THE BIOLOGY OF *AUSTROGLANIS GILLI* AND *AUSTROGLANIS BARNARDI* (SILURIFORMES: AUSTROGLANIDIDAE) IN THE OLIFANTS RIVER SYSTEM, SOUTH AFRICA

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ABSTRACT

Austroglanis gilli and *A. barnardi* are endemic to the Clanwilliam-Olifants System in the Western Cape, South Africa. The populations of each of these species are considered to be threatened by various anthropogenic activities, which include inappropriate agricultural practice and impacts of alien invasive fish species. The purpose of this thesis was to assess the life-history of these two endangered species in order to contribute to understanding their biology. Such information is vital for the development of strategies for their conservation.

Marginal zone and marginal increment analyses from sectioned lapilliar otoliths of both *A. gilli* and *A. barnardi* showed a unimodal peak, suggesting a single annulus formation. The oldest specimens of *A. gilli* and *A. barnardi* were 12+ and 14+ years, respectively. The growth of *A. gilli* and *A. barnardi* was relatively slow and was best described by the von Bertalanffy growth curve as: $L_t = 131.56(1 - \exp(-0.27(t - 1.18)))$ for male and $L_t = 113.86(1 - \exp(-0.43(t - 0.74)))$ for female *A. gilli* from the Rondegat River. In the Noordhoeks River, growth was $L_t = 99.67(1 - \exp(-0.53(t - 0.35)))$ for male and $L_t = 96.60(1 - \exp(-0.64(t - 0.11)))$ for female *A. gilli*, and $L_t = 71.02(1 - \exp(-0.26(t - 3.07)))$ and $L_t = 69.50(1 - \exp(-0.36(t - 1.88)))$ for male and female *A. barnardi*, respectively.

The average natural mortality for the combined sexes was estimated using catch curve analysis at 0.37 ± 0.12 per year for *A. gilli* from the Rondegat River, and at 0.71 ± 0.05 and 0.39 ± 0.04 per year for *A. gilli* and *A. barnardi* from the Noordhoeks River, respectively.

For *A. gilli* in the Rondegat River, the first maturity was estimated at 3.3 years (97.3 mm SL) for males and 3.1 years (94.3 mm SL) for females. In the Noordhoeks River, the first maturity for *A. gilli* was estimated at 2.0 years (71.9 mm SL) for males and 1.7 (66.4 mm SL) for females, and for *A. barnardi* at 2.0 (55.0 mm SL) for males and 2.9 years (58.9 mm SL) for females. Maturity corresponded closely to the asymptotic sizes from the von Bertalanffy curves, suggesting a shift in energy use from somatic growth to gonad development.

Macroscopic assessment of the state of gonads, the Gonado-somatic index and histological examinations revealed that both *A. gilli* and *A. barnardi* have a single spawning season. The presence of oocytes in different stages of development in each of the ovaries of mature females collected between November and January suggested asynchronous, iteroperous serial spawning. The resorption of yolk was observed from ovaries collected between February and March and no vitellogenic oocyte was visible from ovaries collected between April and August. A similar trend was observed for males, with spermatozoa filling the lumen between November and January and some residual spermatozoa being present in the lumens soon after the breeding season. Spermatocytes and spermatids were dominant in the testes until October.

An Index of Relative Importance revealed that *A. gilli* feeds predominantly on the benthic macroinvertebrate larvae of Ephemeroptera (particularly Baetidae), Diptera (particularly Chironomidae and Simuliidae) and Trichoptera. *Austroglanis barnardi* feeds predominantly on dipteran larvae (particularly Chironomidae and Simuliidae). Chi square contingency tables showed a significant difference in the dominant prey items of *A. gilli* and *A. barnardi* in the Noordhoeks River ($\chi^2 = 53.79$, d.f. = 4, $p >$

0.001) and *A. gilli* between Rondegat and Noordhoeks rivers ($\chi^2 = 34.74$, d.f. = 4, $p > 0.001$). The Spearman's rank correlation test showed no shifts in the diet of *A. barnardi* from Noordhoeks River and *A. gilli* from Rondegat River with a change in size and season ($p>0.05$). However, there was a shift in the diet of *A. gilli* from the Noordhoeks River which could suggest a distinct patchiness of benthic macro-invertebrates between the riffle feeding areas used by juveniles and other biotopes used by adults.The occurrence, in stomach contents, of other prey items from a wide variety of taxa and the presence of allochthonous material from the terrestrial environment could suggest an opportunistic feeding guild for both *Austroglanis* species.

The life-history traits of *A. gilli* and *A. barnardi,* which are charaterized by slow growth, long life span and low relative fecundity, indicate that both species are relatively precocial and *K*-selected. The population of a precocial species is relatively stable and if population numbers were to be greatly reduced, they would require a long time to rebuild. An urgent conservation intervention is therefore recommended for *Austroglanis* spp. so as to maintain the diversity of populations within these species. The creation of protected river reserves and raising public conservation awareness may minimise activities that result in altered river hydrology and the destruction of complex benthic habitats.

CHAPTER 1: General Introduction

It is generally accepted that humans have negative impacts on the functioning of natural ecosystems, resulting in a great loss of species diversity (Skelton *et al.* 1995; Lévêque 1997; Revenga & Kura 2003). Consequently, the conservation of natural resources is receiving increased attention in order to minimize negative anthropogenic impacts on ecosystems and to prevent extinctions (Lévêque 1997). Human developmental activities need to be managed to minimize their impact and ensure sustainable use of natural resources in ecosystems, which is essential for the continued health and survival of present and future generations.

Freshwater environments are the most impacted ecosystems in the world but their conservation has received less attention than terrestrial and marine ecosystems (Lévêque 1997; Revenga & Kura 2003). Freshwater ecosystems are characterized by their 'patchiness' within each system or river catchment, often as isolated 'islands' with high levels of endemism and low tolerance to environmental alterations (Lévêque 1997).

1.1 The Cape Floristic Region

The Cape Floristic Region (CFR) in the Western Cape Province of South Africa is home to one of the six floral kingdoms and one of the global biodiversity hotspots (Myers *et al.* 2000; Palmer *et al.* 2003). Most of the species of fauna and flora of the CFR are endemic and IUCN red-listed (Palmer *et al.* 2003). The indigenous freshwater ichthyofauna of the CFR comprises the lowest species diversity with the highest level of endemism (Skelton *et al.* 1995) compared to the species diversity of plants and animals in the region (Palmer *et al.* 2003).

Nineteen primary freshwater fish species occur in the region (Table 1.1) and are IUCN-listed: critically endangered (1), endangered (8), vulnerable (3), near threatened (1), least concern (5) and data deficient (1) (IUCN 2009). The conservation priority in this case should be given to species from near threatened to critically endangered categories.

The Olifants River is the most diverse river system, comprising nine primary freshwater species of which eight species are endemic and all are IUCN-listed (IUCN 2009). This fauna is dominated by Cyprinidae, which comprises six species, followed by the Austroglanididae (*Austroglanis gilli* Barnard, 1943 and *Austroglanis barnardi* Skelton, 1981) and one currently-recognized species of Galaxiidae, *Galaxias zebratus* Castelnau, 1861.

Austroglanis gilli is currently listed by the IUCN as 'vulnerable' (Swartz *et al.* 2007a). Since *A. gilli* occurs in more than 10 tributaries of the Olifants with large population numbers in some of the streams, it qualifies as 'vulnerable' (as the number of locations are more than five). *Austroglanis barnardi* is red-listed as 'endangered' (Swartz *et al.* 2007b).

Because of increasing threats to the status of freshwater ecosystems in the CFR, the future survival of the indigenous fish fauna relies on conservation interventions (Skelton

1987; Impson *et al.* 2002, IUCN 2009). In the CFR context, these threats fall into two broad categories: habitat degradation and alien fish invasion.

1.2 Habitat degradation

In South Africa the scarcity of water is of great concern because of the increasing size of the human population (DWAF 1986 cited from Cambray *et al.* 1997). South Africa is naturally a dry country. Freshwater bodies, including rivers, are heavily utilized and increasing human demands are placed on these ecosystems. In the Western Cape Province, for example, freshwater abstraction for agricultural irrigation has resulted in the loss of aquatic habitats and biota (Gaigher *et al.* 1980; Whitehead *et al.* 2007).

Other impacts are caused by bulldozing of rivers to aid in water abstraction during the summer, and reduced winter flooding as a result of water abstraction and other agricultural impacts. Agricultural runoff may contain fertilizers, pesticides and suspensoids (Bruton 1985). Fertilizers and pesticides used in farmlands have adverse effects on the freshwater biota and have been associated with the decline of indigenous fish (Skelton 1987). Agrochemicals, including copper compounds that control the fungus that causes "crinkle–leaf" in citrus and various insecticides, enter the streams through direct spraying and seepage through the soil. Since the tributaries of the Clanwilliam-Olifants system are oligotrophic with low pH and conductivity (Skelton 1988), the results of associated nutrient loading, eutrophication, direct poisoning of biota, changes in turbidity and increased siltation, are likely to cause severe changes in water quality and habitat alterations (Gaigher *et al.* 1980; Bruton 1985).

Table 1.1. A list of primary freshwater fishes of the Western Cape Province, the river systems where they occur, their conservation status, and threats to their status (IUCN 2009).

Species	System	Status	IUCN status	Threats
Austroglanis gilli	Clanwilliam-Olifants	Endemic	Vulnerable	Habitat destruction
A. barnardi	Clanwilliam-Olifants	Endemic	Endangered	Habitat destruction
Galaxias zebratus	Widespread in CFR	Indigenous	Data deficient	Alien invasives[1] and habitat destruction
Sandelia capensis (Cuvier, 1829)	Widespread in Western Cape	Indigenous	Least concern	Alien invasives and habitat destruction
Pseudobarbus phlegethon (Barnard, 1938)	Clanwilliam-Olifants	Endemic	Endangered	Predation by alien invasives, especially *M. dolomieu*, and habitat destruction
Labeo seeberi Gilchrist & (Thompson, 1911)	Clanwilliam-Olifants	Endemic	Endangered	Habitat destruction, dams (reduced water flows) and alien invasive fish.
Barbus calidus (Barnard, 1938)	Clanwilliam-Olifants	Endemic	Vulnerable	Alien invasives and habitat destruction
Barbus erubescens (Skelton, 1974)	Clanwilliam-Olifants	Endemic	Critically endangered	Alien invasives and habitat destruction
Barbus serra (Peters, 1864)	Clanwilliam-Olifants	Endemic	Endangered	Alien invasives and habitat destruction
Labeobarbus capensis (Smith, 1841)	Clanwilliam-Olifants	Endemic	Vulnerable	Alien invasives, habitat destruction and dams
Pseudobarbus tenuis (Barnard, 1938)	Gourits and Keurbooms systems	Indigenous	Near Threatened	Alien invasives, habitat destruction
Pseudobarbus asper (Boulenger, 1911)	Gourits and Gamtoos systems	Indigenous	Endangered	Alien invasives and, habitat destruction
Barbus andrewi (Barnard, 1937)	Berg and Breede systems	Indigenous	Endangered	Alien invasives habitat destruction
Pseudobarbus burgi (Boulenger, 1911)	Berg, Verlorenvlei, Langvlei and Eerste	Indigenous	Endangered	Predation by alien invasives and habitat loss
Pseudobarbus burchelli (Smith, 1841)	Breede and Duiwenhoks systems	Indigenous	Endangered	Alien invasives , pollution and habitat destruction
Pseudobarbus afer (Peters, 1864)	Coastal rivers from Mossel Bay to Sundays River	Indigenous	Least concern	Alien invasives and habitat destruction
Labeo umbratus (Smith, 1841)	Orange system and some larger coastal systems	Indigenous	Least Concern	Alien invasives and habitat destruction
Barbus anoplus (Weber, 1897)	Orange River and many peripheral karoo systems, widespread in South Africa	Indigenous	Least Concern	Alien invasives and habitat destruction
Barbus pallidus (Smith, 1841)	Eastern Cape and Orange systems	Indigenous	Least Concern	Alien invasives and habitat destruction

[1] 'Alien invasives' refers principally to alien invasive fish

Gore *et al.* (1991) noted that flow rate requirements for the spawning of indigenous fishes of the CFR are species-specific. A behavioural study on the Clanwilliam yellow fish, *Labeobarbus capensis* Smith, 1841, indicated that river flow is one of the environmental factors which triggers spawning and that a continuous flow must be maintained for a successful recruitment of this species (Cambray *et al.* 1997). As a result of bulldozing and water abstraction, the normal flow of the rivers of the CFR has been altered and this has long been associated with the decline of the freshwater fishes (Gaigher *et al.* 1980).

1.3 Impacts of alien invasive species

Alien invasive fish disrupt the normal functioning of ecosystems and can cause irreversible damage to ecosystems (Lévêque 1997). In South Africa, alien invasive fish have been introduced into many river systems and some have adapted well and become established in their "new" environments (Skelton 1987). The major impacts of alien invasive fish species include predation on native fish — which, in the rivers of the Western Cape, is mainly due to smallmouth bass *Micropterus dolomieu* Lacepède, 1802 (Skelton 1987; Impson *et al.* 2002; Woodford 2005) — and competition for food as a result of the presence of species such as bluegill *Lepomis macrochirus* Rafinesque, 1819 (de Moor & Bruton 1988). Recent findings have indicated that selective predation of indigenous fish by alien invasive fish has allowed invertebrate algal grazers to dominate, resulting in a depletion of algal-covered substrates (Lowe *et al.* 2008).

A decline in the numbers of indigenous fish species has been observed as a result of invasion. In some cases this has caused local extinctions of species (Gaigher 1973; Impson *et al.* 2000; Woodford *et al.* 2005). For example, *Barbus serra* Peters, 1864 was once recorded in the lower Rondegat River but disappeared from this reach of the river after the introduction of *M. dolomieu,* a piscivorous alien invasive fish (Gaigher 1973).

The Noordhoeks River, where the largest population of *A. barnardi* occurs, has recently been invaded by *Tilapia sparrmanii* Smith, 1840. Since *T. sparrmanii* feeds mainly on plants (particularly the filamentous algae) and invertebrates (Winemiller & Kelso-Winemiller 2003), its presence in the Noordhoeks River is most likely to have a significant impact on the trophic ecology.

Other alien invasive fish that are widespread in the rivers of the Western Cape Province are listed in Table 1.2. The principal impacts of these fish are generally considered to be predation and competition for resources such as food and space (Gaigher 1973).

Negative impacts on the indigenous freshwater fishes of the Western CFR appear to have been accelerated by the combination of alien invasive fish and habitat alteration. In the Jan Dissels River, for example, where the largest population of *A. gilli* occurs, there is also an invasion by the smallmouth bass, *M. dolomieu.* Interestingly, the population of *A. gilli* coexists with this piscivorous fish and appears to be little impacted. This is presumably due to the cobbled riverbed being pristine and unimpacted by agricultural activities such as siltation (IR Bills, pers. comm.). Conversely, in the Rondegat River, which is impacted by various agricultural activities and contains *M. dolomieu*, sedimentation appears to have increased the susceptibility of *A. gilli* to predation. Cobbles, which are an important feature of the habitat and may be used as cover by the catfish to evade predation (Bills 1999), become covered by sediments. This increases the vulnerability of the catfish to predation. As with many impacts, there is often synergy: indigenous species may survive habitat degradation or the presence of alien fishes alone but disappear when subjected to the combined effects of both these impacts.

Table 1.2. Alien invasive freshwater fishes that occur in the Western Cape Province, South Africa (adapted from Impson *et al.* 2000).

Species	Distribution
Labeobarbus aeneus (Burchell, 1822)	Gourits system
Carassius auratus (Linnaus, 1758)	Localised
Clarias gariepinus (Burchell, 1822)	Widespread
Cyprinus carpio (Linnaus, 1758)	Widespread
Gambusia affinis (Baird & Girard, 1853)	Localised
Lepomis macrochirus (Rafinesque, 1819)	Widespread
Micropterus dolomeiu	Widespread
Micropterus punctulatus (Rafinesque, 1819)	Localised
Micropterus salmoides (Lacepède, 1802)	Widespread
Oncorhynchus mykiss (Walbaum, 1792)	Localised
Oreochromis mossambicus (Peters, 1852)	Widespread
Oreochromis aureus (Steindachner, 1840)	Localised
Tilapia sparrmanii	Widespread
Perca fluviatilis (Linnaeus, 1758)	Localised
Salmo trutta (Linnaeus, 1758)	Localised
Tinca tinca (Linnaeus, 1758)	Breede system

1.4 Conservation initiatives

There is no doubt that humans must play a proactive role in maintaining the normal functioning of natural ecosystems so as to conserve the existing biodiversity. The freshwater fish fauna of the CFR has been the focus of a number of conservation efforts. Currently, these include a proposed habitat rehabilitation project on eradicating aliens using rotenone on four rivers, namely the Suurvlei, Rondegat, Krom (Cedarberg) and Krom (Eastern Cape) (Enviro-FishAfrica 2009).

It is recognized that effective management and the prioritization of conservation efforts are dependent on an understanding of the biology and ecology of the target species. However, the lack of biological information of the species concerned constrains the prioritization of conservation efforts as the level of susceptibility of each species within a system may be different (Cambray & Hecht 1995). For this reason, research on the biology and ecology of the endemic fishes in the CFR is urgently needed. This study has therefore focused on the biology and ecology of two endemic catfish species: *A. gilli* and *A. barnardi*.

1.5 Current state of knowledge of *A. gilli* and *A. barnardi*

Fishes of the family Austroglanididae are endemic to Southern Africa (Skelton 2001). This family comprises only one genus, *Austroglanis* Skelton, Risch & De Vos, 1984 comprising three species: *A. gilli* and *A. barnardi,* which are both restricted to the Clanwilliam-Olifants river system, and *Austroglanis sclateri* Boulenger, 1901, which is restricted to the Orange-Vaal river system (Skelton 2001). De Pinna (1993) (as cited by Impson *et al.* 2000) hypothesized that *Austroglanis* is a Gondwana relic whose sister–group, *Horabagrus* Jayaram, 1955 occurs in Southern India. Subsequent study on osteology, however, suggested other sister–groups which include ictalurid, cranoglanidid, ariid and claroteid catfishes (Diogo & Bills 2006). However, the most recent findings contradict these hypotheses and indicate no clear phylogenetic relationship of

Austroglanididae within the Siluriformes, although it is assumed that fishes of this family probably have an African ancestry (Dr J. G. Lundberg, Academy of Natural Sciences, Philladaphia, pers. comm.).

Austroglanis gilli (Fig. 1.1) is olive-grey in colour and typically does not have spots, although there may be some exceptions as the population from Jan Dissels River contains both spotted and non-spotted individuals. *Austroglanis barnardi* (Fig. 1.2) is golden-brown and is typically covered in spots, although some individuals are unspotted. The relatively longer dorsal and pectoral spines of *A. gilli* also distinguish it from *A. barnardi*.

In addition to some morphological differences within each of the *Austroglanis* species (Bills 1999), recent work has shown that there are also genetic variations within populations of each species and between populations of the same species in different streams (E.R. Swartz, M.J. Cunningham & I.R. Bills, unpubl. data). It was, therefore, recommended that management intervention in the Clanwilliam-Olifants river system should not only focus on *Austroglanis* spp. as a whole, but also on the conservation of morphological complexes and genetic variations. *Austroglanis gilli* and *A. barnardi* also differ in habitat preference and distribution: field observations indicate that *A. gilli* juveniles are only found in riffles with substrates of cobbles and clean sand (Fig. 1.3). Adults of this species occur in a wide variety of habitats, with the majority preferring deeper runs and pools (Fig. 1.4) (Bills 1999). Juvenile and adult *A. barnardi* occur exclusively in shallow, slow-flowing water with cobble riffles and clean sand (Fig. 1.3) (Gore *et al.* 1991).

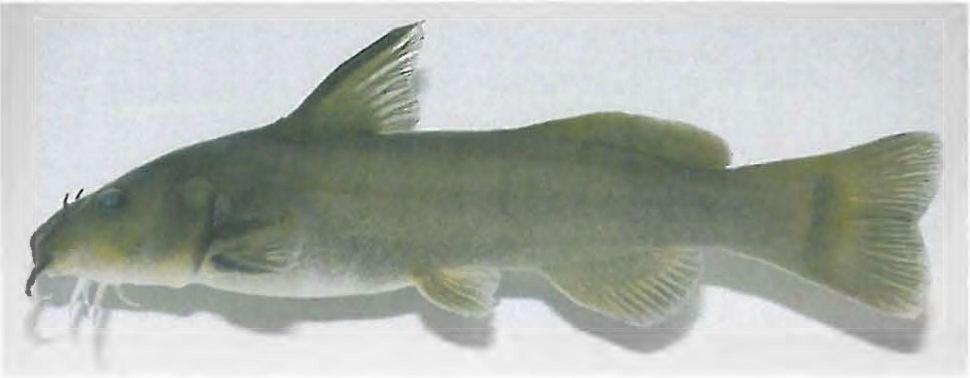


Fig. 1.1. *Austroglanis gilli* from the Rondegat River (near the campsite) (Photograph: I.R. Bills).

Fig. 1.2. *Austroglanis barnardi* from the Thee (upper camp site) (Photograph: I.R. Bills).

Fig. 1.3. A picture showing a typical habitat of *A. barnardi* from the Noordhoeks River (Photograph: I.R. Bills).

Fig. 1.4. A picture showing a typical habitat of *A. gilli* from the Rondegat River. (Photograph: I.R. Bills).

Austroglanis gilli has a wide geographical distribution, occurring in nine Olifants (Oudste, Thee, Noordhoeks, Boontjies, Boskloof, Heks, Rondegat, Jan Dissels, Dwars) and seven Doring (Biedouw, Tra Tra, Eselbank, Matjies, Krom and Breekkrans) basins of the Olifants River system (Swartz *et al.* 2007a). *Austroglanis barnardi* only occurs in three streams, namely, Heks, Noordhoeks and Thee (Fig. 1.5). *Austroglanis gilli* also occurs more widely, typically from the lower to the upper reaches of the river.

1.6 Biology and ecology

Information on the biology and ecology of *Austroglanis gilli* and *A. barnardi* in the Clanwilliam-Olifants System is limited to studies by Bills (1999) and Woodford (2005). Bills (1999) surveyed the distribution limits and habitat preferences of the two *Austroglanis* species and described some aspects of the breeding biology of *A. barnardi*. The study by Woodford (2005) was a precursor to some eradication work carried out during the rehabilitation project of the Rondegat River, which focused on the diet of ichthyofauna. Woodford's study was, however, limited by sample size and lack of seasonal sampling. A search on the literature failed to indicate any further information on the biology such as growth rate, longevity, size and age at maturity and fecundity of either of these species.

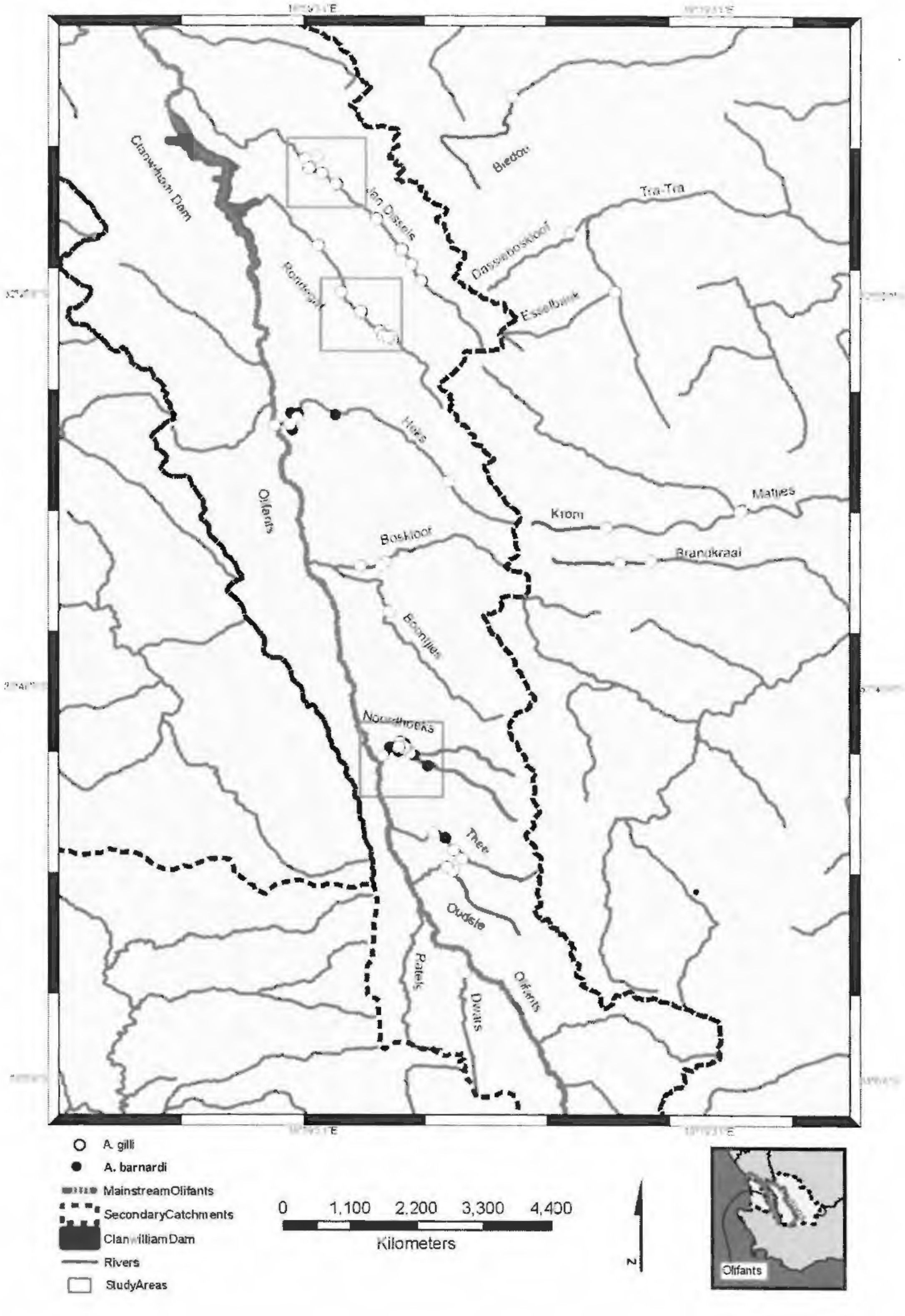


Fig. 1.5. A map showing distribution records of *A. gilli* (open cycles) and *A. barnardi* (dark cycles) in the Clanwilliam-Olifants system in the Western Cape, South Africa.

1.7 Objectives of the thesis

The objective of this study was to undertake a comprehensive assessment of the biology and ecology of two *Austroglanis* spp. in the Clanwilliam-Olifants river system. The study therefore assessed characteristics of the life-history of *A. gilli* and *A.barnardi* in terms of *K*- and *r*-selection life-history continua.

Specific objectives of the study were to:

a) Review the current state of knowledge of the biology and distribution of *A. gilli* and *A. barnardi* (Chap. 1)

b) Formulate a methodology to study the biology of vulnerable and endangered species without causing major detrimental impact on the existing populations of *A. gilli* and *A. barnardi* (Chap. 2)

c) Study age and growth (Chap. 3)
    1. To determine if the two species can be aged using otoliths
    2. To determine whether the two species are short- or long-lived
    3. To determine whether the two species are slow- or fast-growing
    4. To gain an understanding of the population structure and to estimate natural mortality of each of the species

d) Study reproductive biology (Chap. 4) in an attempt to:
    1. Describe gonadal recrudescence
    2. Determine spawning seasonality
    3. Determine age and size at sexual maturity
    4. Understand spawning frequency by determining egg size distribution

    v. Estimate fecundity

e) Study feeding biology (Chap. 5) to determine:
    i. Whether *Austroglanis* spp. are specialists
    ii. Assess the functional aspects of feeding

f) Discuss life-history traits of each of the species (Chap. 6) in attempt to formulate conservation strategy

CHAPTER 2: General Methodology

2.1 Study area

The main study sites were chosen from the Noordhoeks (32°43'32"S, 19°04'30"E) and the Rondegat (32°22'15"S, 19°03'07"E) rivers, which are tributaries of the Clanwilliam-Olifants System (see Chapter 1, Fig. 1.5). These two rivers have their sources in the Cedarberg mountains at elevations above 1000 m above sea level and flow in a north-westerly direction to join the main Olifants River. The tributaries of the Olifants in this region drain from a Table Mountain Sandstone (TMS) substratum and water is characterized by low conductivity (50–150 μS cm^{-1}) and low pH, ranging from 5.5 to 6.5 (Skelton 1988).

