Lake Tana's piscivorous Barbus (Cyprinidae, Ethiopia):

Ecology • Evolution • Exploitation

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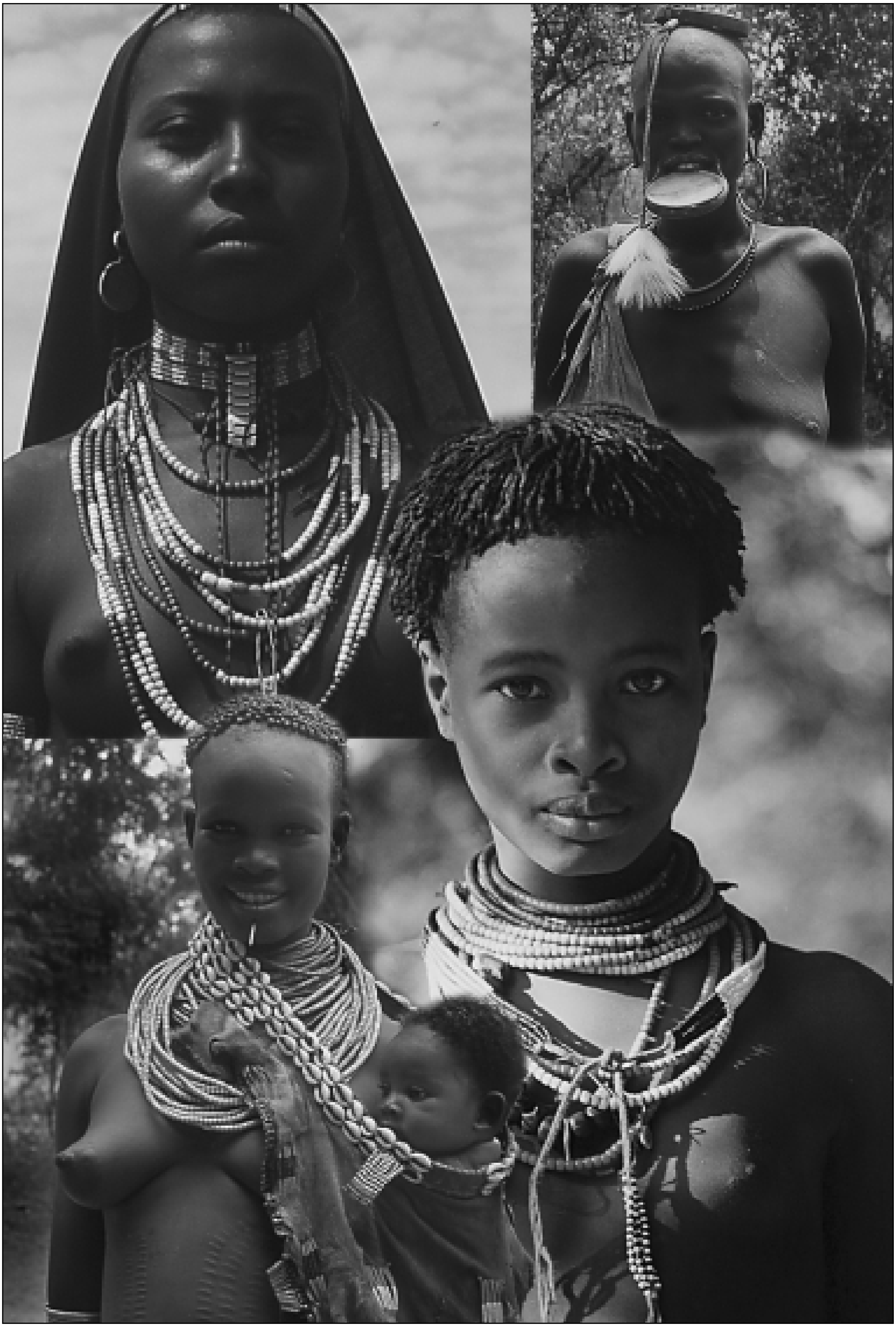

Ethiopia…'The Land of Plenty'

This section of my thesis is optional, but will give the interested reader some background information about Ethiopia and its people.

Ethiopia is rarely in the news in a positive way; the images of starving children as a result of serious droughts during the eighties are engraved into everybody's memory. More recently, the war with their neighbours Eritrea, formerly part of Ethiopia up to 1993 and with whom it still has very close historical and cultural ties, however, did not improve Ethiopia's image in the west as a troubled nation. Unknown to the rest of the world, is Ethiopia's fascinating history, unique culture and traditions, the tremendous diversity of its ethnic people and the high degree of endemic wildlife in its highlands. Ethiopia is the only African country that has its own written language "Ge'ez" and was never successfully colonised by any of the European powers during the "Scramble for Africa" in 1870-1912. The rumoured possession of the Ark of Covenant, resting within the church of Saint Mary of Sion in the ancient city of Axum further adds to its mysteries.

Location and geography

Ethiopia, formally known as Abyssinia, is Africa's 10th largest country with a surface area of approximately 1,1 million km^2. It is located in the north-eastern part of the continent, or the Horn of Africa, lying between 3-18° north and 33-48° east. With the independence of Eritrea on 27 April 1993, Ethiopia became a landlocked country. It is bordered by Kenya in the south, Somalia in the east and south-east, Djibouti in the east, Eritrea in the north and Sudan in the west and north-west. Geographically, the country consists predominantly of rugged mountains and isolated valleys surrounded by lowlands and deserts in the north and east. Two thirds of the country is occupied by the Ethiopian Plateau with an average altitude of 2000 m and approximately 20 peaks over 4000 m. The highest point is Ras Dashen in the Simien Mountains, at 4,620 m it is the 4th highest peak in Africa. The Ethiopian Plateau is divided diagonally from the north-east to the south-west by the Great Rift Valley, which starts at the Red Sea and continues through southern Ethiopia to Mozambique in the south of the African continent. The valley includes the Danakil Depression in the north-east. At 120m below sea level, this desert area is one of the lowest and hottest places on earth. South of the capital Addis Ababa, the Great Rift Valley is notable for a string of lakes including Ziway, Abiata, Langano, Shala, Awasa, Abaya and Chamo.

People, language and religion

Addis Ababa ("new flower"), the capital and largest city of Ethiopia, was founded in 1883 by Emperor Menelik II. It has a population of ca. 2 million people and is located in the heart of the country at an altitude of 2,300 m making it the third highest capital city of the world. The ethnic diversity of Ethiopia's 65 million people is enormous. There are more than 80 ethnic groups (Oromo 40%, Amhara and Tigre 32%, Sidamo 9%, Shankella 6%, Somali 6%, Afar 4%, Gurage 2%, other 1%), speaking 83 languages and over 200 dialects. The official language is Amharic, the other principal spoken languages are Tigrinya, Oromigna, and Guraginya. In general Ethiopian are deeply religious, Ethiopian Orthodox christians (north) and muslims (east/west) each account for ca. 40% of the population while around 20% of the population (south) follow local tribal beliefs (animists). The labour force predominantly consists of (subsistence) agriculture and husbandry (80%), government/services and industry only contribute 10% each. Grim facts are that with ca. 65% of the population living below the poverty line and ca. 10% of the adult population infected with HIV/AIDS, life expectancy at birth is low at 44 years.

Years, months and hours

In 1582 when the Christian world adopted the revised Gregorian calendar, Ethiopia stayed with the Julian calendar. The Ethiopian year is divided into 12 months of 30 days each and a 13th month of five or six days at the end of the year. The first month of the Ethiopian year is September or Meskerem and the New Year start on 11 September in the Gregorian calendar. The Ethiopian leap year follows the same rules as the Gregorian so that the 13th month has six days in a Gregorian leap year. The Ethiopian calendar is seven or eight years behind the Gregorian calendar. In the year we consider to be 2003, it is 1995 in Ethiopia from 1 January to 10 September and 1996 from 11 September to 31 December. The main public holidays are Ethiopian Christmas (Gena) on January 7, Ethiopian Epiphany (Timket) on January 19, Ethiopian New Year (Enketatash), celebrating the end of the raining season on September 11, and the Finding of the True Cross (Meskel) on September 27. Ethiopia also has its own time. An Ethiopian day is divided into 12 hours of daylight, starting at 6:00 and 12 hours of darkness, starting at 18:00. In other words our 9 o'clock in the morning is 3 o'clock in Ethiopian time. Confusingly, urban Ethiopians use both Ethiopian and Western time and calendars. When making appointments in Ethiopia is is necessary to confirm which time and/or calendar system is being used.

Major historical events

The Axumite Empire

The history of Ethiopia and possibly the history of mankind itself started a long time ago. Ethiopia is often referred to as the Cradle of Humankind after the discovery of a 3.5-million-year-old skeleton ("Lucy") a predecessor of modern man. However, the roots of modern Ethiopia lie in the Axumite empire, which was one of the most important and technologically advanced civilisations of its time and was a major force in world trade between the 1st and 7th century AD. The Axumites were literate people who developed the unique language of Ge'ez which is the only written language ever developed in Africa. This ancient language is still used among the Ethiopia Orthodox clergy, whilst the derived languages Amharic and Tigrinya are based on its alphabet. A second important event was the conversion to Christianity as the official state religion during the reign of King Ezanas in the mid-4th century AD. The term Ethiopia or "Aithiopiai" meaning "burnt faces" in ancient Greek, probably originates from this period and was used considerably later to refer to northern highlands of the Horn of Africa and its inhabitants.

Establisment of the 'Solomonic' line

Around 1137 AD a new dynasty, the Zagwe, came to power in Ethiopia's Christian highlands. Staunch Christians, the Zagwe devoted themselves to the construction of new churches and monastries which were often modelled after christian religious edifices in the Holy Land. The most famous are the 11 churches carved in the rocks around their capital city Adefa during the reign of king Lalibela. Nowadays, Adefa is known as Lalibela. The churches are constructed to represent Jerusalem. They are divided in a Northern and Eastern group by a rock-cut channel called Yordannos and connected by narrow and deep passages. Beta Georgis situated apart from the other churches is intricately carved into the shape of a cross. All these churches which originated from the 12th century are still used as places of worship. Despite their architectural achievements there was much resentment against the Zagwe's amongst the people. About 1270 AD Yekuno Amlak, an Amhara nobel, removed the last Zagwe ruler and proclaimed himself king. This new dynasty become known as the 'solomonic' dynasty and its kings claimed descent not only from Aksum but also from King Solomon of Ancient Israel. According to Ethiopian tradition, as written in the 14th century sacred Ethiopian Bible, the "Kebre Negast" or The Glory of Kings, the lineage of Aksumite kings originated with the offspring of an alleged union between King Solomon and the Queen of Sheba, a female ruler of southwest Arabia and/or Aksum. Yakuno Amlak viewed the Zagwe rulers as usurpers without any share in that heritage, so he came to be seen as the legitimate 'restoration' of the Solomonic line. All subsequent Ethiopian kings, including the last ruler, the late Emperor Haile Selassie, traced their legitimacy back to him and, thereby, to Solomon and Sheba

The Battle of Adwa

At the end of the 19th century, much of present-day Ethiopia was united under Emperor Menelik II (1889-1913). The Italians, who were '"awarded" Ethiopia during the Conference of Berlin in 1884-1885, failed to recognize that Menelik had reshaped Ethiopia and, in the process, had created a considerable army. In 1890 the Italians established Eritrea as a colony and in late 1895 they invaded Tigray. The Battle of Adwa on March 1-2 1896, in which Ethiopian troops united by Emperor Menelik II defeated the invading Italian professional army of 20,000 men, was one of the most significant turning points in the history of Ethiopia and modern Africa. It was the first defeat of a European country by a non-white nation at height the "Scramble for Africa" in 1870-1912. It initiated a process of rethinking the Europeans' image of Africans. The victory brought Ethiopia new prestige as well as the recognition by Italy and other major European states of Ethiopia as a sovereign, independent state. In return, Menelik permited the Italians to retain their colony of Eritrea. The victory at Adwa added further pride to Ethiopia's already fiercely nationalistic and proud people. Emperor Tewodros (1855-1889) is said to have ordered the boots of some European visitors to be washed before embarkation on a ship, stating that 'far more precious than jewels is a single drop of Ethiopian soil'. After a second unsuccessful attempt of the Italians to conquer Ethiopia at the start WW II, they were ousted this time out of both Ethiopia and Eritrea and in less than 50 year the country was reunited again. On 2 December 1950, the UN passed a resolution that formally federated Eritrea to Ethiopia.

A negative outcome of the successful campaign in 1896 was that it gave encouragement to isolationist and conservative strains that were deeply rooted in Ethiopian culture. It strengthened the hand of those who were unwilling to adopt technologies imported from the modern west. Both Menelik, and later Emperor Haile Selassie would have to contend with resistance against their efforts to modernize Ethiopia.

Emperor Haile Selassie I (1930-1974)

A cousin of Emperor Menelik II, Emperor Haile Selassie (1930-1974) was born as Tafari Mekonnen on July 23, 1892. Assuming the title Ras Tafari, he named himself regent and heir to the throne. He was crowned king (negus) in 1928 and in November 1930, Ras Tafari was crowned Emperor Haile Selassie I, "Conquering Lion of the Tribe of Judah, Elect of God, and King of Kings of Ethiopia".

Haile Selassie ruled as an absolute monarch, centralizing Ethiopia and instituting a number of reforms (e.g. abolition of slavery) in his attemps to modernize the country. He gained admiration and sympathy of the world with his impassioned plea for aid from the League of Nations after the fascists troops of Benito Mussolini had invaded Ethiopia in 1935. One of his great achievements was that he lobbied the US and Europeans for the reunification, with the blessing of the UN, of Eritrea with Ethiopia. He was skilled in diplomacy and known for his restless efforts against colonisation in Africa. He become a symbol for the independence of the African continent. Unfortunately, he did little to improve the life of ordinary Ethiopians. The lack of infrastructure, increasing poverty, massive famines in the Tigray and Wollo regions in 1973, the cries for land reform by the peasants, fuel crisis and the demand of independence of Eritrea led to civil unrest. Since the 1960s several coups détat were attempted and as a result his rule became increasingly despotic. In 1974 a military junta, the 'Derg', deposed Emperor Haile Selassie and established a socialist state. Whether he died of natural causes or at the hand of Major Mengistu Haile Mariam who supposedly buried him under his toilet remains a mystery. However, 25 years after his death the remains of Ethiopia's late emperor Haile Selassie were finally laid to rest with dignity in the Holy Trinity Cathedral in Addis Ababa.

A curious development was the establishment of a Jamaican messianic movement in the 1930s who named themselves after Haile Selassie's former name Ras Tafari. Rastafarians believe that the only true god is Haile Selassie and Ethiopia is the true Zion. The feeling seemed, however, not mutual, Haile Selassie was a devout Ethiopian Orthdodox christian. Presumably Haile Selassie even dismissed a group of rastafarians who came to Addis Ababa to honour him. However, he did visit Jamaica in 1966 and granted a small group of Jamaican rastaferians some land in Ethiopia. Nowadays there is still a small rastafarian community in Sashemene.

The 'Derg' regime (1974-1991)

Initially the 'Derg' (amharic for 'committee') regime gained mass support from the people as it implemented beneficiary policies for the country, which included land distribution to peasants, nationalising industries and services under public ownership and led Ethiopia into socialism. The popularity did not last long and after internal power struggles the ruthless Major Mengistu Haile Mariam emerged as the undisputed leader. Mengistu adopted a Stalinist policy and declared the Red Terror in 1977 which resulted in imprisoning, torturing and executions of ordinary Ethiopians suspected of supporting opposition groups. Ethiopia entered a new phase of chaos and civil war in Eritrea and Tigray. The 'Derg' regime was finally toppled by a coalition of rebel forces, the Ethiopian People's Revolutionary Democratic Front (EPRDF), in 1991.

People's Democratic Republic of Ethiopia (1991-present)

Tensions, rooted from the division of historic Ethiopia into two parts by the Italian colonizers, culminated in a long civil war (1961-1991) and the eventual secession of Eritrea as an independent state in 1993. The current presidents, Isayas Afewerki of Eritrea linked with the Eritrean People's Liberation Front (EPLF) and Meles Zenawi of Ethiopia linked with the Tigray People's Liberation Front (TPLF), were brothers-in-arms during the struggle that successfully overthrew the bloody communistic 'Derg' regime. The EPLF and TPLF joined forces and initially aimed to remove the 'Derg' from the Eritrean and Tigray regions. Major defeats of the Ethiopian Army in March 1988 lead to the collapse of governmental control in the two regions. The TPLFs victories in Tigray ultimately led to the expansion of the insurgency into the rest of the country and ended the 'Derg' regime in 1991. A constitution was adopted in 1994 and Ethiopia's first multi-party elections were held in 1995. Nevertheless, hostilities started again in May 1999 between the two countries. Ironically the dispute was over the Italian drawn border between the countries. The two and a half year border war with Eritrea, costed an estimated 80.000 lives and ended with a 'shaky' peace treaty on 12 December 2000.

The Ark of Covenant and Lake Tana

Although the fundamentals of the Ethiopian Orthodox Church are indisputably Christian, the rituals are infused with all sorts of archaic Jewish influences. The most intriguing aspect is its relationship with the Ark of Convenant. The most holy item in every Ethiopian Orthodox Church is the tabot (a replica of the Ark or one of the Tablets of Law). Only during Timket (Ethiopian epiphany) are the tabots removed from the holy of holiest and paraded before the people. The original Ark of Covenant is claimed to be in Ethiopian possession, resting within the church of Saint Mary of Sion in the ancient city of Axum.

Whether the Ark of Convenant is really in Ethiopia is doubted, however, Graham Hancock in his book, " The Sign and the Seal" (1992, William Heinemann Ltd), investigated the Ethiopian claim to the Ark and constructed a plausible sequence of events to support it.

The Ark was the very core of Judaism until its disappearance from Jerusalem led to the reforms of Josiah (640-600 BC). According to citations in the bible, the Ark was probably resting in the first temple at least until 701 BC. The Ark was last mentioned in 620 BC, where in two passages King Josiah mentions the absence of the Ark in the temple. The Ark was most likely removed from the temple by Levitical priests during the reign of King Manasseh (687-642 BC). Manasseh was a notorious sinner who was distancing himself from Judaism and installed a pagan idol in the temple. Historical evidence suggests that during the reign of Manasseh, Jews, including Levitical priests, escaping desecration and prosecution founded a colony on Elephantine Island in Egypt. It is strongly possible that these priests took the Ark of Covenant with them. In 650 BC a temple matching precisely the dimensions of Solomon's temple in Jerusalem was built on Elephantine Island, its ruins still visible at present. According to Deuteronomic Law the building of temples outside Jerusalem was strictly forbidden so only extreme circumstances would sanction such an undertaking. Furthermore, a replica temple would be useless at that point in time without serving its primary function, a resting place for the Ark. Ancient documents (Elephantine papyri) confirm the existence of a Jewish temple at that time. In 410 BC, however, the Jewish temple on Elephantine Island was destroyed. At that time, the Ark could not be brought back to Israel because of the Babylonian conquest of Jerusalem in 587-86 BC. Ancient links undoubtedly existed between Israel and Ethiopia. The Ethiopian tribe, known as the Qement appeared to have Jewish origins, and preserved belief and practices (particularly food laws) dating back to the Law of Moses. There is a strong connection between Moses (during his princely upbringing in Egypt) and Ethiopia. After his conquest of the Ethiopian city of Saba, he received an offer of marriage from the Ethiopian king's daughter. These ancient connections and possibly line of Mosaic decedents in Ethiopia would have provided an ideal place of refuge for the Jews and Ark after the destruction of the temple on Elephantine Island. The Ark was apparently brought to Tana Kirkos, a small island in Lake Tana, considered holy by the Ethiopians. Here the Ark rested for 800 years until, King Ezanas, after his conversion to Christianity in the 4th century AD, moved the Ark from Tana Kirkos to Axum, where it still rests today. The monks of Tana Kirkos believe that they are living on the island where the Ark rested and where Levitical-style blood sacrifices were performed. The stone altar that was used for these sacrificial practices is still present today.

1
CHAPTER

The Lake, The Fish, The Project and the Thesis

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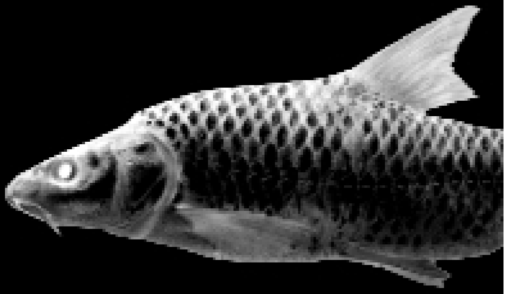
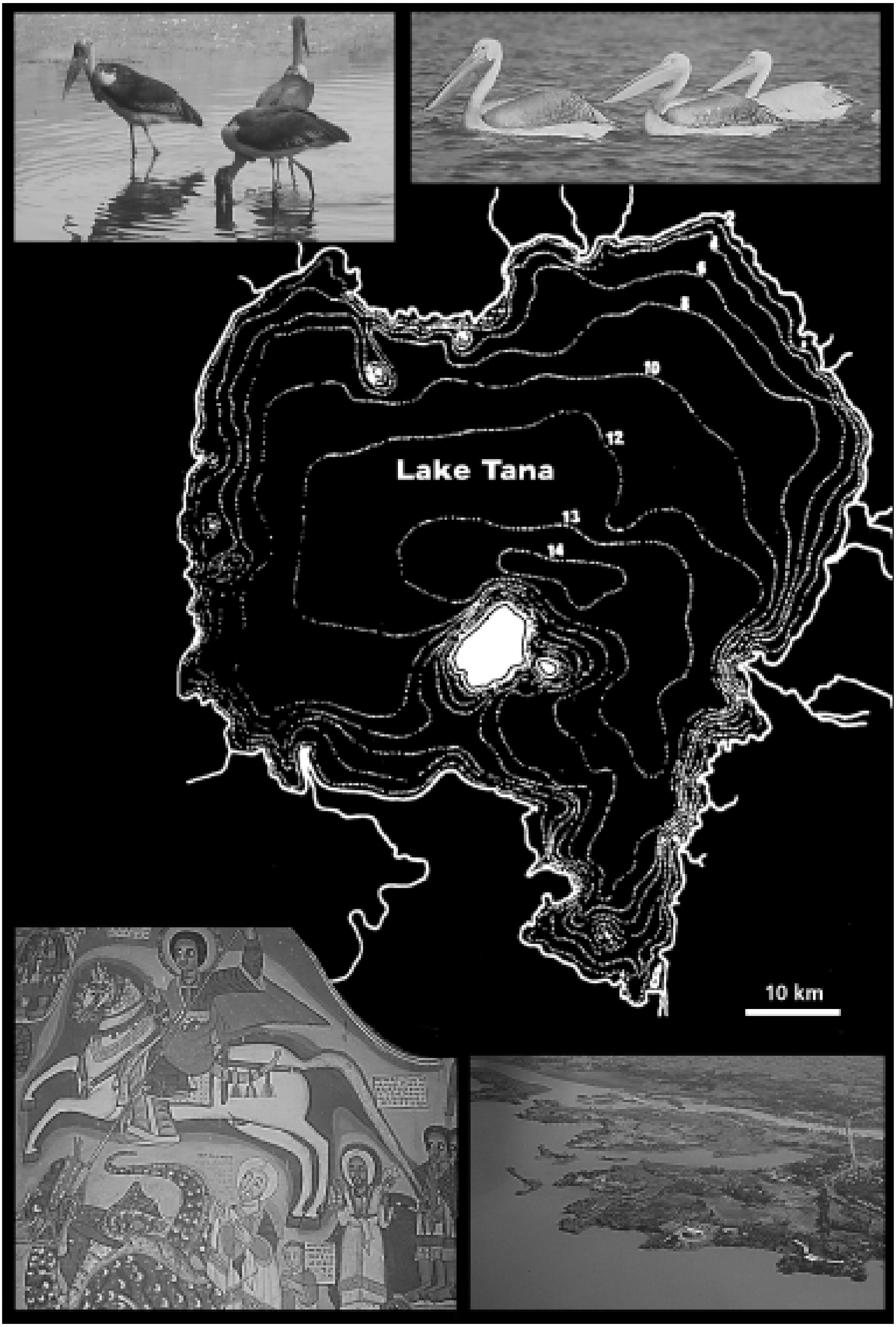



10
12
Lake Tana
13
14
10 km

The Lake

Location and geology

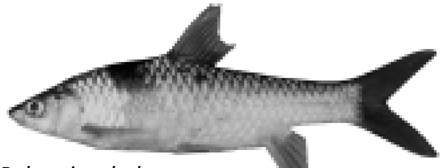
Four major river systems originate in the Ethiopian highlands. The Awash rises in Shoa and flows northwards following the Great Rift Valley where it disappears in the desert near the Djibouti border. The Omo begins in Kafa and drains into Lake Turkana in the south, on the border with Kenya. The Wabe Shebele originates in the Bale Mountains and flows in a south-eastern direction towards Somalia. The most impressive river, however, is the Nile which flows for 4750 km from its source (Lake Tana) to the shores of the Mediterranean. The Nile River is principally fed by two great rivers, the White Nile and the Blue Nile, which fuse at Khartoum, Sudan's capital city. Over the last few centuries several European adventurers have travelled through Ethiopia in search for the source of the Nile. As early as the 1600s Portuguese priests established that the source of the Blue Nile was Lake Tana.

Lake Tana is located at an altitude of 1830 m, in the north-western highlands of Ethiopia, 500 km north of its capital Addis Ababa. The oligo-mesotrophic (chlorophyll *a*, average 6.4 $\mu g \cdot l^{-1}$; Dejen et al. 2003a), shallow lake (average depth 8 m, maximum depth 14 m; Fig. 1) covers an area of c. 3,050 km^2 and is by far Ethiopia’s largest lake, containing half the country’s freshwater supply. Several large permanent rivers feed into the lake (Gelgel Abay River, Gelda River, Gumara River, Rib River) as well as many small seasonal rivers. High waterfalls (40 m) at Tissisat ('smoking waters'), 30 km downstream from the Blue Nile outflow, effectively isolate the lake’s ichtyofauna from the lower Nile basin.

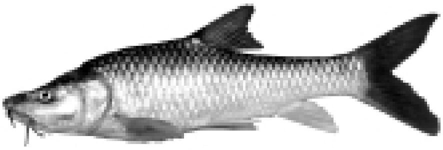
Lake Tana is situated within the Tana basin which covers an area of 16,500 km^2. The Tana basin occurs perched within a large dome (ca. 1000 km) uplifted in the Ethiopian plateau. The time of uplift is uncertain, Late Miocene (Yemane et al 1985) and Quartenary episodes (Chorowicz et al. 1998) are indicated. Subsequent fracturing and subsidence of the dome generated the Tana basin. Also the age of Lake Tana within the basin is uncertain. The lake might owe its present form to damming by a 50-km-long Quartenary basalt flow, which filled the exit channel of the Blue Nile river to a possible depth of 100m. The age of this lava flow is estimated to be some 10,000 B.P. (Grabham and Black 1925). However, according to Chorowicz et al. (1998) the morphology of Lake Tana is not consonant with a lava-dammed, flooded river valley. In his view the present-day morphology expresses a central focus of subsidence, despite fault reactivation and headward down cutting of the Blue Nile. Whether or not the lake was present before or after the blocking of the Nile outlet, the lava most likely created the waterfalls at Tissisat, isolating Lake Tana from the lower Nile drainage. Interestingly, deposits of lignitiferous sediments suggest that the Tana basin was occupied at least partly by a lake around 8 Ma ago, but it is unknown if that lake survived the regional uplift (Chorowicz et al. 1998) during Late Miocene, Quartenary episodes.

The age and history of Lake Tana will hopefully be further clarified as of October 2003, when the University of Wales Aberystwyth will initiate their project entitled “Seismic Survey of Lake Tana, Northern Ethiopia, and the Paleaohydrology of the Blue Nile” (http://www.aber.ca.uk.quartenary).

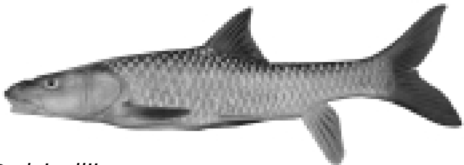
B. acutirostris



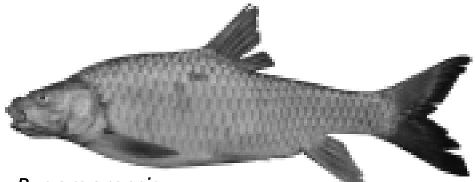
B. brevicephalus



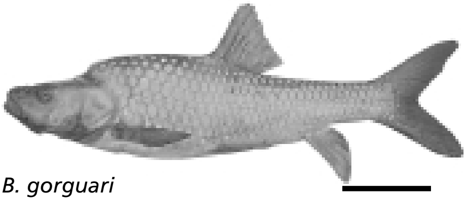
B. crassibarbis



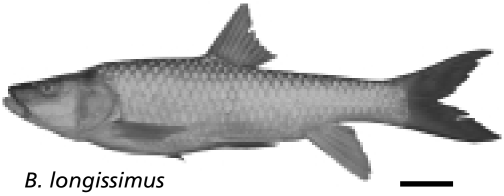
B. dainellii



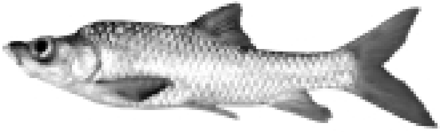
B. gorgorensis



B. gorguari

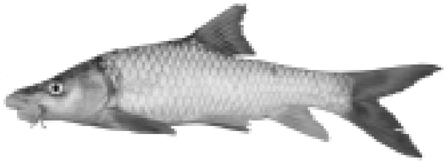


B. longissimus

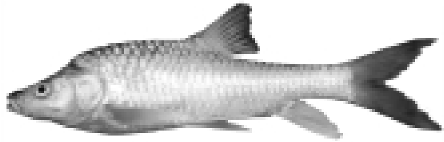


B. macrophtalmus

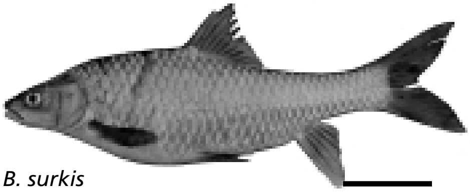
B. megastoma



B. nedgia



B. platydorsus



B. surkis

B. truttiformis

B. tsanensis

Figure 2. The *Barbus* species flock of Lake Tana. Bars indicate 5 cm.

The Fish

Ichthyofauna

In Lake Tana, the families Cichlidae and Clariidae are represented by only one species each, *Oreochromis niloticus* and *Clarias gariepinus*, respectively. Several individuals of the riverine *Nemacheilus abyssinicus* (Balitoridae) (Dgebuadze et al. 1994) were found in the littoral areas of Lake Tana after its first recording in 1993 (Nagelkerke 1997). The largest fish family in the lake are the Cyprinidae, represented by three genera, *Varicorhinus*, *Garra* and *Barbus*. *Varicorhinus*, is represented by a single species *V. beso*. The genus *Garra* is represented by one species in Lake Tana, *G. dembecha*. However, in Lake Tana the bottom-dwelling *Garra* might form a mini-species flock with at least two endemic species "*G. microstoma*" and "*G. tana*" and maybe even more (Getahun 2000). The large (max. 100cm standard length), hexaploid *Barbus* of Lake Tana have been revised several times. Banister (1973) lumped 50 nominal species and subspecies of east-African barbs into one species, *Barbus intermedius*. The large barbs of Lake Tana were considered as belonging to one subspecies *Barbus intermedius intermedius* Rüppell, 1836. The latest revision of Lake Tana's *Barbus* (Fig. 2; Nagelkerke and Sibbing 2000) resulted in the rehabilitation of six species lumped by Bannister as *B. intermedius* intermedius and 9 new species. Three small diploid *Barbus* species (<10 cm standard length) are present in Lake Tana; *B. humilis* (including the previous *B. trispilopleura*, Dejen et al. 2002), *B. pleurogramma* and the recently discovered *B. tanapelagius* (de Graaf et al. 2000).

Development of research

Most work on Lake Tana's ichtyofauna, especially during the 19th and beginning of the 20th century concentrated on the highly intriguing species flock of large barbs and its complicated taxonomy (Rüppell 1836; Boulenger 1911; Bini 1940; Banister 1973). In 1986 the Lake Tana Fisheries Resources Development Program (LTFRDP) started by the EU and ISE-Urk, introducing modern fishing gear and motorised boats. Traditionally, the fisheries in Lake Tana consisted of a subsistence reed boat fishery. The fishermen, limited in their mobility, only had access to the shore areas, using locally made fish traps, hooks and small gillnets (15-20m, 8-10 cm stretched mesh), catching mainly tilapia, *Oreochromis niloticus*. The LTFRDP created new opportunities for the fishermen, extending their fishing area from the shore to deeper, offshore waters and, more importantly, to distant river mouths. The catches of the motorised fisheries roughly consisted of one third *Barbus* species, one third *Oreochromis niloticus* (Cichlidae) and one third *Clarias gariepinus* (Clariidae) and increased rapidly from 39 metric ton 1987 to 323 metric ton in 1993 (LFDP 1997). In 1988 a research project was initiated by the Ethiopian

Ministry of Agriculture, the Ethiopian Orthodox Church and two Dutch NGOs (ISE-Urk and ICCO-Zeist) to characterize the biological and dynamic aspects of the Lake Tana fish resources, to define the current state of fisheries, to identify its impact on the fish community and to assess the options for management of the fish stocks (Wudneh 1998). At the start of the fisheries research project in 1990 the morphological diversity of the barbs in Lake Tana created a stock identification problem. At that time the large barbs were considered as one species, *Barbus intermedius intermedius* (Banister 1973). Cumulative evidence from the analysis of morphology, food niches, spawning patterns and from genome analysis (Dixon et al. 1994, 1996) was so convincing that the 14 *Barbus* morphs were assigned as good biological species, composing a unique species flock (Nagelkerke 1997; Sibbing et al. 1998; Nagelkerke and Sibbing 2000).

The piscivorous Barbus project

The family Cyprinidae is the most species-rich (>2000 species; Nelson, 1994) and widespread of all fresh water fish families and even the largest family among all vertebrates. The largest cyprinid genus, *Barbus* (>800 species) is a polyphyletic assemblage, including diploid, tetraploid and hexaploid species ranging in size from a few cm to over a meter (Howes 1991; Tsigenopoulos et al.,2002). However, Africa's ubiquitous large hexaploid *Barbus* are considered to be of a single recent origin forming a monophyletic group and are classified in the subgenus *Labeobarbus* (Berrebi 1995; Tsigenopoulos et al. 2002 and references therein).

Despite the overwelming abundance of cyprinid fishes throughout the world's fresh water systems, the *Barbus* species of Lake Tana form, as far as we know, the only remaining intact species flock of large cyprinid fishes, since the one in Lake Lanao in the Philippines, has practically disappeared due to anthropogenic activities (Kornfield and Carpenter 1984). One of the most intriguing aspects of Lake Tana's endemic *Barbus* species flock is the large number of piscivores (8 out of 15). Cyprinid fishes do not seem well designed for piscivory, they lack teeth in the oral jaws, have a small slit-shaped pharyngeal cavity and all lack a stomach with low pH for digesting large prey. Cyprinid fish, instead, have their fifth branchial arches modified into powerful pharyngeal jaws and also possess a palatal and sublingual organ lining their pharynx (Sibbing, 1991). The vagal brainlobe in some benthic species is multi-laminary organized like the cortex cerebri of mammals. Both the pharyngeal roof and floor are mapped somatotopically on this brain center for highly localised gustatory control in feeding (Finge, 1981; Lamb and Finger 1995). Using such refined systems, small edible particles are sorted from the mud, thus enabling cyprinids to feed on (micro) benthos (Sibbing 1988). The evolutionary success of cyprinid fish (Howes 1991) is largely attributed to this unprecedented sorting ability as well as their powerful and diversified pharyngeal jaw system (Sibbing 1991). Like in other cyprinid fish, most of Africa's large *Barbus* are benthivorous species. Why then is piscivory, which is rare among cyprinids, so common in Lake Tana's *Barbus* species flock?

Aims and key questions

The three main aims of the project were:

A. To determine the ecological role of each piscivorous *Barbus* species in Lake Tana community.
B. To reconstruct the evolution of piscivory within the *Barbus* species flock using morphological, ecological, behavioural and molecular data.
C. To provide a solid scientific base of fish and fisheries related data required for the development of rational management measures to be taken in order to ensure the sustainability of the fisheries and the unique *Barbus* diversity in Lake Tana.

ad A. Ecology

(1) Along which dimensions (trophic, spatial, temporal) and to what extent are resources partitioned among the piscivorous (and non-piscivorous) *Barbus* species?
(2) At what age and/or size does each species become piscivorous?
(3) How well are these eight fish-eating *Barbus* species actually adapted to piscivory (predation techniques, performance, prey selection), especially compared with piscivores from other fish families?

ad B. Evolution

(1) How did reproductive isolation arise and how was it maintained?
(2) Which process(es) drove speciation of *Barbus* in Lake Tana?
(3) Do Lake Tana's *Barbus* species form a monophyletic group?
(4) What is the age of Lake Tana's *Barbus* species flock?
(5) Does piscivory have one or multiple origins?

ad C. Exploitation

(1) How does size at maturity of the *Barbus* species compare to the size selectivity of the gillnets used by Lake Tana’s commercial fishery?
(2) What has been the impact of the environment and fisheries activities on the development of the *Barbus* stocks during the 1990s?
(3) What are the characteristics of the commercial gillnet fishery and how did it develop since its introduction in 1986?
(4) What is the interaction between the commercial gillnet fishery and the life-history of the three main stocks *C. gariepinus*, *O. niloticus* and *Barbus*?
(5) How can Lake Tana's unique biodiversity be preserved while maintaining a healthy and sustainable fisheries?

The Thesis

Thesis outline

The subsequent chapters and their conclusions will be discussed within the framework of the piscivorous *Barbus* project. In Chapter 2 an overview is given of the major questions on the evolution and exploitation of Lake Tana's *Barbus* species flock. Because this chapter was written at the start of the project it shows how the project itself "evolved". During the past years, based on results and new scientific and social questions, the original (1998) aims have at some points been adjusted (e.g. larger contribution to fisheries related research and inclusion of the non-piscivorous barbs in most chapters) and new questions have been added (phylogenetic analyses using cytochrome *b*). The whole project was done in cooperation with Eshete Dejen, who will defend his thesis also on 2 September 2003.

Ecology

Chapter 3: Barbus tanapelagius, a new abundant small barb species

Previously, it was assumed (Nagelkerke 1997) that all piscivores preyed on the same small barb species, *Barbus humilis*. This view about the evolution of the eight endemic piscivorous *Barbus* species required, already early in the project reconsideration, as the discovery of the highly abundant *B. tanapelagius* created a more complex scenario including several prey species. *Barbus tanapelagius* differs clearly in morphology from the other two small, diploid *Barbus* species known from Lake Tana, *B. humilis* Boulenger 1902 and *B. pleurogramma* Boulenger 1902. Conspicuous differences are its elongated body, large eye diameter, prominent and hooked lower jaw contour and silvery colouration. *Barbus tanapelagius* also differs ecologically from the other small barbs, by its pelagic, strictly zooplanktivorous feeding and its occurrence near the surface in the deeper, offshore waters. The other small *Barbus* species are largely benthic feeders (Dejen et al. 2003b). *Barbus humilis* is dominant in the shallow littoral areas of the lake, while *B. pleurogramma* only occurs among the submerged vegetation at the fringes of the lake.

Chapter 4: Ecological segregation of the piscivorous Barbus

Ecological segregation was studied along three resource dimensions; trophic, spatial and temporal.

Due to fact that these cyprinid piscivores masticate prey fish with their pharyngeal jaws, no whole or partial prey fish are found in the digestive tract for identification. Information about the prey consumed (species and size) was painstakingly reconstructed from the skeletal remains of prey fish in the predators' digestive tract. Spatial distribution patterns were studied in the Bahar Dar Gulf of Lake Tana. Monthly from October 1999 to October 2001, 18 stations (six habitats; littoral rock, littoral sand, sublittoral bottom/surface, and pelagic bottom/surface) were sampled overnight using multimesh gillnets. Three of the pelagic stations (bottom and surface) were also sampled during the day to determine diurnal vertical migration patterns. Time of active feeding was determined in outdoor basins by comparing predation rates during different times of the day. To avoid bias due to ontogenetic (diet) shifts only large (200-400mm FL), adult individuals were used to determine ecological segregation.

Overall the 15 *Barbus* species appear to be well segregated along the three resource dimensions and could roughly be divided into two communities; eight dark coloured littoral species and seven silvery coloured sublittoral-pelagic species. The littoral community consists of the piscivorous *B. dainellii*, *B. gorguari*, *B. acutirostris* and *B. longissimus*, insectivorous *B. nedgia*, molluscivorous/omnivorous *B. intermedius* "shore-complex" (SC), macrophytivorous *B. surkis*, and molluscivorous (bivalves) *B. gorgorensis*. *Barbus dainellii* is restricted to the rocky areas and feeds mainly on *Garra*. *Barbus gorguari* occurs over rock and sandy substrate at the border of the submerged vegetation and the lake, feeding mainly on *Garra*. *Barbus gorguari* is the only species with considerable amounts of *B. pleurogramma* which occurs only among the vegetation in the floodplains at the edge of the lake, in its diet.

Barbus acutirostris is the most abundant piscivore, mainly over sandy bottom feeding on *B. humilis*. *Barbus longissimus* is the only species with a clear habitat shift, smaller (200-300mm FL) individuals are restricted to the littoral rocky areas, larger (300-400 mm FL) are more common near the surface in the sublittoral zones. *Barbus longissimus* feeds high in the water column, compared to the other three littoral species, its diet consisting of *B. humilis* and *B. tanapelagius*. The sublittoral-pelagic community near the surface consists of the piscivores *B. truttiformis*, *B. megastoma*, *B. macrophtalmus* and the zooplanktivorous *B. brevicephalus*. *Barbus truttiformis* is restricted to the pelagic zone and feeds on *B. tanapelagius*. *Barbus macrophtalmus* occurs both in sublittoral and pelagic areas and its diet is a mixture of *B. humilis* and *B. tanapelagius*. In contrast with *B. truttiformis* and *B. megastoma* which are crepuscular hunters, *B. macrophtalmus* is most active at night. Smaller (200-300mm FL) *B. megastoma* have a wider distribution occurring throughout the (sub)littoral and pelagic areas, larger (300-400mm FL) individuals are restricted to the pelagic zone. The diet consists predominantly of *B. tanapelagius*. The benthic community in the sublittoral-pelagic area consists of the piscivorous *B. platydorsus*, the detrivorous *B. crassibarbis* and the insectivorous *B. tsanensis*. The diet of the bottom-dwelling *B. platydorsus* consists of *Garra* and *B. humilis*. No diurnal vertical migration of both predators and prey, takes place between the surface and the bottom in the sublittoral-pelagic areas.

Six of the eight piscivorous *Barbus* species become piscivores at 10-15 cm FL (age 1^+), *B. macrophtalmus* and *B. platydorsus* have a significantly longer ontogenetic trajectory and do not shift towards piscivory until reaching 20 cm FL (age 3^+). The piscivorous *Barbus* follow similar ontogenetic trajectories shifting from zooplankton to insect larvae (*Chaoborus*) and adult insects (especially *B. megastoma*) to fish.

The cichlids of Lake Victoria and the barbs of Lake Tana have some important analogous morphological innovations (decoupling of the maxillary and premaxillary and well-developed pharyngeal jaws) resulting in potentially large diversification of trophic structures and high potential for trophic radiation. A remarkable fact is that although the Lake Victoria haplochromine species flock has a higher diversity per trophic group, the ratio of the species per trophic group (# piscivores: # macrophytivores: # molluscivores: # zooplanktivores: # insectivores: # detrivores) does not differ significantly in both species flocks. Due to the lack of oral teeth some common trophic specializations (aufwuchs feeders, scale scrapers, finbiters) among Lake Victoria's cichlids are lacking in Lake Tana's barbs. Nevertheless, in the absence of modern piscivorous percoid fish as competitors, the cyprinids of Lake Tana used their potential for trophic diversification to the fullest, including the unexpected specialization of piscivory.

Chapter 5: Predation techniques, feeding performance and prey selection

One of the most intriguing aspects of the *Barbus* species flock is the abundance of piscivorous species, a trophic specialization uncommon among cyprinids in general. One of the prominent questions was to determine how well and differently adapted Lake Tana's *Barbus* species are in exploiting fish as prey. A combination of field observations, experiments in indoor tanks and outdoor pools, high-speed recordings of prey capture events and functional morphology was used to determine whether pre- or postcapture factors mediate the observed prey selection (species and size) in the field, which predation techniques are used by the different piscivorous species and why.

Based on an objective as possible a comparison, Lake Tana's piscivorous *Barbus* performed relatively "poor", compared to piscivores from other fish families. The barbs are only able to successfully capture relative small prey (prey-to-predator size ratio PPR, average 0.15 and maximum 0.25) compared to other non-cyprinid freshwater and marine piscivores. Prey size selection is not limited by gape sizes of the feeding apparatus [postcapture factors], but is mediated by differential size-related capture success [precapture factors].

Their limited average and maximum prey size compared with other piscivores is most likely caused by the significantly smaller volume of their oro-pharyngeal cavity, hence restricting the diameter of the ingested water flow generated during suction feeding. The voluminous palatal organ and sublingual organ lining their pharynx, a key innovation in the evolutionary success of cyprinid fishes, came at a cost, of greatly reducing their competitive abilities as piscivores.

Barbs in Lake Tana lack piscivorous competitors, rendering the piscivorous *Barbus* by far the "best" and apparently highly successful. They have adapted to all available macro-habitats, using different techniques (e.g. ambush hunting using velocity suction with protrusion [open water; *B. macrophtalmus*, *B. megastoma*; littoral zone, *B. gorguari*] or volume suction [littoral areas with high structural complexity; *B. dainellii*] and pursuit hunting [open water; *B. longissimus*, *B. truttiformis*]), a unique scenario for barbs.

Evolution

Chapter 6: The role of homing in riverine spawning Barbus

Migration, followed by temporal and spatial reproductive segregation in the upstream reaches of two inflowing rivers (Gumara and Rib) was studied for the endemic *Barbus* species flock of Lake Tana (Ethiopia) over the 1999 and 2000 spawning seasons. Six *Barbus* species where identified that migrate 30-40 km upstream Gumara River in August-October at the end of the rainy season. Spawning occurred in fast flowing, shallow well-oxygenated gravelbeds of four small tributaries of Gumara river, and possibly in the main channel. Eight *Barbus* species were absent from the rivers, or found only incidentally, thus segregating at a larger scale. These missing species might possibly migrate and spawn in upstream areas of other inflowing rivers or maybe even within the lake itself (Chapter 8).

Long distance migration and species specific spawning sites suggest homing, which is common among cyprinid fishes (Mills 1991; Smith 1991; Lucas and Baras 2001). Homing facilitates reproductive isolation (Hendry et al. 2000; Hendry 2001; Quinn et al. 2001) and might have accelerated speciation among these riverine spawning *Barbus* species (Chapter 7). A fine-tuning between homing and gonad development is suggested since females reached spawning maturity only on the actual spawning grounds. This study provided convincing evidence for reproductive isolation and therefore further justified the species status of Lake Tana's large *Barbus*.

Chapter 7: Early morphological divergence and phenotypic plasticity among riverine spawning Barbus

The large, hexaploid African barbs are known for their extensive intra-specific morphological variation, creating a confusing and complicated taxonomical history (Howes 1991). Mina et al. (1996ab, 2001) stated that based on ontogenetic changes in skull characters, the different "morphs" in Lake Tana are best described as "intermedius" undergoing morphological divergence late (>20 cm, age 4-5 years) in ontogeny and that these morphological transformations are triggered by the environment/habitat (phenotypic plasticity; nurture). In 'common garden' experiments we raised progeny of the riverine spawning benthivorous *B. tsanensis* ("intermedius") and the piscivorous *B. truttiformis* and *B. megastoma* all on the same commercial pellet food under similar environmental conditions to test if morphological differentiation would occur (nature). Separately, to study the range of plasticity in the morphological development, part of the juvenile *B. truttiformis* and *B. megastoma* reared in the laboratory were subjected to different diets (commercial pellets or live fish) for a 10-month period.

The results clearly show that the previously published (Nagelkerke and Sibbing 2000) inter-specific morphological differences have a strong genetic base (nature) and morphological divergence occurs already very early in ontogeny (<40 mm SL). The results further support the view that among the riverine spawning *Barbus* species (including *B. tsanensis*, *B. truttiformis*, *B. megastoma*) reproductive isolation is well established and that speciation among these barbs is in an advanced stage. In addition, these barbs retained some small range of intra-specific phenotypic plasticity, an important option for an organism to increase its survival in a heterogeneous or novel environment. Several characters showed distinct phenotypic plasticity, their changes in morphology were clearly induced by the differences in diet.

One of the prerequisites for an organism to successfully invade a new food niche is flexibility in its trophic behaviour and options, partly reflected in its morphological structures (phenotypic plasticity). Whether successful invasion of a new food niche will result in speciation or resource polymorphism is largely dependent on the size of the genetic neighbourhood. Like in many other cyprinid fishes, reproductive homing (Chapter 6) is apparently common among Lake Tana's riverine spawning *Barbus* species. The different species are at present spatially and temporally segregated on the upstream spawning areas (Nagelkerke and Sibbing 1996; Chapter 6). In migrating salmonids, homing reinforces the development of reproductive isolation (sub-populations), facilitating the divergence of other (morphological) traits. Reproductive isolation can arise very quickly (less than 13 generations or 56 years) as has been shown with sockeye salmon (Hendry et al. 2000; Hendry 2001; Quinn et al. 2001). Among the riverine spawning *Barbus* species the genetic fixation of morphological adaptations in trophic structures was probably enhanced and facilitated by philopatry, i.e. homing to natal streams in the ancestral founding population. Prior to the geological formation of Lake Tana, as in salmon, the ancestral riverine barb population was most likely divided in sub-populations due to this reproductive strategy. After the formation of the lake, many new niches became available to the ancestral, riverine spawning barb population. Since random mating did not occur within the ancestral founding population, this might well have resulted in small genetic neighbourhood sizes necessary for sympatric speciation and genetic fixation of trophic morphological adaptations, related to the newly invaded niches in the lacustrine habitat.

Chapter 8: A new reproductive strategy, lacustrine spawning.

The development of assortative mating in Lake Tana's *Barbus* species flock is a key issue when reconstructing how the ancestral *Barbus* population differentiated to occupy separate ecological niches and ultimately became reproductively isolated. As was proposed in Chapter 6 en 7, reproductive homing probably facilitated and enhanced reproductive isolation during adaptive radiation among the riverine spawning *Barbus* species.

To determine when and where the missing *Barbus* species spawned, changes in the gonado-somatic index and abundance of the different *Barbus* species in the mouths of four major affluent rivers was monitored (bi-)monthly during 1999 and 2000. Riverine spawning is characteristic for six (maybe seven) of Lake Tana's 15 contemporary *Barbus* species. Spatial segregation among affluent rivers did not occur among them but significant temporal segregation occurred in aggregating in the river mouths and migrating towards the upstream spawning areas during the breeding season (June-October).

Among the eight missing *Barbus* species peak gonad development occurred generally in the same period as in the riverine spawners. However, these *Barbus* species did not aggregate in the river mouths during the breeding period and were absent from the upstream spawning areas. A derived, novel strategy, lacustrine spawning is hypothesized for these eight *Barbus* species. These species seem to have successfully adapted to the lacustrine environment and probably complete their whole life-history, including reproduction, inside the lake. This hypothesis is further supported by accidental observations of running female fish in the littoral zones far away from any of the affluent rivers.

Lake spawning within the *Barbus* species flock probably evolved following adaptation to divergent ecological environments, i.e. adaptation to the shore areas in the newly formed lake. Most lacustrine spawners are confined to the littoral zone (<10% of the lake surface). Spawning of these specialised *Barbus* species within or near their rocky habitat instead of on faraway rivers, might provide their offspring with suitable habitat, reducing the risk to be forced to live in sub-optimal habitat/trophic conditions. Another characteristic of the littoral-dwelling lacustrine spawning *Barbus* is their body colouration which varies drastically from bright golden yellowish, to green and very dark brown or black. All this is in sharp contrast to the silvery coloured, riverine spawning *Barbus* species that are widely distributed, occurring throughout the sub-littoral and pelagic zones (>90% of the lake surface).

Early ecological divergence, adaptation to macro-habitats, appears to be common in radiations of lacustrine fish groups; e.g. among (1) rock and sand-dwelling clades in Lake Victoria's (Nagl et al. 2000), Lake Malawi's (Danley and Kocher 2001) and Lake Tanganyika's cichlids (Sturmbauer 1998); in the (2) sympatric divergence of benthic and pelagic forms among west African ciclids in crater lakes (Schliewen et al. 1994, 2001; and (3) in the divergence of sticklebacks in benthic and limnetic forms in several North American lakes (Schluter and McPhail 1993). Macro-habitat adaptations of the Lake Tana barbs resulted in a similar divergence of morphological and behavioural characteristics including body coloration, reproductive behaviour and habitat preference (cf. Danley and Kocher, 2001 and references therein).

Further speciation among the littoral-dwelling lacustrine spawning *Barbus* was most likely driven by trophic competition and trophic specialisation within the littoral habitat from a common lacustrine spawning ancestor, probably *B. intermedius* "shore-complex" (SC). The sequence of primary macro-habitat diversification followed by secondary trophic diversification within each macro-habitat has been shown to occur in several lacustrine fish groups; East-African cichlids (Seehausen 1996; Sturmbauer 1998; Danley and Kocher 2001), arctic charr (Snorrason et al. 1989). In such a scenario, trophic specializations like piscivory (for example *B. dainellii*, *B. gorguari*, littoral zone, lacustrine spawners and *B. truttiformis*, *B. macrophtalmus*, offshore pelagic zone, riverine spawners) probably have separate, independent origins within the *Barbus* species flock. Such patterns of parallel trophic morphological evolution in different macro-habitats also occurred among Lake Tanganyika cichlids (Rüber et al. 1999).

Chapter 9: Age and origin of Lake Tana's Barbus species flock

In this chapter we tried to answer a number of prominent questions regarding the cyprinids of Lake Tana: (1) to establish the origin of Lake Tana *Barbus* species, including the recently discovered *B. tanapelagius* (de Graaf et al. 2003), (2) to investigate whether the Lake Tana's large *Barbus* form a monophyletic group, (3) whether historical relationships among species of the Lake Tana *Barbus* species flock can be determined, and (4) to dicuss the age of Lake Tana's species flock. Genetic diversity of Lake Tana's large and small *Barbus* species and other *Barbus* species collected in various lakes and rivers was investigated using cytochrome *b* sequences.

The small, pelagic *B. tanapelagius* apparently recently evolved from the benthic *B. humilis*. Such early ecological splits, i.e. sympatric divergence of benthic and pelagic forms, appear to be common in radiations of lacustrine fish groups (see above Chapter 8), e.g. the existence of specific littoral and sublittoral-pelagic communities among the large *Barbus* species of Lake Tana (Chapter 4).

Barbus pleurogramma and the populations of *B. paludinosus* collected in Ethiopia show high sequence divergence and form distinct clades with a strong, probably geographical, signal. *Barbus pleurogramma* was described by Boulenger in 1902 based on specimens from Lake Tana. *Barbus* *pleurogramma* is characterized by a strongly serrated dorsal spine, and is probably closely related (synonymus?) to the widespread African *B. paludinosus*.

Genetic divergence among the various species of large *Barbus* in Lake Tana is very low, the failure to distinguish species based on cytochrome b sequences is due to the recent origin of the species flock. The haplotypes found in Lake Tana are unique but none are diagnostic for a particular species. The present data supports nor rejects a monophyletic origin of Lake Tana's *Barbus* species flock. The haplotypic variation falls into two distinct Lineages (1 and 2), Tana haplotypes seem to form a subset of the total variation in Lineage 1, adding evidence that the Lake Tana species flock originates from a limited stock of ancestral riverine *B. intermedius*. With a maximum sequence divergence of approximately 1% in Lineage 1 and an estimated sequence divergence of 1.3 to 3.3% per million years, the Tana species flock seems not older than 770,000 to 300,000 years. Other studies have shown that the age of divergence of mitochondrial lineages can be a poor estimate for the age of a species flock in recently formed (refilled) lakes. In Lake Victoria haplochromine cichlids, for instance, lineage diversification at 100,000 years ago (Nagle et al. 2000; Verheyen et al. 2003) is clearly in excess of the age of the (current) lake, estimated at approximately 15,000 years old (Johnson et al. 1996). Circumstantial evidence (geological and climate changes) suggests that the fast radiation of ecomorphological diversity within Lake Tana's *Barbus* species flock occurred as recent as 10,000-25,000 years ago.

Exploitation

Chapter 10: Decline Barbus stocks

A monthly experimental trawl program conducted in 1991-1993 by Wudneh (1998) was repeated in 1999-2001, sampling 12 stations distributed over three habitats differing in depth and distance to the shore. The aim was to compare the total abundance and spatial distribution of the most common *Barbus* species in the Bahar Dar Gulf between both periods. We found a sharp decline (75%) in total abundance, both in number and biomass of the *Barbus* species and even more (90%) in the number of juveniles between the two periods. However, the spatial distribution of the different *Barbus* species over the three habitats had not changed. High natural variability in fish stocks might be expected in environmentally unstable lakes. Although strongly pulsed (seasonal), Lake Tana is a relative stable system. No major differences were found in abiotic parameters during the 1990s that could have caused the dramatic changes in abundance. The most likely explanation is the negative impact of the motorised, commercial gillnet fishery targeting the spawning aggregations of these barbs in the river mouths. Especially the drastic decline in juveniles points towards serious recruitment over-fishing. The results stress the need for the immediate development of a sound management plan focussing on effort restrictions during the *Barbus* breeding season.

Chapter 11: Size at maturity and gillnet selectivity

The dramatic reduction of the adult *Barbus* stocks and the even lower proportion of recruits at the end of the 1990s, stressed the necessity of the development, implementation and control of fisheries legislation in Lake Tana. Both effort restrictions and size restrictions (e.g. mesh size gillnets) might be considered in order to protect the reproductive active part of the *Barbus* populations. One aspect of the reproductive biology of the *Barbus* species, size at maturity, was poorly known. However, the present basic data are essential for advising on future implementation of size restrictions. Size at maturity varied widely among the *Barbus* species, ranging from 18.8 cm FL in *B. brevicephalus* to 44.3 cm FL in *B. crassibarbis*. Males matured at a smaller size and reached a smaller maximum length than females. Estimated selectivity curves fitted closely or were slightly larger than the observed length-frequency distribution of the commercial catch. The vast majority (85%) of barbs landed by the commercial gillnet fishery were mature. Fishing pressure on juvenile, immature fish is unlikely to be the cause of the observed decrease in *Barbus* stocks. Size control regulations like mesh size restrictions, intending to protect the immature part of fish populations are expected to have little positive effects on the *Barbus* stocks and are therefore not recommended. Only effort control regulations limiting the gillnet fishery in the spawning season and/or area will prevent a total collapse of the *Barbus* stocks as has happened to other cyprinids in African lakes.

Chaper 12: Vulnerability of Barbus, Clarias gariepinus and Oreochromis niloticus to fisheries

In addition to the artisanal, predominantly subsistence reedboat fishery, in 1986 a motorised, commercial gillnet fishery was introduced in Lake Tana. The three main fish stocks in Lake Tana are the large *Barbus* spp, African catfish (*C. gariepinus*) and Nile tilapia (*O. niloticus*). The aim of this paper was to discuss the impact of a small-scale commercial gillnet fishery on the development of the three, biologically different, species groups. The catch of the commercial gillnet fishery was monitored for effort allocation, species group composition and spatial/temporal variation. A monthly experimental trawl program conducted in 1991-1993 was repeated in 1999-2001 to compare the abundance and population structure of the three species groups in the Bahar Dar Gulf of Lake Tana.
In 1991-1993 the vast majority of fishing activity took place in the Bahar Dar Gulf (71%) and each species group contributed roughly to one third of the total catch. In 2001, 41% of the effort was allocated to the north-eastern shores of Lake Tana and the catch per unit of effort and the contribution of *O. niloticus* to the total catch had both doubled. The increase in *O. niloticus* was not only due to specific targeting by the commercial gillnet fishery but also to a decline in abundance of especially *Barbus*, which proved to be highly susceptible to the increased fishery. The large barbs are long-lived, ecologically specialised endemics and the commercial gillnet fishery is mainly targeting their spawning aggregations in the river mouths. The collapse of juvenile *Barbus* during the 1990s strongly suggests recruitment-overfishing. The ecologically flexible *C. gariepinus* and especially *O. niloticus* seemed more resilient against the increased fishing pressure. To prevent the extinction of the unique *Barbus* species flock fisheries regulations have to be developed and implemented immediately to protect the vulnerable spawning aggregations.

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CHAPTER

The piscivorous barbs of Lake Tana (Ethiopia): major questions on their evolution and exploitation.

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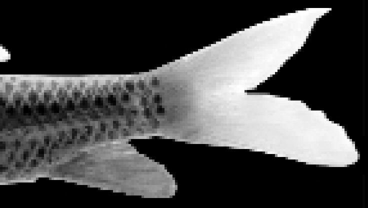

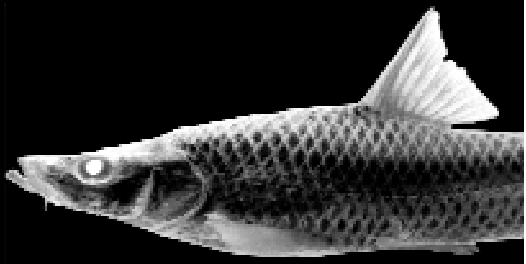

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Abstract

This paper surveys the major questions on the evolution of eigth piscivorous species (*B. acutirostris*, *B. dainellii, B. gorguari, B. longissimus, B. megastoma, B. platydorsus, B. truttiformis* and *B. macrophtalmus*), which together with six other species compose the endemic hexaploid 'large barb' species flock of Lake Tana. Preliminary data suggest that the major prey species of these piscivores (up to 90 cm FL), in contrast to previous reports, is not *B. trispilopleura*, but at least two other diploid 'small barb' species (<10 cm FL). Data obtained from (1) the resource partitioning of the piscivores in the field, (2) aquarium experiments on the predator success in catching different prey species and size classes, (3) analyses on allometric growth and diet shifts in the field and (4) molecular genetic data, will be combined to reconstruct a hypothesis on the process of the evolution of these piscivorous barbs. Being a major target of commercial fisheries, knowledge on these piscivorous fish stocks and their prey species is required for aiming at sustainable fisheries. Preliminary data show a serious decline of *Barbus* stocks, most probably due to overexploiting their spawning grounds.

Introduction

Lake Tana

Lake Tana, source of the Blue Nile, is situated in the northwestern highlands of Ethiopia, 500 km north of Addis Ababa, at an altitude of 1800 m. The lake has probably been formed through volcanic activity, blocking the course of rivers in early Pleistocene times (Mohr 1962). The meso-oligotrophic, shallow lake (average depth 8 m, maximum depth 14 m) now covers an area of approximately 3200 km^2 and is by far Ethiopia's largest lake, containing half of the country's fresh water supply. The lake is isolated from the lower Nile basin ichthyofauna by 40 m high waterfalls, at Tissisat ('smoking waters'), 30 km downstream from the Blue Nile outflow.

Development of fisheries

Before 1986 fishery in Lake Tana was limited to a traditional subsistence reed boat fishery, which had access only to the inshore fish community, catching mainly tilapia, *Oreochromis niloticus* and non-piscivorous 'large' *Barbus* species (Wudneh 1998). Beso (*Varicorhinus beso*) contributed a minor fraction. In 1986 the Lake Tana Fisheries Resources Development Program started, introducing modern fishing gear and motorised boats. As a result, the fishing area extended from the shore to deeper (>5 m), offshore waters and the catch composition changed, now including the African catfish, *Clarias gariepinus* and the large (>90 cm fork length (FL)), commercially important piscivorous species. The catches of the motorised fisheries increased rapidly from 39 metric ton 1987 to 323 metric ton in 1993 (LFDP 1997). In 1990 a research project was initiated to characterize the biological and dynamic aspects of the Lake Tana fish resources, to define the current state of fisheries, to identify its impact on the fish community and to assess the options for management of the fish stocks (Wudneh 1998).

The Barbus species flock

At the start of the fisheries research project in 1990 the morphological diversity of the barbs in Lake Tana created a stock identification problem. At that time the large barbs were considered as one species, *Barbus intermedius* (Rüppell 1836). Evidence from the analysis of morphology (Nagelkerke et al. 1994, 1995a), from the study of food niches (Nagelkerke et al. 1994; Nagelkerke 1997), spawning patterns (Nagelkerke and Sibbing 1996) and from genome analysis (Dixon et al. 1994, 1996) was so convincing that the 14 *Barbus* morphs were assigned as good biological species, composing a unique species flock (Nagelkerke and Sibbing 1997; cf. Nagelkerke and Sibbing 2000). The *Barbus* species flock most likely evolved sympatrically in Lake Tana from an ancestral riverine benthivorous barb species resembling the *B. intermedius* complex commonly found in the Ethiopian highlands (Sibbing et al. 1998). Nowadays such highly variable *B. intermedius* is still found at the shore areas of Lake Tana. Specialisation for different food types and disruptive selection on feeding structures are hypothetised as the evolutionary mechanisms involved in speciation (Nagelkerke and Sibbing 1998), producing the current species representing many trophic types.

Current research questions

One intriguing and important aspect of this endemic *Barbus* species flock, is the large number of piscivorous species (eight, cf. Nagelkerke 1997). Cyprinids seem not to be well designed for piscivory, because they lack teeth in their oral jaws, have a small slit-shaped pharyngeal cavity and all lack a stomach with low pH for digesting large prey. Why is this feeding mode, which is rare among cyprinids in general, so common in Lake Tana? This is most probably due to the abundance of small planktivorous prey fish and the lack of non-cyprinid piscivores, leaving this resource underexploited. For the fishermen, motorised since 1986, these large piscivorous barbs became target species and require rational management. Therefore, data are needed on their role in the ecosystem, their population dynamics and their annual production.

To meet all above questions, the current project on Lake Tana was initiated. The main aims of this project are: (1) to reconstruct the evolution of piscivory within the *Barbus* species flock using morphological, ecological, behavioural and genomic data, and (2) to predict the topdown cascading effects of intensive exploitation of particular piscivores for the composition and community structure of the ecosystem and its production.

Resource partitioning among the piscivores

Resource partitioning is an important mechanism allowing species in an assemblage to coexist and is hypothetised to be the driving factor in their evolutionary divergence. Segregation of coexisting piscivores can occur along trophic, spatial and temporal resource dimensions. Trophic and spatial dimensions are generally more important than the temporal dimension (Ross 1986). Important parameters in spatial segregation in lakes are horizontal distribution over different habitats and vertical distribution over the water column. For the temporal dimension, diurnal and seasonal components are distinguished. With regard to segregation based on feeding behaviour, for piscivores the diurnal component (i.e. the time of active feeding) is probably the most important (Baker and Ross 1981; Jansen and Mackay 1992). The distribution of the different *Barbus* species in space and time has only been studied to a limited extent. Wudneh (1998) mentions the horizontal distribution patterns of only two species, *B. platydorsus* (piscivore) and *B. tsanensis*, based on experimental trawls in three macro-habitats in the southern Bahar Dar Gulf. However, what Wudneh identified as '*B. tsanensis*' appeared actually to be a combination of *B. tsanensis*, *B. brevicephalus*, and the *B. intermedius* 'shore complex' (Wudneh, pers. comm.). Therefore, the only reliable distribution data on piscivores at present are from *B. platydorsus*, which is predominantly found in deeper, offshore waters, irrespective of the type of substrate (Nagelkerke et al. 1994).

Also, the trophic segregation among the piscivores has received little attention. Up to date, the fish remains in the intestines of piscivorous barbs were assumed to belong to only one prey species, *B. trispilopleura* (Nagelkerke and Sibbing 1997). However, de Graaf and Eshete Dejen (pers. obs.) suggest from their current preliminary survey on the small barbs that *B. trispilopleura* is restricted to the shallow, rocky substrate along the shore. Its morphometric data appear to be almost identical with *B. humilis*, suggesting that they may be the same species, only distinguished by colour. The dominant prey species in the majority of the lake's macro-habitats probably are *B. humilis* and '*B. tanapelagius*' (Table 1), the latter species being new to science (de Graaf unpublished data). *Barbus humilis* occurs over muddy/sandy substrate of shallow to intermediate depth (<7 m), while '*B. tanapelagius*', together with *Garra* species, is the only abundant prey species in deep (10-13 m), offshore waters. Juveniles of large barbs, differing from the small barb species in scale structure and first dorsal fin ray, are a minor fraction in our catches (as well as in the barb gut contents) and almost absent from distant deep open water areas (>7 m). It is therefore highly unlikely that all eight piscivorous barb species, most abundant in open deep water, mainly feed on *B. trispilopleura*. The distribution of these prey fish and the niche segregation amongst the piscivorous barbs in Lake Tana remains to be solved along spatial, temporal as well as trophic (size, species) resource dimensions.

Table 1. Species composition of all small prey fish (<10 cm fork length) by number and percentages of the total catch (trawl, October- November 1999) at areas of different depth, taken from the whole water column. The distinction between *B. humilis* and *B. trispilopleura* is made only by their colour.

	Shallow, 2 m		**Intermediate, 5-7 m**		**Deep, 10-13 m**	
Samples	**36**		**35**		**22**	
Species	**N**	**%**	**N**	**%**	**N**	**%**
large barbs	352	9	306	10	18	2
B. trispilopleura	3	0	0	0	0	0
B. humilis	1888	51	1527	49	24	3
B. tanapelagius	1265	34	1074	34	409	47
Garra	155	4	184	6	391	45
O. niloticus	8	0	4	0	0	0
C. gariepinus	0	0	8	0	2	0
Total	3707		3138		866	

Diversification of feeding potentials on an ontogenetic and evolutionary time scale

The 'food-fish model', an ecomorphological model based on functional relationships between food characteristics and fish morphology, has been applied for predicting food resource partitioning in the presumably monophyletic *Barbus* species flock (Nagelkerke 1997). The trophic hierarchy among the 14 *Barbus* species predicted from 33 structural parameters closely resembles the actual resource use. The model lumped all piscivores (> 50 % of volume fish in their gut contents) in two major groups: (a) *B. acutirostris*, *B. dainellii*, *B. gorguari*, *B. longissimus*, and *B. megastoma*, are rather strict piscovores, and (b) *B. platydorsus*, *B. truttiformis* and *B. macrophtalmus*, which are less specialized piscivores. If there are several abundant species and size classes of prey fish distributed over spatially distinctly different habitats, a challenging opportunity arises to increase the resolving power of the food-fish model, by constructing demands and structural profiles for different types of piscivores, depending on their feeding strategies developed for particular prey characteristics (e.g. habitat, size, shape, velocity, mechanical, chemical). The present model already predicted the capture abilities and in-abilities of each piscivore to perform as a pursuit or ambush hunter. However, feeding techniques, performance and prey selection (size/species) remain to be tested in controlled aquarium and outdoor basin experiments.

On the ontogenetic time scale, the adult construction and performance of the piscivores may well develop along different trajectories (cf. Mina et al. 1996). Morphometric measurements to determine coefficients of allometric growth of head and body structures are expected to show how the transformation from juvenile zooplanktivores (and benthivores, like the ancestral *B. intermedius*) towards different piscivores proceeds, the body sizes at which these changes occur and how they are linked to diet or habitat shifts. Such data provide the basis to compare ontogenetic trajectories between the piscivores, elucidate their ecological divergence in the ecosystem and are taken as a starting point for analysing the evolutionary time scale. *Barbus* larvae and small juveniles (<4 cm FL) have not been found yet in the lake itself. Sampling of previously unstudied habitats such as shallow river areas, papyrus beds and floodplains during and after the spawning season will hopefully prove successful. In the mean time, larvae of four different *Barbus* species, collected from their spawning grounds, have been reared in the laboratory. Their study will enable us to follow ontogenetic changes in morphology and performance and to construct an identification key for larvae and juveniles in the field.

Combining ecomorphological and genetic differentiation

Bringing the morphological and ecological differentiation into a phylogenetic framework may provide hypotheses on the evolutionary processes and mechanisms. The large barbs have close genetic similarity, reflecting a recent origin. All large *Barbus* species that have been investigated are hexaploid, have 150 chromosomes and show no karyological differences (Golubtsov and Krysanov 1993; Krysanov and Golubtsov 1996). Allozyme studies (Berrebi and Rab 1998) revealed no diagnostic alleles, but different allele frequencies were observed. However, BainDab alleles from *B. acutirostris*, *B. truttiformis*, *B. nedgia* and *B. tsanensis*, obtained during a study on the evolution of the major histocompatibility complex (MHC) class II b chainencoding genes showed a species specific distribution i.e. no sharing of alleles was found between the four species (Dixon et al. 1994, 1996). A survey of molecular genetic variation using microsatellites revealed that the thirteen ‘morphs’ analyzed do not constitute a single interbreeding population and that some are genetically more distinct than others (Dimmick, pers. comm.). Together, these observations can be considered as a justification of the species status designated to the ‘morphs’ (cf. Nagelkerke and Sibbing 2000). Additional genetic markers are needed to corroborate and extend the results sofar. Important questions remain to be solved: (1) are all large *Barbus* species of Lake Tana a monophyletic group? (2) which are the phylogenetic relationships within the species flock and which are their affinities with the riverine *B. intermedius* and other barbs from the Blue Nile basin? (3) have the piscivores one or multiple origins? (4) does each *Barbus* species in Lake Tana form a single interbreeding stock, i.e. a single unit of management? Phylogenetic relations are currently under investigation using the DNA sequence of the first and second internal transcribed spacer (ITS1-2) of the ribosomal DNA. This technique has been relatively successful in solving similar phylogenetic questions within the salmonid genus *Salvelinus* (Phillps et al. 1994), the subfamily Coregoninae (Sajdak and Phillips 1997) and haplochromine cichlids (Booton et al. 1999). A further survey of molecular genetic variation is planned, using random amplified polymorphic DNA (RAPD). RAPD's proved to be successful in characterizing populations of the cyprinids *Labeo victorianus* and its congeners *L. horie* and *L. coubie* and provided species specific markers (Mwanja, pers. comm.).

Sustainable fisheries

After the 1990-1993 baseline study on the stocks of *B. tsanensis*, *B. platydorsus*, *O. niloticus* and *C. gariepinus* in the southern Bahar Dar Gulf (Wudneh 1998), the catches in Lake Tana kept increasing sharply over the years, from 393 metric tonnes in 1994 to 1470 metric tonnes in 1997 (LFDP 1998). In the same period the commercial fishermen moved to more northern and eastern fishing grounds, concentrating at the river mouths, because they experienced decreasing catches in the southern Bahar Dar Gulf. Comparison of our preliminary data of the experimental trawls in 1999 with the trawls in the same seasons and sites during 1990-1993 by Wudneh, seem to confirm this unwanted development. These results show a serious decline (40-70%) in adult (>17 cm FL) tilapia, both juvenile and adult *B. platydorsus* as well as juvenile and adult *B. tsanensis*. The most likely explanation for this decrease in catches is not the development of motorised fisheries and their efforts over the recent years in the Bahar Dar Gulf. The calculated maximum sustainable yield is much higher (cf. Wudneh 1998) and juveniles should be little affected by lake fisheries. The above decline most probably is caused by over-fishing mature barbs migrating to their spawning rivers and the ongoing use of destructive techniques for catching each single fish at the spawning grounds (Nagelkerke et al. 1994; Nagelkerke et al. 1995b). No detailed catch data are available from the southern Bahar Dar Gulf over the 1994-1997 period. Together with local authorities, current sampling efforts must and will be continued for closely monitoring these developments. Legislation for controlling fishermen efforts is on its way.

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3
CHAPTER

Barbus tanapelagius, a new species from Lake Tana (Ethiopia): it's morphology and ecology.

