nitrates, sulphates, and petrichlor. Changes in the water level affects temperature and availability of food and spawning sites. In the intermittent Groot River the spawning sites are usually only available after flooding (Figure 6).

Gonadosomatic index (GSI)

In some cyprinids the ovaries form 20-30% of total body mass, just before the female spawns (Wootton, 1979). In *P. asper* the GSI can be as high as 28%. The gonadosomatic indices for African *Barbus* and *Pseudobarbus* species range between 15,5% for *P. afer* to as high as 33% for *P. burchelli* (Table 14). Total egg mass represents a massive cytoplasmic investment by females. This investment can be followed by noting seasonal sizes of ovaries which in turn reflect growth of developing oocytes as they accumulate yolk.

Gonadal development of ova for the first spawning *B. anoplus* mainly relied on fat reserves laid down in the previous season (Cambray & Bruton, 1984). Summer food supply fuelled subsequent spawnings. For *P. asper* to spawn as late as April means that not only will larval fish have to develop rapidly before winter but mature females will have to quickly lay down fat reserves for spawning in the following spring. Late spawnings followed by an early winter may result in high adult female mortalities or low reproductive success in the following season. *P. afer* females, on the other hand, will begin to lay down fat reserves as early as February and subsequently would have a higher chance of winter survival.

Egg production during a breeding season may track food availability (Wootton, 1984). Possibly the continual recruitment of oocytes with yolked nuclei in *P. asper* during the breeding season coincided with the food availability in the pools of the Groot River. Water releases from Beervlei Dam contained large quantities of zooplankton which could have triggered the recruitment of more oocytes which would have been ready for spawning during the next release of water. These water releases may have mimicked the natural situation after rainfall when the pools higher up in the system are flushed together with their zooplankton and aquatic invertebrates. *P. afer* do not have that option and their food availability was probably less variable from year to year and within a season.

P. afer not only had larger eggs than *P. asper* but they also invested less per clutch (lower GSI) and had a shorter spawning period. *P. asper* invested less per offspring, more per clutch and more per season.

Egg size and fecundity

Egg size differences are considered in some detail in Chapter 5 and only a brief introduction is given below.

"... the crucial problem of ecology is to determine the environmental factors that influence, in a predictable way, the number of offspring that an individual produces in its lifetime" (Wootton, 1990:4). In multiple spawning fish this quest becomes even more problematical. With *P. afer* and *P. asper* there were seasonal trends in respect to mature ova size. The trend was to a reduction in ova size as the season progressed with the largest mature ova produced at the start of the breeding season (Figures 34 & 35). Data on mature ova from *Barbus trevelyani* also showed a trend to smaller size from the start of the reproductive season in October (Gaigher, 1975; his Figure 8). In *B. anoplus* this trend was not as pronounced (Cambray & Bruton, 1984; their Figure 10).

Breeding season fecundity depends on the number of times a female spawns during a season. Multiple spawning fish such as *P. asper* and *B. anoplus* have the ability to use the area in their small coelomic cavities to accommodate enlarged ovaries. By doing this the fecundity of a fish can be increased (Nikolsky, 1963). Spawning more than once a season decreases the chance of one or more entire year classes being lost due to unfavourable conditions (Nikolsky 1963). On the other hand it reduces the chances of all eggs being spawned under optimal conditions (Gale & Gale, 1977). Very strong and very weak year classes can be overcome by a multiple spawning habit (Gale & Gale, 1977). Cambray & Bruton (1984) listed several other benefits for a multiple spawning habit, such as reduction of intraspecific competition between larval fish. *P. afer* had submaximal clutch volumes which may have indicated that the 'spreading out' of the larval fish during a season would have reduced intraspecific competition for limited food resources in the mountain stream. In the *P. asper* population the clutch was maximal and therefore the food resource was not a limiting factor as they could not physically carry any more mature ova at any one time. That *P. asper* females were allocating maximum resources to their ovaries was also clearly evident when the three October total relative condition values were analyzed and found to be similar (Table 7). Cambray & Bruton (1984) suggested that it may have been beneficial for *B. anoplus* to have a "back-up" spawning at a time of year when most species had completed the breeding cycle. That would reduce interspecific larval fish competition, especially with the fry of the larger cyprinid species in the Orange River. In addition, if spawning localities were limited, multiple spawning would allow more than one use of the site per season.

The number of yolked oocytes present in ovaries at the beginning of the breeding season may not be a reliable indicator of breeding season fecundity, as previously unyolked oocytes become recruited to vitellogenesis during the breeding season (Wootton, 1990). During the breeding season some yolked oocytes may be resorbed by the process of atresia reducing fecundity. The multiple spawning habit presents a problem of assessing fecundity. This applies to many of the small African *Barbus* and *Pseudobarbus* species (Cambray & Bruton, 1984). In African studies there has been a lack of critical examination of the fecundity of fishes with multiple clutches per season. The fecundity studies done on African minnows to date (Table 19) can only be used as first estimates of the reproductive potential of the species until more detailed studies are conducted. Most of the studies relied on the number of yolked eggs in the ovaries at the start of the reproductive season.

Whether variations in batch fecundity are predictable or whether they are a consequence of random events during the maturation of the ovaries, and therefore unpredictable, is the important ecological question (Wootton, 1990). Interaction between genetic and environmental factors would result in predictable variation in batch fecundity as would the effect of the environment on oocyte development and the genetic differences between females. It is difficult to assess the total seasonal fecundity of an individual under natural conditions, especially species such as the broadcast spawning *Pseudobarbus* minnows which breed in mid-channel after rains and the eggs fall between rocks. Cambray (1982) suggested that artificial pond or laboratory experiments on batch spawning may overestimate natural fecundity by providing food to satiation, by the removal of spawned eggs and the lack of competition.

Evidence from GSI values and egg volumes would suggest that there was a wide range in primary commitment of energy and material by the females of *P. afer* and *P. asper*. An optimal set of environmental conditions for the *P. afer* genotype resulted in maximal survival and fecundity. These conditions were provided by stable, low conductivity and clear mountain streams. A reduction in survival would be caused by suboptimal conditions, and here we can assume that the Groot River was suboptimal for *P. afer*. Therefore *P. asper* individuals, the more derived of the sister species, had to make some adaptive responses to the changing Groot River. The adaptive response of *P. asper* was evident in the reduction in egg size which increased fecundity.

Large eggs can only be produced at the cost of a reduced batch fecundity (Wootton, 1990). This was clearly demonstrated between *P. afer* (large eggs, low batch fecundity) and *P. asper* (small eggs, high batch fecundity). Mann & Mills (1985) suggested that, at all levels of reproductive effort, there would be two opposing selection pressures. One would favour the development of large propagules, such as *P. afer* eggs, with a high survival potential. The other would favour the formation of as many propagules as possible, such as the *P. asper* mature ova which were volumetrically up to 4,4 times smaller than those of *P. afer*. The mechanism behind that switch to smaller or larger ova might have been determined by the optimal size of larvae which was determined by the size of the available food particles, the abundance of competitors and the numbers and sizes of predators (Svardson, 1949). *P. afer* had larger larval fish at first feeding than did *P. asper* (Table 18) and the ecological importance of this size difference is discussed in Chapter 5.

P. afer had a submaximal batch volume whereas *P. asper* had a maximal volume with a GSI of up to 28%. *P. afer* in the oligotrophic Wit River were probably not as well "fed" as the *P. asper* in the more enriched Groot River. Subsequently one would expect a lower batch fecundity for *P. afer* even if the eggs of the two species had the same volume. In some fish it has been experimentally shown that the number of spawnings during a breeding season was determined by ration size, with better fed fish spawning more frequently (Wootton, 1977; Fletcher, 1984; Townshend & Wootton, 1985). Oocytes were rapidly resorbed in starving sexually mature European minnows (Mehsin, 1981). Reduced fecundity could also be caused by acidic waters, pollutants or abrupt changes in water level.

Mann *et al.* (1984) studied the number of spawnings in a breeding season of the freshwater cottid, *Cottus gobio*, from two contrasting environments. In an unproductive stream females bred once per season while in a more productive southern site the females spawned several times. Reciprocal transplant experiments showed that the observed differences in spawning characteristics were mainly the result of environmental differences rather than genotypic effects. The Wit River is an unproductive stream compared to the Groot River and, similarly to the cottid results, the fish population in the Wit River had fewer spawnings than did *P. asper* in the productive stream. In sexually mature *P. asper* there was a priority of ovarian growth over somatic growth even as late as March/April. There are therefore distinct differences in the patterns of allocation of energy between the ovaries and the soma of the two redfin minnow species which have important trade-offs on other life-history attributes such as lifespan.

Wootton (1984), in a study of the reproductive attributes of Canadian freshwater fishes, found that there was a strong correlation between total egg volume and body size which suggested that there was limited scope for independent variation of egg size and fecundity. Large egg size is obviously a constraint and lowers fecundity. In Chapter 5 it is suggested that large egg size may be a phylogenetic constraint within the *Pseudobarbus* lineage.

In the relatively harsh environment of the Groot River, which is subject to devastating floods, prolonged droughts and widely fluctuating water chemistry, a multiple spawning habit would be an advantage. If one clutch was lost to desiccation or sudden flash floods then another clutch spawned later may offset this loss within one season. In the more stable Cape Fold Mountain stream breeding early in the season and then laying down fat reserves for the production of large eggs to be shed the following year would be advantageous. The differences in fecundity and spawning frequency have led to a disparity in the quantity and intensity of primary reproductive effort between *P. asper* and *P. afer*.

Fish with small mature ova such as *P. asper* specialize in the quantity not the quality of the progeny. The smaller ova size for *P. asper* allowed for more ova to be present in the ovary at one time and this factor, linked with the potentially longer reproductive season of *P. asper*, would suggest a higher absolute fecundity. The higher percentage of oocytes with yolked nuclei in the *P. afer* collected in February (Figure 37) possibly indicated that the majority of the potential recruitment oocytes had not been recruited whereas many of the smaller oocytes in the *P. asper* ovary had been recruited. The longer reproductive season of *P. asper* would have provided enough time for the remaining oocytes with yolked nuclei to have matured before the end of the breeding season in April. There were mature ova ready for spawning in the *P. asper* female unlike the spent condition of the *P. afer* female (Figure 37).

The higher fecundity of *P. asper* was an important phenotypic option in the dynamic and variable Groot River. More *P. asper* eggs and therefore more offspring per season allowed for more flexibility and chance for survival. Therefore the relatively higher fecundity of *P. asper* compared to *P. afer* would allow for a higher intensity of selection for a desired attribute in *P. asper*. That variability was not necessary in the more stable Wit River.

Gonadal regression

This is the process through which oocytes become atretic, sperm infertile, and the gonads reduce in size as their contents are resorbed. Under natural conditions regression usually occurs at the end of the spawning season and ensures resorption of unspent gametes preventing the initiation of another cycle at the wrong time of year.

Gonadal regression is regulated, at least in part, by environmental factors (Hontela & Stacey, 1990). The environmental factors that control gonadal regression mediate their effects through the hypothalamo-pituitary gonadal axis. Regression occurs several months earlier for *P. afer* than for *P. asper*. In the Wit and Groot Rivers the photoperiods were the same but temperature ranges varied. Factors other than photoperiod and temperature could be involved in initiation of gonadal regression such as food abundance, scarcity or possibly social interactions or presence of young. Diet limitation under laboratory conditions can regress the testes of goldfish in 115 days (Clemens & Reed, 1967). This experimental evidence raises the question as to whether food limitation in the natural environment might initiate regression at certain times of the year. It might help explain why *P. afer* had a shorter spawning season than did *P. asper*. *P. afer* switched from producing eggs to storing reserves earlier than *P. asper*. The oligotrophic mountain stream environment placed a food limitation on *P. afer* which in turn initiated a gonadal regression cycle in February. In the Karoo river the food limitation factor played a minor role in the gonadal regression cycle. In the *P. asper* population the major factors involved in the initiation of gonadal regression were possibly photoperiod and temperature as *P. asper* could produce mature ova as late as April (Cambray, 1991a).

Spawning site

Most cyprinids are broadcast spawners (Breder & Rosen, 1966). They either have adhesive eggs and spawn on plants or have non-adhesive eggs and spawn on gravel or rocks. Most species leave their eggs unattended. Within the nonguarders Balon (1975a, 1981) recognized two main types - egg scatterers (many cyprinids) and the egg hiders (some cyprinids, salmonids). It is typical for the non-guarders to produce large numbers of small eggs. The egg hider group produces fewer, but larger eggs. There are probably a wide variety of factors which have exerted strong selection pressures on the type of spawning substrate used for egg deposition. There are many cyprinids, such as *B. anoplus* (Cambray, 1982), which attach their adhesive eggs to leaves and roots of submerged vegetation. For South African *Barbus* species the spawning substrate is usually of a transient nature, such as flooded terrestrial vegetation.

The nature of the spawning site determines the intensity and type of potential hazards to vulnerable eggs and free-living stages of fishes. Presence of predators and the availability of oxygen are the two main environmental factors responsible for determining the course of reproduction, parental behaviour and subsequent development of larvae (Balon, 1975a). Unguarded fish eggs, free embryos and larval fish are very vulnerable and the spawning site chosen by the parents determines the intensity and nature of the hazards. The most important potential hazards to developing embryos according to Wootton (1990) are: lack of oxygen; danger of being smothered by silt; infection by microorganisms and predation. In drier areas with erratic river flows, such as the Groot River, desiccation is also a serious hazard.

High silt loads of the Groot River may cover developing embryos reducing oxygen availability. The non-adhesive, negatively-buoyant eggs need to be spawned in areas that are cleaned and aerated by water currents. In the riffle spawning sites of the Wit and Groot Rivers there is less chance of eggs being smothered in silt. Subsequent risk of infection by a build-up of bacteria, protozoa and fungal infections is also reduced. The riffle spawning habitat would also provide developing embryos with some protection from potential predators.

Small *Barbus* species with adhesive egg envelopes spawn on vegetation in pools, backwaters and floodplains as described for *B. fasciolatus* and *B. multilineatus* (Axelrod *et al.*, 1984). *B. anoplus* spawned in pools after rain without any increase in water flow (Cambray, 1982) and their adhesive eggs were attached to vegetation. In contrast both *P. afer* and *P. asper* were open substrate, benthic spawners on coarse bottoms (rocks) with non-adhesive eggs deposited in the riffle zone after an increase in water flow.

By spawning in riffle areas the redfin minnows adopted a behaviour which would maximize the number of offspring which in turn may have depended on the behaviour of other fish, either redfins (intraspecific) or other fish species (interspecific), in the population. In an oligotrophic system where food was scarce, such as the Wit River, progeny which were spawned in the riffles would have reduced predation pressure on them. Another Wit River species, the anabantid, *Sandelia capensis*, guards its eggs in the quieter areas of the pool habitats. *S. capensis* are also lurking predators which inhabit the marginal vegetation of the riverine pools and *P. afer* progeny placed in the riffle area would have been safer from their predation.

It is not known whether any of the redfin minnows invest energy in the preparation of a spawning site. It is unlikely as they spawn in shallow riffles in mid-channel of rivers during a spate (Figure 38). These are not the types of spawning habitats which would permit much site preparation by small fish species. The "site preparation" was probably the natural scouring of the rivers. The choice of spawning site by the redfin minnows has evolved to coincide with this "river preparation" of the spawning site during which silt and debris are scoured from the bed. This permitted the highest survival of offspring.

P. asper spawned in the main channel of the Groot River after a flushing flow (56 cumecs) from Beervlei Dam followed by reduced water releases (8 cumecs) (Cambray, 1991a). *P. afer* eggs were collected after rainfall in mid-channel under boulders. Cambray & Meyer (1988) observed the same breeding pattern for the more distantly related *P. quathlambae*.

The three *Pseudobarbus* species (*P. afer*, *P. asper*, *P. quathlambae*) bred during good flows in riffle areas which would have supplied sufficient oxygen. There would be little chance of *P. afer* embryos being smothered by silt, although *P. asper* inhabited a river with high silt loads (Chapter 2). The spawning in mid-channel after a flushing flow would have ensured clean spawning beds. Nothing is known about infection by microorganisms. Possibly the developing embryos of *P. asper* would have less chance of infection in the more saline waters, however these waters are also enriched. Predation by fish would be limited by the non-adhesive eggs falling into crevices between the rocks and boulders of the spawning bed. The photophobic free embryos probably wriggled into crevices as indicated by the laboratory studies on early ontogeny for both species. The most vulnerable stages would have been the free-embryo swim-up stage when fish drifted into their feeding habitat (pools below the riffles). *P. asper* were protected to some degree by the turbidity of the water but *P. afer* were not. The larger size at that swim-up stage would have been advantageous to *P. afer* free embryos in avoiding smaller predators.

Desiccation was a likely hazard for the embryos and photophobic free embryos. Mid-channel spawning would prevent this and all three species, *P. afer*, *P. asper* (Cambray, 1991a) and *P. quathlambae* (Cambray & Meyer, 1988) did spawn in mid-channel. *P. asper* had small eggs and therefore free embryos hatched earlier than the larger embryos of *P. afer*. For *P. asper* this may have been a survival phenotypic option as the flow in the river could be of short duration.

Parental care

After spawning redfin minnows do not seem to show any post-fertilization care of eggs. Non-guarding (that is no parental care of the zygotes) is a primitive style (Balon, 1975a). In 78% of teleostean families there is no parental care (Sargent & Gross, 1986). But in the *P. afer* population there were fewer males (Chapter 6) therefore there was a "relatively high probability of genetic relatedness to the zygotes" which Baylis (1981) and Blumer (1979) have noted as important for the evolution of male parental care. To improve chances of paternity the male may need to drive other fish away from the spawning female. Enlarged tubercles and bright fin colouration may be an advantage to male *P. afer* in behaviourial activities of this sort. It would be worth examining the other *Pseudobarbus* species for signs of parental care. The larger tubercles of *P. burchelli* (Cambray & Stuart, 1985) would indicate that this species would be a good candidate for possible parental care.

Production of demersal eggs is a fundamental step in the evolution of parental care in fish (Potts, 1984:225). Demersal eggs are in general larger and produced in smaller numbers than pelagic eggs (Williams, 1959). Unless demersal eggs are protected they are vulnerable to predation and other environmental hazards. This and other evidence suggests that parental care has tended to evolve in fishes occupying environments that are spatially and temporally unpredictable for developing embryos (Wootton, 1990). The selection, guarding and tending of a spawning site by the parent fish reduces the danger to the zygotes caused by that unpredictability (Krebs & Davies, 1981). In the Wit River the co-occurring *Sandelia capensis* is a male guarder, but this behaviour may have evolved in more unpredictable environments in the past. *P. afer*, *P. asper* and *P. quathlambae* are probably nonguarders of their eggs and young and open substrate benthic spawners on coarse (rocks) bottoms.

Sexual dimorphism

Secondary sexual dimorphism was displayed by both *P. afer* and *P. asper* during the breeding season. Males of both species have a deeper red breeding colouration on their fins and larger tubercles (Chapter 7) than females. Males also have larger pectoral fins which may aid them in clasping females during spawning. Of the two species, *P. afer* males have darker red colouration and larger tubercles which may be directly related to the clear water of the Wit River compared to the more turbid environment of the Groot River.

Behaviourial components of reproduction

Unlike most life-history attributes, there are behaviourial components of reproduction which can be variable, dynamic and used opportunistically by individuals to compete with conspecifics (Gross, 1984). That aspect was considered here because *P. afer* have larger nuptial tubercles and brighter breeding colouration. Balon (1990) has argued that most alternative behaviourial phenotypic options may have their foundations in epigenetic bifurcations and that selectionist interpretations made by Gross (1984) of the bifurcations are incorrect.

Game theory finds a logical structure in evolutionary biology through genetics and natural selection (Gross, 1984). Game theory shows that if fitness payoffs depend both on the style adopted by an individual and on those by other members of the population, the style which evolves need not be optimal. Instead it must be an *Evolutionary Stable Strategy* (*ESS*) (Maynard Smith, 1982), a style (= strategy) which, when adopted by a critical proportion of the population, yields an individual fitness which exceeds that of all alternative styles when these are rare in the population.

Because of the presence of relatively large and numerous tubercles on *P. afer* there is a possibility that there are alternative male mating behaviours (phenotypic options) which would contribute to the reproductive success of their bearers. This type of behaviour is very common in fishes (Gross, 1984). The prevalence of these male alternative phenotypic options in fishes may be due in part to the competitiveness of their mating systems and the widespread occurrence of external fertilization (Gross & Shine, 1981) . The large tubercles and bright red fins may possibly indicate that the redfin minnow mating behaviour is competitive.

It is not necessary for fish to have parental care for an alternative mating behaviour to evolve as, "the primary requisites are only that competition occurs between individuals for access to mates, that this competition is partially Best of a Bad Situation (BBS) or entirely ESS circumvented by alternative behaviours, and the success of these alternatives depends upon the activities of other members of the population" Gross (1984: p.73).

If the alternative reproductive phenotypic options in a population are subject to frequency-dependent selection, then indeterminate growth may contribute to the evolution of discrete alternative life-history pathways (Gross, 1984). Precocial maturity will result in smaller body size. If the smaller males have equivalent fitness Gross (1984) suggested that selection may allow genetic differences to accumulate with specialization into alternative routes. We would not expect to find selection for alternative male reproductive phenotypic options in the turbid waters of the Groot River where the species has smaller and less numerous breeding tubercles than the species occurring in the clear waters. This may be reinforced by the faster growth rate and the shorter life span of *P. asper* males when compared to *P. afer* males. Small male *P. afer* were observed which had bright red fins, few tubercles and they were usually associated with larger males. These males may have been sneakers. This is an avenue of research that should be investigated and is only noted here as a stimulus for future research work on redfin minnows.

Social factors

Social factors are important synchronizing factors for the actual behaviour leading to fertilization. Social cues serve as modifying factors to ensure pair or group synchrony by modulating the rate of gonad development and maturation. The need to reach a critical size before puberty may be regarded as a form of endogenous rhythmicity. This may help to explain why both *P. afer* and *P. asper* have similar 50% sizes at maturity. The genotypes in a population which would be favoured are those which reach their critical size at an appropriate time in a typical year. This would ensure that the trade-off between somatic and reproductive investment (ie the number of potentially successful spawnings and the batch fecundity/spawning) may be optimized.

Multiple spawners, such as *P. asper* (Cambray, 1991a), are opportunists and once mature they will normally breed over a wide range of conditions. Studies should establish the time course of reproductive cycles in the wild, and the relationship of these to environmental cycles. Only then can one design experiments to determine the possible cues for various reproductive events (Munro, 1990b).

Reproductive styles of African minnows

In the small African *Barbus* species there are a variety of breeding styles. Upstream migration for spawning seems rare (Skelton *et al.*, 1991) although this may be due to the lack of research into migrations (Cambray, 1990a). Cambray reviewed migrations of small African *Barbus* and *Pseudobarbus* species and showed that there is very little information. In the Letaba and Elands Rivers the small *Barbus* species (*B. trimaculatus* and *B. unitaeniatus*) were numerically the most dominant species undertaking upstream migrations (Meyer, 1974). *P. asper* were part of an upstream migration of fish species in the Groot River (Cambray, 1990a). *P. asper* were probably migrating upstream during high water flows when farmers were abstracting water (Cambray, 1990b). Although a large number of *P. asper* were collected in an irrigation canal, no co-occurring *B. anoplus* were entrained. This would indicate a difference in migration patterns between these two minnow species as *B. anoplus* do undertake spawning migrations with the males migrating up the rivers first (Cambray, 1982). Massed congregations of breeding fish have been recorded (e.g. *B. calidus* and *B. erubescens* Skelton, 1988). In his book on the freshwater fishes of southern Africa Jubb (1967) has a photo of male and female *Pseudobarbus* congregating below waterfalls in rocky runs for what he assumed was spawning.

Genetic or environmental influence

In their study of the life-history phenotypic options of the sculpin, *Cottus gobio*, Mann *et al.* (1984) were able to do a reciprocal transfer experiment which gave support to the thesis that there was a strong environmental influence upon life-history phenotypic options. Genetically determined life-history phenotypic options were found to be overshadowed by the effects of productivity and temperature. In the present study one may ask, are the two redfin minnows, *P. afer* and *P. asper* geographic variations (ecophenotypes) or is there a genetic explanation for the few morphological and meristic differences? The variability of the reproductive life-history attributes, egg size, fecundity, age at maturity and seasonal and lifetime reproductive effort between *P. afer* and *P. asper* is within the range of some species. The different reproductive styles probably arise from the influence of the environment and not from genetic differences in these two *Pseudobarbus* species. *P. asper* was found to be the more altricial form and *P. afer* the more precocial form. The mechanism for the distinct life-history dichotomy between the two redfin minnow forms may have been 'Alprehost'. In the Groot River the redfin minnow form further along on the precocial life-history trajectory was not as successful as a more altricial form higher up on the same trajectory. This idea is explored in more detail in the general discussion.

Hypothesis

The hypothesis raised in the introduction, that in the more stable mountain stream the *Pseudobarbus* species should have more precocial life-history attributes while the species in the highly variable Karoo stream should be more altricial, is therefore supported by the above findings (Table 20). From the analysis of their reproductive styles *P. afer* and *P. asper* may be ecophenotypes and the differences in their life-history attributes are due to phenotypic variability with shifts in phenotypic options in the two distinct environments (Figure 41).

Table 20. Summary of the differences between the reproductive styles of *P. afer* and *P. asper*.

Attribute	*P. afer*	*P. asper*
GSI	low	high
Fecundity	low	high
Egg size	large	small
Investment per clutch	low	high
No. of clutches per season	less	more
Spawning season	shorter	longer
Reproductive lifespan	longer	shorter
Environmental cues	rainfall photoperiod temperature	water flow photoperiod temperature
Sexual dimorphism	pronounced	reduced
Size at maturity	♂♂ & ♀♀ 39-40 mm SL	♂♂ & ♀♀ 41-42 & 43 mm SL
Spawning site	riffles in mid-channel	riffles in mid-channel
Reproductive guild	lithophils	lithophils

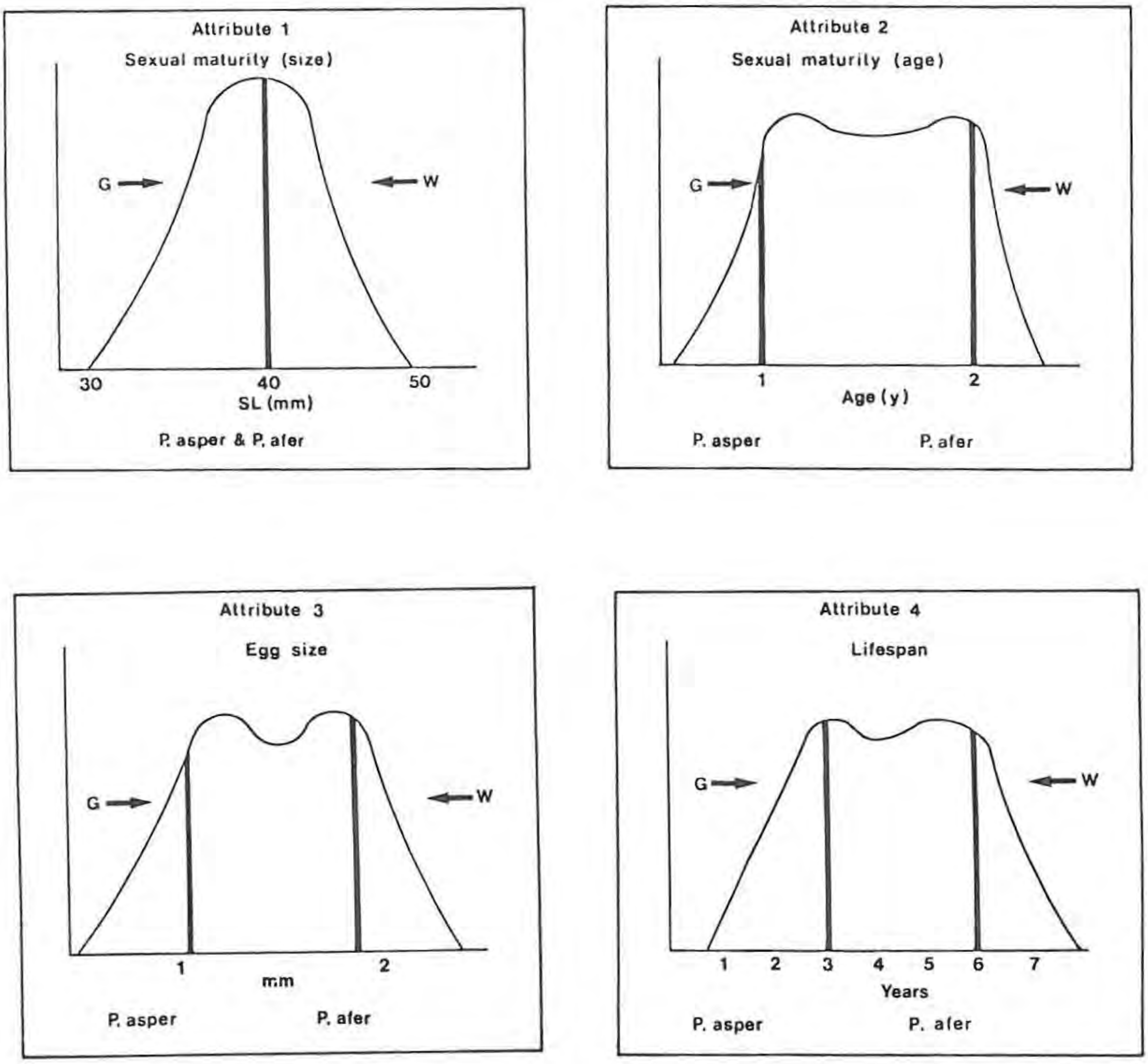


Figure 41. Variability of several of the life history attributes of *P. afer* and *P. asper*. Diagrammatic reproductive style of *P.afer* and *P. asper* showing the range of phenotypic expression (illustrated by the curves) of each attribute for the sister species pair. The phenotypic option expressed by *P. asper* and *P. afer* is shown by the thick vertical lines and depends on the environmental conditions experienced by each of the sister species (G = Groot River; W = Wit River)(modified from Wootton, 1990).

CHAPTER - 5

EVOLUTIONARY TRADE-OFF BETWEEN EGG SIZE AND EGG NUMBER

Introduction

In several comparative studies of egg sizes of freshwater fish it has been found that fish in oligotrophic habitats have larger eggs than those in eutrophic habitats (eg. Coburn, 1986). There are trade-offs between the production of more eggs which will offset high larval mortality and the production of fewer, larger eggs which will increase the fitness of individual larvae.

There are differences in reproductive effort between *P. afer* and *P. asper*, with *P. afer* investing less in gonads and *P. asper* more (see Chapter 4). Based on the findings for North American cyprinids the hypothesis for this chapter is that *P. afer* inhabiting an oligotrophic mountain stream would be expected to have the larger eggs of the two species. In that case, there must be an adaptive significance of a few large eggs spawned in a mountain stream as compared to many small eggs spawned in a turbid, eutrophic stream.

Studies on African minnows of the genera *Barbus* and *Pseudobarbus* have shown that within and between genera there are differences in egg sizes. The adaptive significance of these differences is discussed. In Nearctic minnows, Coburn (1986) established that there was a positive correlation between egg size and vertebral number. This aspect was also investigated for the small data base of African minnows.

Methods

Data on the mean diameters of mature, unspawned ova were taken from collections of *P. afer* and *P. asper* from several sites in the Gamtoos River system (Chapter 4).

Handling fish under laboratory conditions may shut down ovarian physiology for a short time therefore it is better to use mature oocytes from fish fixed in the field than live eggs from laboratory females (Wallace in Heins & Baker, 1988). In eggs, yolk loading ceases as oocytes attain their full mass and begin to ripen (Mashui & Clarke, 1979; Selman & Wallace, 1986) therefore mature oocytes are appropriate for accurate determination of propagule size.

The methods of egg choice and measurement followed those of Coburn (1986). Ripe females were dissected and the mature, deep yellow eggs were removed from the posterior section of the ovaries. Ten eggs from 10 females were measured to the nearest 0,05 mm in diameter with a calibrated ocular micrometer mounted on a Nikon dissecting microscope. The seasonality of the largest ova in the ovaries (Chapter 4) is included in the present discussion. Analyses were done using a two-sample *t* test.

Measurements of fertilized, water-hardened *P. asper* and *P. afer* eggs were made in a separate study on the comparative early ontogeny of the two species. Additional egg measurements were obtained from the literature for African minnows. The egg diameters of the *Barbus* species were compared to those of the *Pseudobarbus*.

The relationship between egg size and tubercle size was examined. The hypothesis tested was: Species with large nuptial tubercles have large eggs.

The relationship between egg size and vertebral number was examined for several small *Barbus* and *Pseudobarbus* species. The hypothesis tested was: Fish with larger eggs will have a higher vertebral count. No attempt was made to match collections of eggs with collections for vertebral studies. Vertebral records were taken from published (Skelton, 1988) and unpublished work (Skelton, 1980a). Mean vertebral counts were calculated from the raw data in Skelton (1980a). A linear regression of vertebrae to egg size was computed for African minnows.

Results

P. afer mature ova were 1,51 mm (S.E. = 0,01; n = 100) in diameter in October 1987 and 1,56 mm (S.E. = 0,01; n = 100) in October 1988. The mean egg size of the *P. afer* sample was significantly different between the two years ($t_{0,05(2),198} = 3{,}10898$ where $P \leq 0{,}002$ of being equal). *P. asper* mature ova were 1,16 mm (S.E. = 0,01; n = 100) in diameter in October 1987 and 1,14 mm (S.E. = 0,007; n = 100) in October 1988. There was no significant difference between the egg sizes in the two years ($t_{0,05(2),198} = 1{,}8601$ where $P \geq 0{,}064$ of being equal). In both years there were highly significant differences between the mean egg diameters of *P. afer* and *P. asper* samples during October (October 1987 $t_{0,05(2),198} = 20{,}6838$; $P < 0{,}0000$; October 1988 $t_{0,05(2),198} = 28{,}2828$; $P < 0{,}0000$).

Mean egg size was compared between the October and December samples for each year for each species. In the *P. afer* sample in December 1987 there was a wide range of eggs measuring 0,7 to 1,7 with a mean size of 1,23 mm (S.E. = 0,02; n = 100). This mean egg diameter was highly significantly different from the October 1987 sample ($t_{0,05(2),198} = 11,3727$; $P \leq 0,0000$). In the December 1988 sample the mean *P. afer* egg diameter was 1,01 mm (S.E. = 0,05; n = 100) which was again highly significantly different from the October mean egg diameter ($t_{0,05(2),198} = 10,9353$; $P \leq 0,0000$). The mean egg diameter for *P. asper* in December 1987 was 1,2 mm (S.E. = 0,006; n = 100) which was significantly larger than the October 1987 sample ($t_{0,05(2),198} = -2,11459$; $P \leq 0,0357$). In comparison, the mean egg diameter of the *P. asper* sample in December 1988 was 0,95 mm (S.E. = 0,022; n = 100) and this was highly significantly smaller than the egg diameter of October 1988 ($t_{0,05(2),198} = 8,21263$; $P \leq 0,0000$).

Table 21 includes a review of the mean mature ova sizes of seven *Barbus* and four *Pseudobarbus* species giving their corresponding volume. In the *Barbus* species egg size ranged from 0,8 mm (0,27 mm^3) to 1,4 mm (1,44 mm^3). In the *Pseudobarbus* species egg size ranged from 1,1 mm (0,7 mm^3) to 1,5 mm (1,77 mm^3).

Fertilized, water-hardened eggs of *P. afer* were spheroid and 2,06 ± 0,14 SD mm and the yolk was 1,74 ± 0,11 SD mm (n = 10) in diameter. *P. asper* fertilized, water-hardened eggs were 1,48 ± 0,07 SD mm and the yolk measured 1,12 ± 0,05 SD mm (n = 21). In Table 22 fertilized egg sizes are reviewed for the African minnows with the largest recorded being 1,74 mm (2,76 mm^3) for *P. afer*.

The average size of mature ova diameters of *Barbus* species was 0,98mm (n = 8; SD = 0,08) and for *Pseudobarbus* species this figure was 1,38 (n = 4; SD = 0,19). At the 95% confidence level they were highly significantly different ($t_{0,05(2)10} = -5,19047$ $P \leq 0,0004$). *Barbus* species had an average volume of 0,5 mm^3 (n = 8; SD = 0,14) compared to *Pseudobarbus* with an average volume of 1,29 mm^3 (n = 4; SD = 0,47) which was highly significantly different ($t_{0,05(2),10} = -4,60378$; $P < 0,001$).

The mean vertebral number ranges from 33,2 in *B. viviparus* to 39,3 in *P. quathlambae* (Table 23). The details of the linear regression of vertebrae to egg size for the *Barbus* and *Pseudobarbus* species are:

$Vert. = 29,7469 + 5,13429(ED)$

$r = 0,758$

$d.f. = 7$

where $Vert.$ = number of vertebrae

ED = egg diameter

Table 21. A review of the egg sizes of African minnow species.

Species	Mature ova* Diameter (mm)	Volume (mm^3)	Reference
Barbus anoplus	1,0	0,52	Cambray (1983)
B. kerstenii	1,0	0,52	Welcomme (1969)
B. liberiensis	1,0	0,52	Payne (1975)
B. paludinosus	1,0	0,52	Crass (1964)
B. paludinosus	0,8-1,0	0,27-0,52	Welcomme (1969)
B. trevelyani	1,0-1,3	0,52-1,15	Gaigher (1975)
B. trevelyani	1,2-1,4	0,9-1,44	Cambray (1985)
B. trimaculatus	0,9	0,38	Crass (1964)
B. viviparus	0,9	0,38	Crass (1964)
Pseudobarbus afer	1,5	1,77	This study
P. asper	1,1	0,7	This study
P. burchelli	1,5	1,77	Cambray & Stuart (1985)
P. quathlambae	1,35-1,43	1,29-1,53	Cambray & Meyer (1988)

* Mature ova before water hardening, except for *P. quathlambae* where the yolk diameter of the water hardened eggs was used.

Discussion

Reproductive output of teleost fish is determined by body size (Elgar, 1990). The way in which the reproductive output is partitioned between the size and number of offspring varies independently of body size. Elgar (1990) calculated that clutch size is significantly and negatively correlated with relative egg volume. This is consistent with the view that for any body size, increases in clutch size are accompanied by decreases in egg size (Duarte & Alcaraz, 1989; Mitton & Lewis, 1989).

A survey of 101 marine and 33 freshwater fish species from Europe indicated that the frequency distribution of egg diameters is skewed towards the smaller diameters. Wootton (1990) interpreted this as an evolutionary tendency in fish to minimize egg size. Since the volume of the abdominal cavity that can accommodate ripe eggs is limited, minimization of egg size would tend to maximize fecundity.

Table 22. A review of the fertilized egg sizes of African minnow species.

Species	Fertilized eggs* Diameter (mm)	Fertilized eggs* Volume (mm^3)	Reference
Barbus anoplus	0,8-1,0	0,27-0,52	Cambray (1983)
B. kerstenii	1,0	0,52	Welcomme (1969)
B. liberiensis	1,0	0,52	Payne (1975)
B. paludinosus	1,0	0,52	Crass (1964)
B. paludinosus	0,8-1,0	0,27-0,52	Welcomme (1969)
B. trevelyani	1,0-1,3	0,52-1,15	Gaigher (1975)
B. trevelyani	1,2-1,4	0,9 -1,44	Cambray (1985)
B. trimaculatus	0,9	0,38	Crass (1964)
B. viviparus	0,9	0,38	Crass (1964)
Pseudobarbus afer	1,74	2,76	This study
P. asper	1,12	0,74	This study
P. quathlambae	1,31	1,17	Rondorf (1976)
P. quathlambae	1,35-1,43	1,29-1,53	Cambray & Meyer (1988)

* Fertilized eggs after water hardening, only the width of the yolk is included, not the width of the egg envelope.

Table 23. Mean vertebral number for some African minnows for which there is data on egg diameters. Mean vertebral number calculated from raw data in Skelton (1980a).

Species	Sample size	Total vertebrae range	Mean vertebrae
Barbus anoplus	35	33-36	34,7
B. paludinosus	50	33-36	34,6
B. trevelyani	29	35-37	36,3
B. trimaculatus	29	34-36	35,1
B. viviparus	38	33-34	33,2
Pseudobarbus afer (Gamtoos)	50	36-37	36,5
P. asper	114	35-38	36,3
P. burchelli	167	34-38	36,3
P. quathlambae	31	38-40	39,3

Seasonal changes in egg size

Within a fish population, mean egg size has been found to vary from one year to the next (Mann & Mills, 1985) or could vary within the same year (Bagenal, 1971). In the *Pseudobarbus* species there was a seasonality in the sizes of eggs (Figures 34 & 35). The analysis indicated that in both December 1987 and December 1988 the mean egg diameter of *P. afer* eggs was significantly smaller than in October of those same years. This was probably due to differences in spawning times of individual fish as some fish may have recently shed eggs and were beginning their next cycle of yolk loading. In October the range of eggs measured was narrower. In the December sample some fish had several large (1,8 mm) eggs with the remainder as small as 0,2 mm. The eggs of *P. asper* in December 1987 were larger than those of the October sample whereas in October 1988 the eggs had a larger mean egg size than in December 1988.

P. asper had less variability in their egg sizes in the December periods (range 1987 - 1,1 - 1,4 mm; range 1988 - 0,5 - 1,2 mm) compared to *P. afer* (range 1987 - 0,7 - 1,7 mm; range 1988 - 0,2 - 1,8 mm).

In the October 1988 sample *P. afer* eggs were significantly larger than those of October 1987. In contrast there was no significant difference between the *P. asper* eggs collected in October 1987 and October 1988. The consistency of egg size in *P. asper* was unexpected in such a variable environment. Seasonal change as well as year to year changes in *P. afer* egg size may have been an adaptive response to the change in conditions that confronted the fish.

Ware (1975) developed a model which predicted that optimal egg size would decrease as incubation period (time from fertilization to hatching) decreased. Since there would be a decrease in incubation time with an increase in temperature during summer there should be a decrease in egg size. A change in egg size would also allow the size of the larvae to match the size spectrum of available prey (Jones & Hall, 1974; Ware, 1975). In the orangethroat darter, *Etheostoma spectabile*, the size of ripe eggs varies over the course of a single breeding season and also among females collected at the same time and place (Hubbs, 1967; Hubbs *et al.*, 1968; Marsh, 1984). The orangethroat darter had larger eggs at the start of the breeding season followed by a decrease in egg size near the end of the breeding season (Marsh, 1986). The egg size appears to track the water temperature with the largest eggs being produced during the coldest period.

In a review of egg sizes of marine and freshwater fishes Bagenal (1971) found that seasonal change within a species was a biological rather than a physical phenomenon which was not dependent on seasonal changes in temperature or salinity. In freshwater species geographic and seasonal variations were attributed to food availability. There was a clearer pattern for marine species as egg size appears to be controlled by seasonal availability of food (Bagenal, 1971; Ware, 1975, 1977). Marsh (1986) considered the effects of photoperiod, temperature and food availability on components of offspring fitness which may be used to predict expected patterns of seasonal egg size variation, if egg size variation was being selected to counter or enhance these environmental effects on offspring. For the orangethroat darter, *E. spectabile,* Marsh found that temperature affected offspring fitness, as warmer temperatures caused earlier hatching, faster growth and more rapid starvation. The seasonal cycles of food availability possibly also favoured the production of larger eggs at colder temperatures in the orangethroat darter populations. Although if fish had more smaller eggs there could be more competition for the limited resources amongst offspring which may lower offspring fitness. Therefore a seasonally varying competitive environment could mediate selection for seasonal variations in egg size (Marsh, 1986).