An annual rainfall of approximately 1400 mm is received by the mountain range of which the majority falls in winter between May and September, February being the driest month (King & Tharme 1993). Rivers on the western side of the Cedarberg mountains appear to receive the most rainfall as opposed to the rivers of the eastern side, which fall in the rain shadow of the mountains and receive less rainfall. Since rainfall is seasonal (Fig. 2.1), summer is characterized by slow river flow (Fig. 2.2), while heavy rains in winter transform rivers into turbid torrents (Fig. 2.3). Winter flooding is, however, periodic and may last for one to two days after the rain. Winter flows may sometimes be similar to summer flows.

Temperatures of the CFR are also subject to seasonal changes: water temperatures in the tributaries of the Clanwilliam-Olifants system exceed 20°C in summer and drop below 10°C in winter (Fig. 2.4).

2.2 Overview of study sites

The Rondegat River is a short mountain stream of about 25 km long. Originating from the Cedaberg mountains (32°24′S; 19°05′E) near the Uitkyk pass above Algeria, it flows in a north-westerly direction and joins the main Olifants River at Clanwilliam Dam. The upper reaches of the river are mostly pristine. The middle reach is impacted by some anthropogenic activities as it passes through a campsite and citrus orchards with no apparent barrier preventing pollution from entering the stream. The lower reach is dominated by *M. dolomieu,* an alien invasive fish that has penetrated from the Clanwilliam Dam upstream to a waterfall that acts as a natural barrier (32°16′40"S; 18°58′46"E), preventing further upstream invasion by this species. The middle and lower reaches of the river have alien invasive plants including black wattle (*Acacia mearnsii*) and, to some extent, are also impacted by cattle damage to the banks and riparian vegetation. Cattle cause widening of beds and major sedimentation, both of which result in a reduction in habitat complexity.

The Noordhoeks River, comprising two major tributaries and its catchment, appears to be relatively large compared to the Rondegat River. Since the Noordhoeks River faces west, it probably receives more rainfall than does the Rondegat, which probably falls in the rain shadow of the range of mountains to its west. The middle and lower reaches are impacted

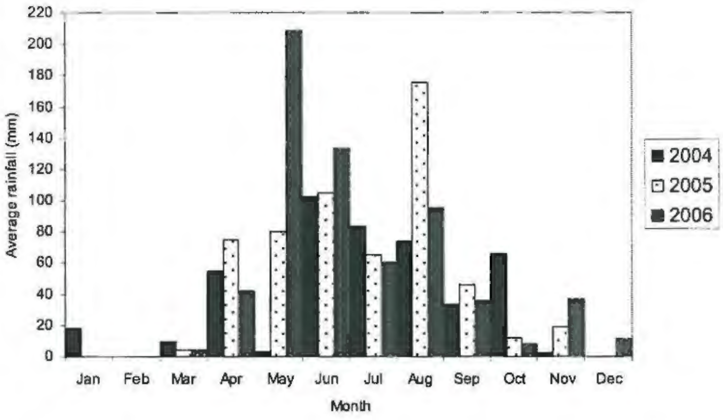


Fig. 2.1. Monthly average rainfall of the Rondegat River recorded during 2004 to 2006 at Algeria in the Western Cape Province of South Africa (Weather SA, http:www.weathersa.co.za).

Fig. 2.2. The appearance of streams of the Olifants River system during the summer season. This picture was taken at Jan Dissels River (Coordinates from Google Earth: 32°14' 26" S 19° 00' 25" E) (Photograph: I.R. Bills).

Fig. 2.3. The appearance of streams of the Olifants River system during the winter season. This picture was taken at Rondegat River at the bridge near Algeria (32° 22' 13.6" S 19° 03' 13.0" E). (Photograph: I.R. Bills).

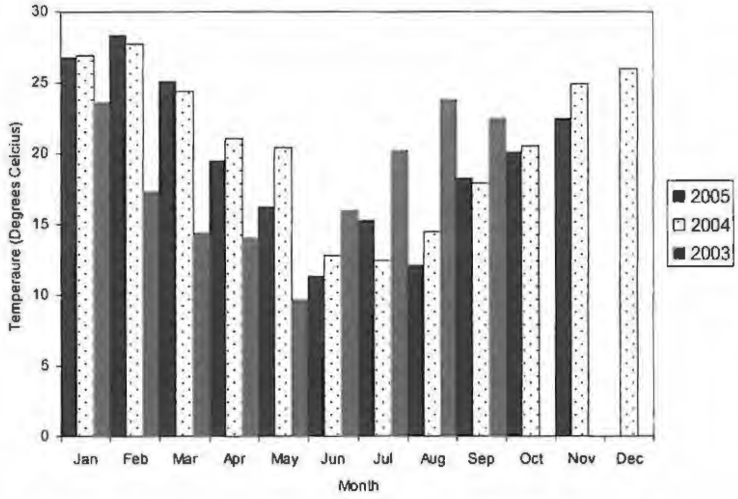


Fig. 2.4. Average monthly temperature recorded in the Driehoeks River, one of the tributaries of the Clanwilliam-Olifants River system: 2003 to 2005 (data supplied by B. R. Paxton, University of Cape Town).

by agricultural activities as the river passes through farmland with some orchards. Irrigation and anti-flooding modifications by farmers — which involve bulldozing of the lower river bed, damming of the river during summer, and irrigation piping — have enabled the invasion of *T. sparrmanii* into the river from the mainstream Olifants River.

Each of the study sites was dominated by riffle habitats, with a few deep pools of depths between 1 to 2 m. The substrata of both Rondegat and Noordhoeks rivers were largely dominated by boulders, cobbles and coarse clean sand, although the Rondegat River appeared to have moderate substrata with dark sediments.

The Noordhoeks River was chosen for this study because it is the most diverse tributary of the Clanwilliam–Olifants, containing both *A. gilli* and *A. barnardi* (Table 2.1). The Rondegat River was a potential site for the proposed alien fish eradication project using rotenone, hence the studies by Woodford (2005) and the present one. A few samples of *A.*

gilli were also collected from the Jan Dissels River as it is the largest of the study sites, with the largest maximum individual size of *A. gilli*. All three study sites have been invaded by alien invasive fish, *M. dolomieu* having been introduced to both Rondegat and Jan Dissels rivers, and *T. sparrmanii* to the Noordhoeks River.

Table 2.1. List of ichthyofauna occurring in the Rondegat and the Noordhoeks rivers in the Western Cape Province, South Africa (compiled from SAIAB's database) (+ indicates that the species has been recorded in the river).

Species	Noordhoeks River	Rondegat River
Galaxias zebratus	+	+
Barbus calidus	+	+
Labeobarbus capensis	+	+
Pseudobarbus phlegethon	+	+
Austroglanis barnardi	+	
Austroglanis gilli	+	+
Micropterus dolomieu		+
Tilapia sparrmanii	+	

2.3. General sampling methods

Austroglanis gilli and *A. barnardi* specimens, which were collected during surveys between 1996 and 2004 and housed in the South African Institute for Aquatic Biodiversity (SAIAB) collection, were used for feeding and reproductive assessments. In the interests of conservation, additional monthly samples of these IUCN-listed species were not collected. An assessment of existing samples indicated, however, that the otoliths had deteriorated as a result of using formalin as a fixative. Additional samples were therefore collected on a seasonal basis for the purpose of using otoliths for age estimates. These collections — which also targeted key seasonal gaps in the sampling programme, — were carried out in January 2005, April 2005, July 2005, September 2006 and November 2006. Fish were sampled by means of electrofishing with back pack Samus and Deka fishing gear in association with seine nets.

Due to low water conductivity it was found that fishing downstream into nets proved to be effective and most fish were caught in a seine net that was set up downstream of the spot where the electrofisher was employed. Adult *A. gilli* were mainly caught in various pools and deeper runs, while juveniles were caught in riffle habitats. Adult and juvenile *A. barnardi* were collected exclusively in shallow riffle habitats.

Only small numbers of fish were caught at a time, identified to species level, and their lengths measured to the nearest 0.1 millimeter. Subsequently, the gular region of the fish was cut through, thereby freeing the lower jaw. The skin and tissue in front of the pharyngeal basket was scraped forward, exposing the bone of the lower head. Each of the lapilliar otoliths was clearly visible on the left and right hand sides. The bone covering the otoliths on each side was chipped using the sharp end of a scalpel blade. The otolith was extracted using sharp forceps and stored dry in gelatin capsules. Fish were then labeled, cross referenced with otolith tubes, and fixed in 10% formalin for two to three weeks and later transferred to 60% propanol. Fish specimens were accessioned into the SAIAB fish collection. Specimens collected during surveys between 1996 and 2004 were also used to estimate population structure, gonad assessments and diet analyses. A complete list of specimens used is presented in Table 2.2.

Table 2.2. A list of SAIAB museum specimens used for biological studies of *Austroglanis gilli* and *Austroglanis barnardi*.

SAIAB No.	Collection Date	Species	Locality	No. of specimens	Length range (mm SL)	Analysis
75313	17/01/2005	*A. gilli*	Rondegat	20	22-58	o, p, g, d
58370	27/02/1998	*A. gilli*	Rondegat	20	21-130	p, g, d
51365	11/03/1996	*A. gilli*	Rondegat	07	22-110	p, g, d
58919	28/03/1998	*A. gilli*	Rondegat	12	31-125	p, g, d
75734	18/04/2005	*A. gilli*	Rondegat	22	37-120	p, g, d
58936	27/05/1998	*A. gilli*	Rondegat	21	35-123	p, g, d
77307	19/07/2005	*A. gilli*	Rondegat	13	44-114	o, p, g, d
77308	21/07/2005	*A. gilli*	Rondegat	2	78-88	o, p, g, d
77309	19/07/2005	*A. gilli*	Rondegat	9	37-115	o, p, g, d
77310	19/07/2005	*A. gilli*	Rondegat	8	36-113	o, p, g, d
58944	06/08/1998	*A. gilli*	Rondegat	17	76-129	p, g, d
75689	13/09/2004	*A. gilli*	Rondegat	22	44-116	p, g, d
79035	06/09/2006	*A. gilli*	Rondegat	19	44-116	o, p, g, d
79034	05/09/2006	*A. gilli*	Rondegat	08	37-120	o, p, g, d
59643	08/10/1998	*A. gilli*	Rondegat	30	39-128	p, g, d
59574	03/11/1998	*A. gilli*	Rondegat	7	42-123	p, g, d
59631	03/11/1998	*A. gilli*	Rondegat	8	40-123	p, g, d
79042	14/11/2006	*A. gilli*	Rondegat	19	34-120	o, p, g, d
79043	15/11/2006	*A. gilli*	Rondegat	19	66-109	o, p, g, d
54117	23/12/1996	*A. gilli*	Rondegat	15	43-116	p, g, d
75314	18/01/2005	*A. gilli*	Noordhoeks	4	83-91	o, p, g, d
58400	03/02/1998	*A. gilli*	Noordhoeks	11	44-118	p, g, d
58403	04/03/1998	*A. gilli*	Noordhoeks	17	48-101	p, g, d
51372	13/03/1996	*A. gilli*	Noordhoeks	9	30-101	p, g, d
58925	01/04/1998	*A. gilli*	Noordhoeks	20	49-113	p, g, d
58932	26/05/1998	*A. gilli*	Noordhoeks	18	51-99	p, g, d
78400	17/07/2005	*A. gilli*	Noordhoeks	15	37-107	o, p, g, d
78401	18/07/2005	*A. gilli*	Noordhoeks	9	36-106	o, p, g, d
58940	05/08/1998	*A. gilli*	Noordhoeks	14	36-108	p, g, d
75735	14/09/2004	*A. gilli*	Noordhoeks	21	37-117	p, g, d
79039	03/09/2006	*A. gilli*	Noordhoeks	9	39-110.9	o, p, g, d
59638	07/10/1998	*A. gilli*	Noordhoeks	17	43-106	p, g, d
53467	10/11/1996	*A. gilli*	Noordhoeks	6	35-99	p, g, d
59625	24/11/1998	*A. gilli*	Noordhoeks	4	75-102	p, g, d
54114	22/12/1996	*A. gilli*	Noordhoeks	16	44-92	p, g, d
79040	17/11/2006	*A. gilli*	Noordhoeks	7	44-96	o, p, g, d
79044	15/11/2006	*A. gilli*	Jan Dissels	21	49-175	o
75315	18/01/2005	*A. barnardi*	Noordhoeks	21	48-68	o, p, g, d
58401	03/02/1998	*A. barnardi*	Noordhoeks	22	45-69	p, g, d
58404	04/03/1998	*A. barnardi*	Noordhoeks	21	32-72	p, g, d
58924	01/04/1998	*A. barnardi*	Noordhoeks	20	32-70	p, g, d
75562	16/04/2005	*A. barnardi*	Noordhoeks	14	37-69	o
58933	26/05/1998	*A. barnardi*	Noordhoeks	26	35-68	p, g, d
78399	17/07/2005	*A. barnardi*	Noordhoeks	10	52-71	o, p, g, d
78399	18/07/2005	*A. barnardi*	Noordhoeks	33	33-86	o, p, g, d
58939	05/08/1998	*A. barnardi*	Noordhoeks	31	32-68	p, g, d

Table 2.2. Cont.

SAIAB No.	Collection Date	Species	Locality	No. of specimens	Length range (mm SL)	Analysis
75781	14/09/2004	*A. barnardi*	Noordhoeks	22	41-69	p, g, d
79037	03/09/2006	*A. barnardi*	Noordhoeks	14	36-72	o, p, g, d
79038	04/09/2006	*A. barnardi*	Noordhoeks	22	46-72	o, p, g, d
59642	07/10 1998	*A. barnardi*	Noordhoeks	27	37-68	p, g, d
59624	24/11/1998	*A. barnardi*	Noordhoeks	10	42-70	p, g, d
59626	24/11/1998	*A. barnardi*	Noordhoeks	11	44-75	p, g, d
79041	17/11/2006	*A. barnardi*	Noordhoeks	40	37-75	o, p, g, d

a = otoliths for age & growth; p = population structure; r = gonads for reproductive assessments; d = guts removed for diet analysis.

CHAPTER 3: Age, Growth, Mortality and Population Structure

3.1 Introduction

Information on age and growth is one of the most important aspects of fish biology because it allows for the assessment of the rate of biological processes such as growth rate, longevity, mortality rate and age at sexual maturity (Campana & Therrold 2001). Information on age at length of all size classes of a fish stock can be used in growth function models, such as the von Bertalanffy growth function, to obtain growth parameters (Cope & Punt 2007). Age and growth information is also an important component of population viability analysis (PVA) (Vélez-Espino 2005). This analysis gives an indication of the possibility of extinction of a particular taxon and can be used to assign a specific level of threat. For *A. gilli* and *A. barnardi*, age and growth parameters were required to understand population dynamics of both species and also to assess their potential recovery capability for conservation decision-making purposes.

Calcified structures in fishes — such as otoliths, vertebrae, spines and scales — have growth rings or checks that are formed at regular intervals, making them useful for age and growth estimations (Beamish & McFarlane 1983; Campana 2001). Some of these structures may have distinct advantages over others. For example, scales and spines can be used without having to sacrifice a fish. Some of these techniques have, however, been criticized for underestimating longevity and overestimating growth rates, and also for low accuracy and precision between readers (Campana 2001). For example, the resorption of growth zones as the lumen of spines grows with age has been a subject of concern in studies of African Siluriformes, which include *Clarias gariepinus* (Bruton 1977) and *Schilbe intermedius* Rüppell, 1832 (Hecht 1980), and of ictalurids in other regions (Nash & Irwin 1999). Nevertheless, the use of spines in age and growth studies on *Chiloglanis pretoriae* van der Horst, 1931 was considered by de Villiers (1991) as all otoliths were damaged by the use of formalin as a fixative.

While the use of otoliths in age and growth studies of vulnerable and endangered fishes has the disadvantage of having to sacrifice the fish, this method remains the most accurate and precise means of estimating age (Campana & Neilson 1985; Casselman 1990). Despite also being time consuming and labour intensive, the examination of sectioned otoliths provides a high level of precision and accuracy in the readability of all age classes when comparing results from different readers (Campana 2001). An examination of sectioned lapilliar otoliths to determine age and growth of *A. gilli* and *A. barnardi* was therefore undertaken in the present study.

3.2 Materials and methods

Age and growth

Samples of *A. gilli* and *A. barnardi* were collected in January 2005, April 2005, July 2005, September 2006 and November 2006 from Rondegat and Noordhoeks rivers using electrofishing and seine nets, as described in the previous chapter. Some otolith samples of *A. gilli* were also collected from Jan Dissels River. Adults and juveniles of *A. barnardi* were collected exclusively in riffle habitats. Fish samples were identified to species and their lengths measured to the nearest 0.1 mm standard length (SL) fork length (FL) and total length (TL). Both lapilliar otoliths were removed and fishes were labeled, cross referenced with their corresponding otoliths, fixed in 10% formalin, and accessioned into the SAIAB collection. Otoliths were stored dry in gelatin capsules. Fish eviscerated mass (EM) was measured to the nearest 0.1 g.

Preparation of otoliths for examination

In the laboratory, either the left or the right otolith was randomly selected for ageing as there was no obvious asymmetry between them. Otolith length (OL), i.e. distance from anterior tip of rostrum to antirostrum, was measured using a calibrated ocular eyepiece fitted to a compound microscope (as each length was less than 2 mm). This otolith was then set in clear polyester casting resin. Each otolith was then ground transversely on both sides, using a 100 grit sandpaper, to expose its nucleus and to create a section for further examination. Towards the end of the process, 800 grit sandpaper was used to smooth the sections. During grinding, the otolith was checked at regular intervals under a dissecting microscope to ensure that annuli were not ground through and grinding was halted when hyaline and opaque zones became visible. If one otolith was damaged, or if hyaline and opaque zones were not clearly visible, the second one from the pair was prepared. The otolith sections were then mounted on slides using DPX mountant and viewed under a dissecting microscope at 10X magnification. The series of alternating hyaline and opaque zones was interpreted as growth increments (Fig. 3.1). Each of the otoliths was read two times, at an interval of at least two weeks between readings, without reference to the previous reading or the length and mass of the fish being examined.

If the number of growth increments for both readings was equal, the count was accepted. If the number of growth increments did not coincide, a third count was made and if the number of growth increment of any two counts were equal, the count was accepted from those two readings. If all three readings differed by one increment (e.g. 1, 2, 3), the median age (2) was accepted. If the three readings differed by more than one growth increment, (e.g. 1, 2, 4) that otolith was rejected as unreadable. The precision of age determination was assessed using the average percentage error (APE) method (Beamish & Fournier 1981; Campana 2001).

Due to the limited sample size (n = 21) of otoliths for *A. gilli* from the Jan Dissels River, it was not possible to perform further growth analysis, except for the determination of longevity. Only samples from the main study sites (Rondegat and Noordhoeks rivers) were analyzed further for age and growth studies.

Age validation

The first attempt at validating increment deposition in *Austroglanis* spp. was made in 2005 when samples of live fish from the Noordhoeks River were transported to Grahamstown for an oxytetracycline (OTC) mark of captive experiment (Campana 2001). However, these fish died in the second month of acclimation to the captive environment. Chemical mark and recapture methods using chemicals such as oxytetracycline were therefore not considered further because of the limited time available for the study and the low probability of recapture of marked individuals from the wild.

In the present study an attempt was made to validate daily increment deposition. Four lapilliar otoliths of each of the species *A. gilli* and *A. barnardi,* of two or more years in age, were randomly selected. The otoliths were ground using sandpaper until growth increments became visible, after which etching was carried out using 0.5% EDTA for two minutes. The etched otoliths were then rinsed in distilled water and glued into metal stubs before being gold-coated for viewing under SEM. No daily growth increments were, however, visible under SEM for any of the otoliths examined.

The periodicity of growth increment formation was subsequently validated using two indirect methods: (1) marginal zone analysis (MZA) and (2) marginal increment analysis (MIA) (Campana 2001). When using MZA, the appearance of the otolith edge was examined to assess whether it contained hyaline or opaque zones and this was expressed as a percentage of the monthly sample. When using MIA, the distance from the last opaque zone to the edge of the otolith was measured. Only otoliths of fish with age ranging from one to five years for *A. gilli* from Rondegat and one to 11 years for *A. barnardi* from Noordhoeks rivers with an easy readability of the last annulus were considered for MIA analyses. Due to the limited sample size, MZA and MIA could not be performed for *A. gilli* from the Noordhoeks River.

Age modeling

Results using MIA and MZA methods confirmed the annual deposition of a single opaque and hyaline growth zone per year (Fig. 3.2). These could therefore be considered annuli for further analysis. Since the breeding period of *A. gilli* and *A. barnardi* extended from November to January (Chap. 4), it was assumed that all otolith specimens were spawned in December and their ages were adjusted according to the spawning date. The observed length-at-age of male and female *A. gilli* and *A. barnardi* from Rondegat and Noordhoeks rivers was modeled using the von Bertalanffy Growth Function, which is described as $L_t = L_\infty(1 - \exp(-(t - t_0)))$, where L_t is length at time t, L_∞ is the theoretical asymptotic length, K is the Brody growth coefficient and t_0 is the age of a zero-length fish (Ricker 1975; Cope & Punt 2007). The error associated with each growth parameter was determined using the methods described by Cope & Punt (2007).

Population structure

An estimate of an instantaneous rate of natural mortality (Z) was obtained by catch curve analysis (Ricker 1975). Length frequency data from SAIAB specimens collected during surveys between 1996 and 2004 were converted into age frequencies using age length keys obtained from otolith readings (Tables 3.2, 3.3 & 3.4). Subsequently, the natural logarithm of frequency In(f) was plotted against the relative age of the fish t. The negative of a slope of the resultant linear regression line through the descending data points gives a first approximation of Z.

Growth performance

The parameter phi prime (Pauly & Munro 1984) was used to compare the growth performance indexes of both *A. gilli* and *A. barnardi,* with those of other small selected catfishes.

3.3 Results

Morphometrics

Morphometric relationships between OL, SL, FL, TL, and EM were determined using regression analysis (Fig. 3.3). Otolith length and fish length showed a linear relationship. Fish length and mass of both species showed an isometric relationship.

Age and growth

Alternating hyaline and opaque zones were visible under transmitted light for both *A. gilli* and *A. barnardi* (Fig. 3.1). Hyaline zones were broader than opaque zones. Although otolith samples were not available for every month, marginal zone analysis indicated a seasonal pattern with a unimodal distribution of opaque and hyaline zones on the margin of the otolith. Marginal increment analysis also showed a progression of the distance between the last opaque zone and the margin of the otolith (Fig. 3.2). Otoliths with opaque zones on the margin peaked in November for both *Austroglanis* spp. The peak for MIA was observed in July and September for *A. barnardi*.and *A. gilli*, respectively. There was a huge variation in MIA, which was probably due to a wide gap between age groups used. These data suggest a single annual deposition of opaque and hyaline zones for both species. Growth zones could, therefore, be interpreted as annuli and were used to obtain estimates of age for *A. gilli* and *A. barnardi*.

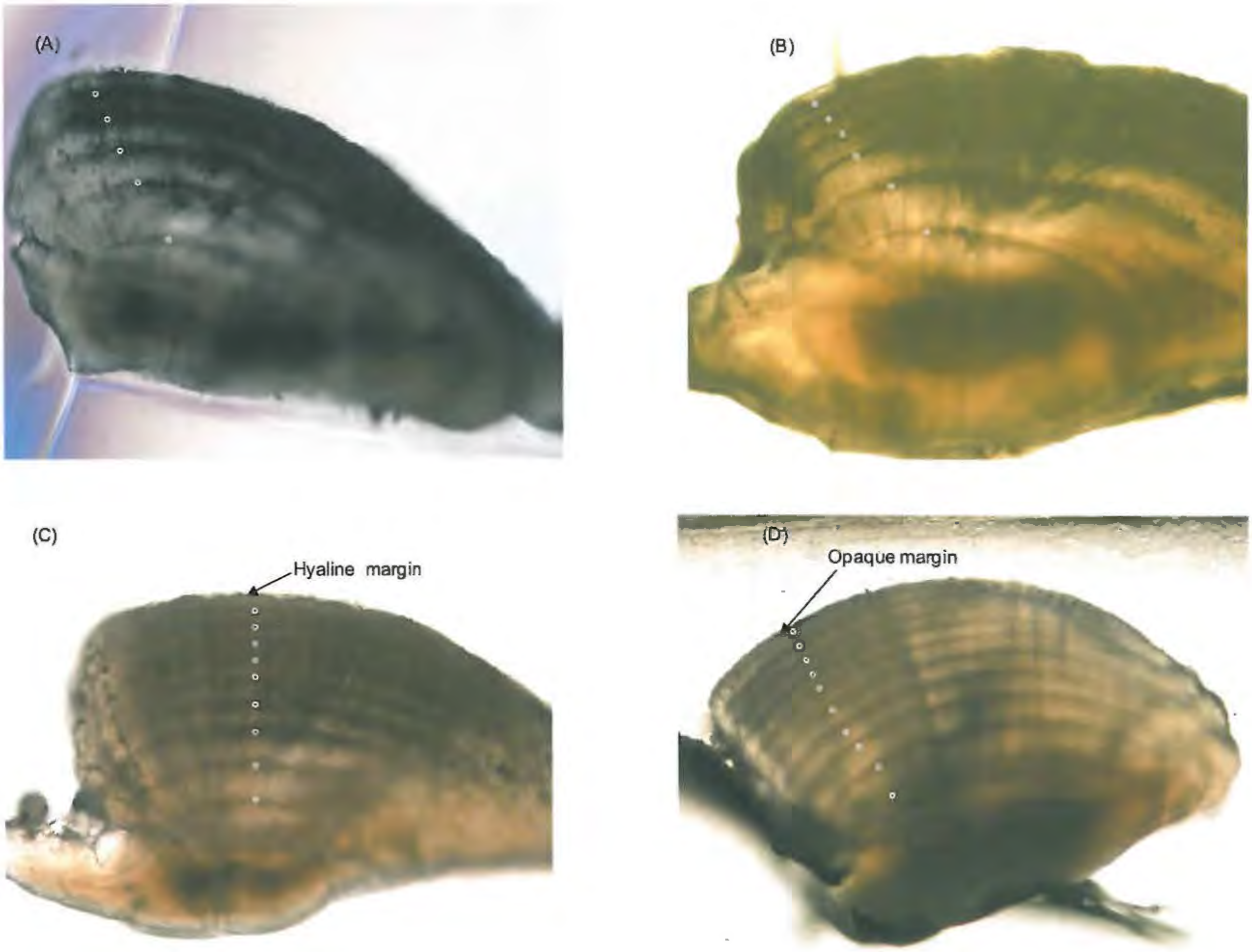
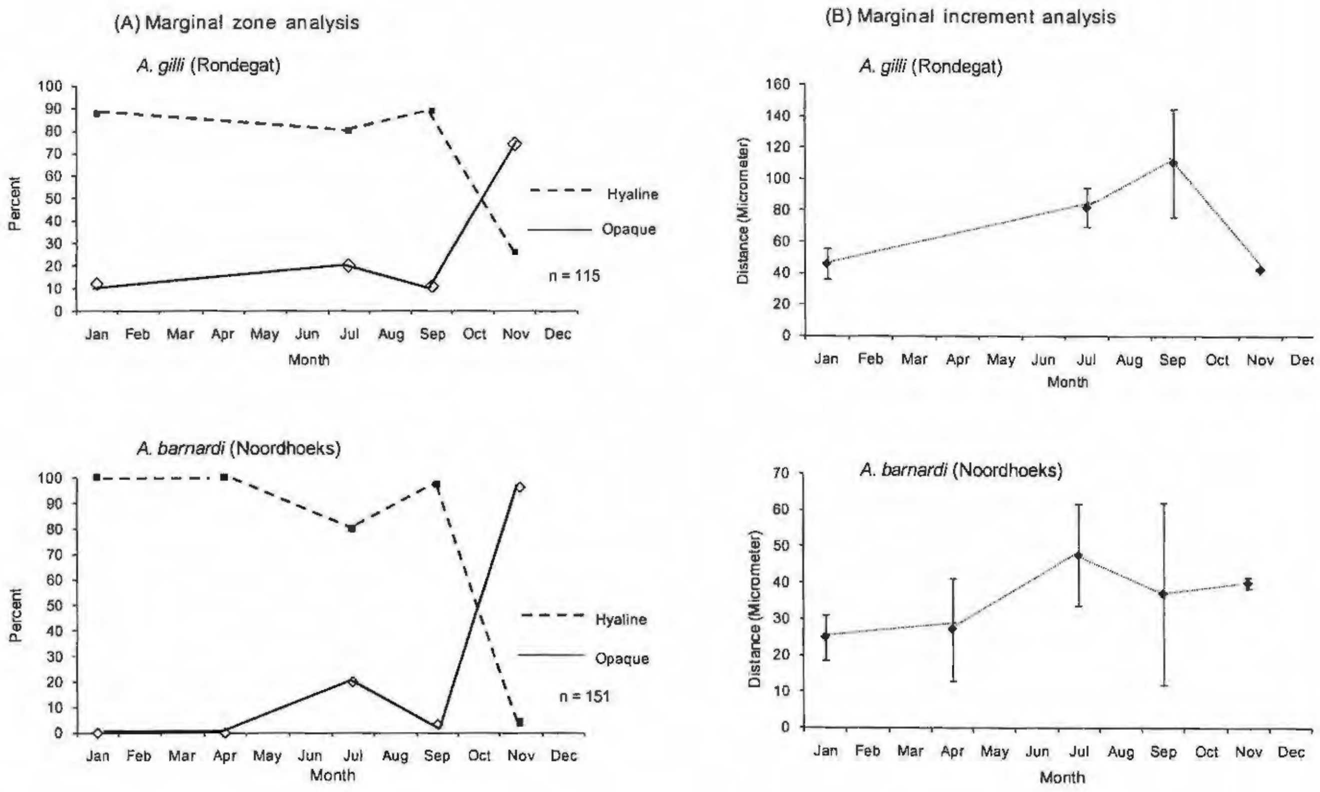


Fig. 3.1. Photomicrographs of sectioned lapilliar otoliths of (A) 111.3 mm SL of a five year old female *A. gilli* from the Rondegat River, (B) 142.9 mm SL of a six year old female *A. gilli* from Jan Dissels River, (C) 106.2 mm SL of a nine year old male *A. gilli* from the Noordhoeks River and (D) 66.0 mm SL of a 10 year old female *A. barnardi* from the Noordhoeks River.


