Development of Protocols for Acute Fish Toxicity Bioassays, Using Suitable Indigenous Freshwater Fish Species

Report to the
Water Research Commission

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EXECUTIVE SUMMARY

The *Poecilia reticulata* (Guppy) test is currently the preferred fish toxicity test being used in most South African laboratories. There are, however, several issues which hamper the successful use of the Guppy test in bioassays (i.e. it is an exotic species and may therefore not be representative of the fish fauna in South African ecosystems, significant variation was detected in results between laboratories and disease result in frequent loss of brood stock). The above emphasizes not only the need for nationally standardized fish bioassay protocols, but also for the use of indigenous fish representing receptor organisms actually present in aquatic ecosystems will ensure the extrapolation of meaningful, relevant and ecologically significant results and management objectives from ecotoxicity tests. This is specifically significant in view of the increasingly important role that toxicity-testing plays in water resource management in South Africa.

With this in mind the current project was initiated during August 2002, with the following aims:

- To develop capacity to ensure the continued production of adequate test organisms to be provided to research and consulting facilities in South Africa in order to meet market requirements;
- to establish protocols for the laboratory culturing and maintenance of selected indigenous freshwater fish species, for use in ecotoxicity testing;
- to establish fish bioassay protocols which will provide representative data for ecosystems in the South African context.

An initial desktop evaluation was conducted in order to assess the potential of different South African freshwater fish species for use in bioassays. Potentially useful fish species were selected, based on their distribution, habitat use specificity, perceived amenability to routine laboratory culturing and maintenance, ecological importance and relative sensitivity. The suitability of fish species for use in bioassays was assessed using a weighted ranking approach. Based on available information, the following indigenous fish species were indicated to have the highest potential suitability for use in bioassays (in order of potential suitability): *Barbus trimaculatus, Barbus anoplus*, *Chiloglanis pretoriae*, *Opsaridium peringueyi, Barbus eutaenia, Tilapia sparrmanii*, *Aplocheilichthys katangae, Barbus paludinosus* and *Chiloglanis paratus.*

The above species (with the exception of the *Chiloglanis* species) were further evaluated in terms of laboratory maintenance (tank stocking specifications, filtration, water quality and photoperiod, maintenance, food and feeding, disease control) and forced spawning behaviour and conditions (design and preparation of spawning tanks, spawning and fry development).

Juveniles of species responding positively to laboratory culturing (*Barbus trimaculatus, B. anoplus, B. paludinosus, B. eutaenia* and *Tilapia sparrmanii*) were used for bioassays in order to assess their suitability for use in toxicological testing. During this phase of the study the response characteristics of the selected species to potassium dichromate ($K_2Cr_2O_7$), sodium chloride (NaCl) and sodium fluoride (NaF) were investigated. This evaluation was based on a preliminary assessment of their response sensitivity towards the selected reference chemicals, as well as the consistency of their response. Consistency or reproducibility of results is a most important factor to consider in standardized species selection, particularly for regulatory purposes.

Results obtained from the breeding trials and bioassays was used to re-evaluate each of the selected freshwater fish species in terms of their suitability for use in bioassays, using the same weighted ranking approach followed during the initial desktop assessment. *Barbus trimaculatus* was indicated to have the highest suitability for use in toxicity bioassays, followed by *Barbus paludinosus* and *Tilapia sparrmanii*.

Barbus trimaculatus is relatively small (SL = 150 mm). This, together with its non-aggressive social behaviour (Skelton, 2001) resulted in it not requiring large holding or spawning facilities. During the present investigation 90 ℓ tanks were successfully used for spawning. *Barbus trimaculatus* were not particularly sensitive to handling and adapted very well to their captive conditions in the laboratory, with very few mortalities recorded. They responded very well to artificially induced ques to stimulate spawning throughout the year (i.e. increased protein in their diet, simulated stream conditions in the spawning tank and manipulation of water hardness to simulate a rain event). They are not very susceptible to disease and are widely distributed. They displayed a sensitive response towards NaCl and NaF, but were less sensitive towards $K_2Cr_2O_7$. More importantly though, they were consistent in their response towards the reference chemicals.

Based on an evaluation of its distribution, habitat requirements, ease of culturing, ecological importance and sensitivity, *Barbus paludinosus* was indicated to be the second most suitable species for use in toxicity bioassays. They did not require large holding or spawning facilities. They were not particularly sensitive to handling and adapted very well to their captive conditions in the laboratory, with very few mortalities recorded. They responded very well to artificially induced ques to stimulate spawning. *Barbus paludinosus* displayed a similar response sensitivity and response consistency than *B. trimaculatus*.

Tilapia sparrmanii is relatively small (230 mm SL) and despite its aggressive behaviour towards other fish during breeding, it was possible to breed the species making use of small

spawning facilities (90 ℓ tanks). Placing a divider in the spawning tank to divide the tank into two equal sized breeding compartments also proved successful, and doubled the effort within the same space. They were not particularly sensitive to handling, adapted very well to their captive conditions in the laboratory and are not susceptible to disease. This species however does require close monitoring and management of breeding cultures, which is time consuming. The latter is a factor to take into account when considering the use of this species in routine laboratory testing. *Tilapia sparrmanii* was found to be tolerant towards NaCl, as could be expected, but was sensitive in its response towards the other reference chemicals ($K_2Cr_2O_7$ and NaF). The reproducibility of data obtained with NaCl and NaF were excellent with Coefficient of Variance (CV) values of 5.8% and 13.9%, respectively.

Results obtained during this investigation also indicated *Barbus anoplus* and *Barbus eutaenia* as potential species for use mainly in site-specific water resource management studies (i.e. ecological risk assessment, refinement of ECOSPECS, etc.). They are relatively small and therefore do not requiring large holding facilities. *Barbus anoplus* were not particularly sensitive to handling and adapted very well to their captive conditions in the laboratory, with very few mortalities recorded. *Barbus eutaenia* was indicted to be slightly sensitive to handling and nitrate-related water quality changes resulting from live foods have led to some mortalities, but they nevertheless adapted very well to laboratory conditions. Both species responded very well to artificially induced ques to stimulate spawning. They displayed the same trend that the other *Barbus* species in their response towards the reference chemicals, being sensitive in their response towards NaCl and tolerant towards $K_2Cr_2O_7$. Both *B. eutaenia* and *B. anoplus* were consistent in their response to the chemicals. When using *B. anoplus* in laboratory bioassays, care should be taken to use fish from the same source to prevent differences in sensitivity as a result of possible genetic differences between different isolated populations.

The breeding system developed for the *Barbus* species evaluated as part of this investigation was not expensive and similar in design. The same protocol, with minor modifications for each species, can be used to breed *Barbus trimaculatus*, *B. paludinosus*, *B. eutaenia*, *B. anoplus*, and probably other *Barbus* species as well. The protocols are easy to implement and do not require large infrastructure. The effort and infrastructure required are comparable with that of other standard protocols (such as *Poecilia reticulata* and *Danio rerio*).

The variation in the toxic response especially between the Cyprinid and Cichlid species confirmed that there is no single species suitable for all applications. It is therefore important to conduct tests with several species, from different taxonomic groups, to get some indication of the natural variability in levels causing an effect. This is especially

important in terms of ecological risk assessment studies, the refinement of ECOSPECS and derivation of site-specific water quality criteria. The species selected for testing may differ from ecosystem to ecosystem, and the selection will often have to be based on site-specific considerations. A standard toxicity test may therefore not be appropriate for answering specific questions regarding a particular aquatic ecosystem. Assessing the hazard of a particular chemical to an indigenous fish community in a specified aquatic ecosystem will therefore require species representative of that system and it will not be useful to employ standard test species that are not normally present in the specific aquatic system. This is clearly demonstrated with the results obtained as part of the present investigation. The *Barbus* species were much less tolerant towards NaCl than *Poecilia reticulata*. Since increased salinization of our water resources is one of the greatest challenges water quality managers in South Africa are faced with, the use of a salt tolerant species such as *P. reticulata* in toxicity bioassays may therefore not provide adequate protection of our water resources.

Based on the findings of this investigation, the following are recommended:

- The protocols developed during this study have important application, especially with regard to ecological risk assessment studies, the refinement of ECOSPECS and derivation of site-specific water quality criteria. The culture techniques developed during this investigation should now be refined further through consultation with other laboratories implementing the procedures.
- *Barbus trimaculatus* displayed the highest suitability for use in toxicity bioassays. The ability of this test organism to generate similar results in different laboratories should be assessed, with the possibility of standardizing the protocol.
- A range of chemicals should be evaluated in order to evaluate the test species' sensitivity and response consistency over a wide spectrum (to include organic and inorganic chemicals).
- The brood stock used in this investigation was collected from wild populations. Future production facilities must take care to sample initial stock from the same areas in order to prevent introduction of different genetic strains, as this may affect test results. A very stringent collection, husbandry, brood stock integrity procedure will have to be designed and implemented to ensure that facilities providing test organisms are managed properly, to ensure a consistent supply of fish that would provide scientifically defensible results. A certification procedure will have to be implemented for these suppliers to ensure compliance with international husbandry practices and standards.
- The use of the above oviparous species in early life stage toxicity tests (e.g. eggs, embryos) has the potential for further development.

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INTRODUCTION

1.1. BACKGROUND

The *Poecilia reticulata* (Guppy) test is currently the preferred fish toxicity test being used in most South African laboratories. There are, however, several issues which hamper the successful use of the Guppy test in bioassays:

- *Poecilia reticulata* is an exotic species and can therefore not be regarded as representative of the fish fauna in any of the South African ecosystems. Results obtained from these tests may therefore either over- or under assess the toxicity of tested effluents on indigenous fish.
- Significant variation was recorded in *P. reticulata* test results amongst South African laboratories (*P. reticulata* acute toxicity test Proficiency Scheme results, Report Numbers 2002/2, 2002/5, 2002/8, 2003/2, 2003/5, 2003/8, 2003/11, 2004/2, 2004/8, 2004/11, 2005/1). This is mainly ascribed to the fact that this test, although internationally validated and standardized, is not yet properly standardized for use in the South African context (mainly with regard to the age of test organisms, dilution water, organism maintenance and source of test organisms).
- *Poecilia reticulata* is currently obtained from varied sources, which may result in the introduction of disease and frequent loss of Guppy brood stock.

The above emphasizes the need for nationally standardized fish bioassay protocols. Furthermore, the use of indigenous fish representing receptor organisms actually present in aquatic ecosystems will ensure the extrapolation of meaningful, relevant and ecologically significant results and management objectives from ecotoxicity tests. This is specifically significant in view of the increasingly important role that toxicity-testing plays in water resource management (WRC Workshop, 10 May 2000). The decision by the Department of Water Affairs and Forestry (DWAF) to include Whole Effluent Toxicity (WET) testing as part of its toxic effluent management policy has necessitated the establishment of suitable procedures for use in the South African context (WRC Report No 453/1/98). Although the Guppy test was proposed as a standard test for local application (WRC Report No 358/1/98), Slabbert and co-workers identified the need to rather use indigenous fish species. This need was again emphasized during a WRC Workshop (10 May 2000) on the future direction of research and implementation of ecotoxicology and bioassaying in South Africa.

In order for fish bioassays to be applicable and relevant not only to aquatic ecosystems in South Africa as a whole, but also to different eco-regions within the country, it is important

to use indigenous fish species. The development of protocols for the use of indigenous fish species will fill the existing gap in toxicity bioassays and will present the water resources manager, regulatory bodies, etc. with scientifically defensible results, representing actual receptor species which may be subjected to potential effects.

With this in mind the current project was initiated during August 2002.

1.2. AIMS OF THE PROJECT

- To develop capacity to ensure the continued production of adequate test organisms to be provided to research and consulting facilities in South Africa in order to meet market requirements
- To establish protocols for the laboratory culturing and maintenance of selected indigenous freshwater fish species, for use in ecotoxicity testing
- To establish fish bioassay protocols which will provide representative data for ecosystems in the South African context

1.3. LITERATURE CITED

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CHAPTER 2
DESKTOP REVIEW AND SELECTION OF INDIGENOUS FRESHWATER FISH SPECIES FOR USE IN ROUTINE BIOASSAYS

2.1. BACKGROUND

This section of the study is aimed at assessing the potential of different South African freshwater fish species for use in bioassays. This was achieved by listing species considered as potentially useful as standard test organisms. In order to achieve this, an extensive literature review was conducted (WATERLIT, FISHLIT and Internet search engines). Potentially useful fish species were selected, based on the following criteria:

- Distribution: each species was evaluated based on its occurrence in each of the biogeographical fish zones (Skelton, 2001) within South Africa
- Habitat use specificity
- Amenability to routine laboratory culturing and maintenance (including size of species and subsequent size of holding facilities that would be required, sensitivity to handling, spawning behaviour and breeding requirements, ease of culturing, susceptibility to disease, food and feeding, behaviour)
- Ecological importance
- Relative sensitivity (resilience and resistance to perturbations)

2.2. SUITABILITY ASSESSMENT

The suitability of fish species for use in bioassays was assessed using a weighted ranking approach. The identified criteria were ranked and a relative importance was assigned to each in terms of a numerical weight, the sum of which was to be 100 (based on the method described by Dean & Nishry (1965) (Table 2.1). The relative importance assigned to each criterion was based on professional judgment with the intention to facilitate the selection process and was substantiated through general agreement resulting from discussion between team members.

Table 2.1: Ranking matrix for each of the identified criteria. Each criterion was rated against each other. Rating: less important = 0, as important = 1, more important = 2. The rating was used to obtain a weighting out of 100.

Criterion	Distribution	Habitat	Culturing	Importance	Sensitivity	Dummy	Total	Percentage
Distribution		2	1	2	1	2	8	26
Habitat	0		2	1	0	2	5	17
Culturing	1	0		2	1	2	6	20
Importance	0	1	0		0	2	3	10
Sensitivity	1	2	1	2		2	8	27
Dummy	0	0	0	0	0		30	100

The suitability of each fish species was then rated in terms of each criterion on the following basis:

- 1-2: Not Suitable
- 3-5: Low Suitability
- 6-8: Moderate Suitability
- 9-10: High Suitability

Each score for each species for each criterion was then separately multiplied by the weight of each criterion to give the suitability of a species for a specific criterion. These scores are added to give the total suitability of a specific species for all criteria. Highest scores translate to highest suitability.

These results were discussed with various fish specialists in order to verify the scores allocated and to finalize habitat use specificity-, ecological importance- and sensitivity scores for those species where some uncertainty existed.

Table 2.2: Potential suitability of different indigenous freshwater fish species for use in bioassays

	Distribution	Habitat	Culturing	Importance	Sensitivity	TOTAL
WEIGHT	26	17	20	10	27	
CYPRINIDAE						
Mesobola brevianalis	6	6	5	7	5	563
Opsaridium peringueyi	4	3	8	8	9	638
Pseudobarbus burchelli	1	4	3	5	7	393
Pseudobarbus burgi	1	4	3	5	7	393
Pseudobarbus afer	1	2	3	5	7	359
Pseudobarbus asper	2	4	3	6	7	429
Pseudobarbus phlegethon	1	4	3	5	7	393
Pseudobarbus tenuis	1	4	3	6	7	403
Barbus anoplus	9	4	8	6	5	657
Barbus gurneyi	2	2	7	4	7	455
Barbus motebensis	2	4	7	7	6	492
Barbus amatolicus	1	?	7	?	?	
Barbus treurensis	1	1	5	7	8	429
Barbus annectens	2	5	5	6	6	459
Barbus lineomaculatus	2	2	5	6	8	462
Barbus brevipinnis	2	3	7	8	8	539
Barbus neefi	4	3	7	7	7	554
Barbus pallidus	2	3	7	4	6	445
Barbus unitaeniatus	5	7	8	2	3	510
Barbus bifrenatus	3	5	5	7	6	495
Barbus viviparus	6	6	8	3	4	556
Barbus toppini	3	6	5	6	5	475
Barbus radiatus	4	6	5	6	4	474
Barbus trimaculatus	8	8	8	6	4	672
Barbus callidus	1	3	3	5	7	376
Barbus erubescens	1	3	3	5	7	376
Barbus eutaenia	5	1	8	7	9	620
Barbus hospes	1	?	3	?	?	
Barbus trevelyani	1	4	3	4	6	356
Barbus argenteus	3	1	4	7	9	488
Barbus paludinosus	8	8	8	2	3	605
Barbus mattozi	3	4	2	7	6	418
Barbus afrohamiltoni	4	2	4	6	6	440
Barbus serra	1	4	2	5	7	373

Barbus andrewi	1	2	2	5	6	312
Labeobarbus kimberleyensis	5	3	1	2	7	410
Labeobarbus aeneus	5	6	2	5	6	484
Labeobarbus natalensis	3	6	2	5	6	432
Labeobarbus polylepis	3	3	2	7	6	401
Labeobarbus capensis	1	4	2	3	6	326
Labeobarbus marequensis	5	5	3	4	5	450
Varincorhinus nelspruitensis	3	3	2	7	8	455
Labeo umbratus	7	7	2	4	4	489
Labeo capensis	4	4	2	3	6	404
Labeo rubromaculatus	1	5	2	5	6	363
Labeo seeberi	1	4	2	4	6	336
Labeo rosae	4	5	2	4	5	404
Labeo ruddi	4	5	2	4	5	434
Labeo congoro	4	4	2	7	6	444
Labeo cylindricus	5	5	2	7	6	487
Labeo molybdinus	6	4	2	7	6	496
CHARACIDAE						
Brycinus imberi	3	7	5	3	4	435
Bryninus lateralis	1	?	5	?	?	
Micralestes acutidens	5	7	5	2	4	477
Hydrocynus vittatus	3	5	1	10	7	472
AUSTROGLANIDIDAE						
Austroglanis barnardi	1	1	1	4	9	346
Austroglanis gilli	1	3	1	4	8	353
Austroglanis sclateri	4	5	1	6	5	404
AMPHILIIDAE						
Amphilius natalensis	3	1	1	9	10	475
Amphilius uranoscopus	5	1	1	9	10	527
SCHILBEIDAE						
Schilbe intermedius	5	7	4	5	4	487
CLARIIDAE						
Clarias gariepinus	9	8	2	2	2	484
Clarias ngamensis	2	?	2	?	?	
Clarias theodorae	2	4	4	6	4	368
MOCHOKIDAE						

Chiloglanis anoterus	2	1	7	10	10	579
Chiloglanis bifurcus	1	1	4	10	10	493
Chiloglanis emarginatus	2	1	4	1	10	453
Chiloglanis paratus	4	4	7	10	7	601
Chiloglanis pretoriae	5	1	7	10	10	657
Chiloglanis swierstrai	3	1	4	10	9	518
Synodontis zambezensis	4	5	7	3	4	467
GALAXIIDAE						
Galaxies zebratus	3	4	5	5	6	458
APLOCHEILIDAE						
Notobranchius orthonotus	1	1	4	10	9	376
Notobranchius rachovii	1	1	4	10	8	349
POECILIIDAE						
Aplocheilichthys johnstoni	3	3	9	4	6	511
Aplocheilichthys myaposae	3	4	9	4	7	555
Aplocheilichthys katangae	5	1	9	9	7	606
CICHLIDAE						
Pseudocrenilabrus philander	8	8	5	2	2	518
Chetia brevis	1	7	5	7	4	423
Chetia flaviventris	4	7	5	7	4	501
Serranochromis meridianus	1	5	2	9	6	403
Tilapia sparrmanii	8	8	9	3	2	608
Tilapia rendalli	4	6	5	2	3	407
Oreochromis mossambicus	7	7	5	2	2	475

? = not known

Those species with highest scores (i.e. highest suitability) are shaded

Based on available information, the following indigenous fish species were indicated to have the highest potential suitability for use in bioassays:

- *Barbus trimaculatus* (672)
- *Barbus anoplus* (657)
- *Chiloglanis pretoriae* (657)
- *Opsaridium peringueyi* (638)
- *Barbus eutaenia* (620)
- *Tilapia sparrmanii* (608)
- *Aplocheilichthys katangae* (606)
- *Barbus paludinosus* (605)
- *Chiloglanis paratus* (601)

These species were evaluated to select those adapting best to laboratory culturing (Phase 2 of the project) and will be discussed separately in Chapters 4 to 10, in order of potential suitability.

A complete account on the maintenance and captive breeding of *Chiloglanis pretoriae* is given in De Villiers & Hecht (1990) and De Villiers (1991). Because of their similar habitat preferences and spawning requirements it may be possible to apply similar spawning and rearing techniques to *Chiloglanis paratus*. Although no attempt was made to breed the *Chiloglanis* species during the present investigation, they will be included in a summary evaluation of their suitability for use as a test species in bioassays (Chapter 12), together with the other species assessed during this investigation, based on the information generated by De Villiers & Hecht (1990) and De Villiers (1991).

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CHAPTER 3
DEVELOPMENT OF CLIMATE CONTROLLED BREEDING FACILITY

3.1. BACKGROUND

Not all environmental laboratories are equipped to breed their own test cultures (limited space, financial constraints, lack of expertise, etc.). In view of the increasing demand for toxicity testing in water resource management, one of the outputs of this study was therefore to set up a national facility (Lydenburg Aquatic Research Unit, Mpumalanga Parks Board) as a producer of test cultures (young fish) and brood stock of selected indigenous fish species.

3.2. DEVELOPMENT OF BREEDING FACILITY

In view of the above, a climate controlled holding facility was developed at Mpumalanga Parks Board's Aquatic Research Unit in Lydenburg. The walls of the facility were isolated so that temperature and photoperiod could be controlled. Five 500 liter tanks were prepared with underground filters and 200 watt heaters. Water temperatures were stabilised between 20° and 23° Celsius. The tanks were allowed to settle for a week before fish were introduced. The tanks were also equipped with suitable aquarium lighting to promote natural growth of plants. All five tanks were later equipped with trickle filters as density and sensitivity of fish to nitrite required more sophisticated filtering systems to ensure maintenance of water quality. A 14 hour light cycle was adopted to simulate summer light conditions. Six 50 liter breeding tanks with sponge filters were prepared with a slightly higher temperature (25° C) and one large square tank was modified to form a riffle and a backwater for use as a breeding tank. Spanish moss was obtained from local fish breeders to serve as a breeding medium in breeding tanks and backwaters.

In most of the literature, a constant supply of live food is considered essential to condition fish for breeding. For this purpose five circulating tanks were prepared to maintain a constant supply of live food (see APPENDIX A).

3.3. HOTHOUSE

A small hothouse was constructed to house the two large aquatan tanks and ten other 1000 liter tanks. This ensured that a larger stock of fish and a greater variety of species could be kept, while the climate-controlled facility could be used primarily for breeding purposes.

The hothouse also promoted natural growth of waterplants for spawning medium for breeding trials and assisted in stimulation of natural algal blooms in the brine shrimp culture.

3.4. MANAGEMENT AND SUPPLY OF BROOD STOCK AND TEST FISH

3.4.1. Management of brood stock and juvenile fish

Fish species selected for breeding trials were sampled from rivers with a high ecological integrity, to ensure that the fish were not genetically altered or "adapted" to pollutants. Evidence of genetic selection in the natural environment has been observed in mosquito fish after exposure to high levels of insecticides and in some crustaceans exposed to metals (Rand, 1995), resulting in such organisms being less sensitive to the specific pollutants or chemical agents.

Newly introduced fish were treated with a mixture of Acriflavine and Methylene Blue (1g each/100-150 liter (Geisler, 1982)) to prevent the outbreak of disease in the facility. They were acclimatized and conditioned for at least two months before they were used in breeding trials.

The age of test organisms is an important parameter when doing toxicity testing. The breeding protocols followed at the Lydenburg facility allowed recording of the hatching date (± 1 day) of embryos. Fry of similar age were separated and kept together.

3.4.2. Transportation of juvenile test fish

Juvenile fish (two to three weeks old) were transported in plastic containers via road from Lydenburg to Johannesburg. During transport, air was supplied continuously to each container by a battery-operated pump. Temperature was monitored and maintained at the temperature of the holding tank from which the juveniles were collected ± 2ºC. Juvenile fish were transported at a ratio of approximately 100 fish per 20 liters of water, to prevent possible water quality problems as a result of nutrient build up. This method resulted in mortality of <0.1%.

The logistics of transporting juvenile fish over longer distances was not assessed during the present investigation. It is however assumed that the fish can be transported without difficulty provided they are not crowded, dissolved oxygen is maintained above 5 mg/l, they do not receive a temperature shock (temperature should not be allowed to vary by more than 2ºC), and the transit time does not exceed 8 hours. When transporting fish over longer distances it is recommended that they are placed in plastic bags, inflated with compressed oxygen. These bags should then be transported in insulated containers to minimize

temperature fluctuations. See Hattingh *et al.* (1975) for a complete description of requirements when transporting fish.

3.4.3. Initial laboratory handling of juvenile test fish

Juvenile fish arriving at the laboratory were allowed to acclimate to holding temperature by placing the fish, in their original shipping containers, in environmental temperature- and illumination-controlled rooms (21±1ºC and 14h/10h light: dark cycle). In order to minimize stress to the juvenile fish, they were kept in the shipping containers for two to three days, after which they were used in toxicity bioassays.