Habitat

Some clues to explain the production of a few large or many small offspring in fish can be found in differences in habitat. An analysis done by Elgar (1990) on both marine and freshwater fish families provided evidence of an evolutionary trade-off between egg size and number. In the data set freshwater fish were found to have significantly larger and fewer eggs than marine fish. That difference was not attributable to differences in body sizes between freshwater and marine fish. Elgar (1990) suggested that there was a possibility that variation in fecundity rates across fish taxa may be correlated with mortality schedules as in birds and mammals (Partridge & Harvey, 1988).

The marine environment favoured the production of larger clutches of small eggs as a result of low survival rates at juvenile stages (Elgar, 1990). In the present comparative study the mountain stream redfin, *P. afer,* had few large eggs compared to the many smaller eggs of *P. asper* which inhabits the more saline Karoo waters. It is suggested here that low survival rates of the embryonic period of *P. asper* may have influenced egg size. Smaller (therefore more eggs) may increase *P. asper* progeny fitness in an intermittent Karoo stream.

Egg size varied across taxonomic groups and associated habitats of New World cyprinids (Coburn, 1986). Coburn found that cyprinids from montane or upland habitats typically developed larger oocytes than those from lowland habitats, larger rivers and lakes. The lowland species usually produced oocytes of 0,86 mm whereas the upland taxa have a mean mature oocyte diameter of 1,36 mm. It was also found that oocyte size and territoriality were significantly associated. Species with parental care and territoriality had the largest eggs generally. In the gobies egg size differences were not related to simple habitat differences (Miller, 1984).

Egg size has been shown to vary intraspecifically as well as interspecifically (Bagenal, 1971; Ware, 1975). The interpopulation differences in egg size of the North Atlantic herring studied by Blaxter (1969) were probably related to seasonal changes in density, size spectrum and spatial distribution of the planktonic prey of larval herring. Mills & Eloranta (1985) found that the eggs of the northern population of the cobitid, *Noemacheilus barbatulus*, had a volume 2,5 times larger than those of a southern population. Intraspecific variation in bestowal has also been observed between different geographical populations of gobies (Miller, 1984).

In salmonids the species with the longest freshwater cycle had the largest eggs (Rounsefell, 1957). It was also found that *Oncorhynchus* species which spawned in rivers have larger eggs than those that spawn in lakes. Bagenal (1969) demonstrated that alevins from large eggs have a significantly higher survival rate than those from small eggs. In carp, *Cyprinus carpio*, Hulata *et al.* (1974) found that 11 month old hatchery reared females produced considerably smaller eggs which had a volume (1,25 mm; 1,02 mm^3) of less than half that of 23 month old fish (1,68 mm; 2,48 mm^3) even though some of the fish were the same size. They found very little further increase in egg size with age. That work indicated that the ages of the fish from which eggs are compared should be known. In wild collected eggs that may not be possible for many species.

P. afer in the oligotrophic mountain stream had larger eggs (1,5 mm ± 0,12 SD) compared to *P. asper* in the eutrophic Karoo stream with significantly smaller eggs (1,16 mm ± 0,12 SD). The optimal egg size for both *P. afer* and *P. asper* would be one that maximized the number of offspring surviving to become reproductively active. That was the egg size at which fecundity and juvenile survival was at a maximum (Sibly & Calow, 1986).

The larger *P. afer* larvae may have a higher rate of success in capturing prey in an environment where food is scarce. The influence of initial egg size on subsequent survival and development has important ecological implications. In the *P. afer / P. asper* study egg size differences between the two species were interpreted as a very important life-history phenotypic option for survival in two different environments.

Predation

A large yolk sac reduces locomotor performance and gives the fish a hydrodynamic disadvantage (Blaxter, 1969), but extends the period of switching from endogenous to exogenous feeding. Any factor that produces larger larvae would be likely to reduce the risk of predation.

Heavy mortality might not be at the egg stage for some fish species but at hatching and swim-up, or at first feeding. Free embryos with small yolk sacs may be better able to move to a preferred habitat to avoid predators in the period before the yolk sac is fully absorbed. However, the larger and the more developed the free embryo, the fewer predators there will be as the fish switches habitats, from the relatively safe refuge of the spawning beds to the pool environments. Any factors that are likely to cause early improvement in growth and size are likely to reduce the range of predators more rapidly. Larger eggs produce larger and better developed free embryos and first-feeding larval fish which are less vulnerable to predation. The larger size and better development is of ecological importance as it allows for faster swimming, better escape and a better ability to catch prey and the large larvae have a larger selection of food items (Flegler-Balon, 1989). The large *P. afer* larvae may counter predation pressure from *Sandelia capensis*, other *P. afer* or invertebrates in the clear mountain streams. In the turbid water environment *P. asper* larval fish would be less visible to predators and large size would not be as advantageous as in the clear water habitat.

Relationship of egg size to fecundity

Egg number is a function of gonad size and is a major component of reproductive fitness (Bagenal, 1967, 1969). Typically the fecundity of a species producing large eggs is lower, thus egg size and fecundity tend to be inversely related (Blaxter 1969; Marsh, 1986). In the present study egg size and fecundity were found to be inversely related (see chapter 4). *P. afer* has the larger eggs but a lower fecundity compared to the smaller eggs and higher fecundity of *P. asper*. In theoretical models developed to examine egg size, the relationship of offspring fitness to egg size and the trade-off between egg size and egg number are key assumptions (Pianka, 1976; Wilbur, 1977).

In *P. afer* there appears to be a trade-off between lower reproductive effort per season and longer lifespan (Chapters 4 and 9). In *P. asper* the reverse was found. Whether investment per offspring is selected for simultaneously with reproductive effort is not known. Possibly the selection forces are different. A larger *P. afer* offspring in the mountain stream has a higher fitness whereas less investment per clutch could result in longer survival of the female to breed another season. Both these phenotypic options would also reduce offspring competition for a scarce food resource and spread it over time.

Parental care and egg size

Among invertebrates and cold-blooded vertebrates egg size is often positively correlated with the amount of parental care given to the developing embryos (Shine, 1978). Egg size and parental care are expected to coevolve, with an increase in egg size there is an expected increase in parental care (Nussbaum & Schultz, 1989). This may be a reflection of parental investment per offspring and in total investment per clutch. In fish "with increasing parental care - from egg scatterers to brood hiders to external and internal bearers - the eggs become yolkier and more numerous" (Flegler-Balon, 1989: 71). The greater amount of yolk enables the young to grow to a larger size and be more developed at hatching and at first feeding. In some fish species parental care of developing embryos would be a means of offsetting the reduction in fitness resulting from increases in egg size (Nussbaum & Schultz, 1989). They suggested that parental care may evolve as a consequence of selection for increased propagule (offspring) size. This would explain the positive correlation between egg size and parental care which has been observed in many groups (Nussbaum, 1985, 1987). However, Nussbaum & Schultz (1989) concluded that no general prediction can be made about the pattern of correlation between egg size and parental care among populations. In anurans fecundity was highest and egg sizes smallest in species which have a generalized reproductive mode, in contrast to specialized species which have a reduced fecundity but larger eggs and/or parental care (Duellman, 1989). In the North American minnows the species with territorial and/or nesting behaviours had larger eggs (mean 1,32 mm) compared to those with no specialized reproductive behaviour (mean 0,87 mm) (Coburn, 1986). As far as is known there is no parental care of either *P. afer* or *P. asper* offspring except for the initial choice of a spawning bed.

Egg size and first feeding larval size

There are a number of reasons to explain why a change in egg size can be selected for independently of parental care. The developing embryo has to depend on the yolk for reserves of energy and nutrients until it is able to start feeding exogenously. Ovum size (= calories available for development) is the primary physical factor which influences the size at hatching and the stage of development. There is a positive correlation between egg and larval size, bigger eggs produce bigger larvae (Blaxter & Hempel, 1963; Ware, 1975; Marsh, 1986). With an increase in egg size there is likely to be an increase in juvenile survival (=offspring fitness). There is therefore a relationship between size and stage at hatching and endogenous and exogenous sources of nutrition. In an experimental study orangethroat darter individuals were larger at hatching from large eggs but they did not take longer to hatch than those from smaller eggs (Marsh, 1986). Larger larvae have a higher survival because they are able to take a wider range of prey sizes and may be able to survive periods of food shortage better than smaller larval fish (Blaxter & Hempel, 1963; Marsh, 1986) and would also have fewer predators (Wootton, 1990). Darter offspring from larger eggs took an average of 3,25 days longer to starve than those from small eggs and were therefore at an advantage especially under colder water conditions (Marsh, 1986). The higher survival of larger larvae has also been shown for brown trout (Bagenal, 1969) and for herring (Blaxter & Hempel, 1963). Thus the large eggs of *P. afer* would have an adaptive advantage if the food supply for the larvae is sparse or variable, or if the period spent in the egg stage is long or relatively unpredictable. This occurs in the northern species of grunion, *Leuresthes tenuis*, which has bigger eggs which might be an adaptation to the more irregular tides than the more southerly species (Wootton, 1990). In the redfin minnow populations it is probably the food supply which is important for the selection of large *P. afer* eggs. In the mountain stream environment the offspring must be larger than in the Karoo river to obtain resources for themselves which plays a vital role in their success in this environment. The larger larvae of *P. afer* would have a higher resistance to starvation and thus be able to survive longer under colder conditions where food is scarce.

Species either produce a small number of large eggs which are adapted for high survival and by-pass or reduce the time in the vulnerable stage (*P. afer*) or produce large numbers of small eggs with low survival but enough eggs to absorb the risk (*P. asper*). Both *P. afer* and *P. asper* have indirect development with a larval period. *P. afer* begins this period at a larger size than do *P. asper*. A primary response to stress (eg, scarce food supply in the Wit River) is a selection for larger egg size. This reduces juvenile mortality and the reduction in the proportion of biomass allocated to reproduction is a secondary response (Stearns & Crandall, 1984). *P. afer* follow this pattern as they have larger eggs as well as a reduced biomass allocated to reproduction.

There are more and smaller food particles in the Karoo river as compared to the mountain stream. The amount of food resources available to the offspring would depend on both the size of the offspring as well as the size and frequency of the appropriate food. Increased egg size in herring, *Clupea harengus*, resulted in a larger jaw size (Blaxter & Hempel, 1963) which would imply that they could handle larger food items. Competition and predation could also be influenced by the size of the offspring. Larger larvae may also result in greater aggressiveness and competitive ability (Magnuson, 1962). The size difference at hatching or first feeding between fish from small eggs and those from large eggs may disappear after a month or more when the fish are fed to satiation (Fowler, 1972; Reagan & Conley, 1977). This would support the idea that it is size at first feeding which is a crucial developmental time (Balon, 1990).

Although the effect of predation on the early stages may also influence size.

Egg size and spawning habitat

In the Karoo stream *P. asper* spawning beds dry up and are covered with silt for long periods (Cambray, 1991a). After short duration flows, due to localized thunderstorms, small eggs which develop quickly would have a higher survival rate and therefore the female which spawns smaller eggs would have a higher fitness than one spawning large eggs. *P. afer* have silt-free spawning beds which are covered with water throughout the year. The chance of survival is high even if development is slow which means investing more energy per offspring which leads to a higher survival rate.

Total embryo mortality is the product of the instantaneous rate of embryo mortality and the duration of the embryonic period. Larger eggs require more time to develop (Nussbaum, 1985; Sargent *et al.*, 1987) which may result in reduced fitness because of increases in the chances of embryonic mortality, although this is not always the case, as egg size of the orangethroat darter had no effect on hatching time (Marsh, 1986). In the developmental studies *P. afer* took three days longer to become first-feeding larval fish compared to *P. asper* (Chapter 4). With *P. afer* the trade-off cost was exacerbated, because larger egg size meant that fewer eggs could be produced. It is suggested here that it was not egg size as such, but rather first feeding larval size that was being selected for in the mountain stream. Offspring fitness for *P. afer* increases with larger first feeding larval fish. If food is in short supply then larger larvae would survive longer before starvation, which Marsh (1986) found to be the case with orangethroat darters.

Adaptive significance of egg size

At all levels of reproductive effort there will be two opposing selection pressures. One will favour the development of large propagules and the other will favour the formation of as many propagules as possible (Mann & Mills, 1985). The phenotypic options used by an individual fish within a population in response to any changes in environmental conditions will have an underlying adaptive component (Mills, 1991). The small eggs of a *P. asper* female will therefore have an underlying adaptive component. In the discussion of reproductive guilds of fishes Balon (1990) noted the evolutionary trajectory towards larger eggs and that the larger free embryos and larval fish produced would have a better chance of escaping predation and also have more effective feeding. Wootton (1990) has suggested that there may be an evolutionary tendency to minimize egg size and maximize fecundity. This latter trend may be due to the historical legacy of a marine environment and there has to be strong selection pressures to overcome it. Wootton (1990) is therefore noting an evolutionary constraint to small egg size, whereas Balon (1990) is noting an evolutionary direction along a trajectory towards larger egg size.

Heins & Baker (1988) noted that accurate determinations of propagule size are critical to interpretations of the adaptive significance of reproductive styles in fishes. It has been suggested that the interplay between egg size and mean vertebral number, habitat and spawning behaviour, may be valuable in formulating ideas on life-history styles, evolutionary trends and adaptive significance (Coburn, 1986). He found that mean egg diameter was strongly conserved with relatively little variation in several taxa (subgenera and genera) of eastern North American cyprinids. As the origin of the North American cyprinid genera pre-dates the Pleistocene, the observed constancy of egg diameter and vertebral number over this time span involving many climatic shifts indicated to Coburn (1986) that important aspects of larval ecology, breeding behaviour, and habitat, which are all likely to be related to egg size, are probably also conserved. It was also established that, as egg diameter increases, there was more variation among species.

Co-evolved phenotypic options (trade-offs) may not exist at the intraspecific level as suggested from work done on mosquito fish where no correlation was found between offspring size and brood size (Stearns, 1980). Marsh (1986) suggested that we keep an open mind on intraspecific trade-offs. It is accepted here that since there can be considerable egg size differences between females taken at the same time in some species one needs to be cautious with any interpretations based on a few specimens. That there is variability in egg size is well documented and the concept of an optimum egg size is not supported by empirical data (Kaplan & Cooper, 1984; Heins & Baker, 1988). Studies done on the interclutch variation in egg size among females collected at the same time and at different times (Marsh, 1984; Heins & Baker, 1987; Heins & Baker, 1988) have demonstrated that oviparous fishes produced eggs that varied in size which may have been an adaption to a variable environment (Capinera, 1979).

The suggestion of Wootton (1990) that there is an evolutionary tendency to minimize egg size and maximize fecundity needs to be considered together with Balon's (1990) suggestion of an evolutionary trajectory towards larger eggs. Marsh (1986) could find no advantage for an orangethroat darter coming from a small egg. Hulata *et al.* (1974) also found it difficult to understand why the eggs from yearling carp were so much smaller than those of older females. They could only suggest that the yearling female did not have enough time for the eggs to develop to their full potential size. If this reasoning is correct it may also explain why there are seasonal shifts from larger to smaller eggs in some species. Within the *Pseudobarbus* species which inhabit mountain streams (eg. Wit River) the tendency to minimize egg size is being selected against. In addition, within the *Pseudobarbus*, there is a tendency towards large egg size which may indicate a phylogenetic constraint in this lineage however Balon (1990) might say that this follows the evolutionary trajectory to larger eggs. Only in the Groot River was small egg size advantageous for a *Pseudobarbus* species to increase offspring fitness. In a Karoo stream the suggested evolutionary tendency of Wootton (1990) towards minimisation of egg size has been selected for in *P. asper*. If selection favoured increased egg size for any reason at all, then changes in life-history attributes that offset the potential increase in embryonic mortality are of selective advantage if these changes are less costly than the benefits that accrued from them. There are trade-off constraints as was clearly seen in the higher number of eggs which could be produced by *P. asper* although *P. afer* invested less per clutch than did *P. asper*. Therefore *P. asper* have increased their fecundity not only by reducing investment per offspring but also by investing more per clutch than *P. afer*. The mechanism behind the switch to smaller or larger ova might have been determined by the optimal size of larvae which was determined by the size of the available food particles, the abundance of competitors and the numbers and sizes of predators (Svardson, 1949). If the small, first feeding larval fish originating from small eggs could not locate enough small prey then they would be selected against. Marsh (1986) found that low levels of food availability for offspring may represent an overriding environmental factor which favours larger eggs. The evolution of a large egg size may have also been associated with low temperatures and/or possibly with variability in stream conditions (Coburn, 1986). The riparian forest along the Wit River shades the river and therefore the river is cooler compared to the open Groot River. The Wit River is also oligotrophic compared to the enriched Groot River.

Review of the egg sizes of small African Barbus *and* Pseudobarbus *species*

Bagenal (1978) cautioned that eggs should be at the same developmental stage if they are to be compared by size. It was recommended that the eggs are best measured after fertilization. In most of the studies done on the African minnows the exact stage of development of the measured ovarian eggs has not been noted. This may be an important starting point for future work in this field. It is therefore with caution that the following comparison is undertaken and hopefully future studies will be able to critically review these findings (Table 21). In a second table only those records are included in which either laboratory fertilized or wild collected eggs are mentioned (Table 22).

Mature ova sizes of African minnows range from 0,8 to 1,5 mm in diameter (Table 21). The small *Barbus* species have mature ova ranging in size from 0,8 to 1,3 mm in diameter with the majority of the species with a diameter of 1,0 mm (Table 21). This was comparable to what Coburn (1986) found for New World cyprinids from lowland habitats. In contrast the eggs of the four *Pseudobarbus* species were bigger and ranged from 1,1 for the lowland species *P. asper* and 1,35 to 1,5 mm for the mountain stream species, which was more comparable to the upland taxa of the New World cyprinids (1,36 mm; Coburn, 1986). If these diameters were converted to volume then the 1,0 mm *Barbus* ova were 0,52 mm^3 and the 1,5 mm *Pseudobarbus* ova had a volume of 1,77 mm^3. Therefore for a 0,67 fold (0,5 mm) diameter increase there was a 3,4 fold (1,25 mm^3) volumetric increase. Therefore a small *Barbus* species could theoretically have up to 3,4 times the mature ova per batch of a *Pseudobarbus* species like *P. afer*. Three of the four *Pseudobarbus* species were bestowing considerably more per offspring than were the majority of the small *Barbus* species (Table 21).

The smallest *Barbus* eggs were recorded for *B. paludinosus* in a stream flowing into Lake Victoria (Welcomme, 1969). The largest of the *Barbus* eggs were those of *B. trevelyani* in the Tyume River which is a tributary of the Keiskamma River (Gaigher, 1975).

There may be a lower and upper limit to the size of the *Barbus* and *Pseudobarbus* eggs which is genetically controlled. Within this range there can be variability (0,8 - 1,3 mm for *Barbus* species and 1,1 - 1,5 for *Pseudobarbus* species) which is under the influence of the local environment. Pooled data demonstrated that overall *Pseudobarbus* eggs are significantly bigger than those of *Barbus* species. Coburn (1986) recorded an ova size range of 0,7 - 2,0 mm (0,18-4,18 mm^3) which is a volume increase of 23 times for Nearctic cyprinid species.

Pseudobarbus species have a tendency towards larger eggs. The majority of the species inhabit oligotrophic streams which have silt-free spawning beds. The early larval fish have to be large enough to successfully hunt for large food items in the clear riverine pools. They also have to be mobile enough to escape predation by other fish.

There must be a balance between the egg number and the egg size (Svärdsen, 1949). If there was not then the parents with small eggs and therefore higher fecundity would leave more progeny and dominate the population. This is an example of what has possibly occurred in the Groot River for *P. asper* and shows the evolutionary tendency to minimize egg size when possible whereas a similar reproductive style in the Wit River would not be successful.

Egg size relationship to tubercle size within the small Barbus *and* Pseudobarbus *species*

In general the *Pseudobarbus* have large tubercles [which Skelton (1980a) recognised as a synapomorphy] and large eggs whereas small *Barbus* species have small or no tubercles and small eggs. It was therefore considered important to assess if there is any correlation between egg size and tubercle size. Within the *Pseudobarbus* there is a trend between *P. afer* (large eggs, large tubercles) and *P. asper* (small eggs, smaller tubercles). Skelton (1980a) reviewed the occurrence of tubercles in some southern African *Barbus* species. The species were grouped with regard to the basal diameter of the head tubercles and tubercle pattern. *B. trimaculatus* had small tubercles (< 0,5 mm) and they also have small eggs (Table 21). *B. trevelyani* have small tubercles (< 0,5 mm) which cover much of the head and the body surface. According to the hypothesis being tested *B. trevelyani* should have medium sized tubercles and they do not. *B. anoplus* were found to have large tubercles (> 0,5 mm) and therefore should have large eggs but do not. There is no mention of other species in Skelton (1980a) for which there is egg size data. From this limited data set there appears to be no positive correlation of egg size with tubercle size except in the trend noted within the *Pseudobarbus* species.

Egg diameter variation correlation to vertebral number of Barbus *and* Pseudobarbus *species*

Coburn (1986) suggested that investigators looking for evolutionary characters should be impressed by the interplay among hard and soft anatomical features (eg. vertebrae and egg diameter) and behaviour. Vertebral counts are a frequently used taxonomic tool (eg. Skelton's 1988 work on the *Pseudobarbus*) and these counts may imply correlated changes in other quantifiable characters, such as egg size. Correlations are usually done between egg diameters and female size or environmental parameters. Cyprinids exhibit pleomerism, or a positive correlation between maximum body size and vertebral number (Lindsey, 1975). Coburn (1986) found a positive correlation between egg size and vertebral count that cut across species of different phyletic lines, habitats, and niches [*Vert.* = 31,4 + 5,88(*Egg diam.*); n = 61; r^2 = 0,657, P < 0,001]. For the small data set available for African minnows the linear regression was *Vert.* = 29,75 + 5,13(*Egg diam.*) (n = 8; r^2 = 0,575, P < 0,018).

Skelton (1980a) suggested that the high vertebral count (36-38) of the flexible-rayed redfins (*Pseudobarbus*) is in accordance with Jordan's Rule (Lindsey, 1975). It is also well-established that fish species with larger eggs tend to have more vertebrae (Lindsey & Ali, 1971), spawn in cooler water (Ware, 1977) and are more likely to build nests (Shine, 1978) than related species with smaller eggs. It is also of comparative interest that the only *Barbus* species with a large egg, *B. trevelyani*, also has a higher vertebral count (36,3) than the other small *Barbus* species (33,2 - 35,1). It would appear from this limited data set that the correlation Coburn (1986) found between egg size and vertebral number in North American minnows may well hold for African minnows and is worth further investigation.

Coburn (1986) reviewed the mechanisms controlling vertebral numbers in teleosts. He concluded that there is strong evidence in support of environmental factors, such as temperature, which induce vertebral number variations around a modal value. Coburn (1986:117) posed the question "Can small changes in egg diameter also account for variations around the modal value, or a shift to a new modal value?" In the eastern North American minnows studied by Coburn the slope of the regression line (5,88) predicts a change of one vertebra with a change in egg diameter of about 0,17 mm. In the African minnow data this figure is one vertebra for a change in egg diameter of about 0,2 mm. Using the American minnow data this would mean that the difference in vertebral number between *B. anoplus* and *B. trevelyani* (1,6 vertebrae) would predict an egg size of 1,27 (1mm plus 1,6 x 0,17) for *B. trevelyani* which is similar to the empirical data of Cambray (1985; Table 21). In the *Pseudobarbus* species, *P. asper* has 36,3 vertebrae and 1,1 mm eggs as compared to 39,3 vertebrae and 1,43 mm eggs for *P. quathlambae*. The other *Pseudobarbus* species have vertebral counts close to *P. asper* but egg sizes closer to *P. quathlambae*. The question posed by Coburn (1986) above may be answered here within the *Pseudobarbus*. *P. asper* modal vertebral number has not shifted to a new modal value with the observed shift in egg size. If we ignore the more atypical and derived of the *Pseudobarbus*, that is *P. asper*, then *P. quathlambae* should have the largest eggs. This also is not the case. Within the *Barbus* species the trend to more vertebrae with larger egg size is clearer (Table 23).

Hypothesis

The hypothesis raised in the introduction is therefore supported. *P. afer* inhabiting an oligotrophic stream has larger eggs compared to its sister species, *P. asper*, which inhabits a more productive system and has smaller eggs. The adaptive significance of few, larger eggs is probably related to larger size at first feeding in a low nutrient environment. Larger size at hatching may also reduce predation pressure. The adaptive significance of many small eggs in the Karoo stream may have been to offset high larval mortalities, and to hatch earlier in order to avoid desiccation in the spawning beds of this intermittent system.

CHAPTER - 6

ADAPTIVE SIGNIFICANCE OF SEX RATIO DIFFERENCES

Introduction

The hypothetical distribution of one male to one female (1:1) in a population holds true for the majority of fish species (Nikolsky, 1963) and indicates that selection favours evolution towards a sex ratio equilibrium (Bull, 1981). It has been suggested that a 1:1 ratio is the most effective way to maintain genetic stability (Tave, 1984). This 1:1 equal allocation of sons and daughters implies that each sex is of equal value (Gross, 1984) and is an evolutionarily stable equilibrium sex ratio (Maynard Smith, 1978).

Since most natural populations of fish have a sex ratio of 1:1 (Reay, 1984:297) it is reasonable to assume that a sex-ratio in favour of either males or females would indicate that there is an adaptive significance to producing more males or females. There has been very little discussion of the adaptive significance of any deviation from this 1:1 ratio for African minnows.

P. afer and *P. asper* were good study species to assess sex ratio differences and their environmental or genetic cause.

Hypothesis

It is assumed that both *P. afer* and *P. asper* have a 1:1 sex ratio.

Methods

Collections

P. afer were collected in the Wit River and *P. asper* were collected in the Groot River. Fish were collected with a minnow seine net (Chapter 4). All fish were fixed in the field in 10 % formalin and returned to the laboratory for further analysis.

The sex of 3212 *P. afer* and 4478 *P. asper* was determined by internal inspection either macroscopically or with the use of a low power (10X) dissecting microscope. The chi-square test was used to examine the deviations from unity for total catch, monthly collections and different length groups.

To avoid confusion all sex ratios are expressed as ♂♂:♀♀ and the following definitions are used for primary, secondary and tertiary sex ratios.

The *primary sex ratio*, that is the relative numbers of males and females soon after fertilization, is controlled genetically. The probability of equal numbers of males and females at "birth" is very high especially if the species has a high fecundity (Volodin & Grechanov, 1985). *P. asper* has a considerably higher fecundity than *P. afer* (Chapter 4) and therefore has a higher hypothetical probability of a 1:1 sex ratio.

The *secondary sex ratio* is that at the end of parental investment (Greenwood & Adams, 1987). Both *P. afer* and *P. asper* release their gametes into the spawning area and there is no subsequent parental care (Cambray, 1991a; and Chapter 4). Therefore in *P. afer* and *P. asper* the secondary sex ratio is the relative number of males and females at hatching.

The *tertiary sex ratio* is the balance of the adult individuals in the population (Greenwood & Adams, 1987). This can differ from the secondary sex ratio due to sex-related differences in ecology and longevity. To overcome some of the problems of differential mortality of the sexes with age juveniles as well as adults were examined.

Results

Of the 3212 *P. afer* sexed, 1199 were males and 2013 were females, giving a ratio of 1:1,68, which deviates significantly from the hypothetical distribution of 1:1 ($X^2 = 206{,}2877$; $p < 0{,}001$ of a 1:1) in favour of females (Table 24).

Of the 4478 *P. asper* sexed, 2334 were males and 2144 were females, giving a ratio of 1:0,92, which deviates significantly from the hypothetical distribution of 1:1 ($X^2 = 8{,}0616$; $p < 0{,}005$ of a 1:1) in favour of males (Table 24).

In all length groups of *P. afer*, except for fish greater than 80,5 mm SL (n = 1), there were significantly more females than males (Table 24). In the *P. asper* collections the sex ratios were more variable in the different length groups (Table 24). There were significantly more males in the smaller length groups (< 40,4 mm SL) and significantly more females in the length classes greater than 50,5 mm SL, except in fish over 80,5 mm SL (n = 1) (Table 24).

Table 24. Sex ratios for *Pseudobarbus afer* (n = 3212) collected in the Wit River between 24 September 1984 and 23 April 1989 and *Pseudobarbus asper* (n = 4478) collected in the Groot River (Smithskraal and Beerpoort combined) between 26 October 1986 and the 24 April 1989.

SL (mm)	*P. afer* ♂♂ : ♀♀	X^2	*P. asper* ♂♂ : ♀♀	X^2
20,0-30,4	1 : 2,0	28,0000***	1 : 0,77	12,2993***
30,5-40,4	1 : 1,40	28,8343***	1 : 0,73	42,4269***
40,5-50,4	1 : 1,66	65,9473***	1 : 0,88	4,8719*
50,5-60,4	1 : 2,02	72,6921***	1 : 1,52	26,2564***
60,5-70,4	1 : 1,83	16,6615***	1 : 3,02	51,7512***
70,5-80,4	1 : 5,75	13,3704***	1 : 8,33	17,2857***
> 80,5	0 : 1,0	1,0000^n.s.	1 : 0,0	1,0000^n.s.
Total	1 : 1,68	206,2877***	1 : 0,92	8,0616**

Probability of a 1:1 ratio
n.s. non-significant
* < 0,05
** < 0,01
*** < 0,001

In 24 out of 46 monthly collections there were significantly more female than male *P. afer* (Tables 25 & 27). In the *P. asper* collections there were significantly more males in only 3 of the 28 monthly collections and significantly more females in 5 of the 28 months (Table 26 & 27). In the earlier period of the study, during the drought, there were more females than males and this situation was reversed after the river flooded and Beervlei Dam filled in February/March 1988 (Table 26).

Table 25. Monthly sex ratios of *Pseudobarbus afer* collected from the Wit River.

Date	n	*P. afer* ♂♂ : ♀♀	X^2	>♂♂ or >♀♀	Probability 1 ♂♂ : 1 ♀♀
Sept. 1984	44	1: 3,4	13,0909**	♀♀	P < 0,001
Oct.	116	1: 1,58	5,8276*	♀♀	0,01 < P < 0,025
Nov.	79	1: 1,82	6,6962*	♀♀	0,005 < P < 0,01
Jan. 1985	79	1: 1,93	7,9114*	♀♀	0,001 < P < 0,005
Feb.	99	1: 1,48	3,6465		n.s.
Mar.	95	1: 1,11	0,2632		n.s.
Apr.	138	1: 1,03	0,0290		n.s.
Aug.	70	1: 1,92	6,9143*	♀♀	0,005 < P < 0,01
Sept.	64	1: 1,56	3,0625		n.s.
Nov.	134	1: 2,44	23,4030**	♀♀	P < 0,001
Dec.	83	1: 3,61	26,6145**	♀♀	P < 0,001
Jan. 1986	58	1: 3,14	15,5172**	♀♀	P < 0,001
Feb.	71	1: 1,96	7,4507*	♀♀	0,005 < P < 0,01
Mar.	67	1: 2,35	10,8806**	♀♀	P < 0,001
Apr.	97	1: 2,03	11,2268**	♀♀	P < 0,001
June	61	1: 1,10	0,1475		n.s.
Aug.	45	1: 2,46	8,0222*	♀♀	0,001 < P < 0,005
Sept.	63	1: 1,10	0,1429		n.s.
Oct.	71	1: 1,84	6,2113*	♀♀	0,01 < P < 0,025
Nov.	58	1: 1,90	5,5862*	♀♀	0,01 < P < 0,025
Dec.	41	1: 3,10	10,7561*	♀♀	0,001 < P < 0,005
Jan. 1987	50	1: 1,5	2,0000		n.s.
Feb.	76	1: 1,0	0,0000		n.s.
Mar.	26	1: 1,6	1,3846		n.s.
Apr.	63	1: 1,52	2,6825		n.s.
May	46	1: 0,77	0,7826		n.s.
July	40	1: 0,74	0,9000		n.s.
Aug.	83	1: 3,61	26,6145**	♀♀	P < 0,001
Sept.	55	1: 2,44	9,6182*	♀♀	0,001 < P < 0,005
Oct.	138	1: 1,26	1,8551		n.s.
Nov.	39	1: 1,60	2,0769		n.s.
Dec.	49	1: 3,08	12,7551**	♀♀	P < 0,001
Jan. 1988	41	1: 1,28	0,6098		n.s.
Feb.	93	1: 1,38	2,4194		n.s.

Table 25 (cont.)

Date	n	*P. afer* ♂♂ : ♀♀	X²	>♂♂ or >♀♀	Probability 1 ♂♂ : 1 ♀♀
Mar.	54	1: 3,91	18,9630**	♀♀	P < 0,001
Apr.	57	1: 3,07	14,7544**	♀♀	P < 0,001
May	79	1: 1,26	1,0253		n.s.
June	66	1: 1,75	4,9091*	♀♀	0,025 < P < 0,05
July	103	1: 1,29	1,6408		n.s.
Aug.	53	1: 1,79	4,2453*	♀♀	0,025 < P < 0,05
Sept.	52	1: 1,60	2,7692		n.s.
Oct.	49	1: 1,45	1,6531		n.s.
Nov.	39	1: 1,60	2,0769		n.s.
Dec.	28	1: 1,55	1,2857		n.s.
Jan. 1989	43	1: 1,53	1,8837		n.s.
Feb.	37	1: 2,08	4,5676*	♀♀	0,025 < P < 0,05
Mar.	66	1: 1,64	3,8788*	♀♀	0,025 < P < 0,05
Apr.	54	1: 1,25	0,6667		n.s.
TOTAL	3212	1: 1,68	206,2877**	♀♀	P < 0,001

n.s. not significantly different (P > 0,05) from 1:1 ratio
* statistically significant
** highly statistically significant

The monthly sex ratios of the *P. afer* collections (in which there were ≥ 30 specimens) varied from 1: 0,77 to 1: 3,91 (Table 25). The monthly sex ratio of *P. asper* collections (≥ 30 specimens) varied from 1: 0,58 to 1: 2,28 (Table 26).

The sex ratios of *P. afer* and *P. asper* were also examined during the main spawning season (October to February) for several years (Table 28). Between 1984/85 and 1988/89 there were significantly more females than males in the *P. afer* population for each season and the overall ratio for this period was 1: 1,88. During the rest of the year there were more females than males (Table 29). However, overall there were significantly fewer females in the non-spawning season (P < 0,001). In the *P. asper* population there were significantly more females in one spawning season and in the following season significantly more males (Table 28). Overall for the three seasons there were more male *P. asper* (1:0,9). During the rest of the year (March to September) there were significantly more females in 1988 and in 1989 significantly more males (Table 29) which is similar to the spawning season (Table 28). The overall sex ratio for *P. asper* during the

resting period was 1: 0,98 which is not significantly different from a 1:1 ratio (Table 29). There was no significant difference between the spawning season and the rest of the year for *P. asper*.

Table 26. Monthly sex ratios of *Pseudobarbus asper* collected from the Groot River.

Date	*P.* n	*asper* ♂♂ : ♀♀	X^2	>♂♂ or >♀♀	Probability 1 ♂♂ : 1 ♀♀
Oct.1986	37	1: 0,95	0,0270		n.s.
Nov.	75	1: 1,34	1,6133		n.s.
Dec.	95	1: 2,28	$14,4105^{**}$	♀♀	P < 0,001
Jan.1987	84	1: 0,68	3,0476		n.s.
Feb.	177	1: 1,01	0,0056		n.s.
Mar.	90	1: 1,05	0,0444		n.s.
Apr.	104	1: 1,17	0,6154		n.s.
May	3	1: 0,50	0,3333		n.s.
July	59	1: 0,69	2,0508		n.s.
Aug.	111	1: 1,06	0,0811		n.s.
Sept.	149	1: 1,29	2,4228		n.s.
Oct.	101	1: 1,06	0,0891		n.s.
Nov.	88	1: 1,32	1,6364		n.s.
Dec.	142	1: 2,16	$19,0423^{**}$	♀♀	P < 0,001
Jan.1988	114	1: 1,07	0,1404		n.s.
Feb.	135	1: 2,21	$19,2667^{**}$	♀♀	P < 0,001
Mar.	24	1: 2,43	$4,1667^{*}$	♀♀	0,025 < P < 0,05
Apr.	73	1: 1,70	$4,9452^{*}$	♀♀	0,025 < P < 0,05
May	114	1: 0,97	0,0351		n.s.
June	109	1: 1,18	0,7431		n.s.
July	78	1: 1,36	1,8462		n.s.
Aug.	88	1: 1,26	1,1364		n.s.
Sept.	73	1: 0,74	1,6575		n.s.
Oct.	174	1: 0,78	2,7816		n.s.
Nov.	110	1: 1,16	0,5818		n.s.
Dec.	507	1: 0,66	$20,9250^{**}$	♂♂	P < 0,001
Jan.1989	514	1: 0,62	$28,0156^{**}$	♂♂	P < 0,001
Feb.	322	1: 0,58	$22,9689^{**}$	♂♂	P < 0,001
Mar.	472	1: 0,77	$7,6271^{*}$	♀♀	0,005 < P < 0,01
Apr	256	1: 0,80	3,0625		n.s.
TOTAL	4478	1: 0,92	$8,0616^{*}$	♂♂	0,001 < P < 0,005

n.s. not significantly different (P>0,05) from 1:1 ratio

* Statistically significant

** Highly statistically significant

Table 27. Summary statistics for monthly sex ratio data of *Pseudobarbus afer* (n = 46 months) and *P. asper* (n = 28 months) for different probabilities. Only months with collections ≥ 30 specimens are included in the table.

Probability **of** **1 ♂♂ : 1 ♀♀**	***P. afer*** **Significantly more** **♂♂**	 **♀♀**	 **1:1**	***P. asper*** **Significantly more** **♂♂**	 **♀♀**	 **1:1**
< 0,05	0	24	22	3	5	20
< 0,025	0	20	26	3	4	21
< 0,01	0	17	29	3	4	21
< 0,005	0	14	32	3	3	22
< 0,001	0	10	36	3	3	22

Table 28. Sex ratios of *P. afer* and *P. asper* during the spawning season from October to February.

Date Oct.-Feb.	**n**	**♂♂:♀♀**	**X^2**	**> ♂♂ or > ♀♀**	**Probability 1♂♂ : 1♀♀**
			Pseudobarbus afer		
1984-85	377	1:2,43	65,382	♀♀	P < 0,001
1985-86	410	1:2,42	70,4878	♀♀	P < 0,001
1986-87	359	1:1,51	14,844	♀♀	P < 0,001
1987-88	415	1:1,58	20,841	♀♀	P < 0,001
1988-89	248	1:1,61	13,5645	♀♀	P < 0,001
Total	1809	1:1,88	167,8281	♀♀	P < 0,001
			Pseudobarbus asper		
1986-87	468	1:1,15	2,188	♀♀	n.s.
1987-88	729	1:1,48	27,2716	♀♀	P < 0,001
1988-89	1627	1:0,67	64,1235	♂♂	P < 0,001
Total	2824	1:0,9	7,9674	♂♂	0,001 < P < 0,005

Table 29. Sex ratios of *P. afer* and *P. asper* during the resting season from March to September.

Date March-September	n	♂♂:♀♀	X^2	> ♂♂ or > ♀♀	Probability 1♂♂ : 1♀♀
			Pseudobarbus afer		
1985	367	1:1,27	5,0381	♀♀	0,01 < P < 0,025
1986	333	1:1,69	21,6967	♀♀	P < 0,001
1987	313	1:1,65	18,9425	♀♀	P < 0,001
1988	464	1:1,76	35,3103	♀♀	P < 0,001
1989	120	1:1,45	4,0333	♀♀	0,025 < P < 0,05
Total	1597	1:1,57	78,9136	♀♀	P < 0,001
			Pseudobarbus asper		
1987	516	1:1,08	0,7752	♀♀	n.s.
1988	559	1:1,18	3,9517	♀♀	0,025 < P < 0,05
1989	728	1:0,78	10,6374	♂♂	P < 0,001
Total	1803	1:0,98	0,2446	♂♂	n.s.

A review of the sex ratios of small African *Barbus* and *Barbus*-like species indicates that, of the 12 studies on seven species, the sex ratio was significantly different from a 1:1 ratio in six of the studies (Table 30). In three of the studies there were significantly more males and in the other three significantly more females.

Discussion

Bulmer & Bull (1982) have recommended that, when considering the evolution of sex ratio, a researcher must keep in mind the sex determining mechanism and its effect on the supply of genetic variation in the sex ratio. There is very little known concerning the genetic basis of sex ratios in vertebrates with labile sex determination (Conover & Heins, 1987a). The secondary sex ratio of fishes varies considerably from species to species, but in the majority of species it is close to 1:1 (Nikolsky, 1963). However, the sex ratio can vary within populations of the same species,

Table 30. Review of the sex ratios of African *Barbus* and *Pseudobarbus* species.

Species	n	♂♂:♀♀	X^2	Reference
Barbus anoplus	22 920	1:1	0,0000$^{n.s.}$	Cambray (1982)
B. treurensis	64	1:0,6	4,000*	Kleynhans (1987)
B.trevelyani	474	1:0,67	18,6414**	Gaigher (1975)
Pseudobarbus afer	3 212	1:1,68	206,2877**	This study
P. asper	4 478	1:0,92	8,0616*	This study
P. asper	932	1:1,22	9,0815*	Cambray (1990)
P. burchelli	319	1:1,44	10,1850*	Cambray & Stuart (1985)
P. burchelli	119	1:1,53	3,8348$^{n.s.}$	Esterhuizen (1978)
P. quathlambae	30	1:0,58	2,1333$^{n.s.}$	Gephard (1978)
P. quathlambae	17	0,41:1	2,8824$^{n.s.}$	Gephard (1978)
P. quathlambae	13	0,75:1	0,0769$^{n.s.}$	Gephard (1978)
P. quathlambae	43	1:1	0,0000$^{n.s.}$	Rondorf (1976)

n.s. not significantly different from 1:1 ratio
* significantly different, probability of 1:1 ratio, $P<0{,}05$
** highly significantly different, probability of 1:1 ratio, $P<0{,}001$.

and may vary from year to year within the same population as was seen in the present study for the *P. asper* population in the Groot River. Nikolsky (1963) gives an example of the common perch, *Perca fluviatilis*, in which the sex ratio varies from 1:1 to 1:9 in different lakes. On a seasonal basis the sex ratios of the collections of *P. asper* varied from 1: 0,58 to 1: 2,28 (Table 26) whereas in the *P. afer* population the seasonal ratios were 1:1 or in favour of females (except for May 1987, Table 25).

Fish populations with sex ratios of 1:1 are most effective in maintaining genetic stability (Tave, 1984). Selection is said to favour the elimination of variance in the sex ratio (Bulmer & Bull, 1982). Sex ratio has probably evolved as an Evolutionary Stable Strategy (ESS) (Maynard Smith, 1982; Gross, 1984). If the ESS is adopted by a critical proportion of the population, it yields an individual fitness which exceeds that of all alternative life-history styles (=strategies) when these are rare in the population. In many populations a 1:1 equal allocation between sons and daughters has evolved, which implies a ratio in which each sex is of equal value. When no alternative sex ratio can do better when rare, then this is an ESS.

An equal sex ratio may arise as a consequence of heterogametic chromosomal sex determination (Cockburn, 1991). This would make it difficult to discern whether the 1:1 sex ratio is an artefact of the sex determining mechanism or whether the sex determining mechanism is a way of achieving the optimum sex ratios (Williams, 1979; Maynard Smith, 1980).

In a study of the entrainment of *P. asper* in the Groot River Cambray (1990b) sexed 932 specimens, with a size range of 20,5 to 79,8 mm SL of which 45,1% were males giving a sex ratio of 1:1,22 ($P < 0{,}005$ of a 1:1). These fish were collected by an irrigation pump when the river was flowing in April 1989. The 256 *P. asper* collected with a seine net several days prior to the entrainment collection had a ratio of 1: 0,8 (n.s.; Table 26). Therefore the migrating fish collected in the entrainment study had a higher proportion of females which indicated that sampling during this migration might have been biased.