(A) Marginal zone analysis

(B) Marginal increment analysis

Fig. 3.2. (A) The seasonal percentage of occurrence of opaque and hyaline margins in otoliths and (B) Marginal increment plot for *A. gilli* from Rondegat River and *A barnardi* from Noordhoeks River sampled in 2005 and 2006.

One hundred and fifteen otoliths of *A. gilli* from the Rondegat River were read successfully. Of the 40 otoliths of *A. gilli* from the Noordhoeks River, 1 (2.5%) was rejected, and of the 156 otoliths of *A. barnardi*, 5 (3.2%) were rejected as unreadable. Ageing APE for *A. gilli* from the Rondegat River was 5.0%, and 3.5% and 9.9% for *A. gilli* and *A. barnardi,* respectively, from the Noordhoeks River. Age-length-keys for *A. gilli* and *A. barnardi* are provided in Tables 3.1–3.3.

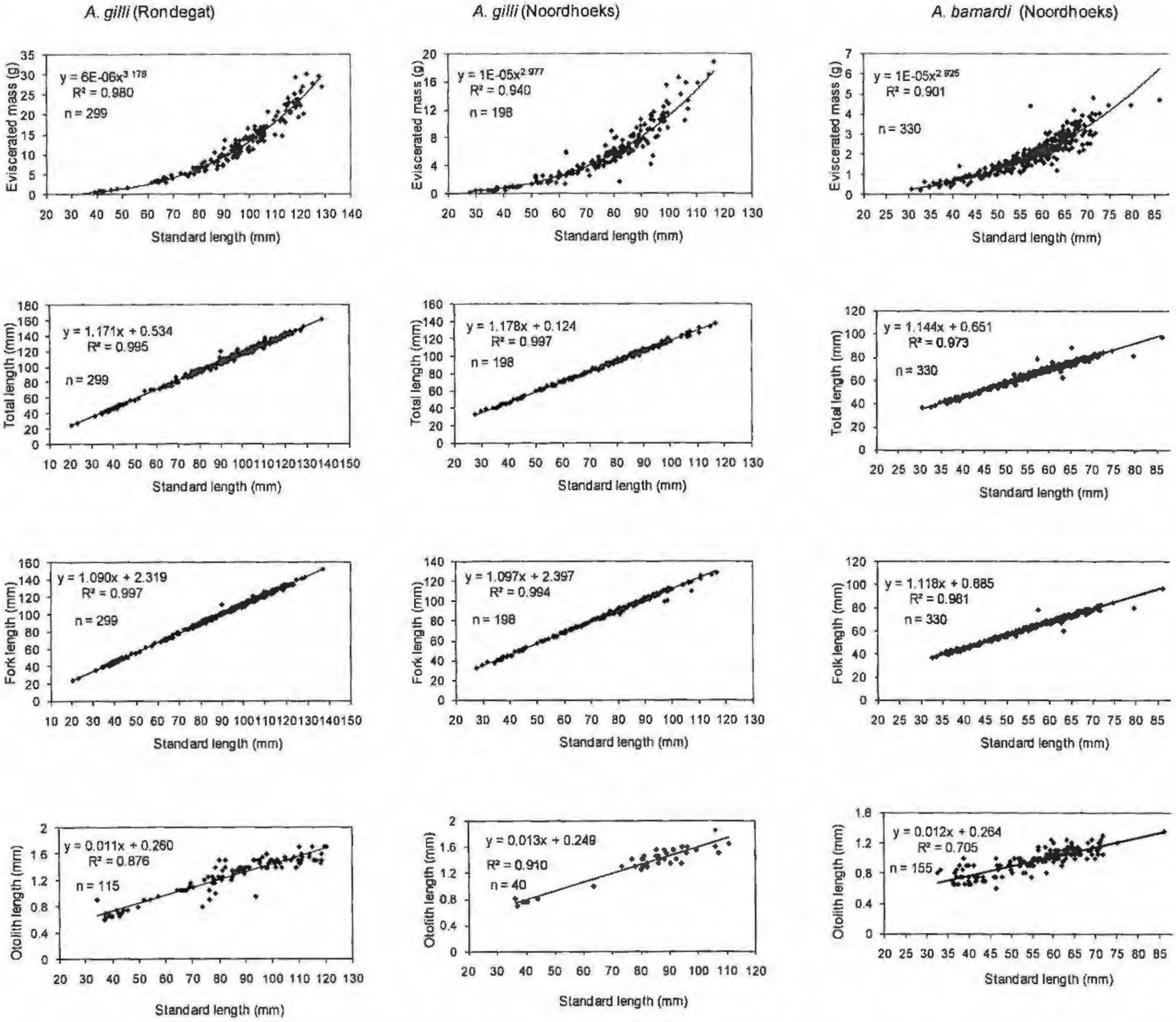


Fig. 3.3. Morphometric relationships derived by regression analysis between otolith length, standard length, fork lengths and eviscerated mass of *A. gilli* and *A. barnardi* sampled from the Rondegat and Noordhoeks rivers.

Table 3.1. Age-length-keys for *A. gilli* sampled from Rondegat River. Age was estimated from sectioned lapilliar otoliths. Samples were collected in 2005 and 2006.

Length class (mm SL)	Age (years)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
30-34.9													
35-39.9													
40-44.9	8												
45-49.9	7												
50-54.9	2												
55-59.9	2												
60-64.9		1											
65-69.9		1											
70-74.9	1	4	2										
75-79.9		1	1										
80-84.9	1	5	4	1									
85-89.9		2	3	3									
90-94.9		2	6	4	1								
95-99.9		1	1	4									
100-104.9		1	1	5	4	2							
105-109.9			1	7	1	0							1
110-114.9				2	1	3	1						
115-119.9					1	5	3						
120-124.9			1			5	1						
125-129.9							1						
N	21	18	20	26	8	15	6						1

Table 3.2. Age-length-keys for *A. gilli* sampled from Noordhoeks River. Age was estimated from sectioned lapilliar otoliths. Samples were collected in 2005 and 2006.

Length class (mm SL)	Age (years)									
	0	1	2	3	4	5	6	7	8	9
30-34.9										
35-39.9	4									
40-44.9	2									
45-49.9										
50-54.9										
55-59.9										
60-64.9		1								
65-69.9										
70-74.9			1							
75-79.9			2							
80-84.9			3	3	2					
85-89.9			2	3	4	1				
90-94.9				1	2	1	1			
95-99.9				2	1					
100-104.9										
105-109.9				1	1					1
N	6	1	8	10	10	2	1			1

Table 3.3. Age-length-keys for *A. barnardi* sampled from Noordhoeks River. Age was estimated from sectioned lapilliar otoliths. Samples were collected in 2005 and 2006.

Length class	Age (years)														
(mm SL)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
30-34.9	2														
35-39.9	15														
40-44.9	13														
45-49.9	7	4	3												
50-54.9	4	8	5	2	1										
55-59.9		2	6	10	5	2									
60-64.9		1	3	4	12	8	1	3	2						
65-69.9				1	1	3	5	2	1	2		3			
70-74.9				1		2	1		1			1	1		1
75-79.9											1				
80-84.9															
85-89.9													1		
N	41	15	17	18	19	15	7	5	4	2	1	4	2		1

The length-at-age of each sex of *A. gilli* and *A. barnardi* fitted to the von Bertalanffy growth curve are presented in Figure 3.4. Growth of both species was rapid until age three and slowed down in subsequent years.

Von Bertalanffy growth parameters and error terms calculated using random error methods (Cope & Punt 2007) are summarized in Table 3.4.

Growth performance

Growth performance of both *Austroglanis* species, including few representations from other species of small catfishes, is presented in Table 3.5. Growth performances of *A. gilli* and *A. barnardi* were similar to those of other small catlets (*t*-test).

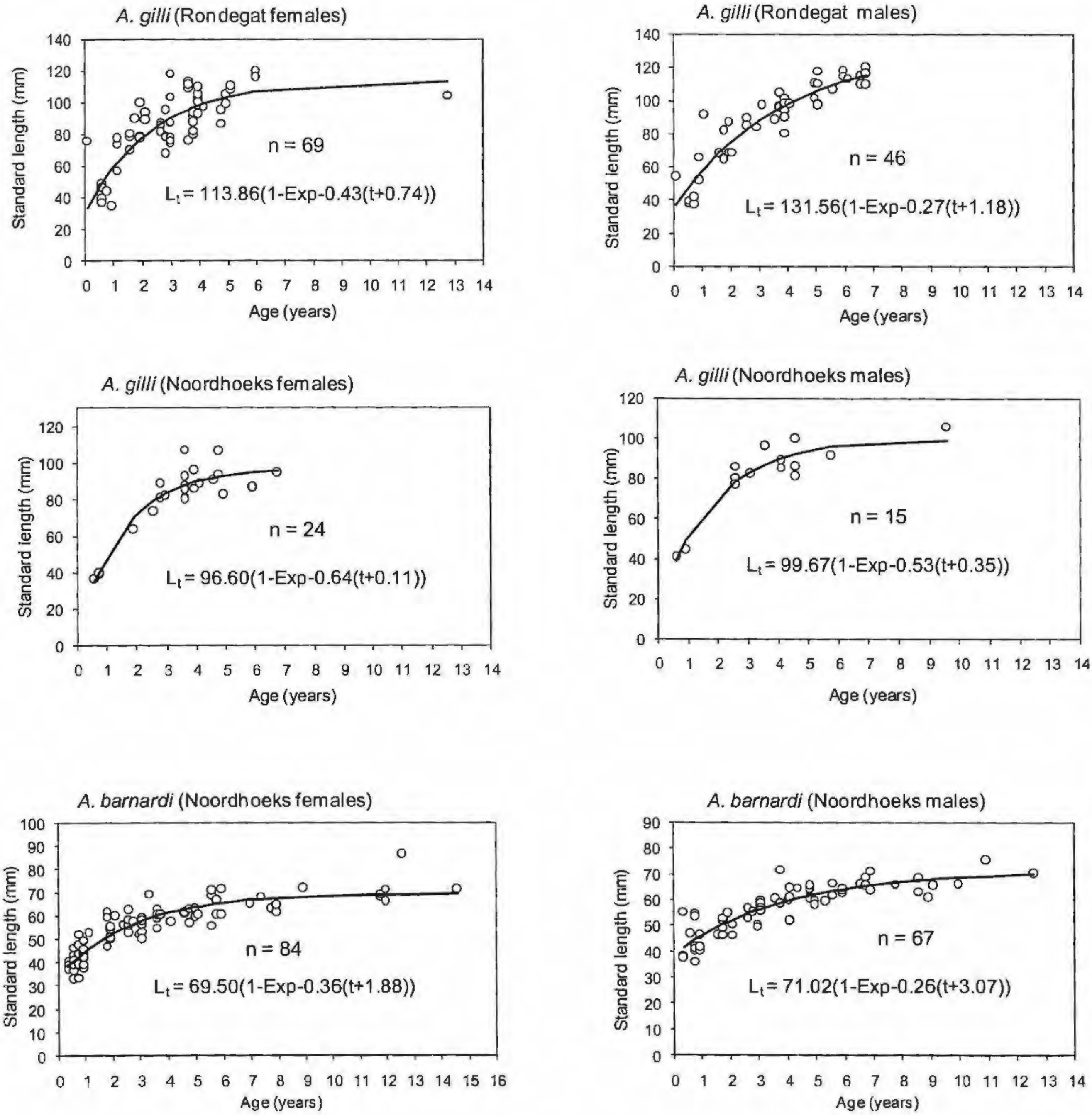


Fig. 3.4. Observed individual lengths-at-age of *A. gilli* and *A. barnardi* using sectioned lapillar otoliths sampled from Noordhoeks and Rondegat rivers.

Table 3.4. A summary of Von Bertalanffy growth parameters and error terms calculated using random error methods (Cope & Punt 2007) for *A. gilli* and *A. barnardi* sampled from Rondegat and Noordhoeks Rivers.

Species	L_∞		k		t_0		CV
	mean	sd	mean	sd	Mean	sd	Length error
			A. gilli (Rondegat)				
female	112	5.01	0.528	0.113	-0.30	0.20	0.10
male	119	4.8	0.368	0.050	-0.88	0.15	0.10
combined	116	6.29	0.381	0.069	-0.94	0.20	0.10
			A. gilli (Noordhoeks)				
female	99.5	7.28	0.525	0.158	-0.36	0.26	0.10
male	99.7	4.94	0.539	0.108	-0.26	0.14	0.10
combined	99.6	6.96	0.540	0.149	-0.22	0.18	0.10
combined	99.6	6.96	0.540	0.149	-0.22	0.18	0.10
			A. barnardi (Noordhoeks)				
female	66.9	2	0.493	0.107	-1.35	0.37	0.10
male	70.9	3.92	0.254	0.073	-3.22	0.87	0.10
combined	69.2	2.01	0.325	0.055	-2.33	0.41	0.10

Table 3.5. Comparison of combined sexes of *A gilli*, and *A barnardi* Von Bertalanffy growth function parameters (t_o, K and L_∞) and calculated phi-prime (Pauly and Munro 1984) derived from this study and a few selected small catfishes that inhabit upstream environments.

Family	Species	t_o	K	L_∞	Φ'	Reference
Austroglanididae	*A. gilli* (Rondegat)	- 0.88	0.37	119.0	3.72	This study
"	*A. gilli* (Noordhoeks)	-0.26	0.54	99.5	3.73	This study
"	*A. barnardi* (Noordhoeks)	-233	0.33	69.2	3.19	This study
Ictaluridae	*Noturus phaeus*	- 1.98	0.27	132.0	3.67	Chan & Parsons (2000)
Mochokidae	*Chiloglanis pretoriae*	- 1.15	0.423	72.7	3.36	de Villiers (1991)

Population structure

Nine age classes for *A. gilli* and 14 for *A. barnardi* were observed. The oldest specimens of *A. gilli* from Rondegat and Jan Dissels rivers were 12+ years. The oldest specimen from the Noordhoeks River was nine years. Age classes between 0+ and 3+ years dominated each of the *A. gilli* populations. *Austroglanis barnardi* was mainly dominated by age classes between 0+ and 5+ years. The oldest male and female specimens of *A. gilli* from the Rondegat River were 6+ and 12+ years, measuring 120.3 and 104.7 mm SL, respectively. In the Noordhoeks River, the oldest male of *A. gilli* was 9+ years, measuring 106.2 mm SL and female was 6+ years measuring 94.5 mm SL. The oldest male of *A. barnardi* from the Noordhoeks was 12+ years, measuring 70.0 mm SL; the oldest female of this species was 14+ years, measuring 71.2 mm SL.

A summary of natural mortality, estimated using catch curve analysis, is given in Table 3.6. Figures showing the best fit catch curves are provided in Figure 3.5. *Austroglanis gilli* from Rondegat River showed similar rates of instantaneous mortality to *A. barnardi* from the Noordhoeks River. In the Noordhoeks River, an instantaneous mortality rate for *A. gilli* was nearly double that of *A. barnardi*. The average total mortality rate estimates for the combined sexes of *A. gilli* from Rondegat River were estimated at 0.37 ± 0.12 yr^{-1}, and *A. gilli* and *A. barnardi* from Noordhoeks River at 0.71 ± 0.05 yr^{-1} and, 0.39 ± 0.04 yr^{-1} respectively.

3.4 Discussion

The processes that occur within otolith microstructures that result in growth zone formation are not yet fully understood but are thought to be a response to exogenous, physiological and endogenous factors (Stewart & Hughes 2007). Growth in otoliths occurs by differential deposition of calcium carbonate and protein during an endogenous circadian rhythm (Campana & Neilson 1985). As a result, a series of alternating hyaline and opaque zones in otoliths are formed and these zones were clearly visible in otoliths in both *A. gilli* and *A. barnardi*. The alternating zones suggest that both species have distinct seasons of slow and fast growth within a year.

Table 3.6. Estimates of total mortality rates (*Z*) derived by using catch curve analyses (Ricker 1975) for *A. gilli* and *A. barnardi* sampled from the Rondegat and Noordhoeks rivers.

Year	Male			Female			Combined sexes		
	Z yr^{-1}	Data points used (age)	R^2	*Z* yr^{-1}	Data points used (age)	R^2	*Z* yr^{-1}	Data points used (age)	R^2
				A. gilli (Rondegat)					
1996	0.37	1–6	0.70	0.33	0–3	0.70	0.56	1–5	0.89
1998	0.33	3–5	0.24	0.24	0–12	0.70	0.31	3–12	0.84
2004	0.31	3–6	0.42	0.25	2–12	0.96	0.31	3–12	0.70
2005	0.39	0–2	0.81	0.21	0–12	0.67	0.27	0–12	0.82
2006	0.18	1–4	0.16	0.29	1–12	0.96	0.38	1–12	0.94
Mean ± std	0.32 ± 0.08			0.26 ± 0.05			0.37 ± 0.12		
				A. gilli (Noordhoeks)					
1996	*			0.55	3–6	0.71	0.71	2–6	0.86
1998	0.28	2–9	0.75	0.72	2–6	0.80	0.69	2–6	0.93
2004	0.23	2–9	0.55	0.62	2–6	0.77	0.80	2–6	0.75
2005	*			0.72	3–6	0.86	0.66	2–6	0.84
2006	*			0.84	3–6	0.88	0.68	3–6	0.80
Mean ± std	0.26 ± 0.04			0.69 ± 0.11			0.71 ± 0.05		
				A. barnardi (Noordhoeks)					
1998	0.45	2–7	0.60	0.350	0–8	0.68	0.38	5–9	0.96
2004	0.22	4–9	0.41	0.382	3–7	0.46	0.40	5–9	0.84
2005	0.36	4–9	0.60	0.342	0–8	0.67	0.43	5–9	0.93
2006	0.20	0–12	0.63	0.315	0–8	0.69	0.34	5–9	0.90
Mean ± std	0.31 ± 0.12			0.35 ± 0.03			0.39 ± 0.04		

* Very small sample size so catch curve analysis could not be applied.

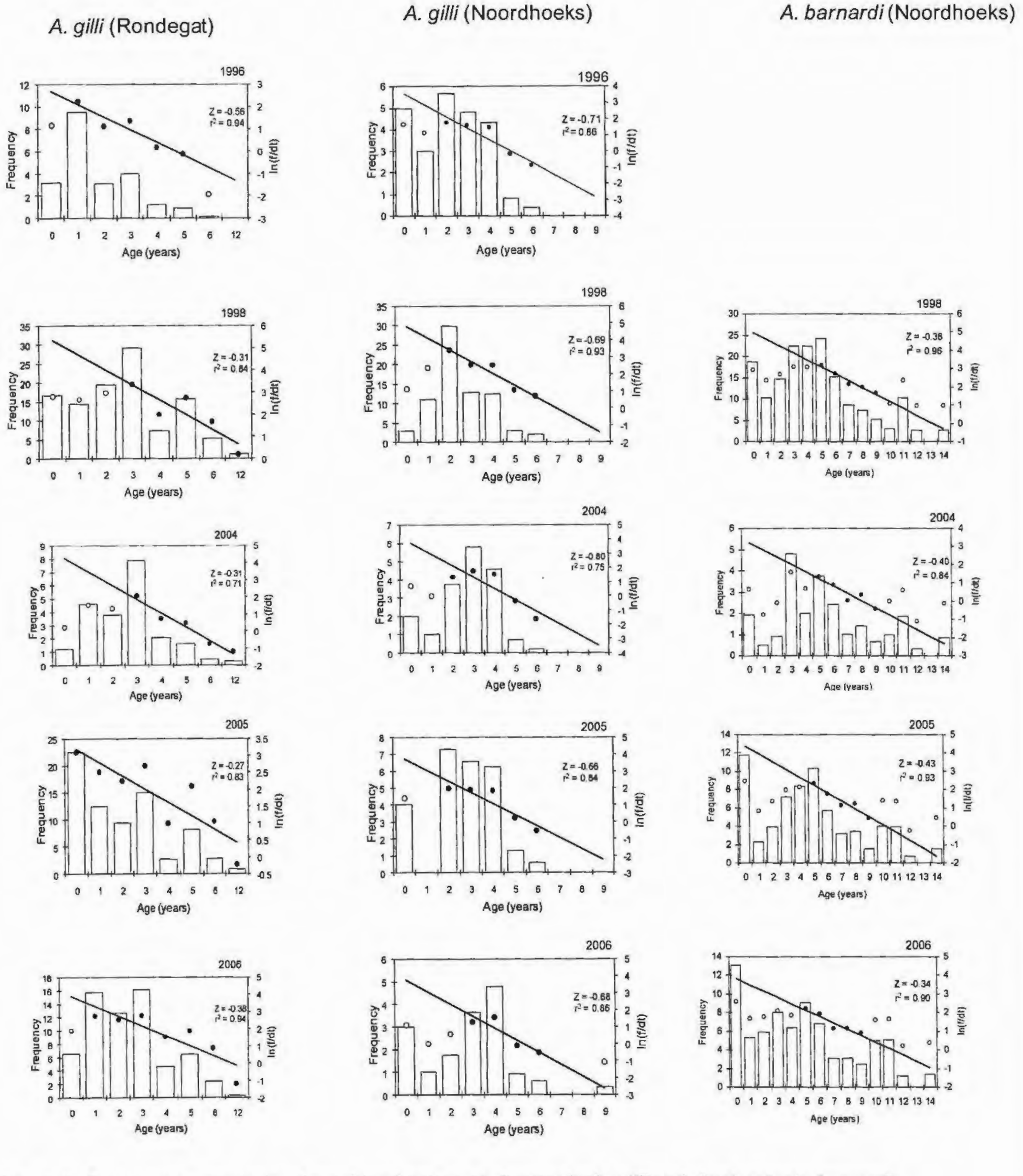


Fig. 3.5. Catch curve analysis of combined sexes of *Austroglanis gilli* and *Austroglanis barnardi* sampled from Rondegat and Noordhoeks Rivers.

The Mediterranean-type climate in the CFR, which is strongly seasonal, brings about changes to the temperature and flow rates in the Olifants River system (Fig. 3.6). This is known to induce physiological changes in fishes resulting in the formation of growth zones (Campana & Neilson 1985; Hecht 1980; Gauldie & Nelson 1990). A change of growth rates in fishes may also be interpreted as a response to a shift of energy use from somatic growth to gonad maturation (Hecht 1980). This is probably true for *A. gilli* and *A. barnardi* as they only have one spawning season, which is in summer (see Chap. 4). The presence of a single growth zone, however, indicates that temperature appears to be an overriding factor influencing annulus formation. Furthermore, it was also noted that the growth rates in each of the species starts slowing down as the individual reaches sexual maturity.

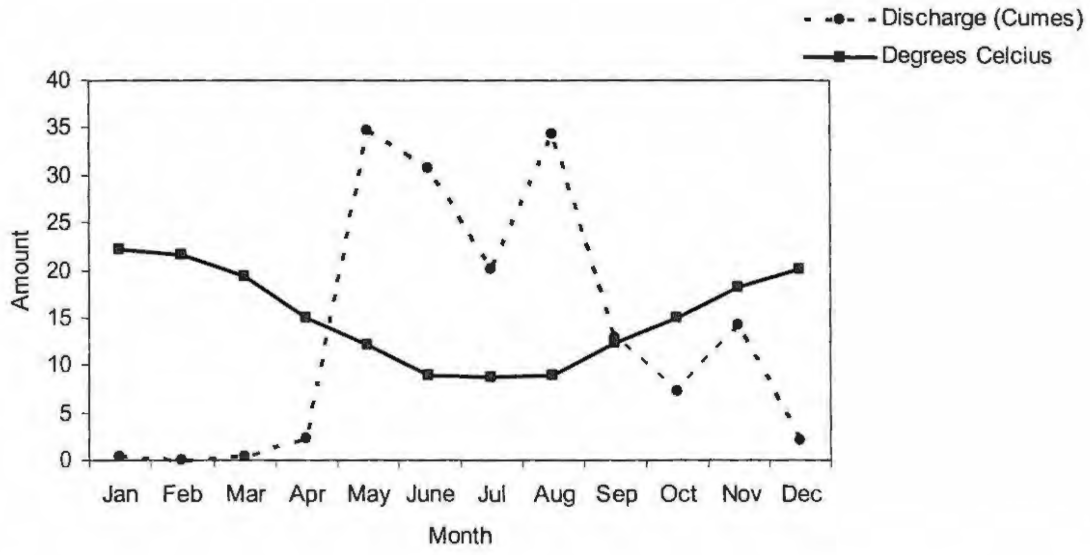


Fig. 3.6. Monthly variation in water temperature and discharge of the Olifants River in the Western Cape Province (DWAF, http:www.dwaf.gov.za).

Other studies on age and growth of African catfishes have also reported single annulus formation in otoliths (Quick & Bruton 1984; Chikou *et al.* 2003; Khumalo 2006) using indirect age validation methods. De Villiers (1991) confirmed a single annulus deposition in a year in *C. pretoriae* using fish of known age. The most recent findings by Weyl & Booth (2008), who undertook a study of chemically-marked wild fish, also indicated that a single annulus is deposited each year in the otoliths of African sharptooth catfish *C. gariepinus*. Although direct methods for validating growth increment deposition in fishes are preferred (Campana 2001), indirect methods using MZA and MIA were used in this study due to logistic constraints. It is, however, recommended that annulus formation be validated by direct methods as soon as possible as indirect methods are somewhat subjective.