3.5. CONCLUSIONS

The Lydenburg facility was successful in constantly supplying healthy juvenile test fish of the desired age to the testing laboratory. The supply of bulk numbers is therefore regarded as viable provided that strict guidelines regarding the type of species, husbandry practices, origin and integrity of the brood stock is ensured and control measures implemented. Depending on the demand, commercial suppliers can also be expected to mobilize and start production of specific species within a relatively short period.

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CHAPTER 4
MAINTENANCE AND BREEDING OF *BARBUS TRIMACULATUS* (THREESPOT BARB), PETERS 1852

4.1. BACKGROUND

Barbus trimaculatus was indicated to be a potentially suitable species for use in bioassays, based on certain key criteria (refer to Chapter 2 for a list of these criteria). In this chapter *Barbus trimaculatus* is evaluated in more detail in terms of its suitability for use in routine laboratory bioassays, based on its amenability to routine laboratory culturing and maintenance (including size of species and subsequent size of holding facilities that would be required, sensitivity to handling, spawning behaviour and breeding requirements, ease of culturing, susceptibility to disease, behaviour, food and feeding). This was based on a survey of available literature and information, as well as experimental evaluation of suitable culturing and breeding requirements.

4.2. MORPHOLOGY AND TAXONOMY

The classification of *Barbus trimaculatus* (California Academy of Sciences Catalog of Fishes, 2005; Fishbase, 2005):

Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Barbus*
Species: *Barbus trimaculatus* Peters, 1852

Barbus trimaculatus attains 150 mm Standard Length (SL) (Skelton, 2001). Cambray (1984) found the largest *B. trimaculatus* male in the Orange River to be 76 mm Fork Length (FL) and the largest female to be 90 mm FL. The largest individual measured by Skelton (1981) in the lower Orange River was 98 mm SL.

The body is robust with two long barbels extending from the mouth. The fish is silvery, but tinged with gold when in breeding condition. It usually has three clear black spots on the body and base of the caudal peduncle. These three lateral spots, otherwise characteristic of this species, are not always evident. Some populations, such as that in the lower Orange River do not show these spots when alive. The round caudal spot is however, present.

Skelton (1981) found that on preservation in formalin the three spots became evident in small individuals.

The dorsal fin is made up of three true spines with eight branched rays, whilst the anal fin consists of three unbranched segmented rays, with five branched rays. There are 31-33 scales in the lateral line series, with 13-16 scales around the caudal peduncle (Skelton, 2001).

4.3. DISTRIBUTION

Barbus trimaculatus is a widespread African species (Gaigher, 1973; Skelton, 1981). This species occurs on the East coast from Ruvuma, Tanzania, to Umvoti in KwaZulu-Natal, as well as in the Orange, Cunene and Zambian Congo Systems. It was replaced in the Okavango, upper Zambezi and Kafue by Dashtail barb (*B. poechii*) (Skelton, 2001). The species does not appear to be abundant in the middle and lower Orange River (Skelton, 1981).

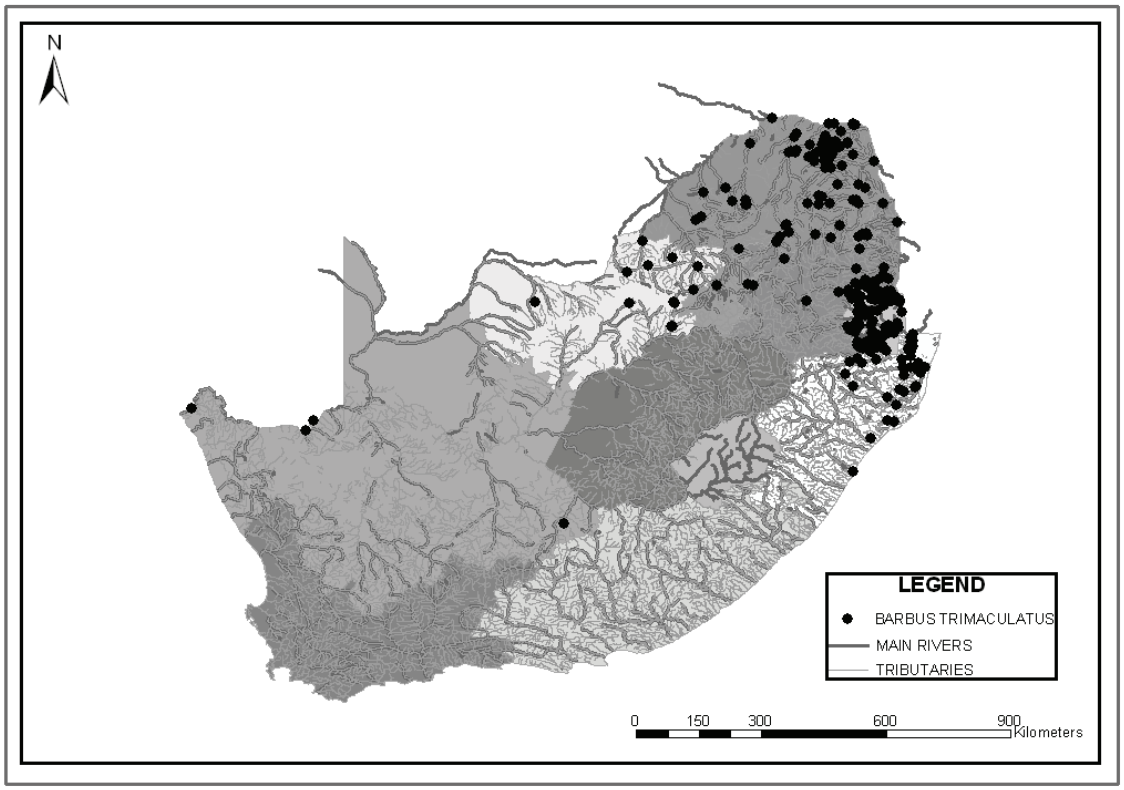

Figure 4.1: Distribution of *Barbus trimaculatus* in South Africa (data courtesy of the South African Institute for Aquatic Biodiversity, SAIAB, Mr. Willem Coetzer)

4.4. RELATIVE SENSITIVITY AND WATER QUALITY REQUIREMENTS

Barbus trimaculatus is hardy and common (Skelton, 2001). It is regarded as a moderately tolerant species, with an overall intolerance rating of 2.2 (Kleynhans, 2003). It is tolerant towards changes in habitat and is capable of surviving wide fluctuations in water quality (Lèvêque & Quensière, 1988, in Brummet & Katambalika, 1996).

4.5. HABITAT USE SPECIFICITY

The species is found in a wide variety of habitats, especially in quiet or slow flowing, well vegetated areas (Gaigher 1973; Skelton, 1981; 2001). Its preferred habitats are quiet, vegetated areas (Cambray, 1984). Crass (1960) reported that *B. trimaculatus* was characteristic of smaller streams rather than large rivers.

4.6. LABORATORY MAINTENANCE OF *B. TRIMACULATUS* CULTURES

About 50 *Barbus trimaculatus* (Threespot barb) individuals were collected during September 2002 from the Sabie River near Hoxanne Weir (25.01867 S, 31.018672 E) and introduced into the temperature controlled holding facility at Lydenburg. Although the species is widely distributed, the Sabie River population was preferred due to the high ecological status of this river.

4.6.1. Culture stock and tank stocking specifications

The breeding fish at the Lydenburg facility were kept in a 1.5 x 0.5 x 0.57 meter glass tank (400 ℓ). The tank was divided with a fine mesh into two equal sized compartments and the sexes were kept separate. The size of the abdominal development in individuals was used to separate the sexes. The population density was maintained at about 30 (15 males and 15 females), i.e. approximately one fish per 13 ℓ of water. By keeping sexes apart but in the same tank ensured that pheromones could assist in the development of gonads. A trickle filter directed flow from males towards the females to ensure that females were constantly exposed to pheromones from the males. Fish were fed regularly on available live foods and vegetable matter to improve their condition and to further induce the development of gonads. Three hundred milliliters of agricultural dolomite were added to the tank to increase hardness and alkalinity of the water in the tank and stabilise the pH between 7 and 8. This was also done because a decrease in hardness can potentially be a stimulus to induce spawning.

Once gonads were fully developed, suitable males and females were selected for breeding trails and placed into breeding tanks. Spawning colours and behaviour were noted within two hours after placing fish into breeding tanks.

At ECOSUN brood stock was kept in a 0.915 x 0.36 x 0.32 meter glass tank (90 ℓ). The tank was also divided with a fine mesh into two equal sized compartments to keep males and females separate. Because of the smaller size of the holding tank, it was not necessary to direct flow from the males to the females in order to enhance gonad development. The population density was maintained at about 16 (8 males and 8 females), i.e. approximately one fish per 6 ℓ of water. Increasing the density resulted in increased mortalities.

Additional specimens were kept in a 2000 ℓ plastic tank in a hothouse at the Lydenburg facility to replace any casualties.

4.6.2. Filtration

The 400 ℓ holding tank at the Lydenburg facility was prepared with coarse sand (0,5-1.0 mm) on the bottom and an under-gravel filter. A flow of about 300 ℓ/hour was maintained through the under-gravel filter operated by an airlift. A filter unit, consisting of a 5 ℓ rectangular tank, divided into four compartments, was also mounted above the fish tank. Water was pumped out of the fish tank into the first compartment of the filter unit and gravitated back into the fish tank via an outlet on the top of the fourth compartment. A flow of approximately 300 ℓ/hour was maintained through the filter unit. Water in the filter unit was allowed to flow from the first and third compartments to the next compartment via a slot on the bottom of the divisions. Water flowed from the second compartment into the third via a slot on the top the division. The first and third compartments were filled with pieces of fine meshed shade cloth and the second compartment was filled with filtration wool. The fourth compartment contained a layer of filtration wool and activated charcoal.

The 90 ℓ holding tank at ECOSUN was fitted with an under gravel filter and a TRIO2000 mechanical and biological internal filtration system.

4.6.3. Water quality and photoperiod

Water temperatures were stabilized between 23°C and 25°C with a 200-watt heater. *Barbus trimaculatus* is a tropical species usually occurring at temperatures ranging between 24ºC and 26°C (Fishbase, 2005). The hardness of the water was slightly increased by placing about 300 mℓ agricultural dolomite in the tank and alkalinity stabilized at about 80 to 120 parts per million (ppm). Alkalinity was measured with commercially available HTH Quick

Test Pool test strips. A photoperiod of 14 hours per day was maintained (to simulate summer conditions).

4.6.4. Maintenance

The gravel in the fish tanks was siphoned weekly to prevent the build-up of nutrients in the tank and the water in the tank was topped-up. The filter material in the filter unit at the Lydenburg facility was washed every second week and the filter wool and activated charcoal was replaced every two months. Although this species is not very susceptible to the build-up of nutrients, the tank still needed to be completely cleaned and gravel washed about every six months. Bottom feeding fish such as *Ancistrus* spp. (*Plecostomus* spp.), freely available in the pet trade, were introduced into all tanks to remove excess food and algae. This was done mainly to avoid potential water quality problems.

Fifty percent of the water in the holding tank at ECOSUN was replaced every second week, and the under gravel filter was cleaned once every second month. The TRIO2000 mechanical and biological internal filtration system was cleaned and the carbon replaced every month. The gravel in the tanks were siphoned every second day to prevent build-up of nutrients in the tank, where after the water in the tank was topped up with dechlorinated tap water of the same temperature. As this tank is much smaller than the tank used at the Lydenburg facility, it required more frequent cleaning.

4.6.5. Food and feeding

Under natural conditions *B. trimaculatus* is zooplanktivorous, eating insects and other small organisms (Skelton, 2001). Their primary food is micro crustaceans, however, they will take advantage of a wide range of foods as available (Brummet & Katambalika, 1996; Cambray 1983). In the laboratory, fish were fed daily on a diet consisting of high quality and well-balanced commercially available fish food, available live foods and green vegetable matter. Live foods consisted mainly of *Daphnia*, cyclops, ostracods, bloodworms, mosquito larvae, sliced earthworms and brine shrimp (see APPENDIX A). Conditioning fish with enriched live food cultures was achieved by adding a mixture of 10 ml milk and 500 mg omega-3 fish oils into the water of the collected *Daphnia* two hours before feeding the fish. Vegetable matter consisted mainly of duckweed (*Lemna major* and *Wolfia arhiza*) and small pieces of fresh lettuce.

4.6.6. Disease control

As a result of the huge trout industry in the Lydenburg area, white spot (*Ichthyophthirius multifiliis*) is a serious threat to any fish introduced into the facility. Precautionary treatment consisting of a mixture of Acriflavine and Methylene Blue (1g each/100-150ℓ, Geisler, 1982) was added to the tank whenever an outbreak of disease was suspected or when new fish were introduced. Nets also needed to be sterilized on a regular basis by soaking them in a solution of 2% formaldehyde overnight.

4.7. NATURAL SPAWNING BEHAVIOUR AND CONDITIONS

Barbus trimaculatus breeds in summer, with shoals of ripe adults moving upstream in spate after rain. Females produce as many as 8000 eggs (Skelton, 2001). Cambray (1984) found *B. trimaculatus* in the Orange River to be resting during March and ripe in September. Ripe males had tubercles located on the dorsal side of the head.

4.8. FORCED SPAWNING BEHAVIOUR AND CONDITIONS

4.8.1. Preparation of spawning tanks

Best results at the Lydenburg breeding facility were obtained by using a 200 ℓ tank (0.8 x 0.7 x 0.4 meter), partially divided with a 0.6 x 0.4 x 0.02 meter polystyrene sheet in two unequal compartments (0.8 x 0.25 x 0.4 and 0.8 x 0.45 x 0.4) (see APPENDIX B). The two compartments were connected on the one side by a 200 mm gap between the glass and the one end of polystyrene sheet. A 500 ℓ/hour pump was used to draw water through a 0.3 x 0.04 x 0.04 meter sponge filter in the larger compartment and release into the closed end of the smaller compartment to create a slow current. An air-stone was placed near the outlet of the pump to create increased turbulence. Rounded stones (200-300 mm) and coarse gravel (5-10 mm) was placed in the smaller compartment to create stream conditions. To simulate backwater conditions, coarse gravel was placed on the whole bottom of the larger compartment and a small quantity of submerged water plants was introduced into one corner as spawning medium. The gravel is important for protecting the eggs after spawning, as the adults will actively hunt and forage eggs. The preferred spawning medium is Java moss but the remains of marine hydroid species (i.e. *Amphisbetia aperculata*), was also found to be an excellent spawning medium. The tanks were filled with dechlorinated tap water, which is softer than the water in the holding tanks. Alkalinity was used as a surrogate measure of hardness and water in breeding tanks normally had an alkalinity of less than 40ppm in comparison to 80-120ppm in holding tanks. Breeding tanks were left for at least a day to

heat and stabilize before fish were introduced. Temperatures were maintained at about 25ºC-27ºC with a 100-watt heater. The preferred temperatures for breeding, based on personal observations in the Sabie and Komati Rivers was above 22ºC.

Since laboratory space is an important consideration with regard to the suitability of this species for use in routine laboratory bioassays, a smaller 90 ℓ spawning tank (0.36 x 0.32 x 0.915 m) was designed at the ECOSUN Environmental Laboratory (see APPENDIX B). The design concept was similar to the tank used at the Lydenburg facility. The tank was partially divided with a 0.36 x 0.762 m glass sheet into two equal compartments of 0.36 x 0.16 x 0.915 m. The two compartments were connected on the one side by a 100 mm gap. A 500 ℓ/hour pump was used to draw water through a filter in the one compartment and released into the closed end of the other compartment to create a current (ranging between 0.02 m/s to 0.2 m/s) in the one compartment and a backwater area (0 m/s) in the other compartment. An air stone was placed near the outlet of the pump, to create increased turbulence. Round stones (200-250 mm) and coarse gravel (5-10 mm) were placed in the compartment into which the water was released to create stream conditions. To simulate backwater conditions, coarse gravel was placed on the bottom of the "stream" compartment and a small quantity of Java moss was introduced in the backwater area to act as a spawning medium.

4.8.2. Spawning

After the preparation of the spawning tanks, the sides of the tank were completely covered with cardboard and/or black cloth to avoid disturbance to the fish during spawning. Two males were introduced into the tank in the morning and two selected females in the afternoon in both the 200 ℓ and 90 ℓ tanks. Sizeable portions of live food, preferably *Daphnia* and or adult brine shrimp were placed in the tank to sustain fish. Two large leaves of lettuce submerged for about a minute in boiling water were placed into the tank to stimulate the formation of infusoria in the tank. The fish were left for two days and were removed on the morning of the second day to prevent hunting and foraging of eggs after spawning. The air-stone was removed to reduce turbulence in the tank and all covers were removed. A filter pump was kept running to maintain flow and water quality.

4.8.3. Fry development

The first fry appear within 3 to 4 days after the introduction of the fish and becomes free-swimming within 24 hours. Commercially available Liquifry No. 1 and micro-worm (*Anguillula silusiae*) were used to raise the fry. Infusoria grown on small amounts of grass cuttings proved useful, but the presence of certain cyclops species proved to be dangerous and killed fish fry rapidly. Lettuce soaked in boiling water for a few minutes to encourage the formation of infusoria was therefore preferred. The number of fry depended on the size,

age and development of gonads in the individuals used, with approximately 200 to 400 fry developed with each successful spawn. The fry were left in the spawning tank for two to three weeks before being transferred to other holding tanks, in order to minimize handling. After two to three weeks the juveniles were fed on Tetramin Baby®.

4.9. CONCLUSIONS

Barbus trimaculatus is relatively small (SL = 150 mm) and easy to handle. This, together with its non-aggressive social behaviour (Skelton, 2001), resulted in it not requiring large holding facilities. They are not particularly sensitive to handling and adapted very well to their captive conditions in the laboratory, with very few mortalities recorded. They responded very well to artificially induced ques to stimulate spawning throughout the year (i.e. increased protein in their diet, simulated stream conditions in the spawning tank and manipulation of water hardness to simulate a rain event). They are not very susceptible to disease.

This study indicated that *B. trimaculatus* did not require large holding or spawning facilities. The spawning tank was relatively inexpensive to construct, and once the protocol was developed, *B. trimaculatus* proved not difficult to breed throughout the year.

Based on its amenability to routine laboratory culturing and maintenance, it can therefore be concluded that *B. trimaculatus* has potential for use as a test species in both site-specific water resource management studies (i.e. ecological risk assessment, refinement of ECOSPECS, etc.) and routine laboratory bioassays.

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CHAPTER 5
MAINTENANCE AND BREEDING OF *BARBUS ANOPLUS* (CHUBBYHEAD BARB), WEBER 1897

5.1. BACKGROUND

Barbus anoplus was indicated to be a potentially suitable species for use in bioassays, based on certain key criteria (refer to Chapter 2 for a list of these criteria). In this chapter *Barbus anoplus* is evaluated in more detail in terms of its suitability for use in routine laboratory bioassays, based on its amenability to routine laboratory culturing and maintenance (including size of species and subsequent size of holding facilities that would be required, sensitivity to handling, spawning behaviour and breeding requirements, ease of culturing, susceptibility to disease, behaviour, food and feeding). This was based on a survey of available literature and information, as well as experimental evaluation of suitable culturing and breeding requirements.

5.2. MORPHOLOGY AND TAXONOMY

The classification of *Barbus anoplus* (California Academy of Sciences Catalog of Fishes, 2005; Fishbase, 2005):

Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Barbus*
Species: *Barbus anoplus* Weber, 1897

The head of *B. anoplus* is typically blunt or rounded. The mouth is small, usually with a single pair of short barbels. Females attain a larger maximum size (120 mm SL) and age (3-4 years) than males (100 mm SL, 2-3 years). The latter obtain a golden-yellow breeding coloration. Females and non-breeding males are grayish green above with a small dot at the mid-base of the caudal fin. Males sometimes have a broad dark band along the body. The scales have numerous radial striations, 33-37 in the lateral line series. The dorsal fin consists of three unbranched segmented rays and seven branched rays, the anal fin consists of three unbranched segmented rays and five branched rays. The primary dorsal ray is simple and flexible (Skelton, 2001).

The early developmental stages of *B anoplus* are well studied and documented (Cambray, 1983b). This study focused on the eggs, early development, the newly hatched larvae, mouth and feeding, growth rates, development of pigmentation and larval behaviour.

5.3. DISTRIBUTION

Barbus anoplus was initially described from the Buffels River (Gouritz River System) in the Cape (Jubb, 1967; 1968). The species is generally widespread and common in South Africa (Jubb 1967). It is in fact the most widespread freshwater fish species south of the Limpopo River (Jubb, 1967; 1968; Skelton, 1980 in Cambray 1983a). *Barbus anoplus* is widely distributed from the Highveld Limpopo to upland KwaZulu-Natal, Transkei and the middle and upper Orange basin, including the Karoo. It is absent from the lower Orange River. *Barbus anoplus* is present in large coastal rivers of the eastern and Western Cape (Olifants, Gourits, Gamtoos, Sundays and Great Fish) but is absent from Cape fold mountain streams (Skelton, 2001).

Skelton & Cambray (1981) reported the distribution of the minnow in the Orange River as puzzling. Albany Museum records indicate that it is common in the southern tributaries of the Orange River within the former Cape Province, as well as in the Free State and the former Transvaal Catchment regions of the Vaal-Orange system. It was found to be common in the marginal areas of both Gariep Dam and Vanderkloof Dam (Cambray, et al., 1978; Cambray & Hahndiek, 1980). Skelton & Cambray (1981) reported very limited occurrence of this species in the lower Orange River, with only two specimens collected from a pool at Prieska. The authors speculated that this phenomenon might be temperature related, with *B. anoplus* being a more temperate species. Crass (1964) and Gaigher (1973) found *B. anoplus* to be mostly limited to altitudes above 900 m in KwaZulu-Natal and the former Transvaal.

In view of the present study it is important to note here that different geographically isolated populations exist and that the genetic differences between these populations are complex. Morphological differences between geographically subdivided *B. anoplus* populations were first detected by Barnard (1943) who divided the northerly Chubbyhead barbs into two species, namely *B karkensis* from Natal and *B. anoplus* for the rest of its distribution. Barnard (1943) further subdivided *B. anoplus* into three geographically isolated forms (Orange, Olifants and Gouritz River Systems) substantiated by morphological differences among the three forms. The genetic differences found between the *B. anoplus* populations found in the study by Engelbrecht & Van der Bank (1997) suggest that the morphological subdivision by Barnard (1943) may be substantiated by a more detailed study of the genetic differences between these geographically subdivided *B. anoplus* populations. Results obtained by Engelbrecht & Van der Bank (1997) also suggest that the dispersion and

isolation of these fish species into different rivers of Southern Africa have created ideal conditions for genetic divergence and speciation.

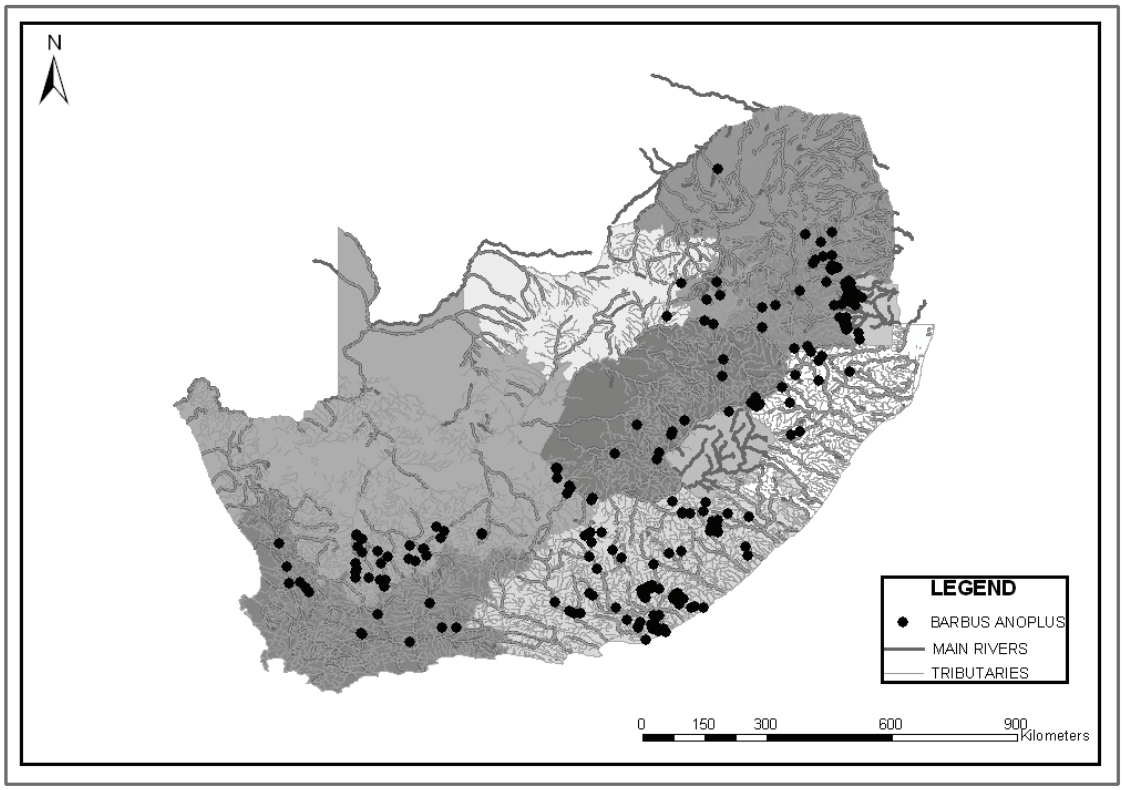

Figure 5.1: The distribution of *Barbus anoplus* in South Africa (data courtesy of SAIAB, Mr. Willem Coetzer)

5.4. RELATIVE SENSITIVITY AND WATER QUALITY REQUIREMENTS

Barbus anoplus is moderately sensitive towards changes in water quality and habitat with an overall intolerance rating of 2.6 (Kleynhans, 2003). *Barbus anoplus* is reported to be the most widespread species south of the Limpopo (Jubb, 1967; 1968; Skelton, 1980 in Cambray 1983). This success is attributed, amongst others, to:

- The unspecialized facultative feeding biology of *B. anoplus* (Cambray, 1983; Tomasson et al., 1983)
- Its high seasonal reproductive potential (Cambray & Bruton, 1985).
- Its pioneering and opportunistic nature, being quick to colonize a new environment and increase greatly in numbers (Jackson et al., 1983; Cambray and Bruton, 1985).