In some African *Barbus* and *Pseudobarbus* species the ratio is more frequently biased towards the female component (Table 30). Many studies on southern African fish species have given an overall sex ratio and thus one cannot assess if there has been differential mortality. Overall sex ratios can be very biased according to the length group one collects. In five of these studies less than 100 specimens were sexed and this may give unreliable sex ratios for the population examined. Esterhuizen (1978) studied a population of *P. burchelli* in the Keisers River of the Breede River system. The overall sex ratio was 1:1,5 (n = 119). In some studies it has been more informative to split the males and females into discrete size classes. In a population of *P. burchelli* in the Breede River of the southern Cape, Cambray & Stuart (1985) calculated a 1:0,72 (n = 31) sex ratio for the length group 0-50 mm FL, 1:0,7 (n = 51) for fish 51-70 mm FL, 1:1,78 (n = 165) for fish 71-90 mm FL and 1:2,03 for fish over 90 mm FL. The relatively large number of fish in the 51-70 mm FL category biased the overall results. The overall sex ratio for this species was 1:1,44 (Table 30). In a population of *B. trevelyani* in the Tyume River in the eastern Cape, Gaigher (1975) calculated a 1:0,42 (n = 61) sex ratio for the length group 0-50 mm FL, 1:0,59 (n = 337) for fish 51-70 mm FL, 1:1,71 (n = 76) for the over 70 mm FL group giving an overall ratio of 1:0,67 (Table 30). Gaigher (1975) could find no reason why the ratio was not 1:1 nor why males were favoured in the smaller fish. However, in the fish over 70 mm FL the females predominated probably due to a higher survival rate than males. In a study involving over 20 000 *B. anoplus* Cambray (1982) found that the ratio was 1:1. However, on a monthly basis for the 25 month study he found that the sex ratio differed most significantly from unity during the early part of the breeding season (November to December) in comparison to the rest of the year. In three different sites within Lake le Roux the sex ratio of *B. anoplus* was

1:3,1; 1:4,4 and 1:2,1 whereas the riverine site had considerably more males, 1:0,1. The data suggested that there was a movement of reproductive males from the lake to the rivers during this time of the year. Collections made only at this time of the year would give biased sex ratios towards females in the lake and biased towards males in the rivers. Sampling throughout the year in a number of habitats gave the expected 1:1. This study on *B. anoplus* highlights the importance of analyzing enough data before assessing the population sex ratio.

The sex ratio of males to females in both *P. afer* and *P. asper* varies inversely with length. There are more females to males in fish over 50,5 mm SL (Table 24). This pattern of more females is especially clear in the length group over 70,5 mm SL (Table 24). Sex ratios frequently vary with age (Greenwood & Adams, 1987). In *P. afer* differential predation may influence the tertiary sex ratio as adult males are very conspicuous during the reproductive season due to their nuptial red fin colouration. Visually cued predators, such as kingfishers, may select males because of their higher visibility.

In a study on a larger African cyprinid, *Labeo capensis*, Gaigher *et al.* (1980) found that after the length at which females reached sexual maturity the tertiary sex ratio changed in favour of females. These authors did not work out chi-square analyses of their data but worked with percentages. From their data even in the smaller length group (0-20 cm; n = 9251; 48,1% ♂♂) there was a highly significant biased sex ratio in favour of females (probability of a 1:1 was < 0,0003). In the fish over 36 cm the probability of a 1:1 ratio was < 0,0000. They suggested that the adaptive significance of this skewed tertiary sex ratio was related to the environmental conditions during spawning. The conditions do not allow for extended breeding activities such as physical competition for females, therefore large males are not selected for. They reasoned that the success of spawning was independent of the size of males, with males becoming sexually mature at a smaller size than females and that most died before reaching a size at which the females matured. It was assumed that this differential mortality reduced intraspecific competition in the larger fish and the females would therefore have a better chance of growth which would have allowed for an increase in the mean mass and fecundity of females. Females matured at six years whereas males matured at four years.

Labile sex determination can be advantageous if the fitness of an individual in a particular set of environmental conditions is gender-dependent (Conover & Heins, 1987a). This would occur for example if the environment of the Groot River increased the fitness of the female *P. asper* more than the males during dry years. Alternatively the wet years appear to increase the fitness of the male *P. asper* (Table 26).

The sex ratio of *P. afer* is strongly skewed towards females (Tables 24 & 25). Other populations of *P. afer* may have a similar pattern and this should be investigated. A skewed sex ratio can be the result of either genetic or environmental sex determination. Genetic sex determination can result from, for example, low fecundity, male or female mortality during and after spawning, selfish genetic elements and hermaphroditism. Environmental sex determination may be the result of spawning ground temperature or water quality, pH, salinity etc. A difference in sex ratio from the hypothetical 1:1 can also be the result of sampling bias (Table 31).

Table 31. Some causes of distorted sex ratios.

Cause	Result	Reference
Natural	Hypothetical 1:1	Nikolsky (1963)
Fecundity	High favours 1:1	Volodin & Grechanov, (1985)
Male mortality during and after spawning	Favours females	Pappantoniou *et al.* (1982)
Selfish genetic elements	Favours females	Warren *et al.* (1988)
Spawning ground temperature	Favours males (high °C) or females (low °C)	Pluzhenko (1971) in Volodin & Grechanov (1985)
Hermaphroditism	Favours males or females	Shapiro (1984)

Genetic sex determination (GSD)

Since every offspring has one mother and one father, then frequency-dependent sex ratio selection will lead to the evolution of genetic mechanisms of sex determination that ensure a 1:1 sex ratio in most cases (Fisher, 1958). The evolutionary reason for the secondary ratio of 1:1 is known as *Fisher's sex ratio theory* (Fisher, 1958). The Fisher model has several possible restrictive assumptions one of which is random mating where complete mixing of the population occurs. *P. afer* and *P. asper* probably do mate randomly. Natural selection will act in favour of the rarer sex, if there is one, and as such the outcome is an equilibrium sex ratio of 1:1 since it cannot be bettered by an alternative 'strategy'. It has been demonstrated that *Fisher's sex ratio theory* is invalid if brothers compete with each other for the same females (Hamilton, 1967). One male fertilizes many females and the mother leaves the most grandchildren by producing one son and many daughters. *P. afer* males have large nuptial tubercles which may be used for competition for females which indicates that *Fisher's sex ratio theory* may be invalid for this species. Male competition often leads to a female-biased offspring sex ratio (Hamilton, 1967).

The size-fecundity hypothesis predicts that mothers which are capable of producing large offspring should bias sex allocation towards daughters (Cockburn, 1991). *P. afer* have the largest eggs and this may indicate that there would be a bias towards daughters.

Bulmer & Bull (1982) noted several ways in which genetic variability for sex ratio can arise. Firstly, in species with sex chromosomes and female heterogamety there may be differential segregation of the sex chromosomes to ova and polar bodies. Secondly, the sex of the zygotes of fish species with polygenic sex determining mechanisms (Kosswig, 1964) is determined by the sum of the genetic effects over many loci. The sex determining genes are said to be fundamentally the same as sex ratio modifiers acting in the zygote. Thirdly, in some haplodiploids the sex of each egg depends on whether the "mother has allowed" it to be fertilized. Fourthly, in some species sex is not fixed at conception, but is determined in the embryo in response to its immediate environment. The sex ratio is therefore determined by the mother's choice of nest site and the embryo's sex differentiation in response to temperature. There may be a genetic variation for both of these.

According to Fisher's theory there should be a 1:1 secondary sex ratio at the end of parental care. If there was a 1:1 sex ratio then this would imply that the potential reproductive success of a son is equal to that of a daughter. Greenwood & Adams (1987: 33) paraphrased Fisher's theory and suggested "invest equally in both sexes." Since Fisher's theory assumes that individuals mate at random in the population it is probably applicable to broadcast spawners such as *P. afer* and *P. asper* (Cambray, 1991a; Chapter 4). Although the large nuptial tubercles and breeding colouration of mature *P. afer* males indicated that this species may not have mated at random (see above) there was no supporting evidence for non-random mating.

The primary sex ratio is controlled genetically. The probability of equal numbers of males and females at "birth" is very high especially if the species has a high fecundity (Volodin & Grechanov, 1985). *P. asper* has a considerably higher fecundity than *P. afer* (Chapter 4) and therefore has a higher hypothetical probability of a 1:1 sex ratio. The present data set supports this assumption.

Male mortality

Even before the *P. afer* males were sexually mature the sex ratio was skewed towards females (Table 24). A higher mortality of males after spawning is known in certain species. In a study of the cutlips minnow the sex ratios were skewed towards females (1:1,32) but not significantly according to a chi-square goodness of fit test ($p > 0,05$ of a 1:1) (Pappantoniou *et al.*, 1982). In this species the male minnow builds and defends a large stone nest. It was thought that this spawning behaviour may have caused higher mortality amongst males than females. Males were observed to be more "bruised" after spawning than females and therefore more susceptible to infections, predation and may have had a higher mortality. After spawning in the Wit River the larger *P. afer* males often have a number of scales missing (personal observations) which may eventually have caused mortalities. Loss of scales on female *P. afer* and male and female *P. asper* was not observed. The larger and more numerous nuptial tubercles on male *P. afer* as compared to male *P. asper* (Chapter 7) would also have indicated that male *P. afer* may have used the tubercles when defending a spawning area from other males.

Selfish genetic elements

There are genetic elements in a wide range of species which are known to distort the sex ratio in ways which are advantageous to the transmission of this element (Werren *et al.*, 1988). In animals cytoplasmic genes are typically inherited through egg cytoplasm not through the sperm. This asymmetric inheritance pattern encourages sex ratio distortions in favour of female offspring. Werren *et al.* (1988:302) posed the question "How do selfish elements influence the evolutionary trajectory of species?" There is the possibility that these selfish genetic elements could cause species extinction. The population of *P. afer* in the Wit River did have significantly more females so the existence of selfish genetic elements could not be ruled out.

Sampling bias

Differences in behaviour, ecology and the catchability of males and females can make it difficult to obtain a sample that is an accurate estimate of the population sex ratio. Sampling during male or female only spawning migrations would give a biased sex ratio. This was evident in the *B. anoplus* study where, during a spawning run, males were abundant in the river and females in the lake (Cambray, 1982). Sampling in only one season or one year might also bias results (see Table 26). The sampling of entrained *P. asper* in an irrigation furrow gave a biased sex ratio for *P. asper* (Cambray, 1990b). This was a short term study and showed sampling bias compared to the bigger data set. In both the redfin minnows the sex ratios were calculated over a long sampling period which included periods when the fish were not reproductively active. It was therefore unlikely that the reason for the observed skewed sex ratios of either species was a sampling artefact.

Natural mortality during early development

In the case of gonochoristic species any deviation in the sex ratio from 1:1 can be caused by higher mortality of one of the two sexes during embryonic or early larval development, when the coefficient of natural mortality is the highest. It is not known if the sex ratio was distorted at that early stage for *P. afer* or for *P. asper.*

Environmental sex determination (ESD)

ESD is a mechanism in which sex is determined in the embryo in response to some environmental stimulus. In some species the sex may not be determined at spawning but at some point later in life in response to an environmental cue. ESD occurs when factors such as temperature irreversibly influence the primary sex determination during development (Conover & Kynard, 1981). Temperature-dependent sex determination (TSD) is well known in reptiles (Bull, 1980). Greenwood & Adams (1987) listed several well documented cases of ESD such as *Menidia menidia* for which the environmental cue is sea temperature, and the result is that there is a higher ratio of females early in the season. Stearns (1982) suggested that the environmentally induced production of alternate forms, in this case another sex, seems to be selected when there are discontinuities in the environment and when environments vary mostly between, rather than within, generations. There may be an underlying distribution of genotypes, alleles at each locus which has a weak influence on whether the zygote becomes male or female in a particular environment. This aspect is discussed later, as early spawned *P. afer* would be exposed to lower temperatures and this may influence the determination of their sex during development.

In general the major cause of ESD in vertebrates is temperature (Bull, 1980, 1983). Sex ratios in fish can be distorted by the temperature of the water during spawning and early development (Volodin & Grechanov, 1985). One of the important parameters of population structure which is determined during the embryonic period is the initial sex ratio. The secondary sex ratio (ie. the relative number of males and females at hatching) probably depends on many factors (Makeeva & Nikolsky, 1965 in Volodin & Grechanov, 1985). One of these factors is the temperature of the water during embryonic development. Pluzhenko (1971 in Volodin & Grechanov, 1985) incubated common carp eggs at different temperatures. At 23 °C he obtained 94% males, at 16 °C there were 78% females and there were equal numbers of males and females at 19 °C. Temperature during development also directly affected the sex ratio of the progeny of the Atlantic silverside, *Menidia menidia*, (Conover & Heins, 1987a). Conover & Heins (*op cit.*) determined that there was a curvilinear relationship of sex ratio to temperature with more females produced at the start of the breeding season than males. More males were produced towards the end of the breeding season when temperatures rose. Therefore at the end of the breeding season the females would be larger than the males because of the longer growing season. In this species large size was more important for female reproductive success. The adaptive significance was that individuals spawned at the start of the breeding season would maximize fitness as females whereas at the end of the breeding season they would do so by developing as males (Conover, 1984). Adjustments of sex ratio in response to temperature are those that would be predicted by adaptive sex ratio theory (Conover & Heins, 1987b). The result is that there is an altering of environmental as opposed to genetic control. Therefore these studies indicate that fluctuations in temperature in the spawning grounds during egg development can alter the sex ratio of the new generation. Population sex ratio is therefore tracking environmental changes, in this case temperature. However, photoperiod should also be considered (Conover & Heins, 1987a).

It can only be postulated that there may be an adaptive significance of the cool water temperature at the spawning sites of the *P. afer* populations which alters the sex ratios. Further collections of *P. afer* spawned both early in the breeding season as compared to later in the season should be investigated. The major spawn was the first spawn in the *P. afer* population and possibly these were mainly females. The cooler embryonic incubation temperatures may have enhanced individual fitness more for a female than for a male *P. afer* as in some lizards (Bulmer & Bull, 1982). The more labile sex ratio of *P. asper* may be related to the wet/dry cycle which would also have had a strong temperature component. Not enough data were available. In dry years one would expect higher water temperatures which may result in more females. In wetter years temperatures would be lowered and more males might be the end result (Table 26). Bull (1981) and Bulmer & Bull (1982) suggested that environmental sex determination was selected against in a fluctuating environment if fitness among females and males did not correlate differently with the environmental parameter determining sex. The variance increases in a fluctuating environment which reduces the extent of environmental sex determination.

Bull (1981) considered the evolution of the environmental cue controlling sex ratio. He argued that the cue appeared to be highly correlated with environmental circumstances where there was a major difference in fitness between becoming a male or a female. Whatever the environmental cue in the Wit River was it may have influenced the sex ratio control. There must have been a differential benefit to females over males in the Wit River and this differential benefit did not occur in the Groot River for *P. asper* females. That was why selection has "over-produced" females which are benefitted most by the Wit River environment.

A sex ratio in favour of female *P. asper* in the drought years would have ensured that, in the highly variable Groot River environment, there were enough eggs spawned to overcome the high mortality rates. The very low water flow would have ensured that a smaller number of males could have adequately fertilised the eggs. In the wet years more males might have been required to ensure fertilisation in the larger spawning habitats and the faster flows. One of the problems with this proposal would be the time-lag required between being spawned (when sex would be determined) and actually being part of the spawning schools the following year when the environment would be very different. Possibly the phenotypic variability of *P. asper* individuals explains their labile sex ratios. The Groot River during a drought cycle might well have a gender-dependent influence on fitness. In this case then ESD was adaptive in the life history of *P. asper* with more females during drought years and more males during wet years. In comparison the female dominated *P. afer* population occurred in a relatively stable mountain stream environment. The dominance of the population by females may have been the result of ESD and the mechanism would be TSD.

ESD probably played a more important role in the sex ratio of *P. afer* as compared to *P. asper*.

The sex ratio of *P. asper* would probably be more under the control of GSD which would explain the many collections which are not significantly different from the 1:1 hypothetical ratio. The small mountain stream habitats of *P. afer* with less variable temperatures and flow patterns than the Groot River habitat of *P. asper* should be investigated in more detail to determine whether these factors determine the distorted sex ratio. The Wit River environment probably selects for an increased level of ESD compared to GSD and the reverse holds true for the Groot River.

As both *P. afer* and *P. asper* are multiple spawners (Cambray, 1991a; Chapter 4) there is a range of temperatures to which the developing embryos are exposed to each year. However, the inter-annual as well as annual range of temperatures to which the *P. asper* are exposed would be greater than *P. afer* as *P. asper* has a longer breeding season and the spawning habitats are not shaded.

Sex ratios in some cichlids can be greatly skewed by water pH (Heiligenberg, 1965). This may possibly occur by affecting sex differentiation after the early life history stages are hatched. There is a difference in the pH between the two sections of the Gamtoos River system (Chapter 2). Early developmental stages of *P. afer* are subjected to slightly acidic to neutral waters whereas the early life stages of *P. asper* in the Groot River are exposed to fluctuating, alkaline water. In the *P. afer* population there are more females in each length group. The skewed sex ratio might exist early in this species' life history and water quality may be a causative factor.

The reproductive effort of males may vary less with size so that females in poor condition should produce a daughter biased sex ratio. *P. afer* females have submaximal clutch volumes compared to *P. asper* which may indicate a relatively poor reproduction condition and therefore they produce more daughters.

In his modelling of sex ratios Bull (1981) considered both constant and fluctuating environments. He reasoned that results for constant environment models do not necessarily extrapolate to fluctuating environments. In the constant environment of the Wit River *P. afer* has a female biased sex ratio whereas in the fluctuating environment of the Groot River the *P. asper* have a 1:1 ratio and at times a male biased ratio. This appears to indicate that a constant versus a fluctuating environment may cause a difference between the sex ratios.

GSD combined with ESD

Conover & Kynard (1981) found that sex determination in the atherinid, *Menidia menidia*, was under both environmental and genetic control during a critical phase of larval development. Progeny from different females had highly skewed, highly variable sex ratios and differed in their response to temperature. That was the first reported case among fishes where sex was determined by environmental factors after conception (Price, 1984). *P. afer* and *P. asper* are genetically close (sister species) however they inhabit two very different environments. That would tend to suggest that the difference in sex ratios was not chromosomally determined but environmentally determined for the two redfin minnows.

GSD versus ESD

Charnov & Bull (1977) argued that if under certain circumstances selection will favour becoming male (or female) then selection might favour a genetic system which permits an animal to do this. In this way ESD may be favoured over GSD because it would enable the embryo to 'control' its sex response to the environment. It was possible that this was happening in the Wit River with *P. afer*. Bull (1981) and Bulmer & Bull (1982) developed population genetic models which depicted the transition between ESD and GSD. The sex determining mechanism and the sex ratio of a species are linked. Bulmer & Bull (1982) considered a model in which sex is determined in the zygote by the sum of genetic effects at many loci plus an environmental effect. If a pair of chromosomes determines sex then the primary sex ratio is controlled by the segregation ratio of these chromosomes in the heterogametic sex. However, if an environmental cue determines an embryo's sex then the primary sex ratio is controlled by the distribution of environmental effects on the population (Bulmer & Bull, 1982). In other cases frequency-dependent sex ratio selection leads to GSD. In the different populations of *M. menidia*, studied by Conover & Heins (1987a&b), the adjustments in sex ratio and in the sex-determining mechanism of populations from different environments are good examples that illustrate the adaptive basis of ESD and GSD.

It has been suggested that sex should be environmentally determined whenever the offspring enters a patchy environment away from the parent, in which some patches strongly favour males over females or females over males, and in this case neither offspring nor parent has control over the patch type the offspring enters (Charnov & Bull, 1977). Cockburn (1991) noted that

the success of sex ratio theory has not been matched by research on how the adaptive manipulation may have come about. Male or female is a clear bifurcation and epigenetic mechanisms, triggered by spawning ground temperature, may be responsible in many cases where the sex ratio is biased.

Hypothesis

The hypothesis which was raised in the introduction was not verified by the findings of the present study. *P. afer* was found to have a female biased sex ratio which differed significantly from the hypothetical 1:1 ratio. The sex ratio of *P. asper* was closer to unity but was slightly in favour of males. In the clear mountain streams, a biased sex ratio in favour of female *P. afer* was probably optimum for the production of fertilized eggs. Cool water temperatures during spawning of *P. afer* may have been the main cause of a female biased sex ratio. Although social behaviour might have also had an influence. The enlarged breeding tubercles and enhanced breeding colours of the male *P. afer* would indicate that there is competition for females.

CHAPTER - 7

SEASONAL OCCURRENCE, MICROWEAR AND DISTRIBUTION OF NUPTIAL TUBERCLES

Introduction

Epidermal breeding tubercles are temporary roughened structures which have evolved in primitive euteleostean fishes and have been recorded in at least 25 families of bony fishes in 7 orders (Wiley & Collette, 1970). In addition tubercles occur in some species of the Coregonidae, Thymallidae (Salmoniformes) and Gadidae (Gadiformes) (Kratt & Smith, 1978; Vladykov *et al.*, 1985; Witkowski, 1982 in Witkowski & Rogowska, 1991). Structural differences indicate that tubercles have evolved independently in a number of fish groups (Collette, 1977). Tubercle patterns can be used to infer similarities or differences in behaviour patterns (Wiley & Collette, 1970). The head and forepart of the body of many species of the family Cyprinidae may be covered with excrescences (Breder & Rosen, 1966) and have a bright nuptial colouration during the spawning season (Banarescu & Coad, 1991). Tubercles might have evolved to enable spawning individuals to maintain contact in fluviatile environments. Tubercles may also be used by some fish species for the defense of nests and territories and possibly for the stimulation of females to breed (Wiley & Collette, 1970; Collette, 1977).

As in other cyprinids the tubercles of the flexible-rayed redfin minnows are deciduous, hypertrophied, epidermal structures with a distinct keratin cap (Skelton, 1980a, 1988). Tubercles can be valuable systematic characters in cyprinids (Wiley & Collette, 1970; Collette, 1977). The distribution, number, size, and developmental patterns of the head tubercles represent the most critical characters in the separation of some species (Lachner & Jenkins, 1971a). Tubercles have also been considered as important evolutionary characters (Lachner & Jenkins, 1971a).

Barnard (1943) and Jubb (1967) both recorded the presence of tubercles and smaller 'pimples' on redfin minnow species. Both authors used the presence or absence of tubercles as a major character to separate species in southern Africa. In the original description of the redfin minnow, *P. quathlambae*, Barnard (1938) mentioned that conical tubercles occurred on both sexes. Skelton (1988) described the tubercle structure and pattern of distribution on the flexible-rayed redfins and considered tubercles to be one of the outstanding characters of these species which include the two sister species *P. afer* and *P. asper*.

The seasonal occurrence of these structures has not been studied in any detail for *P. afer* and *P. asper*. Skelton (1974) gave a brief account of the seasonality of the tubercles for oreodaimon (*P. quathlambae*) and Cambray & Stuart (1985) noted the seasonality of the head tubercles for *P. burchelli* over a 12 month period. A SEM micrograph of a male head tubercle scar in the species *P. quathlambae* was given by Skelton (1974). Data on the seasonal occurrence of breeding tubercles are rarely collected (Collette, (pers. comm., October 10, 1985). Most studies treat tubercles as a two-state character, present or absent. The usual seasonal sequence for breeding tubercles is their appearance before the spawning season and just prior to the spawning season they reach their maximum extent. Following spawning the structures break off, slough, become eroded, or gradually regress.

Hypothesis

If the head tubercles of the *Pseudobarbus* species are used for any visual signal, such as sex or species recognition, then a clear water species (*P. afer*) should have larger and more tubercles than a species inhabiting a turbid system (*P. asper*).

Methods

Collection and analysis

Fish were collected with a minnow seine net, fixed in 10% formalin and taken to the laboratory for analysis. *P. afer* were collected from the clear Wit River and *P. asper* from the turbid Groot River in the Gamtoos River system (Figure 4).

Conical tubercle formation was studied on a monthly basis for the males and females of both species over a 22 month period (July 1987 to April 1989). Fish were examined each month from a size range covering the largest fish in the sample to the smallest without any tubercles. A total of 248 male and 118 female *P. afer* and 320 male and 157 female *P. asper* were examined (Tables 32 - 35). The height and basal width of the conical tubercles were measured with an eyepiece micrometer on a stereoscopic microscope (accuracy 0,05mm). Instead of comparing height or basal width, the volume of the tubercles was considered a better comparative measurement. This would especially be the case if the tubercles are used for visual display. The volume of the tubercles was calculated from the height and basal width data using the formula for a cone:

$$\left(V = \frac{\pi r^2 h}{3}\right)$$

Table 32. Summary of the seasonal pattern of tubercle formation and wear in 248 *P. afer* males collected over a 22 month period in the Wit River, Gamtoos River system.

Date	n	Size range* SL (mm)	Head tubercles[1]	Scales[2]	Rays[3]
Jul. 1987	11	49,2-71,1	B+Sp	A	A
August	3	45,6-51,8	B+Sp	A	A
September	12	39,7-62,8	D+W	P	P
October	31	40,3-78,2	D+W	P	P
November	12	37,3-70,5	D+W	P	P
December	8	45,9-63,4	D+W+S	P	P
Jan. 1988	7	46,0-59,2	D+W+S	A	P (few)
February	12	47,2-68,1	W+S	A	P (few)
March	6	42,8-63,1	W	A	P (few)
April	12	39,1-66,0	W+S	A	P
May	15	43,1-65,8	W+S	A	A
June	5	46,7-58,0	B+S+Sp	A	A
July	9	51,6-72,5	B+W+S+Sp	A	A
August	7	42,2-64,7	B+Sp	P (few)	A
September	11	40,7-63,2	D	P	P
October	12	40,6-62,5	D+W	P	P
November	14	43,5-66,2	D+W+S	P	P
December	5	44,2-70,1	D+W	P	P
Jan. 1989	14	37,2-69,6	B+W+S	A	P
February	9	38,0-70,5	B+W	P (few)	P
March	19	41,4-68,2	B+W+S	A	P
April	14	38,9-67,6	B+W+S	A	P
TOTAL	248				

* Sometimes only on largest specimens

[1] B = bud; D = definitive; S = scars; Sp = spots; W = worn

[2] A = absent; P = present

[3] A = absent; P = present

Table 33. Summary of the seasonal pattern of tubercle formation and wear in 320 *P. asper* males collected over a 22 month period in the Groot River, Gamtoos River system.

Date	n	Size range* SL (mm)	Head tubercles[1]	Scales[2]	Rays[3]
July 1987	8	58,6-67,8	B+S	A	A
August	7	58,8-83,4	B+S	A	A
September	14	43,3-64,0	B	P (few)	P (few)
October	15	41,7-64,9	B+D+W	P	P
November	20	40,4-72,3	D	P	P
December	14	45,7-69,9	D	P	P
Jan. 1988	18	45,4-63,1	W	P	P
February	11	42,4-62,0	D+W	P	P
March	6	50,3-53,7	D	P	P
April	18	34,1-57,6	D+W	P	P
May	10	47,0-58,3	W	A	A
June	16	49,9-65,4	B+S+W	A	A
July	4	48,5-54,5	B+W	A	A
August	10	48,1-70,4	B+S	A	P
September	29	42,8-62,4	B+D	P (few)	P
October	15	42,4-53,9	D+W	P	P
November	20	44,2-63,6	W	P	P
December	16	36,0-64,5	D	P	P
Jan. 1989	19	37,9-61,2	D	P	P
February	13	45,0-66,1	W	P	P
March	12	48,6-71,9	B+D	P	P
April	25	41,7-66,7	B+W	P (few)	P
TOTAL	320				

* Sometimes tubercles were present only on largest specimens
1. B = bud; D = definitive; S = scars; Sp = spots; W = worn
2. A = absent; P = present
3. A = absent; P = present

The terminology used by Lachner & Jenkins (1971a) was followed with several modifications to describe tubercle development. Five stages were identified, the *spot, bud, nuptial definitive tubercle, worn tubercle* and *scar*. The *spots* were defined as distinct light areas which approach the epidermal surface. Tubercle *buds* were small pimples which have a rounded or pointed tip. *Nuptial definitive tubercles* were the mature structures which were hard, pointed and cornified. *Worn tubercles* were definitive tubercles which had lost their points and were rounded or flat but still raised above the surface. *Scars* were left when the tubercles were lost. Tubercles were easily distinguished from dermal sense organs simply by lifting the tubercles from the skin with a needle and a small round depression would remain (Branson, 1962).

Table 34. Summary of the seasonal pattern of tubercle formation and wear in 118 *P. afer* females collected over a 22 month period in the Wit River, Gamtoos River System.

Date	n	Size range* SL (mm)	Tubercles[1]	Scales[2]	Rays[3]
July 1987	5	52,4-62,5	A	A	A
August	5	57,6-63,0	Sp (few)	A	A
September	5	60,8-70,7	B+Sp	A	A
October	8	67,0-76,1	B+Sp	A	A
November	5	61,0-68,0	W+Sp	A	A
December	5	64,3-68,7	Sp	A	A
Jan. 1988	5	46,8-51,8	A	A	A
February	5	56,8-72,4	B	A	A
March	5	53,4-68,7	B	A	P (few)
May	5	54,2-61,2	A	A	A
June	5	55,5-59,8	W (few)	A	A
July	5	59,1-72,8	Sp (few)	A	A
August	5	54,9-68,2	A	A	A
September	5	60,6-64,5	B (few)	A	A
October	5	56,4-61,1	B (few)	A	A
November	5	53,2-64,9	A	A	A
December	5	53,4-70,1	Sp	A	A
Jan. 1989	4	52,7-61,1	A	A	A
February	5	54,0-70,5	A	A	A
March	11	58,0-79,9	B	A	P (few)
April	5	60,3-65,4	B	A	A
TOTAL	118				

* Sometimes tubercles were present only on largest specimens
1. B = bud; D = definitive; S = scars; Sp = spots; W = worn
2. A = absent; P = present
3. A = absent; P = present

SEM micrographs were taken with a JEOL Scanning Electron Microscope operating at 10 mV. The eruption and wear of tubercles were noted. Pre-spawning and post-spawning head tubercles were removed to study the microwear on the surfaces of the tubercles using standard SEM techniques. The microwear on the pectoral fin tubercles was also studied. Microwear is a term used in archaeology to describe the wear on stone, bone or wooden tools by friction and wear patterns caused by the different uses of the tools. It was thought this method could be used to determine if the tubercles had been used for butting females, fighting other males or for digging and making nests. It was hoped that these activities would cause different wear patterns on the tubercles.

Table 35. Summary of the seasonal pattern of tubercle formation and wear in 157 *P. asper* females collected over a 22 month period in the Groot River, Gamtoos River System.

Date	n	Size range* SL (mm)	Tubercles[1]	Scales[2]	Rays[3]
July 1987	3	54,1-56,0	A	A	A
August	3	69,8-76,0	A	A	A
September	11	50,1-69,6	B	A	A
October	4	42,9-57,4	B	A	A
November	6	49,3-63,2	B+Sp	A	A
December	4	50,4-74,3	B+Sp	A	A
Jan. 1988	5	64,7-70,8	Sp	A	A
February	15	50,1-74,1	B+Sp	P (few)	P (few)
March	13	45,1-57,8	B+Sp	A	A
April	8	56,7-61,9	B (few)	A	P (few)
May	4	50,8-60,1	A	A	A
June	5	67,8-72,3	A	A	A
July	4	54,0-63,5	A	A	A
August	4	61,9-74,4	A	A	A
September	5	60,5-79,9	B	A	A
October	5	51,3-70,0	B+Sp	A	A
November	8	65,5-72,2	B+Sp	P (few)	P (few)
December	16	54,1-70,7	B+Sp	A	A
Jan. 1989	5	54,5-70,9	B+Sp	A	A
February	9	60,7-76,5	B+Sp	A	A
March	12	62,2-75,2	B+Sp+S	A	A
April	8	59,1-70,9	B+Sp	A	A
TOTAL	157				

* Sometimes tubercles were present only on largest specimens

1. B = bud; D = definitive; S = scars; Sp = spots; W = worn
2. A = absent; P = present
3. A = absent; P = present

Tubercles were divided into clusters which Skelton (1988) used to describe the pattern of tubercles in the flexible-rayed redfin minnows. These clusters are shown in Figure 42. In each of the paired clusters the left and right sides were counted separately to see if there were different growth and/or wear patterns correlated to side. Regression analyses were done for the total number of tubercles versus length (SL mm) for each sex of each species. Tests of difference of slopes of the regression lines of the total tubercle number for *P. afer* and *P. asper* males were performed by analysis of covariance (Zar, 1984). The F values yielding probabilities greater than 0,95 were considered to be significant.

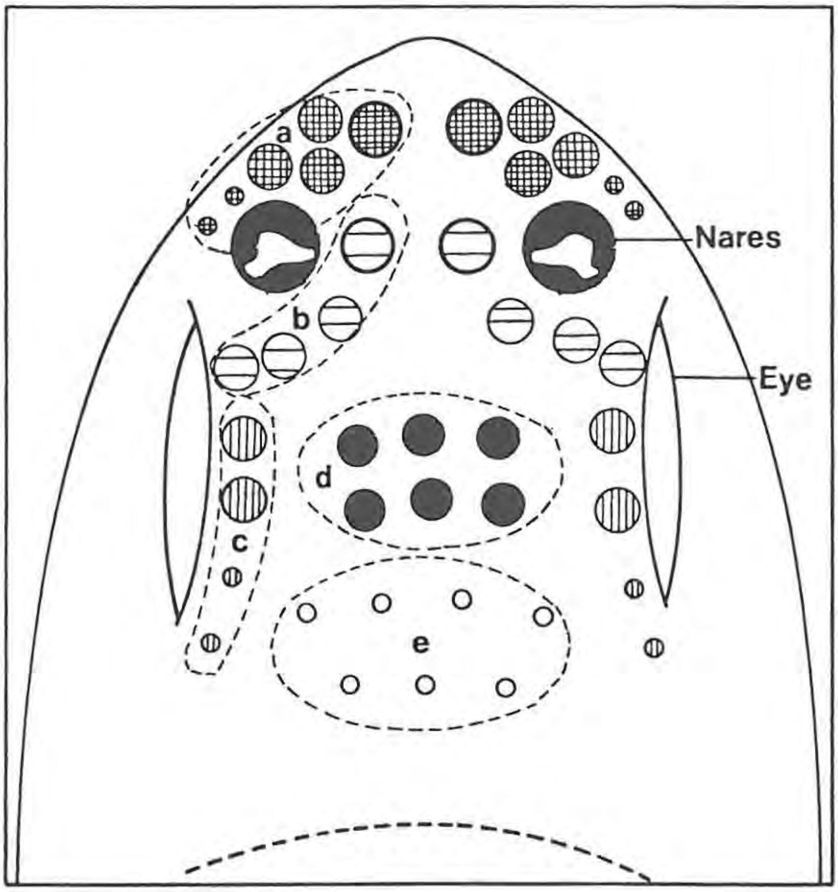


Figure 42. Diagrammatic representation of the pattern of head tubercles on *P. afer* and *P. asper*: (a) - cluster on snout; (b) - row above nares; (c) - row above orbit; (d) - anterior dorsal cluster; (e) - posterior dorsal cluster, (from Skelton, 1988).

A comparison of the presence or absence of definitive snout tubercles and the presence of buds on male *P. afer* and *P. asper* was graphed with the mean monthly gonadosomatic indices to show the relationship between the tubercles and increases in gonad mass. The presence of definitive tubercles was given the value 1, the presence of buds the value 0,5 and worn tubercles and scars were given the value 0.

All fins were examined for tubercle formation and presence or absence was recorded over the 22 month period. The number of rows of tubercles on the rays of the pectoral fins was also recorded. This was not necessary for the other fins since there was only a single row of tubercles on each ray. Scales were examined for tubercle formation and presence or absence was recorded for the 838 fish sampled.

Results

The number of specimens examined, the size range, and presence of tubercles (scars, buds, worn or definitive) for the males and females of both species over 22 months, has been summarized in Tables 32-35.

The seasonality of the occurrence of the tubercles was evident for both males and females of both species but was more clearly defined in the males of the two species (Tables 32-33 and Figure 43). Definitive snout tubercles were only present on the males of the two species. In *P. afer* males definitive snout tubercles were first observed in September 1987 and in September 1988 (Table 32) and there was a close relationship with the seasonal variation in the gonadosomatic indices (Figure 44). Definitive snout tubercles in *P. asper* males were first observed in October 1987 and in September 1988 (Table 33) and as with the *P. afer* males there was a close relationship of the seasonal formation of tubercles and the seasonal pattern of the gonadosomatic indices (Figure 45). The seasonal pattern was also evident in the presence and absence of scale and ray tubercles for both species. The longer potential spawning season of *P. asper* compared to *P. afer* was evident from the presence of definitive tubercles as late as April for *P. asper* males (Figures 44 & 45). In *P. afer* females buds first erupted in September 1987 and in September 1988 (Table 34). In *P. asper* females buds were first observed in September 1987 and again in September 1988 (Table 35). Tubercles were a dominant feature on the scales and fin rays of the males of both species (Tables 32 & 33) whereas very few females of either species had scale or fin ray tubercles (Tables 34 & 35).

The smallest male *P. afer* with tubercles was 41,2 mm SL, collected in October 1987, with five buds in the posterior dorsal section whilst the pectoral fin rays had tubercles. The smallest male

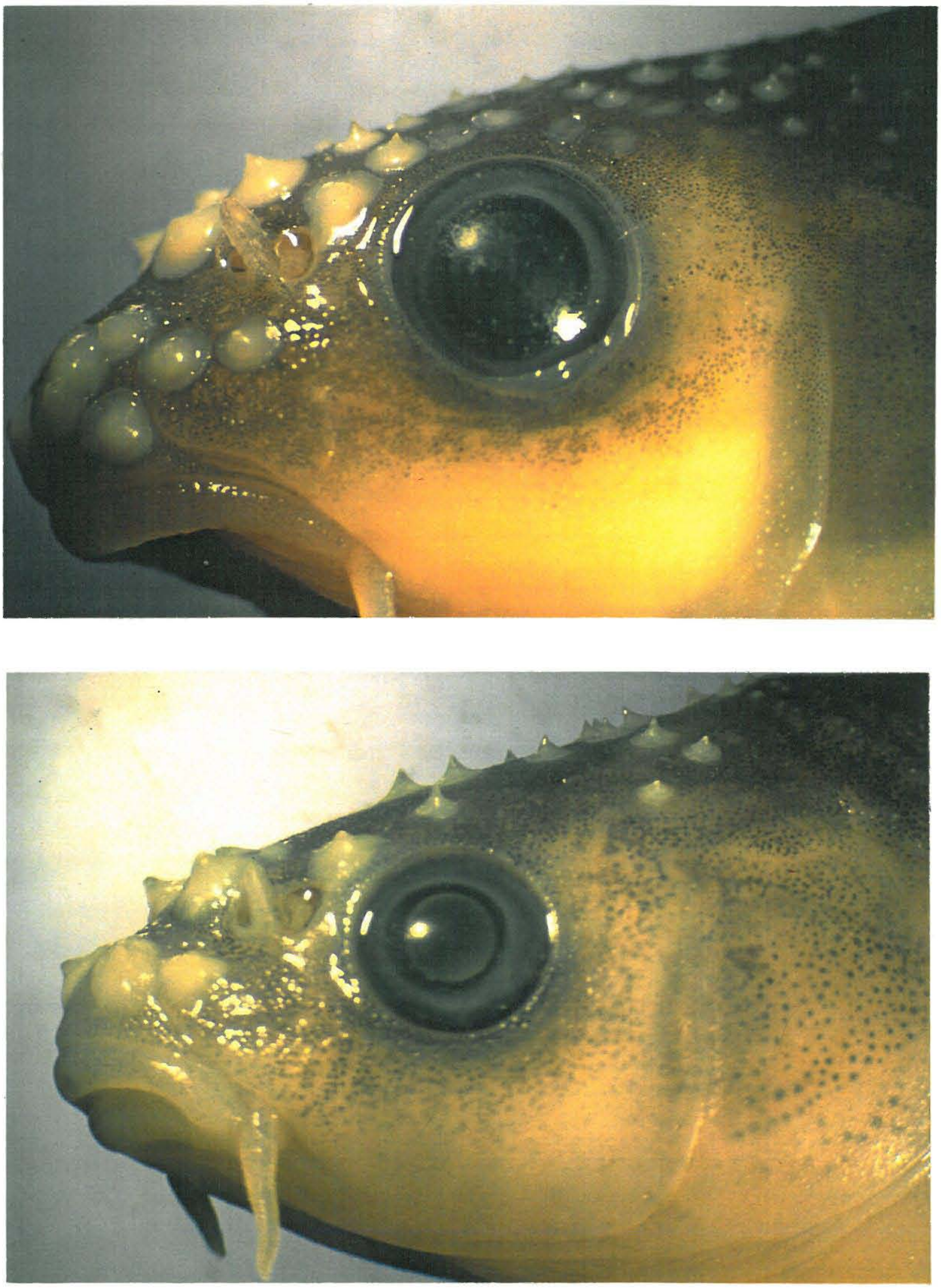


Figure 43. Definitive tubercles on males of *P. afer* (above) and *P. asper* (below).

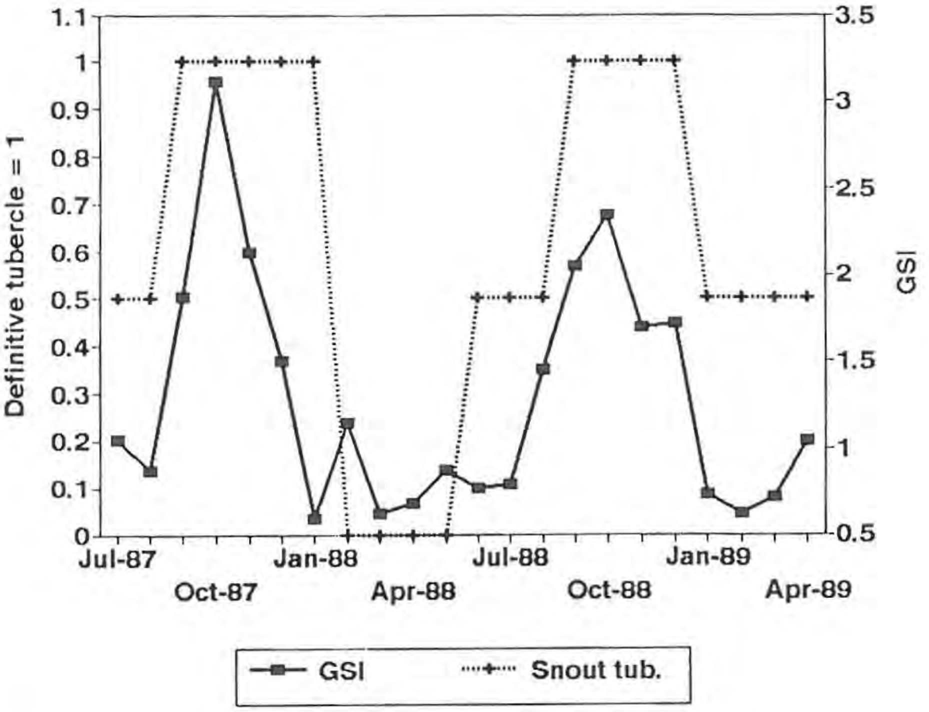


Figure 44. Comparison of the seasonal occurrence of definitive snout tubercles on male *P. afer* (n=145) with the mean monthly gonadosomatic index (GSI) of male *P. afer* for the period July 1987 to April 1989. (Definitive snout tubercle = 1; bud snout tubercle = 0,5).

