THE ECOLOGY AND CULTURE OF THE ROCK CATLET *CHILOGLANIS PRETORIAE* (PISCES : MOCHOKIDAE).

THESIS

Submitted in fulfilment of the
requirements for the Degree of
MASTER OF SCIENCE
of Rhodes University

by

PIERRE DE VILLIERS

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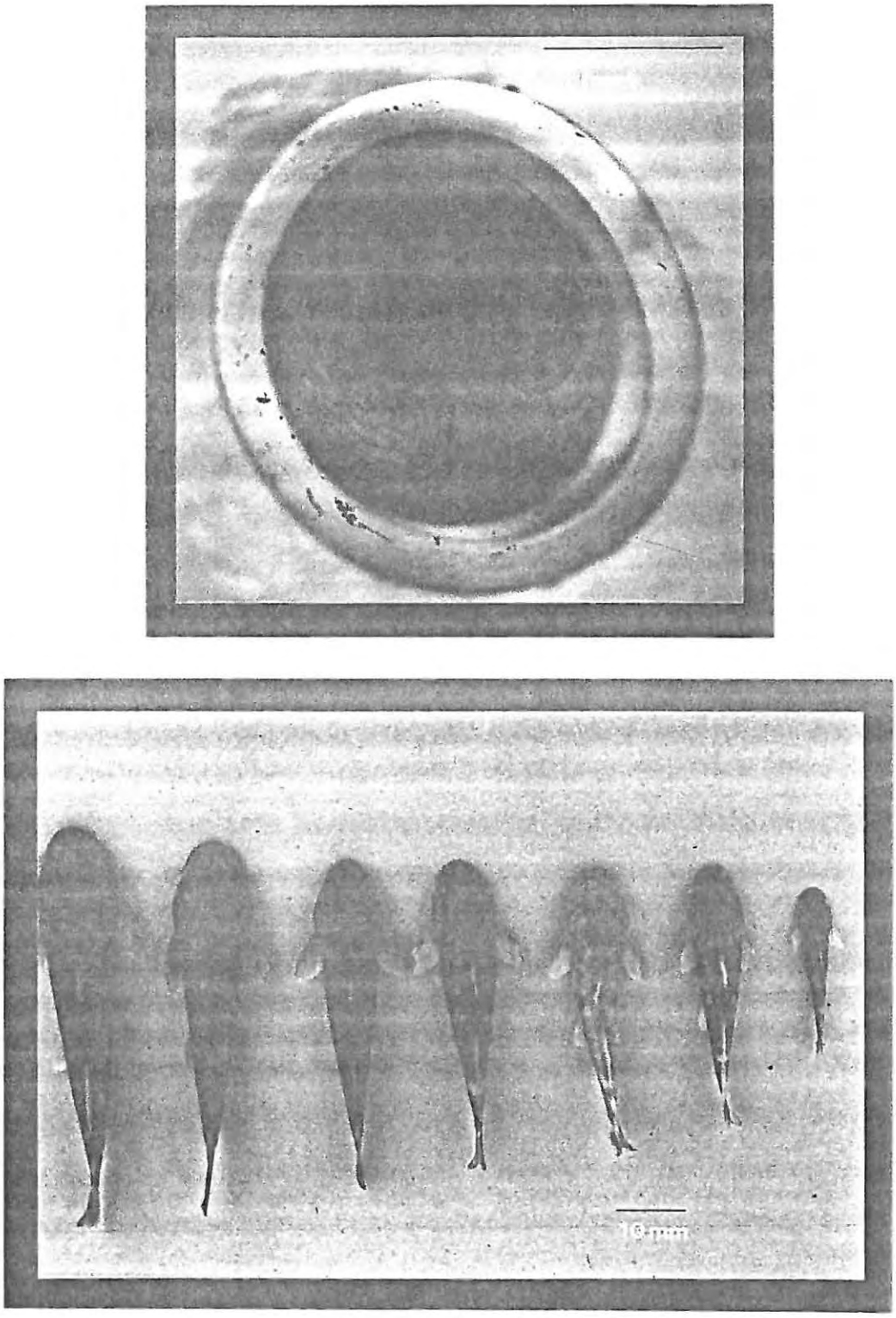

Frontispiece : the rock catlet, *Chiloglanis pretoriae*.

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ABSTRACT

Chiloglanis pretoriae is a rock catlet, indigenous to southern Africa. The aim of the study was to develop a technique to culture *C. pretoriae* as an alternative to harvesting and selling wild caught fish on the international aquarium trade. As nothing was known about the culture of African rock catlets an investigation into the biology and ecology of the species was necessary to develop the culture protocol.

Chiloglanis pretoriae inhabits fast flowing rapids (current speeds over 0.6 metres per second). It is a serial interstitial gravel spawner, that spawns during the summer months. *Chiloglanis pretoriae* is a carnivorous fish species, feeding on aquatic insects. The natural growth rate is relatively fast in the first two years where after it levels off. Sexual maturity (50%) is attained within the first year (44mm total length). From the four cell stage, embryos took seven days to hatch, 16 days to first feeding and 75 days to reach the juvenile phase. The free embryos were well developed and readily accepted artificial feed at first feeding.

The fish spawned readily, without hormone induction, in a continuous raceway. Spawning in the 80l retangular glass aquaria was irregular. The substrate within the raceway consisted of gravel and large rocks. The current was maintained at 0.6m/sec, temperature at 26± 0.6^{o}C, dissolved oxygen concentrations at 7.1± 0.3mg/l, pH at 6.9± 0.2 and photoperiod at 16L:8D. Conductivity was monitored and remained within the acceptable range of *C. pretoriae* (84± 10uS/m).

The optimum broodstock density for maximum egg production was found to be 20 fish per 350l raceway at a sex ratio of 1:1. Larval survival rates were highest when fertilised eggs were artificially incubated in gauze trays suspended in well aerated glass tanks.

Oxheart was found to satisfy the dietary requirements of adult *C. pretoriae*. In addition, *Daphnia* were provided, when available, to supply a "live food" element. The larvae were successfully reared on Tetra Larval Food while juveniles readily accepted oxheart. *Chiloglanis pretoriae* was successfully reared from egg to market size (40mm total length) in 98 days.

TABLE OF CONTENTS

CHAPTER 1.

INTRODUCTION

The international ornamental fish trade is a lucrative one. It is aimed at a luxury market and producers obtain high prices for top quality products (Brown and Gratzek, 1980 ; Hecht and Britz, 1990 ; Edmonds, 1990). In the United States of America the pet industry sales of fish and fish-related products during 1984, totalled 2,9 billion dollars (Myburg, 1986). This is no small wonder as aquarium keeping has for several years now been the second most popular hobby in the U.S.A., while it is generally viewed as the most popular hobby in Europe (Andrews, 1989). In the U.S.A. alone, 10-20 million aquarium enthusiasts keep about 95 million tropical aquarium fish and aquaria are found in seven percent of US homes (Winfree, 1989).

The world's traditional suppliers of farmed ornamental fish are presently experiencing serious difficulties due to industrial pollution and the allocation of farm land for industrial and residential development (Andrews, 1989 ; Hecht and Britz, 1990). These problems have created opportunities for other countries (Andrews, 1989). In 1985, ornamental fish wholesalers reported that approximately 90% of freshwater ornamental fish sold in South Africa were imported (Impson and Bruton, 1985). This has now decreased to approximately 60% which reflects the upsurge in the local production of ornamental fish (Hecht and Britz, 1990). When projected to retail level, the value of locally produced fish during 1987 was estimated at between R4.5 and R5.5 million (Andrews, 1989). During 1988 the retail value had increased to approximately R6.7 million (Hecht and Britz, 1990). In addition to the above increases in production, 10% of locally produced fish are now being exported (Hecht and Britz, 1990). To date, no indigenous South African species are being cultured (Andrews, 1989).

Catfishes such as those of the genus *Corydoras*, have been popular aquarium fish for a long time (Brymer, 1954). Recently however, a major upsurge in the popularity of catfish as aquarium species has been noted (Burgess, 1989). A factor which has direct implications for this study is that a number of indigenous African catfishes are amongst the desired species (Burgess, 1989). According to Sands (1986) some of the more common *Synodontis* species are already available in the international aquarium fish trade, eg. *S. angelicus*, *S. nigromaculatus*, *S. brichardi*, *S. flavitaeniatus*, *S. schall*, *S. decorus*, *S. alberti* and *S. robertai*. Publications on catfishes in the popular tropical fish magazines and the existence of organisations such as the Catfish Association of Great Britain, which was created to disseminate information on catfishes and to promote them as aquarium species, have served to promote them on a large scale.

Although a demand for ornamental catfishes exists internationally, local entrepreneurs, unlike those in other African countries (eg. Zaire and Malawi), have not exploited this market and indigenous catfishes have not been exported. Zaire and Malawi export wild caught fish and there are a number of reasons preventing this in South Africa. Given the demand of the international aquarium trade it is highly unlikely that the coveted South African species would be able to sustain the rate of exploitation (Andrews, 1989). Fortunately, regional conservation ordinances exist which protect indigenous fish from being captured and sold without a permit. Thus the only alternative to harvesting wild stock is the development of a culture protocol for candidate ornamental species. To date in South Africa only a few of the indigenous potential trade species have been successfully bred in captivity. These include *Barbus trevelyani* (Bok and Heard, 1982 ; Cambray, 1985), *Barbus anoplus* (Cambray, 1983), *Aplocheilichthys johnstoni* (Haigh, 1990) and *Pseudocrenilabrus philander* (Ribbink in prep). In general however, very little is known about the captive spawning requirements of indigenous South African fish species, and the

development of techniques for the large scale farming of these fish is likely to take years (Andrews, 1989). If the technology is developed, a lucrative export market with the added bonus of minimal foreign competition can be created.

The incorrect choice of candidate species has in the past resulted in a waste of time and money in aquaculture worldwide (Bruton and Safriel, 1984). Therefore the first step in assessing the suitability of a candidate species, is to establish whether there is a demand for such an animal (Hecht, 1984). Given the popularity of small catfish species on the international aquarium fish market and after consultation with leading producers and retailers, the rock catlet *Chiloglanis pretoriae* was identified as a potential candidate species. Producers and retailers in South Africa have claimed that "the demand for African catlets is so big internationally that almost anything that can be supplied stands a good chance of being sold" (Andrews and Stallard, pers. comm. Amatikulu Hatchery Pty. Ltd. Gingindlovu).

There is only one way of supplying indigenous species in South Africa to the ornamental trade, and that is by culturing them. Aquaculture often depends on the ease with which fish will spawn in captivity (Stickney, 1979). This is especially the case with respect to ornamental fish culture, which generally relies on natural spawning methods. Its success depends on the identification and simulation of the necessary spawning conditions. An ornamental fish culturist must therefore have an adequate knowledge of the biology and environmental requirements of the candidate species. A number of examples exist where a candidate species (eg. the bumblebee catfish *Leiocassis siamensis* and *L. poecilopterus*), has not been spawned in captivity because its natural spawning conditions are poorly documented and understood (Burgess, 1989). Based on both the above evidence and the fact that next to nothing is known of the ecology of the species, the habitat of *C. pretoriae* was identified, studied and characterised. Factors such as water depth, flow rate and substratum type were investigated with the aim of recreating a

similar habitat under artificial conditions in the laboratory on the assumption that a fish would stand more chance of spawning in familiar surroundings. The water quality was monitored on a monthly basis in order to establish seasonal trends and fluctuations and to correlate changes in water quality with cyclical biological events, eg. gonadal maturation.

A detailed study of the biology of the species was therefore also undertaken. However it was not the intention to undertake the investigation with the aim to interpret its life history style in terms of modern theories (eg. Balon, 1990). The study was undertaken purely to examine the biology of the species as an aid towards the development of a culture protocol. Thus the study of the reproductive biology was undertaken to establish what environmental parameters stimulated gonadal recrudescence and when the animal spawns, to establish its natural sex ratio, the length at sexual maturity and to determine the nature of its spawning and its relative fecundity. It was hoped that these data would provide information essential to the design and management of a successful culture system.

To successfully feed fish under culture conditions and to satisfy their nutritional requirements in order to effect gonadal recrudescence and good growth and condition, the natural diet of *C. pretoriae* was examined. Based on the nutritional composition of the natural diet it was hoped to find a suitable substitute. A study of the age and growth was also undertaken to determine at what age fish attain sexual maturity and to calculate the time it would take for the fish to attain marketable size. An attempt was also made to identify the spawning grounds of *C. pretoriae* in order to establish the conditions under which the eggs develop naturally. It was also decided to study the early development of the species in order to establish the rate at which *C. pretoriae* progresses through various possible vulnerable stages, and to establish whether there are any large scale mortalities at hatching or first feeding. It was hoped that the results of these investigations would assist in the design and operation of a

system in which the catlets would feed, grow and reproduce.

The thesis has been divided into two sections. The first section deals with the biology and ecology of the species and the results of the respective investigations are briefly summarised at the end of each chapter. This section is concluded by a discussion of the biology and ecology of the species with particular emphasis on and the implications of these findings to the culture of *C. pretoriae*. The second section of the thesis describes the development of a culture protocol for the species. This includes a description of the culture system and the different feeding and spawning experiments carried out. Once successful feeding and spawning techniques had been developed for the species a preliminary investigation into the primary nursing stage and final production rates was planned. The latter would ultimately be used as a basis upon which the feasibility of the culture of *C. pretoriae* for the ornamental fish trade could be assessed. The study as a whole is concluded by discussing the culture of *C. pretoriae* and placing the research into a broader context.

CHAPTER 2.

NOTES ON THE DISTRIBUTION AND TAXONOMIC STATUS OF *CHILOGLANIS PRETORIAE*.

Order : Siluriformes
Family : Mochokidae
Genus : Chiloglanis Peters, 1968
Species : *Chiloglanis pretoriae* v.d Horst 1931
Common name : Rock catlet

The rock catlet, *C. pretoriae*, belongs to the family Mochokidae which consists of nine genera and 155 species all of which are endemic to tropical Africa and the Nile valley (Berra, 1981). To date the only information available on this family is of a taxonomic and distributional nature and some anecdotal notes on habitat preference (Crass, 1964 ; Bell-Cross, 1972).

The natural distributional range of *C. pretoriae* includes the Limpopo, Incomati and Pongola River systems (Jubb and Le Roux, 1969). More detailed information on its distribution within these river systems is also available. Bell-Cross (1972) stated that *C. pretoriae* are not found in high altitude streams (above 1372m above sea level (ASL)) within the Limpopo River system. However, Hecht and Scholtz (1983) documented the distributional range of *C. pretoriae* in the Steelpoort tributary of the Olifants River (Limpopo River system) to be from 1750m ASL to 457m ASL. During the collecting trips carried out by the author, this species was collected in Louw's Creek at 1600m ASL. Changing altitude has a noticeable effect on water temperature. *Chiloglanis pretoriae* were collected in locations where the minimum temperature was found to be 12.4 $\pm$2.1 ^{o}C at an altitude of approximately 1750m ASL and in other locations where a maximum temperature of 31^{o}C was recorded at an altitude of approximately 457m ASL (Hecht and

Scholtz, 1983). Kleynhans (1984), documented the temperature range for *C. pretoriae* to be between 11 ^{o}C and 24 ^{o}C. Thus available evidence suggests that *C. pretoriae* can tolerate water temperatures of 11^{o}C to 31 ^{o}C and its distribution within the above stated river systems extends from approximately 457m ASL to 1750m ASL.

Although *C. pretoriae* appears to occur naturally within a range of climatological regions, its habitat preference within these regions is relatively specialised (Crass, 1964 ; Bell-Cross, 1972). The preferred habitat of this species consists of rocky rapids and riffles, and it is confined to these to such an extent that it may even disappear from stretches of river where flow ceases (Gaigher,1973 and Kleynhans, 1984). This was also evident during the collecting trips carried out for this study. Fish were found only in fast flowing rapids and riffles (current speeds greater than 0.6 m/sec). Hecht and Scholtz (1983) collected *C. pretoriae* in cascades and stickles where the substratum consisted of rocks, pebbles and sand. The collection stations consisted of relatively clear flowing water (between 30% and 50% turbidity) with high dissolved oxygen concentrations (90 and 108% saturation).

Chiloglanis pretoriae is a small mochokid which rarely exceeds 75mm in length. It has three pairs of barbels, a maxillary pair and two pairs of mandibular barbels, the latter incorporated into an elaborate "beard-like" sensory structure (see Figure 2.1). The mandibular barbels are short, often looking more like points on the large lips. *Chiloglanis pretoriae* has a well developed sucker-like mouth (see Figure 2.1) which it uses to suck and hold onto rocky surfaces enabling it to inhabit fast flowing streams and rivers. This catlet possesses lockable dorsal and pectoral spines. The dorsal fin has one spine and five to six soft rays. The dorsal fin spine is about the length of the head, and is unserrated (Jubb, 1967). The usual colour of this catlet is olive brown with olive yellow markings, the rayed fins having dark bands across them.

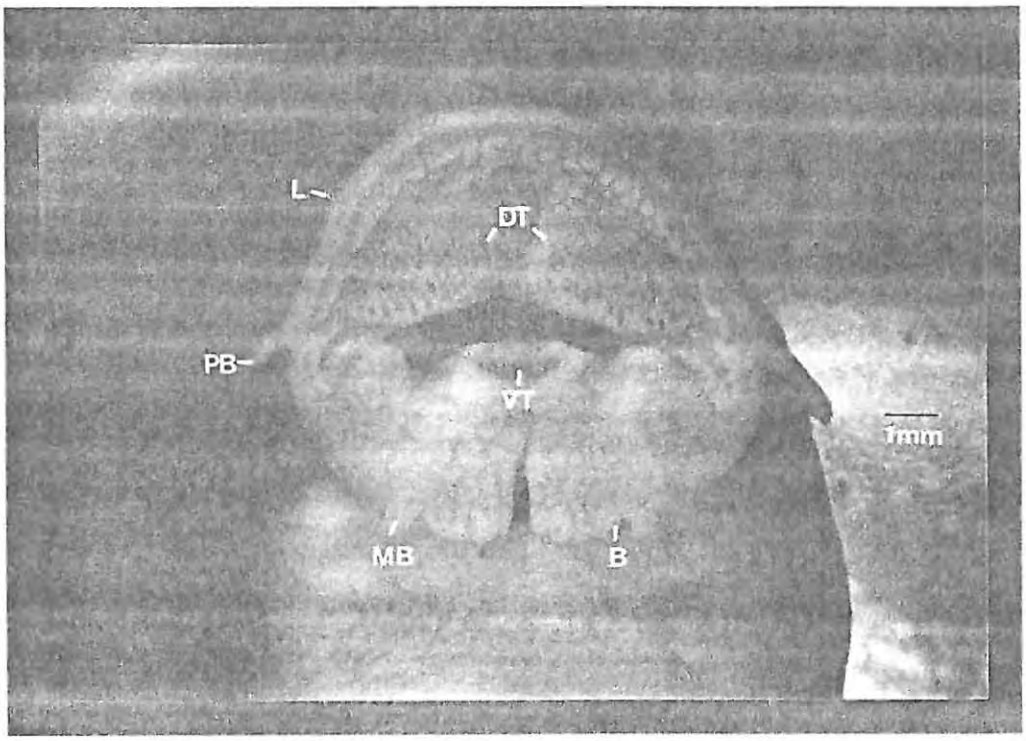

Figure 2.1. The characteristic beard like sensory system showing the short premaxillary pair of barbels (PB) which have been incorporated into the top "lip" structure (L) and the two pairs of mandibular barbels (MB), which have been incorporated into the sensory "beard-like" structure (B). Note the two dorsal tooth pads (DT) and the ventral row of teeth on the lower jaw structure (VT).

Jubb and Le Roux (1967) describe *C. pretoriae* as being a morphologically variable species within its distributional range. Limpopo River fish have a dorsal spine length of 12% - 15% of the standard length, the dorsal spine being shorter in specimens from the Blyde River, and very short in the specimens from the Incomati River system where the dorsal spine is only 5-10% of the standard length. This variability within a species and the similarity between the different *Chiloglanis* species can result in identification problems. For example, *C. anoterus* differs from *C. pretoriae* chiefly in the form of the caudal fin. The medial rays of the caudal fin of mature males are elongated to give the fin a pennant-like appearance (Gaigher, 1973). However, this elongation is barely noticeable in some specimens, while in female *C. anoterus*, elongated median rays in the caudal fin are totally absent. Therefore, without knowing their origin it is virtually impossible to separate the females of the two species. In a few cases other morphological characteristics may be used to determine the difference between the two species. In the Incomati River system for instance, *C. pretoriae* have shorter spines which results in a positive identification of the two species (Gaigher, 1973).

CHAPTER 3.

HABITAT DESCRIPTION, WATER QUALITY AND COLLECTION OF MATERIAL

3.1. INTRODUCTION

The primary aim of this part of the study was to identify and study the physical and chemical characteristics of the natural habitat of *C. pretoriae*. The physical aspects of this habitat were studied with the aim of recreating a similar environment in the laboratory. In addition to this, a study of the water quality and photoperiod was also undertaken in order to identify those environmental parameters that exhibited seasonal variation. It was hoped that by comparing these trends with trends in the natural reproductive cycle of this species, the environmental parameters responsible for inducing gonadal recrudescence would be identified. It was also anticipated that if *C. pretoriae* was provided with the necessary conditions it might be induced to spawn in captivity.

The fieldwork was carried out at Louw's Creek (25^{o} 45' S; 31^{o} 15' E) in Kangwane (see Figure 3.1). The stream originates in the Sondeza Mountain Range (see Figure 3.2) and drains into the Kaap River, a tributary of the Crocodile River. Louw's Creek has a total catchment area of 50 km^2 and has a mean annual runoff of 4.9 Mm^3 (Mulder, 1986).

A tunnel transfers water into Louw's Creek from the Shiyalongubo Dam thereby creating an unnatural flow regime. A concrete canal at a weir below the chosen study site diverts large volumes of water for irrigation purposes. Consequently, the natural fast flowing rocky nature of the stream below the weir is altered to a

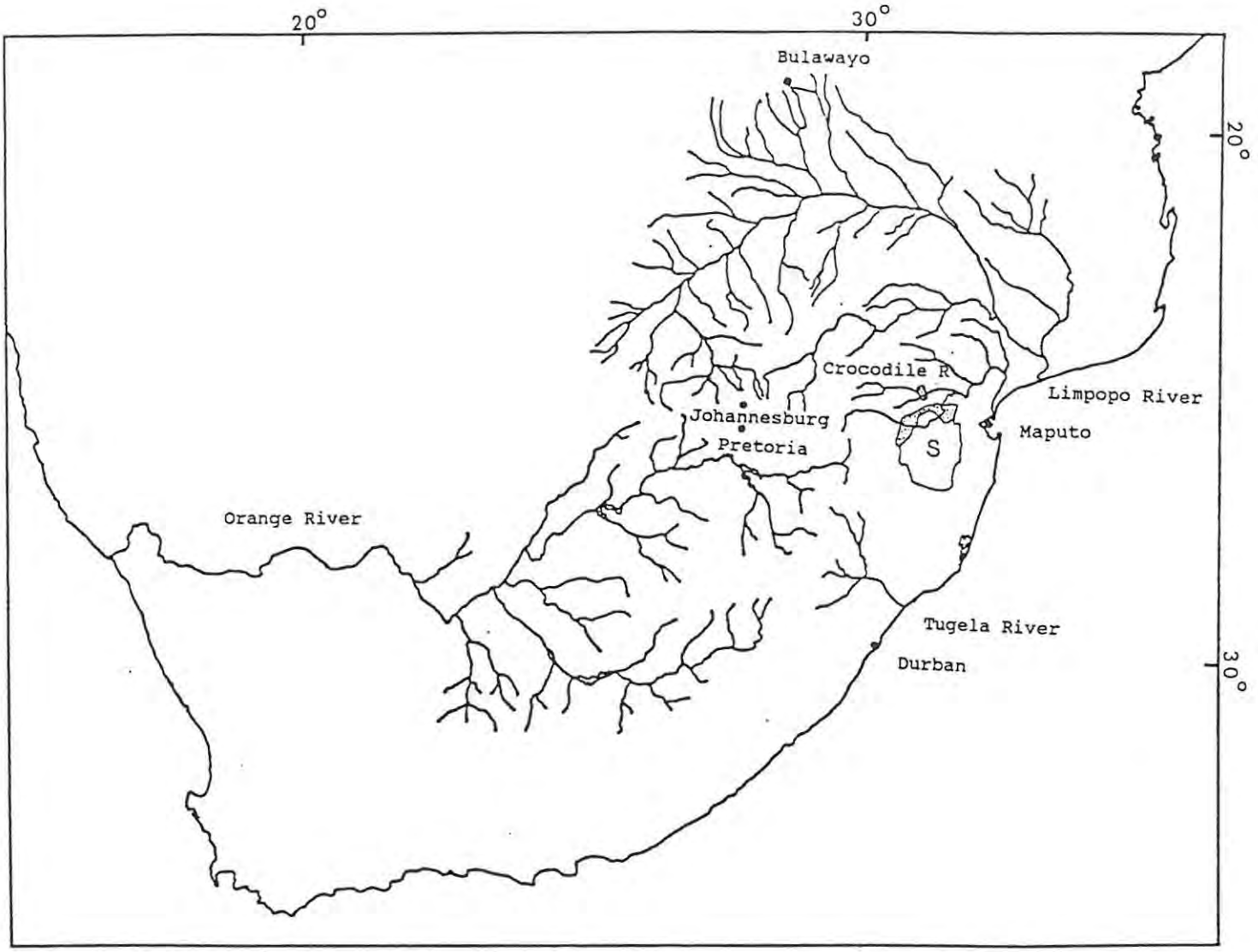

Figure 3.1. A map of South Africa showing the relative position of Kangwane (shaded area) with respect to the Crocodile River and Swaziland (S).

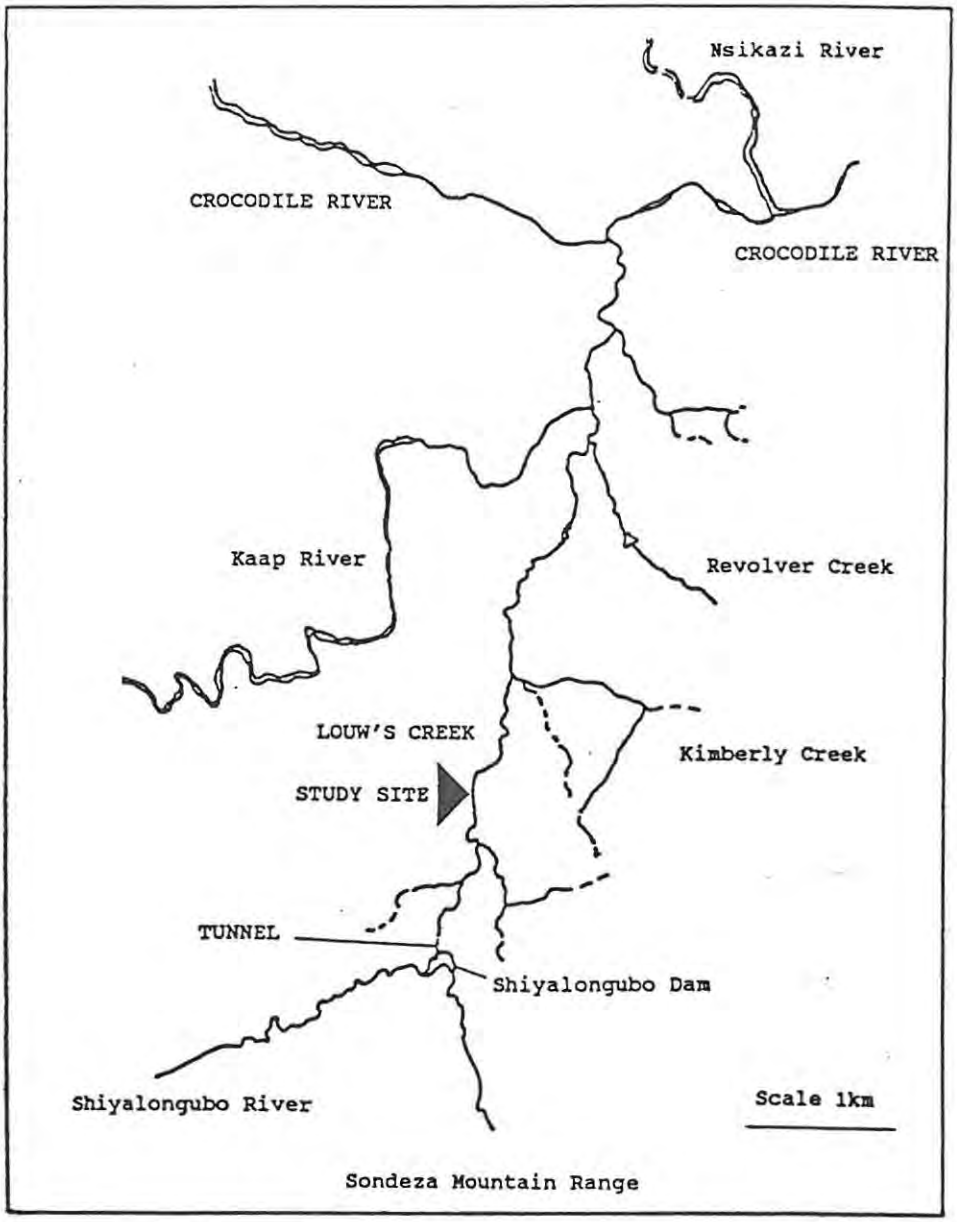

Figure 3.2. The location of Louw's Creek showing its source, flow direction and its confluence with the Crocodile River.

sluggish sandy bottomed stream until it enters the Kaap River. On isolated occasions during the rainy season, torrential rainfalls cause the weir to overflow and the trickle becomes a stream for a few days.

The study site selected for this investigation began a few metres upstream from the weir and extended some 400m upstream. Study site selection was based on the following criteria:

(i) An ichthyological survey carried out by the Kangwane Parks Board (Heymans, 1987), which established the occurrence of *C. pretoriae* in Louw's Creek.

(ii) Accessibility to the study site. This was essential as the project demanded monthly fish and water quality sampling. Louw's Creek fulfilled this requirement and furthermore, was in close proximity to the working areas of both the Kangwane Parks Board and Kangwane Public Works personnel who assisted with the monthly collection of fish and water quality samples respectively.

