THE BIOLOGICAL CONTROL OF MALARIA MOSQUITO LARVAE USING SMALLER INDIGENOUS FRESHWATER FISH SPECIES

BY

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ABSTRACT

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This thesis concerns an investigation into the possibilities of using indigenous Cyprinodontidae for mosquito larvae control in an integrated malaria control programme in South Africa. *Nothobranchius orthonotus* and *N. rachovii* were considered but the investigation shows that they will not be successful in local habitats to effectively control mosquito larvae. *Aplocheilichthys katangae* and *Aplocheilichthys johnstonii* are also investigated and *A. johnstonii* is shown to be the most suitable candidate. Mass breeding techniques are discussed for this latter species as well as feeding preferences in the laboratory and in the field. Peak feeding times are ascertained and the gut content analysis of fish from two populations, one from the Transvaal and the other from Namibia, are investigated and discussed. The toxicity to *A. johnstonii* of insecticides used in the malaria control programme is investigated and the feasability of using this species is discussed.

INTRODUCTION

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INTRODUCTION

Climatologically South Africa is divided into a number of distinct regions such as temperate and dry areas; winter and summer rainfall areas; Highveld-, Middleveld- and Lowveld regions (Rautenbach, 1982). The so called Lowveld areas are by and large located within the summer rainfall belt which is characterised by dry winter periods followed by a summer rainy season consisting mainly of convectional thunderstorms. The rainy season is from November to March with the highest rainfall occurring in January.

The malarious areas of South Africa are mainly confined to the Lowveld areas from the Limpopo in the north to the Tugela River in the south, bordered to the west by the Drakensberg range. Anopheline mosquitoes responsible for transmitting malaria are normally restricted to the Lowveld region (Figure 1).

Malaria remains a serious disease in South Africa characterised by local epidemics and during wet years, favourable to the breeding of the mosquito, a large number of cases may be reported as can be seen from the following figures of officially identified malaria infections in the Northern Transvaal and Republic of Venda. (National Institute for Tropical Diseases, unpublished data).

TABLE 1 : POSITIVELY IDENTIFIED CASES OF MALARIA IN THE TRANSVAAL AND THE REPUBLIC OF VENDA : 1978-1985.

MALARIA SEASON	NUMBER OF CASES
JULY 1977-JUNE 1978	5 381
78 79	2 068
79 80	2 263
80 81	1 827
81 82	1 961
82 83	1 367
83 84	2 579
JULY 84 - JUNE 85	5 109

Malaria control in South Africa consists of an attempt to locate and treat people with *Plasmodium spp.* infections and to control the vector populations primarily by application of residual chemical insecticides to the interior of dwellings. In recent years (Zahar, 1984) there has been a renewed interest in the use of biological control agents and their possible role in supplementing the present control methods in several countries throughout the world. Presently there are no organised biological control methods being used in South Africa.

Although chemical control is still the most effective means of controlling malaria vectors, problems such as:
- the increased costs of insecticides, equipment,etc.,
- vectors developing resistance to the insecticides,
- the lack of suitably qualified expertise in many developing countries to run and supervise control programmes,have been experienced.

Possibly biological control could need minimal checking once the chosen agent has been released into the environment. However, since one is dealing with a human disease maximum possible control is desired, therefore maximum effect could

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be achieved by the use of both chemical and biological control in an integrated control programme.

It was for this reason that the present study was undertaken in order to investigate the feasability of using indigenous larvivorous fish for the biological control of mosquito larvae in malarious areas of Southern Africa.

CHAPTER 1

A BIBLIOGRAPHICAL REVIEW OF THE USE OF LARVIVOROUS FISH FOR MALARIA CONTROL

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A BIBLIOGRAPHICAL REVIEW OF THE USE OF LARVIVOROUS FISH FOR MOSQUITO CONTROL.

1.1 A WORLDWIDE REVIEW

"Since the end of the nineteenth century fish have been suggested for biological control of mosquitoes." Russell in 1892 mentions carp being placed in storage tanks for the elimination of mosquito larvae (Sweetman, 1958). The most effective larvicidal fish belong to the order Cyprinodontiformes. Of the many Cyprinodontiformes investigated the most widely used fish is one of the North American Poeciliidae, *Gambusia affinis* Baird and Girard, 1854.