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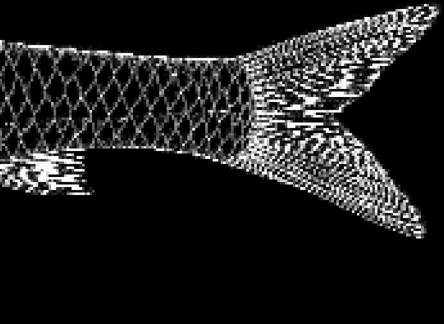

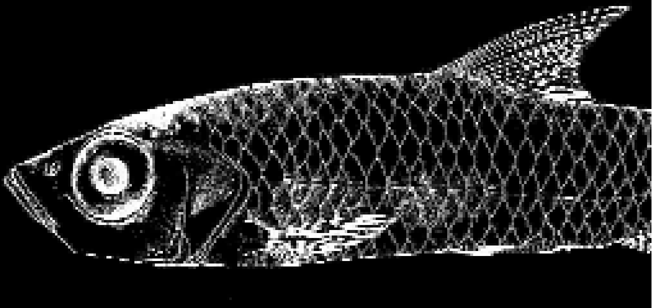

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Abstract

The endemic cyprinid species flock in Lake Tana comprises of 15 species of large hexaploid barbs, eight of which are piscivorous. Previously, it was assumed that all piscivores preyed on the same small barb species, *Barbus trispilopleura*. In this paper we present a description of morphology and ecology of a new abundant small barb species, *Barbus tanapelagius* spec. nova (holotype RMNH 33731) from Lake Tana, Ethiopia, which appears to be the major prey species for the large pelagic piscivorous barbs. *Barbus tanapelagius* differs clearly in morphology from the other three small, diploid *Barbus* species known from Lake Tana, *B. trispilopleura* Boulenger 1902, *B. humilis* Boulenger 1902 and *B. pleurogramma* Boulenger 1902. Conspicuous differences are its elongated body, large eye diameter, prominent and hooked lower jaw contour and colouration. Preliminairy data suggest that *B. tanapelagius* also differs ecologically from the other small *Barbus* spp. by its pelagic, strictly zooplanktivorous feeding and its occurrence mainly in the deeper, offshore waters. The other small *Barbus* species are most probably largely benthic feeders and dominant in the shallow inshore waters. Previous views about the evolution of the present eight endemic piscivorous large barb species therefore require reconsideration, as the present paper shows a more complex scenario including several prey species.

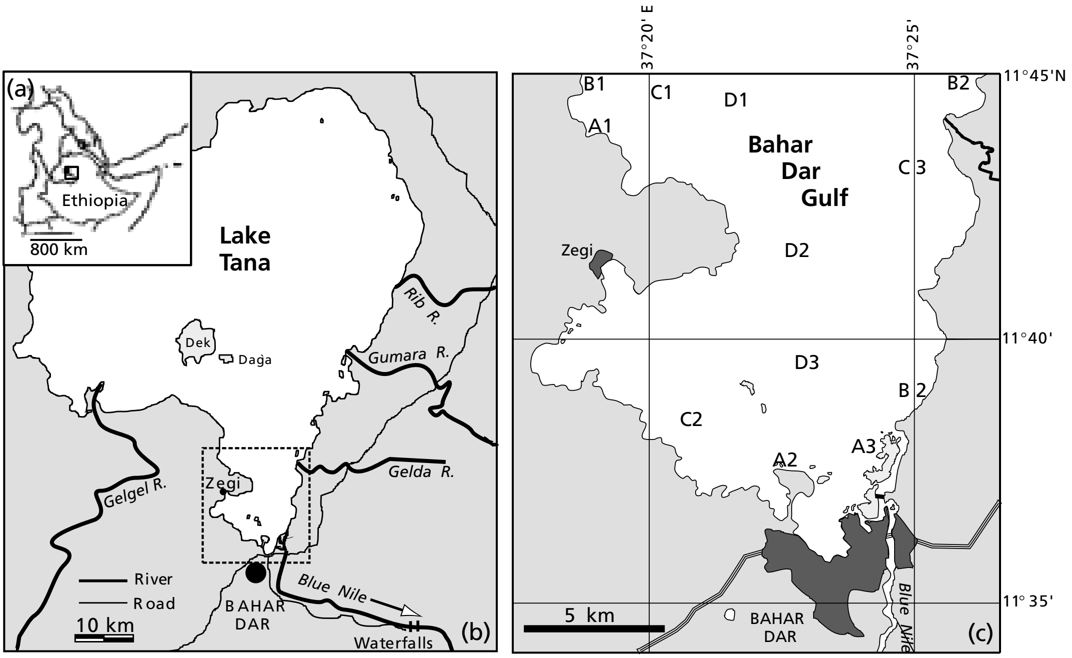

Figure 1. (a) Location of Ethiopia in the Horn of Africa, (b) Lake Tana and its main in- and outflowing rivers, and (c) the positions of the sampling stations in the Bahar Dar Gulf. Habitat A: A1, A2, A, Habitat B: B1, B2, B3, Habitat C: C1, C2, C3 and Habitat D: D1, D2, D3 (cf. Figure 3).

Introduction

Lake Tana is situated in the north-west of Ethiopia at an altitude of 1830 m. It is a turbid, meso-oligo trophic shallow lake (average depth 8 m, maximum 14 m) covering an area of 3200 km^2 and is the source of the Blue Nile. The lake is isolated from the lower Blue Nile basin by 40 m high waterfalls, 30 km downstream from the Blue Nile outflow (Fig. 1).

The largest fish family in the lake are the cyprinids, represented by three genera, *Varicorhinus*, *Garra* and *Barbus*. *Varicorhinus* Rüppell 1836, is represented by a single species *V. beso* Rüppell 1836. The genus *Garra* Hamilton 1822 (formerly *Discognathus*, Heckel 1846), according to Boulenger (1911) has two species in Lake Tana, *G. quadrimaculata* (Rüppell 1836) and *G. dembeensis* (Rüppell 1836) and maybe even more (Abebe Getahun, pers. comm.). The genus *Barbus* Cuvier & Cloquet 1816, has been revised several times. The latest revision of the large hexaploid barbs of Lake Tana was completed by Nagelkerke and Sibbing (1997a, 2000), which resulted in the distinction of 15 different species, of which eight were new.

Boulenger (1911) mentioned three small diploid barb species (<10 cm SL), which are not only distinct from the large barbs (up to 100 cm SL) by their size, but also by their morphology. The small *Barbus* species can without difficulty be separated from juvenile large *Barbus* species by a weakly developed first dorsal spine and radiating striae on the scales. Bini (1940) considered these small barbs as belonging to the subgenus *Enteromius* Cope 1869, and mentioned the same three species.

Most recent studies in Lake Tana have focused on the large, hexaploid *Barbus* species (Golubtsov and Krysanov 1993; Mina et al. 1996ab; Nagelkerke and Sibbing 2000). One intriguing and interesting aspect of this endemic species flock of large barbs, is the high number of piscivorous species (8). Cyprinids are not well equipped for piscivory because they lack oral teeth, have a slit-shaped narrow pharyngeal cavity and lack a distinct stomach with low pH for digesting large prey. Why is this feeding mode, quite rare among cyprinids in general, so common in Lake Tana barbs? How have they evolved and what factors were driving this process? Piscivorous specialists from other genera appear to be absent in Lake Tana, leaving this niche open for cyprinids (Sibbing et al. 1998). The morphological differences between both the piscivorous and non-piscivorous species and among the piscivorous species themselves are mainly found in the head and feeding apparatus. Specialisation for different prey fish might be an option to investigate.

After the introduction of motorized fisheries in 1986, the large piscivorous barbs became a target species of the commercial fisheries in Lake Tana. The large barbs form roughly a third of the total commercial catch (Wudneh 1998a). Also, for rational management of the piscivorous barbs data are required on their, function in the ecosystem, recruitment, annual production and population dynamics. Such data would enhance the predictions of possible top-down cascading effects of exploitation of particular piscivores for the fish community.

To answer these biological and fisheries related questions on prey-predator interactions in the Lake Tana food web, we decided to study also the identity and ecology of the small barb species, being the main prey fish. Preliminary surveys were conducted to gain insight in the distribution and abundance of the small barbs in the Bahar Dar Gulf of Lake Tana in the beginning of 1999. Next to the three small *Barbus* species mentioned by Boulenger (1911) and Bini (1940), *B. trispilopleura*, *B. humilis* and *B. pleurogramma*, we found a fourth previously unknown small barb in large numbers (up to 500 individuals in a single 15 min trawl) predominantly in the unexplored offshore, deep waters of the lake. We have compared its characters with the species described by Bini (1940) and Boulenger (1911) and concluded that one more species of small *Barbus* is abundant in the lake.

This paper provides a description of the morphology and ecology of *Barbus tanapelagius* and will briefly discuss some possible consequences of its discovery for existing hypotheses as to the food web of Lake Tana and the evolution of piscivory within the species flock of large *Barbus*.

Materials and methods

Collection of type material and morphometrics

Barbus tanapelagius were caught in July 1999 by trawl (cod-end 5 mm and 10 mm bar mesh) in the middle of the Bahar Dar Gulf of Lake Tana between sampling stations d1, as defined below, (North Mid Gulf, 11° 45′ latitude (N), 37° 22′ longitude (E)) and d2 (Mehal Zegi, 11° 42′ latitude (N), 37° 23′ longitude (E)) in July 1999.

Fifty-five parameters were used to describe the species. For sources and descriptions of the counts, measurements and coded qualitative characters, see Nagelkerke and Sibbing (2000). Fifteen fresh specimens ranging between 43 mm and 52.4 mm standard length (SL) were used for morphometric analysis. Gut length was determined in 15 fresh additional specimens ranging in length between 39 and 51 mm SL, to prevent the deposited type material from being damaged. The specimens described in this study, have been deposited at the Naturalis Museum, Leiden, The Netherlands (RMNH 33731-33745). The specimens were preserved in 4%, pH neutral formaldehyde solution. After at least one month, the specimens were rinsed and transferred to ethanol solutions of gradually increasing concentration (from 30-70%).

Spatial distribution sampling program

In the Bahar Dar Gulf of Lake Tana four habitats (A-D) were selected, differing in substrate, depth and distance to the shore (see Fig. 3). Three sampling stations (a1, a2, a3-d1, d2, d3) were chosen within each of these habitats (Fig. 1). The area covered by the littoral zones (habitat A and B) is approximately 10% of the total area sampled. The fraction covered by the intermediate (habitat C) and deep (habitat D) zone is estimated to be 20% and 70%, respectively. Data on fish distribution were collected using multi-mesh monofilament gillnets with five different mesh sizes: 5, 6.25, 8, 10, and 12.5 mm bar mesh respectively. Panel length and depth were 3 m and 1.5 m respectively for each mesh. Three multi-mesh bottom nets were set simultaneously at each site. At the deep and intermediate sites three additional nets were set at the surface. Nets were set from 18:00 to 21:00 hours and from 6:00 to 9:00 hours the following morning, monthly from October to December 1999. The fish from the evening catch were stored in 4% formaline before analysis the next day, the fish from the morning setting were transported fresh and analysed. The catches from the different nets, settings, sampling dates and stations were pooled per habitat during analysis.

Analysis of gut content

Six stations (b1-2, c1-2, d1-2, see Figure 1 and 3) were sampled once using a bottom trawl (5 mm bar mesh cod-end) at daytime between October and December 1999. Five fish per site were selected to determine diet composition and stored in 4%, pH neutral formaldehyde. The relative biovolumes of food items in the gut were estimated according to the points method (Hynes 1950) using a microscope. The following food categories were distinguished: calanoid copepods, cyclopoid copepods, Daphnia, Bosminia, Daphniosoma, Ceriodaphnia, insects, chironomid larvae, detritus and unidentified zooplankton. The gut contents of all fish (n=30) were pooled during analysis.

Results

Morphology

The results of the morphometric analysis of *B. tanapelagius* spec. nov (Fig. 2) are listed in Table 1.

Material. - Holotype, 49,4 mm SL, RMNH 33731 Mehal Zegi, Lake Tana, Ethiopia, 5.vii.1999, M. de Graaf; paratypes (14), 43-52.4 mm SL, RMNH 33732 - 33745, Mehal Zegi and North Mid Gulf, Lake Tana, Ethiopia, 5.vii.1999 - 29.vii.1999, M. de Graaf.

Etymology. - The name is derived from the Latin 'pelagius' and the name of Lake Tana. The name refers to the habitat of the fish in the lake, the offshore deep waters.

Habitus and diagnosis. - A small sized (maximally 68 mm SL), shallow and narrow-bodied barb. Head depth (18% SL) and body depth (20.5% SL) are about equal. The smaller depth of the caudal peduncle renders the fish slender, gradually tapering towards the tail. The dorsal head inclination is small (21°) and the dorsal profile is nearly straight. The eyes are large (eye diameter 34% HL > snout length 27% HL), with a small interorbital width (interorbital width 28.3% HL < eye diameter 34% HL). The lower jaw is rather long (43.3% HL), has a high inclination (>40°) and makes a pronounced angle with the ventral head profile. The lower jaw extends beyond the upper jaw with the mouth closed. The barbels are short (anterior barbel length 4.7% HL << eye diameter 34% HL). The oral gape (31-35.5% HL) is large and (sub)terminal (protrusion angle 14°). The pharyngeal tooth formula is 5-3-2:2-3-5.

Colouration. - Living specimens are in general silvery white and the dorsal side of the head is blackish. The dorsal body scales bear many dispersed pigment cells rendering its dorsal side greyish. The ventral parts are silvery white, as are the fins. The silvery colour disappears in 4%, pH neutral formaldehyde solution and the whole fish turns white except for the darker spots on the head and dorsal scales.

Size range. - Specimens from 15 to 68 mm SL have been observed.

Table 1. Morphometry of *Barbus tanapelagius*. Measurements according to Nagelkerke and Sibbing (2000).

	Mean	**Median**	**Min**	**Max**	**Std**	**n**
Meristic measures						
Dorsal fin rays (simple)	3.1	3.0	3	4	0.26	15
Dorsal fin rays (branched)	8.9	8.0	8	9	0.35	15
Anal fin rays (simple)	3.0	3.0	3	3	0	15
Anal fin rays (branched)	6.1	6.0	5	8	0.59	15
Pectoral fin rays (total)	13.7	14.0	11	15	1.05	15
Ventral fin rays (total)	8.0	8.0	8	8	0	15
Scales on lateral line (total)	30.3	30.0	28	33	1.53	15
Predorsal scales	11.0	11.0	10	12	0.76	15
Scales from dorsal fin to lateral line	4.5	4.5	4.5	4.5	0	15
Scales from anal fin to lateral line	2.9	3.0	2	3	0.26	15
Scales from ventral fin to lateral line	3.0	3.0	3	3	0	15
Circumpeduncular scales	11.1	11.0	11	13	0.52	15
Circumference scales	17.8	18.0	16	18	0.56	15
Metric measures						
In % standard length						
Head length	26.6	26.7	25.1	28.2	1.06	15
Body depth	20.5	20.1	17.6	23.4	1.62	15
Body width	12.1	12.3	9.8	13.6	1.02	15
Caudal peduncle length	31.3	31.1	29.6	34.1	1.33	15
Caudal peduncle depth	10.3	10.5	8.8	11.1	0.56	15
Predorsal length	48.9	48.8	46.5	52.5	1.60	15
Preanal length	68.9	69.3	67.4	70.5	0.95	15
Preventral length	48.4	48.7	45.2	51.0	1.43	15
Pectoral-ventral length	22.2	22.5	17.9	25.3	1.76	15
Ventral anal length	22.2	22.0	20.7	23.8	0.97	15
Dorsal spine length	21.5	21.6	18.9	23.1	1.17	15
Dorsal fin base length	10.7	10.9	9.8	11.7	0.64	15
Anal fin length	13.2	13.4	12.1	13.9	0.67	15
Anal fin base length	6.7	6.6	6.1	7.6	0.41	15
Pectoral fin length	16.4	16.7	13.4	17.7	1.13	15
Ventral fin length	15.8	15.9	13.6	16.9	1.05	15
Upper lobe length of caudal fin	29.2	29.0	27.5	31.4	1.38	15
Lower lobe length of caudal fin	29.0	29.0	27.2	30.8	1.15	15
Gut length	82.4	85.1	60.5	100.0	11.62	15
In % of head length:						
Head depth at occiput	66.9	66.7	62.8	73.3	2.53	15
Head width	50.3	49.5	47.7	54.7	1.85	15
Snout length	27.2	26.9	24.4	31.5	2.32	15
Snout width	35.1	34.5	30.2	41.1	2.78	15
Eye diameter	34.0	34.1	31.2	38.2	1.87	15
Orbit diameter	36.3	36.6	34.1	39.7	1.47	15
Postorbital length	37.6	37.8	35.3	39.8	1.55	15
Interorbital width	28.3	28.2	23.8	31.2	1.61	15
Operculum depth	35.3	34.6	30.2	40.5	3.24	15
Anterior barbel length	4.7	4.5	3.1	6.4	1.04	15
Upper jaw length	30.1	29.2	25.0	36.3	2.85	15
Lower jaw length	43.3	43.2	40.3	49.4	2.49	15
Angular measures						
Dorsal head inclination (°)	20.8	22.0	16	27	3.53	15
Gape inclination (°)	42.0	41.0	38	46	2.75	15
Coded qualitative measurements						
Head profile	2.73	3	2	3	0.46	15
Nuchal hump development	1.33	1	1	2	0.49	15
Upper lip development	1	1	1	1	0	15
Lower lip development	1	1	1	1	0	15
Anterior extension of lower jaw	3.87	4	3	4	0.35	15
Protrusion measures						
In % of head length						
Protrusion length	32.1	32.7	26.9	35.1	2.30	15
Horizontal oral gape	31.0	30.5	24.9	35.6	2.98	15
Vertical oral gape	35.5	35.3	31.5	40.0	2.13	15
Protrusion angle (°)	14.1	14.0	9	20	3.86	15

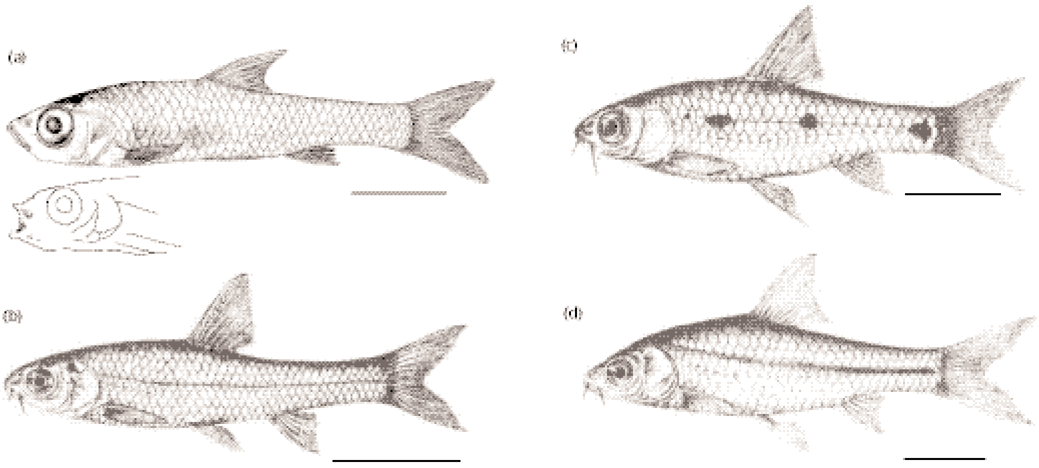

Figure 2. The small *Barbus* species of Lake Tana. (a) *Barbus tanapelagius* spec. nov. Holotype, RMNH 33731, SL of 49,4 mm, lateral view with expanded head contours beneath, (b) *B. pleurogramma* (after Boulenger 1911), (c) *B. trispilopleura* (after Boulenger 1911) and (d) *B. humilis* (after Boulenger 1911). Scales equal 10 mm. (Size scales in b,c and d were derived from Boulenger's drawings supposing his scale lines indicate real total length in mm)

Spatial distribution

The four most important fish species in the gill net catches were *B. tanapelagius*, *B. humilis*, *Garra* spp. and juvenile large *Barbus* spp. Together these species composed 99.8% of the numerical proportion. *Clarias gariepinus, Oreochromis niloticus, B. trispilopleura, B. pleurogramma* and *Varicorhinus beso* were only caught occasionally and were not included in further study. The species composition of the gillnet catches (Fig. 3) shows the numerical dominance of *B. humilis* in the shallow inshore habitats A and B. The intermediate and especially the offshore habitat are dominated by *B. tanapelagius*. *Garra* and juvenile large *Barbus* spp. contribute minor fractions in all the four habitats. The overall fish densities are four times higher in the littoral habitats A and B compared to the intermediate and offshore habitats C and D.

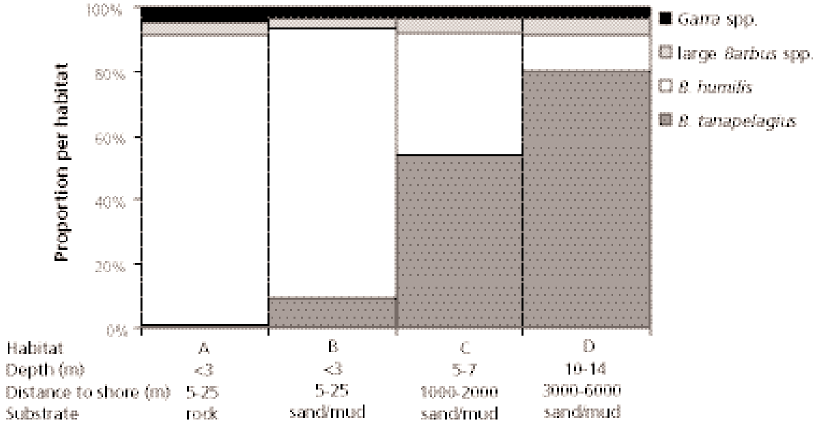

Figure 3. Species composition (%) per habitat. Total number of specimens per habitat: A=5597, B=6909, C=3000, D=3144.

Diet composition

Barbus tanapelagius is a strict zooplanktivore (Fig. 4). Its diet consists mainly of cyclopoid and calanoid copepods and Daphnia.

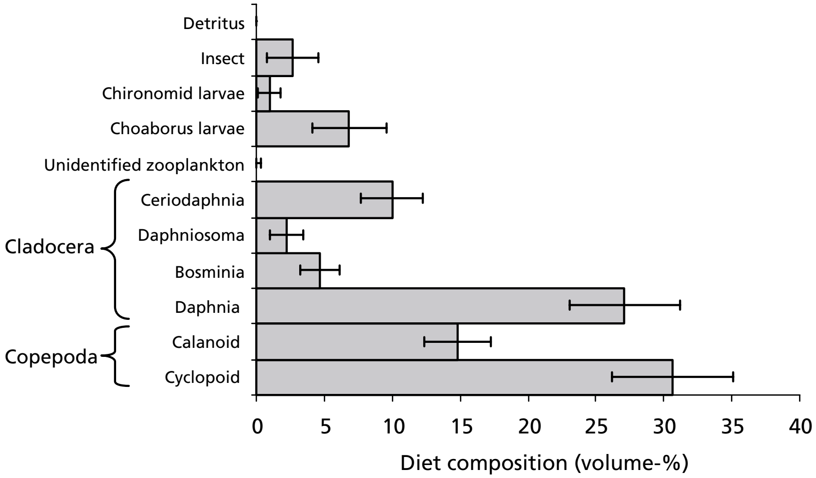

Figure 4. Diet composition (volume-%) of *B. tanapelagius* (n=30) with standard error, pooled from habitats B, C and D over the period October-December 1999.

Discussion

It is no surprise that this small but abundant *Barbus* species from Lake Tana was not found by ichtyologists to date. Firstly, most work has concentrated on the highly intriguing species flock of large barbs and its complicated taxonomy (Rüppell 1836; Boulenger 1911; Bini 1940; Banister 1973; Golubtsov and Krysanov 1993; Mina et al. 1996ab; Nagelkerke and Sibbing 1997a, 2000;). Secondly, the habitat of *B. tanapelagius*, the offshore deep waters was not accessible by scientists in the 19th and early 20th century.

The typical diploid small barbs of Lake Tana can easily be distinguished from the large hexaploid barbs by a small size (<10 cm SL), a weakly developed first dorsal spine and radiating striae on the scales. *Barbus tanapelagius* differs clearly in morphology from the other three small barbs *B. trispilopleura*, *B. pleurogramma* and *B. humilis*, occurring in Lake Tana, by its distinctive elongated body, its large eye diameter, its small anterior barbel length, its pronounced and hooked lower jaw and its light body colouration. Species descriptions of *B. trispilopleura* and *B. humilis* by Boulenger (1911) are, however, insufficient for their distinction. Preliminary evidence suggests that *B. trispilopleura* and *B. humilis* are conspecifics (Dejen, unpublished data). A thorough morphometric analysis of these species and a revision of the small barbs of Lake Tana is urgently needed and currently being executed.

The discovery of *B. tanapelagius* may have important implications for the food web of Lake Tana, as reconstructed by Nagelkerke and Sibbing (1997b) and affects the current scenario's about the evolution of piscivory among the large barbs. Nagelkerke and Sibbing (1997b) suggested that *B. trispilopleura* is a key species in the Lake Tana food web, supposing that the most important flow of energy and mass to the large *Barbus* biomass production, leads from phytoplankton, via zooplankton, especially via *B. trispilopleura*. Benthos was considered to be far less important. These authors suggested further that *B. trispilopleura* occurs in all habitats and is the major prey fish for the eight species of piscivorous large *Barbus* (*B. acutirostris*, *B. dainellii*, *B. gorguari*, *B. longissimus*, *B. macrophtalmus*, *B. megastoma*, *B. platydorsus* and *B. truttiformis*). This scenario probably is, however, oversimplified.

The dominant prey species in the four selected habitats in Lake Tana are *B. humilis* and *B. tanapelagius*. *Barbus humilis* occurs mainly in the shallow shore areas (0-4 m depth) over both rocky and sandy/muddy substrate and decreases sharply in abundance with increasing depth and distance from the shore. *Barbus tanapelagius*, however, is the abundant prey species in deep (8-14 m), offshore waters and decreases sharply in abundance with decreasing depth and distance to the shore. *Barbus pleurogramma* seems to occur only in the affluent rivers of the lake between the shore vegetation and floodplains at depths shallower than 1 meter (M. de Graaf, personal observation).

Benthic organisms and detritus were not found in *B. tanapelagius* and their diet consisted mainly of copepods and cladocerans in the period October-December 1999. The copepods (mean density 32 l^{-1}), containing two cyclopoid species *Mesocyclops* sp. and *Thermocyclops* sp. and one calanoid species *Thermodiaptomus galebi*, constituted the major zooplankton fauna followed by the Cladocera (mean density 20 l^{-1}) in the Bahar Dar Gulf (Wudneh 1998b). In general, the spatial fluctuations in zooplankton densities is larger than their seasonal fluctuations. Densities of copepods and cladocerans were highest in the littoral zone of the lake during March-May prior to the start of the rainy season. *Barbus tanapelagius* is most likely a strict zooplanktivore as these prey organisms are available year round in the intermediate and deeper waters in similar or higher densities compared with the period October-December covered in this study (Wudneh 1998b).

Therefore the energy flow to the large piscivorous *Barbus* species and the main source of their biomass production is more likely to run from phytoplankton, via zooplankton, via the pelagic feeding zooplanktivore *B. tanapelagius* in the intermediate and deeper waters. Preliminary evidence suggests that in the littoral zone an important energy flow to large piscivorous *Barbus* biomass runs from benthos, via *B. humilis*. The diet of 30 *B. humilis* and 30 *B. trispilopleura* collected between October and December 1999 consisted mainly of insects, chironomid larvae and detritus, suggesting a benthic feeding mode for these species (Dejen unpublished data).

The process of adaptive radiation of eight endemic, piscivorous barb species within Lake Tana is still unknown. It seems unlikely that they all specialised on feeding on the same prey item, *B. trispilopleura/humilis*, because of differences in trophic morphology (Nagelkerke and Sibbing 1997b). Present knowledge on the differences in morphology, distribution and ecology between the potential prey species leaves options open for specialisation, segregation and speciation of varied piscivores within Lake Tana. The first aim of current research on the evolution of piscivory within the large

Barbus species flock is to find evidence (morphological, ecological, and behavioural) linking the diversity of the piscivores with the diversity of their prey.

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4

CHAPTER

Ecological differentiation among the eight piscivores within the Barbus species flock (Pisces, Cyprinidae) of Lake Tana, Ethiopia.

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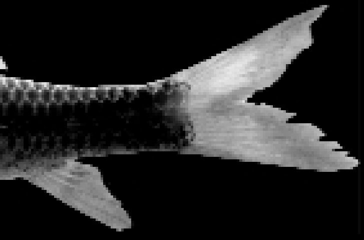
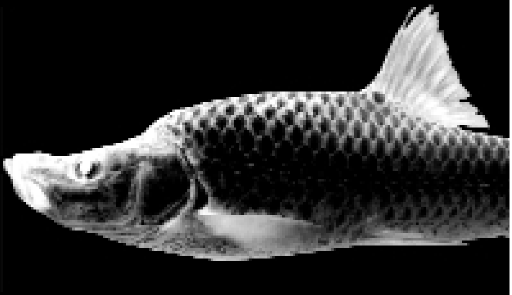




Abstract

The endemic *Barbus* species flock of Lake Tana (15 species) contains a large number of piscivorous species, a unexpected specialisation among the highly successful mainly benthivorous Cyprinidae, the most species-rich (>2000) family of freshwater fish. Overall, Lake Tana's *Barbus* species are well segregated along the three studied resource dimensions; trophic, spatial and temporal. Among the eight piscivores considerable differences exists in prey species composition. However, relative prey size was small (prey-to-predator length ratio (PPR) average 0.15) and the same for all piscivore/prey species combinations. Six of the eight piscivorous *Barbus* species become piscivores at 10-15 cm FL (age 1^+), *B. macrophtalmus* and *B. platydorsus* have a significantly longer ontogenetic trajectory and do not shift towards piscivory until reaching 20 cm FL (age 3^+).

The cichlids of Lake Victoria and the barbs from Lake Tana have some important similar morphological innovations resulting in potentially large diversification of trophic structures and high potential for trophic radiation. Although Lake Victoria haplochromine species flock has a higher diversity per trophic group, the proportion of species per trophic group is the same in both species flocks. Due to the lack of oral teeth some cichlid trophic specializations (e.g. aufwuchs feeders) are lacking in Lake Tana's barbs. Nevertheless, in the absence of common African specialist piscivores or generalist riverine cichlids as competitors, the cyprinids of Lake Tana used their potential for trophic diversification to the fullest, including the unexpected specialisation of piscivory.

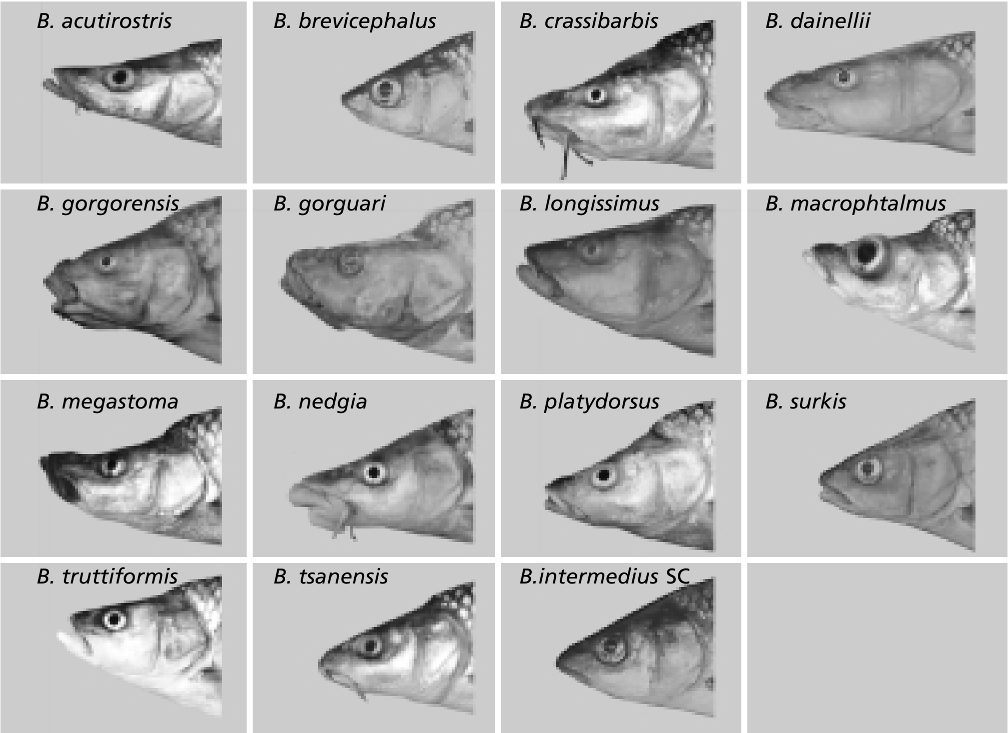


Figure 1. Heads of the 15 *Barbus* species of Lake Tana.

Introduction

Lake Tana in the north-western highlands of Ethiopia, contains an extraordinary diversity of cyprinid fishes. It is currently estimated that at least 15 large, hexaploid endemic *Barbus* species are present (Fig. 1; Nagelkerke et al. 1994; Nagelkerke and Sibbing 2000). The family Cyprinidae is the most widespread and has the highest diversity (>2000 species; Nelson 1994) among all fresh water fish families and even among vertebrates. The largest cyprinid genus *Barbus* (>800 species) is a polyphyletic assemblage (Howes 1987; Tsigenopoulos et al. 2002). However, Africa's ubiquitous large hexaploid *Barbus* are of a single recent origin forming a monophyletic group and are classified in the subgenus *Labeobarbus* (Berrebi 1995; Tsigenopoulos et al. 2002 and references therein).

Despite the overwelming abundance of cyprinid fishes throughout the world's fresh water systems, the *Barbus* species of Lake Tana form, as far as we know, the only remaining intact species flock of large cyprinid fishes, since the one in Lake Lanao in the Philippines, has practically disappeared due to anthropogenic activities (Kornfield and Carpenter 1984). One of the most intriguing aspects of Lake Tana's endemic *Barbus* species flock is the large number of piscivores (8 out of 15). Cyprinid fishes seem not well designed for piscivory, they lack teeth in the oral jaw, have a small slit-shaped pharyngeal cavity and all lack a stomach with low pH for digesting large prey. Most of Africa large *Barbus* are benthivorous species, so why then is piscivory, which is rare among cyprinids, so common in Lake Tana *Barbus*?

Lake Tana's fish fauna is isolated from the lower Nile basin by 40 m high waterfalls which were presumably formed around 10.000 years ago (Chorowicz et al. 1998 and references therein), 35 km downstream from the only outflowing river the Blue Nile. The *Barbus* species flock radiated sympatrically within Lake Tana within a relatively short period and is hypothesised to have originated from an ancestral riverine benthivorous barb species resembling *B. intermedius* commonly found in the Ethiopian highlands (Sibbing et al. 1998; Chapters 7, 8 and 9). Nowadays, a similar highly variable *B. intermedius* "shore-complex" (SC) is still found on the shore areas of Lake Tana. These conditions make of Lake Tana and its *Barbus* species flock a natural laboratory and provide a unique opportunity to study the selective forces driving speciation and diversity in freshwater fish communities. The limited size of Lake Tana's *Barbus* species flock is a further advantage over the species-rich cichlids species flocks of Eastern African lakes (Lake Victoria, 500+ species, Seehausen 1996; Lake Tanganyika 250+ species, Snoeks 2000; Lake Malawi 800+ species, Snoeks 2000) when studying adaptive radiation and speciation, because all 15 *Barbus* species can easily be studied simultaneously.

Many of Lake Tana's *Barbus* species have evolved unique anatomical features, presumably specialisations and adaptations to different food resources, since the radiation of Lake Tana's barbs was hypothesised to be driven by trophic resource partitioning (Sibbing et al 1998). Based on morphological predictions and gut content analysis five trophic groups were distinguished (Sibbing and Nagelkerke 2001): four benthivores, one molluscivore, one macrophytivore, one zooplanktivore-insectivore, and eight piscivores. The evolutionary origin and means of coexistence of especially these eight piscivorous *Barbus* species remains unclear to date.

The objectives of this study are therefore to: (a) determine the extent of ecological segregation along each of the three resource dimensions (trophic, spatial, temporal) within the whole *Barbus* species flock but especially among the piscivores; (b) study the ontogenetic trajectories towards piscivory among the eight piscivorous species; (c) discuss generalities in ecological structuring of the *Barbus* species flock, and (d) discuss the similarities in trophic differentiation between the cyprinid and cichlid species flocks of East Africa.

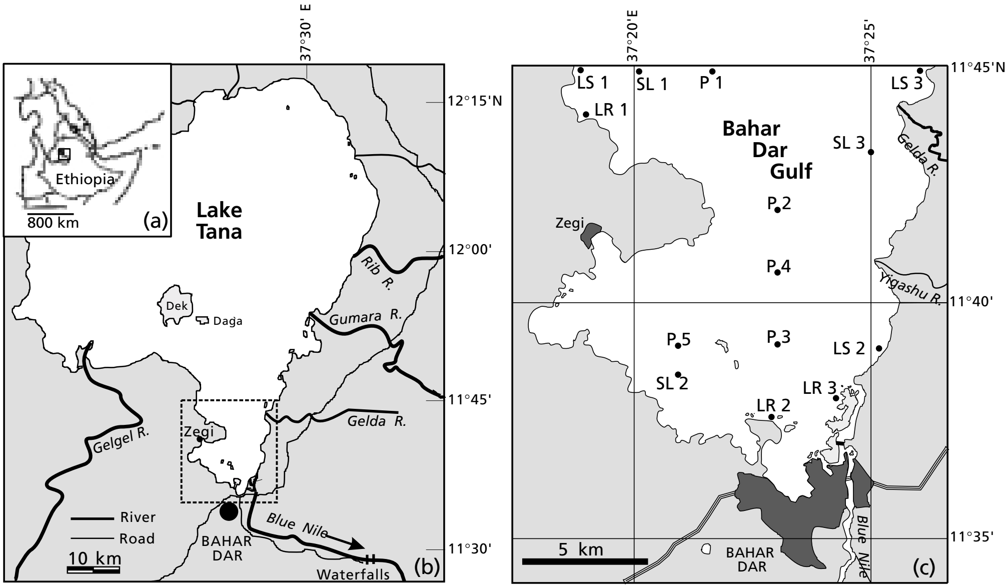


Figure 2. (a) Location of Ethiopia in the Horn of Africa, (b) Lake Tana and its main in- and outflowing rivers, and (c) the locations of the sampling stations in the Bahar Dar Gulf (cf. Table 2)

Materials and Methods

Study Area

Lake Tana is located at an altitude of 1830 m, in the north-western highlands of Ethiopia, 500 km north of the capital city Addis Ababa. The meso-oligotrophic (chlorophyll *a*, average 6.4 $\mu g \cdot l^{-1}$; Dejen et al. 2003), shallow lake (average depth 8 m, maximum depth 14 m) covers an area of c. 3,050 km^2 and is Ethiopia's largest lake, containing half the country's freshwater. Several perennial rivers feed into the lake. Lake Tana is the source of the Blue Nile, which is the only river flowing out of the lake. High waterfalls (40 m) at Tissisat ('smoking waters'), 30 km downstream from the Blue Nile outflow, effectively isolate the lake's ichtyofauna from the lower Nile basin (Fig. 2a).

Three fish families occur in Lake Tana. The Cichlidae and Clariidae are represented by only one species each, *Oreochromis niloticus* and *Clarias gariepinus*, respectively. The largest fish family in the lake are the cyprinids, represented by three genera, *Varicorhinus*, *Garra* and *Barbus*. *Varicorhinus*, is represented by a single species *V. beso*. According to Boulenger (1911) the genus *Garra* is represented by two species in Lake Tana, *G. dembecha* and *Garra quadrimaculata*, but the descriptions are inconsistent (Nagelkerke 1997). However, in Lake Tana *Garra* might form a mini-species flock with at least two endemic species "*G. microstoma*" and "*G. tana*" and maybe even more (Getahun, 2000). The genus *Barbus* has been revised several times. The latest revision of the large hexaploid barbs of Lake Tana (Nagelkerke and Sibbing (2000), distinguished 15 different species. Three small diploid *Barbus* species (<10 cm SL) are present in Lake Tana; *B. humilis* (including the previous *B. trispilopleura*, Dejen et al. 2002), *B. pleurogramma* and the recently discovered *B. tanapelagius* (de Graaf et al. 2000).

Rainfall, water level, water temperature and vertical transparency all follow seasonal patterns (see de Graaf et al. 2003 for details). Rainfall peaks in July-August, followed by a raise in the water level of the lake, peaking in September-October. During the rainy season the vertical transparency in the Bahar Dar Gulf is reduced (from 0.7 m to 0.4 m) due to the inflow of large amounts of silt, resulting from severe erosion, by the affluent rivers. Annual variation in water temperature (20-24°C) shows two peaks, the first just before the rainy season in May (24°C) and the second around October-November (22°C) at the start of the dry, winter season.

Niche overlap and niche breadth

The formula's of Schoener (1968) and Levins (1968) were used to calculate niche overlap and niche breadth, respectively because they are the most straightforward and have the advantage of compatibility with other published studies. Niche overlap (O_{jk}) was calculated using the similarity index of Schoener (1968) where:

$$O_{jk} = 1 - 0.5 \sum_{i=1}^{n} |P_{ij} - P_{ik}| = \sum_{i=1}^{n} \min (P_{ij}, P_{ik})$$

with n = number of resource categories, p_{ij} = mean proportion of resource category i in the spectrum of species j, p_{ik} = mean proportion of resource category i in the spectrum of species k. Similarity values range between 0 (complete segregation of resources) to 1 (complete overlap of resources). Although this index is not open to direct statistical testing, arbitrary values less than 0.4 or greater than 0.6 have been considered biologically significant (Schoener 1968; Ross 1986). Niche breadth was calculated using the formula of Levins (1968), defined as:

$$NW = 1/\sum_{i=1} P_i^2$$

where p_i is the proportion of the i^{th} item in the diet of a species. A species was classified as specialist or generalist from its niche breadth relative to a scale constructed by using as upper and lower bounds the extreme values found for of all species/size classes combined (Colwell and Futuyma 1971; Piet et al. 1999).

Trophic dimension

Diet Categories

Between January 1999 and August 2001 piscivorous *Barbus* were collected using a variety of methods (gillnets, see section Spatial Dimension; trawl, see de Graaf et al. 2003) in the southern Bahar Dar Gulf of Lake Tana and individuals of rare species were purchased from local fishermen. Only fish with a gut fullness >50% were used for analyses; around 95% of the piscivorous *Barbus* handled in the lab had near empty guts. Fish were dissected immediately upon arrival in the lab. Gut contents were transferred to a petri-dish and the contributions of the dietary categories by percentage of volume was estimated to the nearest 5%. The following diet categories were distinguished: Fish (Fis), Macrophytes (Mac), Detritus (Det), Gastropods (Gas), Bivalves (Biv), Zooplankton (Zoo), Insects (Ins), Insect larvae (Lar; mainly chaoborus), Phytoplankton (Phy). These nine categories contained more than 95% of the food in all piscivorous and non-piscivorous *Barbus* species. Data on the diet composition of the seven non-piscivorous *Barbus* spp (*B. brevicephalus*, *C. crassibarbis*, *B. gorgorensis*, *B. nedgia*, *B. surkis*, *B. tsanensis* and *B. intermedius* "shore-complex" [SC]) were obtained from Sibbing and Nagelkerke (2001).

Prey Fish Categories

Table 1. Species, genus, family, size range (mm FL) and sample size (n) of prey fish used to construct regression lines of bone length versus fork length.

Species	Genus	Family	Size Range	n
B. humilis	*Barbus*	Cyprinidae	20.7 – 99.9	183
B. tanapelagius	*Barbus*	Cyprinidae	20.2 – 76.2	93
B. pleurogramma	*Barbus*	Cyprinidae	22.0 – 66.0	34
juvenile 'large' *Barbus* spp.	*Barbus*	Cyprinidae	28.1– 128.9	91
Garra spp.	*Garra*	Cyprinidae	25.5 – 156.2	92
V. beso	*Varicorhinus*	Cyprinidae	48.7 – 85.8	17
O. niloticus	*Oreochromis*	Cichlidae	21.5 – 128.0	47

Due to fact that these cyprinid piscivores masticate prey fish with their pharyngeal jaw, no whole or partial prey fish are found in the digestive tract for identification. Information about prey consumed (species and size) had to be reconstructed from the skeletal elements of prey fish in the digestive tract of the predators. Even when the digestive process is advanced, the slower digestion of bone material and the constant relation between bone length and fish length enable reliable identification and size reconstruction for most prey fish. Over 500 fresh prey fish representing four genera in two families ranging in length from 20 to 150 mm fork length (FL) were measured and dissected (Table 1). Fish were collected as part of several experimental gillnet and trawl programs conducted all over the Bahar Dar Gulf of Lake Tana during 1999 and 2000. After capture, fish were immediately transported to the laboratory. External measurements of the fresh fish were completed to the nearest 0.1 mm with sliding callipers. To select potentially diagnostic bones, fish were macerated using a washing detergent solution (Biotex). After 1 to 4 weeks, depending on the size of the fish, bones were cleaned, measured to the nearest 0,01 mm using a binocular and ocular micrometer and stored in 1% formaline-solution. Least square regression equations were generated to predict original fork length (mm) from measurements of the maximum linear length of the following 14 skeletal elements (premaxillary, maxillary, dentary, articular, hyomandibular, urohyale, preoperculum, operculum, pharyngeal jaw, cleithrum, parasphenoid, quadratum, palatine, and dorsal spine, only *B. pleurogramma*). All skeletal elements were also carefully examined and measured to identify distinguishing characteristics that may be potentially useful for identification of each prey fish species. In addition several scales were removed to test their usefulness for identification.

Statistical Analysis Trophic Dimension

Possible significant inter- and intraspecific differences in diet overlap (Diet Categories and Prey Fish Categories) were investigated using a randomisation test (Bouton et al 1997, Sevenster and Bouton 1998). The first step of the testing procedure is to calculate test statistics for the observed similarity between two groups. The percentage overlap between individuals *a* and *b* was calculated as $O_{ab}=\Sigma_i \min(p_{ai}, p_{bi})$, where p_{ai} is the proportion of resources used by *a* belonging to resource *i*; and min (x, y) is the smallest of x and y. The statistic for interspecific overlap is the mean of the overlaps in all possible heterospecific pairs of individuals. Next step is the randomisation that generates the null-distribution for the test statistics. All *n*1 + *n*2 individuals are pooled and randomly re-divided into two groups of *n*1 and *n*2 individuals. Then the statistics characterising the overlap between groups are recalculated and stored. The randomisation was repeated 1000 times (to stabilise the average). In the final step, the observed average overlap between groups are compared to their null-distributions. If less than the conventional 5% (one-tailed tests) of the simulated interspecific overlaps are smaller than the observed interspecific overlap, then the diets of the groups differ significantly more than expected if all individuals belonged to the same dietary background. All calculations were performed in Matlab 6.1.

The diet overlap (Diet Categories [nine] and Prey Fish Categories [eight]) was calculated using the similarity index of Schoener (1968) and diet breadth (Diet Categories and Prey Fish Categories) using the formula of Levins (1968; see Section Niche overlap and Niche breadth).

To analyse differences in prey size among the piscivorous *Barbus* species ANOVAs were performed. If ANOVAs were significant, multiple-comparisons (Bonferroni adjusted) were used.

Spatial dimension

Horizontal and vertical distribution

In the Bahar Dar Gulf of Lake Tana six habitats (littoral rock, LR; littoral sand, LS; sublittoral bottom, SLB; sublittoral surface, SLS; pelagic bottom, PB; pelagic surface PS) were selected, differing in substrate, depth, and distance to shore. Three sampling stations were chosen within each of these habitats (Fig. 2b [LR 1, LR 2, LR 3 – P 1, P 2, P 3], Table 3). The surface area covered by the littoral zones (0-4 m depth, habitat LR and LS) in Lake Tana is approximately 10% of the total surface area. The fraction covered by the sublittoral (4-8 m depth) and pelagic zone (>8 m depth) is estimated to be 20% and 70% respectively. Data on fish distribution were collected using gillnets with four different mesh sizes: 30, 40, 50 and 60 mm bar mesh. Panel length and depth were 200 m and 3 m respectively for each mesh. At the sublittoral and pelagic sampling stations a set of gillnets was set simultaneously at the bottom and at the surface. Nets were set overnight from 18:00 to 6:00 hours, monthly from October 1999 - October 2001. In the morning the fish were transported fresh to the laboratory, all *Barbus* species were identified as belonging to one of the fifteen species, following Nagelkerke and Sibbing (2000) and FL was measured to the nearest cm. The catches from the different nets, sampling dates and stations were pooled per habitat during analysis.

Statistical Analysis Spatial Dimension

Principal component analysis (PCA) biplots were used to describe (size-)specific habitat use of each of the piscivorous and non-piscivorous *Barbus* species and of their potential prey fish species according to Piet et al. (1999). Possible significant inter- and intraspecific differences in the frequencies of habitat use were analysed using G-tests (Fowler et al. 1998). The critical values of significance of differences at χ^2= 0.05 were corrected (P<[0.05/n], n= the total number of comparisons) due to the multiple comparisons, in order not to overestimate the number of significant differences (Cooper 1968). Overlap in habitat use (six categories) was calculated using the similarity index of Schoener (1968) and habitat breadth using the formula of Levins (1968; see Section Niche overlap and Niche breadth).

Table 2. Description of habitat and sampling stations in the Bahar Dar Gulf of Lake Tana (cf. Figure 2): their code, local name, depth, distance to shore, substrate [S] (r=rock, m=mud, s=sand), border land-water [B] (p= papyrus, fp= floodplain, temporary submerged vegetation, r=rock, t=tree, al=agricultural crop land) and coordinates (each minute 1.8 km).

Habitat	Station		Depth (m)	Distance (m)	S	B	Coordinates	
	Code	Local name					(N)	(E)
Littoral Rock	LR 1	Mushe	1.5-3	5-25	r	p	11°44′	37°19′
	LR 2	Gerima	1.5-3	5-25	r	p	11°37′	37°23′
	LR 3	Blue Nile	1.5-3	5-25	r	r/t	11°38′	37°24′
Littoral Sand	LS 1	Menkir Dinga	1.5-3	5-25	m	p	11°46′	37°19′
	LS 2	Gumatris	1.5-3	5-25	m	fp	11°39′	37°25′
	LS 3	Bosit	1.5-3	5-25	m/s	r/al	11°45′	37°26′
Sublittoral	SL 1	East Ambo Bahir	4-7	500-1000	m	-	11°45′	37°20′
	SL 2	Airport	4-7	500-1000	m	-	11°38′	37°21′
	SL 3	Boled	4-7	500-1000	m	-	11°43′	37°25′
Pelagic	P 1	North Mid Gulf	10-14	2000-5000	m/s	-	11°45′	37°22′
	P 2	Mehal Zegi	10-13	2000-5000	m	-	11°42′	37°23′
	P 3	Kentefami	10-11	2000-5000	m/s	-	11°39′	37°23′
	P 4	Mehal Zegi-Kentafami	10-12	2000-5000	m/s	-	11°41′	37°23′
	P 5	Kibran- Kentafami	10-12	2000-5000	m/s	-	11°39′	37°21′

Table 3. Samples size and length ranges (cm FL) of the piscivorous and non-piscivorous *Barbus* species used to determine niche segregation along trophic (Diet Categories = DC; Prey Fish Categories = PFC) and spatial dimensions. If n < 15samples were neglected, indicate by '-'.

		Trophic Dimension					Spatial Dimension	
		5-10	10-15	15-20	20-30	30-40	20-30	30-40
B. acutirostris	DC	33	85	37	147	58	1738	246
	PFC				45	97		
B. dainellii	DC	-	19	-	24	19	72	31
	PFC				14	16		
B. gorguari	DC	22	49	-	36	15	166	44
	PFC				12	20		
B. longissimus	DC	-	-	-	18	34	103	157
	PFC				13	28		
B. macrophtalmus	DC	54	102	32	69	59	980	211
	PFC				31	30		
B. megastoma	DC	24	28	26	54	50	557	610
	PFC				36	38		
B. platydorsus	DC	20	50	28	70	29	425	107
	PFC				37	23		
B. truttiformis	DC	-	23	-	17	59	383	434
	PFC				17	46		

	8-25	15-25	20-40
B. brevicephalus	184		6137
B. crassibarbis		20	142
B. gorgorensis		25	156
B. nedgia		75	398
B. surkis		57	903
B. tsanensis		95	3024
B. intermedius SC		40	4154

Temporal dimension

Diurnal vertical migration

In order to investigate vertical migartion six sampling stations were chosen within the deep, offshore pelagic habitat. Three stations (PB/S 1, PB/S 2, PB/S 3) were sampled at night time (±18:00-6:00 hours) monthly from October 1999-September 2001 and 3 stations (PB/S 3, PB/S 4, PB/S 5) at day time (±6:00-18:00 hours) monthly from December 2000-December 2001 (Table 3 and Fig. 2b) . A previously described arrangement of gillnets (see 2.1.1 Horizontal and vertical distribution) was set simultaneously at the bottom and at the surface during sampling. The proportional composition of the eight piscivorous *Barbus* species at time-space combinations (night/day, bottom/surface) were compared using χ^2 *r x c* contingency table analysis (Fowler et al. 1998).

Time of active feeding

Similar sized *B. acutirostris* (256 ,264, 269, 272, and 301 mm FL), *B. macrophtalmus* (278, 286, 291, 294, and 297 mm FL), *B. megastoma* (292, 295, 303, and 332 mm FL), and *B. truttiformis* (272, 281, 304, and 309 mm FL) were caught using hook-and-line, fish traps or bottom trawl in Lake Tana and its affluent rivers. The fish were acclimatised for at least one month before the start of the experiments. Predators were individually housed before trials in 250-l tanks (90x45x45 cm). Each 250-l tank was partitioned by a PVC-slate into two equal parts and one predator was held per section (45x45x45 cm). Predators were daily fed with life prey fish (*B. humilis* of ±45 mm FL) which were captured daily in the lake using dipnets, sorted according to size and held in 250-l tanks. Prey fish were fed with commercial flake food. The 16 indoor 250-l tanks were part of a semi-closed system; part of the water was replaced every day with water pumped directly from the lake. The aquarium room was not illuminated by artificial light but followed the natural light cycle. The experiments were conducted in six outdoor pools (diameter 300 cm, height 100 cm) with a water depth of 50 cm from February 2001 to August 2001. The pools were located in an open shed adjacent to the lake, the sides were only covered by nets to prevent piscivorous birds from entering the pool area. The pools were part of an open system. Water was pumped directly from the lake, distributed over the experimental pools and flowed back to the lake. Water temperature, oxygen levels and transparency were similar to lake conditions (see de Graaf et al. 2003). To determine the time of active feeding predators were allowed to forage for four hours on a school of 30 prey fish during four different periods of day; dawn (4:00-8:00), dusk (16:00-20:00), midday (10:00-14:00) and midnight (22:00-2:00). A total of 2160 *B. humilis* and 18 predators were used in the experiment. Fine-meshed nets (3x1 mm) adjusted to the shape of the pools were placed inside each pool. The net was lifted at the end of each trial, facilitating the quick removal of the predator and counting of the remaining prey fish. Predators were randomly assigned to a pool and were trained until they foraged willingly despite the regular handling during lifting of the net. Next the predators were exposed to a random sequence of the four different forage periods. A minimum of 12 hours fasting was maintained between the experimental forage periods. To analyse the effect of time on feeding activity within subject ANOVAs were performed. The dusk and dawn data were pooled before analysis. If ANOVAs were significant, multiple-comparisons (Bonferroni adjusted) were used.

Results

Trophic dimension

Diet Categories

Ontogeny of piscivory

Size-specific diet (shifts) of the piscivorous *Barbus* species (Diet Categories) is presented in Figure 3. The piscivorous *Barbus* were divided in five size classes and only those size classes with n>15 were used in the analysis (Table 3). *Barbus acutirostris* is already piscivorous at 10-15 cm FL (54% fish), smaller individuals (5-10 cm FL) consume considerable amounts of insect larvae (31%). The proportion of fish in the diet increases to ca. 80% in the larger size classes (>20 cm FL). *Barbus dainellii* is piscivorous at 10-15 cm FL (52% fish) but its diet also contains adult insects (17%). The proportion of fish increases to more than 70% in large fish (>20 cm FL). The diet of small (5-10cm FL) *B. gorguari* consists predominantly of larval (38%) and adult insects (23%). The proportion of fish increases rapidly from 49% (10-15cm FL) to more than 70% in fish larger than 20 cm FL. Unfortunately not enough *B. longissimus* smaller than 20cm FL were collected to study ontogenetic diet shifts. In the larger specimens the proportion of fish in the diet is high, around 90%. *Barbus macrophtalmus* is one of the species that shifts towards piscivory late in ontogeny. At 5-10 cm FL the diet consists mainly of larval (30%) and adult insects (30%) and zooplankton (22%). At 10-15 and 15-20 cm FL insect larvae form the main part of their diet (33% and 48%, respectively). Only individuals larger than 20 cm FL are piscivorous (60% fish). The diet of the smallest *B. megastoma* (5-10cm FL) consists mainly of insects (38%) and insect larvae (27%). Although more or less piscivorous at 10-15 cm FL (despite the low percentage of fish (32%) in size class 15-20 cm FL), *B. megastoma* is the only species were adult insects form consistently a part of the diet (5-10 cm FL, 38%; 10-15 cm FL, 19%; 15-20 cm FL, 44%; 20-30 cm FL 11%). *Barbus platydorsus* is the second species with a late shift towards piscivory during ontogeny. Insect larvae (73%) form by far the most important part of the diet of small (5-10 cm FL) *B. platydorsus*. Between 10 and 20 cm FL, the percentage of insect larvae decreases and the percentage of fish and especially detritus increases (51%, 15-20cm FL). After 20 cm FL *B. platydorsus* is piscivorous (>50% fish), the percentage of fish the diet increases >70% in large (>30cm FL) mature individuals. *Barbus truttiformis* of 10-15 cm FL is already piscivorous (59% fish), the larger size classes have the highest percentages of fish (20-30 cm FL, 98% and 30-40 cm FL, 95%).

Among the piscivorous *Barbus* species all main ontogenetic diet shifts occurred before 20 cm FL, their immature, juvenile stage (de Graaf et al. 2003). To determine the extent of ecological segregation among the piscivorous *Barbus* we decided to focus on adult specimens between 20-40 cm FL because: (a) in this size range individuals of the different piscivorous *Barbus* spp are in the same ontogenetic stage, and (b) the size class 20-40 cm FL formed more than 90% of all the fish caught in the multi-mesh experimental gillnet program.

Diet segregation (Diet Categories) of the non-piscivorous *Barbus* species is presented in Figure 3. Each non-piscivorous *Barbus* species was pooled into one large size-class (Table 3). The macrophytivore, *B. surkis* (81% macrophytes) and the molluscivore *B. gorgorensis*, the only species with a considerable

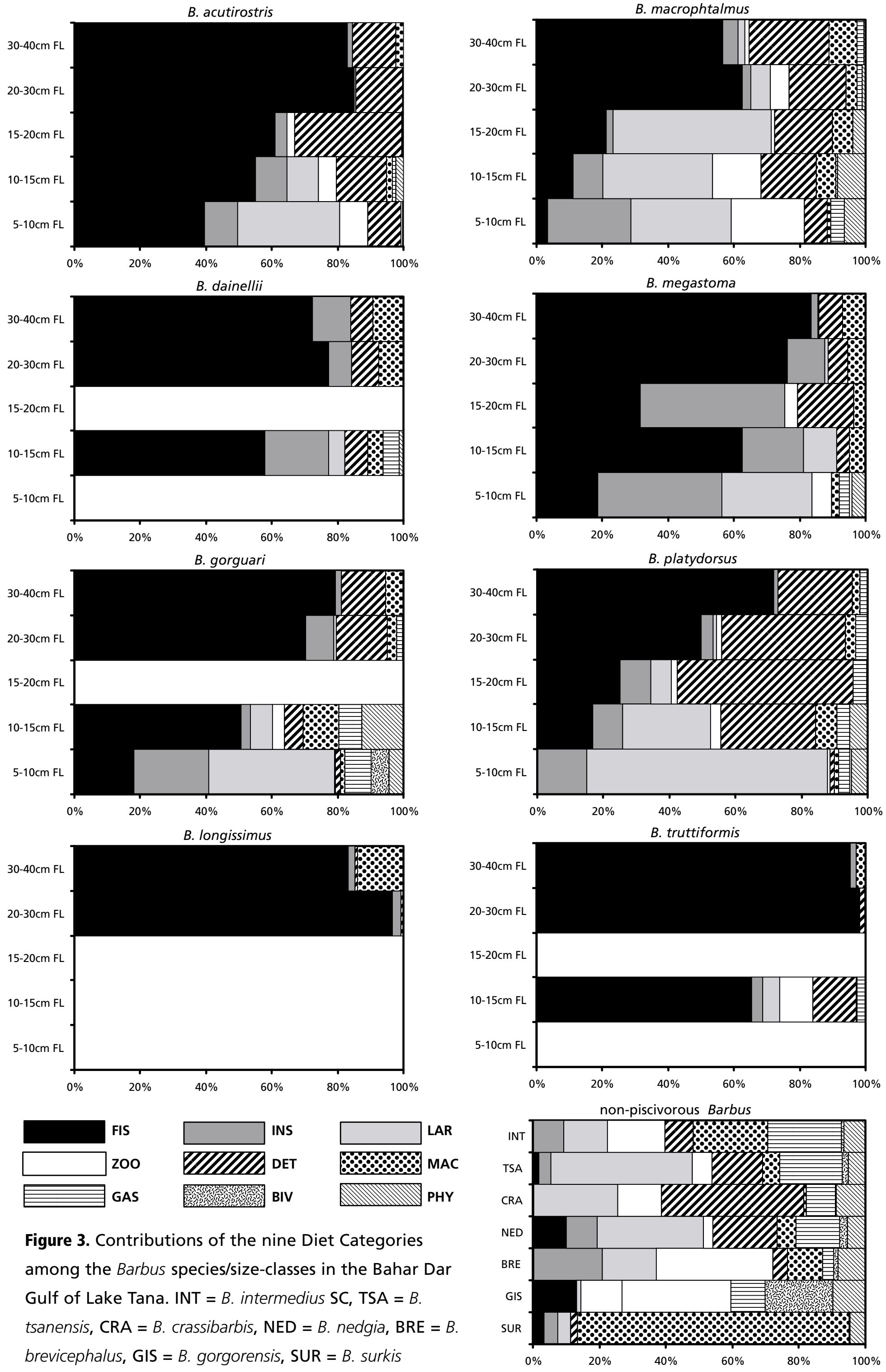
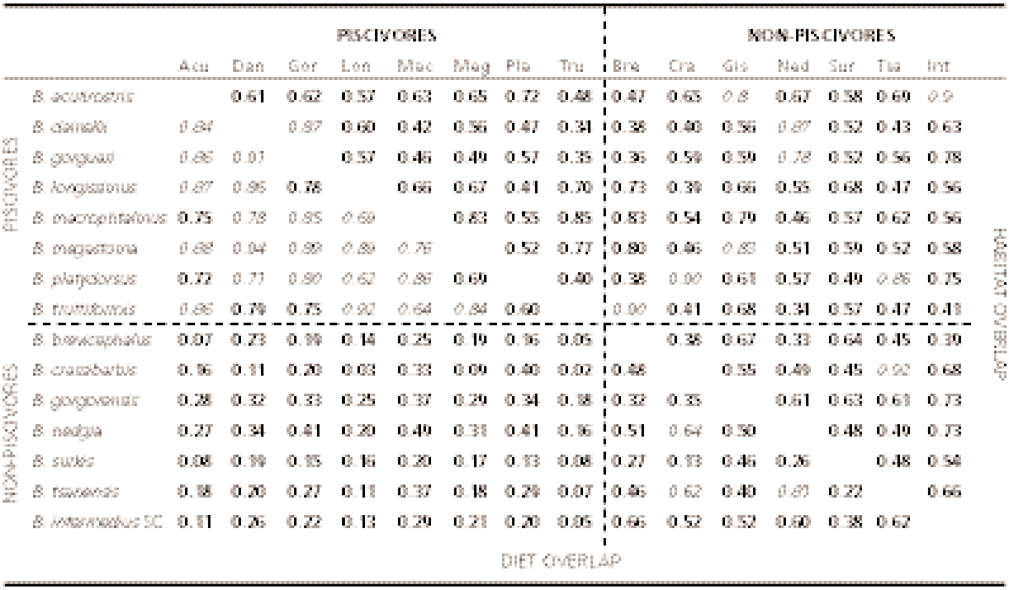


Figure 3. Contributions of the nine Diet Categories among the *Barbus* species/size-classes in the Bahar Dar Gulf of Lake Tana. INT = *B. intermedius* SC, TSA = *B. tsanensis*, CRA = *B. crassibarbis*, NED = *B. nedgia*, BRE = *B. brevicephalus*, GIS = *B. gorgorensis*, SUR = *B. surkis*

amount of bivalves in its diet (19%) are clearly segregated from the other five non-piscivorous *Barbus* species. Zooplankton (34%) and insects (20%) form a major part of the diet of only *B. brevicephalus*. Of the four benthivorous species *B. intermedius* SC has the least specialised diet of the non-piscivorous species. Insect larvae form the dominant part in the diets of *B. nedgia* (31%) and *B. tsanensis* (42%). *Barbus crassibarbis* has the highest proportion of detritus (42%) in its diet.

Table 4. Niche overlap value along the trophic (Diet Categories) and spatial dimensions. Left half: inter-specific diet overlap value, values that differ significantly (*P*<0.01; randomisation tests) are printed in bold face. Right half: interspecific habitat overlap value, values that differ significantly (*P*<0.01; G-tests) are printed in bold face. Note that values less than 0.4 ("insignificant" overlap) or greater than 0.6 ("significant" overlap) have been considered



		PISCIVORES								NON-PISCIVORES						
		Acu	Dan	Gor	Lon	Mac	Mag	Pla	Tru	Bre	Cra	Gis	Ned	Sur	Tsa	Int
PISCIVORES	*B. acutirostris*		0.61	0.62	0.57	0.63	0.65	0.72	0.48	0.47	0.65	*0.8*	0.67	0.58	0.69	*0.9*
	B. dainellii	*0.84*		*0.87*	0.60	0.42	0.56	0.47	0.31	0.38	0.40	0.56	*0.87*	0.52	0.43	0.63
	B. gorguari	*0.86*	*0.87*		0.57	0.46	0.49	0.57	0.35	0.36	0.59	0.59	*0.78*	0.52	0.56	0.78
	B. longissimus	*0.87*	*0.95*	0.78		0.66	0.67	0.41	0.70	0.73	0.39	0.66	0.55	0.68	0.47	0.56
	B. macrophtalmus	0.75	*0.78*	*0.85*	*0.69*		0.83	0.55	0.85	0.83	0.54	0.79	0.46	0.57	0.62	0.56
	B. megastoma	*0.88*	*0.94*	*0.89*	*0.89*	*0.76*		0.52	0.77	0.80	0.46	*0.83*	0.51	0.59	0.52	0.58
	B. platydorsus	0.72	*0.71*	*0.90*	*0.62*	*0.86*	0.69		0.40	0.38	*0.90*	0.61	0.57	0.49	*0.86*	0.75
	B. truttiformis	*0.86*	0.79	0.75	*0.92*	*0.64*	*0.84*	0.60		*0.90*	0.41	0.68	0.31	0.57	0.47	0.41
NON-PISCIVORES	*B. brevicephalus*	0.07	0.23	0.19	0.14	0.25	0.19	0.16	0.05		0.38	0.67	0.33	0.64	0.45	0.39
	B. crassibarbis	0.16	0.11	0.20	0.03	0.33	0.09	0.40	0.02	0.48		0.55	0.49	0.45	*0.92*	0.68
	B. gorgorensis	0.28	0.32	0.33	0.25	0.37	0.29	0.34	0.18	0.32	0.35		0.61	0.63	0.61	0.73
	B. nedgia	0.27	0.34	0.41	0.20	0.49	0.31	0.41	0.16	0.51	*0.64*	0.50		0.48	0.49	0.73
	B. surkis	0.08	0.19	0.15	0.16	0.20	0.17	0.13	0.08	0.27	0.13	0.46	0.26		0.48	0.54
	B. tsanensis	0.18	0.20	0.27	0.11	0.37	0.18	0.29	0.07	0.46	*0.62*	0.40	*0.87*	0.22		0.66
	B. intermedius SC	0.11	0.26	0.22	0.13	0.29	0.21	0.20	0.05	0.66	0.52	0.52	0.60	0.38	0.62	

DIET OVERLAP

HABITAT OVERLAP

Diet overlap and diet breadth

Diet overlap values between all the piscivorous and non-piscivorous *Barbus* species are summarised in Table 4. Eighty-one of the 105 pairwise interspecific comparisons were significantly different. Among the non-piscivorous *Barbus* species the diets of *B. nedgia*, *B. tsanensis* and *B. crassibarbis* did not differ significantly. Insect larvae, detritus and gastropod molluscs form the main part of the diet of all three species.

Niche breadth along the trophic dimension (Diet Categories) is much wider among the non-piscivorous than among the piscivores *Barbus* species (Fig. 4a). *Barbus intermedius* SC has the most diversified diet within the *Barbus* species flock. Based on Levins Index for niche breadth, the macrophytivore *B. surkis* is the only food specialist among the non-piscivorous *Barbus* species (Fig. 4a). When looking at diet categories, all the piscivores are more or less specialists with narrow diet breadths compared with the non-piscivorous species. Food categories other than fish are rare in the diet of the piscivorous *Barbus* species larger than 20 cm FL. This results therefore in high diet overlap values and few significant differences between these eight *Barbus* species in this respect. To further investigate ecological segregation between these piscivorous *Barbus* species it was necessary to determine exactly which prey species (and sizes) contributed to their diet.

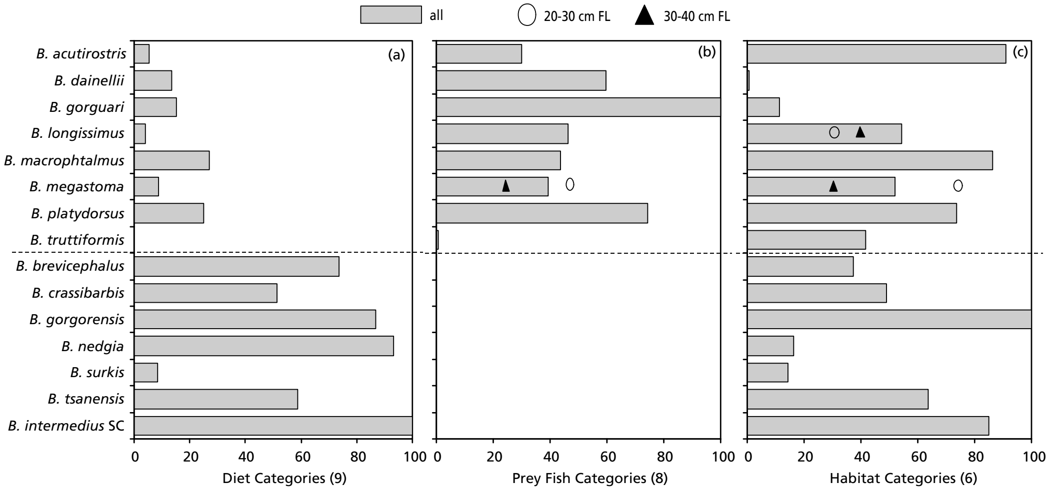


Figure 4. Niche breadth per species/size-class along the trophic (Diet Categories and Prey Species Categories) and spatial (Habitat Categories) dimensions. The value for niche breadth is scaled relative to the extremes of all species/size-classes combined. (a) Diet breadth (Diet Categories) for all *Barbus* species (bars), (b) Diet breadth (Prey Species Categories) for the eight piscivorous *Barbus* species (bars) and per size class (*B. megastoma*; circle 20-30 cm FL, triangle 30-40 cm FL), and (c) Habitat breadth for all *Barbus* species (bars) and per size class (*B. longissimus* and *B. megastoma*; circles 20-30 cm FL, triangles 30-40 cm FL).

Prey Fish Categories

Calibration and Identification

Barbus humilis is by far the most abundant prey species in the Bahar Dar Gulf, followed by *B. tanapelagius* (Figure 5). *Garra* spp. and juvenile (<10cm FL) *Barbus* spp and *O. niloticus* were regularly caught but only in small numbers. *Barbus pleurogramma*, and juvenile *C. gariepinus* and *V. beso* are rare in the lake. The latter three species/size-classes occur predominantly amongst the submerged vegetation in the floodplains and upstream inflowing rivers and are hardly available as prey for the piscivorous *Barbus* species in the lake.

The potential value of scale morphology as a tool for identification of prey fish from the intestines of piscivorous *Barbus* was limited. Scale type, shape and number of striae only partially resolve the identification of the prey species. Major disadvantages of scales in comparison with bones are that, (a) it is not possible to reconstruct prey size and number of prey fish eaten, and (b) scales are less resistant against degradation. Often scales were not present in the cleaned gut contents and if present usually severely damaged so that they could not be "read". Only in few instances could scales be used for prey species identification.

From the 14 head bones studied and measured to determine species specific morphological markers, species specific ratio's, which were consistent over the whole size range were identified on the

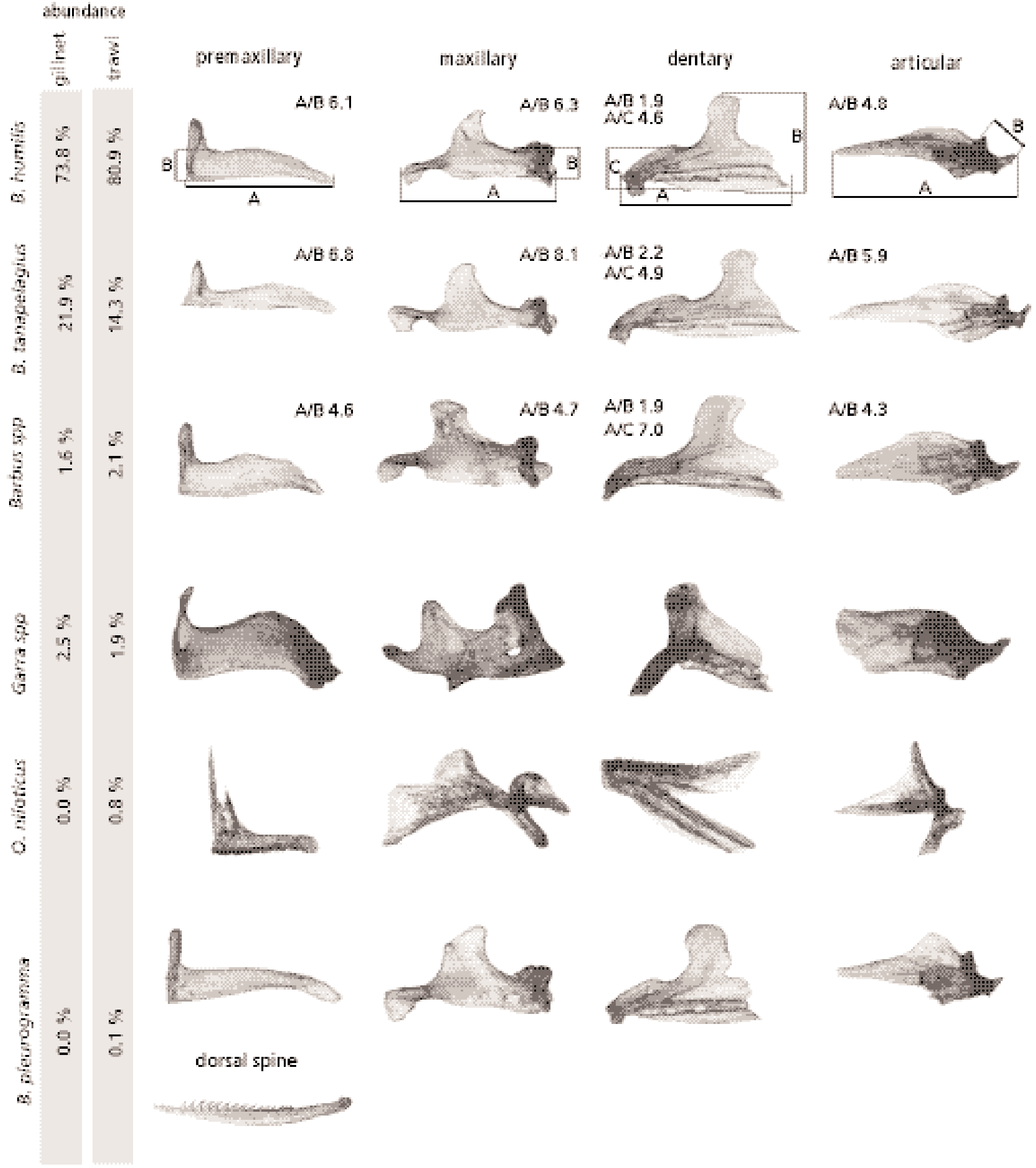


Figure 5. Left premaxillary, maxillary, dentary, and articular of *B. humilis*, *B. tanapelagius*, juvenile large *Barbus* spp, *Garra* spp, juvenile *O. niloticus* and *B. pleurogramma*. Premaxillary, dentary and articular are presented in a lateral view (exterior), the left maxillary is viewed from the interior. The diagnostic characters, used for identifying species from skeletal elements is shown in *B. humilis*. The average value for each ratio in *B. humilis*, *B. tanapelagius* and *Barbus* spp is given at the top of each bone (cf. Table 8). Abundance of potential prey species (<10 cm FL) in the Bahar Dar Gulf of Lake Tana, data from Dejen et al., in prep. Gillnet, 5-12.5 mm bar mesh; habitats LR, LS, SLB, SLS, PB, and PS; total n=75,305 fish, *Varicorhinus beso,* 0.0% and *Clarias gariepinus*, 0.0%). Trawl, codend 5 mm bar mesh; habitats LS, SLB, and PB; total n=235,795 fish, *Varicorhinus beso* 0.0% and *Clarias gariepinus* 0.0%).

premaxillary, maxillary, dentary and articular of *B. humilis*, *B. tanapelagius* and juvenile large *Barbus* spp (Fig. 5, Table 6). The bones of *O. niloticus* and *Garra* spp are easy to identify (Fig. 5). *Barbus pleurogramma* is rare in the lake and has a conspicuously serrated dorsal spine, which is lacking in the other *Barbus* species. Small sized (<10 cm FL or TL) *V. beso* and *C. gariepinus* do not occur in the lake. Regressions relating measurements of the 14 skeletal elements to standard length of potential prey species/size-classes were all highly significant (*P*<0.001) with r^2 values ranging from 0.86 to 0.99 (Table 5). Original prey species and sizes could now easily and accurately be reconstructed from the ingested intestinal remains of the piscivorous *Barbus* spp.