- The different adaptations exhibited by the protolarvae to ensure their survival (Tomasson et al., 1983)

It does however seem unable to co-exist with similar sized juveniles belonging to the same family, probably failing to compete with them for food and space (Cambray, 1982).

5.5. HABITAT USE SPECIFICITY

Barbus anoplus prefers cooler, well-oxygenated waters, occurring in a wide variety of habitats from small streams to large rivers and lakes (Gaigher, 1973; Skelton, 2001). They have a high preference for slow-deep and slow-shallow habitat types, and are usually associated with marginal or aquatic vegetation cover or in shelter such as fallen logs and brushwood (Skelton, 2001). Cambray et al. (1978), Jackson et al. (1983) and Jackson et al. (1982) reported *B. anoplus* to be opportunistic, inhabiting any sheltered place. Jackson et al. (1982) also found *B. anoplus* in pelagic areas in Vanderkloof Dam, even in the upper waters of the dam's center, over a kilometer from any shore. Although they occurred widely in such situations they were never abundant in the open waters.

5.6. LABORATORY MAINTENANCE OF *B. ANOPLUS* CULTURES

Individuals were collected from the upper Crocodile River in Verloren Vallei Nature Reserve and introduced into the facility. Genetic variation within this species between catchments may warrant several separate species. However, potential pollution, habitat degradation and desiccation by introduced alien species within its distribution range, made it difficult to select other suitable populations.

5.6.1. Culture stock and tank stocking specifications

The breeding fish at the Lydenburg facility were maintained in a 1.5 x 0.5 x 0.57 meter glass tank (400 ℓ). The tank was divided with a fine mesh into two equal sized compartments and the sexes were kept separate. The size of the abdominal development in individuals was used to separate the sexes. The population density was maintained at approximately 40 (15 males and 25 females), i.e. one fish per 10 ℓ of water. Keeping sexes apart but in the same tank ensured that pheromones could assist in the development of gonads. A trickle filter directed flow from males towards the females to ensure that females were constantly exposed to pheromones from the males. Fish were fed regularly on available live foods and

vegetable matter to improve their condition and improve the development of gonads. One hundred and fifty milliliters of agricultural dolomite was added to the tank to increase hardness and alkalinity of the water in the tank and to stabilise the pH between 7 and 8. This was done as a decrease in hardness potentially can be a stimulus to induce spawning. As soon as gonads were fully developed, suitable males and females were selected for breeding trials and placed in breeding tanks. Spawning colours and behaviour were noted within two hours after placing fish into breeding tanks.

Additional specimens were kept in a 2000 ℓ plastic tank in a hothouse at the Lydenburg facility to replace any casualties.

5.6.2. Filtration

The tank was prepared with coarse gravel on the bottom and an under-gravel filter. A filter unit, consisting of a 0.4 x 0.05 x 0.05 sponge and a 500 ℓ/hour pump supplied some additional filtration and flow to the system.

5.6.3. Water quality and photoperiod

Water temperatures were stabilized between 20°C and 23°C with a 200-watt heater to simulate optimum breeding conditions. The hardness and alkalinity of the water was increased slightly by placing about 150 ml agricultural dolomite in the tank. The alkalinity was used as a surrogate measure of hardness and was measured with HTH Quick Test Pool Test strips and normally stabilized at about 40 to 80 ppm and the pH between 7 and 8. A photoperiod of 14 hours per day was maintained in order to simulate summer conditions.

5.6.4. Maintenance

The gravel in the fish tanks was siphoned weekly to prevent the build-up of nutrients in the tank and the water in the tank was topped-up. At the Lydenburg facility, the filter material in the filter unit was washed every second week. This species is moderately susceptible to the build-up of nutrients and the tanks needed to be completely cleaned and gravel washed about every four months. In an attempt to avoid potential water quality problems, bottom feeding fish such as *Ancistrus* spp (*Plecostomus* spp), freely available in the pet trade, were introduced into all tanks to remove excess food and algae.

5.6.5. Food and feeding

Under natural conditions *B. anoplus* is a highly opportunistic feeder, with the diet reflecting its habitat (Cambray, 1983: Tomasson et al., 1983). It is however, generally omnivorous, feeding on insects, zooplankton, seeds, green algae and diatoms (Cambray, 1983; Rose et al., 1987; Skelton, 2001).

Barbus anoplus taken from the open-water habitat in Vanderkloof Dam, fed mainly on copepods and cladocerans, whereas those living in a small stream fed mainly on aquatic insect larvae and had a more varied diet. Seasonal shifts in the diet for both juvenile and adult minnows were observed. The smaller length groups change from benthic feeding in spring to mainly mid-water feeding in summer. The larger fish prey on corixids in summer but switch to mainly chironomidae larvae and zooplankton in winter. The minnows fed actively during the day with peak feeding in mid-morning and at dusk. It is suggested that the euryphagous feeding behaviour of *B. anoplus* is partially responsible for the widespread distribution of this species in southern Africa. (Cambray, 1983; Tomasson et al., 1983).

In the laboratory, fish were fed daily on a diet consisting of high quality and well-balanced commercially available fish food (TetraMin®), available live foods and green vegetable matter. Live foods consisted mainly of *Daphnia*, cyclops, ostracods, bloodworms, mosquito larvae, sliced earthworms and brine shrimp (see APPENDIX A). Conditioning of fish with enriched live food cultures was achieved by adding a mixture of 10 mℓ milk and 500 mg omega-3 fish oils into the water of the collected *Daphnia* two hour prior to feeding the fish. Vegetable matter consisted mainly of duckweed (*Lemna major* and *Wolfia arhiza*) and small pieces of fresh lettuce.

5.6.6. Disease control

As a result of the huge trout industry in the Lydenburg area, white spot (*Ichthyophthirius multifiliis*) is a serious threat to any fish introduced into the facility. Precautionary treatment consisting of a mixture of Acriflavine and Methylene Blue (1g each/100-150 liter; Geisler, 1982) was added to the tank whenever an outbreak of disease was suspected or when new fish were introduced. Nets also needed to be sterilized on a regular basis by soaking them in a solution of 2% formaldehyde overnight.

5.7. NATURAL SPAWNING BEHAVIOUR AND CONDITIONS

Cambray & Bruton (1984) observed sexual development in *B. anoplus* to commence in July to August and to reach a peak by late October to November, which coincided with the first

spawning. The males at this time were a bright golden color and had pointed tubercles. After January, the tubercles became rounded and the golden coloration was less brilliant. Small degenerating (rounded) tubercles were still present in large males during April, but were completely eroded by May. Tubercles have not been observed in the northern population used in the present breeding trials.

The reproductive cycle of *B. anoplus* is based on an annual periodicity. These recurring cycles are typical of fish that live in freshwaters in the cold temperate zones, where habitats are dominated by annual cycles of environmental variables such as day-length, temperature and food availability. Spawning appears to be triggered by periods of steady rainfall at times when water temperatures are at or above 20°C. (Tomasson et al., 1983; Cambray & Bruton, 1984; Skelton, 2001). During a study of the biology of *B. anoplus* in Vanderkloof Dam, Cambray & Bruton (1984) found a marked increase in the gonad weight in females when photoperiod increased in July and August. This increase in gonad development, however, was not necessarily triggered by the increase in photoperiod. Initiation of spawning did not occur until November, when there were at least 13h of daylight, and the second spawning occurred when there was still approximately 12h of daylight. The first spawning occurred when water temperatures were approximately 20°C and the second at 22°C.

Barbus anoplus adults migrate into shallow, temporary areas prior to breeding (Cambray 1982), where they lay adhesive eggs amidst vegetation (Skelton, 2001). Cambray & Bruton (1984) found the main breeding population to be around 40 to 50 mm FL. *Barbus anoplus* has two major spawning runs per season, one in spring or early summer and another in February or March (Cambray, 1978; 1983b; 1985 Tomasson et al., 1983; Cambray & Bruton, 1984). There is also the possibility of minor spawning runs throughout the summer because mature ova occur in the ovaries from October to March. (Cambray 1983b; Tomasson et al., 1983; Cambray & Bruton, 1984).

Fish from the first spawning attain a length of 40 mm FL during their first summer, and they are able to participate in the first spawning of their second summer. The length at sexual maturity for both males and females is between 38 and 40 mm FL. In multiple spawning species it is difficult to assess absolute fecundity, which may also vary considerably between years (Tomasson et al., 1983). During the Vanderkloof Dam study yolked ova of 40, 55 and 70 mm FL females counted before the first spawning were 417, 1396 and 3486 in 1979/1980. Corresponding counts were 577, 2317 and 6638 a year later, possibly reflecting improved living conditions (Tomasson et al., 1983). Cambray & Bruton (1984), recorded the highest Gonadal Somatic Index (GSI) values in females during October/November just before the first spawning. After the peak periods there was a gradual decline in GSI values. The GSI values for mature females ranged from less to 1% in spent or undeveloped individuals, to over 20% in ripe fish. The GSI values for males followed a similar early

developmental pattern, but the prolonged spawning period was not as pronounced in males, especially in the first year.

Diameters of the ripe unshed ova range in size from 0.7-1.05 mm. At temperatures between 19° and 21°C the larvae hatch in 53 hours and at temperatures between 24°C and 25°C they hatch in 28 hours (Tomasson et al., 1983). The larvae begin to swim and feed after 6-7 days (Skelton, 2001). Hatched larvae are over 3.0 mm in length. (Cambray, 1983b).

The protolarvae have three adaptations to ensure their survival. Some larvae are pelagic, while others swim to the surface periodically then sink passively, and others adhere to rocks or vegetation with a mucous secretion on the dorsal surface of their head. These adaptations probably evolved to prevent suffocation in the bottom mud (Tomasson et al., 1983). Cambray (1983) observed that fish, which spawned in November, reach the sexual mature length of 38-40 mm FL by March or April. The second spawn fish attain a length of 21-22 mm FL by the end of the growing season, and do not attain a sexually mature length until the mid-summer of the following year. However, the longer-lived minnows are usually from the second spawn. This difference in longevity between the two broods enables the spawn from one year to live into an additional year. Females (maximum 4 years) are longer-lived than males (maximum 3 years) with the majority of the population dying off after one year (Cambray, 1983a; 1985; Tomasson et al., 1983).

Jackson (1973).observed sexually mature male *B anoplus* in the Gariep Dam to outnumber females by a factor of two (55 to 29). The males were however on the average smaller in size, all specimens over 57 mm in length being females.

5.8. FORCED SPAWNING BEHAVIOUR AND CONDITIONS

Cambray (1983b) successfully hatched *B. anoplus* using the method described by Bok & Heard (1982). After the larvae had switched from endogenous to exogenous nutrition, they were fed daily on cooked egg yolk and a commercial fish food (Liquifry, 4.6% protein) at 08h00 and 16h00. The development of the fish was stunted under laboratory conditions. They reached 30 mm FL after 13 months. Their comparable year class under natural conditions would have reached 45 to 50 mm FL (Cambray, 1982).

Cambray & Bruton (1984) carried out a pool-breeding experiment to establish whether or not a spawning migration is necessary for the successful spawning of *B. anoplus*. Plastic pools were used, two 3 m wide and 0.5 m deep and one 2.5 m by 0.5 m. One half of the floor of each pool was covered with gravel, the other half with sand. Fish were collected from Vanderkloof Dam and introduced into the pools in different male to female ratios. *B.*

anoplus bred in the pools, which indicated that this species does not require a spawning migration for successful breeding. The authors however speculated that the transference of the fishes into the pool may have provided a physiological shock similar to that experienced during migration.

5.8.1. Preparation of spawning tank

Several spawning trials were conducted in a 200 ℓ tank (0.8 x 0.7 x 0.4 meter), partially divided with a 0.6 x 0.4 x 0.02 meter polystyrene sheet in two unequal compartments (0.8 x 0.25 x 0.4 and 0.8 x 0.45 x 0.4) (see Spawning Tank 1, APPENDIX B). The two compartments were connected on the one side by a 200 mm gap between the glass and the one end of polystyrene sheet. A 500 ℓ/hour pump was used to draw water through a 0.3 x 0.04 x 0.04 meter sponge filter in the larger compartment and released into the closed end of the smaller compartment to create a slow current. An air-stone was placed near the outlet of the pump to create increased turbulence. Rounded stones (200-300 mm) and coarse gravel (5-10 mm) were placed in the smaller compartment to create stream conditions. To simulate a backwater condition, coarse gravel was placed on the whole bottom of the larger compartment and a small quantity of submerged water plants was introduced into one corner as spawning medium. The gravel is important for protecting the eggs after spawning, as the adults will actively hunt and forage for eggs. The preferred spawning medium is Java moss but the remains of marine hydroids (i.e. *Amphisbetia aperculata*), were also found to be a good spawning medium. The tank was filled with clean tap water, which had an alkalinity of less than 40ppm. Alkalinity was used as a surrogate measure for hardness and therefore represented softer water than the water in the holding tanks. Tanks were left for at least a day to heat and stabilize. Temperatures were maintained at 23ºC-25ºC with a 100-watt heater to represent optimum breeding conditions. Personal field observations suggest that *Barbus anoplus* often breed in shallow, newly inundated, floodplain wetlands where temperatures often exceeded 25ºC. The rate of development of the fry seemed to be temperature related with fry developing more rapidly in water with a higher temperature (also refer to Section 5.7).

5.8.2. Spawning

After the preparation of the spawning tank, the sides of the tank were completely covered with cardboard to avoid disturbance to the fish during spawning. Two males were introduced into the tank in the morning and two selected females in the afternoon. Sizeable portions of live food, preferably *Daphnia* and or adult brine shrimp were placed in the tank to sustain the fish. Two large leaves of lettuce submerged for about a minute in boiling water was placed into the tank to stimulate the formation of infusoria in the tank. The fish were

left for only one day and were removed on the morning of the next day to prevent fish hunting and foraging for eggs after spawning. This was also deemed essential as fry could already appear on the second day with the temperatures maintained in the tank. The air-stone was removed to reduce turbulence in the tank and all covers were removed. The pump and filter system was kept running to maintain water quality and some flow in the tank.

5.8.3. Fry development

The first fry appeared within a day or two after removing of the breeding fish and became free-swimming within 24 hours. The fry were fed twice a day on commercially available Liquifry No. 1 and/or micro-worm (*Anguillula silusiae*). Lettuce soaked in boiling water for a few minutes to encourage the formation of infusoria to ensure a range of available food sources. The number of fry depended on the size, age and development of gonads in the individuals used, but about 150 to 200 fry developed with each successful spawn. The fry were left in the spawning tank for two to three weeks before being transferred to other holding tanks, in order to minimize handling. After three weeks the juveniles were fed on Tetramin Baby®.

5.9. CONCLUSIONS

Barbus anoplus is relatively small (SL = 100 mm). This, together with its non-aggressive social behaviour resulted in it not requiring large holding facilities. They are not particularly sensitive to handling and adapted very well to their captive conditions in the laboratory, with very few mortalities recorded. They responded very well to artificially induced ques to stimulate spawning throughout the year (i.e. increased protein in their diet, simulated stream conditions in the spawning tank and manipulation of water hardness to simulate a rain event). They are not very susceptible to disease.

This study indicated that *B. anoplus* did not require large holding or spawning facilities. The spawning tank was relatively inexpensive to construct, and once the protocol was developed, *B. anoplus* was not difficult to breed.

Based on its amenability to routine laboratory culturing and maintenance, it can therefore be concluded that *B. anoplus* has potential for use as a test species in water resource management studies (i.e. ecological risk assessment, refinement of ECOSPECS) and routine bioassays. Should this species be used in routine laboratory toxicity testing, care should be taken to use fish from the same source to prevent differences in sensitivity as a result of possible genetic differences between different isolated populations.

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CHAPTER 6
MAINTENANCE AND BREEDING OF *OPSARIDIUM PERINGUEYI* (SOUTHERN BARRED MINNOW), (GILCHRIST AND THOMPSON, 1913)

6.1. BACKGROUND

Opsaridium peringueyi was indicated to be a potentially suitable species for use in bioassays, based on certain key criteria (refer to Chapter 2 for a list of these criteria). In this chapter *Opsaridium peringueyi* is evaluated in more detail in terms of its suitability for use in routine laboratory bioassays, based on its amenability to routine laboratory culturing and maintenance (including size of species and subsequent size of holding facilities that would be required, sensitivity to handling, spawning behaviour and breeding requirements, ease of culturing, susceptibility to disease, food and feeding, behaviour). This was based on a survey of available literature and information, as well as experimental evaluation of suitable culturing and breeding requirements.

6.2. MORPHOLOGY AND TAXONOMY

The classification of *Opsaridium peringueyi* (California Academy of Sciences Catalog of Fishes, 2005; Fishbase, 2005):

Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Opsaridium*
Species: *Opsaridium peringueyi* (Gilchrist & Thompson, 1913)

Opsaridium peringueyi attains 90 mm Standard Length (SL) (Skelton, 2001). It has a large oblique mouth without barbels (Crass, 1964). The body is spindle shaped and streamlined, the dorsal fin ray is situated partly above anal fin, and the anal fin is large and elongated in front in males. The head is pointed, eyes large and the lower jaw extends to below orbit (Skelton, 2001). Flanks are barred (7-9 blue-black bars), and the head, ventral parts and some fins are reddish (Crass, 1964; Skelton, 1987). The lateral line is situated low down on the flank (Crass, 1964), with 40-44 scales, usually 14 around caudal peduncle (Skelton, 2001). Mature males have tubercles on the lower jaw, paired fins and on scales behind the pelvic fins (Skelton, 2001). The dorsal fin consists of three unbranched segmented rays with 10-11 soft rays. The anal fin consists of three unbranched segmented rays with 14-16 soft rays (Fishbase , 2005).

6.3. DISTRIBUTION

Opsaridium peringueyi occurs in the Save River system in Zimbabwe, the Limpopo, Incomati and Phongola River systems (Skelton, 2001; Fishbase, 2005). The species is generally confined to an altitude below 1200 m along the lower escarpment and Lowveld of the Northern Province and Mpumalanga, Swaziland and KwaZulu-Natal. A relict population occurs in the Lephalala River, Limpopo River system in the Waterberg, Northern Province (Fishbase, 2005).

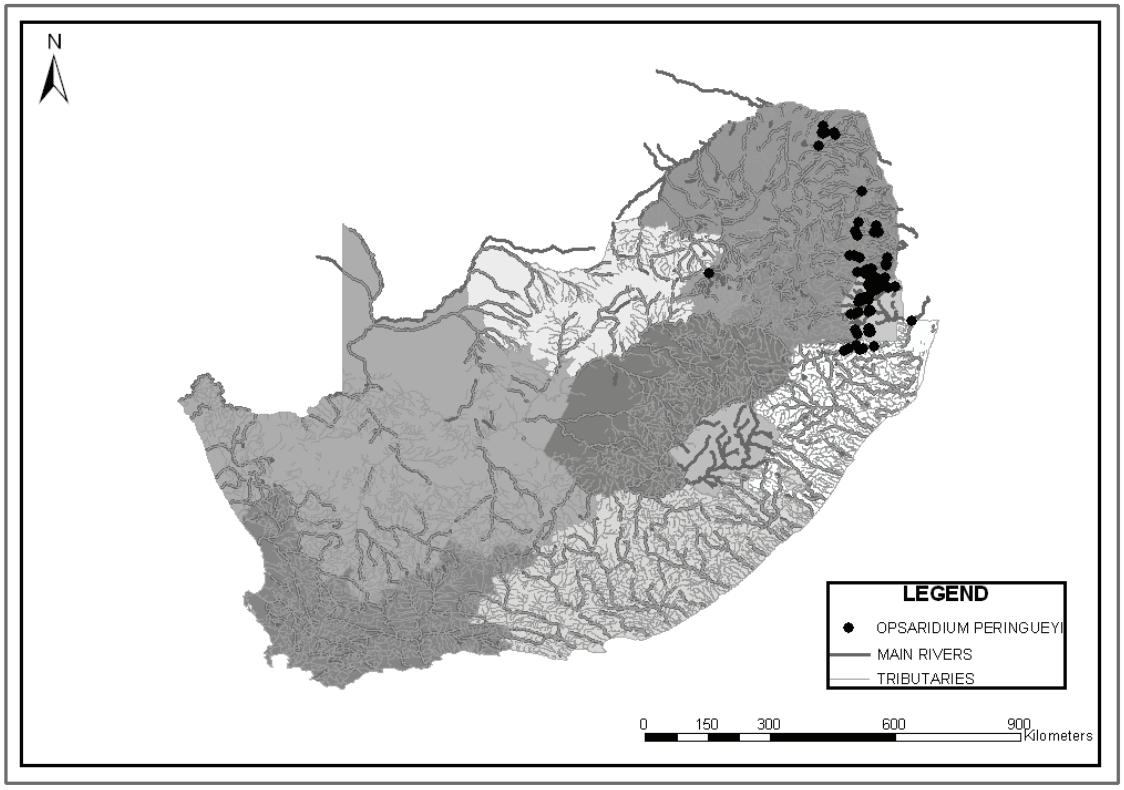

Figure 6.1: Distribution of *Opsaridium peringueyi* in South Africa (data courtesy of SAIAB, Mr. Willem Coetzer)

6.4. RELATIVE SENSITIVITY AND WATER QUALITY REQUIREMENTS

Opsaridium peringueyi is a warm-cold tolerant species (Weeks *et al*, 1996), which is highly sensitive towards water quality and habitat changes. It has an overall sensitivity rating of 4.5 (Kleynhans, 2003). It is sensitive to low oxygen in captivity (Weeks *et al*, 1996).

6.5. HABITAT USE SPECIFICITY

The species is confined to shallow, clear, flowing waters of perennial middle and lowveld rivers, frequenting pools below rapids (Gaigher, 1973; Skelton, 2001). It has a high preference for water column cover and good quality water. It avoids standing or muddy turbid water (Gaigher, 1973; Pienaar, 1987; Coke, 1993). Coke (1993) recorded *O. peringueyi* at an altitude of 120 to 970 meters above sea level (masl). Rocks, sand and gravel are the preferred substrate. They occur in shoals and live 5-6 years (Skelton, 2001).

Habitat changes as a result of dams and water abstraction have greatly reduced the range of this species. It has suffered reductions in distribution over the last three decades both at the scale of lowveld rivers (Olifants River) and within reaches of others (Sand River) (Weeks *et al*, 1996). It is reported as possibly extinct in Zimbabwe (Skelton, 2001). *O. peringueyi* is Red Data listed in the South African Red Data Book – Fishes as Indeterminate – Rare (Skelton, 1987). It is not IUCN Red listed. However, recent surveys suggest that this species has increased in both abundance and distribution during the last few years associated with an increase in shallow sandy runs due to greater sediment depositions in rivers.

6.6. LABORATORY MAINTENANCE OF *O. PERINGUEYI* CULTURES

Approximately 40 *Opsaridium peringueyi* (Southern Barred minnow) individuals were collected during September 2002 from the Sabie River near Hoxanne Weir (25.01867 S, 31.018672 E) and introduced into the temperature-controlled holding facility. However, most of the fish collected developed secondary gill infections (myxobacteria) and died within a week after introduction, most likely due to internal haemorrhaging of gills caused through sampling by means of electro shocking. Microscopic examination of the gills revealed lesions similar to gas-bubble disease and an abundance of myxobacteria. Subsequently, another 40 individuals were collected using a small seine net and were introduced into the same facility. No secondary gill infections developed in these fish and breeding behaviour was observed within a month, induced by live foods. A power failure during December 2002 caused an increase in the nitrate (NO_2) levels in the tank causing huge losses of brood stock, which suggested that the under gravel filter may not have been sufficient to deal with waste products under stressful conditions. Subsequently, a trickle filter was developed and introduced into this tank to avoid nitrogen build-up in the tank. However, the sensitivity of the fish to white spot and myxobacteria as well as to any conventional treatments remained a problem, causing total mortality of stocks. This species also showed hyper sensitivity to most commercially available medicines.