It is a native of the Mississippi Valley and was first distributed from Texas to Hawaii in 1905 (Motabar, 1978) and has since been extensively distributed to the Philippines (1913), Spain and Italy (1920's) and thence to the U.S.S.R., Iran, Afghanistan and North Africa (Gerberich and Laird, 1966).

G. affinis meets most of the requirements of a fish for mosquito larval control in fecundity, abundance, rapid development (first young produced at 6 weeks), small adult size (up to 65mm), an ability to penetrate plant growth which forms cover for mosquito larvae, surface feeding, a natural preference for mosquito larvae as food. Lastly, they are hardy, adaptable and easy to transport.

Because *Gambusia* also eats small fish fry in addition to mosquito larvae and is so adaptable it may have a deleterious effect on indigenous fish

populations (Jubb, 1965). Legendre (1937) dealt with this harmful effect of both *Gambusia affinis* as well as *Lebistes reticulatus* Peters (syn: *Girardinus guppyi* = *Poecilia reticulata* (Peters), and suggested greater use of indigenous fishes for mosquito control. Many authors have since commented on the negative effect of *Gambusia* on the local environment (Hamon, 1970; Bay, 1972; Smith, 1973; Motabar, 1978) whereas others consider the environmental impact as of minor importance in comparison with the beneficial effect (Rubutsov in Mel'nikov, 1973; Green and Imber, 1977; Gerberich and Laird, 1985).

On analysing the literature two facts become apparent:

- Most of the studies on larvivorous fish have used *Gambusia affinis*, although many fish have been mentioned as larvivores. Sweetman (1958) stated that 216 different species of fish have been used for the control of 35 species of mosquitoes in 41 countries. Some papers have reported good success with *G. affinis* (Ronchetti, 1968; Hoy and Reed, 1970; Tabibzadah et al., 1970; Harrison, 1978) whereas others have reported limited success (Bay, 1967, 1972; Menon and Rajagopalan, 1978; Haas and Pal, 1984).

- Instances where limited success was reported were possibly due to a lack of sufficient ecological data on one or more of the following:

  - the aquatic vegetation being too thick for the fish to penetrate
  - the influence of water quality
  - the lack of alternative food once the mosquito larvae were all consumed.
  - fish killed by chemical insecticides.

With the recent increase of interest in larvivorous fish, *Gambusia affinis* is no longer the primary fish of choice (WHO, 1980, 1982; Gerberich and Laird, 1985). Local fish are being more closely studied and sometimes found to be better suited for the purpose than *Gambusia* e.g. in Indian indigenous species like *Aplocheilus blochii* Arnold, and *Oryzias melastigma* McClelland, have been shown to be more effective than *G. affinis* (Menon, 1976; Menon and Rajagopalan, 1978).

Two subspecies of *Gambusia affinis* viz *Gambusia affinis affinis* Baird & Gerald and *Gambusia affinis holbrooki* Gunther are recognised. The two subspecies are differentiated by minor variations on the 3rd and 4th anal rays (Motabar, 1978). Unfortunately most papers on larvivorous fish do not differentiate between the two subspecies. This aspect has been emphasised by Gerberich and Laird (1966, 1985) and therefore no attempt has been made to differentiate between the two subspecies in the present literature survey.

1.2 SOUTHERN AFRICAN REVIEW

Little work has been done in Southern Africa on the use of fish for mosquito control and especially for malaria control. In 1912 there was an unsuccessful attempt to introduce *Poecilia reticulata* (Peters) (syn *Lebistes poeciliodes)* from Barbados to South Africa. This attempt produced the only scientific reference for many years (Gilchrist, 1913). Perhaps the reason for the lack of interest in studying fish for malaria control was that the South African Department of Health did not place much faith in fish for achieving this goal. Dr. S. Annecke believed that they were of limited use and could not, on their own, control malaria vectors

(Annecke, unpublished data, 1934). Annecke also believed that local "top feeding minnows" were just as efficient as *G. affinis*.