3.2. HABITAT DESCRIPTION

The average gradient of the chosen 400m stretch of stream was 1:6 metres. Based on water flow rates, substratum type and mean depth, three different habitat types were identified within the study site. Current speed was measured in metres per second (m/sec) using a flow meter (Oceans Mechanical Flow Meter - Model 2030R), a visual assessment of the substratum was made (the clear water facilitated this) and water depth was measured in centimetres (cm) with a graduated measuring pole. The stretch of Louw's Creek which was studied consisted of a number of alternating sections of rapids, riffles and deeper pools. Based on the above criteria each habitat was identified and the length of each stretch was measured in metres using a tape measure. The overall percentage of the respective habitat types was calculated as a percentage of the total 400m stretch.

Forty eight percent of the study site consisted of rapids (see Figure 3.3). These had a current speed which ranged from 0.8 to 1m/sec. The substratum consisted of rocks, pebbles and gravel and the mean depth was 10±8cm. Thirty five percent of the study site consisted of riffles (see Figure 3.4). These had a current that ranged between 0.5 and 0.8m/sec. The substratum consisted of rocks, pebbles and gravel and the mean depth was 20±11cm. Finally, 14% of the study site consisted of relatively deep pools with a current speed that ranged between 0.2 and 0.5m/sec (see Figure 3.5). The substratum of the pools consisted of gravel and fine sand and the mean depth was 100±35cm. A commercial mining venture upstream of the sampling site has resulted in silt deposition in these slower moving pools and eddies.

During this study *C. pretoriae* were only collected in the rapids and riffles. This supports the statement made by Crass (1964) and Bell-Cross (1972) that *C. pretoriae* has very specific habitat requirements. Available evidence suggests that this species prefers a physical habitat that has a relatively strong current speed and a substratum that consists of rocks, pebbles and gravel. The possible effects of siltation on the spawning success of *C. pretoriae* will be discussed later.

In order to describe Louw's Creek as a functional ecosystem the following information is also provided. Louw's Creek is flanked by steep mountain slopes. These are covered in dense evergreen forests which are dominated by *Podocarpus sp.* This riparian vegetation deposits large amounts of organic debris in the river. This allochthonous material together with autochthonous material and the dead and decaying animal matter, gives rise to a potentially rich food supply for herbivorous, omnivorous and carnivorous micro- and macrovores.

Other fish species found within the study site were *Amphilius natalensis* and *Barbus eutaenia*. The former favour the riffles while the latter inhabit the more protected pools and eddies.

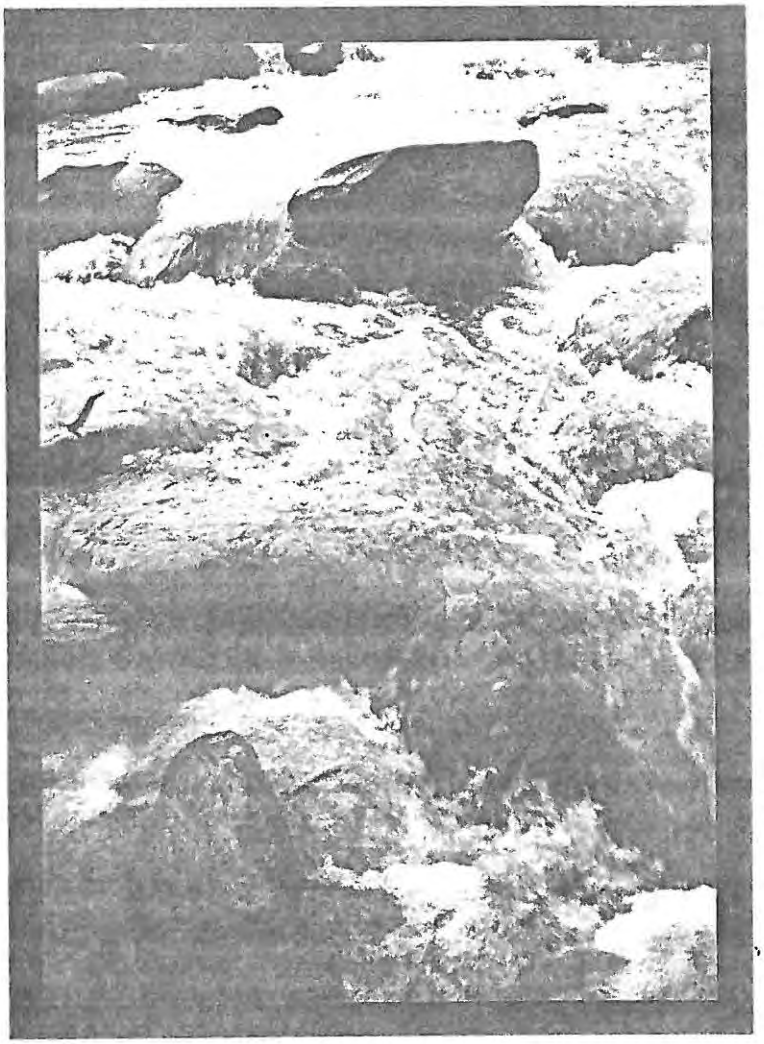

Figure 3.3. The rocky rapid habitat preferred by *Chiloglanis pretoriae*.

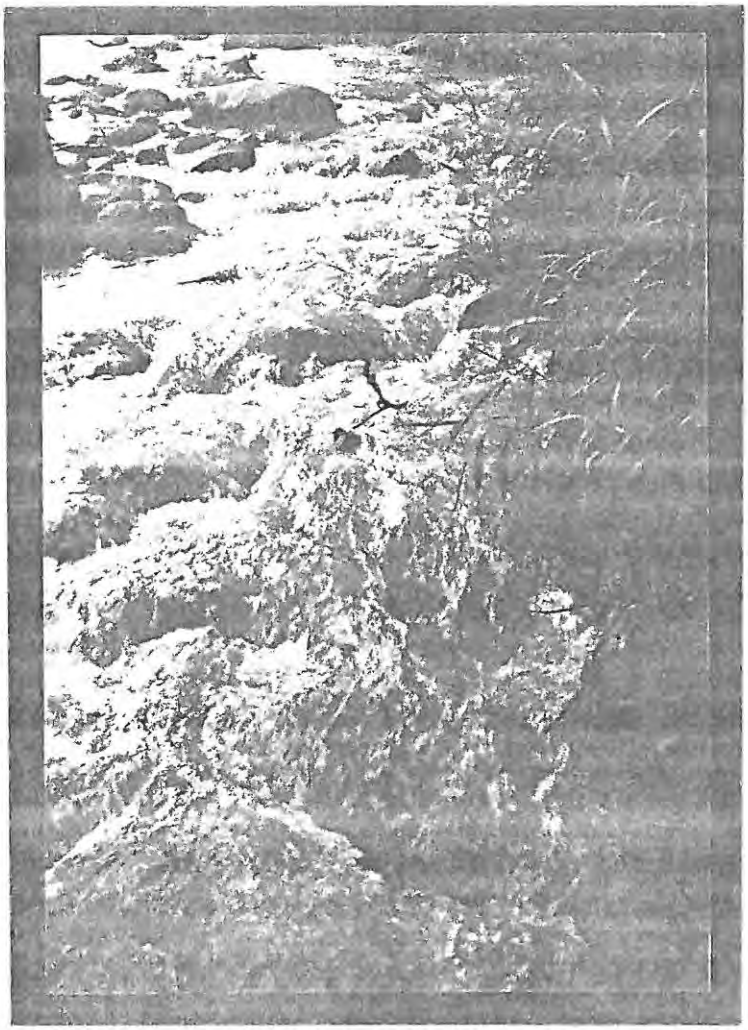

Figure 3.4. The riffle habitat dominated by *Amphilius natalensis* but also inhabited by *Chiloglanis pretoriae*.

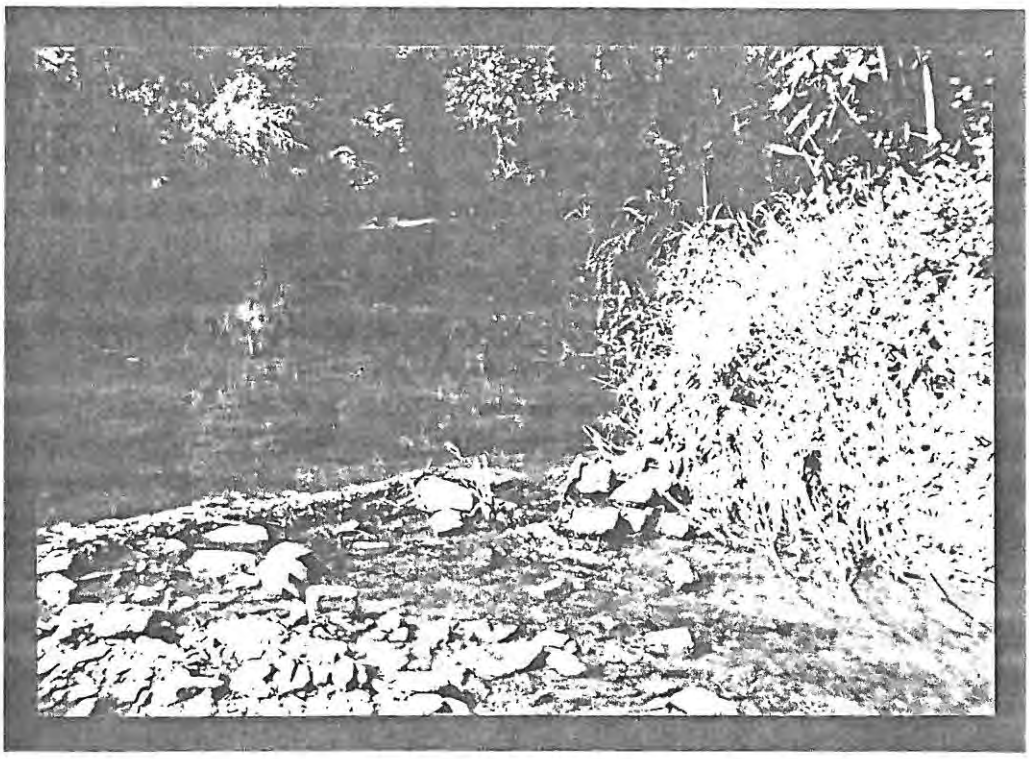

Figure 3.5. An example of a relatively still pool in which no *Chiloglanis pretoriae* were collected.

Appendix 1 illustrates the species diversity in Louw's Creek in general. However, these species are not generally found in the fast flowing headwaters and are also prevented from reaching the study site by the weir and concrete canal which diverts most of Louw's Creeks water for irrigation purposes. Eels (*Anquilla mossambica*), crabs and frogs are the only other dominant macrofauna which inhabit the upper reaches of this river (pers. obs.).

3.3. WATER QUALITY

Water quality in Louw's Creek was monitored on a monthly basis from January 1989 to January 1990. The following parameters were monitored : temperature, dissolved oxygen, pH, conductivity, and turbidity. For the duration of the study, the following instrumentation was used to obtain the water quality data :

1. Temperature : The water temperature was measured in degrees celsius using a glass thermometer (Mercury in glass thermometer accurate to $1^{o}C$)
2. Dissolved oxygen : The dissolved oxygen concentrations were measured in milligrams per litre (mg/l) using a portable oxygen meter (Yellow Springs Inc. Model 57).
3. pH : The pH was measured using a portable pH meter (Orion Research Model 201).
4. Conductivity : Conductivity was measured in millisiemens per metre (mS/m) using a portable conductivity meter (Hach Portable Model 16300).
5. Turbidity : Turbidity was measured in nephelometric turbidity units (NTU) using a portable turbidity meter (Hach Portable Model 16800). Nephelometric turbidity units describe the optical properties of the water rather than the type of particles suspended in the water column.
6. Photoperiod : Data on the natural photoperiod of the Kangwane area was provided by the Barberton Weather Bureau. Photoperiod was measured as the number of hours (hrs) of daylight between

sunrise and sunset.

Water temperatures (Figure 3.6) were lowest (18 ^{o}C) during the the winter months from May to August and began to increase noticeably in September. Water temperature peaked in October (24 ^{o}C) and remained relatively constant through to March, after which it declined.

The pH values (Figure 3.7) remained relatively constant throughout the year and ranged between 6.3 and 7.6.

Due to the current speed and the efficient oxygenating effect of riffles and rapids the dissolved oxygen (DO) levels ranged from 6mg/l to 8.4 mg/l (Figure 3.7) remaining near saturation throughout the study period.

During the winter and spring months the water remained relatively clear with NTU values ranging between 1.7 and 2.9 (see Figure 3.8). However, during the late spring and summer months the water became turbid (2.9 NTU to 47 NTU). The increase in turbidity is associated with the summer rains which wash substantial amounts of silt into the river. A potentially more important factor but closely associated with turbidity is the "settling velocity" of the suspended silt particles (Wilber, 1983). Large quantities of silt are constantly settling out in Louw's Creek altering sections of the river from clear rocky habitats to turbid muddy habitats. The implications for organisms that are dependent on clear rocky habitats for the successful completion of their life history cycle is potentially disastrous.

During the winter months conductivity remained relatively high (52mS/m) (see Figure 3.8). During dry periods ground water is the primary source of water for perennial streams. Sorenson, *et al.* (1977) stated that the conductivity in streams during relatively dry periods is high due to the amount of organic residues and salts existing in the inflowing ground water. These organic

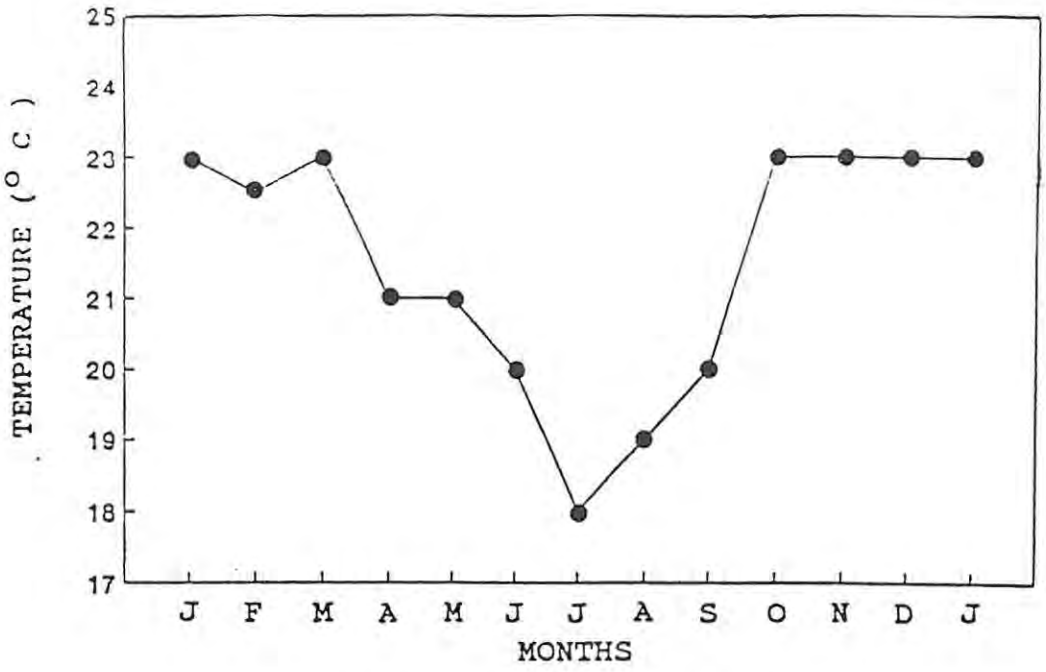

Figure 3.6. Monthly water temperatures in Louw's Creek during the period January 1989 to January 1990.

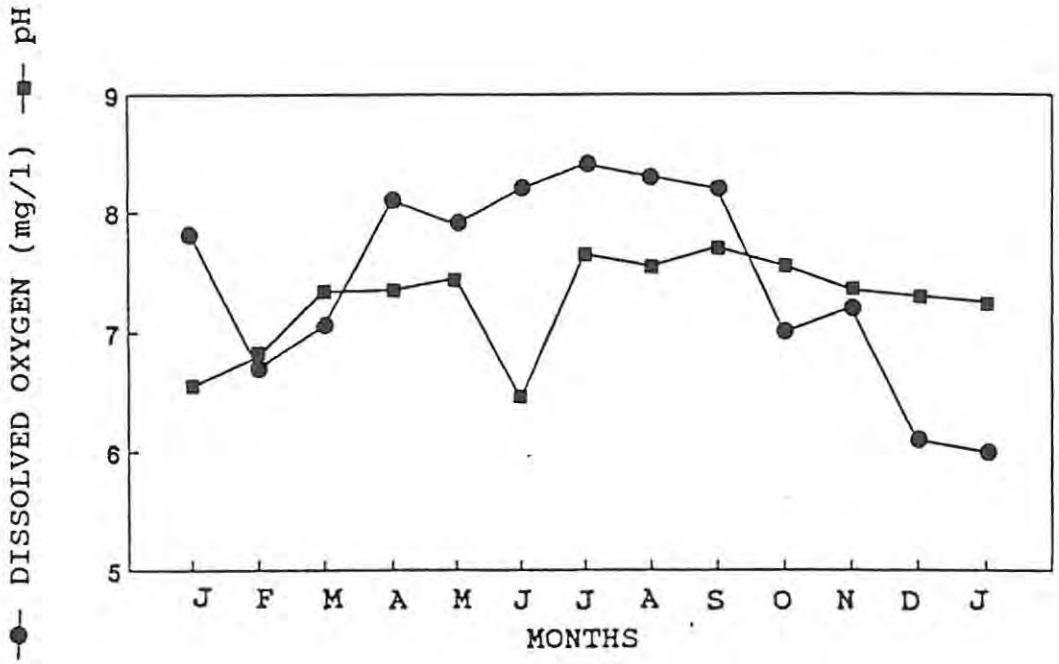

Figure 3.7. Monthly dissolves oxygen concentrations and pH values in Louw's Creek during the period January 1989 to January 1990.

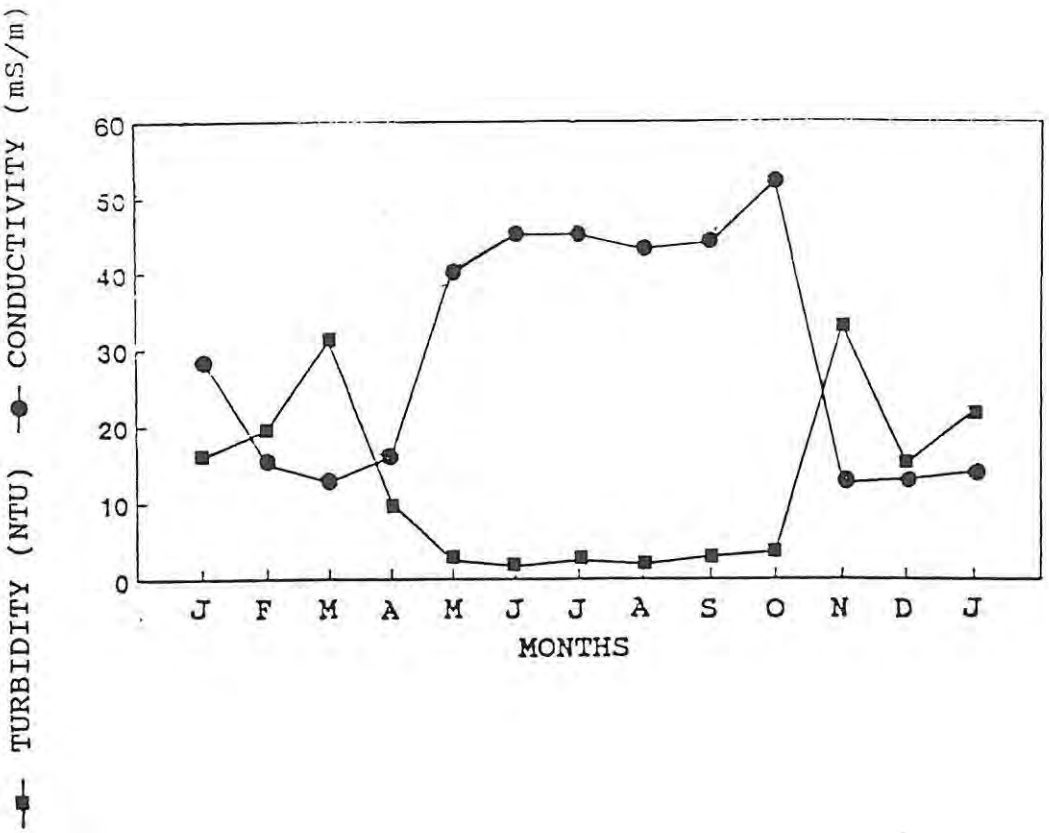

Figure 3.8. Monthly turbidity and conductivity values in Louw's Creek during the period January 1989 to January 1990.

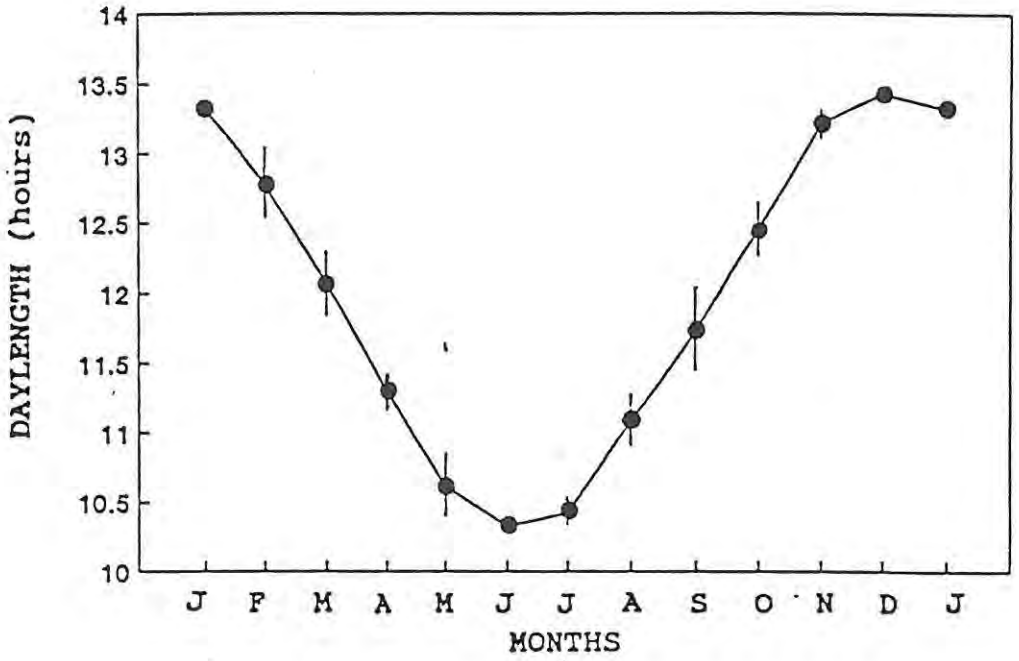

Figure 3.9. Monthly photoperiod during 1989. The vertical lines represent the standard deviation.

residues and salts have been leached from the surrounding vegetation and bed rock. Available evidence suggests that the spring rains increase the turbidity of Louw's Creek, but a decrease in conductivity occurs simultaneously (see Figure 3.8). Thus it may be assumed that the particles causing the increase in turbidity are uncharged particles otherwise the conductivity would also increase during this period.

Photoperiod began to increase in July and peaked in January (see Figure 3.9). The maximum daylength was documented at 13.7 hours, however an additional two hours may be added to compensate for the period of light before sunrise and after sunset (pers. obs.).

3.4. COLLECTION OF MATERIAL

Electro-fishing gear (220V AC Suzuki Generator SE 700A with two electrodes attached to a 30m electric cable) was used. The operator of the electro-fishing apparatus, wearing rubber waders, worked in an upstream direction holding the two poles approximately 0.5m apart. The stunned fish surfaced and were caught with hand-held dip nets. Fishes not caught by the dip nets were caught by a stop net placed downstream. No fish were killed using the above method. Sampling by use of electrofishing in the shallow rocky habitat was shown to be a most satisfactory method of collecting this species of fish.

Immediately after capture those fish required for biological analysis were fixed in 10% buffered formalin and were sent to Grahamstown. Those collected as broodstock were transported live back to Grahamstown.

The following method was found to be most effective for the transportation of live *C. pretoriae* : 50l plastic drums with sealing lids were used as containers. The rounded shape of the bottom of the barrel resulted in good water circulation throughout the 18 hour trips from Kangwane to Grahamstown. The

fish were transported in bore-hole water. The water was oxygenated with medical oxygen on the first trip, and later normal air pumps were tested. Prior to departure fish were purged by keeping them in holding tanks without food for two days. A 0.1% NaCl solution was used as the transport medium to reduce any osmotic imbalance due to stress (Piper *et al.*, 1982). A 100% survival rate resulted on all trips, irrespective of whether oxygen or compressed air was used.

3.5. SUMMARY

The results of this investigation showed that *Chiloglanis pretoriae* prefer fast flowing (0.8m/sec to 1m/sec) rocky rapids which were well oxygenated (above 6mg/l) with a pH range from 6.3 to 7.6. The turbidity readings in Louw's Creek ranged between 1.7 NTU and 47 NTU but it was assessed that the high values were artificial due to the excessive build up of silt from the mining activities upstream. The excessive build up of silt due to inefficient farming methods has also been described as artificial by Sorenson *et al.* (1977) and Moss (1988). It was therefore assumed that relatively clear aquarium water would not prove to be suboptimal for the spawning of *C. pretoriae*. Conductivity ranged between 52 mS/m and 12 mS/m, with the lowest readings measured over the summer months. A literature review revealed that very little is known about the effect of conductivity on fish. Therefore, the most likely option available with respect to the development of a spawning technique for *C. pretoriae* was to use the conductivity range that coincided with its natural spawning season. Photoperiod (daylength) and water temperature increased through spring towards their respective peaks in summer. The primary nutrient source for this river system was the riparian vegetation which deposited large amounts of allochthonous material into the river. This provided the basis for secondary production in this river system.

CHAPTER 4.

REPRODUCTIVE BIOLOGY

4.1 INTRODUCTION

The effect of environmental parameters such as photoperiod, water temperature and feeding on gonadal maturation and ovulation have been widely documented in the literature (Bromage *et al.*, 1982 ; Baggerman, 1982 ; Marsh *et al.*, 1986 ; Carrillo *et al.*, 1989). These and other authors have shown that gonadal activity has a cyclic pattern and that there is a reproductive rhythm that may be triggered by environmental factors. The success of the trigger mechanism depends on a particular timing mechanism, the endocrine system, through the production of several hormones (Bearden and Fuquay, 1980). The use of biological and ecological data to assist in the development of successful spawning techniques for fish in captivity is shown in papers by Bruton (1979), Noakes and Balon (1982), Bruton (1983), Saint-Paul (1986), Neophitou (1986), Dwivedi and Reddy (1986) and Burt *et al.* (1988) among others. Studies on the reproductive biology of wild fish have yielded information on natural sex ratio, length-at-maturity, spawning periodicity, physical and chemical spawning requirements, and the spawning strategy of many fish species (*inter alia* Bruton, 1979 ; Noakes and Balon, 1982 ; Bruton, 1983 ; Saint-Paul, 1986 ; Neophitou, 1986 ; Dwivedi and Reddy, 1986 ; Burt *et al.*, 1988). The aim of this investigation was therefore to examine the reproductive biology of *C. pretoriae* as a contribution towards an understanding of the natural life history of the species, and also as a basis for developing a spawning technique.

Fish samples were collected on a monthly basis and their gonads were dissected out and examined. Particular attention was paid to the identification of the length at which the species attained

sexual maturity and establishing whether it exhibited any seasonal trends in gonadal activity. An investigation into the size frequency distribution of ova and the relative fecundity was also undertaken as it was hoped that this would shed some light on the spawning strategy of the species.

The length at which *C. pretoriae* becomes sexually active, together with the relationship between fish length and fecundity, provides the basis for size selection of broodstock. Spawning seasonality was correlated with environmental data to investigate the possible effects of changing environmental parameters on gonadal recrudescence, and so provide pertinent information for the final system design. The size frequency distribution of the ova may indicate whether the particular species is a total or multiple spawner (Conover, 1985). Relative fecundity was only calculated once the size frequency distribution of ova had been established, as a possibility existed that *C. pretoriae*, like *C. anoterus* (Kleynhans, 1984), had at least two egg size classes which would indicate that it may be a serial spawner. If this was indeed so, the calculation of relative fecundity should ideally incorporate data on batch size and spawning frequency (Conover, 1985 and Burt *et al.*, 1988). The spawning strategy of *C. pretoriae* together with its relative fecundity provided an estimate of the possible number of eggs a female can produce under specific conditions over a set period of time. This knowledge was deemed to be essential in developing a culture protocol.

4.2 MATERIALS AND METHODS

After the fish had been measured, weighed and sexed, their gonads were carefully dissected out and weighed to the nearest 0.01g. Males and females could be distinguished externally from each other as males had a clearly visible genital papilla (Figure 4.1). During this investigation biological analysis was restricted to the ovaries as testes did not show clear

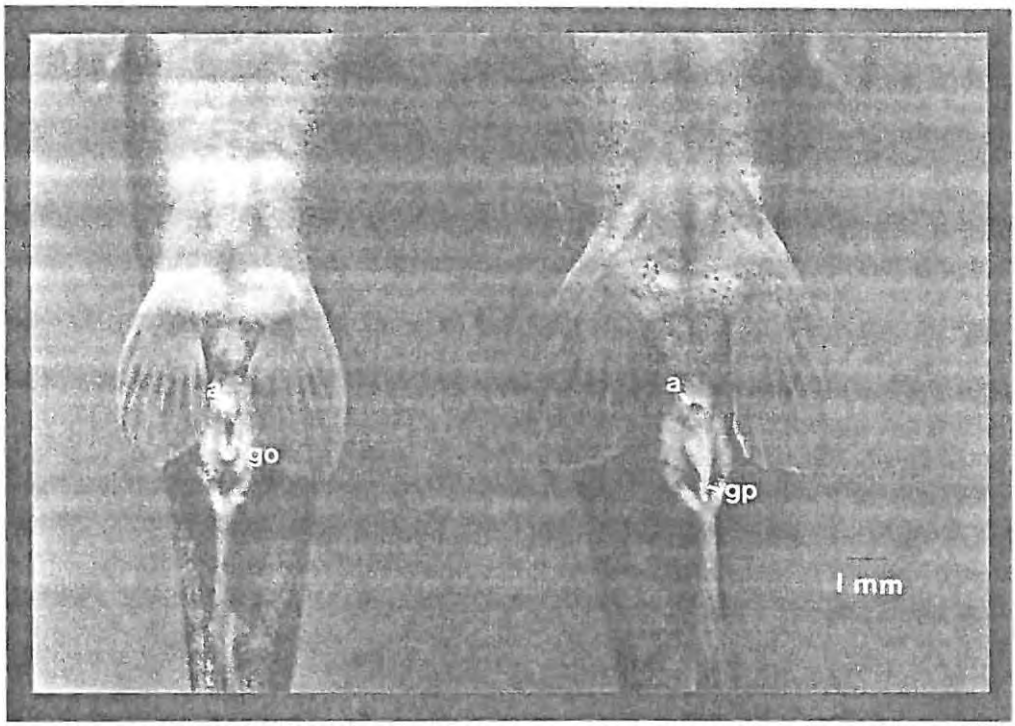

Figure 4.1. A male (right) and female (left) *Chiloglanis pretoriae*. Note the genital papillae of the male (a-anus, gp-genital papillae, go-genital opening).

macroscopic differentiation between the different maturity stages. However, it may be assumed that gonadal maturation in both sexes is synchronised in order to facilitate successful spawning. Aquarium studies showed this to be the case.