Table 5. Regression statistics (Y = aX + b) relating measurements (mm) of skeletal elements (X) to standard length (Y).

		Premaxillary	**Maxillary**	**Dentary**	**Articular**	**Urohyal**	**Hyomandibular**	**Preoperculum**	**Operculum**	**Pharyngeal jaw**	**Cleithrum**	**Parasphenoid**	**Quadratum**	**Palatine**	**Dorsal spine**
B. humilis	a	20.12	17.2	16.15	18.72	11.71	10.25	8.56	9.48	12.15	6.31	6.76	25.74	25.33	
	b	-8.26	-3.73	-8.37	-7.07	0.5	0.74	-3.23	1.24	-1.29	-1.03	-8.63	-11.33	-1.51	
	r^2	0.97	0.98	0.98	0.98	0.96	0.98	0.98	0.98	0.97	0.99	0.98	0.95	0.97	
B. tanapelagius	a	17.46	17.56	14.1	17.47	12.26	11.24	9.06	10.51	15.27	7.16	7.66	26.26	25.99	
	b	-7.84	-8.68	-10.28	-10.74	-3.84	-2.4	-7.79	-2.08	-9.55	-5.3	-14.96	-15.15	-4.82	
	r^2	0.91	0.9	0.91	0.91	0.96	0.95	0.93	0.96	0.94	0.97	0.95	0.86	0.92	
B. pleurogramma	a	18.7	16.17	16.02	17.94	10.12	11.01	8.02	10.22	11.43	6.24	6.72	21.61	28.42	4.49
	b	-3.05	-0.64	-4.94	-1.23	4.1	-0.44	-0.7	-0.81	-0.77	-1.02	-6.58	-5.51	1.17	9.52
	r^2	0.93	0.95	0.94	0.9	0.9	0.95	0.95	0.96	0.96	0.98	0.97	0.93	0.9	0.92
Juvenile ‘large’ *Barbus* spp	a	18.38	15.47	13.71	16.8	13.01	9.99	8.32	9.31	10.92	6.43	5.72	21.21	24.34	
	b	-4.67	-1.03	-3.47	-4.45	-5.18	2.36	-3.36	3.64	1.45	-0.63	-7.93	-1.54	-1.54	
	r^2	0.96	0.95	0.97	0.96	0.97	0.97	0.98	0.98	0.97	0.97	0.98	0.95	0.96	
Garra spp	a	22.37	20.21	22.06	24.97	17.13	15.86	14.16	15.24	22.09	9.69	8.76	21.8	26.13	
	b	-3.85	-9.15	-5.77	-6.69	-10.77	-8.61	-10.26	-7.61	-17.98	-11.59	-16.46	-6.1	-3.59	
	r^2	0.89	0.88	0.84	0.97	0.91	0.97	0.97	0.96	0.96	0.97	0.97	0.96	0.97	
O. niloticus	a	13.63	13.47	13.33	15	8.99	8.23	5.11	6.78	7.76	3.77	4.78	13.14	15.73	
	b	2.65	0.45	0.22	-4.31	-0.28	-0.71	-0.33	2.42	1.54	-0.98	-2.49	-2.52	0.49	
	r^2	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.99	0.98	0.99	0.99	

Table 6. Description of species specific ratio's of premaxillary, maxillary, dentary and articular (see also Fig. 5) 95% CI = 95% confidence intervals, min = minimum value, max = maximum value, n = sample size.

	ratio		mean	95% CI	min	max	n
Premaxillary	A/B	*B. humilis*	6.1	0.09	5.1	7.4	124
		B. tanapelagius	6.8	0.19	5.6	9.6	57
		Barbus spp	4.6	0.07	4.0	5.8	76
Maxillary	A/B	*B. humilis*	6.3	0.09	5.0	8.2	159
		B. tanapelagius	8.1	0.22	6.3	12.2	75
		Barbus spp	4.7	0.11	3.6	5.8	76
Dentary	A/B	*B. humilis*	1.9	0.02	1.7	2.2	162
		B. tanapelagius	2.2	0.03	1.9	2.6	74
		Barbus spp	1.9	0.01	1.7	2.2	76
	A/C	*B. humilis*	4.6	0.09	3.8	7.8	128
		B. tanapelagius	4.9	0.09	4.0	5.6	59
		Barbus spp	7.0	0.19	5.3	8.9	76
Articular	A/B	*B. humilis*	4.8	0.05	3.7	5.5	156
		B. tanapelagius	5.9	0.11	5	7.1	73
		Barbus spp	4.3	0.07	3.5	4.8	75

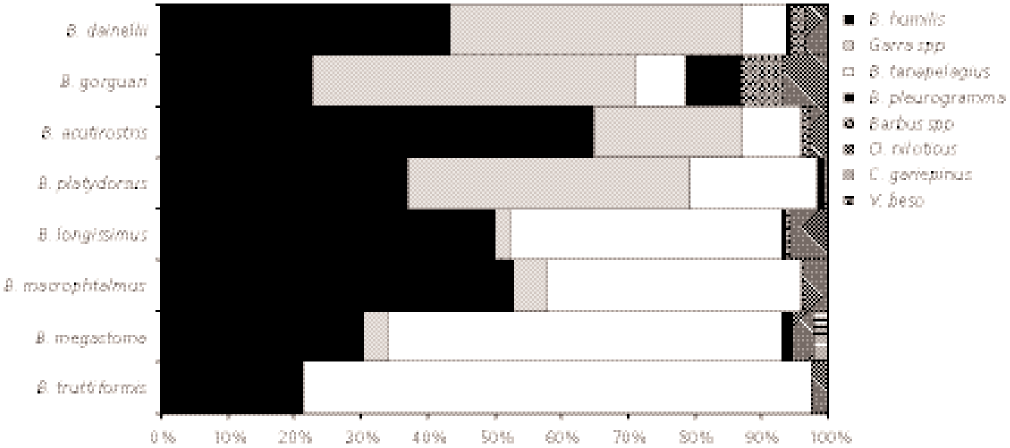


Figure 6. Prey species composition of the eight piscivorous *Barbus* species (20-40 cm FL) as reconstructed from the skeletal remains (see Table 3 for details on sample size).

Table 7. Inter-specific diet overlap (Prey Species Categories). Values that differ significantly (*P*<0.01; randomisation tests) are printed in bold face. (see also Fig. 6)

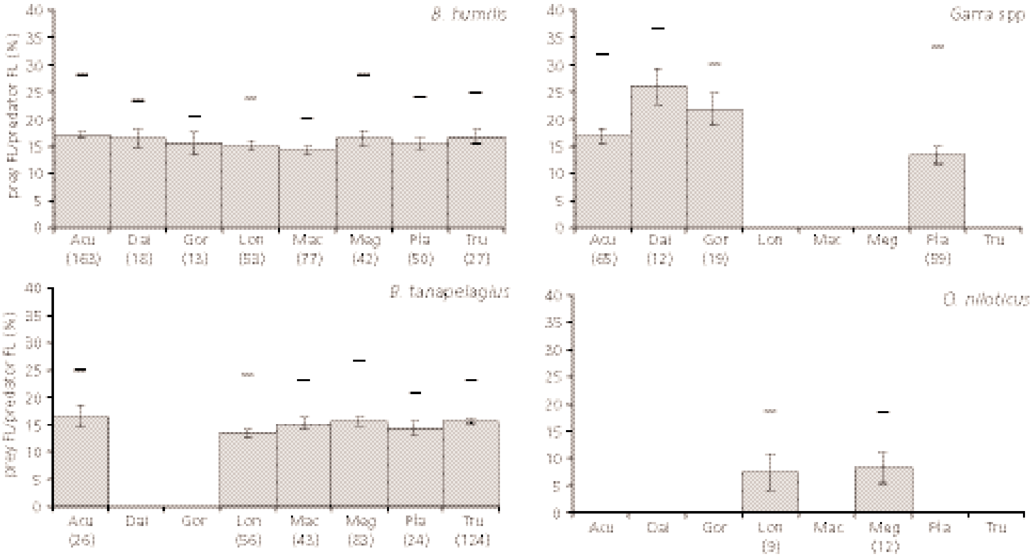
	PISCIVORES							
	Acu	Dan	Gor	Lon	Mac	Meg	Pla	Tru
B. acutirostris		*0.76*	**0.57**	**0.65**	**0.70**	**0.46**	**0.68**	**0.33**
B. dainellii			*0.79*	**0.57**	**0.59**	**0.45**	*0.87*	**0.31**
B. gorguari				**0.40**	**0.39**	**0.39**	*0.74*	**0.32**
B. longissimus					*0.95*	*0.77*	**0.59**	**0.65**
B. macrophtalmus						*0.75*	**0.61**	**0.63**
B. megastoma							**0.55**	*0.83*
B. platydorsus								**0.40**
B. truttiformis								

Prey species composition, diet overlap and diet breadth

Barbus megastoma is the only species with a highly significant ($P<0.001$; randomisation test) difference in prey species composition between the two adult size classes. Smaller specimens (20-30 cm FL) have a wider diet breadth consisting mainly of *B. humilis* (49%) and *B. tanapelagius* (42%). In larger *B. megastoma* diet breadth narrows (Fig 4b) and *B. tanapelagius* increases from 42 to 67% in the diet. To take into account the size-specific difference in diet by small and large *B. megastoma*, the diet use was calculated from the weighted diet use of the two different size classes following Piet et al (1999). *Barbus truttiformis* has the narrowest diet breadth (Fig. 4b) and feeds almost exclusively on *B. tanapelagius* very much like *B. megastoma* (Fig. 6). The prey composition is significantly different from all other piscivorous *Barbus* (Table 7) *Barbus longissimus* and *B. macrophtalmus* both eat almost equal amounts of *B. humilis* and *B. tanapelagius*. The diets of *B. dainellii*, *B. gorguari* and *B. platydorsus* do not differ significantly and consist predominantly of *B. humilis* and *Garra*. However, *B. dainellii* and *B. gorguari* probably feed on *G. dembecha* which is common in the littoral areas (see Fig. 8b), while *B. platydorsus* most likely preys upon "*G. tana*" and "*G. microstoma*". *Barbus gorguari* has the broadest diet and is the only piscivorous species with reasonable amounts (5-10%) of *B. pleurogramma*, juvenile *O. niloticus* and juvenile large *Barbus* spp in its diet. The diet of the abundant bottom-dwelling *B. acutirostris* consists mainly of *B. humilis*.

Relative prey size

In contrast to the large and significant difference in prey species composition, the size of the ingested prey compared to predator size is remarkably similar among all the piscivorous species (ca. 15% FL, Fig 7). However significantly ($F_{3,\ 151}=19.6$; $P<0.001$) larger *Garra* is eaten by *B. dainellii* (26% FL) and *B. gorguari* (22% FL) than by *B. acutirostris* (17% FL) and *B. platydorsus* (13% FL). On the other hand, the average size of ingested *O. niloticus* is clearly smaller (ca. 7% FL) than that of the other prey species.



Spatial dimension

Horizontal and vertical distribution

Size-specific resource use of the (non-)piscivorous *Barbus* and potential prey species along the spatial dimension is presented in PCA biplots (Fig. 8). This technique clearly distinghuises groups of species/size-classes that share the same habitat. The rare *B. dainellii* and *B. gorguari* are most abundant in the littoral zone over rocky substrate (57% and 54% respectively). *B. platydorsus* is a bottom-dwelling species in the sublittoral (20%) and pelagic (35%) areas. *Barbus acutirostris* has a wide distribution covering the littoral (36%), sublittoral (14%) and pelagic (13%) zones near the bottom. In contrast to *B. platydorsus* the abundance of *B. acutirostris* decreases with distance to shore. Small and large adult *B. longissimus* and *B. megastoma* have highly significantly ($P<0.001$, G-test) different spatial distributions. Small *B. longissimus* occurs predominantly above rocky substrate (50%) in the littoral zone while large individuals move towards the surface area of the sublittoral (43%). Small *B. megastoma* are widely distributed throughout the littoral, sublittoral and pelagic zones near the surface. However, larger *B. megastoma* have a much narrower distributed and are predominantly found in the pelagic surface area (48%). *Barbus truttiformis* has a rather narrow distribution and occurs mainly in the sublittoral (29%) and pelagic (42%) near the surface. The distribution of *B. macrophtalmus* is similar to *B. truttiformis* although more regularly caught near the bottom in the sublittoral and even in the littoral zones than *B. truttiformis*.

The abundant *B. tanapelagius* is the only common prey species at the surface in the sublittoral (32%) and pelagic (36%) zones of Lake Tana (Fig. 8b). *Barbus pleurogramma* (72%) and *G. dembecha* (54%) are restricted to the littoral rocky areas. The highly abundant *B. humilis* occurs predominantly in the littoral rocky (72%) area and to a lesser extend near the bottom in the sublittoral (16%). "*G. microstoma*" and "*G. tana*" are bottom-dwelling species that occur over sandy/muddy substrate from the littoral to pelagic zones.

The non-piscivorous *B. nedgia* occurs predominantly over rocky substrate (51%) in the littoral zones (Fig. 8c). *Barbus brevicephalus* is by far the most common species near the surface in the sublittoral (34%) and pelagic (39%) areas. The common benthic *B. intermedius* SC occurs throughout the lake but its abundance decreases with distance to shore. *Barbus tsanensis* and *B. crassibarbis* occur near the bottom in the sublittoral and pelagic areas. *Barbus surkis* is most common near the surface in the sublittoral (53%). *Barbus gorgorensis* has a wide distribution throughout the lake's habitats but is regularly caught in littoral zone over sandy/muddy substrate (30%).

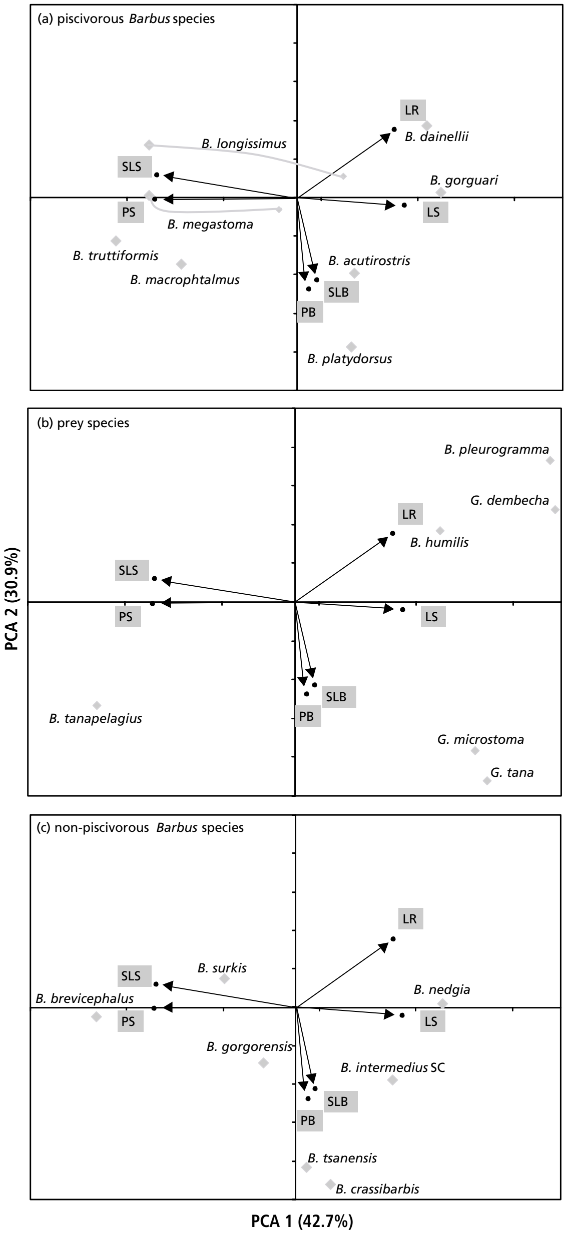


Figure 8 Habitat segregation among the different species/size-classes of the piscivores *Barbus* (a), potential prey species (b), and non-piscivorous *Barbus* (c). The PC-scores of the species on the first two principal components (grey rhombus; x-axis 0.275 to -0.275, y-axis 0.25 to -0.25) and the factor loadings of the resource states (arrows; x-axis 2.75 to -2.75, y-axis 2.5 to -2.5) are plotted. Two adult size classes (20-30 cm FL and 30-40 cm FL) of *B. longissimus* and *B. megastoma* are indicated by increasing size of the rhombus with increasing size range, and are connected with a line. Resource states: LR, littoral rock; LS, littoral sand; SLB, sublittoral bottom; SLS, sublittoral surface; PB, pelagic bottom; PS, pelagic surface.

Habitat overlap and breadth

To take into account the size-specific differences in habitat use by small and large *B. longissimus* and *B. megastoma*, their overall habitat use of was calculated from the weighted habitat use of the different size classes according to Piet et al. (1999). Habitat overlap values between all the piscivorous and non-piscivorous *Barbus* species are summarised in Table 4. Ninety-five of the 105 pairwise interspecific comparisons were significantly different (*P*<0.01, G-tests). The piscivorous *B. gorguari*, *B. dainellii* and the non-piscivorous *B. nedgia* all have a narrow habitat breadth (Fig 4c). These three species are restricted to the rocky substrate in the littoral and did not differ significantly in spatial distribution. On the other the three most widely distributed species *B. acutirostris*, *B. gorgorensis* and *B. intermedius* SC (Fig. 8ac and Fig. 4c) did also not differ significantly. The same holds for the most common piscivore (*B. truttiformis*) and non-piscivore (*B. brevicephalus*) species in the sublittoral and pelagic surface areas. Figure 4c further illustrates the difference in habitat shifts between small and large adult *B. longissimus* and *B. megastoma*. In *B. megastoma* a clear narrowing of the habitat breadth occurs, while in *B. longissimus* a actual shift occurs from littoral rocky to sublittoral surface which does not effect the habitat breadth.

Temporal dimension

Vertical Migration

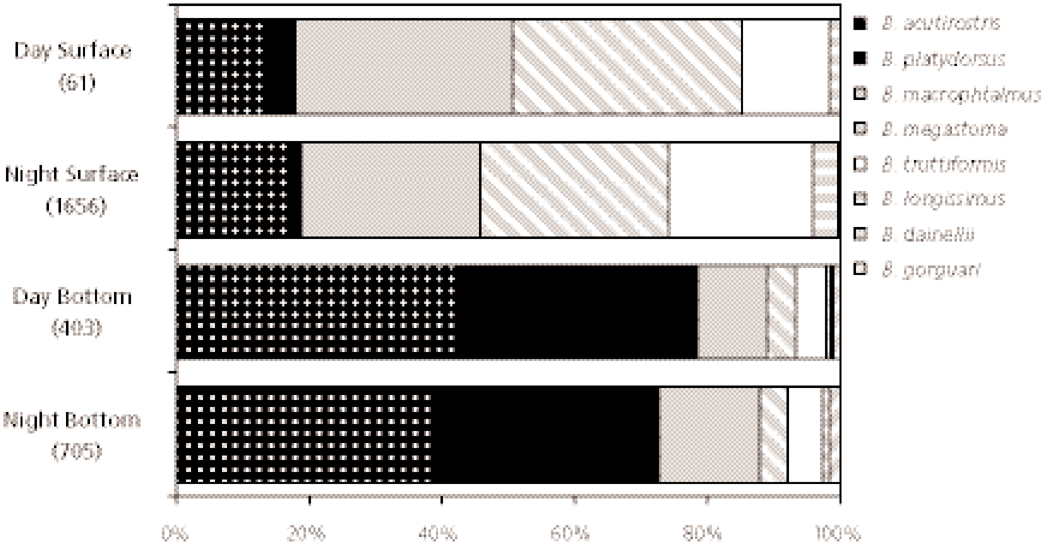


Figure 9. Species composition of the eight piscivorous *Barbus* species as a function of time (day or night) and position in the water column (surface or bottom). Percentages are taken from the total number of piscivorous barbs, indicated between parentheses. Note the highly significant differences in species composition between Night Bottom – Night Surface ($\chi^2_{0.05,\,7}$=735; *P*<0.001) and between Day Bottom-Day Surface ($\chi^2_{0.05,\,7}$=106; *P*<0.001).

The benthic piscivorous community in the sublittoral-pelagic zone of the Bahar Dar Gulf of Lake Tana is dominated by *B. acutirostris* and *B. platydorsus*, while the surface community is dominated by *B. macrophtalmus*, *B. megastoma* and *B. truttiformis* (Fig. 9). The difference in piscivorous species composition between the bottom and surface are highly significantly both at night and at daytime. No differences occur in species composition within the benthic community when comparing night with daytime, the same holds for the surface species community. No diurnal vertical migration occurred by any of the piscivorous *Barbus* species in the sublittoral-pelagic zone. Both habitats, i.e. surface and bottom, have their own specific, separate piscivorous species composition, which do not interact directly. *Barbus dainellii*, *B. gorguari* and to a lesser extend *B. longissimus* are rare in the sublittoral-pelagic zone both at the bottom and at the surface.

Time of active feeding

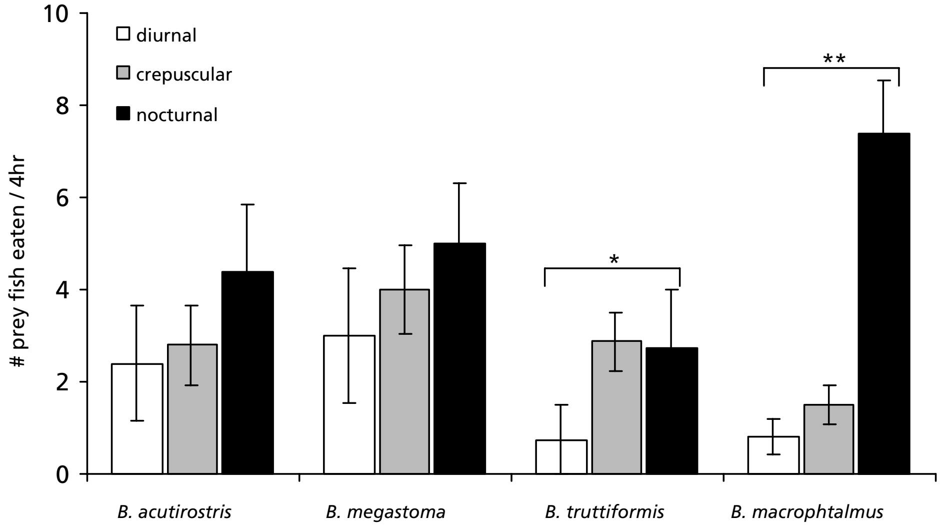


Figure 10. Diurnal, crepuscular and nocturnal feeding activity (# of prey fish eaten) of *B. acutirotris*, *B. megastoma*, *B. truttiformis* and *B. macrophtalmus.* Asterisks indicate significance levels (within subject ANOVAs; *= $P<0.05$, **=$P<0.01$).

No significant effect of period (Fig. 10) was found on the feeding activity of *B. acutirostris* ($F_{2,8}=1.02$, $P=0.41$) and *B. megastoma* ($F_{2,6}=3.27$, $P=0.11$), although feeding activity seemed to be the lowest during the middle of the day (10:00 – 14:00). This pattern of low feeding activity during the day compared to the twilight and night is significant in *B. truttiformis* ($F_{2,6}=5.06$, $P=0.05$). *Barbus macrophtalmus* is highly significantly ($F_{2,8}=19.19$, $P<0.001$) more active during the night compared to midday and twilight. *B. macrophtalmus* is clearly a nocturnal piscivore.

Discussion

Ontogeny of piscivory

During growth piscivorous species usually undergo ontogenetic diet shifts, feeding on progressively larger invertebrate prey before being large enough to feed on other fish (Werner and Gilliam 1984). The length of this ontogenetic trajectory and number of shifts is highly variable among piscivorous fish species, ranging from as short as a few weeks (40mm TL, *Pomatomus saltatrix*, Marks and Conover 1993) to many years (Mittelbach and Persson 1998). Among 27 species of European and North American piscivorous fish, 15 became piscivorous (>50% fish) as young-of-the-year (YOY) fishes at an average total length of around 80 mm (Mittelbach and Persson 1998). The other 12 species shifted towards piscivory at an age of 2^+-3^+ and an average total length of 160 mm. Six of the eight piscivorous *Barbus* species in Lake Tana are piscivores at 10-15 cm FL, *B. macrophtalmus* and *B. platydorsus* have a significantly longer ontogenetic trajectory and do not shift towards piscivory until reaching 20 cm FL. The only available growth estimates of Lake Tana barbs are for the benthivorous *B. tsanensis* (1^+ average 90 mm FL, range 74-103 mm FL; 2^+ average 150 mm FL, range 139-174 mm FL; 3^+ average 200mm FL, range 181-225 mm FL; 4^+ average 250 mm, range 246-260 mm FL; Mina et al. 1996). Based on these growth estimates *B. macrophtalmus* and *B. platydorsus* shift to piscivory at age 3^+, the other six piscivorous *Barbus* species shift at age 1^+.

Lake Tana's piscivorous *Barbus* follow similar ontogenetic trajectories shifting from zooplankton to insect larvae (Chaoborus) and adult insects (especially *B. megastoma*) to fish. Despite the fact that resource segregation among the adult stages might be well established and prevents competition for food, interspecific competition during ontogeny might reduce recruitment to larger size-classes, resulting in the so-called juvenile competitive bottleneck (Werner 1986; Persson and Greenberg 1990). Ignoring such ontogenetic diet shifts when studying ecological segregation will bias (widen) diet breadth and affects distinction between generalists and specialists. It will readily overestimate potential competition (Piet et al. 1999). In their efforts to predict resource partitioning among the Tana barbs from fish morphometrics and food characteristics, Sibbing and Nagelkerke (2001) pooled fish larger than 15 cm FL during morphometric and gut content analysis. Most piscivorous *Barbus* species at that size (>15 cm FL) are functional piscivores (>50% fish), however, *B. macrophtalmus* and *B. platydorsus* are still in the middle of there ontogenetic trajectory towards piscivory. The Food-Fish Model predicted, based on 35 morphological parameters, *B. acutirostris*, *B. dainellii*, *B. gorguari*, *B. longissimus* to be ambush and/or pursuit hunting piscivores. Their model did not fully recognize, the specialist piscivore (>95% fish) *B. truttiformis*, which was predicted to be a facultative piscivore, a trophic generalist. The inclusion of juvenile *B. platydorsus* and *B. macrophtalmus* in the morphological and gut content analysis resulted in incorrect ecological descriptions. *Barbus platydorsus* was initially predicted to be an ambush hunting, facultative piscivore, however, larger individuals (>30 cm FL; 72% fish) are, according to their own definition, obligate piscivores (>65% fish). *Barbus macrophtalmus* was described as a pump-filterfeeder on zooplankton (predicted from morphology) or a polyphagous barb feeding on most food categories (based on gut content analysis). However, it is now clear that after 20 cm FL *B. macrophtalmus* are truly piscivorous (60% fish).

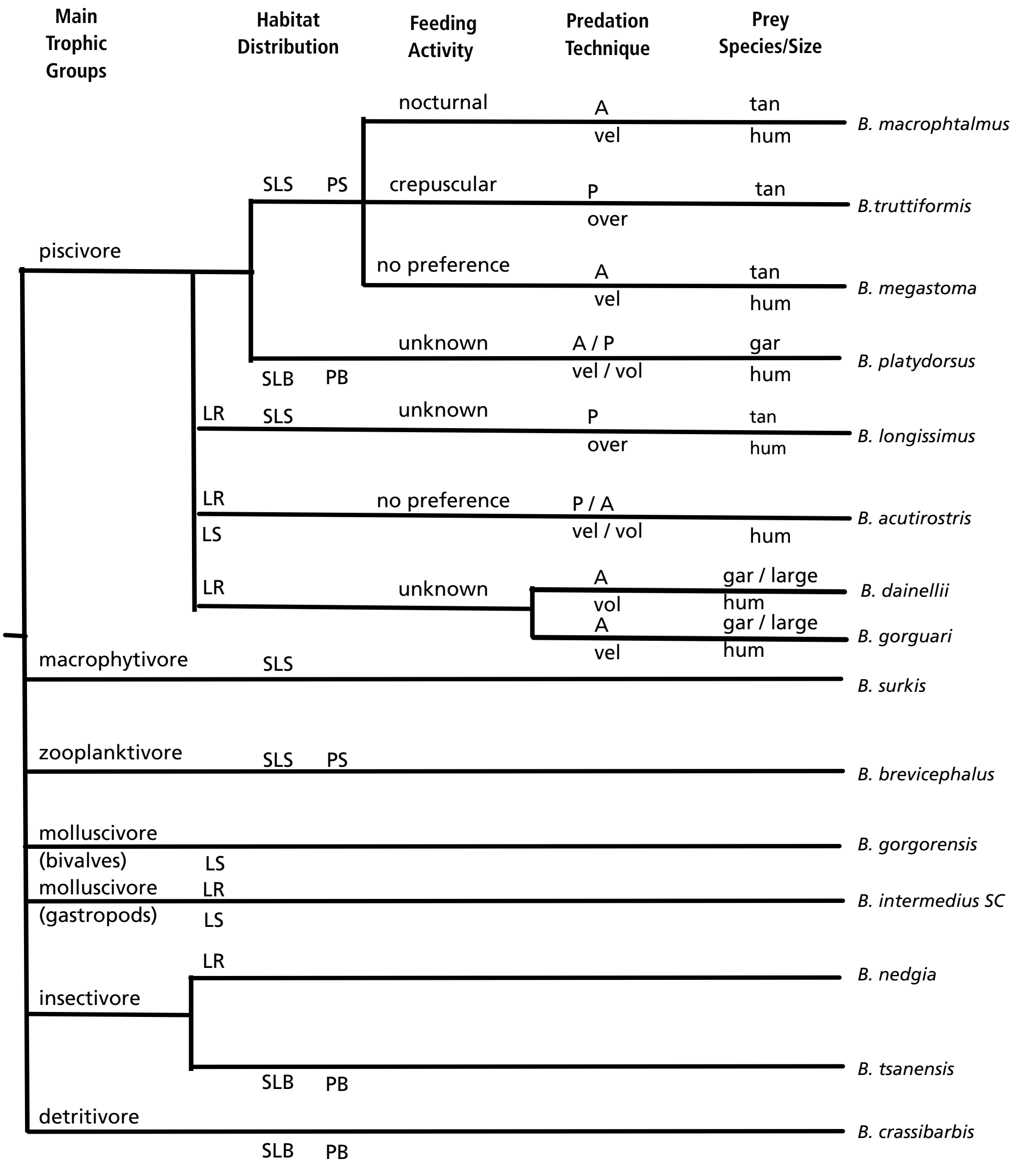


Figure 11. Ecological key to the *Barbus* species flock based on resource use along trophic, spatial and temporal dimensions (this paper) and predation techniques, including type of suction feeding (Chapter 5). Habitats: LR = littoral rock, LS = littoral sand, SLB = sublittoral bottom, SLS = sublittoral surface, PB = pelagic bottom, PS = pelagic surface. Predation techniques: A = ambush, P = pursuit, type of suction feeding (following Muller and Osse, 1984; Chapter 5) vel = velocity suction with protrusion, over = overswimming with little suction, vol = volume suction (with protrusion). Prey Species/Size: hum = *B. humilis*, tan = *B. tanapelagius*, gar = *Garra* spp; all medium prey size (15% predator FL), large = large prey size (25% predator FL).

Ecological differentiation

Resource partitioning is an important mechanism to reduce competition and allowing species in an assemblage to coexist. Segregation of coexisting species can occur along trophic, spatial, and/or temporal resource dimensions. In fish assemblages trophic and spatial dimensions are generally the most important (Ross 1986). Based on resource use along these three dimensions and differences in feeding techniques, an ecological "key" to the adult piscivorous and non-piscivorous *Barbus* of Lake Tana's cyprinid species flock was developed (Fig 11). Ecological differentiation appears to be well developed within the *Barbus* species flock, almost all species differ significantly along the trophic and/or spatial dimensions (Table 4, 7).

Among the non-piscivorous *Barbus* species *B. surkis*, *B. brevicephalus* and *B. gorgorensis* are clearly segregated along the trophic and spatial dimensions. However, the benthivores *B. nedgia*, *B. intermedius* SC, *B. crassibarbis* and *B. tsanensis* have more similar diets. *Barbus intermedius* SC is a generalist along the trophic dimension. *Barbus nedgia* with its fleshy, flaccid lips with large median lobes predominantly feeds on insect larvae (31%) on rocky shores in the littoral areas. Its morphology is highly similar to the fleshy lipped cichlids of Lake Malawi which use their lips to seal off cracks in the rocks before sucking out insect larvae and or other invertebrates (Ribbink et al. 1983; Eccles and Trewavas 1989). Both *B. tsanensis* and *B. crassibarbis* occur mainly over the sandy/muddy bottom in the sublittoral and pelagic areas of the lake and are spatially segregated from the other two benthivorous species. The diets of *B. tsanensis* and *B. crassibarbis* do not differ significantly although *B. tsanensis* has considerably more gastropod molluscs (19%) and *Chaoborus* larvae (42%) in its diet while the diet of *B. crassibarbis* consists mainly of detritus (42%).

Among the piscivorous *Barbus* species ecological segregation is most pronounced along the spatial dimension (Fig. 8). *Barbus dainellii*, *B. gorguari* and small (20-40 cm FL) adult *B. longissimus* are rare species (Table 3) restricted to rocky shores (low spatial niche breadth, Fig. 4c) although larger *B. longissimus* move towards the sublittoral surface areas (43% SLS). *Barbus dainellii* and *B. gorguari* feed on large *Garra* (0.25 PPR) and *B. humilis* while *B. longissimus* is predicted to function more as a pursuit hunter (Chapter 5) feeding on *B. humilis* and *B. tanapelagius*, especially the larger specimens that live near the surface in the sublittoral. The benthic *B. dainellii* has the most pronounced downward protrusion (Chapter 5; Nagelkerke and Sibbing 2000) and large fleshy lips, suggesting a specialisation towards predating ("sucking") *G. dembecha* from crevices in the rocks. *Barbus gorguari* occurs over rocky and sandy substrate at the border of the submerged vegetation and the lake, feeding mainly on *Garra*. *Barbus gorguari* is the only species with considerable amounts of *B. pleurogramma*, which occurs only among the vegetation in the floodplains at the edge of the lake, in its diet. *Barbus acutirostris* is the most common piscivore in Lake Tana and is regularly caught in all six habitats (high spatial niche breadth, Fig. 4c), however, *B. acutirostris* is especially abundant in the littoral zones over sandy/muddy bottom.

Barbus platydorsus is a bottom-dwelling piscivore in the sublittoral and pelagic zones, feeding on *Garra* and *B. humilis*. *Barbus macrophtlamus*, large (30-40 cm FL) *B. megastoma* and *B. truttiformis* have high overlap values along the spatial dimension (0.77-0.85; Table 6), occurring predominantly near the surface in the sublittoral and pelagic zones. All three species have similar diets consisting for a large part of *B. tanapelagius*, the only common prey at the surface in the sub-littoral and pelagic areas (Fig. 8). However, clear differences do exist in predation techniques between these three species. *B. macrophtalmus* is a nocturnal (ambush) predator, *B. truttiformis* is a crepuscular pursuit hunter and *B. megastoma* is an ambush hunter active throughout the day (Chapter 5).

Both pelagic habitats, i.e. surface and bottom, have their own specific, community of piscivorous barbs that seem not to interact directly (interference competition). Exploitative competition might still occur if prey items do migrate between the pelagic and benthic and are eaten by both groups of piscivores. However, *B. tanapelagius* does migrate downward in water column during the day but the majority of the population did not move more than 1-2 m below the surface. *Garra* spp remained at the bottom during day and night.

Except for *B. dainellii* and *B. gorguari*, which are able to eat relative large *Garra*, little segregation is observed in prey size among the piscivores. Overall relative prey size is small (15% FL) and highly similar among all eight piscivorous *Barbus* species. Whether this is the result of active prey choice, gape limitations of the feeding apparatus (oral- and/or pharyngeal gape) or that prey selection is mediated by size-related capture success, remains at this point to be solved (Chapter 5).

A comparison of trophic radiation in cichlid and cyprinid species flocks

Two major innovations within the body plan of cichlids may well be responsible for the large diversification of trophic structures and the massive extent of trophic diverisification (e.g. planktivores, aufwuchs feeders, molluscivores, zooplanktivores, lepidophages, piscivores, peadophages; Witte 1981, Barel 1983; Ribbink et al. 1983; Eccles and Trewawas 1989; Witte and van Oijen 1990, 1995) which spans a range usually occupied by several families of fishes. The restructuring of the pharyngeal jaw apparatus and the decoupling of oral jaw elements increased the number of independent elements ("degrees of freedom"). A body plan with a large number of independent elements, (a) increases the number of potential solutions for biomechanical problems, and (b) is more easily modified and diversified than those with fewer independent elements (Galis and Metz, 1998).

The body plans of cyprinid fishes and cichlid fishes have some important analogous morphological innovations. Cyprinid fish, have their fifth branchial arches modified into powerful pharyngeal jaws. In cyprinids the upper parts of the pharyngeal jaws are lost, these are functionally replaced by the skull base, which is used as an anvil absorbing high stresses. These pharyngeal jaws aid in the transport of food but more importantly, like in cichlids (Liem 1974) the cyprinid pharyngeal jaws have specialized in mechanical food processing (shearing, cutting, lacerating, puncturing, crushing, grinding; Sibbing 1986, 1991ab). Epaxial and hypaxial body muscles provide the power in cyprinid food mastication (Sibbing 1986). The development of the food processing role by the pharyngeal jaw apparatus freed the way for the oral jaws to diversify and specialize on solemnly food collection instead of the ancestral dual function of collecting and processing. The decoupling of the maxillary and premaxillary in the upper jaw allows for independent movement of these two elements, and presumably also resulted in cyprinids for an increased diversity of jaw movement like in cichlids (Liem 1980).

Table 8. Comparison of the trophic groups and their species diversity within the haplochromine cichlid species flock of Lake Victoria and the *Barbus* species flock of Lake Tana. Haplochromine data from Witte and van Oijen (1995) and references cited therein. The dominant food category was taken as decisive of the trophic classification (Witte 1981). For the *Barbus* spp the second most important food category is given between brackets when feeding on several food types occurred. Ins = insects, Gas = gastropod molluscs, Det = detritus, Mac = macrophytes.

	Lake Victoria		Lake Tana	
Trophic groups	haplochromines	Other	*Barbus*	Other
piscivores (ambush, pursuit)	115+		8 *Barbus* spp	
aufwuchs feeders (epilithic, epiphytic)	35+		-	V. *beso* Garra spp
macrophytivore	2		*B. surkis*	
phytoplanktivores (suspension or bottom)	-	*Oreochromis* spp (littoral)		*O. niloticus* (littoral)
phytoplanktivore / detritivores	25+ (sublittoral, pelagic)		*B. crassibarbis* [Ins] (sublittoral, pelagic)	
zooplanktivore	25+		*B. brevicephalus* [Ins]	
insectivore (mainly chaoborus, chironomid larvae)	65+		*B. tsanensis* [Gas] *B. nedgia* [Det]	
molluscivore (mainly Gastropods)	30+ (pharyngeal crusher, oral crusher/sheller)		*B. gorgorensis* [Mac] (Bivalves) *B. intermedius* SC [Mac] (Gastropods)	
paedophages	20+		-	
prawn-eaters	10+		-	
crab-eaters	1		-	
parasite eaters	2		-	
scale eaters	1		-	

Because of these analogous morphological innovations and similar potentials for trophic radiation, it is not surprising that the haplochromine cichlids in Lake Victoria and the *Barbus* spp in Lake Tana show a similar range of trophic diversifications (Table 9). The lack of oral teeth limits the trophic repertoire (e.g. biting, grabbing, scraping) of the *Barbus* species in Lake Tana and is the reason why some trophic groups are absent; aufwuchs feeders, scale-eaters, and parasite-eaters. In Lake Tana the important niche of aufwuch feeders is occupied by other specialist cyprinids, *V. beso* and *Garra* spp. Although Lake Victoria haplochromine species flock has a higher diversity per trophic group, the distribution of the species over the trophic groups (no. piscivores: no. macrophtyvores: no. molluscivores: no. zooplanktivores: no. insectivores: no. detritivores) does not differ significantly ($\chi^2_{0.05,\,5}$ = 5.8; ns) in both species flocks.

The higher species diversity is probably for a large part the result of replicated evolution of the same trophic specializations among these small-sized haplochromines with usually limited home ranges (Rüber et al. 1999). Secondly, Lake Victoria is much larger and deeper than Lake Tana creating the opportunity for extensive spatial segregation and speciation within each trophic group. Unlike the plain coloured (silvery), sexually unimorphic *Barbus*, the haplochromine cichlids of Lake Victoria are very colourful, especially the males, and there is convincing evidence that sexual selection (female mate choice) is a driving force reproductively isolating incipient (ecologically highly similar) species (Seehausen et al. 1997). Under such circumstances, ecological differentiation is not a prerequisite for speciation. The coexistence of sibling species without apparent niche differentiation supports the evolutionary mechanism of sexual selection as a diversifying force (Genner et al 1999)

Despite their similar and high morphological potential for trophic diversification and rapid speciation only few cyprinid species flocks exist. Unlike cichlid fishes, cyprinids possess a palatal and sublingual organ lining their pharynx. The vagal brainlobe in some benthic species is multilaminary organized like the cortex cerebri of mammals. Both the pharyngeal roof and floor are mapped somatotopically on this brain center for highly localised gustatory control in feeding (Sibbing 1991ab; Sibbing and Uribe 1985; Finger 1988). Using such refined systems, small edible particles are sorted from the mud, thus enabling cyprinids to feed on (micro) benthos. The evolutionary success and high competitive abilities as benthivores of cyprinid fish is largely attributed to this unprecedented sorting ability as well as their powerful and diversified pharyngeal jaw system. Benthivores cyprinids are common throughout Africa's lakes and rivers, including the cichlid-rich lakes of Victoria, Malawi and Tanganyika. However, this key innovation in the evolutionary success of cyprinid fishes, came at a cost, it greatly reduced the competitive abilities to function as e.g. piscivores (Chapter 5). The absence of common African specialist piscivores like nile perch (*Lates* spp.), tiger fish (*Hydrocynus*) or generalist riverine cichlids in Lake Tana during its formation formed the basic prerequisite for the evolution of piscivorous *Barbus*. Apparently, only in the absence of competitors are cyprinids able to use their retained potential for trophic diversification to the fullest, including the unexpected specialization of piscivory.

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5
CHAPTER

Feeding performance and predation techniques among segregating piscivores in Lake Tana's Barbus species flock (Cyprinidae; East-Africa).

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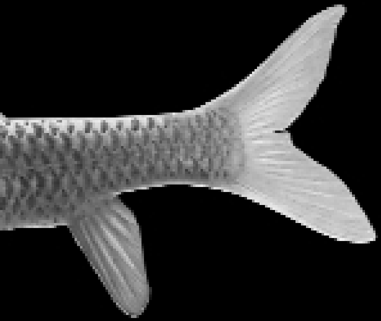
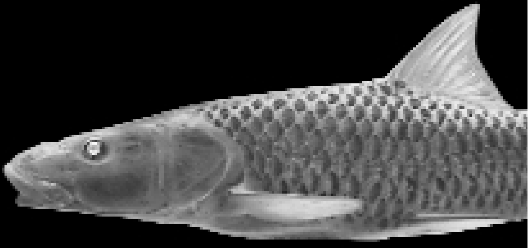




Abstract

With more than 2000 fish species the Cyprinidae is the largest family of vertebrates. Lake Tana (Ethiopia) harbours, as far as we know the only remaining intact species flock of large (max. 100 cm FL) cyprinid fishes (15 *Barbus* spp.). One of the most intriguing aspects of this endemic *Barbus* species flock is the large number of piscivores (8). Cyprinid fishes do not seem well designed for piscivory. They lack teeth in the oral jaws, have a small slit-shaped pharyngeal cavity and lack a stomach with low pH for digesting large prey. Why then is piscivory, which is rare among cyprinids, so common in Lake Tana's *Barbus*? We studied (1) prey size related handling times, (2) the effect of prey size and structural complexity on feeding performance in indoor tanks and outdoor pools, (3) prey capture events using high-speed recordings, and (4) prey selection in the field in order to determine and (5) compare prey selection, feeding performance and predation techniques of these piscivorous *Barbus* with known piscivores from other fish families. The abilities and limitations of the cyprinid fishes to function as piscivores were explained by a functional morphology analysis of their feeding system. Overall, Lake Tana's piscivorous *Barbus* perform relatively "poor", compared to piscivores from other fish families. The barbs are able to successfully capture only relatively small prey (prey-to-predator length ratio PPR, average 0.15 and maximum 0.25) compared to other freshwater and marine piscivores. Prey size selection is not limited by gape sizes of the feeding apparatus (postcapture factors), but is mediated by size-related capture success (precapture factors). Their limited average and maximum prey size compared with other piscivores is most likely caused by the relatively small volume of their oro-pharyngeal-opercular cavity. The voluminous palatal organ and sublingual organ lining their pharynx, a key innovation in the evolutionary success of cyprinid fishes, came at a cost, it greatly reduced the competitive abilities to function as piscivores. However, Lake Tana lacks potential piscivorous competitors, rendering the piscivorous *Barbus* by far the "best" and apparently highly successful. They have adapted to all available macro-habitats, using different techniques, a unique scenario for barbs.

Introduction

The Cyprinidae are cosmopolitic and the most species-rich (>2000 species; Nelson 1994) group of fish among all fresh water fish families. Cyprinid fishes have a highly developed palatal and sublingual organ lining their pharynx resulting in highly localised gustatory control in feeding (Sibbing 1991ab; Sibbing and Uribe 1985; Finger 1988). Using these refined systems, small edible particles are sorted from the soft substrate, thus enabling cyprinids to feed on (micro) benthos. The evolutionary success and high competitive abilities as benthivores of cyprinid fish is largely attributed to this unprecedented sorting ability as well as their powerful and diversified pharyngeal jaw system (Sibbing 1991a). The largest cyprinid genus, *Barbus* (>800 species) is a polyphyletic assemblage (Howes 1987; Tsigenopoulos et al. 2002), however, Africa's large hexaploid *Barbus* are of a single origin forming a monophyletic group and are classified in the subgenus *Labeobarbus* (Tsigenopoulos et al. 2002 and references therein). Despite the enormous abundance of cyprinid fishes throughout the world's lakes and rivers, the *Barbus* species of Lake Tana form, as far as we know, the only remaining intact species flock of large cyprinid fishes, since the one in Lake Lanao in the Philippines, has practically disappeared due to anthropogenic activities (Kornfield and Carpenter 1984). One of the most intriguing aspects of Lake Tana's endemic *Barbus* species flock is the large number of piscivorous species (8 out of 15). Piscivory is rare amongst the highly successful but predominantly benthivorous, cyprinid fishes. Cyprinids seem not well designed for piscivory, they lack teeth in the oral jaw, have a small slit-shaped pharyngeal cavity and all lack a stomach with low pH for digesting large prey. Due to the apparent lack of proper tools for piscivory the question rises, how well adapted these barbs actually are to piscivory?

Predation by piscivorous fish can affect the structure and dynamics of prey populations in many ways. By directly reducing the abundance or recruitment of prey species and/or size classes or indirectly by influencing a variety of behavioural repertoires like habitat use, activity pattern, foraging tactics or reproductive behaviour (Greenberg et al. 1995 and references cited therein). Predation success of a piscivorous species is influenced by predator and prey characteristics and by the environment. Environments with high structural complexity (vegetation or rocks) can reduce predation success by restricting movement of the larger predator compared with the small prey and by decreasing a predators vision, reducing encounter rates (Christensen and Persson 1993). The size range of prey fish that a piscivore might feed on is constrained by its abilities to detect, capture and process the prey (Sibbing and Nagelkerke 2001). Lower prey size limits might be set by the ability of a predator to encounter, detect and retain prey (Lundvall et al. 1999 and references cited therein). Upper prey size limits might be set by gape limitations of the feeding apparatus (northern pike, Nilsson and Brönmark 2000) and handling time [postcapture factors]. However, instead of being the result of active choice of the predator, prey size in a predators diet might also be the result of passive process and mainly reflect the interplay between size-related capture success and prey anti-predator behaviour [precapture factor] (Juanes 1994; Juanes and Conover 1994). Capture success depends strongly on the prey-to-predator length ratios and has been shown to decrease clearly with increasing ratios (Scharf et al. 1998; Lundvall et al. 1999). In addition to increased escape speed with increasing size in prey fish, a range of other prey-species-specific anti-predation behaviours (schooling, hiding, diurnal migrations in water column, evasive jumps, reduced activity) will decrease predation success and affect a predator's diet (Christensen and Persson 1993; Christensen 1996). Prey size may also be dependent on the predation technique used by a predator, sedentary ambush hunters are thought to encounter large, fast-moving prey more often than small slow-moving prey (Greene 1983, 1985), resulting in larger average prey sizes compared with pursuit hunters. The prey species is also dependent on predation technique, e.g. specialistic pursuit hunters are restricted to the open water and its prey species (Muller and Osse 1984).

Chapter 4 showed that the average prey size of Lake Tana's piscivorous *Barbus* is small (predator-to-prey length ratio [PPR] ca. 0.15) and highly similar in almost all predator/prey species combinations. Here, we combine field observations with an experimental approach in order to describe predator-prey interactions and explain the general abilities and limitations of Lake Tana's *Barbus* to function as piscivores. The questions addressed in this study are: (1) How does prey size and environment (structural complexity) affect feeding performance of Lake Tana's piscivorous *Barbus*?; (2) Is prey size selection in Lake Tana's piscivorous *Barbus* constrained by postcapture factors (oral-/pharyngeal gape limitations) or by precapture factors (size-related capture success)?; (3) Which predation techniques are used by the different piscivorous *Barbus* species?; and (4) How well do Lake Tana's *Barbus* species perform and function as piscivores compared with common freshwater and marine piscivores of other fish families.

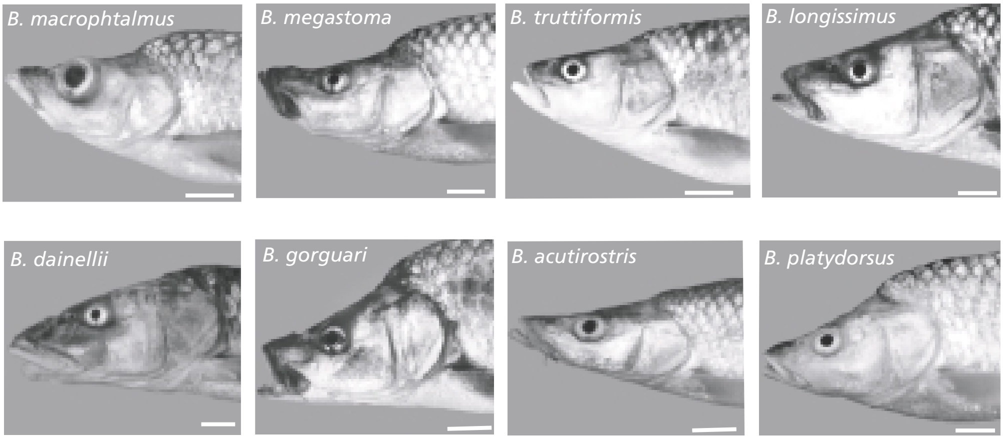


Figure 1. Heads of the eight piscivores in Lake Tana's endemic *Barbus* species flock. Bars are 2 cm.

Materials and Methods

Collection of fish

Piscivorous *Barbus* (200-400 mm SL) were caught using hook-and-line, fish traps or bottom trawl in Lake Tana and its affluent rivers. The fish were acclimatized for at least one month before starting any of the experiments. Predators were housed in 250-l tanks (90x45x45). Each 250-l tank was partitioned by a PVC-slate into two equal parts and one predator was held per section (45x45x45 cm). Predators were fed life prey fish (*B. humilis*) which were captured daily, sorted according to size and held in 250-l tanks. Prey fish were fed commercial flake food (Trouvit, Trouw, Putten). *Barbus humilis* was used as prey in all feeding performance experiments as it is the most common prey species in Lake Tana and forms a significant part of the diet of almost all piscivorous *Barbus* species. The 16 indoor 250-l tanks were part of a semi-closed system (water temperature 21.8 °C, oxygen 6.6 mg l^{-1}); ca. 10% of the water was replaced every day by water pumped directly from the lake. The aquarium room was not illuminated by artificial light but followed the natural light cycle. An overview of the number, size and species of piscivorous *Barbus* used during the different experiments in this study is given in Table 1.

Table 1. Size and number of piscivorous *Barbus* species used in the experiments.

	Indoor Tanks						Outdoor Pools	
Species	SL (mm)	FL (mm)	handling time	captures success	predation rate	predation technique	predation rate	behavioural observations
B. acutirostris	238	253	x	x	x		x	x
B. acutirostris	245	260					x	
B. acutirostris	250	269	x	x	x		x	x
B. acutirostris	251	271	x					
B. acutirostris	253	274	x	x	x		x	x
B. acutirostris	280	300					x	
B. dainellii	254	270	x					
B. gorguari	244	262	x					
B. gorguari	296	315	x					
B. longissimus	201	214	x					
B. longissimus	213	230	x					
B. longissimus	258	276	x	x				
B. longissimus	296	318		x				
B. macrophtalmus	245	263	x					
B. macrophtalmus	260	278	x	x	x		x	
B. macrophtalmus	268	287	x					
B. macrophtalmus	268	288	x	x	x	x	x	
B. macrophtalmus	273	290		x	x	x	x	
B. macrophtalmus	275	295					x	
B. macrophtalmus	278	303	x				x	
B. megastoma	272	288					x	
B. megastoma	275	293					x	
B. megastoma	283	305	x	x	x	x	x	
B. megastoma	290	312	x					
B. megastoma	310	332	x	x	x	x	x	
B. truttiformis	247	260		x	x			
B. truttiformis	255	270					x	
B. truttiformis	263	282	x	x	x	x	x	
B. truttiformis	285	305	x	x	x	x	x	
B. truttiformis	290	308	x	x	x		x	
B. truttiformis	302	328	x					
B. truttiformis	305	320	x					x

Prey handling time

Similarly sized specimens of seven different piscivorous *Barbus* species (Table 1) were used to determine prey handling time. During the experiments predators were fed 1-4 prey fish (B. humilis) per day depending on the size of the prey to avoid satiation. Handling time was measured as the time from capture until cessation of pharyngeal mastication, as apparent from externally visible head and opercular movements (Sibbing et al. 1986; Nagelkerke and Sibbing 1996). Handling time typically consisted of a series of chewing periods (ca. 10s) and included rest periods (ca. 1-2 min), since only the smallest prey size (PPR <0.05) was swallowed after one chewing period. Handling time was recorded with a stopwatch by an observer located behind a screen to avoid disturbance. During analyses predator species were pooled due to low number of individuals per species and fitted to a nonlinear least squares regression model ($y=ae^{bx}$).

Predator performance

Part of the feeding trials was conducted in two indoor tanks (180x50x45 cm), with a water depth of 30 cm. The water in each tank was filtered (Eheim 2215, 620 l/hr) and constantly aerated. Fifty percent of the water was weekly refreshed with water from the semi-open aquarium system. Artificial light (4x36 Watt fluorescent tubes, Philips) was placed 20 cm above the tank and a light/dark regime was set following the natural cycle (day light from ca. 7:00hr-18:00hr). After introduction in the experimental tank, predators were trained (4-7 days) until they foraged willingly despite the regular disturbances associated with conducting the trials. Next, the predators were exposed to a random sequence of the five different prey size and habitat treatments. To determine the effect of prey size on predation rate, predators were allowed to forage for a 10-min period on a school of 20 prey fish (*B. humilis*) of three prey-to-predator size ratios (PPR) 8%, 16% and 24%. To determine the effect of structural complexity on predation rate, predators were allowed to forage for a 10 min period on a school of 20 prey fish (*B. humilis*, PPR 16%) while ca. a third of the tank area was covered by ‘vegetation’ or ‘rock’. The ‘vegetation’ substrate consisted of 50 floating, 30 cm long, green floating strings (diameter 1 cm) tied to a plastified wire metal frame (60x46 cm), placed on the bottom of the tank. The ‘rock’ substrate consisted of four sets of two clay bricks (25x13x6 cm) that were placed vertically on the bottom of the tank at equal distances from each other and the sides of the tank. Each brick had four holes (3.5 cm diameter).

At the start of a trial aeration cubes were removed and pumps were shut down. The tank was divided using a net (3 mm bar mesh), the largest part (2/3) of the tank was used to acclimitize the prey for 10 min. After removal of the net, trials were run for 10 min commencing from the first attack. An attack was defined as a direct strike at the prey with opening of the mouth. The trial was recorded with a digital video-camera (JVC GR-DVL9700) at 100 images s^{-1}. Capture success was calculated as the proportion of attacks that resulted in prey consumption. Predation rate was defined as the total number of prey caught per 10-min trial.

The advantage of the feeding trials in the indoor tanks was that feeding behaviour could easily be recorded. However, the constrained space might influence the predator’s feeding success and/or the prey’s escape possibilities. Therefore, similar feeding trials were conducted in large outdoor pools.

However, due to the natural murkiness of Lake Tana's water it was not possible to make behavioural observations during these trials. The feeding trials were conducted in six outdoor pools (diameter 300 cm, height 100 cm) with a water depth of 50 cm. The pools were located in an open shed adjacent to the lake, the sides were only covered by nets to prevent piscivorous birds from entering the pool area. The pools were part of an open system. Water was pumped directly from the lake, distributed over the experimental pools and flowed back to the lake. Water temperature, oxygen levels and transparency were similar to lake conditions (see de Graaf et al. 2003).

To determine the effect of prey size on predation rate, predators were allowed to forage for 24 hours on a school of 30 prey fish (*B. humilis*) of three different PPRs 8%, 16% and 24%. To determine the effect of structural complexity on predation rate, predators were allowed to forage for 24 hours on a school of 30 prey fish (*B. humilis*, PPR 16%) while ca. 20% of the pool area was covered by 'vegetation' or 'rock'. The 'vegetation' substrate consisted of 220 floating, 50 cm long, green floating strings (diameter 1 cm) tied to a plastified wire metal frame (1.5 m2), placed on the bottom of the pool. The 'rock' substrate consisted of six sets of three, 30 cm long, horizontally placed PVC tubes (diameter 4 cm) and six sets of three, 30 cm long, horizontally placed PVC tubes (diameter 11 cm) tied to a plastified wire metal frame (1.5 m^2), placed on the bottom of the pool. Fine-meshed (3x1 mm) nets adjusted to the shape of the pools were placed inside each pool. The net was lifted at the end of each trial, facilitating the quick removal of the predator and counting of the remaining prey fish. Predators were randomly assigned to a pool and were trained until they foraged willingly despite the regular handling during net lifting. Next, the predators were exposed to a random sequence of the five different prey size and habitat treatments. A minimum of 24 hours fasting was maintained between the trials. Predation rate was defined as the total number of prey eaten per 24-hour trial.

Due to the limited number of individuals, the results of different species were pooled before analysis. The relationship between capture success and PPR in the experimental indoor tanks was analyzed using least squares regression. To analyse the effect of prey size and habitat on predation rate in the experimental indoor tanks and outdoor pools, within subject ANOVAs were performed. If ANOVAs were significant, multiple-comparisons (Bonferroni adjusted) were used. Data were arcsinh-transformed to normalize distribution and homogenize variances (Fowler et al. 1990).

Prey behaviour

To assess possible mechanisms for differential vulnerability of the prey, behavioural observations were conducted. Due to the high turbidity of Lake Tana's water it was not possible to observe predator/prey behaviour in the experimental set-up (open system) described above. In July-August 2001 one pool was disconnected from the open system. The bottom of the pool was marked with a 0.25 m^2 grid and filled with 30 cm of water from the lake. To achieve enough transparency for behavioural observations the water was continuously filtered (Eheim 480 l/hr). Every week the pool was flushed with lake water. Observer 1 was located 3 m above the water surface on a platform in the roof of the open shed and recorded each trial with a Sony (DCR-TRV120E) digital video camera. Observer 2 was located behind a screen on a small platform, 1.5 m above the water surface adjacent to the basin. Four different trial series were used: (1) 30 prey (B. humilis), no structural complexity and no predator, (2) 30 prey (*B. humilis*), structural complexity 'rock' and no predator, (3) 30 prey (*B. humilis* and *G. dembecha*), no structural complexity and one predator (*B. acutirostris*) , and (4) 30 prey (*B. humilis* and *G. dembecha*), structural complexity 'rock' and one predator (*B. acutirostris*). The PPR was ca. 16%. It appeared to be impossible to keep individuals of *B. tanapelagius*, the third main prey species in the diet of Lake Tana's piscivorous barbs (Chapter 4), alive in the laboratory. The video camera could not cover the entire area of the pool. In trials with both prey and predator present observer 1 focussed on recording the behaviour of the predator while observer 2 focussed on the behaviour (location, schooling) of the prey. Observer 2 called out every 5 seconds the position (grid number) of the main school and the organisation of the prey (I = 1 school >25 prey fish, II = two schools, III = no schooling). In trials with only prey fish, observer 1 focused on the location of the largest school.

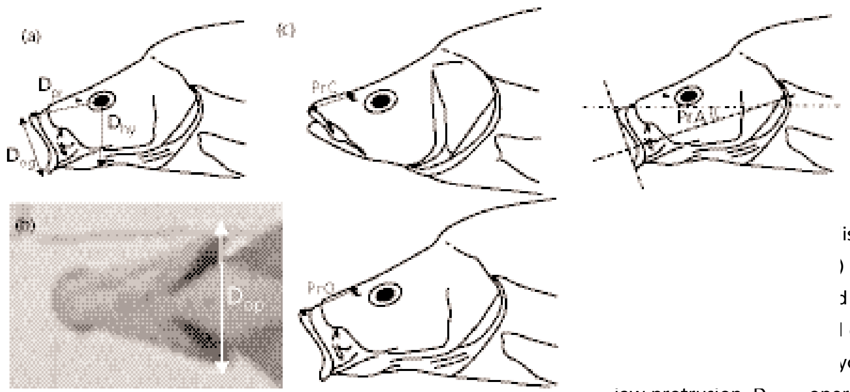
New prey fish were used in each trial. Due to the lack of predators, the same three *B. acutirostris* were used in the prey/predator trials. To minimize the disturbance, four different trials were randomly assigned to the first predator before the predator was replaced by the next. All observations were made during the day, between 9:00hr and 11:00 hr. To analyse effects of predator and substrate on the behaviour of *B. humilis*, ANOVAs were performed. To analyse effects of substrate on the behaviour of *G. dembecha*, independent samples T-tests were performed. Proportions were arcsin $\sqrt{x}$-transformed to normalize distribution and homogenize variances. Some treatments had zero variances, in these circumstances non-parametric tests were used.

Prey selection in the field

Piscivorous *Barbus* (200-400 mm FL) and prey species (0-100 mm FL) were collected in the Bahar Dar Gulf of Lake Tana between August 1999 and September 2001. Piscivorous *Barbus* and prey species were captured using multimesh gillnets (piscivorous *Barbus*, 3, 4, 5 and 6 cm bar mesh; prey 0.5, 0.625, 0.8, 1.25 cm bar mesh) in six different habitats (littoral rock (LR), littoral sand (LS), sublittoral bottom (SLB), sublittoral surface (SLS), pelagic bottom (PB), pelagic surface (PS); three stations per habitat) and by using bottom trawls (piscivorous *Barbus*, codend 4 cm bar mesh; prey, codend 0.5 cm bar mesh) in three different habitats (LS, SLB, PB; four stations per habitat). Specific station locations, sampling dates and details on sampling gear are outlined in Chapter 4 and de Graaf et al. (2003). Because these cyprinid piscivores masticate prey fish with their pharyngeal jaw, no whole or partial prey fish were found in the digestive tract for identification. Information about prey species and prey size was reconstructed from the skeletal elements of prey fish in the digestive tract of the predators (see Chapter 4 for details).

Predation techniques

Barbus macrophtalmus, *B. megastoma* and *B. truttiformis* have similar distribution patterns in the Bahar Dar Gulf of Lake Tana (Chapter 4), all three species occur near the surface in the sublittoral and pelagic areas. Differences in prey species and/or predation techniques might explain the co-existence of these ecologically similar but distinct species. After introduction in the experimental tank (90x45x45), predators were trained until they foraged willingly despite the regular disturbances associated with conducting the feeding trials. Prey capture events were recorded at 200 images s^{-1} with a digital camera (JVC GR-DVL9700) using daylight. A mirror was placed at 45 degrees directly below the position where prey was presented to the predator to obtain an additional ventral view of the capture sequence. Both lateral and ventral views were filmed in the same image. The back and the bottom of the experimental tank were marked with a 2.5 cm grid. Additional markers were placed at the front of the tank near the bottom. A freshly killed prey fish (*B. humilis*, PPR 0.07-0.25) was presented in a fixed horizontal position 5 cm of the bottom of the tank. A thin thread (0.08mm diameter) was attached to the body of the prey fish just in front of the dorsal fin, in such a way that when captured by a predator it would easily come free. To quantify the movement of the prey and predator the images were analyzed frame by frame using Media100 for Macintosh and Motionanalist. Around 20 frames (100 ms total elapsed time) were analyzed for each feeding sequence. Seven points on the predator (lateral view: the anterior tip of both the upper and lower jaw, the center of the eye, the position of the hyoid perpendicular to the center of the eye; ventral view, posterior tips of the operculi, anterior tip of the lower jaw), six points on the prey (ventral/lateral views: anterior end of the head, middle of the body [estimated], posterior end of the tail) and three reference points of the grid at the back of the tank, were digitized. For all sequences, T_0 (time 0) was determined as the time at which the mouth first began to open. After the frames had been digitized, the x and y coordinates of 16 landmarks on the lateral and ventral view of the predator and prey were used to calculate the variables of interest. In total nine kinematic variables (five related to timing and four related to displacement) were analyzed: (1) T_{ogmax} = time (ms) to maximum gape, (2) T_{op} = time (ms) of opercular valve opening and forming of opercular slits, (3) T_{opmax} = time (ms) of maximum opercular abduction, (4) T_{in} = time (ms) that the center of the prey passed the oral gape aperture, (5) T_{cl} = time (ms) to closure of the oral gape, (6) D_{og} = oral gape opening, distance between upper and lower jaw (in % SL, Fig. 2a), (7) D_{hy} = depression of the hyoid (in % SL, Fig. 2a), (8) D_{pr} = upper jaw protrusion (in % SL, Fig. 2a), and (9) D_{op} = opercular abduction (in % SL, Fig. 2b).



isplacement) and ventral (b) d during prey l gape opening, yoid, D_{pr} = upper jaw protrusion, D_{op} = opercular abduction) and (c) the position of the morphological parameters used in Table 4 (PrA = protrusion angle. PrC = protrusion chain length closed, PrO = protrusion chain length open; protrusion length = PrO-PrC)

Results

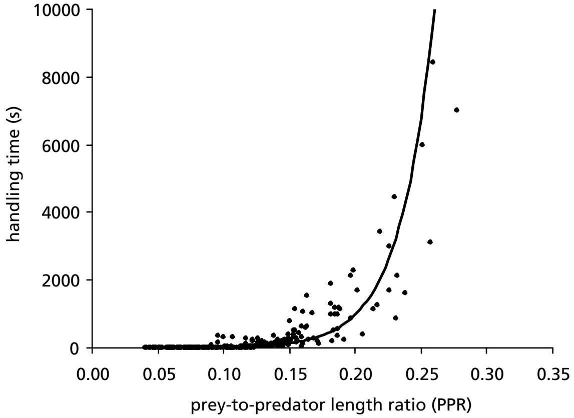


Figure 3. Prey handling time as a function of PPR for Lake Tana's piscivorous *Barbus* feeding on *B. humilis*. (handling time (sec) $=0.43e^{38.7PPR}$, $P<0.001$, $r^2=0.85$, n=242)

Handling time

Twenty-three similarly sized piscivorous *Barbus* (seven species, Table 1) and 242 prey (PPR 0.04-0.28) were used to determine the relationship between prey handling time and prey size. *Barbus* handling times increased exponentially with increasing PPR for *B. humilis* (handling time = $0.43e^{38.7PPR}$, $P<0.001$, $r^2=0.85$, n=242; Fig. 3).

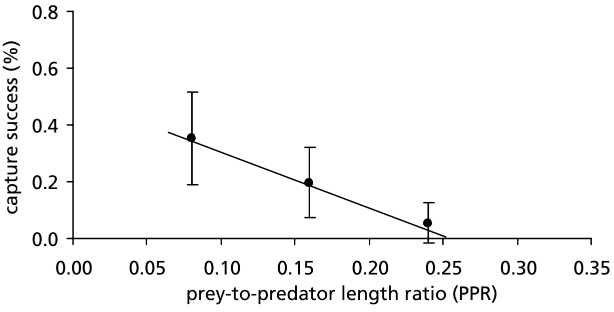


Figure 4. Proportion of successful captures as a function of PPR for Lake Tana's piscivorous *Barbus* feeding on *B. humilis*. (capture success = -1.95PPR + 0.5, $P<0.001$, $r^2=0.49$, n=46). Means (± SD) were calculated for the three PPR classes (small, 0.08 [0.04-0.12]; medium 0.16 [0.12-0.20] and large 0.24 [0.20-0.28]) and are plotted in the figure.

Capture success

A total of 46 successful feeding trials using 14 piscivorous *Barbus* (five species, Table 1) were conducted in the two experimental indoor tanks to determine capture success, PPR's ranged from 0.06 to 0.25. *Barbus* capture success declined linearly with increasing PPR when feeding on *B. humilis* (capture success = -1.95PPR + 0.5, $P<0.001$, $r^2=0.49$, n=46; Fig. 4), the most common and abundant prey species in Lake Tana. Means (±1 SE) were calculated for the three PPR classes (small, 0.08 [0.04-0.12]; medium 0.16 [0.12-0.20] and large 0.24 [0.20-0.28]) and are plotted in Figure 4.

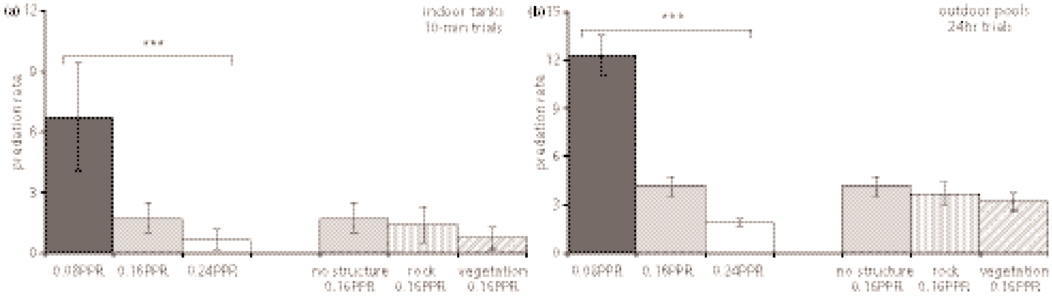


Figure 5. Effect of prey size (0.08, 0.16, 0.24 PPR) and structural complexity ('rock', 'vegetation') on predation rate (means ±95% CI) of piscivorous *Barbus* feeding on *B. humilis* in indoor tanks (a) and outdoor pools (b). Asterisks indicate significance levels (within subject ANOVAs; ***=*P*<0.001). Note the highly significant effect of prey size and the insignificant effect of structural complexity on the predation rate in both experimental designs.

Predation rate

Only piscivorous *Barbus* (four species, Table 1) were used in the analyses for which data of all five different treatments were obtained. Highly significant effects of prey size on predation rate were found for Lake Tana's piscivorous *Barbus* in both the indoor tanks (within subject ANOVA, $F_{2,22}$=26.84, *P*<0.001; Fig. 5a) and the outdoor pools (within subject ANOVA, $F_{2,36}$=47.96, *P*<0.001; Fig. 5b). In both experimental designs predation rate decreased significantly with increasing PPR. Structural complexity, both 'rock' and 'vegetation' had no significant effect on predation rate in both experimental designs (indoor tanks, within subject ANOVA, $F_{2,22}$=2.699, *P*=0.09, Fig. 5a and outdoor pools, within subject ANOVA, $F_{2,36}$=1.027, *P*=0.37, Fig. 5b).

Table 2. Two-way ANOVAs of the effect of predator and structural complexity on swimming speed and the proportion of total time spend in the open water by *B. humilis* (see Fig. 6abc).

Source of variation	SS	F	df	P
swimming speed				
substrate	0.013	0.36	1, 12	0.57
predator	0.270	7.20	1, 12	0.03
substrate x predator	0.083	2.22	1, 12	0.17
distribution				
substrate	43.32	2.70	1, 12	0.14
predator	4.08	0.25	1, 12	0.63
substrate x predator	25.81	1.61	1, 12	0.24

Prey behaviour

For *B. humilis* (n=3), the presence of structural complexity and/or a predator in the experimental pool had little effect on its swimming speed (Table 2), distribution (Table 2) or schooling behaviour (Kruskal-Wallis test, *P*=0.21) (Figs. 6 abc). Only average swimming speed was lower (*P*=0.03, Table 2) in the presence of the predator, *B. acutirostris*. In the trials with structural complexity *B. humilis* never swam through or hid in the PVC tubes, even when under attack from the predator, *B. acutirostris*. For *G. dembecha* (n=3), the presence of structural complexity significantly effected swimming speed (*P*<0.001, independent samples T-test; Fig. 6d), distribution pattern *(P*<0.001, independent samples T-test; Fig. 6e) and schooling behaviour (*P*<0.001, independent samples T-tests; Fig. 6f). If substrate was present *Garra* dispersed and hid in and under the PVC tubes during the whole trial.