Gambusia affinis affinis has established itself in the Cape Province and Jubb (1967) reported on its release in the Northern Transvaal for mosquito control. Jubb (1976) also reported that *G. affinis* was introduced to South Africa in 1936 as "fodder fish" for bass as well as for mosquito larval control. No reference could be found as to the locality and date of these releases. The Department of Health does not appear to have any record of release of fish for mosquito control. Apparently it was recorded that fish were released at Sibasa (Republic of Venda) into ponds by an official of the Department of Agriculture. The fish were described as "mosquito fish characterised by their blue eyes". These fish did not establish themselves and the type of fish is unknown but may possibly have been *Aplocheilichthys*. Attempts to trace this report were unsuccessful.

A single specimen of *G. affinis* was caught in the Limpopo River, at Messina, in the early 1960's (Skelton, pers comm.). Kleynhans (pers. comm.) has reported *G. affinis* from Bon Accord Dam near Pretor - this is the first record of a *G. affinis* wild population in the Transvaal. *G. affinis* is fairly widely distributed in the Eastern Cape. (Jubb, 1967)

Due to lack of work that has been done on the use of fish for mosquito control in South Africa, information on different genera used world wide must be extrapolated and interpreted for relevance to indigenous species under local conditions.

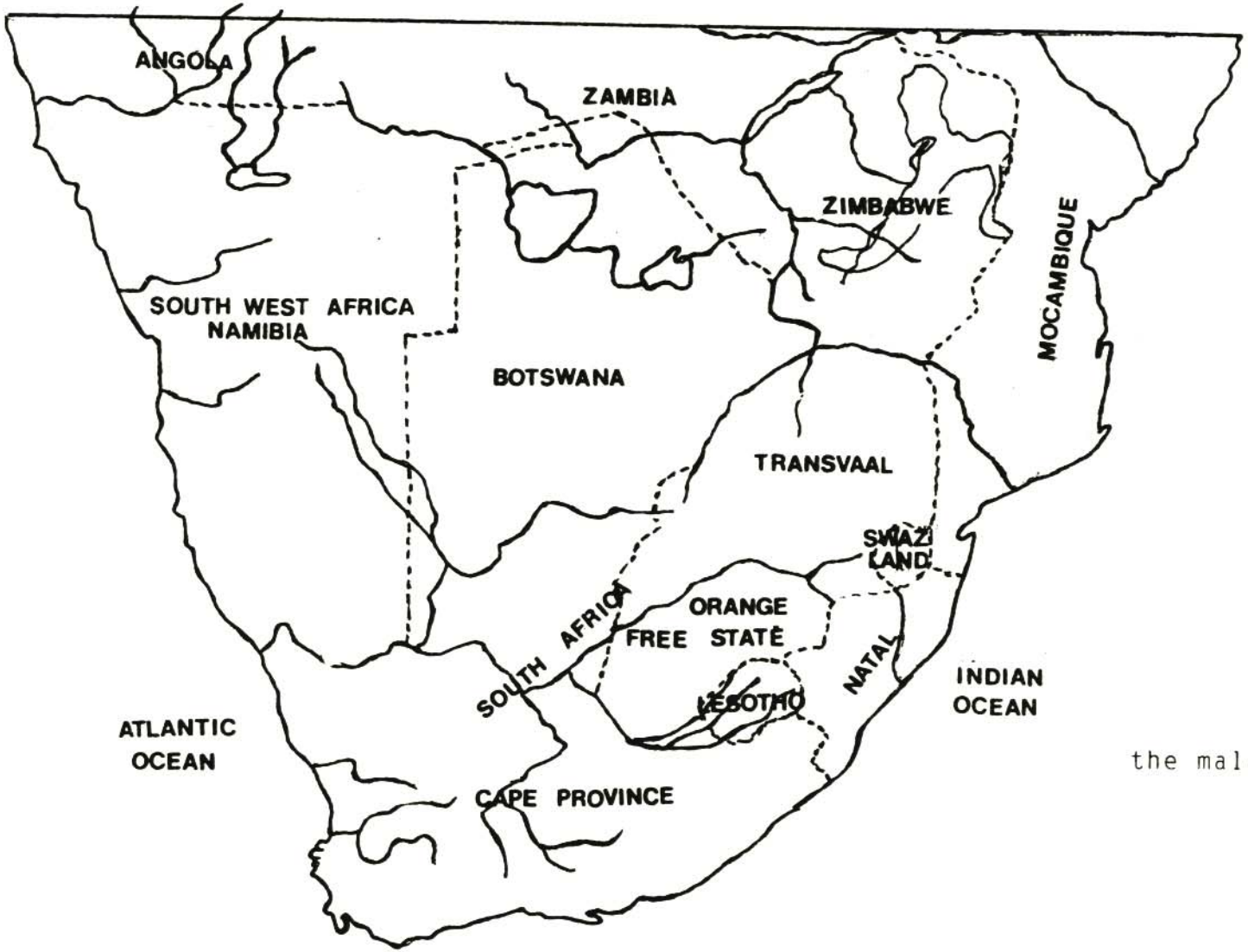
1.3 A BRIEF BACKGROUND OF MALARIA VECTORS IN SOUTH AFRICA.