Table 4.1 represents the maturity stages allocated during this project. Fish with gonads in stage one were regarded as virgin (Buxton, 1988) or immature fish. Those with gonads in stages two, three and four were regarded as sexually mature. Figure 4.2 demonstrates the difference in size between a ripe and a spent ovary (ovaries were taken from fish of similar length) and also shows a ripe testis.

A record of the state of maturity of fish of different length groups was used to establish the length-at-maturity of *C. pretoriae* and to establish what proportion of the fish in each of the monthly samples were reproductively active (Ricker, 1975). Length-at maturity was that length at which gonadal recrudescence was observed for the first time and was calculated by plotting total length against the accumulated percentage of mature fish within that size range (Van der Elst, 1976 ; Elder, 1976). Immature (Stage one) and mature (Stage two to four) fish were clearly distinguishable from each other (see Table 4.1).

Reproductive seasonality was determined by calculating the monthly Gonadosomatic Index (GSI) values. These were calculated using the following formula : (after Meien, 1927)

$$GSI = \frac{\text{gonad mass}}{\text{total fish mass}} \times 100$$

To determine the size frequency distribution of ova, all the eggs in the mature gonads were measured with a microscope eyepiece micrometer accurate to 0.01 mm. The data were used to construct a

Table 4.1. Classification of the gonadal maturity stages of male and female *Chiloglanis pretoriae*.

Maturity stage	Description
1. Virgin	Sexual organs small. Both testis and ovaries are white. No eggs visible in the ovary.
2. Developing	Increase in size of both male and female gonads indicating sexual maturity Testis and ovaries are white to cream in colour, and eggs are visible in the ovary. Different egg sizes exist in the ovary comprising recruitment and developing eggs.
3. Ripe	Increase in size of both testis and ovary. The testis gives the impression of being swollen and is cream in colour. The ovary shows a dramatic increase in width and length giving the appearance of being swollen and occupying approximately 75% of the abdominal cavity. Large yellow eggs are visible through the ovary wall.
4. Spent	Marginal decrease in size of testis which are still cream in colour. Ovary shows a decrease in size with a decrease in the number of eggs over 1mm in diameter, i.e. developing and ripe eggs. The ovary is cream in colour with the smaller eggs visible through the ovary wall. It now occupies less than 50% of the abdominal cavity.

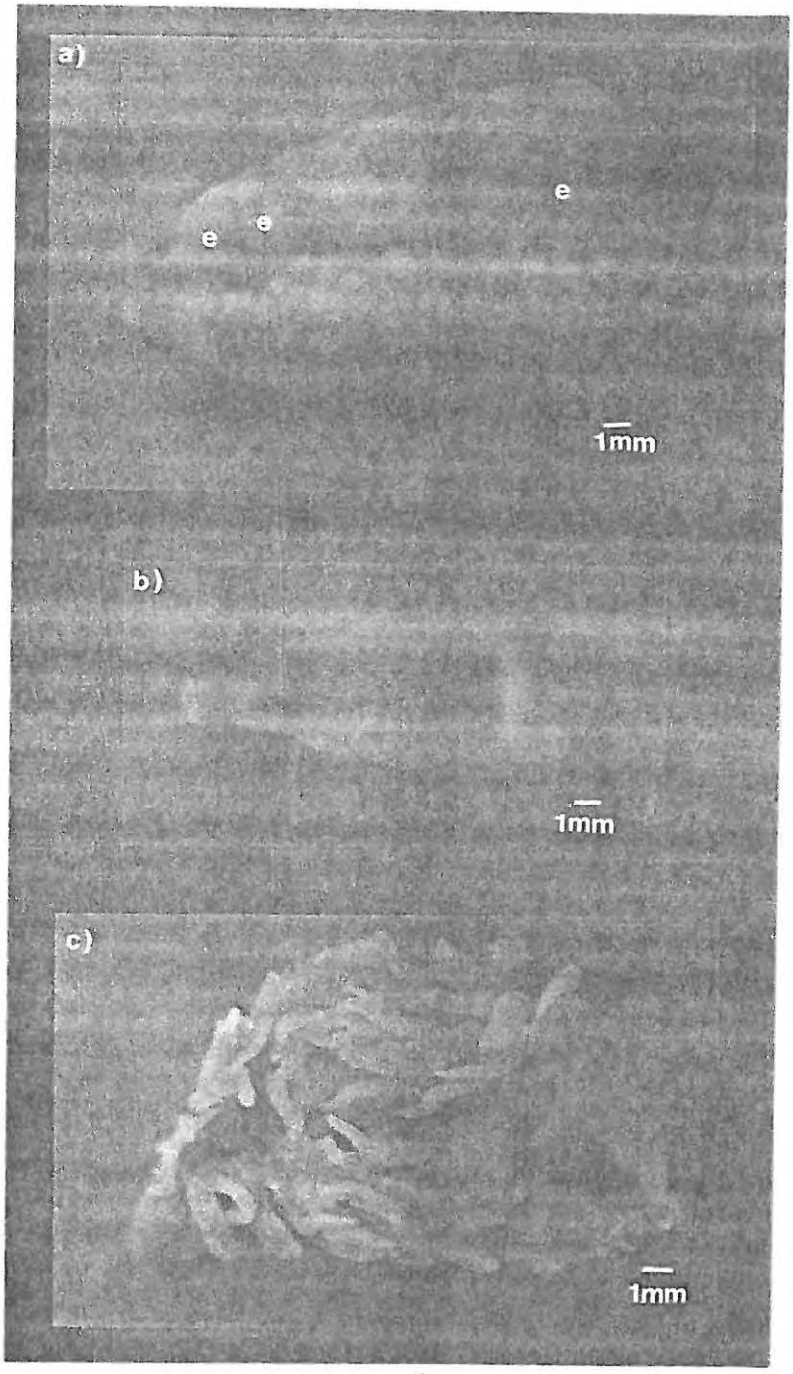

Figure 4.2. a) A ripe female gonad (e-ripe eggs) b) A spent female gonad and c) A male gonad. It was difficult to distinguish between ripe, developing and spent male gonads.

size frequency histogram.

Histological preparations were made, according to the methods used by Humason (1979) and were used to verify the macroscopic stages of egg development. Gonadal tissue was dehydrated through a series of increasing alcohol concentrations, cleared in toluene and impregnated with paraplast wax. Impregnated gonadal tissue was imbedded in blocks of wax to facilitate the sectioning process. Material was sectioned at six micrometres using a Lipshaw rotary microtome (Model 45). The sections were mounted onto glass slides and stained with Gill's Heamatoxylin, a protein stain, and two cytoplasmic stains, Orange G and EA 65, after which they were left to dry. Coverslips were then placed over the stained material using DPX mounting medium. The tissue was examined using a Nikon Microscope with a Nikon Microflex AFX-II photographic attachment. Histological sections were interpreted according to the methods used by Buxton (1990).

Relative fecundity is defined as the number of ova per gram of fish (Bagenal, 1967). The relative fecundity of *C. pretoriae* was calculated as the number of eggs (excluding the recruitment eggs) per gram of fish at the time of capture. For obvious reasons, only those fish collected over the spawning season were used in the calculation of relative fecundity for the species.

Regression analysis was used to establish the relationship between fish length and fecundity, which in this case was restricted to the number of developing and ripe eggs present in the ovary during the spawning season.

4.3 RESULTS

Figure 4.3 shows the length at 50% and 100% sexual maturity. Fifty percent maturity occurs at 44mm and 100% maturity occurs at 50mm. From October to March, (n=45) between 70% and 87% of the monthly fish sampled exhibited ripe gonads (Stage three). Only

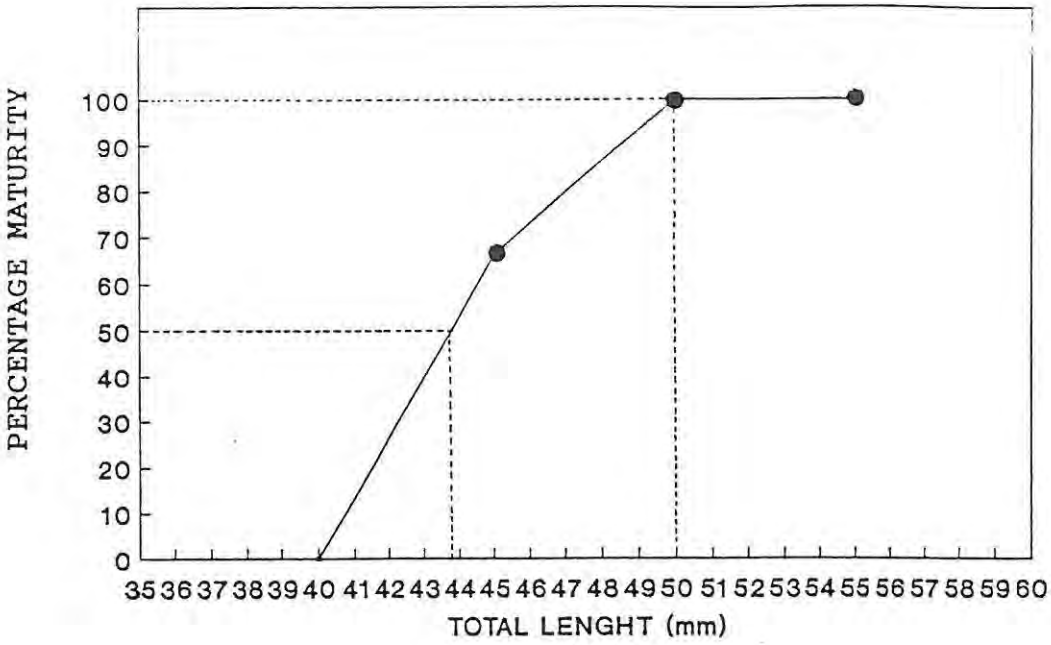

Figure 4.3. The length at sexual maturity of female *Chiloglanis pretoriae*.

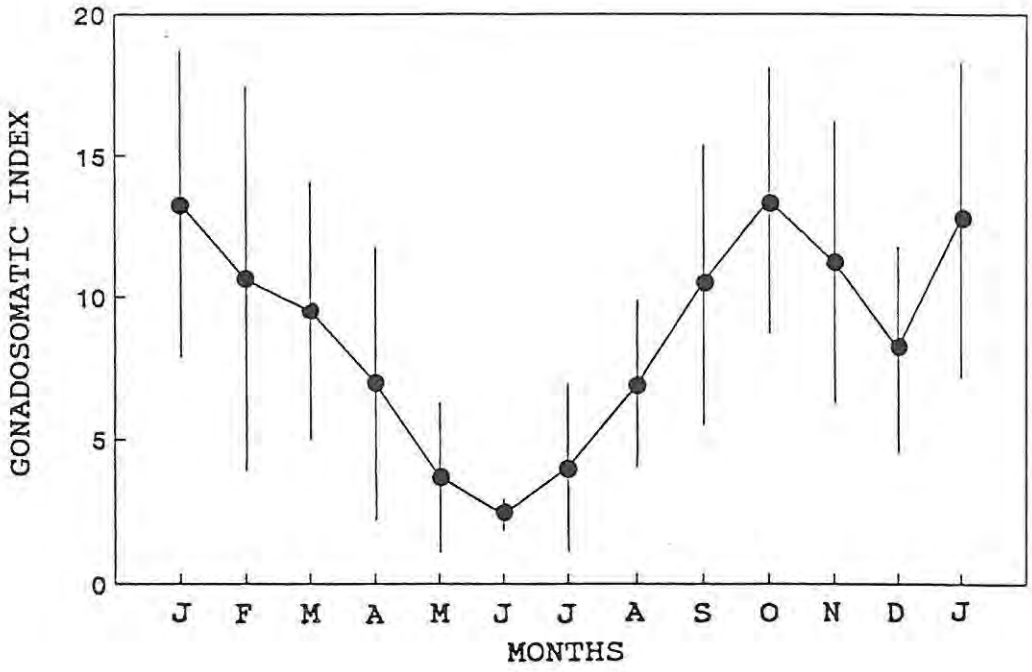

Figure 4.4. Seasonal changes in mean monthly gonadosomatic index values of *Chiloglanis pretoriae*, calculated from the monthly samples of fish collected in Louw's Creek during the period January 1989 to January 1990. The vertical bars represent the standard deviation.

25% of the fish sampled in September (n=8) exhibited gonads in stage three which suggests that the spawning season for *C. pretoriae* effectively begins in October. Only 46% of the fish sampled during April (n=9) exhibited gonads in stage three, while 94% of the fish sampled during May (n=7) exhibited spent gonads (Stage four).

The mean monthly Gonadosomatic Index values are shown in Figure 4.4 and reveal an extended breeding season from October to March after which there is a distinct decline during April and May.

Different egg size classes were identified in the ovaries of *C. pretoriae* (see Figure 4.5). Ovaries in stage three contained three distinct egg size classes, namely eggs smaller than one millimetre in diameter, eggs between one and two millimetres in diameter and eggs between two and three millimetres in diameter. Ovaries in stage two contained eggs in the former two egg size classes while ovaries in stage four only contained eggs in the first size class. The difference between the three egg size classes was substantiated by the results of the histological investigation.

The results from the histological investigation indicated that eggs less than one millimetre in diameter were recruitment eggs which showed no signs of yolk development. Eggs which ranged between one and two millimetres in diameter were classified as developing eggs as they were in various stages of vitellogenisis. Eggs over two millimetres in diameter exhibited large dense yolks and were therefore classified as mature eggs. Figure 4.6 demonstrates the differences between recruitment eggs, developing eggs and ripe eggs.

The relative fecundity of *C. pretoriae* varied between 15 eggs per gram fish mass to 40 eggs per gram fish mass. The variation may be attributed to the serial spawning nature of the fish. It is suggested that the sampled fish were in different stages of their annual reproductive cycle. In order to calculate a theoretical

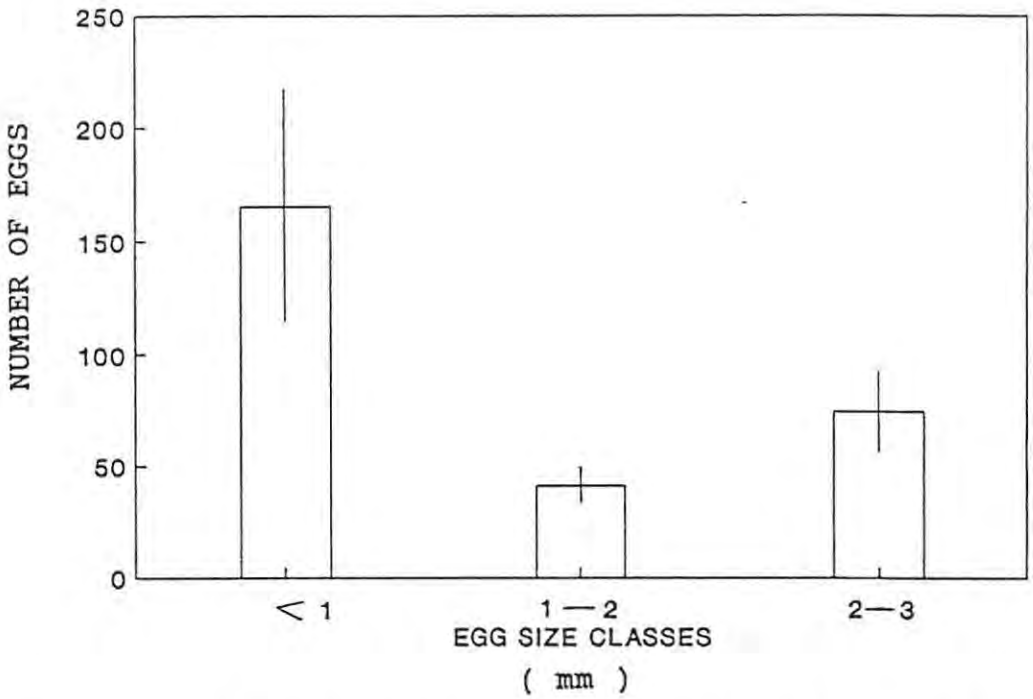

Figure 4.5. The size frequency distribution of eggs in the ripe ovaries (Stage three) of *Chiloglanis pretoriae*. The vertical bars represent the standard deviation.

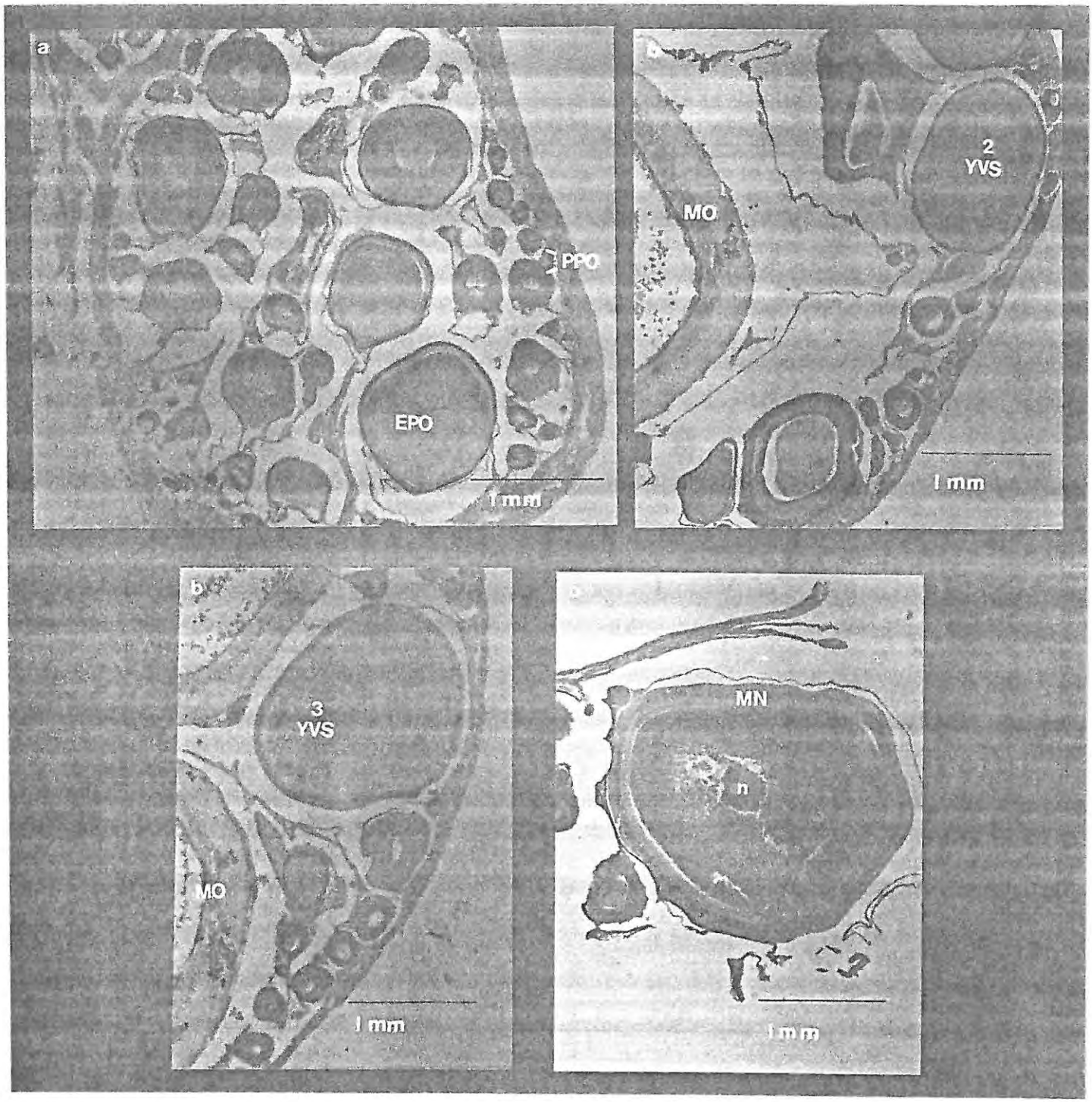

Figure 4.6. Oocyte development in *Chiloglanis pretoriae*. (a) A transverse section through a spent ovary showing PPO=pre-perinuclear oocytes, EPO=early perinuclear oocytes (both PPO and EPO were termed recruitment eggs during this study). (b) A transverse section through a ripe ovary (Stage three) showing 2^{o}YVS=secondary yolk vesicle stage, 3^{o}YVS=tertiary yolk vesicle stage, MO=mature egg that has collapsed during preparation and MN=the migratory nucleus stage (n=nucleus) just prior to final maturation. For the duration of this study the eggs in the yolk vesicle stage were termed developing eggs, while those eggs in the nucleus migratory stage and the final mature stage were collectively termed mature eggs.

relative fecundity value for the species the following formulae were used :

1. The relationship between total length and number of developing and ripe eggs (see Figure 4.8).

Number of eggs = -114.6 + 3.1(TL)

2. The relationship between total length and body mass (see Figure 6.4).

Mass = $0.0000048\ (TL^{3.2})$

3. A theoretical number of eggs was calculated for a fish of a specific total length. The calculated number of eggs was then divided by the theoretical mass calculated for a fish of the same total length. The results of the above methods produced a theoretical relative fecundity for fish of known lengths.

This method was used to calculate the relative fecundity of fish 50mm, 60mm and 70mm (TL). A mean relative fecundity could then be calculated for the species (see Table 4.2).

Table 4.2. The relative fecundity calculated for *Chiloglanis pretoriae* using the above formulae.

Total length (mm)	Relative fecundity (eggs/g fish mass)
50	31.25
60	30.64
70	26.75
MEAN	29.50

The mean relative fecundity of *C. pretoriae* is 29.5 eggs per gram of fish mass.

As stated earlier the relative fecundity was calculated as the number of developing and ripe eggs in the gonad. During the winter months when the fish were not reproductively active their gonads contained no eggs belonging to the two larger egg size classes, hence the low values over this period (see Figure 4.7). This information may also be used to provide an indication of the reproductive seasonality of *C. pretoriae* (see Figure 4.7).

The number of eggs increased with increasing fish length (see Figure 4.8). A fish of 50mm (TL) has a theoretical fecundity of 41 eggs (including developing eggs), while a fish of 70mm (TL) has a theoretical fecundity of 103 eggs (including developing eggs).

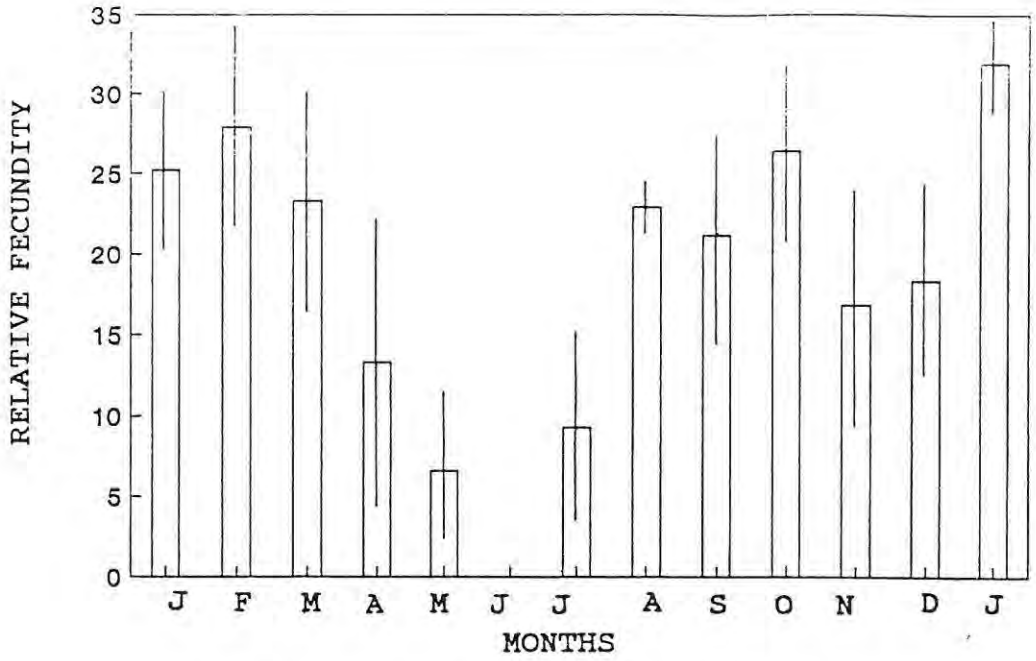

Figure 4.7. Seasonal changes in the mean relative fecundity of *Chiloglanis pretoriae*, calculated from the monthly samples of fish collected in Louw's Creek during the period January 1989 to January 1990. The vertical bars represent the standard deviation.

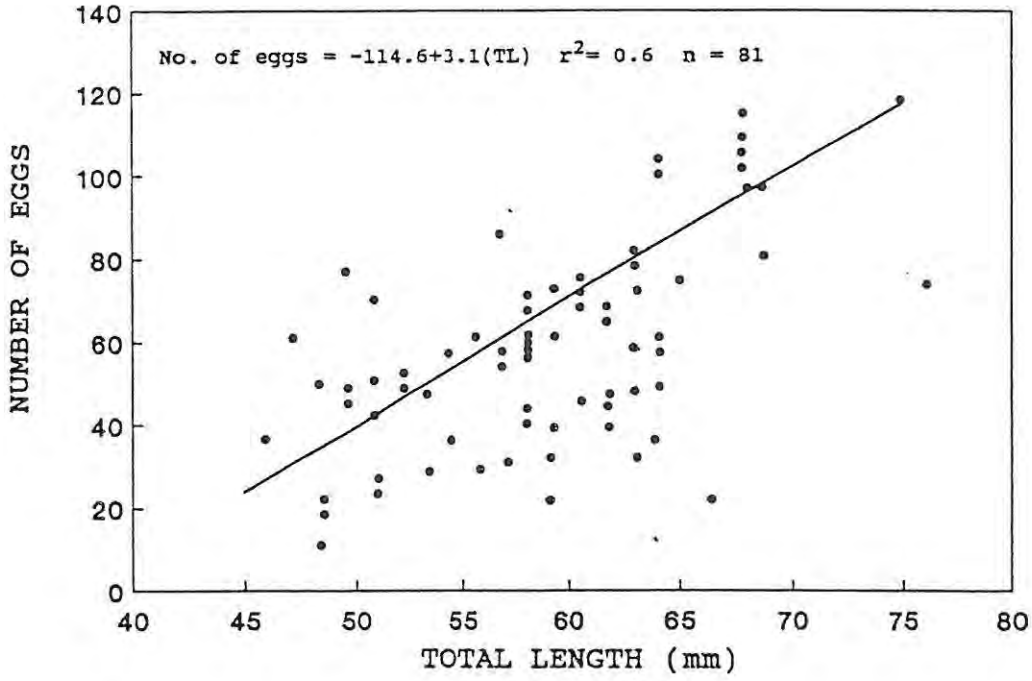

Figure 4.8. The relationship between fish length and the number of developing and ripe eggs.

4.4. SUMMARY

Chiloglanis pretoriae attains 50% sexually maturity at 44mm in length and 100% sexual maturity at 50mm in length. This indicates that all broodstock for the proposed culture programme should be larger that 50mm (TL). The available data suggests that the spawning season of *C. pretoriae* extends over the summer months from October to March. If one is relying on natural spawning techniques, then the summer conditions would have to be maintained for extended periods to provide for maximum production rates. The natural extension of the spawning season also suggests that, if provided with the necessary conditions, it may be extended further. There was a definite correlation between photoperiod, water temperature and seasonal changes in gonadal activity, indicating that these two environmental parameters play a major role in the reproductive cycle of *C. pretoriae*. Thus the spawning system should include and control these two parameters in order to stimulate natural spawning.

Available evidence suggests that this catlet is a serial spawner that lays a few relatively large eggs which are replaced by developing ova in the same season. The yolk size and density and the absence of an oil droplet suggests that the eggs are negatively buoyant. This may have implications for the egg harvesting and incubation methods. The large eggs will probably facilitate the development of large larvae which has implications for the larval feeding programme.

When calculating the relative fecundity of *C. pretoriae*, all the figures used included developing eggs and therefore all calculated figures were based on the assumption that the developing eggs were spawned during that spawning season.

The relative fecundity of the species varied throughout the extended spawning season. However, the theoretical value of 29.5 is regarded as a fair representation of the relative fecundity of the species. This value is low when compared to *Heterobranchus*

longifilis which has a relative fecundity of up to 90 eggs per gram of fish mass (Legendre, 1986). However, *C. pretoriae* has a similar relative fecundity to *Clarias gariepinus* which has been documented to have a relative fecundity of 33.5 eggs per gram mass of fish (Hecht *et al.*, 1982). *Clarias gariepinus* is an established culture species which indicates that a fish species exhibiting a low relative fecundity is not necessarily unsuitable for aquaculture. Available information on the relative fecundity of aquarium species is limited and does not provide for a meaningful comparison, eg. female *Synodontis brichardi*, approximately 250mm in length, have been documented to produce between 3000 to 4000 eggs (Brichard, 1978). The mass of these fish was not given.

The total fecundity of this species, pending further investigation, is low but does increase with fish length. A species that exhibits a low fecundity is not necessarily unsuitable for culture as the problem may be overcome by extending its spawning season.

CHAPTER 5.

FEEDING BIOLOGY

5.1 INTRODUCTION

If *C. pretoriae* is physiologically, morphologically and behaviourally adapted to best exploit its natural food resources, then an investigation of how and what it eats should provide some indication upon which the choice of an artificial food (live or formulated) may be based.

How and what this fish eats may be examined in terms of :

i) Where in the water column does it feed and what is the gross morphology of the mouth?

ii) The structure the alimentary canal.

iii) What does it feed upon and what is the proximate nutritional value of these food items?

iv) What quantities of food does it eat, what effect does feeding have on the condition of the fish and are there any seasonal variations?

In this chapter the above questions are answered by means of an investigation into the natural feeding biology of *C. pretoriae* in Louw's Creek, and a literature review of the proximate nutritional composition of natural fish food items. If the opening statement is assumed to be true then tentative conclusions can be made as to the nutritional requirements of *C. pretoriae*. A theoretical diet could be created and used as a basis upon which existing artificial feeds could be selected and tested.