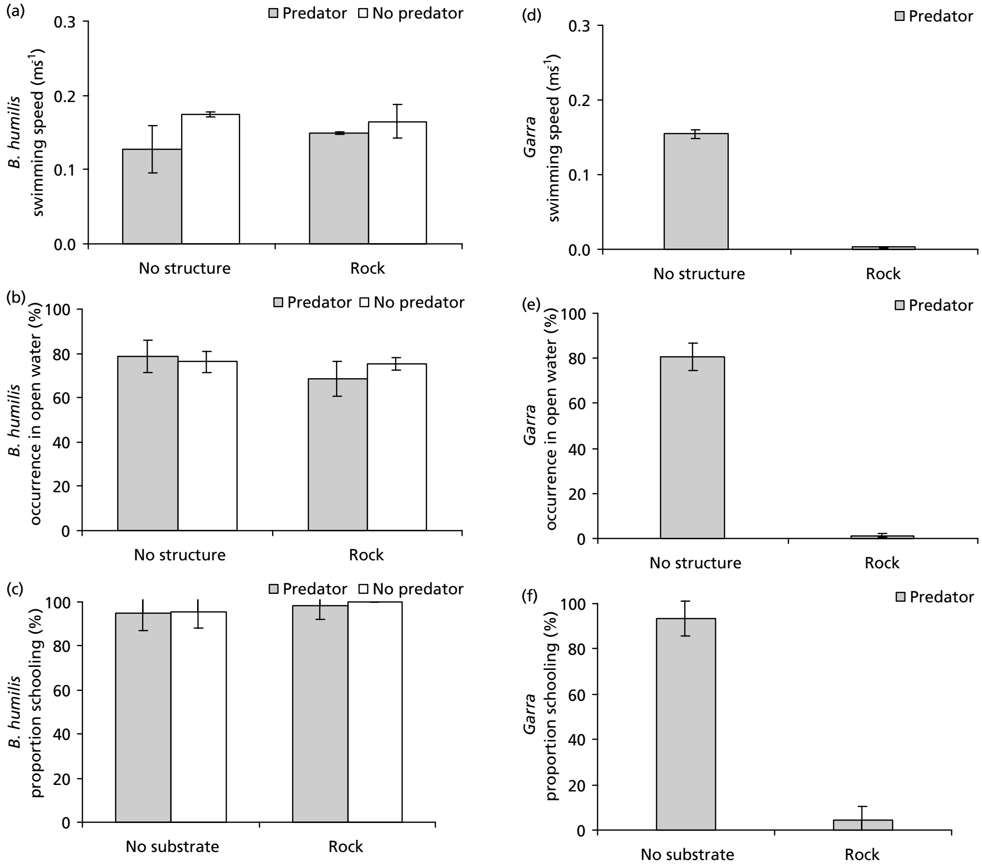


Figure 6. *Barbus humilis*, effect of structural complexity on the (a) swimming speed (mean ± SD), (b) distribution pattern (mean ± SD) and (c) schooling behaviour (mean ± SD) in the presence (grey bars) or absence (open bars) of a predator. *Garra*, effect of structural complexity in the presence of a predator on (d) the swimming speed (mean ± SD), (e) distribution pattern (mean ± SD) and (f) schooling behaviour (mean ± SD).

Prey selection in the field

Large numbers of prey fish (0-100 mm FL) were collected in the three habitats littoral (n=148491), sublittoral-pelagic bottom (n=142708) and sublittoral-pelagic surface (n=15967) in the Bahar Dar Gulf of Lake Tana between August 1999 and September 2001. Prey species and prey sizes were successfully reconstructed from the gut contents of 136 *B. acutirostris* (81 littoral and 55 sublittoral-pelagic bottom), 27 *B. dainellii*, 18 *B. gorguari*, 52 *B. longissimus* (34 littoral and 18 sublittoral-pelagic surface), 23 *B. macrophtalmus*, 66 *B. megastoma* (31 littoral and 35 sublittoral-pelagic surface), 49 *B. platydorsus* and 43 *B. truttiformis*. All piscivorous barbs were of similar size (average 303 mm FL, 2.4 mm standard error) and were collected at the same sites as the prey fish during the same period.

In the littoral area *B. humilis* is by far the most dominant prey species (92%) (Fig. 7a). The diets of especially, *B. gorguari* (42%) and *B. dainellii* (37%) contain considerably more *Garra* than expected from its abundance in the littoral zone (2.5%). *Barbus humilis* (68%) forms the major part of the diet

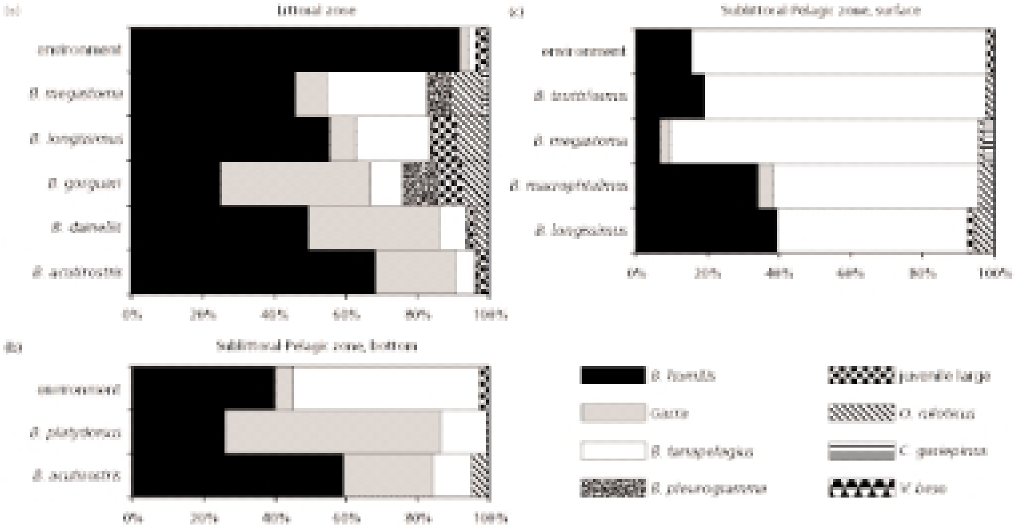


Figure 7. Relative probability of encounter for specific prey species in each of the three environments (a) littoral zone, (b) sublittoral-pelagic zone near the bottom, and (c) sublittoral-pelagic zone near the surface and the observed relative distribution of prey species occuring in the diets of the piscivorous *Barbus* species in the same three environments in the Bahar Dar Gulf of Lake Tana.

of *B. acutirostris*. Smaller specimens (200-300 mm FL) of *B. longissimus* and *B. megastoma* are regularly found in the littoral zones of the lake (Chapter 4) and are therefore included in Fig. 7a. Their diets are similar containing *B. humilis* and the 'rare' *B. tanapelagius*, in contrast to *B. dainellii*, *B. gorguari* and even *B. acutirostris*, *Garra* only forms a small part of their diet. *Barbus* acutirostris and especially *B. platydorsus* are the main benthic piscivores in the sublittoral-pelagic area. The diet of *B. platydorsus* predominantly consists of *Garra* (60%) while this prey species is far less abundant (5%) than *B. humilis* (36%) and *B. tanapelagius* (53%) (Fig. 7b). The diet of *B. acutirostris* in the sublittoral-pelagic area is similar to its diet in the littoral zone and consists of *B. humilis* (59%) and *Garra* (25%). The species composition of the diets of the surface-dwelling pelagic *B. truttiformis* and *B. megastoma* are highly similar with the prey species composition in that environment (Fig. 7c). *Barbus macrophtalmus* and *B. longissimus* contain a little more *B. humilis* than the environment offered, however, these species have a broader distribution occurring frequently in the sublittoral areas where *B. humilis* is more common.

For each prey species, piscivorous *Barbus* and prey length distributions observed in the field from September 1999 to October 2001 were combined to generate a probability distribution of prey-to-predator length ratios, following Scharf et al. (1998). Frequency distributions of PPRs occurring in the environment peaked at 0.15-0.18 PPR for *B. humilis* (Fig. 8 *B. humilis* a). The observed length frequency distributions of the PPRs in the diet of the piscivorous *Barbus* species peaked at 0.15 PPR (Fig. 8 *B. humilis* c). In *B. humilis*, multiplying the probability distributions of length ratios occurring in the environment by the capture success function yielded higher relative frequencies at lower PPRs in the resulting expected frequency distribution (Fig. 8 *B. humilis* c). PPRs peaked at 0.15 in both the predicted and observed frequency distributions when feeding on *B. humilis* (Fig. 8 *B. humilis* bc). Probability distributions of PPRs occurring in the environment peaked at 0.18 PPR for *B. tanapelagius* (Fig. 8 *B. tanapelagius* d). The observed length frequency distributions of the PPRs in the diet of the piscivorous *Barbus* species peaked at lower ratios (0.15 PPR; Fig. 8 *B. tanapelagius* e) similar to those of *B. humilis* (Fig. 8 *B. humilis* c).

Probability distributions of PPRs occurring in the environment were more equally distributed over the whole size range for *Garra* and showed two small peaks at 0.18 PPR and 0.35 PPR (Fig. 8 *Garra* f). The observed length frequency distributions of the PPRs in the diets of the *B. acutirostris* and *B. platydorsus* peaked again at 0.15 PPR (Fig. 8 *Garra* g) similar to those of *B. humilis* (Fig. 8 *B. humilis* c) and *B. tanapelagius* (Fig. 8 *B. tanapelagius* e). However, the observed length frequency distributions of the PPRs in the diet of the *B. dainellii* and *B. gorguari* peaked at higher ratios, 0.28 PPR (Fig. 8 *Garra* h).

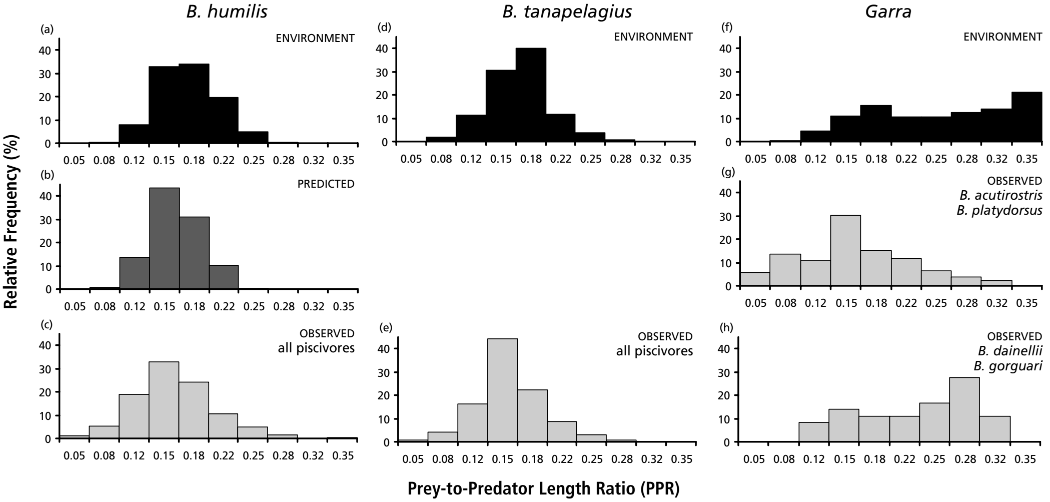


Figure 8. Relative frequency distributions of prey-to-predator length ratios (PPRs) for piscivorous *Barbus* species when feeding on *B. humilis* (abc), *B. tanapelagius* (de) and *Garra* (fgh) in the Bahar Dar Gulf during September 1999 to October 2001.

B. humilis: Distribution (a) [Environment] represents the relative probability of encounter for specific PPRs. Distribution (b) [Predicted] is generated through multiplication of distribution a with laboratory-derived capture success function for all piscivorous barbs when feeding on *B. humilis* and represents the expected relative frequency distribution of PPRs occurring in the diets of piscivorous barbs. Distribution (c) [Observed] represents the relative frequency distribution of PPR's in the diets of piscivorous *Barbus*.

Barbus tanapelagius: Distribution (d) [Environment] represents the relative probability of encounter for specific PPRs. Distribution (e) [Observed] represents the relative frequency distribution of PPR's in the diets of piscivorous *Barbus*.

Garra: Distribution (f) [Environment] represents the relative probability of encounter for specific PPRs. Distribution (g) [Observed] represents the relative frequency distribution of PPR's in the diets of the piscivorous *B. acutirostris* and *B. platydorsus*. Distribution (h) [Observed] represents the relative frequency distribution of PPR's in the diets of the piscivorous *B. dainellii* and *B. gorguari*.

Predation techniques

In general, the piscivorous large barbs were difficult to obtain alive in the field and proved to be easily stressed during handling in the laboratory, resulting in the refusal to eat for days or even weeks. Due to these constraints, successful prey capture events were recorded for only two individuals each of *B. macrophtalmus*, *B. megastoma* and *B. truttiformis*. The displacement and timing of the head structures, our kinematic variables, during prey capture are highly similar in *B. macrophtalmus* and *B. megastoma* (Table 3; Fig. 9ab). The variables T_{ogmax} and T_{opmax} did not differ between the three species, however, T_{op}-T_{in} and Dpr deviated markedly in *B. truttiformis* compared to the two other species. In *B. truttiformis*, the forming of the opercular slits occurred earlier, in many events even before the prey had passed the oral gape (Table 3) and the protrusion length during prey capture was shorter (Fig. 9c, Table 4).

Table 3. A comparison of kinematic variables for piscivorous *Barbus* (Cyprinidae) with typical representatives (Muller and Osse, 1984) of the different types of suction feeding, velocity suction with protrusion in *Pterois russellii,* overswimming with little suction in *Oncorhynchus mykiss* and volume suction in *Gadus morhua*. SL = standard length, T_{ogmax} = time (ms) to maximum gape, T_{opmax} = time (ms) to maximum opercular abduction T_{op} = time (ms) of opercular valve opening and forming of opercular slits, T_{in} = time (ms) that the center of the prey passed the oral gape aperture, Min = minimum value, SD = standard deviation, n = number of successful capture events recorded per individual. References are indicated in brackets: a = van Leeuwen (1984), b = Muller and Osse (1984), c = van Oostenbrugge (unpubl. data), d = van de Weerd et al. (1999), and e = van de Weerd (unpubl. data).

	Species	SL (mm)	prey	T_{ogmax} (ms)			T_{opmax} (ms)			T_{op}-T_{in} (ms)			
				min	mean	SD	min	mean	SD	min	mean	SD	n
velocity suction with protrusion	*B. macrophtalmus*	273	fish	**25**	39.5	11.3	**50**	70.5	16.7	**10**	16.4	6.7	11
	B. macrophtalmus	268	fish	**20**	33.1	7.8	**45**	58	11.0	**0**	9.4	6.0	27
	B. megastoma	310	fish	**30**	50.6	9.0	**50**	82.8	11.8	**10**	16.5	6.1	33
	B. megastoma	283	fish	**30**	40.0	8.2	**65**	86.3	39.7	**0**	27.5	22.5	4
	B. gorguari [d]	350	fish							**7**	9.5	3.5	2
	Pterois russelli (Scorpaenidae) [b]	138	fish	**15**	24.4	7.6	**23**	40	12.3	**-1**	6.2	5.1	7
overswimming	*B. truttiformis*	285	fish	**30**	56.1	16.7	**55**	89.4	28.2	**-25**	4.2	12.7	18
	B. truttiformis	263	fish	**60**	97.8	19.5	**110**	143	23.6	**-35**	1.9	12.8	18
	Aspius aspius (Cyprinidae) [c]	348	fish	**30**			**75**			**-18**			
	Oncorhynchus mykiss (Salmonidae) [a]	*345*	*meat*	**55**			**70**			**-17**			
volume suction (with protrusion)	*B. dainellii* [e]	254	fish	**55**	119	37.6	**120**	210.3	35.1	**0**	43.3	18.8	21
	B. acutirostris [e]	254	fish	**25**	53.6	22.1	**55**	100	34.8	**10**	25	19.1	7
	B. acutirostris [e]	250	fish	**25**	67.5	44.3	**50**	146	77.9	**15**	26	13.9	5
	B. acutirostris [d]	250	fish							**50**			1
	B. acutirostris [d]	268	fish							**33**			
	Gadus morhua (Gadidae)	*375*	*meat*	**28**	*32.1*	*3.6*	**79**	*91*	*10.6*	**60**	*65.1*	*5.2*	*3*

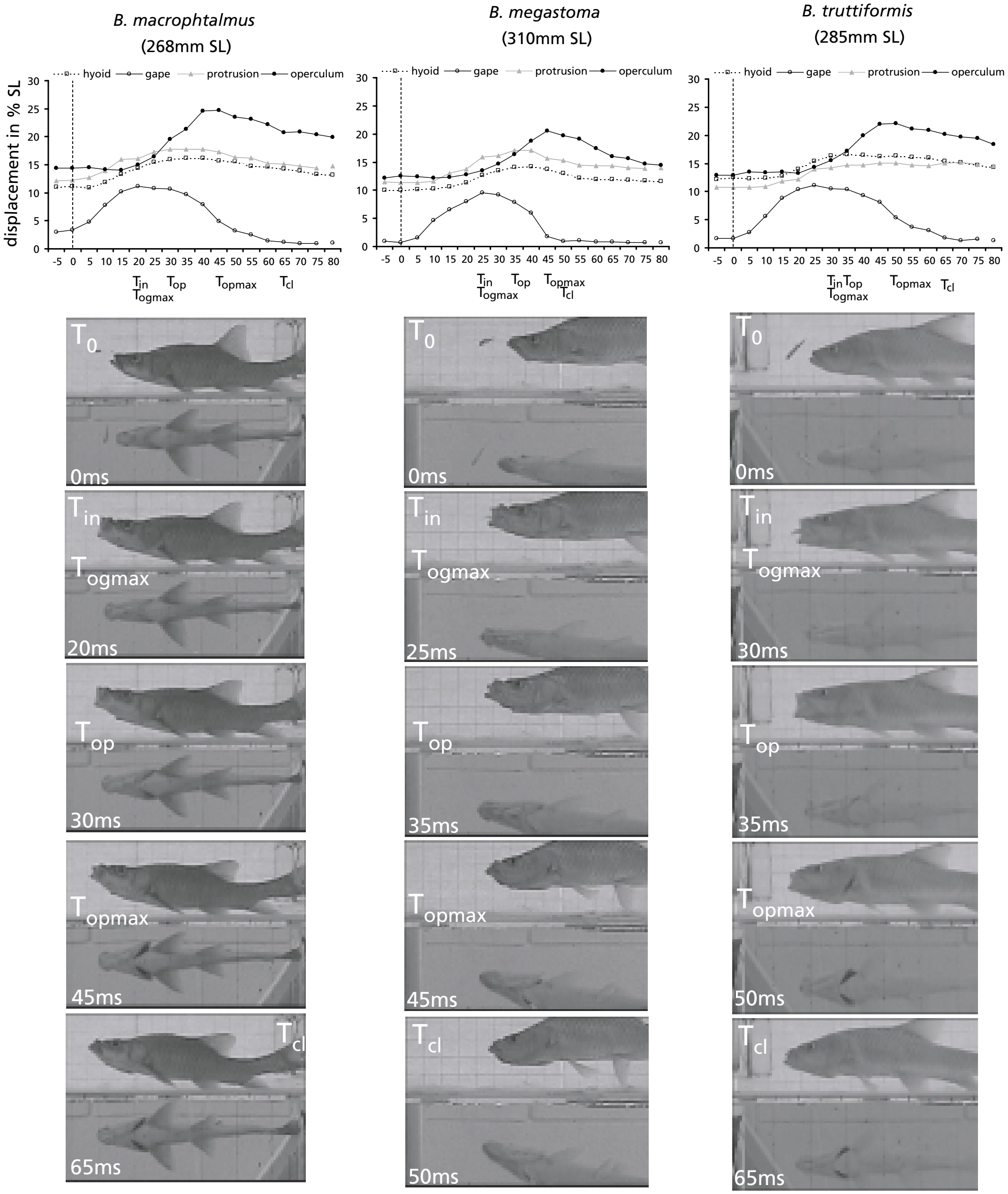


Figure 9. Kinematic profiles of the four measured variables and selected video frames (200 frames s^{-1}) from a prey capture event for (a) *B. macrophtalmus*, (b) *B. megastoma* and (c) *B. truttiformis*. T_0, the onset of mouth opening is indicated by a vertical dashed line. Displacement is expressed in percentage standard length. T_{ogmax} = time (ms) to maximum gape, T_{in} = time (ms) that the center of the prey passed the oral gape aperture, T_{op} = time (ms) of opercular valve opening and forming of opercular slits, T_{opmax} = time (ms) of maximum opercular abduction, T_{cl} = time (ms) to closure of the oral gape,

Discussion

Prey behaviour

The presence of structural complexity (both 'vegetation' and 'rock') had no effect on predation rate by the piscivorous *Barbus* when feeding on *B. humilis*, neither in the indoor tanks nor in outdoor pools. In studies with similar experimental designs, the presence of structural complexity decreased predation rate in zander, *Stizostedion lucioperca* (Greenberg et al. 1995) and in perch, *Perca fluviatilis* (Christensen and Persson 1993). In these studies, prey (crucian carp *Carassius carassius*; roach *Rutilus rutilus*; juvenile perch *Perca fluviatilis*) increased their time hiding in the artificial 'vegetation' or 'rock' substrate in the presence of a predator, reducing encounter and predation rates. For the ambush hunting northern pike, *Esox lucius* (Greenberg et al. 1995) presence of artificial vegetation even increased predation rate. Like rudd (*Scardinius erythrophtalmus*) in the experiments by Greenberg et al. (1995), *B. humilis* swam rapidly, throughout the pool in a tight shoal and spent little time in the area with structural complexity, even in the presence of a predator. Therefore, the encounter and predation rates were similar in the trials with or without structural complexity. The reduction in swimming speed, activity level, in *B. humilis* in the presence of a predator might be a mechanism to avoid detection and decrease encounter rate (Christensen 1996 and references therein). *Barbus humilis* seems to use predominantly schooling as an anti-predator behaviour. *Garra dembecha* behaved completely different, in the presence of 'rock', the shoal dispersed immediately and hiding in crevices prevailed. *Garra* rarely moved during these trials. Without artificial rock *G. dembecha* stayed in a shoal and darted nervously over the bottom of the experimental pool.

In the Bahar Dar Gulf of Lake Tana, *Garra* species only occur at the bottom (sublittoral and pelagic zones; '*G. microstoma*' and '*G. tana*') or between rocks and submerged vegetation in the littoral areas (*G. dembecha*) (Chapter 4). *Barbus humilis* is distributed throughout the water column in the littoral-sublittoral areas, while shoals of *B. tanapelagius* are restricted to the top layer of the water column, close to the surface in the sublittoral-pelagic areas (Dejen et al. 2003; Chapter 4).

Divergence of predation techniques

Four of Lake Tana's eight piscivorous species occur predominantly near the surface in the sublittoral-pelagic area. *Barbus truttiformis*, *B. megastoma* and *B. macrophtalmus* are found more towards the pelagic zone while *B. longissimus* is more common near the surface in the sublittoral areas (Chapter 4). All four species forage high in the water column, which explains the high proportion of *B. tanapelagius* and the lack of *Garra* in their diet. Furthermore, all four species have a very small protrusion angle, hence a nearly terminal mouth opening in comparison with the four benthic feeding piscivores (Table 4). Two different predation techniques are deployed by these pelagic piscivores. *Barbus truttiformis* is a typical pursuit hunter using overswimming (e.g. *Oncorhynchus mykiss*; Muller and Osse 1984; Table 3), characterized by the early formation of opercular slits during prey capture (Table 3) and small protrusion (Table 4). Although no prey capture events for *B. longissimus* were recorded, its similarity in (trophic) morphology to *B. truttiformis* (Table 4; Nagelkerke and Sibbing 2000; Sibbing and Nagelkerke 2001), its distribution in the lake and its diet

Table 4. Hypothesized main predation techniques of the eight piscivorous *Barbus* species. Predictions by the Food-Fish Model (FFM) (fig. 7, p. 419; Sibbing and Nagelkerke (2001) ++ = good, + = reasonable, 0 = neutral abilities, Am = ambush, Pu = pursuit. Spatial distribution LR = littoral rock, LS = littoral surface, SLB = sublittoral bottom, SLS = sublittoral surface. Diet: G = *Garra* (benthic), H = *B. humilis* (whole water column), T = *B. tanapelagius* (top layer water column). Morphology: PrA = protrusion angle in degrees (see Fig. 2), PROT = protrusion length in percentage fork length (FL) (see Fig. 2c). References: 1 = Sibbing and Nagelkerke (2001), 2 = Chapter 4, 3 = van de Weerd et al. (1999), and 4 = van de Weerd, (unpubl. data).

Species	**PREDICTED FFM**[1]		**OBSERVED**					**main predation technique**	
	Am	**Pu**	**distribution**[2]	**diet**	**PrA° mean ±95%CI**	**PROT mean ±95% CI**	**type of suction feeding**		
B. dainellii	++	++	LR	G/H	44 ±4.3	4.1 ±0.5	volume suction [4] (with protrusion)	ambush volume suction (with protrusion)	
B. gorguari	+	+	LR/LS	G/H	10 ±4.9	2.5 ±0.7	velocity suction [3] with protrusion	ambush velocity suction with protrusion	
B. acutirostris	0	++	LS	H	17 ±2.8	1.9 ±0.2	intermediate [4] volume/velocity suction	pursuit / ambush intermediate volume/velocity suction	**benthic**
B. platydorsus	++	0	SLB/PB	G	17 ±3.9	2.0 ±0.3		ambush / pursuit intermediate volume/velocity suction	
B. macrophtalmus	0	0	SLS/PS	T/H	6 ±2.0	3.4 ±0.3	velocity suction with protrusion	'pelagic' ambush velocity suction with protrusion	
B. megastoma	0	++	SLS/PS	T/H	2 ±1.3	2.8 ±0.3	velocity suction with protrusion	'pelagic'ambush velocity suction with protrusion	**surface**
B. longissimus	+	++	LR/SLS	T/H	4 ±3.2	1.5 ±0.4		pursuit overswimming	
B. truttiformis	+	0	SLS/PS	T	3 ±1.2	1.5 ±0.2	overswimming	pursuit overswimming	

composition strongly suggest that *B. longissimus* is also a pursuit hunter using overswimming. Pursuit hunting with overswimming is restricted to the open water (Muller and Osse 1984) and not suitable to capture prey in an environment with high structural complexity, i.e. between rocks or dense vegetation. The smaller specimens (200-300mm FL) of *B. longissimus* that do occur in the littoral zone, forage therefore high in the water column and their diet consists of *B. humilis* and *B. tanapelagius*, the bottom-dwelling *Garra* consequently is rare in their diet. Based on the analysis of the high speed recordings of prey capture events and their ecology (Table 4), *B. megastoma* and *B. macrophtalmus*, are 'pelagic' ambush hunters using velocity suction with protrusion (cf. *Pterois russelli* Muller and Osse (1984), Table 3) in Lake Tana's turbid water. Their capture events are characterized by a shorter duration of the whole event (T_{opmax}), later formation of the opercular slits and larger protrusion distance (Table 3 and 4) compared with pursuit hunting *B. truttiformis*. Again the lack of the bottom-dwelling *Garra* in the diet of smaller (200-300mm FL) *B. megastoma* in the littoral zones shows that their specialization (pelagic ambush hunting using velocity suction with protrusion, terminal oral gape) comes at a cost, i.e. the inability to capture elusive prey from the bottom.

The four benthic predators *B. dainellii*, *B. gorguari*, *B. acutirostris* and *B. platydorsus* are characterized by their downward protrusion (large protrusion angles; Table 4) and the lack of the surface-dwelling *B. tanapelagius* in their diets. *Barbus dainellii* and *B. gorguari* both occur in habitats with high structural complexity, rocky shores (both species) and between the stems of submerged vegetation (*B. gorguari*) at the edge of the lake (Chapter 4), both environments are unfavourable for pursuit hunting using overswimming (Muller and Osse 1984). Among the eight piscivorous *Barbus*, the type of suction feeding of *B. dainellii* shows the most resemblance with *Gadus morhua*, volume suction (Table 3). On average the duration of the whole capture event is long and the formation of opercular slits occurs late. Volume suction (with protrusion) might enable *B. dainellii* to capture prey from a corner or between substrate, e.g. 'sucking' *Garra* from crevices. The stationary *B. gorguari* is an ambush hunter using velocity suction with protrusion (Table 3 and 4). The *B. acutirostris* and *B. platydorsus* both occur predominantly above sandy/muddy substrates in littoral and sublittoral/pelagic zones, respectively. Based on their diet (*B. acutirostris* 70% *B. humilis*; *B. platydorsus* 60% *Garra*), habitat (sandy bottom with no structural complexity), intermediate protrusion (Table 4) and intermediate type of suction feeding (*B. acutirostris*, Table 3), both predators are probably behavioural opportunists possibly switching between ambush and pursuit hunting with intermediate volume/velocity suction with protrusion. The agile *B. acutirostris* seems to lean more towards pursuit hunting, while *B. platydorsus* seems to lean more towards ambush hunting (FFM, Table 4).

Predicting the potential food niche of fish from their structural specialisations in the feeding apparatus, as in the Food-Fish model using food properties and functional morphology (Sibbing and Nagelkerke 2001), appeared reliable at the level of major food categories (e.g., phytoplankton, microcrustaceans, molluscs, insect larvae, fish). To assess the different capture techniques in piscivory (e.g. types of suction feeding) needs behavioural and ecological data and dynamic parameters like velocity in head-expansion and timing of opercular slit opening. Such is beyond the resolving power of the current Food-Fish Model, which needs further refinement to predict this from structural specialisation.

What determines prey size selection?

Postcapture factors

The high similarity in average and maximum prey size between the eight piscivorous species is remarkable (see Chapter 4, Fig. 6) and suggests that prey size among Lake Tana's piscivorous *Barbus* is somehow limited by a 'common' factor. Their average and maximum relative prey size is considerably smaller than that of non-cyprinid piscivores (Table 5). The observed differences between the piscivorous *Barbus* from Lake Tana and non-cyprinid predators might be set by gape limitations of the feeding apparatus. For some marine non-cyprinid predators (Scharf et al. 2000), variability in gape size to predator size ratios partly explained the differences in relative prey sizes (invertebrates and fish). However, the gape/predator size ratios of Lake Tana's *Barbus* are similar or only slightly smaller than most of the piscivorous species listed in Table 4 and oral gape size is therefore unlikely to cause the large differences in relative prey size. Maximum prey size is limited by gape size in the ambush hunting northern pike (Nilsson and Brömark 2000). However, in the *Barbus* species maximum prey length is considerably smaller than predicted from their maximal oral gape (Fig. 10) suggesting that other factors limit prey size among Lake Tana's barbs. The presence of pharyngeal jaws and the palatal organ, narrowing the pharyngeal slit seems much more likely to restrict prey size among the piscivorous *Barbus* than the oral gape (cf. Sibbing 1991). However, the observed maximum prey size is considerably larger than the maximum prey size predicted from pharyngeal gape measurements (Fig. 10). Pharyngeal mastication of prey does seriously affect the handling time of prey, at 0.15 PPR prey handling time is 1.5-70 times longer in the piscivorous *Barbus* compared with other piscivorous species (Table 5).

Table 5. Mean and maximum relative prey size (prey-to-predator length ratio, PPR), capture success and handling time at 0.15 PPR and oral gape ratio among some freshwater and marine piscivorous species. OG ratio = oral gape / predator length ratio. Source: 1 = Mittelbach and Persson (1998) and references therein; 2 = Scharf et al. (2000); 3 = van Oijen (1982), 4 = Lundvall et al. (1999), 5= Scharf et al. (1998), 6 = Abée-Lund et al. (1996), 7 = Hartman (2000), 8= Nilsson and Brönmark (2000), 9 = Winemiller and Kelso-Winemiller (1994).

	PPR		Capture success (0.15 PPR)	handling time (sec) (0.15 PPR)	OG ratio
Species	mean	max			
fresh water					
Barbus (Lake Tana)	0.16	0.24	21%	143	8.6
	(0.14-0.17)	(0.20-0.28)			(8.2 –9.0)
haplochromine Cichlidae	0.25[3]	0.50[3]			
Hepsetus odoe (African pike)	0.40[9]	0.72[9]			
Hydrocynus forskahlii (tigerfish)	0.26[9]	0.509			
Esox lucius (northern pike)	0.25[1]	0.50[1]		25[1]	9.7[8]
		0.58[8]			
Salvelinus fontinalis (brown trout)	0.25[1]	0.50[1]			9[1]
	0.37[6]	0.48[6]			
Salvelinus alpinus (arctic char)	0.25[1]	0.45[1]			7[1]
Micropterus salmoides (largemouth bass)	0.20[1]	0.40[1]		2[1]	16[1]
Perca flavescens (yellow perch)	0.25[1]	0.50[1]		100[1]	8[1]
Perca fluviatilis (eurasian perch)	0.20[1]	0.40[1]	80%[4]	40[1]	12[1]
salt water					
Morone saxatilis (striped bass)	0.14[7]	0.42[7]	59%[7]	2[7]	
Pomatomus saltatrix (blue fish)	0.28[2]	0.60[2]	100%[5]	3[5]	13[2]
Merluccius bilinearis (silver hake)	0.23[2]	0.72[2]			13[2]
Hemitripterus americanus (sea raven)	0.45[2]	0.80[2]			20[2]

Observations of the prey capture events in the indoor tanks, nevertheless, revealed that the piscivorous *Barbus* are capable of capturing a second (or more) prey fish before the long process of masticating the first prey is completed. Overall, postcapture factors do not explain the considerably differences in average and maximum prey size observed between the piscivours *Barbus* and non-cyprinid marine and temperate piscivores.

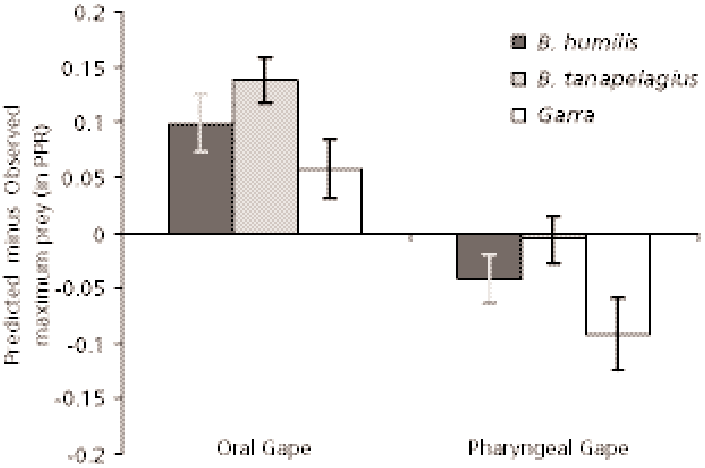


Figure 10. Comparison of predicted maximum relative prey length based on oral gape diameter and pharyngeal gape diameter and observed maximum relative prey length of Lake Tana's piscivorous *Barbus* when feeding on the *B. humilis*, *B. tanapelagius* and *Garra*. Error bars indicate 95% confidence intervals; data on oral gape/FL, head length/FL and pharyngeal gape/FL relationships piscivorous *Barbus* from Sibbing and Nagelkerke (2001); body height/FL relationship *B. humilis*, from Dejen et al. (2001); body height/FL relationship *B. tanapelagius* from de Graaf et al. (2000); body height/FL relationship *Garra* from de Graaf (unpubl. data). Note that the observed maximum prey length is much smaller than predicted by oral gape diameter but larger than predicted by the diameter of the pharyngeal slit.

Precapture factors

The close match between predicted and observed distributions of prey-to-predator length ratios (0.15 PPR, Fig. 8 *B. humilis*) in the diet of Lake Tana's piscivorous *Barbus* indicate that size-dependent capture success (precapture factors) may be largely responsible for apparent predator's prey size 'selection'. Like in other piscivorous species (blue fish, *Pomatomus saltatrix*, Scharf et al. 1998; striped bass, *Morone saxatilis*, Hartman 2000), the observed prey selection by Lake Tana's piscivorous *Barbus* is most likely a passive process mediated by differential size-related capture success rather than active choice or preference (Juanes 1994). *Barbus dainellii* and *B. gorguari* were the only species with a relative higher prey length ratio than 0.15 PPR when feeding on *Garra*. This might be a consequence of their predation technique, i.e. stationary, ambush hunting. Ambush hunters might be expected to encounter large fast-moving prey more frequently than small slow-moving prey, because of the dependence of prey movements on encounter rates (Greene 1983, 1985). The largest relative prey size were also found in typical stationary ambush hunters (goose fish, *Lophius americanus*; sea raven, *Hemitripterus americanus*) among the 18 species of marine predators studied by Scharf et al. (2000).

Size-dependent capture success might be responsible for the observed length distribution of prey. The sharp decrease in capture success with increasing PPR (mean capture success at 0.16 PPR, Table 5) and the considerable smaller average and maximum prey size (Table 5) than that of other non-cyprinid piscivores needs further explanation.

Limitations of the cyprinid 'body plan'

Suction feeding in fishes is the result of a highly coordinated explosive expansion of the oro-pharyngeal and opercular cavity, together the internal head volume, that results in a rapid drop in pressure (Osse 1969). Prey are drawn into the mouth by a flow of water that is generated by this expansion (Muller and Osse 1984; van Leeuwen 1984). Elusive prey elicit a more powerful prey capture act, i.e. strikes with a larger and faster reduction in pressure, than non-elusive prey (Elshoud-Oldenhave 1979; Nemeth 1997). Because escape speed ('elusiveness') of prey increases with body size (Lundvall et al. 1999; *B. humilis*, escape speed (ms^{-1}) = 0.07FL + 0.9, r^2=0.69, *P*=<0.001, de Graaf unpubl. data), the maximum prey size that can be captured by a predator is therefore strongly related to its capacity to develop negative pressure and the orientation of the water flow during suction feeding. The amount of negative pressure that can be generated by a predator is dependent on the speed of the opening of the oral gape (T_{ogmax}), the surface of the oral gape area, the acceleration and velocity of the head expansion and the rate of change of the internal head volume. The highest speed of the opening of the oral gape (T_{ogmax}) is similar (ca. 30ms) among the piscivorous *Barbus* of Lake Tana and the representatives of piscivores of other fish families (Table 3). Therefore, based on oral expansion time during suction feeding no differences are expected in prey size between the barbs and the other piscivores. The maximum volume of the expanded oro-pharyngeal cavity with still closed opercular slits, on the other hand, is significantly different between non-piscivorous *Barbus*, piscivorous *Barbus* and representatives of other piscivorous families (Fig. 11). The maximum internal head volume of Lake Tana's piscivorous *Barbus* is larger (Mann-Whitney U-test, *P*=0.007) than that of the non-piscivorous Barbus. But, more importantly, it is almost two times smaller (Mann-Whitney U-test, *P*<0.001) than the average maximum internal head volume of six temperate marine piscivores (Fig. 11). The relative small maximum internal head volume of Lake Tana's piscivorous *Barbus* is most likely the reason for the differences in prey size with other freshwater and marine piscivorous species. For example, comparing the capture events of two species with equal forward swimming speed, equal expansion times of the head but with different capacities of internal head volume change (volume change of species A is half that of species B) will result in species A in the reduction (factor $\sqrt{2}$) of the width between the asymptotes of the dividing streamline that separates the flow towards the mouth and the flow that passes along the forwardly moving fish (cf. van Leeuwen 1984). The chance of successful prey capture reduces more rapidly in species A when the position of the prey is located (or moves) out of the center of the 'tube-shape' ingested water flow. The key to the evolutionary success of cyprinid fishes and their competitive abilities as benthivores, is their powerful and diversified pharyngeal jaw system in combination with a highly developed palatal and sublingual organ lining their pharynx resulting in: (a) a powerful system to break down tough prey items, and (b) a highly localised gustatory control of edible and non-edible particles during feeding (Sibbing 1991ab; Sibbing and Uribe 1985; Finger 1988). However, these refined systems with tastbud densities up to 800/mm^2, occupy a large amount of space in the oro-pharyngeal cavity, reducing its volume considerably. Therefore, this key innovation of cyprinid fishes seems to have come at a cost, their reduced competitive abilities to function as piscivores and to potentially invade this niche in aquatic systems where representatives of other piscivorous fish families are present. The lack of oral teeth further limits their competitive abilities to function as piscivores, as they are not able to bite pieces of large prey and will have more difficulties preventing prey from escaping after capture.

However, Lake Tana lacks potential piscivorous competitors, rendering the piscivorous *Barbus* by far the "best" and apparently highly successful. They have adapted to all available macro-habitats, using different predation techniques, a unique scenario for barbs.

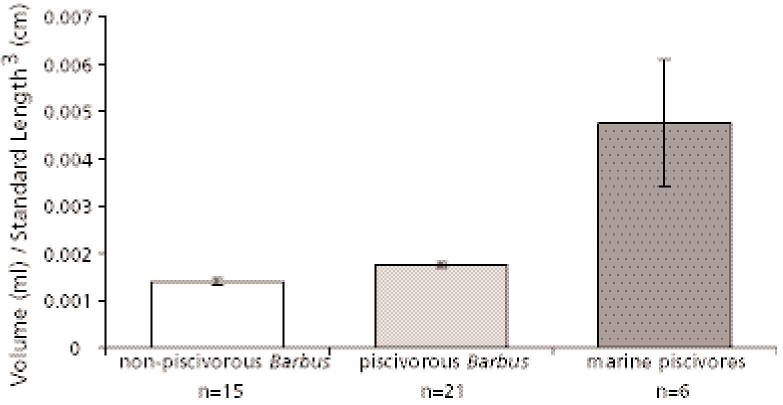


Figure 11. Volume of the oro-pharyngeal-opercular cavity in non-piscivorous *Barbus*, piscivorous *Barbus* and some temperate marine piscivorous species (*Merlangius merlangus* (whiting), *Dicentrarchus labrax* (sea bass), *Scomber scombrus* (mackerel), *Scophthalmus maximus* (tarbut) and *Chelidonichthys lucerna* (tub gunnard). Unpublished data from van de Weerd (piscivorous *Barbus*), Helmes (piscivorous and non-piscivorous *Barbus*) and Muller (temperate marine piscivores). Note that van de Weerd, Helmes and Muller used the same method to determine the volume of the oro-pharyngeal-opercular cavities.

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Figure 1. (a) Local fishermen lifting a basket trap in Gumara river (33 km upstream) at maximum water level, (b) Facing a rapid rise of water level in Kizen tributary, (c) Outflow of Wanzuma tributary into the main channel of Gumara River, and (d) Transport of basket traps, (e) detail of basket trap.

6

CHAPTER

Riverine spawning and reproductive segregation in a lacustrine cyprinid species flock: facilitated by homing?

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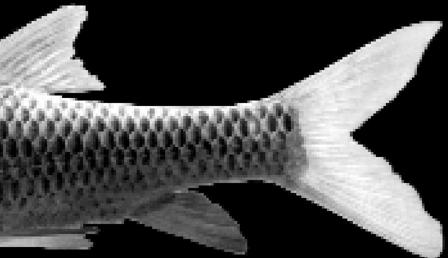
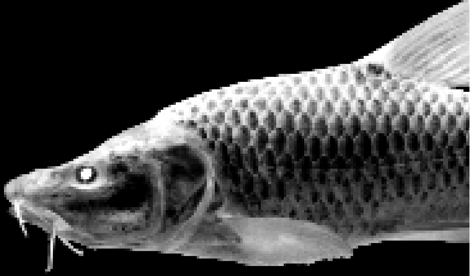




Submitted to *Ecology of Freshwater Fish*

Abstract

Migration, followed by temporal and spatial reproductive segregation in the upstream reaches of two inflowing rivers was studied for the endemic *Barbus* species flock of Lake Tana (Ethiopia) over the 1999 and 2000 spawning seasons. Physical events that may trigger lacustrine migration and characterise suitability of spawning grounds were analysed. Six species migrate 30-40 km upstream Gumara River when water levels have subsided, just after the rainy season. Spawning occurs in the well-oxygenated gravelbeds of four Gumara tributaries. Eight large barb species were absent from the rivers, or found only incidentally, thus segregating at a larger scale. These missing species might possibly migrate and spawn in upstream areas of other inflowing rivers or maybe even within the lake itself. Long distance migration and species specific spawning sites suggest homing, which might have facilitated reproductive isolation and accelerated speciation. A fine-tuning between homing and gonad development is suggested since females reach spawning maturity only at the spawning grounds. This study provides convincing evidence for reproductive isolation and therefore justifies the species status of Lake Tana's large *Barbus*.

Introduction

The diversified large *Barbus* of Lake Tana challenged conflicting hypotheses on their evolutionary status (Banister 1973; Nagelkerke et al. 1994; Mina et al. 1996ab; Nagelkerke and Sibbing 1996; Sibbing et al. 1998; Berrebi and Valiushok 1998; Dgebuadze et al. 1999). The latest revision (Nagelkerke and Sibbing 2000) distinguished 15 biological species composing an endemic species flock of *Barbus* (*Labeobarbus* following Berrebi 1995). Arguments for their species status are increasing over years: (1) their distinct morphometrics (Nagelkerke et al. 1994, 1995a; Nagelkerke 1997; Nagelkerke and Sibbing 2000), (2) their segregation in food niches (Nagelkerke et al. 1994; Nagelkerke 1997; Sibbing and Nagelkerke 2001; Chapter 4 and 5), (3) their distribution patterns (Nagelkerke et al. 1994; Chapter 4), (4) the maximal body size they attain (Nagelkerke and Sibbing 1996) and (5) indications of spawning segregation (Nagelkerke and Sibbing 1996).

Mina et al. (1996ab) and Dgebuadze et al. (1999) have suggested a combination of some distinct species and several phenotypically plastic species. Dgebuadze et al. (1999) stated that there were no evident differences in time and place of spawning among barbs representing the different morphotypes, although sampling in the same area and period as Nagelkerke and Sibbing (1996). The few field data on spawning segregation among Lake Tana *Barbus* (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999) were highly fragmentary, ambiguous, and lacking for many species, due to the difficult environment and logistics. In particular, heavy rainfalls and high water levels complicate access to remote areas and fish sampling (Fig. 1). Previously, no efforts were made to distinguish between tributaries of the main rivers (data were even pooled in Dgebuadze et al. 1999) although there were indications that these were used as spawning grounds (Alekseyev et al. 1996; Dgebuadze et al. 1999). The available data do not provide a solid base for conclusions about pre-mating mechanisms of reproductive isolation, although such knowledge is crucial for the proposed hypotheses. All known African lacustrine large *Barbus* species migrate towards rivers for reproduction (Tómasson et al. 1984; Skelton et al. 1991). Homing is widespread among migratory fishes (review in Lucas and Baras 2001). As documented for salmon (Quinn et al. 2001), homing may serve as a means of reproductive isolation. Reproductive migration of Lake Tana large *Barbus* was reported by previous studies (Nagelkerke et al. 1995a, 1996). In the spawning season local fishermen even gathered at the river mouths for the most profitable catches (Wudneh 1998; Chapter 11 and 12). In order to clarify ambiguities, this study investigated reproductive isolation more systematically, analysing spawning segregation along temporal and spatial dimensions on micro-scale (twice a week sampling of main rivers and small tributaries), throughout the spawning season. Solid scientific data on their reproductive biology are urgently required to protect the reproductive part of the population and to stop the decline of Lake Tana's unique cyprinid fish stocks (Nagelkerke et al. 1995b; de Graaf et al. 2003b). In the end, this contributes to rendering fisheries sustainable and protecting the biodiversity of this natural laboratory.The aim of this paper is to determine patterns in spawning segregation among upstream migrating riverine spawning *Barbus* species of Lake Tana. Does each species have its own separate spawning grounds, e.g. by utilising a specific tributary? Does temporal segregation exist among riverine spawning *Barbus*? Abiotic parameters were measured, in search of possible triggers of upstream spawning migration and to determine the suitability of riverine spawning grounds.

Materials and methods

Study area

Lake Tana is situated in the north-western highlands of Ethiopia at an altitude of 1830 m. It covers an area of approximately 3150 km^2 and is relatively shallow (average depth 8 m, maximum 14 m). The lake area has a tropical highland climate with mild temperatures (extremes 7-31 °C). The dry season is from October until May, with the rains peaking in July and August (Nagelkerke and Sibbing 1996; Wudneh 1998). The lake water has an average temperature of 21.7°C with a low annual variation (<5°C, Wudneh 1998). Four permanent rivers (Rib, Gumara, Gelda and Gelgel Abbay; Fig. 2a) feed Lake Tana, three smaller inflowing rivers are in the north. The Blue Nile is the only outflowing river. High falls (40 m) situated 30 km downstream its outflow isolate Lake Tana from the lower Blue Nile basin.

Upstream sampling areas

Upstream sites in Rib and Gumara River and three of the latter's tributaries (Kizen, Dukolit and Wanzuma) were sampled during the 1999 and 2000 rainy season (Fig. 2b, Table 1).

Rib (Fig. 2a) is approximately 90 km long and originates at an altitude of 2400m. During the rainy season Rib is about 10 m wide near the bridge (R I in Fig. 2a), up to 3 m deep and its banks are composed of clay and sand. Close to the bridge, Rib is joined by a shallow 3 m wide tributary (0.2-1 m deep) with a muddy substrate. Rib has a low decline and, according to local fishermen, does not posses upstream waterfalls or gravelbeds in its main stream or tributaries. In the rainy season, the lower part of Rib is connected to floodplain reservoirs.

Gumara (Fig. 2a) is approximately 104 km long and originates at an altitude of 2500-3000 m. In its main channel, four sites were sampled (Fig. 2b; Table 1). Near the bridge (G I) Gumara is during the rainy season 25-30 m wide, up to 3 m deep with a bed composed of clay. Gumara River at G II narrows to 15-20 m wide and its bed is muddy. Further upstream, near the village Wanzaye (G III), a series of deep pools terminate in wide riffles (0.1-0.4 m deep) of bedrock, boulders and cobble. Upstream of Wanzaye (G IV), a deep pool is located in the main channel. Upstream from this pool the river is characterised by rapids running in bedrock from two waterfalls (Fig. 2a, 43 km upstream), the first being about 5 m high and the second 12 m high.

Four temporary tributaries join Gumara between the bridge (G I) and the first waterfall (43 km upstream). Kizen tributary (Fig. 2b) is 3-5 m wide, up to 1.5 m deep and has a muddy mouth. Further upstream this tributary narrows and its bed is composed of cobble and gravel. Wanzuma is a small streamlet (about 2 m wide) with pools (up to 1.5 m deep) separated by flat gravel beds. Dukolit is a shallow streamlet (3 m wide, 0.1-0.7 m deep) running over a bed of cobble and gravel. A 6 m high waterfall is located about 100 meters upstream from the mouth. Chan tributary (Fig. 2b) is about 7 m wide, 0.1-1 m deep and runs over bedrock and gravel.

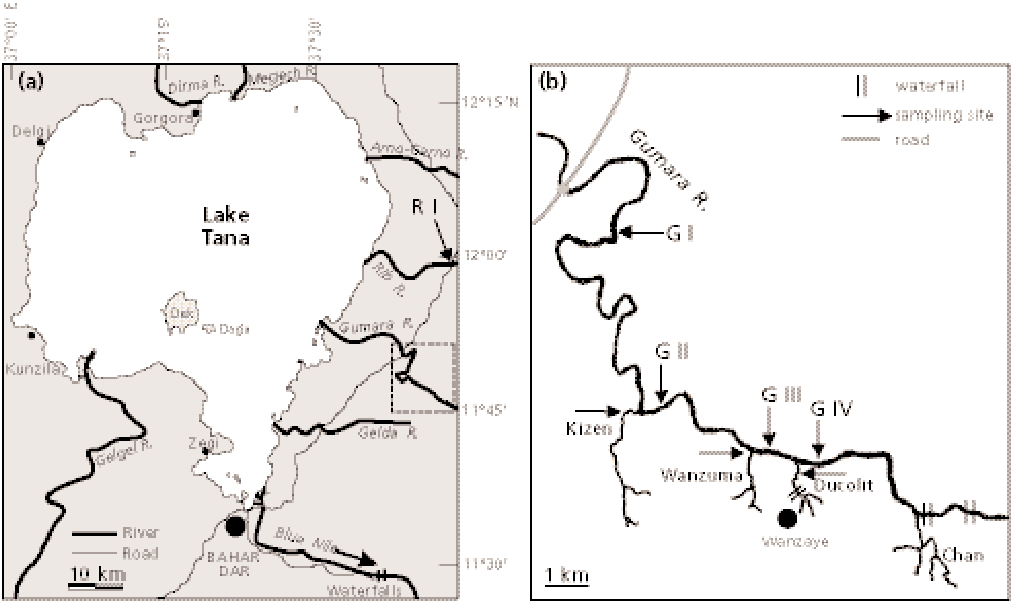


Figure 2. (a) Lake Tana and its major rivers, (b) *Barbus* spawning grounds in Gumara river: sampling sites in its main channel (G I-IV) and in its tributaries Kizen, Wanzuma and Dukolit. Symbols explained in the legend

Table 1. Sampling sites at main rivers and their tributaries (italics), their location upstream from Lake Tana, sampling periods and type of fishing gear used. G I-IV (Gumara) and R I (Rib) refer to the site numbers in Figure 2b.

Site	Code	Km upstream	Period	Gear
Spawning season 1999				
Gumara	G III	40	08/09-01/10	basket traps
Kizen		38	11/09-01/10	Gillnet
Dukolit		41	02/09-15/09	Gillnet
Spawning season 2000				
Gumara	G I	33	05/08-30/10	basket traps, hook and line
	G II	38	18/08-29/08	hook and line
	G III	40	19/08-30/10	basket traps, scoopnet, hook and line
	G IV	42	08/09-30/10	scoopnet, hook and line
Kizen		38	19/08-16/09	Fyke
Wanzuma		40	19/08-16/09	Fyke
Dukolit		41	19/08-16/09	Fyke
Rib	R I	19	11/08-25/09	basket traps

Sampling program and gear

During the spawning season of 1999 (August and September; Table 1), the mouths of Gumara's tributaries Kizen and Dukolit were sampled twice a week with 50-m long gillnets (bar mesh size 40 mm) that were set overnight. From Gumara's main channel catches of local fishermen were monitored. They fished overnight with large (2-m long) handmade basket traps (average bar mesh size 55 mm) which were anchored along the shore of Gumara (site G III; Fig. 1).

During the 2000 spawning season, the sampling program was intensified including Rib River, three more sampling sites along Gumara's main channel (G I, II and IV) and Gumara's tributary Wanzuma. The program was extended to three months (early August – late October; Table 1). Local fishermen were hired to catch fish with both small and large meshsize basket traps (average bar mesh resp. 17 mm and 55 mm) in the main channel of Gumara (G I, G III) and Rib. Gumara's tributaries were sampled overnight using two large fykes (length 5.2 m, 8 compartments) and one small fyke (polyfilament twine; meshbar 5 mm, length 3.5 m, 6 compartments). The entrance of the tributaries was blocked completely, using 4.5 - 9 m long nets (bar mesh size 5 mm) leaving the entrance of the fyke. Also catches of local fishermen using large basket traps, scoopnets or hook and line were monitored (Table 1). Dukolit was observed at night for spawning activity. Sampling was halted after tributaries Kizen, Wanzuma and Dukolit dried (September 16, 2000).

In the morning at 6.00 hr, catches were collected. Barbs were identified as belonging to one of the fifteen species, following Nagelkerke and Sibbing (2000). Their sex was determined and their spawning condition was investigated, i.e. running (large amounts of sperm or eggs can be extruded by pressing the abdomen) or not running (De Silva et al. 1985; Pet et al. 1996). The presence of running females was used to specify the timing of spawning.

Abiotic environmental variables

In the tributaries, the oxygen content (mgl^{-1}), water temperature (°C) and water transparency (Secchi-depth in m) were measured after removing the gillnets or fykes. At Rib and Gumara bridges water level was measured by reference to the distance between the water surface and the bridge. The smallest distance was taken as maximum level.

Data processing

(a) Comparison between Rib and Gumara river. Over the spawning season 2000, basket trap catches at the bridge sites G I and R I were compared (Fig. 3). Catch per unit effort (CpUE) was defined as the number of individuals belonging to genus *Barbus* or *Clarias* per hour per trap.

(b) Temporal segregation in Gumara river and its tributaries. Basket trap catches at the bridge (G I) and fyke-catches at the tributaries of the spawning season 2000 (Table 1) were used.

(c) Spatial segregation in Gumara river and its tributaries. During the 1999 spawning season, species composition data from basket traps (bar mesh 55 mm) at Gumara (Table 1, site G III) and gillnet data from tributary Kizen were compared. The small species *B. brevicephalus* was able to escape through the fishing gear and therefore absent in the catches. For analysing spatial segregation over the spawning season 2000, pooled data representing catches of all gears (traps, fykes, scoopnets and hook and line) were used. Temporal and spatial differences in the frequency of occurrence of the *Barbus* species (rows listed in Table 3) were compared among species using χ^2 *r x c* contingency tables. Statistically significant differences ($P<0.05$) indicate segregation in area or time of spawning. In multiple comparisons between the species, the level of significance was taken as $P<[0.05/n]$ (in which n is the total number of comparisons), in order not to overestimate the number of significant differences (Cooper, 1968).

(d) The oxygen content (mgl^{-1}), temperature (°C) and vertical transparency (m) of the different water bodies were compared through one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc tests for multiple comparisons if significant variance was found. Null hypotheses were rejected at $P<0.05$. In order to meet the assumptions for analysis of variance, transparency data were natural-log transformed.

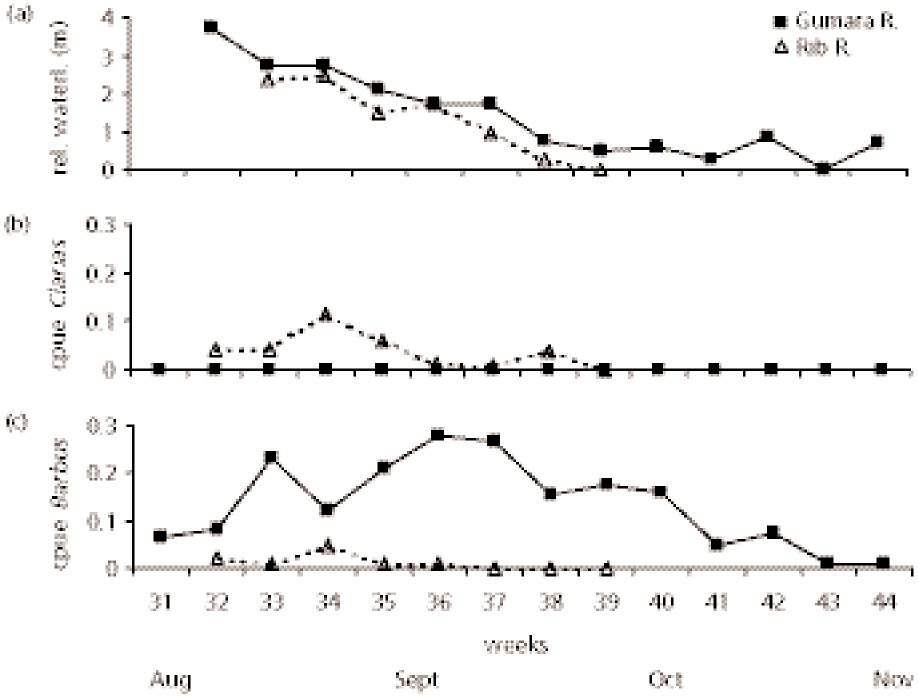


Figure 3. Weekly average in abiotic and biotic characters compared between Gumara (■) and Rib (Δ) river (bridge sites G I and R I; Fig. 2b) during August – November 2000. (a) relative water level in Gumara and Rib are similarly decreasing over time; (b) catch per unit effort (CpUE) of *Clarias* in Gumara (only two specimens) is lower than in Rib; (c) catch per unit effort of *Barbus* in Gumara is much higher than in Rib.

Results

Physical differences among rivers and streams

The specific water body had a highly significant overall effect on oxygen ($F_{(4,\ 55)}$ = 15.64; *P*<0.001), water temperature ($F_{(4,\ 55)}$ = 4.96; *P*<0.002) and vertical transparency ($F_{(3,\ 46)}$ = 26.43; *P*<0.001) (Table 2).

Comparison between Rib and Gumara River (Fig. 3) shows that waterlevels display similar patterns (Fig. 3a). After heavy rains in upstream areas during July, the river level gradually decreases 4 m over August-October. The abundance of *Barbus* and *C. gariepinus* peaks shortly after the highest waterlevels. Dissolved oxygen during the sampling period in Gumara (mean 7.2 mgl^{-1}± 0.07 SE) was higher (*P*<0.001) than in Rib (mean 6.5 mgl^{-1}± 0.09 SE; Fig. 4b), and so is the saturation (79.4% in Gumara, 72.2% in Rib) since the water temperatures are similar (*P*>0.05). Transparency is very low (<0.1 m) and similar (*P*>0.05) in the sediment-loaded Rib and Gumara rivers. Gumara's tributaries have much higher transparency than Gumara's main channel (*P*<0.001; Fig. 4a). Wanzuma has the lowest (*P*<0.01) temperature (18.7 °C ± 0.3 SE; Fig. 4c). At the end of September all three tributaries ran dry.

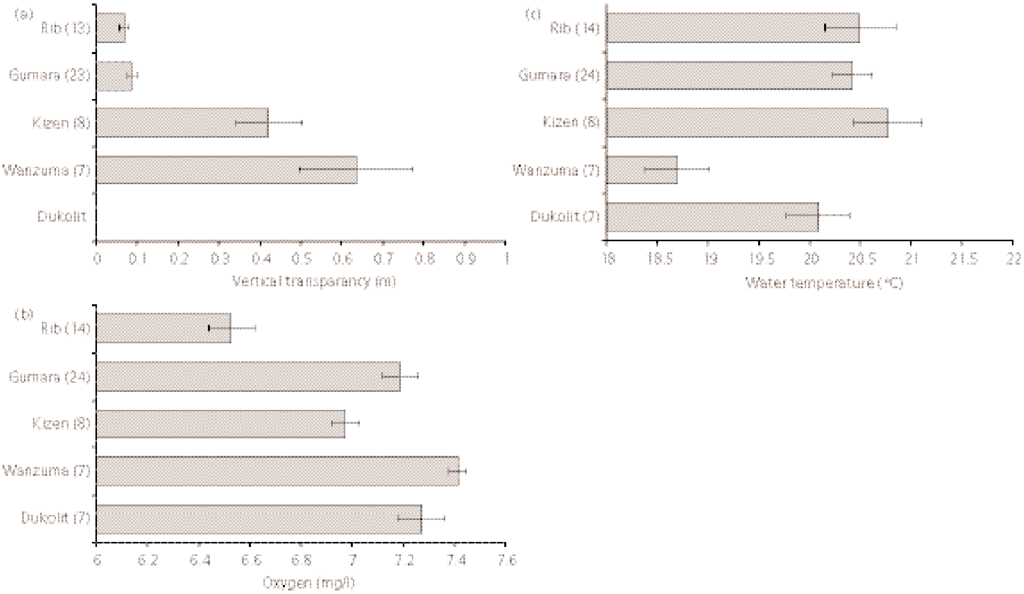
Table 2a. Abiotic parameters, compared among rivers and streams; their mean, standard error (SE) and number of observations (N).

	Oxygen (mgl^{-1})			**Water temperature (°C)**			**Vertical transparency (m)**		
	MEAN	SE	N	MEAN	SE	N	MEAN	SE	N
Rib	6.5	0.09	14	20.5	0.4	14	0.07	0.01	13
Gumara	7.2	0.07	24	20.4	0.2	24	0.09	0.01	23
Kizen	7.0	0.05	8	20.8	0.3	8	0.4	0.08	7
Wanzuma	7.4	0.03	7	18.7	0.3	7	0.6	0.1	7
Dukolit	7.3	0.09	7	20.1	0.3	7	-	-	-

Table 2b. Pairwise comparisons of oxygen (mgl^{-1}; left lower half), water temperature (°C; right upper half) and vertical transparancy (m; below) between the waterbodies (* = *P*<0.05; ** = *P*<0.01; *** = *P*<0.001; ns = not significant *P*>0.05).

Temperature **Oxygen**	Rib	Gumara	Kizen	Wanzuma	Dukolit
Rib	X	ns	ns	**	ns
Gumara	***	X	ns	**	ns
Kizen	*	ns	X	**	ns
Wanzuma	***	ns	ns	X	ns
Dukolit	***	ns	ns	ns	X

Vertical transparency	Rib	Gumara	Kizen	Wanzuma
Rib	X			
Gumara	ns	X		
Kizen	***	***	X	
Wanzuma	***	***	ns	X



Species compositions

A total of 232 fishes were caught in Rib and 4985 in Gumara during all sampling in 1999 and 2000. In Rib, genus *Barbus* contributed 22.4 % of the total catch. Species of other genera were *Clarias gariepinus* (76.2%), *Varicorhinus beso* (0.43%) and *Oreochromis niloticus* (0.43%). In Gumara, genus *Barbus* contributed 98.3% of the total catch. Species of other genera were *Clarias gariepinus* (0.36%), *Varicorhinus beso* (1.08%), *Garra dembeensis* (0.28%) and *Oreochromis niloticus* (0.02%).

Clarias is dominant over *Barbus* in Rib River, whereas *Barbus* is by far dominant (Figs. 3bc) in Gumara River. After two months of sampling, the number of barbs remained very low in Rib and we shifted all efforts to Gumara and its tributaries.

In the *Barbus* catch composition of Gumara and its tributaries (n= 4897), six species contributed to over 99% of the catch (Table 3). Analyses were restricted to these six most abundant species.

Table 3. Temporal and spatial composition of the *Barbus* species on the upstream spawning area of Gumara River. Data represent absolute numbers of specimens.

	Temporal			Spatial						
	Aug	Sep	Oct	G I	G II	G III	G IV	Kizen	Wanzuma	Dukolit
B. acutirostris	32	29	51	23	0	18	48	4	11	8
B. brevicephalus	590	543	119	323	3	26	81	271	230	318
B. macrophtalmus	3	67	140	93	0	10	106	1	0	0
B. megastoma	27	74	11	14	7	51	31	6	3	0
B. truttiformis	85	32	0	67	1	16	6	26	0	1
B. tsanensis	1002	703	79	486	122	424	188	129	110	325

Temporal segregation

Distribution patterns of *Barbus* species over the three spawning months in 2000 (all 7 sampling stations pooled) differ significantly (χ^2, *P*<0.001) between the six most abundant species (Table 3), except for *B. tsanensis* and *B. truttiformis*. Peak abundance and spawning gradually shifted among the species from August towards October (Fig. 5). *Barbus megastoma* was the first river spawner, peaking in week 33-34, with most females in week 34. *Barbus acutirostris* had a clear peak with most females in week 34. *Barbus tsanensis* peaked in week 34 (CpUE of >11 in Dukolit), but the highest numbers of running females were seen during week 35. This pattern was also observed in *Barbus truttiformis* with a major catch of running females in Kizen (CpUE of 0.94). *Barbus brevicephalus* peaked in week 34-36 (e.g. CpUE of 17.2 in Dukolit), and the running females were most abundant during weeks week 35-36 at the same location. *Barbus macrophtalmus* showed the most distinct spawning pattern, since the running females did not peak before week 40. Even higher numbers (n=29, 53% of the total catch) were present in scoopnet catches far upstream in Gumara (G IV) during late October (week 43).

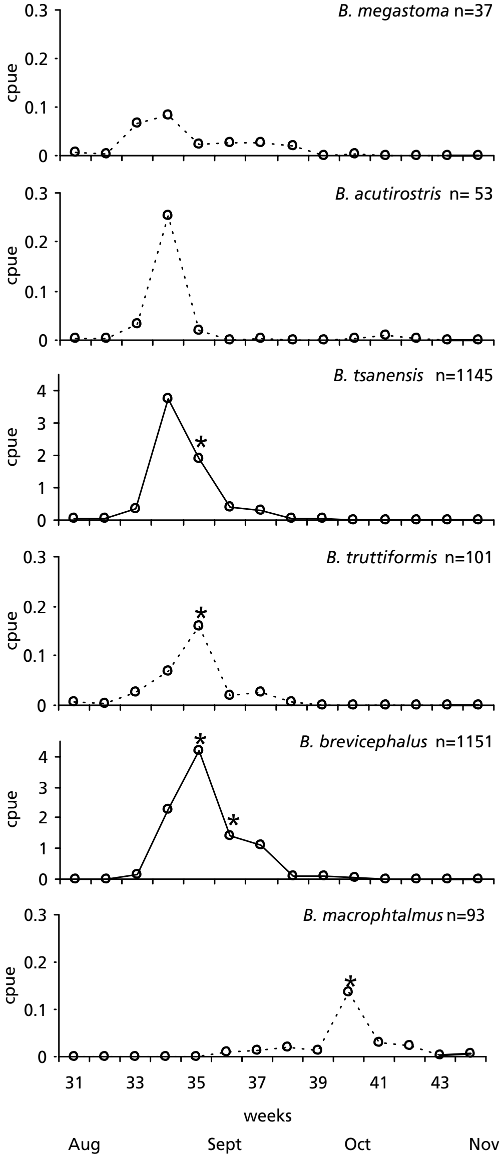


Figure 5. Temporal segregation among *Barbus* species in the Gumara area (trap catches from the main channel and fyke catches from its tributaries pooled) during August-November 2000. CpUE was defined as the number of specific *Barbus* per hour, respectively per trap or per fyke. Note that the CpUE-scale for *B. tsanensis* and *B. brevicephalus* (solid lines) is 15 times higher than for the other species (dashed lines). Asterisks mark peaks in running females.

Spatial segregation

The distribution patterns among the seven Gumara area sampling sites (all sampling weeks in 2000 pooled) also differed significantly (χ^2, $P<0.001$) between the six most abundant species of *Barbus* (Table 3). Comparing the species composition in Kizen tributary with upstream Gumara (G III) in 1999 (Fig. 6) shows that *B. truttiformis* used Kizen tributary as their main spawning ground. In August 2000, Gumara downstream of Kizen's rivermouth (Fig. 7, G I), showed a high abundance of *B. truttiformis*. In Kizen tributary, *B. truttiformis* was abundant in the second half of August and even more in September. Upstream in Gumara (G II) they were almost absent, reconfirming their utilisation of Kizen as spawning ground. The late-spawning *Barbus macrophtalmus* was present on the rocky part in Gumara's main channel upstream from the tributaries (Fig. 7). Most probably, its spawning ground is Chan tributary, since high waterfalls block further upstream migration in the main channel and other tributaries ran dry in October. The same applies to *B. megastoma* and *B. acutirostris* (Fig. 7), although a small number was present in Kizen, Wanzuma and Dukolit earlier in the season. In 1999, *B. megastoma* contributed 29% of the total catch (n=76) in Dukolit during 2-15 September. No clear spatial (or temporal) segregation between these two large species was observed, although Chan tributary was not further investigated.

Running females of the most abundant species *B. tsanensis* and *B. brevicephalus* were scarce in the main channel of the Gumara, but they were abundant in all tributaries, thereby suggesting that they did not show any marked preference for spawning in a special location, provided that it was in a tributary. Catches of running females were especially large in Dukolit.

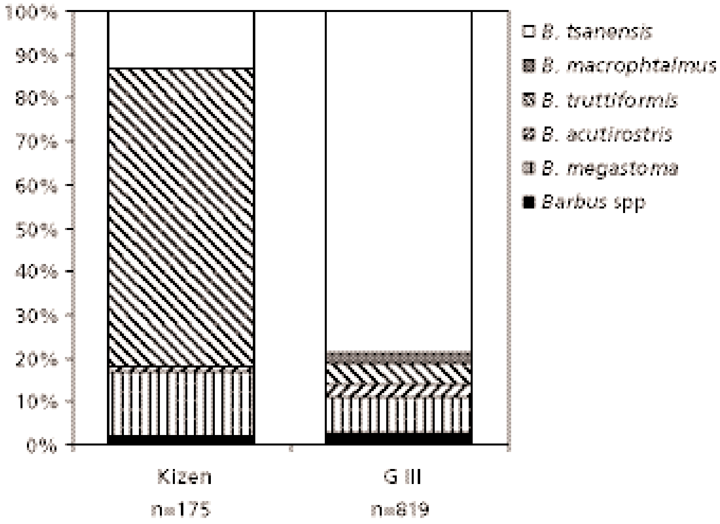


Figure 6. Species composition at Kizen tributary (gill nets) and Gumara's main channel upstream (site G III; all samples pooled) during 8-25 September 1999. Both types of gear have a comparable mesh size (bar mesh 55 mm). *B. brevicephalus* was omitted because it could escape from the fishing gear. *Barbus truttiformis* apparently utilizes Kizen tributary.

Discussion

Migration

Most migratory fish species in the intertropical regions make their upstream runs during the rainy period or period of high flow (Lowe-McConnell 1975; Payne 1986; Wootton 1990, Winemiller and Jepsen, 1998; Lucas and Baras 2001). This pattern is also largely shared by the African barbs (reviews in Tómasson et al. 1984; Cambray et al. 1997). Based on this study and former studies of the ecology and behaviour of the Lake Tana barbs, their migration pattern can be partitioned in three major stages: (1) migration from the foraging area in the lake to the rivermouth, (2) swimming upstream the river's main channel, (3) entering a tributary for spawning after sunset (water level of the tributary rises following late afternoon rainfalls).

ad (1) Heavy rainfall starting in May and peaking in July-August increased river levels by 4 m, causing massive erosion. This resulted in a highly turbid inflow of sediment and dissolved inorganic compounds into the lake. Either increased water level or turbidity or a combination of both might serve as environmental cues that trigger barb spawners to migrate towards river mouths (Whitehead 1959; Sibbing et al. 1998) where they aggregate for the next phase (Nagelkerke and Sibbing 1996; Wudneh 1998).

ad (2) Upstream migration in the river's main channel occurred only after the peak of the rainy season, when average water level and flow velocity in the main rivers had already decreased. This might be due to the difficulty for migrants to ascend rivers and streams with fast currents. Additionally, there is no imperious need to start an ascent earlier than necessary in a system where fish migrate 30-40 km which can be travelled within a few days.

ad (3) The final stage of the migration is short, since the barbs ascend the tributary, spawn and return to the main channel within less than one day. This diel pattern appears to be a response to the local daily rainfall pattern. Around Lake Tana, rains usually start late in the afternoon and causing a fast rise in the tributaries' water level. The following morning the water level has decreased again. This might be a major reason why Lake Tana barbs are nocturnal spawners, unlike other barbs that may spawn at different times of the day (e.g. *Barbus barbus*, Baras 1995). Other factors, especially the lower risk of predation in shallow waters during the night (Dgebuadze et al. 1999), might also concur to the predominance of nocturnal spawning. Except for the daily periodicity, the spawning behaviour of *B. brevicephalus* closely resembled that described for the European barbel *Barbus barbus* (Baras 1994; Poncin 1994, Poncin et al. 1994).

Physical conditions of the spawning grounds

Highly oxygenated water and gravel beds are general requirements for *Barbus* spawning (Rodriguez-Ruiz and Granado-Lorencio 1992; Baras 1994; Baras et al. 1996) due to their critical importance in the development of eggs and larvae (Tómasson et al. 1984). Neither condition was fulfilled in Rib River, which might account for the absence of spawning barbs (Fig. 3c), but also for the dominance of *Clarias gariepinus*, which is highly tolerant to low oxygen levels. Gumara river is well oxygenated by rapids and waterfalls in its upstream reaches and these are for 99% populated by *Barbus* spp.

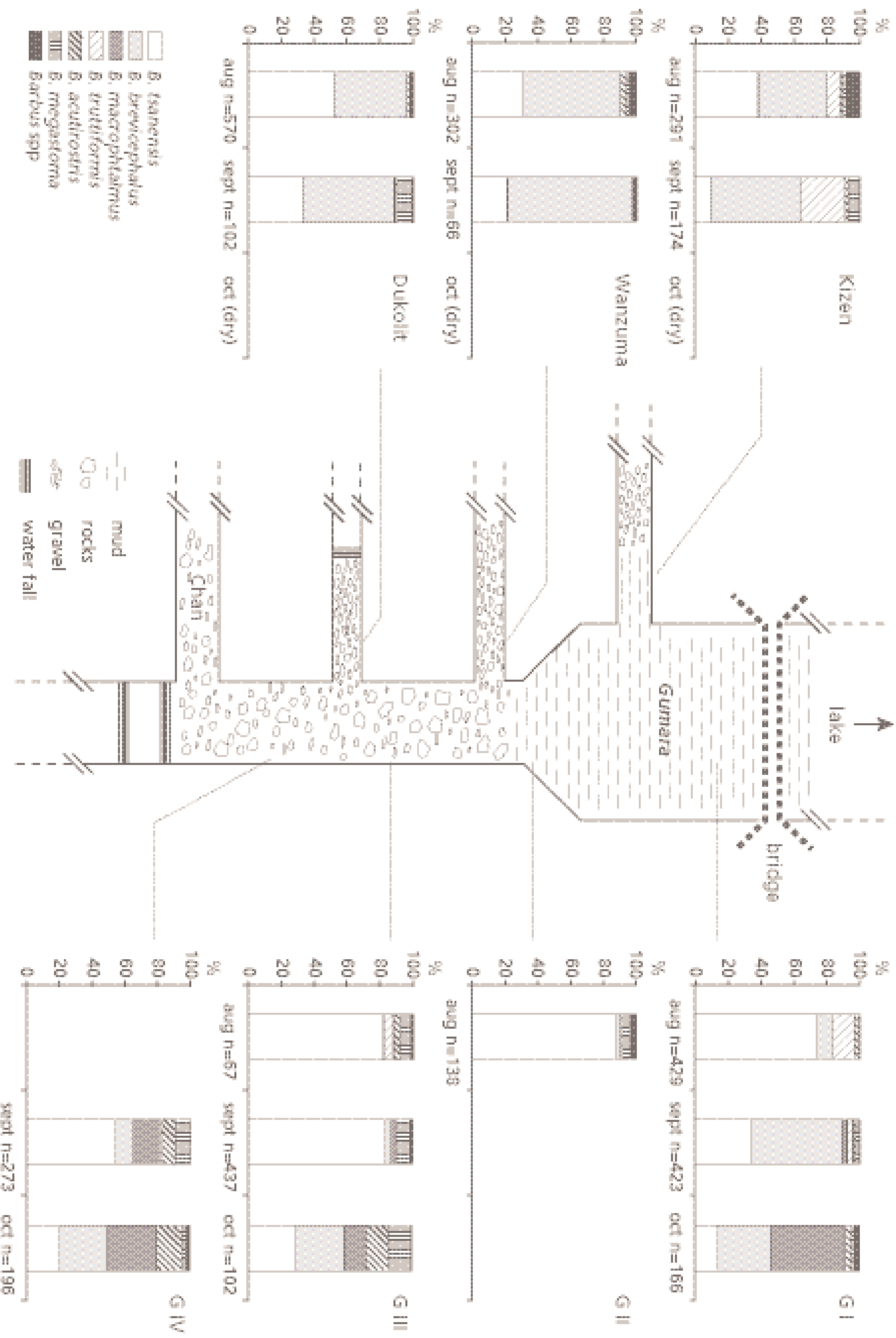


Figure 7. Schematic view of Gumara river and its four tributaries. Relative species composition (all gear data pooled) during the spawning season August – November 2000 at Kizen, Wanzuma, Dukolit, Gumara bridge (site G I), Gumara between Kizen and Wanzuma (site G II), Gumara Wanzaye (site G III) and Gumara Chan (site G IV). N refers to the total number of fishes. The tributaries ran almost dry at the end of September. Site G II was not sampled during September and October, site G IV was not sampled during August.

