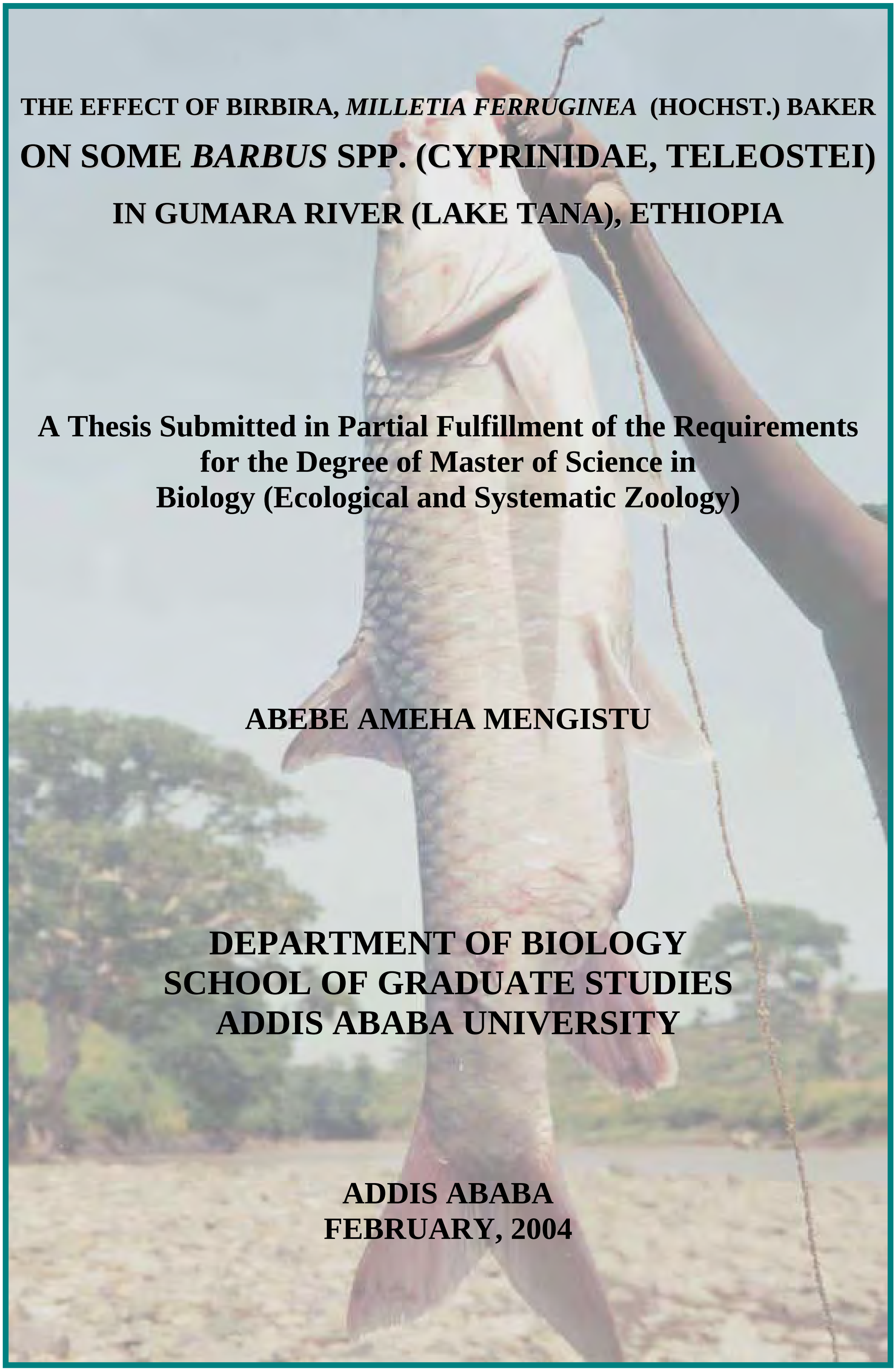

THE EFFECT OF BIRBIRA, *MILLETIA FERRUGINEA* (HOCHST.) BAKER ON SOME *BARBUS* SPP. (CYPRINIDAE, TELEOSTEI) IN GUMARA RIVER (LAKE TANA), ETHIOPIA

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Ecological and Systematic Zoology)

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Dedication

Especially to Tiruwork Mekuriya (Mami).

Also to: Mengistu Hailu (my grandfather),

Mulu Ambaw (my step-mom) and,

Ameha Mengistu (my father).

Declaration

I, the undersigned, declare that this is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

Abebe Ameha Mengistu

Signature ______________________

Addis Ababa University

Addis Ababa

February, 2004

This thesis has been submitted for examination with my approval as research advisor.

Dr. Abebe Getahun

Signature _______________________

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Abebe Ameha Mengistu

Department of Biology, 2004

Abstract

To assess the possible effect(s) of birbira, Milletia ferruginea *(Leguminosae) on the barbs,* Barbus *spp. (Cyprinidae) from Gumara River (Ethiopia), aquarium and field study was conducted during August to September, 2002. The effects of birbira at concentrations of 0.02, 0.04, 0.1, 0.2, 0.3 and 0.4 g/l were studied to conduct experiments on different species, sizes and sexes of fish. The amount of crude powders of birbira seeds actually applied by the fishermen at Dukalit stream (a tributary of Gumara River) was estimated to be about 0.4 g per liter of water. This amount was found to be excessive as only 0.02 g/l (a 5% dilution) was enough to bring about total fish kills in about an hour. It was observed that the amount of oxygen in the birbira solution remained almost intact after all of the fish died, supporting the idea that rotenone does not deplete oxygen dissolved in the water but affects mechanism of oxygen uptake by fish. The findings of the study revealed that when using birbira concentrations of 0.02 to 0.4 g/l, all of the fish died in about 30 to 60 minutes. Comparison of different species showed that* B. brevicephalus *and juvenile 'large' barbs (weighing < 50 g) had relatively better tolerance than had another close relative,* Varicorhinus beso *of similar weight. Female* B. brevicephalus *(usually ≥ 100 g) with running (ripe) gonads of tolerated the poison better than their male (usually < 100 g) counterparts. Fertilized eggs, presumed to have been spawned by* B. brevicephalus*, were collected from Dukalit stream and poisoned with 0.4 g/l birbira for 15 minutes, and then incubated in clean water. None of these eggs hatched while all of the controls (unpoisoned eggs) did after 3 days of collection from the* *river. The findings of this study are believed to lay a basis for further research and consequently to solve problems related to poisoning fish with birbira and/or other herbs on Gumara River and other waters.*

Key words/phrases: birbira (*Milletia ferruginea*); Gumara River (Lake Tana); 'large' *Barbus* spp.; survival time.

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Abstract

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1. Introduction

Ethiopia has a rich diversity of ichthyofauna in its lakes, rivers and reservoirs, although they are poorly known (Abebe Getahun and Stiassny, 1998). In addition, fishing is an age-old practice in these waters; and Lake Tana and its tributaries account for about half of the total water area of the country (Reyntjens *et al*., 1998). We find different species of fish in Lake Tana, *Barbus* Cuvier and Cloquet, 1816, *Oreochromis niloticus* Linnaeus, 1766, and *Clarias gariepinus* Burchell, 1822 being economically the most important (Abebe Ameha and Alemu Assefa, 2002). The ‘large’ barbs (*Barbus* spp.) migrate upstream to spawn during the main rainy season (Nagelkerke, 1997; Nagelkerke and Sibbing, 2000). Fishermen catch migrating fish using traditional fishing techniques (Reyntjens *et al*., 1998; Abebe Ameha and Alemu Assefa, 2002). One of the techniques that have been exercised for ages is poisoning the fish with powders of birbira (*Milletia ferruginea* (Hochst.) Baker) seeds (Rüppell, 1836 in Nagelkerke and Sibbing, 1996).

Using poisons is an age-old method of capturing fish worldwide; the most commonly used chemical being rotenone (Lagler, 1978). Birbira is an indigenous tree in Ethiopia and, among other uses, it is commonly used as a fish toxicant (Legesse Negash, 1995, 2002; MacLachlan, 2001). Despite its application on Gumara River and its tributary streams for ages, we still find spawner barbs performing their annual migration (Nagelkerke and Sibbing, 1996; Abebe Ameha and Alemu Assefa, 2002). Whether or not these fish or their eggs have developed an adaptive mechanism to tolerate the poison is not known. Or else, some species already could have been wiped out even before they are known to science. Studies have shown that the ingredient responsible for the toxicity of birbira seeds could be rotenone, a widely known herbal pesticide and piscicide extracted from various tropical plants (MacLachlan, 2001). Hence, the use of birbira to intoxicate fish is to be discouraged as it could harm fish and other non-target organisms. Alternatively, it could be possible to detoxify the poison based on determination of the median lethal concentration and well-planned procedures. Some believe the effect of other destructive fishing gear is more serious than that of birbira; however, this can not be ruled out unless established by research.

The present study deals with the effect of birbira seed powders on some 'large' barbs in Gumara River, a tributary of Lake Tana. Special attention has been given to the killing effect of birbira. Attempt is made 1) to estimate the concentration of birbira used by fishermen, and experimentally investigate its efficacy on some species of fish sampled from the river and, 2) to study, in aquaria, the effect of birbira concentrations lower than that used by fishermen, and compare the response (time of death) of various groups of fish. *B. brevicephalus* Nagelkerke and Sibbing, 2000 is the target species in this study as it is vulnerable to poisoning every year. Some other species also are considered for comparison purpose. The study was conducted on an outdoor experimental setup by the riverside. This was hoped to simulate major natural conditions. The duration of the field work was from August to September 2002 in the main rainy season. We found Wanzaye as the ideal site for conducting the poisoning experiment, as it is one of the places where the problem exists and where easy access to resources is possible. We used laboratory, aquaria and other experimental facilities previously supplied to the Fisheries Research Center in Bahir Dar by the Lake Fisheries Development Project (LFDP) and Wageningen University. Besides the major objectives set for this study, the present findings could also lay a basis for further research, as there is almost no published scientific evidence to date with regard to the effect of poison fishing on the ichthyofauna of Ethiopia.

2. Objectives

2.1. General objective

To lay a basis for avoiding or minimizing the dangers of fish poisoning on the management of the fisheries and conservation and production of fish resources in Ethiopia in general, and of the *Barbus* spp. of Lake Tana (and Gumara River) in particular.

2.2. Specific objectives

1) To determine the time it takes to kill 'large' *Barbus* spp. from Gumara River exposed to different concentrations of powders of birbira seeds, and

2) To see if there exists any significant difference in the responses of different groups (species, sizes, or sexes) of 'large' *Barbus* spp. to different concentrations of birbira solution.

3. Literature Review

3.1. The ‘large’ *Barbus* spp. of Lake Tana

Fishermen as well as researchers have known the ‘large’ hexaploid barbs of Lake Tana, for a long time. Although there are no indications as to when fishing actually started on Lake Tana and rivers around it, scientific description of these fish dates back to the nineteenth century (Rüppell, 1836 in Nagelkerke, 1997). Recently, 15 distinct species of ‘large’ *Barbus* have been identified from Lake Tana, and these are believed to form a unique *Barbus* species flock (Nagelkerke *et al*., 1995; Nagelkerke, 1997; Nagelkerke and Sibbing 2000). However, the criteria for a species flock (Greenwood, 1984) do not seem to have been met. The status as distinct species of these fish is also debated by other workers (Mina *et al.*, 1996; Dgebuadze *et al.*, 1999).

The ‘large’ barbs (Fig. 1) are referred to as “large” to distinguish them from the small-sized (max. adult fork length, FL < 12 cm), diploid, ‘small’ *Barbus* spp; there are recent studies made on the evolution, ecology and fisheries potential of the ‘small’ barbs of Lake Tana (De Graaf, 2003; Eshete Dejen, 2003). The ‘large’ barbs also differ from their tetraploid European counterpart (*B. barbus* L., 1758). Instead, the ‘large’ barbs of Lake Tana seem to be more closely related to another hexaploid, *V. beso* (Nagelkerke, 1997).

Apparently, most of the ‘large’ barbs migrate upstream to spawn on the tributary rivers, one of which is Gumara, during the main rainy season (Mina *et al.*, 1996; Nagelkerke and Sibbing, 1996; Nagelkerke, 1997; Mina *et al.*, 1998; Dgebuadze *et al.*, 1999; Abebe Ameha and Alemu Assefa, 2002). There could also be some permanent resident species in the river, although this is not supported by published information. At this time, fishermen employ traditional fishing techniques (at the river mouth and upstream) to catch these fish for commercial as well as household consumption purposes. The ‘large’ barbs constitute the bulk of the commercial catches from Gumara River (Index of Relative Importance, IRI = 96.9%).

Fig. 1. A ‘large’ *Barbus* specimen (mature, running female, fork length = 78.7 cm, total weight = 5.5 kg) from Gumara River.

Although the catches from Gumara contribute to about 5% of the total annual catch from Lake Tana, 50-100% of them are ripe (running) adults (Abebe Ameha and Alemu Assefa, 2002). The major fishing techniques used on Gumara River include scoop nets (plus fencing the river), hook and line, and poisoning with powders of birbira seeds. Recently, the total annual catch of barbs from Gumara River has decreased tremendously; many years’ of data on average weight and catch per unit effort also indicate declining catches suggesting the danger cast on the fishery as well as the resource and its biodiversity (Abebe Ameha and Alemu Assefa, 2002). This could be a result of the combined effect of the various fishing techniques employed.