6.6.1. Culture stock and tank stocking specifications

The breeding stock was kept in a completely glass covered 1.5 x 0.5 x 0.57 meter glass tanks (400 ℓ). The size of individuals, as well as the degree of abdominal development was used to separate the sexes. However, the high ratio of male to female (9:1) made it impractical to divide the sexes.

6.6.2. Filtration

The tank was prepared with an under gravel filter covered with coarse gravel (5-10 mm) and a surface-skimmer, packed with a layer of filter wool and charcoal. A flow of about 300 ℓ/hour was maintained through this filter with a pump. This filtered water was then passed through an ultra-violet sterilizer before being returned on the opposite side of the tank to create some flow. A filter unit, consisting of a 0.4 x 0.05 x 0.05 sponge and a 500 ℓ/hour pump supplied additional filtration and flow to the system. An air-stone was also installed near the outlet of the above-mentioned filter to increase the dissolved oxygen level in the tank.

6.6.3. Water quality and photoperiod

Water temperatures were stabilized between 20°C and 23°C with a 200-watt heater to simulate optimum conditions. The hardness and alkalinity of the water was slightly increased by placing about 200 ml agricultural dolomite in the tank. The alkalinity was used as a surrogate measure of hardness and was measured with HTH Quick Test Pool Test strips and normally stabilized at about 80 to 120 ppm. A photoperiod of 14 hours per day was maintained in order to simulate summer conditions.

6.6.4. Maintenance

The gravel in the fish tanks was siphoned weekly in order to prevent the build-up of waste in the tank and the water in the tank was topped-up. This species is very susceptible to the build-up of nutrients and the tanks needed to be completely cleaned and gravel washed on a monthly basis. The sponge filter material was washed every week and the filter wool and activated charcoal in the surface-skimmer were cleaned weekly and replaced about every second month.

6.6.5. Food and feeding

Opsaridium peringueyi feeds on benthic and drifting insects and small organisms (Skelton, 2001). In captivity, Gratwicke (2000) found the species to feed in the mid/surface water in the aquarium on most foods offered except pellets. They especially enjoyed live food.

In the laboratory *O. peringueyi* were fed daily on a diet consisting of high quality and well-balanced commercially available fish food (TetraMin®), available live foods and green vegetable matter. Live foods consisted mainly of *Daphnia*, cyclops, ostracods, bloodworms, mosquito larvae, sliced earthworms and brine shrimp.

6.6.6. Disease control

This species is exceptionally vulnerable to secondary gill infections (myxobacteria) and white spot *(Ichthyophthirius multifiliis)*. It is also highly sensitive to conventional treatments with malachite green or most of the commercially available medicines, as 100% mortality have been inflicted within 12 hours after treatment even with half the recommended dosage. However, precautionary treatment consisting of a mixture of Acriflavine and Methylene Blue (1g each/100-150 ℓ; Geisler, 1982) did not harm the fish. Nets also needed to be sterilized on a regular basis by soaking them in a solution of 2% formaldehyde overnight. The water in the tank was constantly sterilized with an UV filter and the tank was almost completely covered with glass. The latter also necessitated the introduction of an air-stone to drive out stagnant air trapped below the glass

6.7. NATURAL SPAWNING BEHAVIOUR AND CONDITIONS

Opsaridium peringueyi is a multiple spawner, which breeds during spring and summer (Skelton, 2001). Males become flushed with orange-red on head, body and fins in breeding dress (Skelton, 2001). Personal observation suggests that this species spawns in runs with loose moving sand and that the juvenile fish aggregate in the marginal vegetation associated with flowing water.

6.8. FORCED SPAWNING BEHAVIOUR AND CONDITIONS

Opsaridium peringueyi was successfully bred in aquaria on two occasions, once at the Aquatic Research Unit, Mpumalanga Parks Board, and once at the South African Institute for Aquatic Biodiversity (formerly known as the JLB Smith Institute of Ichthyology). Both

these breeding attempts were recorded on video (JS Engelbrecht, pers com; R Bills, pers com).

6.8.1. Preparation of spawning tank

The best results were obtained by using a 200 ℓ tank (1.2 x 0.4 x 0.4 meter). The bottom of the tank was covered in a 20 cm layer of coarse sand (0.5-1.0 mm). A 500 ℓ/hour pump was used to draw water through a 0.3 x 0.04 x 0.04-meter sponge filter from the one side of the tank and was released on the opposite side to create slow flow. The outlet was angled downwards to create a constant disturbance in the sand. An air-stone was placed near the outlet of the pump to create increased turbulence. The coarseness of the sand is important for protecting the eggs after spawning, as the adults will actively hunt and forage eggs. The tanks were filled with clean dechlorinated tap water, which is softer than the water in the holding tanks and left for at least a day to heat-up and stabilize. Temperatures were maintained at 23ºC-25ºC with a 100-watt heater to simulate optimum breeding conditions.

6.8.2. Spawning

After the preparation of the spawning tank, the sides of the tank were completely covered with cardboard to avoid disturbance to the fish during spawning. This species was successfully spawned by placing a single male and female in the spawning tank. Sizeable portions of live food, preferably daphnia and/or adult brine shrimp were placed in the tank to sustain the fish. A large lettuce leaf submerged for about a minute in boiling water was placed into the tank to stimulate the formation of infusoria in the tank. The fish were left for two days and were removed on the morning of the second day to prevent fish hunting and foraging for eggs after spawning. The air-stone was removed to reduce turbulence in the tank and all covers were removed. The fish were observed to spawn in the constantly moving sand and low numbers of fish larvae was observed within 3 days.

6.8.3. Fry development

The first fry appeared within 4 days after the introduction of the fish and became free-swimming within 24 hours. Commercially available Liquifry No. 1 and micro-worm (*Anguillula silusiae*) were used to raise some fry successfully. The low recruitment can be ascribed to several possibilities:

- Relative small females with low fecundity
- Partial spawner
- Group spawner, possibly needing >2 males/female

- Size of gravel too small and most of the eggs were lost to foraging adults.

6.9. CONCLUSIONS

Opsaridium peringueyi is relatively small (SL = 90 mm). The males were much more abundant in all collections made and demonstrated aggressive social behaviour, thereby reducing the number of specimens that could be kept in the holding facilities. They were particularly sensitive to handling but adapted very well to their captive conditions in the laboratory for short periods. They responded very well to artificially induced cues to stimulate spawning (i.e. increased protein in their diet, simulated stream conditions in the spawning tank and manipulation of water hardness to simulate a rain event). However, they were very susceptible to disease and incidence of mortalities and their hyper sensitivity to treatment made them difficult to keep. This study indicated that *Opsaridium peringueyi* was not difficult to breed, but they require extremely stringent husbandry practices to prevent introduction of disease and any decrease in water quality. Larger holding facilities may also be required to produce large numbers of fry for testing purposes.

Based on the above it can be concluded that *Opsaridium peringueyi* may nevertheless have some potential for use as a test species in site-specific water resource management studies (i.e. ecological risk assessments, refinement of ECOSPECS, etc.), but is not regarded as suitable for use in routine laboratory bioassays.

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CHAPTER 7
MAINTENANCE AND BREEDING OF *BARBUS EUTAENIA* (ORANGEFIN BARB), BOULENGER 1904

7.1. BACKGROUND

Barbus eutaenia was indicated to be a potentially suitable species for use in bioassays, based on certain key criteria (refer to Chapter 2 for a list of these criteria). In this chapter *Barbus eutaenia* is evaluated in more detail in terms of its suitability for use in routine laboratory bioassays, based on its amenability to routine laboratory culturing and maintenance (including size of species and subsequent size of holding facilities that would be required, sensitivity to handling, spawning behaviour and breeding requirements, ease of culturing, susceptibility to disease, food and feeding, behaviour). This was based on a survey of available literature and information, as well as experimental evaluation of suitable culturing and breeding requirements.

7.2. MORPHOLOGY AND TAXONOMY

The classification of *Barbus eutaenia* (California Academy of Sciences Catalog of Fishes, 2005; Fishbase, 2005):

Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Barbus*
Species: *Barbus eutaenia* Boulenger (1904)

Barbus eutaenia attains 140 mm Standard Length (SL). The body is stocky, fusiform and the mouth large with two pairs of barbels. There is a sheath of large black scales at the base of the dorsal fin. The fish is olive above, silvery white below, and the fins are yellow or orange. A broad ragged-edged black band runs from the snout through to the mid-caudal rays. "Shadow" bands occur above and below the main band in well-marked specimens. *Barbus eutaenia* has 24-27 scales in the lateral line and 12 around the caudal peduncle. The dorsal fin consists of three spines and eight branched rays. The anal fin consists of three unbranched segmented rays and five branched rays (Skelton, 2001).

7.3. DISTRIBUTION

Barbus eutaenia is found in the Ruanda Urundi Rivers, Lake Mweru (Fishbase, 2005), Cunene, Okavango and Zambezi Rivers, and is present in the east coast system south to the Phongolo system. They also occur in the Cuanza (Angola), Congo system and Lake Tanganyika (Skelton, 2001; Fishbase 2005). They are distributed somewhat patchily in lower order streams of the east coast to the Incomati System (Skelton, 2001).

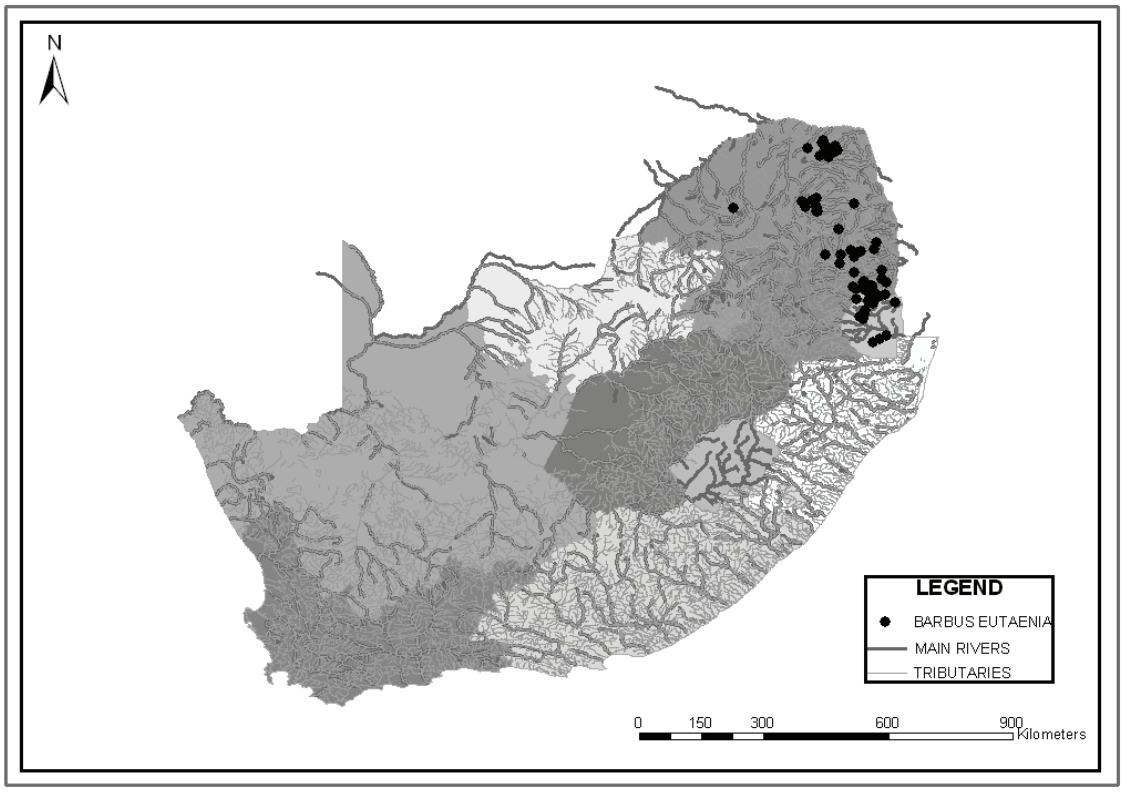

Figure 7.1: Distribution of *Barbus eutaenia* in South Africa (data courtesy of SAIAB, Mr. Willem Coetzer)

7.4. RELATIVE SENSITIVITY AND WATER QUALITY REQUIREMENTS

Barbus eutaenia is highly sensitive towards changes in habitat and water quality, with an overall intolerance rating of 4.3 (Kleynhans, 2003).

7.5. HABITAT USE SPECIFICITY

Barbus eutaenia is a eurythermal species with a very high preference for fast-deep and fast-shallow habitat types. They prefer headwater streams with rocky habitats (Weeks *et al*, 1996; Skelton, 2001). It has a preference for overhanging vegetation, substrate and undercut bank cover. *Barbus eutaenia* is known to ascend tributaries and move onto floodplains only in rainy seasons (Fishbase, 2005).

7.6. LABORATORY MAINTENANCE OF *B. EUTAENIA* CULTURES

About 50 *Barbus eutaenia* (Orangefin barb) individuals were collected during September 2002 from the Sabie River near Hoxanne Weir (25.01867 S, 31.018672 E) and introduced into the temperature-controlled holding facility at Lydenburg. Although the species is widely distributed the Sabie River population was preferred because of the high ecological status of the river.

7.6.1. Culture stock and tank stocking specifications

The fish being prepared for breeding trials were kept in a 1.5 x 0.5 x 0.57 meter glass tank (400 ℓ). The tank was divided with fine mesh into two equal sized compartments and the sexes were kept separate. The size of the abdominal development in individuals was used to separate the sexes. The population density was maintained at 40 (10 males and 30 females), i.e. one fish per 10 ℓ water. Keeping sexes apart, but in the same tank ensured that pheromones could assist in the development of gonads. A trickle filter directed flow from the males to the females to ensure that females were constantly exposed to pheromones from the males. Fish were fed regularly on available live foods and vegetable matter to improve their condition and further induce the development of gonads.

At ECOSUN broodstock was kept in a 0.915 x 0.36 x 0.32 meter glass tank (90 ℓ). The tank was also divided with fine mesh into two equal sized compartments to keep males and females separate. The population density was maintained at 16 individuals (8 males and 8 females), i.e. approximately one fish per 6 liter of water. Increasing the density resulted in increased mortalities. Because of the smaller size of the holding tank, it was not necessary to direct flow from the males to the females in order to enhance gonad development.

Additional specimens were kept in a 2000 ℓ plastic tank in a hothouse at the Lydenburg facility to replace any casualties. Fry from breeding trials with low success were placed in a 1000 ℓ plastic tank in the hothouse to be raised as replacement breeding stock.

7.6.2. Filtration

The holding tank at the Lydenburg facility was prepared with coarse gravel on an under-gravel filter. A flow of about 300 ℓ/hour was maintained through the under-gravel filter operated by an airlift. A filter unit, consisting of a 5 ℓ rectangular tank, which was divided into four compartments, was also mounted above the fish tank. Water was pumped out of the fish tank into the first compartment of the filter unit and gravitated back into the fish tank via an outlet on the top of the fourth compartment. A flow of about 300 ℓ/hour was maintained through the filter unit. Water in the filter unit was allowed to flow from the first and third compartments to the next compartment, via a slot on the bottom of the divisions. Water flowed from the second compartment into the third via a slot on the top of the division. The first and third compartments were filled with pieces of fine meshed shade cloth and the second compartment was filled with filtration wool. The fourth compartment contained a layer of filtration wool and activated charcoal.

The 90 ℓ holding tank at ECOSUN was fitted with an under-gravel filter and a TRIO2000 mechanical and biological internal filtration system.

7.6.3. Water quality and photoperiod

Water temperatures were stabilized between 20°C and 23°C with a 200-watt heater to simulate optimum conditions. *Barbus eutaenia* juveniles are known to inhabit slow flowing backwaters with a temperature of approximately 22° Celsius. The hardness and alkalinity of the water was increased slightly and the pH was stabilized by placing about 150 ml agricultural dolomite in the tank. The alkalinity was used as a surrogate measure of hardness and was measured with HTH Quick Test Pool Test strips and normally stabilized at about 40 to 80 ppm with a pH of 6 to 7. A photoperiod of 14 hours per day was maintained in order to simulate summer conditions.

7.6.4. Maintenance

The gravel in the holding tanks at the Lydenburg facility was siphoned weekly to prevent the build-up of nutrients in the tank and the water in the tank was topped-up. This species is very susceptible to the build-up of wastes and tanks required a comprehensive clean-up every two months, which included thorough washing of the gravel. Because *Barbus eutaenia* showed sensitivity towards nitrite build-up, a trickle filter was also developed for this tank to ensure maintenance of water quality even during power failures. The filter material in the filter unit was washed every week and the filter wool and activated charcoal were replaced every month. Bottom feeding fish such as *Ancistrus* spp. (*Plecostomus* spp.),

freely available in the pet trade, were introduced into all tanks to remove access food and algae. This was done primarily to avoid potential water quality problems.

Fifty percent of the water in the holding tank at ECOSUN was replaced every second week, and the under gravel filter was cleaned once per month. The TRIO2000 mechanical and biological internal filtration system was cleaned and the carbon replaced every second month. The gravel in the tanks was siphoned every second day to prevent build-up of waste in the tank, where after the water in the tank was topped up with dechlorinated tap water of the same temperature. As this tank is much smaller than the tank used at the Lydenburg facility, it required more frequent cleaning.

7.6.5. Food and feeding

Under natural conditions *B. eutaenia* was observed to feed on insects and other small animals (Bell-Cross, 1976; Skelton, 2001; Fishbase, 2005). The laboratory cultures were fed on dried flakes and a variety of life food and small water plants cultivated for this purpose in order to condition the fish for breeding. Fish were fed daily on a diet consisting of high quality and well-balanced commercially available fish food (TertaMin®), available live foods and green vegetable matter. Live foods consisted mainly of *Daphnia*, cyclops, ostracods, bloodworms, mosquito larvae, sliced earthworms and brine shrimp. Conditioning of fish with enriched life food cultures was achieved by adding a mixture of 10 ml milk and 500 mg omega-3 fish oils into the water of the collected *Daphnia* two hours before feeding the fish. Vegetable matter consisted mainly of duckweed (*Lemna major* and *Wolfia arhiza*) and small pieces of fresh lettuce.

7.6.6. Disease control

As a result of the huge trout industry in the Lydenburg area, white spot (*Ichthyophthirius multifiliis*) is a serious threat to any fish introduced into the facility. Precautionary treatment consisting of a mixture of Acriflavine and Methylene Blue (1g each/100-150 ℓ; Geisler, 1982) was added to the tank whenever an outbreak of disease was suspected or when new fish were introduced. Nets also needed to be sterilized on a regular basis by soaking them in a solution of 2% formaldehyde overnight.

7.7. FORCED SPAWNING BEHAVIOUR & CONDITIONS

7.7.1. Preparation of spawning tank

At the Lydenburg facility a 200-liter tank (0.8 x 0.7 x 0.4 meter) was prepared, partially divided with a 0.6 x 0.4 x 0.02 meter polystyrene sheet in two unequal compartments (0.8 x 0.25 x 0.4 and 0.8 x 0.45 x 0.4) (see Spawning Tank 1, APPENDIX B). The two compartments were connected on the one side by a 200 mm gap between the glass and the one end of polystyrene sheet. A 500 ℓ /hour pump was used to draw water through a 0.3 x 0.04 x 0.04 meter sponge filter in the larger compartment and released into the closed end of the smaller compartment to create a slow current. An air-stone was placed near the outlet of the pump to create increased turbulence. Rounded stones (200-300 mm) and coarse gravel (5-10 mm) were placed in the smaller compartment to create stream conditions. To simulate backwater conditions, coarse gravel was placed on the whole bottom of the larger compartment and a small quantity of submerged water plants were introduced into one corner as spawning medium. The gravel is important for protecting the eggs after spawning, as the adults will actively hunt and forage for eggs. The preferred spawning medium is Java moss but the remains of the marine hydroid (*Amphisbetia aperculata*), were also found to be good spawning medium. The tanks were filled with clean tap water, which had an alkalinity of less than 40ppm. As alkalinity was used as a surrogate measure for hardness, the spawning tank contained softer water than the water in the holding tanks (40 to 80ppm). Tanks were left for at least a day to heat and stabilize. Temperatures were maintained at about 22ºC to 24ºC with a 100-watt heater, because juveniles have been collected in October in slow flowing backwaters of the Sabie River with a temperature of approximately 22°C.

Since laboratory space is an important consideration with regard to the suitability of this species for use in routine laboratory bioassays, a smaller 90 ℓ tank (0.36 x 0.32 x 0.915 m) was designed at the ECOSUN Environmental Laboratory (see Spawning Tank 2, APPENDIX B). The design concept was similar to the tank used at the Lydenburg facility. The tank was partially divided with a 0.36 x 0.762 m glass sheet into two equal compartments of 0.36 x 0.16 x 0.915 m. The two compartments were connected on the one side by a 100 mm gap. A 500 ℓ/hour submersible pump was used to draw water through a filter in the one compartment and released into the closed end of the other compartment to create a current (ranging between 0.02 m/s and 0.2 m/s) in the one compartment and a backwater area (0 m/s) in the other compartment. An air stone was placed near the outlet of the pump, to create increased turbulence. Round stones (200-250 mm) and coarse gravel (5-10 mm) were placed in the compartment into which the water was released to simulate stream conditions. To simulate backwater conditions, coarse gravel was placed on the bottom of the “stream” compartment and a small amount of Java moss was introduced in the backwater area to act as a spawning medium.

7.7.2. Spawning

After the preparation of the spawning tanks, the sides of the tanks were completely covered with cardboard and/or black cloth to avoid disturbance of the fish during spawning. Two males were introduced into the tank in the morning and two selected females in the afternoon, in both the 200 ℓ and 90 ℓ tanks. Sizeable portions of live food, preferably *Daphnia* and or adult brine shrimp (*Artemia*) were placed in the tank to sustain the fish. Two large leaves of lettuce submerged for about a minute in boiling water were placed into the tank to stimulate the formation of infusoria in the tank. The fish were left for two days and were removed on the morning of the second day to prevent fish hunting and foraging for eggs after spawning. The air-stone was removed to reduce turbulence in the tank and all covers were removed. The pump filter system was kept running to maintain water quality and some flow in the tank.

7.7.3. Fry development

The first fry appeared within three to five days after removing of the breeding fish and became free-swimming within 24 hours. The fry were fed twice a day on commercially available Liquifry No. 1 and/or micro-worm (*Anguillula silusiae*). Lettuce soaked in boiling water for a few minutes to encourage the formation of infusoria was also placed in the tank to ensure extended availability of food. The number of fry produced depended on the size, age and development of gonads in the individuals used, with approximately 200 to 400 fry developed with each successful spawn. The fry were left in the spawning tank for two to three weeks before being transferred to other holding tanks, in order to minimize handling. After three weeks the juveniles were fed on Tetramin Baby®.

7.8. CONCLUSIONS

Barbus eutaenia is relatively small (SL = 140 mm). This, together with its non-aggressive social behaviour resulted in it not requiring large holding facilities. They are slightly sensitive to handling but adapted very well to their captive conditions in the laboratory, with very few mortalities recorded. They responded very well to artificially induced cues to stimulate spawning (i.e. increased protein in their diet, simulated stream conditions in the spawning tank and manipulation of water hardness to simulate a rain event). Although *B. eutaenia* was not very susceptible to disease, nitrate related water quality changes resulting from live foods did lead to some mortalities. However, frequent water changes normally rectified this problem. The spawning tank was relatively inexpensive to construct, and once the protocol was developed, *B. eutaenia* proved not difficult to breed.

Based on its amenability to routine laboratory culturing and maintenance, it can therefore be concluded that *B. eutaenia* has potential for use as a test species in both site-specific water resource management studies (i.e. risk assessment, refinement of ECOSPECS, etc.) and routine laboratory bioassays.

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CHAPTER 8

MAINTENANCE AND BREEDING OF *TILAPIA SPARRMANII* (BANDED TILAPIA), SMITH, 1840

8.1. BACKGROUND

Tilapia sparrmanii was indicated to be a potentially suitable species for use in bioassays, based on certain key criteria (refer to Chapter 2 for a list of these criteria). In this chapter *Tilapia sparrmanii* is evaluated in more detail in terms of its suitability for use in routine laboratory bioassays, based on its amenability to routine laboratory culturing and maintenance (including size of species and subsequent size of holding facilities that would be required, sensitivity to handling, spawning behaviour and breeding requirements, ease of culturing, susceptibility to disease, food and feeding, behaviour). This was based on a survey of available literature and information, as well as experimental evaluation of suitable culturing and breeding requirements.