Human malaria in South Africa, as in the rest of the world, is transmitted by anopheline mosquitoes. Prior to 1963 the vectors were considered to be, primarily, *Anopheles gambiae* Giles, 1902, and *Anopheles funestus* Giles, 1900, (Gillies and de Meillon, 1968). Davidson (1964) and Paterson (1964) showed that *Anopheles gambiae* constitutes a complex of six different species, each with its own behavioural pattern. Coluzzi (1968) described polytene chromosomes from the salivary glands of fourth instar larvae which could be used to differentiate the members of this species complex. This technique has been superceded by the examination of ovarian polytene chromosomes (Colluzzi, 1968; Hunt, 1973). Mahon et al. (1976) used isozyme identification which enables both male and female adults of all ages to be examined rather than merely the half gravid females used for ovarian polytenes.

Gillies and de Meillon (1968) described the larval sites of members of the *Anopheles gambiae complex* as shallow, open, sun-lit pools. This is the accepted habitat although Holstein (1954) questioned their preference for temporary pools. Isozyme analysis has shown that the classical larval site for *Anopheles gambiae* in South Africa contains *Anopheles quadriannulatus* (Theobald, 1911) - a non-vector member of the *Anopheles gambiae* complex- whereas *An. arabiensis* Patton, 1905 larvae have been found in larger flood pans with associated floating and marginal vegetation (N.I.T.D. unpublished data). Service (1976) emphasizes the utilisation of rice fields by *An. arabiensis* larvae, especially during the earlier growing period of the rice.

Since the implementation of an intradomicillary spraying programme, *An. gambiae* s.s. and *An. funestus* have virtually disappeared and the principal vector of malaria in Southern Africa is *An. arabiensis*. Due to its exophilic/endophilic as well as zoophilic/anthropophilic behaviour eradication with the above control method is not completely successful, because it is aimed at only one stage of the life cycle of the vector. Alternative methods of control therefore need to be investigated, especially methods aimed at the larval stages of the vector. One possible method is biological control using larvivorous fish.

Figure 1 : The Malarious Areas of Southern Africa



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CHAPTER 2

POSSIBLE CANDIDATE FISH SPECIES FOR MALARIA MOSQUITO CONTROL IN SOUTHERN AFRICA

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2.1 REQUIRED CHARACTERISTICS

The ideal larvivorous fish should have the following characteristics:

- larvivorous habit
- characteristic high population density
- small adult size
- overlapping generations within a season, allowing a rapid geometric increase.
- a wide tolerance for various limiting physical and chemical parameters of water quality.
- a range of diet so that if mosquito larvae are eradicated from the habitat the fish can survive.
- fairly resistant to insecticides used for agricultural and environmental health projects
- should be compatible with the biological components of local habitats.
- must not encourage parasitological effects in a particular environment.

It cannot be expected that one single species would comply with all the above criteria and therefore some compromise would be necessary in choosing candidate species.