3.2. Birbira (*M. ferruginea*)

3.2.1. Description

The genus *Milletia* (Leguminosae, Papilonoideae) constitutes about 200 species in tropical and subtropical Africa, Asia and Australia (Thulin, 1983). The seeds and roots of these plants are used as insecticides and piscicides in many parts of the world, and rotenone is responsible for their toxicity (Holman, 1940 in Amha Bekele, 1988; MacLachlan, 2001).

M. ferruginea (Amharic: ‘Birbira’; spelled as ‘Berbera’ in Amha Bekele (1988), ‘Brbrra’ in MacLachlan (2001)) is an indigenous plant species found only in Ethiopia. It occurs commonly between 1,000 and 2,500 m above sea level (Legesse Negash, 1995; MacLachlan, 2001). Two subspecies of the plant are known to occur: subspecies *ferruginea* from northern Ethiopia, and subspecies *darassana* occurring in the southern provinces (particularly in Sidamo). The hybrid of the two subspecies is believed to be found in the central and western parts of the country (Thulin, 1983). The tree is umbrella-shaped or flat-topped, and grows up to a height of 25 m (MacLachlan, 2001) to 35 m (Bekele Jembere, 2002). The seed is dark red to orange colored, somewhat like a flattened disk or almost square with rounded corners (MacLachlan, 2001).

3.2.2. Habitat and uses

Birbira is commonly found in areas where water is accessible, such as near streams or in rain forests (MacLachlan, 2001). This easy access of fishermen to birbira too could have contributed to its wide application in fishing. The crushed and pulverized seeds serve as fish poisons (Legesse Negash, 2002). As a nitrogen-fixing species, birbira has been integrated with crops and coffee growing in Ethiopia (MacLachlan, 2001).

Table 1. Prioritized list of benefits accrued from retaining *M. ferruginea* trees on farmlands in the Gedeo, southern Ethiopia (Legesse Negash, 2002).

Benefits	Number of respondents	Percentage of respondents
Increased crop yield	170	100
Soil fertility improvement	170	100
Fodder for ruminants	170	100
Erosion control	170	100
Fuelwood supply	167	98
Shade for coffee trees	165	97
Bee forage	158	93
Cash generation through sell of fuelwood	128	75
Medicinal uses (includes fishing)	89	52
Building material	5	3
Tool handles	2	1
Fencing	2	1

The soil near birbira tree is found to be rich in nutrients (Tadesse Hailu *et al*., 2000). Its wood is used for firewood, the flowers serve as feed for bees; fodder for ruminants, erosion control, and shade are its other uses (MacLachlan, 2001). Legesse Negash (2002) presented a reproduced summary of the uses of birbira trees based on interviews made in Gedeo, southern Ethiopia (Table 1).

3.3. Rotenone

3.3.1. Description

Rotenone is a crystalline, water-insoluble, poisonous heterocyclic compound, $C_{23}H_{22}O_6$, used chiefly as the active ingredient of certain insecticides and in medicine in the treatment of chiggers and scabies (Stein, 1973). It is a naturally occurring substance found in the roots and stems of several tropical plants such as Jewel Vine (*Derris* spp.), Lacepod (*Lonchocarpus* spp.), and Hoary Pea (*Tephrosia* spp.) (Brunson, 1954). It is also found in the genera *Milletia*, *Mundulea*, *Spoatholobus*, and *Pachyrhizus* (Hayes, 1982; Gosselin *et al*., 1984).

One of the sources for rotenone from Ethiopian plants is *M. ferruginea* (Nabiel, 1992). Phytochemical studies on the seeds of *M. ferruginea* indicated the presence of three rotenoids: rotenone, tephrosin and dehydrodeguelin (Clarke, 1943 in Amha Bekele, 1988). Bekele Jembere (2002) also has shown that among the different plant materials, it was the seeds of *M. ferruginea* that showed significant toxicity effect on the maize weevil, *Sitophilus zeamais* (Motschulsky).

Rotenone (Fig. 2) is a colorless, crystalline solid with a melting point of 165-166 ^{o}C, and has a very low solubility in water (0.00002 g/100 ml) at ambient temperatures (WHO, 1992; Bekele Jembere, 2002; Cornell University, 2002). It is soluble in acetone, carbon disulfide, ethyl acetate and chloroform. It is less soluble in ether, carbon tetrachloride, and petroleum solvents. Solutions are readily oxidized, in the presence of light and alkali, to products with weaker insecticidal properties (WHO, 1992).

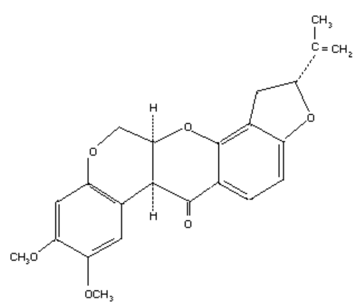


Fig. 2. Chemical structure of rotenone (chemical formula: $C_{23}H_{22}0_6$) (WHO, 1992)

3.3.2. Uses

Rotenoids, the rotenone-related materials (Dewick, 1982), have been used as crop pesticides since 1848, when they were applied to plants to control leaf-eating caterpillars; however, they have been used for centuries before that (at least since 1649) in South America to catch fish by paralyzing and causing them to surface (Ware, 1988).

In the U.S.A., rotenone and antimycin are registered pesticides for eradication of fish. Only rotenone is economically feasible for complete eradication of fish populations (Ware, 1988), and hence is a more commonly used compound (Brunson, 1954). Annual usage of rotenone reaches 50,000 to 120,000 lb (or 22,680 to 54, 430 kg) (Cornell University, 2002). In order to be effective, rotenone concentration that is added to a lake or pond must be from 0.5 to 5.0 ppm of a product containing only 5% rotenone in water (Prentiss Incorporated, 2000). In recent years, agricultural uses of rotenone have declined in the face of new synthetic pesticides that can be mass produced but fishery needs have continued. It is a selective non-systemic insecticide used in agriculture, livestock, pets and household (Cornell University, 2002); it has also been used on humans for external treatment of chiggers (2% lotion) and scabies (10% emulsion) (WHO, 1992). Since rotenone is not persistent in the environment, and because fish reproduce at a much slower rate than insects, acquired resistance has not been a problem (Mbi *et al*., 1990).

3.3.3. Mechanism of action

Rotenone works by inhibiting a biochemical process in the fish cells, resulting in an inability of fish to use oxygen in the release of energy during normal body processes (Brunson, 1954; WHO, 1992; Cornell University, 2002). In effect, the fish suffocate due to lack of oxygen. Rotenone inhibits the oxidation of NADH to NAD, and blocks the oxidation by NAD of substances such as glutamate, alpha-ketoglutarate and pyruvate (Goodman *et al*., 1985; Rockstein, 1978). Concerning tolerance, however, different species of fish respond variously to rotenone (Brunson, 1954).

3.3.4. Toxicity

Toxicity effects of insecticides to higher animals could be acute oral, dermal, inhalation, or chronic toxicity (Fenemore and Prakash, 1992). Rotenone is highly toxic for aquatic life, especially to fish (Pesticide Information Profile, 1993); most values for the 96-h LC_{50} for different fish species and for daphnids lie in the range of 0.02-0.2 mg/liter (WHO, 1992). In general, most common aquatic invertebrates (such as snails and clams) are less sensitive than fish to rotenone but some zooplanktons are equally sensitive. With respect to fish, trout and salmon are the most sensitive, while catfish are the most resistant. Because fish absorb rotenone rapidly through their gills, they absorb a lethal dose more quickly (Prentiss Incorporated, 2000; American Fisheries Society, 2001). Fish eggs are much more resistant to rotenone treatments than larval or adult stages (Prentiss Incorporated, 2000). For example, newly fertilized rainbow trout eggs were 41 to 106 times more resistant; salmon eggs are 10 times more resistant than the fish, and carp eggs are 50 times more resistant. While adult frogs and other amphibians would not be seriously affected, tadpoles and juvenile salamanders probably would be killed. Using levels of up to 75 ppm rotenone in the daily diet of rats through two generations showed no effects on sexual performance, fertility, pregnancy, fetal development, or on the ability to sire or to produce normal offspring (Prentiss Incorporated, 2000). Also, in two-year feed studies, there is evidence of carcinogenic activity of rotenone for male rats; however, there was no evidence of carcinogenic activity for male or female mice fed diets containing 600 or 1200 ppm rotenone for two years (TR-320, 1988).

Laboratory studies on intravenous administration of rotenone in rats revealed the appearance of limb tremors and occasional rigidity, symptoms like that of Parkinson’s disease (Betarbet *et al*., 2000). Parkinson’s disease results in a lost function of the brain cells that produce dopamine, required to transmit signals in the brain (Giasson and Lee, 2000). However, results from a chronic feeding study with rats using rotenone found no Parkinson's-like anatomical or behavioral symptoms (Marking, 1988). Rotenone is safe for all livestock except swine; and its toxicity to humans is extremely low (Brunson, 1954). Normal exposure to rotenone in humans from its use in fisheries management would be ingestion, inhalation or through the skin, but not directly through the blood stream (American Fisheries Society, 2001). It is more toxic when inhaled than when ingested (National Library of Medicine, 1992); and it is hundreds of times more toxic intravenously than orally (Gosselin *et al.*, 1984). The mean lethal oral dose in humans is from 0.3 to 0.5 g/kg (Gosselin *et al.*, 1984). Fine powders are much more toxic than coarse particles (National Library of Medicine, 1992). Fats and oils increase absorption of rotenone (Gosselin, 1984). Rotenone is highly irritating to the skin in rabbits, and there is no evidence that rotenone causes mutations or chromosome damage. Rotenone is non-toxic to plants (Hayes, 1982; Windholz, 1983; Reynolds, 1989; WHO, 1992) as well as to bees (The Agrochemicals Handbook, 1991).

3.3.5. Detoxification and elimination

Fish, insects, birds and mammals have natural enzymes to detoxify sub-lethal amounts of rotenone. If orally administered into mammalian body, it could go on biotransformation, or metabolic disposition (Griffiths, 1982). The compound is metabolized by the mammalian liver; most of the ingested compound is metabolized and excreted by the liver and kidney (Rahde, 1989; Finlayson *et al*., 2000), or eliminated in the faeces (WHO, 1992). Surviving fish quickly eliminate rotenone residues from their bodies. Cooking destroys rotenone so there would be a further loss of any residues during cooking. However, since the maximum acceptable residue level permitted in fish for human consumption is not established, human consumption of fish killed by rotenone cannot be recommended. If fish are removed from treated waters quickly enough, they can often be revived by placing them in fresh water

(Brunson, 1954). To neutralize rotenone, charcoal filtration, potassium permanganate or chlorine can be used at 1:1 or 2:1 ratio with concentration of rotenone applied (Brunson, 1954). Toxicity can also be reversed by placing the affected fish in a water solution of methylene blue.

In 1981, the United States EPA concluded that “there was no reason to restrict the use of rotenone in waters intended for irrigation, livestock consumption, and recreational swimming uses” (Cornell University, 2002).

3.3.6. Persistence

Rotenone is an unstable compound that rapidly breaks down when exposed to the environment (Finlayson *et al*., 2000) with a half-life of 1-3 days for both aerobic aquatic and anaerobic aquatic soils (Ware, 1988; Cornell University, 2002). It is ultimately converted to carbon dioxide and water. Heat, light, oxygen, and alkalinity affect this process (Brunson, 1954). In natural waters, factors like the presence of organic debris, turbidity, lake shape and depth, dilution by inlets and runoff, and the dosage used also influence the degradation rate. In most types of soils, rotenone leaches up to 2 cm, an exception being in sandy soils where it can penetrate down to 8 cm (Cornell University, 2002).

4. Materials and methods

4.1 The study area

4.1.1. Location

Gumara is one of the big perennial rivers flowing into Lake Tana. It is situated on the eastern side of the lake bordering two Woredas: Fogera and Dera (in South Gondar). The local people tell that its source is at the Debre-Tabor massifs (somewhere in Mount Guna). The river is located at 37°30'E, 11°53'N at Megenagna (the river mouth) and at 37°45'E, 11°45'N at Wanzaye (about 40 km upstream) (Fig. 3). The Bahir Dar-Gondar highway crosses Gumara about 25 km upstream from the river mouth. Some of Gumara's tributaries include rivers and streams like Chan, Dukalit, Kizin and Guanta. Far upstream, Gumara runs through the highlands, and then falls down into a deep rocky gorge to form its biggest waterfall: Durrba. Just below Wanzaye area, the river runs through the Fogera Plain until it joins the lake between Christos Semra and Tana Kirkos.

4.1.2. Morphometry

Gumara River has a length of about 44 km from Durrba Fall to the river mouth, and a maximum width of about 30 m (during the rainy season). Durrba Fall (Fig. 5B) is Gumara's biggest waterfall dropping down to about 15 m, and having upper and lower parts. There are patchy pools upstream, one of them being Rga-rga (about 14 m deep) at Wanzaye. The rhithron (upper, steep, fast flowing part of the river) has rocky banks, while the potamon (lower, flat, sluggish part) is subject to riverside crop farming. The substratum is covered with pebbles (upstream) and/or mud (downstream). Eroded soil from the nearby lands makes the water highly turbid especially during the rainy season. This has led to very high siltation at and near the river mouth, and consequently to the formation of (about 2 km) newly inundated fertile land in the inshore area of Lake Tana.