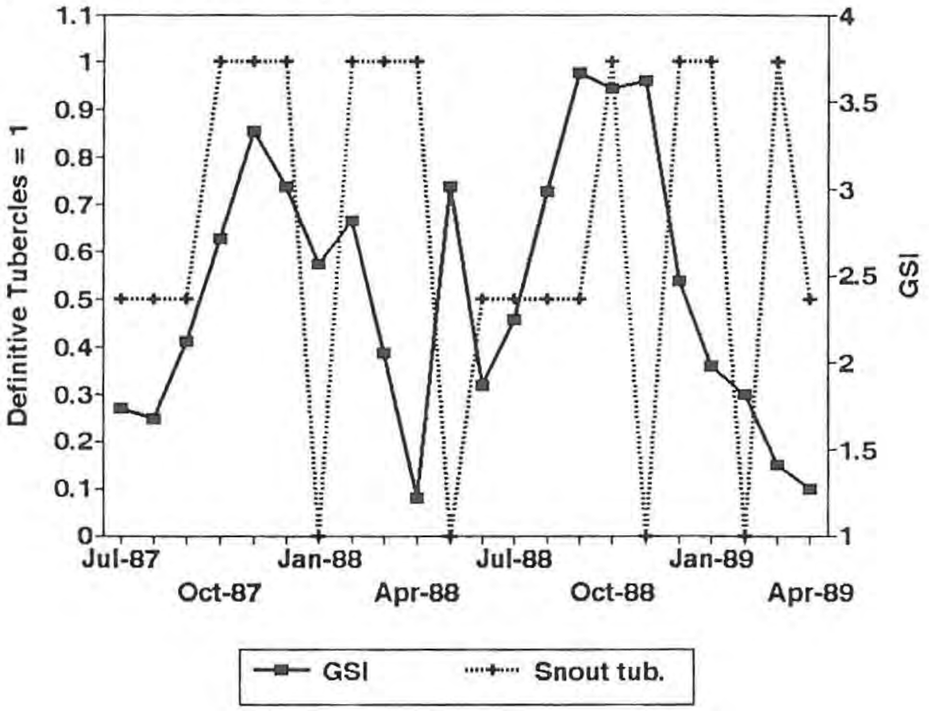


Figure 45. Comparison of the seasonal occurrence of definitive snout tubercles on male *P. asper* (n=204) with the mean monthly gonadosomatic index (GSI) of male *P. asper* for the period July 1987 to April 1989. (Definitive snout tubercle = 1; bud snout tubercle = 0,5).

P. asper was 39,3 mm SL, collected in January 1989, with three tubercle buds (1 snout and 2 nares).

The smallest female *P. afer* with tubercles (= spots) was 56,5 mm SL collected in December 1988. Two spots were present, one in the nares position and one in posterior dorsal position. The smallest female *P. afer* with buds was a 56,8 mm SL specimen collected in January 1988. There were 6 buds and all were in the posterior dorsal position. In comparison the smallest female *P. asper* with tubercles (= spots) was 49,4 mm SL collected in March 1988. The 4 spots were all in the posterior dorsal position. The smallest female *P. asper* with buds was a 49,5 mm SL specimen collected in October 1987. All 6 buds were in the posterior dorsal position.

A 70,5 mm SL male *P. afer* had the most tubercles of all the males examined for both species. There was a total of 88 tubercles (14 snout; 7 nares; 12 above orbits; 6 anterior dorsal; 49 posterior dorsal)(Table 36). The biggest tubercles were on the snout and the largest had a volume of 0,89 mm^3 (b = 1,5 mm; h = 1,5 mm) (Table 37). In comparison the highest number of tubercles found on male *P. asper* was 53 (6 nares; 3 above orbits; 4 anterior dorsal; 34 posterior dorsal) on a 69,4 mm SL specimen collected during March 1989 (Table 38). There was a highly significant difference (t= 5,50985, d.f. = 18, $P < 0,001$ of having equal numbers) between number of tubercles on *P. afer* males compared to *P. asper* males. The largest snout tubercle for *P. asper* males had a volume of 0,26 mm^3 (Table 39). There was a highly significant difference (t = 12,5016, df = 18, $P < 0,001$ of having an equal volume) between volume sizes of *P. afer* male tubercles compared to the largest *P. asper* tubercles.

There was an increase in the number of tubercles with increasing length for both males (Figure 46) and females of the two species. The lower correlation coefficient for *P. asper* males and the very low values for the females of both species probably indicated the lack of significance of tubercles for these groups compared to *P. afer* males.

Males *P. afer* $y = -81,2053 + 1,99929x$ ($n = 145$; $r^2 = 0,878$)
P. asper $y = -43,659 + 1,20292x$ ($n = 204$; $r^2 = 0,722$)
Females *P. afer* $y = -12,1037 + 0,233274x$ ($n = 60$; $r^2 = 0,323$)
P. asper $y = -33,3291 + 0,694969x$ ($n = 91$; $r^2 = 0,583$)
where $x = SL$ (mm); y = number of tubercles

Table 36. Summary of ten *P. afer* males with the highest number of head tubercles and their position.

Date	SL (mm)	Snout	Nares	Above orbits	Ant. dorsal	Post. dorsal	Total
Nov. 1987	70,5	14	7	12	6	49	88
Dec. 1988	70,1	7	6	10	5	39	67
Oct. 1987	70,8	10	5	8	5	34	62
Feb. 1989	70,5	9	8	7	5	33	62
Oct. 1987	78,2	11	7	7	7	29	61
Feb. 1989	66,2	8	6	8	5	34	61
Sept.1987	71,5	6	6	6	4	38	60
Sept.1988	70,5	6	6	7	7	31	57
Jan. 1989	69,6	6	6	10	4	31	57
Dec. 1987	63,4	9	6	5	7	27	54
Means	70,1	8,6	6,3	8	5,5	34,5	62,9
SD	3,8	2,6	0,8	2,1	1,2	6,3	9,5

Table 37. Summary of the ten largest snout tubercles found on *P. afer* males collected in the Wit River, Gamtoos River System.

Date	SL (mm)	Base (mm)	Height (mm)	Volume (mm^3)
Oct. 1987	78,2	1,5	1,5	0,89
Nov. 1987	58,0	1,6	1,0	0,67
Nov. 1987	70,5	1,3	1,5	0,67
Sept.1987	63,2	1,25	1,05	0,43
Dec. 1987	63,4	1,1	1,1	0,35
Dec. 1987	58,1	1,2	0,9	0,34
Dec. 1987	49,5	1,2	0,85	0,32
Oct. 1987	60,9	1,2	0,7	0,26
Oct. 1987	57,3	1,2	0,6	0,23
Nov. 1988	59,7	1,1	0,6	0,19
Means	61,9	1,27	0,98	0,44
SD	7,9	0,16	0,33	0,23

Table 38. Summary of ten *P. asper* males with the highest number of head tubercles and their position.

Date	SL (mm)	Snout	Nares	Above orbits	Ant. dorsal	Post. dorsal	Total
Mar. 1989	69,4	6	6	3	4	34	53
Dec. 1987	69,9	6	6	3	6	30	51
Nov. 1987	72,3	8	6	2	2	31	49
Dec. 1987	64,6	6	6	4	4	24	44
Jan. 1988	63,1	6	6	1	1	29	43
Aug. 1988	70,4	8	6	3	5	20	42
Sep. 1987	62,4	6	6	4	1	24	41
Apr. 1989	66,6	6	6	5	2	21	40
Dec. 1988	59,7	6	6	1	8	18	39
Feb. 1989	65,9	8	6	3	0	22	39
Means	66,4	6,6	6	2,9	3,3	25,3	44,1
SD	4,0	1,0	0,0	1,3	2,5	5,4	5,1

Table 39. Summary of the ten largest snout tubercles found on *P. asper* males collected in the Groot River, Gamtoos River System.

Date	SL (mm)	Base (mm)	Height (mm)	Volume (mm^3)
Nov. 1987	72,3	1,2	0,7	0,26
Dec. 1988	64,5	1,2	0,7	0,26
Feb. 1989	66,1	1,0	0,9	0,24
Jan. 1989	55,5	1,0	0,8	0,21
Sep. 1988	58,8	1,0	0,7	0,18
Dec. 1988	60,4	1,0	0,7	0,18
Dec. 1988	56,4	0,9	0,8	0,17
Feb. 1988	56,0	1,0	0,5	0,13
Dec. 1988	62,3	0,8	0,7	0,12
Dec. 1988	59,7	0,8	0,7	0,12
Means	61,2	1,0	0,72	0,19
SD	5,3	0,14	0,1	0,05

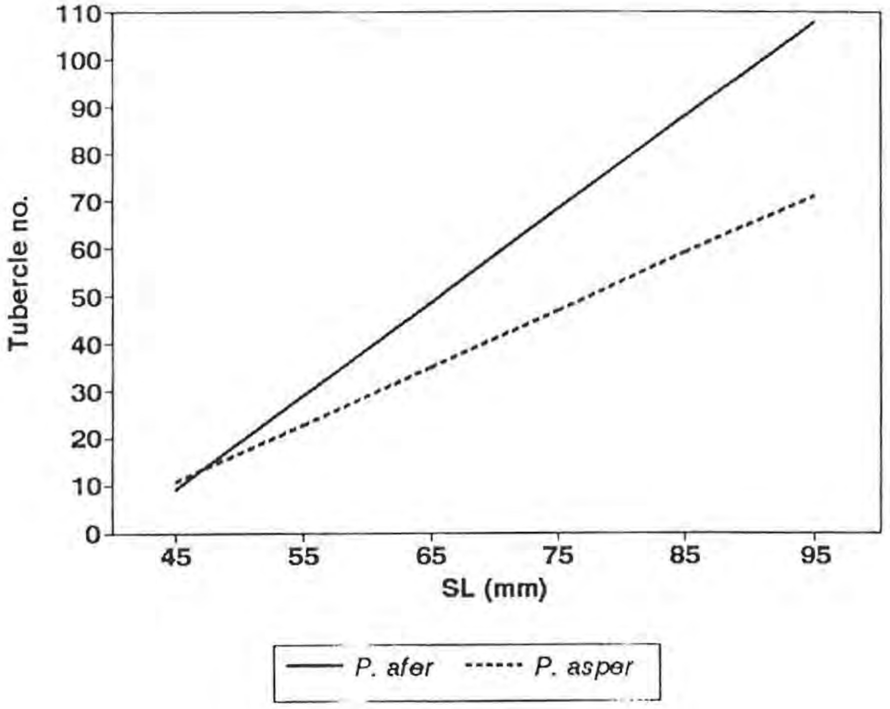


Figure 46. The relationship between body length and the number of head tubercles in male *P. afer* (n=145) and *P. asper* (n=204) collected between September and February 1987/1988 and 1988/1989. The regression equations are given in the text.

For each separate cluster of tubercles the regression analysis is shown in Tables 40 & 41. The highest correlation coefficient in *P. afer* males was in the snout tubercles to length and the lowest in the anterior dorsal cluster. Similarly in the tubercle clusters on the head of *P. asper* the snout tubercles had the highest and the anterior dorsal cluster had the lowest correlations with length. In the females of both species there were low correlations for most clusters which had tubercles (Table 41).

The slope of the regression of total tubercle number on standard length of *P. afer* males was significantly different from *P. asper* males (F = 29,5848; d.f. = 345; $P<0,001$; Figure 46).

In the female sample a 72,4 mm SL *P. afer* had the most tubercles (= spots) for that species. There were 29 spots of which 3 were snout, 4 nares, 4 above orbits, 0 anterior dorsal and 18 posterior dorsal. In the *P. asper* female sample a 72,0 mm SL specimen collected in November 1988 had the most tubercles (including spots). There were 43 spots and buds positioned as follows, 3 (spots) snout, 7 (spots) nares, and 33 (buds) in the posterior dorsal position.

Table 40. Linear regression analyses of the number of head tubercles in the different clusters (see text) correlated against length of male *P. afer* and *P. asper*.

Tubercle cluster	n	a	b	r
P. afer ♂♂				
Total	143	-80,332	1,980	0,878
Snout	143	-12,664	0,307	0,883
Nare	143	-7,951	0,233	0,748
Orbit	143	-12,968	0,296	0,867
Ant. dorsal	143	-7,784	0,181	0,739
Post. dorsal	143	-38,965	0,964	0,773
P. asper ♂♂				
Total	202	-44,017	1,210	0,722
Snout	202	-6,366	0,206	0,685
Nare	202	-6,127	0,195	0,601
Orbit	202	-3,052	0,067	0,504
Ant. dorsal	202	-3,257	0,075	0,407
Post. dorsal	202	-25,216	0,666	0,643

Table 41. Linear regression analyses of the number of head tubercles in the different clusters (see text) correlated against length of female *P. afer* and *P. asper*.

Tubercle cluster	n	a	b	r
P. afer ♀♀				
Total	60	-12,104	0,233	0,323
Snout	60	-2,195	0,040	0,404
Nare	60	-6,463	0,117	0,525
Orbit	60	-33,329	0,695	0,583
P. asper ♀♀				
Total	91	-33,329	0,695	0,583
Snout	91	-5,558	0,113	0,484
Nare	91	-4,587	0,093	0,431
Post. dorsal	91	-22,671	0,479	0,523

The largest snout tubercle measured was found in the *P. afer* male sample. The volume of the tubercle was 0,89 mm^3 on a 78,2 mm SL specimen (Table 37). In comparison the largest tubercle in the *P. asper* sample was on a 72,3 mm SL male collected in November 1987. The volume of that snout tubercle was 0,26 mm^3 (Table 39). The largest *P. afer* snout tubercle was therefore 3,42 times the volume of the largest *P. asper* tubercle.

In contrast to the males the female *P. asper* had the most numerous tubercles (= spots or buds) (Tables 42 & 43). The mean number of tubercles for *P. asper* sample was 30,7 (SD = 6,3) (Table 43) as compared to 12,1 (SD = 7,7) for *P. afer* females (Table 42). Buds or spots were present in the snout, nares and posterior dorsal positions for most specimens. Few spots or buds for both species were observed in the above orbits and interior dorsal positions (Tables 42 & 43). Volumetric calculations were not made for the females as the buds are usually less than 0,002 mm^3. The largest bud measured on a female was on a specimen of *P. asper* ($v = 0,012$ mm^3, $b = 0,3$; $h = 0,5$).

The development of tubercles was not always symmetrical on both sides. In the *P. afer* males the snout tubercles ranged up to 7 on both sides (70,5 mm SL). Of the 248 specimens 72 had more tubercles on one side than the other ($X^2 = 145,806$, 1 d.f., $P < 0,0000$). In only two specimens was the difference greater than one. Forty-two of the 72 specimens had more tubercles on the left side than on the right ($X^2 = 2,0$, 1 d.f., $P < 0,157299$). In the *P. asper* males the snout tubercles on the left side ranged up to 4 and 4 on the right side (70,4 & 72,3 mm SL). Of the 320 specimens examined 80 had more tubercles on one side than the other ($X^2 = 200,0$, 1 d.f., $P < 0,0000$). In three specimens the difference was two tubercles. Thirty-seven of the specimens had more tubercles on the left side than on the right side ($X^2 = 0,225$, 1 d.f., $P < 0,635256$).

Individuals can have both spots and buds. Tuberculation on the head increases with an increase in body length.

The first set of tubercles to show any wear were the snout tubercles followed by the nare clusters for both species. The posterior dorsal tubercles often did not show any sign of wear.

No further deductions could be made in the SEM microwear part of this study as no clear patterns of differences in wear were revealed.

Table 42. Summary of ten *P. afer* females from Wit River, Gamtoos River system, with the highest number of head tubercles and their position.

Date		SL (mm)	Snout	Nares	Above orbits	Ant. dorsal	Post. dorsal	Total
Feb.	1988	72,4	3	4	4	0	18	29
Dec.	1987	68,4	2	8	2	2	6	20
Feb.	1988	57,0	0	0	0	0	16	16
Mar.	1989	70,7	2	5	0	1	6	14
Feb.	1988	58,1	0	0	0	0	10	10
Mar.	1989	63,9	2	4	0	0	1	7
Sep.	1987	61,7	2	3	0	0	2	7
Feb.	1988	56,8	0	0	0	0	6	6
Sep.	1987	70,3	0	4	1	0	1	6
Oct.	1987	72,6	2	4	0	0	0	6
Mean		65,2	1,3	3,2	0,7	0,3	6,6	12,1
SD		6,4	1,2	2,6	1,3	0,7	6,3	7,7

Table 43. Summary of ten *P. asper* females from the Groot River, Gamtoos River system, with the highest number of head tubercles and their position.

Date		SL (mm)	Snout	Nares	Above orbits	Ant. dorsal	Post. dorsal	Total
Nov.	1988	72,0	3	7	0	0	33	43
Mar.	1989	68,5	6	6	0	0	29	41
Feb.	1988	74,1	2	4	0	0	27	33
Nov.	1988	72,2	4	3	4	0	18	29
Feb.	1989	76,5	6	4	0	0	18	28
Feb.	1988	66,4	2	4	0	0	21	27
Feb.	1988	65,2	2	3	0	0	22	27
Nov.	1988	70,1	6	4	0	0	17	27
Mar.	1989	75,2	4	4	0	0	19	27
Sep.	1987	68,0	2	4	0	0	19	25
Means		70,8	3,7	4,3	0,4	0	22,3	30,7
SD		3,8	1,8	1,3	1,3	0	5,5	6,3

Table 44. Possible functions of the breeding tubercles of male *P. afer* and *P. asper*.

Function	*P. afer* (clear water)	*P. asper* (turbid)
Maintain contact during spawning (tactile)	Possibly	Possibly
Holding to the substrate during spawning in swift flowing waters	Possibly	Possibly
Stimulation of females	Possibly	Possibly
Excavating a nest	Unlikely	Unlikely
Defense of nests	Unlikely	Unlikely
Fighting other males	Possibly	Possibly
Visual threat	Possibly	Unlikely
Sex recognition	Possibly	Unlikely
Species recognition	Possibly	Unlikely

Discussion

Nuptial or breeding tubercles, which have an epidermal origin, can develop on the head, fins and bodies of fish. They are present in regions which come into contact with either another male or female fish. The arrangement of the tubercles on various species is diversified (Witkowski & Rogowska, 1991). If the tubercles are present on both sexes then they are usually better developed on the male (Branson, 1962). In addition both Kratt & Smith (1978) and Witkowski (1982 in Witkowski & Ragowska, 1991) found that tubercles were better developed on those fish which have spawned several times than on first time spawners. The structures were better developed on males of both *P. afer* and *P. asper* than females. Secondary sexual characteristics, such as epidermal tubercles and breeding colouration, all require resources which must be diverted away from growth and survival requirements (Miller, 1979; Wootton, 1985). The formation of and the subsequent behaviour of fish with tubercles would probably be energetically costly which would reduce growth. If females developed large tubercles with associated behaviour then the allocation of energy to the secondary sexual characteristics would reduce the future production of offspring. This would favour the development of these characters on males (Wootton, 1990).

The presence of these structures could be for a number of reasons: fighting other males or invaders, the white colour of the tubercles denotes spawning readiness, spawning activities (maintaining body contact during spawning), protect the body and fins from damage during nest construction, tubercles on the ventral surface of females may protect the anus and urogenital papillae against mechanical damage during egg laying, holding to the substrate in swift waters during the spawning act, and taking care of the eggs, nest and young (Hubbs & Cooper, 1936; Raney, 1940; Fabricius & Gustafson, 1955; Branson, 1961, 1962; Wiley & Collette, 1970; Collette, 1977; Kratt & Smith, 1978). As in some other fish species the tubercles of the redfin minnows reflected a pearlescent hue. In *Nocomis* species shining tubercles may attract females to the nest. Since there are different patterns for different species, tubercles may be an aid for species recognition and for sex recognition. For a *Notropis* species (Koehn, 1965) and for a species of *Etheostoma* (Braasch & Smith, 1967) there was evidence that the tubercles could be used by males to stimulate females through contact. Collette (1977) suggested that breeding tubercles originally evolved to enable breeding individuals to maintain close contact during spawning. In fishes which breed in fast-flowing waters close contact would ensure fertilization of the eggs. The redfin minnows are also small species and therefore would find it more difficult to maintain position in swift waters as compared to larger species. The nuptial tubercles on the pectoral fins of some cyprinids are probably used in a 'pectoral fin lock' in which the male clasps the female (Branson, 1962). The tubercles on the fin can be recurved which would facilitate clasping. Boulenger (1911) noted that there was sexual dimorphism of the pectoral fins of *P. asper* with males having longer pectoral fins than females. Sexual dimorphism of the fins, length of pectorals relative to the base of the pelvics, was found to be evident in most of the flexible-rayed redfins (Barnard, 1943). Male *P. afer* and *P. asper* have broader and more rounded pectoral fins than females (Skelton, 1988). That would indicate that the larger, longer and broader pectoral fins with more tubercles of the males are used for clasping (pectoral fin lock) the female during spawning. Cambray & Meyer (1988) and Cambray (1991a) noted that some of the *Pseudobarbus* species breed in mid-channel in the riffles. Clasping of the pectoral fins, aided by increased size and tubercles on the rays of these fins would be advantageous to ensure fertilization of the eggs especially in fast-flowing water. Tubercles on the scales would also help to maintain a spawning embrace. The large tubercles on the head may have a different purpose and are not used for clasping.

The tubercles may be for interactions between males to acquire mates (intrasexual selection) or for interactions between the sexes to choose mates (intersexual selection). The dorsal tubercles of the head and trunk in many species are usually fairly well developed, possibly because they are used for fighting invaders and other males as well as to nudge females during spawning. Tubercle rows over the orbits might be a protective mechanism during agonistic behaviour of *P. afer* and *P. asper* males, as was suggested for some *Nocomis* species by Lachner & Jenkins

(1971a). Because of the highly turbid waters in which *P. asper* lives, it would be unlikely that this species used head tubercles for visual threat, sex recognition or species recognition (Table 44). But as *P. afer* inhabits clear waters they could be used for the above functions (Table 44). If the tubercles were used to nudge the female during spawning then that would not help to explain the smaller tubercles of *P. asper* males. There was no obvious evidence for the use of tubercles in the flexible-rayed redfins. The microwear study did not elucidate the cause of the observed wear of the tubercles during the season.

Witkowski & Rogowska (1991) studied tubercles on 7 cyprinid species in different ecological breeding groups. They found that fish which spawned on plants had few tubercles on their ventral surfaces and the tubercles were most numerous on the lateral surface. In comparison species which spawned on stony, gravely or sandy substratums had the densest and the largest tubercles on the lateral surface below the lateral line and on the ventral side of the caudal peduncle.

The tubercles could also have made the fish more conspicuous to predators which would increase their risk of predation. Tubercles combined with the breeding coloration of *P. afer* would at first appear to decrease the survivability of their bearers. Darwin (1872) reasoned that these characters must confer an advantage to the individual, which would balance or outweigh their deleterious effects on survival. In his theory of sexual selection he wrote, "This form of selection depends, not on a struggle for existence in relation to other organic beings or to external conditions, but on a struggle between the individuals of one sex, generally the males, for the possession of the other sex" (Darwin, 1872: 64).

The quantitative differences in the secondary sexual characters of a species may even play a role in mate discrimination in reinforcing speciation once populations that had diverged in allopatry came back into contact (Dobzhansky, 1940). The noted differences in secondary sexual characters of *P. afer* and *P. asper* males may reinforce speciation in the Groot River.

Lande (1982) has suggested that incipient speciation in a population occupying a continuous range is modeled as the joint evolution of geographic variation in female mating preferences and also in the quantitative secondary sexual character of males. Even in the absence of genetic instability, the evolution of female mating preferences can greatly amplify large-scale geographic variation in male secondary sexual characters and produce widespread sexual isolation with no geographical discontinuity. The smaller and less numerous tubercles on *P. asper* males may have helped in the past to produce sexual isolation of populations of *Pseudobarbus* in the Gamtoos River system with large tubercles, associated with the clear mountain streams and smaller tubercles and duller breeding colouration associated with the turbid Karoo stream.

The lifetime reproductive success of individuals may be influenced by the individual variation in tubercle number and/or size. In *P. afer* the tubercles were both volumetrically larger as well as more numerous than was shown for *P. asper*. That would suggest that in the clear water habitat of *P. afer* size and number of tubercles were important for reproductive success and may have influenced mate choice. The presence of tubercles may signal information about the genetic quality of the carrier, or it may only be that the presence of large tubercles and bright red colouration makes the bearer more likely to be chosen by a potential mate (Bradbury & Andersson, 1987). The ultimate factors which have lead to the evolution of the secondary sexual characters, such as tubercles, are still a subject of debate (Wootton, 1990). In the New World cyprinids the sexual differences are usually more pronounced in those species that are highly territorial, have some form of parental care, or carry out their life cycles in high-gradient and clear rivers (Mayden, 1991). There is no known parental care in *Pseudobarbus* species but most of the species, such as *P. afer* and *P. burchelli*, inhabit clear water systems.

Value in systematics

The pattern of the distribution of breeding tubercles can be useful in furthering our knowledge of phyletic relationships (Vladykov, 1935; Hubbs & Black, 1947; Lachner, 1952; Gibbs, 1957; Collette, 1965, 1977; Géry, 1966). In the Cyprinidae there are many studies which have used tuberculation as a systematic character (e.g. Wiley & Collette, 1970: Skelton, 1988). In all species of *Nocomis* tuberculation has been considered an important evolutionary character (Lachner & Jenkins, 1971a & b). Similarly, tubercles have been considered one of the outstanding characters of the flexible-rayed redfin species which include the two sister species *P. afer* and *P. asper* (Skelton, 1988). Behaviour and life-history data should be known if tubercle patterns are to be used as indicative characters of phylogeny (Branson, 1962). The present study on the life history of the two closely related redfin minnows helps to interpret the variability of this character due to environmental variables.

Skelton (1980a) noted the presence, size and patterns of tubercles on 25 *Barbus* species from southern Africa. Conical tubercles occur in all the flexible-rayed redfins including *P. afer* and

P. asper. In comparison to the *Barbus* species, the flexible-rayed redfin tubercle pattern consists of large tubercles and there are also tubercles on the borders of the scales which occur in regular rows (Skelton, 1980a). The tubercles in the seven redfin minnow species have a common distribution pattern (Figure 42). The size and the pattern of the tubercles in *P. burchelli*, *P. burgi*, *P. afer*, *P. asper*, *P. tenuis* and vestigially in *P. phlegethon* was found to be quite unique for African fish and represents a very distinct synapomorphy (Skelton, 1988). There is a size difference pattern within the *Pseudobarbus* species although there has previously not been a thorough seasonal study on the appearance and development of the nuptial tubercles of *P. afer* and *P. asper* to show the size differences. Tubercle size and number is probably a variable attribute amongst the *Pseudobarbus* species.

Branson (1961) was able to correlate tubercle patterns with habits and ecological 'station'. It was concluded from this study that 'tubercular characters should be used in conjunction with other characters and only if the life histories and ecological stations are understood' (Branson, 1961:537).

Because of the presence of relatively large and numerous tubercles on *P. afer* there is a possibility that there are alternative male mating behaviours (phenotypic options) which would contribute to the reproductive success of their bearers. This type of behaviour is very common in fishes (Gross, 1984). The prevalence of these male alternative phenotypic options in fishes may be due in part to the competiveness of their mating systems and widespread occurrence of external fertilization (Gross & Shine, 1981). The large head tubercles may possibly indicate that the redfin minnow mating behaviour is competitive.

If the alternative reproductive phenotypic options in a population are subject to frequency-dependent selection, then indeterminate growth may contribute to the evolution of discrete alternative life-history pathways (Gross, 1984). Precocial maturity will result in smaller body size. If the smaller males have equivalent fitness, selection may allow genetic differences to accumulate with specialization into alternative routes. In some cases bright red fins were observed on small *P. afer* males and these may be individuals which have undergone precocial maturity. In the turbid waters of the Groot River and with the smaller, less numerous breeding tubercles of *P. asper* we would not expect to find selection for alternative male reproductive phenotypic options. This may be reinforced by the faster growth rate and the shorter life span of *P. asper* males when compared to *P. afer* males. This is an avenue of research that should be investigated and is only noted here as a stimulus for future work on redfin minnows.

It is not necessary for fish to have parental care for an alternative mating behaviour to evolve, "the primary requisites are only that competition occurs between individuals for access to mates, that this competition is partially (BBS) or entirely (ESS) circumvented by alternative behaviours, and the success of these alternatives depends upon the activities of other members of the population" (Gross, 1984: 73). The large tubercles of male *P. afer* and the female-biased sex ratio (Chapter 6) would indicate that competition between males for mates does occur.

The seasonal occurrence, distribution, number, size, and developmental patterns

In the primitive condition head tubercles were arranged in definite rows whereas a scattered pattern indicated an evolutionary advance (Gibbs, 1957; Branson, 1961). With redfin minnows the majority of tubercles are scattered, although there are rows on the snout and above the orbits (Figure 42). Since these structures are concerned with reproduction, it is reasonable to assume that they should vary according to the habits of particular species (Branson, 1961). Caution is needed as the tubercles could be used in different ways by different species in the same habitat. Branson (1961) observed that since tubercles were only present during the breeding season then their development was endocrine influenced. Unlike the head tubercles, the lateral tubercles on the fins and scales are likely to be involved in body contact during spawning (Wiley & Collette, 1970; Collette, 1977).

The keratinized tips of the definitive tubercles on both of the redfin minnow species were pointed and curved posteriorly (Figure 43). In both species the largest tubercles were found in the males and the larger females had only rudimentary tubercles. The largest tubercles were found on the *P. afer* males and were up to 3,42 times the volume of the largest tubercles on male *P. asper* (Table 37 & 39).

Of the snout, nare, above orbit, anterior dorsal and posterior dorsal sets of tubercles the snout and nare tubercles were generally the largest followed by above orbits, anterior dorsal and the smallest were the posterior dorsal ones. Seasonal wear first appeared on the snout and nares tubercles. The small posterior dorsal tubercles remained pointed for the longest period during the summer breeding season for both species.

Why should the larger females have invested any energy into producing tubercles? The females with tubercles had high gonadosomatic indexes and therefore were not senescent. When the tubercles occurred on the head they followed the pattern exhibited on the male fish. That very few females were found to have tubercles on their rays indicated that they did not use them for maintaining position during mating and the occurrence of tubercles on the large females may merely indicate a hormonal change as the fish grew older. *P. asper* females had more head tubercles than *P. afer* females. There is no obvious adaptive significance to the bearer and if anything the allocation of resources to produce weakly developed tubercles would decrease fitness. It can only be suggested here that in the turbid waters the female *P. asper* might use the head tubercles to 'hold' on to a rock (under a boulder or in a crevice) to maintain position while spawning. In the clear waters this would not be as advantageous as the females could visually orientate on reproductive males.

In *Nocomis* species head tubercles were deciduous on postnuptial males (Lachner & Jenkins, 1971a). If they were not broken off during the spawning period they would fall off shortly after spawning. In the redfin minnows the tubercles became worn after a spawning session and worn tubercles remained until the end of the reproductive season.

One of the most notable characters of sexually mature male *P. burchelli* were the large conical head tubercles (Cambray & Stuart, 1985) which were more highly developed than most other *Pseudobarbus* and small *Barbus* species (Skelton, 1980a). Cambray & Stuart (1985) have noted the seasonal occurrence, wear, number and size of the breeding tubercles of both male and female *P. burchelli*. The tubercles were more developed on males as compared to females. The large conical tubercles on the heads of the males (> 80 mm FL) began to erupt in May. The tubercles were found to erupt through the worn tubercle bases of the previous season. Some tubercles were well formed by August and the first signs of wear occurred in November which coincided with the spawning season. In *P. burchelli* the wearing and/or sloughing off of the tubercles continued until April when only the base of the tubercles was visible. Cambray & Stuart (1985) suggested that this seasonal cycle of tubercle formation and wear added support to the hypothesis that the tubercles were associated with reproduction in redfin minnows (Skelton, 1980a). It was proposed by Cambray & Stuart (1985) that the wearing as the season progressed suggested that the tubercles are of use when defending a territory and are biggest on the largest males. The keratin caps get worn off as the breeding season progresses.

In another flexible-rayed redfin minnow, *P. quathlambae*, several specimens were examined which had been collected at different times of the year (Skelton, 1974). In April (1972) there was minor tuberculation on the head but not on the scales. Specimens collected in November had small head tubercles and microscopic tubercles on the scales. Two male specimens collected in

January had various developmental stages for tubercles depending on the size of the fish.

P. afer (Chapter 4), *P. asper* (Cambray, 1991a) and *P. quathlambae* (Cambray & Meyer, 1988) spawned after an increase in river flow and their spawning habitat was mid-channel amongst boulders. Ozarkian minnows which were very active spawners or which spawned in rapidly flowing waters were found to have more extensively developed, scattered tubercles than sedentary species which had tubercles in lines (Branson, 1962). Lachner & Jenkins (1971a) have noted that cyprinids with unspecialized breeding habits usually have small tubercles distributed over the head in no particular pattern. This may indicate that in the *Pseudobarbus* species with large tubercles, such as *P. afer* and *P. burchelli*, there may be more specialized breeding habits compared to species such as *P. asper* and especially *P. phlegethon* which have smaller or vestigial tubercles.

The possible functions of the nuptial tubercles for male *P. afer* and *P. asper* are given in Table 44. The males of both *P. afer* and *P. asper* had larger definitive head tubercles than did the females. *P. afer* males had the most numerous and largest tubercles when compared to *P. asper* males. Tubercles are important taxonomic characters in the monophyletic *Pseudobarbus* lineage. The seasonal occurrence and disappearance of tubercles for both species follows the reproductive cycle and is probably under seasonal hormonal control (Figures 44 & 45). Scanning electron microscopy of the microwear of the keratinized cap of the tubercles was uninformative. It is suggested that the head tubercles are used for display prior to breeding, whereas during breeding the tubercles on the scales and fin rays are for maintaining contact with females, as are the larger pectoral fins of the males, in the riffle spawning habitat. The fewer and smaller sizes of the tubercles on *P. asper* males may be due to the turbid waters where visual characters would have less of a selective advantage than in a clear mountain stream (Table 44). That the head tubercles are still relatively large in *P. asper* may be a phylogenetic constraint within the sister species pair. Large tubercles may be primitive for the *Pseudobarbus* species monophyletic lineage and small tubercles more derived. Only the large females of both species have head tubercles which are never definitive and their presence on reproductively active females is difficult to explain. That no tubercles form on the smaller females indicates an expected trade-off constraint, in that the energy is put into egg production and not sexually dimorphic characters. The smaller pectoral fins of the females which had few if any tubercles also indicated that it was more beneficial for the female to put reproductive effort into progeny.

Hypothesis

The hypothesis which was raised in the introduction has been verified. The clear water species has larger and more head tubercles than does the species inhabiting the turbid water. The large breeding tubercles on the heads of male *P. afer* are probably used as visual signals during breeding. In *P. asper* males inhabiting a turbid water environment the head tubercles are reduced both in size and in number. Head tubercle size and density is therefore a variable character within the sister species.

CHAPTER - 8

COMPARATIVE NEUROECOLOGY RELATED TO TURBIDITY

Introduction

Studies of many different species of fish and vertebrate taxa have revealed that their ecological characteristics are reflected in the various structures of their brains. The assumption is that a larger size of a section of the brain means better performance of the functions controlled by that section. Fish are useful for comparative studies as the primary targets of sensory modalities are distinct brain divisions which can be measured in the intact brain (Huber & Rylander, 1992). There have been good correlations between brain pattern and function in many fish species (Tuge *et al.*, 1968). How a fish feeds, what kind of an environment they inhabit, what kind of movements they make etc. all have effects on the structure of the brain. The behaviour of fishes is to a large extent dependent on a highly developed nervous system. The main benefit of this study of brain patterns is to understand sensory specializations at the species level (Miller & Evans, 1965).

Neuroecology can be used to elucidate and predict ecological potentials, radiations of vertebrate taxa and to generate novel evolutionary and functional hypotheses (Kotrschal & Palzenberger, 1992). Neuroanatomical characters may be useful for and contribute to phylogenetic reconstructions (Lauder & Liem, 1983). In specious and closely related vertebrate groups quantitative, comparative brain morphological techniques can reveal within group trends of sensory diversification, and the findings can be interpreted ecologically and evolutionarily (Bullock, 1983; Northcutt, 1988; Goldschmid & Kotrschal, 1989; Huber & Rylander, 1992; Kotrschal & Palzenberger, 1992).

In a morphological and meristic study Skelton (1988) found that the only differences separating *P. afer* and *P. asper* were scale size, the length of the gut and pigment pattern (Chapter 3). Both species attained a similar absolute size (Chapter 9). Since *P. asper* occurred in the highly turbid section of the river system and *P. afer* inhabited the clear flowing mountain streams, they were ideal candidates to investigate the relationship of brain morphology and turbidity preference. The patterns of brain morphological variation and their biological basis are considered here for *P. afer* and *P. asper*. The ecology of most of the redfin minnows is poorly known. An understanding of their brain morphologies on a comparative basis may provide some information on the ecology of these species.

Hypothesis

It was hypothesized that in clear water vision should be superior to all other sensory modalities for evaluating the environment and as such larger eyes and optic lobes would be expected in *P. afer*. With increasing turbidity other senses such as taste, smell or touch, larger barbels and the brain areas associated with these senses should be larger in *P. asper*.

Methods

The *P. afer* sample was collected in the Wit River, a clear, perennial stream which occurs in the Cape Fold Mountains. The *P. asper* were collected from the Groot River, a turbid, intermittent river (Figure 4).

A total of 85 *P.afer* (29,2 - 67,3 mm SL: 36 males) and 63 *P. asper* (27,7 - 71,6 mm SL; 30 males) were examined. An additional collection of only adult *P. afer* (n = 30 ♂♂; n = 30 ♀♀) and *P. asper* (n = 30 ♂♂; n = 30 ♀♀) between 54,0 and 57,0 mm SL was dissected and measured. It was assumed that fish in this narrow length range would have the same behaviour and that there would be no functional shifts in sensory developments within this length range. Males and females of *P. afer* and *P. asper* were analyzed separately as there can be intersexual differences in the size of some brain structures (Table 45).

All the fish were fixed in 10 % formalin and then preserved in buffered 5 % formalin. They were dissected after at least six months preservation to allow for tissue shrinkage. Morphometric data were taken as suggested by Hubbs & Lagler (1947). Each specimen was measured to the nearest 0,5 mm SL and sexed by examining the gonads. Standard length was used in this study as body mass varies with seasonal gonad development and other physiological and nutritional factors (Chapter 4). The eye diameter (along the nasal-temporal axis) and length of barbel were measured to the nearest 0,05 mm. The fish were placed in a V-notched dissecting block on the stage of a dissecting microscope. The dorsal section of the neurocranium was carefully dissected and removed and the brain was exposed from the anterior olfactory bulbs to the rostral end of the spinal cord. The lobes were cleared of any tissue by using sharply pointed forceps to improve accuracy of measurements. The length and width of the olfactory bulbs, olfactory lobes, optic lobes, cerebellum, facial lobe and vagal lobes were measured to the nearest 0,05 mm at 10 X magnification using a calibrated ocular micrometer. The length and width measurements of the paired structures, that is the olfactory bulbs and lobes, the optic bulbs, and the vagal lobes were taken from the structure on the right side of the brain (Figure 47). In some of the *P. asper* specimens the longitudinal enlargements of the olfactory lobes formed over part of the olfactory bulbs. The lobes were measured first and then lifted so that the bulbs could be measured. The size of each neural structure was estimated by the product of length and width of the structure (Huber & Rylander, 1992). These measurements provide a quantitative basis for comparison of the sizes of the various neural structures. The two-sample *t*-test in STATGRAPHICS (ver. 5.0) was used to analyze the differences between the brain structures for: ♂♂ *P. afer* to ♀♀ *P. afer*; ♂♂ *P. asper* to ♀♀ *P. asper*; ♂♂ *P. afer* to ♂♂ *P. asper*; ♀♀ *P. afer* to ♀♀ *P. asper*; ♂♂ *P. afer* to ♀♀ *P. asper* and ♂♂ *P. asper* to ♀♀ *P. afer*.

Table 45. Several potential problems of comparative neuroecological studies and how they were overcome in the present study.

Potential problem	*P. afer*	*P. asper*
Ontogenetic, allometry	- adult brain	- adult brain
Size differences	- mean size ♂♂ - 55,6 mm SL ♀♀ - 55,6 mm SL	- mean size ♂♂ - 55,65 mm SL ♀♀ - 55,4 mm SL
Difference between sexes	- ♂♂ and ♀♀ separate analysis	- ♂♂ and ♀♀ separate analysis
Not closely related	- sister species	- sister species
Change in functional importance of sensory modalities	- assumed none as same size	- assumed none as same size
Shrinkage during preservation	- sampled after 6 or more months in buffered 5 % formalin	- sampled after 6 or more months in buffered 5 % formalin

Results

Results of the regression analyses of the two species are given in Table 46. Males and females were combined as the smaller specimens were not sexed. There are good correlation coefficients (r) for all structures of both species with length at 95% confidence levels.

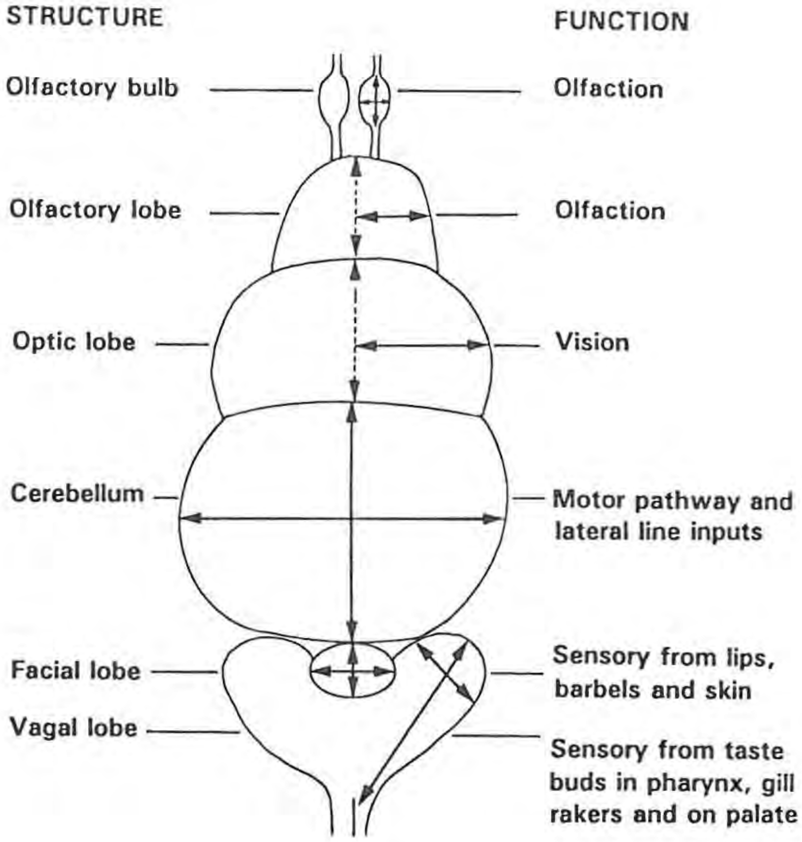


Figure 47. Schematic diagram of the dorsal view of a *P. afer* brain showing how the measurements were taken for paired and unpaired neural structures.

Summary statistics of the lengths and widths of the structures are given in Table 47. Summary statistics of the products of length times width for each of the neural structures are given in Table 48 and the trends are depicted in Figure 48.