Growth of both *A. gilli* and *A. barnardi* showed a pattern typical of teleost growth which is size-dependent and decreases with an increase in age. The populations of *A. gilli* from both the Rondegat and Noordhoeks rivers attained asymptotic lengths after three years, with the former attaining larger body size. It was also noted that the individual size of *A. gilli* from Jan Dissels River is nearly three times that of the individual size of *A. gilli* from the Noordhoeks River, and two times the individual size of *A. gilli* from the Rondegat River. The Noordhoeks and Jan Dissels rivers are relatively large (probably provide adequate feeding niches). Since there could be a positive correlation of fish size to water body size (Woolnough *et al.* 2009), one would have expected Noordhoeks River to comprise larger individual size of *A. gilli* than the Rondegat River.

In the Noordhoeks River, for example, there may be competition for limited food resources as this river has the highest diversity of indigenous ichthyofauna (six species) and *A. barnardi* may compete with *A. gilli*. The impacts of competition, however, seem to have been minimized by the ability of adult *A. gilli* to occupy a wide variety of habitats, which could provide a greater variety of shelter and food sources. The Noordhoeks River is largely dominated by riffles, which could provide inferior feeding conditions for *A. gilli*. The lower diversity of indigenous ichthyofauna (five species) in the Rondegat River than that found in the Noordhoeks River might explain the larger individual size of *A. gilli*. A similar inference could be made for the Jan Dissels River, which comprises three indigenous species (*A. gilli*, *B. calidus* and *G. zebratus*) of which the individual size of *A. gilli* is nearly triple that found for this species in the Noordhoeks River. There could also be different feeding conditions in these rivers, probably due to different invertebrate abundance and diversity (de Moor & Barber-James, unpubl. report). Nevertheless, the observed difference in individual sizes is probably due to biotic and abiotic factors, both of which influence growth in fishes (Campana & Neilson 1985).

Austroglanis gilli and *A. barnardi* are long-lived species whose life expectancy exceeds 10 years. A comparison between phi-prime values showed that the growth performances of both *A. gilli* and *A. barnardi* were similar to other small catfishes (Table 3.5). This may suggest an adaptability of both *Austroglanis* spp. to meet nutritional requirements in the oligotrophic upstream environment of the CFR.

Despite different individual sizes in the populations of *A. gilli,* all populations appeared to have similar longevity, as the oldest individuals from both the Rondegat and Jan Dissels rivers aged 12+ years, and 9+ years from the Noordhoeks River. *Austroglanis barnardi* from the Noordhoeks River attained a maximum of 14+ years, suggesting a better adaptability than *A. gilli.* Otters, which hunt in the deeper runs and pools than in the riffles, may select for the larger *A. gilli* that inhabit these pools and the resultant higher mortality may explain the lower maximum age and higher natural mortality rate.

The life histories of both *Austroglanis* species are characterized by slow growth, long life and low adult natural mortality. These suggest characteristics of species that are both relatively *K*-selected (Adams 1980). This observation also suggests that both *Austroglanis* species have stable populations that, when depleted, will take a long time to recover (Adams 1980).

CHAPTER 4: Reproduction

4.1 Introduction

The study of reproduction is important in terms of understanding the ecology, population dynamics and life history styles of fishes (Wootton 1998). Fishes may tend towards precocial or altrical life strategies to maximize their reproductive potential and to enhance offspring survival (Hodgkiss & Mann 1977; Adams 1980). Generally, species that are relatively precocial are characterized by delayed sexual maturity, long lifespan, low mortality rates and low relative fecundity but high parental investment in offspring (Adams 1980). Precocial life-histories are common in fishes living in stable environments (Adams 1980). Conversely, species that are relatively altricial are characterized by early sexual maturity, high fecundity, low longevity and high mortality rates; this lifestyle is common to fishes living in abiotically-harsh environments (Adams 1980).

Catfishes are well represented in all the reproductive guilds found in teleosts and they are considered to have evolved various strategies that enable them to succeed in different environments (Bruton 1996). Although the majority of catfish families are considered to be relatively altricial (Bruton 1996), others such as *C. pretoriae* (de Villiers 1991), *Mystus macropterus* Bleeker, 1870 (Wang & Luo 1992) and some loricariids (Trajano & Bichuette 2007) are considered to have evolved towards a more precocial life-history style. For example, *Phyllonemus filinemus* Worthington & Ricardo, 1937 and *Auchenoglanis occidentalis* Valenciennes, 1840 are mouth brooders (Ochi *et al.* 2001 a,b,c). Many ariids are mouth brooders and *Bagrus meridionalis* Günther, 1894 from Lake Malawi is a guarder (McKaye 1985).

Due to the diversity of reproductive strategies in fishes, information on the life-history of a species is necessary to guide conservation measures. Life history strategies influence the vulnerability of species to human impacts on ecosystems and management interventions, for the conservation of particular species, need to take this into account. For example, populations of relatively precocial species are typically stable but once these populations are reduced they generally take longer to recover than would be the case for altricial species (Adams 1980). In addition, dispersal through larval drift is likely to be reduced in less fecund precocial species.

The paucity of comprehensive information on reproduction biology makes it difficult to position *Austroglanis gilli* and *A. barnardi* in the altricial or precocial life-history continuum. The present study, therefore, investigated aspects of reproduction, which include spawning periodicity, length and age at sexual maturity, egg size, and fecundity.

4.2 Materials and methods

The spawning season of *A. gilli* and *A. barnardi* was determined by visual assessment of the maturity state of the gonads and by monthly progression of the Gonado-somatic Index (GSI).

Validation of macroscopic staging criteria

As there was no documented information on macroscopic assessment of gonad recrudescence for *A. gilli* or *A. barnardi*, visual assessment criteria required validation. Six macroscopic stages — to represent ‘immature’, ‘resting’, ‘developing’, ‘maturing’, ‘ripe, and ‘spent’ stages — were identified. Due to the inherent subjectivity of macroscopic evaluation of gonad stages, these visual assessments were validated using histological techniques. Twelve samples were processed personally at Rhodes University Department of Ichthyology and Fisheries Science by dehydrating them through a series of increasing alcohol concentrations, clearing in xylene, and then impregnating them with paraffin wax. Subsequently, they were sectioned to 8 μm using a Lipshaw rotary microtome, mounted onto glass slides, stained using Gill’s Haematoxylin and Papanicoloau’s Eosin, and allowed to dry. To increase sample size, a further 151 representative gonads were sent to the National Health Laboratory Service[1] and Amanzi Biosecurity[2] for histological preparation and sectioning and staining using the same techniques.

Gonad sections were viewed under a compound light microscope at various magnifications. Oocyte stages were identified according to the criteria given by West (1990) and Marriot *et al.* (1997).

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[1] National Health Laboratory Services, Port Elizabeth.

[2] Amanzi Biosecurity, Private Bag 15, Suite 190, Hermanus, 7200.

Visual assessment of gonads

After validation, gonads could be macroscopically scored. The progression of fish in the population, from nesting to ripe and then spent, was used as an indicator of spawning season.

Gonado-somatic index

To assess for the timing of the spawning season, characterized by increasing development and size of the gonads, a GSI was calculated for monthly samples (de Vlamming *et al.* 1982). The eviscerated fish and gonads of fish larger than length at 50% maturity of both *A. gilli* and *A. barnardi* were weighed to the nearest 0.001g. Gonado-somatic index was then calculated as: $\mathrm{GSI} = \left[\frac{\text{Gonad mass(g)}}{\text{Eviscerated mass(g)}}\right] \times 100$.

Length at maturity

The length-at-50%-maturity (l_{50}) was determined by fitting a logistic function of the form $P_a = \left(1 + \exp^{-(l - l_{50/\delta})}\right)^{-1}$ to the proportion of reproductively-active female fish in each 5 mm size classes (stages 3, 4, 5 and 6: Table 1) collected during the spawning season between October and January. In this equation, P_a is the percentage of mature fish at length l, l_{50} the length at which 50% of the fish in the size/age class are sexually mature, and δ the steepness of the ogive. Age at sexual maturity was determined by converting length at maturity stage into age using age-length keys and then fitting the logistic function to maturity-at-age data (Chap. 3, Table 3.2).

Sex ratio

The sex ratio of each of the *Austroglanis* species was estimated from all sexually mature fish (stages 3 to 6) sampled during the spawning season (October to January). The male to female ratio was tested for unity using a chi-square test.

Fecundity

The left and right ovaries of the mature females collected in November were each weighed to the nearest 0.001 g and all eggs were counted. In cases where the right ovary was kept for histological analysis, egg counts were only done for the left ovary.

For egg counts, each ovary was dissected under a dissecting microscope. Dissecting needles were used to tease connective tissues apart and this facilitated the release of eggs (Fig. 4.1). Eggs were separated according to size classes. Subsequently, the maximum and minimum diameters of eggs from a subsample were measured to the nearest 0.1 millimeter, using a calibrated ocular eyepiece fitted to a compound microscope; the average value of the two diameters was taken as the diameter of the egg (West 1990). Relative fecundity was estimated by dividing the number of all vitellogenic oocytes by fish mass.

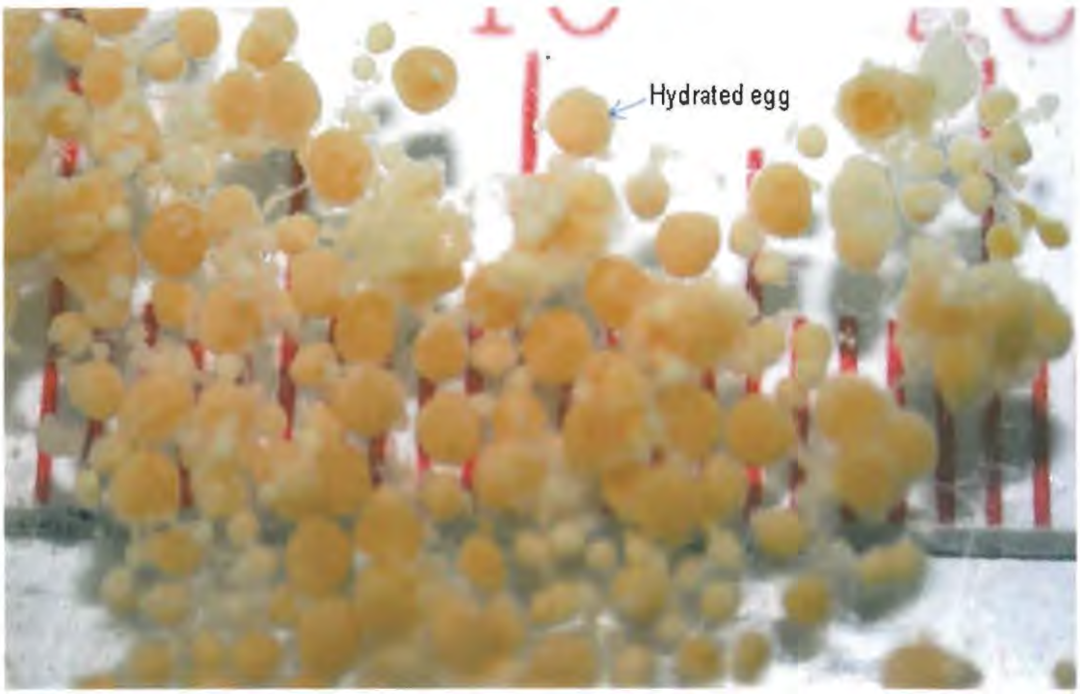


Fig. 4.1. A photograph showing eggs of *A. gilli,* which were teased apart from connective tissue of an ovary and are ready to be sorted, counted and measured. Red ruler bars are 1 mm apart.

4.3 Results

Validation of macroscopic staging criteria

Ovaries and testes of each individual specimen of *A. gilli* and *A. barnardi* appeared as paired structures (Fig. 4.2, 4.3, 4.4, 4.5) that were suspended in the body cavity above the stomach and intestines. Ovaries appeared as two equal lobes. During the summer season, ovaries of adult specimens increased in size and oocytes of different sizes were clearly visible under the naked eye. Testes also appeared as bilobed structures, each lobe comprising multi-lobular structures. Testes followed a similar developmental trend to that of ovaries, their lobes becoming thickened during the summer season and remaining relatively deflated for the rest of the year.

Description of gonadal recrudescence

Gonadal recrudescence was similar for both *Austroglanis gilli* and *A. barnardi* and descriptions of macroscopic stages and histological appearance are summarized in Tables 4.1, 4.2, 4.3 and 4.4. It was noted, however, that there were discrepancies in assigning the correct stages in some of the gonads.

Ovaries

Incorrect visual staging was made for ovaries collected in January as they appeared deflated, but histological examination showed that they contained hydrated oocytes, a condition consistent with the criteria for the spawning stage. This may indicate partial spawning by the species. Mature ovaries of samples collected between April and August were macroscopically classified as 'spent' but the histology was consistent with a maturing-stage ovary, containing mainly the primary yolk vesicle oocytes.

The previtellogenic stages of female gonads were mainly dominated by early and late perinuclear oocytes. Ovaries in the first stage ('developing') were identified by the presence of primary yolk vesicle oocytes, which contained cortical alveoli and zona radiata. As the primary yolk vesicle oocytes grew in size, they developed yolk, forming the secondary yolk vesicle oocytes, and these were consistent with 'maturing' stage of gonad development. The late stage of oocyte development, the 'spawning' stage, was marked by the presence of tertiary yolk vesicle oocytes, containing yolk globules in the entire ooplasm. The migration of nucleus and dissolution of membrane of the tertiary yolk vesicle oocyte was also a characteristic of this stage, which was observed from all mature fish collected in November, December and January. The degeneration of all vitellogenic oocytes occurs in February and March, leaving perinuclear, primary yolk vesicles and post ovulatory follicle oocytes in the ovary.

Testes

Testes were very difficult to stage with accuracy using macroscopic assessment. Large lobules, which appeared whitish in colour suggesting the presence of spermatozoa, sometimes contained spermatids. Histological examination of male gonads indicated that spermatogenesis was initiated by the multiplication of spermatogonia surrounding large, empty lumen from immature fish. Spermatogonia developed and became primary spermatocytes, which subsequently developed into secondary spermatocytes. Secondary spermatocytes gave rise to spermatids. During the breeding season, spermatids were released into the lobule lumen, where they finally matured as spermatozoa. As a result of such inconsistencies in histology and the macroscopic stage in testes, visual staging cannot be recommended as an indicator of gonadal recrudescence in these fishes.

Spawning seasonality

Histology (Figs. 4.2 – 4.5) and macroscopic assessments of gonads (Fig. 4.6) indicated that both *A. gilli* and *A. barnardi* spawn only during the summer season. The female GSI of the two *Austroglanis* species remains relatively low, (< 1%) before the summer season. It then increases to 1.2 ± 0.5% from October onwards, peaking in November (4.3 ± 2.1). It decreases again from January and remains relatively low for the rest of the year (Fig. 4.7). A similar seasonal trend was also observed in males.

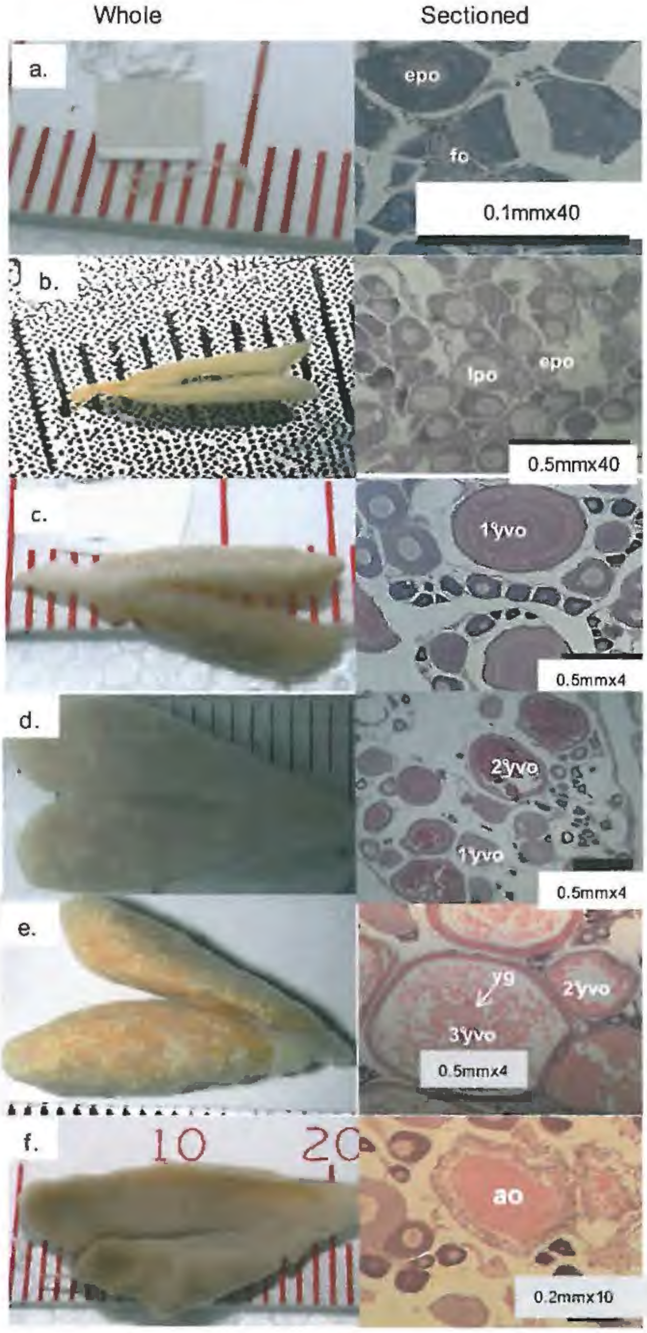


Fig. 4.2. *Austroglanis. gilli,* whole and transverse-sectioned ovaries that were macroscopically and histologically classified as: a – 'immature', containing early and late perinuclear oocytes surrounded by follicle cells (fc). b – 'resting', containing all stages of perinuclear oocytes. c – 'developing', containing the primary yolk vesicle oocyte (1°yvo) with cortical alveoli (ca) and the development of zona radiata (zr). d – 'maturing', with the appearance of secondary yolk vesicle oocyte (2°yvo). e – 'spawning', containing fully developed oocytes with yolk globule (yg). f – 'spent'.

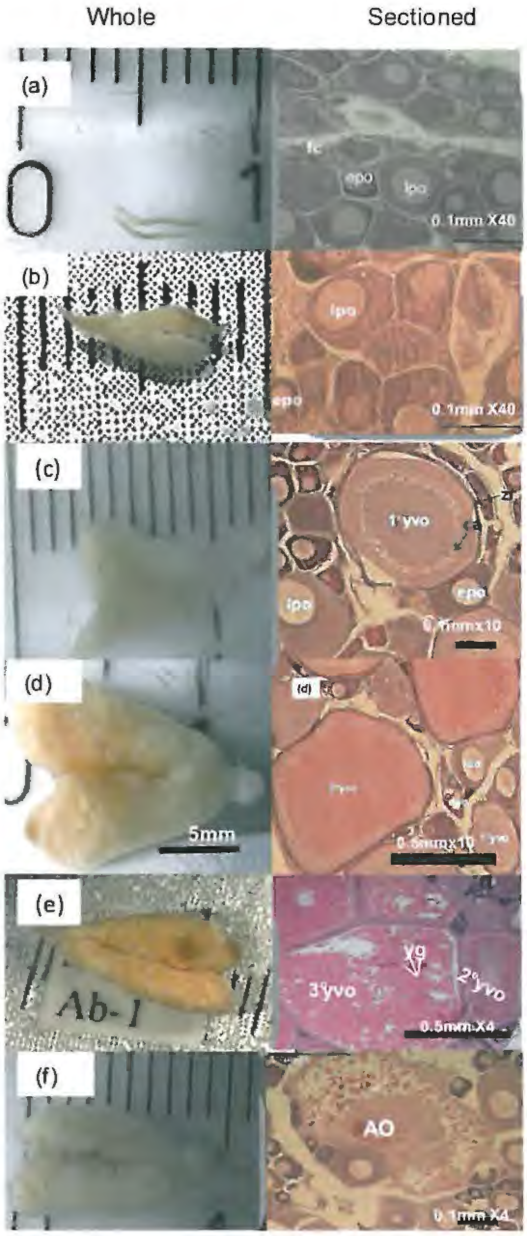


Fig. 4.3. *Austroglanis. barnardi,* whole and transverse-sectioned ovaries that were macroscopically and histologically classified as: a – 'immature', containing early and late perinuclear oocytes surrounded by follicle cells (fc). b – 'resting', containing all stages of perinuclear oocytes. c – 'developing', containing the primary yolk vesicle oocyte (1°yvo) with cortical alveoli (ca) and the development of zona radiata (zr). d – 'maturing', with the appearance of secondary yolk vesicle oocyte (2°yvo). e – 'spawning', containing fully developed oocytes with yolk globule (yg). f – 'spent'.

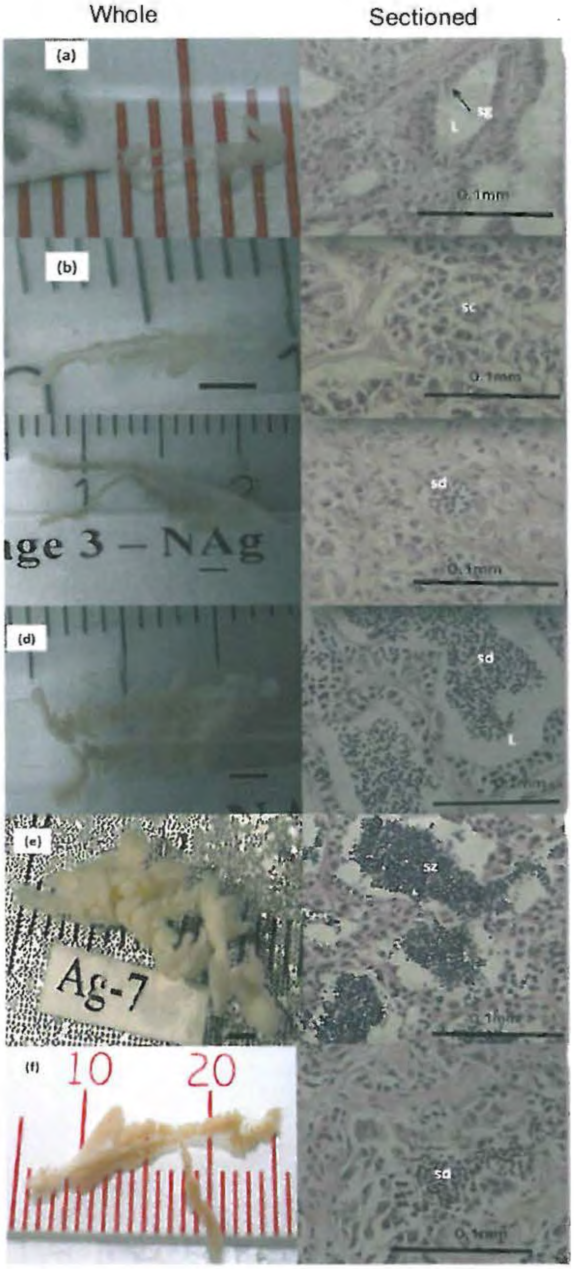


Fig. 4.4. Transverse sections through testes of *A. gilli*, illustrating stages of spermatogenesis, that were macroscopically and histologically classified as: a –'immature', an empty lumen (L) is surrounded by spermatogonia. b – 'developing', testes predominantly comprising spermatocytes. c – 'maturing', spermatids present in the histology[1]. d – 'ripe' but histology[1] may comprise only the spermatids (sd) or spermatozoa (sz). e – 'spawning' comprising spermatozoa but histology[1] may comprise only the spermatids, suggesting the ripe stage. f – 'spent' but histological appearance is consistent with maturing. All scale bars were taken at 40X magnification.

[1] The term 'histology' indicates that a more detailed histological examination revealed the structures described.

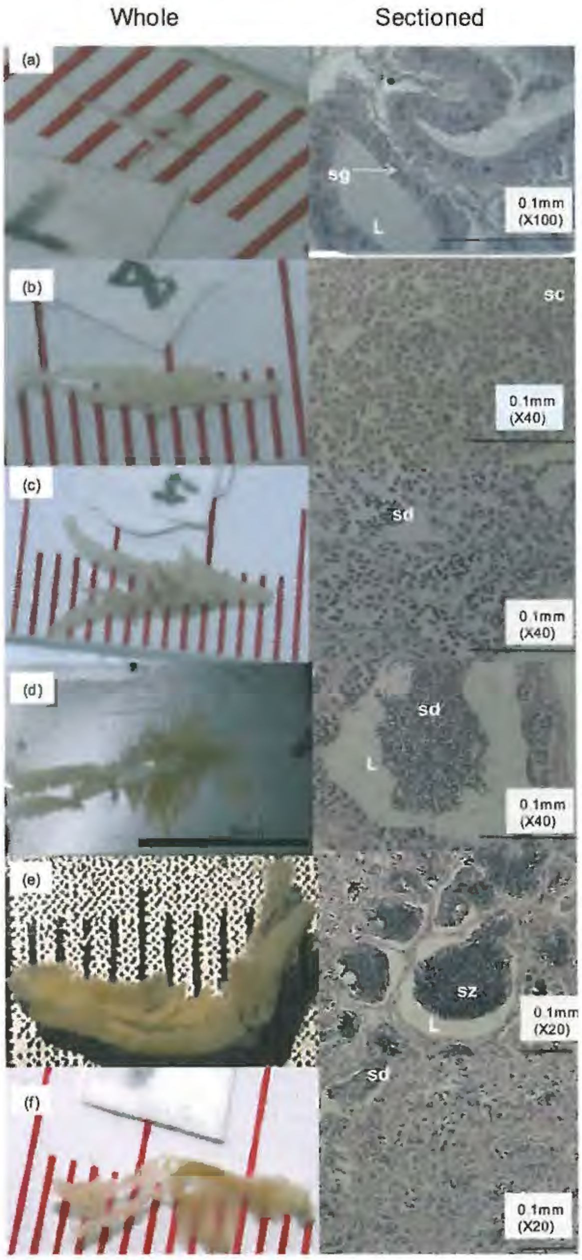


Fig. 4.5. Transverse sections through testes of *A. barnardi* illustrating stages of spermatogenesis that were macroscopically and histologically classified as: a –'immature', an empty lumen (L) is surrounded by spermatogonia. b – 'developing', testes predominantly comprising spermatocytes. c – 'maturing', spermatids present in the histology[1]. d – 'ripe' but histology[1] may comprise only the spermatids (sd) or spermatozoa (sz). e – 'spawning' comprising spermatozoa but histology[1] may comprise only the spermatids, suggesting the ripe stage. f – 'spent' but histological appearance is consistent with maturing.

[1] The term 'histology' indicates that a more detailed histological examination revealed the structures described above

Table 4.1. Gonad developmental stages and the equivalent macroscopic and histological characteristics for female *A. gilli* from the Rondegat River.

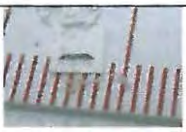
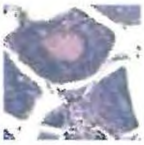
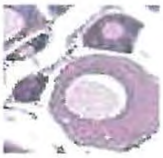
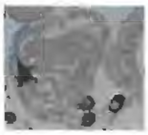
Stage	Staging	Macroscopic characteristics	Visual photograph	Histological characteristics	Histological photograph
1	Immature	Hard to distinguish sex with the naked eyes; gonad of each of the sexes appears as thin gelatinous strips		Follicle cells, early perinuclear oocytes, late perinuclear oocytes dominate ovary	
2	Resting	Sexes distinguishable, ovary is a little bigger than the previous stage; eggs in the ovary are not visible to naked eye		Ovary is dominated by early perinuclear oocytes and late perinuclear oocytes	
3	Developing	Whitish eggs clearly visible to naked eye; ovaries occupy half the body size		Primary yolk vesicle, cortical alveoli, zona radiata, zona granulosa	
4	Maturing	Yellowish eggs are visible to the naked eye but do not appear hydrated; ovaries occupy nearly three quarters of body cavity		Secondary yolk vesicle. Non-vitellogenic oocytes are also present	
5	Spawning	Hydrated orange eggs; ovaries occupy most of the body cavity; oocytes of different sizes are clearly visible in an ovary		Hydrated oocytes with yolk globules which are evenly dispersed throughout cytoplasm	
6	Spent	Ovaries appear deflated and occupy less body cavity than in the previous stage		Atretic oocytes, but mainly dominated by oocytes of cortical alveoli stage	

Table 4.2. Gonad developmental stages and the equivalent histological and macroscopic characteristics for male *A. gilli* from the Rondegat River.