Deposition of eggs in gravel beds prevents them from being washed away, and clear water will not cover them with a film of sediment obstructing the diffusion of oxygen. Final maturation and spawning of *Barbus* occur in the tributaries and for some large species possibly at gravel areas in the far upper reaches of Gumara's main channel. This is deduced from the distribution of running females.

Observations on spawning

Spawning was observed and video-taped (using infrared light source) at night for *B. brevicephalus* in Wanzuma and Dukolit tributaries, both in 1999 and 2000. Immediately after sunset, migration from Gumara's main channel up to the spawning sites in the tributary occurred. Spawning took place on shallow (depth 0.10 m) gravelbeds under clear water conditions in the rivermouth of Wanzuma and from the rivermouth to the waterfall in Dukolit. Although it was observed from 19.00 hr until 1.00 hr, spawning may have continued later into the night. The examination of gillnets and fykes between 5.00 and 6.00 hr revealed a massive migration out of the tributaries suggesting that spawning had stopped by the end of the night. Densities of individuals were high, hundreds of fish splashing in the shallow water.

For growth experiments, we stripped running barbs and fertilized the eggs by mixing them with conspecific sperm. Viable batches of larvae were cultured during 1999 and 2000 (Chapter 7). Large batches of *B. tsanensis* (5) and *B. brevicephalus* (3) were obtained from Dukolit. Kizen provided two batches of *B. truttiformis*. The two batches of *B. megastoma* larvae obtained from running individuals in the Gumara river (1999) support the presumed spawning of this species upstream in the main channel.

Reproductive segregation

Pairwise comparisons of abundance over time and space showed highly significant differences among all *Barbus* species (Tabel 3; χ^2, $P<0.001$). Reproductive segregation is most distinct for *B. macrophtalmus* being the only species spawning in October (week 40-43), probably in Chan tributary (or Gumara's upstream gravel beds) like *B. megastoma* and *B. acutirostris* at the end of August. Sampling in the remote Chan tributary could provide the answers. Most barbs are abundant and running from mid-August until mid-September (week 33-36). Although there is considerable overlap, the peak of most species gradually shifts (Fig. 5).

Spatial segregation is most distinct for *B. truttiformis* utilizing only Kizen tributary as spawning ground. There is no evidence yet for segregation between *Barbus tsanensis* (week 34-35) and *B. brevicephalus* (week 34-36), they overlap both in time and place. They spawn in all tributaries, with some preference for Dukolit, close to its waterfall. However, size-dependent assortative mating (Nagel and Schluter 1998; Schliewen et al. 2001) may isolate *B. tsanensis* ($FL_{50\%}$ 26.0cm FL) and *B. brevicephalus* ($FL_{50\%}$ 18.2 cm FL) and possibly also other riverine spawning species as $FL_{50\%}$ varies highly (de Graaf, 2003a). Little is known about other pre-mating mechanisms in barbs (Dgebuadze et al. 1999), that may minimize hybridisation and contribute to assortative mating. Sexual dimorphism cf. the colourfull African cichlid species (Seehausen 1999), is not observed in barbs.

Missing species in upstream reaches

Although the present fishing efforts were quite extensive and systematically done throughout the rainy season, catching ca. 5000 large barbs, evidence for riverine spawning was provided for only six species of barbs (Table 4). This corroborates with previous reports on upstream reaches of Lake Tana's rivers (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999) and suggests (macro) segregation in reproduction of the large Lake Tana barbs. Three other species were only incidentally found in Gumara's upstream reaches: *B. platydorsus*, *B. gorgorensis* and *B. crassibarbis* (Table 4). Five species (*B. dainellii*, *B. gorguari*, *B. surkis*, *B. nedgia* and *B. longissimus*) were completely absent in Gumara river, in its tributaries and in the river mouth (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999). These species apparently spawn at times and/or places different from what was covered in this study. Systematic year-round sampling programs on species abundance and maturity in Lake Tana and all its rivermouths will clarify this issue.

Table 4. *Barbus* species composition in the upstream spawning area of Gumara River. Note that in all studies the same five species were absent on the spawning grounds.

Source	present study	Dgebuadze et al. (1999)	Nagelkerke and Sibbing (1996)
Sampling period	2 Sep - 1 Oct 1999 5 Aug – 30 Oct 2000	8-24 Oct 1994	15 Sep 1994
Sampling frequency	1-2 times per week (100)	several (10)	once
Sampling area	3 tributaries and 4 sites main channel	1 tributary and 1 site main channel	1 site main channel
Number	4896	540	70
Species composition (%)			
B. tsanensis	52	33[a]	42
B. brevicephalus	29	36[b]	27
B. truttiformis	5·6	0·5	19
B. macrophtalmus	5.0	19[c]	0
B. megastoma	4·9	2	8
B. acutirostris	3.0	8	0
B. platydorsus	0·7	2	2
B. gorgorensis	0·2	0·2	2
B. crassibarbis	0·1	0·3	0
B. dainellii	0	0	0
B. gorguari	0	0	0
B. surkis	0	0	0
B. nedgia	0	0	0
B. longissimus	0	0	0

[a] referred to zas morphotype "normal intermedius" in Dgebuadze et al. (1999) but presumably *B. tsanensis* according to Nagelkerke and Sibbing (2000).

[b] referred to as morphotype "precocious intermedius" in Dgebuadze et al. (1999) but presumably *B. brevicephalus* according to Nagelkerke and Sibbing (2000).

[c] High abundance of *B. macrophtalmus* because these authors sampled late (October) in the spawning season.

In conclusion, present results show distinct reproductive segregation among the Lake Tana barbs: (1) highly significant differences in abundance over spawning time and space, pairwise among all *Barbus* species, (2) on a temporal scale (e.g. *B. macrophtalmus* in October), (3) on a micro-spatial scale (e.g. *B. truttiformis* in Kizen tributary) and (4) on a macro-spatial scale since only six species were spawning in the river (Table 4). The presence of segregation is confirmed by genetic data suggesting separate gene pools in at least ten of the *Barbus* spp. (Dixon et al. 1996; Kruiswijk et al. 2002) and substantial differences in allele frequencies among eleven of the *Barbus* spp. (Berrebi and Valiushok 1998).

Reproductive segregation and speciation, facilitated by homing?

In view of the results of this and previous studies (Whitehead 1959; Ochumba and Manyala 1992; Rodriquez-Ruiz and Granado-Lorencio 1992; Lucas and Baras 2001), it is suggested that barbs might show a strong fidelity to a particular river, tributary or set of tributaries, and possibly reproductive homing (sensu lato). Homing brings spawners back to conditions which are deemed favourable, since they permitted their own survival, and in places where they meet other mature spawners, thereby restricting the search for mates. Homing facilitates and accelerates reproductive isolation (Quinn et al. 2001). In migrating salmonids, it has been suggested that this process can proceed very quickly (less than 13 generations or 56 years in sockeye salmon: Hendry et al; 2000; Hendry 2001; Quinn et al; 2001). We hypothesize that the founder population of the riverine ancestral *Barbus* species used homing to spawn in specific tributaries, and was therefore, probably already divided in reproductively segregated subpopulations prior to the geologic formation of Lake Tana. If, and how homing might have enhanced the genetic fixation of adaptations in trophic structures among the present riverine spawning *Barbus* species, is one of the main aims of current research (Chapter 7).

Implications for fisheries

Collective migration and riverine spawning of *Barbus* species makes them very vulnerable for overfishing at the spawning areas. The development of a motorised commercial gillnet fisheries at the end of the 1980s, which targeted the spawning aggregations in the rivermouths, seems to be one of the main causes of the observed dramatic decline of Lake Tana's *Barbus* stocks (de Graaf et al. 2003b). Between July and October farmers traditionally catch *Barbus* on the upstream spawning grounds using a variety of fishing techniques like barriers, basket traps, hooks, scoop nets and even poisoning of the shallow water upstream using the dried and crushed seeds of the berberra tree (*Milletia ferruginea*, Leguminosae) (Nagelkerke and Sibbing, 1996). This type of seasonal fishing on migrating barbs has taken place for at least 200 years, and most probably for hundreds of years more, as fishing with poison was already observed and described around 1770 by the Scottish explorer James Bruce during his travels around Lake Tana. Unfortunately no data are available on the impact of this traditional, seasonal riverine fisheries on the *Barbus* stocks. In Lake Victoria a similar indigenous cyprinid fishery, using barriers and basket traps along the rivers, did not have a particular deleterious effect on the *Labeo* and *Barbus* stocks (Ogutu-Ohwayo 1990). However, preventing fishing activities both in the river mouths and the upstream spawning grounds during the peak spawning period (August-September), is of immenent importance in order to keep the *Barbus* fishery sustainable and to protect Lake Tana's unique biodiversity.

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7

CHAPTER

Early morphological divergence and phenotypic plasticity among Lake Tana's riverine spawning Barbus species: implications for their evolution.

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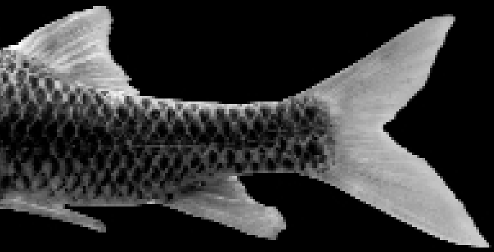

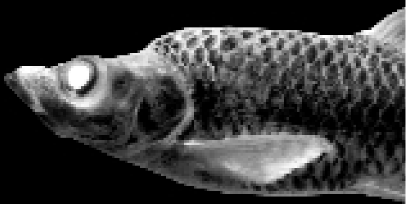

Abstract

In 'common garden' experiments we raised progeny of the riverine spawning benthivorous *B. tsanensis* ("intermedius") and the piscivorous *B. truttiformis* and *B. megastoma* all on the same commercial pellet food under similar environmental conditions to test if morphological differentiation would occur. To study the range of plasticity in the morphological development, part of the juvenile *B. truttiformis* and *B. megastoma* reared in the laboratory were subjected to different diets (commercial pellets or live fish) for a 10-month period. Inter-specific morphological differences have a strong genetic base and divergence occurs already early in ontogeny (<40 mm SL), supporting the view that among the riverine spawning *Barbus* species reproductive isolation and speciation is well established. In the diet experiments, several characters showed distinct adaptive phenotypic plasticity. These piscivorous barbs still retained some adaptive phenotypic plasticity, which is an important option to increase their survival in a heterogeneous or novel environments. We hypothesize that among the riverine spawning *Barbus* species the genetic fixation of morphological adaptations in trophic structures was probably enhanced and facilitated by reproductive homing to natal streams in the ancestral founding population. Prior to the geological formation of Lake Tana, the ancestral riverine barb population was most likely divided in sub-populations due to this reproductive strategy. After the formation of the lake, many new niches became available to the ancestral, riverine spawning barb population. Since mating did not occur randomly within the ancestral founding population, this resulted in small genetic neighbourhood sizes necessary for sympatric speciation and genetic fixation of trophic morphological adaptations, related to the newly invaded niches in the lake habitat.

Introduction

Phenotypic plasticity, i.e. environmentally induced phenotypic variation, is an option for an organism to increase its survival in a heterogeneous environment (Via et al. 1995). Discrete phenotypic responses induced by a (novel) environmental condition, might be followed by genetic changes in the same direction ('genetic assimilation') and for that reason even contribute to speciation, although its importance is debatable (Waddington 1953, 1956; West-Eberhard 1989; Williams 1966; Schlichting and Pigliucci 1998; Chapman et al. 2000, Bouton et al. 2002). Genetic assimilation could result in a decrease or 'canalization' (Williams 1966) of the reaction norm - linear or curved relationship between an environmental signal and its phenotype (Stearns 1989)- of an organisms phenotypic plasticity. Assimilation can, nevertheless, also lead to a situation where only a shift occurs in the reaction norm instead of an unfavourable reduction in flexibility (Schlichting and Pigliucci 1998). The role of phenotypic plasticity in speciation events of e.g. fresh water fish like cichlids is controversial. According to Bouton et al. (2002) in Lake Victoria's haplochromine cichlids, 'phenotypic plasticity contributes to allopatric speciation by helping individuals to survive and establish populations after migration between islands'. However, as plastic traits evolve slower than canalized traits (Sultan 1987), Meyer (1987) argued that 'plasticity may be a form of inertia against speciation' in Central American cichlids.

The cichlids are perhaps the most famous, but not the only family of African fresh water fish that underwent rapid speciation events forming species flocks (Fryer and Iles 1972; Keenleyside 1991). Lake Tana in the north-western highlands of Ethiopia, contains an extraordinary diversity of cyprinid fishes. This lake forms an ideal system to study the processes driving speciation due to the apparently recent age and relative limited number of species (15) in this presumably monophyletic species flock (Chapter 9).

The large (max 100 cm SL), hexaploid (Golubtsov and Krysanov 1993) African barbs are known for their extensive intra-specific morphological variation, creating a confusing and complicated taxonomical history (Howes 1991). Banister (1973) lumped 50 nominal species and subspecies of East-African barbs into one species, *Barbus intermedius*. The large barbs of Lake Tana were considered as belonging to one subspecies *Barbus intermedius intermedius* Rüppell 1836. The latest revision of Lake Tana's *Barbus* (Nagelkerke and Sibbing 2000) resulted in the rehabilitation of six species lumped by Banister as *B. intermedius intermedius* and nine new species. However, Mina et al. (1996ab, 2001) stated that based on ontogenetic changes in skull characters, the different "morphs" are best described as "intermedius" undergoing morphological divergence late (>20 cm, age 4-5 years) in ontogeny. These morphological transformations are triggered by the environment/habitat (phenotypic plasticity; nurture) in which a juvenile barb happens to end up, but is not genetically determined (Mina, pers. comm.). Therefore, early divergence of morphological traits characteristic for adult specimens among progeny raised in common laboratory environment, would strongly point towards a genetic base (nature) for morphological differences, instead of being the result of phenotypic plasticity.

In this study we raised the benthivorous *B. tsanensis* ("intermedius") and the piscivorous *B. truttiformis* and *B. megastoma* (Fig.1) all on the same commercial pellet food under similar environmental conditions to test if morphological differentiation would occur. To further study the range of plasticity in the morphological development, part of the juvenile *B. truttiformis* and *B. megastoma* reared in the laboratory were subjected to different diets (commercial pellets or live fish) for a 10 month period. Our aim was to answer the following questions: (a) Does morphological differentiation among the Tana barbs have a pure genetic base (nature)?, (b) If so, at what size (age) does morphological divergence occur?, and (c) To what extent do *Barbus* species show phenotypic plasticity in response to different food-types? The results will be related to the ecology and reproductive behaviour of Lake Tana's barbs and we will discuss the implications for the evolution of the *Barbus* species flock.

Materials and Methods

Fish collection

In 1999 and 2000 spatial and temporal segregation of spawning among the riverine spawning *Barbus* species was intensively studied on the upstream (40km) spawning grounds of the Gumara River (Chapter 6). Abundance and distribution of ripe *Barbus* in the main channel and the small tributaries was studied twice a week during August-October using gillnets, fykes and basket traps. Running males and females were hand-stripped. The cloaca was dried with a tissue before sperm or eggs were extracted by gently pressing the abdomen repeatedly from pectoral fin to anal fin. Sperm was collected in a syringe and eggs in a 9 cm petri-dish. Sperm was mixed with the eggs using a soft dry feather before water was added to induce activation of the eggs. The mixture was shaken for 30s and transferred to a plastic bag containing river water. During transport (2-4 hours) to the laboratory oxygen was supplied by battery operated air pumps. In the laboratory the fertilized eggs were transferred to small hatching containers (20x10x10 cm) in one of the tanks (90x45x45 cm) within the semi-open aquarium system (16 tanks; water temperature 21.8 °C, oxygen 6.6 mg l^{-1}). All tanks were connected to the same filter system, daily 10% of the water in the whole system was refreshed with water pumped directly from the lake.

In this way a large batch of larvae was obtained from *B. truttiformis* captured in Kizen River (cross #11: fertilization 19 September 1999; hatching 23 September 1999) and from *B. megastoma* captured in the main channel of the Gumara River (cross #14: fertilization 22 September 1999; hatching 26 September 1999). Several small batches of *B. tsanensis* were obtained during the 1999 and 2000 breeding seasons (cross #2: Dukolit; fertilization 8 September 1999, hatching 12 September 1999; cross #3: Dukolit; fertilization 8 September 1999, hatching 12 September 1999; cross #10: Kizen; fertilization 9 September 2000; hatching 13 September 2000).

The first three days after hatching fish were fed *Paramecium*. From 3 to 10 days after hatching the proportion of zooplankton increased from 0 to 100% and between day 11 and 21 after hatching, the proportion of commercial food (Trouvit, Trouw, Putten, The Netherlands) was gradually increased to 100%. Pellet size was increased during growth and fish were fed *ad libitum*.

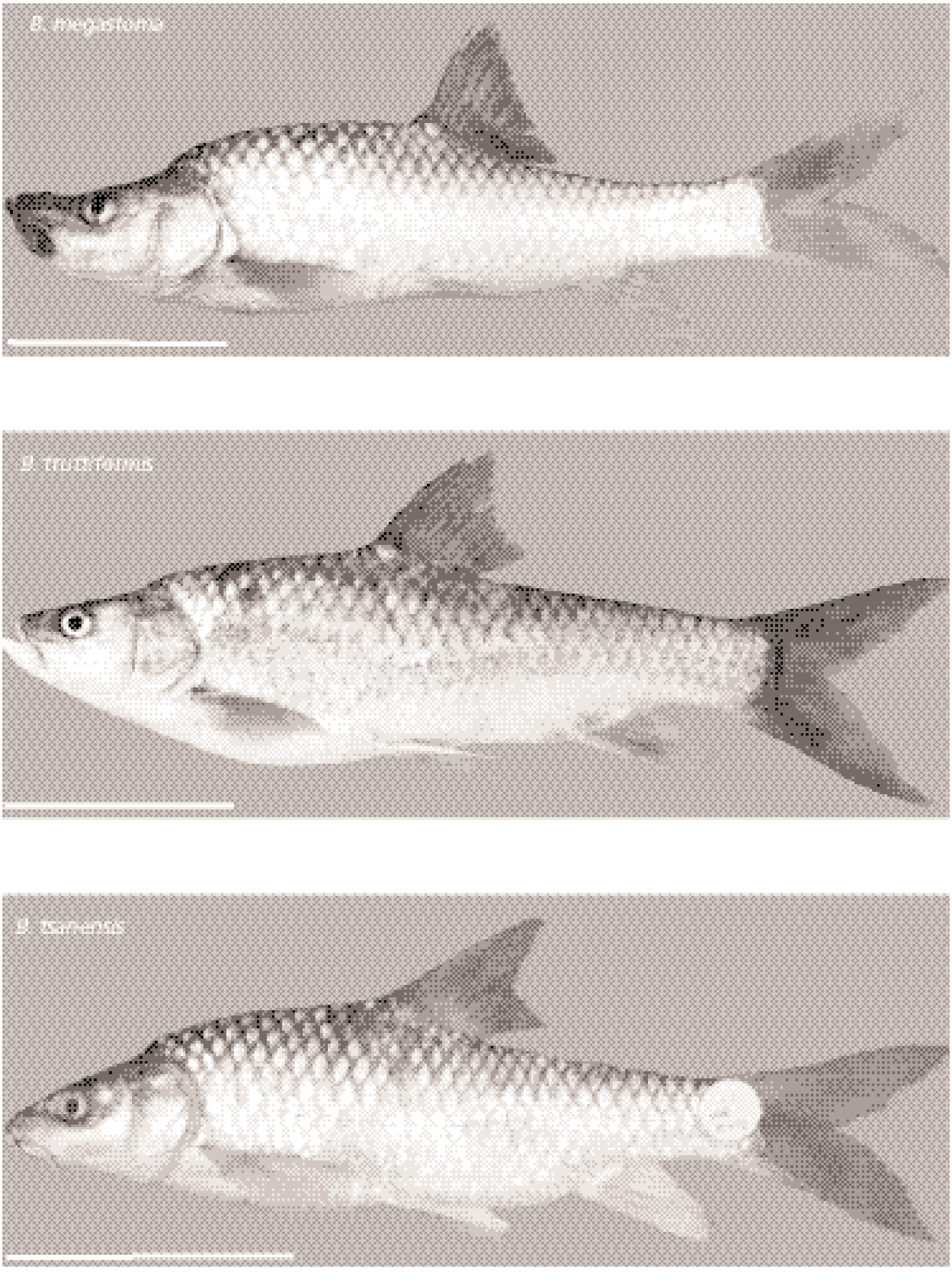

Figure 1. *Barbus megastoma*, *B. truttiformis* and *B. tsanensis*. Bars indicate 10 cm.

Table 1. Species, sampling date, sample size and size range used to study morphological divergence.

	hatching date	Sampling		
		date	n	size range (mm SL)
B. megastoma	26 Sep 99	1 Jan 00	10	14.5-20
		1 Mar 00	10	25-35
		1 May 00	5	40-47.5
		3 Aug 00	4	46-56.5
		11 Nov 00	8	59-73
		22 Aug 01	10	90.5-105.5
B. truttiformis	23 Sep 99	1 Jan 00	10	16-21
		1 Mar 00	10	27-34
		1 May 00	10	36-48
		3 Aug 00	5	50-60.5
		9 Nov 00	3	61.5-74
		22 Aug 01	9	92-111
B. tsanensis #2	12 Sep 99	3 Jan 00	3	18.5-23.5
B. tsanensis #3	12 Sep 99	22 Nov 00	4	95-107
		13 Aug 01	3	120-131
B. tsanensis #10	13 Sep 00	23 Aug 01	11	37-55.5

Table 2. Characters measured in *Barbus* specimens and their abbreviation. The location of each measured characteristic is illustrated in Figure 2.

	Abbreviation	**Morphological divergence**	**Phenotypic plasticity**
Standard Length	SL	x	x
Head Length	HL	x	x
Body Depth	BD	x	x
Body Width	BW	x	x
Caudal Peduncle Length	CPL	x	x
Caudal Peduncle Depth	CPD	x	x
Head Depth	HD	x	x
Head Width	HW	x	x
Snout Length	SnL	x	x
Snout Width	SnW	x	x
Eye Diameter	ED	x	x
Post-Orbital Length	POrL	x	x
Interorbital Width	IOW	x	x
Operculum Depth	OpD		x
Anterior Barbel Length	ABL		x
Upper Jaw Length	UJL		x
Lower Jaw Length	LJL		x
Protrusion Chain Length, Closed	PrC		x
Protrusion Chain Length, Open	PrO		x
Gape Height	GH		x
Gape Width	GW		x
Anal Fin Length	AFL	x	
Trunk Length	TrL	x	
Gut Length	GL		x

Morphological divergence

Between September 1999 and August 2001 regular samples were taken from cultivated batches of *B. megastoma*, *B. truttiformis*, *B. tsanensis* #2, *B. tsanensis* #3 and *B. tsanensis* #10 (Table 1). Samples were stored in 5% buffered formaldehyde solution. For the analysis of ontogeny 15 external parameters (Table 2 and Fig. 2) which are associated with species (Nagelkerke and Sibbing 2000; Sibbing and Nagelkerke 2001) were determined to the nearest 0.1 mm using a sliding caliper or a binocular and calibrated eyepiece.

To analyze the effects associated with species (Nagelkerke and Sibbing 2000; Sibbing and Nagelkerke 2001) we firstly compared the different species per size class by an analysis of variance (ANOVA) of all characters (in percentage of SL), followed by multiple comparisons between the means. The significance level (α) was adjusted for unplanned multiple comparisons according to Bonferroni's method (Sokal and Rohlf 1995). In our study we used the a priori recognized species - size class combinations as classes (size classes were $\leq$ 40; 41-80; 81-120 mm SL). The characters, which were measured in all specimens, were log-transformed and used in a canonical discriminant analysis (CDA). CDA is a dimension-reduction technique which produces canonical variables. These are linear combinations of the original parameters that summarise the variation between (a priori chosen) classes . These combinations are chosen so as to maximize the quotient of between-group and within-group variance. This results in the first canonical variable.

The second canonical variable is obtained by finding the linear combination uncorrelated with the first canonical variable that has the highest quotient of between-group and within-group variances etc. The loading factors of the canonical variables show the correlation of the original parameter with canonical variable.

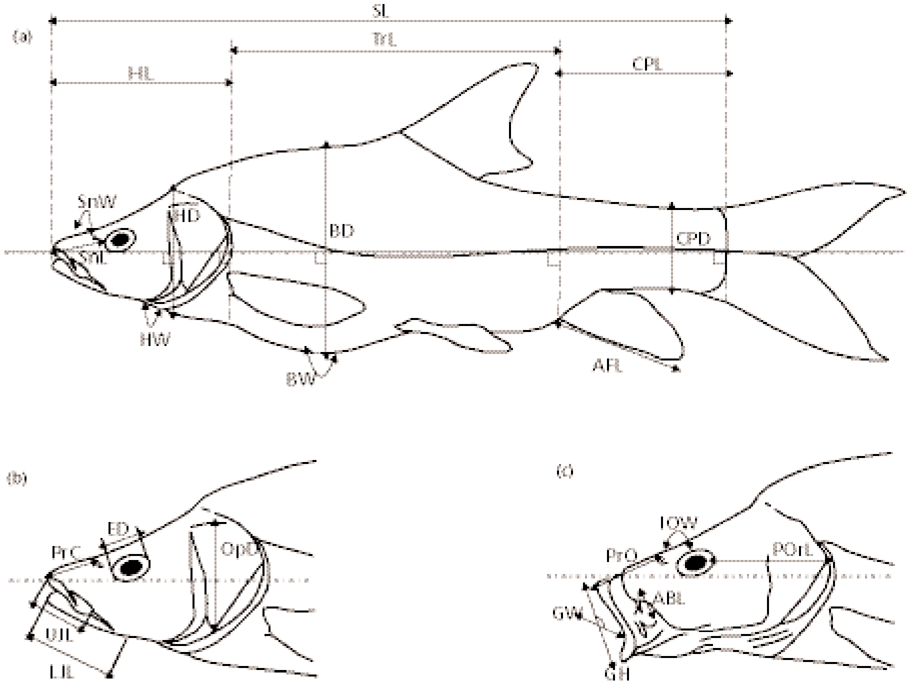

Figure 2. External parameters as measured on the barbs: (a) whole fish, (b) head with mouth closed, and (c) head with mouth open, protruded mouth. Abbreviations refer to descriptions in Table 2.

Phenotypic plasticity

To keep genetic variation to a minimum, only one large batch of *B. truttiformis* (cross #11) and one large batch of *B. megastoma* (cross #14) taken from parents caught in the wild were used. All juveniles were fed the same commercial food from shortly after hatching at the end of September 1999 until the start of the experiment in October 2000. After this period of common food, 20 *B. truttiformis* and 20 *B. megastoma* of ca. 60 mm standard length (SL) were taken from the stock tanks and each group was divided over two tanks (90x45x45 cm) in the semi-open aquarium system. For each species two different diet treatments were used: commercial pellets and live prey fish (*B. humilis*, 10-20 mm SL). Experiments were initiated on 19 October 2000 and lasted 10 months (until 14 August 2001). Twenty-one external (Table 2 and Fig. 2) and one internal (gut length) character that are associated with diet and/or species (Nagelkerke and Sibbing 2000; Sibbing and Nagelkerke 2001), were determined to the nearest 0.1 mm using a sliding caliper on fresh specimens.

To analyze the effects associated with diet and/or species (Nagelkerke and Sibbing 2000; Sibbing and Nagelkerke 2001) we compared the different species-diet combinations by an analysis of variance (ANOVA) of all characters (in percentage of SL), followed by multiple comparisons between the means. The significance level (α) was adjusted for unplanned multiple comparisons according to Bonferroni's method (Sokal and Rohlf 1995).

In both experiments we measured a minimum of six individuals per class (pseudo-replicates). Unfortunately we were not able to obtain replicates from the treatments themselves due to the limited number of tanks in the laboratory at Lake Tana. It is therefore arguable that differences found between treatments are the effect of differences between tanks. To keep such possible effects to a minimum, all other factors were kept constant and all aquariums were connected to the same water-filtering system (cf. Bouton et al., 2002). In addition, the direction of anatomical changes could be predicted based on functional morphology (Sibbing and Nagelkerke 2001).

Results

Table 3: Characters used for the canonical discriminant analysis in the "Morphological Divergence" experiment and the loading of the canonical variables (see Table 1 and Fig. 2).

Character	Loading factors canonical variables	
	Can 1	Can 2
Head Length	-4.72	2.89
Body Depth	3.28	-4.42
Body Width	-1.03	-1.09
Caudal Peduncle Length	-1.41	2.85
Caudal Peduncle Depth	2.08	-5.24
Head Depth	1.66	2.37
Head Width	-2.19	-3.96
Snout Length	1.29	-0.31
Snout Width	2.88	0.15
Eye Diameter	0.31	-0.18
Post-Orbital Length	-0.95	-0.96
Inter-Orbital Width	0.76	1.23
Anal Fin Length	-3.11	-1.15
Trunk Length	2.22	-7.50

Morphological divergence

Nine, 13 and 11 of the 15 measured characters were highly significantly different among the three *Barbus* species in the size-classes <40 mm SL, 40-80 mm SL and 80-120 mm SL, respectively (ANOVA; $P < 0.01$). From the canonical discriminant analysis of the 14 log-transformed measurements it was found that the first two canonical variables accounted for 77.4% of the variance. Head length, body depth, anal fin length, and snout width are the most important variables along the first canonical axis and trunk length, caudal peduncle depth, body depth, and head width along the second canonical axis (Table 3). The morphological differences between the three species were clear in all three size-classes (Fig. 3). Differences between size-classes of all species were most pronounced along the first canonical axis. With size all species especially developed relatively shorter heads, shorter anal fins, wider snouts, and deeper bodies and caudal peduncles. Differences between species were clearest along the second canonical axis. In the size-classes <40 mm and 40-80 mm *B. tsanensis* had the relatively longest trunk, narrowest head and smallest caudal peduncle and body depth, while *B. truttiformis* had a short trunk, wide head, and deep caudal peduncle and body. *Barbus* megastoma was intermediate.

We found the same differences between *B. truttiformis* and *B. megastoma* in the size-class of 80-120 mm, but the position of *B. tsanensis* in relation to the other species was aberrant compared to the smaller size-classes. Here *B. tsanensis* had the same position along the second canonical axis as *B. truttiformis*, but it differed along the first canonical axis (through its generally shorter head , shorter anal fin, wider snout, and deeper body).

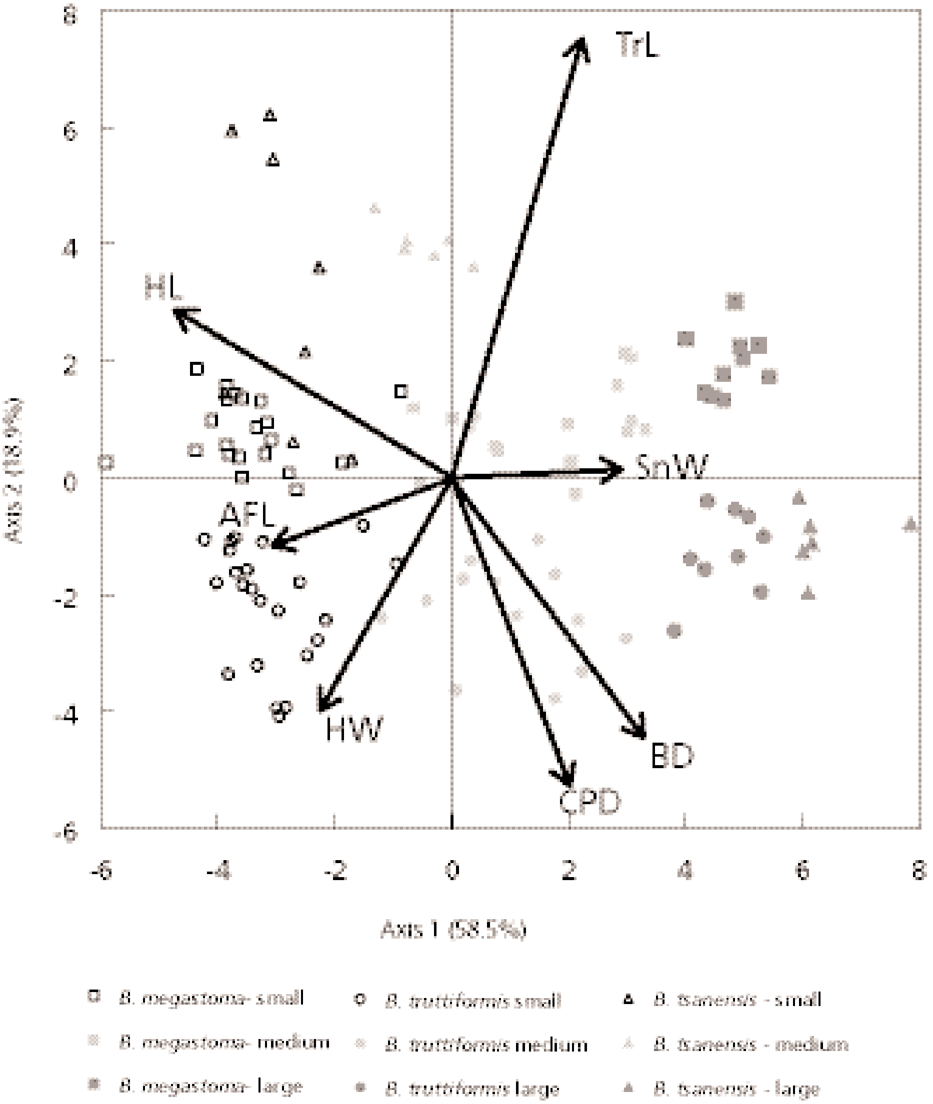

Figure 3. Segregation of the species/size-classes along the first 2 canonical axis. The abbreviations (see Table 2) along the arrows represent the characters with the highest loading factors (Table 3) and indicate in which direction these characters increase; small = ≤ 40 mm SL; medium = 41-80; large = 81-120 mm SL.

Phenotypic plasticity

Growth, measured as mm SL at the end of the experiment, was similar between the four groups (Table 4). Four fish escaped from their experimental tanks during the 10-month period and were found dead on the floor. Note that netting between all the tanks in the indoor aquarium system prevented a fish entering a neighbouring tank, should they escape. Table 4 shows the morphological characters that were consistently different when comparing the effect of species (*B. megastoma* "fish" vs. *B. truttiformis* "fish" and *B. megastoma* "pellet" vs. *B. truttiformis* "pellet") or diet (*B. megastoma* "fish" vs. *B. megastoma* "pellet" and *B. truttiformis* "fish" vs. *B. truttiformis* "pellet"). The effect of species (13 significant differences) on overall morphology was at ca. 100 mm SL considerably larger than the effect of diet (four, predicted significant differences).

Table 4. The lower left part of the table shows the comparisons between the diets within each species, the upper right part the comparisons between the species within each diet. Only parameters that are significantly different (P<0.01) are indicated; a '-' sign indicates that the parameter has a smaller value for Species/Diet column than Species/Diet row; CI = 95% confidence interval.

	***B. megastoma* Fish**	***B. megastoma* Pellet**	***B. truttiformis* Fish**	***B. truttiformis* Pellet**
B. megastoma **Fish** 100.3mm SL ±9.05 CI n=8	x		-HL -HD -BD -BW -CPD -HW -SnW -POrL -IOW -OpD -LJL ProtL -GW	
B. megastoma **Pellet** 106.5mm SL ±5.0 CI n=10	-HL BW -POrL GL	x		HL -HD -BD -BW -CPD -HW -SnW -POrL -IOW -OpD -LJL ProtL -GW
B. truttiformis **Fish** 93.1mm SL ±6.6 CI n=8			x	
B. truttiformis **Pellet** 107mm SL ±3.8 CI n=10			-HL BW -POrL GL	x

Discussion

Morphological divergence

The statement of Mina et al. (1993, 1996) that morphological divergence most frequently starts between 20-25 cm SL was already questioned by Nagelkerke et al. (1995). These authors showed that several of the 15 *Barbus* species could already be morphologically distinguished at 10-15 cm SL and argued that the divergence of the barbs is not due to phenotypic plasticity alone. Although their arguments were reasonably convincing, the evidence was "circumstantial" because all the fish they used were still caught in the wild. Therefore, the morphological differences between their barbs could still have been the result of different environmental conditions experienced during growth. They stated, however, that "juvenile barbs do not occupy clearly different habitats or trophic niches". This is not true, when looking at habitat distribution in the Bahar Dar Gulf of Lake Tana. Juvenile barbs (10-15 cm SL) are already clearly segregated over the six main habitats (littoral rock /sand, sub-littoral bottom/surface, pelagic bottom/surface). For example, among the riverine spawning species, *B. tsanensis* (62%, n=79) predominantly occurs near the bottom in the sub-littoral/pelagic areas, while *B. truttiformis* (75%, n=12) and *B. brevicephalus* (63%, n=749) are mainly found near the surface in the sub-littoral/pelagic zone (de Graaf, unpubl. data). Similar differences are also already apparent when looking at the diet composition of juvenile barbs (Chapter 4).

The present "common garden" experiments were not conducted in the past due to the difficulty of obtaining running fish on the upstream spawning areas and the lack of proper laboratory facilities and a reliable aquarium system in Bahar Dar. The results clearly show that the previously published (Nagelkerke and Sibbing 2000) inter-specific morphological differences have a strong genetic base (nature) and divergence occurs already very early in ontogeny (<40 mm SL). In contrast to *B. truttiformis* and *B. megastoma*, *B. tsanensis* showed allometric growth during early ontogeny (Fig. 3). Large (81-120 mm SL) *B. tsanensis* had a markedly smaller head length, larger trunk length, larger body depth and larger body width in comparison with individuals <80mm SL.

The results further support the view that among the seven riverine spawning *Barbus* species (including *B. tsanensis*, *B. truttiformis*, *B. megastoma*) reproductive isolation is well established (Chapter 6 and 8) and that speciation is in an advanced stage.

Phenotypic plasticity

The results of the feeding experiment provided further proof that the majority of the morphological differences between *B. truttiformis* and *B. megastoma* have a strong genetic base. The majority (11) of the inter-specific differences between juvenile *B. megastoma* and *B. truttiformis* are consistent over the whole size range. Only two characters show allometric growth and sharply decrease in relative size. Lower jaw length and head length are larger in juvenile *B. truttiformis*, however, when comparing adults these two characters are larger in *B. megastoma* (Nagelkerke and Sibbing 2000).

Few characters (GL, BW, HL and POrL) showed distinct phenotypic plasticity, their changes in morphology were clearly induced by the differences in diet. In general, the relative gut length decreases with increasing quality/digestibility of the food (Wootton 1990; Verigina 1991). Low quality food with many fibers and plant materials, requires long intestines and increased digestion time. Specialised piscivorous fish have the shortest intestines ranging from 0.7-1 x SL, the intestines of omnivores range from 1.2-2.2 x SL while the largest intestines are found in herbivores and detrivores, ranging from 5-29 x SL (Kramer and Bryant 1995). The significant ($P<0.001$) differences in gut length between the two diets show the same phenomenon, in both *B. truttiformis* and *B. megastoma*, the intestines of the "fish" group being smaller than the "pellet" group. The longer, more voluminous intestines in the "pellet" groups might require a larger body cavity, possibly explaining the significant increase ($P<0.001$) in body width. The significant increase ($P<0.01$) in head length and post-orbital length in the "fish" groups, is most likely related to an improved suction performance when feeding on fast evasive prey compared to pellets (Sibbing and Nagelkerke 2001). The observed plasticity in these piscivorous characteristics might be advantageous in the field to react on the availability of prey fish. Both *B. truttiformis* and *B. megastoma* become piscivorous around ca. 100 mm FL (Chapter 4). Fish that are able to switch to piscivory at smaller sizes have higher growth rates and eventually become larger (Mittelbach and Persson 1998).

Phenotypic plasticity, genetic neighbourhood and sympatric speciation

One of the prerequisites for an organism to successfully invade a new food niche is flexibility in its trophic morphological structures (phenotypic plasticity). Whether successful invasion of a new food niche will result in speciation or resource polymorphism is largely dependent on the size of the genetic neighbourhood. Kawata (2002) developed a simulation model to examine how phenotypic variation for resource use (i.e. food sizes) and genetic neighbourhood size, effect the population dynamics of 'invasion of a vacant niche' and subsequent sympatric speciation. In his simulations populations with small phenotypic plasticity and large genetic neighbourhood (random mating) could not invade the vacant niche (large food size). Populations with large phenotypic plasticity but large genetic neighbourhoods (near random mating) developed resource polymorphisms, but reproductive isolation was still not achieved. In these populations the large genetic neighbourhood size apparently neutralized the ability of natural selection to drive sympatric speciation, keeping the genetic content of the population close to that of the initial conditions. However, if the genetic neighbourhood was small, so that within-group dispersal of genes was inhibited, complete speciation did occur (probability 0.15). A sub-population came into existence that used the unexploited larger food sizes and became reproductively isolated from the sub-population using the smaller food sizes.

The body plans of the African cichlids in East-Africas great lakes and the barbs of Lake Tana contain similar morphological innovations (well-developed pharyngeal jaw apparatus, decoupling of oral jaw elements) resulting in large phenotypic plasticity of trophic structures and high potential for trophic radiation (Fryer and Iles 1972; Liem 1980; Keenleyside 1991; Witte and van Oijen 1995; Galis and Metz 1998; Danley and Kocher 2001 and references cited therein; Chapter 4; Sibbing and Nagelkerke 2001). The small-sized, stenotopic rock-dwelling haplochromine cichlids in Lake Victoria further lack pre-mating migration and larval dispersal (Bouton et al. 2002), resulting in small genetic neighbourhoods, and therefore enhancing the chance of sympatric speciation according to Kawata (2002). In contrast, most of Lake Tana's large-sized barbs are not stenotopic and undergo, like other African large barbs

(Skelton et al. 1991; Tómasson et al. 1984) an annual reproductive migration upstream inflowing rivers to spawn on shallow gravel beds in fast flowing, small tributaries. At first sight, Lake Tana's barbs seem to have a large genetic neighbourhood size, thereby not fulfilling both requirements necessary for sympatric speciation according to the previously described model.

What mechanism facilitated the origin, development and maintenance of reproductive isolation (small genetic neighbourhood size) during adaptive radiation among these large-sized, mobile, widely dispersed riverine spawning *Barbus* species? Like in many other cyprinid fishes (Mills 1991; Smith 1991; Lucas and Baras 2001), reproductive homing is common among Lake Tana's riverine spawning *Barbus* species (Chapter 6). The different species are at present spatially and temporally segregated on the upstream spawning areas (Nagelkerke and Sibbing 1996; Chapter 6). In migrating salmonids, homing reinforces the development of reproductive isolation (sub-populations), facilitating the divergence of other (morphological) traits. Reproductive isolation can arise very quickly (less than 13 generations or 56 years) as has been shown in sockeye salmon (Hendry et al. 2000; Hendry 2001; Quinn et al. 2001). Among the riverine spawning *Barbus* species the genetic fixation of morphological adaptations in trophic structures was probably enhanced and facilitated by philopatry, i.e. homing to natal streams in the ancestral, founding population. Before the geological formation of Lake Tana, as in salmon, the ancestral riverine barb was most likely divided in sub-population due to this reproductive strategy. After the formation of the lake, many new niches became available to the ancestral, riverine spawning barb population. Since mating did not occur randomly within the ancestral founding population, this resulted in small genetic neighbourhood sizes necessary for sympatric speciation and genetic fixation of trophic morphological adaptations, related to the newly invaded niches in the lake habitat.

Among the riverine spawning *Barbus* species, size at maturity varied widely, ranging from 19 cm FL in the zooplanktivorous *B. brevicephalus*, 26 cm FL in the insectivorous *B. tsanensis* to 35 cm FL in the piscivorous *B. megastoma* (de Graaf et al. 2003). Differences in resource use by the diverging sub-populations might have led to differences in growth and in size at maturity. Size-assorative mating (Schliewen et al. 2001) might have further enhanced reproductive isolation between the diverging barb populations.

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8
CHAPTER

Lacustrine spawning, a new reproductive strategy among 'large' African cyprinid fishes?

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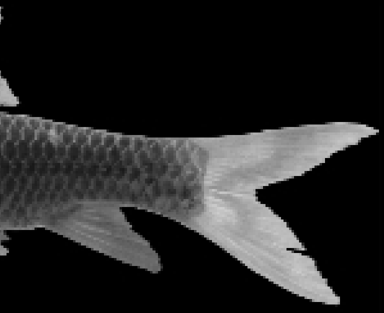

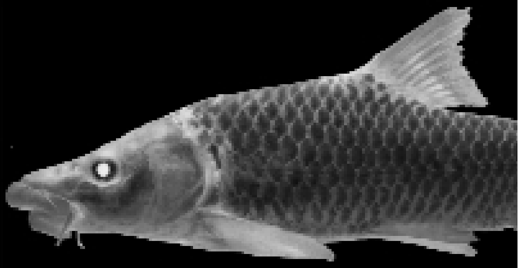

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Abstract

The development of assortative mating in the only remaining species flock of 'large' cyprinid fishes in Lake Tana (Ethiopia) is a key issue when reconstructing how the *Barbus* populations differentiated to occupy different ecological niches and ultimately become reproductively isolated. Changes in the gonado-somatic index and abundance of the different *Barbus* species in the mouths of four major affluent rivers was monitored (bi)monthly during 1999 and 2000. Riverine spawning is characteristic for seven of Lake Tana's 15 contemporary *Barbus* species. Spatial segregation among affluent rivers did not occur among them but significant temporal segregation occurred in aggregating in the river mouths and migrating towards the upstream spawning areas during the breeding season (June-October).

Among the eight other species peak gonad development occurred generally in the same period as in the riverine spawners. However, these species did not aggregate in the river mouths during the breeding period and were absent from the upstream spawning areas. A derived, novel strategy, lacustrine spawning is hypothesized for these eight *Barbus* species. This hypothesis is further supported by observations of running females in the littoral zones far away from any of the affluent rivers. This derived strategy is only common among the littoral-dwelling *Barbus* species with restricted distribution patterns. The present hypothesized scenario is that sequential waves of speciation, i.e. habitat divergence followed by trophic specialization, shaped the diversity of Lake Tana barbs.

Introduction

With the extinction of most of the endemic cyprinid species in Lake Lanao in the Philippines (Kornfield and Carpenter 1984), the *Barbus* species of Lake Tana (Ethiopia) form, as far as we know, the only remaining intact species flock of 'large' cyprinid fishes (max. 100 cm fork length [FL]) left in the world (Nagelkerke et al. 1994; Nagelkerke and Sibbing 2000). The taxonomic status of the Lake Tana barbs has been fiercely debated over the last decade. Alternative hypotheses have suggested that the 15 *Barbus* morphotypes distinguished by Nagelkerke et al. (1994): (a) all belong to one phenotypically plastic species, *B. intermedius* Rüppell (Banister 1973), (b) are a combination of some distinct species and several phenotypically plastic morphs, which show drastic changes in patterns of relative growth late in ontogeny at sizes larger than 15 cm FL (Mina et al. 1996a, 1996b, 1998, 2001), and (c) are all distinct, species (Nagelkerke and Sibbing 2000).

The latest revision described 15 good biological species of which eight were new to science (Nagelkerke and Sibbing 2000). The *Barbus* species flock of Lake Tana, an isolated lake of presumably recent origin (10,000 years, Chorowicz et al. 1998 and references therein; Chapter 9), consists of a limited number of closely related species of unresolved phylogenetic relationships, which differ in resource utilization as well as size and age of reproduction (Nagelkerke and Sibbing 1996; Sibbing and Nagelkerke 2001; de Graaf et al. 2003a). These conditions make Lake Tana a natural laboratory, ideal to study the hypotheses on the selective forces driving speciation and diversity in freshwater fish communities (Schliewen et al. 2001). Trophic specialization was hypothetized to be the main driving factor of speciation among Lake Tana barbs (Nagelkerke and Sibbing 1994; Sibbing et al. 1998; Sibbing and Nagelkerke 2001). In order to understand how populations differentiate to occupy different niches and eventually become reproductively isolated during adaptive radiation, the origin, development and maintenance of reproductive isolation is a central problem.

In the present *Barbus* study we focus on mechanisms that might prevent interbreeding between Lake Tana's contemporary *Barbus* species and on the origin and evolution of reproductive isolation. Most African barbs occur in rivers and generally 'large' *Barbus*, including lake dwelling species, are considered to be riverine spawners, that migrate annually upstream to spawn on shallow gravel beds in fast flowing, small rivers during short periods (Tómasson et al. 1984; Skelton et al. 1991). In migrating salmonids, it has been suggested that homing might reinforce the development of reproductive isolation, and that this isolation can arise very quickly (less than 13 generations or 56 years) as has been shown in sockeye salmon (Hendry et al. 2000; Hendry 2001; Quinn et al. 2001).

In order to explain how assortative mating may have developed among the endemic *Barbus* species in Lake Tana, one needs to investigate the prevailing, actual differences in their reproductive characteristics and spawning patterns. Detailed information on gonad development, peak breeding period, spawning area and size at maturity of each of the 15 species was, until recently, scarce, fragmented and sometimes unreliable due to limited sampling (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999), misidentification or lumping of species (Wudneh 1998). Difficult local circumstances and transportation problems prevented extensive sampling in time and space when studying reproductive segregation in the field and this probably caused some of the ambiguous and contradicting results (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999).

Only recently (Chapter 6) we managed to sample intensively the upstream spawning areas in Gumara River. Comparing the distribution and abundance in the main channel and several small tributaries, twice a week for three consecutive months, showed reproductive segregation along micro-temporal and micro-spatial dimensions among up to seven riverine spawning *Barbus* species (*B. acutirostris*, *B. brevicephalus*, *B. macrophtalmus*, *B. megastoma*, *B. platydorsus*, *B. truttiformis*, *B. tsanensis*). All three previous studies on temporal and spatial segregation focused on Gumara River during August-October and found spawning aggregations of the same seven (or less) species upstream and/or in the river mouth.

To date still no information on spawning area and breeding period exists for the other half of the *Barbus* species flock. These missing species might spawn during another season and/or migrate upstream another affluent perennial river. For reconstructing the evolution of the *Barbus* species flock, but also for the development of fisheries management and the protection of the reproductive active part of the *Barbus* populations, it is of utmost importance to determine the spawning area and breeding season of the remaining species.

The following questions will be addressed in this paper: (1) Do the *Barbus* species have the same breeding period; does temporal segregation occur within the *Barbus* species flock?, (2) Do all *Barbus* species aggregate in river mouths during the breeding period and undertake an upstream spawning migration; are all species riverine spawners?, (3) Does spatial segregation occur among riverine spawners within the *Barbus* species flock; do these *Barbus* species aggregate in the mouths of same affluent rivers?, (4) Does temporal segregation occur among the riverine spawners within the *Barbus* species flock; do these *Barbus* species aggregate simultaneously in the mouths of the affluent rivers?, and (5) To what extent does reproductive isolation exist within the *Barbus* species flock?

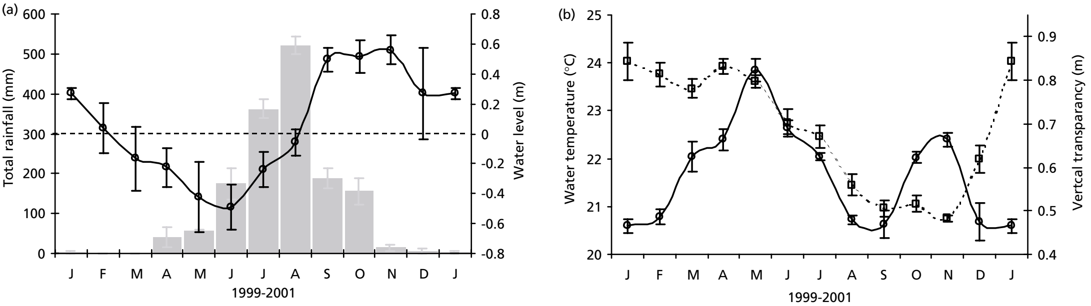


Figure 2. (a) Relative lake level (solid line) and total rainfall per month (grey bars) in Bahar Dar during 1999-2001 (mean ± SE; data from National Meteorology Office, National Water Resources Department, Addis Ababa, Ethiopia), and (b) average monthly water temperature (solid line, solid circles) and average monthly vertical transparancy (Secchi depth, dotted line, open squares) during 1999-2001 [mean ± SE; data from de Graaf et al. (2003b)].

Materials and Methods

Study area and environment

Figure 1. Lake Tana and its main in- and outflowing rivers.

Lake Tana is situated at an altitude of approximately 1,800 m in the north-western highlands of Ethiopia, 500 km north of the capital Addis Ababa. The meso-oligotrophic (Dejen 2003), shallow lake (average depth 8 m, maximum depth 14 m) covers an area of c. 3,050 km^2 and is Ethiopia's largest lake. Seven perennial rivers tribute to Lake Tana (Fig. 1). The lake's ichtyofauna is isolated from the lower Nile basin by 40 m high waterfalls, 30 km downstream from the Blue Nile outflow at Tissisat ('smoking water').

Rainfall, lake water level, water temperature and vertical transparency, measured by Secchi disk, all showed strong seasonal patterns (Fig. 2). Rainfall peaked in July-August, followed by a raise in the lake's water level in the lake, peaking one month later in September-October (Fig. 2a). During the rainy season the vertical transparency in the Bahar Dar Gulf is reduced due to a large inflow of silt, resulting from severe erosion, by the rivers Gumara, Gelgel Abbay, Rib and Gelda (Fig. 2b). Annual variation in water temperature showed two peaks, the first just before the rainy season in May and the second around October-November at the start of the dry, winter season (Fig. 2b). These seasonal patterns of abiotic parameters during 1999-2001 did not differ from patterns in 1990-1993 (Wudneh 1998) and 1993-1994 (Nagelkerke and Sibbing 1996).

Gonado-Somatic Index

Between January 1999 and April 2001, more than 26,000 freshly caught fish were analysed in the laboratory to assess the reproductive characteristics (size at maturity, gonad development) for each of the 15 *Barbus* spp. Of each fish the fork length (FL, to the nearest mm), total wet weight (TW, to the nearest 0.1 g) and gonad weight (GW, to the nearest 0.01g) was measured and the gonad developmental stage (1-7) was determined (Nagelkerke and Sibbing 1996). The fish were collected monthly using a variety of methods (gillnets, trawl, hook-and-line, fish traps) in the southern Bahar Dar Gulf of Lake Tana, in the mouths of the Gelgel Abbay, Rib, Gumara and Gelda river (see next section for details), upstream Gumara river (August-September 1999; August-October 2000) and fish were purchased from local fishermen.

The Gonado-Somatic Index (GSI), also known as the maturity coefficient, is the weight of the gonad expressed as a percentage of the body weight (Wootton 1990). The index is used to assess the state of gonad maturation. For each species only adult individuals (FL ≥ FL at which 50% of the fish are mature ($FL_{50\%}$), de Graaf et al. 2003a) were used in the analysis, since immature fish would not be expected to show temporal variation in their monthly GSI. Monthly changes in average male and female GSI were recorded over 24 consecutive months from January 1999 till January 2001 for all *Barbus* species except *B. intermedius* "shore-complex" (SC) which was studied for 23 months from May 1999 till April 2001.

Abundance and distribution in the river mouths

The river mouths of four permanent in-flowing rivers (Gelgel Abbay, Gelda, Gumara and Rib; Fig. 1) were sampled once a month from May 1999 through to December 2000 and even twice a month in the rainy seasons, June 1999-October 1999 and July 2000-September 2000. Sampling occurred during the first week of each month, and also in the third week during the rainy season. Data on fish distribution was collected using polyfilament gillnets with four different mesh sizes: 60, 80, 100 and 120 mm stretched mesh respectively. Panel length and depth were 200 m and 3 m respectively for each mesh. Nets were set at the bottom (3-6 m depth), within 500 m from the river mouth overnight from 18:00 to 6:00 hours. In the morning the fish were transported fresh to the laboratory, where each fish was identified to species level and FL, TW and GW were measured. Analysis of distribution and abundance patterns of *Barbus* species in the river mouths was performed comparing the number of fish caught per overnight gillnet setting per species, month and river mouth. The catch data of 1999 and 2000 and the catch data of the four major river mouths were pooled during analysis unless stated otherwise.

In the first place, we determined which *Barbus* species aggregated in the river mouths during the three months of peak breeding (based on GSI values). *Barbus* species that were rare in the river mouths during the peak breeding period were left out of further analysis of spatial and temporal spawning segregation (see Fig. 3).

Secondly, to determine if a *Barbus* species only formed aggregations during its breeding period, the abundance during its peak breeding period (three months) was compared with the non-breeding season (nine months, Mann-Whitney U-test, Table 1).

Finally, spatial and temporal distribution patterns of *Barbus* species in the river mouths during the main breeding period (July-October) were studied through analysis of variance (ANOVA). The catch data were arcsinh transformed (Fowler et al. 1998) before ANOVA could be applied. The *Barbus* catch data were analysed for differences among river mouths (Gelda, Gelgel Abbay, Gumara and Rib), months (July-August, September and October) and the interaction between river mouth and month (Table 2).

Table 1. Abundance (number of fish per gillnet setting) in the river mouths of each *Barbus* species over the year (see also Fig. 3). Result of Mann-Whitney U tests testing the hypothesis that the abundance of a particular *Barbus* species in the river mouths was the same (*P* > 0.05) during the three peak breeding months when compared to the other nine months within a year. Mean = number of fish per over night gillnet setting, SE = standard error, N = number of overnight gillnet settings; *P* = significance level: *** = *P* < 0.001; * = *P* < 0.05; ns = not significant (*P* > 0.05).

	Peak breeding season			Non-breeding season			
	Period	Mean ±SE	N	Period	Mean ±SE	N	P
B. brevicephalus	Aug-Oct	24.8 ±3.4	44	Nov-Jul	11.1 ±2.2	68	***
B. macrophtalmus	Aug-Oct	8.0 ±1.3	44	Nov-Jul	2.8 ±0.5	68	***
B. truttiformis	Jul-Sep	4.2 ±1.0	48	Oct-Jun	0.6 ±0.1	64	***
B. tsanensis	Jul-Sep	25.7 ±3.7	48	Oct-Jun	10.0 ±2.0	64	***
B. acutirostris	Aug-Oct	11.2 ±1.8	44	Nov-Jul	10.0 ±1.0	68	ns
B. megastoma	Jul-Sep	3.3 ±0.7	48	Oct-Jun	3.5 ±0.4	64	ns
B. platydorsus	Aug-Oct	6.2 ±1.1	44	Nov-Jul	4.2 ±0.6	68	*
	"High" breeding season			"Low" breeding season			
B. intermedius SC	Mar-Sep	65.8 ±7.5	76	Oct-Feb	54.2 ±6.2	36	ns

Table 2. Results of the analysis of variance. The degrees (df), the mean squares (ms), and the level of significance (*P*): *** = *P*<0.001; ns = not significant (*P*>0.05)] of the sources of variation are indicated (see also Fig. 4).

	B. brevicephalus			*B. macrophtalmus*			*B. truttiformis*			*B. tsanensis*		
Source of variation	df	ms	*P*	df	ms	*P*	df	ms	*P*	df	ms	*P*
Month	3	19.75	***	3	8.45	***	3	8.32	***	3	15.93	***
River	3	0.74	ns	3	0.62	ns	3	0.77	ns	3	2.02	ns
Interaction	9	0.68	ns	9	0.82	ns	9	0.39	ns	9	0.25	ns
Error	44	1.63		44	1.47		44	0.78		44	1.02	
R^2		0.49			0.35			0.47			0.56	

	B. acutirostris			*B. megastoma*			*B. platydorsus*			*B. intermedius* SC		
Source of variation	df	ms	*P*	df	ms	*P*	df	ms	*P*	df	ms	*P*
Month	3	0.41	ns	3	0.20	ns	3	2.33	ns	3	0.64	ns
River	3	0.11	ns	3	0.82	ns	3	0.60	ns	3	0.94	ns
Interaction	9	0.95	ns	9	0.99	ns	9	1.27	ns	9	0.45	ns
Error	44	0.99		44	0.78		44	0.90		44	0.54	
R^2		0.19			0.26			0.34			0.28	

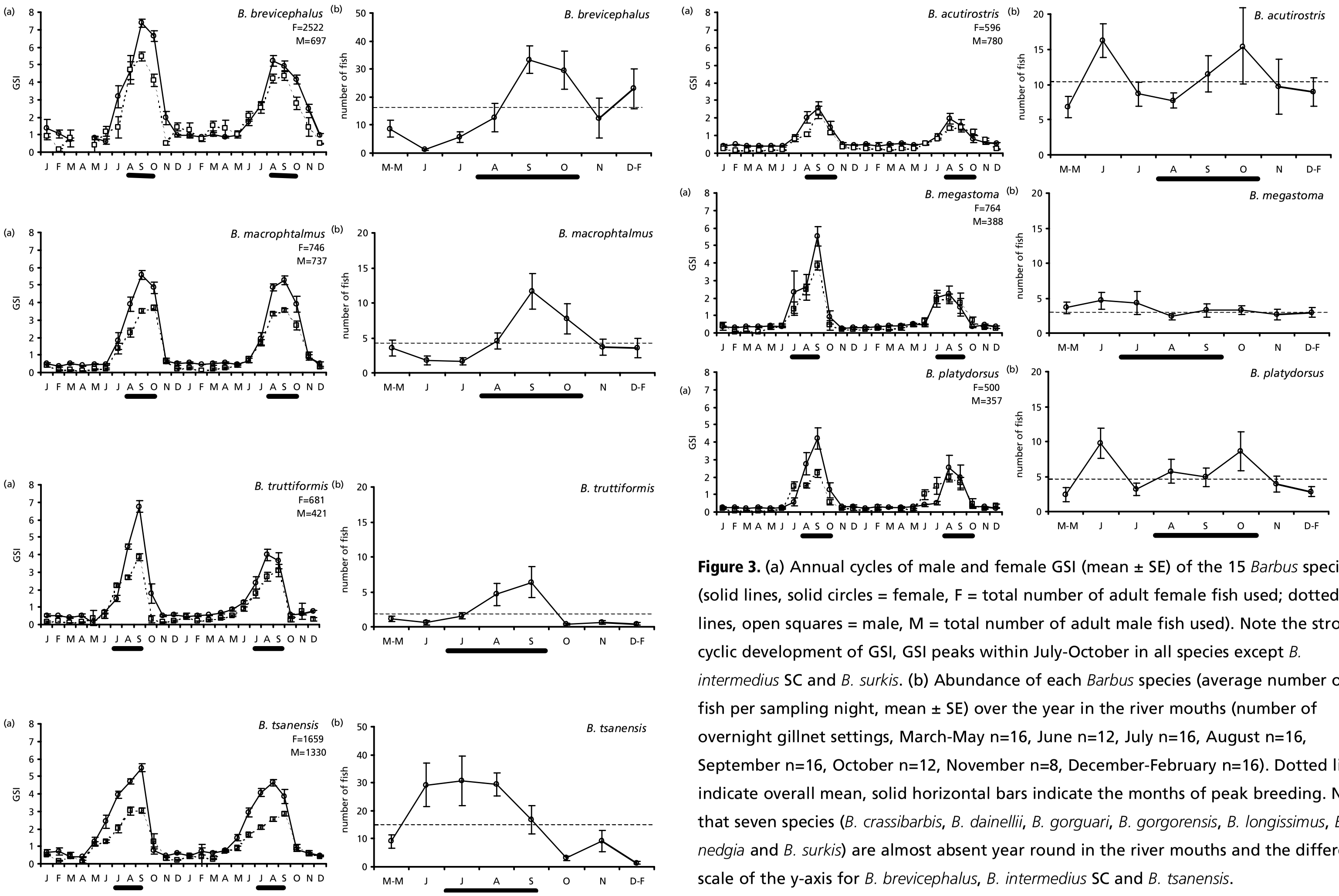
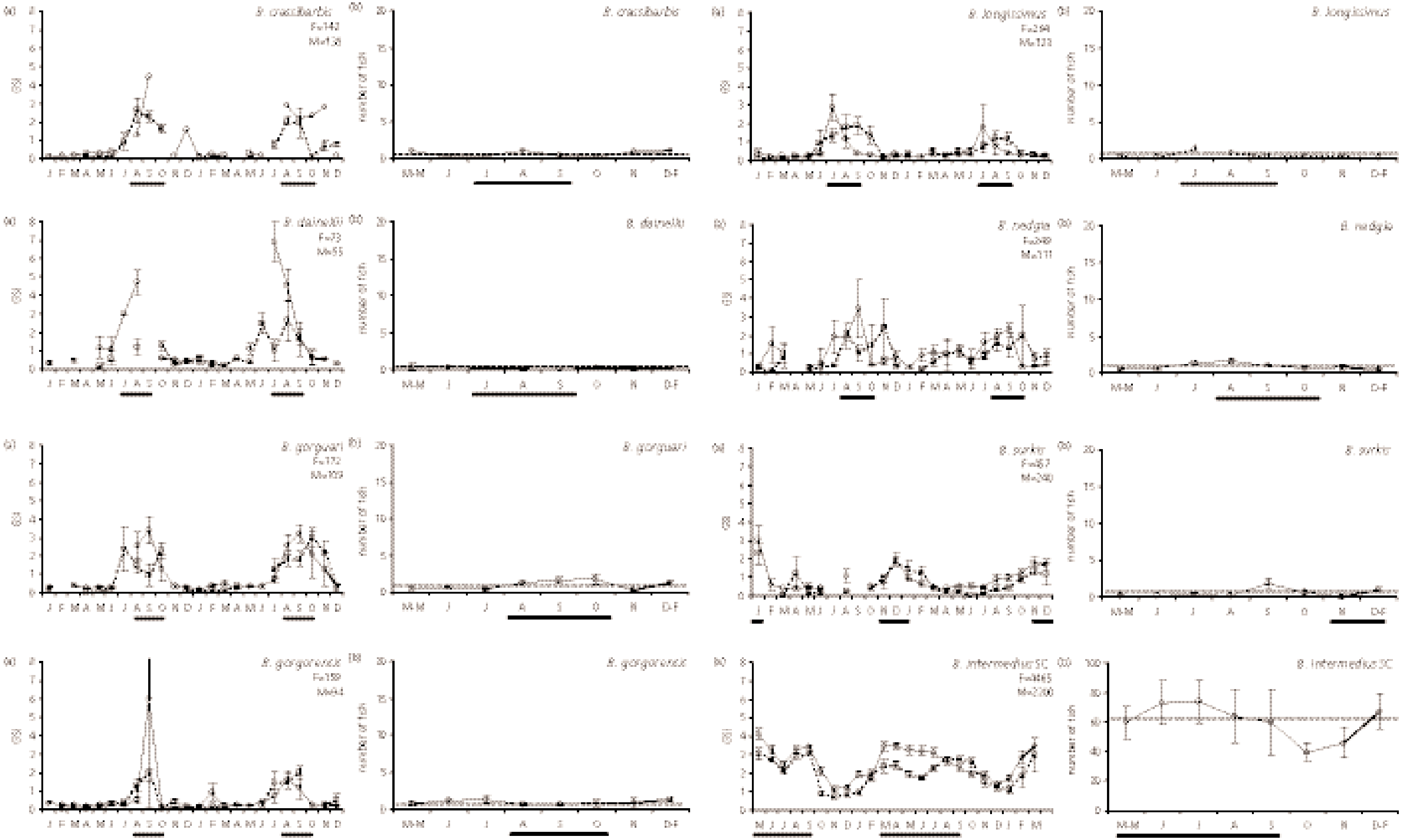


Figure 3. (a) Annual cycles of male and female GSI (mean ± SE) of the 15 *Barbus* species (solid lines, solid circles = female, F = total number of adult female fish used; dotted lines, open squares = male, M = total number of adult male fish used). Note the strong cyclic development of GSI, GSI peaks within July-October in all species except *B. intermedius* SC and *B. surkis*. (b) Abundance of each *Barbus* species (average number of fish per sampling night, mean ± SE) over the year in the river mouths (number of overnight gillnet settings, March-May n=16, June n=12, July n=16, August n=16, September n=16, October n=12, November n=8, December-February n=16). Dotted lines indicate overall mean, solid horizontal bars indicate the months of peak breeding. Note that seven species (*B. crassibarbis*, *B. dainellii*, *B. gorguari*, *B. gorgorensis*, *B. longissimus*, *B. nedgia* and *B. surkis*) are almost absent year round in the river mouths and the different scale of the y-axis for *B. brevicephalus*, *B. intermedius* SC and *B. tsanensis*.


B. crassibarbis
F=142
M=138
GSI
B. crassibarbis
number of fish
B. longissimus
F=294
M=133
GSI
B. longissimus
number of fish
B. dainellii
F=73
M=55
GSI
B. dainellii
number of fish
B. nedgia
F=349
M=111
GSI
B. nedgia
number of fish
B. gorguari
F=172
M=109
GSI
B. gorguari
number of fish
B. surkis
F=487
M=240
GSI
B. surkis
number of fish
B. gorgorensis
F=159
M=64
GSI
B. gorgorensis
number of fish
B. intermedius SC
F=4865
M=2200
GSI
B. intermedius SC
number of fish
J F M A M J J A S O N D J F M A M J J A S O N D
M-M J J A S O N D-F

Results

Gonado-Somatic Index

Do the *Barbus* species have the same breeding period?

In general, the highest GSI values were recorded from July to October and the lowest from November until June (Fig. 3a). In both 1999 and 2000 13 of the 15 *Barbus* species had the highest GSI development between July and October. *Barbus surkis* was the only species with high GSI development from November till January in both years. The GSI development showed the existence of a short reproductive period, peaking in August-September or December-January (*B. surkis*) in 1999 and 2000, for all but one of the *Barbus* species. The morphologically variable *B. intermedius* SC was the only species with an extended spawning season lasting from March till September with only a low GSI development from October to February. However, ripe male and female *B. intermedius* SC were present throughout the year.

Abundance and distribution in river mouths

Do all *Barbus* species aggregate in river mouths during the breeding period?

The development of the GSI throughout the year showed a short breeding season lasting from July to October but peaking generally in August-September for 13 out of 15 *Barbus* species. We focused on distribution and abundance patterns in the river mouths during the three months of peak gonad development. Based on the GSI values of 1999-2001 (Fig. 3a) the peak breeding season was July-August-September for *B. dainellii*, *B. longissimus*, *B.megastoma*, *B. truttiformis* and *B. tsanensis*, August-September-October for *B. acutirostris*, *B. brevicephalus*, *B. crassibarbis*, *B. gorgorensis*, *B. gorguari*, *B. macrophtlamus*, B. nedgia and *B. platydorsus*, and November-December-January for *B. surkis*. The abundance of *B. intermedius* SC in the river mouths was compared between March-September ("high" breeding season) and October-February ("low" breeding season). Seven species were rare in the river mouths during their peak breeding season (Fig. 3b): *B. crassibarbis* (0.6 ±0.2 SE), *B. dainellii* (0.1 ±0.1SE), *B. gorguari* (1.6 ±0.2SE), *B. gorgorensis* (0.7 ±0.2SE), *B. longissimus* (0.8 ±0.3SE), *B. nedgia* (1.1 ±0.2SE), and *B. surkis* (0.5 ±0.2SE). These *Barbus* species apparently did not form spawning aggregations and were left out of further analysis of macro-spatial and temporal segregation in the river mouths. *Barbus brevicephalus*, *B. macrophtalmus*, *B. truttiformis*, *B. tsanensis*, *B. acutirostris*, *B. megastoma*, *B. platydorsus*, and *B. intermedius* SC were common in the river mouths during their peak breeding season (Fig. 3b). To test whether these *Barbus* species aggregated in the river mouths only during the breeding season, the abundance in the river mouths during their peak breeding season was compared with the abundance in the river mouths in the non-breeding season (Table 1). *Barbus brevicephalus*, *B. macrophtalmus*, *B. truttiformis* and *B. tsanensis* exhibited migration patterns, forming spawning aggregations in the river mouths. These four *Barbus* species were highly significantly ($P< 0.001$) more abundant in the river mouths during their peak breeding season (Table 1, Fig. 3b) compared to the rest of the year. *Barbus acutirostris*, *B. megastoma*, *B. platydorsus*, and *B. intermedius* SC were relatively abundant in the river mouths throughout the year, masking possible aggregations of these species in the river mouths during the peak spawning months (Fig. 3b).

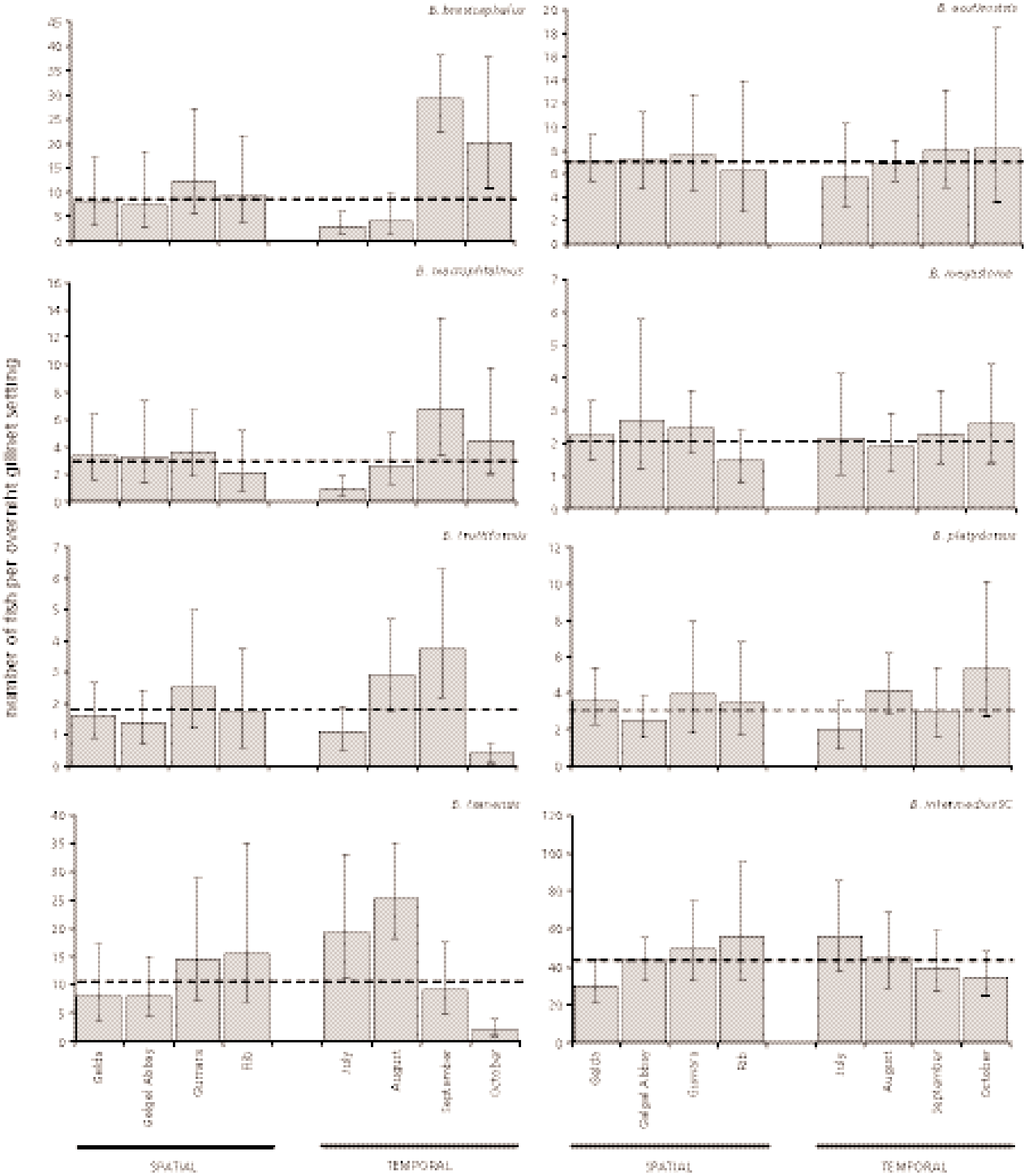


Figure 4. Mean abundance (number of fish per overnight gillnet setting) ± 95% confidence intervals of eight *Barbus* species per river mouth and per month in Lake Tana. Dotted lines indicate the overall mean of 60 gillnet settings per *Barbus* species. Note the different scales of the y-axes.