5.2 MATERIALS AND METHODS

The position of the mouth of *C. pretoriae* was studied in order to establish what type of feeder it was, eg. benthic or pelagic. The structure of the mouth parts were studied in order to establish how it feeds. Light microscopy studies using a Nikon Stereo microscope with a fitted camera mounting were used to identify the gross morphology while scanning microscope studies were used to investigate the surface area of the lip and "beard-like" structures in detail. During the scanning electron microscope investigation, the mouth structure of preserved specimens were dissected and tissue samples were prepared using the preparation techniques described by Cross (1985). Tissue was viewed using a JOEL JSM 840 scanning microscope. During this study, cognisance was taken of the behaviour of the aquatic insects preyed upon by *C. pretoriae*.

The alimentary canal of the fish collected on a monthly basis were removed for intestine length measurements, stomach content analysis and stomach fullness ratings. A total of 224 fish were used.

The length of each intestine was measured from the end of the stomach (pyloric sphincter) to the anus. Regression analysis was used to establish the relationship between fish length and intestine length. Low relative intestine lengths generally indicate that a fish is carnivorous while high relative intestine lengths generally indicate that a fish is herbivorous or detritivorous (Nikolsky, 1963 ; Weatherly, 1972 ; Kruger and Mulder, 1973 ; Ribble and Smith, 1983).

Of the numerous methods that exist for stomach content analysis, three were chosen for this investigation, namely the numerical method, the frequency of occurrence method, and the volumetric method. Each of these methods have inherent faults, criticism of which are outlined in Hynes (1950), Windell and Bowden (1978) and

Hyslop (1980). Hynes (1950) stated that the use of any one technique is a compromise, and that a combination of methods is important so that the number and bulk (mass or volume) of any organism is measured.

Gut content analysis was restricted to the food items in the stomach as the food in the intestine was at an advanced stage of digestion and individual items could no longer be identified. In the laboratory, stomach contents of the fish were examined under a stereo dissecting microscope at variable magnification. The three techniques used to assess the stomach contents of *C. pretoriae* are briefly described below.

a. Numerical method (Allen, 1938): The number of individuals of each food type in each stomach was counted. These were summed to give totals for each type of food item. The overall total of all food items was also calculated. A percentage representation for each type of food item was then calculated.

b. Frequency of occurrence method (Allen, 1935): Individual food items were identified and sorted. The number of stomachs in which each food item occurred were recorded and expressed as a percentage of the total number of stomachs which contained food.

c. Volumetric method (Modified from Allen, 1938): A visual estimate of the volumetric abundance of each food type in each stomach was made on a percentage basis, and this was recorded as a percentage of the entire stomach contents.

In order to express the relative importance of each food item using a combination of the data from all three methods, an index of relative importance (IRI) was calculated. An index for expressing the relative importance of each food category was calculated as follows :

I.R.I. = (%N + %V)(%F) (Pinkas *et al.*, 1971)

N = numerical value
V = volumetric value
F = frequency of occurrence value

With the use of published data on the energy content and proximate nutritional composition of natural fish food organisms (Long, 1961 ; Philips, 1972 ; Yurkowski and Tabachek, 1979; Piper *et al.*, 1982) the approximate nutritional composition of the natural food of *C. pretoriae* was calculated. This could be used as a basis upon which to select different artificial feeds for the culture of the species.

The stomachs were also rated for fullness using the following point allocation (Modified from Swynnerton and Worthington, 1940) :

Visual estimation of fullness	Points
0	0
0-20%	1
20-40%	2
40-60%	3
60-80%	4
80-100%	5

The condition of the fish was calculated on a monthly basis. The condition factor gives an indication of the feeding intensity of a fish by revealing its "fatness" (Tesch, 1968) and can be used to investigate seasonal differences in feeding rate and food availability (Tesch,1968). A condition factor with and without the inclusion of the gonad mass was calculated for each fish in an effort to establish the effect of reproduction on fish condition.

Monthly condition factor values were calculated using the following formula: (after Tesch, 1968)

$$CF = \frac{W}{L^b} \times 100$$

W - weight (g)
L - length (cm)
b - exponent derived from the length weight relationship

5.3 RESULTS

Figure 2.1 (Chapter 2) shows the mouth structure of *C. pretoriae*. It is ventrally situated (inferior) indicating that this species is a benthic feeder. The pointed teeth indicate that this catlet feeds on live animals which it impales using the premaxillary teeth in the two tooth pads and then scoops the food into the buccal cavity using the mandibular row of teeth. An electron microscope investigation showed that the surface area of the "lip", barbel and "beard-like" structures were lined with sensory cells, which in turn were covered in microvilli (see Figure 5.1). Thus it may be assumed that *C. pretoriae* actually tastes its food before the food is taken into the buccal cavity.

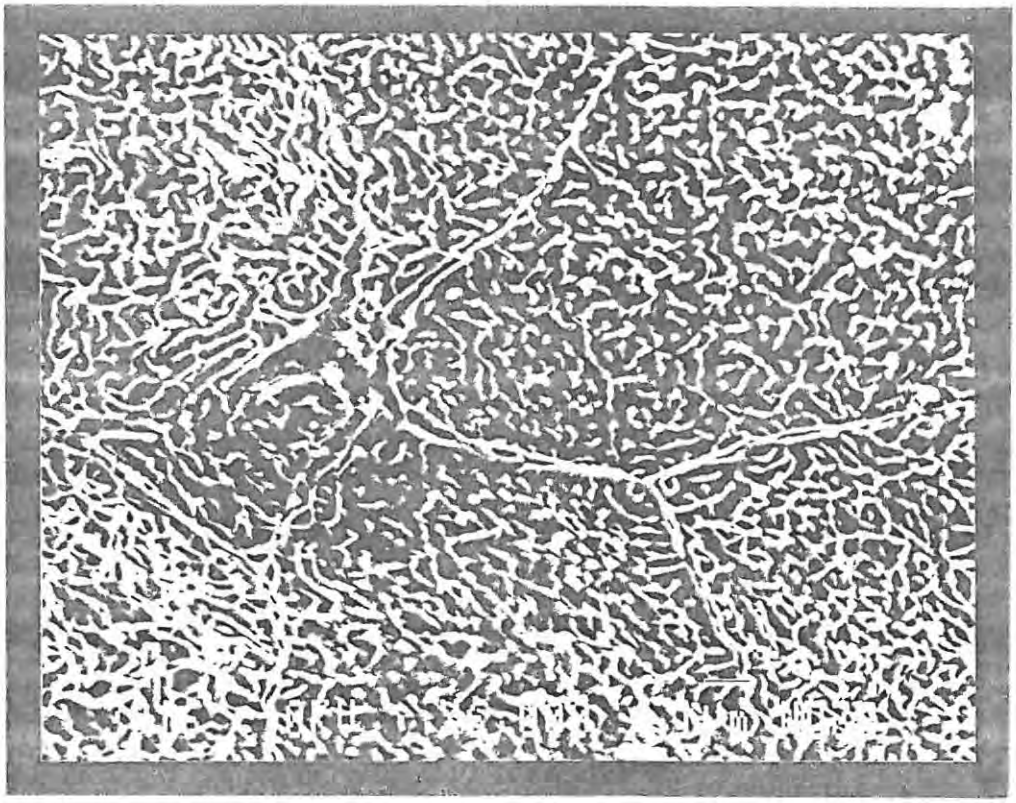

Figure 5.1. The sensory microvilli that line the mouth and beard structure of *Chiloglanis pretoriae*, creating a very sensitive feeding structure.

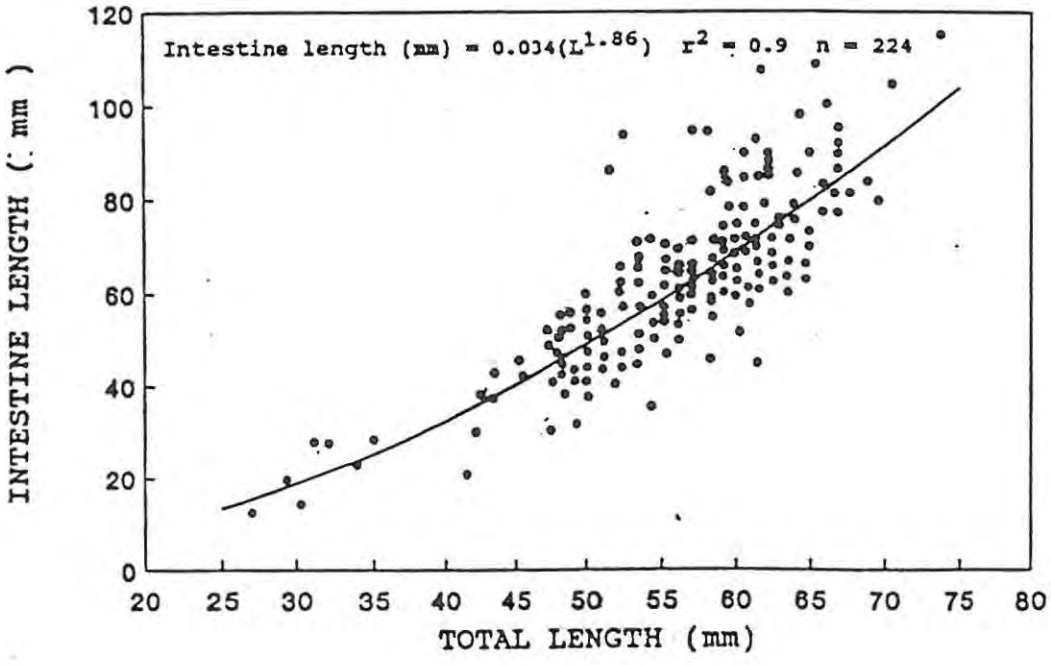

Figure 5.2. The relationship between fish length and intestine length.

Intestine length increases at a faster rate than the total fish length (see Figure 5.2) and results in a ratio of 1.3:1 respectively for adult fish. The ratio calculated for adult *Oreochromis mossambicus* is 10:1 (de Moor *et al.*, 1986). *Oreochromis mossambicus* is a herbivore (Bowen, 1981 ; de Moor *et al.*, 1986). Weatherly (1972) states that a carnivorous fish has a relatively short intestine, which is the case with *C. pretoriae*. Based on the above evidence, captive *C. pretoriae* should be fed a diet which includes animal protein.

Fifteen percent of all the stomachs analysed were empty. During December 1989 a large amount of silt and detritus was found in all the stomachs. However, Heymans (pers. comm. Sogenvelo Nature Reserve, Kangwane) stated that there had been a massive increase in the amount of silt deposited into Louw's Creek during the same month. This unnaturally high silt and detrital component was therefore emitted from the stomach content analysis results, as it was felt that it would create an unnatural bias towards silt and detritus in the natural diet of *C. pretoriae*. In Louw's Creek the diet of *C. pretoriae* consisted largely of aquatic insects. As no information existed on the diversity and abundance of aquatic invertebrates in Louw's Creek, no conclusions could be made as to the feeding selectivity of *C. pretoriae*. The results indicated that *C. pretoriae* fed primarily on Chironomidae and Baetidae larvae (see Figure 5.3). Table 5.1 provides a detailed list of the dietary organisms taken by *C. pretoriae*.

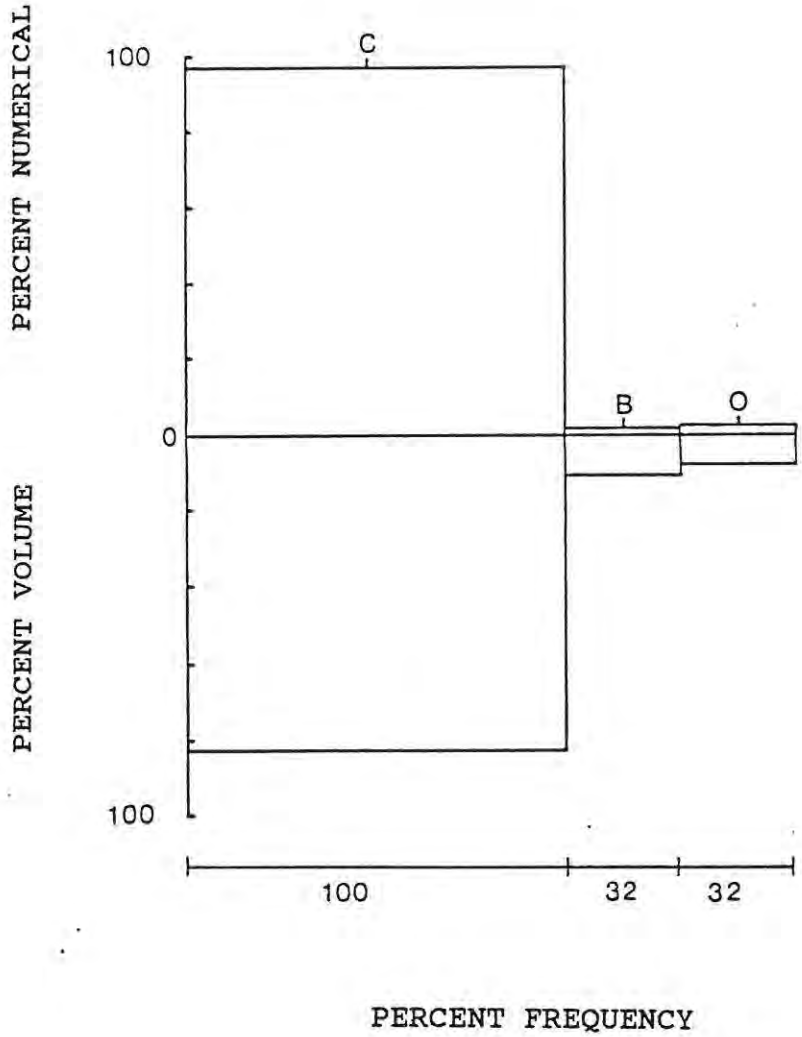

Figure 5.3. The composition of the principal prey items of *Chiloglanis pretoriae* in Louw's Creek (C-Chironomidae, B-Baetidae, O-Other).

Table 5.1. The percentage numerical, percentage volume, percentage frequency of occurrence and the index of relative importance (IRI) of the prey items taken by *Chiloglanis pretoriae*.

	% Number	% Volume	% Frequency	IRI
Ephemeroptera				
Baetidae	1.3	10.6	32.9	379.9
Diptera				
Chironomidae	97.1	82.9	100	18000
Ephydridae	0.01	0.3	0.5	0.16
Simulidae	0.04	0.03	1.4	0.1
Empididae	0.01	0.1	0.5	0.06
Tipulidae	0.2	0.8	7.1	7.4
Trichoptera				
Hydropsychidae	0.1	0.4	2.4	1.2
Polycentropodidae	0.01	0.02	0.5	0.02
Hydroptilidae	0.01	0.02	0.5	0.01
Lepidoptera				
Pyralidae	0.02	0.2	1	0.2
Mollusca				
Ancylidae	1.16	5.4	17.5	115

The data provided by Long (1961), Philips (1972), Yurkowski and Tabachek (1979) and Piper *et al.* (1982) were used to calculate the approximate nutritional requirements of *C. pretoriae* based on the proximate nutritional analysis of its natural food (see Table 5.2). Using dry mass and based on the relative importance of the different food groups one can calculate the relative amount of

Table 5.2 Estimated proximate composition of the natural food organisms taken by *Chiloglanis pretoriae*. (IRI represents the index of relative importance of the respective food groups).

	IRI	%PROTEIN	%LIPID	%CARBO-HYDRATES	%CHITIN	UTILISABLE ENERGY(KJ/g)
DRY MASS ANALYSIS						
CHIRONOMID LARVAE	18000	42-68	2-14	4-23	4	14.5
COMPOSITE (INSECTS)	389	34-66	13-32	2-23	9	19.1
MOLLUSCS	108	60	6-12	<1	<1	13.0
WET MASS ANALYSIS						
AQUATIC ORGANISMS (75% WATER)	-	12-15	3-7	1	1-4	-

All values have been rounded off to the nearest percentage. Dry mass values of chironomid larvae and the composite values were based on those given for related organisms by Yurkowski and Tabachek (1979). Dry mass values for molluscs were based on values given by (Long, 1961) and the wet mass composite values were based on those given for aquatic organisms by Piper *et al.* (1982). Estimates of utilisable energy were calculated using 16.7 KJ/g total protein, 37.6 KJ/g lipid and 16.7 KJ/g carbohydrate on a total dry solids basis (Philips, 1972).

each food type in the diet of this catlet, eg. the ratio of molluscs:composite:chironomids was calculated by dividing the IRI value of each food group by the smallest IRI value (115).

Molluscs : 115/115=1, composite : 389/115=3.4 and chironomids : 18000/115=157.

The proximate nutritional values for the respective food groups was then multiplied by the calculated factors. Mid-range values were used for this calculation. For example the calculated nutrient values for the chironomids would be : Protein (157 x 55), lipid (157 x 8), carbohydrate (157 x 13.5), chitin (157 x 4) and gross utilisable energy (157 x 14.5). This was done for all three food groups. A total value was then calculated for the protein (8635), lipid (1341.5), carbohydrate (2163), chitin (659.6) and gross utilisable energy (2354.4) in the diet.

This theoretical diet was now weighted in favour of the most important food groups. In order to express the value of each nutrient as a percentage, the above totals were divided by the sum of the above factors, eg. 1, 3.4 and 157 (161). The final theoretical diet consisted of 55.1% protein, 8.3% lipid, 13.4% carbohydrate, 4.1% chitin and 14.6 KJ/g gross utilisable energy.

When using wet mass, the diet of *C. pretoriae* had a moisture content of 70-80%, 12-15% protein, 3-7% fat, 1-4% ash and 1% carbohydrate (see Table 5.1).

The above data may be used as a basis upon which to select existing artificial feeds with similar proportions of protein, lipids, carbohydrates, chitin and gross utilisable energy. The suitability of these chosen feeds could then be tested during the development of the culture protocol for the species (see Chapter 11).

The stomach fullness index values calculated for *C. pretoriae* during this study are shown in Figure 5.4. There is a noticeable

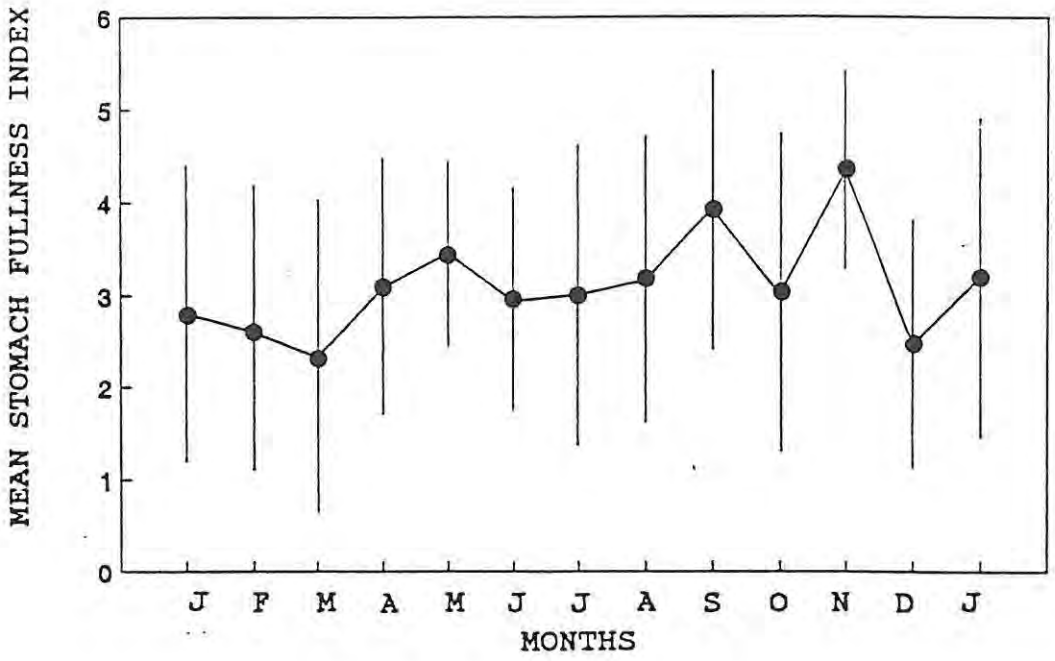

Figure 5.4. The mean monthly stomach fullness index values of *Chiloglanis pretoriae*, calculated from the monthly samples of fish collected in Louw's Creek during the period January 1989 to January 1990. The vertical bars represent the standard deviation.

increase in feeding rate just prior the spawning season (September). The condition factor of the fish throughout the study period is depicted in Figure 5.5. An increase in the condition factor prior to the spawning season substantiates the trend shown by the stomach fullness index values.

During December the condition factor (including gonad mass) decreased noticeably. However the condition factor (excluding gonad mass) remained relatively constant. A possible explanation, is that during December (as mentioned before) excessively high amounts of silt and detritus were found in the stomachs of the fish. Thus it may be assumed that while fish can survive on a diet consisting of silt and detrital matter, detritus does not provide sufficient nutrients to facilitate gonadal maturation.

4.4 SUMMARY

According to the description of different foraging guilds provided by Calliet *et al.* (1986), *C. pretoriae* may be termed a demersal microcarnivore, feeding on benthic invertebrates. It has a relatively short intestine which indicates that it requires a diet with a high animal protein content. The protein type together with its foraging guild indicate that *C. pretoriae* should be provided with a sinking artificial feed that has an animal protein component.

Kleynhans (pers. comm., Transvaal Department of Nature Conservation, Pretoria) has described *C. pretoriae* as being an opportunistic feeder. However, in Louw's Creek available evidence suggests that it selected for the more sedentary chironomid larvae. The proximate nutritional analysis of the aquatic organisms consumed by *C. pretoriae* should be regarded as speculative because of the large variation in the proximate composition of natural food organisms, even within a species (Yurkowski and Tabachek, 1979). If however mid-range values are used to calculate the percentage composition of the various

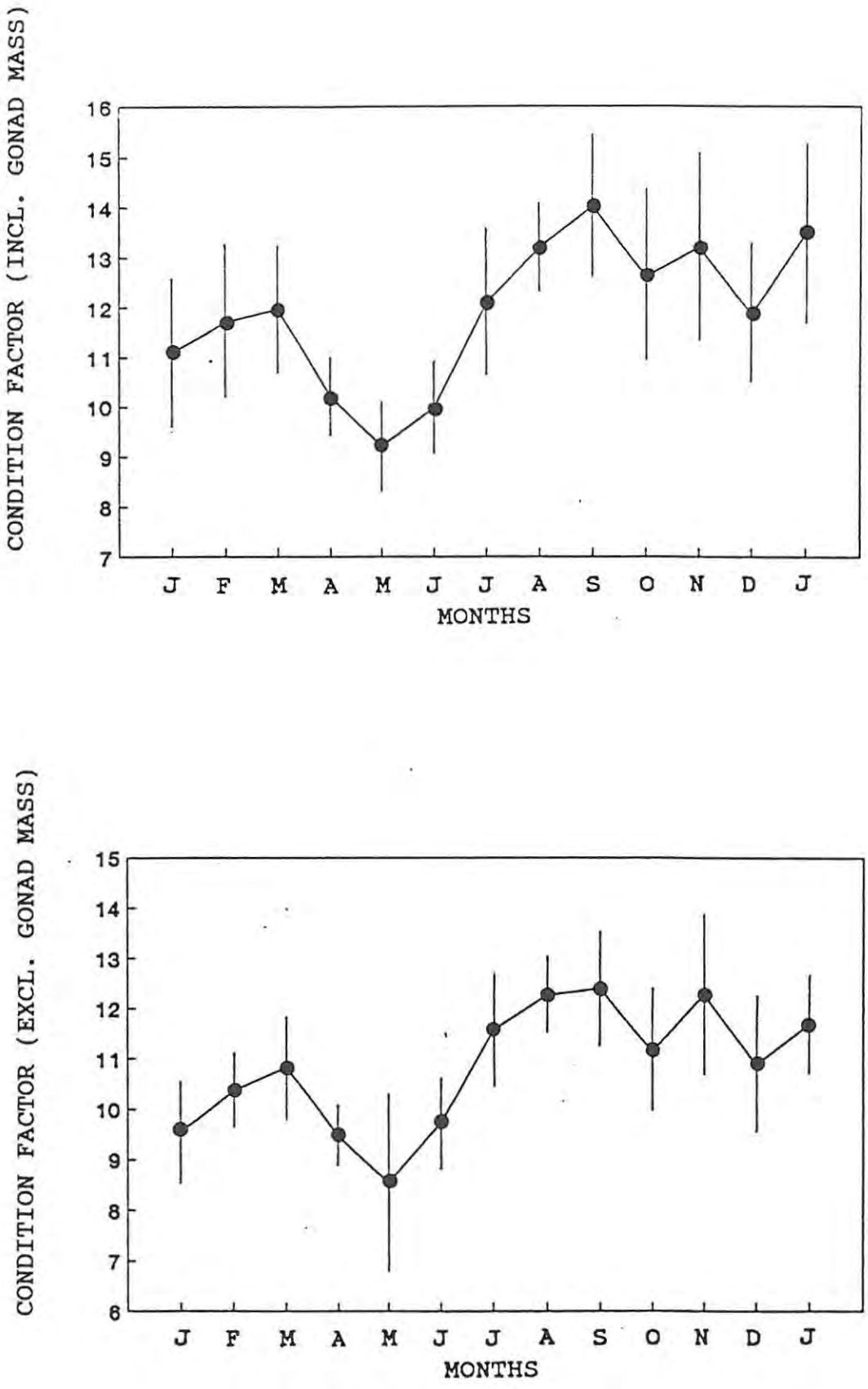

Figure 5.5. The condition factor of *Chiloglanis pretoriae*, calculated from the monthly samples of fish collected in Louw's Creek during the period January 1989 to January 1990. The vertical bars represent the standard deviation.

components, the source of error may be somewhat offset. Although the data does not provide the proximate analysis of the natural diet of *C. pretoriae* at the time of sampling, it does however provide an indication of what it may be. This could be used as a basis upon which to select available artificial feeds during the development of the culture protocol for *C. pretoriae* (see Chapter 11).

The average monthly stomach fullness index values showed peaks from September which indicated that additional energy was required for gonadal maturation. It would therefore be important to feed broodstock optimal feeding rations to facilitate gonadal maturation.

Chiloglanis pretoriae reaches peak condition in spring just prior to the spawning season and retains its condition until the end of the spawning season in April and May. This indicates the need for a feeding method that operates at optimal levels for extended periods. Detritus provides a suboptimal diet for *C. pretoriae* substantiating the fact that this catlet (especially broodstock) requires a diet consisting of a large animal protein component.

CHAPTER 6.

AGE AND GROWTH

6.1 INTRODUCTION

A knowledge of age and growth is fundamental to the understanding of a fish population in its natural environment as well as in captivity (Bruton and Allanson, 1980). Growth rate and the age at which the fish attains sexual maturity provide the basis for both fisheries management and for judging the suitability of a candidate species for aquaculture. When studying a candidate species for aquaculture the assessment of its natural growth rate and age-at-maturity is of the utmost importance (Bruton and Safriel, 1984). A fast growth rate results in a relatively quick product turnover rate which in turn decreases feed and maintenance costs and increases production rates (Meade, 1989). Age-at-maturity, on the other hand, indicates how long fish will have to be kept before they can be used as broodstock. Bearing these points in mind, the aim of this investigation was to estimate the natural growth rate of *C. pretoriae* and then to calculate its age-at-maturity. The length-mass relationship was also calculated as it may be used to describe the fishes growth as being isometric or allometric (Ricker, 1975) and to calculate its condition factor.

The method most frequently used to age fish is the interpretation and counting of growth zones or checks on their hard parts. These zones or checks are formed during periods of faster or slower somatic growth and reflect various environmental or internal influences (Bilton, 1974 ; Simkiss, 1974 ; Calliet *et al.*, 1986). Age determination of siluroid catfishes has usually been based on the interpretation of the growth zones on vertebrae, spines or otoliths (Quick and Bruton, 1984).

6.2 MATERIALS AND METHODS

Spines (n=18) and otoliths (n=18) were collected from the first of the monthly fish samples and a comparison was made between the use of these hardparts for the ageing of *C. pretoriae*. Otoliths were difficult to locate, and 11 (61%) were found to be detrimentally affected by the 10% buffered formalin fixative. These were very soft and disintegrated during the dissection process. The growth rings in the remaining nine otoliths lacked the clarity of those in the pectoral spine sections. Studies that have been carried out on other catfish indicate that pectoral spines provide a clear and accurate assessment of the age of the fish (Hall and Jenkins, 1952 ; Marzolf, 1955 ; Donnelly and Caulton, 1969 ; Gaigher, 1969 ; van der Waal and Schoonbee, 1975 ; Bruton and Allanson, 1980 ; Clay, 1982 ; Quick and Bruton, 1984). During this study pectoral spines were found to be easily accessible, they were not detrimentally affected by the fixative and showed clear growth zones. Spines were therefore used to age *C. pretoriae*.

The pectoral spines were dissected from the fish during the measuring and weighing procedure. A total of 224 spines were used during this investigation. Each spine was individually embedded in clear epoxy resin using a rubber mould consisting of small rectangular units, each of which housed a single spine. Spines were cross- referenced with the respective fish number in order to eliminate any possible subjective bias.

Spines were sectioned at the distal end of the basal groove as suggested by Sneed (1951), Marzolf (1955) and Hecht (1980). Sectioning was carried out using a twin blade diamond edged otolith cutting machine (Rauk, 1976). This machine produced thin, highly polished sections of consistent thickness (0.5mm) which served to enhanced the clarity of the rings. The spine sections were then mounted on glass slides using DPX Mounting Medium. All sections were examined under a compound microscope using

transmitted light. When examined under the above conditions a central lumen with alternating opaque and hyaline zones were observed. "Known age" fish grown in excess of one year were used to validate zone deposition. Fish that were reared in a raceway under summer environmental conditions laid down a single opaque zone. Based on this finding it was assumed that one opaque and one hyaline zone was deposited per year.

The relationship between spine diameter and fish length should be linear before one may use these hardparts for the aging of a fish (Hecht, 1980). The diameter of the sectioned spines was measured using a Nikon Microscope fitted with a eyepiece micrometer. The relationship between spine diameter and fish length for *C. pretoriae* was calculated using regression analysis.

An important aspect to note with respect to age and growth studies carried out on spines is the increasing size of the spine lumen with age. The possibility that it might resorb a number of the early growth rings was investigated during this study. Incorrect data would result in a false impression of the natural growth rate of the species.

Once it had been established that the spines were suitable for aging *C. pretoriae*, each section was read. This was done on four separate occasions. Once to gain familiarity with the ring pattern, and a further three times to record the number of opaque and hyaline zones. Only those counts occurring at least twice for each spine section were accepted.