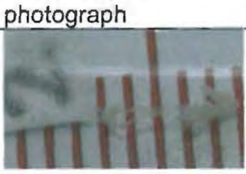
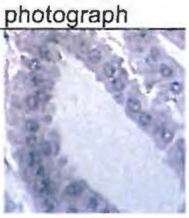
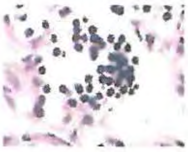
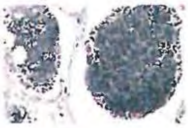
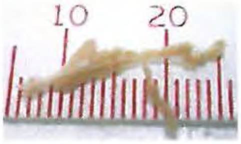
Stage	Staging	Macroscopic characteristics	Macroscopic photograph	Histological characteristics	Microscopic photograph
1	Immature	Hard to distinguish sex with the naked eyes; gonad of each of the sexes appears as thin gelatinous strips		Large empty lumen surrounded by lobules containing spermatogonia.	
2	Resting	Testes distinguishable, as lobules are clearly visible for the entire strip of the branch; testes occupy larger space than in the previous stage		Spermatocytes dominate lobules; lumen is still empty	
3	Developing	Testes are greatly enlarged, with lobule appearing partially thick		Spermatocytes dominate; spermatids present in the lobules; lumens still empty	
4	Maturing	Lobules of the testes appear partially swollen		Spermatids fill the lumen	
5	Spawning	Lobules of the testes are fully swollen and whitish in colour		The lumen is dominated by spermatozoa	
6	Spent	Testes appear deflated with lobules appearing as stage three. However, the sizes of the testes are enlarged		Empty lumen, sometimes contains residual spermatozoa	

Table 4.3. Gonad developmental stages and the equivalent histological and macroscopic characteristics for female *A. barnardi* from the Noordhoeks River.

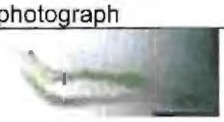
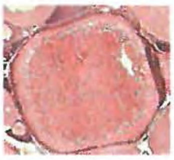
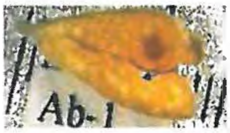
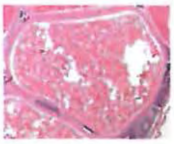
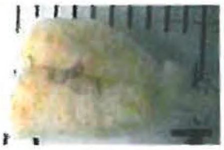
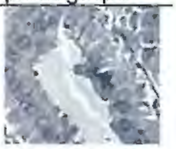
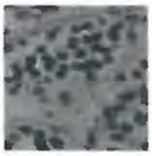
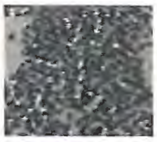
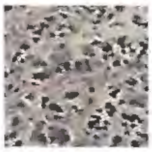
Stage	Staging	Macroscopic characteristics	Macroscopic photograph	Histological characteristics	Microscopic photograph
1	Immature	Hard to distinguish sex with the naked eyes; gonad of each of the sexes appears as thin gelatinous strips		Follicle cells, early perinuclear oocytes, late perinuclear oocytes dominate ovary	
2	Resting	Sexes distinguishable, ovary is a little bigger than the previous stage; eggs in the ovary are not visible to naked eye		Ovary is dominated by early perinuclear oocytes and late perinuclear oocytes	
3	Developing	Whitish eggs clearly visible to naked eye, ovaries occupy half the body size		Primary yolk vesicle, cortical alveoli, zona radiata, zona granulosa. Perinuclear oocytes also present	
4	Maturing	Yellowish eggs are visible to the naked eye but do not appear hydrated; Ovaries occupy nearly three quarters of body cavity		Secondary yolk vesicle; primary and perinuclear oocytes also present	
5	Spawning	Hydrated orange eggs, ovaries occupy most of the body cavity; oocytes of different sizes are clearly visible in an ovary		Hydrated oocytes with yolk globules which are evenly dispersed throughout cytoplasm	
6	Spent	Ovaries appear deflated and occupy less body cavity than in the previous stage		Atretic oocytes, but mainly dominated by oocytes of stage 3	

Table 4.4. Gonad developmental stages and the equivalent histological and macroscopic characteristics for male *A. barnardi* from the Noordhoeks River.

Stage	Staging	Macroscopic characteristics	Histological characteristics	Macroscopic photograph	Microscopic photograph
1	Immature	Hard to distinguish sex with the naked eyes; gonad of each of the sexes appears as thin gelatinous strips		Large empty lumen surrounded by lobules containing spermatogonia	
2	Resting	Testes distinguishable, as lobules are clearly visible for the entire strip of the branch; testes occupy larger space than in the previous stage		Spermatocytes dominate lobules; lumen is still empty	
3	Developing	Testes are greatly enlarged, with lobule appearing partially thick		Spermatocytes dominate. Spermatids present in the lobules; lumens still empty	
4	Maturing	Lobules of the testes appear partially swollen		Spermatids fill the lumen	
5	Spawning	Lobules of the testes are fully swollen and whitish in colour		The lumen is dominated by spermatozoa	
6	Spent	Testes appear deflated with lobules appearing as stage three. However, the sizes of the testes are enlarged.		Empty lumen, sometimes contains residual spermatozoa	

Maturity

The percent maturity at length and the fitted logistic ogive for *A. gilli* and *A. barnardi* sampled from the Rondegat and Noordhoeks Rivers are summarized in Figure 4.8. In the Rondegat River, the smallest mature *A. gilli* males and females were 61.8 mm SL and 62.9 mm SL, respectively, and 50% maturity was attained in males and females at 97.3 mm SL (corresponding to 3.3 years from the von Bertalanffy curve) and 94.4 mm SL (3.1 years), respectively. In the Noordhoeks River, the smallest mature *A. gilli* males and females were 57.1 mm SL and 60.2 mm SL, respectively, and 50% maturity was attained in males and females at 71.6 mm (2.0 years) SL and 66.4 mm SL (1.7 years), respectively. The smallest mature *A. barnardi* males and females were 46.6 mm SL and 49.6 mm SL, repectively, and 50% maturity was attained in males and females at at 58.9 mm SL (2.9 years) and 55.0 mm SL (2.0), respectively.

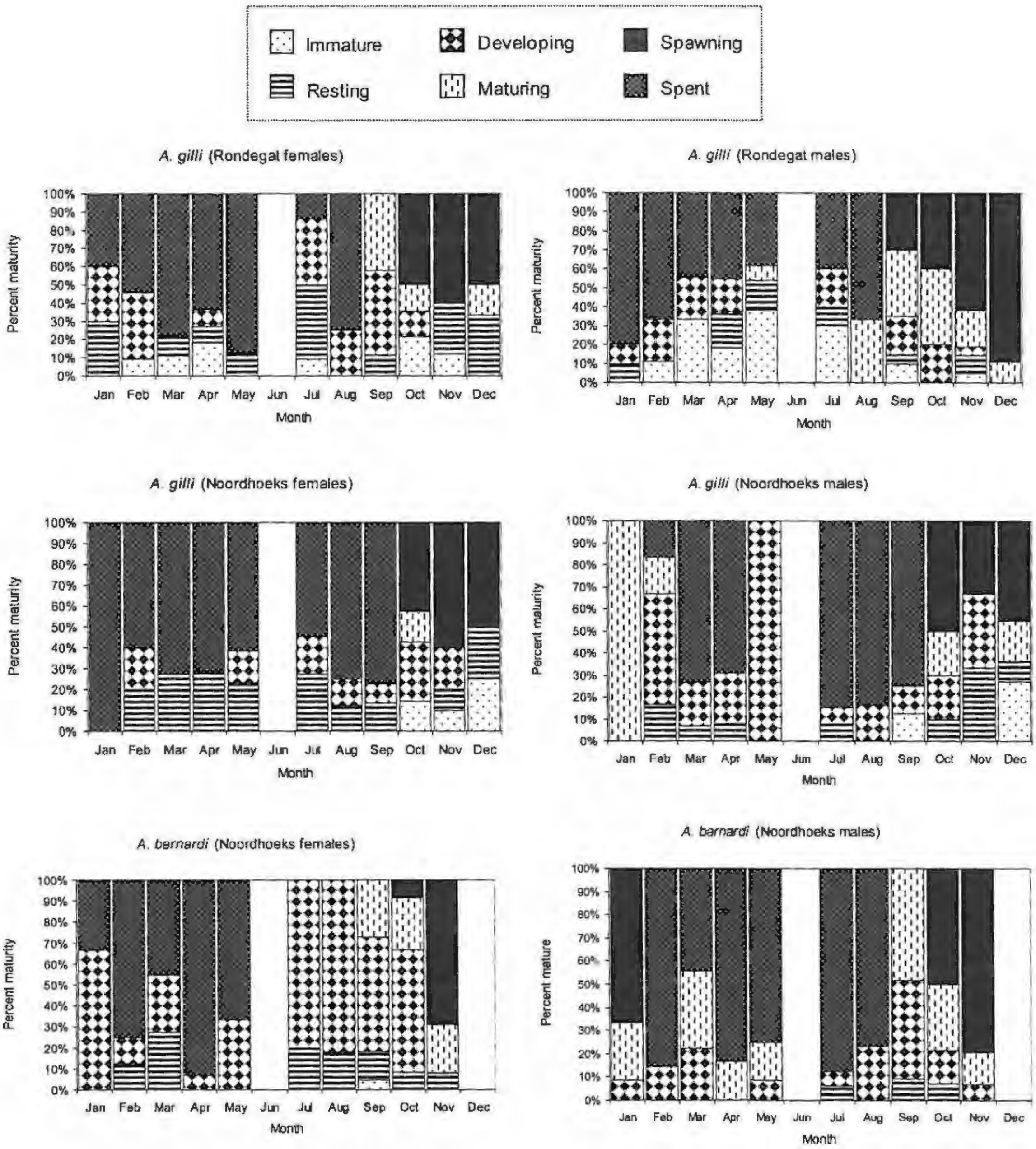


Fig. 4.6. Visual assessment of the state of gonads assigned to the gonads of *A. gilli* and *A. barnardi* sampled from Rondegat and Noordhoeks Rivers. Fish samples were collected on a monthly basis in different years between 1996 and 2006.

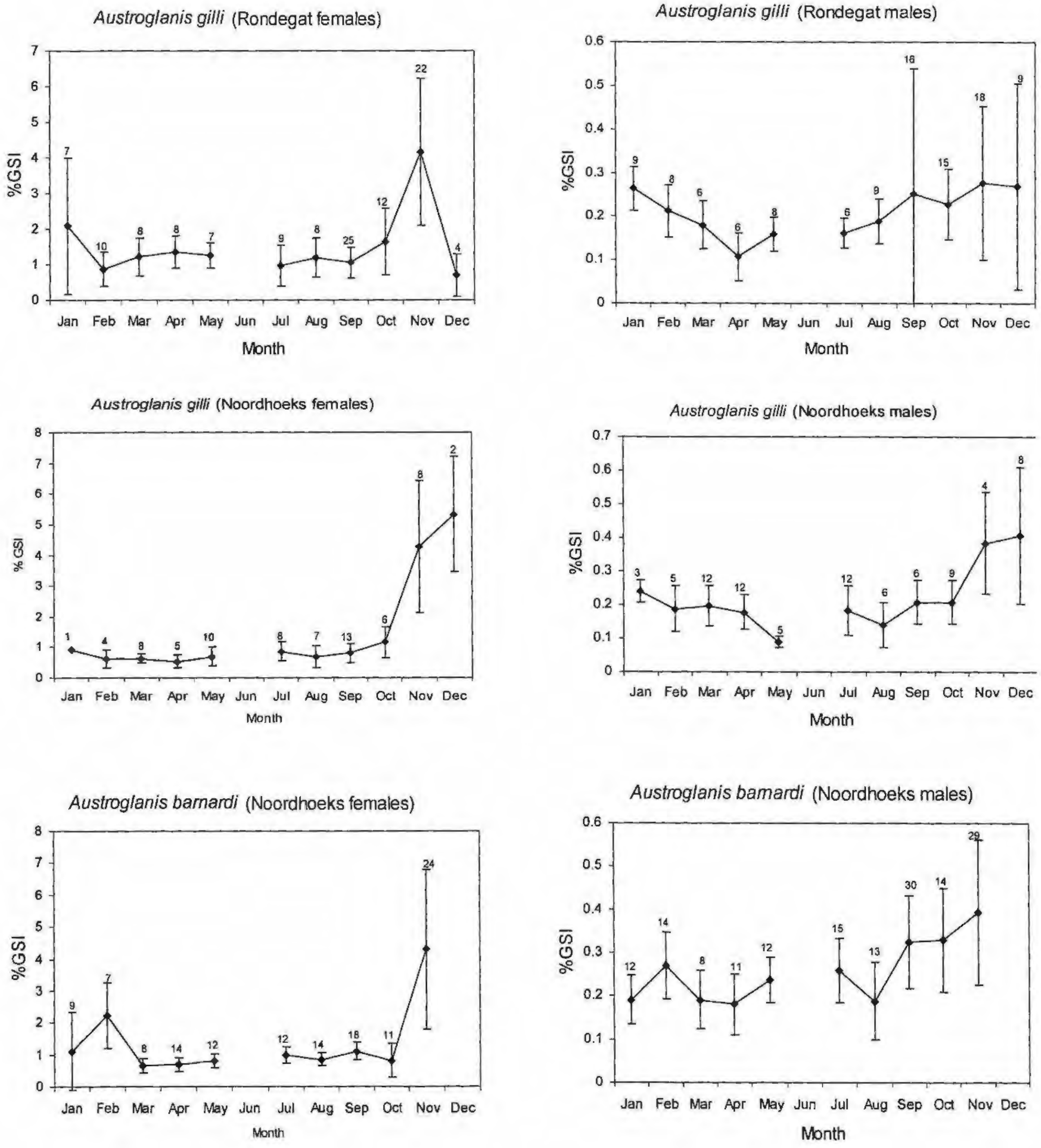


Fig. 4.7. Variation in mean (±standard deviation) gonado-somatic indices for both male and female *A. gilli* and *A. barnardi* sampled from Rondegat and Noordhoeks Rivers between 1996 and 2006. Numbers above error bars indicate sample size.

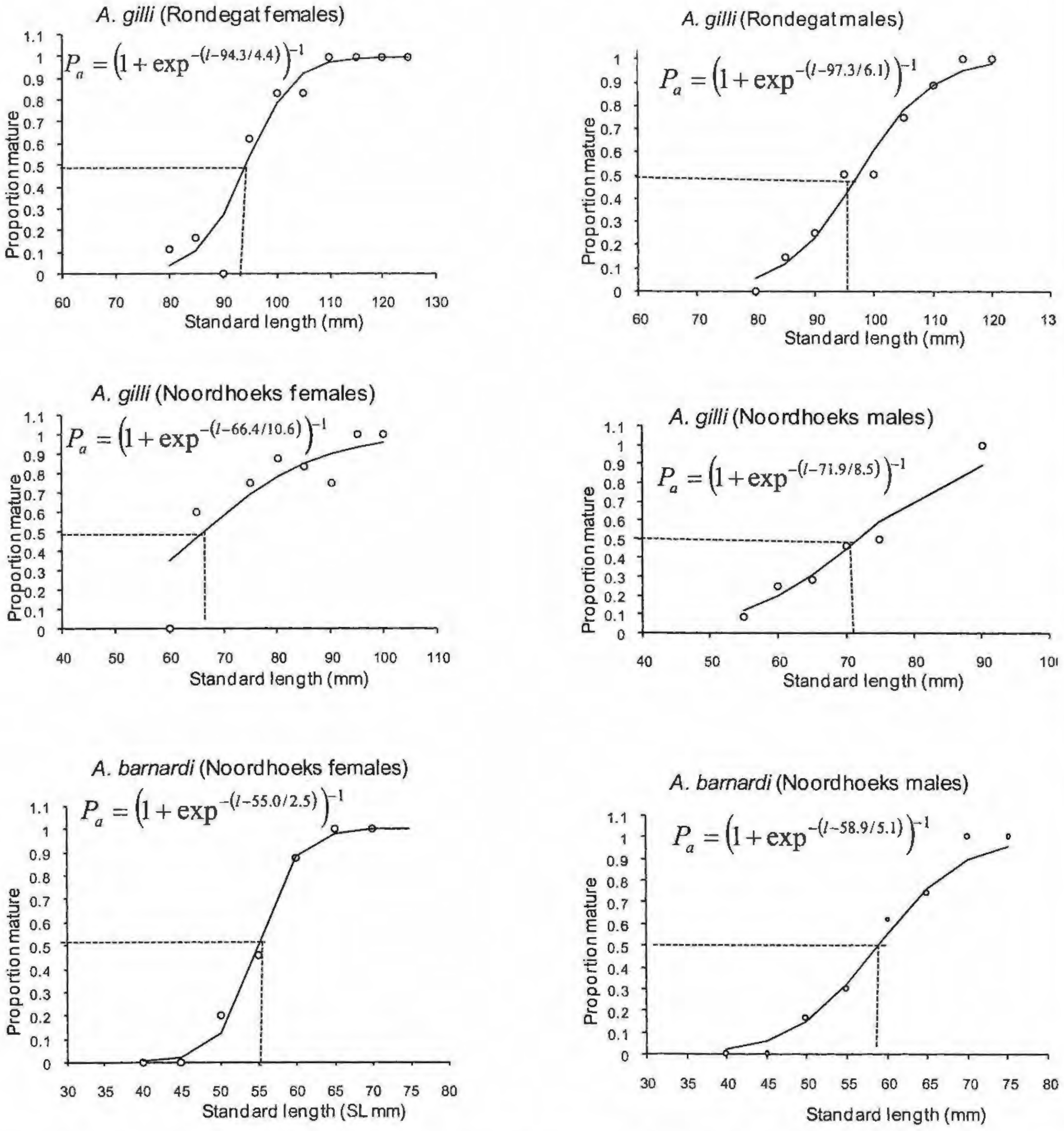


Fig. 4.8. Length at 50% maturity of *A. gilli* and *A. barnardi* females from Rondegat and Noordhoeks rivers samples, determined from logistic ogive.

During the breeding season, the sex ratio in a mature population of *A. gilli* from the Rondegat River was 1: 1.2 in favour of females but was not significantly different from unity ($\chi^2 = 0.57$, d.f. = 1, $p > 0.05$). The population of *A. gilli* from the Noordhoeks River followed a similar pattern, skewing at 1: 1.3 in favour of females, but also not differing significantly from unity ($\chi^2 = 0.76$, d.f. = 1, $p > 0.05$). The population of *A. barnardi* from the Noordhoeks River had a skew sex ratio of 1:1.6 in favour of males, differing significantly from unity ($\chi^2 = 4.05$, d.f. = 1, $p < 0.05$).

Fecundity

Fecundity is defined as the number of ripe eggs before spawning and is a measure of reproduction potential of a fish species. The number of vitellogenic oocytes per fish of *A. gilli* from the Rondegat River ranged from 152 to 1474. In the Noordhoeks River, this range was counted at 164 – 640 oocytes/fish and 28 – 238 oocytes/fish for *A. gilli* and *A. barnardi*, respectively. Counts of vitellogenic oocytes of both *A. gilli* and *A. barnardi* are presented in Table 4.5. Relative fecundity (number of vitellogenic oocytes per gram of eviscerated fish mass) of *A. gilli* from Rondegat, and *A. gilli* and *A. barnardi* from Noordhoeks were estimated at 60 ± 18 ova/fish g, 62 ± 18 ova/fish g and 65 ± 24 ova/fish g, respectively. Fish size and fecundity were positively correlated for *A. gilli* but there was no clear relationship for *A. barnardi* (Fig.4.9).

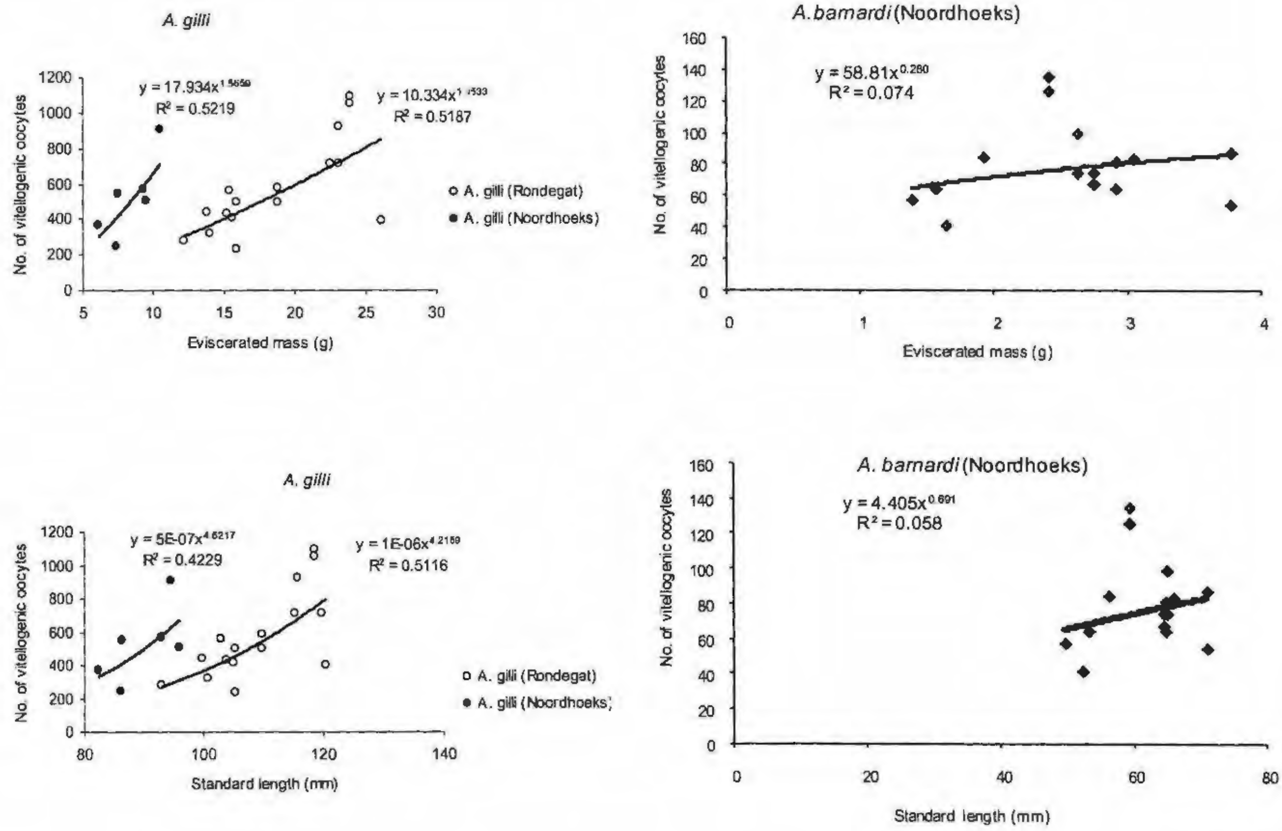


Fig. 4.9. The relationships between fish mass, length and number of ova for *A. gilli* and *A. barnardi* sampled from the Rondegat and Noordhoeks rivers.

The largest oocyte diameters of *A. barnardi* and *A. gilli* measured 2.0 and 1.8 mm, respectively. There was no significant difference between the egg sizes of *A. gilli* and those of *A. barnardi* in the Noordhoeks River (Kruskal-Wallis test, H = 3.56, p = 0.06) and between egg sizes found in *A. gilli* populations in the Rondegat and Noordhoeks rivers (Kruskal-Wallis test, H = 2.90, p = 0.09).

Table 4.5. A summary of egg counts, batch and relative fecundity for the female gonads of *A. gilli* and *A. barnardi* with well hydrated oocytes collected from the Rondegat and Noordhoeks rivers in November.

Ovary side	Fish length (mm SL)	Fish eviscerated mass (g)	Ovary mass (g)	No. hydrated oocytes	No. all vitellogenic oocytes per ovary	Relative fecundity (No. all vitellogenic oocytes/fish g)
			A. gilli (Rondegat)			
Left	93	12.2	0.107	141	281	46.1
Left	99.9	13.8	0.299	313	443	64.0
Left	100.9	14.0	0.167	139	321	45.7
Left	103	15.4	0.304	342	562	72.8
Left	103.8	15.2	0.334	238	436	57.3
Left	105.1	15.6	0.304	198	413	52.8
Left	105.3	15.9	0.202	296	503	63.3
Right	105.3	15.9	0.314	149	237	29.8
Left	109.7	18.8	0.513	388	583	62.2
Left	109.7	18.8	0.443	311	498	53.1
Left	115.2	22.5	0.513	353	714	63.6
Left	115.7	23.1	0.704	681	924	79.9
Left	118.6	23.9	0.875	737	1059	88.5
Right	118.6	23.9	0.834	727	1099	91.9
Left	119.6	23.1	0.578	398	719	62.3
Left	120.4	26.1	0.628	225	792	30.3
Average	109.83± 8.3	18.6 ± 4.5	0.44 ± 0.23	352.3 ± 198.5	599 ± 264.3	60.2 ± 17.8
			A. gilli (Noordhoeks)			
Left	96	9.5	0.205	186	510	53.5
Left	86.4	7.5	0.159	181	550	73.3
Left	86.1	7.4	0.124	82	248	33.6
Left	82.4	6.1	0.100	112	372	61.0
Left	94.6	10.5	0.363	320	914	87.2
Left	93.1	9.3	0.181	151	568	61.1
Average	89.8 ± 5.5	8.4±1.6	0.20 ± 10	172.0±82.9	527 ± 225.4	61.6 ± 18.1
			A. barnardi (Noordhoeks)			
Left	56.2	1.9	0.056	62	84	87.5
Left	53.2	1.6	0.023	23	64	82.1
Left	71	3.8	0.096	58	87	46.0
Right	71	3.8	0.079	34	54	28.6
Left	64.8	2.9	0.058	41	81	55.5
Right	64.8	2.9	0.048	28	64	43.8
Left	64.5	2.8	0.064	41	74	53.8
Right	64.5	2.8	0.061	36	67	48.7
Left	65	2.6	0.050	44	74	56.3
Right	65	2.6	0.058	63	99	75.3
Left	52.3	1.6	0.027	14	41	50.0
Left	49.6	1.4	0.026	19	57	82.6
Left	66	3.1	0.086	55	83	54.4
Left	59.3	2.4	0.063	82	135	112.6
Right	59.3	2.4	0.061	79	126	104.6
Average	61.8 ± 6.5	2.6 ± 0.7	0.057 ± 0.02	45.3 ± 20.8	79.3 ± 25.4	65.4±23.9

4.4. Discussion

Prior to the current study, information on the reproduction biology of *A. gilli* and *A. barnardi* was limited to the investigational report written by Bills (1999). On the basis of back calculation of juvenile daily rings of *A. barnardi*, Bills (1999) established that those juveniles were spawned in November and used that information to spawn *A. barnardi* in captivity. After 48 hours of injecting with pituitary hormones, adult females of *A. barnardi* produced 30-40 adhesive eggs in a clump; each egg was 2 mm in diameter. The clump of adhesive eggs could suggest a nesting behaviour in *A. barnardi* (Bills 1999), which is also the case with other small catfishes such as *Auchenoglanis occidentalis* Valenciennes, 1840 (Baras & Lalèyè 2008) and *Pseudobagrus ichikawai* Okada & Kubota, 1957 (Watanabe 2008). There is currently no information on the fertilization rate of *A. barnardi* as no fertilized eggs were obtained in Bills's (1999) experiment.