8.2. MORPHOLOGY AND TAXONOMY

The classification of *Tilapia sparrmanii* (California Academy of Sciences Catalog of Fishes, 2005; Fishbase, 2005):

Class: Actinopterygii
Order: Pesciformes
Family: Cichlidae
Subfamily: Pseudocrenilabrinae
Genus: *Tilapia*
Species: *Tilapia sparrmanii* Smith, 1840

The body shape of *T. sparrmanii* is variable, usually moderately deep with an ovoid, straight or concave predorsal profile. The mouth is small with fine teeth. There are 9 to 12 short well-spaced gill rakes on the first arch (Skelton, 2001). They are silver grey to olive-green in colour with eight to nine vertical bars on their side. The colour tends to darken when habitat conditions are good, as well as during the spawning season. The eye is red with a prominent black stripe through it (McVeigh, 1980). Breeding males have a bright red margin to the dorsal and caudal fins, and a grey-black throat and chest (Skelton, 2001). The dorsal fin carries the tilapia spot for about the first three years of its life, after which it fades slowly. This tilapia spot is found on most of the tilapia group and is a black and white eye-type marking at the end of the fin (McVeigh, 1980). Juveniles have characteristic light

"bubbles" behind the tilapia mark on the soft dorsal fin. The species also has a dark spot on the gill cover surrounded by iridescent green or blue scales, and is iridescent blue along the lower jaw (Skelton, 2001). Banded tilapia are generally small fish, reaching not much more than 230 mm SL (Skelton, 2001). The dorsal fin is made up of 13-15 spines with 9-11 branched rays, while the anal fin consists of three unbranched segmented rays, with 9-10 branched rays (Skelton, 2001; Fishbase, 2005).

8.3. DISTRIBUTION

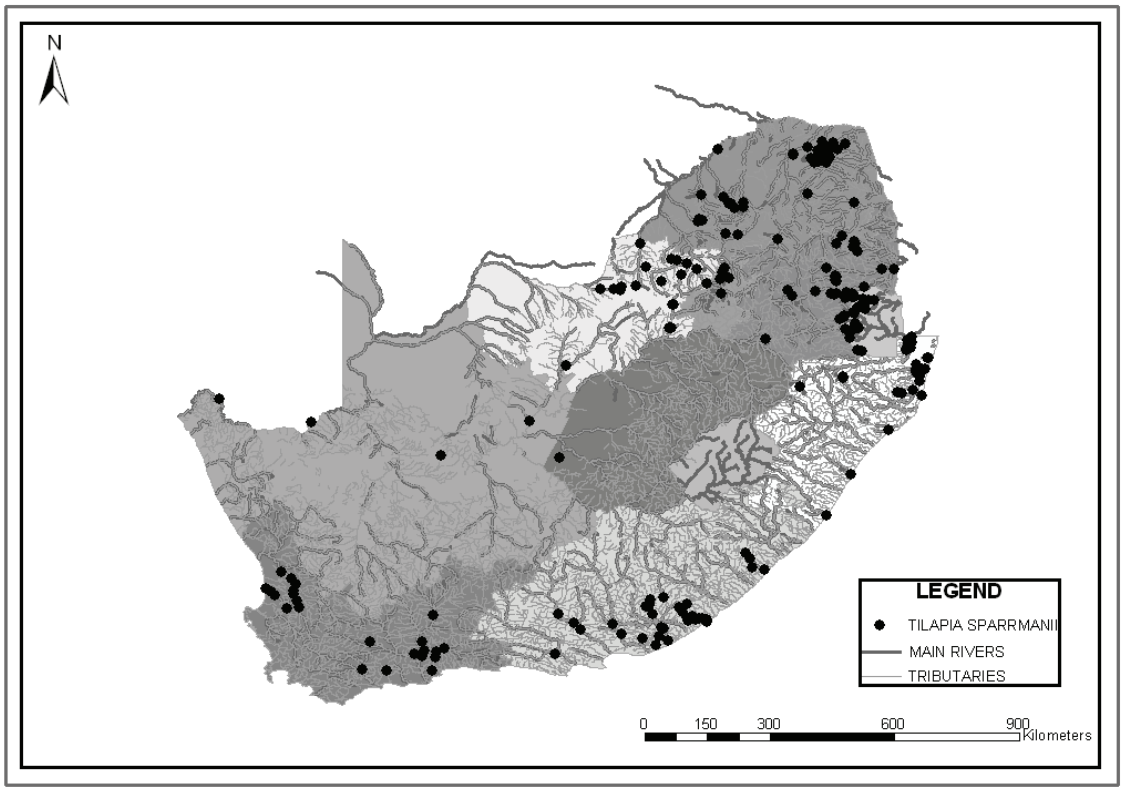

Figure 8.1: Distribution of *Tilapia sparrmanii* in South Africa (data courtesy of SAIAB, Mr. Willem Coetzer)

Tilapia sparrmanii was originally described from the Hartz River, in the Orange River system (Jackson et al., 1983). Their natural distribution is however mostly tropical/sub-tropical. They occur from the Orange River and KwaZulu-Natal south coast northwards to the upper reaches of the southern Congo tributaries, Lake Malawi and the Zambezi system. (Skelton, 2001). Skelton & Cambray (1981) found *T. sparrmanii* to be widespread but not common in both the middle and lower Orange River. They are also extensively translocated south of the Orange River in the Cape (Skelton, 2001).

8.4. RELATIVE SENSITIVITY AND WATER QUALITY REQUIREMENTS

Tilapia sparrmanii is the more tolerant species of the tilapia group (Rose et al., 1987). It is not sensitive towards changes in habitat and water quality, with an overall intolerance rating of 1.3 (Kleynhans, 2003). The species has a wide habitat preference (Gaigher, 1973).

Tilapia sparrmanii is a tropical species, generally occurring in waters with a temperature between 22°C-25°C (Fishbase, 2005), although larger fish have reduced tolerances towards lower temperatures (Jackson et al., 1983). *Tilapia sparrmanii* does not reach the same size in colder areas than in warmer tropical regions (Jackson et al., 1983). However, the distribution of this species suggests it is more restricted by high (above 32°C) than by low temperatures (Skelton, 2001).

8.5. HABITAT USE SPECIFICITY

Tilapia sparrmanii is tolerant to a wide range of habitats, but has a preference for slow flowing or standing waters with aquatic macrophytes-, overhanging and/or marginal vegetation cover (Gaigher, 1973; Skelton, 2001). *Tilapia sparrmanii* prefers shallow, sheltered waters and does not colonize the open water of large lakes (Fishbase, 2005)

8.6. LABORATORY MAINTENANCE OF *T. SPARRMANII* CULTURES

Tilapia sparrmanii has been used extensively in research projects (King et al., 1984; Grobler et al., 1989; Wepener, 1990; Grobler-Van Heerden et al., 1991; Wepener et al., 1992; Du Preez et al., 1993) because of its adaptability to laboratory conditions.

Approximately 50 individuals were collected from the captive population at the Aquatic Research Unit, Mpumalanga Parks Board, and introduced into the temperature controlled holding facility at ECOSUN.

8.6.1. Culture stock and tank stocking specifications

A 2000 ℓ plastic aquatan tank was used to keep broodstock. This tank was filled with dechlorinated (Tetra Aqua Safe® or Nutrafin Aqua Plus®) aged tap water, and no substrate was used in the tank in order to prevent spawning behaviour and to facilitate cleaning of the tank. *Tilapia sparrmanii* individuals were kept at a density of one fish per 50 ℓ of water.

8.6.2. Filtration

The 2000 ℓ tank was fitted with an Ultra Zap filter system with a flow through capacity of 4000 ℓ, driven by a Penguin 8500 submersible pump.

The tanks in which the sexually mature fish and breeding pairs were kept (see Section 8.9) were fitted with an under gravel filter and a TRIO2000 mechanical and biological internal filtration system.

8.6.3. Water quality and photoperiod

Water temperatures in the holding tank were stabilized between 25°C and 28°C with a 100-watt heater. The pH ranged between 7.4 and 8.2, while alkalinity ranged between 40 and 60ppm. Alkalinity, used as a surrogate for hardness, was measured with commercially available HTH Quick Test pool test strips. A photoperiod of 14 hours per day was maintained to simulate summer conditions (also refer to Section 8.9).

8.6.4. Maintenance

The tanks in which the sexually mature fish and breeding pairs were kept were siphoned twice per week, underwent a 30% replacement of water once a week and the tanks and under gravel filters were cleaned once per month in order to prevent the build-up of waste. Aged dechlorinated tap water of the same temperature was used as replacement water.

8.6.5. Food and feeding

Under natural conditions *T. sparrmanii* is an omnivore, feeding on available foods including zooplankton, crustacea, small invertebrates such as insects, vegetation, algae and even small fish (Skelton, 2001; Mc Veigh, 1980). In the laboratory they were fed on a diet consisting of high quality and well-balanced commercially available fish food (Tetramin®, Marltons Tropical Flakes and Nutron Cichlid Sticks), available live foods and green vegetable matter. Live foods consisted mainly of *Daphnia*, cyclops, ostracods, bloodworms and mosquito larvae. Vegetable matter consisted of small pieces of fresh lettuce.

8.6.6. Disease control

Tilapia sparrmanii is not very susceptible to disease. However, as a precautionary measure they were treated with a mixture of Acriflavine and Methylene Blue (1g each/100-150 ℓ;

Geisler, 1982) when introduced into the holding facility. Nets with which the fish were handled were sterilized on a regular basis by soaking them in a solution of 2% formaldehyde overnight.

8.7. NATURAL SPAWNING BEHAVIOUR AND CONDITIONS

Tilapia sparrmanii is strongly territorial and forms a male/female pair-bond (unlike related tilapiine groups which are polyandrous). During the breeding season the fins develop a light red edging. The spawning season is from October to December, and the male will choose a territory where he will dig a saucer-shaped nest in the sand or mud on the bottom of the water body. These nests are usually made in shallow water. The female lays her eggs in the nest and, after fertilization, both parents guard the nest during the entire hatching period. Although fecundity is relatively low, survival of young is high, due to parental guarding (Rothbard, 1979; McVeigh, 1980; Jackson et al., 1983, Skelton, 2001).

Newly hatched larvae attach to the substrate by head glands and wriggle constantly for aeration. After 7-8 days the fry are free-swimming, remaining in a shoal guarded by the parents for several weeks (Skelton, 2001). The fry become bolder, venturing a little further from the nest each day. One to two months after hatching, the young *T. sparrmanii* is old and strong enough to fend for themselves. They are about 15 mm long and at this stage the family shoal begins to break up. The parent fish will move off, remaining together to spawn again during the season, if time and temperature allow (Rothbard, 1979; McVeigh, 1980; Jackson et al., 1983).

8.8. FORCED SPAWNING BEHAVIOUR AND CONDITIONS

Rothbard (1979) studied the reproductive behaviour of several tilapia species under aquarium conditions. He found fish activity to increase upon a rise in temperature and spawning occurred at 26°C to 29°C.

Stutterheim (1981) studied the environmental factors affecting the breeding frequency in *Tilapia sparrmanii*. This species is a substrate-breeding Cichlid and as such, the following reproductive stages were distinguished:

1. The schooling stage which is characteristic of immature Cichlids but which also occurs during periods of unfavourable physical and social conditions among mature Cichlids
2. The territorial stage which is indicative of the beginning of the reproductive cycle, the length of which is dependent on the prevailing physical and social environment.

3. The paired stage which is a territorial stage characterized by the existence of a pairbond that cumulates in egg laying.
4. The parental stage which is dependent on the growth and development of the young. If stage four is artificially stopped, stages two and three will follow each other at regular intervals depending on environmental conditions.

In this study Stutterheim (1981) attempted to determine the acidity, thermal and salinity requirements of *T sparrmanii* with regard to reproduction, distinguishing between conditions under which a species can survive and those under which they can complete their reproductive cycle. All environmental conditions were kept within certain limits, varying only one condition at a time. Variations in the breeding interval and production of eggs were noted, indicating that temperature was the most important factor determining the breeding rate. Salinity was of secondary importance, while breeding was possible over a wide acidity range (pH 4 to 9) correlating closely with the acidity range the species is able to survive.

8.8.1. Preparation of spawning tank

A substrate of sand (2-3 mm) was placed on the bottom of a 90 ℓ tank (0.36 x 0.32 x 0.915 m). Adult *T. sparrmanii* showing signs of sexual maturity (colour intensification) were transferred to the 90 ℓ glass tanks at a ratio of five males and five females per tank, i.e. approximately one fish per nine liters of water. Dechlorinated (with Tetra Aqua Safe® or Nutrafin Aqua Plus®) aged tap water was used. The bottom of the tank was covered with coarse sand (2-3 mm diameter), to stimulate spawning behaviour.

Temperatures were maintained at 25°C-28°C with a 100-watt heater, based on the preferred temperatures for breeding obtained from literature (Rothbard, 1979).

8.8.2. Spawning

The above procedure allowed for the easy identification of breeding pairs. Once the fish paired off they were aggressive towards the other fish in the tank, pushing them away from their chosen territory. Breeding pairs were removed and placed in separate 90 ℓ tanks, with a thick layer of coarse sand (10 cm-15 cm) to allow for nest building. No additional features were placed in the spawning tank. The breeding pair started nest construction within a few days after being transferred. The male took position just above the sand and moved his pectoral fins to create a saucer shaped nest. Immediately after the female laid the eggs they were fertilized by the male. The breeding pair was very sensitive to disturbance and the tanks were therefore covered with black cloth during this period. There were approximately 200-300 large olive-coloured eggs per breeding attempt. Fry was not successfully raised when left with the breeding pair. Removing the adults from the tank as soon as the eggs were fertilized resulted in a high success rate (approximately 60% to 70%). Adults were

returned to the tank containing the other sexually mature fish. The eggs were left in the breeding tank to hatch.

Placing a divider in the breeding tank to divide the tank into two equal sized breeding compartments also proved successful, and doubled the spawning effort within the same space.

8.8.3. Fry development

The eggs hatched within two days of fertilization. The fry was free-swimming 10-11 days after hatching. The juveniles were left in the breeding tank until they were three weeks old, in order to minimize handling. The fry were initially raised on micro-worms (*Anguillula silusiae*) and TetraMin® Baby. *Daphnia*, bloodworms, mosquito larvae and vegetable matter (lettuce leaves) were introduced after one month.

8.9. CONCLUSIONS

Tilapia sparrmanii is not particularly sensitive to handling, adapted very well to captive conditions in the laboratory and were not susceptible to disease. The protocol is cost-effective in that the spawning tanks were relatively inexpensive to construct, and once the protocol was developed, *T. sparrmanii* was easy to breed.

Tilapia sparrmanii is relatively small (230 mm SL) and despite its aggressive behaviour towards other fish during breeding, was possible to breed making use of small spawning facilities (90 ℓ tanks). Placing a divider in the spawning tank to divide the tank into two equal sized breeding compartments also proved successful, and doubled the effort within the same space.

This species however does require close monitoring and management of breeding cultures, which is time consuming. The latter is a factor to take into account when considering the use of this species in routine laboratory testing.

However, based on its amenability to routine laboratory culturing and maintenance, it can be concluded that *Tilapia sparrmanii* has potential for use as a test species in both site-specific water resource management studies (i.e. ecological risk assessment, refinement of ECOSPECS, etc.) and routine laboratory bioassays.

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CHAPTER 9
MAINTENANCE AND BREEDING OF *APLOCHEILICHTHYS KATANGAE* (STRIPED TOPMINNOW) (BOULENGER, 1912)

9. 1. BACKGROUND

In Chapter 2, *Aplocheilichthys katangae* was indicated to be a potentially suitable species for use in bioassays, based on certain key criteria. In this chapter *A. katangae* is evaluated in more detail in terms of its suitability for use in routine laboratory bioassays, based on its amenability to routine laboratory culturing and maintenance (including size of species and subsequent size of holding facilities that would be required, sensitivity to handling, spawning behaviour and breeding requirements, ease of culturing, susceptibility to disease, food and feeding, behaviour). This was based on a survey of available literature and information, as well as experimental evaluation of suitable culturing and breeding requirements.

9.2. MORPHOLOGY AND TAXONOMY

The classification of (California Academy of Sciences Catalog of Fishes, 2005; Fishbase, 2005):

Class: Actinopterygii
Order: Cyprinodontiformes
Family: Poecilidae
Subfamily: Aplocheilichthyinae
Genus: *Aplocheilichthys*
Species: *Aplocheilichthys katangae* (Boulenger, 1912)

Aplocheilichthys katangae is moderately deep bodied, with a distinctive zigzag black band along the body. The abdomen and lower head are white. The iris, upper gill cover and scattered midbody scales are iridescent blue-turquoise, while the fins are clear or light yellow. It has 25 to 28 scales in lateral series. The species attains 50 mm TL (Skelton, 2001). The dorsal fin consists of 8 to 10 branched rays and the anal fin of 14 to 15 branched rays (Skelton, 2001)

9.3. DISTRIBUTION

Kleynhans (1986) recorded *Aplocheilichthys katangae* in the Matlapitsi River, in a tributary of the Nyl River, the Groot Nyl River and in two tributaries of the Mogalakwena River, the Sterk and Mmadikiri Rivers, but was not found in the Apies River. This species is also known from Natal (Crass, 1964), South West Africa, Angola, Zaire, Zimbabwe, Mozambique, Zambia, Botswana and Malawi (Bell-Cross, 1976; Skelton 2001), and occurs at an altitude of between 465 and 1200 meters above sea level (Kleynhans, 1986).

Owing to its restricted distribution Kleynhans (1986) considered *A. katangae* to be rare in the former Transvaal. This species is regarded as safe in the Matlapitsi River, which lies within the Wolkberg Wilderness area. Its future in the Groot Nyl, Sterk and Mmadikiri Rivers, which lie in an area where crops are grown along the riverbanks, was however, indicated as uncertain. The excessive extraction of water, most notably during drought periods as well as the use of pesticides, may pose a threat to the survival of the species (Kleynhans, 1986).

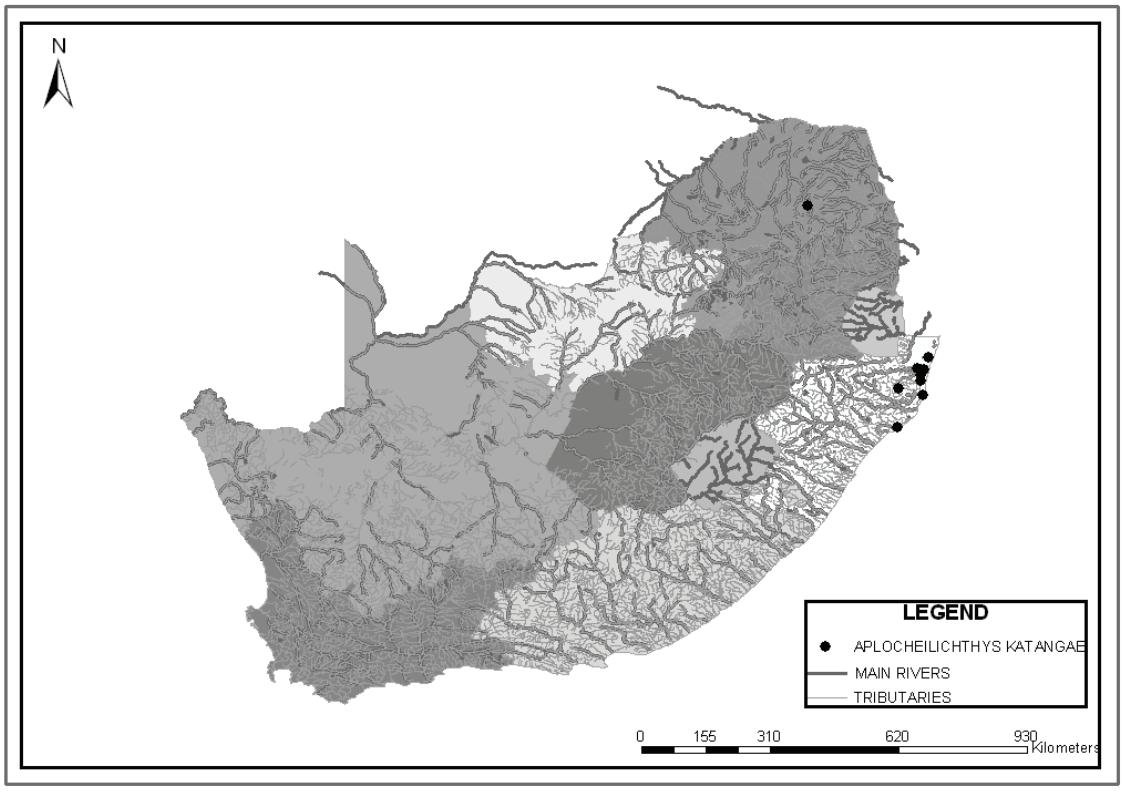

Figure 9.1: Distribution of *Aplocheilichthys katangae* in South Africa (data courtesy of SAIAB, Mr. Willem Coetzer)

9.4. RELATIVE SENSITIVITY AND WATER QUALITY REQUIREMENTS

Aplocheilichthys katangae is moderately sensitive towards changes in habitat and water quality, with an overall intolerance rating of 3.3 (Kleynhans, 2003).

9.5. HABITAT USE SPECIFICITY

Aplocheilichthys katangae is highly habitat specific. It has a preference for pools and backwaters, with aquatic, semi-aquatic and marginal vegetation cover (Kleynhans, 1986; Skelton 2001). It is however, uncommon in floodplains (Skelton, 2001).

9.6. LABORATORY MAINTENANCE OF *A. KATANGAE CULTURES*

Aplocheilichthys katangae (Striped topminnow) individuals were collected from the Molaphitsi River. Approximately 60 individuals were introduced into the holding facility at Lydenburg during September 2002. They were kept in a 2000 ℓ aquatan tank with abundant vegetation (*Ceratophyllum demersum*) in the hothouse. The fish were mainly fed on a variety of life foods but the species showed a noted preference for mosquito larvae. The fish were not keen to accept dried foods but readily accepted frozen foods. This species bred prolifically in this holding tank during summer by depositing single large eggs on vegetation. Fecundity, predation and partial spawning seemed to be the main problems prohibiting the harvesting of large numbers of offspring. It was attempted to overcome these problems, by using enough individuals in smaller breeding tanks so as to produce a constant supply of similar aged offspring. Water temperatures were maintained above 20ºC.

Subsequently, approximately 20 individuals were transported to ECOSUN where they were kept in a 0.915 x 0.36 x 0.32 meter glass tank (90 ℓ) (see Section 9.6.1).

9.6.1. Culture stock and tank stocking specifications

Additional brood stock was kept in a 2000 ℓ plastic tank in the hothouse at the Lydenburg facility and in a small permanently flowing fishpond outside. The plastic tank was densely stocked with submerged aquatic macrophytes (*Ceratophyllum demersum*). The fishpond contained dense stands of emergent aquatic macrophytes such as *Juncus lomatophyllus* and *Nymphaea nouchalii*.

At ECOSUN the brood stock was kept in a 0.915 x 0.36 x 0.32 meter glass tank (90 ℓ), i.e. one individual per 4.5 ℓ of water. Temperature was maintained at 25ºC-26ºC. A cage was constructed in the tank with PVC material and 60% shade cloth (see Spawning Tank 3, APPENDIX B). The size of the openings was big enough to let fry through, but small enough to prevent adult fish from moving through. The cage was suspended from the top of the tank, allowing a space of approximately 5 cm at the bottom of the tank. The upper section of the cage was above the water level to prevent fish from escaping into the tank. Plant material was placed in the cage (Java moss) to act as spawning medium.

9.6.2. Filtration

A filter unit, consisting of a 0.4 x 0.05 x 0.05 meter sponge and a 500 ℓ/hour pump supplied filtration and flow to the system at the Lydenburg facility. Due to the fact that the fishpond outside has slow perennial flow, no filtration was needed.

The small 90 ℓ tanks were fitted with under gravel filters and a TRIO2000 mechanical and biological internal filtration system.

9.6.3. Water quality and photoperiod

Water temperatures at the Lydenburg facility were maintained in the hothouse above 16°C and the hardness of the water was increased slightly by placing about 500 ml agricultural dolomite in the tank. The water temperatures in the fishpond dropped below 12°C during winter and seldom exceeded 22°C in summer. Photoperiod was dependent on the season.

The water temperature in the holding and spawning tanks at ECOSUN were maintained at 25°C-26°C. Alkalinity was used as a surrogate measure for hardness and was maintained at 40 to 80ppm. A photoperiod of 14 hours per day was maintained (to simulate summer conditions).

9.6.4 Maintenance

At the Lydenburg facility the holding tank was regularly topped-up and allowed to overflow, to prevent the build-up of waste. The filter material in the filter unit was washed every second week.