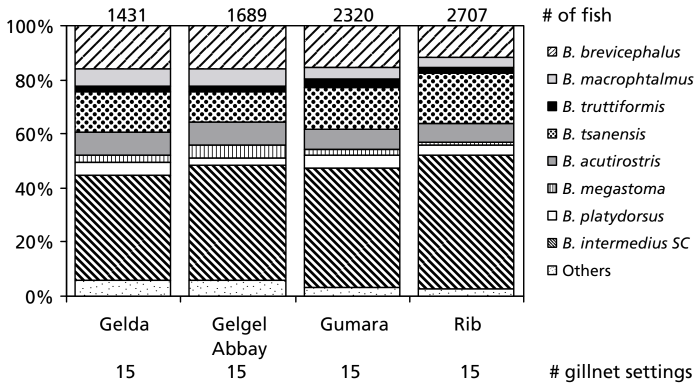
Does macro-spatial segregation occur among affluent rivers?

The abundance of *B. acutirostris*, *B. brevicephalus*, *B. macrophtalmus*, *B. megastoma*, *B. platydorsus*, *B. truttiformis*, *B. tsanensis* and *B. intermedius* SC in the Gumara, Gelda, Rib and Gelgel Abbay river mouths was compared during the breeding season (July-October) in order to determine possible preferences for one or more rivers during their annual breeding migration (Fig. 4). No significant geographic effect was found among any of the eight *Barbus* species, densities of each *Barbus* species were similar in all four river mouths (Table 2, Figs. 4 and 5a).

Does temporal segregation occur within the breeding period?

Aggregation in the river mouths did not occur simultaneously among the eight different *Barbus* species within the breeding season in 1999-2000. A highly significant temporal effect ($P<0.001$, Table 2) on abundance in the river mouths was observed for half of the eight *Barbus* species. *Barbus tsanensis* was the first to appear in the river mouths in June-July, followed by *B. truttiformis* in August-September. *Barbus macrophtalmus* and *B. brevicephalus* were the last species to aggregate in the river mouths during the breeding season, with the highest densities in September-October (Figs. 3b, 4 and 5b).

(a) spatial segregation



(b) temporal segregation

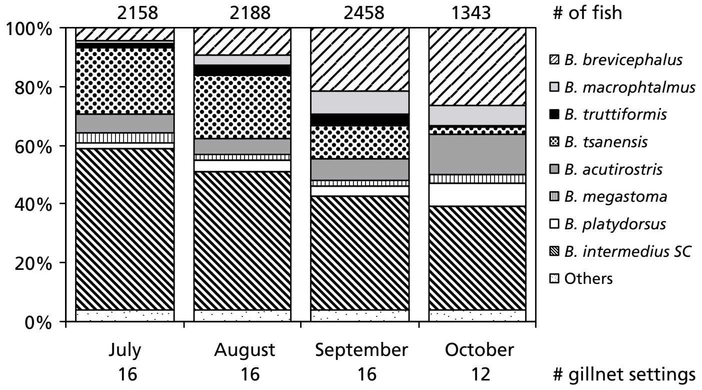


Figure 5. Species composition of the overnight gillnet settings (a) as a function of geographic location (i.e. in four different river mouths) during Jul-Oct breeding period, and (b) as a function of time (i.e. in four different months) in the river mouths. Note the similarity of species composition between the different river mouths (a) and the changes in species composition over the breeding season (b).

Discussion

Breeding season

Previous studies on reproductive segregation among Lake Tana barbs (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999; Chapter 6) reported spawning aggregations of ripe and running fish for maximally seven of the 15 *Barbus* species. The remaining eight species were never or only incidentally caught (<1%, Table 3) on the upstream spawning areas. Because all sampled within the same three month period (August-October) a (first) good explanation might be that the eight "missing" *Barbus* species matured and spawned during another season.

Year round observation of the gonad development revealed, however, that in all species except *B. intermedius* SC and *B. surkis* maturation of the gonads starts in May-June at the beginning of the rainy season. Ripening of the gonads is most likely triggered and possibly synchronised by decreasing water temperature, increasing turbidity and/or increasing water level in the lake after the beginning of the rainy season in May-June (Figs. 2 and 3a). The pre-spawning aggregations in the river mouths may effect synchronisation of the gametogenesis as individual gonadial cycles might not coincide in such widely dispersed stocks. The reproductive period is short, peaking in August-September (most barbs) or December-January (B. surkis). *Barbus intermedius* SC is the only exception having an extended spawning season lasting from March till September. However, ripe individuals were found throughout the year.

Comparison of the development of the GSI clearly proved that in the past the absence of spawning aggregations of many *Barbus* species in river mouths and/or the upstream spawning areas was not caused by temporal differences in peak breeding periods. In 14 *Barbus* species (including seven of the "missing" species) spawning occurs in August-September which overlapped with the sampling periods of Nagelkerke and Sibbing (1996), Dgebuadze et al. (1999), and Chapter 6. Only the absence of *B. surkis* can be explained by its late maturation and breeding season, from November to January (Fig. 3a).

Spawning rivers

The studies of Nagelkerke and Sibbing (1996), Dgebuadze et al. (1999), and Chapter 6 all focused on the Gumara river. Therefore, a second explanation might be that the eight "missing" *Barbus* species aggregated for spawning in other affluent rivers, e.g. Gelgel Abbay river on the western shore and Rib river and Gelda river on the eastern shore (Fig. 1). The experimental gillnet program showed that the seven "missing" *Barbus* species did not aggregate in the four sampled river mouths during breeding (Fig. 3b). The large numbers of *B. intermedius* SC in the river mouths seems unrelated to reproductive activities as this species was virtually absent on the upstream spawning areas (Table 3). This barb is common in all littoral zones, including river mouths, throughout the lake.

Among the seven riverine spawning *Barbus* species, no evidence was found for spatial segregation, e.g. each species choosing its own river (Figs. 4 and 5a), Table 2). These results do not support the conclusion of Nagelkerke and Sibbing (1996) claiming spatial segregation among Gumara, Rib, Gelda and Gelgel Abbay river mouths. However, their sample size was very small (1-5 samples per river) and, more importantly, collected over a long period [early September (Gelda) - late October (Gumara)]. Significant changes in species abundance in the river mouths (temporal segregation) does, however, occur within the breeding period (Figs. 3, 4 and 5b, Table 2). The "spatial" effect as claimed by Nagelkerke and Sibbing (1996) may in fact simply be temporal segregation.

Consistent patterns were observed in the formation of spawning aggregations in the river mouths and the arrival on the upstream spawning area within the July-October breeding season (Chapter 6). Early (*B. tsanensis*), intermediate (*B. acutirostris*, *B. megastoma*, *B. platydorsus*, *B. truttiformis*) and late (*B. brevicephalus*, *B. macrophtalmus*) riverine spawners could distinctly be identified. The *Barbus* species composition of the catch of the commercial gillnet fishery in 2001 matched the abundance patterns as found in the experimental gillnet program, i.e. a sequential dominance of the different riverine spawning *Barbus* species from July to October, and the absence of the "missing" *Barbus* species (Chapter 12).

Two rivers on the northern shore, Megech and Dirma, and one river on the northeastern shore, Arno-Garno, could not regularly be sampled due to the distance (Fig. 1). The probability, however, that the "missing" *Barbus* species only use these northern rivers for spawning is small. Incidental gillnet catches by us and interviews with local fishery experts and fishermen revealed that barbs do aggregate in the mouths of these northern rivers but that these *Barbus* are the same species that aggregate in Rib, Gumara, Gelda and Gelgel Abbay rivers. Since no geographic effect on *Barbus* densities was found between the four regularly sampled rivers, it is unlikely to occur between these and the three northern rivers. In conclusion, despite the intensive 'year round and lake round' sampling programs in the river mouths and upstream spawning areas it remains unclear where the "missing" *Barbus* species spawn.

Lacustrine spawning

African 'large' *Barbus* species predominantly occur in rivers, and although some species are lacustrine, all are riverine spawners (Tómasson et al. 1984). These 'large' *Barbus* perform one single annual mass upstream breeding migration and spawning occurs in clean, well circulated gravel beds in fast flowing, shallow rivers where a salmon-like red is made (Skelton 1991).

This ancestral reproductive strategy appears characteristic for at least seven of the *Barbus* species in Lake Tana as discussed above. By far the most intriguing and unique aspect of the reproductive biology of Lake Tana barbs is that half of the *Barbus* species do not migrate upstream affluent rivers to spawn (Table 3, light grey block). These species seem to have successfully adapted to the lacustrine environment and probably complete their whole life-history, including reproduction, inside the lake. The small upstream tributaries used by the riverine spawning *Barbus* species are only available for a short period at the end of the rainy season (Sep-Oct). The rest of the year, including the breeding seasons of *B. intermedius* SC (Mar-Sep) and *B. surkis* (Nov-Jan), these small tributaries fall dry. Therefore especially for these two species lake spawning seems the only probable option. The most likely scenario is that the "missing" *Barbus* species breed in the lake and/or adjacent floodplains and deposit their eggs on sand or rocks, near roots of plants or on aquatic or flooded terrestrial vegetation as is common in many other cyprinid genera (Mills 1991).

Table 3. Overview of *Barbus* species composition in river mouths and upstream Gumara River spawning areas.

	River Mouth		**Upstream Gumara River**		
Source	this paper	Nagelkerke and Sibbing (1996)	Chapter 6	Dgebuadze et al. (1999)	Nagelkerke and Sibbing (1996)
Sampling period	Jul-Oct 1999 Jul-Oct 2000	Sep-Oct 1993 Sep-Oct 1994	Sep-Oct 1999	Sep-Nov 1994 Aug-Oct 2000	Sep 1994
Sampling frequency	Twice per month	3 times Sep 4 times Oct	Twice per week	few times	once
Sampling area	Gumara, Gelda, Rib, and Gelgel Abbay	Gumara	3 tributaries and 4 locations in main channel	1 tributary and 1 location in main channel	1 location in main channel
Number	8147	1530	4950	540	70
B. tsanensis	15·6	18	48·5	33[a]	42
B. brevicephalus	14·5	46	27·4	36[b]	27
B. truttiformis	2·5	1	5·5	0·5	19
B. megastoma	2·4	3	4·9	2	8
B. macrophtalmus	4·7	23	4·7	19	0
B. acutirostris	7·7	5.5	2·8	8	0
B. platydorsus	4·0	1	0·6	2	2
B. gorgorensis	0·7	0·5	0·2	0·2	2
B. crassibarbis	0·4	0·5	0·3	0·3	0
B. dainellii	0·1	0	0	0	0
B. gorguari	0·9	0·3	0	0	0
B. surkis	0·5	0·5	0	0	0
B. nedgia	0·9	0·5	0	0	0
B. longissimus	0·5	0	0	0	0
B. intermedius SC	44·7	0	0	0	0

[a] referred to as morphotype "normal intermedius" in Dgebuadze et al. (1999) but presumably *B. tsanensis* according to Nagelkerke and Sibbing (2000).

[b] referred to as morphotype "precocious intermedius" in Dgebuadze et al. (1999) but presumably *B. brevicephalus* according to Nagelkerke and Sibbing (2000).

Using the marginal vegetation as spawning sites will provide both shelter from predators, and high densities of prey for larvae and juveniles. However, these spawning sites also more likely suffer from oxygen depletion (Mills 1991). Several observations of running females provide further evidence for spawning activities within the lake. In November 1992 a trawl catch near the floodplains consisted mainly of running *B. surkis* (Wudneh pers. comm.). On 11 October 1995 running *B. dainellii* were found in the littoral zone far from any river (Nagelkerke and Sibbing 1996) and on 9 November 2000 we caught a few hundred running male and female *B. intermedius* SC close to the papyrus fields to the west of Bahar Dar. In contrast, running females of the river spawning *Barbus* species were never observed in the lake. They have only been found close to or in the affluent rivers with the upstream spawning areas.

Although lacustrine spawning is a novelty among 'large' African *Barbus*, a few anecdotes reported on lacustrine spawning among *Barbus* from the Middle East (*B. longiceps*; Fishelson et al. 1995). The spawning grounds of *B. sharpeyi* are confined to the lakes and marshes in the alluvial plain and, to a lesser extent, to the lower reaches of the Tigris and Euphrates rivers (Al-Hamed 1972). *Barbus sharpeyi* deposits the eggs on submerged parts of aquatic vegetation or other objects, from the surface down to a depth of 1 m.

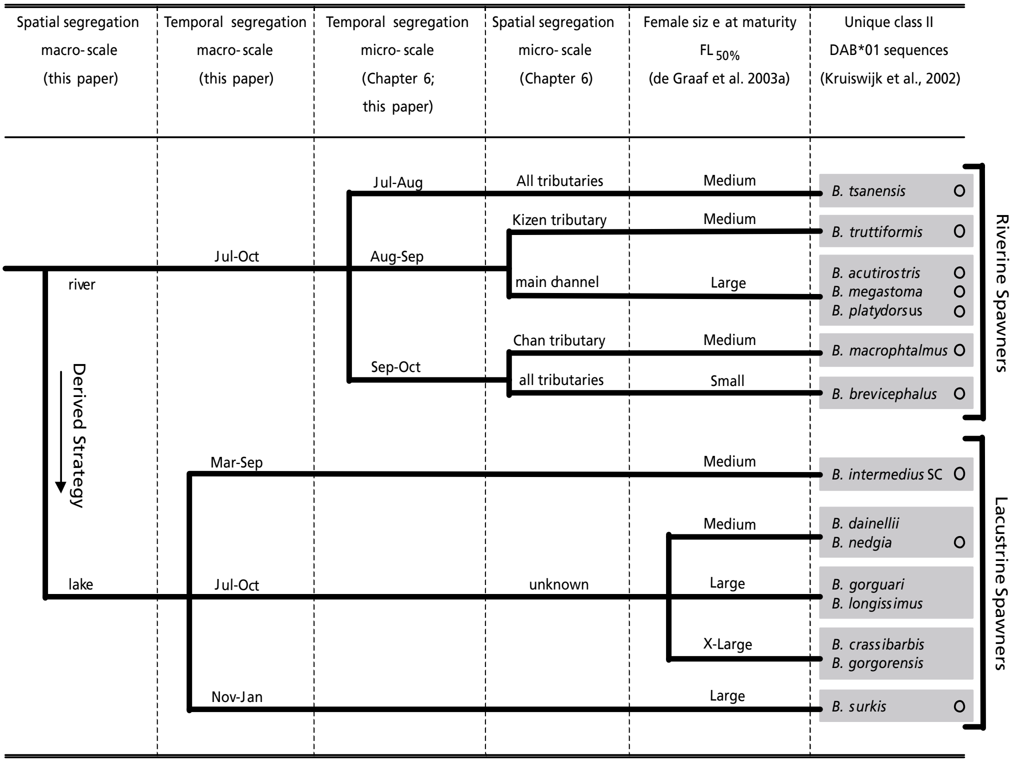


Figure 6. Overview of the differences in reproductive characteristics and spawning patterns of Lake Tana's *Barbus* species (including data from Chapter 6 and de Graaf et al. (2003a), small = 15-20 cm $FL_{50\%}$, medium = 22.5-27.5 cm $FL_{50\%}$, large = 30-37.5 cm $FL_{50\%}$, x-large = 40-47.5 cm $FL_{50\%}$). Solid circles indicate presence of unique class II DAB*01 sequences (Kruiswijk et al. 2002); *B. dainellii*, *B. gorguari*, *B. longissimus*, *B. crassibarbis* and *B. gorgorensis* were not tested.

Reproductive isolation

In order to explain how assortative mating and reproductive isolation have evolved within the *Barbus* species flock, we need to highlight the current differences in reproductive characteristics and spawning patterns. All data on spatial and temporal spawning patterns and reproductive characteristics (size at maturity) are used to construct a flow chart visualising the origin and extent of genetic segregation within the *Barbus* species flock (Fig. 6). An important limitation of gene flow among the members of the species flock is the macro-spatial divergence of spawning area among the barbs, separating the riverine spawners (ancestral) and the lacustrine spawners (derived). The occurrence of micro-spatial segregation among riverine spawners on the upstream spawning areas was demonstrated in Chapter 6. The most striking example is *B. truttiformis*, which only spawned in the first upstream tributary (Kizen) of the Gumara River. Colouration and morphology are not sexually dimorphic among any of Lake Tana's 'large' *Barbus* species, however, body size and average size at maturity ($FL_{50\%}$) varied greatly between the species (from 18 cm FL in female *B. brevicephalus* to 44 cm FL in female *B. crassibarbis*; de Graaf et al. 2003a). Reproductive isolation may be achieved by size-assortative mating despite eventual temporal-spatial overlap in breeding areas. The tendency for size-assortative mating is known for several fish species like cichlids (Schliewen et al. 2001) and closely related species pairs of sticklebacks (Nagel and Schluter 1998). Overall, the high degree of assortative mating within the species flock as described above, is further supported by the fact that class II DAB*01 sequences were not shared (reproductive isolation) among any of the 10 *Barbus* species (seven riverine spawners and three lacustrine spawners) tested by Kruiswijk et al. (2002).

Evolutionary origin lacustrine spawning

Lake spawning within the *Barbus* species flock probably evolved following adaptation to divergent ecological environments, i.e. adaptation to the shore areas in the newly formed lake. Most lacustrine spawners are confined to the littoral zone (<5% of the lake surface) either over sandy and/or rocky substrate (Fig. 7). Especially the *Barbus* species associated with rocky substrate, like *B. dainellii*, *B. gorguari*, *B. longissimus* and *B. nedgia* (Nagelkerke and Sibbing 2000; Chapter 4) have a limited and patchy distribution (<2% of the lake surface). Spawning of these highly specialised *Barbus* species within or near their rocky habitat instead of on faraway rivers, might provide their offspring with suitable habitat, reducing the risk to be forced to live in sub-optimal habitat/trophic conditions. Another characteristic of the littoral-dwelling lacustrine spawning *Barbus* is their body colouration which varies drastically from bright golden yellowish to green and very dark brown to black (Nagelkerke and Sibbing 2000; Fig. 7). This is in sharp contrast to the riverine spawning, "open-water" *Barbus* species that are widely distributed, occurring throughout the sub-littoral and offshore waters (>95% of the lake surface, Fig. 7) and have a silvery white body colouration with a slightly darker back.

Early ecological divergence, adaptation to macro-habitats, appears to be common in radiations of lacustrine fish groups; rock- and sand-dwelling clades in Lake Victoria's (Nagl et al. 2000), Lake Malawi's (Danley and Kocher 2001) and Lake Tanganyika's cichlids (Sturmbauer 1998); sympatric divergence of benthic and pelagic forms among west African ciclids in crater lakes (Schliewen et al. 1994, 2001; divergence of sticklebacks in benthic and limnetic forms in several North American lakes (Schluter and McPhail 1993). Similarly, macro-habitat adaptations resulted in the divergence of morphological and behavioural characteristics including body colouration, reproductive behaviour and habitat preference (Danley and Kocher 2001 and references therein).

Further speciation among the littoral-dwelling lacustrine spawning *Barbus* most likely resulted from trophic competition and trophic specialisation within the littoral habitat from a common lacustrine spawning ancestor, probably *B. intermedius* SC. The sequence of primary macro-habitat diversification followed by secondary trophic diversification within each macro-habitat has been shown to occur in several lacustrine fish groups; East African cichlids (Seehausen 1996; Sturmbauer 1998; Danley and Kocher 2001), arctic charr (Snorrason et al. 1989). In such a scenario, trophic specializations like piscivory (for example *B. dainellii*, *B. gorguari*, littoral zone, lacustrine spawners and *B. truttiformis*, *B. macrophtalmus*, offshore pelagic zone, riverine spawners) probably have separate, independent origins within the *Barbus* species flock (Fig. 7). Such patterns of parallel trophic morphological evolution in different macro-habitats also occurred among Lake Tanganyika cichlids (Rüber et al. 1999).

Current research focuses on the reconstruction of the phylogenetic history of the *Barbus* species flock to test such hypothesized evolutionary scenario's. Interesting questions that need further attention are the role of adaptation to ecological divergent environments and the role of philopatry (homing to natal streams) on the development of assortative mating, reproductive isolation and speciation within Lake Tana *Barbus* species flock.

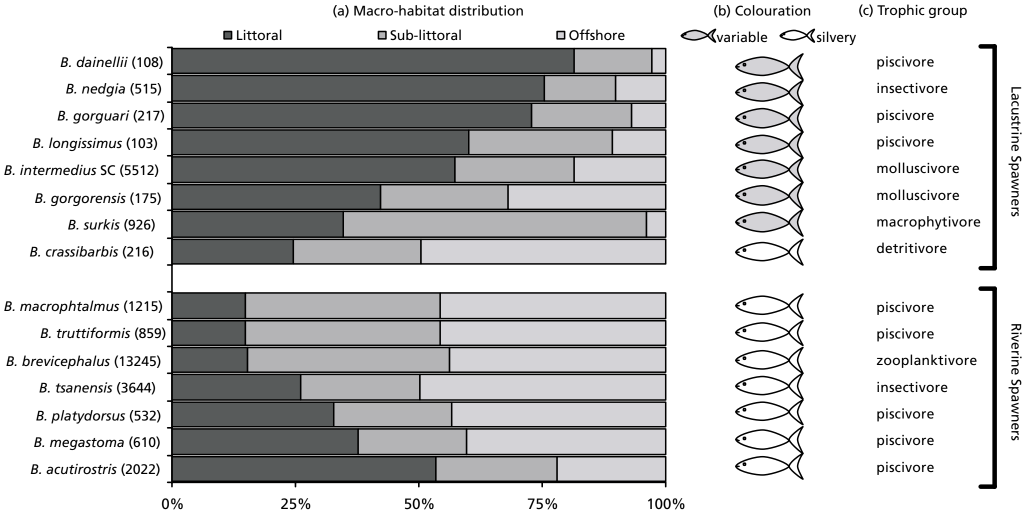


Figure 7. (a) Distribution of the *Barbus* species over three macro-habitats (Littoral, depth < 4 m, distance to shore < 100 m; Sub-littoral, depth 4-8 m, distance to shore 100-1000 m; Offshore, depth > 8 m, distance to shore 1000-5000 m) from the overnight experimental gillnet catches between October 1999 and September 2000 [data from Chapter 4]. The percentage per macro-habitat is given for each *Barbus* species, n per *Barbus* species is indicated in parentheses. (b) Overall body colouration of the *Barbus* species. (c) Tropic groups (see Chapter 4).

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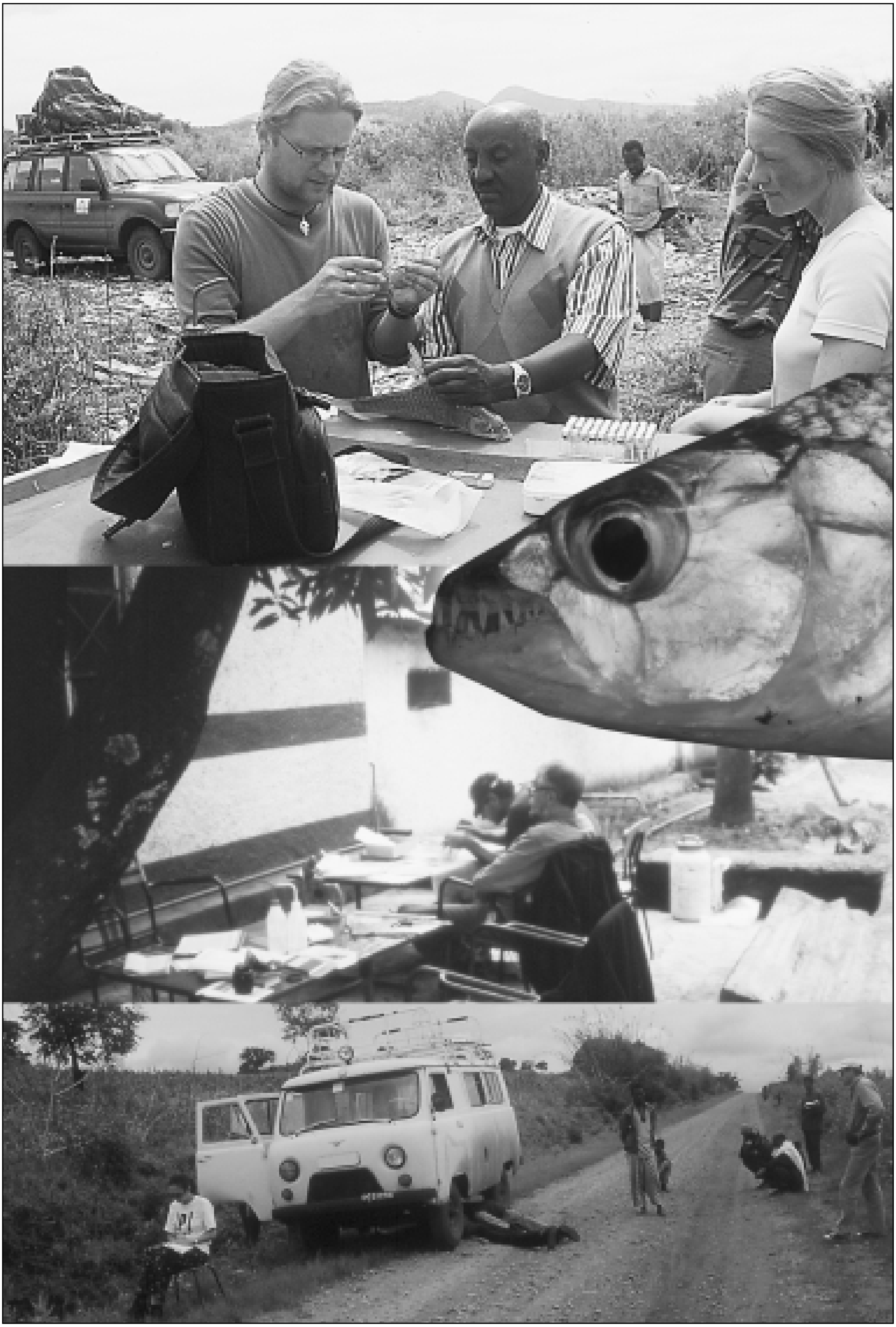

9
CHAPTER

Rapid speciation of Lake Tana's Barbus (Cyprinidae; East-Africa) as inferred from mtDNA markers.

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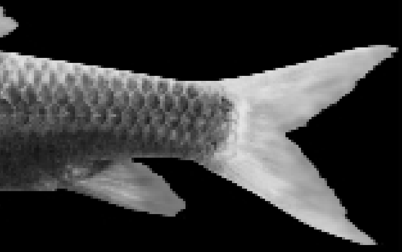

Abstract

Lake Tana harbors the only remaining intact species flock of cyprinid fishes. The 15 large (max 100 cm FL) hexaploid *Barbus* species have exploited their potential for trophic diversification to the fullest, as exemplified by the unexpected specialization for piscivory. Three small (<10cm FL), diploid *Barbus* species also occur in Lake Tana. The small, pelagic *B. tanapelagius* has recently evolved from a small benthic species in Lake Tana, *B. humilis*. Genetic diversity of the mtDNA cytochrome *b* gene among Lake Tana's large *Barbus* species is very low. The failure to distinguish between species is possibly due to the recent origin of the species flock. The haplotypes found in Lake Tana are unique but none is diagnostic for a particular species. The present data supports nor rejects a monophyletic origin of Lake Tana's *Barbus* species flock. The haplotypic variation falls into two distinct Lineages (1 and 2). Tana haplotypes seem to form a subset of the total variation in Lineage 1, adding evidence to the notion that the Lake Tana species flock originates from a limited stock of ancestral riverine *B. intermedius*. With a maximum sequence divergence of approximately 1% in Lineage 1 and an estimated sequence divergence of 1.3 to 3.3% per million years, the Tana species flock seems not older than 770,000 to 300,000 years. Other studies have shown that the age of divergence of mitochondrial lineages can be a poor estimate for the age of a species flock in recently formed (refilled) lakes. Circumstancial evidence suggests that the fast radiation of ecomorphological diversity within Lake Tana's *Barbus* species flock maybe occurred as recent as 10,000-25,000 years ago.

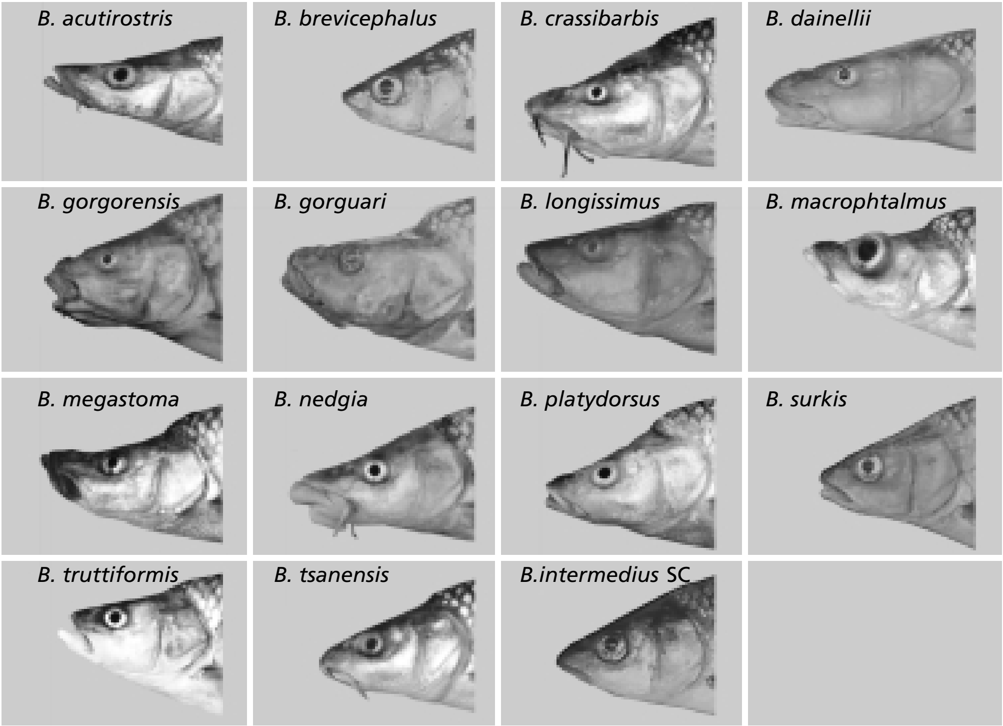

Figure 1. Heads of the 15 *Barbus* species of Lake Tana.

Introduction

The highly diverse cichlid species flocks in the East-African Great Lakes (Victoria, Malawi, Tanganyika) are famous examples of explosive adaptive radiations and rapid speciation events (Fryer and Iles 1972; Keenleyside 1991). Not as well known, but therefore not less spectacular, is the extraordinary diversity of cyprinid fishes in Lake Tana, a large lake (3,050 km^2) situated at an altitude of 1830 m in the north-western highlands of Ethiopia. Lake Tana is the source of the Blue Nile. High waterfalls (40 m) at Tissisat ('smoking waters'), 30 km downstream from the Blue Nile outflow, effectively isolate the lake's ichtyofauna from the lower Nile basin. Despite the overwhelming abundance (>2000 species; Nelson 1994) of cyprinid fishes throughout the world's fresh water systems, the *Barbus* species of Lake Tana (Fig. 1) form, as far as we know, the only remaining intact species flock of large cyprinid fishes, since the one in Lake Lanao in the Philippines, has practically disappeared due to human activities (Kornfield and Carpenter 1984).

The 15 large, hexaploid (Golubtsov and Krysanov 1993) endemic *Barbus* species (Nagelkerke et al. 1994; Nagelkerke and Sibbing 2000) have exploited their potential for trophic diversification to the fullest, including the for cyprinids unexpected specialization for piscivory (Sibbing and Nagelkerke 2001; Chapter 4 and 5). The range of successfully invaded trophic niches is almost similar to that of Lake Victoria's haplochromine species flock (Chapter 4). According to Nagl et al. (2000) and Verheyen et al. (2003), the major lineage divergence took place about 100,000 years ago, but the vast radiation of ecomorphological diversity within Lake Victoria's haplochromine species flock occurred in the last 15,000 years (Johnson et al. 1996). The age and origin of the assumed monophyletic (Nagelkerke and Sibbing, 1998) *Barbus* species flock in Lake Tana is at present unknown. Estimates for the age of Lake Tana range from as recent as 10,000 BP (Grabham and Black 1925) to as old as 2 Ma (early Pleisocene, Mohr, 1962) and 6 Ma (late Miocene, Chorowicz et al. 1998 and references therein) years ago. The possible recent age of Lake Tana limits the choice of markers for a phylogenetic study. Compared to nuclear DNA, mitochondrial DNA has a substitution rate that is about ten times higher in vertebrates (e.g. Moriyama and Powell 1997), allowing for a much faster accumulation of (phylogenetically relevant) base changes. In addition, due to a smaller effective population size and effective haploidy, coalescence times of mitochondrial haplotypes are theoretically four times shorter compared to nuclear alleles. This means that mitochondrial genes have a much higher likelihood of correctly tracking species evolution (Avise 1994), and the level of shared ancestral polymorphism will be much lower (Moriyama and Powell 1997). Cytochrome *b* has been widely used to address phylogenetic (e.g. Tsigenopoulos and Berrebi 2000 and Tsigenopoulos et al. 2002) and phylogeographic questions (e.g. Kotlík and Berrebi 2001) in the genus *Barbus*. The use of this mitochondrial gene in the present study allows easy comparison with results from these earlier studies.

In this paper we describe the first comprehensive investigation to discern historical patterns in the barbs of Lake Tana using mtDNA, including all the endemic *Barbus* species as well as populations from other Ethiopian rivers and lakes. Specifically, we try to answer a number of prominent questions regarding the evolution of cyprinids of Lake Tana. Firstly, we try to establish the origin of Lake Tana *Barbus* species, including the recently discovered small *B. tanapelagius* (de Graaf et al.

2003). Furthermore, we investigate whether the Lake Tana's large barbs form a monophyletic group, and whether historical relationships among species of the Lake Tana *Barbus* species flock can be determined. Finally, we aim to age the Lake Tana species flock.

Material and Methods

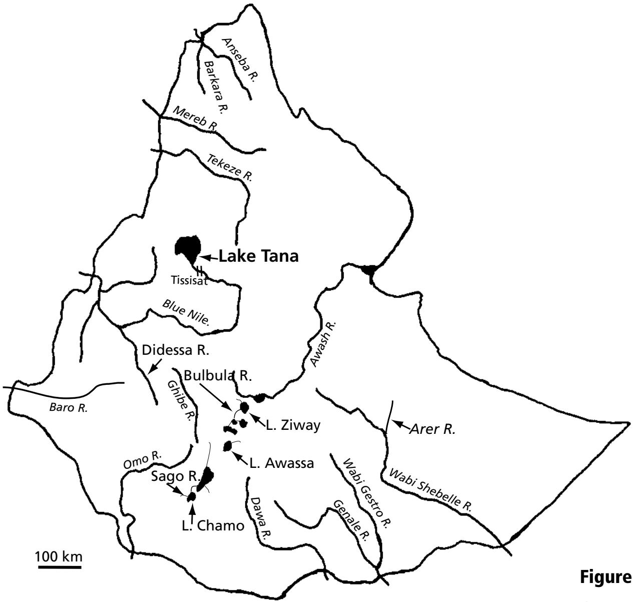

Figure 2. Major river systems and lakes in Ethiopia. Arrows indicate the localities at which *Barbus* spp investigated in this study were collected.

Collection and isolation of DNA

Barbus species were collected in Lake Tana and several rivers and lakes of different drainage systems during 1999-2001 (Fig. 2). The complete cytochrome *b* sequences of the collected Ethiopian barbs as well as sequences of other cyprinid species recovered from the GenBank/EMBL were included in the analyses. The specimens from Sago (1 specimen), Arer (1 specimen), Bulbula (1 specimen), Lugo (3 specimens) and Dabena (1 specimen) river were collected by Abebe Getahun (Addis Ababa University, Ethiopia) and the sequences were provided to us by William Jordan (British Museum of Natural History, London, UK). Pieces of the dorsal fin from collected specimens were preserved in 70% ethanol and DNA was extracted following the Technical manual Wizard Genomic DNA Purification Kit of Promega.

Polymerase chain reaction, amplification and sequencing

For PCR amplification we used primers L15267 (5'- AAT GAC TTG AAG AAC CAC CGT - 3') and H16461 (5'-CTT CGG ATT ACA AGA CC- 3') (Briolay et all, 1998). Numbers refer to the position of the 3' end of the primers in the complete mitochondrial DNA sequence of the carp *Cyprinus carpio* (GenBank accession no. X61010). The use of L15267 and H16461 in the PCR reactions resulted in the amplification of a complete of cytochrome *b* gene (in total 1140 basepairs (bp). The volume of each

PCR was 50 µl and consisted of 1.8 mM $MgCl_2$ (Promega), 1 µM of each primer, 1 µl of 10 mM dNTP-mix of Deoxynucleoside Triphoshate set PCR Grade (Roche-1.969.064), 0,3 µl of 5.0 Units / µl Taq DNA Polymerase (Sigma-D6677) and 1 x of amplification buffer (Promega). Amplification conditions involved a first denaturation at 94 °C for 2min., annealing at 50 °C for 1min., extension at 71 °C for 1min, and then 30 cycles of denaturation at 94 °C 30 sec., annealing at 50 °C for 1 min. 30 sec. and extension at 71 °C for 1 min. (followed by a final extension at 71 °C for 3 min.). For visualization, 5 µl of each PCR product was run on a 1.2 % TAE-agarose gel and stained with Ethidium-Bromide. The remaining amplified product was purified using a Zymo-DNA Clean & Concentrator / Spin I -column kit following the manufacture's protocol, Zymo Research Inc. The Sequencing reaction consisted of denaturation at 95 °C for 1 min. and 25 cycles at 96 °C for 10 sec., at 50 °C for 5 sec. and at 60 °C for 4 min.

The volume of the sequencing reaction was 5µl and consisted 0.5 µl PCR Primer 1.6 pmol / µl, 2.5 µl 10 ng / µl DNA, 1 µl 0.5 x Big Dye and 1 µl Terminator Ready Reaction Mix following the manufacture's protocol of ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kits of Applied Biosystems. Products were run out on a 5 % denaturing acrylamide gel Sanver Tech) and visualized on a ABI-377 automated sequencer following the manufacturer's instructions. Both strands were sequenced using each one of the two inital PCR primers.

Sequence alignment and analysis

Complete cytochrome *b* sequences obtained from large Lake Tana barbs, *Barbus intermedius*, *B. paludinosus*, *B. pleurogramma*, and *B. tanapelagius* were aligned to the African barb cytochrome *b* sequences reported in Tsigenopolous et al. (2002). This was done for the purpose of determining the phylogenetic position of some species not previously included in phylogenetic studies, as well as to determine the level of sequence divergence between various species and populations in reference to a larger set of taxa. Included in the phylogenetic analysis were positions 71 to 1140 (*B. barbus* cytochrome *b* gene, Tsigenopoulos and Berrebi 2000). Neighbour Joining (NJ) analysis with uncorrected pairwise distances was carried out using PAUP4b10 (Swofford 2002), and a NJ bootstrap analysis was carried out with 2000 replicates.

Cytochrome *b* haplotypes for large Tana barbs and *Barbus intermedius* were established based on sites 45 to 415 and sites 747 to 1098 only, because we were unable to sequence the complete region for all specimens. Traditional methods of phylogenetic inference, such as parsimony, neighbour joining and maximum likelihood, make assumptions that are invalid at the population level, such as absence of 'ancestral' haplotypes. In addition, these methods require reasonably large numbers of variable characters (Clement et al., 2000). Therefore we used TCS v1.13 (Clement et al. 2000), which is a computer program that implements the estimation of gene genealogies from DNA sequences as described by Templeton et al. (1992). This cladogram estimation method is also known as statistical parsimony, not creating dichotomous trees but networks to incorporate the often-times nonbifurcating genealogical information associated with very low level of divergence.

Results

We obtained complete cytochrome *b* sequences for four specimens of *Barbus tanapelagius*, four specimens of *B. humilis* from Lake Tana, twelve specimens of *B. paludinosus* from Lake Awassa, Lake Ziway, and the Didessa River, and four specimens of *B. pleurogramma* from Lake Tana. We furthermore obtained 35 complete and 19 partial cytochrome *b* sequences from all 15 large *Barbus* species from Lake Tana, and from *B. intermedius* from a number of rivers and lakes in Ethiopia (see Table 1).

All specimens for which complete cytochrome *b* sequences were available are included in the NJ analysis. The resulting tree (Fig. 3) is in complete agreement with Tsigenopoulos et al (2000) with regard to the well supported clades. All large *Barbus* species from lake Tana cluster together with Ethiopian *Barbus*, and shows very limited levels of divergence. *Barbus intermedius* from Lake Chamo formed a distinct group from the large barbs from Lake Tana and Didessa River. *Barbus humilis* and *B. tanapelagius* cytochrome *b* sequences clustered together and show very limited sequence divergence. *Barbus pleurogramma* from Lake Tana clusters together with *B. paludinosus* from various other lakes and rivers in Ethiopia.

Fifty-four cytochrome *b* sequences from Ethiopian large *Barbus* spp. were included in the haplotype analysis. Out of 721 bases included in this part of our study, 37 were variable (19 parsimony informative sites), and we identified 22 haplotypes (Table 1). The distribution of haplotypes among the large *Barbus* are shown in Table 2. There are no haplotypes that are diagnostic for any of the large Lake Tana *Barbus* species. In addition, for those species for which multiple specimens were included, only *B. truttiformis* shows the same haplotype for each individual. No haplotypes are shared between individuals from Lake Tana and specimens from other lakes and rivers in Ethiopia. The Lake Tana haplotypes seem therefore, based on our sample, unique.

A haplotype network was constructed and is shown in Figure 4. Haplotypes h1 to h17 and h18 to h22 (arbitrarilly named) each fall into separate, well-defined lineages (Lineage 1 and 2). In particular within Lineage 1 few reliable relationships can be established. The number of pairwise nucleotide differences for which the MP criterion is justified with 95% confidence (Templeton et al. 1992, as implemented in TCS) was determined to be 11. The maximum number of pairwise differences is 15, but within Lineage 1 the maximum number is 7, within Lineage 2 that number is 9. Lake Tana large *Barbus* species only contain haplotypes from Lineage 1. Furthermore, almost all haplotypes from the Didessa River and its tributaries fall within Lineage 1.

Figure 3. Neighbour-joining tree of the Ethiopian *Barbus* spp and other predominantly African cyrpinid fishes (taken from Tsigenopoulos et al. 2002) based on cytochrome *b* sequence data. Branch lengths are proportional to the mean number of substitutes per site (see scale bar). Numbers above nodes indicate bootstrap values higher than 50%. Sequences of two catostomid taxa were used as an outgroup.

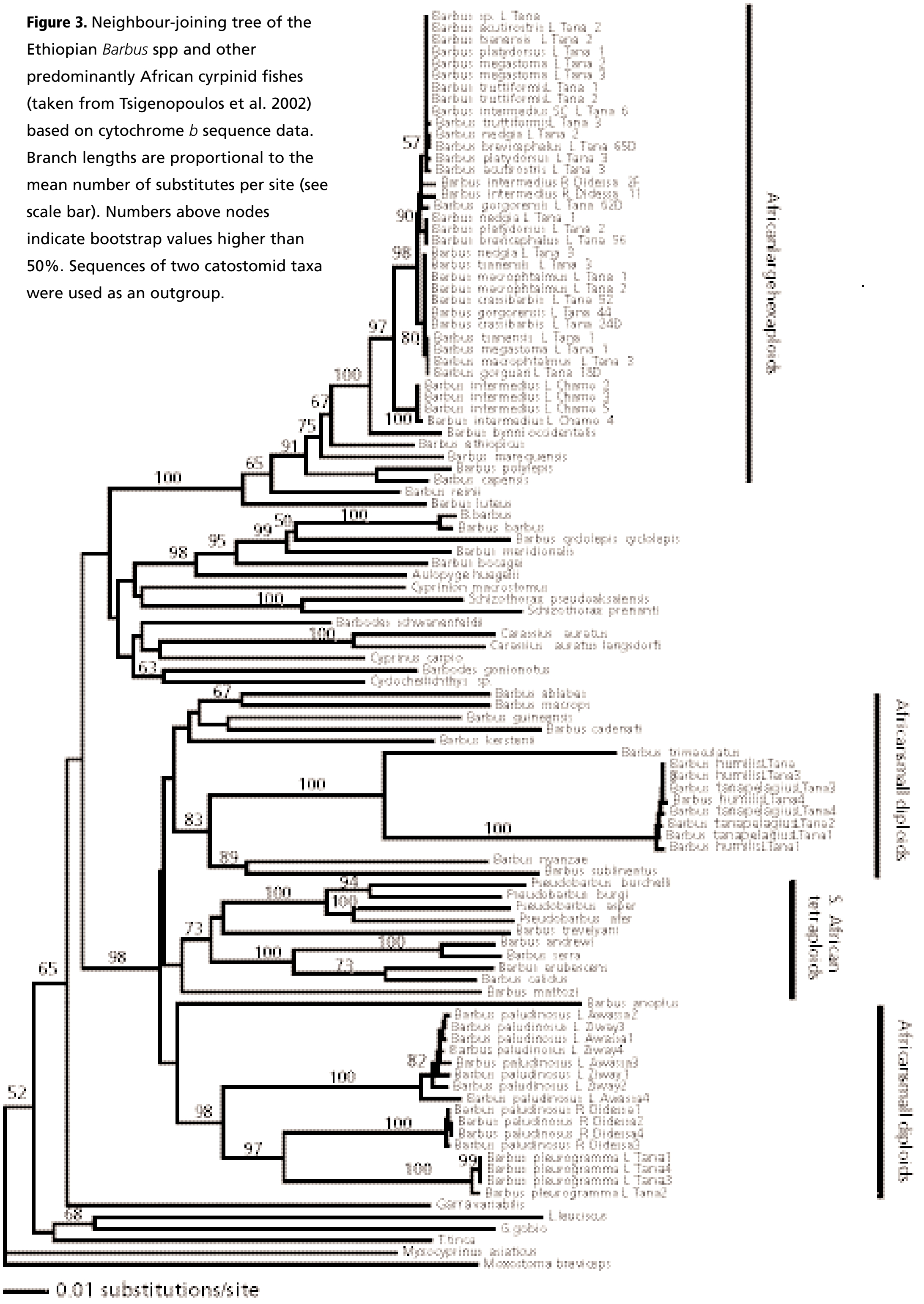

Table 1. Haplotypes found in the endemic large *Barbus* spp from Lake Tana and *B. intermedius* collected in several river and lakes in Ethiopia. Position numbers refer to the *B. barbus* cytochrome *b* sequence (AF112123; Tsigenopoulos and Berrebi 2000). Included in the haplotype analysis were positions 45 to 415 and sites 747 to 1098.

	Position of the variable site																																			
	6	7	8	1	1	1	2	2	2	2	2	3	3	3	3	3	3	4	7	8	8	8	8	9	9	9	9	9	9	9	9	9	9	1	1	1	1
	1	8	5	5	6	8	2	5	5	7	8	1	1	2	4	5	9	0	5	0	5	5	8	0	0	1	1	2	3	8	8	9	9	0	0	0	0
				9	5	6	2	1	8	0	5	5	8	7	2	2	0	2	0	7	2	8	8	1	3	2	9	7	6	5	7	0	7	3	4	7	9
																																		8	4	8	8
H1	T	C	A	A	T	A	C	C	A	C	C	A	T	T	T	G	C	G	A	G	T	C	T	T	G	G	C	C	A	G	A	A	G	G	C	G	T
H2			G			G				T									G																		
H3						G				T									G																		
H4																									A												
H5			G			G				T				C					G																		
H6			G			G				T							T		G																		
H7																																		A			C
H8		A																												A							
H9			G			G				T																											
H10			G			G														A																	
H11			G	G		G				T																											
H12			G			G				T		G											C														
H13			G			G				T		G											C					G									
H14	?	?	?	?	?	?		T		T		G											N														
H15	?	?	?			G				T							N														G						
H16	C		G			G				T											C															A	
H17	C		G			G	T			T											C															A	
H18			G		C	G				T			C		C	A		A							A	A			G				A	A			
H19			G		C	G				T			C		C	A		A							A	A			G				A	A	A		
H20	?	?	?		C	G				T			C		C										A	A	T		G				A	A			
H21	?	?	?		C	G				T			C		C							T	C	C		A			G			C	A				
H22	?	?	?			G			G	T	T		C		C											A			G					A			

Table 2: Frequency distribution of the 22 identified (see Table 1) haplotypes among the large *Barbus* spp. collected the different lake and rivers in Ethiopia. No = number of specimens.

			Haplotypes																					
Species	Location	no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
B. acutirostris	L. Tana	3		1	2																			
B. brevicephalus	L. Tana	2						1				1												
B. crassibarbis	L. Tana	3	2						1															
B. dainellii	L. Tana	1				1																		
B. gorgorensis	L. Tana	4	1							1	1	1												
B. gorguari	L. Tana	1				1																		
B. intermedius SC	L. Tana	2		1		1																		
B. longissimus	L. Tana	1		1																				
B. macrophtalmus	L. Tana	3	2			1																		
B. megastoma	L. Tana	3		2		1																		
B. nedgia	L. Tana	3	1					1				1												
B. platydorsus	L. Tana	3		1			1					1												
B. surkis	L. Tana	1	1																					
B. truttiformis	L. Tana	3		3																				
B. tsanensis	L. Tana	3	1	1		1																		
B. intermedius	Didessa R.	4											1	1	1		1							
B. intermedius	Didessa R. (Lugo trib.)	3											1			1								1
B. intermedius	Didessa R. (Dabena trib.)	1													1									
B. intermedius	L. Awassa	3																2	1					
B. intermedius	Bulbula R.	1																				1		
B. intermedius	L. Chamo	4																		3	1			
B. intermedius	L. Chamo (Sago trib.)	1																					1	
B. intermedius	Arer R.	1																					1	
		54	8	10	2	6	1	2	1	1	1	4	2	2	1	1	1	2	1	3	2	2	1	1

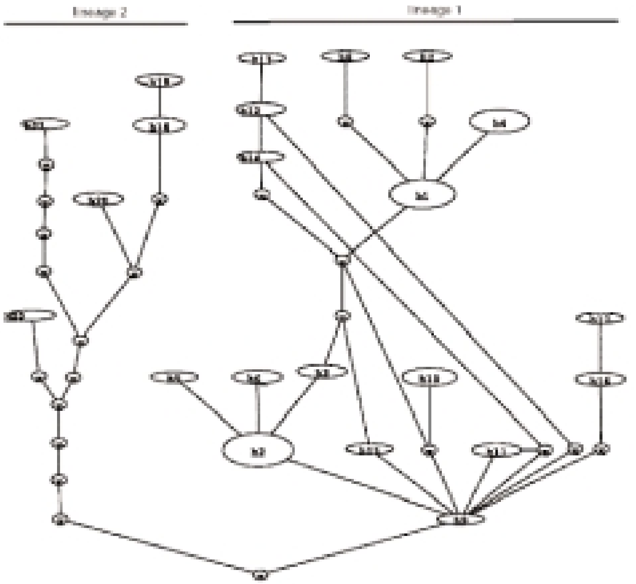

Figure 4. Unrooted haplotype network of the 22 haplotypes found in Lake Tana large *Barbus* spp. and Ethiopian *B. intermedius*. Ovals represent haplotypes, size of the ovals is proportional to the number of individuals sharing that haplotype. Small circles (x) represent hypothetical, 'missing' haplotypes. Each branch represent a single base change (see also Table 1 and 2).

Discussion

Lake Tana's large, hexaploid barbs

Genetic divergence among the endemic large *Barbus* species of Lake Tana is very low, as is indicated by the tree in Figure 3. Ten cytochrome *b* haplotypes are present in Lake Tana, but none is diagnostic for a particular species. Furthermore, for those species for which multiple specimens were included, only *B. truttiformis* shows a single haplotype. Haplotypic lineages (or individual haplotypes) have clearly not coalesced in the species of the species flock. Cumulative evidence from their ecology (Chapter 4 and 5; Sibbing and Nagelkerke 2001), reproductive behaviour (Chapter 6 and 8; Nagelkerke and Sibbing 1996), morphology (Chapter 7; Nagelkerke et al. 1994; Nagelkerke and Sibbing 2000) and genetics (Berrebi and Valiushok 1998; Dixon et al. 1996; Kruiswijk et al. 2002) indicate that the Lake Tana barbs are indeed 'good species'. The retention of haplotypic variation in these species could be explained by large population sizes during speciation or high levels of gene flow between species. Such assumptions, however, seem difficult to reconcile with sympatric speciation. Rather, there is evidence that suggests that 'genetic neighbourhood' (Kawata 2002), at least for a number of species, is fairly small (non-random mating, Chapter 7). An alternative hypothesis, which would be notably plausible in the light of such a limited gene pool, is that the failure to distinguish species based on cytochrome *b* sequences is due to the recent origin of the species flock.

The haplotypes found in Lake Tana are not found in rivers and lakes elsewhere in Ethiopia based on the sample we present in this study. Conversely, none of the haplotypes found outside occur in Lake Tana. This suggests a partitioning of haplotypic variation that could either be explained by a single incursion of ancestral *B. intermedius* in the Lake Tana drainage basin, or the disruption of an ancient continuous riverine population by the emergence of the Tissisat waterfalls. Yet, the present data neither supports nor rejects a monophyletic origin of Lake Tana's *Barbus* species flock. Although the haplotypes of the Tana barbs do not overlap with those from outside the lake, the relations between the haplotypes (Fig. 4) would suggest that haplotypes from Didessa River might cluster within those from Lake Tana. However, the inferred relationships are very unreliable as indicated by the network analysis.

The haplotypic variation falls into two distinct lineages (1 and 2; Fig. 4). There appears to be no clear-cut phylogeographic division between Lineage 1 and 2, although many (but not all) of the haplotypes from southern Ethiopian lakes and rivers fall within Lineage 2, and most (but not all) of the haplotypes derived from the Didessa River and its tributaries fall within Lineage 1. By contrast, the Tana haplotypes seem to form a subset of the total variation in Lineage 1, adding evidence that the Lake Tana species flock originates from a limited stock of riverine *B. intermedius*.

Similar to the East-African haplochromine monophyletic superflock (Nagl et al. 2000; Verheyen et al. 2003), among the barbs there is a striking discrepancy between molecular and morphological variation. On the one hand in Lake Tana the barbs diverged morphologically to the degree of clearly distinct species. At the molecular level, by contrast, the members of the flock display considerable trans-specifically shared haplotypes but no fixed substitutions differentiating the distinct morphological forms. Determining the age of the species flock by a molecular clock analysis is a trivial pursuit because of the likelihood that shared ancestral polymorphies remain in the populations studied. At best, we can determine the maximum age of the haplotypic variation in the lake. With a maximum sequence divergence of approximately 1% in Lineage 1 (fig 3) and an estimated sequence divergence of 1.3 to 3.3% per million years (Anabel et al. 2002 and references therein; Burridge and White 2000 and references therein) the haplotypes in Lineage 1 (and therefore the Tana species flock) seems not older than 770,000 to 300,000 years. This contrasts with Dixon et al. (1996), who found that lineages in the MHC class 2β could be up to 5 Ma old.

Other studies have shown that the age of divergence of mitochondrial lineages can be a poor estimate for the age of a species flock in recently formed (refilled) lakes. In Lake Victoria haplochromine cichlids, for instance, lineage diversification at 100,000 years ago (Nagle et al. 2000; Verheyen et al. 2003) is clearly in excess of the age of the (current) lake, estimated at approximately 15,000 years old (Johnson et al. 1996). Given the poor phylogenetic structure between Didessa River and Lake Tana haplotypes, it seems plausible that the haplotypic variation of Lake Tana did not originate after the lake emerged. The upper bound of 770,000 years BP, therefore, is likely to be an overestimation of the age of (the species flock in) Lake Tana.

There is circumstantial evidence that suggests that the fast radiation of ecomorphological diversity within Lake Tana's *Barbus* species flock occurred in the last 10,000-25,000 years. Firstly, East-Africa is prone to large drastic changes in climate and rainfall, resulting in frequent fluctuations in the lake's water levels in this region (Owen et al. 1990). In Lake Tanganyika (max depth 1400 m, average depth 570 m) water levels were 600 m below the current level approximately 25,000 years ago (Danley and Kocher 2001), while Lake Victoria (max depth 70 m, average depth 40 m) might have dried out completely around 15,000 years ago (Johnson et al. 1996). Compared to these other East African lakes, the saucer-shaped Lake Tana is extremely shallow (max depth 14 m, average depth 8 m) and it is therefore not unlikely that Lake Tana might have dried up once or more in recent history. Secondly, the lake might owe its present form to damming by a 50-km-long Quartenary basalt flow, which filled the exit channel of the Blue Nile river to a possible depth of 100m (Grabham and Black 1925; Jespen and Athearn 1961). The age of this lava flow is estimated to be some 10,000 B.P. (Grabham and Black 1925). However, according to Chorowicz et al. (1998) the morphology of Lake Tana is not consonant with a lava-dammed, flooded river valley. In his view the present-day morphology expresses a central focus of subsidence, despite fault reactivation and headward downcutting of the Blue Nile. Whether or not the lake was present before or after the blocking of the Nile outlet, the lava flow did create the waterfalls at Tissisat, isolating Lake Tana from the lower Nile drainage. The age and geological history of Lake Tana will hopefully be further clarified as of October 2003, when the University of Wales Aberystwyth will initiate their project entitled "Seismic survey of Lake Tana, northern Ethiopia, and the paleaohydrology of the Blue Nile".

Lake Tana's small, diploid barbs

Barbus pleurogramma and populations of *B. paludinosus* collected in Ethiopia show high sequence divergence and form distinct clades with a strong, probably geographical, signal (cf. Figs. 2, 3). *Barbus pleurogramma* was described by Boulenger in 1902 based on specimens from Lake Tana. *Barbus pleurogramma* is characterized by a strongly serrated dorsal spine, and is probably very much related (synonymus?) to the widespread African *B. paludinosus*.

The endemic pelagic, zooplanktivorous *B. tanapelagius*, which has only recently been described (de Graaf et al. 2003), apparently evolved recently from the benthic *B. humilis*. Such ecological splits, i.e. sympatric divergence of benthic and pelagic forms, appear to be common in radiations of lacustrine fish groups; as shown by the (1) existence of specific littoral and sublittoral-pelagic communities among the large *Barbus* species of Lake Tana (Chapter 4 and 8), (2) sympatric divergence of benthic and pelagic forms among west African ciclids in crater lakes (Schliewen et al. 1994, 2001), (3) divergence of sticklebacks in benthic and limnetic forms in several North American lakes (Schluter and McPhail 1993), and (4) macro-habitat divergence of several fish groups in temperate lakes such as lake whitefish, brown trout, rainbow smelts (reviewed by Schluter 1998) and arctic charr (Snorrason et al. 1989). *Barbus tanapelagius* represents, next to the endemic large barbs, an independent, second case of rapid adaptation to lacustrine conditions within one and the same lake.

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10

CHAPTER

Declining stocks of Lake Tana's endemic Barbus species flock (Pisces, Cyprinidae): natural variation or human impact?

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Abstract

The only remaining species flock of endemic, large cyprinid fishes is found in Lake Tana, Ethiopia. A monthly experimental trawl program was conducted in 1991-1993 and 1999-2001, sampling 12 stations distributed over three habitats differing in depth and distance to shore. The aim was to compare the total abundance, spatial distribution and proportion of juveniles of the most common *Barbus* species in the Bahar Dar Gulf between both periods. We found a sharp reduction (75%) in total abundance, both in number and biomass of the *Barbus* species and even more (90%) in the number of juveniles between the two periods. However, the spatial distribution of the different *Barbus* species over the three habitats had not changed. High natural variability in fish stocks might be expected in environmentally unstable lakes. Although strongly pulsed (seasonal), Lake Tana is a relative stable system. No major differences were found in abiotic parameters in 1990s that could have caused the dramatic changes in abundance. The most likely explanation is the negative impact of the motorised, commercial gillnet fishery targeting the spawning aggregations of these barbs. Especially the drastic decline in juveniles points towards serious recruitment over-fishing. The results stress the need for the immediate development of a sound management plan focussing on effort restrictions during the *Barbus* breeding season.

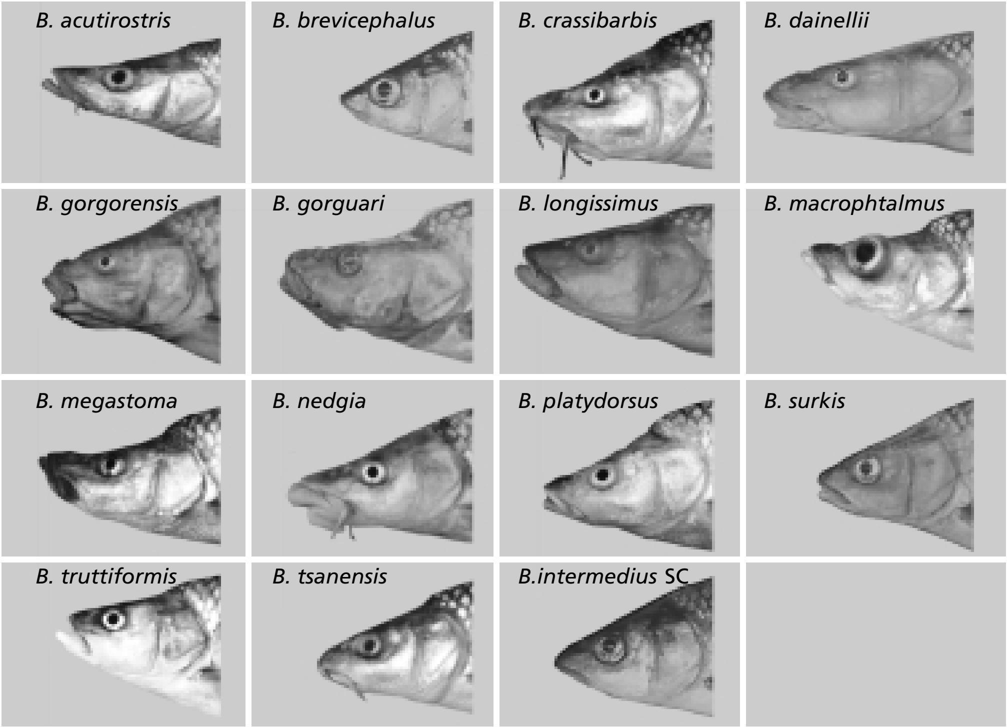

Figure 1. Heads of the 15 *Barbus* species of Lake Tana.

Introduction

Generally, the large cyprinids of Africa are riverine spawners, undertaking a single annual breeding migration up rivers (Tómasson et al. 1984). Although the vast majority of cyprinids primarily occur in rivers, some large *Barbus* and *Labeo* species are adapted to a lacustrine environment (Skelton et al. 1991). These lake-dwelling cyprinids only appear in the rivers for short periods to reproduce after forming large spawning aggregations in the river mouths.

Lake Tana, a large shallow lake in the northwestern highlands of Ethiopia, harbours the only known, intact species flock of large (max. 100 cm fork length), hexaploid cyprinids (Nagelkerke and Sibbing 2000; Fig. 1). The 15 *Barbus* species appear to have similar reproductive strategies as the other large African cyprinids. Peak spawning occurs at the end of the rainy season around August-September. At least eight species aggregate in river mouths and migrate up to 40 km upstream to spawn on gravel beds in clear, small and fast-flowing tributaries (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999; Chapter 6).

During this century, the vulnerability of Africa's large cyprinids for modern fisheries targeting these spawning aggregations in river mouths has been proven time and again with the collapse of several *Barbus* and *Labeo* fisheries throughout the continent. Intense overfishing of *Barbus altianalis* and *Labeo victorianus* in Lake Victoria, has reduced populations of cyprinids that were once abundant (Ogutu-Ohwayo 1990; Ochumba and Manyala 1992). The stocks of *Labeo mesops* in Lake Malawi and *Labeo altivelis* in Lake Mweru have declined drastically after the introduction of nylon netting and increased fishing pressures in the 1950s and 1960s (Skelton et al. 1991). In general, after the introduction of more efficient gillnets compared to traditional fishing gear, unregulated fisheries has had severe impact on the stocks of these riverine spawning, lake dwelling cyprinids. Gillnets were set near river mouths, effectively blocking them off from the lake, preventing mature individuals from reaching the upstream spawning areas.

Modernisation of Lake Tana's fishery did not occur until the end of the 1980s. Traditionally, the fisheries in Lake Tana consisted of a subsistence reed boat fishery. The fishermen, limited in their mobility, only had access to the shore areas, using locally made fish traps, hooks and small gillnets (15-20 m). In 1986 motorised boats and modern, nylon gillnets were introduced as part of the Lake Tana Fisheries Resources Development Program which was initiated by the Ethiopian Ministry of Agriculture, the Ethiopian Orthodox Church and two Dutch NGOs (ISE-Urk and ICCO-Zeist). This created new opportunities for the fishermen, extending their fishing area from the shore to deeper, offshore waters and, more importantly, to distant river mouths. The commercial gillnet fishery of Lake Tana developed rapidly, total catches increased from 39 MT in 1987 to 360 MT in 1997 (Wudneh 1998). The annual catch roughly consisted of one third *Barbus* species, one third *Oreochromis niloticus* (Cichlidae) and one third *Clarias gariepinus* (Clariidae) (Wudneh 1998).

However, the commercial gillnet fishery on *Barbus* is highly seasonal and mainly targets the spawning aggregations, as more than 50% of the annual catch is obtained in the river mouths during August and September (Nagelkerke et al. 1995; Wudneh 1998). During the 1990s *Barbus* catch per unit of effort (CpUE) of the commercial gillnet fishery decreased significantly from 63 kg/trip in 1991-1993 to

28 kg/trip in 2001 (Chapter 12). These characteristics of Lake Tana's gillnet fishery raises great concern about the sustainability of the fishery on the *Barbus* stocks as similar African cyprinid fisheries have collapsed.

The objectives of this study are to: (a) describe, quantify, and compare the abundance, distribution and proportion of juveniles of the dominant *Barbus* species in the Bahar Dar Gulf of Lake Tana between 1991-1993 and 1999-2001; (b) determine and discuss the possible effects of the commercial gillnet fishery and of the natural environmental variability on the development of the *Barbus* stocks during the 1990s; and (c) suggest management measures to be taken in order to ensure the sustainability of the commercial gillnet fishery and the *Barbus* diversity in Lake Tana.

Materials and Methods

Lake Tana

Lake Tana is a turbid, shallow lake (average depth 8 m, maximum depth 14 m), located at an altitude of 1830 m, in the northwestern highlands of Ethiopia. It is Ethiopia's largest lake, covering an area of c. 3,050 km^2 and the source of the Blue Nile. High waterfalls (40 m) at Tissisat ('smoking waters'), 30 km downstream from the Blue Nile outflow, effectively isolate the lake's ichtyofauna from the lower Nile basin (Fig. 2a).

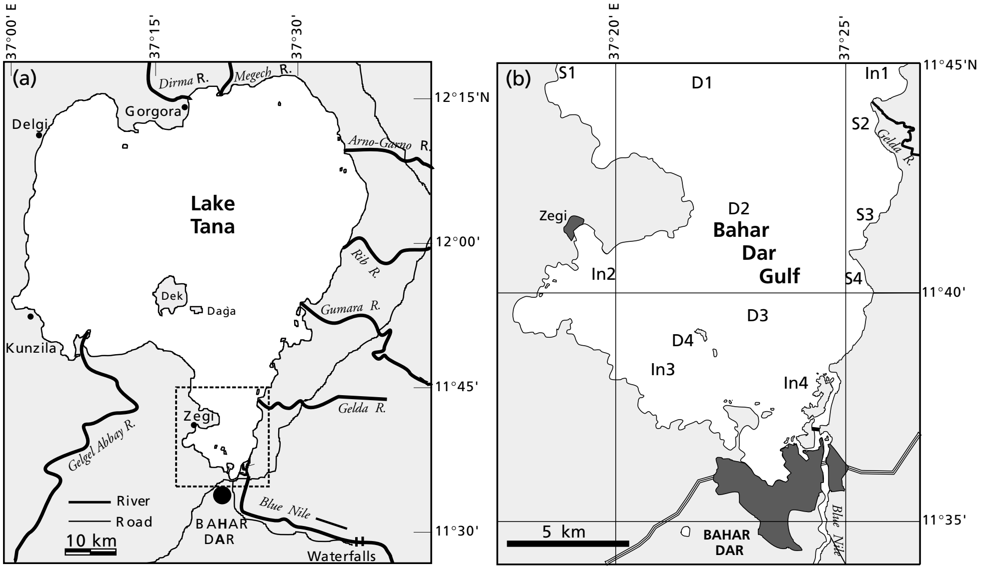

Figure 2. (a) Lake Tana and its main in- and outflowing rivers, and (b) the location of the sampling stations in the Bahar Dar Gulf. Habitat shallow (S1, S2, S3, S4), depth < 3 m, distance to shore < 100 m; habitat intermediate (In1, In2, In3, In4), depth 3-6 m, distance to shore 100-1000 m; habitat deep (D1, D2, D3, D4), depth 6-12 m, distance to shore 1000-5000 m.

Sampling program

A bottom trawl, made of multifilament twine with a ply ranging from 210/12 to 210/46, with a 15 m head rope and 17 m foot rope was used in the experimental fishing program. Mesh sizes ranged from 45 to 20 mm bar mesh at the codend. The trawl net had a vertical opening of 1-1.5 m and a horizontal opening of 7.5-8 m during operation. Trawling was conducted monthly on four consecutive mornings from ca. 7 am to 12 noon (three stations per morning) between September 1991 and August 1993 (91/93) and September 1999 and August 2001 (99/01). In the Bahar Dar Gulf three habitats (four sampling stations per habitat) were selected differing in depth and distance to the shore (see Fig. 2b). A 30 min trawl at 1 $m \cdot sec^{-1}$ was carried out at each of the twelve sampling stations. Note that the sampling stations, the boat, the bottom trawl and the crew in 91/93 and 99/01 were the same in order to maximise the comparability of the obtained data.

At the start of each trawl water depth (m), vertical transparency (Secchi disk, 20 cm diameter), and water temperature (°C) were recorded. Data on monthly rainfall in the Bahar Dar area between 1984 and 2001 were obtained from the National Meteorology Office, National Water Resources Department, Addis Ababa, Ethiopia.

The fish caught were sorted to species level immediately on deck, total weight per species was taken and fork length (FL) was measured individually to the nearest cm above. *Barbus brevicephalus*, *B. intermedius* 'shore-complex' (SC) and *B. tsanensis* were not distinguished in 91/93 due to lack of knowledge of the species diversity of the barbs and were therefore pooled, also in 99/01, during analysis and referred to as BrInTs.

Data analysis

The most important *Barbus* species in the trawl catches, composing around 95% in number and 90% in weight of the total *Barbus* catch in both sampling periods, were BrInTs and the piscivorous species *B. acutirostris*, *B. macrophtalmus* and *B. platydorsus*. The remaining nine *Barbus* species were only caught occasionally and were excluded from further study.

The CpUE per species [number/weight (kg)$\cdot$haul^{-1}] was analysed to determine (a) temporal variation in CpUE between the two sampling periods (91/93 and 99/01; Mann-Whitney U-test, $P<0.05$), and (b) spatial variation in CpUE between the three habitats within each sampling period (Shallow, Intermediate, Deep; Kruskal-Wallis test, $P<0.05$). Spatial variation was only analysed for the three piscivorous *Barbus* species.

Temporal variation in the proportion of juvenile (age range 0^+ - 2^+; Mina et al. 1996; Wudneh 1998), immature fish (<20 cm FL, based on minimum size at maturity; de Graaf et al. 2003) between the two periods (91/93 and 99/01) was calculated for each of the three piscivorous species using χ^2 *2x2* contingency table analysis.

Results

Abiotic parameters

Rainfall, lake water level, water temperature and vertical transparency all exhibited strong, seasonal patterns, which were highly similar between the two sampling periods (Fig. 3). In both periods, average rainfall peaked in July-August (Fig. 3a), followed by a rise in the water level in the lake, during September-October (Fig. 3b). Annual variation in water temperature showed two peaks in the Bahar Dar Gulf during 91/93 and 99/01. The first peak occurred at the end of the dry season in May and the second around October-November at the end of the wet season (Fig. 3c). During the rainy seasons (June-October) of 91/93 and 99/01, the vertical transparency decreased (Fig. 3d) due to the inflow of large amounts of silt by the rivers Gumara, Gelgel, Rib and Gelda (Fig. 2a).

The annual deviation (%) in rainfall from the 18 year (1984-2001) average (1363 mm) in Bahar Dar showed a cyclic trend of 4-5 years of relative high rainfall alternating with 4-5 years of relative low rainfall. Both sampling periods fell in relative wet periods that had started 1-2 years earlier.

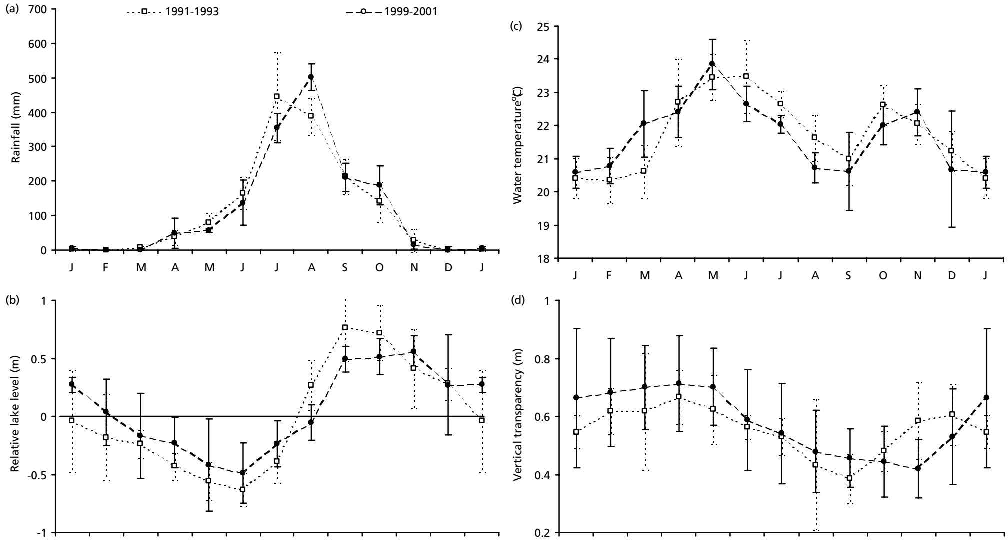

Figure 3. Monthly changes (mean ± SE) in (a) rainfall, (b) lake water level, (c) water temperature, and (d) vertical transparency (Secchi depth) in the Bahar Dar Gulf during 91/93 (dotted lines, open squares) and 99/01 (dotted lines, solid circles).

Abundance

Barbus acutirostris, *B. macrophtalmus*, *B. platydorsus* and BrInTs decreased highly significantly ($P<0.001$) in abundance (both in kg and in number per haul) in the Bahar Dar Gulf between the two sampling periods (Fig. 4). Furthermore, the CpUE of *B. acutirostris*, *B. macrophtalmus*, *B. platydorsus* and BrInTs decreased in similar proportions, on average 75% (67% - 86%) in biomass and on average 80% (71% - 84%) in number.