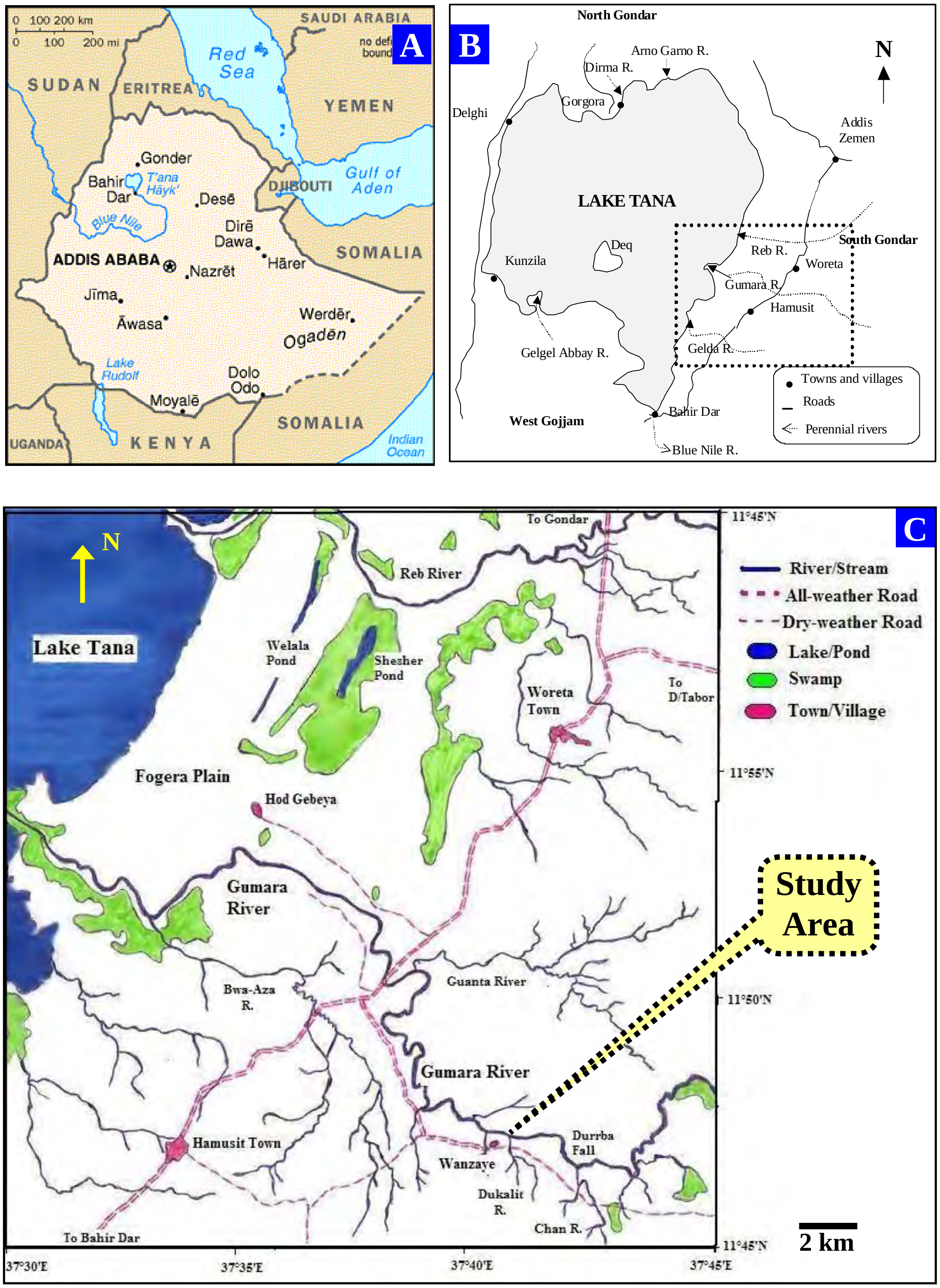


Fig. 3. Map of (A) Ethiopia, (B) Lake Tana, and (C) Gumara River showing the study area at Wanzaye (Source: A) Britannica, 2003, B) LFDP, 1997, and C) reproduced from Ethiopian Mapping Agency, 1987).

Dukalit Stream: Dukalit is one of the streams joining Gumara at Wanzaye (Fig. 4). It has a length of about 225 m from its waterfall to Gumara with a maximum width of about 8 m. It is one of the major spawning grounds for some barbs of Lake Tana. The substratum is covered with stones and pebbles; bushy and grass vegetation plus some farmland compose the bank and catchment area. Underneath Dukalit Fall (Fig. 5A) there is a small (mean max. depth 2 m) pool (described in Section 4.4. below) that further runs down (to Gumara) with a maximum depth of 50 cm during the main rainy season. During the dry season, this part of Dukalit dries up and some water drains under the riverbed. The water is relatively clearer and warmer as compared to the more turbid and colder conditions in Gumara.

Fig. 4. Rear and front views of Dukalit-Gumara interface at Wanzaye (September, 2002). The clear and shallow water of Dukalit joins the turbid and deeper water of Gumara.

Fig. 5. (A) Dukalit, and (B) Durrba Falls where powders of birbira seeds are applied to stupefy and catch the barbs of Gumara River (Lake Tana). Note that Durrba has a larger volume of water and the fishermen apply about 10 times more powders of birbira seeds than that used at Dukalit.

4.1.3. Climate

Gumara River area has, as other parts of Lake Tana, a tropical highland climate, with moderated temperatures, because of the altitude (Nagelkerke, 1997). According to 10 years (1993-2002) climatic data (National Meteorology Service Agency, 2003), the main rainy season (*kiremt*) lasts from June/July to September/October, with a maximum monthly rainfall reaching up to 550 mm (July 2000). The dry season (*bega*) is long, extending from October/November to April/May, and interrupted by a small rainy season (*belg*). The total annual rainfall is below 1700 mm. Mean minimum air temperature ranges from 8.4 ^{o}C (November 2000) to about 18 ^{o}C (October 2001); the mean maximum air temperature range is from 24 ^{o}C (July 1994) to about 34 ^{o}C (April and May 2002).

4.1.4. Vegetation and macrofauna

The catchment area in the upper part is covered with ruins of shrubs and a few indigenous trees that are leftover from the once forested area; the lower part is grass-covered with some patches of eucalyptus tree. Some major mammalian fauna that depend on Gumara River include Vervet (Grivet/Green) Monkey (*Cercopithecus aethiops* L., 1758), Olive (Anubis) Baboon (*Papio cynocephalus anubis* L., 1766), Golden (Common/Asiatic) Jackal (*Canis aureus* L., 1758), Spotted (Laughing) Hyena (*Crocuta crocuta* Erxleben, 1777), Rock Hyrax (*Procavia capensis* Pallas, 1766), Common Hippopotamus (*Hippopotamus amphibious* L., 1758), and domestic cattle. There is also report by local people of encountering a mammal fitting with the description of otters. The avifauna include Egyptian Goose (*Alopochen aegyptiacus* L., 1766), African Darter (*Anhinga rufa* (*melanogaster*) Daudin, 1802), White (Great White) Pelican (*Pelecanus onocrotalus* L., 1766), African Fish-Eagle (*Haliaeetus vocifer* Daudin, 1800), Hammerkop (*Scopus umbretta* Gmelin, 1789), and others. The Nile Monitor (*Varanus niloticus* L., 1766) is the biggest reptile in the area. Identification of these mammals, birds and reptiles was based on Alden *et al* (2001). The major fish fauna of Gumara River and its mouth include 'large' and 'small' barbs (*Barbus* spp.), Beso (*V. beso*), African Catfish (*C. gariepinus*), *Garra* Hamilton, 1822, and Nile Tilapia (*O. niloticus*).

4.1.5. Importance for fisheries

Gumara River in general is one of the major spawning grounds for the 'large' barbs (*Barbus* spp.) of Lake Tana; Dukalit in particular is one of the major breeding sites for 'Shorthead Barb' (*B. brevicephalus*) in September. The local people use traditional fishing techniques such as gill nets, scoop nets, cast nets, fish traps, hook and line, and fish poisoning (with birbira) to catch mainly the 'large' barbs. The catches are marketed mainly at the nearest towns of Woreta and Hamusit. Recently, a drastic decline in the catch levels is reported and several factors are thought to be responsible for this, poisoning with birbira being one of them (Abebe Ameha and Alemu Assefa, 2002). Fishing with birbira on Gumara River is an occasional (but regular) activity exercised by people coming from the highlands (personal observation).

4.2. Experimental setup

Toxicology study is risky to be experimented in natural water bodies directly as it could result in unpredictable deleterious effects on the organisms living inside. Hence, the current experiment was conducted in the field, but in aquaria set by the riverside. An ideal experimental design in the laboratory would have incorporated well-controlled hydrological conditions (water temperature, dissolved oxygen, etc.), predetermined experimental groups (species, size categories, etc.), equal number of test organisms per treatment, fixed number of replications, etc. In an effort to satisfy these criteria, the present study has faced the following limitations:

1) relatively short duration of *Barbus* migration that does not allow to get large sample sizes and treat them in successive concentrations,

2) unpredictable and limited availability of the various species and/or sizes of fish during the migration period and,

3) shortage/lack of equipment and facilities (thermostat, aquaria, transportation, etc.).

To surpass these limitations, the experimental study was designed to simulate the actual mechanism exercised by the fishermen during application of birbira into the river.

We constructed a 3m x 3m shelter (Fig. 6) by the riverside of Gumara at Wanzaye to protect the aquarium setting from sunlight, wind, rain and other possible dangers by cattle and the likes. The distance from the shelter to the fishing sites ranged from 2 m (at Gumara River) to 200 m (at Dukalit Fall). The shelter was made of dry eucalyptus wooden poles, dry papyrus mats and polyvinyl sheets joined with iron nails and ropes. We set three glass aquaria (each with size of 70 cm x 30 cm x 40 cm) on cement blocks (to elevate from the ground), and arranged them along their length for easy observation during experimentation.

Fig. 6. Aquarium setting in a shelter built by the side of Gumara River (at Wanzaye during the main rainy season in 2002) for the experiment on poisoning fish with powders of birbira seed.

It is assumed that the fish migrate far upstream to spawn due to demands such as clear, oxygenated water (Nagelkerke and Sibbing, 1996). Hence, to fulfill this requirement, three portable oxygen pumps (NS-POCKET Dry Battery Pump, and ATOM-2) were put beneath each aquarium. Air passes from the pumps to the aquaria through a plastic tube at the end of which is attached an air stone.

During the experiments we added 48 liters of clear (unturbid) riverine water into each aquarium. One of the aquaria was for control while the other two were treatment aquaria with different concentrations of the toxic material. Other important materials including oxygenmeter and thermometer (OxyGuard), digital stopwatch (CASIO-DATABANK 300), digital balance (TEFAL, 4 kg, 0.1 g), measuring board (wooden, 100 cm), dissecting kit, and data collection forms were also prepared. A camcorder (JVC, VHS-ET) and photograph camera (KONICA Z-up 70) were prepared to record motion- and photograph pictures of the most important phenomena.

4.3. Collection and handling of fish specimens

The period of the experiment (August 10 to September 30, 2002) was in the main rainy season. Fish specimens were collected from Gumara and Dukalit rivers using scoop nets and seine. We used the traditional scoop net as well as our own modified version of (green colored) scoop net-like mosquito net (Fig. 5A) so as to catch small as well as big specimens. The seine was stretched wide open at its mouth and dragged slowly by two persons in pools of the river against the current. We repeated this procedure until enough number of specimens was caught for a particular test. The specimens were put in plastic buckets half-filled with river water, and aerated by portable pumps, and then transferred to temporary containers (with similar supplies) to be acclimatized to the aquaria conditions. After some minutes, selected specimens were transferred to test aquaria using dip nets. Specimens were handled as carefully as possible to avoid injuries and stress.

4.4. Preparation of powders and solutions of birbira seed

The intention in the experiment was to simulate the real situations of poisoning fish with powders of birbira seed. So, we tried to follow almost the same procedure as that of the traditional fishermen during preparation of birbira seed powder as well as application of the birbira solutions. Ripe seeds were collected from birbira trees around Hamusit town (South Gondar) in April 2002, dried in shaded area, and stored at room temperature until August 2002. Then the dried seeds were pound into fine powder with locally made wooden mortar and pestle. To maintain the toxic power of the powder, we performed the treatments within 24 hours of pounding, that is following the technique used for birbira in Muzeyi (2002).