Fifty percent of the water in the holding tank at ECOSUN was replaced every second week, and the under gravel filter was cleaned once per month. The TRIO2000 mechanical and biological internal filtration system was cleaned and the carbon replaced every second

month. The gravel in the tanks was siphoned every second day to prevent build-up of waste in the tank, where after the water in the tank was topped up with dechlorinated tap water of the same temperature.

9.6.5. Food & feeding

Under natural conditions *A. katangae* utilizes the upper 10 cm of the water column and it obtains its food (insect larvae, *Daphnia* and other small invertebrates) from the water surface (Kleynhans, 1986; Skelton, 2001).

In the laboratory the fish were fed daily on a diet consisting of high quality and well-balanced commercially available fish food and available live foods. Live foods consisted mainly of *Daphnia,* cyclops, ostracods, bloodworms, mosquito larvae, sliced earthworms and brine shrimp. The fish however showed a definite preference to mosquito larvae.

9.6.6. Disease control

As a result of the huge trout industry in the Lydenburg area, white spot (*Ichthyophthirius multifiliis*) is a serious threat to any fish introduced into the facility. Precautionary treatment consisting of a mixture of acriflavine and methylene blue (1g each/100-150 ℓ; Geisler, 1982) was added to a separate treatment tank whenever an outbreak of disease was suspected or when new fish were introduced. A separate tank was preferred for treatment as aquatic macrophytes are susceptible to this medicine. Nets also needed to be sterilized on a regular basis by soaking them in a solution of 2% formaldehyde overnight.

9.7. NATURAL SPAWNING BEHAVIOUR AND CONDITIONS

Aplocheilichthys katangae is a typical serial spawner, laying eggs on vegetation (Skelton, 2001). It could be expected that similar to its relative *A. johnstoni*, breeding could occur daily over an extended period (practically year round), when food is abundant.

9.8. FORCED SPAWNING BEHAVIOUR AND CONDITIONS

9.8.1. Preparation of spawning tanks

See Sections 9.6.1. to 9.6.3

9.8.2. Spawning

This species was allowed to breed in their holding tank at the Lydenburg facility. They bred prolifically especially during summer by depositing single large eggs on vegetation. Much higher recruitment was evident in the fishpond. Fecundity, predation and partial spawning seemed to be the main problems prohibiting the harvesting of large numbers of offspring.

Aplocheilichthys katangae laid their eggs on the vegetation in the "cage". The eggs hatched after about two days, where after the fry attached themselves to the glass of the spawning tank. At this stage the adults were placed in another tank, where they continued to breed.

9.8.3. Fry development

The fry were free-swimming after about 4-5 days. Although the spawning was more controlled in the 90 ℓ spawning tanks, the number of offspring was low. The low fecundity and partial spawning habit of *A. katangae* result in the need for large numbers of broodstock in order to obtain adequate numbers of juveniles for use in routine laboratory testing. The relative large size of the fry (2-3 mm) made it possible to feed these fish on powdered commercially available fish flakes (TetraMin Baby®, Tertamin Growth®) and any other small live foods available.

9.10. CONCLUSIONS

Aplocheilichthys katangae did not adapt well to their captive conditions in the laboratory, losing condition rapidly, despite being fed on live foods. The fish kept in the 2000 ℓ tank in the hothouse at the Lydenburg facility remained healthy and bred profusely. It was however not possible to manage the culture properly in terms of the number and age of the offspring, both of which are crucial when using fish in bioassays. Although the spawning was more controlled in the 90 ℓ spawning tanks, the number of offspring was low. Due to the low fecundity and partial spawning habit of *A. katangae* large numbers of broodstock will be required to obtain adequate numbers of juveniles for use in routine laboratory testing.

Based on the above it can be concluded that *A. katangae* is not suitable as a test species for use in bioassays.

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CHAPTER 10
MAINTENANCE AND BREEDING OF *BARBUS PALUDINOSUS* (STRAIGHTFIN BARB), PETERS 1852

10.1. BACKGROUND

Barbus paludinosus was indicated to be a potentially suitable species for use in bioassays, based on certain key criteria (refer to Chapter 2 for a list of these criteria). In this chapter *Barbus paludinosus* is evaluated in more detail in terms of its suitability for use in routine laboratory bioassays, based on its amenability to routine laboratory culturing and maintenance (including size of species and subsequent size of holding facilities that would be required, sensitivity to handling, spawning behaviour and breeding requirements, ease of culturing, susceptibility to disease, food and feeding, behaviour). This was based on a survey of available literature and information, as well as experimental evaluation of suitable culturing and breeding requirements.

10.2. MORPHOLOGY AND TAXONOMY

The classification of *Barbus paludinosus* (California Academy of Sciences Catalog of Fishes, 2005; Fishbase, 2005):

Class: Actinopterygii
Order: Cypriniformes
Family: Cyprinidae
Genus: *Barbus*
Species: *Barbus paludinosus,* Peters, 1852

The head of *B. paludinosus* is pointed and the mouth small with two pairs of short barbels. *Barbus paludinosus* is plain olive grey or silvery in turbid waters, a thin sideline sometimes present. Females generally attain a larger size than males (Skelton, 2001). The largest female specimen collected by Cambray (1984) in the Orange River was 68 mm Fork Length (FL) and the largest male was 63 mm FL. Skelton (2001) reported *B. paludinosus* to attain 150 mm SL.

Barbus paludinosus has 32-36 scales in the lateral line and 16 around the caudal peduncle. The primary dorsal fin ray is serrated on the distal half and the hind margin of the erect dorsal fin is vertical (Skelton, 2001). The dorsal fin is made up of three spines with seven

branched rays. The anal fin consists of three unbranched segmented rays, with five branched rays (Skelton, 2001).

10.3. DISTRIBUTION

Barbus paludinosus is a widespread species in Africa. It occurs in east coastal rivers from East Africa south to the Vungu, KwaZulu-Natal, and from the southern Congo tributaries and the Quanza in Angola to the Orange River (Skelton, 2001).

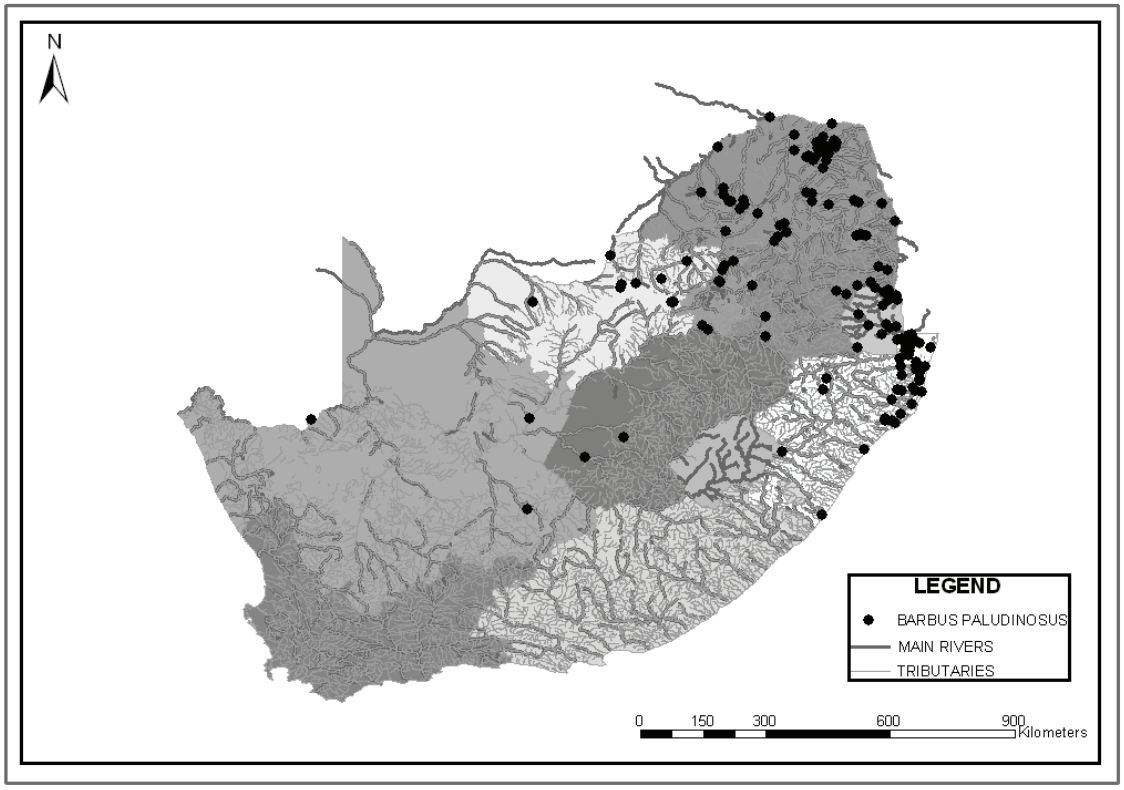

Figure 10.1: Distribution of *Barbus paludinosus* in South Africa (data courtesy of SAIAB, Mr. Willem Coetzer)

10.4. RELATIVE SENSITIVITY AND WATER QUALITY REQUIREMENTS

Barbus paludinosus is a hardy species capable of surviving wide fluctuations in water quality and changes in habitat (Lèvêque & Quensière, 1988, in Brummet & Katambalika, 1996). It has an overall intolerance rating of 1.8 (Kleynhans, 2003). In slightly saline

conditions, unsuitable for other *Barbus* species, *B. paludinosus* can become extremely abundant (Tweddle, 1996).

10.5. HABITAT USE SPECIFICITY

Barbus paludinosus occurs in a wide variety of habitats from the sand-bottomed shores of Lake Victoria and the shallow marshy Lake Chilwa, to the highly turbid and rocky Orange River (Cambray, 1983). *Barbus paludinosus* prefers quiet, well-vegetated waters in lakes, swamps and marshes or marginal areas of larger rivers, but has a preference for slow-flowing streams (Skelton, 2001; Fishbase, 2005). The species shows a preference for well-protected habitats with overhanging vegetation, aquatic macrophyte cover and water column cover.

10.6. LABORATORY MAINTENANCE OF *B. PALUDINOSUS* CULTURES

Approximately 20 individuals were collected from the upper Tshukhutse Stream (S:25°36'41.7"; E:27°35'10.3") and introduced into the temperature-controlled holding facility at Lydenburg. This species is widely distributed and very common on the Highveld, where streams are potentially polluted. Breeding stock could also be collected from the Sabie or Lomati Rivers due to the high ecological status of these rivers.

10.6.1. Culture stock and tank and stocking specifications

The breeding stock was maintained in a 1.5 x 0.5 x 0.57 meter glass tank (400 ℓ) at the Lydenburg facility. The tank was divided with fine mesh into two equal sized compartments and the sexes were kept separate. The size of the abdominal development in individuals was used to separate the sexes. The population density was 18 individuals (8 males and 10 females), i.e. one fish per 22 liters of water. Keeping sexes apart but in the same tank ensured that pheromones could assist in the development of gonads.

10.6.2. Filtration

The tank was prepared with coarse gravel on the bottom and an under-gravel filter. A flow of about 300 ℓ/hour was maintained through the under-gravel filter operated by an airlift. A filter unit, consisting of a 5 ℓ rectangular tank, which was divided into four compartments, was also mounted above the fish tank. Water was pumped out of the fish tank into the first compartment of the filter unit and gravitated back into the fish tank via an outlet on the top of the fourth compartment. A flow of about 300 ℓ/hour was maintained through the filter unit. Water in the filter unit was allowed to flow from the first and third compartments to the next compartment, via a slot on the bottom of the divisions. Water flowed from the second

compartment into the third via a slot on the top the division. The first and third compartments were filled with pieces of fine meshed shade cloth and the second compartment was filled with filtration wool. The fourth compartment contained a layer of filtration wool and activated charcoal.

10.6.3. Water quality and photoperiod

Water temperatures were stabilized between 20°C and 23°C with a 200-watt heater to simulate optimum conditions. The hardness and alkalinity of the water was increased slightly by placing about 300 ml agricultural dolomite in the tank. The alkalinity was used as a surrogate measure of hardness and was measured with HTH Quick Test Pool Test strips and normally stabilized at about 120 to 180 ppm with a pH of 7 to 8. A photoperiod of 14 hours per day was maintained in order to simulate summer conditions.

10.6.4. Maintenance

The gravel in the fish tanks was siphoned weekly to prevent the build-up of waste in the tanks and the water in the tanks was topped-up. The filter material in the filter unit was washed every second week and the filter wool and activated charcoal were replaced every two months. Although this species was not very susceptible to the build-up of waste, but the tanks still needed to be completely cleaned and gravel washed about every six months.

10.6.5. Food and feeding

Barbus paludinosus shows little specialization in its diet (Cambray, 1983). Their primary food is micro crustaceans, but they will take advantage of a wide range of other foods as available, including insects, small snails, algae, diatoms and detritus (Cambray 1983; Polling et al., 1992; Kaunda, 1994; Brummet & Katambalika, 1996; Skelton, 2001). The studies of Cockson & Bourn (1973) (in Cambray, 1983) on the digestive enzymes of *B. paludinosus* confirmed that this minnow is able to utilize a wide spectrum of foods and is capable of digesting plant matter.

The change in the ratio of gut length to standard length suggests a change in diet as the fish grow older (Kruger & Mulder, 1973, in Polling et al., 1992). The smaller *B paludinosus* (<40 mm) were found to feed predominantly on zooplankton, whereas the larger fish had a more varied diet. Bourn (1974) found that *B. paludinosus* fed less actively during the night than during daylight hours.

Polling et al. (1992) suggested that *B. paludinosus* fed mainly in midwater areas. Groenewald (1957), Welcome (1969) and Kirk (1976) (in Cambray 1983) on the other hand found *B. paludinosus* to have a bottom feeding habit. Gratwicke (2000) found *B. paludinosus,* which was kept in aquaria, to forage in the substrate. This author also noted that the species fed effective on pellets and larger food particles.

In the laboratory fish were fed daily on a diet consisting of high quality and well-balanced commercially available fish food (i.e. Sera Vipan, TetraMin®), available live foods and green vegetable matter. Live foods consisted mainly of *Daphnia,* cyclops, ostracods, bloodworms, mosquito larvae, sliced earthworms and brine shrimp. Conditioning of fish with enriched life food cultures was achieved by adding a mixture of 10 ml milk and 500 mg omega-3 fish oils into the water of the collected *Daphnia* two hours before feeding the fish. Vegetable matter consisted mainly of duckweed (*Lemna major* and *Wolfia arhiza*) and small pieces of fresh lettuce.

10.6.6. Disease control

As a result of the huge trout industry in the area, white spot (*Ichthyophthirius multifiliis*) is a serious threat to any fish introduced into the facility. Precautionary treatment utilizing a mixture of acriflavine and methylene blue (1g each/100-150 ℓ; Geisler, 1982) was added to the tank whenever an outbreak of disease was suspected or when new fish were introduced. Nets also needed to be sterilized on a regular basis by soaking them in a solution of 2% formaldehyde overnight.

10.7. NATURAL SPAWNING BEHAVIOUR AND CONDITIONS

The species spawns amongst vegetation during summer. Females are multiple spawners, laying 250-800 eggs at 50-60 mm SL to as many as 2500 eggs at 112 mm SL (Skelton, 2001). Males develop minute tubercles on their heads and pectoral fins when ripe, usually in spring (Cambray, 1984).

10.8. FORCED SPAWNING BEHAVIOUR AND CONDITIONS

10.8.1. Preparation of spawning tank

Best results were obtained by using a 200 ℓ tank (0.8 x 0.7 x 0.4 meter), partially divided with a 0.6 x 0.4 x 0.02 meter polystyrene sheet in two unequal compartments (0.8 x 0.25 x 0.4 and 0.8 x 0.45 x 0.4 meter) (see Spawning Tank 1, APPENDIX B). The two

compartments were connected on the one side by a 200 mm gap between the glass and the one end of polystyrene sheet. Two separate 300 ℓ/hour pumps were used to draw water through two separate 0.3 x 0.04 x 0.04 meter sponge filters in the larger compartment and released into the closed end of the smaller compartment to create a slow current. An air-stone was placed near the outlet of the pump to create increased turbulence. Rounded stones (200-300 mm) and coarse gravel (5-10 mm) was placed in the smaller compartment to simulate stream conditions. To simulate backwater conditions, coarse gravel was placed on the whole bottom of the larger compartment and a small quantity of submerged water plants was introduced into one corner as spawning medium. The gravel is important for protecting the eggs after spawning as the adults will actively hunt and forage eggs. The preferred spawning medium is Java moss but the remains of the marine hydroid (*Amphisbetia aperculata*), was also found to be good spawning medium. The tanks were filled with clean tap water, which is softer than the water in the holding tanks, and left for at least one day heat and stabilize. Temperatures were maintained at 20ºC-25ºC with a 100-watt heater to present optimum breeding conditions. Personal field observations suggest that *Barbus paludinosus* often breed in shallow newly inundated floodplain wetlands where temperatures often exceeded 25ºC.

10.8.2. Spawning

After the preparation of the spawning tank, the sides of the tank were completely covered with cardboard to avoid disturbance to the fish during spawning. Both pumps were switched on to create a noticeable slow current. Two males were introduced into the tank in the morning and two females were selected and introduced in the afternoon. Sizeable portions of live food, preferably *Daphnia* and/or adult brine shrimp, were placed in the tank to sustain fish. Two large leaves of lettuce submerged for about a minute in boiling water was placed into the tank to stimulate the formation of infusoria in the tank. The one pump was switched off the next morning to reduce the current. The fish were left for two days and were removed on the morning of the second day to prevent fish hunting and foraging for eggs after spawning. The air-stone was removed to reduce turbulence in the tank and all covers were removed. The pump filter system was kept running to maintain water quality and some flow in the tank.

10.8.3. Fry development

The first fry appeared within 4 days after the introduction of the fish and became free-swimming within 24 hours. The fry were fed twice a day on commercially available Liquifry No. 1 and/or micro-worm (*Anguillula silusiae*). Lettuce soaked in boiling water for a few minutes to encourage the formation of infusoria was also placed in the tank to ensure

extended availability of food. The number of fry produced depended on the size, age and development of gonads in the individuals used, with about 200 to 300 fry developed with each successful spawn. The fry were left in the spawning tank for two to three weeks before being transferred to other holding tanks, in order to minimize handling. After three weeks the juveniles were fed on Tetramin Baby®.

10.9. CONCLUSIONS

Barbus paludinosus is relatively small (SL = 150 mm). This, together with its non-aggressive social behaviour, resulted in it not requiring large holding facilities. It is not particularly sensitive to handling and adapted very well to captive conditions in the laboratory, with very few mortalities recorded. They responded very well to artificially induced cues to stimulate spawning (i.e. increased protein in their diet, simulated stream conditions in the spawning tank and manipulation of water hardness to simulate a rain event). Additionally, *B. paludinosus* was found not to be very susceptible to disease.

This study indicated that *B. paludinosus* did not require large holding or spawning facilities. The spawning tank was relatively inexpensive to construct, and once the protocol was developed, *B. paludinosus* proved not difficult to breed.

Based on its amenability to routine laboratory culturing and maintenance, it can therefore be concluded that *B. paludinosus* has potential for use as a test species in both site-specific water resource management studies (i.e. ecological risk assessment, refinement of ECOSPECS, etc.) and routine laboratory bioassays.

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CHAPTER 11
ASSESSMENT OF THE TOXICITY OF POTASSIUM DICHROMATE ($K_2Cr_2O_7$), SODIUM CHLORIDE (NaCl) AND SODIUM FLUORIDE (NaF) TOWARDS SELECTED INDIGENOUS CYPRINID AND CICHLID SPECIES

11.1 INTRODUCTION

Chemical and physical parameters alone are not sufficient to assess the effects of pollutants on an aquatic ecosystem, as the integrated effects of pollutants on the biota are not taken into consideration. Biological tests and toxicological end points, on the other hand, provide rapid, reliable and unequivocal, integrated measures of environmental effects or their potential (Rand, 1995). For this reason aquatic toxicity testing is currently being built into policies, strategies, guidelines and regulations in South Africa. Examples include

- ⇒ *The management of complex industrial waste discharges: Direct Estimate of Ecological Effects Potential (DEEEP).* A discussion document published in June 2003 by the Institute for Water Quality Studies, Department of Water Affairs and Forestry.
- ⇒ *National Toxicity Monitoring Programme (NTMP). Phase 1: A Needs Assessment and Development Framework for a Tested Implementation Plan for the Initialisation and Execution of the NTMP*. Published by the Department of Water Affairs and Forestry in June 2003
- ⇒ *Hazard-Based Water Quality Ecospecs for the ecological Reserve in freshwater surface water resources.* Published by the IWQS, Department of Water Affairs and Forestry in 2002

Against this background it became necessary for the development of suitable toxicity testing protocols, which represent receptor organisms occurring in our aquatic systems and which will be applicable to South African conditions. The Centre for Aquatic Toxicology (renamed Unilever Centre for Environmental Water Quality), Institute for Water Research, Rhodes University, started research in this field in the early 1990's. Their research included establishing procedures for the selection and maintenance of indigenous stream macroinvertebrates, and the design of suitable experimental procedures (DWAF, 2000). Research conducted by this group contributed significantly towards refinement of the South African Water Quality Guidelines for the protection of Aquatic Ecosystems, as well as water

quality management in South Africa (Haigh and Davies-Coleman, 1997; Palmer et al., 1996; Palmer et al., 2004; Palmer & Rossouw, 2000; Scherman et al., 2003). Suitable protocols for fish species are however still lacking.

One of the aims of this study was therefore to assess the suitability of selected indigenous freshwater fish species for use in toxicological testing. During this phase of the study the response characteristics of selected species to potassium dichromate ($K_2Cr_2O_7$), sodium chloride (NaCl) and sodium fluoride (NaF) were investigated. The evaluation of the species selected for this phase of the project is based on a preliminary assessment of their response sensitivity and response consistency.

11.2 METHODS

11.2.1. Source and handling of test organisms

Toxicity testing was conducted on fry of fish species responding positively to laboratory maintenance and culturing (*Barbus trimaculatus*, *B. eutaenia, B. anoplus, B. paludinosus* and *Tilapia sparrmanii)*. Parallel tests were conducted with the Guppy, *Poecilia reticulata*.

The test fish used during the laboratory investigations were obtained from the breeding facility at Lydenburg as well as in-house cultures at ECOSUN. The brood stock used for the supply of juvenile fish was obtained from specific populations from minimally impaired rivers to prevent genetic encoding as a result of anthropogenic activities. The sensitivity of juveniles to the reference toxicants is therefore assumed to be representative.

Juvenile fish arriving at the laboratory were allowed to acclimate to holding temperature by placing the fish, in their original shipping containers, in environmental temperature- and illumination-controlled rooms (21±1ºC and 14h/10h light: dark cycle). In order to minimize stress to the juvenile fish, they were kept in the shipping containers for two to three days, after which they were used in toxicity bioassays.

Juvenile fish from in-house cultures at ECOSUN were kept in the spawning tank for the entire three week period after hatching. The temperature was gradually reduced in these tanks after two weeks to acclimate the juvenile fish to 21±1ºC, which is the temperature at which bioassays was conducted. A temperature regime of 20ºC-24ºC is recognized as suitable for testing of warm water fish species (Hansen, 1979).

The *Barbus* species and *Tilapia sparrmanii* juvenile test fish were used for bioassays during the juvenile stage (all fin rays were in place and the finfold was almost completely regressed). The fish were approximately three to four weeks old at this stage. This allowed for comparison between the *Poecilia reticulata* (which is a live bearer), the *Barbus* species and *Tilapia sparrmanii* (which are oviparous) (Cambray, pers. com). *Poecilia reticulata* test organisms were typically between 7 and 21 days old. The juveniles were collected using a small fine-meshed net and 5 individuals were placed into each of the testing containers. Care was taken not to physically damage the test organisms during transfer.

11.2.2. Toxicity Testing Procedures

Standard acute tests have been developed to obtain concentration-response relationships for fish exposed to chemicals. Acute tests are usually the first step in evaluating the effects of an effluent or chemical on aquatic organisms. These tests are designed to evaluate the relative toxicity of a chemical to selected aquatic organisms upon short-term exposure to various concentrations of the test chemical (Rand, 1995). The acute toxicity testing procedures followed during the present investigation were based on those described by the US EPA (1993, 1996).

Static acute toxicity tests were conducted with a selection of the following reference toxicants:

- ⇒ Potassium dichromate ($K_2Cr_2O_7$)
- ⇒ Sodium chloride (NaCl)
- ⇒ Sodium fluoride (NaF)

Factors influencing the toxicity of reference toxicants include its physical and chemical properties such as solubility, vapor pressure, pH and lipophilicity. These factors affect the persistence, transformation, bioavailability and ultimate fate of the chemical in the water (Rand, 1995). The selected chemicals were evaluated in terms of their suitability for use as reference toxicants, specifically in terms of solubility, using the following:

- ⇒ solubility tables contained in the *CRC Handbook of Chemistry and Physics*, CRC Press
- ⇒ solubility tables contained in *Solubilities of Inorganic and Organic Compounds*, H. Stephen and T. Stephen, eds., Pergamon Press
- ⇒ Merck Index

The evaluation took account of the moderately hard water system to be used as dilution medium. The solubility tables suggest that no solubility/precipitation problems shall be experienced for the salts of Cr(VI) or NaCl.