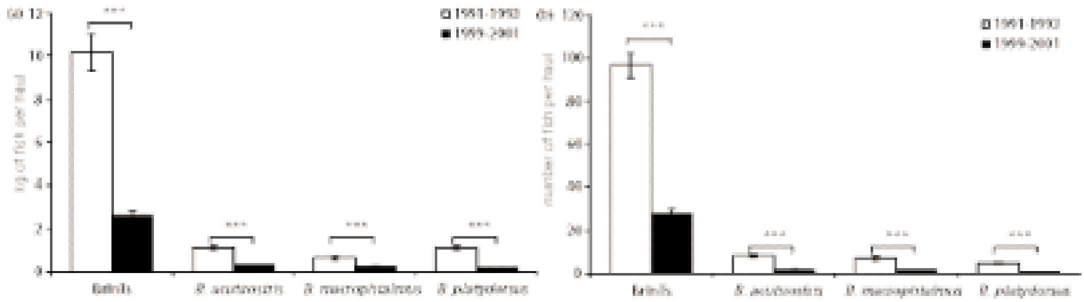

Figure 4. (a) Weight and (b) number of *Barbus* per haul (mean ± SE) during 91/93 (white bars) and 99/01 (black bars). Three asterisks indicate significance at the 0.001 level (Mann-Whitney U-test; n1 = 288, n2 = 288).

Spatial distribution

The drastic decrease in abundance had little impact on the spatial distribution of the three *Barbus* species in the Bahar Dar Gulf (Fig. 5). *Barbus macrophtalmus* and *B. platydorsus* predominantly occur in the deeper, offshore waters during both sampling periods. *Barbus acutirostris* is more widely distributed throughout the Bahar Dar Gulf and is the only species of the three that regularly occurs in the shallow, littoral zone. This distribution pattern is especially clear during 1999-2001 when *B. acutirostris* is evenly distributed over the three habitats (Figs. 5c and 5d).

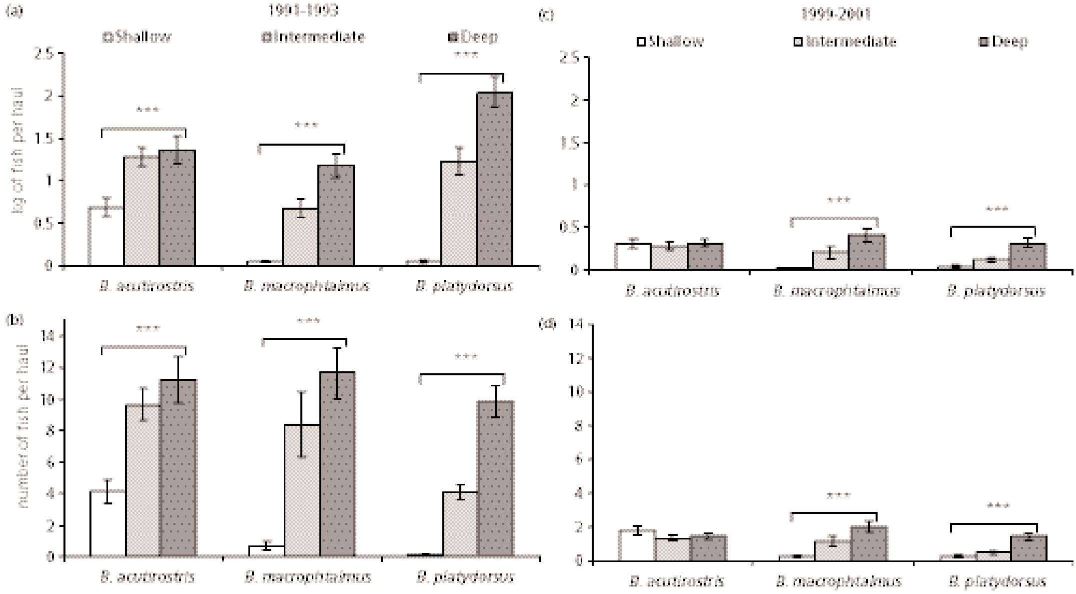

Figure 5. Distribution of *B. acutirostris, B. macrophtalmus* and *B. platydorsus* over the three habitats, Shallow (white bars), Intermediate (light grey bars) and Deep (dark grey bars) during the two sampling periods (mean ± SE), (a) weight 91/93, (b) number 91/93, (c) weight 99/01, and (d) number 99/01. Three asterisks indicate significance at the 0.001 level (Kruskal-Wallis test; n = 288, df = 2). Note different scales on the Y-axis in 91/93 and 99/01.

Proportion of juveniles

The large decrease in overall population size of the different *Barbus* species did not occur equally over the different size classes. In general, the length frequency distribution of the different *Barbus* species caught during the experimental trawl program has changed markedly, from being dominated by juveniles (≤20 cm FL) in 91/93 to being dominated by (sub) adults (>20 cm FL) in 99/01 (Fig. 6). This change in the proportion of juveniles between the two periods is highly significant ($P<0.001$) in all three piscivores, *B. acutirostris*, *B. macrophtalmus* and *B. platydorsus* (Table 1). The proportion of juvenile, immature *B. acutirostris*, *B. macrophtalmus* and *B. platydorsus* was reduced from 57% to 21%, 77% to 49%, and 56% to 37%, respectively between 91/93 and 99/01 (Fig. 6d). The changes in juvenile/adult ratio suggest recruitment limitation of these *Barbus* species in the Bahar Dar Gulf in 99/01.

Table 1. Results of the χ^2 *2x2* contingency table analysis testing the hypothesis that the proportion of juveniles is independent of the sampling period. Juv. = juvenile fish; <20 cm FL, age range 0^+ - 2^+; ad. = adult fish, >20 cm FL; *P* = significance level: ***= *P*<0.001.

		1991-1993 Number	1999-2001 Number	χ^2	P
B. acutirostris	juv.	1363	93	190.7	***
	ad.	1038	349		
B. macrophtalmus	juv.	1530	163	109.2	***
	ad.	458	167		
B. platydorsus	juv.	749	77	26.1	***
	ad.	599	133		

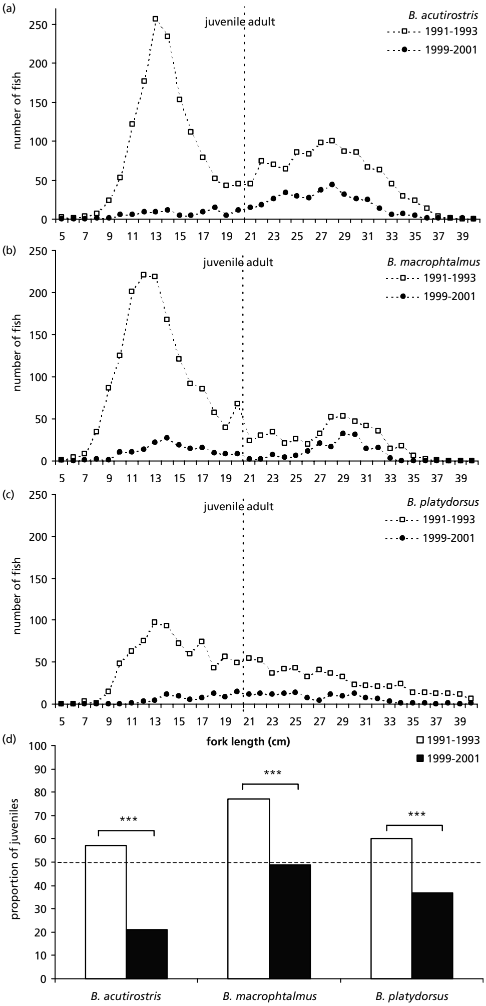

Figure 6. Length frequency distribution of (a) *B. acutirostris*, (b) *B. macrophtalmus,* (c) *B. platydorsus*, in 91/93 (dotted line, open squares) compared to 99/01 (dotted line, solid circles), and (d) proportion of juveniles (91/93 white bars, 99/01 black bars). Three asterisks indicate significance at the 0.001 level (χ^2 *2X2* contingency table analysis, Table 1).

Discussion

Natural environmental variability

Lake Tana's hydrological characteristics such as a high flushing time (6.1 year; Wudneh 1998 and references therein), small average depth (8 m; Wudneh 1998 and references therein), high seasonal changes in water level (high RLLF[s], Table 2) and a year round mixed water column, categorise it as a seasonally pulsed system, an allotrophic riverine lake (Kolding 1994; Jul-Larsen et al. 2003). In such water bodies biological productivity is mainly externally driven by nutrient inputs through seasonal fluctuations in water level and river inflow, including flooding of the lake margins and floodplains (Jul-Larsen et al. 2003). The influx of nutrients is often unpredictable and variable between years (high RLLF[a]) thus creating unstable environments. In pulsed, unstable systems like Lake Mweru, Lake Chilwa and Lake Bangweulu (Table 2) biological productivity is highly variable by nature. Their fish communities are dominated by ecologically flexible, unspecialised species with *r*-selected life histories. They live under highly variable abiotic conditions resulting in fluctuating and unpredictable natural mortality, and high inter-annual variability in biomass (Jul-Larsen et al. 2003 and references therein).

In Lake Tana, however, the variation of the annual influx of nutrients was low from year to year between 1980-2001 ((low RLLF[a]), Table 2) and the timing was highly predictable. Lake levels always peaked in September-October. Lake Tana can therefore be considered, at least for the period under consideration, as a stable environment despite the fact that nutrient influx is externally driven. On the African continent Lake Tangayika, Lake Malawi and Lake Victoria are examples of other stable systems. However, the biological production in these lakes is largely driven by an internal supply of nutrients through vertical mixing. The fish faunas of lakes with constant abiotic conditions and internal energy pathways are characterised by a high biodiversity, high number of ecologically specialised endemics, *K*-selected life history, and stable and predictable natural mortality. Lake Tana's fish fauna consists partly of resilient, flexible species like *O. niloticus*, *C. gariepinus* and the short-lived, small-sized (max 10 cm FL) diploid cyprinids, *B. humilis* (benthic) and *B. tanapelagius* (pelagic) (de Graaf et al. 2000ab; Dejen et al. 2002). However, the large *Barbus* are a species-rich, endemic, and ecologically specialised group of fish more characteristic of stable systems like the endemic cichlid species flocks of Lake Victoria, Lake Tanganyika and Lake Malawi (Nagelkerke et al. 1994; Sibbing and Nagelkerke 2001; Jul-Larsen et al. 2003; Chapter 4). Generalised implications for fisheries exploiting ecologically specialised fish, such as Lake Tana's *Barbus* species flock, are that these fish populations have a low regenerative capacity, high susceptibility to increased fishing mortality but low inter-annual natural variability in biomass and hence catches (Jul-Larsen et al. 2003 and references therein). The fact that Lake Tana is a strongly pulsed but relatively stable system with an expected small inter-annual natural variability in biological production makes it easier to relate trends in fish stocks to fishing activities. The large reduction in *Barbus* stocks in the Bahar Dar Gulf observed during the 1990s (Fig. 4) is therefore unlikely to be the result of natural variability driven by water level and river inflow.

Table 2. Differences in seasonal [s] and inter-annual [a] Relative Lake Level Fluctuations (RLLF) among several African lakes.

	S (km^2)	D (m)	FT (yrs)	Water Level Change (m)		Relative Lake Level Fluctuation		Lake Characteristics	
				Seasonal ΔWL [s]	Inter-annual ΔWL [a]	Seasonal RLLF[s]	Inter-annual RLLF[a]	Seasonal Pulse	Inter-annual Stability
Tana	3100	8	6	1.44	0.08	18.00	1.00	+	+
Tangayika	32600	580	7000	0.78	0.22	0.14	0.04	--	++
Malawi	28800	290	750	0.97	0.28	0.30	0.10	-	+
Victoria	68800	40	140	0.44	0.22	1.10	0.60	-	+
Chilwa	680	3	-	1.19	0.53	39.70	17.80	++	--
Bangweulu	2733	3.5	-	1.20	0.26	34.30	7.40	++	-
Mweru	4650	8	4	2.05	0.58	25.70	7.20	+	-

S = surface, D = mean depth, FT = flushing time, ΔWL = mean change in water level (m); RLLF[s]or[a] = ΔWL [s]or[a] / mean depth * 100 (data Lake Tana (1980-2001) from Wudneh (1998) and references therein, Nagelkerke and Sibbing (1996), Fig. 3b and National Meteorology Office, National Water Resources Department, Addis Ababa, Ethiopia; data other African lakes from Jul-Larssen et al., (2003) and references therein).

Development of commercial gillnet fishery

All fish faunas contain species that are more susceptible to human exploitation than others. In Lake Tana's fish community *O. niloticus* and *C. gariepinus* can be considered as resilient species while as *Barbus* species have to be categorised as highly susceptible (Jul-Larssen et al. 2003; Chapter 12). The first two species are both widely distributed and are rather unspecialised ecologically flexible species that reproduce in the safety of heavily vegetated floodplains. On the other hand the large *Barbus* species are largely specialised endemics and at least half of the species form aggregations in river mouths and migrate upstream to spawning grounds (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999), rendering them vulnerable to over-exploitation especially during their breeding period. The most likely explanation for the decrease in the *Barbus* stocks is not due to natural environmental variation or the traditional, predominantly subsistence, fisheries but the development and modernisation of the commercial gillnet fishery in the Bahar Dar Gulf since the late 1980s.

Traditionally most Ethiopians are no fish eaters. Fish is not highly valued as a source of cheap protein and mainly eaten during fasting. The local markets around Lake Tana are sufficiently supplied with fish by the artisanal reed boat fishery and the seasonal riverine fishery. The ca. 400 papyrus reed boats are restricted to the littoral zones near the few towns and villages that border Lake Tana's shores and target mainly *O. niloticus* using small gillnets or basket traps placed among the vegetation in the floodplains (Wudneh 1998). Between July and October farmers traditionally catch *Barbus* on the upstream spawning grounds using a variety of fishing techniques such as barriers, basket traps, hooks, scoop nets and even poisoning of the shallow water upstream using the dried and crushed seeds of the berberra tree (*Milletia ferruginea*, Leguminosae) (Nagelkerke and Sibbing 1996). This type of seasonal fishing on migrating barbs has taken place for at least 200 years, and most probably for hundreds of years more, as fishing with poison was already observed and described around 1770 by the Scottish explorer James Bruce during his travels around Lake Tana. Unfortunately no data are available on the impact of this traditional, seasonal riverine fisheries on the *Barbus* stocks. In Lake Victoria a similar indigenous cyprinid fishery, using barriers and basket traps along the rivers, did not have a particular deleterious effect on the *Labeo* and *Barbus* stocks (Ogutu-Ohwayo 1990). Overall the characteristics of the traditional fisheries in Lake Tana imply that they are mostly on subsistence level.

In the last few decades a demand for fish was created in the capital city Addis Ababa by foreigners and upper and middle class Ethiopians (Reyntjes et al. 1998). Since 1986 the development of the motorised gillnet fishery in Lake Tana has considerably benefited from external assistance by NGOs (Interchurch Foundation Ethiopia, Interchurch Organisation for Development Cooperation) and the EU sponsored Lake Fisheries Development Projects. During the 1990s, each motorised boat carried around 20 gillnets (10 cm stretched mesh; length 50 m) and 5-7 boats were active daily on the fishing areas. Almost all fish landed by the motorised fishery are purchased by the Fish Production and Marketing Enterprise, processed and transported to the capital. Addis Ababa is the single biggest market for Lake Tana's commercial gillnet fishery, the local markets around the lake are, as was in the past, supplied with fish from the traditional reed boat and riverine fisheries.

Comparing the reproductive biology of *Barbus* spp (time and place of spawning; Fig. 7ab) and spatial-temporal patterns of catch and effort allocation by the commercial gillnet fishery (Fig. 7cd) demonstrates that most landed *Barbus* are caught during the annual mass spawning run. Gonad development of 14 of the *Barbus* spp peaks in July-September (Fig. 7a). The ripe barbs aggregate in the river mouths (Fig. 7b) before migrating towards shallow gravel beds in the upstream areas of the major affluent rivers. The catches of the commercial gillnet fishery peak in the breeding season (Jul-Sep; Fig. 7c). Gumara River is one of the main spawning areas for Lake Tana's *Barbus* and the highest catches of the commercial gillnet fishery come from the mouth of this river (Fig. 7d). Most *Barbus* species are only vulnerable to the commercial gillnet fishery when aggregating in the river mouths during the peak spawning period (Fig. 7e).

The marked reduction in the proportion of juvenile fish of the three piscivorous barbs (Fig. 6) clearly demonstrates the expected negative consequences of a fishery targeting spawning aggregations, i.e. a disturbance of the reproductive process resulting in a dramatic decrease in the number of recruits (Craig 1992; Gabriel et al. 1989; Regier et al. 1999). The sensitivity of cyprinids for a modern, motorised gillnet fishery targeting spawning aggregations is best illustrated by the disappearance of *Labeo* altivelis (mpumbu) from the Mweru-Luapula system within a 20 year period (Gordon 2002). Prior to 1947 traditional fishermen caught *Labeo altivelis* as the fish spawned in the river after flooding in February-March. A new fishery using motorised boats and gillnets started operating in the lagoons where the Luapula River entered Lake Mweru. This expatriate (mostly Greeks) fishery targeted the spawning aggregations of *L. altivelis* in the river mouth and effectively blocked the entrance of the Luapula river. Already in 1949, after huge catches in 1948, *L. altivelis* failed to spawn. Conservation measures were misdirected and discriminated against traditional, riverine fishermen, not the new lake fishery. Not surprisingly, the conservation measures did not prevent the end of the spawning run in the 1950s and *L. altivelis* disappeared from Mweru-Luapula by the early 1970s.

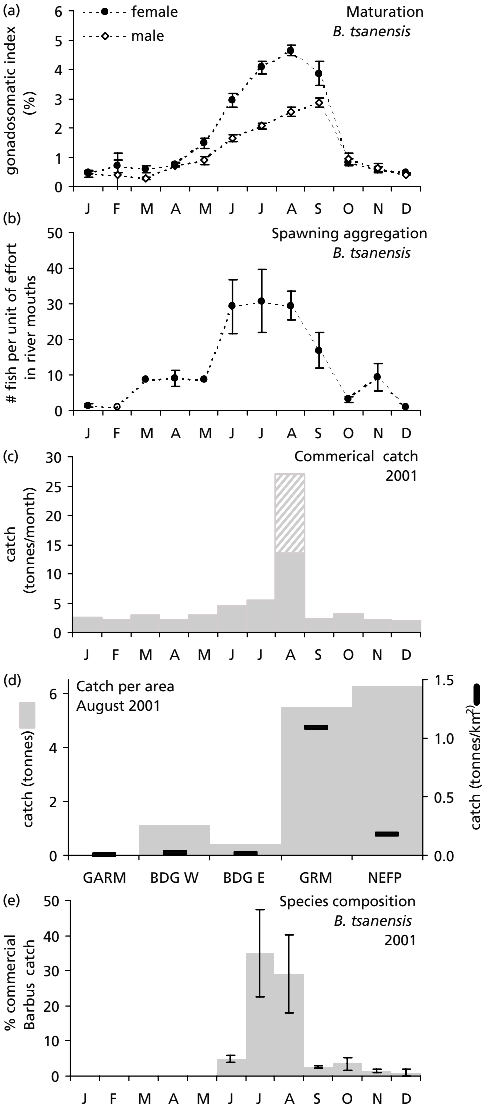

Figure 7. Comparison of spawning patterns of *Barbus* and variation in catch and effort allocation of the commercial gillnet fishery. (a) Monthly gonad development of male and female *B. tsanensis*, a common riverine spawning species, (b) Aggregation of ripe *B. tsanensis* in the river mouths of the four main inflowing rivers during the breeding season, (c) Monthly *Barbus* catch (grey bars, tonnes) of the commercial gillnet fishery in 2001. Hatched area indicates predicted total catch for September if commercial gillnet fishery had not been closed for two weeks; monthly landings peaked in August, (d) Spatial variation in *Barbus* catch (grey bars, tonnes per fishing area; black marks, tonnes per km^2 fishing area) of the commercial gillnet fishery during August 2001; GARM = Gelgel Abbay river mouth (5 km^2), BDG W = Bahar Dar Gulf west coast (50 km^2), BDG E = Bahar Dar Gulf east coast (50 km^2), GRM = Gumara river mouth (5 km^2), NEFP = northeastern floodplains (35 km^2). Note that almost half of the catch comes from Gumara river mouth, this fishing area gives by far the highest yield of *Barbus* per km^2, and (e) Monthly abundance of *B. tsanensis* in the catch of the commercial gillnet fishery; no data for January-May. Error bars indicate standard error. Data Chapter 8 (fig. 7ab) and Chapter 12 (fig. 7cde).

Fisheries management

The drastic changes in overall abundance and especially the sharp decrease in number of recruits of Lake Tana's unique *Barbus* species in just over a decade, stress the necessity of the urgent development and implementation of fisheries regulations. The present results are in sharp contrast with Wudneh (1998) who predicted in the early 1990s that exploitation levels could be increased five to six times. However, two presumed conditions in his simulation to determine maximum sustainable yield were not fulfilled the model, (1) assumed undisturbed and constant reproduction and recruitment, and (2) did not include spatial and temporal variation in fishing effort.

The *Barbus* species flock is a unique source of freshwater biodiversity in Ethiopia. Lake Tana's barbs are worth conserving for future generations, not only for their scientific potential, but more importantly as a cheap source of protein for the local people. An impoverishment of Lake Tana's biodiversity might result in a lower production and a reduced resilience against environmental fluctuations (Loreau et al. 2001 and references therein; Tilman et al. 2001).

Size control regulations, such as an introduction of a minimum mesh size for the commercial gillnet fishery, will have little positive effect on the development of *Barbus* stocks (de Graaf et al. 2003). The most simple, effective and easily controlled measure to protect the reproductively active part of the *Barbus* populations is the restriction of fishing effort around the spawning areas and/or during the spawning season. Such a measure will ensure future recruitment.

An important characteristic of both the commercial gillnet fishery and the riverine fishery is that the catch is landed centrally. The commercial gillnet fleet is stationed in Bahar Dar but operates mainly on distant fishing areas (40-60 km) along the eastern shores. The fishermen stay on the fishing sites continuously for five nights (Monday-Friday) before returning to Bahar Dar. All sell their fish to the same fish buyer who collects the fish daily on the fishing sites and transports the entire catch back to Bahar Dar for further processing. The seasonal riverine fisheries are concentrated on the rivers Gumara and Gelda. The vast majority of the daily catch is offered for sale on the markets of the two nearby small towns.

Several options are available to sustain the commercial gillnet fishery:

(a) closure of fishing around river mouths and upstream rivers in August-September but allow unrestricted fishing in other areas
(b) similar to (a), but closure only every other week
(c) closure of fishing throughout the lake and upstream rivers during August-September
(d) similar to (c), but closure only every other week

Ideally, only limited spatial restrictions need to be implemented in order to protect the spawning aggregations of the *Barbus* species, i.e. closing of fishing near river mouths, surrounding floodplains and upstream spawning sites. Another beneficial effect of spatial restrictions is the limited reduction in income of the fishermen when fishing outside the restricted areas is allowed. Options (a) and (b) have therefore, the least impact on the income of the fishermen but are the most difficult to control by the regional government, especially when these institutions face a chronic lack of manpower, material and budget. It is currently not possible for the local authorities to control the fishermen on the lake or to determine the origin of the fish when landed. Therefore, proposed (temporal) closures of fishing have to be enforced for the whole lake and can not be limited to specific areas. Options (c) and (d) are easy to control, although, option (c) may be too drastic for the fishermen to accept as they potentially lose a considerable part of income. Option (d) seems the best compromise for several reasons. In the first place it will be easy and cheap to implement, enforce and control, as the catches of the commercial gillnet fleet and the riverine fisheries are landed centrally. Furthermore, it will not create a total loss of income for the fishermen during the breeding season and, most importantly, it will result in a significant reduction of fishing pressure on the migrating barbs.

Interestingly, "option (d)" has already been put into practice during the spawning season of 2001. The only fish buyer who collects daily the catch of the commercial gillnet fleet closed down his operation every other week during August and September to prevent being flooded with barbs which are less favoured for consumption than *O. niloticus*. This measure effectively closed down the commercial gillnet fleet, as there are no alternative fish buyers.

In the past no management plan has been developed because of lack of, (1) federal fisheries legislation, (2) data on the characteristics of both the fish stocks (size at maturity, spawning period, spawning areas) and the commercial gillnet fishery (gillnet selectivity, temporal/spatial variation in effort allocation), and (3) knowledge dissemination, i.e. information is published in English in international scientific journals, hence less accessible for local experts, civil servants and policy makers. Recently (January 2003) Ethiopia's first fisheries legislation was approved by the federal government. To anticipate on this positive development and in co-operation with the Amhara Region Agricultural Research Institute, several activities are being planned to disseminate the wealth of knowledge on Lake Tana's fish and fisheries that has been generated in recent years. A workshop is planned for the fall of 2003 to develop management and research strategies with the participation of policy makers, researchers and private stakeholders. At the beginning of 2004 a book will be published on BSc level and in the local language Amharic, containing basic data on the fish stocks and fisheries collected in the last decade and the research and management strategies formulated at the workshop. Continuous monitoring of commercial catches, and the regular independent experimental sampling programs, are of utmost importance to determine the condition of the stocks and to evaluate the consequences of implemented regulations.

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11

CHAPTER

Length at maturity and gillnet selectivity of Lake Tana's Barbus species (Ethiopia): implications for management and conservation.

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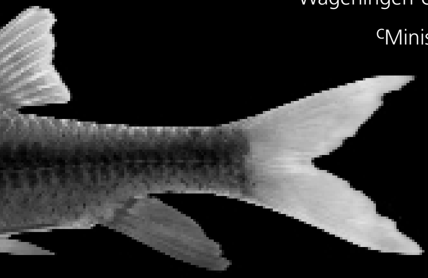

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Abstract

Lake Tana's 15 large *Barbus* species form the only known intact endemic cyprinid species flock left in the world. The barbs contribute around one third of the total annual catch of the motorised commercial gillnet fishery which was introduced in 1986. A dramatic reduction in the adult *Barbus* stocks and the even lower proportion of recruits at the end of the 1990s, stress the necessity of the development, implementation and control of fisheries legislation in Lake Tana. The reproductive biology of the *Barbus* species, essential for fishery management, is poorly known. This paper presents results on size at maturity, size at harvest and gillnet selectivity curves, which can be used to provide a scientific base for management proposals. Size at maturity varied widely among the *Barbus* species, ranging from 18.8 cm FL in *B. brevicephalus* to 44.3 cm FL in *B. crassibarbis*. Males matured at a smaller size and reached a smaller maximum length than females. Estimated selectivity curves fitted closely or were slightly larger than the observed length-frequency distribution of the commercial catch. The vast majority (85%) of barbs landed by the commercial gillnet fishery were mature. Fishing pressure on juvenile, immature fish is unlikely to be the cause of the observed decrease in *Barbus* stocks. Size control regulations like mesh size restrictions, intending to protect the immature part of fish populations are expected to have little positive effects on the *Barbus* stocks and are therefore not recommended. The drastic reduction in barbs during the 1990s is most like due to recruitment overfishing, i.e. poorly regulated high fishing effort by the commercial gillnet fishery on the spawning aggregations of adult barbs during their annual breeding migration in river mouths and surrounding floodplains. Only effort control regulations limiting the gillnet fishery in the spawning season and/or area will prevent a total collapse of the *Barbus* stocks as has happened to other cyprinids in African lakes. Such measures have to be implemented urgently in order to guarantee the conservation of Lake Tana's unique biodiversity as a sustainable source of cheap protein and a natural laboratory to study the evolutionairy processes underlying speciation in freshwater fish.

Introduction

Experimental trawling conducted at 12 stations both in 1991-1993 and 1999-2001 showed a sharp decline (up to 70%) in abundance of the endemic *Barbus* species (Cyprinidae) in the Bahar Dar Gulf of Lake Tana over the last decade (de Graaf et al. 2003). This severe reduction of the *Barbus* stock and the concerns of local fishermen claiming decreased catches in the Bahar Dar Gulf, stress the necessity of the development, implementation and control of fisheries legislation in Lake Tana.

Lake Tana, the source of the Blue Nile, is situated at an altitude of 1830 m, in the north-western highlands of Ethiopia. The meso-oligotrophic, shallow lake (average depth 8 m, maximum depth 14 m) covers an area of c. 3,050 km^2 and is by far Ethiopia's largest lake, containing half the country's fresh water supply. The lake's ichtyofauna is effectively isolated from the lower Nile basin by 40 m high waterfalls, at Tissisat ('smoking waters'), 30 km downstream from the Blue Nile outflow.

Traditionally, the fisheries in Lake Tana consisted of a low impact subsistence fishery, operated from reedboats by the Woito people (ethnic minority) and limited to the shore areas, using locally made fish traps, hooks, fishing spears and small gillnets (length 15-20 m). The introduction of motorised boats and longer, more efficient, nylon gillnets in 1986 offered new opportunities for the fishermen. Most interestingly not the Woito people, the traditional fishing community around Lake Tana, benefited from this development but a new group of Amharic (ethnic majority) fishermen started operating. Their fishing area was extended to deeper, offshore waters and to distant river mouths (Wudneh 1998; Chapter 12). Total catches of the commercial gilllnet fishery increased rapidly from 39 MT in 1987 to 360 MT in 1997 (LFDP 1997; Wudneh 1998). The *Barbus* species, *Oreochromis niloticus* (Cichlidae) and *Clarias gariepinus* (Clariidae) each form around a third of the catch.

The large, hexaploid barbs of Lake Tana form, as far as we know, the only remaining intact species flock of large cyprinid fishes in the world. The latest revision of these unique barbs was recently completed, which resulted in the distinction of 15 *Barbus* species, eight of which were new to science (Nagelkerke and Sibbing 1997, 2000). Although the taxonomy of the large barbs of Lake Tana has received considerable attention of ichthyologists for the last century (Boulenger 1911; Bini 1940; Bannister 1973; Nagelkerke et al. 1994; Mina et al. 1996; Nagelkerke and Sibbing 2000; Sibbing and Nagelkerke 2001), many questions on their reproductive biology are still not answered. Detailed information on gonad development, size at maturity, spawning area, and spawning period of each of the 15 species is scarce, fragmented and sometimes unreliable due to limited sampling, misidentification or lumping of species (Nagelkerke and Sibbing 1996; Wudneh 1998; Dgebuadze et al. 1999). Information on the reproductive characteristics of the *Barbus* species is, however, required in order to advise on management measures to protect the reproductively active part of the population. Such measures could involve the introduction of a minimum mesh size and closed seasons and/or areas. Proper fisheries management requires that fishing gears are used which harvest the large mature fish while allowing the small juveniles to escape (Armstrong et al. 1990), i.e. size at harvest is larger than size at maturity. If the length at first harvest is correctly determined, a stock is more likely to be sustained and the yield optimized.

This paper is part of a larger study to reconstruct the development of the *Barbus* stocks and related fisheries since the 1980s and to compare the characteristics (selectivity, allocation of effort) of the commercial gillnet fishery with the life history traits of the different *Barbus* species. Here we focus on size at maturity of the barbs and size selectivity of gillnets used by Lake Tana's commercial fishery in order to provide a scientific base for advice on mesh size regulations. The results of this study will also provide essential data for gaining insight in the evolutionary processes underlying speciation within Lake Tana *Barbus*. The actual differences in reproductive characteristics and spawning patterns help to explain if and how mechanisms that prevent interbreeding have evolved within the species flock.

Materials and Methods

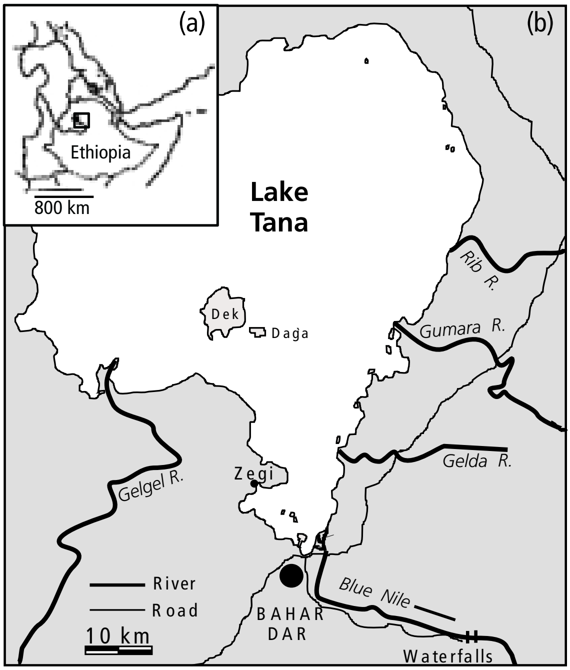

Figure 1. (a) Location of Ethiopia in the Horn of Africa, and (b) Lake Tana and its main in- and outflowing rivers.

Length at maturity

Throughout 1999-2001, monthly experimental gillnet and trawl programs, using a variety of mesh sizes (3.2, 4.4, 6, 8, 10, 12 cm stretched mesh), were conducted for sampling the southern Bahar Dar Gulf and the mouths of the four permanent contributing rivers (Fig. 1). More than 30,000 fish were analysed in the laboratory to determine the reproductive characteristics for each of the 15 *Barbus* species. Fork length (0.1 cm), total weight (0.1 g) and gonad weight (0.01 g) were measured and the gonad developmental stage (1-7) according to De Silva et al (1985) and Pet et al (1996) was determined for each fish. Females and males were considered sexually mature if they had reached gonad developmental stage 4 or higher. The mean fork length at first maturity ($FL_{50\%}$) was defined as the fork length at which 50% of all the females (or males) were sexually mature. Only fish collected in the three peak spawning months of 1999-2001 were used for the analysis (July-September, *B. intermedius* "shore-complex" (SC), *B. dainellii*, *B. longissimus*, *B. megastoma*, *B. truttiformis*, *B. tsanensis*; August-October, *B. acutirostris*, *B. brevicephalus*, *B. crassibarbis*, *B. gorgorensis*, *B. gorguari*, *B. macrophtalmus*, *B. nedgia*, *B. platydorsus*; November-January, *B. surkis;* Chapter 8).

Mature-immature data were analysed for each *Barbus* species and sex separately using logit regression (Ter Braak and Loman 1995). In the generalized linear model, the response variable p_i was defined as:

p_i = n_i(mature)/(n_i(immature)+n_i(mature))

Where: p = response variable (fraction mature fish); n = number of fish; i = fork length

The response variable p_i has a binominal distribution (Sokal and Rolf 1995). The expected value of the response variable (p) was linked to the linear predictor by a logit function g(M):

$g(M) = \ln(p_i/1-p_i)$

Where: g(M) = expected value of mature
fish and M=overall mean.

The linear predictor for the expected fraction of mature fish was defined using the following model:

$g(M) = M + fl_i$

Where fl_i = effect of i^{th} fork length

This procedure was implemented using PROC GENMOD of the SAS software package (SAS Institute Inc., 1990). 95% Confidence limits of the fitted value were calculated as described in the GENMOD procedure manual.

Gillnet selectivity

Gillnet selectivity can be predicted from measurements of a species' head girth and maximum girth if gilling and wedging of the fish are the main ways of capture upon swimming into the net. Theoretical selectivity curves were derived by Sechin (1969) and Kawamura (1972) assuming that (1) all fish are captured whose head girth is smaller, but maximum girth is greater than the mesh perimeter and (2) girth among any particular size class of fish is distributed normally, with a common variance for all length classes. In this study we used an extended Sechin model as described by Pet et al (1995). In this model a constant coefficient of variation in body girth was assumed. Optimum fork length (FL_{opt}) was defined as the length of a fish (FL) predicted to be retained most efficiently in a 10 cm stretched mesh gillnet.

In 1991-1993 Wudneh (1998) estimated girth-length relationships for *O. niloticus*, *C. gariepinus* and most of the *Barbus* species from an experimental trawl program in the southern Bahar Dar Gulf (Table 1). Fork length and girth circumference (maximum, head) were measured to the nearest mm for the individual fish. Not enough girth data were collected to estimate accurate selectivity curves for *B. crassibarbis* and *B. dainellii*. *Barbus brevicephalus* is a small-sized species (max. 25 cm FL) not retained in the gillnets of the commercial fishermen that use 10 cm stretched mesh. It was therefore not analysed. The girth data of *B. tsanensis* and *B. intermedius* SC were pooled, due to identification problems in 1991-1993.

Barbus species composition

Data on species composition of the different *Barbus* species and length-frequency distribution of the different *Barbus* species, *O. niloticus* and *C. gariepinus* in the catch of the commercial gillnet fishery were collected monthly from June 2001 to December 2001. On three consecutive days per month all *Barbus* and a sample of around 300 *O. niloticus* and 200 *C. gariepinus* landed by the motorised boats in Bahar Dar were identified and measured to the nearest 0.5 cm. Roughly 80% of the total annual *Barbus* catch is landed during these seven months (Wudneh 1998; de Graaf et al. 2003; Chapter 12). Additional data on length-frequency distribution for the *Barbus* species were taken from an experimental gillnet program in the period October 1999-September 2001 (Chapter 4). Only fish caught in 10 cm stretched mesh gillnets of the experimental gillnet program were used for the assessment of gillnet retention, as this is the same mesh size used by commercial fishermen. Modal fork length (FL_{mode}) was defined as the length of a fish (FL) observed to be retained most efficiently in a 10 cm stretched mesh gillnet.

Table 1. Sample size (n) and size range (minimum, maximum cm FL) used for girth measurements and calculation of the selectivity curves for each species in 1991-1993 and the sample size (n) of the specimens used to determine the length-frequency distribution of each species in 10 cm stretched mesh gillnets. Note that girth measurements of *B. intermedius* SC and *B. tsanensis* were pooled in 1991-1993 for calculation of the selectivity curves.

	Estimated selectivity curves			**Observed L-F distribution**
	n	min FL	max FL	n
B. acutirostris	415	10	46	567
B. crassibarbis	-	-	-	97
B. dainellii	-	-	-	43
B. gorguari	131	12	59	79
B. gorgorensis	67	18	59	287
B. longissimus	78	9.7	39	142
B. macrophtalmus	265	8	46	807
B. megastoma	114	8.5	77	912
B. nedgia	104	10	56	163
B. platydorsus	277	9.5	55	750
B. surkis	35	9.6	35.5	450
B. truttiformis	131	10	35.5	797
B. tsanenis	-	-	-	1449
B. intermedius SC	-	-	-	2640
B. intermedius SC/tsanensis	1004	5	36	-

Results

Length at maturity

The relation between the proportion of mature fish (≥ stage 4) and the fork length (cm) of the 15 *Barbus* species is given in Figure 2 and Table 2. In general, male *Barbus* mature at a smaller length and attain a smaller maximum length than females. No reliable maturity curves could be estimated for the males of some species. This was probably caused by a lack of small males in the sample (*B. brevicephalus*), a small overall sample size (*B. gorgorensis*, *B. gorguari*, *B. longissimus*, *B. surkis*) and lack of a short peak spawning season (*B. intermedius* SC). The female maturity data is more complete over the whole size range for each species. The estimated $FL_{50\%}$ differs widely between the females of the different *Barbus* species from as small as 18.8 cm FL in *B. brevicephalus* to as large as 44.3 cm FL in *B. crassibarbis*.

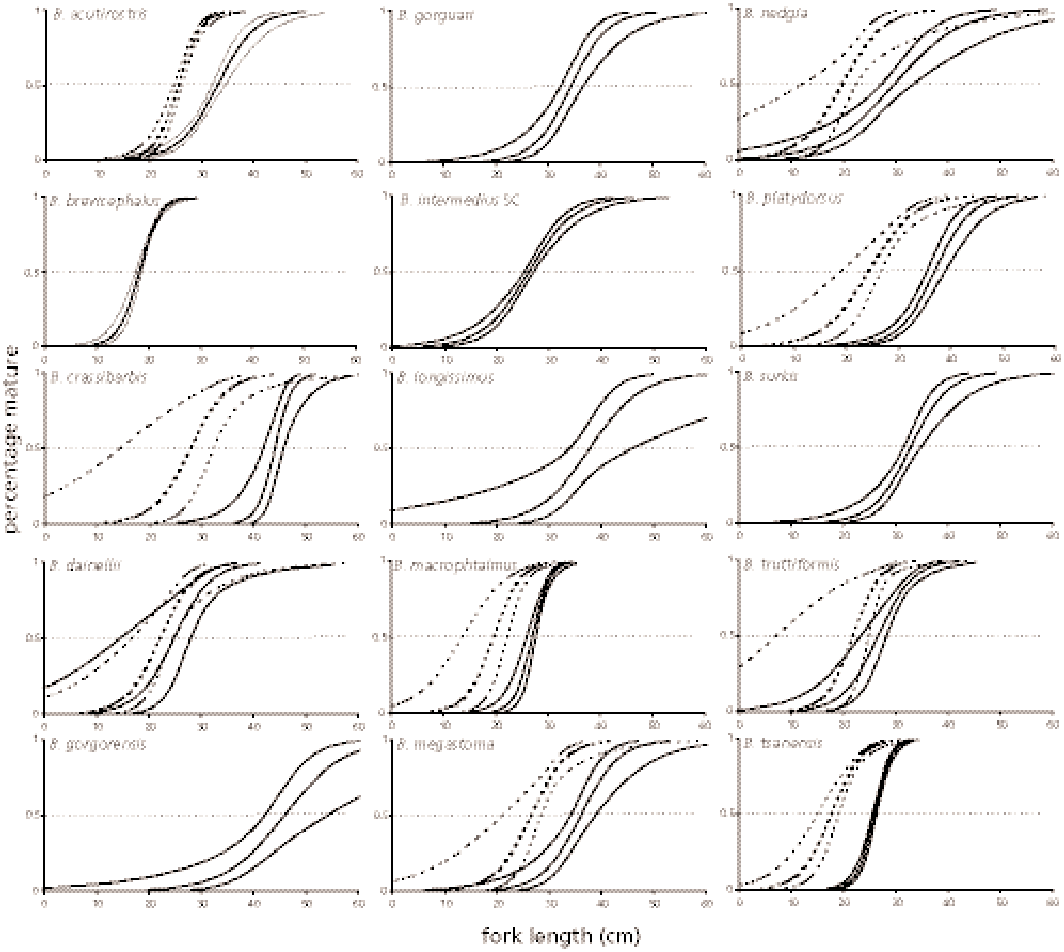

Figure 2. Male (- - - -) and female (——) sexual maturity curves for each of the 15 *Barbus* species during the spawning months of 1999-2001. Thin lines show lower and upper 95% confidence intervals. Percentage mature on the y-axis, barbs fork length on the x-axis. See Table 2 for values sigmoid curves and number of specimens per species and sex.

Table 2. Estimate of the parameters of the sexual maturity curves (male and female) of the *Barbus* species in Lake Tana in 1999-2001. n = number of individuals; a and b = coefficients of the sigmoid curves; *P*= significance levels, ns = not significant, * = *P*<0.05, ** = *P*<0.01, *** = *P*<0.001; FL_{min} = smallest FL (in cm) at which a mature individual of a *Barbus* species was found; $FL_{50\%}$ = FL (in cm) at which 50 % of the males and females are sexually mature; CI = confidence interval

Species	Sex	n	a:	b:	P	FL_{min}	$FL_{50\%}$	95% CI lower	95% CI upper
B. acutirostris	♀	419	0.3	-8.9	***	22.3	33.0	32.0	34.5
	♂	349	0.5	-11.6	***	21.5	25.5	24.6	26.1
B. brevicephalus	♀	954	0.5	-9.1	***	16.4	18.2	17.5	18.7
	♂	324	-	-	ns	13.0	-	-	-
B. crassibarbis	♀	71	0.6	-26.5	***	41.6	44.1	41.9	45.8
	♂	72	0.3	-7.9	*	26.1	28.0	14.5	32.4
B. dainellii	♀	68	0.3	-7.0	*	25.5	24.8	14.5	28.0
	♂	53	0.3	-6.7	**	20.0	22.1	16.6	24.7
B. gorgorensis	♀	61	0.2	-8.1	**	31.0	46.0	42.2	54.2
	♂	34	-	-	ns	27.3	-	-	-
B. gorguari	♀	94	0.3	-9.6	***	26.0	34.3	32.4	36.7
	♂	73	-	-	ns	24.1	-	-	-
B. intermedius SC	♀	1003	0.2	-5.9	***	17.0	26.0	25.2	26.9
	♂	698	-	-	ns	13.2	-	-	-
B. longissimus	♀	77	0.2	-7.6	**	28.5	37.8	33.9	45.9
	♂	34	-	-	ns	26.8	-	-	-
B. macrophtalmus	♀	551	0.6	-16.2	***	26.2	27.0	25.9	27.7
	♂	616	0.4	-7.6	***	21.2	19.6	13.5	22.2
B. megastoma	♀	330	0.3	-9.8	***	27.5	36.1	34.1	38.8
	♂	210	0.3	-7.8	**	27.0	26.5	21.0	28.4
B. nedgia	♀	130	0.2	-4.7	***	19.5	29.7	27.2	33.3
	♂	55	0.2	-4.9	*	17.0	19.8	12.0	22.4
B. platydorsus	♀	257	0.3	-10.8	***	26.3	37.0	35.5	39.0
	♂	162	0.2	-5.7	***	22.5	24.4	19.1	26.7
B. surkis	♀	150	0.3	-9.2	***	25.1	32.9	31.5	34.8
	♂	78	-	-	ns	20.9	-	-	-
B. truttiformis	♀	252	0.3	-7.9	***	25.8	26.6	23.3	28.3
	♂	193	0.4	-8.5	*	23.9	21.8	7.1	24.9
B. tsanensis	♀	812	0.6	-15.8	***	20.6	26.0	25.6	26.3
	♂	486	0.3	-6.1	***	15.7	17.7	15.0	19.1

Gillnet selectivity

The results of the gillnet selectivity estimates, obtained by applying the Sechin method and the observed length-frequency distribution for the various *Barbus* species are presented in Figure 3. The mode of the actual length-frequency distribution is similar (*B. intermedius* SC, *B. gorgorensis*, *B. gorguari*, *B. longissimus*, *B. megastoma*, *B. platydorsus*, or (slightly) smaller (*B. acutirostris*, *B. macrophtalmus*, *B. nedgia*, *B. surkis*, *B. truttiformis* and *B. tsanensis*) than the optimum length predicted by the Sechin method.

Morphological variety is high among the *Barbus* species (cf. Nagelkerke and Sibbing 2000) which is reflected in the FL_{mode} of the different species ranging from 30 cm FL (*B. tsanensis*) to 37 cm FL (*B. longissimus*). Some species (*B. crassibarbis*, *B. dainellii*, *B. gorguari*) displayed more than one mode, most likely due to the relatively low numbers of individuals (n<100) caught for these species.

The $FL_{50\%}$ is larger than FL_{mode} in *B. crassibarbis*, *B. gorgorensis*, *B. gorguari*, *B. longissimus* and *B. platydorsus*, smaller than the FL_{mode} in *B. dainellii*, *B. intermedius* SC, *B. macrophtalmus*, *B. truttiformis*, *B. tsanensis* and roughly equal to the FL_{mode} in the remaining species (Fig. 3, Table 3).

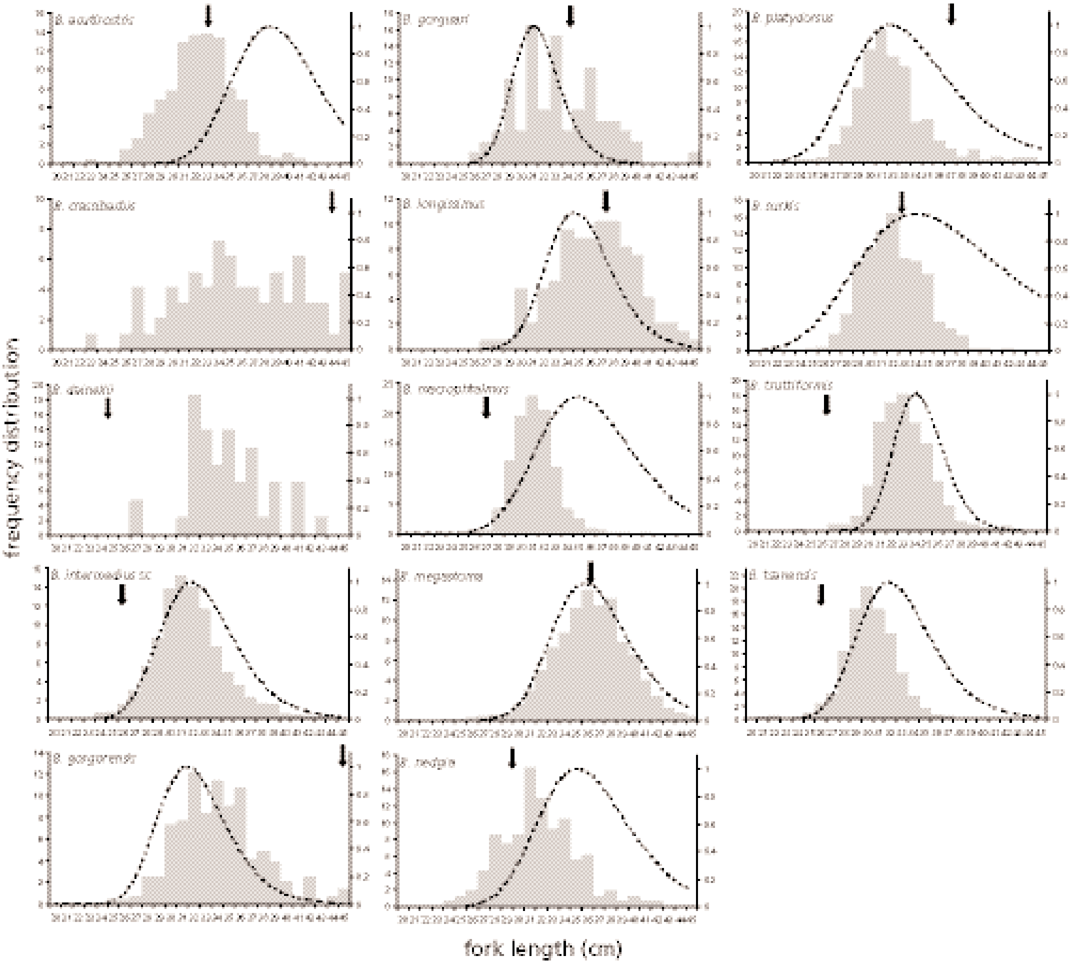

Figure 3. Selectivity curves for 10 cm stretched mesh (dotted line) estimated with the model of Sechin compared with observed length frequency distribution (bars) for the different *Barbus* species. Arrows indicate female $FL_{50\%}$. See Table 1 for n and size range selectivity curves and for n length-frequency distributions.

Barbus species composition

Barbus intermedius SC is in number by far the most dominant species (37.9%) in the catch of the commercial fishery using 10 cm stretched mesh gillnets, followed by *B. tsanensis* (15.4%), *B. platydorsus* (10.3%) and *B. megastoma* (10%) (Table 3). Note that the five species (*B. crassibarbis*, *B. gorgorensis*, *B. gorguari*, *B. longissimus*, *B. platydorsus*) that are predominantly immature fish when harvested ($FL_{mode} < FL_{50\%}$) contribute to only around 15% of the total number of the commercially landed barbs.

Table 3. *Barbus* species composition of the commercial catch (in percentage number; n=5795) during the period June-December 2001 and the comparison of $FL_{50\%}$ (Table 2, Fig. 2) with FL_{mode} (Fig. 3) for each species.

Species	% Commercial catch in numbers	$FL_{50\%}$ compared with FLmode
B. dainellii	0.5	<
B. intermedius SC	37.9	<
B. macrophtalmus	6.5	<
B. truttiformis	6.3	<
B. tsanensis	15.4	<
Total	**66.6**	
B. acutirostris	5.2	≈
B. megastoma	10.0	≈
B. nedgia	1.3	≈
B. surkis	0.6	≈
Total	**17.1**	
B. crassibarbis	0.9	>
B. gorgorensis	3.4	>
B. gorguari	0.6	>
B. longissimus	0.4	>
B. platydorsus	10.3	>
Total	**15.6**	

Discussion

Life-history traits

Increased mortality due to high fishing pressures can result in maturity at a smaller size (Rochet 1998; Chen and Mello 1999; Diamond et al. 1999). An attempt was made during the 1993-1994 spawning seasons to determine the $FL_{50\%}$ for *B. acutirostris* (♀ and ♂), *B. macrophtalmus* (♀), and *B. tsanensis* (♀ and ♂) (Nagelkerke and Sibbing 1996). Unfortunately, an incorrect method was used to calculate the maturity curves and $FL_{50\%}$ values, which made those preliminary results unreliable. Reanalysis of the 1993-1994 maturity data revealed, however, no significant relationships between fork length and percentage mature individuals, due to low overall sample size and lack of small individuals for most species. It was therefore impossible to compare the $FL_{50\%}$ of these barbs between the two periods, 1993-1994 and 1999-2001. The present data, however, form a solid base in order to reliably monitor future changes in this life-history trait.

Size control regulations: minimum mesh size

Currently, the mesh size used by the commercial fishermen is 10 cm stretched mesh. Using this mesh size at least 85% of the barbs harvested by the commercial gillnet fishery are large adults as their size at maturity is much smaller than their size at capture (Table 4, Fig. 3). Size control regulations towards the use of increasing mesh size will hardly reduce the fishing pressure on immature fish as already the vast majority of the fish landed are mature. A reduction in mesh size used might increase the fishing pressure on juveniles. For example, a reduction in mesh size to 8 cm stretched mesh will result in most

Barbus harvested being immature (Table 4). Again, such a scenario only forms a risk in combination with high fishing pressure. In Lake Tana, the development in the near future of high fishing effort on immature barbs is improbable for two reasons. In the first place, on average only 5-7 boats each carrying 2 km of gillnets are operational (Wudneh 1998; Chapter 12). Secondly, the fishing effort on barbs is concentrated during August-September in the river mouths on the eastern shores, targeting the aggregations of large, adult fish. Immature fish do not aggregate in the river mouths during these months.

If, however, mesh-size restrictions are be implemented the question remains if all large *Barbus* species can be lumped and treated as "one". The variation in the expected (FL_{opt}) and observed (FL_{mode}) size at harvest is relatively small (30-37 cm FL) and is the result of differences in head and body morphology among the *Barbus* species (Nagelkerke and Sibbing 2000). Size at maturity on the other hand shows large between- and within-species variation, ranging from 18 to 44 cm FL. This large variation in size at maturity does not allow lumping of all the *Barbus* species and to treat them as "one" when developing mesh size restrictions. Species dependent mesh size restrictions should, ideally, be developed and implemented. However, such species dependent mesh size restrictions will only be possible if fishermen target the different *Barbus* species separately, which is not the case. Furthermore size-selective fishing mortality due to strict mesh size regulations might cause negative genetic changes in population productivity, like reduced growth (Conover and Munch 2002).

Mesh size restrictions will be difficult to implement and their potential positive effect on the sustainability of the *Barbus* fisheries and on the recovery of the drastically reduced stocks is dubious as has been argued above. The current mesh size of 10 cm stretched mesh is adequate, reduction of mesh size does only give reason for concern if accompanied by a sharp increase of fishing effort specifically targeting juveniles barbs. Most importantly, the dramatic decline of *Barbus* stocks in the 1990s (de Graaf et al. 2003) is not caused by "unsustainable" harvesting of juveniles by the commercial gillnet fishery.

Table 4. Predicted relationship between $FL_{50\%}$ and FL_{opt} with decreasing and increasing mesh size (14-6 cm stretched mesh); white, $FL_{50\%} < FL_{opt}$; light grey, $FL_{50\%} \approx FL_{opt}$, bold figures indicate FL_{opt}; dark grey, $FL_{50\%} > FL_{opt}$. Note that the current mesh size used by the commercial gillnet fisheries is 10 cm stretched mesh.

		FL_{opt}								
	$FL_{50\%}$	14	13	12	11	10	9	8	7	6
B. acutirostris	33.0						34.7	▨	▨	▨
B. gorgorensis	46.0	44.0	▨	▨	▨	▨	▨	▨	▨	▨
B. gorguari	34.3				34.2	▨	▨	▨	▨	▨
B. intermedius SC	26.0							25.5	▨	▨
B. longissimus	37.8				38.1	▨	▨	▨	▨	▨
B. macrophtalmus	27.0							28.0	▨	▨
B. megastoma	36.1					35.8	▨	▨	▨	▨
B. nedgia	29.7						31.6	▨	▨	▨
B. platydorsus	37.0			38.2	▨	▨	▨	▨	▨	▨
B. surkis	32.9					34.1	▨	▨	▨	▨
B. truttiformis	26.6							27.3	▨	▨
B. tsanensis	26.0						28.7	▨	▨	▨

Effort control regulations: closed areas/season

Characteristic for Africa's large *Barbus* spp., is that they are primarily riverine species, and although some species occur in lakes, all are riverine spawners, undertaking a single annual upstream breeding migration (Tómasson et al. 1984; Skelton et al. 1991). At least half of Lake Tana's *Barbus* species appear to have a similar reproductive strategy. Gonad development of 14 out of the 15 *Barbus* species peaks during August/September (Chapter 8) and about half of the 15 *Barbus* species aggregate around river mouths during these two months and migrate up the rivers for spawning (Nagelkerke and Sibbing 1996; Dgebuadze et al. 1999; Chapter 6 and 8).

Fishing effort on *Barbus* is not equally distributed in Lake Tana, but varies highly both in time and space. Monthly catches of barbs peak at the end of the rainy season in August/September (spawning period; Chapter 8) when fishermen take their highest catches near river mouths (spawning aggregations; Chapter 6 and 8). Around 50% of the total annual catch during 1991-1993 was landed in just 2 months, August and September. Roughly 90% of these barbs were caught near the river mouths and the surrounding flood plains (Wudneh 1998; Chapter 12).

Recruitment overfishing might occur due to severe and unregulated overfishing of spawning aggregations, resulting in a dramatic decrease of recruits (Craig 1992; Gabriel et al. 1989). In Lake Tana recruitment overfishing potentially threatens the survival of the unique *Barbus* species flock. Recent results show sharp declines in abundance, up to 75% in number and biomass, of different *Barbus* species. More importantly, drastic changes in population structure showing a severe limitation of recruits (de Graaf et al. 2003).

If protective measures are not undertaken soon the future of Lake Tana's barbs and its fisheries might follow the same path as other African cyprinid fisheries. The reproductive strategy of African barbs, i.e. total spawners undertaking single yearly migrations, renders them vulnerable to overexploitation as many cyprinid fisheries are centred on these spawning migrations. The susceptibility of cyprinids for modern fisheries concentrating on spawning runs has been demonstrated repeatedly by the drastic decline of several *Labeo* fisheries (Lake Malawi, Luapula River in Zambia, Lake Victoria) after the introduction of nylon netting and increased fishing pressures in the 1950s and 1960s (Skelton et al. 1991). Lake Victoria's cyprinid fishery deteriorated as a result of intensive gillnetting of gravid female *B. altianalis* and *Labeo victorianus* near river mouths. Unregulated fisheries after the introduction of the more efficient gillnets compared to traditional fishing gear, had severe impact on the populations of these once abundant species as gillnets were set near river mouths, effectively blocking them off from the lake (Ogutu-Ohwayo 1990; Ochumba and Manyala 1992).

Only effort control regulations, limiting the gillnet fishery in spawning seasons and/or areas, will be appropriate in order to prevent the barbs from undergoing the same fate as the cyprinids in other African lakes. A further advantage of a management plan based on effort control regulations is that the *Barbus* species can be lumped and treated as "one", as all species more or less spawn in the same restricted period (August-September) and areas (river mouths and surrounding floodplains).

However, for any policy to be effective in the fishing community around Lake Tana it is important that representatives of the stakeholders and the state (various governmental offices of the Amhara National Regional State) are involved in the development, implementation and control of fisheries management. Continuous monitoring of the commercial catches is a first priority to evaluate the condition of the system and follow the effects of implemented management regulations.

Lake Tana; Ethiopia's biodiversity hot spot

Although Ethiopia hosts a number of endemic birds and mammals above the waterline, Lake Tana has remarkable high number of endemic species per km^2. This unique source of biodiversity is worth conserving for future generations as it yields as a natural laboratory to study evolution a great scientific potential. More importantly, for the local people, it should be a sustainable source of cheap protein. A species-rich ecosystem may produce a higher biomass than expected from the performance of individual species grown alone, due to a more efficient utilisation of the resources by the members of a highly diverse community (Loreau et al. 2001; Tilman et al. 2001). Furthermore biodiversity may provide resilience against environmental fluctuations (Loreau et al. 2001 and references therein).

In addition to providing a scientific base for implementing fisheries regulation in Lake Tana, the results of the present study also shed some light on the origin of Lake Tana's biodiversity, the evolution of the *Barbus* species. Reproductive isolation is a decisive character in the distinction of biological species. Genetically based morphological differences amongst the evolving populations of *Barbus* could only have become fixed by, at least partially, assortative mating. The assumed reproductive segregation among the *Barbus* species is supported by the large, consistent differences in average size at maturity ($FL_{50\%}$). This variety in reproductive characteristics suggests differentiation in life-history traits among the *Barbus* species, such as life span, growth rate and age at first maturity. Genetic differentiation and reproductive isolation could be inferred from such differences in ecology and life-history and therefore instrumental in speciation events (Crawford and Balon 1994; Taylor 1999).

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12

CHAPTER

Vulnerability of Lake Tana's Barbus compared with African catfish and Nile tilapia, to a small-scale commercial fishery: an example of recruitment overfishing.

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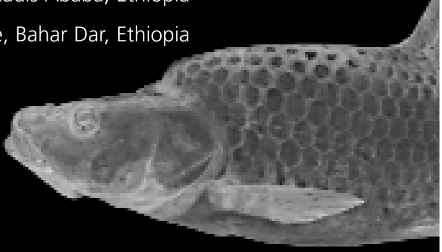


Abstract

In addition to the artisanal, predominantly subsistence fishery conducted from reedboats, in 1986 a motorised, commercial gillnet fishery was introduced in Lake Tana, Ethiopia's largest lake (3050 km^2). The three main species groups in Lake Tana are a species flock of endemic, large *Barbus* spp, African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*).

In 1991-1993 the vast majority of fishing activity took place in the Bahar Dar Gulf (71%) and each species group contributed to roughly one third of the total catch. In 2001, 41% of the effort was allocated to the north-eastern shores of Lake Tana. *Clarias gariepinus* and especially *O. niloticus* seemed resilient against the increased fishing pressure during the 1990s. Both the catch per unit of effort and contribution of *O. niloticus* to the total catch had doubled. The increase in *O. niloticus* was not only due to specific targeting of the commercial gillnet fishery but also to a three-fold decline in the abundance of especially *Barbus*, which proved to be highly susceptible to fishery activities. The large barbs are long-lived, ecologically specialised endemics and the seven riverine spawning *Barbus* species form aggregations in the rivermouths during August to September. These spawning aggregations are easily targeted by the commercial gillnet fishery.

The sharp decrease in abundance of migratory riverine spawning *Barbus* species in the sub-littoral and pelagic zones of the lake, areas where no fishing effort is allocated, and the collapse of juvenile *Barbus* during the 1990s suggests recruitment-overfishing. To prevent extinction of the unique *Barbus* species flock, effort control restrictions near the river mouths during August-September (peak breeding period) have to be implemented immediately to protect the vulnerable spawning aggregations.

Introduction

The susceptibility of large African cyprinids to over-exploitation has repeatedly been proven in the previous century by the collapse of *Labeo mesops* fisheries in Lake Malawi (Skelton et al. 1991), *L. victorianus*, *Barbus altianus* in Lake Victoria (Ogutu-Ohwayo 1990; Ochumba and Manyala 1992) and *L. altivelis* in Lake Mweru (Gordon 2002). Their reproductive strategy is one of the main reasons that make these large African cyprinids vulnerable to fishing activities. In general, large African cyprinids, including lake-dwelling species, are riverine spawners that form spawning aggregations in river mouths before migrating upstream to spawn on shallow gravel beds during brief periods each year (Tómasson et al. 1984; Skelton et al. 1991). The decline of these African *Labeo* and *Barbus* stocks were all attributed primarily to increased fishing pressure after the introduction of more efficient gillnets compared to artisanal fishing gear, targeting ripe females during breeding migration (Skelton et al. 1991; Ogutu-Ohwayo 1990; Ochumba and Manyala 1992).

The 15 *Barbus* species of Lake Tana (Ethiopia) form the only remaining intact species flock of large cyprinid fishes since the extinction of most endemic cyprinid species in Lake Lanao in the Philippines (Kornfield and Carpenter 1984; Nagelkerke et al. 1994; Nagelkerke and Sibbing 2000). In contrast to the other large African cyprinids, Lake Tana *Barbus* show both riverine spawning (ancestral strategy; seven *Barbus* spp, forming 76% of the barbs in number) and lacustrine spawning (derived strategy; eight *Barbus* spp, forming 26% of the barbs in number) (Chapters 4 and 8).

Prior to 1986 Lake Tana fisheries consisted only of an artisanal, predominantly subsistence reedboat fishery, operated by the Woito people. This type of fishery is limited to the shore areas and targets the native Nile Tilapia, *Oreochromis niloticus*, using locally made fish traps and small gillnets (length 15-20 m). In 1992-1993 there were 113 reed boat fishermen with a total of 374 gillnets (each net: length 18m, 8 cm stretched mesh) in the Bahar Dar Gulf (Wudneh 1998). The total number of reedboat fishermen in the lake is estimated at ca. 400.

Fishing has not been an important activity historically. Ethiopians are meat eaters (10 kg/year) and most eat little fish. Fish is not highly valued as a source of cheap protein and mainly eaten during religious fasting periods. The Ethiopian Orthodox Church also prohibits the consumption of the scale-less African Catfish, *Clarias gariepinus*. On average, an Ethiopian only eats 0.1 kg of fish per year, however, the distribution of this consumption is highly skewed towards those areas close to the shores of lakes (8-10 kg/year) and also to Addis Ababa (1.0 kg/year). However, to fulfil the increasing demand for fish from the capital city, Addis Ababa, created by foreigners and Ethiopians with foreign contacts (Reyntjes et al. 1998), motorised boats and modern, more efficient, nylon gillnets were introduced in Lake Tana in 1986. The development of the commercial motorised gillnet fishery in Lake Tana has considerably benefited from external assistance by NGOs and EU sponsored Lake Fisheries Development Projects. Almost all fish landed by the motorised gillnet fishery, which was manned by a new group of approximately 100 Amhara fishermen, are purchased by the Fish Production and Marketing Enterprise, processed and transported to Addis Ababa.

Since its introduction in 1986, little has been documented about the development and characteristics of the commercial gillnet fisheries and development of the three targeted species groups, *Barbus*, *C.*

gariepinus and *O. niloticus*. This lack of knowledge about the natural resources and the impact of the commercial gillnet fishery is one of the main reasons why to date no management plan or fisheries regulations exist in Lake Tana. However, since the mid-1990s fishermen have noted a drastic reduction of their catches in the Bahar Dar Gulf of Lake Tana. This stresses the need for sound data on Lake Tana's fish and fisheries in order to provide a scientific base for advice on development of a management plan and fisheries regulations. The objective of this study is therefore to discuss the effects of the motorised commercial gillnet fishery on the development of the *Barbus*, *C. gariepinus* and *O. niloticus* stocks during the 1990s by: (a) to quantify, describe and compare the variation in catch per unit effort of *Barbus*, *C. gariepinus* and *O. niloticus*, total annual yield and effort allocation by the motorised commercial gillnet fishery in Lake Tana in 1991-1993 and 2001, the two periods for which data exist; and (b) to quantify, describe and compare the abundance of *Barbus*, *C. gariepinus* and *O. niloticus* in the Bahar Dar Gulf of Lake Tana between 1991-1993 and 1999-2001 using a fisheries independent sampling method.

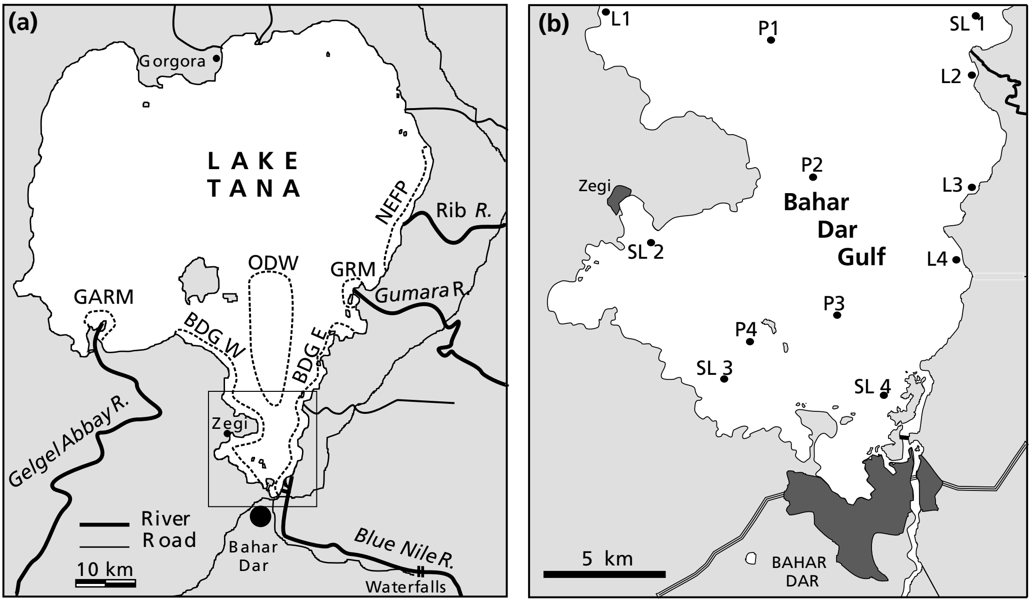


Figure 1. (a) Location of the fishing grounds in Lake Tana. GARM = Gelgel Abbay River Mouth, 5 km^2; BDG W = Bahar Dar Gulf West coast, 50 km^2; BDG E = Bahar Dar Gulf East coast, 50 km^2; GRM= Gumara River Mouth, 5 km^2; NEFP = North-Eastern Flood Plains, 35 km^2; ODW = Offshore Deep Water area, 80 km^2. (b) Location of the sampling stations in the Bahar Dar Gulf. Habitat littoral (L1, L2, L3, L4), depth <3 m, distance to shore <100 m; habitat sublittoral (SL1, SL2, SL3, SL4), depth 3-6 m, distance to shore 100-1000 m; habitat pelagic (P1, P2, P3, P4), depth 6-12 m, distance to shore 1000-5000 m.

Materials and methods

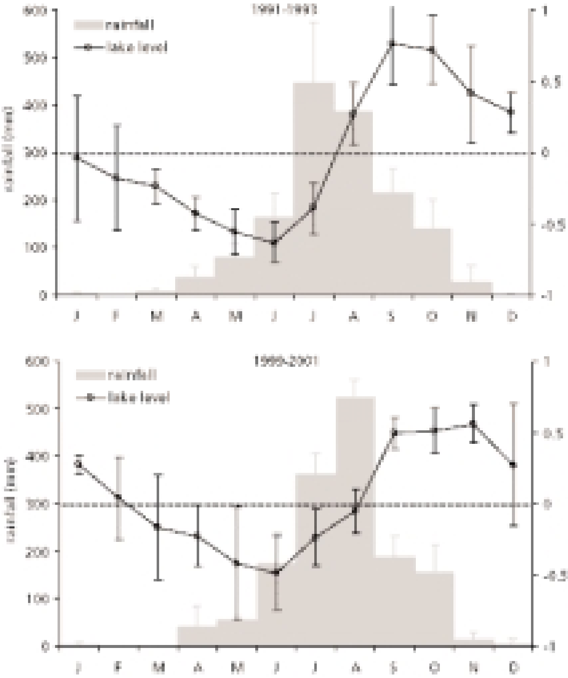


Figure 2. Monthly changes (mean ± SE) in rainfall (mm) and relative lake level (m) during 1991-1993 and 1999-2001.

Lake Tana

Lake Tana is situated at an altitude of approximately 1,800 m in the north-western highlands of Ethiopia, 500 km north of the capital Addis Ababa. The oligo-mesotrophic (chlorophyll *a*, average 6.4 $\mu g \cdot l^{-1}$; Dejen et al. 2003), shallow lake (average depth 8 m, maximum depth 14 m) covers an area of ca. 3,050 km^2 and is Ethiopia's largest lake. Several perennial rivers feed into Lake Tana (Fig. 1a). The lake's ichtyofauna is isolated from the lower Nile basin by 40 m high waterfalls, 30 km downstream from the Blue Nile outflow at Tissisat ('smoking water'). Rainfall, lake water level, water temperature and vertical transparency all follow seasonal patterns (details in de Graaf et al. 2003a). Rainfall peaks in July-August, followed by a raise in the water level of the lake, peaking in September-October (Fig. 2). During the rainy season the vertical transparency in the Bahar Dar Gulf is reduced due to the inflow of large amounts of silt, resulting from severe erosion, by the affluent rivers. Annual variation in water temperature shows two peaks, the first just before the rainy season in May and the second around October-November at the start of the dry, winter season.

Catch and effort commercial gillnet fishery

From December 1991 to October 1993 and from January to December 2001 records were made of the total weight or number of three species groups (*Barbus* spp, *Oreochromis niloticus*, *Clarias gariepinus*) sold to the Fish Production and Marketing Enterprise (FPME) and Giorgis Fish Shop (only in 2001) by individual motorised boats. In 2001 the total catch of all individual motorised boats was recorded daily. In December 1991-October 1993 the total catch of all individual motorised boats was recorded during 5-10 days each month, with the exception of the period March-April 1992. When recording the total catch of an individual motorised boat, additional data were collected by interviewing fishermen about their fishing grounds, number of gillnets, mesh size (cm stretched mesh), and unsold fish being discarded or used for their own consumption. To recalculate weight from number of fish, the average weight used for a *C. gariepinus*, *O. niloticus* and *Barbus* in the commercial gillnet fishery was 0.86, 0.39 and 0.53 kg, respectively, based on the following relations and data: length-weight relationships for *C. gariepinus*, *O. niloticus* and *Barbus* were $W = 0.005\ L^{3.05}$, $W = 0.013\ L^{3.13}$ and W

$= 0.017\ L^{2.96}$, (weight in gr, length in cm) respectively, and mean length of *C. gariepinus*, *O. niloticus* and *Barbus* in the catch of the gillnet fishery was 52, 27 and 33 cm, respectively (Wudneh 1998; de Graaf et al 2003b).

Six fishing areas (Fig .1a) were identified in Lake Tana for statistical analysis, grouping fishing grounds with geographical proximity and/or habitat similarity (river mouths, neighbouring floodplains, depth and distance to shore). To assess a measure of fishing effort, the relationship between total weight per fishing trip (TW) and the number of gillnets (NNETS) carried on the trip was examined. A power curve was fitted for the two sampling periods (i = 1 or 2) separately:

$$\mathrm{TW}_{ij} = \alpha_i \cdot N^{\beta_i} \cdot \varepsilon_{ij}$$

where:
α_i = coefficient of period i
β_i = exponent of period i
ε_{ij} = random term for period i and observation j

For period 1, 1991-1993, the results were $\alpha_1 = 4.9$, $\beta_1 = 1.11$ ($R^2 = 0.46$, n=1560, $P<0.01$) and for period 2, 2001, $\alpha_2 = 19.3$, $\beta_2 = 0.60$ ($R^2 = 0.17$, n=1770, $P<0.01$). The unit of fishing effort, which was used in the further analysis, was a fishing trip with the average number of gillnets (19.5) carried per trip. CpUE, catch per unit of effort is calculated as:

$$\mathrm{CpUE} = \mathrm{W}\left(\frac{19.5}{\mathrm{NNETS}}\right)^{\beta 1 \text{ or } 2}$$

where W is the weight of a particular species category in the catch.

Spatial and temporal patterns in *Barbus*, *C. gariepinus* and *O. niloticus* CpUE were studied through analysis of variance (ANOVA). The catch data were log-transformed before ANOVA could be applied. The CpUE data were analysed for differences among fishing areas (GARM, BDG W, BDG E, GRM, NEFP, ODW), sampling months and the interaction between fishing area and sampling month (see Table 1). Model residuals were always tested for normality, to check if the conditions for parametric analysis of variance were met. Confidence limits (95%) were estimated to compare main group means in case significant effects were found. To analyse effect of sampling period on *Barbus*, *C. gariepinus* and *O. niloticus* CpUE independent samples T-tests were performed on log-transformed data. The proportions of *Barbus* spp, *O. niloticus* and *C. gariepinus* in the commercial catch and the proportions of the five fishing areas in 1991-1993 and 2001 were compared using χ^2 *r x c* contingency table analysis (Fowler et al. 1998).