2.2 CANDIDATE SPECIES

The Cyprinodontoidei have been shown to be the most effective larvicidal fish worldwide and they are often referred to as "mosquito fish". Two families of this suborder are found in South Africa viz. the Cyprinodontidae and the Poeciliidae.

2.2.1 POECILIIDAE

Gambusia affinis affinis, although not indigenous to South Africa, has become established in the Eastern Cape, but because of the possible harmful effects on the natural environment (Haas and Pal, 1984; Legner and Sjogren, 1984) it was not primarily considered for mosquito control in South Africa. It would also not be an acceptable candidate to the conservation authorities in this country.

2.2.2 THE CYPRINODONTIDAE

Two genera of this family are found in South Africa viz. *Nothobranchius* and *Aplocheilichthys*. The following species occur in Southern Africa:

Nothobranchius orthonotus (Peters, 1844)
Nothobranchius rachovii Ahl, 1926
Nothobranchius brieni Poll, 1938
Nothobranchius furzeri Jubb, 1971
Aplocheilichthys johnstonii (Günther, 1893)
Aplocheilichthys katangae (Boulenger, 1912)
Aplocheilichthys myaposae (Boulenger, 1908)

2.3 The Occurrence of *Nothobranchius* in Southern Africa with notes on the Life Cycle.

Nothobranchius are indigenous to Africa. They are commonly called "annual fish" due to their life cycle. The fish are found in temporary water and eggs are deposited in the substrate. Under natural circumstances the water body must dry up and remain dry for several months to allow the eggs to develop. There is a long embryonic development of at least three months. Once the habitat becomes filled with rain water the eggs hatch. The fish

larvae are free swimming as soon as they have hatched and almost immediately start feeding. Fish become sexually mature at about six weeks of age. The adults, due to the natural habitat, survive less than one year (WHO, 1981).

The only member of the genus that has been extensively studied is *N. guentheri* (Pfeffer, 1893) from Tanzania. Markofsky and Perlmutter (1973) investigated the longevity of *N. guentheri* and found the "median" life span to be 14 months. Markofsky and Matias (1977); Matias and Markofsky, (1978) and Markofsky et al. (1979) have shown that temperature and daylight length are important for diapause in the eggs and that, under laboratory conditions, spawning is possible throughout the year. Eggs laid in the same batch do not hatch simultaneously. This delayed hatching response is an advantageous adaptation to the ephemeral habitat of this genus. In the laboratory females of some species can produce up to 45 eggs per day for extended periods (WHO, 1981).

Nothobranchius has been considered for mosquito control due to its habitat preference (Jubb, 1981) and several authors mention a feeding preference for mosquito larvae (Turner and Pafenyk, 1967; Bell-Cross, 1976; Pienaar, 1978; Jubb, 1961).

Four species have been reported from Africa south of the Zambezi Drainage system (Figure 2). Of the four *N. furzeri* has only been found in one locality in the Gona-re Zhou Game Reserve in South-East Zimbabwe (Jubb, 1981). Another species *N. brieni* was reported from East Caprivi (Van der Waal, 1976) but Jubb (1981) refers to the specimens from this locality as a member of the *N. taeniopygus* group. "This Caprivi species cannot

be described scientifically until the researches of Mr. R.H. Wildekamp into the status of what I call the *N. taeniopygus* group have been completed" (Jubb, 1981, P. 46). It would therefore seem safer to consider the locality of *N. brieni* as being the Lualaba River Drainage System, South-Eastern Zaire (Jubb, 1981). The other two species *N. rachovii* and *N. orthonotus* are more widely distributed but appear to be intermittently distributed and restricted to limited suitable habitats (Fig. 2).

In South Africa *N. rachovii* is found in an isolated pan in the Kruger National Park (Pienaar, 1978) and has been relocated elsewhere in the Park in order to increase distribution. The habitat seems to be limited to the Sandveld areas of the Kruger National Park where the pH of the water is 6,7 compared to a range of 7,6-8,3 in the rest of the Park. It is not clear if this pH difference is a limiting factor in the distribution of *N. rachovii* or whether the distribution is dependent on other factors.