The comparative statistics of the four groups analyzed, ♂♂ and ♀♀ *P. afer* and ♂♂ and ♀♀ *P. asper* indicate a significant enlargement of the optic lobes and cerebella of male *P.afer* compared to any of the other groups (Table 49). Male *P. afer* had significantly larger optic lobes and cerebella than female *P. afer* whereas male *P. asper* had larger olfactory bulbs and cerebella than female *P. asper*. In a comparison of males, *P. afer* had larger olfactory bulbs, olfactory lobes, optic lobes, cerebellum, eyes and longer barbels than male *P. asper*. That is, the only two structures which were not significantly different were the facial and vagal lobes. In a comparison of females, *P. afer* had larger olfactory bulbs, olfactory lobes, optic lobes, cerebellum, vagal lobes and eyes and longer barbels than female *P. asper*. Male *P. afer* brains were significantly

larger than female *P. asper* brains in all structures. Male *P. asper* brain and associated structures compared to the female *P. afer* structures were smaller in every respect except the facial lobe and barbel length. Overall male *P. afer* had the largest brain and female *P. asper* the smallest.

Table 46. Linear regression analyses of the neural structures for a length range of *P. afer* (n = 85) and *P. asper* (n = 63).

Structure	a	b	r
		P. afer	
Olfactory bulb	-0,39419	0,020482	0,942
Olfactory lobe	-0,87137	0,078884	0,969
Optic lobe	-0,57799	0,121911	0,967
Cerebellum	-1,39855	0,168248	0,922
Facial lobe	-0,49409	0,032313	0,926
Vagal lobe	-0,22134	0,113199	0,854
Eye diameter	1,2945	0,042496	0,960
Barbel length	-3,12038	0,113199	0,853
		P. asper	
Olfactory bulb	-0,52324	0,022426	0,959
Olfactory lobe	-0,31624	0,053069	0,962
Optic lobe	0,07879	0,081623	0,953
Cerebellum	-0,55074	0,10184	0,971
Facial lobe	-0,18005	0,014088	0,954
Vagal lobe	-0,12068	0,034036	0,860
Eye diameter	1,50148	0,026256	0,949
Barbel length	-2,27093	0,080972	0,942

Discussion

The brain has been shown to be a good indicator of the dominant sensory modalities in fishes. The teleost brain is developed primarily on the reflex level (Papez, 1929). Each reflex handles an important reaction to the environment. Davis & Miller (1967) suggested that this type of brain reflects the correlation between sensory adaptation and the principal modes of activity more clearly than that of the higher vertebrates. Dorsal views of fish brains can permit fairly accurate predictions of feeding habits and habitat preferences of fishes (Davis & Miller, 1967).

Table 47. Summary statistics for the definitive brain morphology of *P. afer* and *P. asper*. All measurements are in millimetres (L = length, W = width).

Neural structure		***P. afer***		***P. asper***	
		♂♂ n = 30	♀♀ n = 30	♂♂ n = 30	♀♀ n = 30
Olfactory bulb	W	0,76	0,79	0,76	0,72
(S.E.)		(0,01)	(0,02)	(0,01)	(0,01)
Olfactory bulb	L	1,11	1,07	0,91	0,85
		(0,03)	(0,02)	(0,02)	(0,03)
Telencephalon	W	1,26	1,27	1,21	1,15
		(0,02)	(0,01)	(0,01)	(0,1)
Telencephalon	L	2,42	2,38	2,22	2,31
		(0,04)	(0,04)	(0,03)	(0,03)
Optic lobe	W	2,17	2,13	1,99	1,94
		(0,02)	(0,02)	(0,02)	(0,02)
Optic lobe	L	2,84	2,71	2,57	2,57
		(0,03)	(0,03)	(0,03)	(0,02)
Cerebellum	W	3,01	2,9	2,68	2,56
		(0,03)	(0,03)	(0,03)	(0,04)
Cerebellum	L	2,51	2,34	2,2	2,13
		(0,03)	(0,03)	(0,03)	(0,03)
Facial lobe	W	1,33	1,31	1,19	1,16
		(0,03)	(0,02)	(0,02)	(0,02)
Facial lobe	L	1,0	0,95	1,07	1,04
		(0,01)	(0,01)	(0,01)	(0,02)
Vagal lobe	W	0,72	0,76	0,7	0,7
		(0,01)	(0,01)	(0,01)	(0,01)
Vagal lobe	L	2,53	2,46	2,5	2,46
		(0,03)	(0,03)	(0,02)	(0,02)
Eye diameter		3,7	3,79	3,1	3,12
		(0,03)	(0,04)	(0,02)	(0,03)
Barbel	L	2,7	2,55	2,36	2,31
		(0,1)	(0,09)	(0,04)	(0,05)

There have been a number of studies which related brain morphology and ecological parameters of fish (e.g. Miller & Evans, 1965; Davis & Miller, 1967; Rao, 1967; Kishida, 1979; Kotrschal & Junger, 1988; Huber & Rylander, 1992; Kotrschal & Palzenberger, 1992). Some of these studies have suffered from a number of problems, such as: comparisons of distantly related taxa, comparisons of members of polyphyletic groups, comparisons of individuals of different size and small sample sizes (Huber & Rylander, 1992)(Table 45). In other studies (eg. Huber & Rylander, 1992) some specimens have been preserved in formalin and others in alcohol. In neuroanatomical studies there are potential effects of ontogenetic changes, such as those listed by Huber & Rylander (1992). These include developmental constraints, allometry, and changes in the functional importance of sensory modalities during development. The definitive brain pattern should be used to avoid the ontogenetic variable (Miller & Evans, 1965). Specimens should be of the same size as in an ontogenetic study on four cyprinid brain morphologies there were considerable brain-internal allometries, which decreased with growth (Kotrschal & Junger, 1988; Brandstätter & Kotrschal, 1990). Huber & Rylander (1992) only used adults of a similar size ranging from 40 to 90 mm in length although in minnow species 50 mm is a considerable difference. To overcome these problems Huber & Rylander (1992) used the residuals of a linear regression on standard length in their analysis of the brains of *Notropis* and related species. For meaningful conclusions to be made from quantitative interspecific comparisons of brain areas the species should be closely related to ensure that there would be a high probability that the compared area would have a comparable functional role (Northcutt, 1988; Goldschmid & Kotrschal, 1989; Kotrschal & Palzenberger, 1992).

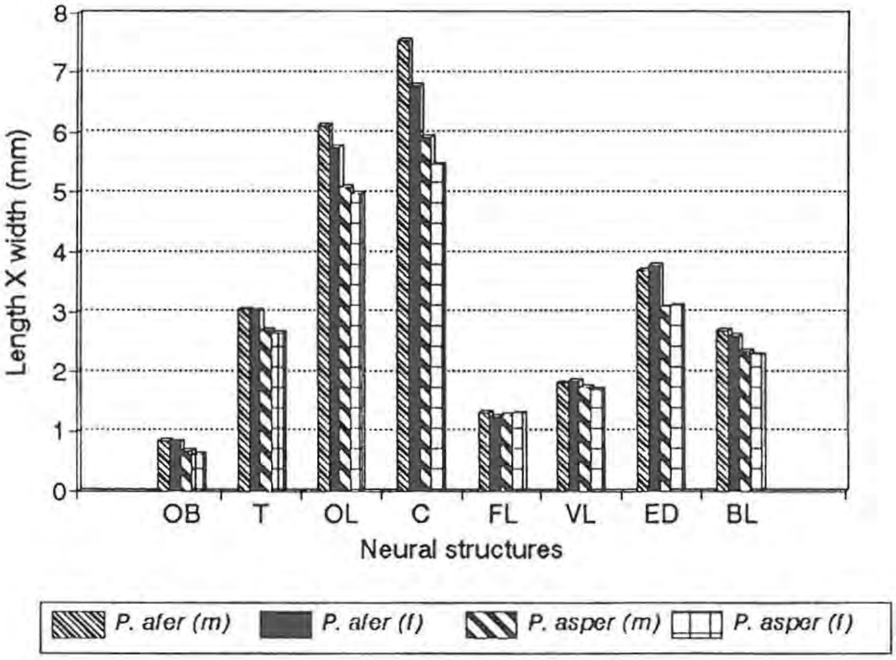


Figure 48. Comparative summary statistics for seven neural structures and the barbel length of *P. afer* (n=30 ♂♂ and 30 ♀♀) and *P. asper* (n=30 ♂♂ and 30 ♀♀). (OB = Olfactory bulb; T = Telencephalon; OL = Optic lobe; C = Cerebellum; FL = Facial lobe; VL = Vagal lobe; ED = Eye diameter; BL = Barbel length).

Table 48. Summary statistics of the products (length X width) of the neural structures of *P. afer* and *P. asper*.

	P. afer		*P. asper*	
	♂♂ n = 30	♀♀ n = 30	♂♂ n = 30	♀♀ n = 30
Mean SL (mm) (S.E.)	55,6 (0,16)	55,6 (0,16)	55,65 (0,16)	55,4 (0,17)
Olfactory bulb	0,85 (0,03)	0,84 (0,02)	0,69 (0,02)	0,61 (0,03)
Telencephalon	3,05 (0,06)	3,01 (0,06)	2,69 (0,04)	2,65 (0,05)
Optic lobe	6,14 (0,09)	5,77 (0,07)	5,11 (0,08)	4,98 (0,03)
Cerebellum	7,56 (0,15)	6,8 (0,13)	5,93 (0,14)	5,48 (0,15)
Facial lobe	1,32 (0,03)	1,25 (0,03)	1,28 (0,03)	1,21 (0,03)
Vagal lobe	1,83 (0,03)	1,86 (0,04)	1,75 (0,03)	1,71 (0,02)
Eye diameter (mm)	3,7 (0,03)	3,79 (0,04)	3,1 (0,02)	3,12 (0,03)
Barbel length (mm)	2,7 (0,1)	2,6 (0,09)	2,36 (0,04)	2,31 (0,05)

The two redfin minnows used for the present study are closely related sister species and were therefore ideal candidates. The problems outlined above did not affect the present analysis as only adults were used, a reasonably large sample size was dissected, enough specimens were available near 55 mm SL to avoid the problems noted by Huber & Rylander (1992) and all the material had been fixed and then preserved in the same way. Davis & Miller (1967) have suggested that width rather than length measurements of brain lobes were better indicators of habitat preference. They reasoned that longitudinal enlargements can displace adjacent lobes whereas lateral expansion is prevented only by available cranial space. Huber & Rylander (1992) used both length and width measurements and this method was followed in the present study.

The gross brain morphology, as viewed in the dorsal aspect, of the two redfin minnows is typical of cyprinid fish such as the false-goby minnow (*Pseudogobius esocinus*) depicted in Tuge *et al.* (1968). Both redfin minnows have a similar configuration but there is a difference in the sizes of the various lobes either as a result of hypertrophy or degeneration (Table 49).

Table 49. Two-sample analysis tests of the neural structures of *P. afer* and *P. asper* (95% confidence levels).

	t statistic	Sig. level	HO: Diff. = 0
Olfactory bulb			
♂♂/♀♀ *P. afer*	0,2268	0,8214	HO: Diff. = 0
♂♂/♀♀ *P. asper*	2,1905	0,0325	reject HO
♂♂ *P. afer* to ♂♂ *P. asper*	4,084	0,0014	reject HO
♀♀ *P. afer* to ♀♀ *P. asper*	6,7301	0,0000	reject HO
♂♂ *P. afer* to ♀♀ *P. asper*	5,8288	0,0000	reject HO
♂♂ *P. asper* to ♀♀ *P. afer*	-4,7429	0,0000	reject HO
Olfactory lobe			
♂♂/♀♀ *P. afer*	0,4862	0,6287	HO: Diff. = 0
♂♂/♀♀ *P. asper*	0,6039	0,5483	HO: Diff. = 0
♂♂ *P. afer* to ♂♂ *P. asper*	4,8489	0,0000	reject HO
♀♀ *P. afer* to ♀♀ *P. asper*	4,4277	0,0000	reject HO
♂♂ *P. afer* to ♀♀ *P. asper*	5,2441	0,0000	reject HO
♂♂ *P. asper* to ♀♀ *P. afer*	-4,0292	0,0002	reject HO
Optic lobe			
♂♂/♀♀ *P. afer*	3,3111	0,0016	reject HO
♂♂/♀♀ *P. asper*	1,1488	0,2553	HO: Diff. = 0
♂♂ *P. afer* to ♂♂ *P. asper*	8,8165	0,0000	reject HO
♀♀ *P. afer* to ♀♀ *P. asper*	7,3485	0,0000	reject HO
♂♂ *P. afer* to ♀♀ *P. asper*	9,8866	0,0000	reject HO
♂♂ *P. asper* to ♀♀ *P. afer*	-6,1702	0,0000	reject HO
Cerebellum			
♂♂/♀♀ *P. afer*	3,9015	0,0000	reject HO
♂♂/♀♀ *P. asper*	2,1999	0,0318	reject HO
♂♂ *P. afer* to ♂♂ *P. asper*	8,0738	0,0000	reject HO
♀♀ *P. afer* to ♀♀ *P. asper*	6,6952	0,0000	reject HO
♂♂ *P. afer* to ♀♀ *P. asper*	9,8543	0,0000	reject HO
♂♂ *P. asper* to ♀♀ *P. afer*	-4,6468	0,0000	reject HO

Figure 49. (cont.)

	t statistic	Sig. level	HO: Diff. = 0
Facial lobe			
♂♂/♀♀ *P. afer*	1,6816	0,098	HO: Diff. = 0
♂♂/♀♀ *P. asper*	1,5718	0,1214	HO: Diff. = 0
♂♂ *P. afer* to ♂♂ *P. asper*	1,052	0,2972	HO: Diff. = 0
♀♀ *P. afer* to ♀♀ *P. asper*	0,0844	0,4022	HO: Diff. = 0
♂♂ *P. afer* to ♀♀ *P. asper*	2,4117	0,0191	reject HO
♂♂ *P. asper* to ♀♀ *P. afer*	0,7319	0,4672	HO: Diff. = 0
Vagal lobe			
♂♂/♀♀ *P. afer*	-0,7377	0,4637	HO: Diff. = 0
♂♂/♀♀ *P. asper*	0,8404	0,4042	HO: Diff. = 0
♂♂ *P. afer* to ♂♂ *P. asper*	1,6533	0,1037	HO: Diff. = 0
♀♀ *P. afer* to ♀♀ *P. asper*	3,5831	0,0007	reject HO
♂♂ *P. afer* to ♀♀ *P. asper*	2,7496	0,0079	reject HO
♂♂ *P. asper* to ♀♀ *P. afer*	-2,3901	0,0201	reject HO
Eye diameter			
♂♂/♀♀ *P. afer*	1,9638	0,0544	HO: Diff. = 0
♂♂/♀♀ *P. asper*	-0,4491	0,6551	HO: Diff. = 0
♂♂ *P. afer* to ♂♂ *P. asper*	16,4756	0,0000	reject HO
♀♀ *P. afer* to ♀♀ *P. asper*	14,6728	0,0000	reject HO
♂♂ *P. afer* to ♀♀ *P. asper*	15,2788	0,0000	reject HO
♂♂ *P. asper* to ♀♀ *P. afer*	-15,5093	0,0000	reject HO
Barbel length			
♂♂/♀♀ *P. afer*	1,1168	0,2687	HO: Diff. = 0
♂♂/♀♀ *P. asper*	0,7993	0,4274	HO: Diff. = 0
♂♂ *P. afer* to ♂♂ *P. asper*	3,244	0,002	reject HO
♀♀ *P. afer* to ♀♀ *P. asper*	2,365	0,0214	reject HO
♂♂ *P. afer* to ♀♀ *P. asper*	3,616	0,0006	reject HO
♂♂ *P. asper* to ♀♀ *P. afer*	-1,9346	0,0579	HO: Diff. = 0

Telencephalon

The telencephalon consists of the olfactory bulbs connected to the olfactory lobes via olfactory tracts. The telencephalon integrates olfactory, visual and gustatory stimuli (Friedlander, 1983; Davis & Kassel, 1983) and is involved with learning, and agnostic or social behaviours (Demski, 1983). Teleost olfaction (olfactory bulb) is used primarily for social communication and in most species is of little or no use in foraging (Finger, 1988). The degree of development of the secondary olfactory centres has a direct relationship with whether the fish has well developed olfaction or not (Tuge *et al.*, 1968).

The olfactory bulbs were very similar for both sexes of *P. afer* but male *P. asper* had larger bulbs than did the females. *P. afer* males and females have larger olfactory bulbs than do *P. asper* males or females (Table 49). The olfactory lobes were similar in both sexes of *P. afer* and both sexes of *P. asper* (Table 49). The olfactory lobes of both sexes of *P. afer* were larger than both sexes of *P. asper*. Therefore the telencephalon of the *P. afer* is better developed than that of *P. asper*. The significantly larger olfactory bulbs and lobes of *P.afer* indicated that this clear water species had a better developed sense of olfaction and that these neural structures in the turbid water species, *P. asper*, have degenerated. This may indicate that in clear water the olfactory bulb was used for social communication and was therefore of more importance than in turbid conditions.

Optic lobes and eyes

Both the eyes and optic lobes are related to vision. The optic lobes are the primary targets of retinal fibres. In species which inhabit clear water a combination of lateral line and vision may be more adaptive than an elaborate chemosensory apparatus such as would be expected in fish which inhabit turbid waters (Huber & Rylander, 1992; Kotrschal & Palzenberger, 1992). *P.afer* is a visual specialist compared to *P. asper*. The larger visual centres of *P. afer* would indicate a more predatory way of life than that of *P. asper*. Since *P. asper* is the more derived species we can assume that there has probably been a degeneration of the optic lobes. This degeneration correlated to the afferent sensory system, the eyes would be understandable considering the two habitats. The male *P. afer* had the biggest optic lobes and eyes when compared to *P. asper* males and females. The optic lobes of male *P. afer* were significantly larger than those of *P. asper* and this may be associated with their behaviour. Male *P. afer* have bright breeding colouration as well as large nuptial tubercles and the large optic lobe may be positively correlated to these features. Male *P. asper* are not as brightly coloured and have considerably smaller and fewer tubercles.

Cerebellum

The cerebellum of a teleost is divided into three major areas, the valvula, corpus and vastibulolateral lobe. The function of the cerebellum was usually thought of as a motor structure (Dow & Moruzzi, 1958). However, in terms of the afferent and efferent connections the cerebellum would be as intimately related to the primary sensory systems as it would be to the common motor pathway (Finger, 1983). The corpus cerebella may be most closely related to motor control functions whereas the vestibulolateral lobe appears to be more involved in processing lateral line sensory input. The cerebellum plays a central role in proprioception, motor coordination, and eye movement (Demski, 1983). The size of the cerebellum taken as a whole from the dorsal aspect may be misleading and the size of the various sections of the cerebellum should be considered where possible. In general the cerebella of fishes which perform quick swimming movements are well endowed (Tuge *et al.*, 1968).

The shape of the corpus cerebella, the posterior portion of the cerebellum, may relate less to function than to morphology of the skull (Finger, 1983). It is assumed here that in the two sister species the morphology of the skull would be very similar.

Overall *P. afer* males have the largest cerebella and female *P. asper* the smallest (Table 49). Therefore the relatively large size of the cerebellum of *P.afer*, compared to that of *P. asper*, is probably due to the perennial flow of the mountain stream, the clarity of the water and the feeding behaviour of the species in this environment. The large cerebellum might be advantageous for this species to quickly and efficiently orientate on food items in the clear, flowing waters below riffles. It has to maintain a high-level of motor activity, which is related to "station-keeping" and thus resisting being displaced in the relatively fast water currents. That male *P. afer* have the largest cerebella may also be due to the importance of quick eye movement and processing of information which may be related to nuptial activities as suggested by the bigger optic lobes of *P. afer* males.

Medulla oblongata

In all vertebrates the cranial nerves which supply connections to the taste receptor cells terminate centrally in the visceral sensory column of the medulla. In fish taste is used for both locating and evaluating food. Sensory nuclei of the nervus glossopharyngeus (IX) receive information about the quality of a food item which innervates taste buds in the back of the mouth and the first gill slit. The primary gustatory nuclei receiving the afferent visceral sensory fibres are located in the facial lobes which are supplied by cranial nerve VII (nervus facialis) and in the vagal lobes supplied by cranial nerve X (nervus vagus). In minnows the facial and vagal lobes are the most prominent (Huber & Rylander, 1992). The vagal lobes receive fibres from the taste buds in the pharynx, on the gill rakers, and on the palate. The facial lobes receive sensory fibres from the lips, barbels and skin and are the main neural structure involved in locating food. Dorsal enlargements or lobes arising from the visceral sensory column are typical of fish with elaborately evolved gustatory systems. These gustatory (facial and vagal) lobes are most evident in some of the cyprinids (including here the two minnows under study) and catfish. In members of the family Cyprinidae there are taste buds which are scattered widely over the body as well as barbels and in these species the lobes in the medulla oblongata are hypertrophied when compared to the brains of other fish species (Tuge *et al.*, 1968).

In a study of catostomid fishes the smallest vagal lobes and largest facial lobes relative to total brain size occurred in fishes inhabiting faster headwater streams (Miller & Evans, 1965). The largest vagal lobes occurred in species in lakes and pools.

The facial lobes were similar for both redfin minnow species. The only significant difference occurred in the larger facial lobe in male *P. afer* compared to female *P. asper*. The swelling of the lobes of the vagal section of male and female *P.afer* were more developed than female *P. asper*. On a comparative basis there is very little difference between the facial and vagal lobes for both species.

Barbels

Barbel size is usually longer in turbid water species of *Hybopsis* when compared to clear water forms (Davis & Miller, 1967). The males of *P. afer*, the clear water species, had significantly longer barbels and female *P. afer* had longer barbels than female *P. asper*. This again may be related to the need to locate food quickly and efficiently in the oligotrophic, clear mountain stream.

Turbid versus clear water species

In clear water species of *Notropis* and related genera the primary optic structures and cerebellum were found to be larger (Huber & Rylander, 1992) and this relationship was also found in the present study. In turbid water species of *Notropis* and related genera the olfactory bulb and the facial lobe were larger. This suggests that there was compensation by taste when vision was impeded by high turbidity. In *P. afer* and *P. asper* the olfactory bulbs were larger in the clear water species and the facial lobes were of similar proportions. Therefore these differences may still be evolving in these two sister species.

As the habitat of American minnows became more variable there was a corresponding increase in the size-configurational pattern of the brain lobes (Davis & Miller, 1967). Species which lived in both clear and turbid habitats had more variability than did species which inhabited constant environments. In a study of the correlation of brain morphology and turbidity in *Notropis* and related genera Huber & Rylander (1992) found that the size of the brain structures concerned with vision, olfaction and gustation was correlated with habitat turbidity. They found that habitat turbidity correlated with the facial lobe but not with the vagal system. The facial lobe system is important for locating a food source in the environment, and the glossopharyngeal-vagal system is important for ingestion of food placed in the mouth (Atema, 1971). In the present study the two gustatory subsystems did not show the trend found in the American minnows. The results of the study done by Huber & Rylander (1992) supported two hypotheses: (1) species of minnows rely on different sensory modalities, which correlate with the physical parameters in their preferred habitat, and (2) the importance of a particular modality is reflected in the size of the corresponding neural structures. Their analysis showed that neither similar turbidity preferences nor shared phylogeny were alone sufficient to explain the observed differences in brain morphology.

In contrast to many studies on fish brain ecomorphology Kotrschal & Palzenberger (1992) concluded that brain structures would not necessarily be a close predictor of ecology and that morphology would be more conservative than behaviour and ecology. That *P. asper* has not developed some larger neural structures to cope with turbidity may indicate that morphology in this case was more conservative than ecology.

In American minnows there was a high correlation between size of cerebellum and visual structures which suggested a functional association between these structures (Huber & Rylander, 1992). The present study supports this finding for two African minnows. A visually orientating species which can pursue faster moving prey may also need a better developed motor coordination than a species relying on taste (Huber & Rylander, 1992). But swimming ability is more developed in species which inhabit fast currents which are frequently clear (Gatz, 1979; Felley, 1984). Therefore swimming ability and vision may be independent adaptations to a clear, fast flowing stream environment (Huber & Rylander, 1992). *P.afer* are frequently seen in the swift water below riffles searching in the water column for prey. In the turbid waters inhabited by *P. asper* the need for quick feeding movements would not be as advantageous. The larger areas of the brain of *P.afer* can possibly be explained by its adaptation to the habitat variables such as clear, perennial flowing mountain streams.

Sight-feeding fishes usually have enlarged optic but small facial and vagal lobes, whereas fishes with enlarged vagal lobes are usually bottom feeders (Davis & Miller, 1967). *P.afer* has enlarged optic lobes but the vagal lobes are similar to those of *P. asper* males. This may be because *P.afer* feeds on the bottom of pools as well as in the water column below riffles (personal observations).

Intersexual differences

Intersexual differences in the size of some brain structures such as the olfactory bulb and cerebellum of some *Notropis* and related genera suggested the existence of intersexual differences in ecology and behaviour (Huber & Rylander, 1992).

Male *P. afer* had larger optic lobes and cerebella than female *P. afer* which may indicate a behaviourial difference as was also suggested by the larger tubercles and brighter red nuptial fin colouration of male *P. afer*. The optic lobes of male and female *P. asper* are similar, however males have larger cerebella than do females. It was noted in the discussion of the cerebellum that it has many different functions and a closer investigation may be required here. Male *P. asper* have larger telencephalons, olfactory bulbs and lobes than do female *P. asper*. It is suggested here that in the turbid environment a larger telencephalon may be more important than larger optic lobes, which is the reverse of what was found for *P. afer* males and females. The olfactory bulbs play a role in social communication and may be important in the turbid river, although it is noted that these structures in *P. asper* are not bigger than those of *P. afer* (Table 49).

Taxonomic character

The morphology of neural structures, such as the valvula cerebella, can be used as a means of identification of otherwise morphologically closely related fish species (Tandon, 1986). Attempts to use brain morphology as a taxonomic character have not always been successful (Lissner, 1923 in Davis & Miller, 1967) because ecological factors strongly influence the brain lobe proportions. Similar habitat preferences may lead to similar brain morphologies and will not necessarily show good phylogenetic differences. Brain pattern should not be used alone as ecological factors influence brain morphology and may obscure phylogenetic relationships (Svetovidov, 1953). True phylogenetic relationships may be revealed in the early ontogeny of a fish before specializations associated with the more recent ecological adaptations make their appearance ontogenically (Miller & Evans, 1965). There are differences in the sizes of neural structures of the sister species *P. afer* and *P. asper* and it is suggested here that these are due to ecological differences.

Hypothesis

The hypothesis raised in the introduction is therefore accepted for *P. afer*, which was found to have larger neural structures related to vision but rejected for *P. asper* which did not show any compensation for inhabiting a turbid environment.

CHAPTER 9

GROWTH AS A SPECIFIC ADAPTIVE PROPERTY OF TWO CLOSELY RELATED REDFIN MINNOWS, *P. AFER* AND *P. ASPER*, IN THE GAMTOOS RIVER SYSTEM.

Introduction

Growth of two redfin minnow species, *P. afer* and *P. asper*, was investigated and the differences in their life-history styles were assessed with respect to growth rates, age at maturity and longevity. Examination of growth aspects in isolation can lead to a lack of understanding of environmental factors that determine growth rates and how natural selection has channelled growth rate potentials in particular directions (Mann, 1991). In this redfin minnow study a knowledge of environmental factors helped to interpret differences in life-history styles between the two minnow species. This is the first account of the growth of these two species in their natural habitats.

A single species such as the European minnow, *Phoxinus phoxinus*, occurs over a wide geographical range (in this case almost the whole of Europe and into northern Asia) and displays a remarkable variability in its life-history (Mills, 1988). There have been very few studies done on the African minnows (Cambray, 1982) and especially on a comparative basis for two closely related species in two different environments. This study of the two redfin minnow sister species is close to this approach.

It was hypothesized that the warmer, more productive waters of the Groot River would lead to a faster growth rate, earlier maturity and shorter lifespan of *P. asper* whereas slower growth rate, delayed maturity and longer lifespan would be expected for *P. afer* in the shaded, oligotrophic Cape Fold Mountain Belt stream that they inhabit.

Methods

Length frequency analysis (Petersen method)

Specimens were collected with the minnow seine net described in Chapter 4 or a scoop net on a D frame (50 cm diameter and a height of 30 cm and mesh of 0,2 mm). Since this was a long-term study, electrofishing was not used as monthly electrofishing may have damaged fish which were not collected.

The length-frequency data were obtained from monthly collections over a period of 31 months for both populations. Length-frequency graphs were then constructed on a monthly basis which showed the influx of new recruits. This method was especially useful in establishing the relatively rapid early growth rate of *P. asper* in comparison to *P. afer*. Early cohorts could be followed as they formed distinct modes. The growth of 0^+ cohorts was estimated by following monthly shifts in the position of the mode of that cohort. Length frequency analysis was hampered by the fish breeding more than once in a season, especially in the *P. asper* population. The cohorts of one year soon became indistinguishable from cohorts of previous years making separation of different cohorts difficult. To separate overlapping cohorts the age and length of individual fish were determined using scales. Size-frequency analysis was used to corroborate ages attained by scale reading.

Scales

Five to six scales were removed from the scale row immediately above the lateral line and in the area below the base of the dorsal fin on the right side of the fish. If there were not enough useable scales on the right side then scales were removed from the left side. On some of the older fish a high number of regenerated scales made it necessary to use scales from both sides. The scales were mounted on microscope slides then covered with a cover slip which was taped to the slide. Each slide was given an unique identifying number which was cross-referenced to the data sheets. Scales were allowed to dry for at least two weeks before they were viewed with a Bell & Howell MT4 microfiche reader at a magnification of 48X.

The ageing of fish by identifying calcareous structures must be validated in case the structures have false checks which could be interpreted as annular marks. False annuli can be caused by starvation, unfavourable temperatures, injury, spawning checks etc. (Van Oosten, 1957). In the present study the annular marks were validated by using samples taken over a 22 month period. The total numbers (*P. afer* n = 1126; *P. asper* n = 1085) and size ranges of the fish from which scales were examined are given in Tables 50 & 51. The number of circuli after the last "annular mark" were counted and this was done throughout the year to validate what was being called the annular marks and to establish when they had formed. The scales of juvenile (0^+) fish collected at the end of their first growing season were examined to enable the larval mark to be distinguished from the first annual mark.

Table 50. Total numbers and size ranges of *Pseudobarbus afer* specimens which were aged. Collections from the Wit River, Gamtoos River system.

Date	Male n	Size Range (SL mm)	Female n	Size Range (SL mm)	Unsexed Juv. n	Size Range (SL mm)	Total
1987							
July	10	35,5-71,1	8	37,3-62,5	9	22,3-34,4	27
Aug.	8	36,1-51,8	28	35,7-63,0	23	21,5-34,6	59
Sept.	15	38,7-63,7	28	38,5-70,2	13	23,0-37,9	56
Oct.	27	35,5-78,2	35	34,6-76,1	17	27,3-34,7	79
Nov.	14	36,7-70,5	23	41,9-66,1	16	26,2-34,5	53
Dec.	12	36,2-63,4	31	36,9-68,7	5	27,2-35,7	48
1988							
Jan.	17	35,0-59,2	18	35,3-51,8	9	22,6-34,5	44
Feb.	21	36,8-68,1	27	35,1-72,4	7	19,1-28,1	55
Mar.	8	36,0-63,1	38	30,7-68,7	8	20,2-33,0	54
Apr.	10	39,1-66,0	26	38,8-63,7	7	23,7-31,4	43
May	25	34,0-65,8	27	30,9-61,2	9	23,4-27,4	61
Jun.	15	30,9-58,4	32	31,7-59,8	11	19,2-31,2	58
Jul.	26	35,2-72,5	30	39,2-72,8	6	26,2-29,7	62
Aug.	12	34,9-64,7	28	33,7-68,2	9	21,1-30,3	49
Sept.	17	37,8-63,2	25	32,9-64,5	7	25,3-32,2	49
Oct.	19	32,5-62,5	25	31,8-61,1	8	24,1-31,1	52
Nov.	15	31,9-66,2	24	31,3-64,9	9	28,3-32,7	48
Dec.	11	34,3-70,1	18	31,4-68,4	0		29
1989							
Jan.	17	32,3-69,6	22	34,2-61,1	4	17,3-32,1	43
Feb.	11	35,9-70,5	20	35,7-64,3	4	22,0-27,8	35
Mar.	21	31,6-68,2	33	32,1-79,9	7	23,2-28,9	61
Apr.	19	34,6-67,6	29	31,7-65,4	13	18,6-29,9	61
Total	350	30,9-78,2	575	30,7-79,9	201	17,3-37,9	1126

The length-at-age data were fitted to several growth models by means of the curve-fitting and statistical procedures developed by (Punt & Hughes, 1989).

Table 51. Total numbers and size ranges of *Pseudobarbus asper* specimens which were aged. Collections from Groot River.

Date	Male n	Size Range (SL mm)	Female n	Size Range (SL mm)	Unsexed Juv. n	Size Range (SL mm)	Total
1987							
July	21	34,1-62,1	13	33,3-56,0	0		34
Aug.	27	33,3-83,4	31	32,3-76,0	1	31,1	59
Sept.	22	35,3-63,0	26	30,6-69,6	0		48
Oct.	19	37,0-64,9	14	36,2-57,4	0		33
Nov.	25	35,8-72,3	19	38,4-63,2	0		44
Dec.	18	42,5-69,9	14	44,9-74,3	7	21,0-33,4	39
1988							
Jan.	25	33,4-63,1	25	35,3-70,2	9	28,7-33,5	59
Feb.	18	32,8-62,0	30	31,8-74,1	0		48
Mar.	5	50,4-53,7	13	49,4-57,8	0		18
Apr.	14	34,1-55,6	32	40,4-60,8	0		46
May	27	40,5-60,4	27	38,6-62,2	0		54
Jun.	28	40,0-65,4	42	40,8-72,3	0		70
Jul.	12	41,3-54,5	18	41,6-63,5	0		30
Aug.	24	35,6-70,4	27	39,0-74,4	0		51
Sept.	22	41,6-62,4	25	39,1-79,9	0		47
Oct.	14	42,4-53,9	7	45,7-70,0	5	21,7-24,0	26
Nov.	16	44,2-63,6	24	46,4-72,0	1	24,3	41
Dec.	33	48,8-62,7	27	39,0-68,2	13	20,4-33,5	73
1989							
Jan.	29	34,3-61,2	20	34,2-70,9	8	24,0-31,6	57
Feb.	31	33,6-66,1	31	33,9-76,5	5	26,3-30,0	67
Mar.	26	34,5-71.9	35	34,8-75,2	9	22,5-33,4	70
Apr.	35	33,6-51,2	28	31,2-70,9	8	26,0-30,3	71
Total	491	32,8-83,4	528	30,6-79,9	66	20,4-33,5	1085

Early life-history

Growth of the early life-history stages was obtained from laboratory work outlined in Chapter 4. The growth for both species was followed under a constant temperature regime (23°C) in aquaria.

Length-frequency analysis (Petersen method)

The length frequency of 5734 *P. afer* and 7521 *P. asper* are shown in Figure 49. There was a similarity in the maximum size attained in each population.

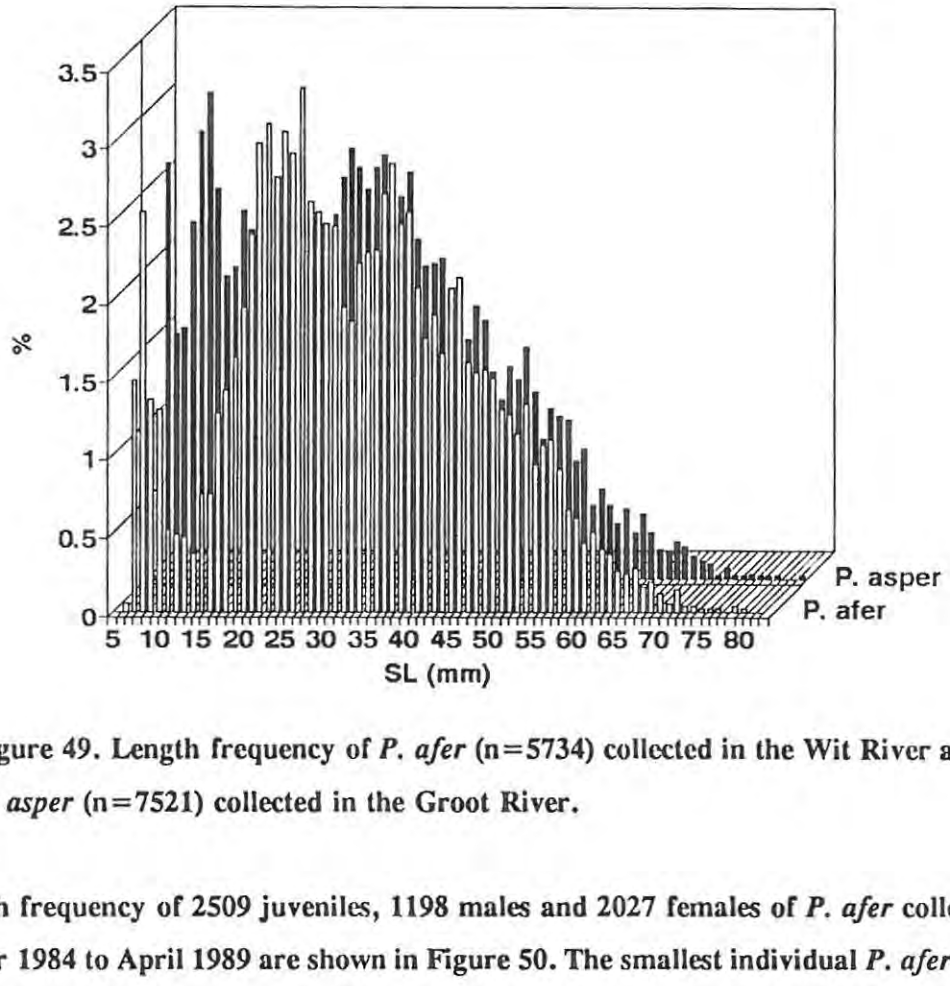


Figure 49. Length frequency of *P. afer* (n=5734) collected in the Wit River and *P. asper* (n=7521) collected in the Groot River.

The length frequency of 2509 juveniles, 1198 males and 2027 females of *P. afer* collected from September 1984 to April 1989 are shown in Figure 50. The smallest individual *P. afer* was a free embryo of 6 mm NL. The largest individual collected was a female of 81 mm SL and the largest male was 78 mm SL.

The length frequency of 3054 juveniles, 2330 males and 2137 females of *P. asper* collected from October 1986 to April 1989 are shown in Figure 51. The smallest was a free embryo of 5 mm NL. The largest individual collected was a male of 83 mm SL and the largest female was 80 mm SL.

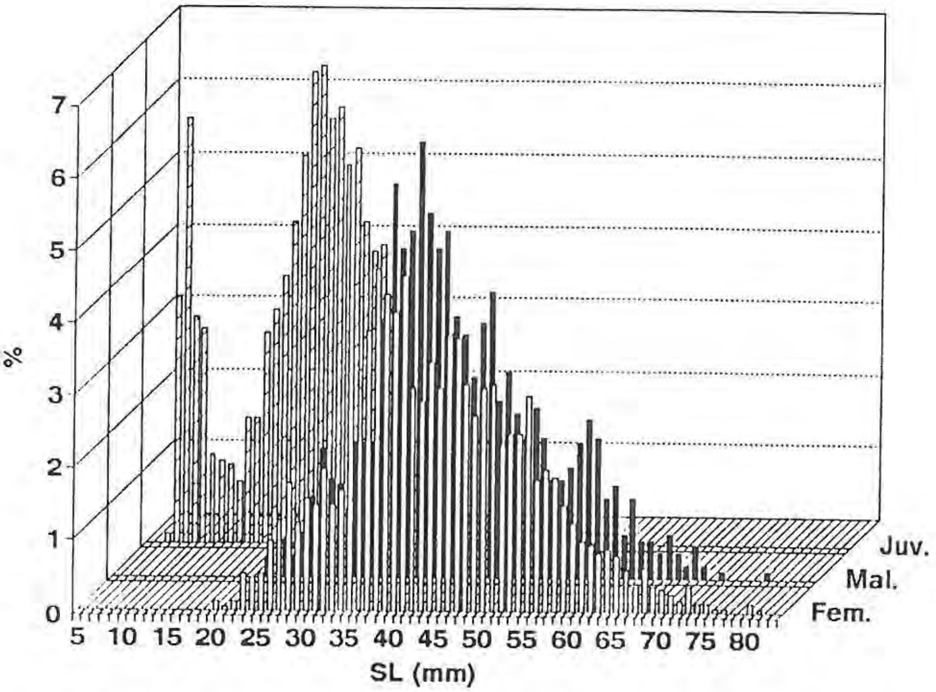


Figure 50. Length frequency of juvenile (n=2509), male (n=1198) and female (n=2027) *P. afer* collected in the Wit River between September 1984 and April 1989.

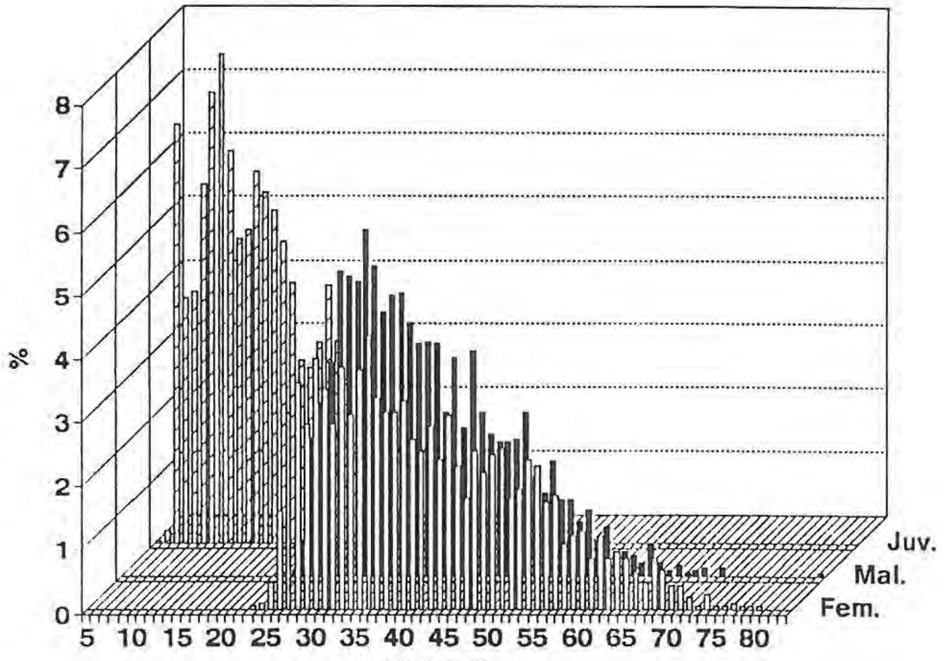


Figure 51. Length frequency of juvenile (n=3054), male (n=2330) and female (n=2137) *P. asper* collected in the Groot River between October 1986 and April 1989.

Length-frequency graphs were constructed for the monthly collections of *P. afer* (Figures 52 - 55) and *P. asper* (Figures 56-58). All specimens were combined whether collected by seine or scoop nets. For *P. asper* the data from the two collection sites, Beerpoort and Smithkraal were combined.