During the actual birbira application, fishermen add 2 ‘kuna’ (or about 10 kg) of fine seed powder into the pool just below Dukalit Fall. We gathered morphometric data of the pool, which is one of the major sites for birbira application by the fishermen. The pool’s surface assumes a kidney shape (composed of two somewhat circular shapes), and its basin has the shape of an inverted cone. The radius (r) of each of the two circles was 250 cm and the respective maximum depth during high-water and low-water levels averaged at 200 cm (=h). Then the mean volume of the water that could be retained in each of the basins at a particular time was estimated using the formula $V = \pi r^2 h/3$. Doubling the result gave us the pool’s total volume, which was found to be about 26,180 liters of water. Thus, the actual birbira concentration applied by the fishermen at Dukalit fall was found to be 10,000 g / 26,180 liter (= about 0.40 g/l). This value is referred to as the 100% concentration (C_{100}). As fresh input of water from upstream and subsequent running of the poisoned water downstream dilutes the original concentration continuously, successive dilutions of C_{75}, C_{50}, C_{25}, C_{10}, C_5 and C_0 (control) were prepared. The respective concentrations (in g/l) for these dilutions were 0.30, 0.20, 0.10, 0.04, 0.02 and 0.

4.5. Testing procedure

In this study, juvenile ‘large’ barbs (JLB) are the ones that Nagelkerke (1997) and Nagelkerke and Sibbing (2000) stated as ‘large’ barbs that could not be identified externally as distinct species below a fork length (FL) of 12 cm. In a recent ecological study, Eshete Dejen (2003) also referred to these groups of barbs as “juvenile large barbs”. We also referred to adults of less abundant ‘large’ barbs (*B. acutirostris, B. intermedius* complex*, B. macrophtalmus, B. megastoma*, and *B. nedgia*) as other ‘large’ barbs (OLB). These fish have a maximum adult size usually greater than that for *B. brevicephalus*, which grows only up to a FL of 25 cm

(Nagelkerke, 1997). Hence, JLB and OLB are treated as separate groups (from *B. brevicephalus*, or *V. beso*) in interspecific comparisons.

4.5.1. Juvenile and adult fish

The time of death (t_d) was taken as a measure of the ultimate effect of poisoning fish with birbira solutions. Death (or failure), here, is defined as ceased fin, mouth, opercular and other external body movements, and can be ascertained by probing the body of the fish with a needle. Dissolved oxygen concentration, [DO_2], (mg/l) and water temperature (^oC) in each aquarium was measured just before adding the test animals (at the initial time, t_0) and after almost all of the poisoned fish failed (after 30 minutes, t_{30}). Other biological variables recorded include FL or total length (TL) (in mm), body weight (W: in g), sex (S), gonadal maturity (GM: 1-5, and 99) (Holden and Raitt, 1979), the concentration of the birbira solution in which each was treated (C: in g/l and in % of dilution), time (t: in min) of some pre-mortem activities (restlessness, inverted position), and time of failure. However, since all of the pre-mortem activities shown did not have a similar pattern (sequence) in all specimens, we did not consider such variables during data analysis. Different species, sizes and sexes of fish were tested in different dilutions until enough numbers of specimens were investigated.

4.5.2. Fish eggs

Some fertilized eggs were collected from Dukalit stream while *B. brevicephalus* was migrating up in huge numbers in September, 2002. There were no other species of fish in the stream, and hence the eggs are presumed to be those of *B. brevicephalus*; fertilized eggs were easily identified by their white dense core and a translucent peripheral part. The stream had a temperature and [DO_2] of 21.6 ^oC and 10.5 mg/l, respectively. We put the eggs in polyethene bags half-filled with fresh water from the river and aerated with pumps. These were immediately transported to the laboratory in Bahir Dar and treated with 0.4 g/l of aqueous solution of birbira for 15 minutes. Then we transferred them into aquaria containing fresh water from Lake Tana (average temperature = 21.3 ^{o}C, average [DO_2] = 10.2 mg/l); due to logistic problems, it was not possible to conduct this particular experiment at Wanzaye. The fate of the eggs (hatching, or not hatching) was recorded in the following days.

4.6. Data processing and analysis

Different species react differently to toxic substances (ILPI, 2003). Hence comparison of failure-time among JLB, *B. brevicephalus*, OLB, and *V. beso* was made at different concentrations. We compared the responses of the different sizes and sexes for *B. brevicephalus* at similar concentrations. Specimens belonging to the same species, size group, and sex were tested to see the effect of varied levels of concentrations of birbira. All statistical tests were made using independent t-tests and ANOVA at a significance level, α, of 0.05. When $p \leq \alpha$, we rejected the null hypothesis about the equality of the means. We also constructed survival curves (Fox, 1993; Pagano and Gauvreau, 1993) to show the trend in failure of fish poisoned with birbira. The survival function, $S(t)$, is the probability that an individual survives beyond time t. Equivalently, for a given t, $S(t)$ specifies the proportion of individuals who have not yet failed (died). Data analysis was performed using the statistical software SPSS 10.0 for Windows.

5. Results

5.1. General observations

5.1.1. Water temperature and dissolved oxygen concentration, [DO_2]

During the study period, the mean daytime water temperature (T) and [DO_2] for Gumara and Dukalit were 23.0 ^oC (S.D. 2.22, n = 36) and 8.8 mg/l (S.D. = 1.8, n = 28), respectively. In the aquaria, almost similar conditions were recorded during the experiment; that is, an average temperature of 23.1 ^oC (range 19.6-26.1 ^oC, S.D. = 1.49, n=42) and average initial [DO_2] of 7.7 mg/l (S.D. = 0.97, n = 25). As a range of 18.5-23.0 ^oC water temperature appears somewhat stable for the barbs of Gumara River (Alekseyev *et al*., 1996), we have considered the treatments made in successive dates as replicates for each level of dilution. The mean change (initial minus final) in the water temperature, ΔT, after 30 minutes of commencement of treatment was found to be -0.18 ^oC (S.D. 0.11, n = 42) for the aquaria with concentrations of 0.1 to 0.4 g/l, and -0.30 ^oC (S.D. = 1.7, n = 12) for the controls. The respective mean change (initial minus final) in dissolved oxygen concentration, $\Delta[DO_2]$, was found to be -0.56 mg/l (S.D. = 0.3, n = 25) for the aquaria with concentrations of 0.1 to 0.4 g/l, and -2.63 mg/l (S.D. = 1.1, n = 12) for the control aquaria.

5.1.2. Availability of fish specimens

Through the whole experimental program, we observed a total of 469 fish specimens of different species, sizes, and sex (Tables 2, 3 and 4). From these tables we can have an overview of the availability (or relative abundance) of the different species in Gumara River during August to September, 2002. The majority of the specimens were caught after mid-September (Table 2). The species available (those caught from the rivers) during this season were *B. acutirostris* Bini, 1940 (Acu), *B. brevicephalus* (Bre), *B. crassibarbis* Nagelkerke and Sibbing, 2000 (Cra), *B. dainellii* Bini, 1940 (Dai), *B. intermedius* Rüppell, 1836 (Int), *B.*

longissimus Nagelkerke and Sibbing, 2000 (Lon), *B. macrophtalmus* Bini, 1940 (Mac), *B. megastoma* Nagelkerke and Sibbing, 2000 (Meg), *B. nedgia* Rüppell, 1836 (Ned), unidentified juvenile 'large' *Barbus* spp. (JLB), *C. gariepinus* (Gar), *G. dembecha* Abebe Getahun, 2000 (Dch), *O. niloticus* (Nil), and *Varicorhinus beso* Rüppell, 1836 (Bes). Of these, the most numerous ones were Bre, JLB, Int and Bes. For statistical analyses, however, we considered only JLB, Bre, OLB and Bes specimens.

Table 2. Summary of the number of fish specimens (by species) that were available and examined during each experimental date.

Date	Species [a] composition (numbers)													
(mm/dd/yy)	JLB	Acu	Bre	Cra	Dai	Int	Lon	Mac	Meg	Ned	Bes	Gar	Nil	Dch
08/28/02						6			1					
08/29/02						3			2					
08/30/02	9	1				2					1			1
09/05/02	10	2							1	1	16			1
09/07/02	2					6			2					2
09/09/02	8				2	4			2					
09/20/02			20							1	2			
09/21/02			53								2			1
09/23/02			32											
09/25/02	10		57											1
09/26/02	9		33											2
09/28/02	21		25			1								
10/02/02	24		6			2		1						
10/06/02		4				3	1	3	1					
10/07/02	16	4		1		14	1	3	4	1				
10/08/02		1				1	1	2	3			1	1	
10/09/02	10		1			2			1			1		
Total	**119**	**12**	**227**	**1**	**2**	**44**	**3**	**9**	**17**	**3**	**21**	**2**	**1**	**8**

[a] See text for complete scientific names of the different species codes.

5.1.3. Composition of different size, sex and gonad maturity categories

The different sizes are tentatively grouped into three weight categories: <50 g, 50-100 g and ≥100 g (Table 3). To convert the weights to length measurements, one can use length-weight relationships; that is, $W=0.013*FL^{2.967}$ for *B. tsanensis* Nagelkereke and Sibbing, 2000 (Tesfaye Wudneh, 1998), and for convenience this can be extrapolated for the other 'large' *Barbus* spp. For Bes, the relationship is $W=0.032*FL^{2.801}$ (Abebe Ameha *et al*., Submitted).

The sex composition of the various species is presented in Table 4. Almost all of the tested fish, except the very small-sized ones weighing less than 50 g (JLB and Bre specimens) had ripe and/or running gonads (Table 5).

Table 3. Summary of the number of fish specimens (by species) within three size (weight) categories.

Weight category (g)	Number of specimens (by species [a])													
	JLB	**Acu**	**Bre**	**Cra**	**Dai**	**Int**	**Lon**	**Mac**	**Meg**	**Ned**	**Bes**	**Gar**	**Nil**	**Dch**
< 50	119		40		2	8				2	21			8
50-100			141			8								
≥100		12	46	1		28	3	9	17	1		2	1	
Total	**119**	**12**	**227**	**1**	**2**	**44**	**3**	**9**	**17**	**3**	**21**	**2**	**1**	**8**

[a] See text for complete scientific names of the different species codes.

Table 4. Summary of sex composition of the fish specimens (by species) examined during the study; unidentifiable sexes are designated as "X".

Sex [a]	Sex composition by species [b]													
	JLB	**Acu**	**Bre**	**Cra**	**Dai**	**Int**	**Lon**	**Mac**	**Meg**	**Ned**	**Bes**	**Gar**	**Nil**	**Dch**
F		3	62	1		12	1	6	7		1	2	1	1
M	5	9	163		2	30	2	3	10	1	2			
X	114		2			2				2	18			
Total	**119**	**12**	**227**	**1**	**2**	**44**	**3**	**9**	**17**	**3**	**21**	**2**	**1**	**8**

[a] The sex of small-sized fish was difficult to identify, and hence is marked as 'X'.
[b] See text for complete scientific names of the different species codes.

Table 5. Summary of the gonad maturity stages (in per cent) of the fish specimens (by species) examined during the study; unidentified gonad stages are designated as "99".

Gonad stages [a]	Per cent composition of gonad stages by species [b] (sample size in brackets).													
	JLB (119)	**Acu (12)**	**Bre (227)**	**Cra (1)**	**Dai (2)**	**Int (44)**	**Lon (3)**	**Mac (9)**	**Meg (17)**	**Ned (3)**	**Bes (21)**	**Gar (2)**	**Nil (1)**	**Dch (8)**
1			1.0											
2	2.0		3.0								14.3	100.0	100.0	
3	0.7				100.0	2.3								
4	0.7	66.7	94.9			75.0	66.7	33.3	82.4					12.5
5	19.6	33.3		100.0		18.2	33.3	66.7	17.6	33.3				
99	77.0		1.0			4.5				66.7	85.7			87.5
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

[a] The gonad maturity of small-sized and some big fish was difficult to identify, and hence designated as '99'
[b] See text for complete scientific names of the different species codes.

5.1.4. Stocking density and responses to poisoning

The stocking density (number of fish specimens treated at a time in an aquarium) varied in different treatments. That was because the number of fish to be added into each aquarium was determined based on the availability and size of the fish specimens (Table 6). Hence, for smaller-sized and highly available specimens we find relatively higher stocking densities, and vice versa.

Table 6. Stocking density (number of fish specimens treated at a time in an aquarium) during the experiment at different concentrations of powders of seeds of birbira.

	Concentration of birbira (g/l)							
	0	**0.02**	**0.04**	**0.10**	**0.20**	**0.30**	**0.40**	**1.00**
Total no. of replications	15	2	2	12	10	8	13	4
Total sample size	30	15	33	123	87	70	104	7
Stocking density:								
Minimum	1	7	16	5	6	5	5	2
Maximum	4	8	17	15	13	12	15	3

All treated fish showed pre-mortem changes in their activities, and finally death followed in all dilutions except in the control. The pre-mortem activity patterns were restlessness, gasping at the surface, quick sudden movements, reduced mouth and opercular movements, sinking and inverted position. In all of the dilutions (except the 0.02 g/l) restlessness started a minute after exposure. The other activities followed then after but showing no clear pattern (sequence) among different individuals. The fastest death was observed after 8 minutes for two JLB weighing 2 g each (FL 50 and 57 mm) treated with 0.2 and 0.3 g/l concentrations, respectively. The slowest death was observed after 137 minutes for a JLB weighing 6 g (FL 77 mm) exposed to 0.02 g/l concentration. Table 7 presents a summary of the mean size and mean time of death for each species and concentration of birbira. Post-mortem observation of the fishes' external body revealed signs of damage to the internal body parts as depicted in the bloodshot at the opercular and fin regions. The control groups are excluded from the forthcoming demonstration of survival curves.

Table 7. Summary of the mean size of fish and mean time of death for each species at different concentrations of birbira.