The present study supports Bills's (1999) observations, as histological studies revealed that ovaries of *A. gilli* and *A. barnardi* collected from November to January contained tertiary yolk vesicle oocytes, confirming summer spawning. The resorption of vitellogenic oocytes observed in the ovaries collected in February and March, and the development of yolk again in September also showed that these catfishes spawn only in summer. The maximum egg size of 2 mm in diameter observed in *Austroglanis* spp. is similar to findings on other small catfishes, which are regarded as relatively precocial (Table 4.6).

Table 4.6. Maximum fish length, size at maturity, oocyte diameter of *Austroglanis gilli* and *Austroglanis barnardi* sampled in the Rondegat and Noordhoeks River, compared with results from similar studies on the reproductive biology of other small catlets that are considered to be relatively precocial.

Species	$L_{maximum}$	$L_{(50\%\ maturity)}$	Relative fecundity(No. eggs/fish g)	Oocyte diameter	Reference
A. gilli (Rondegat)	137.3 mm SL	94.4 mm SL	60.2 ± 17.8	1.8 mm	This study
A. gilli (Rondegat)	116.8 mm SL	71.6 mm SL	61.6 ± 18.1	1.8 mm	This study
A. barnardi (Noordhoeks)	86.2 mm SL	71.9	65.4 ± 23.9	2.0 mm	This study
Chiloglanis bifurcus (Jubb & le Roux 1969)	68 mm SL	25 mm SL	–	–	Kleynhans & James (1995)
C. pretoriae	65 mm SL*	44 mmTL	29.5	2.6 mm	de Villiers (1991)
Amphilius natalensis	125 mm TL*	62.0 mm TL	–	–	Marriot *et al.* (1997)
Chrysichthys nigrodigitatus	640 mm TL	–	13 ± 6**	3.5 mm	Ekanem (2000)
Trichomycterus itacarambiensis (Trajano & de Pinna, 1996)	83.0 mm SL	43.0 mm SL	–	1.7–3.0 mm	Trajano (1997)
Noturus flavus (Rafinesque, 1818)	310 mm TL	97.0 mm TL	15.5	1.9–3.4 mm	Walsh & Burr (1985)
Synodontis nigrita	335 mm TL	160 mm TL	–	0.5 – 1.0	Lalèyè *et al.* 2006
S. schall	343 mm TL	150 mm TL	–	0.6 – 1.2	Lalèyè *et al.* 2006

* This information was obtained from www.fishbase.org.
** This number may vary from 13 to 40 oocytes per g body mass depending on the river.

It is not so clear what caused the population of *A. gilli* from Rondegat River to mature at larger size and age than the population from the Noordhoeks River. Although not tested in this study, differences in size and age at maturity could be attributable to different feeding conditions which include inter-species competition and invertebrate abundance between the two rivers.

The similarity in the GSI and egg size between *A. gilli* and *A. barnardi* was a surprising find due to the large difference in maximum size between the two species. These results are comparable to those from other African catfish such as *Synodontis schall* and *S.*

nigrita from Ouémé River in Bénin, in which the female GSI ranged from 0.43% ± 0.25 to 9.874% ± 7.38 and 0.33% ± 0.01 to 9.88% ± 9.01, respectively (Laléyè *et al.* 2006).

Egg size in fishes is considered as a tradeoff between fecundity and offspring survival (Paugy 2002). Fishes that lay large eggs tend to hatch well-developed offspring that have abbreviated larval or no larval period. *Austroglanis* species in the CFR appear to exhibit this life history style, which may be advantageous to the offspring because they have an extended period to grow rapidly and reach the juvenile stage before the onset of abiotically harsh winter conditions.

Reproduction is also considered as an energy investment because the energy that was used for somatic growth during the early lifetime is shifted to gonad development, thereby slowing down growth. This was observed in *A. gilli* and *A. barnardi* as both species reached 50% maturity when reaching asymptotic length, suggesting slow growth as a result of a shift in energy budget from somatic growth to reproductive output. This is the case with most fishes.

The summer spawning of *A. gilli* and *A. barnardi,* which coincides with an increase in temperature, is similar to that found in other catfishes in South African river systems, including *S. intermedius* (Hecht 1980), *C. gariepinus* (Hecht *et al.* 1982), *C. pretoriae* (de Villiers 1991), *C. bifurcus* (Kleynhans & James 1995), *A. natalensis* (Marriot *et al.*, 1997), *C. emarginatus* (Kleyhans 1997) and *S. intermedius* (Khumalo 2006). The spawning of *A. gilli* and *A. barnardi* also coincides with the season of high macroinvertebrate abundance (King 1983), the main diet of both *A. gilli* and *A. barnardi* (Chap. 5). This is the case with most annual spawning fishes and the strategy ensures the availability of food for both adults and offspring (Schwassmann 1978).

Summer in the CFR is dry with almost no rainfall and flooding (Fig. 4.10). These conditions could enhance fry predation as fish populations become concentrated into small channels. The benefits of summer spawning need to be greater than the costs. This type of assessment was, however, beyond the scope of the present study.

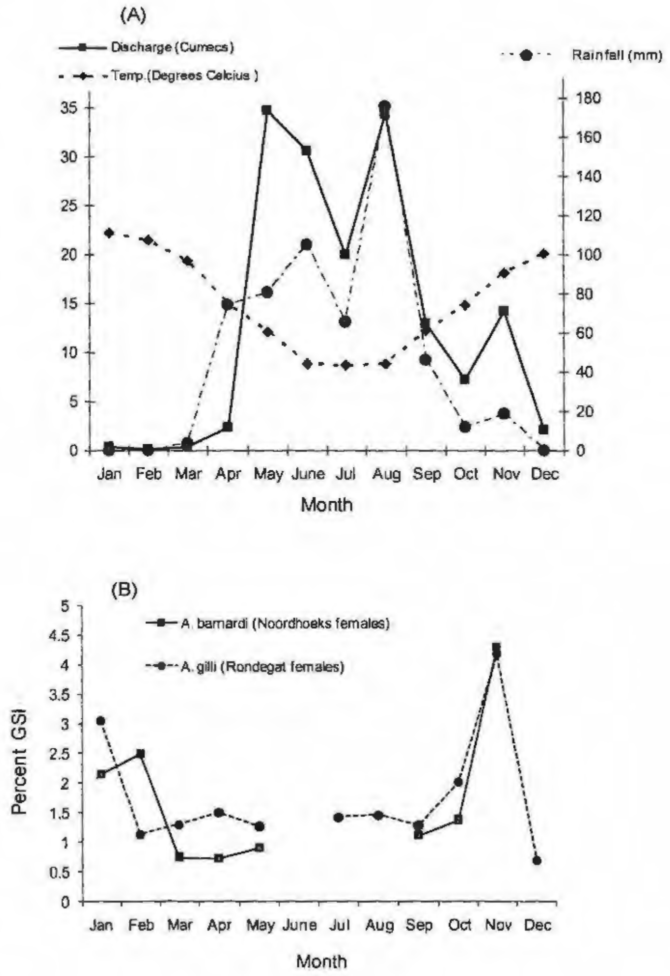


Fig. 4.10. Monthly variation in temperature, discharge and rainfall (A) with respect to gonado-somatic index of *A. gilli* and *A. barnardi* from Rondegat and Noordhoeks rivers (B).

Sex ratio can sometimes be biased due to gear selectivity. It was assumed in this study that electrofishing was not biased and that the sex ratio of each population of the *Austroglanis* spp. was representative of the entire population. *Austroglanis gilli* had a sex ratio that was not significantly different from unity. Rogers (1987) noted a 1:1 sex ratio in the cichlid, *Cichlasoma citrinellum* Günther, 1864 which was associated with monogamy in the Great Lakes of Nicaragua. A sex ratio of 1:1 during the breeding season of *A. gilli* could also suggest a pair spawning behavior.

Austroglanis barnardi had a skew sex ratio in favour of males, differing significantly from unity. This observation may suggest aggressive behaviour, with males defending their young and territories. Male territorial behaviour associated with guarding nests and larvae has been observed in other catfishes such as some of the Brazillian loricariids (Suzuki *et al.* 2000) in. Watanabe (2008) also made a similar observation of territorial behaviour in *P. ichikawai*, where males showed an aggressive behaviour, becoming violent against each other: biting caudal peduncles and pectoral fins, and pushing heads, while engaging in a fight which could last up to half a minute during the breeding season. This bagrid species was also observed attacking co-habiting fishes from other taxa, including gobies and cyprinids, which approached their nesting area (Watanabe 2008).

Similar aggressive behaviour was observed in *A. barnardi* by I.R. Bills (pers. comm.) in an aquarium where individual fish aggressively attacked conspecific intruders into their territory. A comprehensive study on the behavior of *Austroglanis* spp. is, however,

required before any inferences can be made in this regard. It is therefore recommended that future research should focus on the behaviour of *Austroglanis* species.
Female gametogenesis of both *A. gilli* and *A. barnardi* followed a developmental pattern which is similar to that of the mountain catfish, *A. natalensis,* as described by Marriot *et al.* (1997), and of the loricariid, *Loricariichthys spixii* Steindachner, 1881, as described by Duarte *et al.* (2007). The simultaneous occurrence of previtellogenic, primary, secondary and tertiary oocytes in the ripe ovaries of each of the *Austroglanis* species suggests asynchronous, iteroparous and serial spawning strategies (West 1990). Serial spawning over a protracted period has been reported in other small South African catfishes, *C. pretoriae* (de Villiers 1991), *C. bifurcus* (Kleynhans & James 1995), *A. natalensis* (Marriot *et al.* 1997) and *C. emarginatus* (Kleyhans 1997).

A protracted spawning season increases the chance of laying eggs during the most favourable conditions and may minimise intraspecific competition at the beginning of exogenous feeding of the offspring (Bruton 1979; Cussac & Ortubay 2002). It may also be the best strategy to maximise the chances of offspring survival under unpredictable environmental conditions (Cambray & Stuart, 1985). This strategy may, however, be disadvantageous to adult females who risk the chance of being captured by predators before being able to spawn eggs. Despite the lack of information on the factors affecting the recruitment of *A. gilli* and *A. barnardi*, serial spawning would, nevertheless, be considered as the best strategy to enhance offspring survival in these catfishes.

As the environmental conditions in the Cedarberg area are relatively constant in summer, serial spawning over a protracted season suggests bet-hedging against unpredictable events that are more likely to result from biotic factors such as predation or cannibalism than from abiotic factors, such as environmental instability. There was, however, no data available to support this supposition.

The cause of atresia in *A. gilli* and *A. barnardi* is not exactly known but it may be attributed to biotic factors such as a decrease in food abundance. It is also possible that a larger number of vitellogenic oocytes undergo atresia than the number being spawned, or alternatively other vitellogenic oocytes, as in *Bagrus meridionalis* Günther, 1894 (McKaye 1986), are laid to feed the young. The answer to this supposition, however, still needs to be investigated.

The life-history traits of *A. gilli* and *A. barnardi,* which are characterized by delayed sexual maturity, may suggest a precocial *K*-selected strategy that is typical of specialists whose mortality is density-dependent (Adams 1980). This suggests that the numbers of populations of these two species will take a long time to rebuild if they were greatly reduced.

CHAPTER 5: Feeding

5.1 Introduction

An understanding of the feeding biology of particular species of fish is important in terms of providing insights into the habitat requirements of such species (Keevin *et al.* 2007). Environmental threats on prey species, such as the spraying of insecticides, can have negative impacts on predator species. It is, therefore, recognized that successful conservation of threatened species often hinges on gaining knowledge of the diet and feeding behaviour of such species (Sreeraj *et al.* 2006). For example, Keevin *et al.* (2007) showed that information on the feeding habits of sturgeon, *Scaphyrhynchus suttkusi* Williams & Clemmer, 1991 was useful in developing and evaluating recovery plans for this endangered species. Understanding the diet of *A. gilli* and *A. barnardi* is therefore necessary for developing conservation strategies.

Current knowledge on the feeding of the Austroglanididae is based on field observations (Skelton 2001) and a data-limited study (of the contents of 32 stomachs) by Woodford (2005) on the feeding biology of *A. gilli.* These studies as well as an examination of morphological features relating to feeding apparatus (inferior mouths and downward-projecting barbels: Fig. 5.1) indicate that *Austroglanis* species feed on benthic invertebrates, including insects of the orders of Ephemeroptera (mainly Baetidae) and Diptera (mainly Chironomidae).

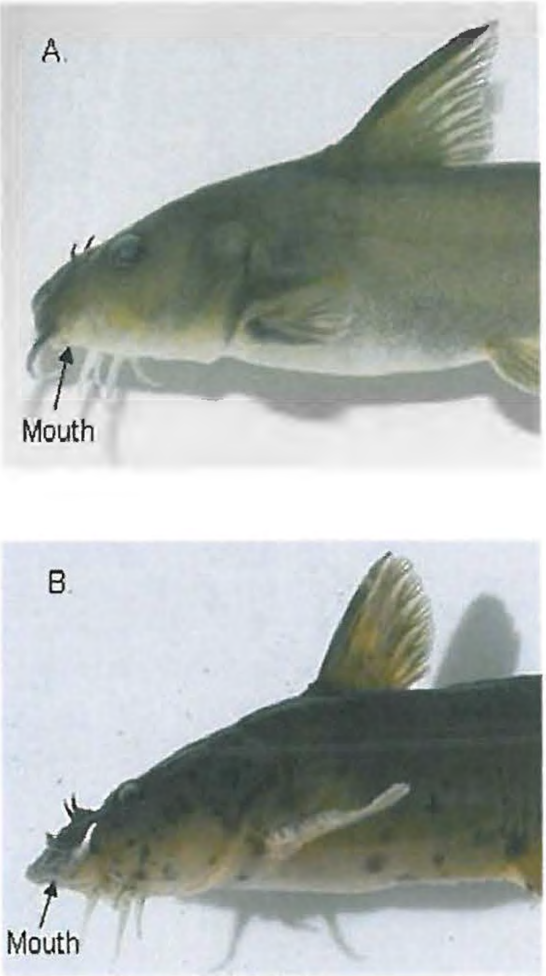


Fig. 5.1. Photographs illustrating the inferior position of the mouth of (A) *A. barnardi* and (B) *A. gilli*. (Photograph: I.R. Bills).

The main objective of this study was to undertake a comprehensive investigation into the diets of *A. gilli* and *A. barnardi*, from Rondegat and Noordhoeks rivers, using specimens — obtained from monthly collections — that are available in the SAIAB fish collection (see Chapter 2, Table 2). Jaw- and tooth structures were also examined in order to gain an understanding of the feeding modes of these two species.

5.2 Materials and methods

A total of 495 specimens of *A. gilli* and 330 specimens of *A. barnardi* — which were collected by electrofishing, fixed immediately in 10% formalin and later preserved in 60% propanol (See Chapter 2, Table 2) — were used for the feeding study. Each of the specimens from the samples was measured to the nearest 0.1 mm SL.

The entire gut was dissected out of each specimen and measured to the nearest 0.1 mm. The relationship between gut length (GL) and fish length was subsequently determined using regression analysis. A condition factor (*K*) was used to give an indication of feeding intensity. This was calculated as: $K = 100w \div l^3$, where w is the weight (g) and l is the length (cm) (Banegal & Tesch 1978).

Jaw and gill raker morphology

In order to determine certain functional aspects of feeding, the gill arch, premaxillae, dentary, lower and upper pharyngeal tooth pads were dissected out from nine specimens of *A. gilli* and seven specimens of *A. barnardi*. Muscle tissue was removed from these bony structures by cutting with a scalpel and pulling with a pair of forceps. The remaining muscle tissue was removed by soaking in 5% trypsin. The bony structures were then dried and gold-splutter coated. Subsequently, they were inspected under Scanning Electron Microscope (SEM) and photographs were taken. Information on these structures was correlated with the diet.

Tooth counts were done along the outer rows of the dentary, premaxillary and lower pharyngeal tooth pads. The outer rows of the upper pharyngeal tooth pads were difficult to count as they were not clearly distinct. Counts were also done on the rows of the edges where the two halves of the lower jaw join together (Fig.5.2). Gill rakers were counted from the first gill arch.

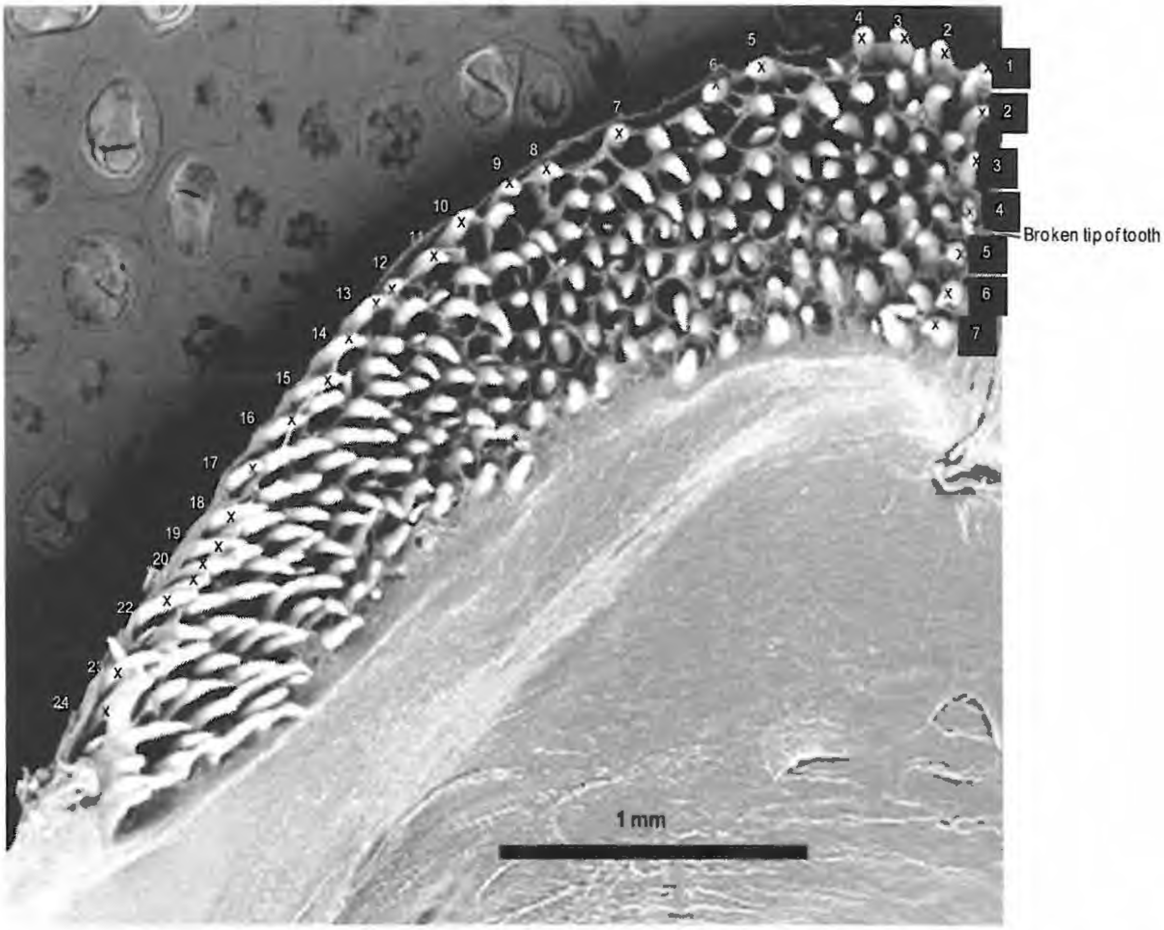


Fig. 5.2. An illustration of the method used for counting the number of teeth of the lower jaw. Teeth marked with 'X' were considered to belong to the same row along the edge.

Gut contents

Each of the stomachs was assessed visually and given a score on the basis of fullness (Table 5.1). Prey items, if present, were removed from buccal cavities and gill rakers and stored in vials. The oesophagus and stomach of each fish was dissected under a dissecting microscope and prey items removed. Only prey items from the buccal cavity, gill rakers, oesophagus, stomach and intestine (until the first U-bend of the intestine) were included in the analysis.

Table 5.1. Criteria used to assign point scores and stomach fullness based on visual assessments of the degree of fullness of stomachs of *A. gilli* and *A. barnardi*, sampled from Rondegat and Noordhoeks Rivers (adapted from Cambray 1983).

Point Score	Degree of fullness	Criteria
0	Empty	Oesophagus and stomach contain no food
1	≤ 25%	Food items are present, either as traces or occupying about one quarter of the total volume of the foregut
2	26% - 50%	Oesophagus and stomach contain food and the walls of the stomach are becoming thick
3	51% - 75%	Oesophagus and stomach nearly filled with food
4	76% - 100%	Oesophagus and stomach completely full of food

Field observations of live specimens indicated that, when placed in a bucket prior to otolith extraction, *Austroglanis* spp. would prey on small cyprinids and galaxiids that were also present in the bucket. In such cases, fresh fish prey was excluded from the gut content analysis because this could bias assessments of the natural diet of *Austroglanis* spp.

Prey items from each of the guts were sorted, identified to the lowest possible taxonomic level, and counted. Since prey items from a wide variety of taxa were recorded in relatively low numbers and low frequencies, some items were combined, for the purpose of analysis, into the following groups: vertebrates (fish or fish scales, frogs), unidentified aquatic insects (unidentified exoskeletons and insect heads), Oligochaeta (various Oligochaete worms), Elmidae (larvae and adults), Diptera larvae (various taxa of dipteran larvae), Coleoptera (terrestrial beetles, unidentified species) and plant material (filamentous algae, detritus, plant twigs and acacia leaves). Various families of Trichoptera that make use of sand cases and could not be identified with certainty were grouped as Trichoptera (sand case) and Hydropsychidae was separated from the group.

Stomach contents were assessed according to recommendations by Hyslop (1980) using an Index of Relative Importance (IRI) expressed as: $\text{IRI} = (\%\text{N} + \%\text{W}) \times \%\text{FO}$, where %N is the number of prey items of a specific type expressed as a percentage of all prey items; %W is the total weight of any prey type as a percentage of the combined weight of all prey and %FO is the frequency of guts containing a prey taxon expressed as a percentage of all guts (Pinkas *et al.* 1971). Because invertebrate prey were all of a similar size, the IRI was modified so that: $\text{IRI} = \%\text{N} \times \%\text{FO}$. This modification was previously applied to an assessment of the gut contents of *Pseudobarbus phlegethon* Barnard, 1938 in the Clanwilliam-Olifants (Whitehead *et al.* 2007). In order to determine ontogenic shifts in the diet, IRI was calculated for 20 mm SL size classes for *A. gilli* and in 10 mm SL size classes for *A. barnardi*.

Spearman rank correlation was used to test for the dependency of diet composition on fish size and season. Chi-square contingency tables (2 species x 5 prey groups) were used to compare the diets of *A. gilli* and *A. barnardi* from the Noordhoeks River and to compare the two *A. gilli* populations from the Rondegat and Noordhoeks rivers.

The Shannon Weiner diversity index was used to test for insect diversity in the guts of both *A. gilli* and *A. barnardi*.

5.3 Results

Morphology

In both *Austroglanis gilli* and *A. barnardi*, the mouth is situated ventrally, with barbels pointing downwards (see Fig. 5.1) and the premaxillary, dentary, upper and lower pharyngeal tooth pads are armed with unicuspid caniniform teeth. Electron micrographs illustrating these tooth pad structures, including the gill rakers of the first gill arch for *A. gilli* and *A. barnardi,* are illustrated in Figs 5.3 and 5.4, respectively. The gill rakers of *A. gilli* were much thicker than those of *A. barnardi* and were very sharp and pointed at the ends, being relatively long at the arm, becoming greatly reduced to about a fifth at the end of the arm; gill raker counts ranged from 11 to 14.

In *A. barnardi* the gill rakers were nearly similar in length, with the last end becoming slightly reduced to about half the size of the raker in the arm with blunt ends; gill raker counts ranged from 8 to 10.

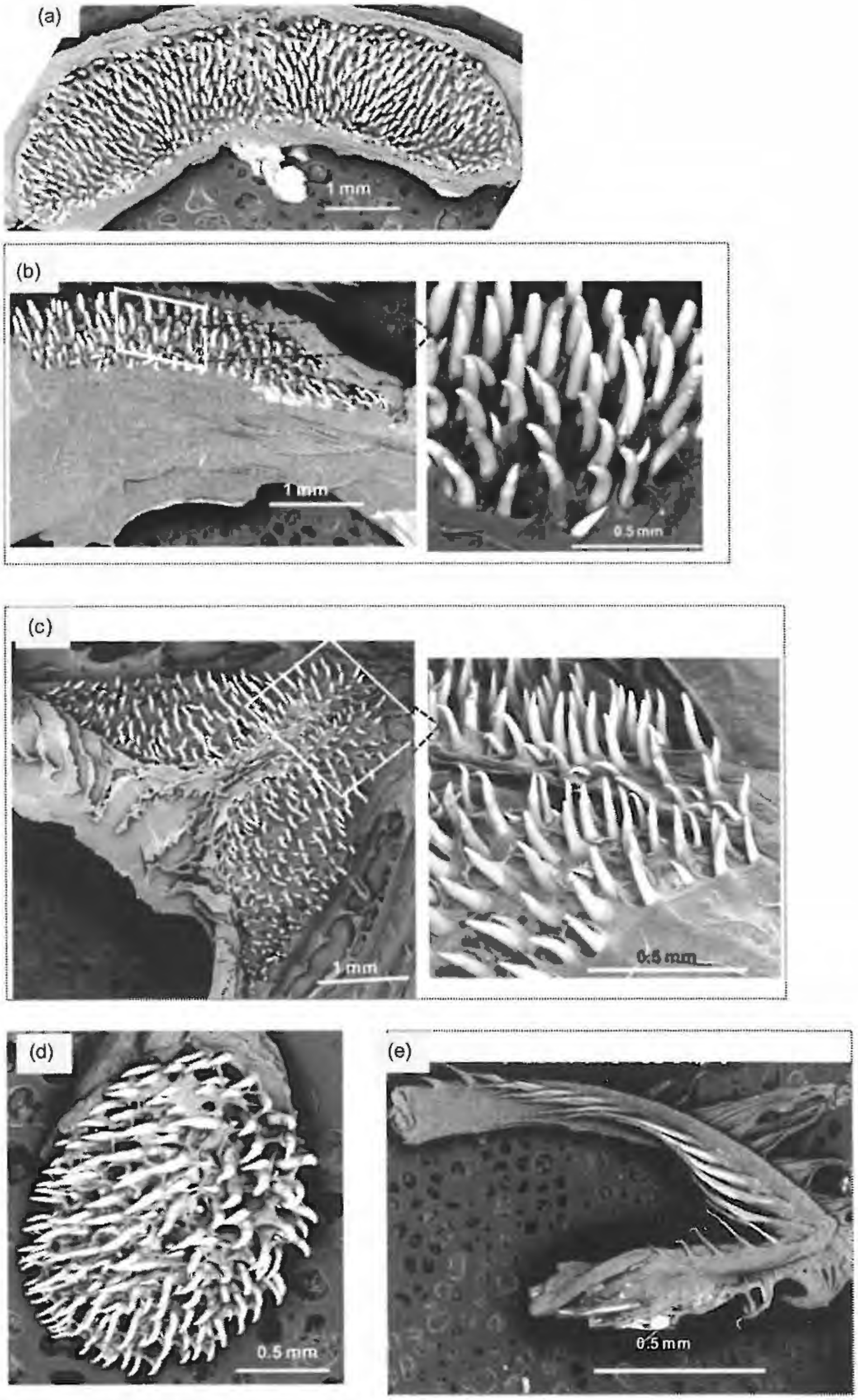


Fig.5.3. Electron micrographs illustrating the dentition of *A. gilli* sampled from the Rondegat River: (a) premaxillary tooth plate, (b) dentary teeth, (c) pair of lower pharyngeal tooth plates with an enlarged, more lateral, view of central teeth, (d) one of the upper pharyngeal tooth pads and (e) an anterior part of the first gill arch showing the form and arrangement of the gill rakers.