Student t tests on the mean length at age of males and females ($P=0.05$) showed no significant differences between the growth rates of the two sexes. The data for the two sexes were therefore combined for the purpose of growth calculations and for the construction of an age-length key. For the calculation of growth, the observed length-at-age data were fitted to the Von Bertalanffy growth equation.

Until recently this method has involved the use of unweighted mean length-at-age data and the use of a Fort Walford plot. However, by using a mean value at each age point one unrealistically assumes that sampling error is zero (Hughes, 1986). Thus an attempt was made to fit the von Bertalanffy equation with more advanced curve fitting techniques that included all length-at-age data. These included the absolute error and the transformed logarithmic error models described in Punt and Hughes (1989).

Using Beverton's method (Beverton, 1954) growth was also calculated as follows :

By plotting a least squares linear regression of L_t against L_{t+1} the Y intercept and X coefficient (k) were calculated. Based on these data L_∞ and K could be calculated.

$$L = \frac{\text{Y intercept}}{1-k}$$

$K = -\log_e k$ (which may also be calculated as the slope of the graph of t plotted against $\log_e (L_\infty - L_t)$

By plotting a least squares linear regression of t against $\log_e(L_\infty - L_t)$ a second Y-intercept was calculated. This value in conjunction with the calculated L_∞ and K values facilitated the calculation of t_o.

$$t_o = \frac{\text{Y intercept}}{K} - \log_e L_\infty$$

The final growth equation took the form of :

$$Lt = L\,(\,1-e^{-k(t-to)}\,)$$ (Ricker, 1975)

where Lt = length at age t
L_{∞} = theoretical asymptotic length
K = Brody's growth coefficient
t_o = theoretical age at zero length

In order to establish the age at 50% and 100% maturity, theoretical lengths-at-age were calculated for years one and two using the Von Bertalanffy growth equation. This data could then be transformed to length-at-maturity data (see Chapter 4) thus establishing the age-at-maturity of *C. pretoriae*.

The length-mass relationship for *C. pretoriae* was calculated using the following formula :

$$W = aL^b$$ (after Tesch, 1968)

where W = mass (g)
L = length (mm)
a and b = constants

6.3 RESULTS

A linear relationship was found to exist between spine diameter and fish length (see Figure 6.1) indicating that spines may be used for growth calculations.

Spines of *C pretoriae* exhibited wide opaque and narrow hyaline zones (see Figure 6.2). One opaque zone and one hyaline zone were assumed to represent the annual growth increment of *C. pretoriae*. This is in accordance with Donnelly and Caulton (1969), who

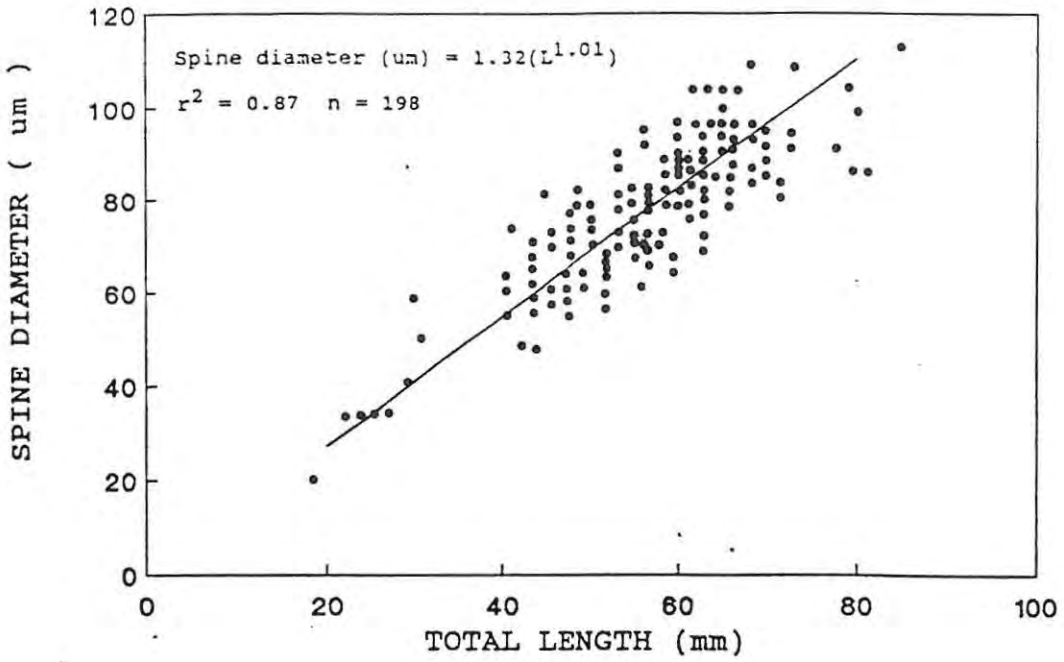

Figure 6.1. The relationship between fish length and spine diameter.

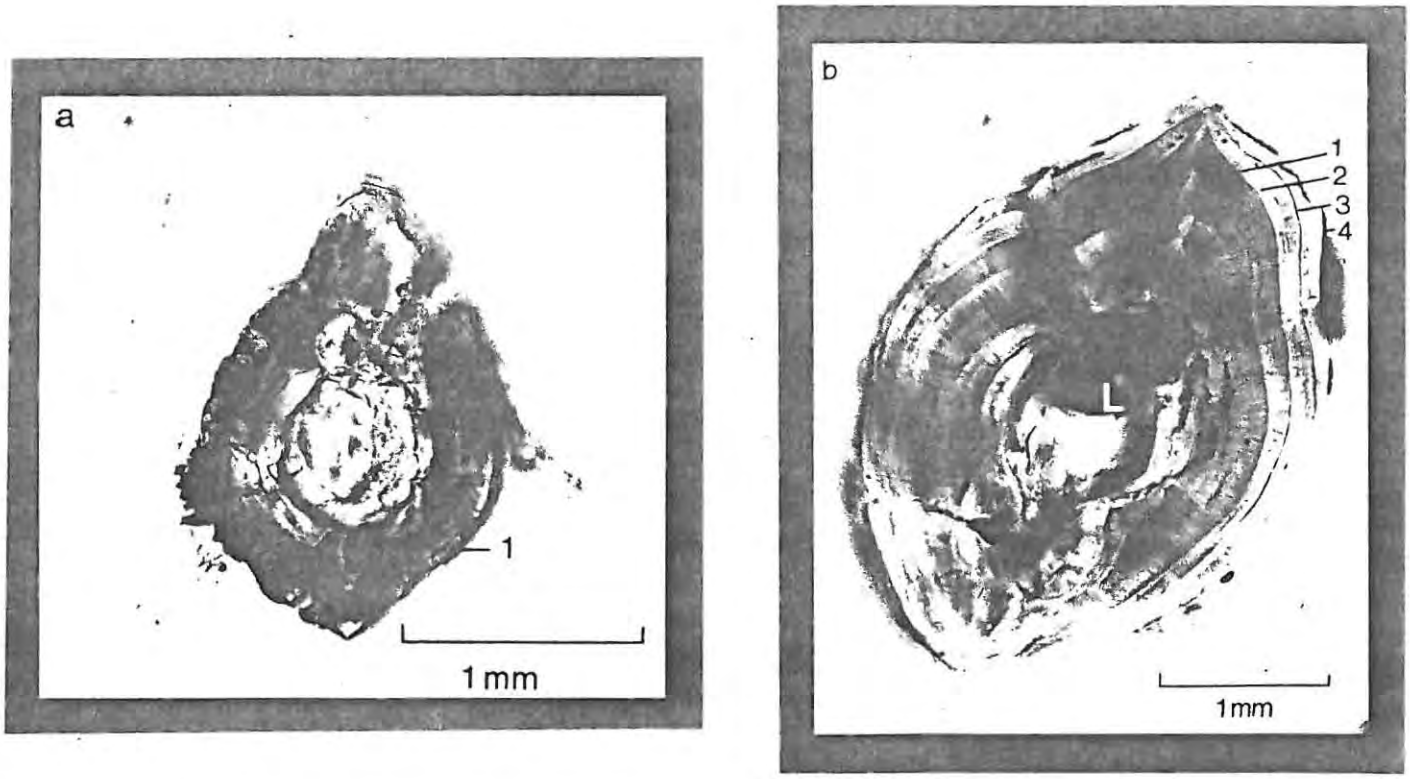

Figure 6.2. A section through a spine of a) a one year old fish and b) a four year old fish showing the distinctive growth zones.

suggested that annual rings are formed on the hardparts of fish occurring in waters where the amplitude of seasonal temperature variation is around 10 ^{o}C.

Of all the spine sections (n=224) 88.4% were successfully read. Most of the sections were clearly legible as illustrated in Figure 6.2. Unsuccessful aging of spine sections occurred when no distinct opaque and hyaline zones could be recognised. The increasing lumen did pose a problem in that it only affected the clarity of the first ring in a few fish. However the first ring was always wider than the other rings and in most cases it was possible to identify it.

The results of the advanced curve fitting techniques described earlier did not pass the error model tests for homoscedasticity and randomness of residuals, which meant that the Von Bertalanffy growth parameters could not be accepted with any degree of confidence (Punt and Hughes, 1989). In view of this the Von Bertalanffy growth equation derived using Beverton's method (Beverton, 1954) was used for all growth calculations. The resultant equation was calculated as follows :

By regressing L_t against L_{t+1}, the y-intercept was calculated as 25.5. K could then be calculated as the negative natural log of the x-coefficient produced by the regression analysis.

$$K=-\log_e 0.649$$

$$K=0.432$$

Using the above data, L_∞ was calculated as follows :

$$L_\infty = 25.5/1-0.649$$

$$L_\infty = 72.7$$

By regressing t against $\log_e(L_\infty - L_t)$, the second y-intercept was calculated as 4.0.

Using the above data t_o was calculated as follows :

$$t_o = 4.0 - \log_e L_\infty / K$$

$$t_o = -1.15$$

The final growth equation was as follows :

$$Lt = 72.7 \left(1 - e^{-0.432(t+1.15)} \right) \text{ mm}$$

The calculated L_∞ value was within the maximum length attained by fish collected in Louw's Creek. The paucity of these large fish (see Table 6.1) indicated that they were of record size rather than of average size. This is in accordance with Ricker (1975) who states that L_∞ is the asymptotic length to which the average uncaptured fish in the population would grow and is not intended to represent those of record size.

Figure 6.3 illustrates the relationship between the observed length-at-age data and the calculated Von Bertalanffy growth curve. The growth rate of *C. pretoriae* is highest in the first two years during which time they attained 60% and 74% of their maximum theoretical length respectively.

Based on the calculation of the theoretical length-at-age, *C. pretoriae* reached 50% sexual maturity at one year of age (44mm) and 100% maturity at two years of age (54mm).

Table 6.1. The length-age key of *Chiloglanis pretoriae* collected in Louw's Creek over the period October 1988 to March 1990.

AGE	1	2	3	4	5	6	7	8	9	n
LENGTH CLASSES (TL mm)										
20-39	3									3
30-39	4									4
40-49	3	12	4							19
50-59		9	41	22	12	1				85
60-69			2	18	39	19	3			81
70-79					2	2		1	1	6
TOTAL	10	21	47	40	53	22	3	1	1	198

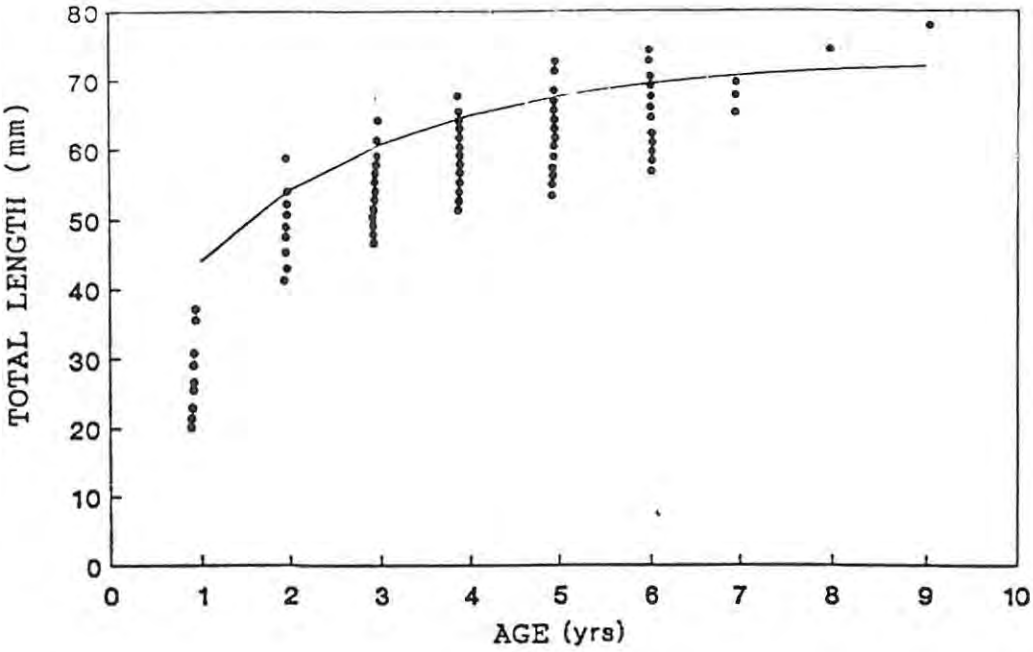

Figure 6.3. The growth rate of *Chiloglanis pretoriae* comparing observed length-at-age with calculated length-at-age.

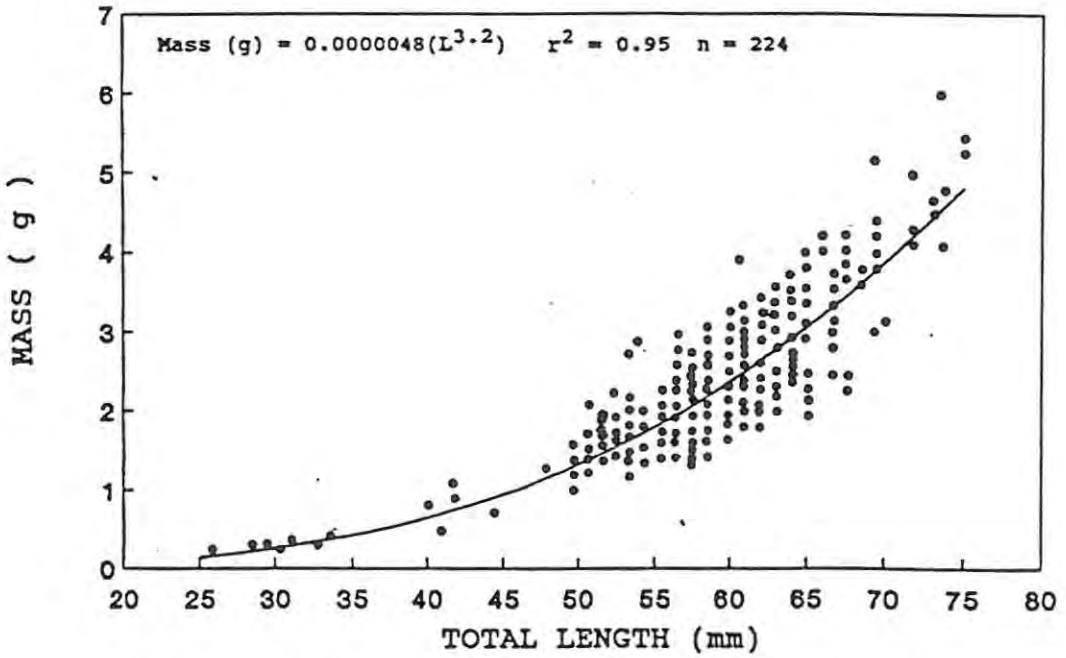

Figure 6.4. The relationship between length and mass of *Chiloglanis pretoriae*.

The length-mass relationship for *C. pretoriae* is described by the equation $W = aL^b$ and is shown in Figure 6.4. The length-mass equation for *C. pretoriae* is as follows:

$$Mass = 0.0000048 \times L^{3.2}$$

As the exponent "b" was approximately equal to three, the growth of *C. pretoriae* could be described as being isometric (Ricker, 1975). The equation used to calculate the condition factor of *C. pretoriae* was as follows :

$$CF = \frac{W}{L^{3.2}} \times 100$$

6.4 SUMMARY

The highest growth rate was recorded in the first and second year during which *C. pretoriae* attained 60% (44mm) and 74% (54mm) of its theoretical asymptotic length respectively. The ideal time to sell this catlet would be after the period during which it exhibited maximum growth. Based on above evidence, *C. pretoriae* should be marketed after one year of growth.

The oldest fish collected was nine years of age. *Chiloglanis pretoriae* attained 50% sexual maturity during its first year (44mm) and 100% sexual maturity during the second year (54mm). In comparison to *C. pretoriae* some cyprinodonts, particularly those that inhabit temporary pools, reach sexual maturity at the age of a few weeks but die after one reproductive season (Simpson, 1979). On the other hand *Eutropius depressirostris* reaches 100% sexual maturity during its second and third year and reaches a maximum age of seven years (Hecht, 1980). This seems to be similar to *C. pretoriae*, however *E. depressirostris* reaches a theoretical asymptotic length of 339mm, compared to 72.8mm for *C.*

pretoriae, indicating that *C. pretoriae* is relatively long lived for such a small fish. Based on the above evidence, broodstock should be selected from fish older than two years or longer than 54mm (TL). Because this species is relatively long lived, older broodstock (three to four years old) should be selected as they are larger and should produce more eggs.

CHAPTER 7.

EARLY DEVELOPMENT

7.1 INTRODUCTION

There is a co-ordinated inter-relationship between the life history of an organism and its environment (Wootton, 1990 and Balon, 1990). If this statement is accepted as being correct, then the reproductive style, egg structure and early developmental rate of an organism should be best suited to the natural conditions under which it exists. During this study, however, no eggs or larvae were collected in the wild and no information was available on the spawning site or the early development of *C. pretoriae*. Thus the primary objective of this investigation was to spawn *C. pretoriae* in captivity, and in doing so, examine its reproductive style, to determine where it spawns and to document the early development.

The above information could be used in designing a culture programme. The reproductive style could shed some light on the possible egg production rate of this species by establishing whether or not it is a serial spawner (De Martini and Fountain, 1981; Burt *et al.*, 1988). Information on the natural spawning site of *C. pretoriae* could be used to develop techniques for the artificial incubation of eggs by identifying conditions under which natural development of eggs takes place (Hogendoorn, 1983; Impson, 1987). The investigation of the developmental rate of embryonic and larval fish under controlled optimum environmental conditions could be used to identify those developmental periods, which, in addition to the optimal environmental conditions, depend on the direct management input in order to maintain high survival rates, eg. first feeding (Yusa, 1974). Finally, the monitoring of larval development necessitates the identification of an acceptable larval feed (Bruton, 1979; Cambray, 1983 and

1985) which can then be used to rear larvae in the culture programme.

7.2 MATERIALS AND METHODS

Broodstock were collected in the wild and transported to Grahamstown (see Chapter 3 for methods). Once in Grahamstown they were transferred to the spawning system where they were allowed to spawn under simulated natural conditions (see Chapter 10 in which the full description of the spawning environment is presented).

Once the fish started to spawn, eggs were collected on a daily basis. *Chiloglanis pretoriae* was found to be an interstitial gravel spawner. Thus the exact time of fertilisation could not be established. Attempts were made to collect eggs throughout the day but only those collections made each morning (06h00 to 08h00) produced any eggs. The lights of the spawning system were only switched on at 06h00 indicating that *C. pretoriae* is a nocturnal spawner. Embryos in the four cell stage of development were the earliest developmental stage collected and were used as time zero for the duration of this study.

The eggs were incubated in specially designed plastic holders that were made from soft plastic vials (each had a volume of 10ml), by cutting out 2 ovals opposite one another and gluing nylon mesh over these holes (see Figure 7.1). These vials were then suspended in the 80l glass incubation tanks, using a perspex sheet with a grid of labeled holes. These holes were the same diameter as the vials (Haigh, 1990)(see Figure 7.1). Each egg was kept in its own labeled vial.

The experimental conditions within these tanks were kept constant (see Table 7.1 below) as factors such as light regime, water temperature and water quality have a definite effect on the developing embryo (Boulekbache, 1981). The incubation tanks were

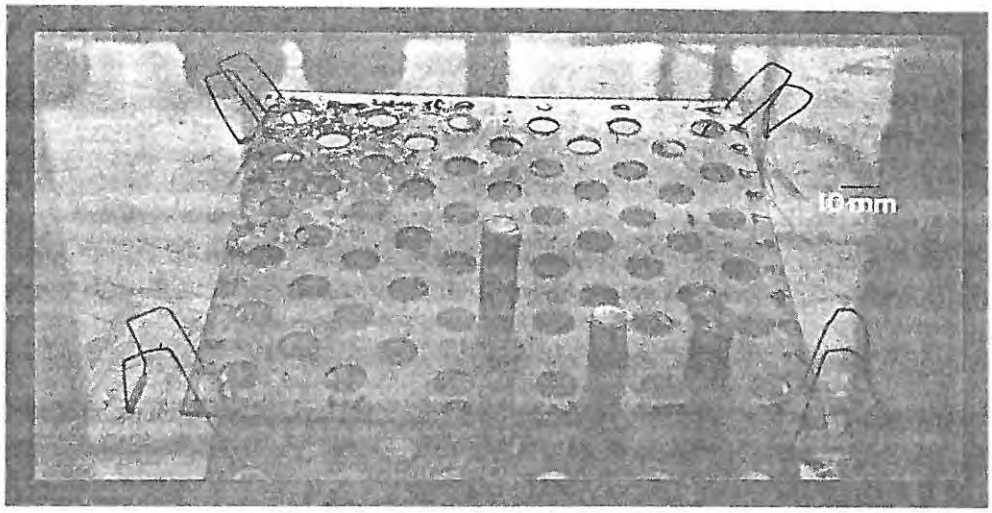

Figure 7.1. The incubation system used to house developing embryos and larvae.

maintained in a controlled temperature room with the photoperiod set at 16L:8D and the temperature set at 25 ^{o}C. The effect of changing environmental conditions on developmental and survival rates was not investigated. Water quality was maintained using three standard 30cm x 30cm undergravel filters. Ammonia, nitrite and nitrate levels were assumed to be negligible as a maximum of 10 eggs or larvae were maintained in a 80l glass aquaria at any one time. A submersible water pump was installed in each incubation tank to create an artificial current and insure good aeration.

Table 7.1 Water quality and photoperiod in the incubation tanks.

TEMPERATURE (^{o}C)	25
DISSOLVED OXYGEN (mg/l)	7-8
pH	7
PHOTOPERIOD (hours)	16:8 (LIGHT:DARK)

Development was monitored using a Nikon Stereo microscope with a double camera mounting. This allowed for both colour and black and white photographs to be taken using a motor drive and a remote shutter release. Development was followed through to the juvenile stage (when the larval finfold was resorbed and the fish possessed the full complement of adult structures (Balon, 1990)). This was done because the larval phase has generally been accepted as the most critical phase in a fishes lifecycle (May, 1974 ; Sifa and Mathias, 1987 ; Wootton, 1990). Visual observations in the form of notes and drawings were made on a $\pm$24 hour basis. In order to describe developmental events, the same embryo was used sequentially, returning it to the incubator

between observations. There was no means of temperature control during the period the embryos and larvae were under observation. Observation time was therefore kept to a minimum and seldom exceeded five minutes. Newly hatched embryos that were still feeding endogenously were termed free embryos while those that began feeding exogenously were termed larvae (Balon, 1990). The larvae were fed Tetra Min Baby Fish Food (45% protein). They were fed to satiation four times per day between 08h00 and 17h00. Larvae were considered satiated when they stopped searching for food.

The developmental process from hatching to the juvenile phase was sub-divided into developmental steps. A review of the literature revealed that steps (or subtitles) are used to focus the reader's attention on specific developmental periods (Balon, 1985 and Paine and Balon, 1985), or on the development of specific organs or attributes (de Silva, 1974 and Cambray, 1985) which the author wishes to highlight depending on the final objectives of the specific study. During this investigation steps were used to highlight different periods within the early development of *C. pretoriae* which were identified as being important by the author. As stated in the introduction the objective of this part of the investigation of the early development of *C. pretoriae* was to establish its developmental rate, and to identify those developmental periods that depend on direct management input for high survival rates. The steps were chosen accordingly.

Step one was from the four cell stage to the hatching stage. The husbandry of this stage involved the use of incubation tanks, egg holders, set environmental conditions and prophylactic treatment against fungus. The period of time that the developing embryos require these specific conditions is important to a prospective culturist as the successful husbandry of these eggs will ensure high survival rates.

Step two was from hatching to first feeding. Of those conditions described above, all but the egg holders are still necessary

during this stage. The embryos are free of the sterile egg envelope so it may be assumed that the maintenance of optimal conditions and the prophylactic treatment of fungus is imperative. The free embryos of *C. pretoriae* were found to be more sensitive to handling than the eggs (see Chapter 13). Once again the period of time during which the developing embryos require the maintenance of specific conditions is important to the prospective culturist as the successful husbandry of these embryos will ensure high survival rates.

Step three described the development of the suction ability of the larval mouth structure, which indicated that the larvae were beginning to feed. Therefore, in addition to the maintenance of set environmental conditions and the prophylactic treatment of fungus, the larvae had to be fed. Supplying larvae with an acceptable diet at first feeding is essential for the success of any rearing programme (see Chapter 13).

Step four described the metamorphosis from the larval phase to the juvenile phase. Careful husbandry is generally required up to the completion of this phase.

7.3 RESULTS

The developmental time is given in days:hours:minutes.

Step 1. Four cell stage to hatching
(time 0 to day 7)

00d00h00min : Four cell stage, with one of the four cells in the process of dividing (This was the earliest developmental stage collected). The fertilised eggs at this developmental stage consisted of an outer egg membrane, a liquid filled area between the outer egg membrane and an inner egg membrane, followed by a perivitelline space and finally the yolk and the

developing cells (see Figure 7.2).

01d00h40min : The outline of the developing embryo was clearly distinguishable from the yolk. Differentiation into head, mid-body and tail regions was evident. When viewed from the side the embryo was observed to protrude above the yolk surface.

02d02h50min : The overall shape of the embryonic fish was recognisable. The larval finfold had developed. Melanophores were visible dorsally at the base of the skull, anterior to the larval fin fold. The post anal trunk-tail region was free of the yolk sac. At this stage the embryo moved. Movement consisted of lateral post-anal body flexions.

02d23h50min : The head region had enlarged considerably and differentiated into a more recognisable shape. This indicated brain and notochord development. A pair of otoliths were evident. The vitelline plexus along with some major blood vessels had been laid down. The vitelline capillary system consisted of the relatively large poorly branched left and right subintestinal vitelline veins. These emptied into a common cardinal vein at their juncture with the sinus venosus. Blood was pumped down the length of the body in a single artery and returned to the heart in a single vein. The caudal vein was supplied by the caudal artery, a post-anal extension of the dorsal aorta. The posterior cardinal vein was present. The blood returned to the heart via the yolk capillary system. The heart, a tube like structure, was beating. The heart beat was recorded at 40 beats per minute. It was situated in a pericardial cavity located in a depression in the yolk under the developing head region. The blood flow rate was uneven and was orange in colour, probably due to the

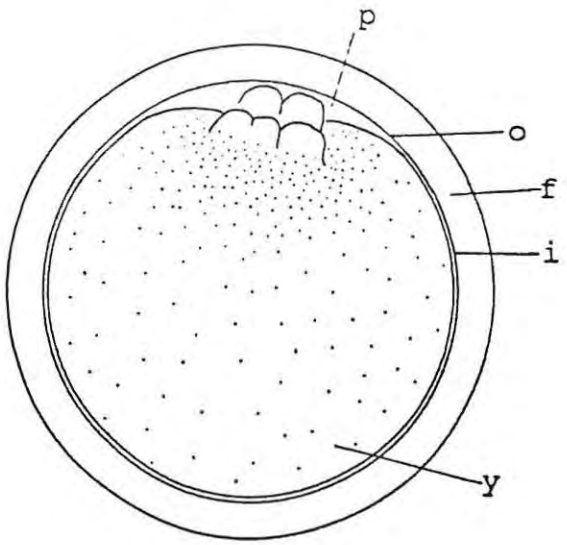

Figure 7.2. The embryo in the four cell stage with one cell dividing. The egg of *Chiloglanis pretoriae* consistes of the following : o-outer egg membrane, f-fluid layer, i-inner egg membrane p-perivitilline space and y-yolk.

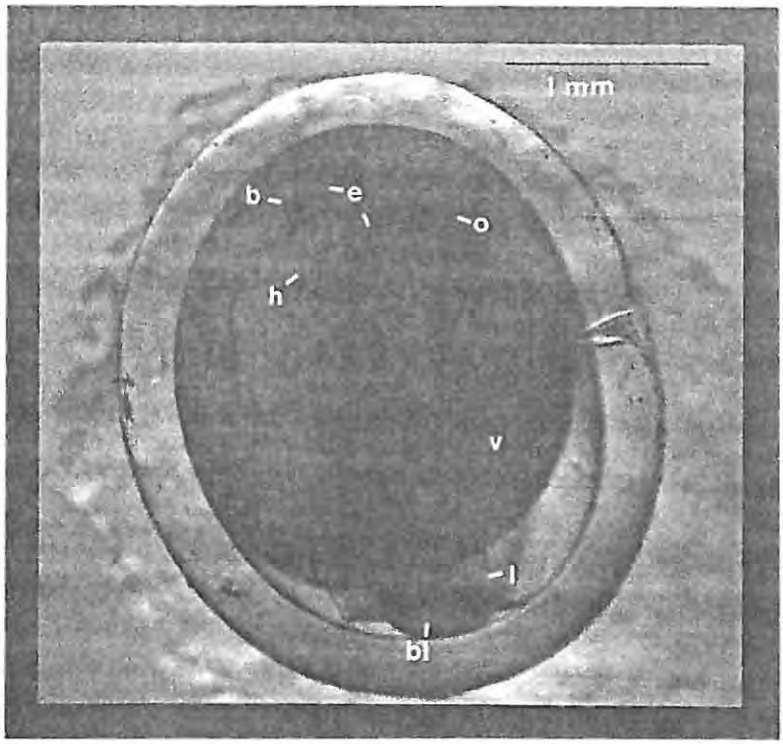

Figure 7.3. A pre-hatching *Chiloglanis pretoriae* embryo (125 hours old) showing the development of e-eyes, o-otolith, b-premaxillary barbels, h-heart, bl-blood system, v-vitelline complex and l-larval finfold.

lack of the full complement of differentiated erythrocytes. Mesenchymatic tissue was present on the larval finfold as it rounded the posterior tip of the developing embryo. This indicated that the caudal fin had begun to differentiate. It had a single unbranched blood supply.