Species code [a]	Mean FL ± SE	Mean W ± SE	N	Mean time of death, t_d ± SD (n) at different concentrations (g/l)							
				0	**0.02**	**0.04**	**0.1**	**0.2**	**0.3**	**0.4**	**1.0**
JLB	63.84±2.01	4.37±0.44	119	ND(7)	60.5±49.5(10)	16.7±5.3(16)	18.9±4.6(20)	14.6±2.8(25)	14.1±3.3(18)	17.1±4.1(23)	
Acu	325.00±4.89	400.42±19.34	12			27.0±1.4(2)	26.3±3.8(4)			22.0±3.6(4)	
Bre	181.96±1.90	74.51±2.26	227	ND(14)			23.3±5.0(86)	22.9±5.6(39)	20.3±3.6(41)	19.5±5.0(46)	
Cra	---	---	1								
Dai	118.50±0.50	---	2					14.0±0.0(2)			
Int	229.57±9.23	195.27±23.87	44	ND(5)	37.0±1.4(2)	25.3±4.7(9)	27.0±4.6(4)	19.0±5.1(10)	28.0±1.4(2)	19.3±2.3(6)	18.3±4.2(6)
Lon	351.00±36.00	587.67±119.29	3								
Mac	317.56±5.19	416.89±20.64	9				26.0±5.7(2)	28.5±12.0(2)	26.7±5.1(3)		
Meg	367.88±9.86	573.82±48.26	17	ND(2)		28.7±1.5(3)	20.0±0.0(2)	22.0±6.5(4)	23.0±8.5(2)	25.0±0.0(2)	
Ned	161.33±88.35	157.33±152.33	3							17.0±1.4(2)	
Bes	85.24±5.02	9.29±2.15	21	ND(2)						15.2±1.2(17)	
Gar [b]	502.50±37.50	791.50±147.5	2								
Nil [b]	---	---	1								
Dch	60.86±8.00	3.29±1.02	8				25.0±6.9(3)	17.5±3.5(2)		19.3±5.1(3)	
			469								

[a] See text for complete scientific names of the different species codes.
[b] For all of the cyprinids length is measured as fork-length (FL), but as total length (TL) for "Nil" and "Gar".
ND = No death
(n) Numbers in parentheses represent the sample sizes for different species at different concentrations, while N is the total number of specimens examined.

5.2. Response by size and/or sex

For inter-size and/or inter-sex comparison of the time of death, only Bre specimens are considered; the other species do not have sufficient numbers of specimens representing each size and/or sex category. It was not possible to identify the sex of JLB, and all of them belong to a single size category (Tables 3 and 4). One-way ANOVA conducted among different sizes (< 50, 50-100, and ≥100 g) of Bre exposed to 0.1, 0.3, and 0.4 g/l concentration resulted in significant differences ($p < 0.01$ in all cases). Larger (≥ 100 g) and smaller (< 100 g) groups of Bre have significantly different mean times of death as seen from the tests done at concentrations of 0.1, 0.3, and 0.4 g/l (Appendix A). In addition, there was also sex-based difference in Bre treated in 0.1, 0.3, and 0.4 g/l dilutions; only the 0.2 g/l medium resulted in no significant difference (Appendix B). A coincidence here is that the larger specimens were females while the smaller ones were males. A previous work by Nagelkerke and Sibbing (1996) also mentioned that in Bre running females are larger than males. Hence, the survival curves constructed to compare < 100 g vs. ≥ 100 g became similar with those for male vs. female Bre (Fig. 7A-D).

Under all concentrations, the first female or male specimen of Bre died after 12 or 16 minutes of exposure. The last female lived no longer than 30 minutes at higher concentrations, but up to 40 minutes at lower ones. The last male survived for about 30 minutes at all levels of treatment. The respective survival curves for the different sexes of Bre indicate that the females could survive for a relatively longer period than the males at concentrations of 0.1 to 0.4 g/l (Fig. 7A-D).

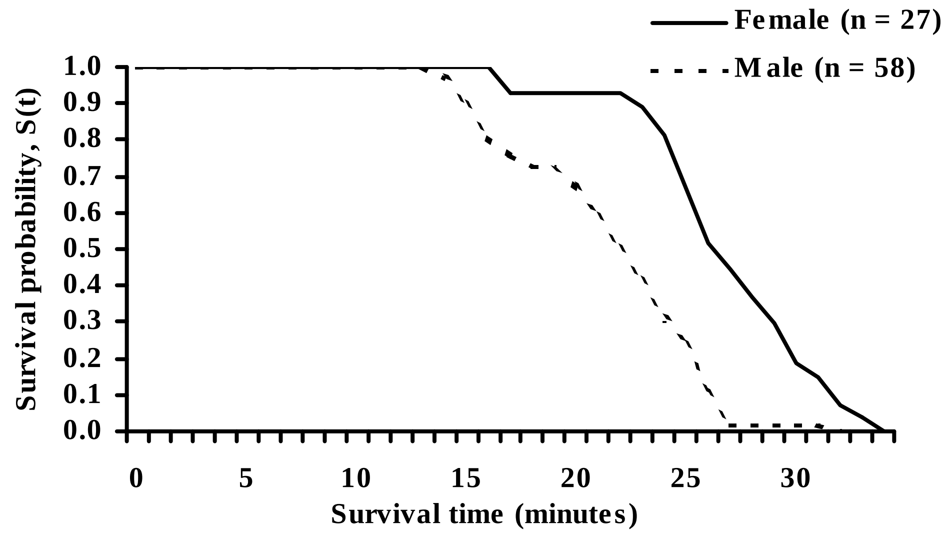


Fig. 7 (A). Survival curves, S(t), for male and female *B. brevicephalus* (Bre) treated in 0.1 g/l birbira solution.

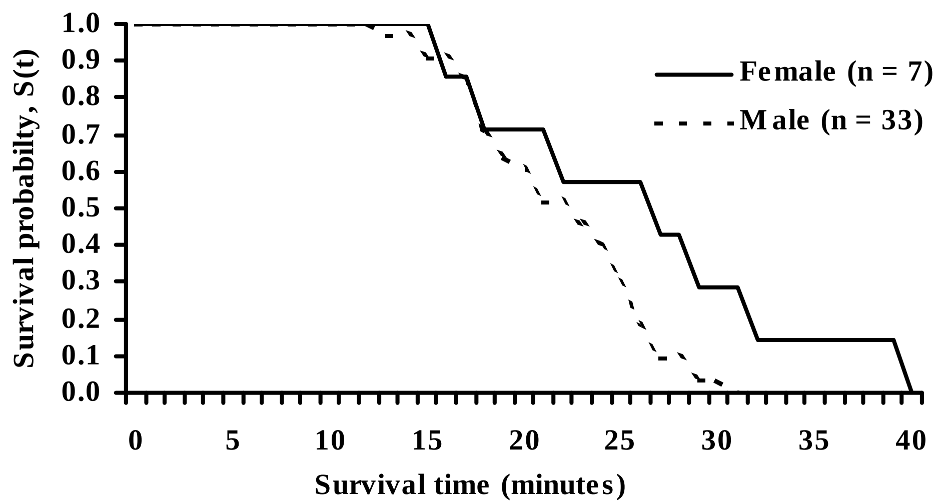


Fig. 7 (B). Survival curves, S(t), for male and female *B. brevicephalus* (Bre) treated in 0.2 g/l birbira solution.

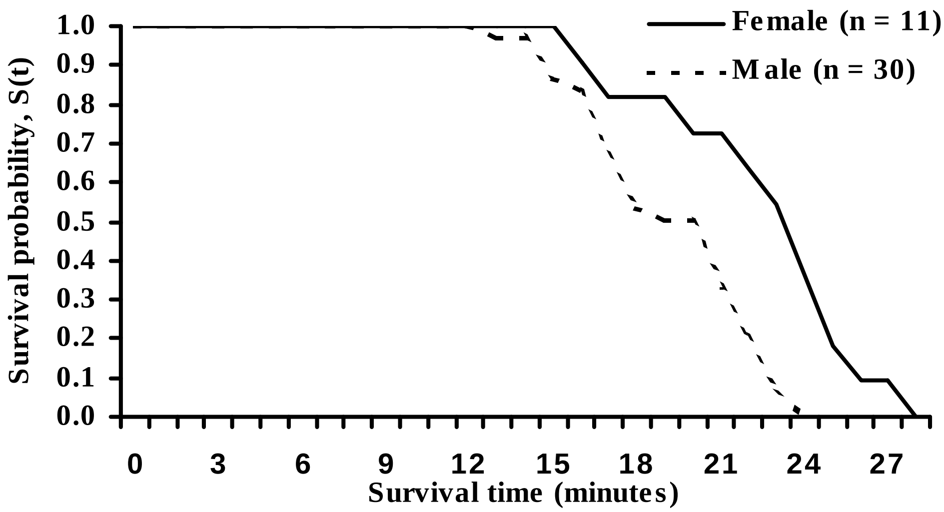


Fig. 7 (C). Survival curves, S(t), for male and female *B. brevicephalus* (Bre) treated in 0.3 g/l birbira solution.

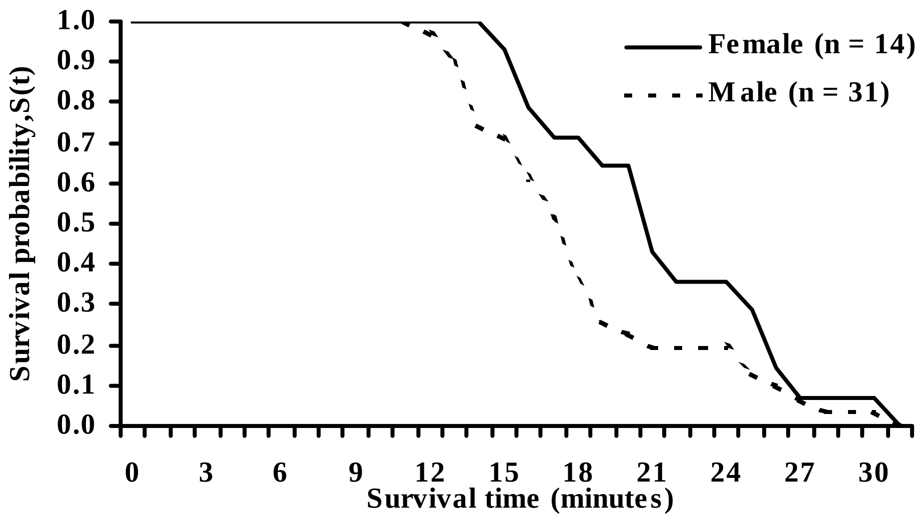


Fig. 7 (D). Survival curves, S(t), for male and female *B. brevicephalus* (Bre) treated in 0.4 g/l birbira solution.

5.3. Interspecific responses

The forthcoming analysis and presentation of interspecific responses is based on the availability of sufficient numbers of specimens of each species (Table 7). That is, only JLB, Bre and Bes are considered in the survival curves. One-way ANOVA conducted among JLB, Bre, and Bes (all weighing < 50 g) exposed to 0.4 g/l concentration resulted in significantly different ($p = 0.008$) mean time of death. However, using independent t-tests, there is no significant difference seen in the responses for JLB vs. Bre. Bes showed a significant difference from JLB as well as from Bre at 0.4 g/l concentration. Larger (weight ≥ 100 g) specimens of Bre vs. OLB treated in different concentrations (0.1, 0.3, and 0.4 g/l) also showed no significant difference. In general, JLB and the OLB have significantly different mean time of death when exposed to different concentrations of birbira. The results of the statistical tests for differences in mean time of death are presented in Appendix C.

As shown in Fig. 8, in all three (JLB, Bre, Bes) cases, the first specimen died after 12 minutes of exposure. The last specimens of JLB and Bre died after 30 minutes while that of Bes lived no longer than 18 minutes. As a result, JLB and Bre showed relatively longer survival than Bes when exposed to a concentration of 0.4 g/l.

5.4. Response by concentration

Comparison of responses of different concentrations was possible only for JLB (< 50 g), Bre (< 100 g) and OLB (≥ 100 g). The other species were represented by very few specimens at each concentration (Table 7), and hence are excluded from this comparison. ANOVA for JLB (all weighing < 50 g) treated with different concentrations (0.02, 0.04, 0.1, 0.2, 0.3, and 0.4 g/l) resulted in significant difference in time of death ($p < 0.001$); particularly, we found significant differences between the responses for the 0.02 g/l and the rest of the higher concentrations. However, most (6 out of 10) of similar tests made for JLB among the 0.04, 0.1, 0.2, 0.3, and 0.4 g/l dilutions showed no significant difference (Appendix D).