N. orthonotus is more widely distributed in South Africa. It was found in the Kruger National Park in the same isolated pan as *N. rachovii* (Pienaar, 1978). It is also distributed in Northern Zululand in Maputaland in a few pans of the Pongola floodplain system (Jubb, 1967; Bruton en Kok, 1980). These habitats are presently threatened by the effects of the Pongolapoort Dam in decreasing the annual extensive flooding that occurred during wet years, as well as the change in the ecology following the effects of the cyclone Domoina in 1984. *N. orthonotus* has not been found in any of the pans in the Ndumu Game Reserve on the Mocambique border since the cyclone (Vermaak 1985 pers. comm.). There is one report of *N. orthonotus* from the Mkuze area in 1934 (Jubb, 1981). In the

Kruger National Park *N. orthonotus* has also been successfully relocated from the original habitat to increase distribution in the Park.

The initial expectancy worldwide in using *Nothobranchius* species for biological control of mosquito larvae has decreased following various problems experienced, especially in breeding (Jubb, 1968; Hamon, 1970; Bailey, 1972; Bay, 1972). During the present study a series of laboratory experiments was set up to test the feasability of using *Nothobranchius spp.* for mosquito larval control. (Chapter 3).

2.4 The occurrence of *Aplocheilichthys* spp. in Southern Africa.

The distribution of *Aplocheilicthys spp.* in Southern Africa is shown in Figure 3.

A. myaposae is only found in South Africa in Northern Natal and Zululand in low lying areas (Jubb, 1967) and is very limited in its distribution. Jubb (1961) reported *A. myaposae* as being widely distributed from the Okavango River to Natal. These distribution records are not mentioned in a later publication (Jubb, 1967), and it would appear that the records referred to in 1961 actually refer to *A. katangae* (Jubb, 1967; Bell-Cross, 1976; Van der Waal, 1976).

A. katangae is widely distributed in the Okavango River, East Caprivi, the whole Zambezi system, parts of Zaire and southwards to Natal (Bell-Cross, 1976).

The only natural habitat for *A. katangae* in the Transvaal would seem to be in the Mohlapitse River (Hecht and Mashego, 1981; Polling 1982). It is common in Maputaland in the Northern Zululand area

(Bruton en Kok, 1980).

A. johnstonii has a similar distribution to *A. katangae* except that in the Transvaal it is found in the Nyl, Mogol, Palala and Levubu Rivers (Kleynhans, pers. comm.; Pienaar, 1978). The present author collected this species in the Botete River in Botswana as well as at Poppa in the Kavango River, Namibia. (Fig. 3).

Due to the wide distribution of *Aplocheilichthys* in South Africa as well as its surface feeding habits and reported preference for mosquito larvae (Jubb, 1961; Bell-Cross, 1976; Pienaar, 1978; Bruton et al., 1982) this genus was selected for further studies.

Since *Nothobranchius brieni* and *N. furzeri* do not occur naturally in South Africa it was decided to concentrate further studies on *N. rachovii* and *N. orthonotus* as representative species of this genus. Similarly *Aplocheilichthys myaposae* is restricted to Natal and Zululand whereas both *A. johnstonii* and *A. katangae* occur throughout the region. Therefore they were chosen as possible candidate species for the current investigation.

A comprehensive survey of available literature indicates very few detailed studies undertaken on either the breeding biology under natural conditions, nutritional studies indicating feeding preferences or other behavioural patterns. As mentioned by Gerberich and Laird (1985) most studies have been done on *Gambusia affinis*. The only reference found to the breeding season of South African *Aplocheilichthys* was Bruton (1979). This lack of knowledge has also been commented on by Bell-Cross (1976). Artificial breeding methods have been reported by aquarists (Roloff, 1975; Wright, 1975;

Wildekamp, 1980) for *Aplocheilichthys*. Artificial spawning of and hatchery procedures with *Nothobranchius* have been more intensively studied (Markofsky and Matias, 1977; Matias and Markofsky 1978; Jubb, 1981; WHO, 1982).

Experimentation on artificial breeding methods has mainly been done to satisfy the aquarist trade and as such is too time consuming and meticulous for the utilization of these species as biological control agents.