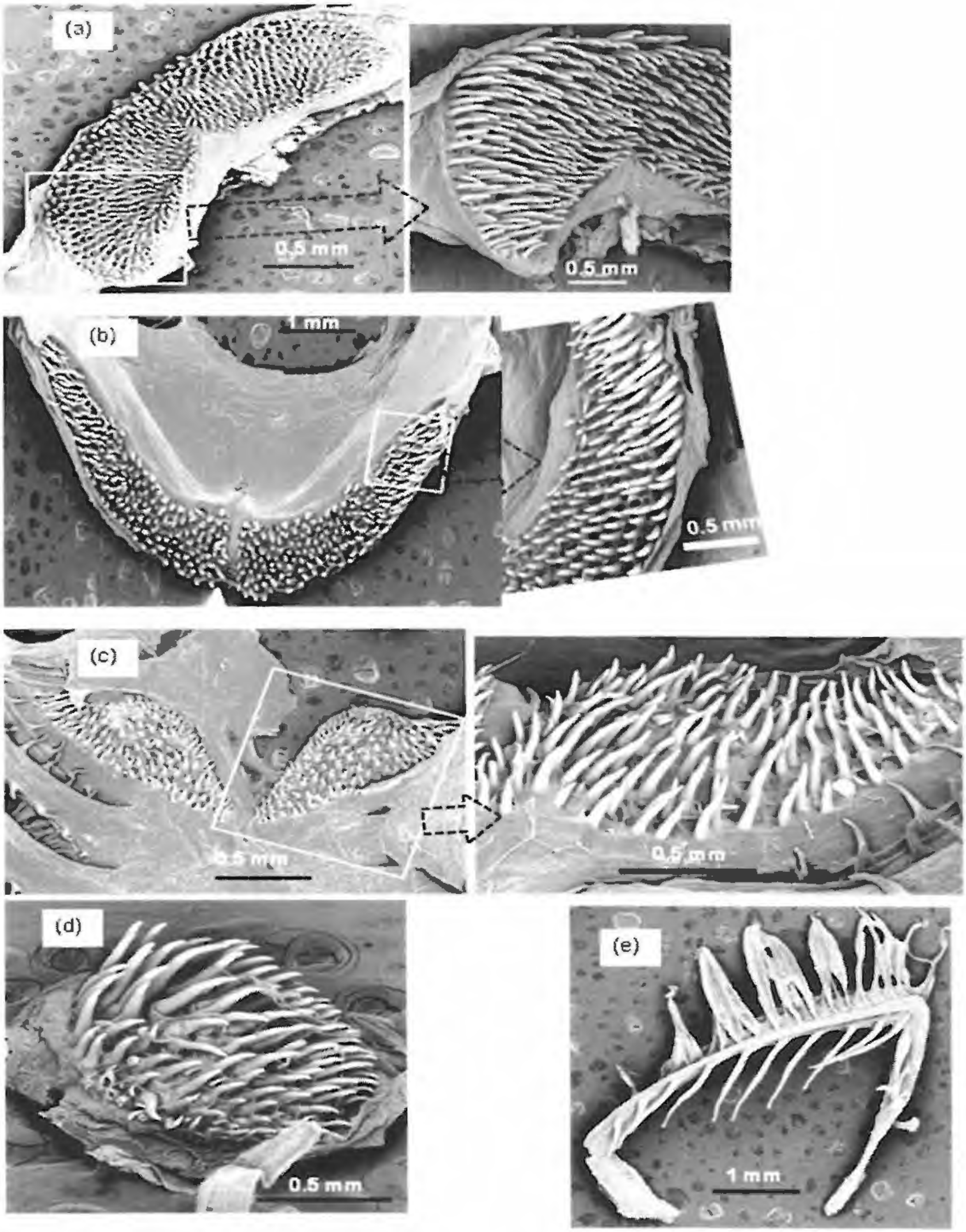


Fig.5.4. Electron micrographs illustrating the dentition of *A. barnardi* sampled from the Noordhoeks River: (a) premaxillary tooth plate and enlarged side view, (b) dentary and enlarged side view, (c) upper pharyngeal tooth pad, (d) pair of lower pharyngeal tooth pads and one enlarged view, and (e) an anterior part of the first gill arch showing the form and arrangement of the gill rakers.

A summary of tooth and raker counts is given in Table 5.2. The number of teeth in the outer row of one half of the lower jaw ranged from 26 to 36 for *A. gilli* and 24 to 29 for *A. barnardi*. Tooth counts along the row where the two halves of the lower jaw meet ranged from six to eight for *A. gilli* and six to seven for *A. barnardi*.

Table 5.2. A summary of tooth and raker counts for *A. gilli* and *A. barnardi* from Rondegat and Noordhoeks rivers.

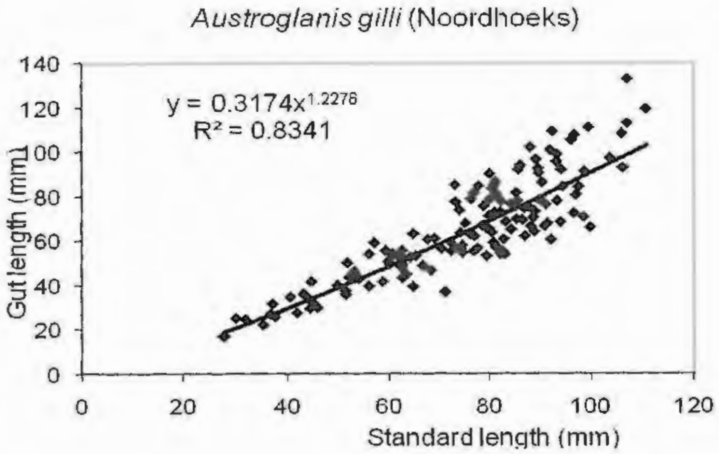
SEM structure	*A. gilli* (Rondegat)	*A. barnardi* (Noordhoeks)
Premaxilla (outer row)	70–76	35–47
One half of the lower jaw (outer row)	26–36	24–29
Row between the joining edges of the lower jaw	6–8	6–7
Lower pharyngeal jaw (outer row)	18–24	10–18
Gill raker counts	11–14	8–10

The individual gut length of each of the *Austroglanis* species was shorter than the standard length. The relationships of Gut Length (GL) to Standard Length (SL) are summarised as follows (also see Fig. 5.5):

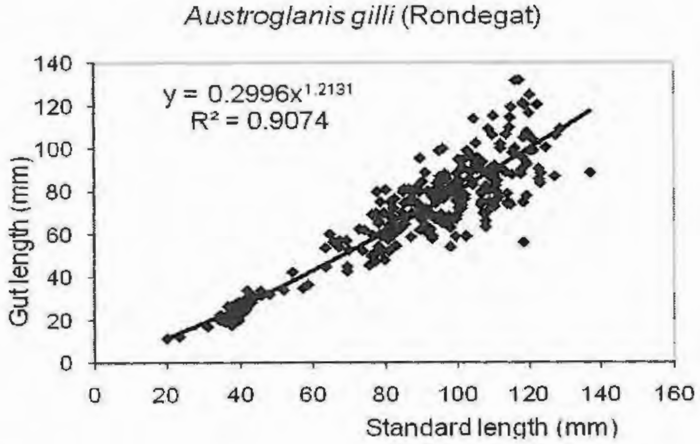
$GL(mm) = 0.2996SL(mm)^{1.213}$ ($r^2 = 0.91$) for *A. gilli* in the Rondegat River;

$GL(mm) = 0.317SL(mm)^{1.227}$ ($r^2 = 0.83$) for *A. gilli* in the Noordhoeks River;

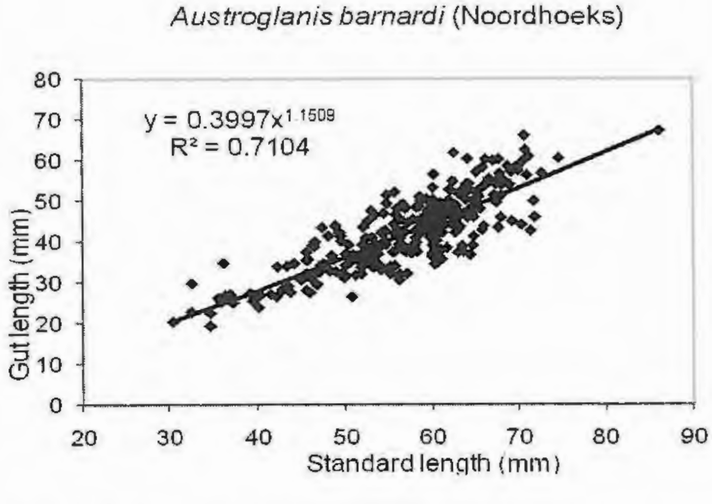
$GL(mm) = 0.408SL(mm)^{1.146}$ ($r^2 = 0.73$) for *A. barnardi* in the Noordhoeks River.



Austroglanis gilli (Noordhoeks)



Austroglanis gilli (Rondegat)



Austroglanis barnardi (Noordhoeks)

Fig. 5.5. Ratio of gut length to standard length of *A. gilli* and *A. barnardi* sampled from Rondegat and Noordhoeks rivers.

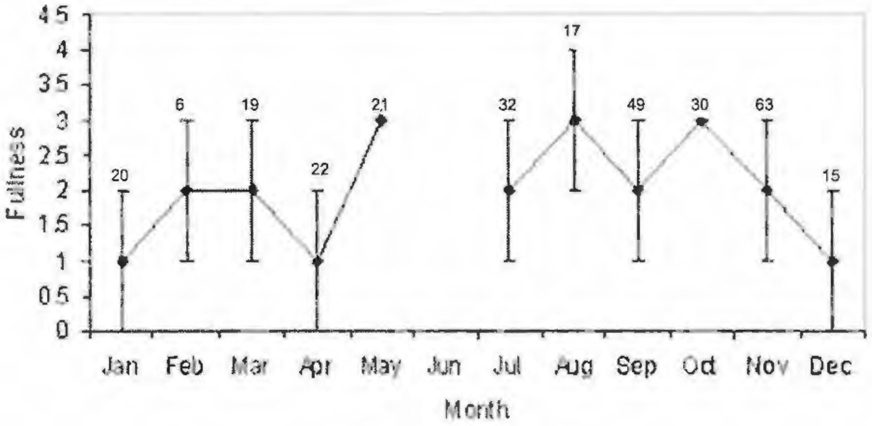
Somach fullness

Monthly stomach fullness indexes of *A. gilli* and *A. barnardi* in the Noordhoeks River dropped sharply in winter, peaked in spring and dropped again in summer (Fig. 5.6). A similar trend was observed for *A. gilli* from the Rondegat River, except that the value peaked in May for the Rondegat population, while dropping sharply for the Noordhoeks populations. Condition factor also showed a seasonal pattern, decreasing to 0.6 in winter, rising sharply and peaking in spring at 0.8, and remaining relatively high in summer and autumn (Fig. 5.7).

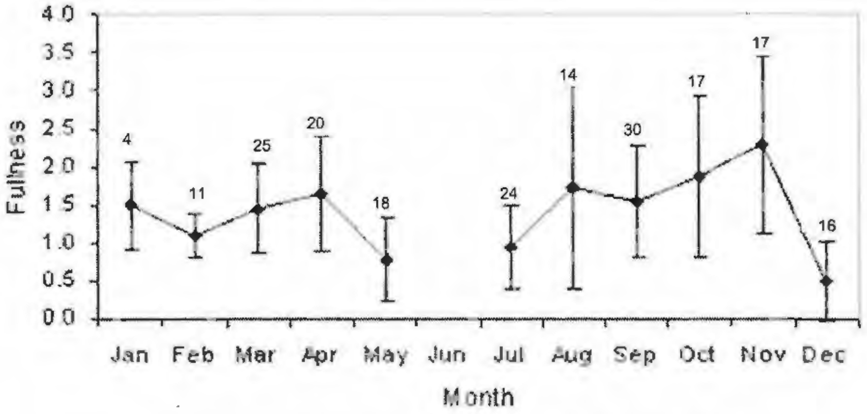
Gut content analysis

A complete dietary analysis for both *A. gilli* and *A. barnardi* is given in Tables 5.3, 5.4 and 5.5. In the Rondegat River, the overall occurrence of Ephemeroptera nymphs (particularly Baetidae) was recorded in 68.1% stomachs. Diptera (Chironomidae and Simuliidae) were respectively recorded in 69.2% and 55.8% stomachs (Table 5.3). The occurrence of these prey items varied seasonally: Baetidae were recorded in 97%, 81% and 32.8% of stomachs in winter, summer and autumn, respectively. The Chironomidae and Simuliidae occurred equally (in 90.5% of stomachs) in winter and summer; the occurrence of Simuliidae was the lowest in autumn when it was recorded in 14.8% stomachs. Other prey items from a wide variety of taxa — including Trichoptera, Oligochaeta, Odonata, Arachnidae (Hydrachnellae), Plecoptera and Megaloptera — occurred in relatively low frequencies.

Austroglanis gilli (Rondegat population)



Austroglanis gilli (Noordhoeks population)



Austroglanis barnardi (Noordhoeks population)

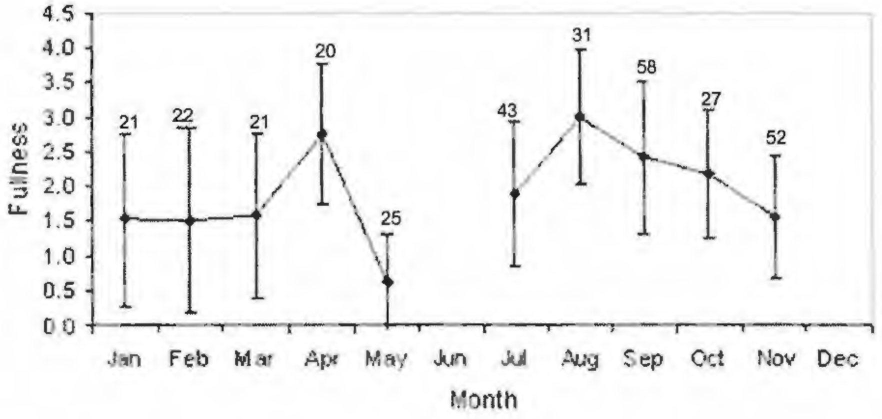


Fig. 5.6. Mean monthly stomach fullness of *A. gilli* and *A. barnardi* sampled from Rondegat and Noordhoeks rivers. Error bars represent standard deviation and the number above each error bar represents sample size.

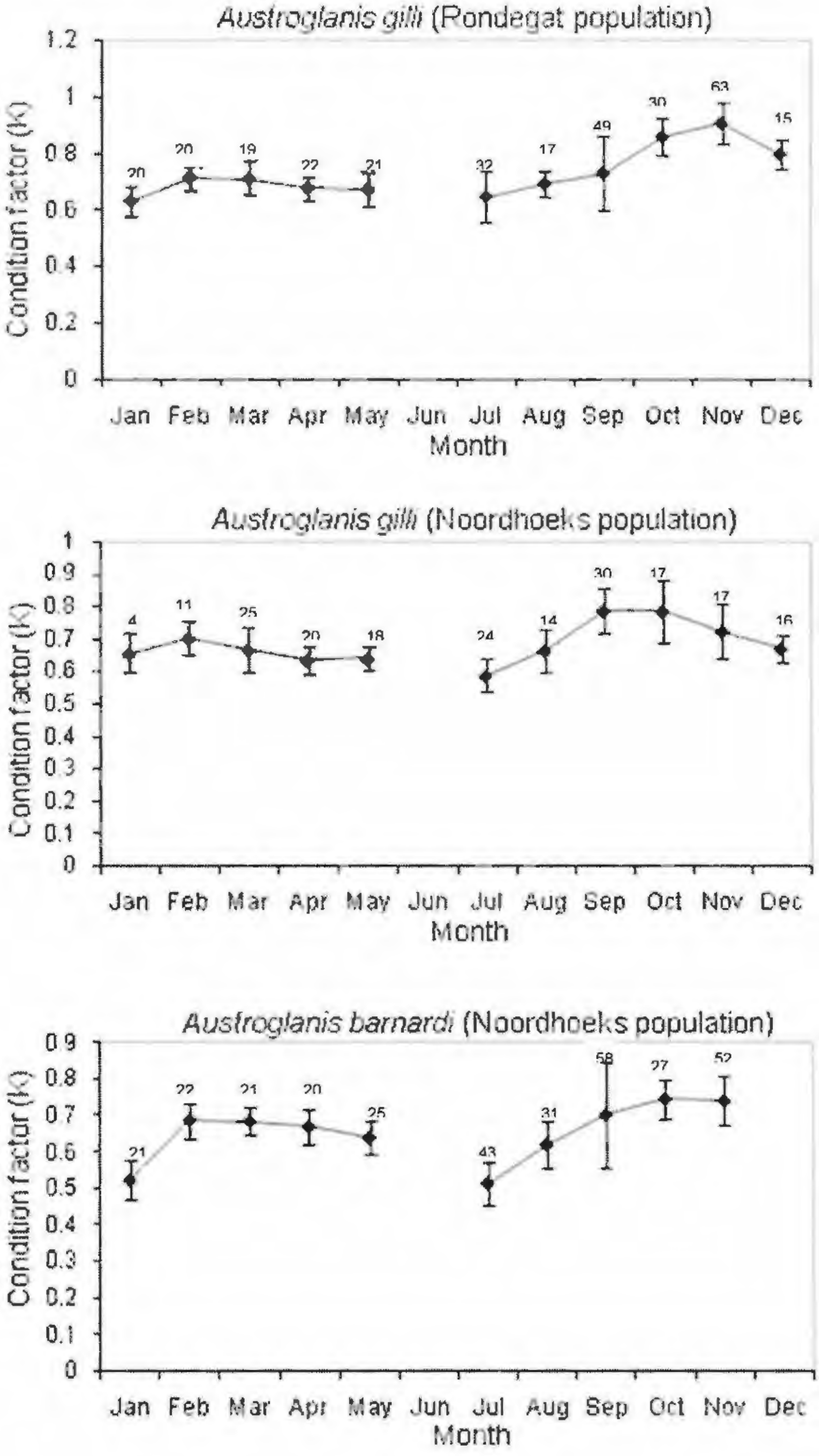


Fig. 5.7. Monthly changes in condition factors of *A. gilli* and *A. barnardi* sampled from the Rondegat and Noordhoeks rivers. Error bars represent standard deviation and the number above each error bar represents sample size.

In the Noordhoeks River, prey items for *A. gilli* followed a similar seasonal trend to that of *A. gilli* from Rondegat River. For *A. barnardi*, the overall occurrence of Chironomidae was 62.8%, with Baetidae and Chironomidae occurring equally at 36.7%. The occurrence of these prey items varied seasonally: Chironomidae and Simuliidae were dominant during winter, occurring at levels of 101.1% and 89.1%, respectively. The lowest occurrence of Simuliidae was in autumn, when it was recorded in 7.8% stomachs. The lowest occurrence of Chironomidae was recorded in spring at 29.8%.

Prey items from families of Trichoptera that made use of sand cases were recorded in 31.9% stomachs. This excluded the Hydropsychidae, which were found in 13.4% stomachs. In the order of Coleoptera, Helodidae and Elmidae occurred in 19.9% and 14.9% stomachs, respectively. Detritus and plant matter occurred in 32.6% stomachs. Other prey items from a wide variety of taxa — including Oligochaeta, Odonata, Arachnidae (Hydrachnellae), Plecoptera and Megaloptera — occurred at relatively low frequencies.

The Spearman rank correlation test showed a correlation ($p > 0.5$) in the diet of different size classes for *A. gilli* from the Rondegat River and *A. barnardi* from the Noordhoeks River (Fig. 5.8). There was also a seasonal correlation in the diet of these two populations. For *A. gilli* in the Noordhoeks, however, the Spearman rank correlation test showed no correlation in different size classes but there was a seasonal correlation.

Table 5.3. Percentage frequency of occurrence, percentage number, and %IRI of various food categories found in the stomachs of *A. gilli* sampled from Rondegat River.

Food category	FO%					N%					%IRI				
INSECTA	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All
Unidentified aquatic insects	16.4	13.5	4.5	18.9	12.3	1.4	0.2	0.2	1.0	0.4	0.5	0.0	0.0	0.3	0.1
Ephemeroptera															
Baetidae	32.8	97.3	60.2	81.1	68.1	16.0	60.8	48.9	34.1	50.9	12.0	65.1	55.0	36.4	55.7
Leptophlebiidae	3.3	32.4	9.1	24.5	17.0	0.2	0.5	0.5	1.7	0.7	0.0	0.2	0.1	0.6	0.2
Teloganodidae	0.0	17.6	1.1	9.4	6.9	0.0	0.5	0.0	0.5	0.4	0.0	0.1	0.0	0.1	0.0
Diptera															
Chironomidae	49.2	90.5	53.4	88.7	69.2	68.5	16.3	9.6	46.6	23.8	77.0	16.2	9.6	54.4	26.4
Simuliidae	14.8	90.5	54.5	56.6	55.8	2.7	16.1	32.1	8.9	17.0	0.9	16.1	32.6	6.6	15.3
Diptera	1.6	8.1	2.3	0.0	3.3	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Ceratopogonidae	0.0	0.0	6.8	0.0	2.2	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Odonata															
Odonata	14.8	2.7	9.1	9.4	8.7	1.2	0.0	0.3	0.4	0.2	0.4	0.0	0.1	0.1	0.0
Aeshnidae	0.0	1.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trichoptera															
Trichoptera sand	13.1	56.8	25.0	30.2	31.9	1.9	2.0	2.4	1.3	1.9	0.6	1.2	1.1	0.5	1.0
Hydropsychidae	3.3	29.7	10.2	7.5	13.4	0.3	0.5	0.6	0.3	0.5	0.0	0.2	0.1	0.0	0.1
Orthotrichia	0.0	13.5	3.4	7.5	6.2	0.0	0.1	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0
Coleoptera															
Coleoptera	0.0	0.0	3.4	1.9	1.4	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Hydraenidae	1.6	24.3	10.2	1.9	10.5	0.1	0.4	0.5	0.1	0.4	0.0	0.1	0.1	0.0	0.1
Helodidae	8.2	41.9	13.6	13.2	19.9	1.4	1.5	1.0	1.7	1.4	0.3	0.7	0.2	0.3	0.5
Elmidae	3.3	21.6	10.2	26.4	14.9	0.3	0.3	0.6	1.7	0.6	0.0	0.1	0.1	0.6	0.1
Blephariceridae	0.0	1.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydrophilidae	0.0	0.0	0.0	1.9	0.4	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Terrestrial beetle	0.0	1.4	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5.3. Cont.

Food category	FO%					N%					%IRI				
	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All
Megaloptera															
Megaloptera	0.0	4.1	8.0	0.0	3.6	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Plecoptera															
Plecoptera	0.0	5.4	5.7	9.4	5.1	0.0	0.1	0.3	0.5	0.2	0.0	0.0	0.0	0.1	0.0
ARACHNIDA															
Hydrachnellae	1.6	4.1	6.8	0.0	3.6	0.1	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0
CRUSTACEA															
Crab	1.6	1.4	3.4	1.9	2.2	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
Amphipoda	0.0	0.0	1.1	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ANNELIDA															
Oligochaeta	4.9	2.7	6.8	7.5	5.4	0.3	0.0	0.3	0.3	0.2	0.0	0.0	0.0	0.0	0.0
VERTEBRATA															
Vertebrates	1.6	0.0	1.1	3.8	1.4	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
PLANTS															
Detritus and Plant material	70.5	14.9	31.8	15.1	32.6	5.1	0.3	1.3	0.5	0.8	8.2	0.0	0.8	0.1	0.4

Table 5.4. Percentage frequency of occurrence, percentage number and %IRI of of various food categories found in the stomachs of *A. gilli* sampled from Noordhoeks River.

Food category	FO%					N%					%IRI				
INSECTA	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All
Unidentified aquatic insects	1.3	6.3	5.4	2.6	3.5	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1
Ephemeroptera															
Baetidae	32.0	66.7	59.5	21.1	43.4	6.4	12.3	47.1	0.8	16.5	3.1	9.9	53.5	0.2	16.5
Leptophlebiidae	1.3	0.0	0.0	5.3	1.5	0.1	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.1
Heptageniidae	0.0	0.0	0.0	5.3	1.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Teloganodidae	1.3	0.0	0.0	2.6	1.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Diptera															
Chironomidae	84.0	93.8	67.6	73.7	81.3	65.0	33.5	20.7	72.7	48.1	84.1	37.9	26.7	72.1	48.1
Simuliidae	42.7	87.5	40.5	81.6	60.6	6.6	47.8	17.5	25.0	24.7	4.3	50.5	13.6	27.5	24.7
Diptera	1.3	16.7	2.7	0.0	5.1	0.0	1.1	0.0	0.0	0.3	0.0	0.2	0.0	0.0	0.3
Ceratopogonidae	18.7	6.3	27.0	2.6	14.1	1.8	0.1	10.5	0.1	3.1	0.5	0.0	5.4	0.0	3.1
Odonata															
Odonata	16.0	0.0	5.4	0.0	7.1	0.9	0.0	0.1	0.0	0.2	0.2	0.0	0.0	0.0	0.2
Anisoptera	0.0	0.0	2.7	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aeshnidae	2.7	10.4	2.7	2.6	4.5	0.1	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1
Trichoptera															
Trichoptera sand	41.3	14.6	24.3	15.8	26.8	2.4	0.5	0.4	0.6	0.9	1.5	0.1	0.2	0.1	0.9
Orthotrichia	16.0	10.4	5.4	0.0	9.6	0.9	0.4	0.1	0.0	0.3	0.2	0.0	0.0	0.0	0.3
Hydroptila	32.0	2.1	0.0	0.0	12.6	9.6	0.0	0.0	0.0	2.2	4.7	0.0	0.0	0.0	2.2
Glossomatidae	0.0	0.0	5.4	0.0	1.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydropsychidae	21.3	20.8	5.4	7.9	15.7	1.7	0.7	0.5	0.2	0.7	0.5	0.2	0.1	0.0	0.7
Coleoptera															
Coleoptera	0.0	2.1	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Helodidae	17.3	43.8	0.0	0.0	17.2	1.2	1.8	0.0	0.0	0.7	0.3	1.0	0.0	0.0	0.7
Hydraenidae	6.7	6.3	10.8	7.9	7.6	0.6	0.1	0.3	0.1	0.3	0.1	0.0	0.1	0.0	0.3
Elmidae	9.3	0.0	0.0	0.0	3.5	0.6	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1

Table 5.4. Cont.

Food category	FO%					N%					%IRI				
	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All
Coleoptera															
Gyrinidae	2.7	6.3	10.8	2.6	5.1	0.2	0.1	1.7	0.0	0.5	0.0	0.0	0.3	0.0	0.5
Ptylodachthtylidae	4.0	10.4	2.7	0.0	4.5	0.1	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1
Hydrophilidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dytiscidae sp.	4.0	0.0	0.0	0.0	1.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Plecoptera															
Plecoptera	1.3	0.0	2.7	0.0	1.0	0.1	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.1
Hemiptera															
Hemiptera	1.3	2.1	2.7	0.0	1.5	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1
Lepidoptera															
Lepidoptera sp.	0.0	4.2	0.0	0.0	1.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CRUSTACEA															
Crab	0.0	4.2	0.0	2.6	1.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ANNELIDA															
Oligochaeta	6.7	18.8	5.4	10.5	10.1	0.5	0.4	0.1	0.2	0.3	0.1	0.1	0.0	0.0	0.3
ARACHNIDA															
Hydrachnellae	2.7	0.0	5.4	0.0	2.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Arachnida sp.	0.0	2.1	5.4	0.0	1.5	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PLANTS															
Detritus and Plant material	14.7	2.1	5.4	0.0	7.1	0.6	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.2
Other	1.3	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5.5. Percentage frequency of occurrence, percentage number and %IRI of various food categories found in the stomachs of *A. barnardi* sampled from Noordhoeks River.