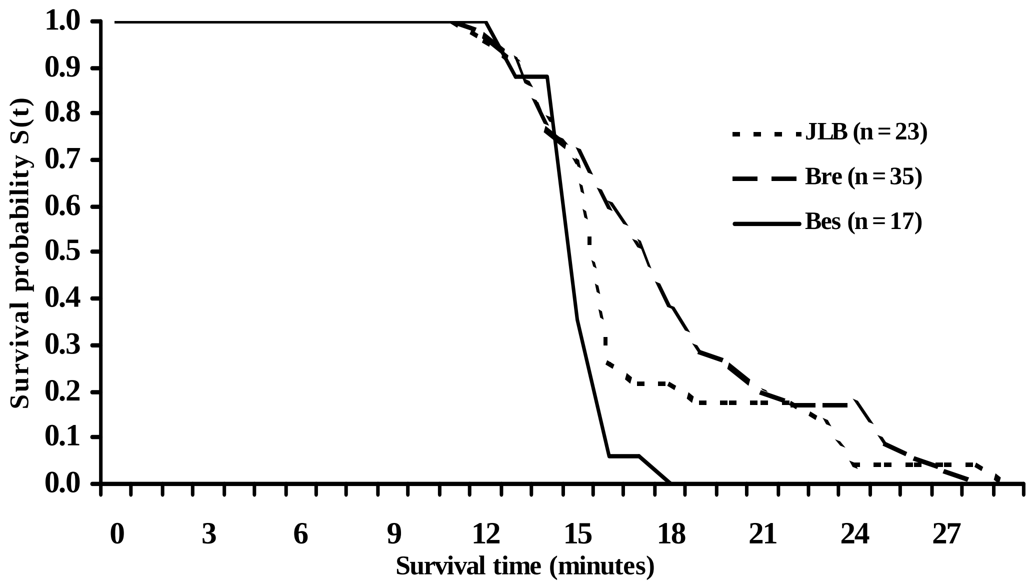
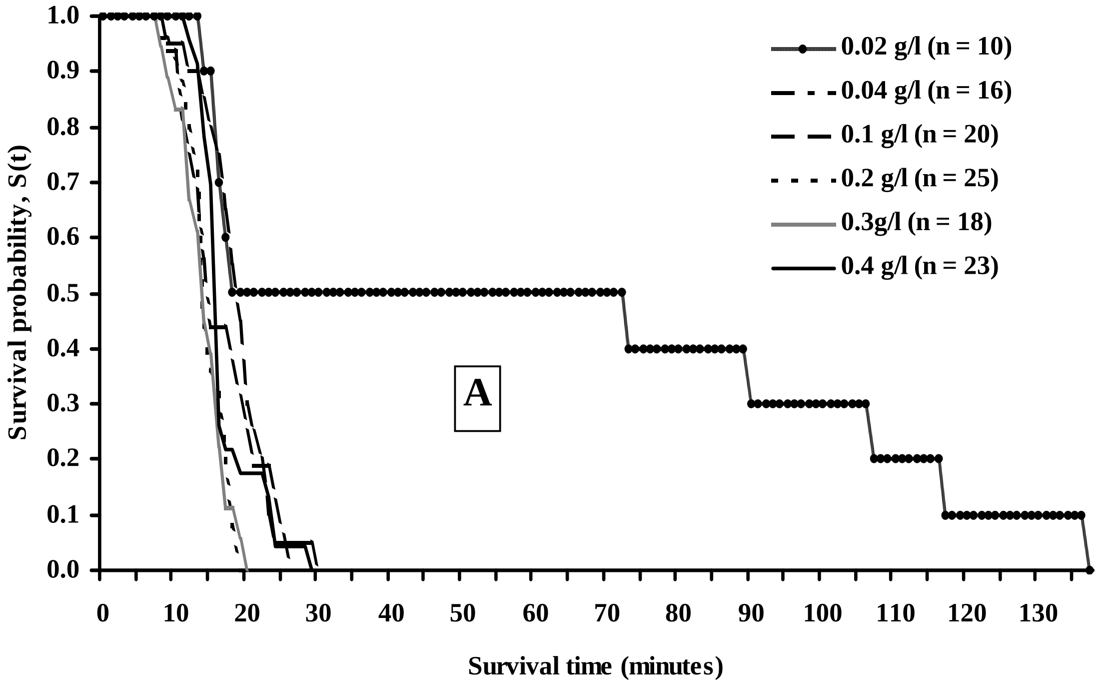


Fig. 8. Survival curves, S(t), for < 50 g juvenile 'large' barbs (JLB), < 100 g *B. brevicephalus* (Bre), and < 50 g *V. beso* (Bes) treated in 0.4 g/l birbira solution (note that Bre of weight < 50 g and those weighing 50-100 g are merged here since there is no significant difference in their responses).

Bre of similar size group (< 100 g) tested with different concentrations showed significant differences in most (4 out of 6) of the tests. OLB (≥ 100 g) tested with 0.04, 0.1, 0.2, 0.3, and 0.4 g/l concentrations also resulted in significant difference ($p < 0.01$); but particular independent t-tests made between paired groups resulted in no significant differences (Appendix D). Hence OLB are excluded from analysis of survival curves.

The survival curves constructed indicate that JLB specimens could survive for about six times longer period of time in 0.02 g/l birbira solution than in 0.04 to 0.4 g/l concentrations (Fig. 9A). In the 0.02 g/l medium, 50% of the JLB specimens survived for more than an hour, and



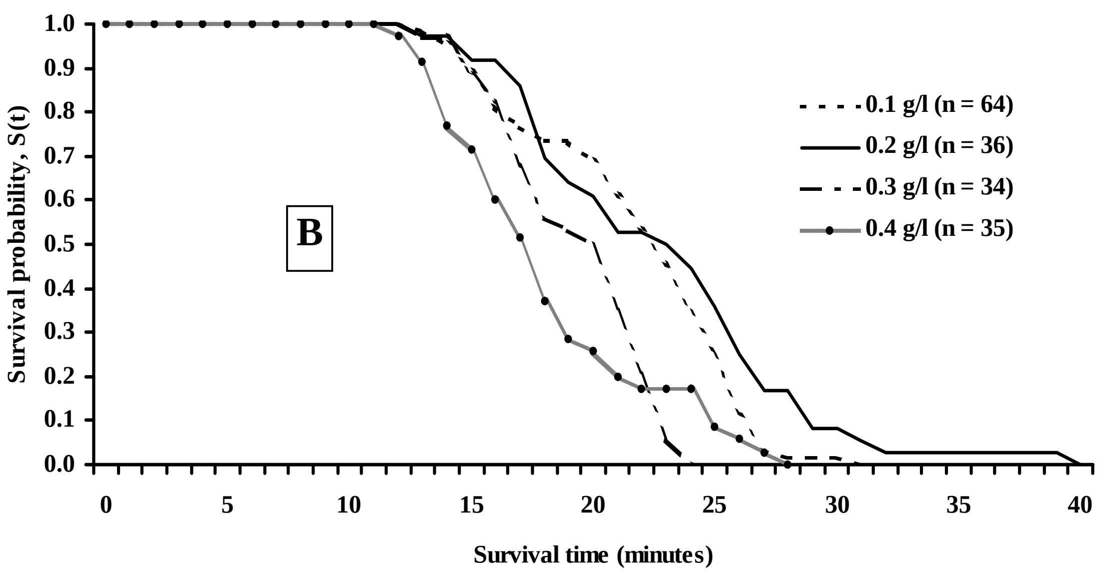


Fig. 9. Survival curves, S(t), (A) for < 50 g juvenile large barbs (JLB), treated in 0.02, 0.04, 0.1, 0.2, 0.3, and 0.4 g/l birbira solution, and (B) for < 100 g *B. brevicephalus* (Bre) treated in 0.1, 0.2, 0.3, and 0.4 g/l birbira solution.

the last ones died after 2 hours period. Both JLB and Bre treated in 0.1 to 0.4 g/l solution resulted in a total death in about 30 minutes of exposure (Figs. 9A and B). Bre of < 100 g weight (almost all males) died at significantly different times in dilutions of 0.1 to 0.4 g/l.

5.5. Response of fertilized fish eggs

The average water temperature and [DO_2] of the aquaria in the laboratory was 21.3 ^oC (S.D. = 0.4, n =28) and 10.2 mg/l (S.D. = 0.9, n = 26), respectively, that is very close to the conditions in Dukalit stream. None of the eggs that were poisoned with 0.4 g/l of birbira solution for 15 minutes hatched. All of the controls, however, hatched after 3 days of incubation under similar conditions. Unfortunately, the exact time period (number of days) that passed between fertilization of the eggs and the day we collected them from the river could not be recorded. The average size of the newly hatched fingerlings was 8 mm. These were taken care of in the laboratory at the Fisheries Research Center (Bahir Dar) for a few weeks.

6. Discussion

6.1. General effects

Oxygen consumption rate (OCR) is the amount of oxygen a fish requires for oxidative (aerobic) metabolic processes over a given length of time; four of the most significant factors that can affect OCR can be body weight, level of activity, environmental temperature, and feeding (Moyle and Cech, 1996). From the current study on the ‘large’ barbs of Gumara River, we can see that solutions of birbira seed powder affect oxygen uptake by the fish. This is evidenced by the almost intact (unchanged) amount of dissolved oxygen left in the solutions after the fish died whereas a similar initial amount in the birbira-free water was found depleted later on. That is, the amount of dissolved oxygen in the water at the beginning of each experiment is not lost after 30 minutes of treatment with birbira. This supports the idea that rotenone, an ingredient in seeds of birbira, does not remove oxygen from the water (Brunson, 1954). However, we do not mean that only birbira solely affects oxygen uptake by all of the fish under natural conditions. Other factors could also be responsible for reduced total oxygen uptake; an example could be the pathological branchial conditions in the freshwater catfish, *Wallago attu*, where parasitic infestation hindered normal respiration (Ojha and Hughes, 2001).

As in the case for other pesticides tested on fish, such as Diazinone 60 EC on *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus* (Rahman *et al*., 2002), birbira solutions resulted in abnormal pre-mortem activities such as restlessness, sudden quick movements, rolling movements, swimming on the back, and settling at the bottom (Fig. 10). Some areas like the internal parts of the fins and opercula of the barbs poisoned with birbira became bloodshot. Rahman *et al*. (2002) also has noticed hemorrhagic areas in the liver and kidney of fish as a response for Diazinone 60 EC. This leads to propose the need for further examination of the effects of birbira on the structure and function (physiology) of these fish.

Fig. 10. Large barbs from Gumara River poisoned with powders of birbira seeds and showing pre-mortem activities.

6.2. Effects related to species, size, or sex factors

From the results of this experiment, it appears that different 'large' *Barbus* spp. of similar sizes do not respond differently when exposed to similar concentrations of birbira solution. This could be attributed to their close phylogenetic as well as shared environmental situations that in turn could have resulted in similar levels of tolerance to birbira. *V. beso*, a closely related (to the large' barbs) hexaploid cyprinid, appears to be less tolerant to birbira solutions than are 'large' *Barbus* specimens of similar sizes. *V. beso* has a rare occurrence (Index of Relative Importance = 1.1%) in Gumara River (Abebe Ameha and Alemu Assefa, 2002), Lake Tana (Abebe Ameha *et al*., submitted) and its other tributaries (personal observation). Its less tolerance to birbira poisoning by the fishermen could be one of the reasons that contributed to its scarcity in this area. However, whether *V. beso* of Gumara River migrate or not is not known.

The data show that there is no difference in the time of death between different size groups of *B. brevicephalus* exposed to similar concentrations of birbira. The amount of birbira we applied during the experiment simulates that used by the fishermen, which is an excessive application relative to that required to kill all of the fish in about an hour. The high concentration we used could have hidden a possible varied response by different sizes of fish. There were response similarities between groups of < 50 g and 50-100 g fish. These size groups, however, responded differently when compared with fishes of size ≥ 100 g. We did not consider this difference as an effect of difference in size. Rather, *B. brevicephalus* weighing <100 g were found to be males while those with ≥ 100 g size were females. So we attributed this difference in responses as an effect of sexual difference. Moreover, female Bre appear to have better tolerance to lower concentrations than to higher ones, while male Bre show no difference in tolerance for concentrations of 0.1 to 0.4 g/l.

Within the same size and sex categories of *B. brevicephalus*, different birbira concentrations above 0.04 g/l do not seem to show difference in the time of death. It is only the 0.02 g/l concentration that resulted in considerable divergence (in the responses) from other higher concentrations. That is, the maximum effective concentration seems to be found between 0.02 and 0.04 g/l. This leads to suggest that the amount of birbira needed to kill all of these fish in an hour's time could be not more than 0.04 g/l. Even at a concentration of 0.02 g/l, the longest survivor lasted only for about a couple of hours, proving the high toxic effect of birbira to the barbs in Gumara River. The reason why the fishermen apply excessive amounts (up to 0.4 g/l) of the toxic powder could be to compensate for the amount that may be lost being washed down, or diluted by incoming fresh water. This is supported by the fact that after adding the powders into the pools of water, the fishermen run downstream for an hour or more to catch the stupefied fish.

Although it is very likely that stupefying the barbs in Gumara River with birbira could play a role in damaging the fish resources, it is also equally likely that other factors could contribute to this effect. To mention some, high fishing effort (number of fishermen, gear, time, etc), the use of insecticides (such as DDT) and agrochemicals, disturbing breeding and nursery grounds, and deforestation are commonly observed recent phenomena. Experiences in other parts of the world also support this idea. Examples could be shallow spawning areas and acidic runoff restricting recruitment in brown trout, *Salmo trutta*, in Hovvatn, southern Norway (Barlup *et al*., 1998), and possible effect of pollutants resulting in vertebral deformities in Gobiidae from Tagus estuary (Da Cunha, 1999). Pesticides at high concentrations are known to reduce the survival, growth and reproduction of fish (Mckim *et al*., 1975). Very limited work has been done on the histo-biological effects of pesticides on fishes (Rahman *et al*., 2002). About 70% of the total annual catch from Lake Tana is contributed by the traditional fishery, of which 5% comes from Gumara River (Abebe Ameha and Alemu Assefa, 2002). Hence, although the motorized boat fishery at the river mouths can have its own negative impact on the fishery, greater attention is needed towards the traditional riverine fishing in which poison fishing is a component.