04d00h50min : Premaxillary barbel buds were visible. The vitelline capillary system had developed into an intricate network encompassing an increasingly large proportion (±50%) of the yolk surface area. The density of melanophores had increased along the dorsal ridge of the embryo.

05d02h50min : Black pigment had developed in each retina. Approximately 20% of the dorsal section of both retinas was pigmented. Yolk capillaries encompassed the entire yolk surface area (see Figure 7.3). The blood was now dark red and was circulating in the embryo at an even flow rate.

06d02h30min : 50% of retina was pigmented. Opercular movement became evident (very slow and erratic, once every three to four seconds). Artery and vein intersegmentals had begun to form and were made visible by blood cells passing through them. The pectoral fin buds were visible. Precursors of the extensive caudal plexus were established. Small loops appeared in the caudal vein.

06d23h50min : A lens had developed in each eye. The lower jaw was developing, but remained attached to the yolk. A pair of mandibular barbel buds had developed. Opercular movement was measured at 60 per minute. Four gill arches were visible on each side of the head. Nostril indentations were visible dorsally above the developing mouth. The heart rate was

recorded at 80 beats per minute. The developing hind gut was visible. The dorsal fin was differentiating and was distinguishable from the dorsal fin fold. Fin ray buds were visible in the developing dorsal fin. The embryo was wriggling vigorously. Embryos hatched (see Figure 7.4). They were 6.7 to 7.1 mm in length.

Step 2. Post hatching to first feeding (day 8 to day 16)

08d00h00min : Gill arches were visible with gill filament buds developing on each. The lower jaw was no longer attached to the yolk surface, but could not close. The lower jaw, the gill arches and the operculae which were interconnected showed rudimentary signs of functioning as a gaseous exchange unit. Nostrils were mere olfactory pits in the forehead region. The urinary bladder was developing. The rudimentary gut had developed up to the anus, but did not possess a lumen. Melanophore development was in close association with the development of a blood supply to the specific body regions. Vigorous wriggling occurred. The free embryos were 7.1 to 7.6 mm in length.

09d00h00min : There was an increase (70%) in the amount of pigmentation in the retinas. The yolk was approximately half its original size. Rays were visible in the caudal fin. The free embryos were approximately 7.5 to 7.7mm in length

10d00h00min : A second pair of mandibular barbel buds were visible. The nostrils had developed a pair of external receptors at each original olfactory pit. The liver had begun to develop. As the feeding structures (mouth, barbels, liver and gut) and the

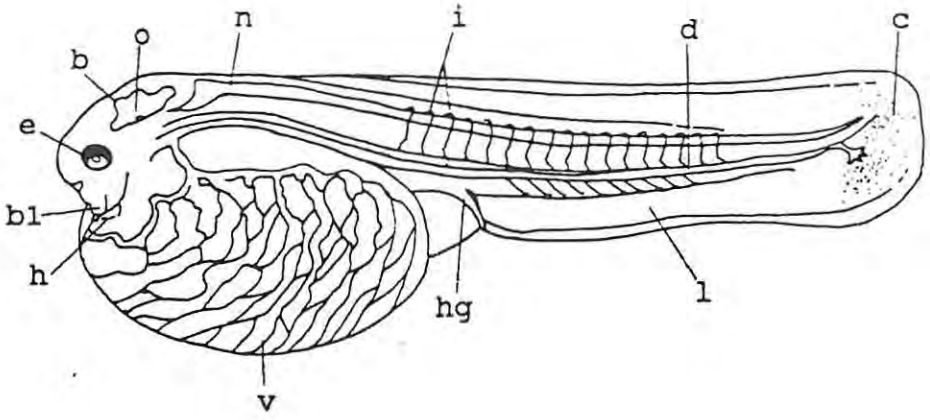

Figure 7.4. Seven day old *Chiloglanis pretoriae* free embryo after hatching (e-eye , b-brain, o-otolith, n-notochord, b1-premaxillary barbel bud, h-heart, i-intersegmentales blood system, d-dorsal aorta, c-differentiating caudal fin, hg-hind gut, v-vitelline complex, l-larval finfold).

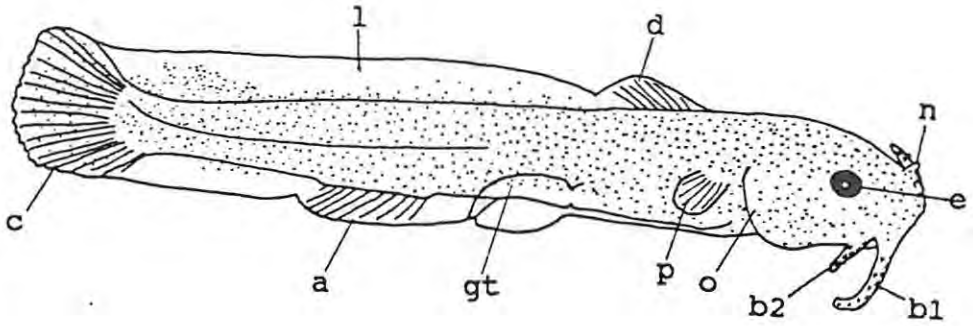

Figure 7.5. A *Chiloblanis pretoriae* larva at first feeding, 16 days after the four cell stage (b1-premaxillary barbel , b2-mandibular barbel , e-eye, p-pectoral fin with rays, a-anal fin with rays, d-dorsal fin with rays, c-caudal fin with rays, gt-gut, o-operculum, n-external structure of nostril, l-larval finfold).

branchial respiratory system (gills) developed so the yolk rapidly decreased to 25% of its original size. There was no vascularisation of the finfold. Free embryos were 7.8 to 8.1mm in length.

11d00h00min : Transparent lenses were visible in the centre of each developing eye. The bile duct had developed. There was yellow bile in gut. A network of blood capillaries were developing in the caudal fin with a capillary loop along each ray. Fin ray buds were visible in the anal fin. An increase in the number and density of the melanophores had resulted in the embryo having a overall brown colour. Free embryos were 8.5 to 8.8mm in length.

12d00h00min : The eyes were able to move. A small (10% of the retina) ventrally situated unpigmented wedge existed in both retinas. The lower jaw could open and close. Each gill arch was lined with gill filament buds which were elongating. Fin rays were developing in pectoral fins. The dorsal fin was beginning to differentiate from the dorsal fin fold. Free embryos were 8.9 to 9.1 mm in length.

13d00h00min : Sensory cells (taste buds?) were visible on the upper lip region and on the barbels. The gill filaments had increased dramatically in length. The gut lumen was visible and developing toward anus. The liver had enlarged. The caudal fin had its full complement of fin rays and had differentiated away from the finfold, developing a serrated edge. A dorsal spine was visible. It was developing within a spine envelope. Free embryos were 9.3 to 9.6mm in length.

14d00h00min : The lens in each eye was protruding above the retina. Premaxillary tooth pads and a line of

mandibular teeth were visible. Gill arches which were lined with gill filaments which in turn were lined with gill lamellae were nearing completion. The yolk comprised 50% (by volume) of the abdominal cavity, in which it was situated. The vitelline capillary complex was resorbed. The gut had differentiated into a stomach and an intestine. Papillae were visible on the stomach wall. Free embryos were 9.7 to 9.9mm in length. The width of the skull at the operculae was 1.3 to 1.5 mm.

15d00h00min : The lenses were becoming more prominent. Sensory cells existed on all barbels and the forward facing edge of pelvic fins. The top lip region had thickened (sensory function ?). The external structures of each pair of nostrils were nearing completion indicating that they could be relied upon to locate food. The yolk comprised 2/5 of the abdominal cavity. Peristaltic movement took place in the gut. The lumen was connected to the anus. Free embryos were 10.2 to 10.5 mm in length.

16d00h00min : The surface area of the gills had drastically increased. The stomach had enlarged and the yolk had been completely absorbed. There was food in the gut. There were fin rays in the dorsal fin. Larvae were 10.5 to 10.7 mm in length (see Figure 7.5).

Step 3. The development of the mouth structure and function. (day 17 to day 30)

Once larvae start moving around in search for food they will require a mouth which is developed for tasting and "sucking", in order to select feed items and to cope with the current, respectively. The development of other sensory functions such as sight

and smell undergo simultaneous development to facilitate both prey and predator detection.

17d00h00min : Sensory cells (taste buds?) visible on all mouth parts. The width of the larval fin fold was diminishing. Pectoral spines were developing in spine envelopes. The longitudinal growth rate seemed to have slowed down and there was a noticeable increase in head width with respect to body length. Larvae were 10.7 to 10.9 mm in length.

18d00h00min : There was one spine and 4-5 fin rays in the dorsal fin. Larvae were 10.9 to 11 mm in length.

19d00h00min : Larvae were 10.9 to 11.1 mm in length.

20d00h00min : A muscle structure was developing at the base of each pectoral fin. There is a possible functional connection between this and the mouth because the increase in size of the muscular structure coincided with an increase in the suction ability of the mouth. In adult fish the development of the muscle was very pronounced. Larvae were 11.3 to 11.5 mm in length.

21d00h00min : An iridescent zone surrounding each lens had developed in each retina (night vision which is present in the adult fish (pers. obs.)?). The muscle at the base of the pectoral fins had increased in size. Larvae were 11.4 to 11.6 mm in length.

22d00h00min : All barbels had stopped elongating and were thickening at the base. Cellular differentiation between the 2 pairs of mandibular barbels indicated the beginning of the adult "beard like" sensory structure attached to the lower jaw. The larvae were 11.5 to 11.7 mm in length.

23d00h00min : The adipose fin was beginning to differentiate. The larvae were 11.9 to 12.2 mm in length.

24d00h00min : The mandibular barbels were being incorporated into the "beard like" structure below the lower jaw. The mouth exhibited the ability to suck weakly onto the substrate. Larvae were 12 to 12.2 mm in length.

25d00h00min : The lens in each eye was made up of what appeared to be other smaller lenses. The nostrils were fully developed. The head region relative to the the barbels and the trunk region had enlarged noticeably. Larvae were 12 to 12.2 mm in length.

26d00h00min : The two pairs of mandibular barbels were almost totally incorporated into the "beard like" structure. The premaxillary pair of barbels was still relatively long. A single pelvic fin bud was visible. There were capillaries visible in the mouth parts, the tooth pads and in the upper lip region. Larvae were 12.4 to 12.7 mm in length.

28d00h00min : Larvae were 12.6 to 12.8 mm in length.

30d00h00min : The iridescent area of the retina was enlarging. The mouth structure was almost fully developed and its suction ability could hold its own body weight suspended on a vertically held glass slide. The muscle at the base of the pectoral fins was large, and was increasing in size with increasing age. Larvae were 13 to 13.3 mm in length. The width of the skull at the operculae was 2.9 to 3 mm wide. At this stage the mouth had developed its full complement of functional structures (see Chapter 2, Figure 2.1). It remained a larva as it possessed remnants of the larval finfold, was short of one pelvic fin and did not have fully developed eyes.

Step 4. Development into the juvenile phase
(day 31 to day 75)

34d00h00min : Larvae were 13.6 to 13.9 mm in length.

38d00h00min : The lenses were no longer transparent. The number of micro lenses had increased and these were slightly pigmented. Larvae were 14.5 to 14.8 mm in length.

42d00h00min : The retinas were fully developed. The second pelvic fin bud was visible. Larvae were 14.6 to 14.9 mm in length.

45d00h00min : Larvae were 15.1 to 15.5 mm in length.

56d00h00min : Traces of the larval finfold were still present. Larvae were 16 to 16.4 mm in length.

63d00h00min : Fin rays were developing in the right pelvic fin. Larvae were 16.5 to 16.8 mm in length.

75d00h00min : The larva had metamorphosed into a fully fledged juvenile. The mouth structure was fully developed and functional. The larval fin fold had been resorbed. Rays had developed in both pelvic fins. The adipose fin was fully developed. All adult structures were present. Juveniles were 16.7 to 17.1 mm in length.

During this investigation it was found that the eggs and free embryos were susceptible to fungal infections. This was controlled by the administeration of a commercially available fungicide, Marpet Anti Ich, at the recommended dosage (one drop per 10l).

7.4 SUMMARY

Chiloglanis pretoriae is a serial spawner that lays large demersal eggs (two to three millimetres in diameter) in the interstices of the gravel beds. Available evidence suggests that this species is a nocturnal spawner. According to Balon's (1990) ecoethological guilds, *C. pretoriae* is a brood-hiding lithophilic spawner (guild A.2.3.). Thus, to culture this species, a gravel spawning substrate must be provided, and a method to harvest the hidden eggs or the larvae has to be perfected.

Of interest was the observation that each egg had a double egg membrane and a yolk which was dark yellow in colour. The double egg membrane buffers and protects the developing embryo against the abrasive or shearing action of water in zones that are relatively turbulent and have high current speeds (Pommeranz, 1974). The dark yellow colour of the yolk indicates a high concentration of carotenoids, which have the potential to supplement external respiration during the early stages of a fishes life (McElman and Balon, 1985). The dark yellow yolk in conjunction with the extensive vitelline plexus suggests that the developing embryos require high levels of dissolved oxygen (Balon, 1985), a factor to keep in mind when designing an egg incubator.

The early development of *C. pretoriae* was indirect and proceeded from a fertilised egg to a developing embryo, to a hatched embryo (still feeding endogenously (Balon, 1990)), to a larva (exogenous feeding), to a juvenile and finally to a sexually mature adult. At a water temperature of 25 ^{o}C, pH of 7 and DO levels between 7 and 8mg/l the embryos hatched seven days after the four cell stage. Compared with *C. gariepinus* which hatched 24 hours after fertilisation at 19 to 33 ^{o}C (Bruton, 1979) and 48 hours after fertilisation at 20 ^{o}C (Hogendoorn, 1979), *C. pretoriae* has an extended embryonic period. This indicates that the eggs of this species should spend at least seven days in egg

incubators under the above mentioned culture conditions.

The large dense yolk supported a free embryo phase for nine days, compared with a 56 hour free embryo phase for *C. gariepinus* (Bruton, 1979). The larger yolk size of *C. pretoriae* gives rise to larger first feeding larvae.*Chiloglanis pretoriae* larvae were 10.7mm in length at first feeding while *C. gariepinus* were only 6.2mm long. Balon (1990) and Wootton (1990) related larval size to food particle size and predation pressure and concluded that larger first feeding larvae have a better chance of survival than smaller ones. A high larval survival rate is a desirable trait for a candidate species for aquaculture (Bruton and Safriel, 1984).

Supplying an acceptable diet to first feeding larvae is a major stumbling block in aquaculture (Lam, 1974 ; Fluchter, 1974 ; Jones *et al.*, 1974 ; Chen, 1976 and Mearns, 1986). During this study however, first feeding larvae readily accepted the prepared food offered to them. Larvae metamorphosed into juveniles 75 days after hatching. No mass mortalities occurred during this phase.

CHAPTER 8

A DISCUSSION OF THE BIOLOGY AND ECOLOGY OF *C. PRETORIAE* AND THE IMPLICATIONS FOR AQUACULTURE

Environmental factors may be subdivided into those that remain relatively constant throughout the year and those that exhibit seasonal variation. The first group of environmental parameters are essential for the survival of *C. pretoriae*. These include current speed, rock and gravel substratum, pH values which fluctuate around seven and dissolved oxygen levels near saturation. The second group exhibit seasonal extremes to which *C. pretoriae* reacts in different ways and includes changes in water temperature, photoperiod and food availability. *Chiloglanis pretoriae* can survive within the entire range of this latter group but particular biological events such as gonadal recrudescence and spawning are restricted to specific sub-ranges. Thus to successfully spawn *C. pretoriae* in captivity, a system had to be designed to maintain an adequate current speed, it had to include a rock and gravel substratum, and to maintain the necessary dissolved oxygen and pH ranges. A mechanism by which water temperature and photoperiod could be regulated was also necessary. Trends in environmental data were compared with trends in biological data in an effort to identify the above mentioned sub-ranges, which could then be simulated in the laboratory in order to spawn *C. pretoriae* in captivity.

Gonadal recrudescence in *C. pretoriae* begins in July. This coincides with the increasing water temperatures and daylength, both of which peak between October and January. For those fish species that originate in the subtropics, changing photoperiod is an important environmental cue for the regulation of reproductive cycles, and it is often associated with changing water temperatures (Skarphedinsson, *et al.*, 1982 ; Pohl *et al.*, 1982 ; Hanyu, 1982 ; Baggerman, 1982 ; Borg, 1982 ; Tay and Lam, 1984).

Photoperiod was noted as being essential for the final maturing and ovulatory processes (Bromage *et al.*, 1982). The actual spawning season of *C. pretoriae* begins in October indicating that the peak values of water temperature and photoperiod are necessary to stimulate final gonadal maturation and spawning.

The size frequency distribution of eggs in a ripe ovary (Stage three) suggests that *C. pretoriae* is a multiple or serial spawner. Similar studies by Clark (1925) and Taylor and DiMichele (1980) noted that the relative abundance of mature, intermediate and immature eggs in *Leuresthes tenuous* and *Fundulus heteroclitus* respectively was relatively constant during the breeding season, and they concluded from this that new oocytes must be continuously produced to replenish those spawned.

Results from the histological investigation indicated that eggs over one millimetre in diameter are developing, and may therefore be included in the estimates of relative fecundity as they could be spawned in that year. In order to calculate the actual fecundity of a serial spawner, a combination of the biological analysis of preserved specimens and experimentation with live fish is essential (Burt *et al.*, 1988). The aquarium studies indicated that *C. pretoriae* lay small batches of between one and six demersal eggs that are not guarded. Available evidence suggests that the fecundity of *C. pretoriae*, even though it can spawn over an extended period, is relatively low and as a result, should one aim at producing these fish for the export market large numbers of broodstock would be required to produce adequate numbers of juveniles.

The number of eggs in the gonads increases with fish length, which indicates that larger broodstock should be selected for the culture programme. The poor correlation coefficient may be explained as follows : At the time of collection all sexually mature females were carrying ripe eggs, but due to the nature of spawning (serial spawning), some females may be at their maximum egg carrying capacity and others may not be.

Chiloglanis pretoriae has an extended spawning season which is generally a characteristic of tropical species (Bye, 1984). Marsh *et al.* (1986) noted that certain Malawian Cichlids exhibited distinct fluctuations in breeding activity when naturally exposed to similar environmental conditions year round and suggested that breeding activity was closely associated with food availability. In other climatological regions, there is often a massive upsurge in the abundance of aquatic insects associated with spring (Wootton, 1979). Wootton (1979) states that the fecundity of many fish species in these regions is dependent on increased food supply to provide the necessary energy for gonadal development and maturation. The ripe gonads of *C. pretoriae* comprised between 10 and 25% of the total body mass providing an indication of the amount of additional energy required during the spawning season. The stomach fullness index values and the condition of the fish peak prior to the spawning season (September). Nutrition is known to have a profound effect on gonadal growth and fecundity (Davey and Chouinard, 1980; Wootton, 1990) as a result the correct diet for *C. pretoriae* broodstock is essential. In addition, the extended nature of the spawning season requires an optimal feed and feeding regime to operate over a similarly extended period under culture conditions.

Chiloglanis pretoriae may be termed a demersal microcarnivore, preying on benthic invertebrates. Its diet is similar to that of three other *Chiloglanis* species studied by Kleynhans (1984). Based on the characteristic benthic feeding method of *C. pretoriae*, a prerequisite for a successful feed for this fish would be that the artificial food should sink quickly. This catlet has a relatively short gut length which is characteristic of most carnivorous fish (Ribble and Smith, 1983). Based on published data on the proximate nutritional analysis of natural fish food organisms (Long, 1961 ; Philips, 1972 ; Yurkowski and Tabachek, 1979 and Piper *et al.*, 1982) the proximate nutritional analysis of the natural dietary organisms of *C. pretoriae*

indicated that the protein content of its natural diet was approximately 55.1% when based on dry mass analysis and between 12% to 15% when based on wet mass analysis. Protein is required for growth and gonadal recrudescence and is therefore an important factor in a fishes diet (Stickney, 1979). Thus, in order to produce ripe fish, an artificial diet with a high animal protein content will have to be supplied to *C. pretoriae*.

During December 1989, 80% of the stomachs analysed contained silt with no traces of aquatic insects. The condition factor values that included gonads showed a distinct decrease indicating that energy is put into gonadal development rather than somatic growth during this period. It also indicated that while detritus could keep fish alive, animal matter was essential to maintain a spawning population of *C. pretoriae*.

According to Wootton (1982) the minimum age at first reproduction is genetically determined, although in a few cases it is possible to infer that environmental factors have led to the evolution of the observed age. However, Alm (1959) observed that within a population, faster growing individuals matured at an earlier age than slower growing ones. Wootton (1990) stated that growth rate is limited by environmental factors such as food supply and temperature. Then according to the above evidence, if fish are maintained under optimal conditions they will grow faster and may therefore mature faster.

The age at 50% and 100% sexual maturity was calculated for *C. pretoriae* using the Von Bertalanffy growth equation. This catlet attained 50% sexual maturity at one year (44mm in length) and 100% sexual maturity at year two (54mm in length). This suggested a minimum age of two years for *C. pretoriae* broodstock. The growth rate of *C. pretoriae* is highest during the first two years of its life during which time it attains 60% and 74% of its theoretical asymptotic length. Quick and Bruton (1983) showed that *C. gariepinus* only attained 17% and 26% of its calculated asymptotic length during its first and second years respectively.

Although *C. pretoriae* is a smaller fish available evidence suggests that its growth rate is suitable for culture purposes. Stickney (1979) suggested that the market size of a fish should ideally be at the time when the initial high growth rate decreases. Based on this idea, the data suggest that *C. pretoriae* should be marketed after one year.

As mentioned in Chapter 7, Balon (1990) divided fishes into 32 ecoethological guilds based on their breeding biology. Three main groups were recognised : nonguarders, guarders and rearers. *C. pretoriae* is a nonguarding, brood-hiding lithophilic spawner (guild A.2.3 of Balon, 1990). Thus to culture *C.pretoriae*, spawning sites would have to be supplied, an egg harvesting method perfected and an egg incubation system developed.

The extensive vitelline complex and the dark yellow colour of the yolk in the developing embryos and larvae indicates the need for good aeration in the incubator. In addition to this, two periods were isolated as being vulnerable even if the embryos and larvae were provided with optimal environmental conditions. These were hatching and first feeding, both of which depended directly on management procedures for high survival rates. When an embryo hatches it is no longer protected by the sterile egg envelope. During this investigation it was found that the free embryos were susceptible to fungal infection which could however be controlled.

Balon (1990) describes larvae as food gathering devices, with the length of the larval period depending on the relative size of the yolk. The large eggs of *C. pretoriae* contained large dense yolks. These sustained embryonic development for seven days until hatching, and then supported a free embryonic phase of a further nine days until the yolk was resorbed. Free embryos then became larvae and began feeding exogenously. May (1974) and Wootton (1990) both describe the first feeding period in a fishes life history as the critical period during which time mass mortalities may occur. However, due to the large eggs produced by *C.*

pretoriae the first feeding larvae were relatively large (10.7mm in length) and readily accepted the prepared food provided. The channel catfish *Ictalurus punctatus* consumes artificial diets immediately after egg sac absorption, enhancing its suitability as a culture species (Stickney, 1979). The identification of a successful feed for larvae during this investigation provided data upon which the development of the larval rearing protocol could be based.

In conclusion, the information provided by the investigation into the biology and ecology of the species provided important information upon which the development of a culture protocol for *C. pretoriae* was based.

CHAPTER 9.

CULTURE PROGRAMME

9.1 INTRODUCTION

Aquaculture has developed in many parts of the world under various natural and socio-economic conditions. Generally the primary interest is directed towards establishing viable industries for the purpose of domestic consumption, export, employment opportunities, income distribution, or a combination of some or all of the above objectives. These development objectives cannot be achieved if a minimum income and profitability are not attained by the producers (Meade, 1989).

As mentioned in the introduction to this study, the ornamental fish trade is a lucrative one. It does not supply an essential food item, but rather aims at a luxury market (Stickney, 1979). The world's traditional suppliers of farmed ornamental fish are presently experiencing serious difficulties due to industrial pollution and the allocation of land for industrial and residential development (Andrews, 1989). This lucrative market has therefore been opened up for exploitation by other countries. African catlets are in demand on the international market, and several species already feature on international dealers price lists (Sands, 1986). To date, however, the only indigenous African fish to reach the international aquarium market have been wild caught specimens (Sands, 1986). Fish stocks indigenous to South Africa would not be able to sustain the harvesting necessary to supply the large international market (Andrews, 1989). Therefore captive breeding programmes have to be developed for selected candidate species before local producers could export fish species indigenous to South Africa.

The suitability of a candidate species for aquaculture can only

be established by a thorough knowledge and understanding of its biology under natural conditions (Hecht, 1984). The biology of *C. pretoriae* under natural conditions was therefore investigated as part of the study and is discussed in Chapter 8.

This part of the study which deals with the development of a culture method has made direct use of the ecological and biological data discussed in the first part of the thesis. The chapters that follow describe the design and functioning of the culture system, the development of an effective filtration system, the identification of a suitable feed and feeding regime for the captive fish, the use of different spawning techniques to successfully spawn the fish, the harvesting and incubation of the eggs produced, and the assessment of the final production rate of marketable sized fish. The aim of the study is to finally provide the information necessary to culture this species for the international aquarium trade.

CHAPTER 10.

SYSTEM DESIGN

10.1 INTRODUCTION

The study of the natural habitat of *C. pretoriae* in Louw's Creek (see Chapter 3) provided an assessment of the conditions under which *C. pretoriae* lives. Louw's Creek is characterised by a typical lotic or fast flowing riverine habitat. This is made up of erosional zones and depositional zones (McCafferty, 1981). *Chiloglanis pretoriae* inhabit erosional zones. An erosional zone is that part of a stream where the flow rate is fast enough to carry away small suspendid particles. In Louw's Creek the current speed within these erosional zones was measured and ranged between 0.6m/sec and 1m/sec. This zone was typified by rapids and riffles, and the bottom was to a large extent devoid of sediment. The substratum characteristically consisted of boulders, stones, gravel and sometimes coarse sand. Based on the available evidence, the ideal spawning tank should incorporate a consistent, fairly strong current (between 0.6 and 1m/sec) with intermittent sheltered areas with reduced current speeds. The substratum should consist of rocks and gravel.

As mentioned in Chapter 8, two types of environmental parameters exist : those that remain fairly constant throughout the year, eg. pH (6.3 to 7.6) and dissolved oxygen (6mg/l to 8.5mg/l), and those that exhibit seasonal fluctuations, eg. photoperiod (minimum of 10.4 hours between sunrise to sunset during winter and a maximum of 13.7 hours between sunrise and sunset in summer), water temperature (a minimum of 18 ^{o}C during winter and a maximum of 23 ^{o}C during summer), turbidity (a minimum of 2.9 NTU during winter and a maximum of 47 NTU during spring) and conductivity (a maximum of 52mS/m during winter and a minimum of

12mS/m during spring). In order to successfully spawn *C. pretoriae* the system must maintain dissolved oxygen and pH values within the above described ranges. Secondly, the seasonal changes exhibited by the other environmental parameters will have to be compared to the available biological data on the seasonality of gonadal activity. Gonadal maturation was shown to begin during spring (September) and peak during summer. Thus, the final spawning system for *C. pretoriae* should be maintained under a photoperiod of at least 13.7 hours light, water temperature of 23 ^{o}C and a conductivity of 12mS/m. Turbidity was not incorporated (see Chapter 3 for an explanation).

Control of the necessary environmental conditions required during this study necessitated the design of a recirculating system which could facilitate the accurate maintenance of these parameters (Spotte, 1979 ; Stickney, 1979 ; Hawkins and Anthony, 1981 ; Piper *et al.*, 1982 ; Meske, 1985). This also entailed the designing of a filtration system to remove accumulated metabolites, notably ammonia and nitrite (biological filter) and the build up of detritus (mechanical filter).

10.2 SYSTEM DESIGN AND WATER QUALITY

Two systems were designed for this study. The first was a continuous raceway and the second a rectangular 80l glass tank.

A continuous raceway was considered to be the ideal tank design as it facilitated a continuous current. Since daily observations were necessary, a glass sided octagonal shaped continuous raceway was designed (see Figures 10.1 and 10.2).

The substratum in the spawning aquarium was based on an erosional zone habitat which consisted of rocks and gravel (McCafferty, 1981). When transferring wild fish to captivity one can match the physical and chemical properties of the water, but one cannot match the familiar home ground and its role in the life of a fish

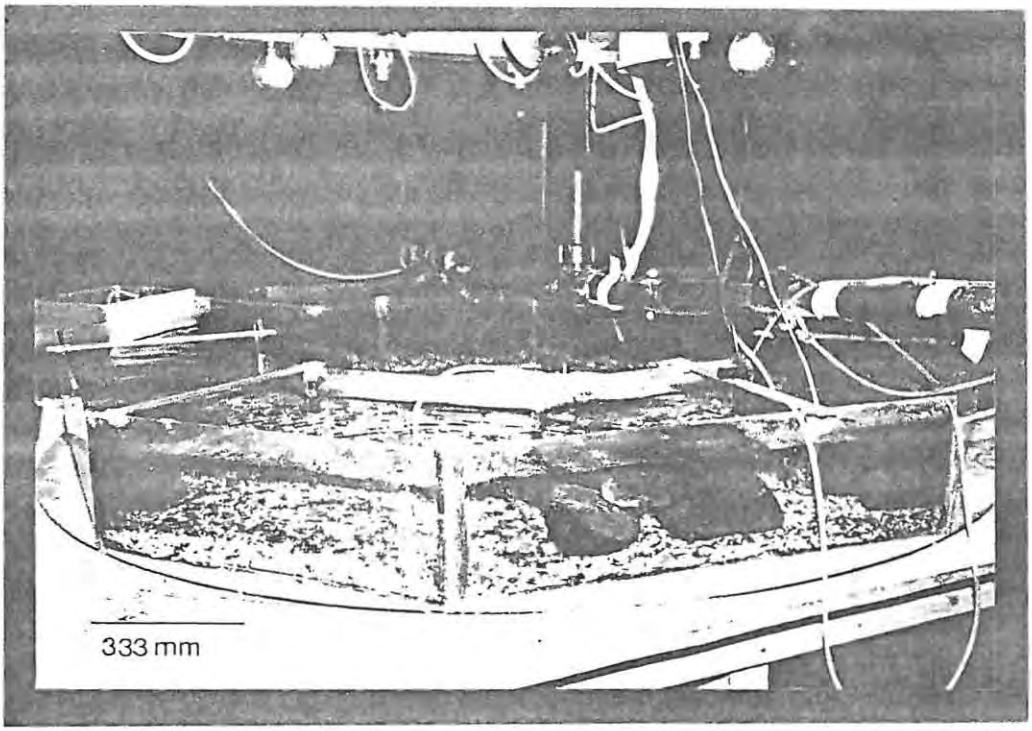

Figure 10.1. The continuous raceway designed for the culture of *Chiloglanis pretoriae*.