P. afer *cohort analysis*

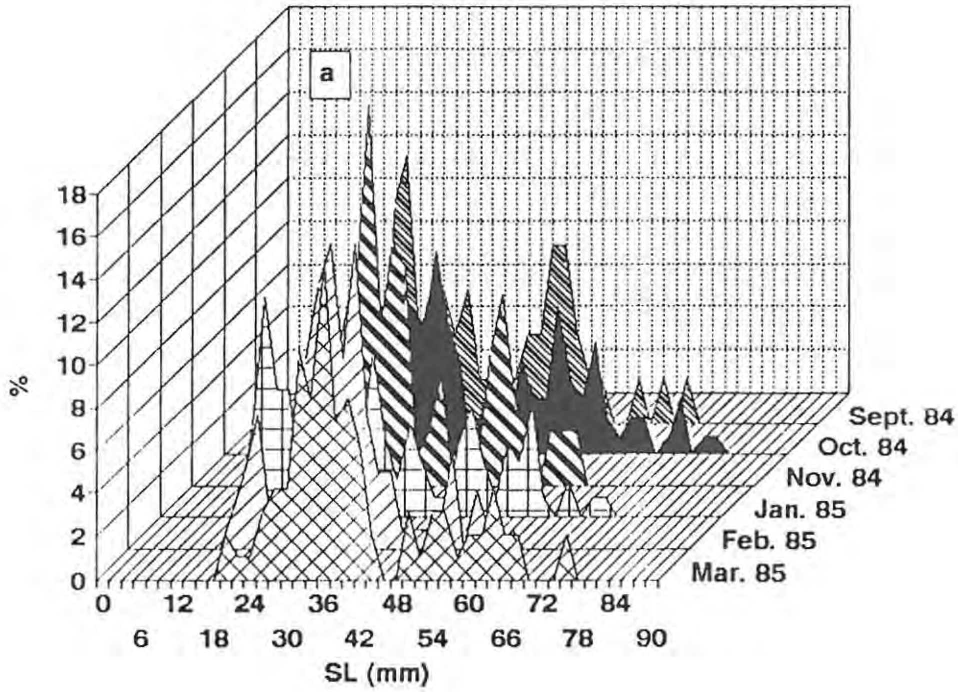
The 1985/1986 summer 0^+ cohort attained a size of 22-34 mm SL by 14 October 1986 (Figures 52b & 53b). The first 1986/1987 0^+ cohort, collected on 18 November 1986 was 8-14 mm SL and by the end of the growing season that cohort peaked at between 28-30 mm SL. The 1985/86 cohort peaked at 38-42 mm SL from February to March 1987, which was also the median size at sexual maturity. By October 1987 the 1986/1987 cohort peaked at 30-32 mm SL (Figure 54a) and by January 1988 that cohort had peaked at 36-38 mm SL (Figure 54b). The new 0^+ cohort appeared at 10-24 mm SL. At the end of that growing season the 0^+ cohort was 20-32 mm SL (Figure 55b 24-4-86). The previous years cohort then peaked at 42 mm SL.

By October 1988 the 0^+ cohort of the previous season had reached a peak of 30-32 mm SL (Figure 55a) and the first recruitment of the 1988/89 year-class could be seen in November 1988 at 8-10 mm SL (Figure 55b). That cohort attained a length of between 18-34 mm SL by April 1989 and the 1987/88 cohort peaked around the sexually mature length of 40 mm SL (Figure 55b).

P. asper *cohort analysis*

The 1985/86 0^+ cohort peaked at 40-48 mm SL by October 1986 (Figure 56). They were therefore at the median size of sexual maturity (♂♂ = 41-42 mm SL; ♀♀ = 43 mm SL; Chapter 4). Recruitment was first observed during October with a single specimen at 14 mm SL, and by November the recruitment was represented by fish of 10-24 mm SL. The monthly growth of the 0^+ cohort could be followed throughout the summer months and reached a peak at 40 mm SL by the end of the growing season (Figure 56b; April 1987). The 1986/87 year-class peaked at 40-46 mm SL (the sexually mature length) by October 1987 (Figure 56b). Recruitment was first observed in November 1987 at 14-16 mm SL and by February 1988 that cohort peaked at 34-36 mm SL (Figures 56b & 57a). More recruitment was apparent in March 1988, at 6-12 mm SL

(Figure 57a). The May 1988 graph showed that the first cohort had merged with the previous year class between 40-50 mm SL and the February cohort peaked at 12-16 mm SL (Figure 57a).



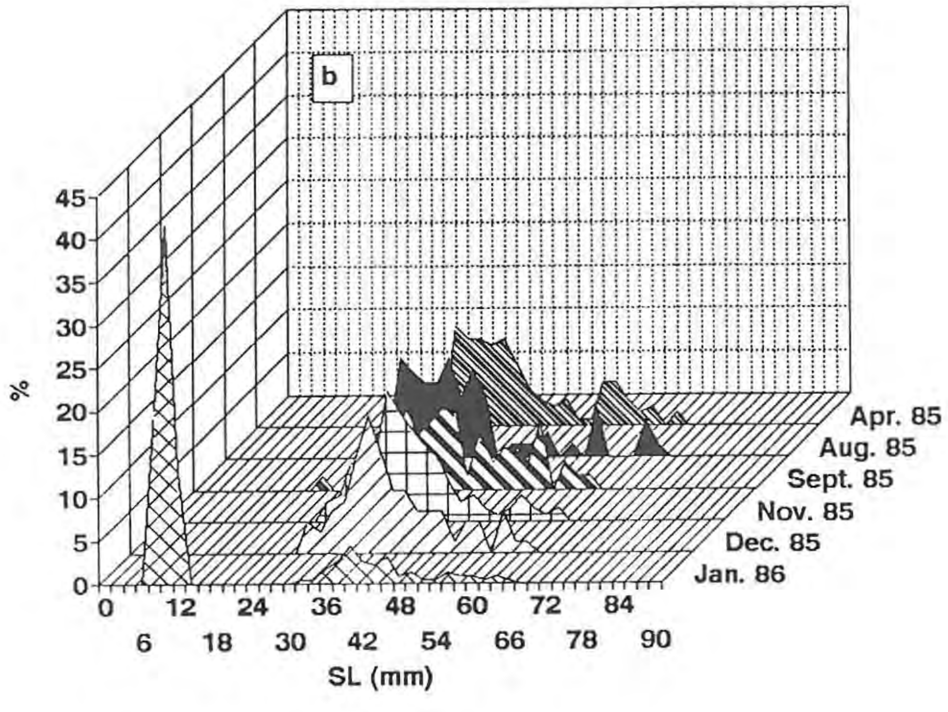


Figure 52. Monthly length frequency for *P. afer* collected in the Wit River (a) September 1984 - March 1985 (n=568), (b) April 1985 - January 1986 (n=720).

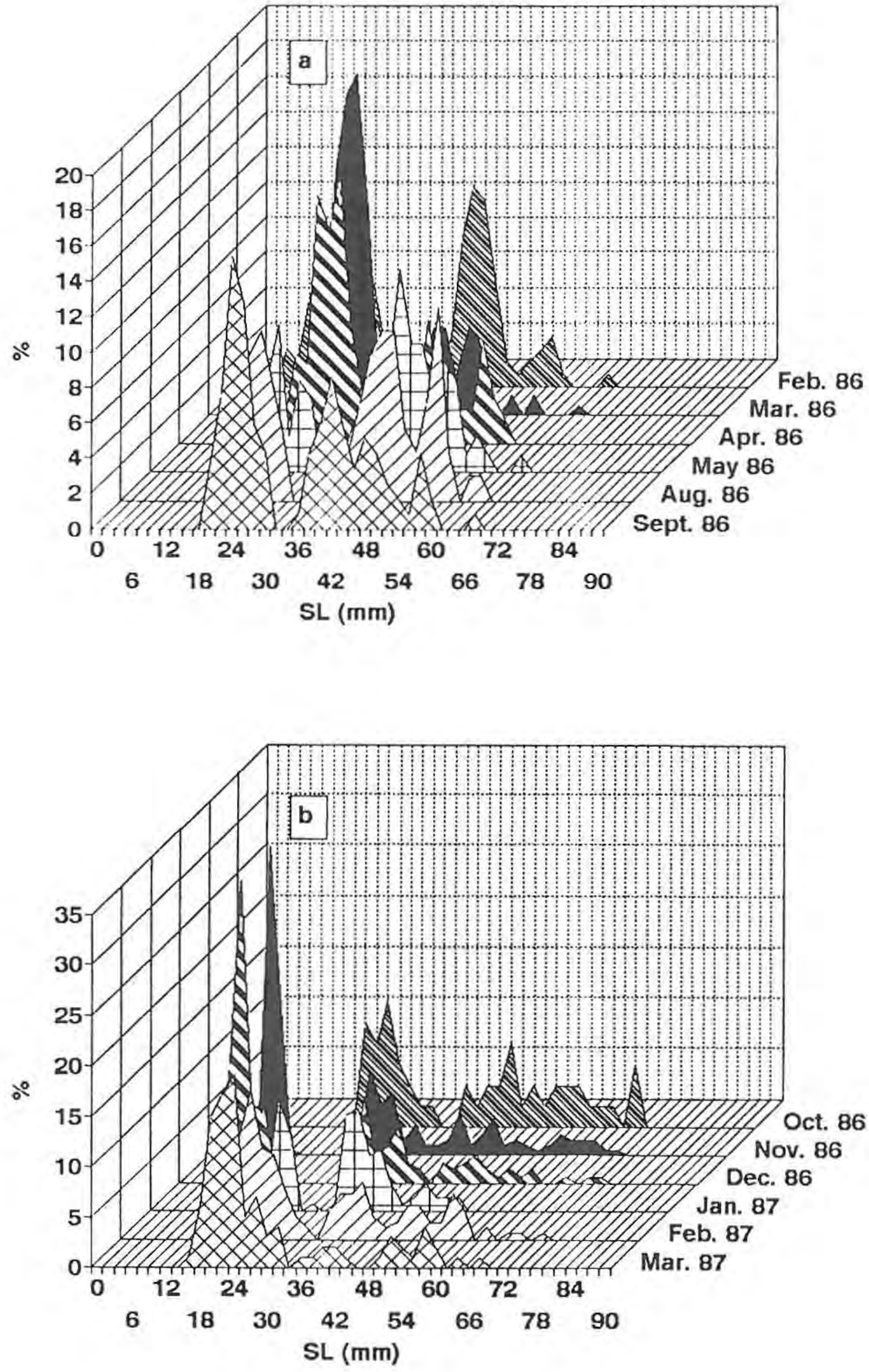
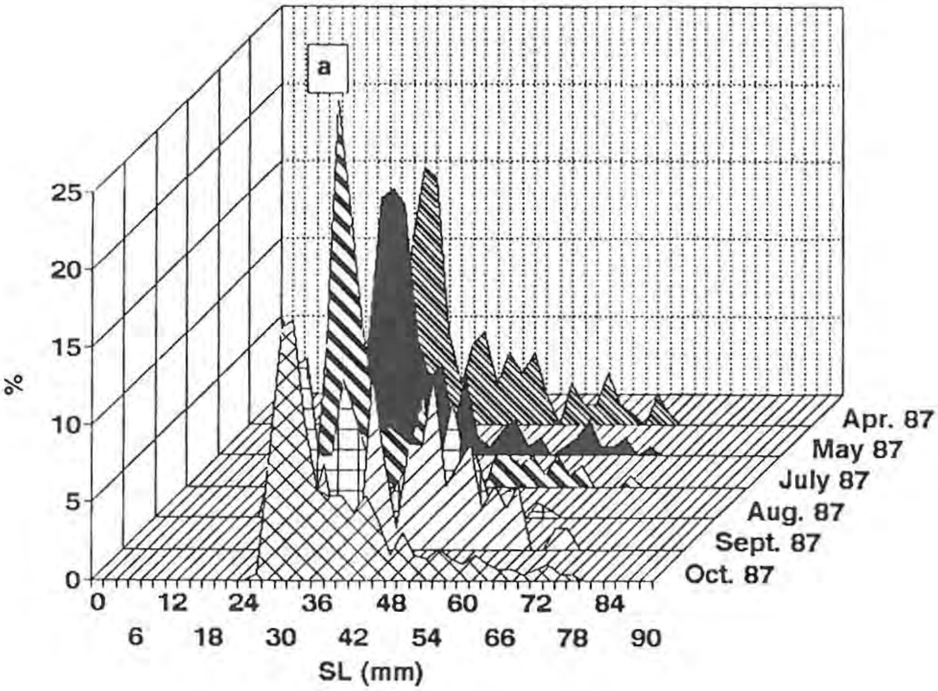


Figure 53. Monthly length frequency for *P. afer* collected in the Wit River (a) February 1986 - September 1986 (n=768), (b) October 1986 - March 1987 (n=808).



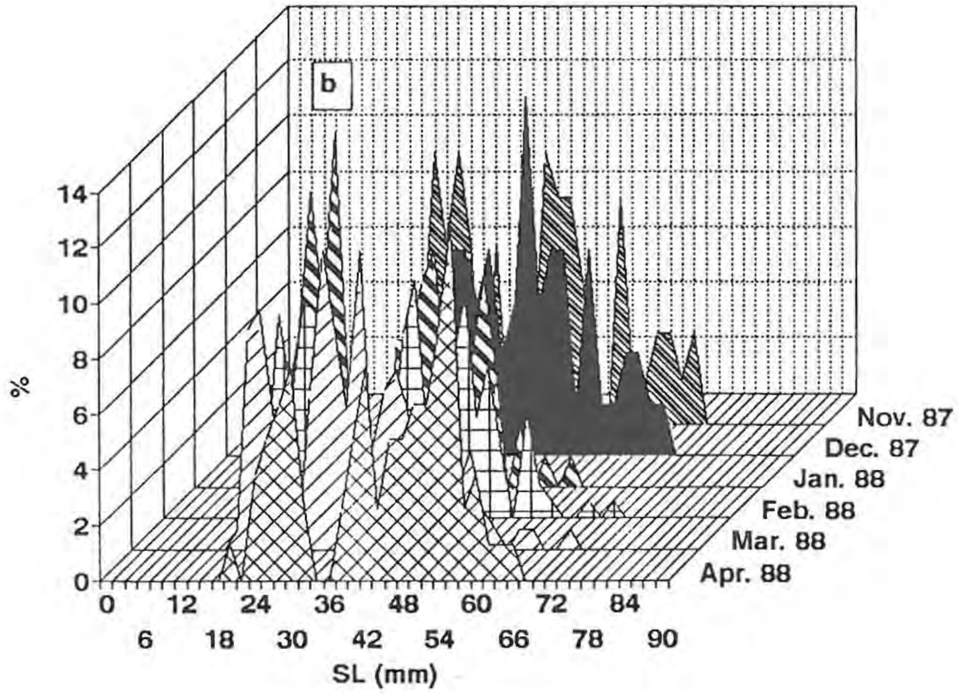
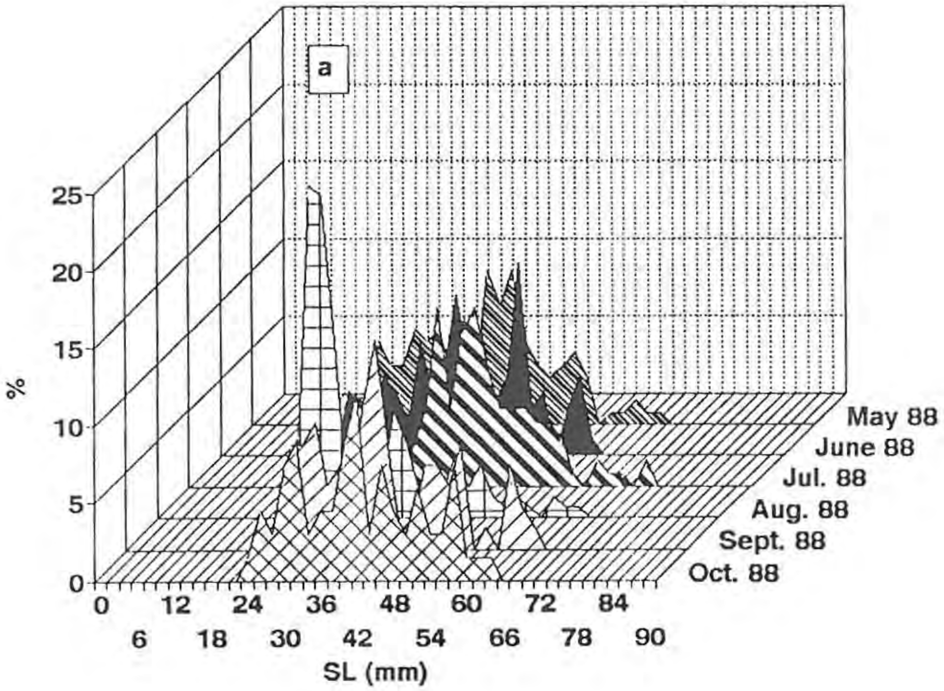


Figure 54. Monthly length frequency for *P. afer* in the Wit River (a) April 1987 - October 1987 (n=1049), (b) November 1987 - April 1988 (n=590).



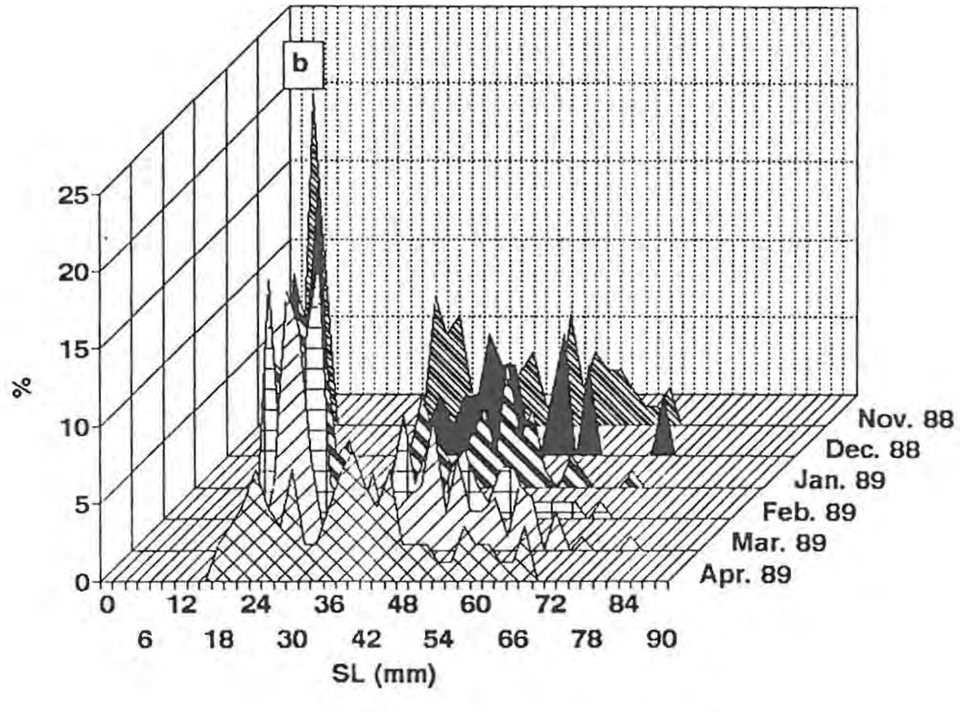
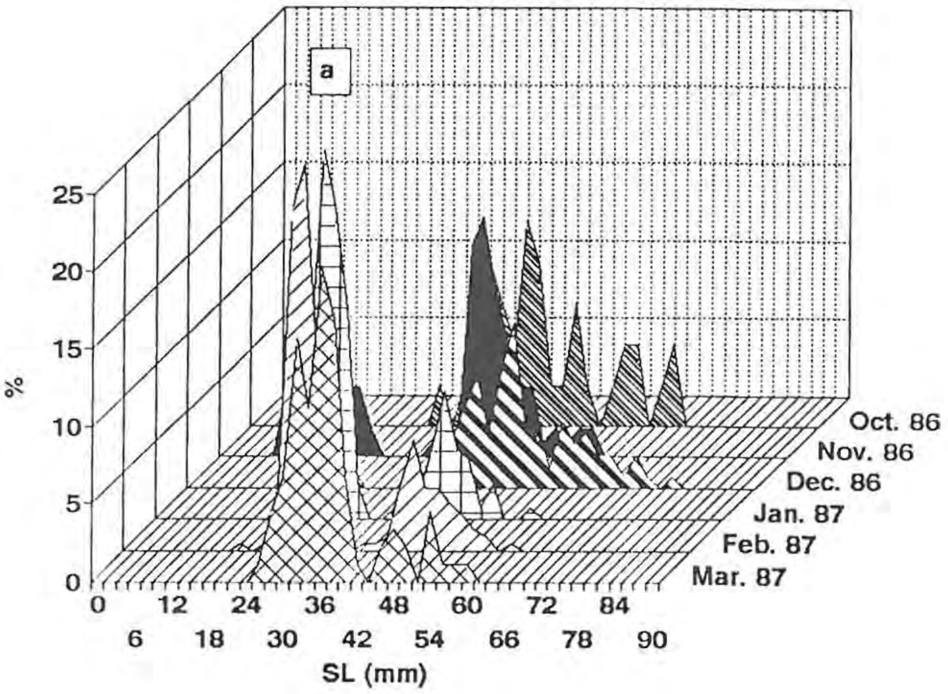


Figure 55. Monthly length frequency for *P. afer* collected in the Wit River (a) May 1988 - October 1988 (n=639), (b) November 1988 - April 1989 (n=531).



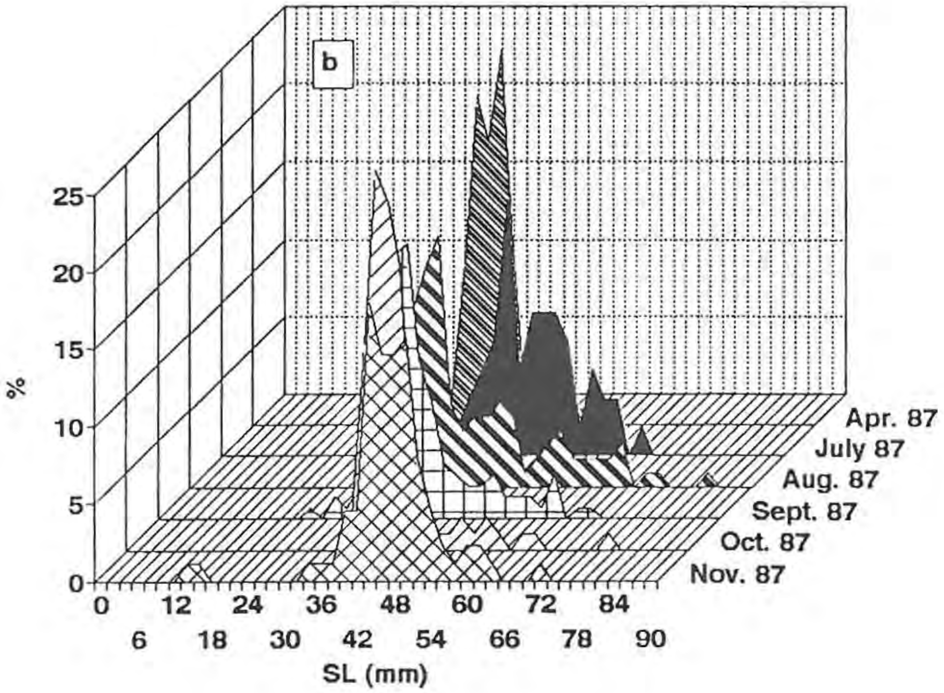
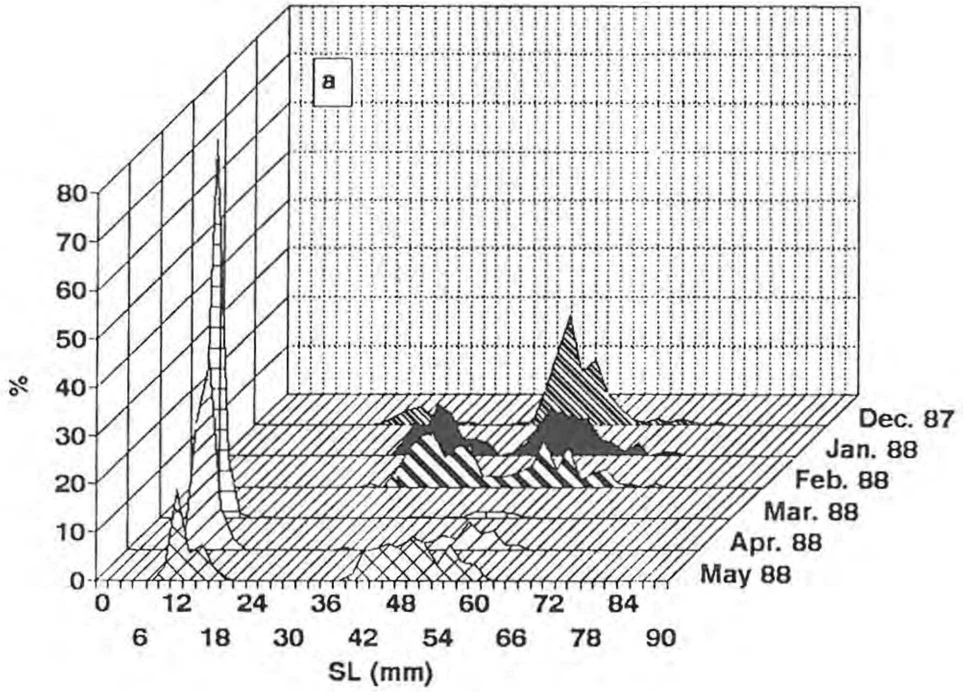


Figure 56. Monthly length frequency for *P. asper* collected in the Groot River (a) October 1986 - March 1987 (n=737), (b) April 1987 - November 1987 (n=551).



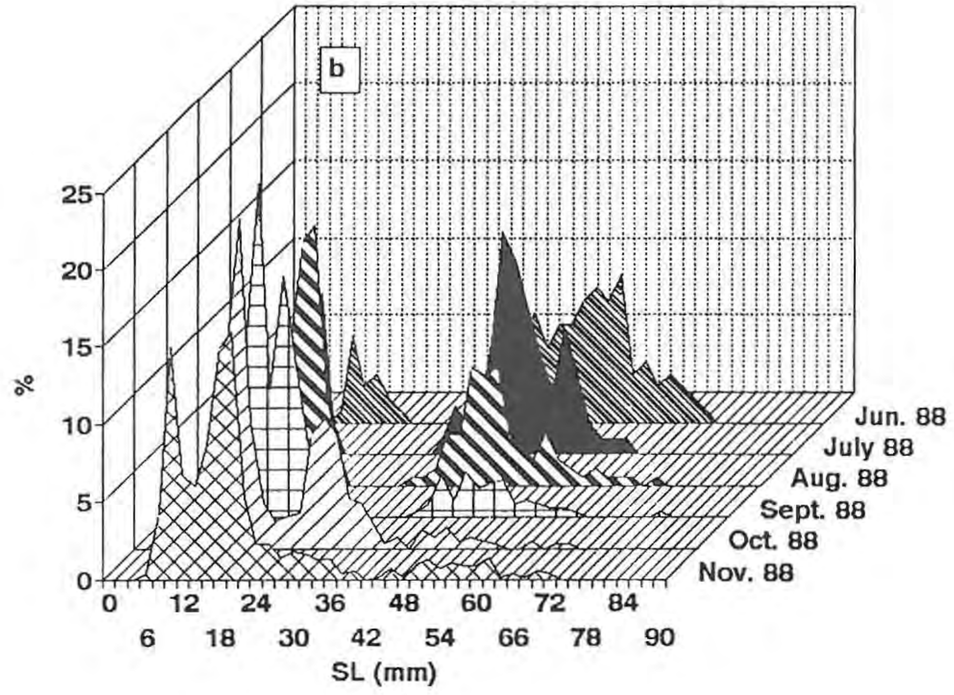


Figure 57. Monthly length frequency for *P. asper* collected in the Groot River (a) December 1987 - May 1988 (n=1253) (b) January 1988 - November 1988 (n=1794).

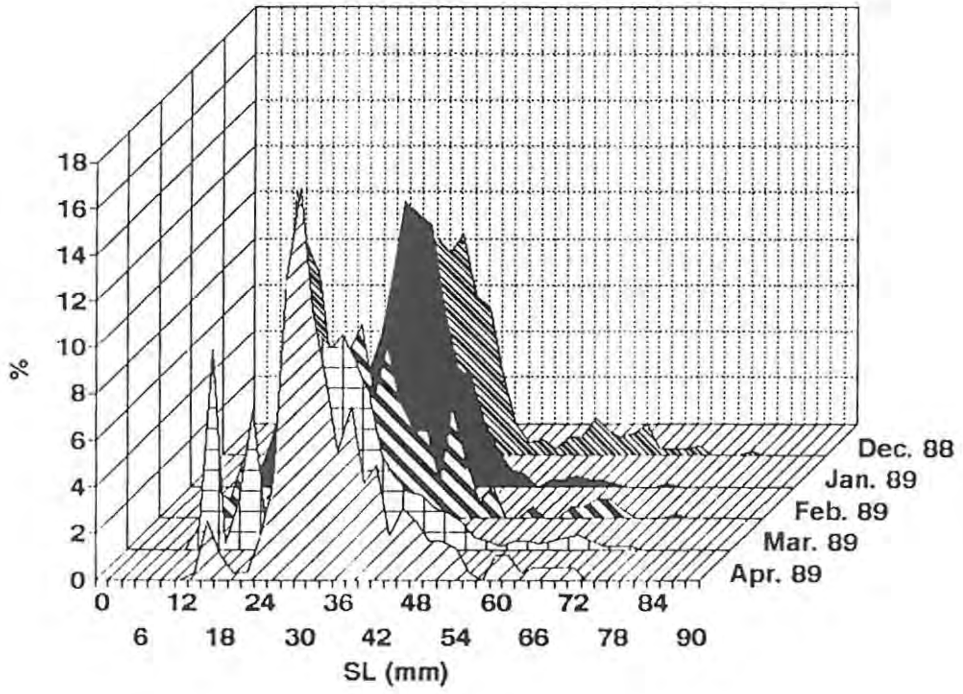


Figure 58. Monthly length frequency for *P. asper* collected in the Groot River (December 1988 - April 1988) (n=3152).

By October 1988 two distinct groups were present. One peaked at 14-16 mm SL and the other at 28-32 mm SL (Figure 57b). The first cohort of the 1987/88 season was not clearly defined but may have peaked at 50 mm SL (Figure 57a October 1988). Recruitment in the 1988/89 season was first observed in November and peaked at 10 mm SL, while the smallest cohort of the previous year was then at 20-22 mm SL (Figure 57b). The 1988/89 cohort attained a length of 28-32 mm SL by April 1989 and the other cohort from the 1987/88 year was 38-42 mm SL and was no longer as well defined (Figure 58).

The sizes attained by the 0^+ cohorts of *P. afer* and *P. asper* by the following October of each year are summarised in Table 52.

Scales

Scales of the two redfin minnows showed the periodicity of scale growth. Scales formed in the normal way of concentric circuli. The inequality of the seasonal growth rates was reflected in the circuli of the scales. The end of a year's growth was shown by an annulus which was more clearly seen in the lateral field. In most cases the annular mark was identifiable by circuli which appeared to cut across other circuli. In some cases a zone of closely spaced circuli was followed by a zone of widely spaced circuli which signalled the resumption of the growth season. In *P. afer* the annular marks were more distinct than in *P. asper*.

Table 52. Size attained by the first 0 cohort from the previous season by the following October for *P. afer* and *P. asper* populations in the Gamtoos River system.

	P. afer	*P. asper*
October 1986	22 - 26 mm SL	38 - 42 mm SL
October 1987	30 - 32 mm SL	40 - 46 mm SL
October 1988	30 - 32 mm SL	approx. 50 mm SL

Validation of annular marks

In both redfin minnows the annual ring on the scales appeared to be a direct result of the retardation of growth during winter as the annular ring appeared in spring (Figures 59-61). Because rings can be laid down at different times of the year for different age groups of the same species (Nikolsky, 1963) this aspect was investigated for the longer lived minnow, *P. afer*. The validation of the *P. afer* annular marks showed that the annular mark was laid down between September and October 1987 and between October and November 1988 for fish between 40-50 mm SL (Figure 61). The same could be seen for all *P. afer* greater than 50 mm SL (Figure 59).

The annular marks for *P. asper* were laid down during October 1987 and during September 1988 for fish over 50 mm SL (Figure 60). Age-length graphs for *P. afer* and *P. asper* males and females are given in Figures 62-65. The oldest *P. afer* was a female of 6^+ years (Figure 63) and males and females of *P. asper* both attained an age of 3^+ years (Figures 64 & 65).

Relationship of scale radius to fish length

The relationship of scale radius to fish length (SL mm) for 1126 *P. afer* and 1085 *P. asper* was calculated as:

P. afer $R = -13{,}2875 + 1{,}3841L$ $r^2 = 93{,}69$

P. asper $R = -7{,}85283 + 0{,}966937L$ $r^2 = 91{,}13$

where R = scale radius in mm X 48
L = standard length in mm

Growth model

The length-at-age data for both species best fitted the three-parameter relative-error Von Bertalanffy special model (Pauly, 1981) which satisfied both the requirements for randomness of residuals and homoscedasticity (Punt & Hughes, 1989). The growth of *P. afer* in the Wit River can be described by the equation:

$$L_t = 92{,}82(1 - e^{-0{,}184(t+0{,}776)})$$

The growth of *P. asper* in the Groot River can be described by the equation:

$$L_t = 88{,}27(1 - e^{-0{,}291(t+0{,}963)})$$

Early life-history

The laboratory growth studies of both species were carried out under a constant temperature regime to facilitate comparison (see Chapter 4). Growth solely on endogenous sources, that is the yolk supply, was probably more reliable as after exogenous feeding commenced food suitability for the two species may have varied and that would be reflected in their growth performances under artificial feeding conditions. Batches 1 & 2 of *P. afer* had similar growth rates (Table 53; Figure 66), whereas Batch 1 of *P. asper* had the highest growth rate and Batch 2 the slowest overall growth rate (Table 53; Figure 66).

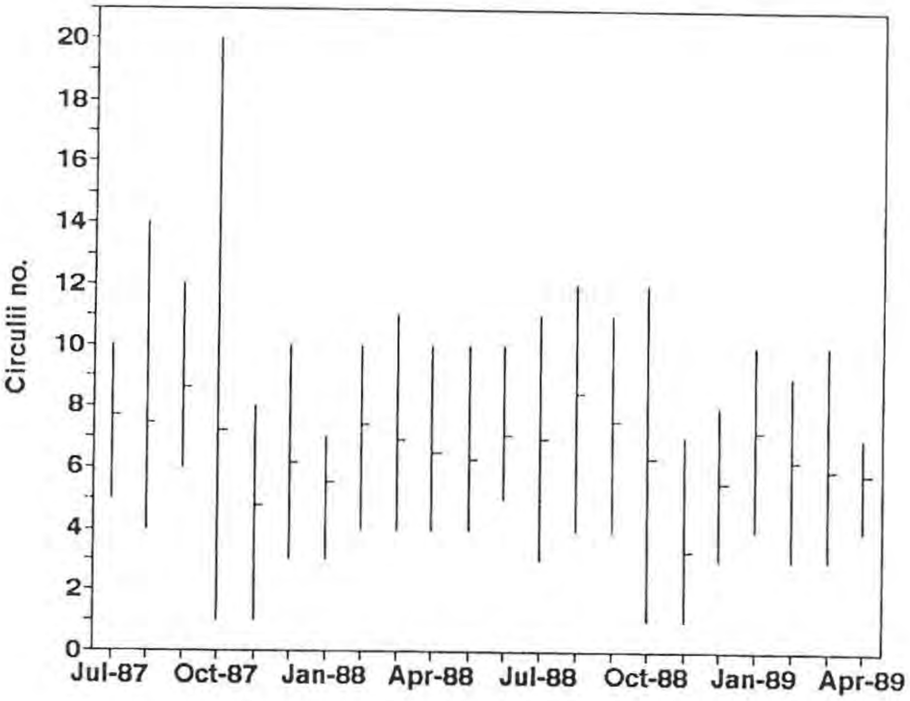


Figure 59 Validation of the annular marks on *P. afer* scales for fish over 50 mm SL (n=378); range and mean).

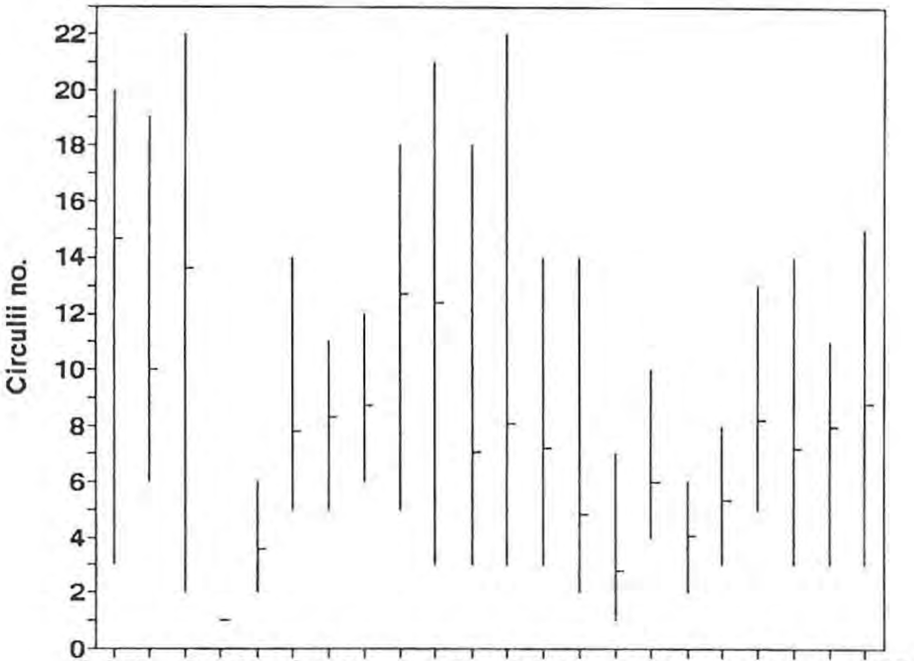


Figure 60. Validation of the annular marks on *P. asper* scales for fish over 50 mm SL (n=470; range and mean).

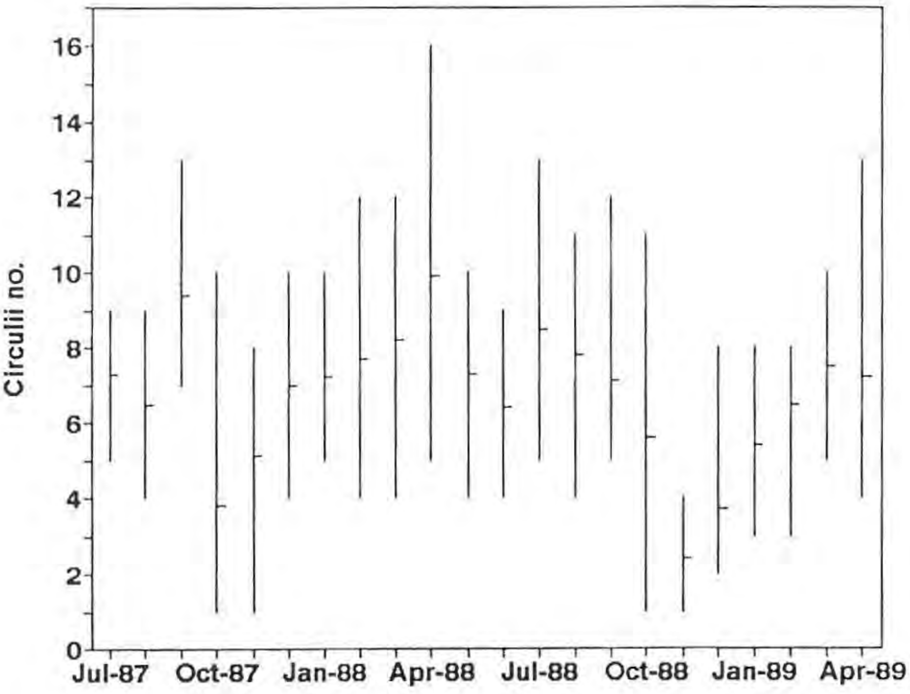


Figure 61. Validation of the annular marks on *P. afer* scales for fish over 40-50mm SL (n=344; range and mean).

Discussion

"Growth is a specific adaptive property, ensured by the unity of the species and its environment" (Wootton, 1990: 187). The unity that the two redfin minnows have with their own distinct environments was expressed very differently with regard to growth. The larger eggs of *P. afer*, compared to *P. asper*, indicated that *P. afer* would have larger first feeding larvae which would be advantageous in the Wit River (Chapter 5). The different growth patterns of the two redfin minnows starting with the differences in the primary anabolic expenditure per egg are probably partly under genetic control and partly the product of phenotypic variability.

Genetic control and phenotypic variability

"Fish have a genetically determined programme or programmes of growth defining a framework within which external factors can cause modifications" (Wootton, 1990:148). For the two redfin minnows the analysis of length frequency and scale readings provided demographic information

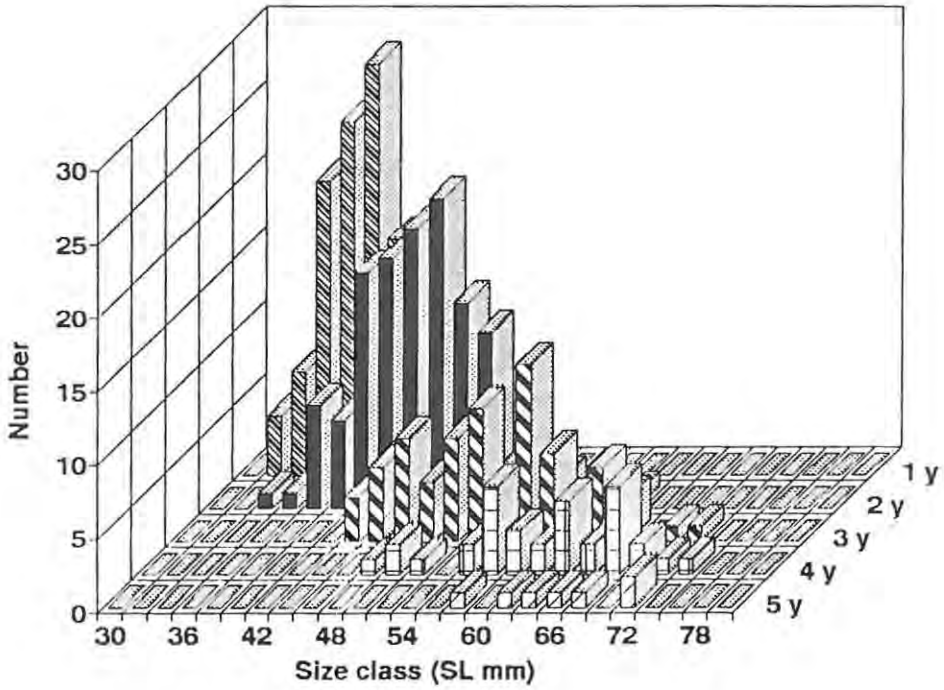


Figure 62. Age-length graph for *P. afer* males collected in the Wit River from August 1987 to April 1989 (n=350).