In general, in this work, the significant difference in the mean time of death for different groups of fish could mean a difference of only a few (5 to 10) minutes. This seems unimportant either to the effect or to the revival of fish poisoned with birbira. But the literature indicates that fish that can get the chance of escaping early from water poisoned with rotenone and entering into fresh water, could revive (Brunson, 1954). Hence, for the barbs in Gumara River, there could be a possibility of revival in a few minutes' swimming to unpoisoned part(s) of the river.

6.3. Effect on fertilized eggs of *B. brevicephalus*

From the current study, much can not be said about the effect of poisoning the eggs of the 'large' barbs in Gumara River with powders of seeds of birbira. However, fertilized eggs of *B. brevicephalus* that were poisoned with 0.4 g/l of aqueous solution of birbira could not hatch, while those in fresh water (control) hatched and were observed surviving for more than a week. The exact nature of this effect on the development of the embryos needs well-controlled laboratory studies. From our earlier field observation, however, we have seen that fecundity of barbs of Gumara River could reach from 10,000 to 100,000. Killing spawner barbs, hence, implies killing millions of eggs and breaking the continuous line of recruitment to the fish stocks in the area. The possibility of hatching eggs of Barbus spp. in the laboratory, on the other hand could be helpful to restock the lake and the rivers, as well as to rear experimental fish for further research on poisoning fish.

6.4. Determination of lethal concentration (LC)

This work could not determine the median lethal concentration (LC_{50}), the concentration at which 50% of the test animals die before a certain time limit (ILPI, 2003), using probit analysis method (Finney, 1971). That is because the concentrations we used represented the actual application of birbira by the fishermen, which seems an extreme overdose beyond the amount (about 0.04 g/l) needed to kill all of the fish in half an hour's time after exposure. Hence, further study on determining the LC_{50} values for the different species is needed. This requires considering very low concentrations (< 0.1 g/l) that are selected systematically to fit into probit analysis methods.

6.5. Fishing with birbira and resource management

Length-based cohort analysis using Thompson and Bell model for 1994/95 and 1995/96 indicated that the barbs of Lake Tana were overexploited; the reason for this was explained to be recruitment overfishing on the rivers (LFDP, 1997). Catch and effort data analysis made for Gumara River until 1998 strengthened this point (Abebe Ameha and Alemu Assefa, 2002). The field observation on this study has revealed that the high physical structure and the strong power of the water at Durrba Fall could not allow further upward migration of these fish. This place is one of the major sites where birbira is applied on Gumara River.

During our study period (August and September, 2002) there was very little fishing activity seen on Gumara River around Wanzaye area. In the nearby Gelda River, however, we observed a lot of fishermen who caught only a few fish after applying birbira (Fig. 11). The Office of Agriculture, Fogera Woreda (South Gondar) also informed us the implementation of a ban on fishing with scoop nets and birbira in this area before three years. A lot of fishermen were scared of the construction of our research shelter by the riverside. That was because they thought it was meant to control illegal fishing. We have seen that this by itself contributed to the safe upward migration, spawning, and return of *B. brevicephalus* in September, 2002. It is not known how these fish locate the most suitable spawning area; but at least we know that *B. brevicephalus* chooses Dukalit stream and other similar waters.

The most recent development in preparation of a fisheries policy in Ethiopia is the release of the proclamation on Fisheries Development and Utilization, Proclamation No. 315/2003 (Federal Negarit Gazeta, 2003). This could serve as a key tool to facilitate execution of management measures. Preventive measures must be initiated now, before an ecological crash occurs (Nagelkerke *et al.*, 1995). One of the most important conditions for sustainability is elaboration and enforcement of fishing regulations in consultation with fishers (Reyntjens *et al.*, 1998).

Fig. 11. Fishermen shy of the camera and disappointed by the absence of fish after applying birbira (*M. ferruginea*) for stupefying the barbs in Gelda River, a tributary of Lake Tana found south of Gumara River (September, 2002).

The idea of neutralizing natural waters poisoned with birbira (or rotenone) seems impracticable, as the detoxifying chemicals themselves could be harmful to the fish. For instance, it is known that chlorine/chloramine irritates the gills of fish and blocks oxygen carrying cells, leading to suffocation (Brunson, 1954). Even arranging use of a maximum allowable concentration (MAC) (Sanockij, 1975) could not be a remedy as it would be difficult to determine possible dangers on the aquatic fauna. Hence, a practical preventive measure could be complete ban on fishing with birbira on Gumara River and other waters facing the problem, thereby facilitating alternative activities for the fishermen.

Although the focus of this study is on the deleterious effect that birbira may have on fish resources, it is equally necessary to consider the need for conserving existing birbira trees and planting additional ones. The basis for this is birbira's endemicity plus the multi-faceted uses it has. Developing management procedures for the species with a view to using it for different purposes is very important (Legesse Negash, 2002). Future practices of aquaculture in Ethiopia could consider using rotenone extracts from seeds of birbira for wiping out unwanted fish stocks (populations) from ponds and dams, or harvesting fish in small aquarium settings. The lack of substantial knowledge on the potential uses of the plant also could attract attention of researchers. To save the possible damage on fish resources, one needs to focus on ways of avoiding (or at least minimizing) improper fishing techniques, including poison fishing with birbira.

In any use of toxicants, adequate attention should be given to unwanted and even unexpected side effects; the use of toxicants in rivers is particularly fraught with danger because of the rapid downstream spread that is possible (Lagler, 1978). Fisheries also require management which may be accomplished either by direct interventions on the fish stock or by legislative or economic activities on the fishermen themselves, the major developmental emphasis being towards replacement activities such as aquaculture (Welcomme, 1985).

7. Conclusion and recommendations

The present study is believed to lay the basis for further work on problems associated with poisoning fish with birbira. The seed of *M. ferruginea* contains a highly toxic material that can cause mass destruction on the *Barbus* spp. in Gumara River. Despite the age-old use of birbira for fishing, the easy breakdown (low persistence) of rotenone could have given unexposed populations of fish the chance to maintain the diversity of barbs we find now in Lake Tana and its rivers. In summary, the following conclusive remarks could be made on the effect of birbira on the 'large' barbs in Gumara River, Lake Tana:

1) Water solution of powders of seeds of *M. ferruginea* does not remove oxygen from the water, but appears to affect oxygen uptake by the 'large' *Barbus* spp.
2) The 'large' *Barbus* spp. probably have relatively higher tolerance to poisoning with *M. ferruginea* than has *V. beso*, a close relative.
3) The minimum concentration of water solution of powders of seeds of *M. ferruginea* needed to kill 100% of exposed 'large' *Barbus* spp. in an hour is about 0.04 g/l.
4) Different size groups of 'large' *Barbus* spp. (at least *B. brevicephalus*) do not appear to show different levels of tolerance to poisoning with *M. ferruginea*.
5) As compared to their male counterparts, ripe females of *B. brevicephalus* show relatively longer survival time when poisoned with *M. ferruginea*.
6) Fertilized eggs of *B. brevicephalus* that are exposed to 0.4 g/l of water solution of powders of seeds of *M. ferruginea* for 15 minutes, and then incubated in aquaria at an average temperature of 21.3 ^{o}C and average DO_2 of 10.2 mg/l, did not hatch while the unpoisoned fertilized eggs (the controls) did.
7) The possibility of hatching eggs and rearing fingerlings of 'large' *Barbus* spp. in aquaria seems promising for restocking Lake Tana and its rivers in an effort to rehabilitate the declining catches and conserve the resource.

Although rotenone is claimed to be responsible for birbira's toxicity to fish, the possible toxic effect of other ingredients found in the seeds is not yet clear. Based on findings of the current study and relating to previous works, the following recommendations are forwarded.

1) It is necessary to conduct well-organized further studies on the toxicity of rotenone and other ingredients of seeds of *M. ferruginea* on the *Barbus* spp. and other fishes of Lake Tana; on the tolerance potential of these fish to seeds of birbira (based on physiological and behavioral studies); on the reproductive biology of the barbs including their embryological development; and on possible effects of birbira on other aquatic fauna that build the food web.
2) Alternative activities to capture fishing need be foreseen prior to implementing closure of fishing area or gear restrictions. For instance, introduction of feasible small-scale aquaculture practices in the fishermen's villages could compensate for the declining catches from natural waters, and serve as a new job opportunity for prospectively 'idle' fishermen.
3) Monitoring of the *Barbus* spp. and other fishes in and around Lake Tana need be strengthened for conservation as well as production purposes; protection and cultivation of birbira as an indigenous and potentially exploitable herbal resource also needs attention.
4) Enforcement of management measures, effective training and extension work should incorporate active participation of the fisher community including the highlander visitors who fish on Gumara River with birbira occasionally.

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Appendices

Appendix A. Inter-size (< 50, 50-100, and ≥ 100 g) comparisons in mean time of death (MTD) for *B. brevicephalus* (Bre) treated with similar birbira concentrations.

Grouping by size		Group statistics				Independent samples test									
						Levene's test for equality of variances			t-test for equality of means						
Conc. (g/l)	Size (weight, g)	n	MTD (min.)	Std. devn. MTD	Std. error MTD	Assumption of variance	F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% C.I. of the difference	
														Lower	Upper
0.4	< 50	6	20.3	3.98	1.63	Equal	0.001	0.981	1.289	33	0.206	2.47	1.92	-1.43	6.37
	50 - 100	29	17.9	4.32	0.80	Unequal			1.363	7	0.212	2.47	1.81	-1.74	6.69
0.3	< 50	10	19.5	3.14	0.99	Equal	0.144	0.707	-0.035	32	0.972	-4.17E-02	1.18	-2.44	2.35
	50 - 100	24	19.5	3.12	0.64	Unequal			-0.035	16	0.972	-4.17E-02	1.18	-2.53	2.45
0.1	< 50	19	21.4	3.99	0.92	Equal	1.284	0.262	-0.680	62	0.499	-0.81	1.19	-3.19	1.57
	50 - 100	45	22.2	4.49	0.67	Unequal			-0.714	37	0.480	-0.81	1.13	-3.10	1.49
0.4	< 50	6	20.3	3.98	1.63	Equal	1.156	0.299	-1.193	15	0.251	-2.94	2.46	-8.19	2.31
	≥ 100	11	23.3	5.24	1.58	Unequal			-1.297	13	0.217	-2.94	2.27	-7.83	1.95
0.3	< 50	10	19.5	3.14	0.99	Equal	0.234	0.636	-2.890	15	0.011	-4.64	1.61	-8.07	-1.22
	≥ 100	7	24.1	3.44	1.30	Unequal			-2.841	12	0.015	-4.64	1.63	-8.20	-1.09
0.1	< 50	19	21.4	3.99	0.92	Equal	0.127	0.723	-4.271	38	0.000	-5.77	1.35	-8.51	-3.04
	≥ 100	21	27.1	4.51	0.98	Unequal			-4.297	37	0.000	-5.77	1.34	-8.49	-3.05
0.4	50 - 100	29	17.9	4.32	0.80	Equal	1.606	0.213	-3.335	38	0.002	-5.41	1.62	-8.69	-2.13
	≥ 100	11	23.3	5.24	1.58	Unequal			-3.055	15	0.008	-5.41	1.77	-9.18	-1.65
0.3	50 - 100	24	19.5	3.12	0.64	Equal	0.923	0.345	-3.359	29	0.002	-4.60	1.37	-7.40	-1.80
	≥ 100	7	24.1	3.44	1.30	Unequal			-3.181	9	0.011	-4.60	1.45	-7.87	-1.33
0.1	50 - 100	45	22.2	4.49	0.67	Equal	0.410	0.524	-4.180	64	0.000	-4.97	1.19	-7.34	-2.59
	≥ 100	21	27.1	4.51	0.98	Unequal			-4.173	38	0.000	-4.97	1.19	-7.37	-2.56

Appendix B. Inter-sex (male, female) comparisons in mean time of death (MTD) for *B. brevicephalus* (Bre) treated with similar birbira concentrations

Grouping by concentration		Group statistics				Independent samples test									
						Levene's test for equality of variances			t-test for equality of means						
Conc. (g/l)	Sex	n	MTD (min.)	Std. devn. MTD	Std. error MTD	Assumption of variance	F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% C.I. of the difference	
														Lower	Upper
0.1	M	57	21.6	4.09	0.54	Equal	0.432	0.513	-5.621	82	0.000	-5.39	0.96	-7.29	-3.48
	F	27	27.0	4.12	0.79	Unequal			-5.605	50	0.000	-5.39	0.96	-7.32	-3.46
0.2	M	32	22.1	4.71	0.83	Equal	4.625	0.038	-1.821	37	0.077	-4.16	2.28	-8.79	0.47
	F	7	26.3	8.38	3.17	Unequal			-1.270	6	0.245	-4.16	3.28	-11.94	3.62
0.3	M	30	19.4	3.11	0.57	Equal	0.051	0.823	-2.849	39	0.007	-3.29	1.16	-5.63	-0.96
	F	11	22.7	3.72	1.12	Unequal			-2.621	15	0.019	-3.29	1.26	-5.97	-0.62
0.4	M	31	18.5	4.87	0.87	Equal	0.042	0.838	-2.040	43	0.048	-3.19	1.56	-6.35	-3.64E-02
	F	14	21.6	4.83	1.29	Unequal			-2.047	25	0.051	-3.19	1.56	-6.40	1.77E-02