Additionally, the tables indicate that fluoride should be secure at concentrations up to approximately 8 ppm, with CaF_2 being the limiting solubility species (limiting solubility is 18 ppm CaF_2). Ca is present in the synthetic hard water at a concentration of 14 mg/ℓ, which equates to a CaF_2 concentration of 27 mg/ℓ. At these levels, CaF_2 is anticipated to precipitate out until it reaches the limiting solubility mentioned above, once NaF is added to the extent to which F exceeds 8 ppm. It is therefore anticipated that this precipitation will occur to the tune of 9 mg/ℓ (of CaF_2), leaving 18 mg/ℓ CaF_2 in solution. Upon making up the solution of NaF in the synthetic hard water, there was no precipitate, providing a possible indication of the Ca being taken up in one of a series of complex equilibria know to be present in multiple ion scenarios.

MgF_2 should also pose no problems, since it has a limiting solubility of approximately 130 ppm, which represents a fluoride concentration of 78 ppm. Mg is present below this limiting solubility, the result of which is that MgF_2 should not precipitate from the solution. Sodium fluoride, on the other hand, has a high solubility of 41.3 g/ℓ.

Different test concentrations were made up from 1000 mg/ℓ stock solutions and were diluted with standard moderately hard dilution water. The moderately hard water is made up according to US EPA (1993) and contains single salts of Ca(II), Mg(II), Na(I) and K(I).
Tests were conducted in 250 mℓ disposable polystyrene airline cups, with the total volume being made up to 200 mℓ in each cup. Testing was conducted in environmentally controlled rooms at a temperature of 21±1ºC and a 14/10 hour light/dark cycle. The individual bioassays consisted of 10 fish per concentration (5 fish per cup). The tests were done with seven to ten concentrations of the reference toxicant and a control. A preliminary range-finding test preceded the definitive 96h test to determine a suitable range of concentrations. Bioassays were conducted over a 96h interval. Test mortalities were recorded at 24h intervals. Total Dissolved Salts (TDS), pH, Dissolved Oxygen (DO) of each test solution were measured prior to- and after completion of the test.

A sub-sample to each test solution was taken prior to, as well as after completion of the test for chemical verification of toxicant concentration. Fluoride, chloride and chromium, which were present as sodium fluoride, sodium chloride and potassium dichromate, respectively, were analytically evaluated. Solutions of the salts mentioned above were made up in deionised water, using known masses of the respective salts. ICP-OES analyses were carried out using sodium and chromium as markers for the respective salts, to ensure correctness of the concentrations. The back calculation was performed making use of the

analytically determined value of sodium in solution (for example of a sodium fluoride solution in water). For this purpose, the exact concentration of sodium in parts per million, ppm, representing the number of milligrams of sodium per liter of solution, was used in the determination. For example, a 100 ppm analysis indicated a concentration of 100 mg Na per liter of solution. Using the molar mass of NaF (41.99 g/mol), together with the known fraction of Na in the NaF (54.75% by mass, which is the same as a mass fraction of 0.5475 attributable to Na), the calculation of the amount of F present is calculated according to the following approach: in the present example, 100 mg per liter of Na calculates back to 182.65 mg NaF per liter (100 ÷ 0.5475). Of the 182.65 mg, 100 mg are ascribable to Na; the example solution therefore contains 82.65 mg F per liter, rounded to 83 mg per liter, or 83 ppm. The same process was followed for the other analytes.

The results obtained from sub-samples taken prior to commencement of tests were used in LC50 calculations, i.e. calculations were based on the concentration of a specific chemical actually present in the test chamber. The results obtained from sub-samples taken on completion of the test was to confirm that test concentrations did not significantly decrease over the duration of the test (i.e. as a result of precipitation).

Test results were regarded as acceptable when survival in the control(s) was equal to or exceeding 90%, as indicated in US EPA (1996).

11.2.3. Statistical analyses of the data

Data obtained from the exposure tests were initially analyzed using the EPA Probit Analysis Program (version 1.4). If the Probit analyses were not suitable to calculate the LC50 value, then the data were analyzed using the Trimmed Spearman-Karber method (version 1.5).

The requirements for the data obtained to be appropriately analyzed using the Probit Method is (according to the USEPA, 1993):

- ⇒ The observed proportion mortalities must bracket 0.5
- ⇒ The Log_{10} of the tolerance is assumed to be normally distributed
- ⇒ Two or more of the observed proportion mortalities must be between zero and one.

The Trimmed Spearman-Karber estimate of the LC50 is obtainable for most experiments. In order to estimate an LC50 the only requirement is that at least one mortality proportion must be less than or equal to 50% and at least one must be greater than or equal to 50%. That is, it

is not possible to calculate the LC50 by the Spearman-Karber Method if the mortality proportions are all less than 50% or all over 50% (Montana State University, 1999).

Toxicity estimated from the two methods of analyses provides approximately similar values and therefore are considered comparable. However since more data points are considered in Probit analyses, greater reliance can be placed on the results obtained from this approach.

LC50 values for the different species of fish were analyzed with the SPSS Programme, Version 11, using both parametric (ANOVA) and non-parametric (Kruskall Wallis) analyses. The Kruskall Wallis test was however considered the better method, because of the small sample sizes.

11.3. RESULTS

Poecilia reticulata, followed by *Tilapia sparrmanii* was found to be the most sensitive species to $K_2Cr_2O_7$. These species also displayed the highest variation in test results with calculated coefficients of variance (CV) of 68.0% and 60.8%, respectively. The *Barbus* species (*Barbus trimaculatus, B. anoplus, B. eutaenia* and *B. paludinosus*) were significantly less sensitive to the toxicant (Figure 11.1) and results from the different tests were also more comparable, with CV values ranging between 9.0% and 37.5%.

High CV values are not uncommon in biological testing. According to the US EPA (1991) intra-laboratory test precision data from 268 acute toxicity tests with four species and five reference toxicants had CV values ranging between 9% and 120%. Coefficient of variance values reported by Jop et al. (1986), Dorn and Rogers (1989) and Hall et al. (1989) ranged from 8% to 41%. Variations in intraspecific test results can be attributed to variations in the experience and skill of the analyst, the age of the test organisms, their condition and sensitivity, the quality of the dilution water and food used, and temperature control (US EPA, 1993). The aim should however be to obtain the smallest possible CV value, when performing toxicity tests with the same species, using the same reference toxicant at the same concentrations, under the same test conditions (i.e. the same test duration, type of dilution water, age of test organisms, feeding, etc.) and using the same data analyses methods.

Comparison of above results with that recorded for other freshwater fish species (obtained from the US EPA ECOTOX database), indicate the *Barbus* species to be in the higher tolerance bracket for $K_2Cr_2O_7$ (Figure 11.2).

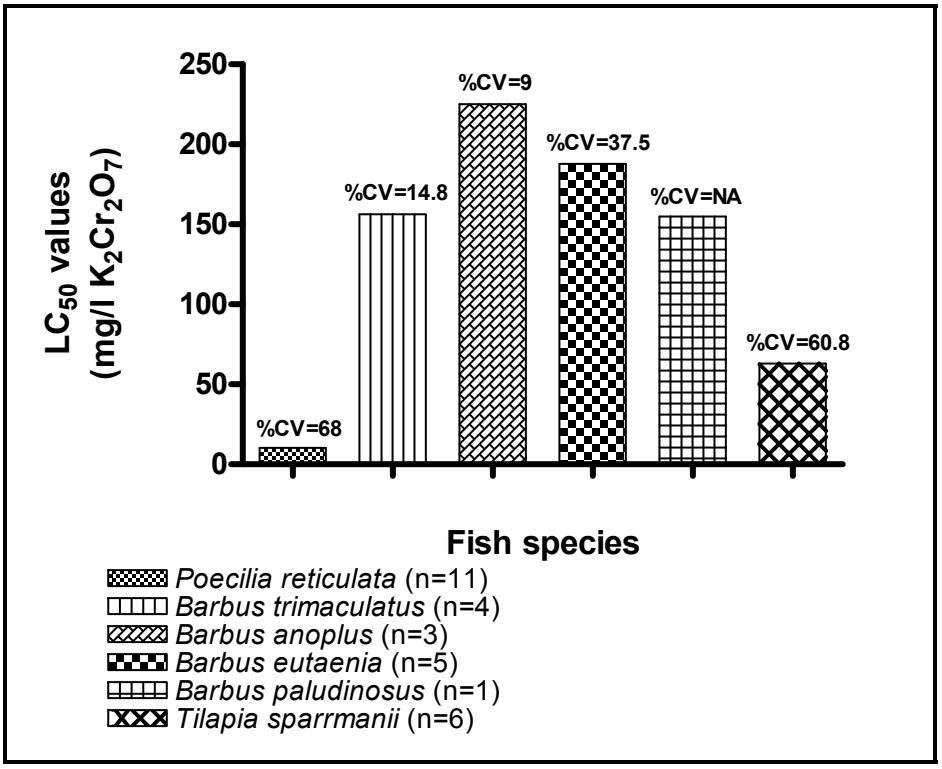

Figure 11.1: Response of selected fish species to $K_2Cr_2O_7$. The Coefficient of Variance (%) for each set of tests is indicated above the bar representing the mean LC50 values. The number of tests is presented in parenthesis in the legend.

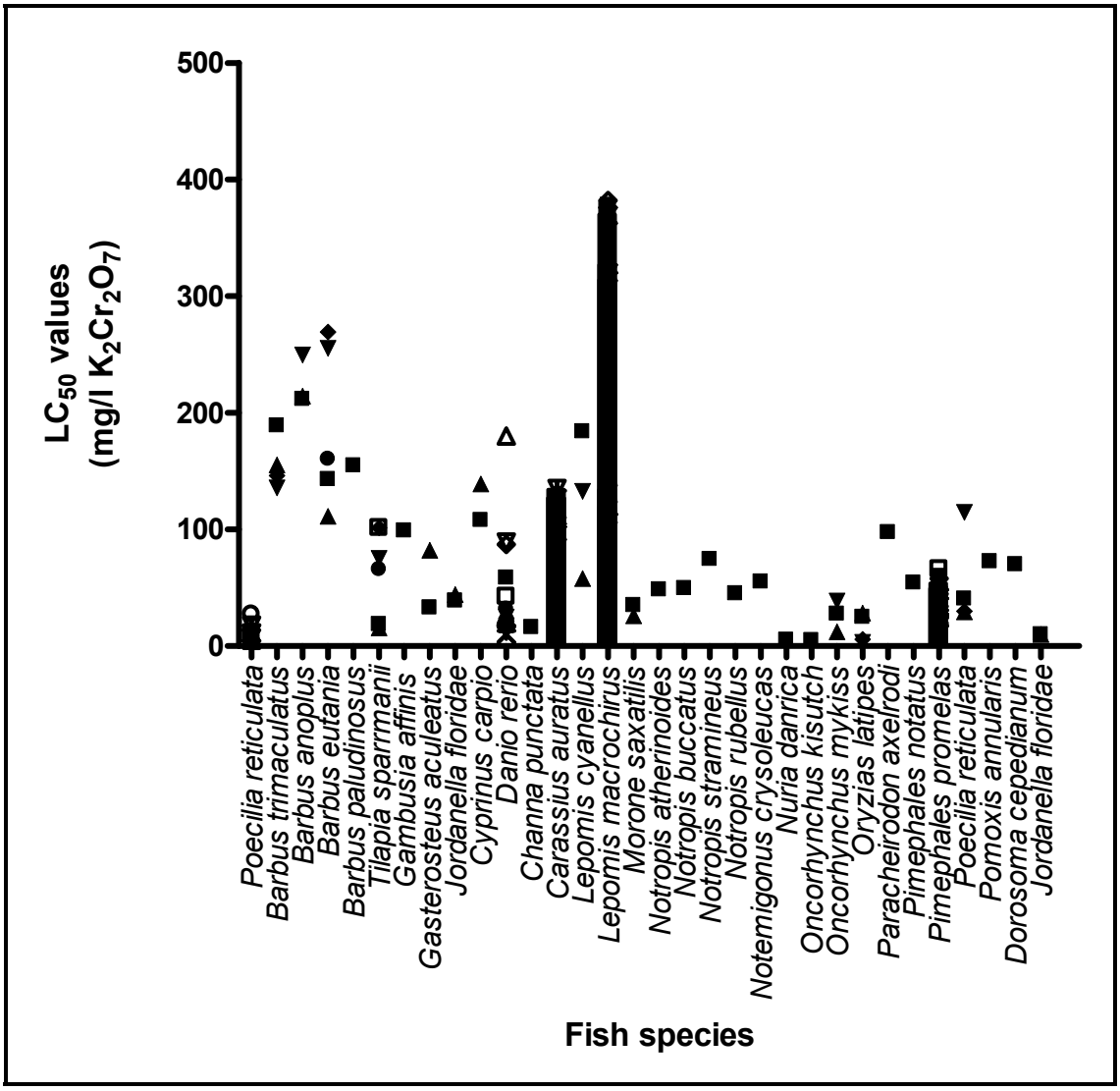

Figure 11.2: Scatter plot of $K_2Cr_2O_7$ LC50 values comparing results obtained during the present investigation with results obtained for other freshwater fish species (comparable results from the US EPA ECOTOX database). Results for the first six species are from the present investigation. Common names for the remainder of the species included on this graph are listed in APPENDIX C.

Results obtained with NaCl yielded exactly the opposite trend than that observed for $K_2Cr_2O_7$. *Poecilia reticulata* and *Tilapia sparrmanii* were less sensitive to NaCl than the *Barbus* species (Figure 11.3). A mean LC50 of 11 337 mg/ℓ NaCl were recorded for *Poecilia reticulata* and a mean LC50 of 12 115 mg/ℓ NaCl for *Tilapia sparrmanii*. The latter species can be expected to display a high tolerance towards NaCl, since cichlids are classed as secondary freshwater fish, being able to withstand relatively high salinity (Skelton, 2001). The LC50 for the *Barbus* species ranged between 3 464 mg/l NaCl and

7 746 mg/ℓ NaCl. The CV values were low (ranging between 5.8% and 15%), indicating good comparability of test results (Figure 11.3).

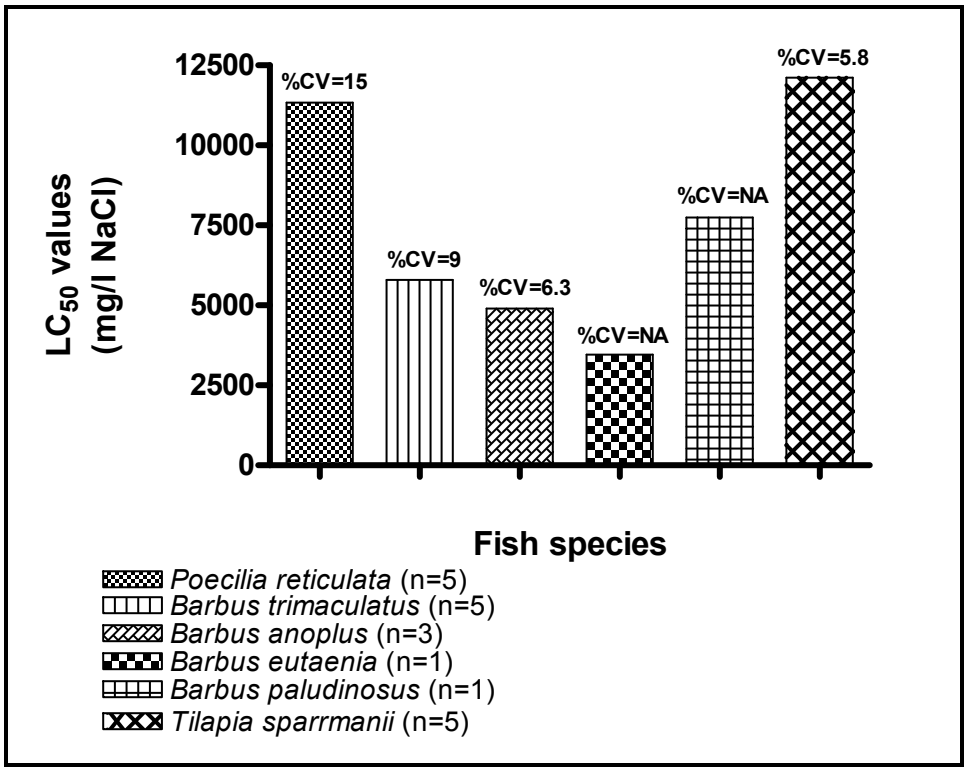

Figure 11.3: Response of selected fish species to NaCl. The Coefficient of Variance (%) for each set of tests is indicated above the bar representing the mean LC50 values. The number of tests is presented in parenthesis in the legend.

Comparison of NaCl LC50 values with that recorded for other freshwater fish species (obtained from the US EPA ECOTOX database), indicate the *Barbus* species to be generally comparable in sensitivity to *Lepomis macrochirus* (Bluegill), *Oncorhynchus mykiss* (Rainbow trout), *Carassius auratus* (Goldfish), *Morone saxsatilis* (Striped bass) and *Pimephales promelas* (Fathead minnow) (Figure 11.4).

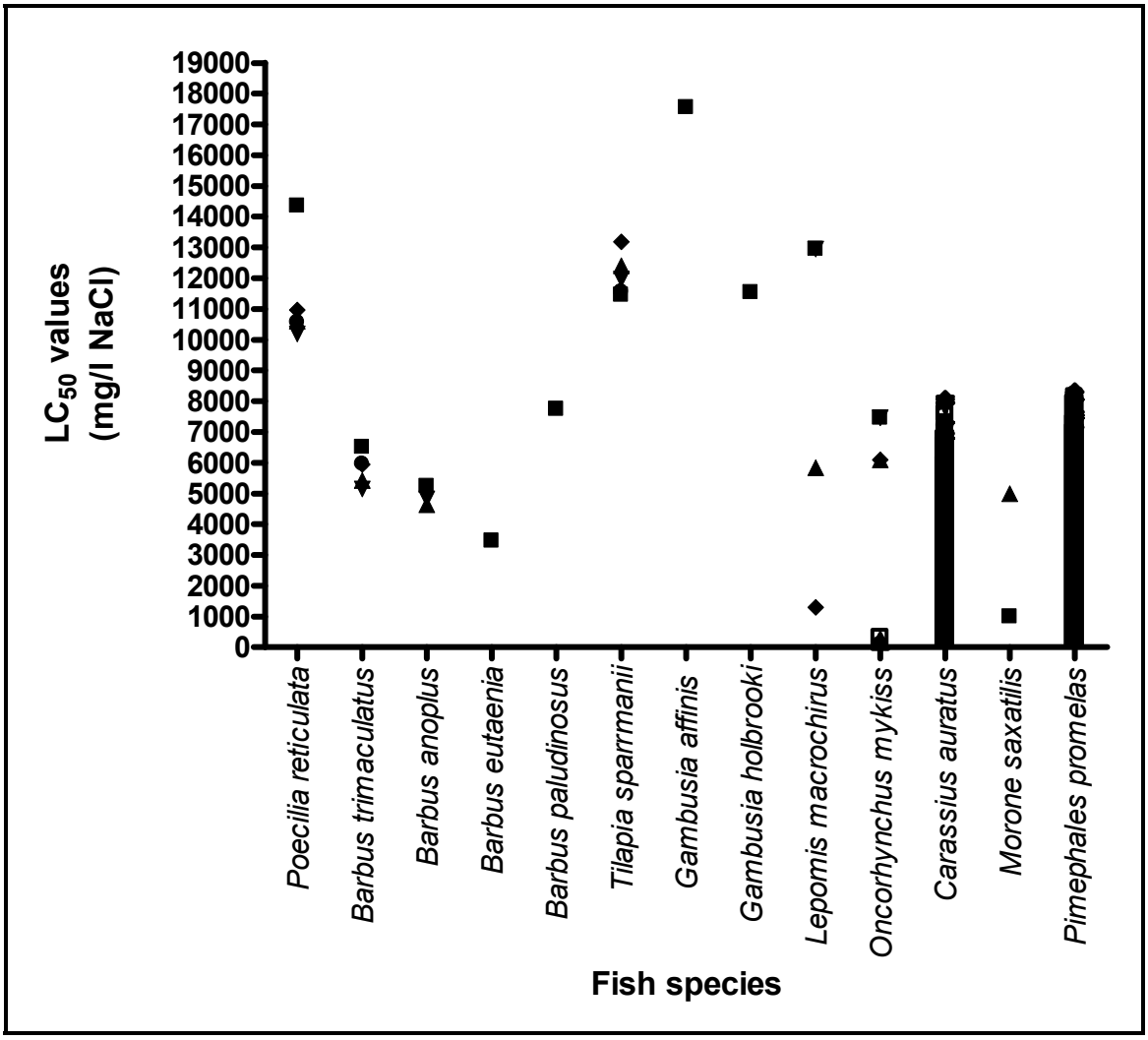

Figure 11.4: Scatter plot of NaCl LC50 values comparing results obtained during the present investigation with results obtained for other freshwater fish species (comparable results from the US EPA ECOTOX database). Results for the first six species are from the present investigation. Common names for the remainder of the species included on this graph are listed in APPENDIX C.

Poecilia reticulata was indicated to be the most sensitive species towards NaF with a mean LC50 of 162 mg/l NaF recorded (Figure 11.5). A CV value of 18.6% indicated good comparability, and hence, reproducibility of test results. *Tilapia sparrmanii* displayed a mean LC50 of 676 mg/l NaF, with an excellent CV value of 13.9%. A mean LC50 of 292 mg/l was recorded for *Barbus trimaculatus* (Figure 11.5). The results however showed some variation, with a CV value of 43.7%.

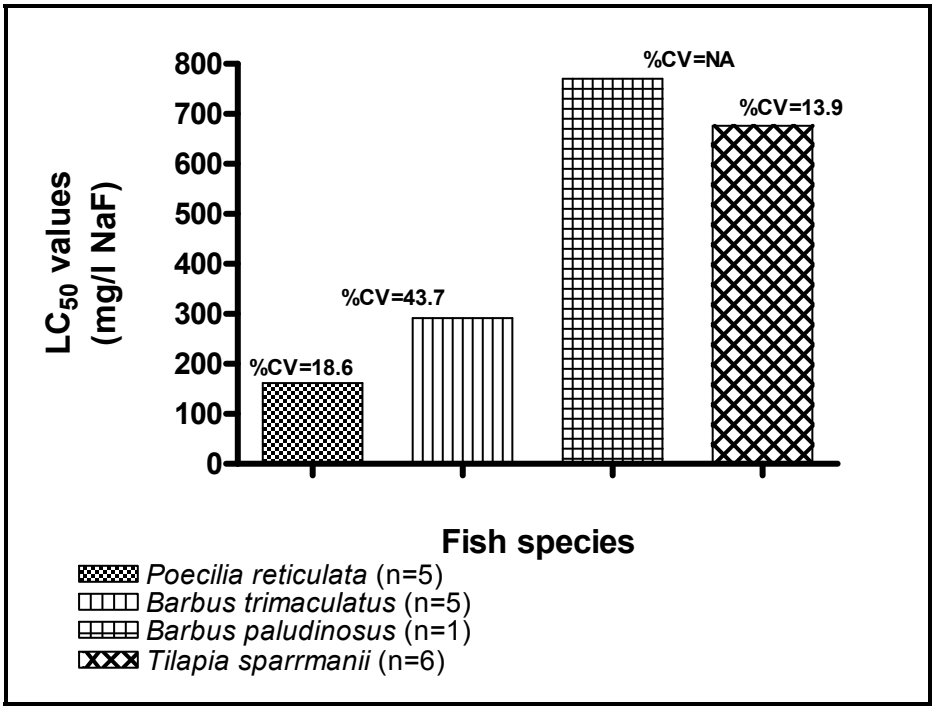

Figure 11.5: Response of selected fish species to NaF. The Coefficient of Variance (%) for each set of tests are indicated above the bar representing the mean LC50 values. The number of tests is presented in parenthesis in the legend.

Comparison of NaF LC50 values with that recorded for other freshwater fish species (obtained from the US EPA ECOTOX database), indicate *Tilapia sparrmanii* and *Barbus paludinosus* to be of the more tolerant species with regard to this specific chemical. *Poecilia reticulata* test organisms were sensitive and *Barbus trimaculatus* slightly more tolerant, generally comparing with results obtained for *Gambusia affinus* (Mosquitofish), *Gasterosteus aculeatus* (Threespine stickleback) *Oncorhynchus mykiss* (Rainbow trout), *Carassius auratus* (Goldfish), *Pimephales promelas* (Fathead minnow) and *Salmo trutta fario* (Brown trout) (Figure 11.6).