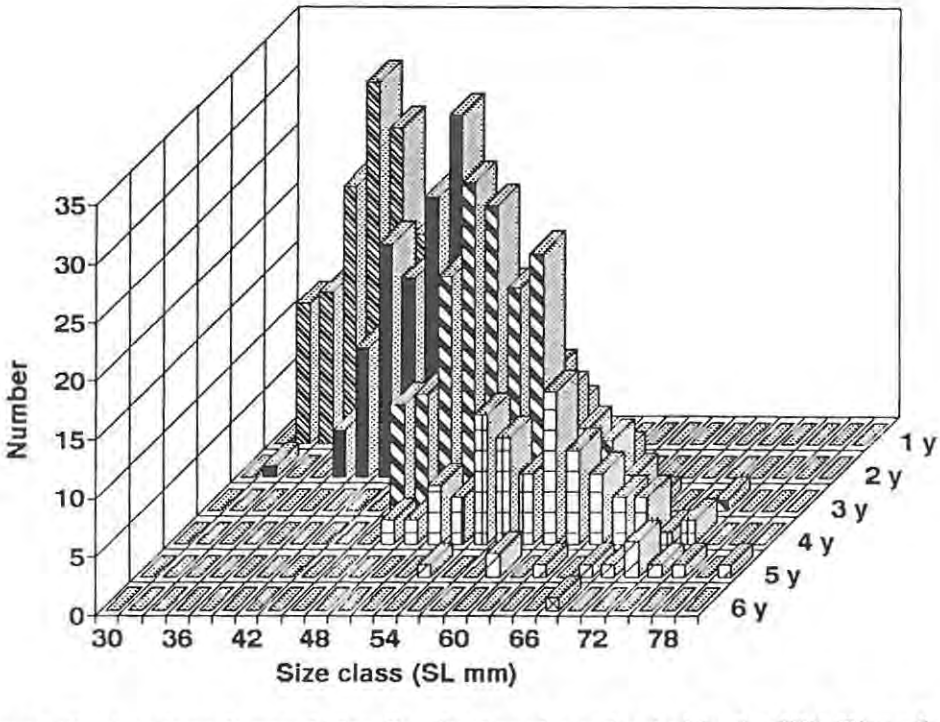


Figure 63. Age-length graph for *P. afer* females collected in the Wit River from August 1987 to April 1989 (n=575)

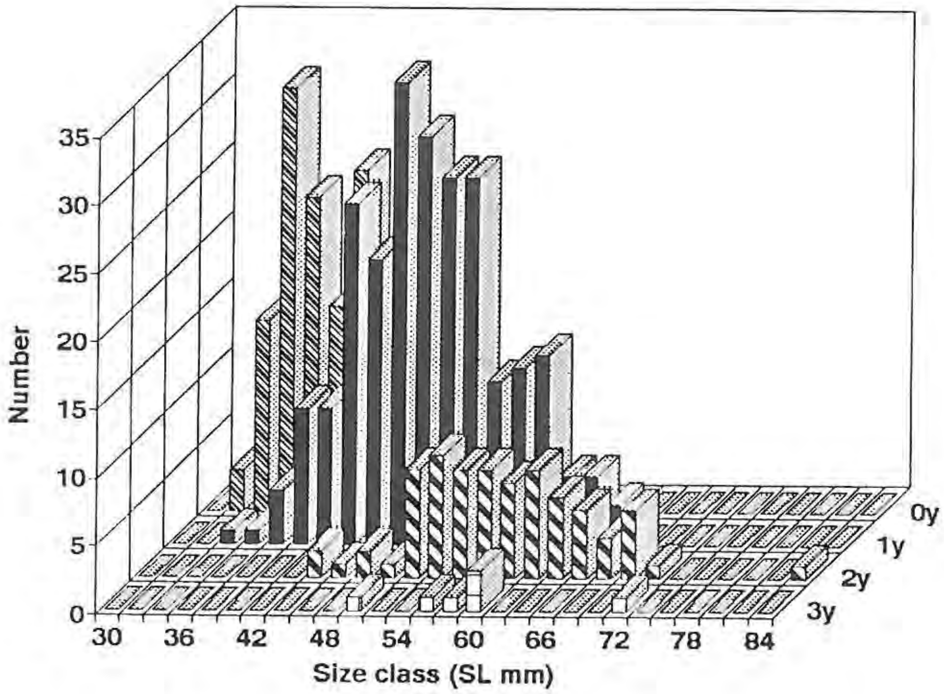


Figure 64. Age-length graph for *P. asper* males collected in the Groot River from August 1987 to April 1989 (n=491).

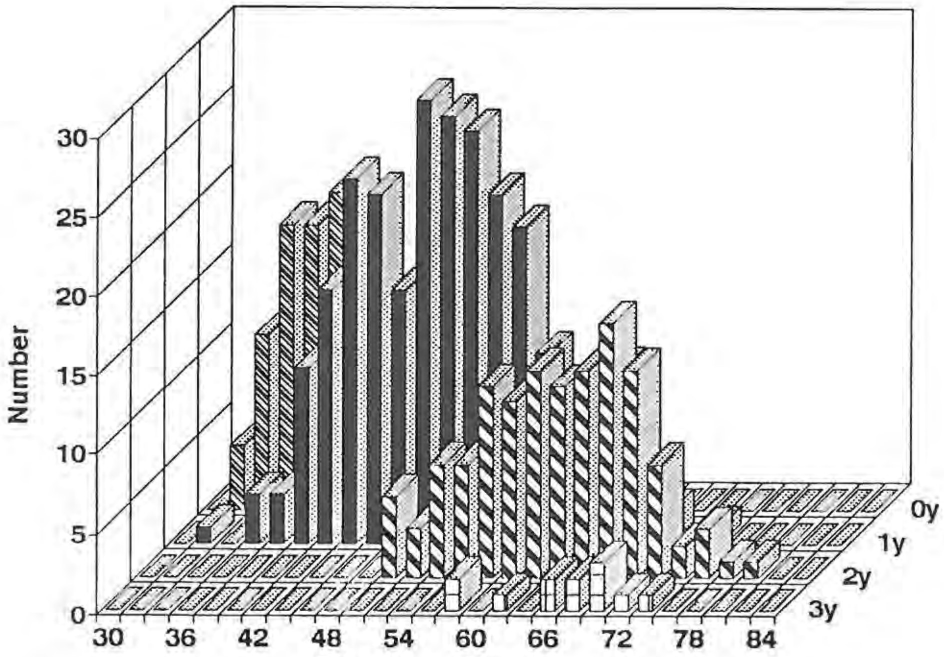


Figure 65. Age-length graph for *P. asper* females collected in the Groot River from August 1987 to April 1989 (n=528)

which helped to interpret the differences in the population dynamics of these two species. There are upper limits of growth which are known to be species specific (Weatherley & Rogers, 1978). *P. afer* and *P. asper* both attained a similar maximum size (Figure 49) and a similar size at maturity (Figures 39 & 40). The main differences were in their growth rates and age at maturity. These differences may be under genetic control and/or due to phenotypic variability of the redfin minnows. *P. asper* attained a higher growth rate than *P. afer*. External factors such as higher temperatures during summer, more food and possibly a slower average water flow rate in the *P. asper* environment may have allowed for a higher growth rate but the genetically determined programme(s) controlled size at maturity and maximum size.

Table 53. Multiplicative regression statistics and age and size at first exogenous feeding of developmental series of *P. afer* and *P. asper*.

	P. afer		***P. asper***	
	Batch 1	**Batch 2**	**Batch 1**	**Batch 2**
	n = 126	n = 666	n = 201	n = 933
a	4,2699	3,9617	2,8664	3,0621
b	0,3004	0,3205	0,418	0,3553
r^2	93,2	93,2	97,03	93,17
Size at first feeding (mm NL)	8,6 n=2 SD=0,64	8,5 n=10 SD=0,35	6,75 n=3 SD=0,17	6,55 n=8 SD=0,21
Age at first feeding (d)	9,04	10,22	7,49	7,25
Size at 35 days (mm SL)	12,4	12,4	12,7	10,8

The relationship between growth rate and age structure may be mainly a phenotypic adaptation to the environment rather than a genetic one (Mills, 1988). Two populations of the minnow *Phoxinus phoxinus*, one in Finland and one in southern England exhibit the inverse relationship between growth and longevity that occurs in many fish species (Mills, 1987, 1988). In southern England many *P. phoxinus* mature after one year and few were found to survive until their third "birthday" (Mills, 1987), which is similar to the *P. asper* population in the Groot River. In Finnish Lapland it was evident that *P. phoxinus* maturity was strongly size-dependent and was therefore delayed until fish reached 5-7 years of age and had a maximum age of 13 years (Mills, 1988). *P. afer* in the Wit River had a delayed maturity when compared to the *P. asper* population, but by only one year. *P. afer* lived to a maximum of six years of age compared to three years for *P. asper*. Therefore the inverse relationship can be seen in the two redfin minnows of the present study. *P. afer* grows more slowly, attains maturity later and has enhanced longevity as compared to the faster growth rate, earlier maturation and shorter adult life of *P. asper*. Limited food supply in the oligotrophic Wit River may have lead to delayed maturity and longevity of *P. afer*, whereas the more abundant food supply and warmer waters in the Groot River increased growth rate at the expense of longevity of *P. asper*. It is suggested that the growth rate adaptations to two contrasting environments in these two redfin minnows are an example of phenotypic variability.

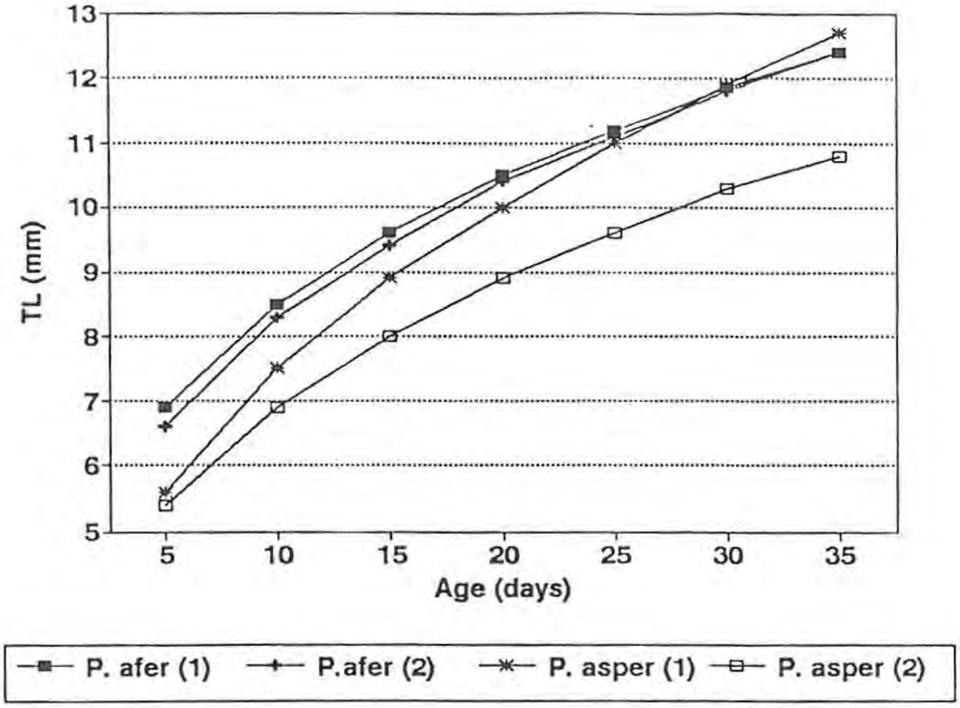


Figure 66. Size and age for four developmental series of *P. afer* (Batch 1 n=126; Batch 2 n=666) and *P asper* (Batch 1 n=201; Batch 2 n=933).

A number of studies have shown that a phylogenetic perspective could provide insight into present day ecological associations (eg Wanntorp *et al.*, 1990; Gotelli & Pyron, 1991). The Mantel test (Mantel, 1967) used on the North American minnows assessed the hypothesis that there was no relationship between phylogenetic distance and life-history differences between species (Gotelli & Pyron, 1991). They found a positive correlation between phylogeny and life-history differences which suggested a phylogenetic association. The more closely related two species are then the more similar their life-history attributes should be from inheritance (Moore

& Gotelli, 1990). Life-history attributes were shown to have an association with phylogeny or with latitude but not with both (Gotelli & Pyron, 1991). Species-typical sizes such as those at maturity are evidence of a genetic component determining the patterns of growth. Maximum potential growth, ultimate size and longevity of each species are under genetic control (Beverton, 1987). The phylogenetic association (genetic input) of *P. afer* and *P. asper* probably controlled the final size and the size at maturity whereas the growth rate was under environmental control.

A study of the life-history attributes of 21 North American minnows with well resolved phylogenies showed that total female body length at maturity and maximum total female body length were more similar for closely related species pairs than for more distantly related species pairs (Gotelli & Pyron, 1991). This finding held true for the *P. afer* /*P. asper* pair and indicated genetic involvement in the determination of total body size and length at maturity. The final size of *P. asper* may not have represented an adaptation to large riverine pools of the Groot River (current environment) but may be inherited from the ancestral taxon of both *P. afer* and *P. asper*. That was what Brooks (1985) termed "historical legacy" and the final size of *P. asper* may not represent a "solution" to a particular current ecological problem. In the *P. afer* and *P. asper* populations, "heritability gives an estimate of the contribution of genetic factors, relative to environmental factors, to the variation shown by a phenotypic trait (such as growth) in a population at a given time" (Wootton, 1990: 145).

Gotelli & Pyron (1991) also found that age at maturity, maximum age, length of spawning season and ova diameter were not correlated with phylogeny. In the present study *P. afer* matured at 2 and *P. asper* at 1 year, the maximum age of *P. afer* was 6 compared to 3 years for *P. asper*, the length of the spawning season for *P. afer* was 4 compared to 6 months for *P. asper* and *P. afer* had larger ova than *P. asper*. These four life-history attributes can probably be interpreted as the function of the current environment in which *P. afer* and *P. asper* live. These variations in life-history attributes are an expression of the phenotypic variability of the sister species whereas the attributes of maximum size and size at maturity appear to reflect a strong historical component (Table 54).

In some cyprinid species there can be a marked intraspecific variability in growth rate between different life-history types and also between different sexes (Mann, 1991). This variation may be a result of genetic adaptations to local environments. Variations may also result from widespread phenotypic variability and evidence for this has been shown by conducting fish transfers (Mann & Steinmetz, 1985). This phenotypic variability allows for a rapid response to changes in environmental conditions in individual growth rates and development to maturity. The two distinct environments of the Gamtoos River system, the mountain stream and the Karoo stream, would permit the expression of such phenotypic variability. In the dynamic Groot River, phenotypic variability would be a necessity. The differences in response to years of drought followed by good flows would be advantageous. A longer term study than the present would determine if there were any differences in the growth rates during droughts and high flow periods in the Groot River population of *P. asper*.

Table 54. Some of the life-history attributes of *P. afer* and *P. asper*, either as a result of genotypic similarities or phenotypic variability.

Attribute	*P. afer*	*P. asper*	Genetic	Phenotypic
Size at maturity (SL mm)	♂♂39-40 ♀♀39-40	♂♂41-42 ♀♀43	X	
Age at maturity (y)	2	1		X
Maximum size (mm SL)	81	83	X	
Maximum age (y)	6	3		X
Length of spawning season (months)	4	6-7		X
Egg size (mm)	1,5	1,1		X

Growth and potential offspring production

Natural selection leads to the evolution of patterns of growth that will tend to maximize lifetime production of offspring (Wootton, 1990). Faster growth in the highly dynamic environment of the Groot River ensured that *P. asper* quickly reached maturity at a size where females would be able to produce as many offspring as possible in good years. In comparison the relatively more stable, perennial flow and oligotrophic conditions in the Wit River enabled *P. afer* individuals with a slower growth rate, relative to *P. asper*, to maximize their lifetime production of offspring by increased longevity.

Exogenous factors affecting growth rates of P. afer *and* P. asper

A combination of exogenous factors, imposed by the environment, and endogenous factors related to genotype and physiological condition of the fish, determine the growth pattern of that fish (Wootton, 1990). Exogenous factors such as food quality and quantity, current speed, pH, salinity, oxygen and temperature may act together or alone to influence the growth rate of a fish. The environment inhabited by *P. afer* differs in the majority of these exogenous factors when compared to the environment inhabited by *P. asper* (Chapter 2).

Exogenous (environmental) factors:

Food quality and quantity

Density-dependent growth in the Cyprinidae is often associated with food availability, although growth inhibitors are sometimes produced under crowded conditions (Mann, 1991). These growth inhibitors might have been produced by the *P. asper* population in the Groot River during severe droughts when the river was reduced to a series of disconnected pools and fish were very crowded.

The extent to which natural populations of fish experience nutritionally sub-optimal diets and the consequences for survival, growth and reproduction has received very little attention (Wootton, 1990). West African *Barbus liberiensis* which fed on an animal diet had a higher growth rate and growth efficiency (Payne, 1979). *P. afer* may have higher quality food (aquatic invertebrates) but that was a scarce resource. *P. asper* had lower quality food but it was more abundant. Further in depth dietary studies of *P. afer* and *P. asper* which will be published elsewhere, will shed some light on this aspect of growth.

There is a relationship between growth of a fish and size spectrum of food (Wootton, 1990). The limited range of prey sizes that a fish will take increases with growth. The profitability of prey of a given size depends on its total energy content in relation to the time and energy required for the fish to detect, pursue and handle a prey of that size. The longer intestinal tract of *P. asper* (relative to *P. afer*) would enable more food to be processed at any one time and allowed food of lower quality to be assimilated. The growth of the fish is affected by the quantity, quality and size of food items. Before maturity the growth of cyprinids is most closely related to food supply and during this time growth is subject to the greatest fluctuations (Nikolsky, 1963). More abundant food in the Groot River as compared to the oligotrophic Wit River may have led to different growth rates reflected in differences in age at maturity and lifespan. After attaining maturity food is allocated to ripening gonads and linear growth. Female *P. asper* had gonads weighing up to 28% of total body weight (Chapter 4) which meant that a considerable amount of energy was directed away from somatic growth after maturity in this species.

Impoverished feeding conditions usually lead to a reduction in growth of the entire population and an increase in variability of growth indicated by the existence of individuals of very different sizes in the same age group. This important adaptation makes available a wider range of usable foods. The somatic condition of female *P. afer* were seen to be significantly different between years whereas there was no significant difference in the somatic or total condition of *P. asper* between years (Chapter 4). These differences in condition in *P. afer* females were interpreted as a result of the oligotrophic mountain stream possibly leading to more variability in growth.

Temperature

Temperature strongly influences growth of fish by altering rates of digestion and assimilation (Backiel 1971, 1979; Webb, 1978) and by affecting swimming activity and therefore frequency of prey encounters (Jezierska, 1979). For both redfin minnows most growth occurred during the highest water temperatures and growth slowed down when water temperatures were low. There was a difference in the environmental temperatures of the Wit and Groot Rivers. The Wit River had a forest canopy shading it whereas the Groot River was open (Figure 3).

Both *P. afer* and *P. asper* were found to be multiple spawners (Chapter 4). Fish spawned at different times of the year would be subjected to different ranges in climatic and environmental conditions. These different cohorts may have had different growth patterns which could be the subject for a further investigation.

Oxygen

The *P. asper* population suffered from oxygen depletion in the crowded pool refuge areas during severe droughts. High densities of fish may result in decreased food consumption rates because of the associated low oxygen levels.

Salinity

The energy costs of osmotic and ionic regulation would mean that less energy could be allocated for growth in fish that experienced fluctuating salinities (Brett, 1979) such as *P. asper*. The desert pupfish, *Cyprinodon macularius*, showed the highest growth rates at 30 °C and a salinity of 40 ppt, but the highest growth efficiencies were at 20 °C and 15 ppt (Kinne, 1960). *P. asper* live in a relatively high and fluctuating salinity environment and therefore there would be certain energy costs that the *P. afer* population would not be subjected to in the Cape Fold Mountain Belt stream.

pH

Slow growth of trout in acidic waters was caused by the direct effect of water quality on growth, temperature regime, a shortage of suitable food in such waters and high population densities which was a result of the presence of good spawning habitats (Campbell, 1971). Similarly the relatively low pH of the Wit River may be one of the causes of a shortage of suitable food items. But there were also high population densities and clean spawning habitats available as compared to the silt-laden Groot River where the pH was alkaline and there was abundant food but a lack of clean spawning sites.

Current speed

Current speed may act as a masking factor for growth, as energy used for maintaining position would not be available for growth (Wootton , 1990). *P. afer* often fed in the riffle - pool interface and had to use energy to maintain position. In contrast *P. asper* often fed in large, stagnant riverine pools in the Groot River. It would therefore be reasonable to assume that *P. afer* used more energy for maintaining position while feeding than *P. asper*. However, this may be too simplistic as major floods occur in the Groot River which may interfere with the growth of some cohorts of *P. asper*, although the floods are usually of a short duration.

Summary of exogenous limiting factors for P. afer *and* P. asper

The possible limiting exogenous factors which influence *P. afer* growth are: food quantity; current speed; pH and temperature. In contrast the possible limiting exogenous factors for *P. asper* growth are: food quality; fluctuating salinity and low oxygen levels in pools during droughts and lack of habitat (Table 55).

Table 55. Comparative exogenous factors which may influence the growth rates of *P. afer* in the Wit River and *P. asper* in the Groot River.

	Food quality and quantity	**Current speed**	**pH**	**Salinity**	**Oxygen**	**Temperature**
P. afer	+ ve - ve	- ve (high)	- ve (<7)	+ ve (low)	+ ve	- ve (low)
P. asper	- ve + ve	+ ve (low)	+ ve (>7)	- ve (high)	- ve	+ ve (high)

Age and growth studies of African minnows

Cambray (1982) listed the life-history attributes of 39 small African *Barbus* species. Age and length at maturity and maximum length and age were known for only four species, *B. anoplus*, *B. liberiensis*, *B. paludinosus* and *B. trevelyani*. Table 56 updates this table and adds several more species from recent studies.

Several studies have found that six years is the maximum lifespan for an African minnow (Table 56). In the Tyume River in the eastern Cape *B. trevelyani* grew rapidly in their first year to a maximum of 40-50 mm FL. The growth rate then decreased and remained approximately linear until the sixth year (80-100 mm FL). Female *B. trevelyani* grew faster than males and reached a length of 103 mm FL. In the population over 70 mm FL 63% were females. All males were sexually mature after their first year whereas females reached sexual maturity in their third year (Gaigher, 1975). This delayed age at maturity occurred in *P. afer* males and females and was unlike any of the other small *Barbus* species (Table 56).

Table 56. Review of the length and age at first sexual maturity and maximum length of various small African *Barbus* and *Pseudobarbus* species, (n.g. = not given).

Species	Locality	Length at maturity (mm FL)		Age at maturity (y)		Maximum		Reference
		♂♂	♀♀	♂♂	♀♀	Length (FL mm)	Age	
B. afrohamiltoni	Zimbabwe	n.g.	n.g.	n.g.	n.g.	115TL	n.g.	Bell-Cross (1976)
B. afrohamiltoni	Natal	<76	<76	n.g.	n.g.	127	n.g.	Crass (1964)
B. afrohamiltoni	-	n.g.	n.g.	n.g.	n.g.	127	n.g.	Jubb (1967)
B. afrovernayi	Zimbabwe	n.g.	n.g.	n.g.	n.g.	45	n.g.	Bell-Cross (1976)
B. afrovernayi	-	n.g.	n.g.	n.g.	n.g.	57	n.g.	Jubb (1967)
B. annectens	Zimbabwe	n.g.	n.g.	n.g.	n.g.	76TL	n.g.	Bell-Cross (1976)
B. annectens	-	n.g.	n.g.	n.g.	n.g.	76	n.g.	Jubb (1967)
B. annectens	Kruger Park	n.g.	n.g.	n.g.	n.g.	75	n.g.	Pienaar (1978)
B. anoplus	P.K. le Roux Lake	40	40	1	1	60♂ 73♀	4	Cambray (1982)
B. anoplus	Grassridge Lake	n.g.	n.g.	n.g.	n.g.	101♀	n.g.	Cambray *et al.* (1977)
B. anoplus	Natal	38	38	n.g.	n.g.	89	n.g.	Crass (1964)
B. anoplus	-	n.g.	n.g.	n.g.	n.g.	89	n.g.	Jubb (1967)
B. apleurogramma	Lake Victoria	32SL	32SL	1	1	n.g.	n.g.	Welcomme (1969)
B. argenteus	Natal	70	70	n.g.	n.g.	197	n.g.	Crass (1964)

Species	Locality	Length at maturity (mm FL)		Age at maturity (y)		Maximum		Reference
		♂♂	♀♀	♂♂	♀♀	Length (FL mm)	Age	
B. argenteus	-	n.g.	n.g.	n.g.	n.g.	197	n.g.	Jubb (1967)
B. argenteus	Kruger Park	70	70	n.g.	n.g.	197	n.g.	Pienaar (1978)
B. aurantiacus	Zambezi	n.g.	n.g.	n.g.	n.g.	76	n.g.	Jubb (1967)
B. barnardi	Zimbabwe	n.g.	n.g.	n.g.	n.g.	76TL	n.g.	Bell-Cross (1976)
B. bellcrossi	Zimbabwe	n.g.	n.g.	n.g.	n.g.	89	n.g.	Jubb (1967)
B. brevipinnis	Incomati R.	n.g.	n.g.	n.g.	n.g.	51	n.g.	Jubb (1967)
B. calidus	Olifants R.	n.g.	n.g.	n.g.	n.g.	95	n.g.	Jubb (1967)
B. eutaenia	Zimbabwe	n.g.	n.g.	n.g.	n.g.	140TL	n.g.	Bell-Cross (1976)
B. eutaenia	-	n.g.	n.g.	n.g.	n.g.	127	n.g.	Jubb (1967)
B. eutaenia	Kruger Park	n.g.	n.g.	n.g.	n.g.	100	n.g.	Pienaar (1978)
B. fasciolatus	Zimbabwe	n.g.	n.g.	n.g.	n.g.	60TL	n.g.	Bell-Cross (1976)
B. fasciolatus	-	n.g.	n.g.	n.g.	n.g.	57	n.g.	Jubb (1967)
B. gurneyi	Tugela	n.g.	n.g.	n.g.	n.g.	102	n.g.	Jubb (1967)
B. gurneyi	Natal	<51	<51	n.g.	n.g.	102	n.g.	Jubb (1967)
B. haasianus	Zambezi	n.g.	n.g.	n.g.	n.g.	32	n.g.	Jubb (1967)
B. kerstenii	Lake Victoria (stream)	34SL	34SL	n.g.	n.g.	68♂SL 75♀SL	n.g.	Welcomme (1969)
B. liberiensis	Sierra Leone (stream)	50-60 TL	50-60 TL	1	1	119TL	4	Payne (1975; 1976)
B. lineomaculatus	Zimbabwe	n.g.	n.g.	n.g.	n.g.	76TL	n.g.	Bell-Cross (1976)

Species	Locality	Length at maturity (mm FL)		Age at maturity (y)		Maximum		Reference
		♂♂	♀♀	♂♂	♀♀	Length (FL mm)	Age	
B. lineomaculatus	Natal	n.g.	n.g.	n.g.	n.g.	83	n.g.	Crass (1964)
B. lineomaculatus	-	n.g.	n.g.	n.g.	n.g.	76,2	n.g.	Jubb (1967)
B. manicensis	Zimbabwe	n.g.	n.g.	n.g.	n.g.	153TL	n.g.	Bell-Cross (1976)
B. manicensis	Zimbabwe	n.g.	n.g.	n.g.	n.g.	152	n.g.	Jubb (1967)
B. motebensis	Limpopo	n.g.	n.g.	n.g.	n.g.	97	n.g.	Jubb (1967)
B. multilineatus	Zimbabwe	n.g.	n.g.	n.g.	n.g.	45TL	n.g.	Bell-Cross (1976)
B. multilineatus	-	n.g.	n.g.	n.g.	n.g.	64	n.g.	Jubb (1967)
B. neefi	Limpopo	n.g.	n.g.	n.g.	n.g.	51	n.g.	Jubb (1967)
B. pallidus	Natal	n.g.	n.g.	n.g.	n.g.	51	n.g.	Crass (1964)
B. pallidus	Cape	44	44	n.g.	n.g.	70	n.g.	Jubb (1967)
B. paludinosus	Zimbabwe	n.g.	n.g.	n.g.	n.g.	130TL	n.g.	Bell-Cross (1976)
B. paludinosus	Natal	51	51	n.g.	n.g.	102	n.g.	Crass (1964)
B. paludinosus	Lake Chilwa	50TL	50TL	1	1	120TL	3	Furse *et al.* (1979)
B. paludinosus	-	n.g.	n.g.	n.g.	n.g.	127	n.g.	Jubb (1967)
B. paludinosus	Lake Chilwa	n.g.	48	n.g.	n.g.	n.g.	n.g.	Kirk (1972)
B. paludinosus	Kruger Park	50	50	n.g.	n.g.	n.g.	n.g.	Pienaar (1978)
B. paludinosus	Lake Victoria	48SL	54SL	n.g.	n.g.	n.g.	n.g.	Welcomme (1969)
B. poechii	Zimbabwe	n.g.	n.g.	n.g.	n.g.	110TL	n.g.	Bell-Cross (1976)
B. poechii	-	n.g.	n.g.	n.g.	n.g.	152	n.g.	Jubb (1967)

Species	Locality	Length at maturity (mm FL) ♂♂	♀♀	Age at maturity (y) ♂♂	♀♀	Maximum Length (FL mm)	Age	Reference
B. puellus	Zambezi	n.g.	n.g.	n.g.	n.g.	38	n.g.	Jubb (1967)
B. radiatus	Zimbabwe	n.g.	n.g.	n.g.	n.g.	80TL	n.g.	Bell-Cross (1976)
B. radiatus	-	n.g.	n.g.	n.g.	n.g.	102	n.g.	Jubb (1967)
B. radiatus	Kruger Park	n.g.	n.g.	n.g.	n.g.	95	n.g.	Pienaar (1978)
B. tangandensis	Zimbabwe	n.g.	n.g.	n.g.	n.g.	65TL	n.g.	Bell-Cross (1976)
B. tangandensis	-	n.g.	n.g.	n.g.	n.g.	76	n.g.	Jubb (1967)
B. thamalakanensis	Zimbabwe	n.g.	n.g.	n.g.	n.g.	50TL	n.g.	Bell-Cross (1976)
B. toppini	Natal	n.g.	n.g.	n.g.	n.g.	38	n.g.	Crass (1964)
B. toppini	-	n.g.	n.g.	n.g.	n.g.	38	n.g.	Jubb (1967)
B. toppini	Kruger Park	n.g.	n.g.	n.g.	n.g.	40	n.g.	Pienaar (1978)
B. treurensis	Blyde R.	n.g.	n.g.	n.g.	n.g.	98♂ 108♀	6	Kleynhans (1987)
B. trevelyani	Tyume R.	40-50	65-75	1	3	103♀	6	Gaigher (1975)
B. trevelyani	-	n.g.	n.g.	n.g.	n.g.	89	n.g.	Jubb (1967)
B. trimaculatus	Zimbabwe	n.g.	n.g.	n.g.	n.g.	140TL	n.g.	Bell-Cross (1976)
B. trimaculatus	Natal	<76	<76	n.g.	n.g.	152	n.g.	Crass (1964)
B. trimaculatus	-	n.g.	n.g.	n.g.	n.g.	152	n.g.	Jubb (1967)
B. trimaculatus	Kruger Park	75	75	n.g.	n.g.	155	n.g.	Pienaar (1978)
B. unitaeniatus	Kruger Park	n.g.	n.g.	n.g.	n.g.	105TL	n.g.	Bell-Cross (1976)

Species	Locality	Length at maturity (mm FL)		Age at maturity (y)		Maximum		Reference
		♂♂	♀♀	♂♂	♀♀	Length (FL mm)	Age	
B. unitaeniatus	-	n.g.	n.g.	n.g.	n.g.	140	n.g.	Jubb (1967)
B. viviparus	Zimbabwe	n.g.	n.g.	n.g.	n.g.	55TL	n.g.	Bell-Cross (1976)
B. viviparus	Natal	38	38	n.g.	n.g.	76	n.g.	Crass (1964)
B. viviparus	-	n.g.	n.g.	n.g.	n.g.	64	n.g.	Jubb (1967)
B. viviparus	Kruger Park	n.g.	n.g.	n.g.	n.g.	65	n.g.	Pienaar (1978)
P. afer	Cape	n.g.	n.g.	n.g.	n.g.	102	n.g.	Jubb (1967)
P. afer	Wit R.	39-40SL	39-40SL	2	2	78♂SL 81♀SL	6	This study
P. asper	Cape	n.g.	n.g.	n.g.	n.g.	117	n.g.	Jubb (1967)
P. asper	Groot R.	41-42SL	43SL	1	1	83♂SL 80♀SL	3	This study
P. burchelli	Breede R.	n.g.	n.g.	n.g.	n.g.	113♂ 123♀		Cambray & Stuart (1985)
P. burchelli	Breede R.	n.g.	n.g.	n.g.	n.g.	90SL	4	Esterhuizen (1978)
P. burchelli	Cape	n.g.	n.g.	n.g.	n.g.	95	n.g.	Jubb (1967)
P. burgi	Cape	64	64	n.g.	n.g.	121	n.g.	Jubb (1967)
P. phlegethon	Olifants R.	n.g.	n.g.	n.g.	n.g.	70	n.g.	Jubb (1967)

Male and female *B. paludinosus* in Lake Chilwa reached maturity in their first year at 50 mm TL although females grew faster than males and matured at a slightly smaller size (Kirk, 1972; Furse, 1979). In Sierra Leone *B. liberiensis* attained maturity in the first year at 50-60 mm TL (Payne, 1975). *P. burchelli* grew rapidly in the first year to 40-50 mm FL whereafter growth slowed down. The oldest specimen examined was a 6$^+$ female of 120 mm FL and the oldest male was 113 mm FL (4$^+$) (Cambray & Stuart, 1985). *B. treurensis* in the Blyde River, Limpopo River system, attained a mean length of approximately 36 % (37 mm FL) of their maximum growth (104 mm FL) during their first year (Kleynhans, 1987). The oldest specimen was 6 years and 104 mm FL.

P. asper growth rate during the first year was similar to other African minnows for which there are data (Table 57). In contrast *P. afer* had a slower growth rate during the first year.

Table 57. Approximate percentage of total growth obtained in the first year of life by four small *Barbus* species and two *Pseudobarbus* species.

Species	Approximate length at end of first year		Approximate percentage of total growth attained in the first year		Reference
	♂♂	♀♀	♂♂	♀♀	
B. anoplus	39	41 mm FL	65*	59*	Cambray & Bruton (1985)
B. liberiensis	69	69 mm TL	62	62	Payne (1975)
B. paludinosus	50	50 mm TL	67	42	Furse (1979)
B. trevelyani	±45	±45 mm FL	46	48	Gaigher (1975)
P. afer	22-32	22-32 mm SL	28-41	27-40	This study
P. asper	38-50	38-50 mm SL	46-60	48-63	This study
P. burchelli	40-50	40-50 mm FL	35-44	33-41	Cambray & Stuart (1985)

* Percentage of first years' growth calculated from largest specimen collected.

In *Barbus* minnow species which have been studied males usually have a shorter life-span (Table 56). The early demise of male *B. liberiensis*, which inhabit the headwater streams where resources are scarce conserved the food supply (Payne, 1975). It was unlikely that the males died in order to conserve food for the females. The cause of their earlier demise may rather have been the result of very active spawning sessions. In Lake Chilwa there is a decrease in male *B. paludinosus* after 45 mm TL which was considered a "significant factor in maintaining a high reproductive effort" but Furse (1979: 188) does not elaborate further.

In teleosts fecundity is usually a positive allometric function of body size, and higher fecundity values can be obtained by growth of females (Blaxter, 1969). That is, larger (older) females are physically capable of carrying more eggs than smaller females (Chapter 4). Several examples are given in Table 58 for African minnows. The benefit of larger/older *P. afer* females was quite clear with a 12 fold increase in yolked eggs but the benefits were not as evident in the *P. asper* females (6,4 fold increase) which may have been related to their lower relative longevity and smaller (relative to *P. afer*) investment per offspring. A small female *P. asper* at an earlier age can carry more smaller eggs than a *P. afer* female of the same size which has larger eggs.

Table 58. Relationship of female size increase to fecundity increase for several African minnows.

Species	Size	Fecundity	Increase in length	Increase in fecundity
Barbus anoplus[1]	40 mm FL	220		
	63 mm FL	3552	X1,6 (23 mm)	X15
Pseudobarbus afer[2]	41 mm SL	233		
	73 mm SL	2784	X1,8 (32 mm)	X12
Pseudobarbus asper[2]	42 mm SL	511		
	70 mm SL	3263	X1,6 (28 mm)	X6,4
Pseudobarbus burchelli[3]	71 mm FL	7433		
	123 mm FL	10678	X1,7 (52 mm)	X14,4

Sources: 1 - Cambray (1982); 2 - This study; 3 - Cambray & Stuart (1985).

The size of reproductive males in *B. anoplus* in the Orange River was not very important and had limited influence on reproductive success (Cambray & Bruton, 1985). In comparison, male *P. afer* have large nuptial tubercles and dark red fins during the breeding season. They inhabit clear mountain streams where large size may influence their reproductive success. *P. asper* males also have relatively large tubercles, as compared to *B. anoplus*, but the tubercles are smaller and less numerous than *P. afer* tubercles and the red colouration of the male is not as enhanced as in *P. afer* (Chapter 7). Cambray & Stuart (1985) documented the seasonality of large tubercle sizes for *P. burchelli* males and these fish also get dark red fins when they are sexually active. Large size may influence their reproductive success. It is suggested that in the *Pseudobarbus* species large males may have higher reproductive success than smaller males especially in clear water habitats.

In species which have an extended spawning season, such as *B. anoplus* (Cambray & Bruton, 1984), it was difficult to identify the different cohorts by size. An understanding of the multiple spawning strategy of *B. anoplus* was important to interpreting the age and growth adaptations of this species (Cambray & Bruton, 1985). The fish in the first and second major spawnings were analyzed separately. The second spawn fish lived longer because their first breeding was delayed compared to the first spawn fish. Cambray (1982) suggested that this was an adaptation to the unstable Orange River environment. There was a rapid turnover of the *B. anoplus* population and the standing populations were mainly dependent on 1^+ males and 1^+ and 2^+ females. *B. anoplus* co-occurred with *P. asper* in the Groot River and appeared to have a similar life-history style with regard to growth.

Early life-history

The early stages of fish are characterised by a high growth rate. Within season growth changes in Cyprinidae are most noticeable among 0^+ fishes, especially during the first two or three months of life (Mann, 1991). The period of highest linear growth occurs before the onset of maturity which is an important adaptation for the individual as small fish are more available to predators than large fish. *P. afer* have larger young than *P. asper* which would be interpreted by Stearns (1976) as being associated with density-dependent selection accompanied by a fall in reproductive effort. In the Wit River population of *P. afer* there was selection for a larger size at hatching or first feeding as compared to *P. asper* in the Groot River. That could be expected as *P. asper* have smaller young and a higher reproductive effort (see Chapter 4).

In laboratory studies two developmental series of *P. afer* and one of *P. asper* attained similar sizes after 35 days under similar temperature regimes (Figure 66). In the field, with differences in temperature and food availability, *P. asper* early life-history stages grew considerably faster than *P. afer*, as was clearly seen in the length frequency data discussed above. In the regression analysis of the developmental series the first batch of *P. asper* showed a higher growth rate than either of the two *P. afer* developmental sequences or the other *P. asper* series (Figure 66).

Growth rates and year class strengths of P. afer *and* P. asper

Many cyprinid populations are dominated by a few year classes (Mann, 1991). Climatic conditions may be the cause of the production of dominant year classes as they are often synchronous in different species and habitats (Mann, 1991). In the Groot River years of high rainfall and flows would produce strong year classes of *P. asper*. Years of good flow combined with warm summers would result in exceptionally strong year classes. The flow would allow enough space and increases the number of spawning opportunities and the warmer temperatures would result in increased food production and therefore growth. This can only be assumed and needs to be tested.

Growth rate, sexual dimorphism and age at first maturity of P. afer *and* P. asper

In teleost fishes, males are generally smaller than females and species in the family Cyprinidae follow this general rule (Mann, 1991). In several African minnows the males have been recorded as smaller than the females (Table 59). Males of *P. afer* and *P. asper* attained a length comparable to that of females, however more females of both species attained a larger size than did males (Figure 67). Female *P. burchelli* attained a larger size and were also more abundant than males in the larger length classes (Cambray & Stuart, 1985).

Natural selection favours females with fast growth rates because larger fish are more fecund than smaller fish of the same species (egg number/fish length) (Mann, 1991). In a review of 32 populations of cyprinids, females were found to grow faster than males in 19 whereas males grew faster in only four populations (Mann, 1991). In the first category females usually became sexually mature 1 year later than males, therefore they had one more year of only somatic growth. Few African minnows have been recorded where either sex delayed maturity although male *B. trevelyani* matured at one year and females delayed maturity to three years (Gaigher, 1975). *P. afer* males and females matured after two years, and male and female *P. asper* both matured within their first year.

Table 59. Recorded maximum lengths for male and female African minnows of the genera *Barbus* and *Pseudobarbus*.

	♂♂	♀♀	Reference
Barbus anoplus	60 mm FL	73 mm FL	Cambray & Bruton (1985)
B. kerstenii	68 mm SL	75 mm SL	Welcomme (1969)
B. paludinosus	48 mm SL	54 mm SL	Welcomme (1969)
B. trevelyani	50 mm FL	75 mm FL	Gaigher (1975)
Pseudobarbus afer	78 mm SL	81 mm SL	This study
P. asper	83 mm SL	80 mm SL	This study
P. burchelli	113 mm FL	123 mm FL	Cambray & Stuart (1985)

In some species fast growing fish of a particular cohort attain maturity at an earlier age than those that grow slowly (Mann, 1991). Many of the widely-distributed freshwater fish species in the Northern Hemisphere exhibit an increase in longevity accompanied by a decline in growth rates with increased age of maturity at various localities (Mills, 1988). The maximum age of walleye (*Stizostedion vitreum*) populations ranged from 3 to 19 years (Colby & Nepszy, 1981) and from 3 to 10 years between populations of the sculpin, *Cottus gobio*, (Mann *et al.*, 1984). It has been shown that in other small-sized species there is a switch to later maturity and from multiple to single clutches in unproductive northern habitats (Mann *et al.*, 1984). For example the loach, *Noemacheilus barbatulus* (L.) switches from large clutches of small eggs to a single small clutch of much larger eggs (Mills & Eloranda, 1985). This trend is present in *P. asper* (large clutches of small eggs) and *P. afer* (small clutches of larger eggs).

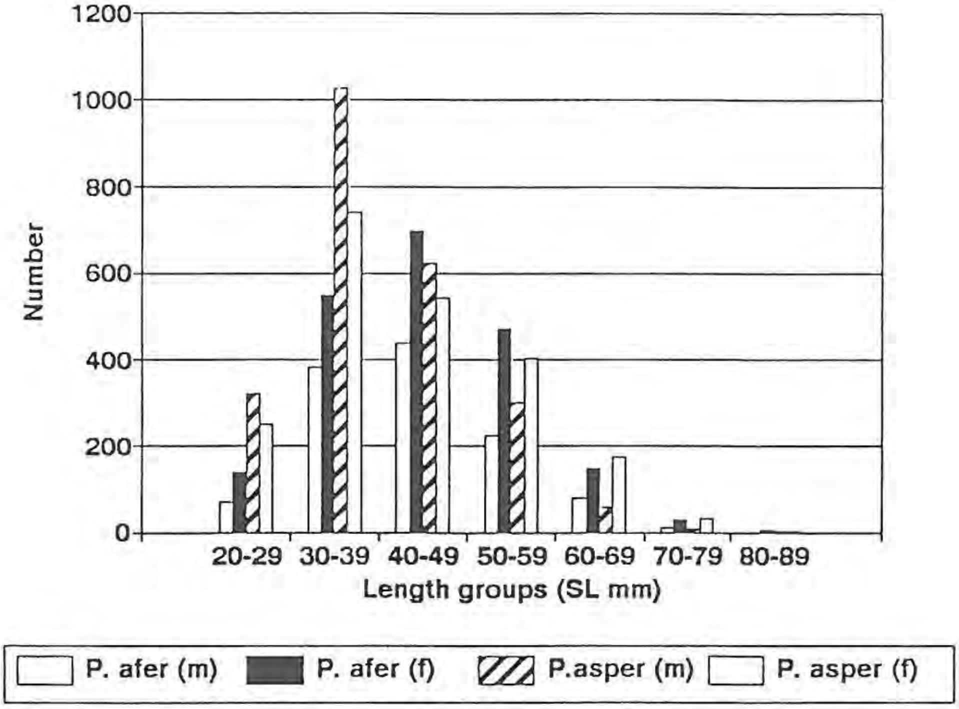


Figure 67. Comparison of the number of fish in each length group of male (n=1198) and female (n=2027) *P. afer* and male (n=2330) and female (n=2137) *P. asper*.