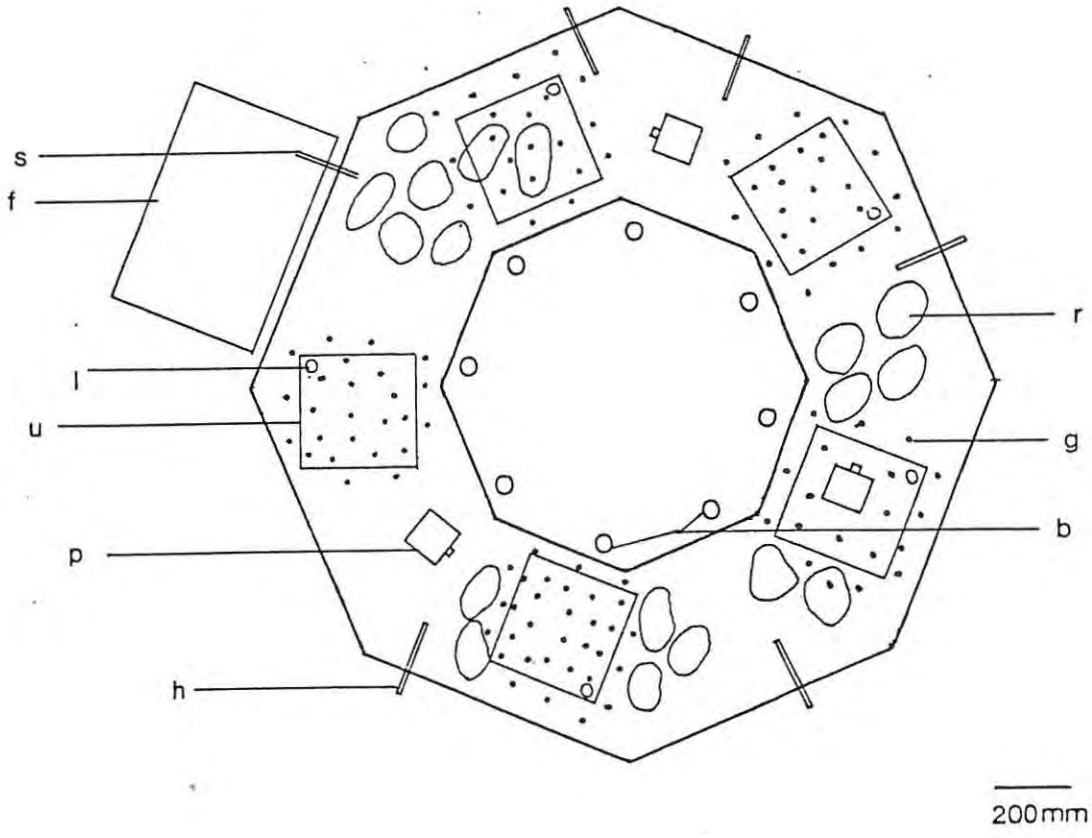

Figure 10.2. A scaled drawing of the continuous raceway showing the mechanical detail (p-submersible water pump, g-gravel, r-rock, u-undergravel filter, l-air-uplift pipe, h-thermostatically controlled heater, a-airstone, b-over head bulbs, f-automatic feeder tank, s-siphon).

(Wardle, 1981). In an attempt to recreate the microhabitats of Louw's Creek, rocks and gravel from the natural environment were used in the system.

Five standard undergravel filters were set up in the raceway. In conjunction with a carrying capacity equation created by Hiryama (1974), de Villiers (1987) established that a standard 30cm by 30cm undergravel filter overlayed with a five centimetre layer of aquarium gravel could maintain over a 100g of three gram fish in a 27l aquaria, fed twice daily with Tetramin flaked fish feed. The stocking density during this study was maintained well below the above capabilities of a single undergravel filter. A single external filter (Aquaclear 150 Power Filter) was used to remove any excessive build up of suspensoids. The acidifying effect caused by the biological oxidation of metabolites was buffered with $CaCO_3$ in the form of oyster shell chips, which were incorporated into all gravel filter beds. In addition to providing the nitrogen fixing bacteria with a surface area which they could colonise, the gravel overlying the undergravel filters supplied a clear, well aerated gravel substratum, similar to that occurring naturally in Louw's Creek.

Three submersible water pumps were installed to create the desired current speed. Each had a maximum output rate of 1600 l/min. Pumps were positioned at equal distances apart, resulting in areas of strong flow alternating with areas of relatively slow flow. The maximum current speed was approximately 0.6m/sec and the minimum current speed was approximately 0.2m/sec. An Oceans Mechanical Flow Meter (2030R) was used to measure the current speed.

The water was aerated by two airstones. In addition the air uplift mechanisms of the undergravel filters aerated the water as did the water pumps which were fitted with a venturi facility. A combination of these aeration methods resulted in the dissolved oxygen being maintained above 6mg/l.

Eight 100W daylight bulbs were used to supply the necessary light. The light intensity at the water surface was 60 lux, recorded using a lux meter (Agrometeorology EL505-106). The light bulbs were connected to an automatic timer set at 16 hours light and eight hours dark. The addition of two hours onto the maximum documented time between sunrise and sunset (13.7 hours) was to allow for the period of light before sunrise and after sunset (pers. obs.)

Five 200W thermostatically controlled heaters were used to maintain water temperature at 26 $\pm$0.6 ^{o}C for the duration of all experiments. This was three degrees higher than the recorded summer temperatures in Louw's Creek. The elevated culture water temperatures were based on personal communications with Kangwane Parks personnel who observed that the water temperatures in Louw's Creek often reached temperatures above 26 ^{o}C during summer.

Temperature, pH and dissolved oxygen were monitored on a daily basis during all experiments (see Table 10.1). Conductivity was monitored on a weekly basis. The concentration of ammonia, nitrite and nitrate were measured over a 21 day experimental period using a Technicon Autoanalyser TMII. The procedures followed were similar to those documented by Demanche *et al.* (1973) and Mostert (1983).

A 10% water exchange was carried out on a weekly basis during which time excessive detritus was also siphoned out. The water depth was maintained at 150mm. This depth is similar to that recorded in the natural habitat of the species. All nets used in the system were sterilised in 10% formalin before and after use.

The 80l rectangular glass aquaria were maintained under the same environmental conditions as the continuous raceway. The only difference being that the shape was different which would theoretically have an effect on the flow dynamics. Each tank was maintained as follows: The substratum consisted of rocks and

Table 10.1. Water quality in the continuous raceway system.

	MAX	MIN	MEAN	STD
TEMP. (° C)	27	25	26	0.6
DO (mg/l)	7.5	6.2	7.1	0.3
pH	7.3	6.5	6.9	0.2
COND (uS/m)	102	61	84	10
NH -N (mg/l)	0.002	0.0005	0.001	0.0005
NO (mg/l)	0.0005	0.0002	0.0003	0.0001
NO (mg/l)	0.065	0.028	0.044	0.015

Table 10.2. The water quality in the 80l glass aquaria.

	MAX	MIN	MEAN	STD
TEMP. (° C)	27	25	26	0.5
DO (mg/l)	7.5	6	7	0.3
pH	7.5	6.5	6.9	0.3
COND (uS/m)	113	71	96.8	9.6
NH -N (mg/l)	0.0016	0.0008	0.0012	0.00027
NO (mg/l)	0.0007	0.0003	0.00048	0.00012
NO (mg/l)	0.1	0.048	0.069	0.019

gravel. Three undergravel filters (30cm x 30cm) were installed to maintain water quality (see Table 10.2). Each tank was aerated by two airstones, and a submersible pump with a maximum output of 1600l/min was used to generate the necessary current speed of 0.6m/sec. The water temperature was maintained at 26 $\pm 0.5^{o}$ by two 200W thermostatically controlled heaters. The photoperiod was set at 16L:8D.

10.3 DISCUSSION

Because *C. pretoriae* spawned naturally in the circular raceway it was concluded that this system successfully simulated the natural environment of the species.

Those environmental parameters which exhibited seasonal variation that coincided with gonadal maturation were identified and successfully maintained at constant levels within the system to ensure constant breeding. As noted in Chapter 8, changing photoperiod is an important environmental cue for the regulation of the reproductive cycles of those fish species that originate in the subtropics, and is often associated with changing water temperatures (Skarphedinsson *et al.*, 1982 ; Phol *et al.*, 1982 ; Hanyu, 1982 ; Baggerman, 1982 ; Borg, 1982 ; Tay and Lam, 1984 among others). Photoperiod was also noted as being essential for the final maturation and ovulation processes (Bromage *et al.*, 1982). As fish are poikilothermic an increase in water temperature results in an increase in metabolic rate, which often provides the stimulus for gonadal recrudescence and spawning migrations (Nikolsky, 1963). There is no evidence available on the effect of conductivity on gonadal activity, thus it was maintained within its natural range during the spawning season of *C. pretoriae*.

The biological oxidation of toxic metabolic by-products such as ammonia and nitrites into relatively harmless nitrates was accomplished using the filtration system described earlier and

maintained within acceptable levels as shown in Table 10.1 and 10.2. A literature review indicated that the toxicity of ammonia varied, depending on the species and its stage of development. Brown and Gratzek (1980) suggest that in general, ammonia concentrations should not exceed 0.1mg/l. At ammonia concentrations of 0.0125mg/l trout showed a reduction in growth rate, with damaged gill, liver and kidney tissue (Piper *et al.*, 1982). Thurston *et al.* (1983) in their work on fathead minnows showed that the ammonia toxicity range for these fish was from 0.75 to 3.4mg/l. Meade (1985) showed that the toxicity of ammonia was related to water temperature and pH. The concentration of the toxic un-ionised form of ammonia increases with increasing temperature and pH. Nitrite, the intermediate product derived from the biological oxidation of ammonia, is also toxic and is known to oxidise haemoglobin to methaemoglobin, a form that is incapable of transporting oxygen to the body tissues (Piper *et al.*, 1982). Hanson and Grizzle (1985) showed how stress induced by nitrite concentrations of 6mg/l increased the susceptibility of channel catfish to bacterial infection. Yearling trout were stressed by 0.15mg/l and were killed by 0.55mg/l ammonia (Piper *et al.*, 1982). The spawning systems designed for *C. pretoriae* maintained the ammonia and nitrite levels well below the stressful and lethal levels documented above.

CHAPTER 11

FEEDING TRIALS

11.1 INTRODUCTION

As the primary aim of this study was to spawn *C. pretoriae* the next step after designing a spawning system, would be to select a suitable feed for the fish. A review of the literature concerning the selection of artificial feeds for cultured fish species revealed that an understanding of the natural feeding biology of the candidate species was imperative to the success of its captive feeding programme. Thus at this stage it would be pertinent to describe some factors related to the feeding biology of fish and which have implications for aquaculture. A fishes mouth structure indicates whether it is a benthic or pelagic feeder (Greenwood, 1984), which in turn will indicate whether the ideal artificial feed should sink or float. Gut length provides an indication of the type of protein the fish requires, eg. carnivorous or herbivorous (Weatherly, 1972 ; Ribble and Smith, 1983). This will provide an indication of the type of protein that must be included in the fishes artificial diet. An analysis of the stomach contents may be used to establish the type and relative bulk of the dietary items selected by fish (Windell, 1968 ; Windell and Bowden, 1978 ; Hyslop, 1980). This information can be used to substantiate the conclusions made during the gut length analysis. It can also be used to calculate the quantity of food eaten and to establish whether the fish selectively feed on any particular food organisms. A proximate nutritional analysis may be carried out on these organisms in order to calculate the proximate nutritional composition of the fishes natural diet (Long, 1961 ; Philips, 1972 ; Yurkowski and Tabachek, 1979 ; Piper *et al.*, 1982). As shown in Chapter 5 *C. pretoriae* was found to be a benthic feeding carnivore living on a diet of aquatic invertebrates. Based on the proximate dry mass analysis

the natural diet of *C. pretoriae* consisted of approximately 55.1% protein, 8.3% lipid, 13.4% carbohydrate, 4.1% chitin and 14.6 KJ/g utilisable energy (see Chapter 5).

The next step would be to establish what artificial feeds have been developed for the aquaculture industry. A literature review revealed that, until comparatively recently, virtually all captive fish held for experimental or other purposes were maintained on natural food sources (Cowey, 1981). However, fish food has since advanced from natural food and granulated parts of slaughtered animals (supplemented by vegetable foodstuffs where necessary), to wet pellets and finally to dried pellets and flakes (Piper *et al.*, 1982). The latter are easily stored, water stable, quality controlled, manufactured on a large scale and distributed with ease (Uys, 1984). The increasing growth of the aquaculture industry has necessitated these developments, as the production of natural food has become impractical and uneconomical (Piper *et al.*, 1982).

The literature also revealed that although the above developments indicate progress in feed formulation, certain circumstances still necessitate the earlier forms of fish feed, eg. when weaning a wild fish onto a pelleted diet granulated meat or wet pellets are often the necessary bridge (Stickney, 1979). In many instances, cultured fish larvae can only survive if given live food (Meske, 1985; Nellen, 1986 ; Sorgeloos, 1986). Within the ornamental fish trade where natural colour, colour intensity and behaviour of the fish are of supreme importance, the granulated meat, live food and "home-made" feeds are still used (Mellen and Lanier, 1935 ; Jocher, 1966 ; Axelrod *et al.*, 1984). A number of rare aquarium species can only be maintained on specific live feeds or supplemented diets (Axelrod *et al.*, 1984). Many culture methods also incorporate natural foods as a supplement to the prepared diet (Meske, 1985 ; Chan, 1990). Most ornamental fish, particularly those in ponds, obtain additional nutrients from phytoplankton, zooplankton and zoobenthos (Brown and Gratzek, 1980).

To formulate a feed for a specific fish species is a detailed process, and beyond the scope of this study. In addition to this, factors such as food intake and assimilation rate vary according to temperature and photoperiod (Cowey, 1981). None of these parameters were investigated during this study. Instead, this investigation was restricted to set feeding trials under the assumed optimum breeding conditions (see Chapter 10). These conditions were identified during the earlier investigations into the biology and ecology of the species (see Chapter 8 for discussion). Similar studies on different larval feeds were carried out on *C. gariepinus* (Hogendoorn, 1980 and Uys, 1984) in order to establish which diet produced the highest growth rates. Assessing the value or suitability of a feed is complex and conclusions must be drawn on the basis of its effectiveness over a given time period with respect to a specific aim (Meske, 1985). The aim of the first part of this investigation was to identify an available feed that was readily accepted by *C. pretoriae* under culture conditions.

Once a suitable feed has been identified an attempt should be made to establish an optimal feeding regime (Meske, 1985). If fish are fed whilst they are still full, food will be ignored and hence wasted (Cowey, 1981). Undigested and uneaten food would result in an increase in suspended and dissolved solids in the culture system which in turn results in an increase in fungal growth and bacterial action and the associated decrease in DO concentrations (Piper *et al.*, 1982). To prevent this one would have to allow for a period between successive feeds during which time the fish can metabolise the last meal. An example which substantiates this idea may be seen in work carried out by Wootton (1990) who showed that trout that had metabolised 75% to 90% of their stomach content showed dramatic increases in appetite.

Although the development of successful feeding regimes are generally associated with growth studies (Hogendoorn, 1980 ;

Hecht, 1982 ; Hecht and Viljoen, 1982 ; Uys, 1984), the aim of the second part of this investigation was to develop a feeding regime that produced broodstock in spawning condition. Should the fish spawn naturally the success of the feeding regime would be confirmed.

The available literature on accurate methods of broodstock nutrition is relatively sparse. Nash and Shehadeh (1980) recommended a diet of 45% protein for *Mugil cephalus* broodstock and a feeding ration (dry artificial feed) of five percent body mass per day. Hogendoorn (1979) states that *C. gariepinus* broodstock can be fed on a specially formulated diet of wet brewery waste, ground cotton seed cakes, blood and dead fish (high protein diet). Brown and Gratzek (1980) suggest the *Ictalurus punctatus* broodstock should be fed two to three percent of their body mass per day, and that a diet consisting of meat was readily utilised by the fish. In the ornamental trade, broodstock are provided with a supplement of live food, generally *Daphnia* species or *Artemia* species (Brown and Gratzek, 1980). Chan (1990) suggested a diet of blood worms, tubifex worms and oxheart for discus (*Symphysodon aequifasciata* and *S. discus*) broodstock.

11.2 MATERIALS AND METHODS

Based on available information from the literature it was assumed that broodstock should be fed to satiation on a high protein diet. A diet supplement in the form of live food should be included where possible. Thus, prepared and live foods were tested during the first part of this investigation. As previously mentioned the aim of the first part of this investigation was to identify a feed that was readily accepted by *C. pretoriae* in an artificial environment.

Ten catlets were placed in each of the three 80l glass aquaria and in the continuous raceway (only one had been constructed at

this stage). Except for the tank shape and volume, all other environmental conditions were kept constant (see Chapter 10). Each feed was tested in all the tanks simultaneously. In order to duplicate the trials carried out in the continuous raceway each experiment had to be repeated. The trials ran for a period of 10 days during which time the catlets were fed to satiation twice a day between 09h00 and 17h00. The fish were described as being satiated when they stopped searching for food and remained motionless under the rocks. Uneaten food was removed each morning.

An artificial diet should provide the cultured fish with all their nutritional requirements. However, as previously stated, the detailed analysis required to establish this was beyond the scope of the study. Thus, as protein is generally the major constituent and the most costly component of aquatic animal diets (Castell *et al.*, 1986), it was used as the principle dietary component upon which the different feeds were evaluated and selected.

The proximate protein content of the natural diet of *C. pretoriae* was calculated in Chapter 5. Based on dry mass analysis, the protein content was found to be 55.1% and based on wet mass analysis it was calculated at 13.5%. These figures were based on mid-range values. The incorporation of both dry and wet analytical methods facilitated the selection of both types of diets. The following feeds were tested :

Tetramin standard mix	(35% protein)
Tetramin conditioning mix	(38% protein)
Freeze dried tubifex worms	(52% protein)
Meal worms	(50% protein)
Daphnia species	(50% protein)
Chironomid larvae	(55% protein)
Oxheart	(57% protein)

Appendix two provides a detailed proximate analysis (where possible) of all the feeds tested.

Although available information on broodstock nutrition does not discuss feeding periodicity in any detail, it is nevertheless an important aspect of a successful feeding strategy. This data may be used to prescribe the exact method of facilitating gonadal maturation in *C. pretoriae*. Thus once the optimal feed had been identified, an attempt was made to establish an optimal feeding frequency. The aim of this part of the investigation was to produce broodstock in spawning condition. Fish with noticeably distended abdomens were assumed to be in spawning condition. However, should the fish spawn naturally the success of the feeding regime would be confirmed.

Four different feeding regimes were tested, each for the duration of 30 days. The same tanks as those described above were used. Five males and five females were placed in each tank. The same feeding regime was tested in all four tanks simultaneously. During the first three feeding regimes tested the fish were fed to satiation once, twice, and three times respectively, between 08h00 and 16h00 each day. The fourth feeding regime tested included four feeds between 09h00 and 21h00. This regime included a night feed.

During the first experimental regime tested, a known amount of food was weighed out for each tank before each feed. The food that was left over after each feed was re-weighed to calculate the mass of the food that had been fed. Food was weighed using a Mettler PJ3000 balance accurate to 0.01g. The total mass of food fed to the fish in each tank was calculated for the 30 day experimental period. The fish in each tank were weighed to calculate the total fish mass in each tank. Fish were weighed using a Mettler PJ3000 balance accurate to 0.01g. Each fish was dabbed dry using a soft paper towel, weighed and returned to a bucket of water. The bucket contained culture water that was well aerated. The total amount of oxheart fed to the fish in each tank

was calculated and then divided by the total number of feeds. This figure was then calculated as a percentage of the total mass of fish in that tank. This calculated amount was then used for the remainder of the experiments.

11.3 RESULTS

The results of the different food trials are expressed in terms of food type, source, cost, acceptability by the fish and remarks based on observations during the respective trials (see Table 11.1). The first five feeds tested proved suboptimal. The two Tetramin feeds consisted of dried flakes which floated on the surface. These feeds were tested as they are generally accepted by most aquarium fish species. However, while a floating feed may be ideal for most ornamental fish species (Brown and Gratzek, 1980) it is suboptimal for benthic feeders. Even when fresh flakes were submerged they were generally ignored by the catlets. Freeze dried tubifex worms, which are also generally eaten by most ornamental fish (Axelrod *et al.*, 1984 ; Chan, 1990), were ignored by *C. pretoriae*. Again, their floating nature is suboptimal with respect to the benthic feeding nature of the species.

The catlets did not eat any meal worms. This trial was terminated after seven days as it was obvious that meal worms were an unsuitable feed for these catlets. The catlets immediately consumed chironomid larvae during the fifth feeding trial. However, this trial was also terminated within seven days as it proved impossible to cultivate or collect sufficient numbers of these creatures to maintain all four experiments.

Daphnia species proved to be the first diet tested where the source was more reliable and the food type readily accepted by the catlets. However no *Daphnia* could be collected on two of the days during the 10 day trial period. This source, as large as it was, proved to be unpredictable.

Table 11.1. The different food types tested during this study.

FOOD ITEM	SOURCE	COST	SUITABILITY	REMARKS
TETRAMIN ST.	COMMERCIALLY AVAILABLE	R123/kg	POOR	FLOATING FOOD FISH IGNORED IT
TETRAMIN CN.	COMMERCIALLY AVAILABLE	R163/kg	POOR	FLOATING FOOD FISH IGNORED IT
FREEZE DRIED TUBIFEX WORMS	COMMERCIALLY AVAILABLE	R228/kg	POOR	FLOATING FOOD FISH IGNORED IT
MEAL WORMS	CULTURED	R5/kg	ZERO	FISH DID NOT EAT ANY
CHIRONOMIDS	COLLECTED	R10/kg	GOOD	READILY EATEN INSUFFICIENT SUPPLY
DAPHNIA	COLLECTED	R10/kg	GOOD	READILY EATEN INCONSISTANT SUPPLY
OX HEART	COMMERCIALLY AVAILABLE	R5/kg	EXELLENT	READILY EATEN CONSISTANT SUPPLY FISH SPAWNED NATURALLY

A suitable food is one that is quickly located and actively consumed by the experimental fish.

Oxheart proved to be the most suitable feed tested. It was commercially available and readily eaten by the catlets. Oxheart sank to the substratum as soon as it was placed into the water. The catlets located it and consumed it immediately and readily.

Oxheart was used in the four feeding regimes tested. Satiation feeding with this feed occurred when the fish were fed approximately five percent of their body mass per feed. Determining spawning condition visually is very subjective as all regimes tested produced similar numbers of fish with swollen abdomens. However, during the fourth feeding regime tested a single spawning occurred in the continuous raceway system. Thus it may be assumed that, feeding *C. pretoriae* approximately 20% body mass of oxheart per day facilitated gonadal maturation.

Chiloglanis pretoriae was observed to feed at night.

11.4 DISCUSSION

The investigation into the natural feeding biology of *C. pretoriae* provided the basis upon which a successful artificial feed could be selected. The most important information discovered was that this catlet is carnivorous. This information, in combination with available literature which suggested a diet of meat (Brown and Gratzek, 1980 ; Chan, 1990), led to the selection of oxheart as a possible feed for *C. pretoriae*. This proved to be an ideal diet for this catlet and, in addition, it was readily available.

During the feeding response a fish must; a) be aroused or become aware of the presence of the food, b) locate and identify it, c) take the food into its mouth and d) accept the food and ingest it or reject it (Cowey, 1981). *C. pretoriae* was aroused to the presence of oxheart in the water and quickly located and consumed it. Available evidence (see Chapter 5) suggests that *C. pretoriae*

tastes the food before taking it into the buccal cavity. Brown and Gratzek (1980) describe taste as one of the most important factors with respect to the development of ornamental fish feeds. During this study oxheart satisfied all four of the above mentioned requirements. In addition, it immediately sank to the aquarium substratum where the benthic feeding catlets could locate it.

However, the problem with oxheart is that its preparation is labour intensive, and it is perishable and has to be kept frozen until use. These factors may present difficulties if one were to culture *C. pretoriae* on a commercial scale. For mass rearing of culture fish an artificial diet is far more practical (Uys, 1984). The artificial aquarium feeds tested during this study however, proved to be unsuitable for *C. pretoriae*. They floated on the surface while *C. pretoriae* feeds on the substratum. Once larger numbers of these catlets are spawned, investigations into the effect of different artificial feed formulations may be initiated. Research into acceptable tastes and odours is imperative. A review of the aquarist literature revealed that no feed specifically for demersal feeding catlets is available. If protein proves to be expensive, experiments into the protein sparing effects of lipids can be initiated. Similar work has successfully been carried out during the formulation of an artificial diet for rainbow trout (*Salmo gairdneri*) (Beamish and Medland, 1986).

The natural spawning that occurred during the fourth feeding regime (i.e. four feeds between 09h00 and 21h00), indicated that this regime provided an adequate amount of food at the assumed optimal intervals to facilitate gonadal maturation. During this experiment *C. pretoriae* were observed to actively feed at night. General activity increased noticeably during the hours of darkness. In his work on *C. gariepinus* Hogendoorn (1980) showed that larvae fed at night exhibited better growth rates than those which were fed the same amount during the day. However, during the present study, the specific effect of night feeding was not

investigated. Fish fed less than four times per day did not spawn. This indicated that they were either provided with insufficient food or the inclusion of the night feed provided a necessary spawning stimulus.

The method used to calculate the approximate feeding ration proved to be effective. Thorpe *et al.* (1990) used a similar technique to establish the approximate feeding level of salmon (*Salmo salar*) when assessing the effect of starvation on this species.

The feeding ration provided to the experimental fish seemed relatively high in comparison to those mentioned in the literature. The daily feeding ration for broodstock using dry feed (45 to 55% protein) has been documented at three percent body mass for *M. cephalus* by Nash and Shehadeh (1980) and for *I. punctatus* by Brown and Gratzek (1980). However, the above feeds are based on dry mass analysis, therefore, each pellet consisted of approximately 55% protein. Each piece of oxheart however, has an approximate protein content of only 17%. Therefore, based on the protein gram equivalent, 10g of dry feed will provide as much protein as 32g of oxheart. A dry feeding ration of five percent body mass per day will therefore be equivalent to a 15% body mass feeding ration of oxheart for the same fish. Thus the diets documented in the literature are comparable to the diet developed for *C. pretoriae* broodstock. With respect to ornamental fish broodstock, the supply of live food cannot be quantified in order to compare it to the diet developed during this study. Based on a review of ornamental fish feeding literature, a diet supplement for *C. pretoriae* consisting of *Daphnia* is recommended.

Based on a diet of oxheart, the annual cost of maintaining 100, 1000 and 10 000 broodstock, would be R109.50, R1095 and R10950 respectively. The above calculations were based on three gram fish being fed 20% body mass per day. These figures can be combined with production rates to ascertain the cost of producing a set number of marketable fish (see Chapter 12 and 13). It must

be born in mind that this is a theoretical calculation which remains to be tested in the field.

CHAPTER 12

SPAWNING

12.1 INTRODUCTION

"The notion that a few fish can be put into a pond or concrete vat and basically left untended to propagate prolifically for easy money happens as often as lead turns to gold" (Brown and Gratzek, 1980). However, successful aquaculture is often dependent on the ease with which culture animals can be reproduced in captivity (Stickney, 1979). Often fish may seem to be acclimated to their captive environment (eg. by eating), but they will not spawn if they are using the available energy to cope with suboptimal environmental conditions (Wardle, 1981). If one assumes that the natural conditions under which a fish spawns are optimal for that fish, then the identification and simulation of these conditions should result in the successful artificial propagation of the species.

As mentioned previously, *C. pretoriae* is a relatively small riverine catlet which attains 100% sexual maturity at a total length of 50mm. This indicates that the broodstock used during spawning experiments should be over 50mm (TL) to ensure that they are sexually mature. *Chiloglanis pretoriae* has a spawning season that extends over the spring and summer months. Summer environmental conditions therefore have to be simulated in order to stimulate seasonal gonadal maturation in captivity. During the spawning season the females lay small batches of relatively large demersal eggs. Available data on the batch size and the total fecundity of the species suggests that the extended spawning is necessary to facilitate the laying of all the developing and ripe eggs present in a mature gonad. The eggs are hidden in the gravel interstices during the spawning process. A suitable spawning substratum is therefore a prerequisite for

successful spawning.

The primary aim of this part of the study was to identify the conditions under which *C. pretoriae* spawns naturally on a continuous and regular basis. Once this had been achieved different broodstock densities could then be subjected to these conditions in order to establish the optimal stocking density. Correct broodstock densities form an integral part of a culture programme (Stickney, 1979 ; Brown and Gratzek, 1980 ; Piper *et al.*, 1982 ; Reay, 1884). An attempt was also made to establish whether the natural spawning season of *C. pretoriae* could be extended. If this part of the study was successful, the extended spawning season would theoretically double the final production rate and so reflect positively on the suitability of *C. pretoriae* as a species for aquaculture.

Generally ornamental fish are small and sensitive and are not suitable for artificial spawning techniques (Brown and Gratzek, 1980). However, Bok and Heard (1982) and Cambray (1985) have successfully induced gonadal maturation in the Border barb *Barbus trevelyani* using homogenised pituitary gland extract (PGE) and human chorionic gonadotrophin respectively showing that hormonal induction of gonadal maturation in small fish species is possible. An attempt was therefore made to ascertain whether the same could be achieved for *C. pretoriae*.

12.2 MATERIALS AND METHODS

The results presented in Chapter 11 showed that oxheart fed at a ration of 20% body mass per day at a constant water temperature of 26 $\pm$ 0.6 ^{o} and a photoperiod of 16L:8D facilitated gonadal maturation under captive conditions. However, while *C. pretoriae* had spawned in captivity (see Chapter 11), the data had not been qualified nor quantified. Similar examples exist in the various aquarist magazines. The aim of these articles is to enlighten the aquarist not the aquaculturist. Therefore, as previously

mentioned, the aim of this part of the investigation was to identify, qualify and quantify the conditions under which *C. pretoriae* spawned on a regular basis.