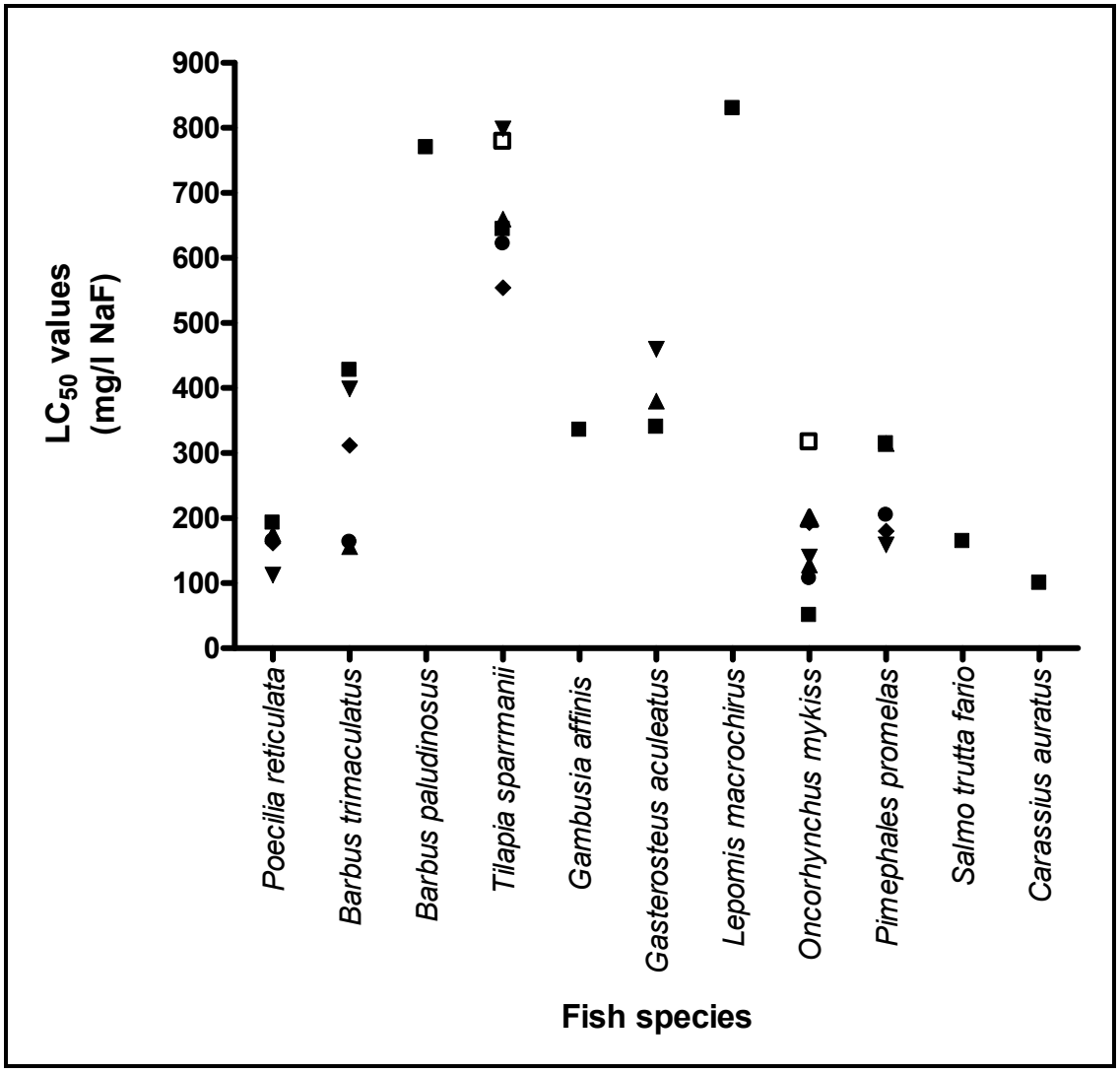

Figure 11.6: Scatter plot of NaF LC50 values comparing results obtained during the present investigation with results obtained for other freshwater fish species (comparable results from the US EPA ECOTOX database). Results for the first four species are from the present investigation. Common names for the remainder of the species included on this graph are listed in APPENDIX C.

Using the Kruskall Wallis test, the different species of fish were found to be significantly different from each other ($P<0.05$) in terms of sensitivity to $K_2Cr_2O_7$, NaCl and NaF.

11.4. DISCUSSION

In general, this phase of the study reaffirms that response levels among species can be, and often are, quite different. However, the main objective of this experimental investigation was to conduct a preliminary assessment of the sensitivities of the different species towards the selected reference chemicals and to assess their consistency in response to these chemicals. Consistency or reproducibility of results is a most important factor to consider in standardized species selection, particularly for regulatory purposes. Tests conducted under identical conditions must yield similar results in order to maintain scientific and legal integrity. Unless reproducible results can be accomplished, sensitivity response levels have little meaning, and most certainly, will not be legally defensible (Hansen, 1979).

The results indicated that different species displayed different consistencies for the different reference chemicals:

- ⇒ Consistency results obtained in the $K_2Cr_2O_7$ tests from most variation to least variation based on the %CV values: *Poecilia reticulata* (68%), *Tilapia sparrmanii* (60.8%), *Barbus eutaenia* (37.5%), *B. trimaculatus* (14.8%) and *B. anoplus* (9%). *Barbus paludinosus* was not included in this assessment since only one data set was available.
- ⇒ Consistency results obtained in the NaCl tests from most variation to least variation, based on the %CV values: *Poecilia reticulata* (15%), *B. trimaculatus* (9%), *B. anoplus* (6.3%) and *Tilapia sparrmanii* (5.8%). It must be noted here that all the above species provided excellent consistency of results for the NaCl. *Barbus eutaenia* and *B. paludinosus* were not included in this assessment since only one data set was available for each species.
- ⇒ Consistency of results obtained in the NaF tests from most variation to least variation, based on the %CV values: *Barbus trimaculatus* (43.7%), *Poecilia reticulata* (18.6%) and *Tilapia sparrmanii* (13.9%).

The same degree of variation was observed in sensitivity data obtained for the different species exposed to the three reference chemicals. The *Barbus* species (*Barbus trimaculatus, B. anoplus, B. eutaenia* and *B. paludinosus*) were indicated to be significantly less sensitive to $K_2Cr_2O_7$ than *P. reticulata* and *T. sparrmanii* during the present investigation, and was also more tolerant than other species of freshwater fish for which available data was obtained from the ECOTOX database (see Figure 11.2). The direct opposite was observed for NaCl. The *Barbus* species were more sensitive to NaCl than the *P. reticulata* and *T. sparrmanii*. Compared to other freshwater fish species (ECOTOX database) the *Barbus*

species seem to be representative of the species within the lower NaCl tolerance bracket. This variation in sensitivity of species to different chemicals have been confirmed in the past and is ascribed to the difference between species as a result of differences in rates and patterns of metabolism and excretion, as well as genetic factors (Rand, 1995).

However, the fact that the *Barbus* species are so much less tolerant towards NaCl than *Poecilia reticulata* is of specific significance, since increased salinization of our water resources is one of the greatest challenges water quality managers in South Africa are faced with (O'Keeffe et al., 1992; CG Palmer et al., 2004). The use of a salt tolerant species such as *P. reticulata* in toxicity bioassays may therefore not provide adequate protection of our water resources.

11.5. CONCLUSION

Based on the sensitivity and consistency results obtained during the experimental investigation it can be concluded that *Barbus trimaculatus, B. anoplus* and *B. eutaenia* has potential for use in standard toxicity bioassays, while all the indigenous species assessed in this chapter also have potential for use in other applications such as ecological risk assessment studies, refinement of ECOSPECS and derivation of site-specific water quality criteria.

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CHAPTER 12

CONCLUSIONS AND RECOMMENDATIONS

In order to extrapolate meaningful, relevant, and ecologically significant results from aquatic toxicity tests, not only appropriate tests, but also appropriate organisms should be used. Several criteria should be considered in selecting organisms for toxicity testing (Rand, 1995):

1. Due to varying sensitivity among species, a group of species representing a broad range of sensitivities should be used whenever possible;
2. Widely available and abundant species should be considered;
3. Whenever possible, species should be studied that are indigenous to or representative of the ecosystem that may receive the impact;
4. Species that are recreationally, commercially, or ecologically important should be included;
5. Species should be amenable to routine laboratory maintenance and techniques should be available for culturing and rearing them in the laboratory so as to facilitate both acute and chronic toxicity tests; and
6. If there is adequate background information on a species (i.e. its physiology, genetics, and behaviour), the data from the test may be more easily interpreted.

Potentially suitable species selected during a desk-top exercise (Chapter 2) were evaluated with the above criteria in mind. Information obtained during the present investigation was used to re-evaluate each of the selected freshwater fish species in terms of their suitability for use in bioassays. Although no attempt was made to breed the *Chiloglanis* species during the present investigation, they are included in this evaluation, together with the other species assessed during this investigation, based on the information generated by De Villiers & Hecht (1990) and De Villiers (1991).

The suitability of each fish species was rated in terms of each criterion on the following basis:

- 1-2: Not Suitable
- 3-5: Low Suitability
- 6-8: Moderate Suitability
- 9-10: High Suitability

Each score for each species for each criterion was then separately multiplied by the weight of a specific criterion to give the suitability of a species for a specific criterion (refer to Chapter 2 for an explanation of the weights allocated to each criterion). These scores were added to give the total suitability of a specific species for all criteria. Highest attained scores translated to highest suitability for use in toxicity bioassays.

Table 12.1: Potential suitability of different indigenous freshwater fish species for use in bioassays, refined based on information obtained during the present investigation

	Distribution	**Habitat**	**Culturing**	**Importance**	**Sensitivity**	**TOTAL**
WEIGHT	26	17	20	10	27	
CYPRINIDAE						
Opsaridium peringueyi	4	3	4	8	9	558
Barbus anoplus	9	4	8	6	6	684
Barbus trimaculatus	8	8	9	6	8	800
Barbus eutaenia	5	1	8	7	8	593
Barbus paludinosus	8	8	8	2	7	713
MOCHOKIDAE						
Chiloglanis paratus	4	4	5	10	7	561
Chiloglanis pretoriae	5	1	5	10	10	617
POECILIIDAE						
Aplocheilichthys katangae	5	1	3	9	7	486
CICHLIDAE						
Tilapia sparrmanii	8	8	8	3	6	696

Barbus trimaculatus was indicated to have the highest suitability for use in toxicity bioassays (with a total score of 800), followed by *Barbus paludinosus* (with a total score of 713) and *Tilapia sparrmanii* (with a total score of 696) (Table 12.1).

Barbus trimaculatus is relatively small (SL = 150 mm). This, together with its non-aggressive social behaviour (Skelton, 2001) resulted in it not requiring large holding or spawning facilities. During the present investigation 90 ℓ tanks were successfully used for spawning. *Barbus trimaculatus* were not particularly sensitive to handling and adapted very well to their captive conditions in the laboratory, with very few mortalities recorded. They responded very well to artificially induced cues to stimulate spawning throughout the year (i.e. increased protein in their diet, simulated stream conditions in the spawning tank and

manipulation of water hardness to simulate a rain event). They are not very susceptible to disease and are widely distributed. They displayed a sensitive response towards NaCl and NaF, but were less sensitive towards $K_2Cr_2O_7$. More importantly though, they were consistent in their response towards the reference chemicals (refer to Chapter 11).

Based on an evaluation of its distribution, habitat requirements, ease of culturing, ecological importance and sensitivity, *Barbus paludinosus* was indicated to be the second most suitable species for use in toxicity bioassays. They did not require large holding or spawning facilities. They were not particularly sensitive to handling and adapted very well to their captive conditions in the laboratory, with few mortalities recorded. They responded very well to artificially induced cues to stimulate spawning. *Barbus paludinosus* displayed a similar response sensitivity and response consistency than *B. trimaculatus*.

Tilapia sparrmanii is relatively small (230 mm SL) and despite its aggressive behaviour towards other fish during breeding, it was possible to breed the species making use of small spawning facilities (90 ℓ tanks). Placing a divider in the spawning tank to divide the tank into two equal sized breeding compartments also proved successful, and doubled the effort within the same space. They were not particularly sensitive to handling, adapted very well to their captive conditions in the laboratory and are not susceptible to disease. This species however does require close monitoring and management of breeding cultures, which is time consuming. The latter is a factor to take into account when considering the use of this species in routine laboratory testing. *Tilapia sparrmanii* was found to be tolerant towards NaCl, as could be expected, but was sensitive in its response towards the other reference chemicals ($K_2Cr_2O_7$ and NaF). The reproducibility of data obtained with NaCl and NaF were excellent with Coefficient of Variance (CV) values of 5.8% and 13.9%, respectively. A CV value of 60.8% was recorded for the $K_2Cr_2O_7$ results. The reason for this variation is unknown.

Results obtained during this investigation also indicated *Barbus anoplus* and *Barbus eutaenia* as potential species for use mainly in site-specific water resource management studies (i.e. ecological risk assessment, refinement of ECOSPECS, etc.). They are relatively small and therefore do not require large holding facilities. *Barbus anoplus* were not particularly sensitive to handling and adapted very well to their captive conditions in the laboratory, with very few mortalities recorded. *Barbus eutaenia* was indicted to be slightly sensitive to handling and nitrate-related water quality changes resulting from live foods have led to some mortalities, but they nevertheless adapted very well to laboratory conditions. Both species responded very well to artificially induced cues to stimulate spawning. They displayed the same trend as that of the other *Barbus* species in their response towards the reference chemicals, being sensitive in their response towards NaCl and tolerant towards

$K_2Cr_2O_7$. Most importantly, they were consistent in their response to the chemicals. When using *B. anoplus* in laboratory bioassays, care should be taken to use fish from the same source to prevent differences in sensitivity as a result of possible genetic differences between different isolated populations.

The breeding system developed for the *Barbus* species evaluated as part of this investigation was not expensive and similar in design. The same protocol, with minor modifications for each species, can be used to breed *Barbus trimaculatus*, *B. paludinosus*, *B. eutaenia*, *B. anoplus*, and probably other *Barbus* species as well. The protocols are easy to implement and does not require large infrastructure. The effort and infrastructure required are comparable with those of other standard protocols (such as *Poecilia reticulata* and *Brachidanio rerio*).

The variation in the toxic response especially between the Cyprinid and Cichlid species confirmed that there is no single species suitable for all applications. It is therefore important to conduct tests with several species, from different taxonomic groups, to get some indication of the natural variability in levels causing an effect (Rand, 1995). This is especially important in terms of ecological risk assessment studies, the refinement of ECOSPECS and derivation of site-specific water quality criteria. The species selected for testing may differ from ecosystem to ecosystem, and the selection will often have to be based on site-specific considerations. A standard toxicity test may therefore not be appropriate for answering specific questions regarding a particular aquatic ecosystem. Assessing the hazard of a particular chemical to an indigenous fish community in a specified aquatic ecosystem will therefore require species representative of that system and it will not be useful to employ standard test species that are not normally present in the specific aquatic system (Rand, 1995)

Based on the findings of this investigation, the following are recommended:

- ⇒ The protocols developed during this study have important application, especially with regard to ecological risk assessment studies, the refinement of ECOSPECS and derivation of site-specific water quality criteria. The culture techniques developed during this investigation should now be refined further through consultation with other laboratories implementing the procedures.
- ⇒ *Barbus trimaculatus* displayed the highest suitability for use in toxicity bioassays. The ability of this test organism to generate similar results in different laboratories should be assessed, with the possibility of standardizing the protocol.

⇒ A range of chemicals should be evaluated in order to evaluate the test species' sensitivity and response consistency over a wide spectrum (to include organic and inorganic chemicals).

⇒ The brood stock used in this investigation were collected from wild populations. Future production facilities must take care to sample initial stock from the same areas in order to prevent introduction of different genetic strains, as this may affect test results. A very stringent collection, husbandry, brood stock integrity procedure will have to be designed and implemented to ensure that facilities providing test organisms are managed properly, to ensure a consistent supply of fish that would provide scientifically defensible results. A certification procedure will have to be implemented for these suppliers to ensure compliance with international husbandry practices and standards.

⇒ The use of the above oviparous species in early life stage toxicity tests (e.g. eggs, embryos) has the potential for further development.

12.1. LITERATURE CITED

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APPENDIX A

LIFE FOOD CULTURES

A constant supply of life food is considered essential by most literature to condition fish for breeding. For this purpose 5 circulating tanks were prepared to maintain a constant supply of crustaceans. One of the tanks was used mainly to fertilise water with chicken manure and was circulated to the other tanks intermittently to avoid spikes that can kill the crustacean population.

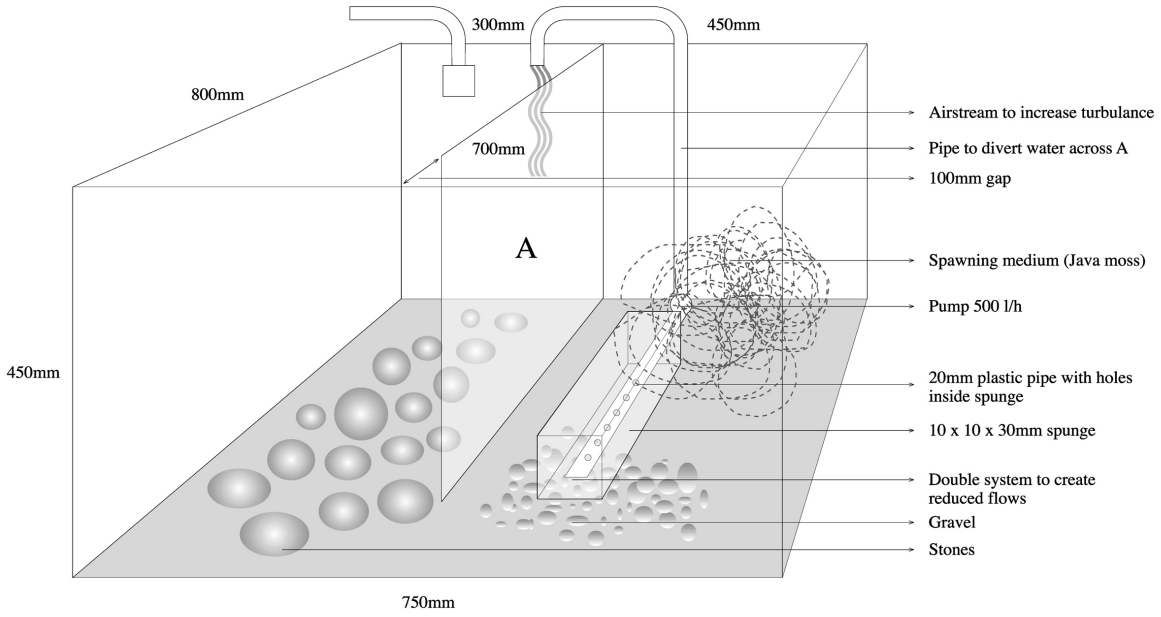
Crustaceans were collected from Highveld pans and stocked into these tanks. The crustaceans introduced consisted mainly of *Daphnia*, cyclops and *Artemia*. Bloodworm normally appears in abundance in well-matured tanks.

This system is successful in maintaining a viable supply of life food. The enrichment of the life food on a regular basis with fatty acids before feeding it to the fish has also been adopted. The application of hormones etc. via this method may also proof to be of some value to stimulate more complete breeding.

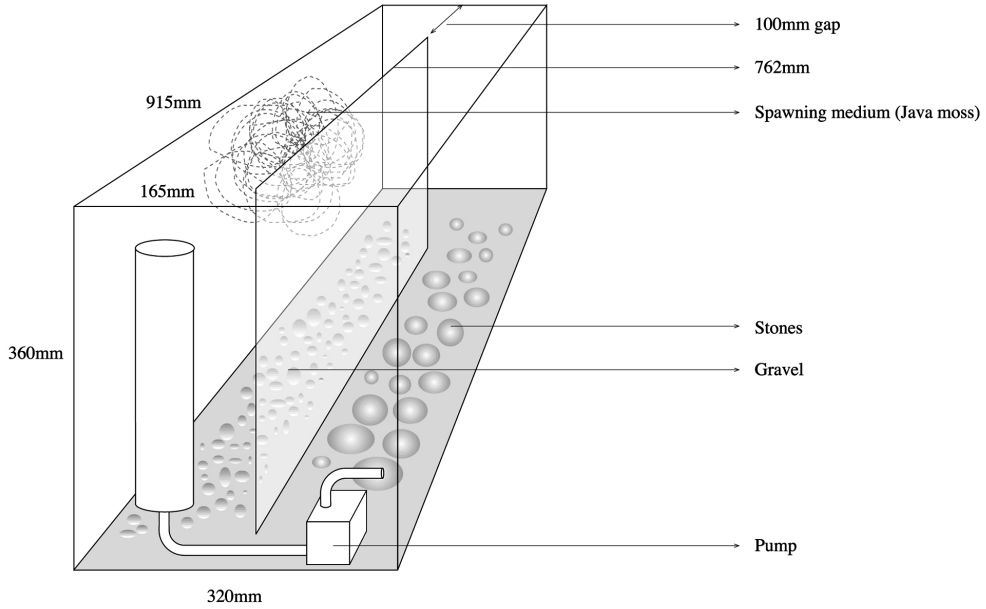
Vegetable matter consisting of *Lemna major* (Duckweed) and *Wolfia arhiza* was cultivated in a 1000 litre tank enriched with organic fertiliser. These two species of aquatic plants were fed at on a regular basis to the majority of the species. An infusoria culture was developed in a 1000 litre tank which was enriched on a regular basis with grass cuttings. The infusoria is an essential food source for feeding the fry of all the species cultivated.

A culture of microworm was maintained and grown on wetted oats and was allowed to migrate onto small pieces of wet filter wool. Microworm was washed of the filter wool in 200 ml of water before being fed to the fish fry.

APPENDIX B

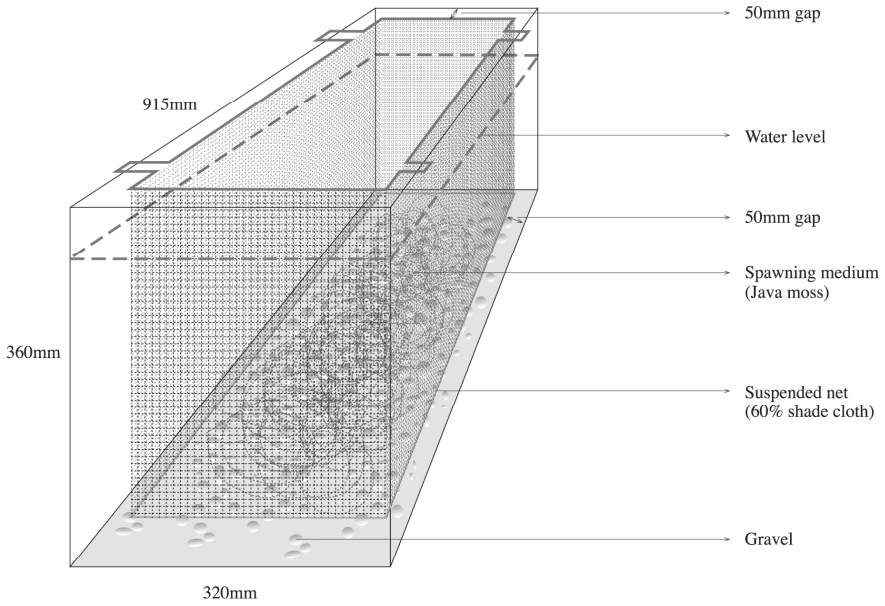


SPAWNING TANK 1



SPAWNING TANK 2

FIGURE PLATE 1: Sketch of a 200 liter spawning tank used in the Lydenburg facility (Spawning Tank 1) and a 90 liter spawning tank used at ECOSUN (Spawning Tank 2)



SPAWNING TANK 3

FIGURE PLATE 2: Photo of a 90 liter spawning tank (Spawning Tank 3) used in *Aplocheilichthys katangae* breeding trials

APPENDIX C

Channa punctata – Snake-head catfish
Carassius auratus – Goldfish
Danio rerio – Zebra danio
Cyprinus carpio – Common carp
Gambusia affinis – Western mosquitofish
Gambusia holbrooki – Eastern mosquitofish
Gasterosteus aculeatus – Threespine stickleback
Jordanella floridae – Flagfish
Lepomis cyanellus – Green sunfish
Lepomis macrochirus – Bluegill
Morone saxatilis – Striped bass
Notemigonus crysoleucas – Golden shiner
Notropis atherinoides – Emerald shiner
Notropis buccatus – Silverjaw shiner
Notropis stramineus – Sand shiner
Nuria danrica – Channelfish
Oncorhynchus kisutch – Coho salmon, silver salmon
Oncorhynchus mykiss – Rainbow trout
Salmo trutta fario – Brown trout
Oryzias latipes – Medaka, high eyes
Paracheirodon axelodi – Neon
Pimephales notatus – Bluntnose minnow
Pimephales promelas – Fathead minnow
Pomoxis annularis – White crappie
Dorosoma cepedianum – Gizzard shad
Notropis rubellus – Rosyface shiner