Conservation

Fishes with a short life cycle and a population which consists of only a few age groups are adapted to living under the conditions of very high and variable mortality (Nikolsky, 1963). Their population dynamics include early maturity, multiple annual spawning, etc., to ensure a rapid replacement of the stock. Fish with these life-history characters usually have years when their numbers can be greatly reduced eg. African minnows (Cambray & Bruton, 1984) and American cyprinodonts (Miller, 1943). These populations can quickly recover due to the high reproductive capacity of the remaining individuals in favourable years. This was witnessed for *P. asper* in the Groot River during the present study. Drought can reduce the Groot River to a few isolated pools of high salinity in which only a nucleus of the minnows survive. When the drought was broken *P. asper* quickly colonised new habitats (Cambray, 1991a).

Fishes such as *P. afer*, which have a later maturity and therefore contain individuals in relatively more age groups than *P. asper*, are adapted to living under conditions of a relatively stable food supply and negligible annual fluctuations in the mortality of mature individuals and comparatively slight effect of predators on older fishes. In this case replacement is slow if a large part of the population is predated upon by alien predacious fish. *P. afer* are more vulnerable to man-made perturbations to the environment than *P. asper*. This is evident in the Baviaanskloof and the Kouga Rivers where introduced bass species have made major impacts on redfin minnow populations (Cambray & Cambray, 1988). The redfin minnows never attain a size great enough to offer a size refuge from bass predation.

Only through integrated studies which include growth and productivity, reproduction, life-history etc will it be possible to achieve rational policies to manage and protect populations of rare species and communities (Mann, 1991). The present study supports this suggestion and it is hoped that some of the present findings will be useful in the conservation of these two redfin minnows and the aquatic communities they inhabit.

Hypothesis

The two redfin minnow populations inhabit two contrasting environments in the same river system and observed differences in growth rates, age at maturity and lifespans were probably phenotypic responses to these two distinct environments, whereas genetic controls determined size at maturity and ultimate size. The hypothesis raised in the introduction is therefore supported by the findings of this study.

CHAPTER 10

GENERAL DISCUSSION

> *"These forms which possess in some considerable degree the character of species, but which are so closely linked to them by intermediate gradations, that naturalists do not like to rank them as distinct species, are in several respects the most important to us" (Darwin, 1859: 47).*

As the discussions in Chapters 2-9 are exhaustive this final chapter has been kept brief. The main findings can best be summarized in tabular format (Table 60) and diagrammatically (Figure 68).

Previously known differences between *P. afer* and *P. asper* included gut length, scale size and pigmentation (Skelton, 1980a, 1988). Further differences have now been determined such as a different suite of life-history phenotypic options suited to each environment, timing of early life-history events, brain size and tubercle number and density (Table 60).

Moore (1920 in Brooks & McLennan, 1991:23) wondered whether we will "... be content to remain zoologists, botanists and foresters, with little understanding of one another's problems, or will we endeavour to become ecologists in the broad sense of the term? The part we will play in science depends upon our reply ... the future is in our own hands."

This "broad sense of the term" approach has necessitated a study of the literature on evolution, palaeoclimates, the Gondawanaland break-up, tracing of forest pattern changes, the connections of palaeo-drainage patterns and to the present day distribution and ecology of *P. afer* and *P. asper*. It is suggested that the ancestor of *P. asper* was similar to *P. afer* at present. These findings are hopefully of use to conservation managers as the ever increasing negative influences of man reduces habitat and causes extinction of some of these *Pseudobarbus* populations which have been around for so many years (Cambray & Cambray, 1988; Cambray, 1990a & b; Cambray, 1991a).

Knowledge of drainage pattern history, changes in climate and forest cover together with the knowledge of the interrelationship of *P. afer* and *P. asper* have greatly assisted in the interpretation of the life history work undertaken in this study. *P. asper* have been shown to be capable of responding opportunistically to the variability of the Groot River. In comparison *P. afer* is conservative in the more stable Wit River.

Table 60. Summary of the differences between *P. afer* and *P. asper.*

	Pseudobarbus afer (Eastern Cape redfin)	*Pseudobarbus asper* (Small-scale redfin)
Morphologically		
- intestinal tract	short	long
Meristically		
- scale size	large	small
Reproductive biology		
- breeding season	short	long
- fecundity	low	high
- GSI	low	high
- egg size	large	small
- egg envelope	honeycomb	honeycomb
- size at first feeding	large	small
- breeding site	mid-channel after high flow	mid-channel after high flow
- tubercle size	large	small
- tubercle number	high	low
- sex ratios	1♂ : 1,68♀♀	1♂ : 0,92♀
- length at maturity	♂♂ 39-40mm SL ♀♀ 39-40mm SL	41-42mm SL 43mm SL
- age at maturity (years)	2	1
- maximum age (years)	5 - 6	3
- relative condition	peaks	extended
Neuroecology		
- olfactory lobe	large	small
- optic lobe	large	small
- cerebellum	large	small
- eye diameter	large	small
- barbel length	long	short

Brundin (1986: 606) noted that "adherence to conservatism - as signified by comparative plesiomorphy in one of two sister species - means insurance of persisting possibilities for breakthrough of the evolutionary potential of the ancestral species, if the experiment symbolized by the derivative, apomorphic sister species proves unsuccessful. Also, in pairs of sister groups, we often are able to observe a dual trend towards conservatism and change. The two alternatives have always functioned as a double insurance of survival.' This 'double insurance' is evident in the *Pseudobarbus* sister species pair, with *P. afer* (conservative) and *P. asper* the derivative, apomorphic sister species.

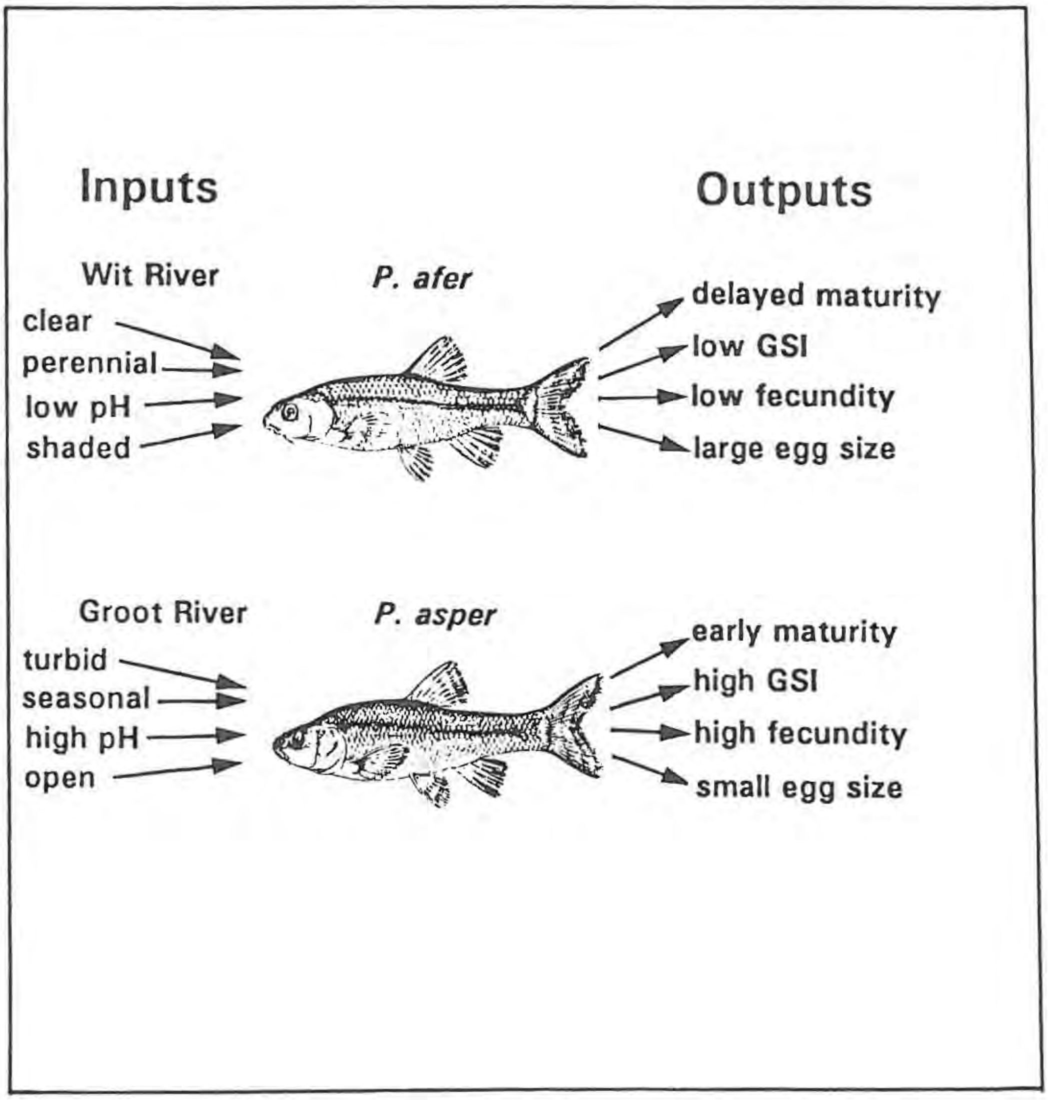


Figure 68. The precocial *P. afer* in the Wit River display different phenotypic options of certain life-history attributes compared to the more altricial *P. asper* in the Groot River.

Skelton (1980a) demonstrated that the *Pseudobarbus* are a monophyletic group and this has been substantiated by a study of karyotypes (Oellermann, 1988; Naran, 1992). The members of this group show characters that are variations on the group-specific themes. Such themes demonstrate the directed constraints that are set on the mutations by the specific design of the genotype of each stem species (Brundin, 1986). The few morphological and meristic differences between *P. afer* and *P. asper* are therefore understandable. Shifts in phenotypic options of life-history attributes coupled with minor morphological and meristic changes followed with changes in the Groot River (Chapter 2). Constraints were obviously strong within the *Pseudobarbus* lineage and this is evident in the many present day populations of *P. afer* which are reproductively isolated and between which there has been no gene flow for a considerable time (Chapter 3).

Selection, like mutations is a directive force and selection may "approve" or "disapprove" of life's continuous experiments with canalized mutations and new species. Brundin (1986: 606) has argued that environmental factors cannot be creative and that they cannot create new properties "..without attention to the metaphysic consequences.... selection stands out as the censorship performed primarily by the intrinsic epigenetic factors and, secondarily, by the extrinsic abiotic and biotic factors of the environment."

> ***"If common ancestors cannot be recognized with reasonable certainty then the evolutionists are in trouble. If they can be then cladists are in trouble" (Hull, 1979: 430).***

Skelton (1980a, 1988) analyzed his data on the *Pseudobarbus* according to the Hennigian model whereby ancestors cannot be recognised or identified but can only be hypothesised. Related taxa were clustered into lineages on the basis of shared derived characters (synapomorphies) with species originating in dichotomous branching sequences. Cladists point out that relationships are not always successfully established since the necessary synapomorphies have not been recognised (Skelton, 1980a). Taxa with unresolved interrelationships remain clustered in groups which are defined on the last recognisable synapomorphy/ies (Greenwood, 1979).

***P. afer* and *P. asper* resemble each other more closely than any other species. They therefore may have one of two kinds of phylogenetic interrelationships. They may either be sister species (as suggested by Skelton, 1980a) or be ancestor and descendant (as suggested here). Cladistic analysis cannot discern an evolutionary phenomenon which actually exists, that is ancestor-**

descendant relationships (Hull, 1979). Hull (*op cit.*) considered this a definite limitation of cladism. In the *P. afer* / *P. asper* sister group it is more informative that the ancestor of *P. asper* (given the biogeographical background of this pair) was like *P. afer*. "Closely similar species could possibly be an ancestor and its descendant" (Ridley, 1986:139). This 'imperfection (if it is one)' of cladism could be incorporated since no classification can be perfect (Ridley, 1986).

Bell (1976) presented a model of speciation using evolutionary mechanisms, the biology of *Gasterosteus* and past climatic change to show that speciation could repeatedly occur, through freshwater isolates from the anadromous ancestor, without significant alteration to the parental species. Bell (1979: 79) argued that "...anadromous *Gasterosteus* has not experienced evolutionary change as a consequence of loss of genetic information to or by competition with its numerous freshwater descendant-sister species." *G. aculeatus* was found to have great phenotypic stability despite the establishment of morphologically divergent freshwater populations. Speciation had therefore not significantly affected this species. Bell (1979: 87) concluded that "all freshwater populations generated from anadromous ones since at least the Pliocene thus are sister (evolutionary) species of anadromous sticklebacks." Bell (1979) noted that his findings would present problems for certain systematists but suggested that their methods must accommodate evolutionary mechanisms if evolutionary relationships are to be obtained for such taxa. If Bell (1979: 87) is correct that "speciation involving the transition from marine to freshwater habitats provides the conditions for repeated speciation without significant alteration of the evolutionary tendencies or historical fate of the parental marine species" then the reverse could also occur. That is, speciation caused by the transition from mountain stream to a relatively highly saline environment with the parental 'fresher' water species (*P. afer*) remaining relatively unchanged.

Relying on the thorough meristic and morphological work on the *Pseudobarbus* (Skelton, 1980a, 1988), the palaeoecological work (van Zinderen Bakker, 1978; Deacon, 1983), changes in climate (Brain, 1985), forest expansions and especially contractions (Geldenhuys, 1989), palaeo-drainage changes (Partridge & Maud, 1987) and present day distribution of *P. afer* populations (Figure 18) it appears that the ancestor of *P. afer* and *P. asper* as depicted in the *Pseudobarbus* cladogram of Skelton (1980a; see Figure 22) has not become *entirely* extinct (sensu Botosaneanu, 1988) but was similar to *P. afer*. According to the distribution studies recorded by Skelton (1988), *P. afer* populations still occur, in the Sundays River; Swartkops River; Baakens River; Maitlands River and in many small, isolated rivers in the Cape Fold Mountain Belt section of the system. *P. afer* is a widely-distributed species with small scattered populations in a number of river systems which in some cases have been separated for a long time (Chapter 2). The key populations lie in the river systems to the east of the Gamtoos, in the Sundays, Swartkops, Baakens and Maitlands Rivers. The palaeo-Groot-Sundays-Kariega River was once distinct from the palaeo-Baviaanskloof-Kouga Rivers. Populations of *P. afer* remain east of the present day Gamtoos system where there are no *P. asper* populations. It therefore seems logical to assume that *P. afer* migrated to the Cape Fold Mountain streams when the palaeo-Baviaanskloof-Kouga River captured the Groot River from the palaeo-Groot-Sundays-Kariega system. Alternatively, *P. afer* moved from river to river when sea levels were lower (which explains many of the isolated populations in small rivers flowing out of the Cape Fold Mountains at present (Figure 18). The climate then became more arid, the forests contracted and fragmented (Geldenhuys, 1989), and the Groot River possibly became more intermittent and saline. Under these changing conditions *P. asper* evolved. *P. afer* populations were left isolated and possibly relatively unchanged as were the forests which also remain as unchanged "island" refugia in the Cape Fold Mountains.

Phylogenetic or genealogical constraints have played an important role in shaping the patterns of biological diversity. Some workers, such as Brooks & Wiley (1988) have suggested that persistent ancestral attributes constrain the scope of the adaptively possible at every point in evolution. The "pull of history" (Brooks & Wiley, 1988; Gould 1989) has probably constrained the many small isolated populations of *P. afer* except those which occurred in the Groot River which had to change to survive in the changing environment.

Traditionally it has been suggested that there must be phenotypic change, the evolution of 'isolating mechanisms', before speciation (Mayr, 1963). One of the isolating mechanisms outlined by Mayr was that potential mates do not meet because of habitat isolation. It is suggested here that *P. asper* remains in the larger river with its high conductivity and turbidity during spawning whereas *P. afer* spawn in clear mountain streams with low conductivity. They are therefore isolated by spawning habitat preferences (water quality) which prevents interspecific crosses. There may also be changes in Specific-Mate Recognition Systems (SMRS) (Paterson, 1985), as *P. asper* occur in the highly turbid Groot River (especially turbid in floods when the fish spawn) and the *P. afer* mate in the clear mountain streams. Density and size differences of nuptial tubercles between the two species and differences in intensities of male nuptial colouration (Chapter 7) indicate that there could be a difference in SMRS.

'Habitat divergence' (Templeton, 1981) may have lead to the speciation of *P. afer* and *P. asper*.

The isolating barrier for the two redfin minnow species is common to many fish species (freshwater, estuarine and marine fishes). That is, salinity tolerance, possibly also combined with turbidity tolerance in the case of the two redfin minnows. Speciation was probably a consequence of a vicariant event, in this case water quality changes. Many evolutionists acknowledge that environmental change and vicariance play an important role in the origin of species (Vrba, 1985). The environmental change, water quality, divided the formerly continuous distribution of the ancestor of *P. afer* and *P. asper* into the mountain stream form and the Karoo stream form. Without this difference in water quality reinforcing reproductive isolation, the *Pseudobarbus* in the Gamtoos River system would be no more than ecophenotypes. Variable life-history attributes changed depending on whether an individual occurred in the Cape Fold Mountain streams (eg few, large eggs) or the Karoo streams (eg many, small eggs).

Possibly the population of *P. afer* which remained in the Groot River gradually adapted to the changing environment, higher turbidity, higher salinity and more intermittent flows. The other populations of *P. afer* in the mountain streams did not change. This is what Mayr (1963) would call phyletic speciation, that is the gradual progression of forms through a single lineage (anagenesis). Therefore it was not the *P. afer* populations in the mountain streams which have budded off a descendant, they have not changed, but the *P. afer* in the Groot River which have undergone phyletic speciation.

As noted above persistent ancestral attributes may be adaptations that originated in the past and they may be slowly evolving attributes which come to characterize genealogical groups of species and they constrain the scope of the adaptively possible during evolution of those groups (Brooks & Wiley, 1988). In the redfin minnows tubercles may be one of these ancestral attributes, others may be spawning habitat and lack of egg envelope adhesion. In the mountain streams these attributes exert a positive influence on fitness whereas in the Groot River they may be not as advantageous. Reproductive energy of the males may be more efficiently used in covering more eggs than in visual threats (large tubercles and brilliant colours). Spawning adhesive eggs on vegetation, after little or no rain in a short-lived minnow species also might be advantageous in the Groot River environment of today. The co-occurring minnow, *B. anoplus*, has this kind of reproductive style (Cambray & Bruton, 1984).

Reproductive conclusions (Chapter 4)

Ware's (1984) analysis of reproductive patterns suggested that no one pattern is universally adaptive and instead there should be selection for a combination of life-history characteristics in a life-history style which confers the highest fitness. The combination of attributes which confer the highest fitness for *P. afer* is not the same combination as for *P. asper* (Table 20) and these differences are directly related to the two distinct environments of the Gamtoos River system.

The ability of the *Pseudobarbus* lineage to respond adaptively to environmental changes is most clearly seen in the *P. asper* population in the Groot River. *P. asper* is meristically and morphologically the more derived of the sister species pair (Skelton, 1980a). In its reproductive style it is also derived and atypical of the other *Pseudobarbus* species studied to date (Cambray & Stuart, 1985; Cambray & Meyer, 1988).

"Each individual fish has a suite of reproductive attributes which are determined by its genotype and hence by the evolutionary history of the gene pool of which the fish is a member" (Wootton, 1990:160). The reproductive style is a combination of the reproductive attributes characteristic of individuals belonging to the same gene pool. This has been diagrammatically represented for *P. afer* and *P. asper* to indicate both the range of the phenotypic expression of the sister species pair and the phenotypic options of each species with their different environmental factors (Figure 41).

There is a cyclical demand for both energy and materials to operate, as Miller (1984) termed it, the "reproductive machinery" of a fish. Within the overall reproductive style there is a temporal pattern in amplitude, frequency and direction of the fuelling of reproduction. The "reproductive machinery" of *P. asper* requires more fuelling (higher GSI) which is channelled into many small ova for a longer reproductive season than *P. afer*. *P. afer* and *P. asper* therefore have distinct differences in the temporal pattern of allocation of resources for reproduction and also in the way this resource is partitioned.

Should the 'starter' environment for the redfin minnows have been a stream similar to the present Cape Fold Mountain stream (which today is a more typical environment for the rest of the *Pseudobarbus* species) then a form with another life-history trajectory either ventured out into the variable environment of the Karoo stream, or alternatively the stream changed over time into a Karoo stream. The *Pseudobarbus* population had to make some changes to their phenotypic options in their reproductive style for that style to be successful in the changing environment of the Karoo stream. Some of these changes occurred in life-history attributes such as a longer reproductive season and higher fecundity (Chapter 4), smaller eggs (Chapter 5) and early maturity (Chapter 9). The adaptive and ecological significance of such changes in phenotypic options of the life-history attributes are directly related to the two distinct environments. These changes in the phenotypic options are what one would expect and the resulting reproductive style of *P. asper* (altricial) has been successful in the variable Groot River. *P. asper* have shown the potential flexibility of the *Pseudobarbus* species in their response to environmental change in life-history attributes such as age at maturity and increased reproductive effort per season. There may be two different selection pressures for multiple spawning. In the *P. asper* population there is a need to spread the risk of losing an entire year class whereas in *P. afer* there is a need to spread the number of larval fish over time with regard to a scarce food resource.

The redfin minnows are non-guarders of their non-adhesive eggs and young, open substrate benthic spawners on coarse bottoms (rocks) and have photophobic free embryos. The riverine spawning sites indicate a conservative tendency and represents a phylogenetic constraint as compared to the more variable attributes such as egg size etc. which are under environmental control within the limits expressed by the genotype.

Since 1957 the Groot River has been managed by putting a flood control dam across its course. Periodic water releases are sufficient to trigger spawning of the highly fecund, multiple spawning *P. asper*, which cycles between ripe, ripe-running, partially spent to ripe-running over a six to seven month period (Cambray, 1991a). In contrast the *P. afer* reproductive style in the relatively stable environment of the perennial Wit River is to have fewer, larger eggs, with resulting lower fecundity and a shorter reproductive season which is a more precocial life-history style. Considered together, the two redfin minnows might be exhibiting all or part of the range in variation that their reproductive attributes can show in the face of the environmental variables offered by the Gamtoos River System. Each of the two distinct sections of the system require a different phenotypic option within each attribute (Figure 41).

Redfin minnows moving from the Wit River into the Groot River would possibly require biochemical and physiological changes for acclimatization. However, it is suggested here that the Groot River was more perennial in the past and also had a lower conductivity and was more like the Wit River today than the present Groot River (Chapter 2). Many fish species have been shown to have a variability of life-history attributes such as fecundity, growth rate, size and age at sexual maturity. Eventually individuals within a population may adapt to a long-term change through alterations in gene and genotype frequencies. The environmental change (Wit River - Groot River or Groot River -past to Groot River - present) may have acted as an evolutionary factor causing changes in the gene pool of the population through the process of natural selection and/or by epigenetic mechanisms.

Egg size (Chapter 5)

In several studies on egg size of freshwater fishes it has been demonstrated that the fish which inhabit the more oligotrophic systems have larger eggs than those from eutrophic waters (Coburn, 1986). The findings presented here are in agreement with these studies. Since three of the four *Pseudobarbus* species, for which there are data, have relatively large eggs, large egg size is probably primitive in this monophyletic lineage and the small egg size of *P. asper* is derived. This finding substantiates other morphological and meristic data which suggested *P. asper* to be more derived in comparison to its sister species *P. afer* (Skelton, 1988). It is difficult to separate the influence of environmental factors on egg size from genetic factors and there is a need to be cautious. There is good empirical evidence that there is no optimum propagule size in some fish species and that egg size variability is an adaptation to a changing environment. As the Groot River became more intermittent and less well vegetated over time a shift to smaller eggs may have increased the offspring fitness of *P. asper* as the free embryos could hatch earlier and escape desiccation in the temporal spawning beds. In addition, smaller sizes meant more *P. asper* offspring could be released at any one time into this highly variable environment. In comparison large egg size for *P. afer* in the mountain stream environment may have increased offspring fitness which was probably related to food availability and predator avoidance. The larger eggs of *P. afer* meant that larger and better developed first-feeding larval fish would be able to avoid predators and have a wider prey spectrum than smaller, less developed larval fish. *P. afer* invested less per clutch than *P. asper* but more per offspring. *P. asper* followed the evolutionary tendency suggested by Wootton (1990) to minimize egg size and maximize fecundity. Whereas *P. afer* followed the evolutionary tendency suggested by Balon (1990) with the more precocial species investing more per progeny. The evolutionary tendency to smaller eggs (Wootton (1990) is probably an evolutionary constraint whereas the evolutionary trajectory of Balon (1990) denotes movement towards larger eggs. Therefore the further one goes back on a lineage the smaller the eggs should be. The differences in investment per offspring between *P. afer* and *P. asper* was considered important in understanding the differences in the life-history trajectories of the two species.

Sex ratio conclusions (Chapter 6)

The sex ratio of *P. afer*, unlike *P. asper*, was highly significantly biased towards females. This would indicate that sex ratio control was probably in response to some environmental and/or behaviourial stimulus which correlated with fitness. During spawning there may be an optimum sex ratio for the production of fertilized eggs which depends on the social behaviour of the species (Reay, 1984). In the turbid waters of the Karoo stream, *P. asper* has a sex ratio which is closer to the hypothetical 1:1 ratio unlike the more skewed female-biased ratio of *P. afer*. In the clear mountain streams, a biased sex ratio in favour of females is probably optimum for the production of fertilized eggs and is evolutionary stable. That social behaviour might have influenced the sex ratio of *P. afer* is indicated by the enlarged breeding tubercles and enhanced breeding colours of the males compared to the less vivid breeding colouration and reduced tubercles of *P. asper* males. In addition the main spawning of *P. afer* occurs in October/November period when the water is still relatively cooler than later in the year which may result in more females. In this crowded, relatively constant environment of the Wit River, in which males may compete for females the female-biased sex ratio correlates with fitness. The production of males in the early spawning will have a relatively lower fitness. However, later in the season with warmer waters more males may be produced but the relative fecundities and thus number of offspring will be less than the earlier spawning.

Even in the smallest length group of *P. afer* there was the tendency towards more females. It is therefore unlikely that differential mortality has biased the secondary sex ratio but it did influence the tertiary sex ratio as is common among small *Barbus* species (Cambray & Bruton, 1984). It is possible that this ratio occurred in the early ontogeny of the species. Compared to the *P. asper* environment of the Groot River, the temperature, pH and salinity are all considerably lower in the Wit River. *P. afer* has a lower fecundity and shorter breeding season than *P. asper*. The Wit River is oligotrophic compared to the eutrophic Groot River which may influence the equilibrium of the sex ratio. Local resource competition between related members of one sex may influence the sex ratio. Related males, but not females, competing for food resources may have an effect on fitness. The secondary sex ratio would then be biased towards females. The reproductive success (fitness) of the mother, is increased by producing more female progeny.

Reproductive output is more frequently limited by the availability of eggs than sperm. This would mean that mate competition would occur more frequently between related males than related females (Greenwood & Adams, 1987). Female *P. afer* may maximise fitness by producing fewer males but this would imply that the daughters would have a higher chance of having their eggs fertilised. The large nuptial tubercles of *P. afer* and the bright red fins might indicate that the more sons produced the more competition there would be for mates in the clear mountain streams, which possibly would reduce female *P. afer* fitness. In this case male production at the expense of females is likely to decrease the number of the F_2 generation.

Nuptial tubercle conclusions (Chapter 7)

Tubercles are important taxonomic characters in the monophyletic *Pseudobarbus* lineage (Skelton, 1980a) where they have mainly been studied as present or absent characters for species. Field studies to note seasonality of tubercle formation and wear have been rare for African cyprinids (Cambray & Stuart, 1985).

The possible functions of the nuptial tubercles for male *P. afer* and *P. asper* are given in Table 44. The males of both *P. afer* and *P. asper* had larger definitive head tubercles than did the females. *P. afer* males had the most numerous and largest tubercles when compared to *P. asper* males. The seasonal occurrence and disappearance of tubercles for both species follows the reproductive cycle and is probably under seasonal hormonal control. Scanning electron microscopy of the microwear of the keratinized cap of the tubercles was uninformative. It is suggested that the enlarged, white head tubercles of *P. afer* are used for display prior to breeding, whereas during breeding the tubercles on the scales and fin rays are for maintaining contact with females, as are the larger pectoral fins of the males, in the riffle spawning habitat. The smaller number and size of the head tubercles on *P. asper* males indicate that in turbid waters visual characters would have less of a selection advantage than in a clear mountain stream (Table 44). That the head tubercles are still relatively large in *P. asper* may be a phylogenetic constraint within the sister species pair. Large tubercles may be primitive for the *Pseudobarbus* species monophyletic lineage and small tubercles more derived. Only the large females of both species have head tubercles which are never definitive and their presence on reproductively active females is difficult to explain. The lack of tubercles on the smaller females indicates an expected trade-off constraint where the energy is put into egg production and not sexually dimorphic characters. The smaller pectoral fins of the females, which had few if any tubercles, also indicated that it was more beneficial for the female to put reproductive effort into progeny. Secondary sexual characteristics of *P. afer* and *P. asper*, epidermal tubercles, breeding colouration and larger pectoral fins all require resources which must be diverted away from growth, survival requirements and the production of gametes, and as such that favours their development on male *P. afer* and *P. asper*. The smaller number and size of tubercles on *P. asper* males would have required less resources and the remainder of the resource would then be available for other reproductive activities. These differences are probably under environmental control and would be expected for ecophenotypes. Paterson (1985) has suggested that if the ancestral Specific-Mate Recognition System (SMRS) persists after speciation then so does the species. However, if tubercles are used for SMR then they could well separate the two species during spawning, even if the redfin minnows occurred together.

Neuroecology conclusions (Chapter 8)

From the neuroanatomical study it could be concluded that *P.afer* which inhabits the clear mountain streams probably has better olfactory and optic senses and a considerably better developed locomotory centre (cerebellum) than does its more derived sister species, *P. asper*, which inhabits the turbid section of the Gamtoos River system. The difference in relative size of the cerebellum was probably the result of greater specialization of *P.afer* in the fast-flowing mountain streams. *P. asper* has probably undergone degeneration of certain neural structures such as the optic lobes which are correlated to the afferent sensory system, the eyes. *P. asper* has not developed those structures which would compensate for it inhabiting a turbid environment.

There were intersexual as well as interspecies differences in some of the neural structures of *P. afer* and *P. asper*. The intersexual differences may be related to the ecology or behaviour of males and females. Overall the largest brain was in male *P. afer* (which also has the largest nuptial tubercles) and the smallest in female *P. asper*.

There is a lack of comparative work on the quantitative analysis of the brain sizes of redfin minnows and in fact on that of African cyprinids. It would therefore be premature to make any further conclusions with regard to generalized or specialized features although this preliminary study was encouraging and indicated that in turbid conditions certain neural structures degenerate. The response of the smaller brain in *P. asper* living in a turbid environment would be expected in an ecophenotype of *P. afer* as well as in a sister species.

Growth conclusions (Chapter 9)

Mann (1991) attributed the success of the Cyprinidae to the flexible response of their growth and other parameters to environmental conditions. A major characteristic of fish growth is flexibility and the same species may show different patterns of growth in different environments, with maturity being reached at different sizes and at different ages (Wootton, 1990). A flexible growth pattern is a mechanism for an adaptive phenotypic response to a changing environment (Wootton, 1990). The differences in the growth rates of *P. afer* and *P. asper* substantiated this statement.

Links between growth, sexual development, sexual size dimorphism and survival are features that identify the life-history phenotypic options of particular species or populations (Mann, 1991). These links between life-history phenotypic options have been found to be quite distinct in the mountain stream population of *P. afer* compared to the turbid stream population of *P. asper*.

Most of the observed changes in the remarkable variability in the life history of *Phoxinus phoxinus* over its wide geographical range are phenotypic responses to contrasting environments (Mills, 1988). The two closely-related redfin minnow populations inhabit two contrasting environments in the same river system and as with *P. phoxinus* the observed changes within life-history attributes, such as growth rate, age at maturity and lifespan, are probably phenotypic responses to these two distinct environments. Whereas genetic controls determined size at maturity and ultimate size, which are similar for both *P. afer* and *P. asper*. These later two attributes are probably due to an historical legacy. The final size and size at maturity of *P. asper* may not be a "solution" to the current environment but inherited from the ancestral taxon. Since the same species can undergo very different growth patterns in different environments then as far as the growth results have shown *P. asper* could be an ecophenotype of *P. afer* or a sister species.

P. afer **was found to be the more precocial species and *P. asper* the more altricial species (Chapter 4). Shifts in parental investment per offspring have resulted in alternative life-history trajectories for *P. afer* and *P. asper*. It was hypothesized that the investment per offspring was important and as noted by Reid (1985: 251) "... the earlier in development a change occurred, the greater the evolutionary saltation might be." Balon (1980, 1981b, 1985, 1990) has suggested that prior to having any evolutionary impact these epigenetically introduced changes lead to alternative life-histories which can occur even in a clutch from the same parent. It has been further suggested that the more pronounced events (eg. phylogenetic divergence) are stronger "reverberations" of the same epigenetic process (Balon, 1990). The amount of parental investment per offspring is the earliest stage which "fuels" development and ultimately influences the life-history trajectory of that individual. The amount invested per offspring by the parent may be synchronised by an environmental cue, such as food availability and/or temperature in the parental environment.**

It is suggested here that a population of *"P. afer"* may have occurred in the Groot River and over time had to co-evolve as the river changed from perennial, low salinity, clear, oligotrophic to an intermittent, highly saline, turbid and eutrophic environment (Chapters 2 & 3). The precocial *P. afer* individual was vulnerable to these changes and by juvenilization the probable eventual extinction of the redfin minnows in this environment was prevented by the survival of the juvenilized ('despecialized') progeny.

As the nature of the Groot River changed individuals with altricial attributes were selected for and survived. The fish had longer guts and as such could utilize smaller and less nutritious food items. The formation of smaller scales may be due to epigenetic factors, such as salinity. Sire & Arnulf (1990) have suggested that there may be epigenetic induction of squamation development in fishes by mechanical constraints. The differences and ranges of scale sizes (meristic counts variability) which Skelton (1980a) found in the *P. afer*/*P. asper* complex may be due to salinity and/or other epigenetic factors in the different environments. With the increase in turbidity and enrichment of the water the investment per progeny of the *"P. afer"* population became less and there were more eggs which resulted in higher relative fecundities. This may have lead to more variability by nature of the number of eggs per spawning and increased number of spawnings per season per fish. In individuals which were adapted in this way the free embryos hatched in a shorter time and therefore more survived as the river flowed

irregularly and for shorter periods. Over time a species with smaller scales (salinity effect?), longer guts (food availability - quality and quantity?) and a different pigmentation pattern (turbidity?) evolved which had a tolerance to the high salinity levels and poor visibility (*P. asper*). It was not necessary for a set of altricial and a set of precocial eggs to be spawned each generation as suggested by Balon (1990). There is size variability in batches of eggs that are spawned but it has not been found here to be in two distinct groups, one altricial set and one precocial set. The dichotomy we see today in the redfin minnows of the Gamtoos River system, *P. afer* and *P. asper*, may not be the result of the production of two forms within each generation but with the production of variability within each generation, which would suggest a range of forms, here called the altricial to precocial range of forms. This is not as simple or as neat as the altricial/precocial pair idea but may be more relevant and a means to ensure the continuation of variability. This variability in egg size in the two environments of the Gamtoos River system should not be overlooked and probably plays an important evolutionary role and is seen as adaptive in a fluctuating environment. The changing Groot River environment with the changing food resources would have favoured a higher survival of the smaller eggs and a higher mortality, due to stranding, of the larger, slower developing eggs. So not only were the food resources leading to smaller eggs, the smaller eggs were being selected for simply because they were less likely to be stranded or smothered by silt than the larger eggs. *P. afer* in the Wit River form was overspecialized to survive in the changing Groot River environment. Despecialization by paedomorphosis (Figure 69) changed this population. *P. asper* is therefore the paedomorphic response to changing environmental conditions which have taken place in the Groot River. The physiological constraints of *P. afer* were therefore overcome and enabled *P. asper* to evolve in the increasingly more saline Groot River.

The bifurcation of the Gamtoos River system into the Cape Fold Mountain streams and the Karoo section of the system offered the *Pseudobarbus* two distinct environments (Figure 1). The variability of the precocial form, *P. afer,* in the changing environment of the Groot River was not sufficient, without paedomorphosis returning more variability to the *Pseudobarbus* and as such they now had enough resilience to survive in the harsher Karoo environment. The environmental cues which drove this may have been temperature and/or food quality and quantity and/or salinity increase. Alberch (1980) has described phenotypes as well buffered (homeostatic) systems which are resilient to environmental and genetic perturbations during their ontogeny. The phenotype of *P. afer* was not sufficiently resilient to the changing environment of the Groot River and the environmental perturbations possibly played a major role in the formation of *P. asper*. In contrast the phenotype of *P. afer* was resilient enough to survive in many isolated rivers which occur in the Cape Fold Mountains (Chapter 3).

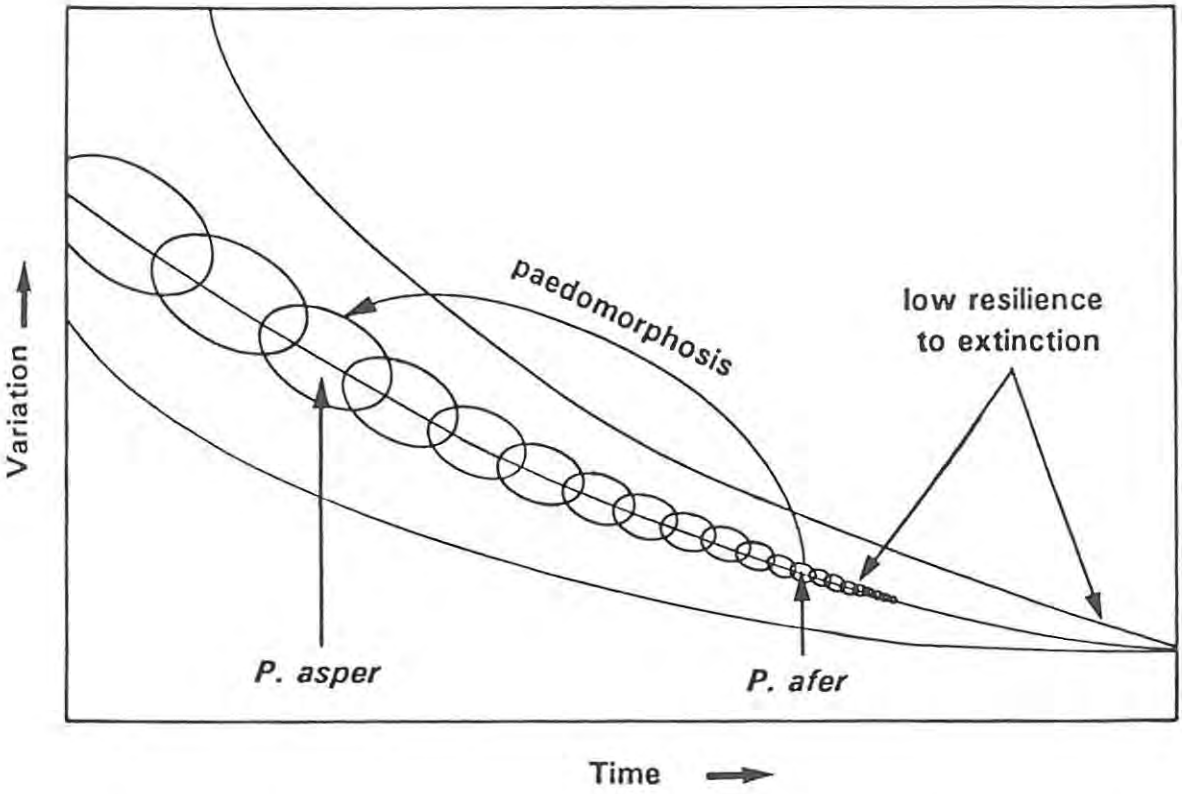


Figure 69. Stabilized trajectory for genetic and epigenetic variation in a succession of reproductive lineages towards a precocial *P. afer*. *P. asper* is hypothesized as having undergone an epigenetic retreat via paedomorphosis to a species that is more altricial and has more variability (modified from Balon, 1990).

The time courses of the two mechanisms of adaptation, phenotypic variability and genetic selection, are different (Wootton, 1990). Quick changes can be made by phenotypic adaptation within a generation. Egg size changes in the *Pseudobarbus* may have been rapid and such phenotypic adaptations to the immediate environment may have occurred within a generation. This would be the phenotypic option used by an individual in response to their encounter with an enriched environment. A phenotype is an information gathering and transmitting device and epigenesis 'creates' new phenotypes (Balon, 1990). The genetic adaptations, such as salinity tolerance to the Groot River would occur between generations. It is a 'strategic' response observable at the level of the population (gene pool), and importantly it is driven by the adaptive differences between individuals as suggested by Wootton (1990).

It is therefore suggested that the food resources utilised by the female parent influenced the size and number of offspring and that within a batch of eggs there was a range of sizes. The current environment selected the ones that would survive. In contrast to what was happening in the Groot River the many scattered and isolated populations of *P. afer* inhabited relatively stable, Cape Fold Mountain stream environments and as such have not speciated. The ancestor of *P. asper* in the Groot River speciated as the environment changed resulting in the *P. asper* population. *P. asper* was the result of paedomorphosis and as such was as Balon (1990) has termed it, an *epigenetic retreat* to a less specialized early ontogeny, with a shorter embryonic period as shown by the early life-history study. The trajectory as depicted in Figure 69 would have led to the extinction of a precocial form of the ancestor of *P. asper* in the present day Groot River.

The physiological tolerances of the two species are viewed as a key character which separates *P. afer* and *P. asper*. Can the salinity tolerance of *P. asper* be explained by epigenetic mechanisms? That in every generation a batch of fish is produced which is a little more tolerant to high salinity levels compared to a second batch is unlikely. It is more likely that the ancestral *Pseudobarbus* species had progeny which were adapted for and survived in increased salinity levels. However, the very fact that paedomorphosis has occurred, may indicate that reverting to the more altricial (more variable) form carried with it the advantage of a higher tolerance to salinity levels. So it is argued here that *P. asper* probably did not have to adapt by a genetic adaptation to higher salinity levels but instead it increased its potential for tolerance by paedomorphosis (ie. the potential tolerance was already in the parental gene pool). This tolerance level was possibly present in the history of the lineage. It is of interest to note here that a member of the proposed outgroup of the *Pseudobarbus*, *Barbus anoplus*, co-occurs with *P. asper* in the Groot River. *B. anoplus* is tolerant to the salinity and fluctuations within the system, and it also does not occur in the Wit River.

In the Gamtoos River system the *Pseudobarbus* were faced with a marked geomorphological bifurcation, the Cape Fold Mountain streams and the Karoo stream. *P. asper* was able to survive due to the ability to become an altricial form away from the more precocial form of *P. afer* which had life-history attributes which were adapted to the stable, clear mountain streams.

Hypothesis

The main hypothesis of this study, as raised in Chapter one, was that the two distinct sections of the Gamtoos River system would exert different selection pressures on the two closely-related *Pseudobarbus* species which would influence their life-history styles. It has been shown in this study that the two distinct habitats have exerted different selection pressures on the life- histories of the two *Pseudobarbus* species which can best be shown diagrammatically (Figure 68). That two species of redfin minnows occur in the Gamtoos River system today is mainly because the river system is bifurcated into distinct mountain stream environments and a distinct Karoo stream environment.

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