Experimental trawl program

A bottom trawl with a 15 m head rope and 17 m foot rope was used in the experimental fishing program. Mesh sizes ranged from 45 to 20 mm bar mesh at the cod-end. The trawl net had a vertical opening of 1-1.5 m and a horizontal opening of 7.5-8 m during operation. Trawling was conducted monthly on four consecutive mornings from ca. 07:00 to 12:00 hours (three stations per morning) between September 1991-August 1993 (91/93) and September 1999-August 2001 (99/01). In the Bahar Dar Gulf three habitats (four sampling stations per habitat) were selected differing in depth and distance to the shore (Fig. 1b). A 30 min trawl at 1 $m \cdot sec^{-1}$ was carried out at each of the twelve sampling stations. The sampling stations, boat, bottom trawl and crew were the same in 91/93 and 99/01. Immediately after a trawl, the fish caught were sorted to species group (*Barbus*, *C. gariepinus*, *O. niloticus*) on deck, total weight per species group was taken and fork length (FL; *Barbus*) or total length (TL; *C. gariepinus*, *O. niloticus*) was measured individually to the nearest cm. The CpUE per species group [number or weight $(kg) \cdot haul^{-1}$] was analysed to determine temporal variation between the two sampling periods (91/93 and 99/01; Mann-Whitney U-test, $P<0.05$) and to determine spatial variation (habitats: littoral, sublittoral, pelagic following Wudneh 1998; Chapter 4) and temporal variation (seasons: Dec-Feb, Mar-May, Jun-Aug, Sep-Nov following Wudneh 1998) within each sampling period (Kruskal-Wallis tests). The frequency of occurrence of the various *C. gariepinus* (small <30 cm TL, medium 30-50 cm TL, large >50 cm TL) and *O. niloticus* (small <20cm TL, large >20 cm TL) size classes in the trawl catches was analysed by a G-test to the *r x c* contingency table (Fowler et al. 1998).

Barbus species composition

Data on the *Barbus* species composition in the commercial gillnet fishery catch were collected monthly from June 2001 to October 2001. During the last week of each month for three consecutive days all barbs landed by the commercial gillnet fishery in Bahar Dar were identified to species level and fork length (FL) was measured to the nearest 0.5 cm. The frequency of occurrence of the various *Barbus* species in the commercial gillnet fishery catch during the five consecutive months was analysed by a G-test to the *r x c* contingency table (Fowler et al. 1998).

Results

Annual yield, total CpUE, effort allocation and catch composition

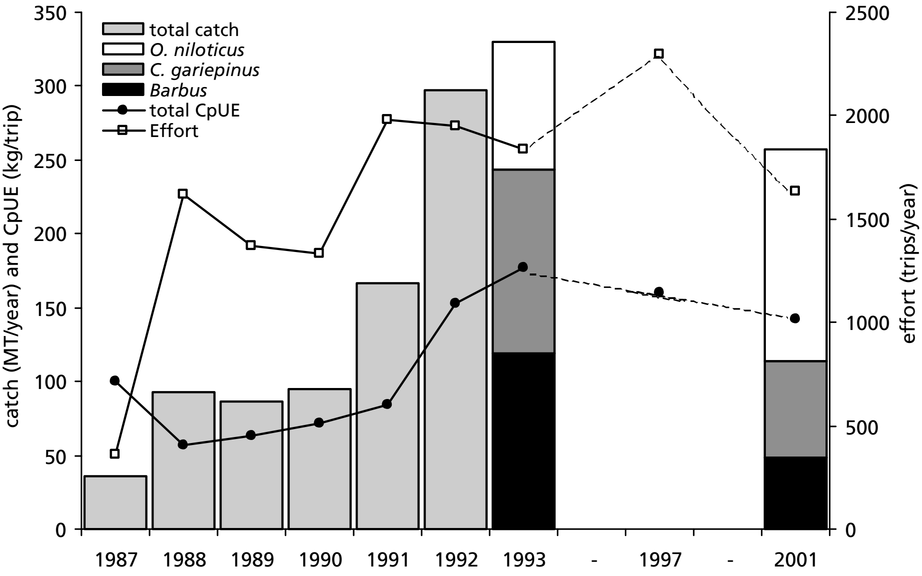
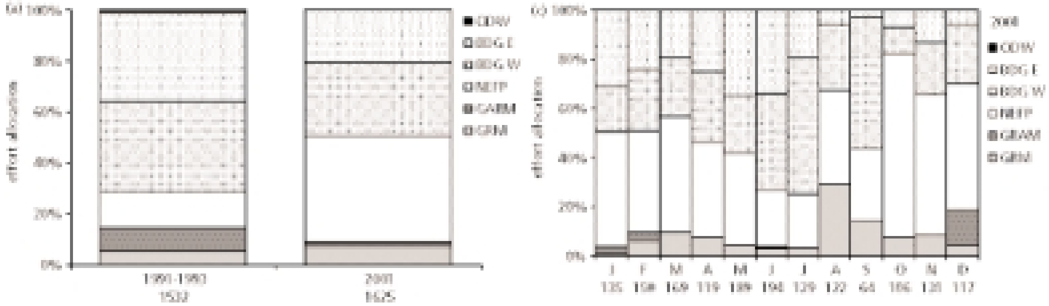


Figure 3. Estimated total annual yield (grey bars, tonnes/year), total effort (open squares, trips/year) and total CpUE (solid circles) of the motorised gillnet fishery in Lake Tana. Date from Fish Production and Marketing Enterprise (1987-1993), Lake Fishery Development Project (1997; LFDP 1997) and the present paper (1993 and 2001). Species group composition of the total annual yield in 1993 and 2001 was constructed from the species composition of the sampled landings of the commercial gillnet fishery. Note the statistically highly significant ($\chi^2_{0.05,\,2} = 50478$; $P<0.001$) change in species group composition between the two periods. The contribution of *O. niloticus* has doubled in 2001.

Between 1987 and 1993, total fishing effort, total CpUE and thus estimated annual yield (MT = metric tonnes) of the commercial gillnet fishery increased (Fig. 3). Annual yield peaked in 1997 (360 MT) but decreased again in 2001 (255 MT). This decrease was caused not only by a lower daily effort (# boats per day: 7 ±0.4 95%CI in 1991-1993, 5 ±0.5 95%CI in 2001) but also by a decrease in total CpUE (177.3 ±6.5 95%CI 1991-1993, 140.5 ±5.2 95%CI 2001) starting in 1993 (Fig. 3). Number of nets (19.6 ±0.4 95%CI 1991-1993, 19.5 ±0.2 95%CI 2001), mesh size (10 cm stretched mesh) and number of fishing days per month (20) did not change during the 1990s.

A significant shift ($\chi^2_{0.05,\,5} = 387$; $P<0.001$) occurred in the effort distribution over the six different fishing areas between 1991-1993 and 2001 (Fig. 4a). In 1991-1993 the vast majority of fishing activity took place in the Bahar Dar Gulf (71%) and only 14% of the effort was allocated to the north-eastern shores. After 1991-1993 the gillnet fishery moved in a northern direction. In 2001 41% of the effort was allocated to the north-eastern shores of Lake Tana adjacent to the floodplains. Within each period there was a considerable increase of fishing effort in Gumara river mouth (GRM) during the months August and September (Figs. 4bc). The offshore, deep-water (ODW) area was an insignificant fishing area for Lake Tana's commercial gillnet fleet during both periods. In 2001 Gelgel Abbay river mouth (GARM) contributed little (1.6%) to the total effort. Therefore, ODW (both periods) and GARM (2001) were excluded from analysis of spatial and temporal patterns in CpUE.

Between 1991-1993 and 2001, a significant shift ($\chi^2_{0.05,\ 2}$ = 50478; *P*<0.001) also occurred in the contributions of the three species groups to the total catch of the gillnet fishery (Fig. 3). In 1991-1993 each group contributed roughly a third of the total catch, however, in 2001 the contribution of *O. niloticus* doubled to more than 50%, while the contribution of both *C. gariepinus* and *Barbus* halved. A similar change occurred in CpUE for each species group between two periods (see Fig. 5).



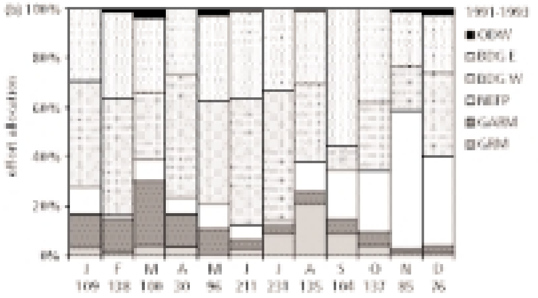


Figure 4. (a) Overall effort allocation during the two periods, (b) monthly changes in effort allocation during 1991-1993, and (c) during 2001. Percentages are taken from the total number of sampled trips, which is indicated below each bar. Note the highly significant change ($\chi^2_{0.05,\ 5}$ = 387; *P*<0.001) in fishing grounds between the two periods, the motorised gillnet fishery moved from the Bahar Dar Gulf (BDG W, BDG E) to the north-eastern shores (NEFP) of Lake Tana.

Barbus

Fisheries dependent

Mean *Barbus* CpUE in the commercial gillnet fishery was significantly ($F_{(2940)}$=333.5; *P*<0.001) lower in 2001 (28 kg/trip) than in 1991-1993 (63 kg/trip) [Table 1; dashed lines Fig. 5]. Although CpUE decreased over all months and fishing areas, the overall spatial and temporal patterns in *Barbus* CpUE remained strikingly similar between the periods. All effects modelled in the ANOVAs were significant at *P*<0.001 (Table 1). CpUE was highest in July-October, peaking in both periods in August (almost three times mean CpUE). Spatial variation of CpUE (Fig. 5) also showed high overlap in patterns between both periods. *Barbus* CpUE is three times higher in Gumara river mouth area than the mean CpUE. The CpUE near the north-eastern floodplains, including the area around Rib River, is also in both periods higher than in the remaining fishing grounds.

Barbus brevicephalus is missing from the commercial catch data (Fig. 7) as this species is too small to be retained in the nets used by the motorised gillnet fishery (de Graaf et al. 2003b). The lacustrine

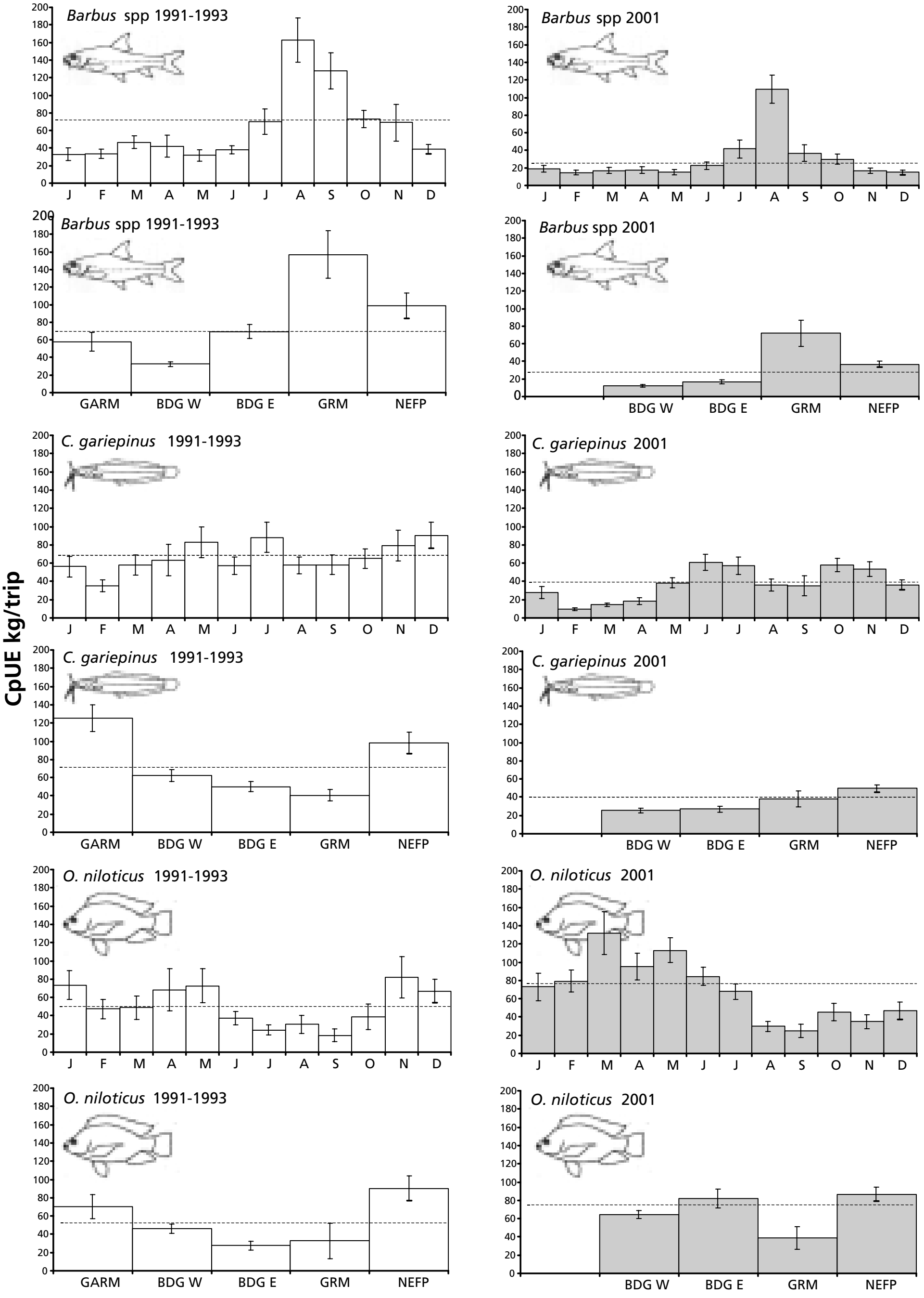


Figure 5. Temporal and spatial variation in *Barbus*, *C. gariepinus* and *O. niloticus* CpUE of the commercial gillnet fishery during 1991-1993 and 2001. Dotted lines indicate overall mean, error bars indicate the 95% confidence intervals. Note that ODW (both periods) and GARM (2001) have not been analysed due to insignificant allocation (0-1.5%) of the total effort to these areas.

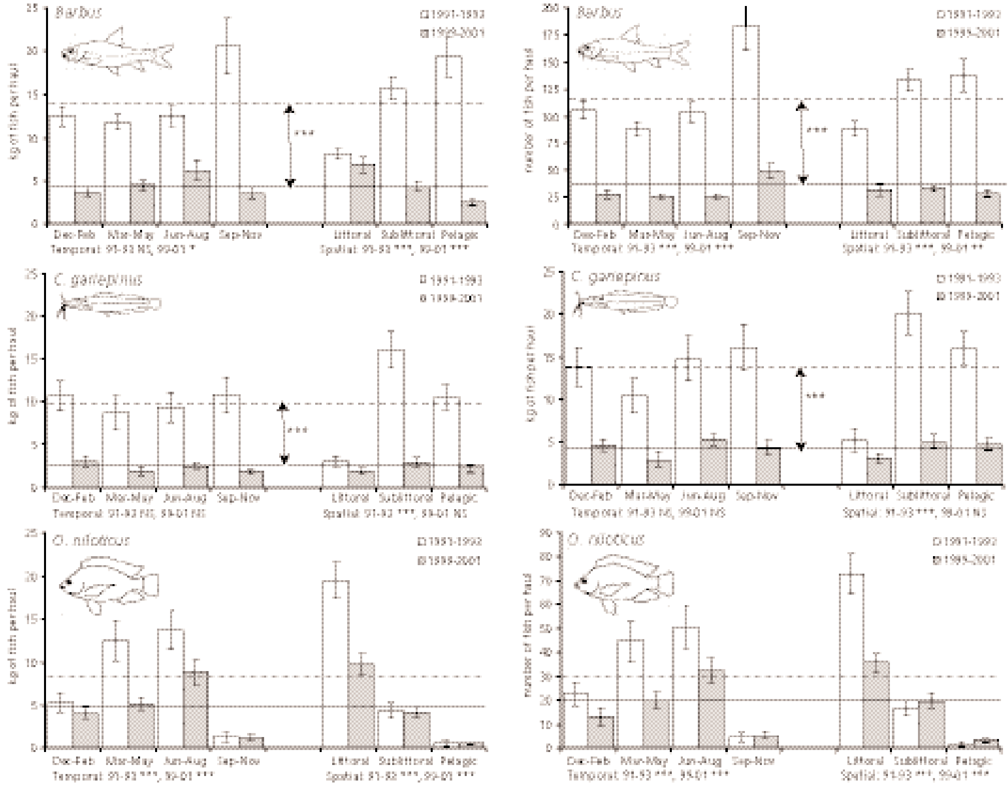


Figure 6. Temporal and spatial variation in abundance (weight and number) of *Barbus*, *C. gariepinus* and *O. niloticus* per haul (mean ± 95% CI) during the experimental trawl program in 91/93 (white bars) and 99/01 (grey bars). The effect of season and habitat on the abundance of each species group in each sampling period is given at the bottom of the graphs [Kruskal-Wallis tests, n=288, df=2 (habitat) or df=3 (season)]. Arrow and three asterisks indicate significance differences at the 0.001 level (Mann-Whitney U-test) in abundance between the sampling periods. Note that (1) the sharp decrease in abundance of *Barbus* and *C. gariepinus* is restricted to the sublittoral and pelagic areas and (2) only *O. niloticus* does not decrease significantly in abundance between both periods.

Table 1. Results of the analysis of variance of *Barbus*, *C. gariepinus* and *O. niloticus* CpUE. The degrees of freedom (df), the mean squares (ms), and the level of significance [*P*: ***= *P*<0·001; ns= not significant (*P*>0·05)] of the sources of variation are indicated (see also Fig. 5).

	Barbus			*Clarias gariepinus*			*Oreochromis niloticus*		
	1991-1993			*1991-1993*			*1991-1993*		
Source of variation	df	ms	P	df	ms	P	df	ms	P
Area	4	4.72	***	4	4.09	***	4	4.79	***
Month	11	3.54	***	11	0.38	ns	11	4.39	***
Interaction	43	0.55	***	43	0.56	***	41	1.31	***
Error	1422	0.16		1424	0.22		1105	0.34	
R^2	0.37			0.22			0.31		
	2001		2001	2001					
Source of variation	df	ms	P	Df	Ms	P	df	ms	P
Area	3	12.37	***	3	9.15	***	3	4.94	***
Month	11	3.93	***	11	4.06	***	11	4.71	***
Interaction	33	0.31	***	33	0.59	***	33	0.54	***
Error	1370	0.16		1477	0.15		1498	0.18	
R^2	0.38			0.37			0.3		

spawners *B. crassibarbis*, *B. dainellii*, *B. gorgorensis*, *B. gorguari*, *B. longissimus*, *B. nedgia*, and *B. surkis* are rare in the catch of the commercial gillnet fishery. Overall these seven *Barbus* species contributed less than 6.4% to the total number of barbs in June-October 2001 and were not further analysed. There is a highly significant temporal effect ($G_{0.05,\ 28}$=100346; *P*<0.001) on the species composition of the landed barbs between June and October (breeding period, Chapter 8). The riverine spawners were abundant in the commercial catch only in July-August (*B. truttiformis*, *B. tsanensis*) or September (*B. macrophtalmus*) when they aggregate in the river mouths (Chapter 8), and were almost absent during the other months (Fig. 7). The lacustrine spawner *B. intermedius* "shore-complex" (SC) is the most common barb in the littoral zone throughout the lake and contributes most to the total amount of landed Barbus, especially outside the peak breeding period (July-September, Chapter 8).

Fisheries independent

Like the CpUE in the commercial gillnet fishery, the fisheries independent trawl CpUE (both in kg and in number per haul) of *Barbus* was significantly lower (*P*<0.001) in the Bahar Dar Gulf between the two sampling periods (Fig. 6). Temporal variation between the four seasons was similar in both sampling periods, CpUE was highest in Sep-Nov. Spatial abundance patterns were markedly different between the two periods. In 1991-1993, CpUE increased with distance to shore while in 1999-2001 CpUE decreased with distance to shore.

Clarias gariepinus

Fisheries dependent

Mean *C. gariepinus* CpUE in the commercial gillnet fishery was significantly ($F_{(3064)}$=145.7; *P*<0.001) lower in 2001 (37 kg/trip) than in 1991-1993 (67 kg/trip) [Table 1; dashed lines Fig. 5]. In 2001 *C. gariepinus* CpUE decreased more or less equally in all months and fishing areas. All effects in the ANOVA model were significant at *P*<0.001, except in 1991-1993 mean CpUE did not differ significantly between the months (Table 1). In 2001, CpUE was higher in the second half of the year. In 1991-1993 larger spatial variation occurred in CpUE, peaking in GARM and NEFP. In 2001, NEFP had the highest CpUE but the difference with GRM and the Bahar Dar Gulf was smaller.

Fisheries independent

Like the CpUE of the commercial gillnet fishery, the trawl CpUE (both in kg and in number per haul) of *C. gariepinus* was highly significantly (*P*<0.001) lower in 1999-2001 (Fig. 6).

Temporal patterns in CpUE were the same in both periods, *C. gariepinus* was caught throughout the year independent of season. In 1991-1993 *C. gariepinus* was significantly more abundant in the sublittoral and bentho-pelagic areas of the Bahar Dar Gulf. In 1999-2001, however, the abundance in these two areas had seriously decreased and was not significantly different from the littoral area.

A significant shift ($P<0.001$) occurred in the proportions of small, medium and large *C. gariepinus* between the two periods (Table 2, Fig. 8). In 1999-2001 large (>50cm TL) *C. gariepinus* were less abundant. Almost 70% of the trawl catches consisted of medium sized (30-50 cm TL) individuals.

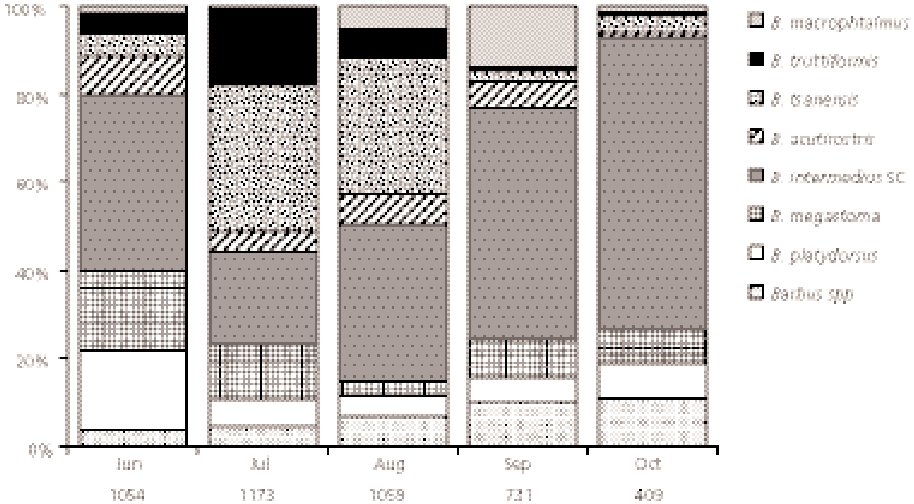


Figure 7. Species composition of the *Barbus* landed by the commercial gillnet fishery as a function of time (month) in 2001. Percentages are taken from the total number of sampled barbs, which is indicated below each bar. Note the statistically highly significant ($G_{0.05,\ 28}=100346$; $P<0.001$) overall temporal effect. The riverine spawning species *B. tsanensis*, *B. truttiformis* and *B. macrophtalmus* only form a significant part of the commercial catch when in July/August and September, respectively when they form spawning aggregations near river mouths.

Oreochromis niloticus

Fisheries dependent

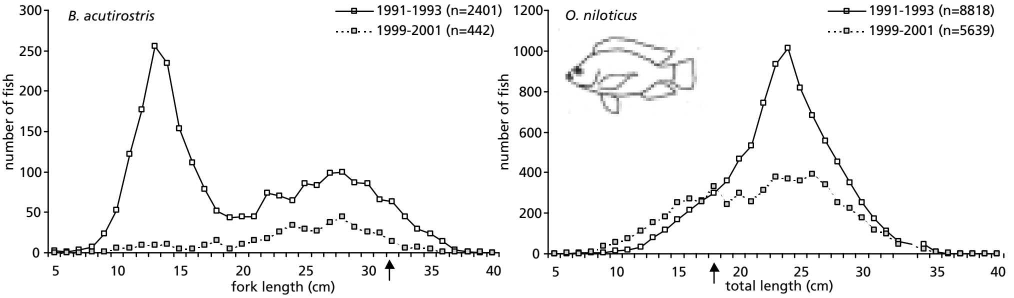
In contrast to *Barbus* and *C. gariepinus*, mean *O. niloticus* CpUE was significantly ($F_{(2741)}=40.8$; $P<0.001$) higher in 2001 (75 kg/trip) than in 1991-1993 (47 kg/trip) [Table 1; dashed lines Fig. 5]. In 2001 *O. niloticus* CpUE increased especially in February-July and in the whole Bahar Dar Gulf compared to 1991-1993. All effects modelled in the ANOVAs were significant ($P<0.001$) (Table 1). The overall temporal patterns in *O. niloticus* CpUE were similar between the periods but were more pronounced in 2001. CpUE was highest in December-June, peaking in 2001 in March-May. Spatial patterns in *O. niloticus* CpUE differed between the periods. In 1991-1993 CpUE in the north-eastern flood plains (NEFP) was two to three times higher than in both Bahar Dar Gulf East/West (BDG E/W) and in Gumara river mouth In 2001 CpUE was the same in NEFP but CpUE in BDG E and BDG W had both doubled, reaching the same level as in NEFP.

Fisheries independent

Oreochromis niloticus is the only species that did not differ significantly in trawl CpUE, both in kg [*P* = 0.72] and in number [*P* = 0.81] per haul, in the Bahar Dar Gulf between the two sampling periods (Fig. 6). However, small (≤ 20 cm TL) *O. niloticus* increased both in proportion (G-test; *P*<0.001) and in absolute number in the 1999-2001 trawl catches (Table 2, Fig. 8). Temporal and spatial distribution patterns were the same in both sampling periods. *Oreochromis niloticus* is most common in the littoral areas of the lake and abundance increased significantly between March and August (low water level).

Table 2. Results of the contingency table analysis (G-test) testing the hypothesis that the proportion of the different size-classes in the trawl catches is independent (*P*>0.05) of the sampling period (see also Fig. 8). *P*= significance level: ***= *P*<0.001.

	1991-1993		1999-2001		G-statistic	*P*
	Number	Proportion	Number	Proportion		
Clarias gariepinus						
<30 cm TL	297	7.4	106	8.5	86.8	***
30-50 cm TL	2293	57.2	866	69.8		
>30 cm TL	1422	34.4	269	21.7		
Oreochromis niloticus						
≤ 20 cm TL	2057	22.1	2280	38.4	462.5	***
> 20 cm TL	7231	77.9	3659	61.6		



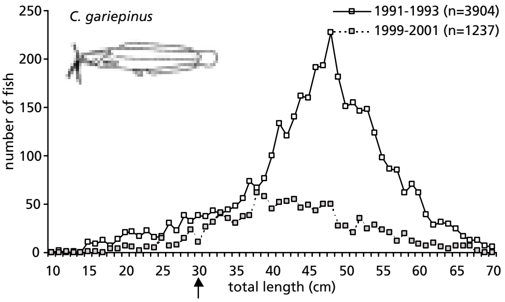


Figure 8. Length frequency distribution of *B. acutirostris* (redrawn from de Graaf et al. 2003), *C. gariepinus*, and *O. niloticus* in the experimental trawl program of 91/93 (solid line, white squares) and of 99/01 (dotted line, grey squares). (see also Table 2). Note the collapse of juvenile *B. acutirostris* in 2001, the proportionally significant stronger decrease of large *C. gariepinus* (>50 cm TL) and *O. niloticus* (>20 cm TL), and the increase of small *O. niloticus* (<20 cm TL) between the two periods. Arrows indicate female size at maturity ($FL_{50\%}$); data *B. acutirostris* (de Graaf et al. 2003b, *C. gariepinus* and *O. niloticus* (Wudneh 1998; de Graaf, unpubl. data).

Discussion

Development of Lake Tana's commercial gillnet fishery

Lake Tana's traditional fishermen belong nowadays to a small ethnic group, the Woito, who used to live around the banks of Lake Tana. However, the Woito were excluded from participation in the modernisation of Lake Tana's fisheries. Initially, the motorised commercial gillnet fishery was therefore run by inexperienced Amhara fishermen. Although CpUE is expected to decline gradually in a newly developing fishery (Goudswaard et al. 2002; and references therein), in Lake Tana CpUE initially increased. This increase might be the result of learning, experience in using fishing gear and increasing knowledge about distribution of fish by the new Amhara fishermen. Since 1993 total CpUE started, as expected, to decrease.

Total effort (number of trips per year) and annual yield increased sharply in the first few years, remained at the same level during the 1990s and started to decrease in 2001. This decrease in effort is in sharp contrast with the general trend of other African lakes, like Victoria (Goudswaard et al. 2002), where the number of fishermen and number of nets used by each fishermen increase annually. After the initial investments in Lake Tana's fisheries at the end of the 1980s, the number of operating boats (maximum 23) started to decrease due to mechanical failure and lack of spare parts. The EU sponsored Lake Fisheries Development Program only enabled the fishermen to use the credits to buy European (Italian) outboard engines. Unfortunately, these engines proved to be unreliable and spare parts were not available. In the first few years, the crews of the motorised boats with outboard engines quickly ceased to operate. Only boats with an inboard diesel engine remained functional during the 1990s. Still the number of boats fishing per day kept decreasing slowly from 7 to 5 between 1993 and 2001. Spare parts for these inboard engines are still difficult to obtain and expensive. The expected income of fishing is too low and the costs of a boat, engine and gillnets too high for new fishermen to start operating. An important characteristic of the commercial gillnet fishery is that the catch is landed centrally. The commercial gillnet fleet is stationed in Bahar Dar but operates often on distant fishing areas (40-60 km) along the north-eastern shores. The fishermen stay on the fishing sites continuously for five nights (Monday-Friday) before returning to Bahar Dar. All boats sell their fish to the only fish buyer (FMPE) who collects the fish daily on the distant fishing sites and transports all catch back to Bahar Dar for further processing. Prices paid to the operating fishermen by the FMPE are even too low (*O. niloticus* ± 0.8 euro kg^{-1}; *Barbus* ± 0.6 euro kg^{-1}; *C. gariepinus* ± 0.4 euro kg^{-1}) to cover maintenance, let alone to save money and invest in new or better gear. To maximise their income, the fishermen specifically target *O. niloticus*. *Oreochromis niloticus* is the most favoured fish for consumption among Ethiopians and fetches the highest price. *Barbus* is less appreciated due to its intra-muscular bones and is therefore mainly used for soup or 'wot', a spicy (chopped) fish sauce. The scale-less *C. gariepinus* is considered unclean according to the Ethiopian Orthodox Church and is almost exclusively eaten by Western and African foreigners in Addis Ababa. Between 1993 and 2001 the proportion of *O. niloticus* in the commercial catch doubled from 26% to 55%. The CpUE of *O. niloticus* also increased sharply in the same period from 47 kg trip-1 to 75 kg trip^{-1}. This increase in *O. niloticus* can largely be explained by a shift in fishing grounds, from the

Bahar Dar Gulf to the 'tilapia-rich' littoral areas adjacent to the extensive floodplains along the north-eastern shores of Lake Tana (Fig. 4). Such shallow, protected areas are the preferred habitat of *O. niloticus* (Kolding 1993). The increase in *O. niloticus* in the commercial catch is not only due to specific targeting (active) of the commercial gillnet fishery but also due to a decline (passive) in abundance of the other two species groups, *Barbus* and *C. gariepinus*.

Life-history and vulnerability to fisheries

Fish species or even populations differ in susceptibility to human exploitation. Generally, under prolonged and/or intensifying fishing pressure the composition of the catch shifts towards the more resilient species of a fish community (Jul-Larsen et al 2003 and references therein). In general, susceptible to fisheries are old segments of population of long-lived species, species with riverine migrations and spawning aggregations, and/or highly specialised endemics. Relatively unspecialised ecologically flexible species distributed widely in rivers and lakes, and adapted to fluctuating environments can be categorised as resilient. The most resilient fish are small sized species, like *Limnothrissa* (Clupeidae; Lake Tanganyika, Lake Kariba) or *Rastrineobola* (Cyprinidae; Lake Victoria), with high population turnover rates. Based on their ecology, reproductive biology and size/age characteristics *Barbus*, *C. gariepinus* and *O. niloticus* can be placed on a scale from susceptible to resilient against increased fishing mortality (Table 3).

Barbus species flock

Within Lake Tana's fish community the *Barbus* species are predicted to be by far the most susceptible to the commercial gillnet fishery (Table 3) as the barbs are: (a) long-lived (Wudneh 1998), (b) form spawning aggregations (Nagelkerke and Sibbing 1996; Chapters 6 and 8), and (c) predominantly specialised endemics (Chapters 4 and 5; Sibbing and Nagelkerke 2001). Catchability is highly variable during the year, CpUE peaked sharply in July, September and especially August in both periods. More than 50% of the annual *Barbus* yield is landed during the three month of peak spawning, July-September, in 1992, 1993 and 2001. The landings of *Barbus* could have even been higher if it was not for the fact that the fishermen ceased their activities each year for two weeks during the second half of September due to the Ethiopian new year ('Unkutatash') on 11 September followed by the 'Finding of the True Cross' (Meskel) on September 27.

Highest CpUE is in the Gumara River mouth where the riverine spawning *Barbus* species form large aggregations (Chapter 8) before migrating 40 km upstream to spawn on the shallow, gravel beds (Chapter 6). The fishermen clearly target these spawning aggregations as is illustrated by dominance, appearance and disappearance of riverine spawning/aggregating *Barbus* species in the catch during these months (Fig. 7). The selective impact of the gillnet fishery on especially the riverine spawning *Barbus* species is further illustrated by the sharp decrease in *Barbus* in the sublittoral and pelagic areas compared with the littoral area (Fig. 6). Lacustrine spawning *Barbus* species occur predominantly in the littoral areas of the lake while the riverine spawning *Barbus* species are restricted to the sublittoral and pelagic areas (Chapter 4). The sharp reduction in abundance of

Table 3. General biology and predicted susceptibility to fishery in Lake Tana of adult *Barbus* , *C. gariepinus* and *O. niloticus*. References (Ref.): 1 = Witte and van Densen (1995); 2 = Wudneh (1998); 3 = Sibbing and Nagelkerke (2001); 4 = Goudswaard et al. (2002); 6 = Chapter 8; 7 = Chapter 6; 8 = Chapter 4; 9 = Duponchelle and Panfili (1998); 10 = Kolding (1993); 11= Winemiller and Kelso-Winemiller (1996); 12 = Twongo (1995).

	***Barbus* species flock**	***Clarias gariepinus***	***Oreochromis niloticus***	**Ref.**
Reproductive biology				
Spawning period	2 months August-September	2 months June-July	throughout the year	6, 2
Spawning aggregation, migration	spawning aggregations , in river mouths of major affluent rivers especially Gumara River	general migration from sublittoral/bentho-pelagic towards littoral areas and floodplains during inundation	general migration from littoral zone into floodplains during inundation	6
Spawning area	riverine spawners: upstream areas major affluent rivers lacustrine spawners:	floodplains wide habitat tolerance for spawning and nursery areas	floodplains wide habitat tolerance for spawning and nursery areas	1, 4, 7, 8, 11, 12
Ecological niches				
Diet	narrow mostly food specialists piscivore, benthivore macrophytivore, molluscivore, insect-zooplanktivore	broad omnivore fish, insects, plankton, plants, molluscs, detritus	intermediate planktivore (opportunist) phyto-, zooplankton, diatoms, detritus, molluscs, insect larvae	1, 3 4,
Habitat	riverine spawners: sublittoral/pelagic, bottom and surface lacustrine spawners: littoral zone	sublittoral/bentho-pelagic zone, bottom dwelling	littoral zone bordering floodplains	2, 8
Growth				
Age at FL$_{50\%}$ maturity	3-4 years	2-3 years	1-2 years, highly flexible	2, 9, 10
Susceptibility to fishery	high, vulnerable especially riverine spawning *Barbus* species	old part population: high, vulnerable young part population: moderate, resilient	low, most-resilient	

Barbus in the sublittoral-pelagic area of the lake is not caused by direct fishing effort (1% in 1991-1993, 0% in 1999-2001) in these habitats but is the result of over-exploitation of the spawning aggregation of these species in the river mouths.

The collapse in the proportion of juvenile fish of the three piscivorous barbs *B. acutirostris* (Fig. 8a), *B. macrophtalmus* and *B. platydorsus* (de Graaf et al. 2003a) demonstrated the expected negative consequences of a fishery targeting spawning aggregations, i.e. a disturbance of the reproductive process resulting in a dramatic decrease in the number of recruits (Craig 1992; Gabriel et al. 1989; Regier et al. 1999). In *C. gariepinus* and *O. niloticus* mainly the larger size classes and older part of the population were reduced and the smaller individuals decreased little (*C. gariepinus*) or even increased in number (*O. niloticus*).

Clarias gariepinus (African catfish)

Clarias gariepinus is an ecologically flexible species. It has a broad diet spectrum and occupies habitats ranging from the offshore to the littoral areas in the lake, to floodplains and to river channels 40 km upstream (Wudneh 1998; Chapter 6). *Clarias gariepinus* is the only species in Lake Tana that tolerates aquatic hypoxia by an accessory breathing organ which enables it to utilize atmospheric oxygen. Catchability of *C. gariepinus* changes throughout the year. During the dry season (Dec-May) the benthic *C. gariepinus* is most common in the sub-littoral and offshore areas of the lake. Landings of *C. gariepinus* are low during this period because the commercial gillnet fishery allocates its effort only in the littoral areas. At the start of the rainy season, *C. gariepinus* moves through the littoral areas towards the inundated floodplains and upstream inflowing rivers to spawn in June-July. While the Gumara with its upstream oxygen-rich shallow gravel beds is used by *Barbus* as a spawning area, *C. gariepinus* is by far the dominant species upstream the turbid Rib river with its extended marginal floodplains and lower aquatic oxygen content (Chapter 6). When the water level starts to decrease (Oct-Dec) *C. gariepinus* migrates back through the littoral zone towards the sub-littoral and pelagic areas. CpUE of the commercial gillnet fishery increases at the beginning and at the end of the rainy, high water season when *C. gariepinus* migrates through the littoral areas. The impact of the gillnet fishery on migrating *C. gariepinus* is illustrated by the sharp decrease in trawl CpUE in the sublittoral and benth- pelagic areas compared with the littoral area (Fig. 6). The decrease in the sublittoral-pelagic area of the lake is not caused by direct fishing effort (1% in 1991-1993, 0% in 1999-2001) in these habitats. *Clarias gariepinus* is intercepted by the commercial gillnet fishery when migrating between the floodplains (spawning areas) and the sublittoral-pelagic areas.

CpUE of both the commercial gillnet fishery and the experimental trawl program decreased sharply in the Bahar Dar Gulf during the 1990s. However, the decrease did not occur equally over the whole size. Especially the more susceptible older and larger (>50 cm TL) individuals decreased in the trawl catches between the two periods. The decrease of especially large *C. gariepinus* is supported by the fact that the abundance of *C. gariepinus* predominantly decreased in the sublittoral and pelagic areas of the Bahar Dar Gulf (Fig. 6) and not in the littoral area. Large, adult *C. gariepinus* prefer deep water while juveniles occur mainly in the littoral zones (Goudswaard and Witte 1997; Wudneh 1998). Although the large, older individuals have been proven to be vulnerable for increased mortality by the commercial gillnet fishery, it is expected that compared with *Barbus*, *C. gariepinus* is only moderately susceptible to future fishing pressure. The resilience of *C. gariepinus* was also illustrated by Goudswaard and Witte (1997) who observed that of the six catfishes species in Lake Victoria, *C. gariepinus* seemed to be the least affected by fisheries activities and the introduction of Nile perch. Furthermore, because of the minimal value and appreciation of this species by the Ethiopians, *C. gariepinus* is not specifically targeted by the commercial gillnet fishery and is mainly landed as bycatch.

Oreochromis niloticus (Nile tilapia)

Of the three species groups *O. niloticus* is expected to be the most resilient against increased mortality induced by the motorised gillnet fishery (Table 3). Catchability of *O. niloticus* is variable and highly correlated with seasonal changes in water level (see Figs. 2, 5 and 6). CpUE decreased significantly just after the start of the rainy season from July-August to November-December when water levels are high. When water levels are high, *O. niloticus* migrates towards the relative safety of the inundated floodplains where the commercial gillnet fishery has no access (Fig. 6). Regular seasonal dispersion of *O. niloticus* associated with inundation of floodplains has been reported for several African lakes (Kolding 1993 and references cited therein). *Oreochromis niloticus* has no well defined breeding period and ripe fish can be found throughout the year. Their reproductive strategy is rather specialised. *O. niloticus* are mouthbrooders with a high investment in parental care. The high reproductive success of *O. niloticus* is further caused by the wide habitat tolerance for spawning and nursery purposes. Phenotypic plasticity in maturation size is remarkably high in *O. niloticus*, ranging from 39 cm TL (Lake Turkana, Kenia; Lowe-McConnell 1958) to 9 cm SL with an age of just six months (Korokara resevoir, Cote d'Ivoire; Duponchelle and Panfili 1998). This phenomenon of stunted growth or 'dwarfing' is suggested to be a unique adaptive mechanism to withstand extremely high mortality rates under adverse biotic or abiotic conditions like predation or desiccation (Iles 1973). This flexibility in size at maturity might also be important to compensate for high mortality induced by fishing pressure. In Lake George a reduction in size at maturity was attributed to intensive fishing (Kolding 1993 and references cited therein).

As expected from its flexibility (Table 3), the abundance of *O. niloticus* is the least affected by the increase in fishing pressure since the introduction of the motorised commercial gillnet fishery. Female size at maturity did not change during the 1990s ($FL_{50\%}$ 18.1 cm TL in 1991-1993, Wudneh 1998; $FL_{50\%}$ 17.6 cm TL, de Graaf unpubl. data) and $FL_{50\%}$ is well below the average size at harvest (26 cm TL Wudneh 1998). In the Bahar Dar the abundance both in number and weight did not decrease significantly during the 1990s. Although the number of large specimen (>20 cm TL) decreased, the number of small individuals increased in contrast to *Barbus* and *C. gariepinus*. In 1995-1997 a low height weir was constructed near the outflow of the Blue Nile at Chara Chara, to regulate the seasonal fluctuation of water discharge to the new hydroelectric plant located ca. 30 km downstream at "Tissisat" waterfalls. The control in water level might have resulted in longer inundation of the floodplains around Lake Tana during the second half of the 1990s. This might have benefited *O.*

niloticus, as the inundated flood plains provide a refuge from fishery, foraging areas, spawning grounds and nursery areas.

Future prospects commercial gillnet fishery

New investments in Lake Tana's commercial gillnet fishery are currently being planned by NGOs. However, it is of utmost importance that first, a sound management plan including conservation goals are formulated and implemented by the (regional) Ethiopian government prior to intensifying the commercial gillnet fishery with the help of foreign donors. In the past no management plan has been developed due to, (1) lack of data on the characteristics of both the fish stocks and the commercial gillnet fishery, (2) lack of federal fisheries legislation, and (3) limited knowledge dissemination, i.e. information is published in English in international scientific journals, hence less accessible for local experts, civil servants and policy makers. However, recently (January 2003) Ethiopia's first fisheries legislation was approved by the federal government and several activities are being planned to disseminate the wealth of knowledge on Lake Tana's fish and fisheries that has been generated in recent years (see for details de Graaf et al. 2003a). Advantageous characteristics of Lake Tana's commercial gillnet fishery are its small scale and the centrally landed catch in Bahar Dar. This enhances the communication and co-operation with fishermen and the monitoring of the catch.

Of the three species groups the situation of the unique *Barbus* species flock is the most critical. To remove the threat of extinction for Lake Tana's endemic *Barbus* species in the near future, protective measures have to be taken immediately. Only effort control regulations limiting the gillnet fishery in the spawning season and/or areas around the river mouths will prevent a total collapse of the *Barbus* stocks as. The drastic and rapid consequences of an unregulated modern gillnet fishery on spawning aggregations of large African cyprinid fishes has became painfully clear with the collapse of *Labeo mesops* fisheries in lake Malawi (Skelton et al. 1991), *L. victorianus* and *Barbus altianus* fisheries in Lake Victoria (Ogutu-Ohwayo 1990; Ochumba and Manyala 1992) and the disappearance of *Labeo altivelis* (mpumbu) from the Mweru-Luapula system within a 20 year period (Gordon 2002).

Continuous monitoring of the catches of the commercial gillnet fishery, and the abundance of the different species groups with experimental sampling programs (trawl), are of utmost importance to follow the development of *Barbus*, *C. gariepinus* and *O. niloticus* stocks and to evaluate the consequences of implemented fisheries regulations.

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Summary

Ethiopia

Ethiopia, often called the Cradle of Humankind after the discovery of a 3.5-million-year-old predecessor ("Lucy") of modern man, is rarely in the news in a positive way. Recently, the war with their neighbours Eritrea, formerly part of Ethiopia up until 1993, did not improve Ethiopia's image in the west. Unknown, however, to most of the world, is Ethiopia's fascinating history, unique culture and traditions and the tremendous diversity of its ethnic people. Ethiopia is the only African country that has its own written language "Ge'ez" and was never successfully colonised by any of the European powers during the "Scramble for Africa" in 1870-1912. The rumoured possession of the Ark of Covenant, resting within the church of Saint Mary of Sion in the ancient city of Axum further adds to its mysteries.

Piscivorous Barbus project

Lake Tana is located at an altitude of 1830 m, in the north-western highlands of Ethiopia, 500 km north of its capital Addis Ababa. The oligo-mesotrophic, shallow lake (average depth 8 m, maximum depth 14 m) covers an area of c. 3,050 km^2 and is by far Ethiopia's largest lake, containing half the country's freshwater supply. Lake Tana contains an extraordinary diversity of cyprinid fishes. It is currently estimated that at least 15 large, endemic *Barbus* species are present. The family Cyprinidae is the most widespread and has the highest diversity (>2000 species) among all fresh water fish families and even among vertebrates. Despite the enormous abundance of cyprinid fishes throughout the world's lakes and rivers, the Barbus species of Lake Tana form, as far as we know, the only remaining intact species flock of large cyprinid fish. One of the most intriguing aspects of Lake Tana's endemic *Barbus* species flock is the large number of piscivorous species (8 out of 15), an unexpected specilisation for cyprinid fish. Cyprinids seem not well designed for piscivory, they lack teeth in the oral jaws, have a small slit-shaped pharyngeal cavity and lack a stomach with low pH for digesting large prey. Cyprinid fishes have a highly developed palatal and sublingual organ lining their pharynx resulting in highly localised gustatory control in feeding. Using these refined systems, small edible particles are sorted from the soft substrate, thus enabling cyprinids to feed on (micro) benthos. The evolutionary success and high competitive abilities as benthivores of cyprinid fish is largely attributed to this unprecedented sorting ability as well as their powerful and diversified pharyngeal jaw system. Like in other cyprinid fish, most of Africa large *Barbus* are benthivorous species, so why then is piscivory, which is rare among cyprinids, so common in Lake Tana *Barbus* species flock?

The first two aims of the piscivorous *Barbus* project were to: (a) determine the ecological role of each piscivorous *Barbus* species in Lake Tana community [Ecology], and (b) reconstruct the evolution of piscivory within the *Barbus* species flock using morphological, ecological, behavioural and molecular data [Evolution]. In 1986 the Lake Tana Fisheries Resources Development Program (LTFRDP) started by the EU and ISE-Urk, introducing modern fishing gear and motorised boats. Traditionally, the fisheries in Lake Tana consisted of a subsistence reed boat fishery. The fishermen, limited in their mobility, only had access to the shore areas, using locally made fish traps, hooks and small gillnets (15-20 m, 8-10 cm strtched mesh), catching mainly tilapia, *Oreochromis niloticus*. The LTFRDP created new opportunities for the fishermen, extending their fishing area from the shore to deeper, offshore waters and, more importantly, to distant river mouths where *Barbus* form large spawning aggregations. The third aim of the project was to provide a solid scientific base of fish and fisheries related data required for the development of rational management measures to be taken in order to ensure the sustainability of the fisheries and the unique *Barbus* diversity in Lake Tana [Exploitation].

Ecology

Experimental designs in the laboratory were used to study the different predation techniques of the piscovorous barbs and their performance. Extensive field observations were done to determine the extend of ecological segregation along three resource dimensions; trophic, spatial and temporal. Due to fact that these cyprinid piscivores masticate prey fish with their pharyngeal jaws, no whole or partial prey fish are found in the digestive tract for identification. Information about the prey consumed (species and size) was painstakingly reconstructed from the skeletal elements of prey fish in the predator's digestive tract.

Overall, the 15 *Barbus* species appeared to be well segregated along the three resource dimensions. The eight piscivorous *Barbus* species have adapted to all available macro-habitats, using different techniques (e.g. ambush hunting using velocity suction with protrusion [open water; *B. macrophtalmus*, *B. megastoma*; littoral zone, *B. gorguari*] or volume suction [littoral areas with high structural complexity; *B. dainellii*] and pursuit hunting [open water; *B. longissimus*, *B. truttiformis*]), a unique scenario for barbs.

The cichlids of Lake Victoria and the barbs of Lake Tana have some important analogous morphological innovations resulting in potentially large diversification of trophic structures and high potential for trophic radiation. A remarkable fact is that although the Lake Victoria haplochromine species flock has a higher diversity per trophic group, the proportion of the species per trophic group is the same in both species flocks. Due to the lack of oral teeth, some common trophic specialisations (aufwuchs feeders, scale scrapers, finbiters) among Lake Victoria's cichlids are lacking in Lake Tana's barbs. Nevertheless, in the absence of modern piscivorous percoid fish as competitors, the cyprinids of Lake Tana use their potential for trophic diversification to the fullest, including the unexpected specialization of piscivory.

Based on an objective as possible comparison, Lake Tana's piscivorous *Barbus* performed relatively "poor", compared to piscivores from other fish families. The barbs are only able to successfully capture relative small prey (prey-to-predator size ratio PPR, average 0.15 and maximum 0.25) compared to other non-cyprinid freshwater and marine piscivores. Prey size selection is not limited by gape sizes of the feeding apparatus [postcapture factors], but is mediated by differential size-related capture success [precapture factors]. Their limited average and maximum prey size compared with other piscivores is most likely caused by the significantly smaller volume of their oro-pharyngeal-opercular cavity, hence restricting the diameter of the ingested water flow generated during suction feeding. The voluminous palatal organ and sublingual organ lining their pharynx, a key innovation in the evolutionary success of cyprinid fishes, came at a cost, their reduced competitive abilities as piscivores. Barbs in Lake Tana lack piscivorous competitors, rendering the piscivorous *Barbus* by far the "best" and apparently highly successful.

Evolution

The development of assortative mating in Lake Tana's *Barbus* species flock is a key issue when reconstructing how the ancestral *Barbus* population differentiated to occupy separate ecological niches and ultimately became reproductively isolated. One of the prerequisites for an organism to successfully invade a new food niche is flexibility in its trophic behaviour and options, partly reflected in its morphological structures. Whether successful invasion of a new food niche will result in speciation or resource polymorphism is largely dependent on the size of the genetic neighbourhood.

Like in many other cyprinid fishes, reproductive homing is apparently common among Lake Tana's seven silvery coloured offshore-dwelling riverine spawning *Barbus* species. The different species are at present spatially and temporally segregated in the upstream spawning areas. In migrating salmonids, homing reinforces the development of reproductive isolation (sub-populations), facilitating the divergence of other (morphological) traits. Among the riverine spawning *Barbus* species the genetic fixation of morphological adaptations in trophic structures was probably enhanced and facilitated by philopatry, i.e. homing to natal streams in the ancestral founding population. Prior to the geological formation of Lake Tana, the ancestral riverine barb population was most likely divided into sub-populations due to this reproductive strategy. After the formation of the lake, many new niches became available to the ancestral, riverine spawning barb population. Since random mating did not occur within the ancestral founding population, this might well have resulted resulted in small genetic neighbourhood sizes necessary for sympatric speciation and genetic fixation of trophic morphological adaptations, related to the newly invaded niches in the lacustrine habitat.

Among the eight predominantly dark coloured, littoral dwelling *Barbus* species peak gonad development occurred generally in the same period as the riverine spawners. However, these *Barbus* species did not aggregate in the river mouths during the breeding period and were absent from the upstream spawning areas. A derived, novel strategy, lacustrine spawning is hypothesized for these eight *Barbus* species. These species seem to have successfully adapted to the lacustrine environment and probably complete their whole life-history, including reproduction, inside the lake. This hypothesis is further supported by accidental observations of running female fish in the littoral zones far away from the affluent rivers. Lake spawning within the *Barbus* species flock probably evolved following adaptation to divergent ecological environments, i.e. sympatric divergence of benthic and pelagic forms. Such early ecological splits appear to be common in radiations of lacustrine fish groups. Further speciation among the littoral-dwelling lacustrine spawning *Barbus* and sublittoral-pelagic-dwelling riverine spawning *Barbus* was most likely driven by trophic competition and trophic specialisation within each of the macro-habitats. The sequence of primary macro-habitat diversification followed by secondary trophic diversification within each macro-habitat has been shown to occur in several lacustrine fish groups

Genetic diversity of Lake Tana's large and small *Barbus* species and other *Barbus* species collected in various lakes and rivers was investigated using cytochrome *b* sequences. The small, pelagic *B. tanapelagius* apparently evolved recently from the benthic *B. humilis*, i.e. sympatric divergence of benthic and pelagic forms similar to the large *Barbus* and other lacustrine fish groups (see above). Genetic divergence, when studying cytochrome *b*, among the various species of large *Barbus* in Lake Tana is very low, the failure to distinguish species based on cytochrome *b* sequences is due to the recent origin of the species flock. The haplotypes found in Lake Tana are unique but none are diagnostic for a particular species. The present data supports nor rejects a monophyletic origin of Lake Tana's *Barbus* species flock. The haplotypic variation of Ethiopian large barbs falls into two distinct Lineages (1 and 2), Tana haplotypes seem to form a subset of the total variation in Lineage 1, adding evidence that the Lake Tana species flock originates from a limited stock of ancestral riverine *B. intermedius*. Following prudent estimates the age of the species flock is somewhere between 700,000 and 10,000 old, clearly needing further study.

Exploitation

In addition to the artisanal, predominantly subsistence reedboat fishery, in 1986 a motorised, commercial gillnet fishery was introduced in Lake Tana. The three main fish stocks in Lake Tana are the large *Barbus* spp, African catfish (*C. gariepinus*) and Nile tilapia (*O. niloticus*). The objective was to determine the effects of the motorised commercial gillnet fishery on the development of the *Barbus*, *C. gariepinus* and *O. niloticus* stocks during the 1990s by: (a) to quantify, describe and compare the variation in catch per unit effort of *Barbus*, *C. gariepinus* and *O. niloticus*, total annual yield and effort allocation by the motorised commercial gillnet fishery in Lake Tana in 1991-1993 and 2001, the two periods for which data exist; and (b) to quantify, describe and compare the abundance of *Barbus*, *C. gariepinus* and *O. niloticus* in the Bahar Dar Gulf of Lake Tana between 1991-1993 and 1999-2001 using a fisheries independent sampling method.

In 1991-1993 the vast majority of fishing activity took place in the Bahar Dar Gulf (71%) and each species group contributed to roughly one third of the total catch. In 2001, 41% of the effort was allocated to the north-eastern shores of Lake Tana and the catch per unit of effort and the contribution of *O. niloticus* to the total catch had both doubled. The increase in *O. niloticus* was not only due to specific targeting of the commercial gillnet fishery but also due to a decline in abundance of especially *Barbus*, which proved to be highly susceptible to the increased fishery. The large barbs are long-lived, ecologically specialised endemics and the commercial gillnet fishery is mainly targeting their spawning aggregations in the river mouths. The collapse of juvenile *Barbus* during the 1990s, strongly suggests recruitment-overfishing. The ecologically flexible *C. gariepinus* and especially *O. niloticus* seemed more resilient against the increased fishing pressure.

High natural variability in fish stocks might be expected in environmentally unstable lakes. Although strongly pulsed (seasonal), Lake Tana is a relative stable system. No major differences were found in abiotic parameters during the 1990s that could have caused the dramatic changes in *Barbus* abundance. The most likely explanation is the negative impact of the motorised, commercial gillnet fishery targeting the spawning aggregations of these barbs in the river mouths. Especially the drastic decline in juveniles suggets serious recruitment over-fishing. To prevent the extinction of the unique Barbus species flock fisheries regulations have to be developed and implemented immediately focussing on effort restrictions during the *Barbus* breeding season to protect the vulnerable spawning aggregations.

Samenvatting

Ethiopië

Ethiopië, soms de Wieg van de Mensheid' genoemd na de ontdekking van een 3,5 miljoen jaar oude voorouder ("Lucy") van de moderne mens, is slechts zelden op een positieve manier in het nieuws. De recente oorlog met het buurland Eritrea dat tot 1993 deel uit maakte van Ethiopië, verbeterde het beeld over Ethiopië in het westen niet. Onbekend voor het grootste deel van de wereld is echter de fascinerende geschiedenis van Ethiopië, de unieke cultuur en tradities en de immense verscheidenheid aan etnische groeperingen. Ethiopië is het enige Afrikaanse land dat zijn eigen schrift "Ge'ez" ontwikkeld heeft en is het enige Afrikaanse land dat nooit succesvol is gekoloniseerd door een Europese macht.

Het Barbus project

Het Tana-meer ligt op een hoogte van ongeveer 1830 m, in de hooglanden van Ethiopië, 500 km ten noorden van de hoofdstad Addis Ababa. Het relatief voedselarme, ondiepe meer (gemiddelde diepte 8 m, maximale diepte 14 m) beslaat een gebied van 3050 km^2. Het is het grootste meer van Ethiopië en bevat de helft van de hele zoetwater voorraad. Alleen de Blauwe Nijl stroomt het Tana-meer uit. Het meer is afgesloten van andere zoetwatersystemen door een 40 m hoge waterval in de Blauwe Nijl op ongeveer 30 km vanaf de uitstroom van het meer. Het Tana-meer huisvest een buitengewone diversiteit aan karperachtige vissen, op z'n minst zwemmen er 15 endemische *Barbus* soorten rond. Vertegenwoordigers van de Cyprinidae familie komen voor op de meeste continenten en de soorten rijkdom (>2000 soorten) is het grootst van alle zoetwater visfamilies en zelfs van alle gewervelden. Ondanks de enorme hoeveelheid aan karperachtige vissen in de rivieren en meren op wereld, vormen de *Barbus* soorten van het Tana-meer, voor zover we weten, de enige complete soortenzwerm van grote karperachtige vissen. Een van de meest bijzondere aspecten van deze endemische *Barbus* soortenzwerm is het onverwacht grote aantal visetende *Barbus* soorten (8 van de 15). Karperachtige vissen zijn namelijk niet goed uitgerust voor het eten van vis, ze hebben geen tanden in hun mond, ze hebben een nauwe keelopening en ze hebben geen maag (met een lage zuurgraad) voor het verteren van grote prooien. Karperachtige vissen hebben een zeer goed ontwikkeld 'proef-smaak' orgaan aan de binnenkant van de mond en keel waarmee ze heel goed in staat zijn om kleine eetbare deeltjes uit het zand en de modder te sorteren. Het evolutionaire succes van de karperachtige vissen wordt voornamelijk toegewezen aan dit ongekende vermogen om eetbare deeltje te sorteren uit de modder maar ook aan de grote verscheidenheid aan krachtige keelkaken bij de verschillende soorten. Zoals te verwachten, eten veel Afrikaanse *Barbus* soorten bodemdieren (insecten, larven, slakken etc.) die ze uit het substraat sorteren. De vraag is nu, waarom er juist in het Tana-meer zoveel visetende *Barbus* soorten zijn?

De eerste twee doelstelling van het *Barbus* project waren: (a) het bepalen van de rol van de visetende *Barbus* soorten in het ecosysteem van het Tana-meer [Ecologie], en (b) het reconstrueren van de evolutie van vis etende barbelen in de soortenzwerm van het Tana-meer door gebruik te maken van morfologische, ecologische, gedrag en genetische data [Evolutie]. In 1986 werd het Tana-meer Visserij-Ontwikkelingsprogramma (LTFRDP) gestart door het Ethiopische Ministerie van Landbouw in samenwerking met Ethiopische Orthodoxe Kerk, de Interkerkelijke Stichting Ethiopië (ISE) uit Urk en de Interkerkelijke Coördinatie-commissie Ontwikkelingsprojecten (ICCO) uit Zeist. Dit ontwikkelingsprogramma introduceerde modern vistuig en gemotoriseerde boten in het Tana-meer. Tot die tijd werd er in het Tana-meer voornamelijk op kleine schaal (levensonderhoud) gevist vanuit bootjes gemaakt uit papyrus door de een kleine deel van de lokale bevolking (de Woito stam). Deze vissers gebruiken kleine kieuwnetten en fuiken om tilapia (*Oreochromis niloticus*) te vangen dicht aan de oever. Het LTFRDP creëerde mogelijkheden voor een nieuwe groep vissers (de Amharen), met hun gemotoriseerde boten en lange moderne kieuwnetten konden ze o.a. vissen op de barbelen die in de paaitijd grote scholen vormen in de mondingen van verafgelegen rivieren. De derde doelstelling van het project was het verkrijgen van wetenschappelijke gegevens gerelateerd aan de visserij en de commerciële vissoorten [Exploitatie]. Zulke gegevens zijn noodzakelijk voor het ontwikkelen van een reëel beleidsplan voor vispopulaties van het Tana-meer. Het ontwikkelen en uitvoeren van een beleidsplan zal hopelijk zorgen voor een gezonde visserij en voor de bescherming van de unieke verscheidenheid aan barbelen in het Tana-meer.

Ecologie

Met behulp van experimenten in het laboratorium in aquaria en kleine zwembaden werd onderzocht op welke manier (vang technieken) de visetende barbelen prooivisjes vangen en hoe efficient ze daar in zijn. Uitgebreid onderzoek in het veld moest uitwijzen op welke manier de visetende barbelen verdeeld zijn over de verschillende habitats in het Tana-meer en welke soorten en grootte prooivissen ze eten.

Omdat de visetende barbelen hun prooi vermalen met hun keelkaken, moest hun dieet worden gereconstrueerd aan de hand van de overgebleven botjes van de gegeten prooivisjes in de darmen. Dit leverde een aardige puzzel op maar uiteindelijk ben ik er in geslaagd om aan de hand van een paar duidelijk herkenbare botjes uit het kopskelet van de prooivisjes, het dieet (soort en grootte) van elke visetende barbeel vast te stellen.

Over het algemeen hebben de 15 grote barbelen de beschikbare ecologische ‘ruimte’ in het Tana-meer netjes verdeeld onder elkaar. Grofweg zijn er twee groepen te onderscheiden; acht donker gekleurde soorten in de oeverzone van het meer (littoraal) en zeven zilverkleurige barbeelsoorten in de diepe wateren (pelagiaal), ver van de oever. De visentende barbelen, die samen voorkomen in een bepaalde habitat, verdelen de beschikbare ecologische 'ruimte' door het eten van verschillende prooivissen en/of door gebruikmaking van verschillende vangtechnieken. Doordat de verschillende soorten allemaal net een ander ecologisch plekje bezetten in het Tana-meer, kunnen ze zonder problemen naast elkaar leven. Op deze manier voorkomen de verschillende soorten dat ze elkaar teveel moeten beconcurreren.

De bonte baarzen (cichliden) in het Victoria-meer en de barbelen in het Tana-meer hebben een aantal belangrijke overeenkomsten in hun lichaamsbouw (keelkaken en losse botstructuren in de bovenkaak). Deze analoge innovaties in het bouwplan van deze niet-verwante visfamilies zijn hoogstwaarschijnlijk één van de belangrijkste oorzaken voor het feit dat zowel de barbelen als de bonte baarzen in potentie zich in korte tijd 'makkelijk' kunnen aanpassen en specialiseren in een grote verscheidenheid aan voedsel (vis, slakken, insecten, planten, plankton etc). Het aantal verschillende voedselbronnen dat de cichliden in het Victoria-meer en de barbelen in het Tana-meer kunnen exploiteren is dan ook vergelijkbaar. Al met al hebben de barbelen in het Tana-meer hun mogelijkheden voor voedsel specialisaties ten volle benut, inclusief de onverwachte specialisatie van het vangen en eten van vis.

In vergelijking met viseters uit andere visfamilies (b.v. snoek, baars, forel, kabeljauw) presteren de barbelen echter relatief 'slecht'. De visetende barbelen zijn enkel in staat om kleine prooivisje te vangen (maximaal 25% van hun eigen lichaamslengte). Viseters uit andere visfamilies zijn in staat om veel grotere prooien te vangen (50-70% van hun lichaamslengte). Dit onvermogen om grote prooivissen te vangen wordt veroorzaakt door het beperkte inwendige volume van hun kop. Dit beperkte inwendige volume zorgt ervoor dat slechts een beperkte hoeveelheid 'water-met-prooi' kan worden aangezogen tijden een aanval. Het volumineuze ‘smaak-proef’ orgaan, de sleutel tot het evolutionaire succes van karperachtige vissen in het algemeen heeft als groot nadeel dat het het vermogen van karperachtige vissen om te concurreren met viseters uit andere visfamilies echter sterk vermindert. In het Tana-meer zijn er echter geen concurrerende viseters uit ander visfamilies zodat de visetende barbelen de 'beste' en meest succesvolle jagers zijn. De visetende barbelen hebben zich uitstekend aangepast en zijn in staat om diverse prooivissen (bodemvissen, scholen vis in het open water) te vangen, op verschillende plekken in het meer (ondiep, aan de oppervlakte of op de bodem), gebruikmakend van uiteenlopende vangtechnieken (vanuit een hinderlaag of achtervolgen; prooi opzuigen of er overheen zuigen). Een daadwerkelijk unieke prestatie voor karperachtige vissen.

Evolutie

Eén van de voorwaarden waar aan moet worden voldaan, wil een organisme in staat zijn om een nieuwe voedselbron aan te boren, is enige flexibiliteit in zijn ‘bouwplan’. Of het succesvol veroveren van een nieuwe voedselbron resulteert in (a) een nieuwe soort, gespecialiseerd in de nieuwe voedselbron (b.v. grote, snelle vis) of (b) in de oude soort die zijn repertoire heeft uitgebreid en nu zowel de oude (b.v. kleine, langzame insecten) als nieuwe voedselbron (grote, snelle vis) aankan, hangt af van het voortplantings systeem. Alleen als de individuen, die de nieuwe voedselbron kunnen benutten, op de een of andere manier op een andere plaats of tijdstip paaien (non-random paaien) en dus reproductief geïsoleerd raken van de rest, kan er een nieuwe soort ontstaan. Als de viseters en de insecteneters met elkaar blijven paaien (random paaien) kan er geen nieuwe soort ontstaan. Kennis van de voortplantings strategieën van de Tana-barbelen was dus van cruciaal belang om te kunnen begrijpen hoe deze 15 soorten zo snel konden evolueren uit één voorouderlijk barbeelsoort.

Zoals gebruikelijk bij vele andere karperachtige vissen, migreren de zeven zilverkleurige, pelagische barbeelsoorten tijdens het paaiseizoen naar hun geboorte rivier ('homing'). Deze soorten zijn tijdens het afpaaien voor een groot deel gescheiden in plaats en/of tijd op de stroomopwaarts gelegen paaigronden. In migrerende zalmen is aangetoond dat 'homing' de ontwikkeling van reproductieve scheiding in plaats en/of tijd tussen populaties, bevordert. Binnen de rivier-paaiende barbelen is het genetisch vastleggen van morfologische aanpassingen aan b.v. de verschillende voedselbronnen naar alle waarschijnlijkheid bevordert door deze voortplantingsstrategie. Voor het onstaan van het Tana-meer was de voorouderlijke (oer)barbeel waarschijnlijk al opgedeeld in sub-populaties, m.a.w. elke sub-populatie paaide in ander riviertje en/of tijdstip. Na het ontstaan van het Tana-meer kwam er opeens een veelvoud aan alternatieve voedselbronnen en habitats beschikbaar voor de rivierpaaiende voorouderlijke barbeelpopulatie. Aangezien het paaien in de voorouderlijke barbeelsoort niet random plaatsvond, werd er dus voldaan aan een van de eerder beschreven voorwaarden voor het ontstaan van een nieuwe soort. Morfologische aanpassingen aan de nieuwe voedselbronnen in het meer werden waarschijnlijk snel genetisch vastgelegd in de subpopulaties van de voorouderlijke barbeelsoort wat uiteindelijk resulteerde in een deel van de huidige (verschillende) barbeelsoorten.

De piek in gonaden ontwikkeling van acht donker kleurige barbelen uit de oeverzone, valt in dezelfde periode als de rivier-paaiers. Deze acht soorten vormen echter geen scholen in de riviermonden tijdens het paaiseizoen en migreren niet naar de stroomopwaarts gelegen paaigronden. Onze hypothese is dat deze acht soorten in de loop der tijd een nieuwe paai strategie hebben ontwikkeld en in het meer paaien. Deze soorten hebben zich succesvol aangepast aan het leven in het Tana-meer en volbrengen hun hele levenscyclus, inclusief de voortplanting, in het meer zelf. Dit idee wordt ondersteund door het feit dat regelmatig paairijpe vrouwtjes van deze soorten werden gevangen in de oeverzones van het meer, ver verwijderd van de riviermonden. Het meer-paaien binnen de soortenzwerm is waarschijnlijk geevolueerd als gevolg van het specialisatie in verschillende ecologische milieus, m.a.w. het divergeren van 'oever' en 'open-water' vormen. Dergelijke ecologische opsplitsingen tijdens soortsvorming zijn vaker waargenomen bij andere visfamilies. Verdere soortsvorming binnen de littorale meerpaaiers en de pelagische rivierpaaiers werd hoogstwaarschijnlijk gedreven door competitie om voedsel en de daaruit voortvloeiende voedsel specialisaties. Deze opeenvolging van in eerste instantie opsplitsing in habitats, gevolgd door opsplitsing in voedsel specialisaties binnen elk habitat afzonderlijk, is ook aangetoond tijdens de soortsvorming van andere visfamilies in zoetwatermeren.

De genetische variatie van de kleine en grote barbelen uit het Tana-meer en van grote barbelen in andere meren en rivieren in Ethiopië werd onderzocht aan de hand van cytochroom *b* sequenties. De kleine, pelagische *B. tanapelagius* is waarschijnlijk recentelijk geëvolueerd uit de kleine bentische *B. humilis*, m.a.w. wederom een voorbeeld van ecologische opsplitsing tijdens soortvorming binnen een meer.

The genetische variatie van het cytochrome *b* gen tussen de grote barbelen uit het Tana-meer was erg laag. Het onvermogen om aan de hand van cytochroom *b* sequencties de verschillende soorten te onderscheiden duidt op een zeer recente oorsprong van de soortenzwerm in het Tana-meer. Aan de hand van voorzichtige schattinggen zou de leeftijd van de soortenzwerm in het Tana-meer ergens tussen de 10.000 en 700.000 jaar oud moeten liggen.

Exploitatie

Tot 1986 werd er in het Tana-meer voornamelijk gevist voor levensonderhoud door een kleine groep vissers (Woito). Deze vissers vingen vanuit papyrus-bootjes voornamelijk tilapia (*Oreochromis niloticus*) dicht bij de oever. In 1986 werd er met steun van buitenlands NGOs modern vistuig (kieuwnetten) en gemotoriseerde boten geïntroduceerd. De drie belangrijkste commercieel interessante vissoorten zijn de grote barbelen, tilapia en de Afrikaanse meerval (*Clarias gariepinus*). Het doel van ons onderzoek was om het effect van deze nieuwe kieuwnetvisserij op de ontwikkeling van de drie commerciële vissoorten in kaart te brengen. Gedurende 1999-2001 werden de vangsten van de commerciële visserij bemonsterd en werd de dichtheid barbelen, tilapia en meerval in de Golf van Bahar Dar bepaald. Deze twee monsterprogramma's waren in 1991-1993 ook al uitgevoerd door Tesfaye Wudneh. Op deze manier kon er een goed beeld worden verkregen van de veranderingen in de visserij en de vispopulaties gedurende de jaren '90.

In 1991-1993 werd er voornamelijk gevist in de Golf van Bahar Dar (71% van de totale vistrips), de vangst bestond voor een derde uit barbelen, tilapia en meerval. In 2001 was de visserij naar het noorden van het meer verschoven, 41% van de totale vistrips vond plaats langs de noordoostelijke oever van het Tana-meer en de vangst bestond voor meer dan de helft uit tilapia. De toename van tilapia in de vangst kwam deels doordat de vissers actief viste in gebieden waar veel tilapia voorkomt. De hoeveelheid barbelen in het Tana-meer nam gedurende de jaren '90 echter dramatisch af (ongeveer 75%!). De barbelen bleken enorm gevoelig voor exploitatie door de nieuwe kieuwnet visserij. De barbelen zijn ecologische specialisten met een nauwe 'niche' en worden pas op late leeftijd geslachtsrijp. Tijdens het korte paaiseizoen in augustus-september vormen ze grote scholen in de monding van de instromende rivieren en zijn dan een gemakkelijke prooi voor de vissers. De helft van de jaarlijkse hoeveelheid gevangen barbelen wordt opgevist in deze twee maanden. De gevolgen van het wegvangen van de Tana-barbelen voordat ze zich kunnen voortplanten wordt duidelijk geïllustreerd door de bijna complete afwezigheid van juvenielen in de barbeelpopulatie aan het eind van de jaren '90. De ecologisch 'flexibele' tilapia en meerval blijken veel beter bestand tegen de toegenomen visserijdruk sinds de introductie van de moderne, commerciële kieuwnetvisserij.

Vispopulatie's kunnen ook enorm fluctueren door verandering in hun omgeving (temperatuur, zuurstofgehalte etc). Het Tana-meer is echter een stabiel aquatisch systeem en we hebben geen veranderingen in het abiotisch milieu kunnen waarnemen in de jaren '90. De meest waarschijnlijke verklaring voor de dramatisch achteruitgang van de barbelen blijft daardoor de negatieve impact van de commerciële kieuwnet visserij door het overvissen van de paaipopulaties. Om te voorkomen dat deze unieke diversiteit aan barbelen in de nabije toekomst uitsterft is het van buitengewoon belang dat er restricties worden opgelegd aan de commerciële kieuwnet visserij om zo de paaipopulaties te beschermen en de aanvoer van juvenielen te garanderen.

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THE B.E.S.T.

Curriculum vitae

Martin de Graaf werd geboren op 23 juni 1971 te Arnhem. In 1989 begon hij met de studie Biologie aan de Rijksuniversiteit Groningen (RuG). Tijdens de doctoraalfase heeft hij stage gelopen bij het Australian Institute of Marine Science en afstudeeronderzoek verricht bij de vakgroep Mariene Biologie (RuG) en Bamfield Marine Station (Bamfield, Vancouver Island, Canada). Beide afstudeerprojecten resulteerden in wetenschappelijke publicaties, de stage in Australië was o.a. het begin van de vriendschap met Erika. In september 1995 studeerde hij af en in december van hetzelfde jaar vertrok hij naar Bonaire (Nederlandse Antillen). In Bonaire verrichtte hij onderzoek naar het voortplantingsgedrag van koralen en de biologie van juveniele papegaaivissen. Hij voorzag in zijn onderhoud met het ontwikkelen en doceren van biologie cursussen aan (duik)toeristen. In mei 1998 werd hij aangesteld als onderzoeker-in-opleiding bij de leerstoel groep Experimentele Zoölogie (Wageningen Universiteit). Na wat 'probleempjes' aan het begin van het *Barbus*-project in Bahar Dar, is hij er samen met Eshete Dejen in geslaagd een geweldig team van Ethiopiërs (BEST = *Barbus* Ecology Survey Team) samen te stellen waardoor het verzamelen van de onderzoeksgegevens op een gegeven moment bijna 'automatisch' verliep en er een fantastische sfeer heerste binnen de groep. Het onderzoek van Martin resulteerde tot op heden in meerdere publicaties in internationale vakbladen. De resultaten van het uitgevoerde onderzoek staan beschreven in dit proefschrift.

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