Two different spawning techniques were attempted : natural spawning and hormone induced artificial spawning. During all experiments fish were fed to satiation on oxheart, four times per 24 hour period (see Chapter 11). Attempts at natural spawning were made in the continuous raceways and in rectangular glass aquaria. Two additional 80l continuous raceways were constructed for this purpose. Each of the smaller continuous raceways was fitted with a single submersible water pump generating a current speed of approximately 0.6m/second. Each continuous raceway was also provided with two air stones, two thermostatically controlled heaters and three undergravel filters. Ammonia, nitrites and nitrates were not measured as the filtration capabilities, water volumes and broodstock densities were similar to those in the 80l aquaria (see Chapter 10). The water quality was therefore presumed to be similar. All other factors being equal, the effect of tank shape on spawning success could be investigated.

(i) Natural spawning

Fish were maintained under optimum summer environmental conditions and fed at the determined optimal feeding regime (see Chapter 10). Where possible the trials were duplicated. With respect to the single 350l continuous raceway, the only form of duplication available was repetition. A comparison was made between the spawning success achieved in the continuous raceways and in the glass aquaria. For the duration of this study spawning success is taken as the production of viable eggs. This was established by harvesting eggs each morning over a 21 day period. The comparison was necessary, to established if the construction of the sophisticated spawning raceways was necessary for the captive large scale propagation of *C. pretoriae*.

A natural sex ratio of 1:1 was maintained. The effect of broodstock density on egg production was tested using the following stocking densities :

350l Continuous raceway

1. 60fish/350l ..... 0.17 fish/litre
2. 40fish/350l ..... 0.11 fish/litre
3. 20fish/350l ..... 0.06 fish/litre

80l continuous raceways and 80l (90cmx30cmx30cm) rectangular glass aquaria

1. 10fish/80l ..... 0.13 fish/litre
2. 4fish/80l ..... 0.05 fish/litre
3. 2fish/80l ..... 0.03 fish/litre

(ii) Artificial spawning

Broodstock were maintained in the 350l continuous raceway. They were fed using the optimum feed and feeding regime (see Chapter 11) and were maintained under the optimal experimental conditions described in Chapter 10.

Pituitary gland extract (PGE) was used in an attempt to induce gonadal maturation in *C. pretoriae*. A review of the literature revealed that pituitary gland extract has been widely used to successfully induce gonadal maturation in different fish species, eg. *Barbus trevelyani* (Bok and Heard, 1982) and *Clarias gariepinus* (Hecht *et al.*, 1982).

A pituitary gland from a 1.2kg ripe running sharptooth catfish *C. gariepinus* female was used. The pituitary gland was preserved in absolute alcohol. The gland was prepared for injecting by

homogenising it in two millilitres of sterile 0.9% physiological saline solution. The dosage was based on that used by Hecht *et al.* (1982) to successfully induce gonadal maturation in *C. gariepinus*. The technique used was similar to that used by Bok and Heard (1982) when these authors successfully induced gonadal maturation in *Barbus trevelyani*.

Two male and two female *C. pretoriae* were used. All four fish had noticeably swollen abdomens. This was taken as an indication that they were in spawning condition. Each fish received a dose of 0.01ml of the homogenised pituitary gland extract. This was injected in the nape region of the dorsal musclature. The four injected catlets were held in gauze cages within the 350l continuous raceway under conditions described in Chapter 10. The fish was examined 24 hours later in order to establish if any were ripe running. The experiment was repeated twice using the same procedure.

12.3 RESULTS

Natural spawning

Table 12.1 shows the egg production rates of the different broodstock densities. It also allowed for a comparison between artificial and natural egg incubation methods. These methods will be discussed in greater detail in Chapter 13, however, in order to understand the table, it is necessary to explain the difference between the natural and tray egg incubation methods. The former method involved leaving the eggs to develop in the gravel beds where they were spawned, while the latter method involved the harvesting and incubation of eggs on gauze trays in incubation tanks.

The production rate of those broodstock densities where the eggs were artificially incubated was calculated as the number of eggs per female over a 21 day period. At all the densities tested, *C.*

Table 12.1. The spawning success of different broodstock densities over a 21 day experimental period. Spawning success was expressed in eggs/day. The different broodstock densities are represented by their respective sex ratios. Included in the results are the respective embryonic survival rates, the resultant number of larvae and the incubation methods used to attain this.

	EGGS/ DAY	TOTAL NO. EGGS/21DAYS	%EMBRYONIC SURVIVAL	NUMBER OF LARVAE	INCUBATION METHOD
3501 RACEWAY					
SEX RATIO					
M30F30	UNKNOWN	UNKNOWN	UNKNOWN	23	NATURAL
M30F30	2.9	61	72	44	TRAY
M20F20	2.67	56	79	44	TRAY
M10F10	3.24	68	69	47	TRAY
801 RACEWAY					
SEX RATIO					
M05F05	UNKNOWN	UNKNOWN	UNKNOWN	9	NATURAL
M02F02	0	0	0	0	0
M01F01	0	0	0	0	0
STD. 801 GLASS TANK					
M05F05	UNKNOWN	UNKNOWN	UNKNOWN	1	NATURAL
M02F02	0	0	0	0	0
M01F01	0	0	0	0	0

pretoriae broodstock produced batches of one to six eggs on a daily basis. Statistical analysis using the student t-test showed that egg production at a broodstock density of M30F30 was the same as that at a broodstock density of M20F20 (P=0.99, t=0.42, df=40). A significantly higher egg production rate was found to occur at a broodstock density of M10F10 (M30F30, P=0.99, t=4.43, df=40 and M20F20, P=0.99, t=3.82, df=40).

Two experiments, one in the 350l (M30F30) continuous raceway and one in the 80l (M05F05) continuous raceway, were undertaken to determine the production rate under natural incubation conditions. The production rate for these experiments was calculated as the number of larvae produced per female over a 21 day period as the eggs and developing embryos could not be seen in the gravel beds. For comparative purposes, the production rates of all the broodstock densities tested was calculated as the number of larvae produced per female over a 21 day period (see Table 12.2).

Table 12.2 The larval production rate of different broodstock densities at a sex ratio of 1:1.

Broodstock Density (fish/litre)	Egg incubation method	Production rate (larvae/female/21days)
0.17	natural	1.1
0.17	artificial	2
0.13	natural	1.8
0.13	artificial	2.8
0.06	artificial	6.8

Thirty males and thirty females maintained in a 350l raceway spawned continuously for a 12 month period. This indicated that it was possible to extend the natural documented six month spawning season for a further six months.

Spawning occurred only once in one of the 80l rectangular glass aquaria. It occurred at the highest density tested (0.13 fish/litre). The resultant spawning rate was calculated as 0.2 eggs/female/21days. The inconsistency of spawning in the 80l rectangular aquaria indicated that these conditions were suboptimal for the regular spawning of this catlet.

Artificial spawning

The use of a PGE injection to induce gonadal maturation in *C. pretoriae* was unsuccessful. When the fish were examined 24 hours after the PGE injection all the females were dead. They exhibited abscesses at the point where the needle was inserted. The males were alive but not ripe running.

12.4 DISCUSSION

Chiloglanis pretoriae spawned naturally under controlled artificial conditions. The continuous raceways proved to be the most effective of the two spawning systems tested while the 80l rectangular glass aquaria proved to be suboptimal.

Many of the of wild caught catlets already on sale on the international aquarium fish market have not been spawned in captivity, eg. *Kryptopterus bicirrhus*, *Synodontis nigriventris*, *Microglanis parahybae*, *Pimelodus clarias* and *Pimelodus gracilis* among many others (Brymer, 1954 and Sands, 1986). Therefore, this study has shown that the need for scientific research in this field is imperative, otherwise the statement "has not been bred in captivity" will remain associated with many species for a

long time to come. Thus, the identification of the natural environmental conditions under which a fish species spawns provides invaluable information for the successful spawning of that species (Bruton, 1979; Crow, 1985; Saint-Paul, 1986; Bailey, 1986).

The administration of hormone injections proved to be unsuccessful. Bagenal (1978) indicated that serial spawning may be explained by physical or morphological constraints, eg. obstruction by unovulated eggs or lack of space preventing simultaneous complete maturation of all eggs. If this is the case, then the natural serial spawning rate of between one and six eggs per day is the optimum number of eggs a female may lay in a day. Similar results were found by Axelrod and Burgess (1973) when observing the natural spawning of the Burundi Killy *Aphyosemion scheeli* (Radda) and by Fromm (1986) when discussing the spawning behaviour of two Killifish, namely *Rivulus xiphidius* and *R. uriflammeus uriflammeus*.

This study has established that 10 males and 10 females in a 350l continuous raceway was the optimal broodstock stocking density. If one were to project these results by multiplying both the volume of the raceway and the number of fish by a factor of five, then 100 broodstock could be housed in a 1750l portapool (continuous raceway). Based on egg production rates, one could produce 340 eggs every 21 days which would result in an annual production of 5905 eggs. The annual cost of feed at current prices for the 100 broodstock (approximate mass of three grams) being fed 20% body mass of oxheart per day would be R109.50. Based on these figures, the cost to produce each egg is R0.02. If one used 10 such portapools with 100 broodstock in each, the annual feed cost will be R1095 to produce 59095 eggs. Chapter 13 will discuss the final production capabilities in more detail, incorporating embryonic survival rates and larval production rates.

CHAPTER 13

PRIMARY NURSING (EGG TO JUVENILE PHASE)

13.1 INTRODUCTION

A review of the literature revealed that larval size (Nash and Shehadeh, 1980; Wootton, 1990), the existence of unknown growth factors (Fluchter, 1974) and metamorphosis (Jones *et al.*, 1974) were the major problem areas in larval rearing. Balon (1990) stated that the larval phase of a fish is vulnerable due to its dependence on very small food particles or organisms. For this reason alone, larval rearing is often the most difficult part of the whole culture operation (Lam, 1974 ; Chen, 1976 ; Nash and Shehadeh, 1980 ; Hecht, 1982 ; Hecht and Viljoen, 1982).

Nash and Shehadeh (1980) fed larval mullet *Mugil cephalus* L. with phytoplankton (undescribed particle size) during the first three to four days of exogenous feeding, after which larvae were fed for a further six days on rotifers (*Brachionus plicatilis*) which had a documented particle size range of 50 to 175 micrometres. Hecht and Viljoen (1982) started feeding larval carp *Cyprinus carpio* on a feed of 150 micrometres for three to four days after which a particle size of 200 micrometres was used for a further five days. Hecht (1982) started feeding larval catfish *Clarias gariepinus* on a feed with a particle size of 200 micrometres, then changed to 250 micrometres at day five. Uys (1984) suggested that the optimal food particle size for *C. gariepinus* was 2.2% of the mean total length.

Metamorphosis from the larval stage to the juvenile stage has also been documented as a vulnerable stage (Fluchter, 1974 ; Jones *et al.*, 1974). Metamorphosis in *C. pretoriae* progressed at

a constant rate, with slight changes or remolding events (see Chapter 7). Therefore, no problems were expected with the growing out of *C. pretoriae* from the egg to the juvenile phase.

The primary nursing trials were carried out in conjunction with the spawning experiments. The time available for this study did not allow for an investigation into the mass rearing of larvae. (conclusions can be drawn from the results).

13.2 MATERIALS AND METHODS

Based on data made available by the investigation into the early development of *C. pretoriae* (see Chapter 7), larvae were relatively large (10-12mm in length) at the start of exogenous feeding and could therefore ingest a relatively wide range of food particle sizes. Based on the method used by Uys (1984) to calculate the food particle size for larval *C. gariepinus* a theoretical food particle size of 230 micrometres may be suitable for larval *C. pretoriae*. The food supplied to growing larvae during the early developmental study was highly successful and was incorporated into the rearing programme.

Three different rearing techniques were tested. These were the free spawning method, the egg harvesting method and the larval harvesting method. Unless otherwise stated all experiments were run under the culture conditions described in Chapter 10.

i) Free Spawning Method

Broodstock were placed in a continuous raceway as described in Chapter 12. Egg laying, hatching, and growing of young to marketable size occurred in the same tank. The embryonic survival rates could not be monitored as the eggs and free embryos remained hidden in the gravel beds. The production rate was therefore calculated as the number of larvae produced over a 21

day period. Larvae (± 13mm total length) were visible and could be counted when moving around and feeding on pieces of oxheart and naturally occurring infusoria.

ii) Egg harvesting method :

Eggs were collected from the gravel beds each day. The gravel was carefully disturbed using a glass rod. Eggs disturbed in this way were siphoned into a plastic container. They were then transferred to 80l glass incubation tanks where they were incubated in specially constructed nylon gauze trays (see Figure 13.1). The incubation tanks were maintained under the same conditions as those described in Chapter 7 (see Table 7.1). Each time new eggs were put into the incubation tanks, prophylactic fungal treatment was carried out as a matter of course. Marpet Anti Ich was used as prescribed (one drop per 10 litres).

First feeding larvae and juvenile *C. pretoriae* were fed Tetra Min Baby Fish Food (45% protein) which they readily accepted. Larvae were fed to satiation five times between 08h00 and 20h00. This resulted in an approximate feeding ration of 20 to 25% body mass per day. These approximate values were calculated using larvae which were sacrificed every 14 days over the measured growth period. Uys (1984) suggested a similar ration of 25% body mass for the first two weeks of larval growth of *C. gariepinus*. Satiation was taken to be when the larvae stopped searching for food and and moved to the side of the incubation trays. Embryonic (egg to larval stage) and larval survival rates were calculated for each batch of eggs produced at the respective broodstock densities.

The growth rate was monitored by measuring the total length of 10 larvae from first feeding to 40mm (TL). Larvae were very sensitive to handling and had to be kept in water for the duration of the measuring procedure. Each larva was placed in a petri-dish which contained water at 26^{o}C. It was then viewed using a dissecting microscope fitted with an eyepiece micrometer.

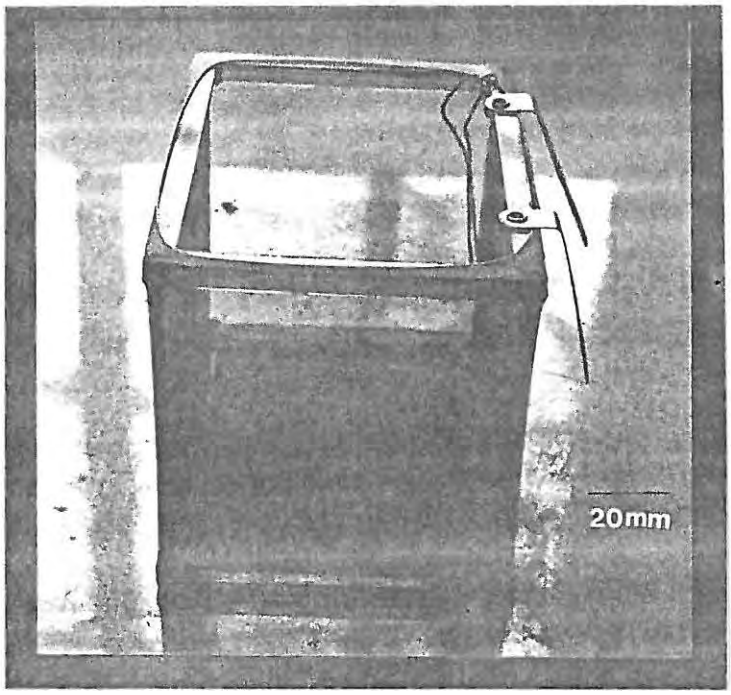

Figure 13.1. Hatching tray.

The graticule was used to measure the total length of each larva to the nearest 0.01mm. Juveniles were measured using a vernier caliper accurate to 0.1mm.

iii) Larval harvesting method :

In this trial, eggs were left to develop and hatch in the raceway. When the larvae reached approximately 12mm to 15mm in length, they were removed and placed into growing tanks. These were standard rectangular 80l glass tanks set up and maintained under environmental conditions described in Chapter 10. Here they were fed Tetramin Baby Fish Food (45%). Larvae were fed to satiation five times between 08h00 and 20h00 each day. Growth and survival rates were monitored as described in the previous section.

13.3 RESULTS

For the purpose of this section, a specific number of eggs produced at a specific broodstock density will be referred to as a batch of eggs.

The results of the free or natural spawning methods may be seen in Tables 12.1 and 12.2. Although cannibalism of larvae by the adult fish was not apparent and no intracohort cannibalism was observed these methods produced fewer larvae than the egg harvesting method. In addition to the lower production rates, the embryonic and larval survival rates and growth rates could not be accurately determined during the free spawning method.

The egg harvesting method was labour intensive but produced more larvae per given broodstock density than the free spawning method. Gravel trays were provided in order to localise spawning sites and thus to maximise egg harvesting rates. The effect of different broodstock densities on the final production of larvae

is apparent from the results shown in Table 12.1. The embryonic survival rates ranged between 69% and 79% for all batches tested (see Table 12.1).

Figure 13.2 shows the growth rate of *C. pretoriae* from 14 day old embryos to marketable size juveniles of 40mm (TL). From the time of egg harvesting, it took approximately 98 days to reach 40mm (TL). The survival rate of larvae from first feeding to the juvenile stage ranged between 95% to 100% for all the batches tested.

The survival rate of the larval harvesting method was less than five percent which is unacceptable for a commercial culture programme.

13.4 DISCUSSION

There is no information on the mass rearing of ornamental aquarium species. All the papers relating to the mass rearing of larvae relate to food fish. Examples of this are *C. gariepinus* (Hogendoorn, 1979 ; Hogendoorn and Vismans, 1980 ; Hogendoorn, 1980 ; Hecht, 1982 ; Uys, 1984), and *M. cephalus* (Nash and Shehadeh, 1980) amongst others. It must be borne in mind however, that in many cases ornamental fishes do not have the high fecundities of the above species and therefore egg and larval production rates are relatively low.

To date, a wide variety of culturing techniques exist for the production of ornamental fish. Each of these is based on the spawning method of the specific fish species. For example, Brown and Gratzek (1980) describe methods to breed a wide variety of ornamental fish from live bearing species, eg. mollies (*Poecilia latepinna*) to fish of the family Callichthyidae, eg. (*Corydoras aeneus*). Mollies are spawned in large ponds (30.5m x 7.6m x 1.8m) and harvested when needed. Corydoras, however, are spawned in glass aquaria and spawn erratically. These fish spawn in pairs or

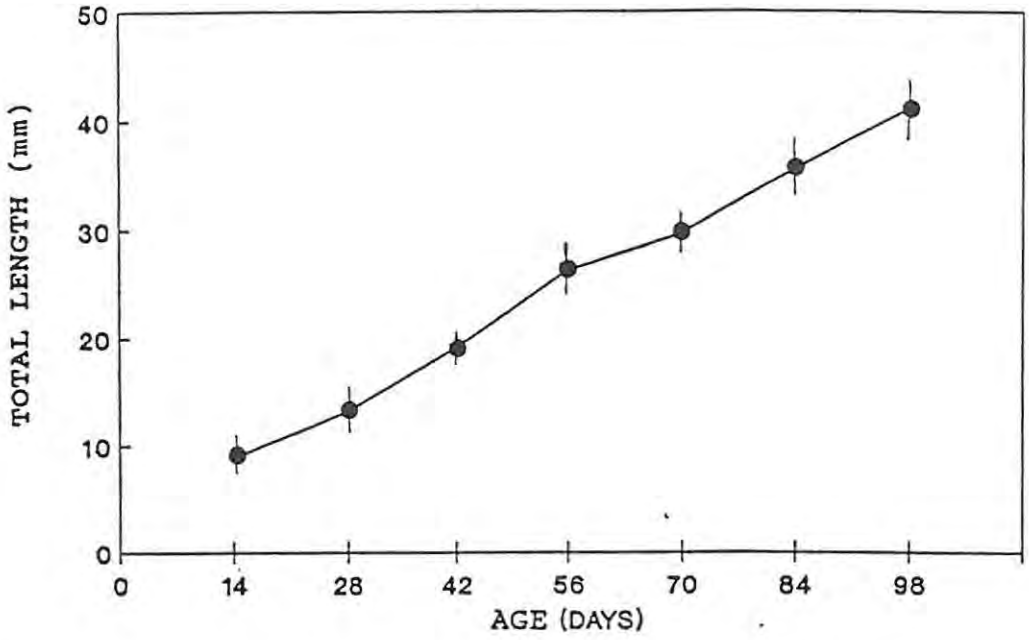

Figure 13.2. The growth of *Chiloglanis pretoriae* from 14 day old larvae to marketable sized juveniles of 40mm (TL). The vertical bars represent the standard deviation.

small colonies, and only produce a few eggs during a spawning. Both types of culture methods are commercially viable operations. The above information suggests that if the existing demand and the market price are such that the producer can make a profit, then that particular fish species is suitable for the ornamental trade. Since the spawning strategy of *C. pretoriae* is similar to that of *C. aeneus* available evidence suggests that it has an acceptable production rate for the commercial culture of the species.

The higher production rate of the egg harvesting method indicates that careful husbandry is necessary to obtain high survival rates. The results show that the eggs of *C. pretoriae* may be harvested, as described earlier, without any obvious damage. However, once hatched the embryos and larvae are very sensitive to handling. This fact was substantiated by the poor survival rates during the larval harvesting method. Thus, in order to ensure acceptable survival rates a culturist can : a) leave the eggs to incubate naturally and only harvest juveniles (similar to the mollie culture described above), b) harvest the eggs and incubate them under artificial conditions. The former method is less labour intensive but unpredictable, while the latter method though labour intensive, produces a predictable number of marketable size fish. For example, if one harvests 50 eggs, approximately 33 market size juveniles will be produced in 98 days ie. based on 70% survival from the egg to the larval phase, and 95% survival from the larval to the juvenile phase.

CHAPTER 14

CONCLUSION

The overall aim of the study was to develop a culture protocol for a fish which was identified as a viable aquarium species in order to protect it from over exploitation in its natural environment. This goal has been achieved and the results show that the rock catlet *C. pretoriae* may be successfully cultured for the aquarium trade. The study has also shown the necessity of investigating the biology and ecology of the candidate species prior to the development of a culture protocol. This information was found to be of inestimable value for the design of the spawning system, the feeding of broodstock, larvae and juveniles, the natural induction of spawning and the primary nursing phase. The following set of physical and chemical environmental factors were found to be optimal for the successful culture of *C. pretoriae*. A continuous current of 0.6m/sec, rock and gravel substratum, photoperiod of 16L:8D, water temperature of $26\pm0.6^{o}C$, dissolved oxygen levels of 7.1 ± 0.3mg/l, pH of 6.9 ± 0.2, conductivity of 84 ± 10uS/m, and a filtration system that maintained low levels of ammonia (0.001mg/l) and nitrites (0.0003). The broodstock were fed to satiation on oxheart four times a day, between 09h00 and 21h00. The optimal stocking density for maximising egg production was found to be 0.06 fish/litre at a sex ratio of 1:1.

Using the data obtained in this study a theoretical production rate could be calculated. As previously mentioned in Chapter 12, 100 *C. pretoriae* broodstock in a simple raceway system at a sex ratio of 1:1, are capable of producing of 5909 eggs per year. If the broodstock are fed 20% body mass of oxheart per day the annual feed costs for the 100 broodstock would be R109.50. Thus one egg would cost R0.02 to produce. Based on a 70% embryonic survival rate, 4136 larvae will be produced. Based on a 95%

larval survival rate, 3929 juveniles will attain the theoretical market size of 40mm (TL) within 98 days of being harvested as eggs.

If these growing fish are fed the equivalent of 20% body mass per day, one would require 8.5kg of larval feed. Each market size fish would therefore cost in the region of R0.58 to produce. If the cost of producing one egg is added to this, the final cost to produce a market size fish will be R0.60. The estimated market value of *C. pretoriae* is R1.20 (Stallard pers. comm. Amatikulu Hatchery Pty. Ltd. Gingindlovu). A culturist would therefore make a 100% profit on every fish sold. This example does not include fixed and other variable costs such as water, tanks, electricity, labour, etc. These factors vary with location and have to be calculated on site by the prospective culturists. However, the above figures do provide an estimate of the profitability of culturing *C. pretoriae* for the aquarium trade.

This study has essentially dealt with the development of a culture protocol for the indigenous rock catlet *C. pretoriae*. However, the research should also be placed in a broader context. *Chiloglanis pretoriae* is one of five subtropical *Chiloglanis* species that are endemic to southern Africa. With the exception of *C. swierstrai* which prefers a sandy substratum (pers. obs.), all the other species are found in rocky riverine habitats (Crass, 1964 ; Jubb and Le Roux, 1969 ; Bell-Cross, 1972 ; Kleynhans, 1984). Because of their similar habitat preferences it may be possible to apply similar spawning and rearing techniques as those developed during this study for *C. pretoriae*.

The success of this study indicates that similar captive propagation programmes may be carried out on threatened fish species to replenish wild stocks. Ribbink (in prep.) has bred an isolated strain of *Pseudocrenilabrus philander* in captivity and has successfully reintroduced aquarium reared specimens into the wild. Internationally, a number of captive spawning and rearing programmes exist to support fisheries and so prevent the

demise of the respective fish stocks. Hatchery programmes in the U.S.A. date back to the nineteenth century. The United States government, through the Commission of Fish and Fisheries, Bureau of Commercial Fisheries, Fish and Wildlife Service and the National Marine Fisheries Service, is responsible for the release of billions of hatchery reared fish into the wild (Stickney, 1979). These captive propagation programmes prevented the total decimation of natural fish populations through excessive fishing pressure (Stickney, 1979).

The successful culture of *C. pretoriae* also has implications for the captive propagation of other ornamental catlet species on a world-wide basis. Edmonds (1990) stated that the captive breeding of *Dianema urostriata* (the flag tailed catlet) is poorly documented, as with many other catfishes. Sands (1986) and Burgess (1989) both provide similar evidence on the difficulty of spawning catlets in captivity. The literature revealed that most attempts to spawn these fish have been carried out in rectangular glass aquaria. While these could successfully maintain *C. pretoriae*, this catlet only spawned in the continuous raceway. Thus if other system designs are experimented with, other catlets may be bred in captivity. The demand for these fish at present is high (Edmonds, 1990).

During this study it was also established that each habitat has a selection of organisms that have evolved within the confines of that specific niche. The structure and function of the particular niche' is imperative to the survival of the co-evolved organisms (Jones, 1987; Bruton, 1989). Vermeij (1986) concluded that habitat destruction and fragmentation, and hunting (harvesting) are the most important causes of extinctions of plants and animals. This culture programme was developed in order to protect the natural fish populations from over exploitation and their possible demise. However, habitat destruction has the same effect. Habitat destruction can take place in many ways. This is evident in Louw's Creek where irrigation and siltation have caused an overall change in the character of the river.

Firstly irrigation, by diverting large volumes of water, has resulted in the destruction of the fast flowing headwater habitat. The stream below the irrigation canal is now a sluggish trickle, and is no longer inhabited by specialist species such as *C. pretoriae* and *A. natalensis*. Secondly, the existence of an upstream mining operation has caused excessive siltation of the riverine habitat. Silt settles on the substratum, smothering crevices between rocks and covering the gravel spawning beds of *C. pretoriae*. Silt also interrupts the natural food chain by smothering the sources of primary and secondary production.

This type of habitat destruction defeats one of the primary objectives of this research programme, namely to culture *C. pretoriae* in order to prevent the depletion of natural populations through harvesting of fish for the international aquarium trade. River systems are finely balanced and very sensitive to human intervention. Habitat destruction in most instances is merely looked upon as a temporary change that may be returned to "normal" later. A world-wide increase in population has resulted in a greater need for agriculture and industry both of which rely on the environment for raw materials, and in some instances destroy the same environment. The conservation of the natural environment is essential, for without this resource base breeding operations would become meaningless.

In conclusion, this study has shown that it is possible to culture the indigenous South African rock catlet for the ornamental fish trade. By culturing this species the natural populations can be protected from indiscriminate harvesting and a new export industry can be created in southern Africa.

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Appendix 1. The species composition of Louw's Creek after Heymans (1987).

Chiloglanis pretoriae
Amphilius natalensis
Anquilla mossambica
Barbus eutaenia
Barbus trimaculatus
Barbus unitaeniatus
Clarias gariepinus
Labeo molbdinus

Appendix 2. An analysis of the prepared feeds used during the culture programme.

Prepared foods

1.Tetramin standard mix

Ingredients : Fish meal, feeding oat meal, wheat germ meal, shrimp meal, torula dried yeast, gluten, soybean oil, lecithin, butylated hydroxytoluene (BTH) preservative, food colouring materials E101 and E172. The guaranteed analysis of the above feed was as follows :

Min. crude protein	35%
Min. crude fat	9%
Max. crude fibre	4%
Max. moisture	8%
Max. sodium chloride	3%

2. Tetramin conditioning mix

Ingredients : Fish meal, torula dried yeast, soybean meal dehulled solvent extracted, feeding oat meal, wheat feed flour, ground brown rice, algae meal (*Spirulina maxima*), casein, glutin. chlorophyll, carotene, lutein from natural sources (leaves and dehydrated alfalfa meal), food colouring materials E101, E102, E132.

Guaranteed analysis :

Min. crude protein	38%
Min. crude fat	3%
Max. crude fibre	9%
Max. moisture	8%
Max. sodium chloride	3%

3. Freeze dried tubifex worms

Tetra product (52% protein - Marpet Analysis)

4. Grated oxheart

Approximate analysis (after Kleiner, 1947) : In 100g

protein	17g (in the form of myoglobin)
fat	4g
carbohydrate	1g
water	78g
Vit. B biotin	7-8 mg
thyemine	400-600 ug
riboflavine	750-900 ug
Vit. A	present
Vit. C	present

Fe	present
Cu	trace present
cytochrome C	high conc.

Live foods

1. Meal worms cultured in the laboratory. The protein content was estimated at 50% based on the combined value for different insects (see Table 5.1).

2. *Chiloglanis pretoriae's* natural diet of aquatic insects captured in nearby streams. The protein content was estimated at 55% based on the value calculated for chironomid larvae in Table 5.1.

3. *Daphnia* species collected in a nearby dam. Freezed dried *Daphnia* consist of approximately 50% protein (Marpet Analysis).

Larval food

Tetra Min Baby Fish Food "E" (for egg layers)

Ingredients : Fish meal,Torula dried yeast, shrimp meal, wheat feed flour, feeding oat meal, ground brown rice, soybean meal, dehulled solvent extracted casein, algae meal, wheat germ meal, wheat germ oil, chlorophyll, carotene and luten from natural sources (leaves and dehydrated alfalfa meal), bixin from natural sources (annatto tree seed) and food colouring materials E101, E102, E127, E132 and E172.

Guaranteed analysis :

Min. crude protein	45%
Min. crude fat	6%
Max. crude fiber	6%
Max. moisture	8%
Max. sodium chloride	2.5%