Food category	FO%					N%					%IRI				
INSECTA	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All
Unidentified aquatic insects	48.4	0.0	9.9	0.0	12.4	5.6	0.0	1.7	0.0	0.3	4.7	0.0	0.8	0.0	0.1
Ephemeroptera															
Baetidae	34.4	54.3	27.5	38.3	36.7	8.5	1.0	28.7	4.8	2.7	5.1	0.5	36.0	2.7	1.7
Teloganodidae	1.6	1.1	0.6	1.7	1.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Leptophlebiidae	3.1	2.2	1.8	3.3	2.3	0.3	0.0	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Diptera															
Chironomidae	82.8	101.1	29.8	76.7	62.8	34.2	95.8	35.7	73.2	89.2	49.2	96.8	48.6	83.1	94.9
Simullidae	7.8	89.1	13.5	53.3	36.7	1.5	2.9	18.0	16.7	4.3	0.2	2.6	11.0	13.2	2.7
Diptera	15.6	16.3	1.8	0.0	7.2	1.9	0.1	0.4	0.0	0.2	0.5	0.0	0.0	0.0	0.0
Ceratopogonidae	4.7	0.0	0.6	0.0	1.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Odonata															
Odonata	0.0	0.0	0.6	0.0	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aeshnidae	1.6	0.0	0.6	0.0	0.5	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trichoptera															
Trichoptera (sand)	60.9	4.3	5.3	18.3	16.3	33.6	0.0	1.8	0.9	1.6	35.6	0.0	0.4	0.2	0.5
Hydropsychidae	29.7	6.5	4.7	6.7	9.6	4.3	0.0	1.9	0.4	0.3	2.2	0.0	0.4	0.0	0.1
Trichoptera none-case	1.6	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hydroptila	9.4	1.1	2.3	11.7	4.7	0.9	0.0	1.6	0.7	0.1	0.1	0.0	0.2	0.1	0.0
Coleoptera															
Coleoptera	6.3	3.3	0.6	3.3	2.6	0.4	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Hydraenidae	0.0	0.0	0.6	0.0	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Helodidae	1.6	3.3	1.2	1.7	1.8	0.1	0.0	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Elmidae	15.6	5.4	1.8	8.3	5.9	1.3	0.0	0.3	0.6	0.1	0.3	0.0	0.0	0.1	0.0
Terrestrial beetle	0.0	0.0	0.6	0.0	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5.5. Cont.

Food category	FO%					N%					%IRI				
	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All	Aut.	Win.	Spr.	Sum.	All
Plecoptera															
Plecoptera	4.7	0.0	0.0	0.0	0.8	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Megaloptera															
Megaloptera	0.0	2.2	2.3	0.0	1.6	0.0	0.0	1.2	0.0	0.1	0.0	0.0	0.1	0.0	0.0
ARACHNIDA															
Hydrachnellae	0.0	1.1	0.6	1.7	0.8	0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0
CRUSTACEA															
Crab	1.6	0.0	1.2	0.0	0.8	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ANNELIDA															
Oligochaete type 1	1.6	3.3	0.6	18.3	4.1	0.3	0.0	0.1	0.9	0.1	0.0	0.0	0.0	0.2	0.0
Oligochaetes type 2	15.6	3.3	1.2	18.3	6.7	1.6	0.0	0.2	0.9	0.1	0.4	0.0	0.0	0.2	0.0
VERTEBRATA															
Vertebrates	0.0	0.0	0.6	0.0	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unidentifiable	25.0	0.0	9.9	0.0	8.5	1.6	0.0	1.7	0.0	0.1	0.7	0.0	0.8	0.0	0.0
PLANTS															
Detritus and plant material	14.1	1.1	7.0	8.3	7.0	2.9	0.0	4.7	0.4	0.4	0.7	0.0	1.5	0.1	0.0

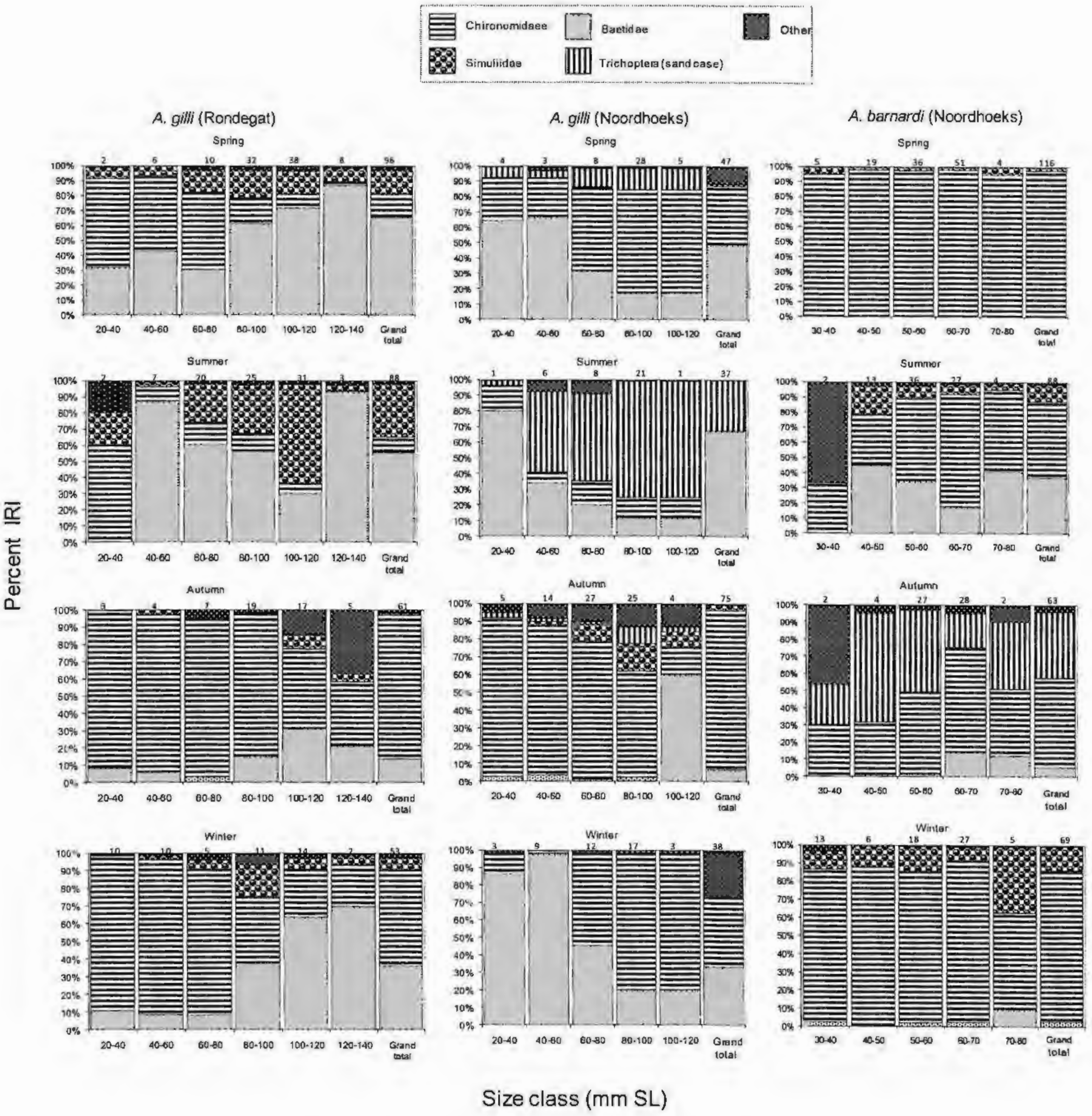


Fig 5.8. Seasonal summary of the gut contents of *A. gilli* and *A. barnardi* from the Rondegat and Noordhoeks Rivers. The numbers above each bar denote fish stomachs.

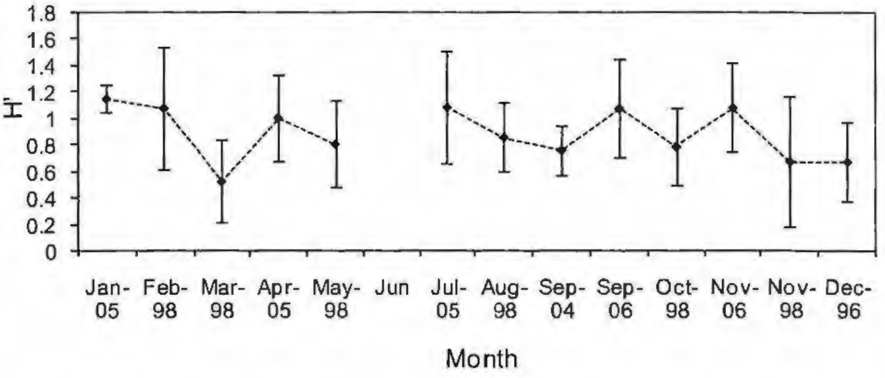
There was a significant difference ($\chi^2 = 53.79$, d.f. = 4, $p > 0.001$) between the prey items of *A. gilli* and *A. barnardi*, and between the prey items of *A. gilli* populations in the Rondegat and Noordhoeks rivers ($\chi^2 = 34.74$, d.f. = 4, $p > 0.001$).

There was a seasonal pattern in insect diversity in the diet of *A. barnardi* from the Noordhoeks River, winter having the lowest invertebrate diversity (Shannon Weiner index: Fig. 5.9). There was no clear seasonal pattern for the diversity of insects from the guts of *A. gilli* (Fig. 5.9).

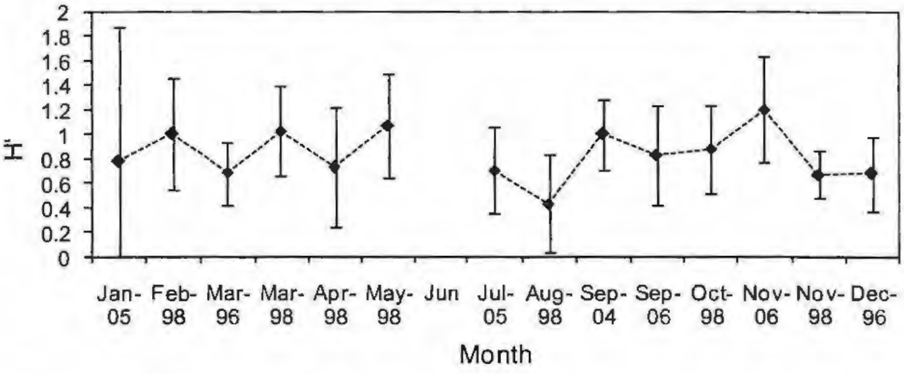
5.4 Discussion

Austroglanis spp. have evolved structural adaptations which include downward-projecting barbels, sensory skin cells, inferior positioning of the mouth and buccal cavity comprising dentition, and premaxillary and pharyngeal pads armed with inwardly-curved teeth, which assist in the location of benthic prey. These features were considered by Abes *et al.* (2001) — in his study of the catfish, *Iheringichthys labrosus* Lütken, 1874 — as special adaptations enabling this species to grip and manipulate benthic prey items from various taxa. *Austroglanis* spp. can exploit nearly every trophic level. Oral and pharyngeal teeth could serve as a piercing, rather than a mastication, function. This was evidenced by the observation that most of the prey items in the stomachs were still intact.

A. gilli (Rondegat)



A. gilli (Noordhoeks)



A. barnardi (Noordhoeks)

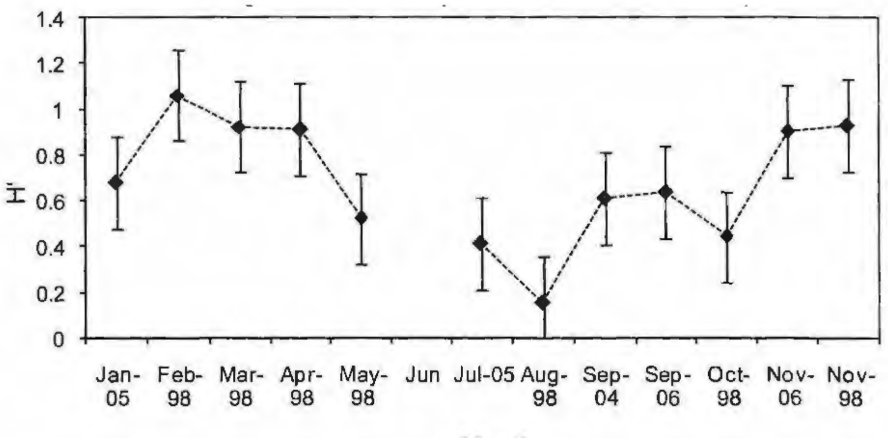


Fig. 5.9. Shannon Weiner diversity Index (H') determined from macroinvertebrates obtained from the guts of *A. gilli* and *A. barnardi* sampled from Rondegat and Noordhoeks rivers. Error bars indicate standard deviations.

Inspection of gut contents of both *A. gilli* and *A. barnardi* showed that the two species feed predominantly on ephemeropteran and dipteran larvae. The observed differences in the prey composition of *A. gilli* between Rondegat and Noordhoeks rivers may suggest differences in invertebrate composition, which is comparable to the findings by de Moor & Barber-James (unpubl. report) who recorded different taxa in the streams of the Clanwilliam-Olifants. Invertebrate patchiness in different biotopes of some of the rivers of the Western Cape Province has also been reported (Dallas 2007), and this could explain the differences in prey compositions between *A. gilli* and *A. barnardi* in the Noordhoeks River. Unfortunately, there were no data available to test this further.

The dominance of ephemeropteran and dipteran insects in the Rondegat River was reported in Lowe *et al.* (2008) and the dominance of these two orders in the guts of *A. gilli* and *A. barnardi* may suggest an opportunistic feeding guild. The presence of aquatic invertebrates from a wide variety of taxa recorded in relatively low frequencies and numbers, including allochthonous materials from terrestrial environment, may support the proposed feeding guild. Opportunistic feeding in *Austroglanis* spp. is comparable to that observed in some other small South African catfishes, including *C. pretoriae* (de Villiers 1991) and *A. natalensis* (Marriot *et al.* 1997), and for the North American ictalurids such as *Noturus eleutherus* Jordan, 1877 (Starnes & Starnes 1985) and *N. flavus* (Walsh & Burr 1985).

In a study of the ecology of catfishes of the Zambezi River, Winemiller & Kelso-Winemiller (1996) identified four feeding categories of catfish namely, benthic insectivores (e.g. *Leptoglanis* spp.), detrivores (some *Synodontis* spp.) and piscivores (e.g. *Clarias* spp.). The diets of *A. gilli* and *A. barnardi*, as well as *C. pretoriae* (de Villiers 1991) and *A. natalensis* (Marriot *et al.* 1997) indicate that these species could be regarded as benthic insectivores.

Benthic insectivory in the *Austroglanis* spp. could also be evidenced by the behaviour of the dominant prey items that occur mainly on the substrates (Table 5.6). Sand particles, which were occasionally recorded in large quantities in the diet of *A. gilli* and *A. barnardi,* could be incidental due to the substrate feeding behaviour. A similar inference on substrate feeding was also made by Whitehead *et al.* (2007) in relation to their studies of *P. phlegethon* in the Noordhoeks River.

Table 5.6. Behaviour and habitat preferences of the major prey items of *A. gilli* and *A. barnardi* (after Gerber and Gabriel 2002) sampled from the Rondegat and Noordhoeks rivers.

Main food group	Behaviour and habitat preference	Abundance
	DIPTERA	
Chironomidae	In silk tubes on any type of substrate in pools and streams	Many species with distinct seasonal trends but group as a whole available throughout the year
Simuliidae	Attached to stones, plants or any solid surface in shallow rapid streams	Often patches of almost 100% cover in very shallow fast flows
	EPHEMEROPTERA	
Baetidae	On the surfaces of rocks, plants and course sand in moderately fast streams	Abundant in summer

Correlation analysis of the diets of different size classes of *A. gilli* from the Rondegat River and *A. barnardi* from the Noordhoeks River (Spearman rank correlation $p > 0.05$) suggests that the different size classes of each population feed on similar diets. This could suggest that both juveniles and adults of each species are foraging mainly in similar niches. These findings are comparable to those of Ikusemiju & Olaniyan (1977) who observed no change in the diet with an increase in size classes of *C. nigrodigitatus* from Lekki Lagoon in Nigeria.

In the case of *A. barnardi*, these findings are not surprising, since all size classes occur exclusively in the shallow riffles and all probably forage in this biotope. Although Chironomidae occur in almost every freshwater environment (McCafferty 1981), their high frequency of occurrence in the guts of *A. barnardi* suggests that there could be correlation of riffle habitats with Chironomidae abundance. Galdean *et al.* (2000) reported the correlation of Chironomidae with sandy substrata in Brazilian streams and these findings can be comparable to the habitat of *A. barnardi* from the Noordhoeks River.

For the population of *A. gilli* from the Rondegat River, the correlation of diet in the different size groups was not expected because of differences in habitat preferences between juveniles and adults. The riffles of the Rondegat River are not, however, as distinct as those of the Noordhoeks River, and such a situation in the Rondegat may enhance the chances of juveniles and adults foraging mainly in the riffles.

The Noordhoeks River, which is mainly dominated by the shallow riffles, may differ from the Rondegat River in terms of invertebrate patchiness. This could explain the differences, observed in the former river, in prey composition in the diets of juvenile and adult *A. gilli.* The Noordhoeks has a more open canopy and may receive more sunlight penetration than is the case for the Rondegat. Primary production is lower in rivers with high canopy cover, resulting in lower secondary production, hence reduced macroinvertebrate abundance (Dineen *et al.* 2007). De Moor & Barber-James (unpubl. report) noted differences, in terms of invertebrate taxa composition, between the Rondegat and Noordhoeks, with the latter being more diverse.

During snorkel surveys *A. gilli* has been observed coming out from under rocks during the night, probably for the purpose of feeding in a different niche (IR Bills pers. comm.). Adult *A. gilli* were probably foraging in the shallow riffles during the night when chances of being preyed upon were reduced. Nocturnal feeding behaviour in small catfishes seems to be common as Watanabe (2008) made similar observations of the bagrid, *P. ichikawai*, while Starnes & Starnes (1985) observed nocturnal feeding in the ictalurid *Noturus eleutherus* Jordan, 1877 coinciding with the time when the majority of invertebrates were drifting. *Austroglanis gilli* has probably evolved a similar nocturnal feeding behaviour and during the night feeds in biotopes other than cobbles. This behaviour could minimize chances of being preyed upon by otters. These suppositions, however, require confirmation.

The observation of *Austroglanis* spp. feeding on the minnows and galaxiids placed together in the bucket and the presence of scales in some of the guts suggests piscivory. Fish scales have also been recorded in other small catfishes such as *Chrysichthys* *filamentosus* Boulenger, 1912 (Nwadiaro & Okorie 1987) and *H. brachysoma* (Sreeraj *et al.* 2006). No cannibalism was observed in *Austroglanis* spp. during this study.

A decrease in feeding intensity during the breeding season observed in *A. gilli* and *A. barnardi* is comparable to results obtained from studies of other catfishes, including *S. schall* (Willoughby 1974 as cited by Nwadiaro & Okorie (1987)). Nwadiaro & Okorie (1987) proposed that a decrease in feeding intensity during the spawning period of *S. schall* was attributable to courtship behaviour and parental care, which impacted on the time allocated to feeding.

Dietary overlap of cohabiting species may suggest competition for food resources (Michaletz 2006). This could be the case for *A. gilli* and *A. barnardi* from the Noordhoeks River, since both species feed predominantly on benthic invertebrates. Future studies could investigate aspects of inter- and intra- species competition between *Austroglanis* spp. so as to make some inferences as to whether these result in increased mortality rates of *A. gilli* (see Chapter 3, Table 3.6).

Since *A. gilli* and *A. barnardi* as well as cohabiting species *B. calidus* (Nthimo 1997), *B. erubescens* (Marriott *et al.* 1997) and *P. phlegethon* (Whitehead *et al.* 2007) all feed predominantly on macroinvertebrates, conservation management of indigenous fish should stress the importance of maintaining invertebrate biodiversity in the study area.

CHAPTER 6: General Discussion

In this chapter, the life-history traits of *A. gilli* and *A. barnardi* assessed in the previous chapters are discussed in relation to conservation. With the available information on the life-history, some management recommendations can be made which will contribute to the maintenance of the existing three *A. barnardi* and 16 *A. gilli* populations (Swartz *et al.* 2007a,b). These recommendations may also contribute to improving their conservation status.

The key findings from this study were that:

- The two *Austroglanis* catfishes displayed longevity, growth, maturity and fecundity traits that are typical of precocial, *K*-selected species.
- *Austroglanis* catfishes live much longer than we expected (maximum age of 12+ years for *A. gilli* and 14+ years for *A. barnardi*).
- They reach late maturity (2+ to 3+ years old) and produce large egg sizes (maximum diameter of 2 mm).
- The relative fecundity of 62 ± 18 g/ova for *A. gilli* and 65 ± 24 g/ova for *A. barnardi* are considerably low for these catfishes.
- Histological analysis showed that not all vitellogenic oocytes are spawned indicating that these fishes have evolved a serial, iteroperous spawning strategy.
- Fish spawn throughout the summer season (November to January). Unfortunately this spawning period coincides with the driest period of the year and the peak water off-take by farmers from the streams for irrigation.

- The diet of both *Austroglanis* spp. is dominated by macro-benthic invertebrates of Insecta.
- The population of *A. gilli* from Rondegat River attains first maturity at a larger size and older age and has lower annual mortality rates than the population at the Noordhoeks River. This population would therefore take longer to rebuild after a deleterious event and is thus of higher conservation priority.
- The spawning period, frequency and diet are common to the other co-habiting indigenous fishes in the Olifants/Doring River system (Table 6.1).

Table 6.1. A list of indigenous fishes of the Olifants System, summarising some information on their biology.

Species	Main diet	Spawning period	Spawning frequency	Longevity (years)	Reference
Labeobarbus capensis	-	October–February	Asynchronous, iteroperous serial spawner	*20+	Cambray *et al.* (1997); *Wolhuter & Impson (2007)
Pseudobarbus phlegethon	Predominatly Chironomidae	October–February	"	-	(Whitehead *et al.* 2007)
Barbus calidus	Predominantly Diptera, Chironomidae and Formicidae	October–February	"	-	Nthimo 1997
Barbus erubescens	Predominantly Diptera, Ephemeroptera and other invertebrates	October–February	"	6+	Marriot (1998)
Barbus serra	-	November–February	"	-	Paxton 2008
Austroglanis gilli	Predominantly Baetidae, Chironomidae and Simuliidae	November–January	"	12+	This study
A. barnardi	Predominantly Chironomidae	November–January	"	14+	This study

Precocial life histories indicate a vulnerability to events that decrease population size through increasing mortality. Because the two *Austroglanis* catfishes are long-lived, late maturing and have low fecundities, it will take a relatively long time for populations to rebuild after they are reduced by human activities. In addition, there is some evidence that there is very low dispersal of these catfishes within the river systems. Genetic assessments of *A. gilli*, for example, showed different alleles present in fishes in almost every kilometer of Jan Dissels River in the Olifants River system (Swartz *et al.* unpubl. data). The concern is that with increasing human impacts on the stream ecosystem some of the smaller populations might be lost.

6.1 Environmental impacts

Understanding the impacts can help to develop mitigation measures and management recommendations. The principal impacts of concern relate to water abstraction, agrochemical pollution and alien invasive fishes.

Water abstraction

Water off-take from streams for irrigation by farmers (mainly over the dry summer which is the peak breeding period), at the very least results in reducing flows. At worst, it involves bulldozing of streambeds and damming of rivers which destroys important fish habitat and completely dries out sections of the river. In conjunction to this, foreign material, such as soil, is often introduced into the streambed resulting in sedimentation of the substrate.

Apart from the direct effects of spawning habitat loss, smothering of eggs with silt and drying out sections of the river, reduced flow rates in summer will also affect the dispersal of these catfishes. Drifting of *Austroglanis* juveniles have been observed during January in the Noordhoeks River (IR Bills pers comm.), reduced flow rates and isolation of pools during this time by damming or streambed dessication will also result in decreased chances of *Austroglanis* spp. to repopulate disturbed environments.

Water off-take results in alterations in river flow which can negatively impact on the distribution and abundance of macro-benthic invertebrate prey (Olomukoro & Ezemonye 2007). For example, the Simuliidae, one of the austroglanid's major prey groups, requires fast-flowing water (Gerber & Gabriel 2002). It has been shown that a great reduction in the flow of the Great Fish River in the Eastern Cape Province can significantly reduce the outbreak of the pest, *Simulium chutteri* Lewis, 1965 (Rivers-Moore & de Moor 2008). The maintenance of the normal river flow should therefore be considered in the conservation measures of *Austroglanis* species.

Agrochemical pollution

The impact of agrochemical pollution, from crop spraying using herbicides and insecticides and by leaching of fertilizers, on the indigenous fishes of the CFR has not been measured directly. In this region sandy soils dominate and drainage of soils is rapid. As farming activities are concentrated on the stream banks it is highly likely that this will result in, at least some, chemical pollution of the streams. This will result in a number of possible negative effects on the austroglanid catfish. As their diet is dominated by aquatic insects, insecticides are likely to directly impact on insect abundance in streams. The

Baetidae, for example, are sensitive to pollution and their absence is considered a good indicator of pollution (Olomukoro & Ezemonye 2007). In addition, herbicides may impact on algal growth and fertilizer run-off may result in nutrient loading which may lead to eutrophication, changes in water quality and in invertebrate and vertebrate community structure (Tubea *et al.* 1981). In addition, agro-chemical pollution can have both lethal and sublethal effects. Sublethal effects include reduced fish growth, condition factor and organ weights. This has been shown to be case with *O. mossambicus* (Jordaan & Reinecke 2009). This may explain the observed disappearance of *Austroglanis* spp. from the systems at or just below the first major farms (IR Bills pers comm.).

Alien invasive fishes

Substantial populations of both *Austroglanis* spp. co-occur with alien bass and trout in some streams e.g. *A. gilli* and *A. barnardi* in the Heks co-exist with piscivorous *M. dolomieu*. The populations of *A. gilli* from Jan Dissels and Krom rivers co-exist with *M. Dolomieu* and *O. mykiss*, respectively. These *Austroglanis* populations have co-existed with piscivorous fish for more than 10 years and have maintained large populations in the extension of more than 10km (Bills pers. comm.). They are thus different to all other CFR species in that they can co-exist with alien fish in the longer term. The general assumption is that *Austroglanis* spp. can continue to survive with bass and trout because they avoid predation by living in between rocks during the day when these predators are active. In the future, it is important that this assumption is tested using PVAs and predator dietary analyses. What is clear, however, is that in areas where there are multiple stressors such as, water abstraction, agricultural runoff and alien species, the numbers of *Austroglanis* are severely reduced (Bills 1999).

6.3 Conservation

A successful conservation of the freshwater biota depends also on the management of the catchment because this can minimise threats related to the altered hydrology (Saunders *et al.* 2002; Filipe *et al.* 2003). Some rivers that pass through protected areas can also benefit from the reserves. The creation of conservancies to increase protected areas would thus be recommended for the conservation of *Austroglanis* species.

The promotion of conservation awareness to the general public, particularly the farmers might minimise chances of further habitat degradation and help restore the complex nature of benthic habitats.

A long-term biomonitoring project should be considered in the conservation measures for *Austroglanis* species. The streams comprising small number of populations do not appear to be of major concern. The monitoring of the lower limit points of populations (numbers of indigenous species) is recommended. The upper limits of agro-impacts and upper limits of alien fishes should also be monitored through habitat assessment indices.

The rivers of high conservation priority in this case would be the three streams containing *A. barnardi* and streams with low to medium levels of development in the catchments such as the Rondegat, Krom and Jan Dissels rivers. Prioritisation of catchments should be based on recommendations derived from a full assessment of the vulnerability of each *Austroglanis* poplation to extinction by applying PVAs. The results from this study are important components of a PVA, but the inclusion of genetic analyses and the modeling of *Austroglanis* vulnerability to extinction using a PVA was however beyond the scope of the present study. The application of a PVA and the development of a long term effective conservation and management strategy could however be achieved through an inter-institutional collaborations in research and implementation.

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