Appendix C. Inter-specific comparisons in mean time of death (MTD) for juvenile large barbs (JLB), *B. brevicephalus* (Bre), other large *Barbus* (OLB), and *V. beso* (Bes) belonging to similar size ranges and treated with similar birbira concentrations

Grouping by species			Group statistics				Independent samples test									
							Levene's test for equality of variances			t-test for equality of means						
Conc. (g/l)	Size (weight, g)	Species code	n	MTD (min.)	Std. devn. MTD	Std. error MTD	Assumption of variance	F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% C.I. of the difference	
															Lower	Upper
0.4	< 50	JLB	23	17.1	4.09	0.85	Equal	9.341	0.004	1.810	38	0.078	1.85	1.02	-0.22	3.92
		Bes	17	15.2	1.15	0.28	Unequal			2.065	26	0.049	1.85	.90	9.91E-03	3.69
0.4	< 50	Bre	6	20.3	3.98	1.63	Equal	25.922	0.000	4.910	21	0.000	5.10	1.04	2.94	7.26
		Bes	17	15.2	1.15	0.28	Unequal			3.090	5	0.025	5.10	1.65	0.93	9.27
0.3	< 50	JLB	18	14.1	3.27	0.77	Equal	0.002	0.964	-4.238	26	0.000	-5.39	1.27	-8.00	-2.78
		Bre	10	19.5	3.14	0.99	Unequal			-4.291	19	0.000	-5.39	1.26	-8.01	-2.76
0.4	< 50	JLB	23	17.1	4.09	0.85	Equal	0.120	0.732	-1.740	27	0.093	-3.25	1.87	-7.07	0.58
		Bre	6	20.3	3.98	1.63	Unequal			-1.768	7	0.115	-3.25	1.84	-7.48	0.99
0.1	< 50	JLB	20	18.9	4.60	1.03	Equal	0.100	0.754	-1.822	37	0.077	-2.52	1.38	-5.32	0.28
		Bre	19	21.4	3.99	0.92	Unequal			-1.829	36	0.076	-2.52	1.38	-5.31	0.27
0.4	≥ 100	Bre	11	23.3	5.24	1.58	Equal	3.181	0.090	1.151	20	0.263	2.18	1.90	-1.77	6.14
		OLB	11	21.1	3.48	1.05	Unequal			1.151	17	0.265	2.18	1.90	-1.81	6.17
0.3	≥ 100	Bre	7	24.1	3.44	1.30	Equal	3.395	0.087	-0.369	14	0.717	-0.86	2.32	-5.83	4.12
		OLB	9	25.0	5.32	1.77	Unequal			-0.390	13	0.702	-0.86	2.20	-5.58	3.87
0.1	≥ 100	Bre	21	27.1	4.51	0.98	Equal	0.501	0.484	0.891	31	0.380	1.48	1.66	-1.90	4.85
		OLB	12	25.7	4.70	1.36	Unequal			0.881	22	0.388	1.48	1.68	-2.00	4.95

Appendix D. Inter-concentration (0.02, 0.04, 0.1, 0.2, 0.3, 0.4 g/l) comparisons in mean time of death (MTD) for juvenile large barbs (JLB), *B. brevicephalus* (Bre), and other large *Barbus* (OLB) with similar size ranges

Grouping by concentration			Group statistics				Independent samples test									
							Levene's test for equality of variances			t-test for equality of means						
Species code	Size (weight, g)	Conc. (g/l)	n	MTD (min.)	Std. devn. MTD	Std. error MTD	Assumption of variance	F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. error difference	95% C.I. of the difference: Lower	95% C.I. of the difference: Upper
JLB	< 50	0.02	10	60.5	49.50	15.65	Equal	92.447	0.000	3.551	24	0.002	43.81	12.34	18.35	69.28
		0.04	16	16.7	5.33	1.33	Unequal			2.789	9	0.021	43.81	15.71	8.35	79.28
JLB	< 50	0.02	10	60.5	49.50	15.65	Equal	120.042	0.000	3.797	28	0.001	41.65	10.97	19.18	64.12
		0.1	20	18.9	4.60	1.03	Unequal			2.655	9	0.026	41.65	15.69	6.21	77.09
JLB	< 50	0.02	10	60.5	49.50	15.65	Equal	167.070	0.000	4.721	33	0.000	45.86	9.71	26.10	65.62
		0.2	25	14.6	2.83	0.57	Unequal			2.928	9	0.017	45.86	15.66	10.44	81.28
JLB	< 50	0.02	10	60.5	49.50	15.65	Equal	116.778	0.000	4.022	26	0.000	46.39	11.53	22.68	70.10
		0.3	18	14.1	3.27	0.77	Unequal			2.960	9	0.016	46.39	15.67	10.96	81.82
JLB	< 50	0.02	10	60.5	49.50	15.65	Equal	142.220	0.000	4.262	31	0.000	43.41	10.19	22.64	64.19
		0.4	23	17.1	4.09	0.85	Unequal			2.769	9	0.022	43.41	15.68	7.98	78.85
JLB	< 50	0.04	16	16.7	5.33	1.33	Equal	1.479	0.232	-1.306	34	0.200	-2.16	1.66	-5.53	1.20
		0.1	20	18.9	4.60	1.03	Unequal			-1.285	29	0.209	-2.16	1.68	-5.60	1.27
JLB	< 50	0.04	16	16.7	5.33	1.33	Equal	12.077	0.001	1.608	39	0.116	2.05	1.27	-0.53	4.62
		0.2	25	14.6	2.83	0.57	Unequal			1.416	20	0.172	2.05	1.45	-0.97	5.06
JLB	< 50	0.04	16	16.7	5.33	1.33	Equal	6.433	0.016	1.721	32	0.095	2.58	1.50	-0.47	5.63
		0.3	18	14.1	3.27	0.77	Unequal			1.675	24	0.107	2.58	1.54	-0.60	5.75
JLB	< 50	0.04	16	16.7	5.33	1.33	Equal	3.330	0.076	-0.265	37	0.792	-0.40	1.51	-3.45	2.65
		0.4	23	17.1	4.09	0.85	Unequal			-0.253	26	0.802	-0.40	1.58	-3.64	2.85
JLB	< 50	0.1	20	18.9	4.60	1.03	Equal	2.505	0.121	3.775	43	0.001	4.21	1.12	1.96	6.46
		0.2	25	14.6	2.83	0.57	Unequal			3.585	30	0.001	4.21	1.17	1.81	6.61
JLB	< 50	0.1	20	18.9	4.60	1.03	Equal	0.906	0.348	3.620	36	0.001	4.74	1.31	2.08	7.39
		0.3	18	14.1	3.27	0.77	Unequal			3.685	34	0.001	4.74	1.29	2.13	7.35
JLB	< 50	0.1	20	18.9	4.60	1.03	Equal	0.251	0.619	1.330	41	0.191	1.76	1.33	-0.91	4.44
		0.4	23	17.1	4.09	0.85	Unequal			1.319	38	0.195	1.76	1.34	-0.94	4.47
JLB	< 50	0.2	25	14.6	2.83	.57	Equal	0.362	0.550	0.567	41	0.574	0.53	0.93	-1.36	2.41
		0.3	18	14.1	3.27	0.77	Unequal			0.553	33	0.584	0.53	0.96	-1.41	2.47
JLB	< 50	0.2	25	14.6	2.83	057	Equal	1.074	0.305	-2.428	46	0.019	-2.45	1.01	-4.48	-0.42
		0.4	23	17.1	4.09	0.85	Unequal			-2.392	38	0.022	-2.45	1.02	-4.52	-0.38
JLB	< 50	0.3	18	14.1	3.27	0.77	Equal	0.206	0.652	-2.519	39	0.016	-2.98	1.18	-5.37	-0.59
		0.4	23	17.1	4.09	0.85	Unequal			-2.589	38	0.013	-2.98	1.15	-5.30	-0.65

Appendix D (…continued)

Grouping by concentration			Group statistics				Independent samples test									
							Levene's test for equality of variances			t-test for equality of means						
Species code	Size (weight, g)	Conc. (g/l)	n	MTD (min.)	Std. devn. MTD	Std. error MTD	Assumption of variance	F	Sig.	t	Df	Sig. (2-tailed)	Mean difference	Std. error difference	95% C.I. of the difference Lower	Upper
Bre	< 100	0.1	64	22.0	4.33	0.54	Equal	3.833	0.053	-1.020	98	0.310	-1.03	1.01	-3.05	0.98
		0.2	36	23.0	5.71	0.95	Unequal			-0.944	57	0.349	-1.03	1.10	-3.23	1.16
Bre	< 100	0.1	64	21.9	4.33	0.54	Equal	3.996	0.048	2.876	96	0.005	2.41	0.84	0.75	4.07
		0.3	34	19.5	3.08	0.53	Unequal			3.185	87	0.002	2.41	0.76	0.91	3.91
Bre	< 100	0.1	64	22.0	4.33	0.54	Equal	0.182	0.670	4.016	97	0.000	3.65	0.91	1.85	5.46
		0.4	35	18.3	4.32	0.73	Unequal			4.020	70	0.000	3.65	0.91	1.84	5.46
Bre	< 100	0.2	36	23.0	5.71	0.95	Equal	11.237	0.001	3.112	68	0.003	3.44	1.11	1.24	5.65
		0.3	34	19.5	3.08	0.53	Unequal			3.162	54	0.003	3.44	1.09	1.26	5.63
Bre	< 100	0.2	36	23.0	5.71	0.95	Equal	3.610	0.062	3.891	69	0.000	4.69	1.20	2.28	7.09
		0.4	35	18.3	4.32	0.73	Unequal			3.907	65	0.000	4.69	1.20	2.29	7.08
Bre	< 100	0.3	34	19.5	3.08	0.53	Equal	1.706	0.196	1.375	67	0.174	1.24	0.90	-0.56	3.05
		0.4	35	18.3	4.32	0.73	Unequal			1.381	61	0.172	1.24	0.90	-0.56	3.04
OLB	≥ 100	0.04	10	29.0	3.09	0.98	Equal	4.057	0.058	1.920	20	0.069	3.33	1.74	-0.29	6.95
		0.1	12	25.7	4.70	1.36	Unequal			1.994	19	0.061	3.33	1.67	-0.16	6.83
OLB	≥ 100	0.04	10	29.0	3.09	0.98	Equal	3.799	0.065	2.961	21	0.007	6.92	2.34	2.06	11.79
		0.2	13	22.1	6.85	1.90	Unequal			3.241	17	0.005	6.92	2.14	2.43	11.42
OLB	≥ 100	0.04	10	29.0	3.09	0.98	Equal	7.381	0.016	2.254	15	0.040	4.86	2.16	0.26	9.45
		0.3	7	24.1	5.79	2.19	Unequal			2.028	8	0.075	4.86	2.40	-0.62	10.33
OLB	≥ 100	0.04	10	29.0	3.09	0.98	Equal	0.740	0.400	5.485	19	0.000	7.91	1.44	4.89	10.93
		0.4	11	21.1	3.48	1.05	Unequal			5.518	18	0.000	7.91	1.43	4.91	10.91
OLB	≥ 100	0.1	12	25.7	4.70	1.36	Equal	0.705	0.410	1.515	23	0.143	3.59	2.37	-1.31	8.49
		0.2	13	22.1	6.85	1.90	Unequal			1.538	21	0.139	3.59	2.33	-1.26	8.44
OLB	≥ 100	0.1	12	25.7	4.70	1.36	Equal	.884	0.360	0.627	17	0.539	1.52	2.43	-3.60	6.65
		0.3	7	24.1	5.79	2.19	Unequal			0.592	10	0.566	1.52	2.57	-4.16	7.21
OLB	≥ 100	0.1	12	25.7	4.70	1.36	Equal	1.983	0.174	2.635	21	0.015	4.58	1.74	0.96	8.19
		0.4	11	21.1	3.48	1.05	Unequal			2.670	20	0.015	4.58	1.71	1.00	8.15
OLB	≥ 100	0.2	13	22.1	6.85	1.90	Equal	0.014	0.907	-0.677	18	0.507	-2.07	3.05	-8.48	4.35
		0.3	7	24.1	5.79	2.19	Unequal			-0.713	14	0.487	-2.07	2.90	-8.26	4.13
OLB	≥ 100	0.2	13	22.1	6.85	1.90	Equal	2.639	0.119	0.432	22	0.670	0.99	2.28	-3.75	5.72
		0.4	11	21.1	3.48	1.05	Unequal			0.454	18	0.655	0.99	2.17	-3.57	5.54
OLB	≥ 100	0.3	7	24.1	5.79	2.19	Equal	5.468	0.033	1.408	16	0.178	3.05	2.17	-1.54	7.65
		0.4	11	21.1	3.48	1.05	Unequal			1.258	8	0.241	3.05	2.43	-2.45	8.56