Because of the above lack of appropriate knowledge it was decided to undertake preliminary experimentation in order to ascertain the suitability of these species for purposes of the present study.

Fig. 2 The Distribution of *Nothobranchius spp.* in Southern Africa.

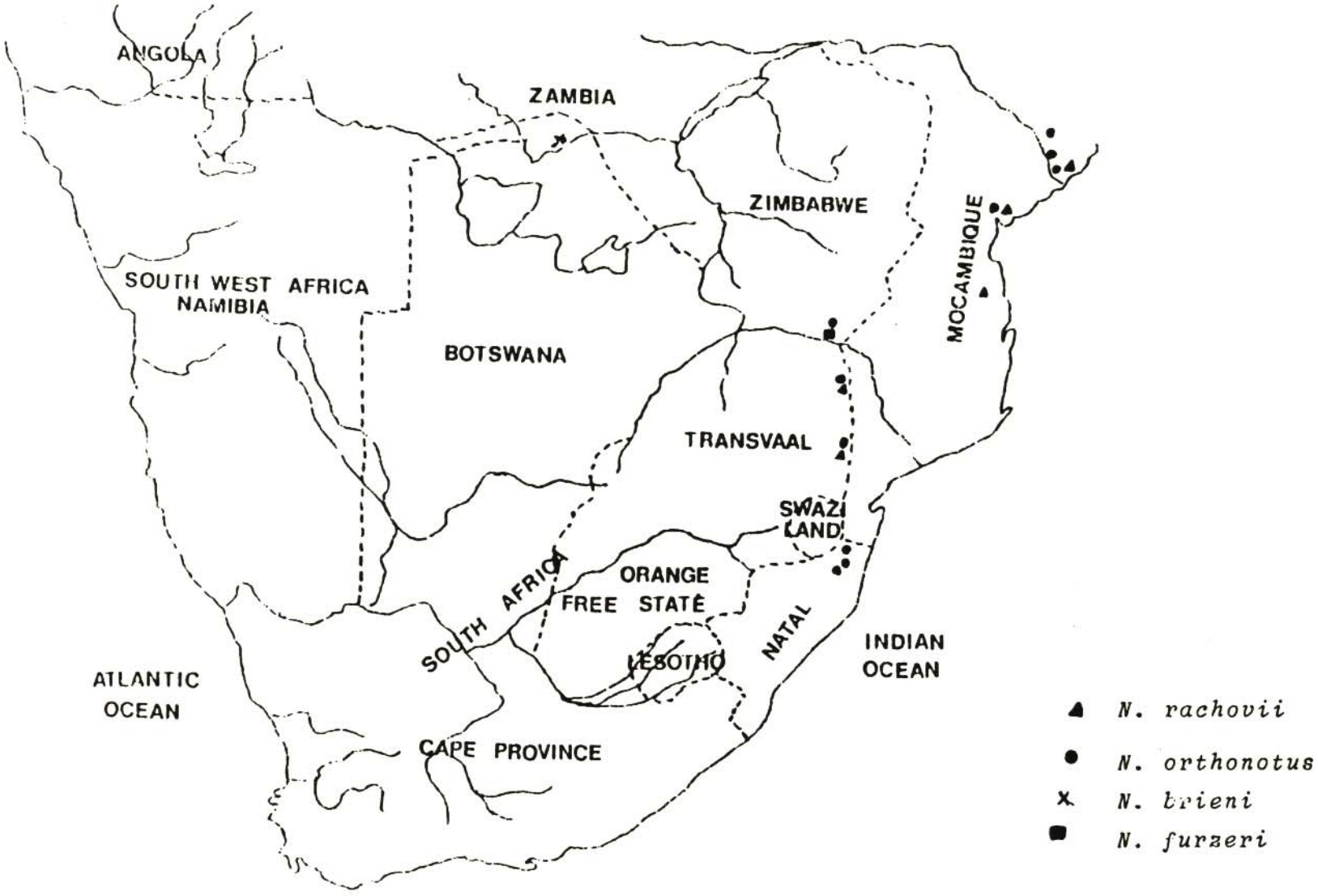
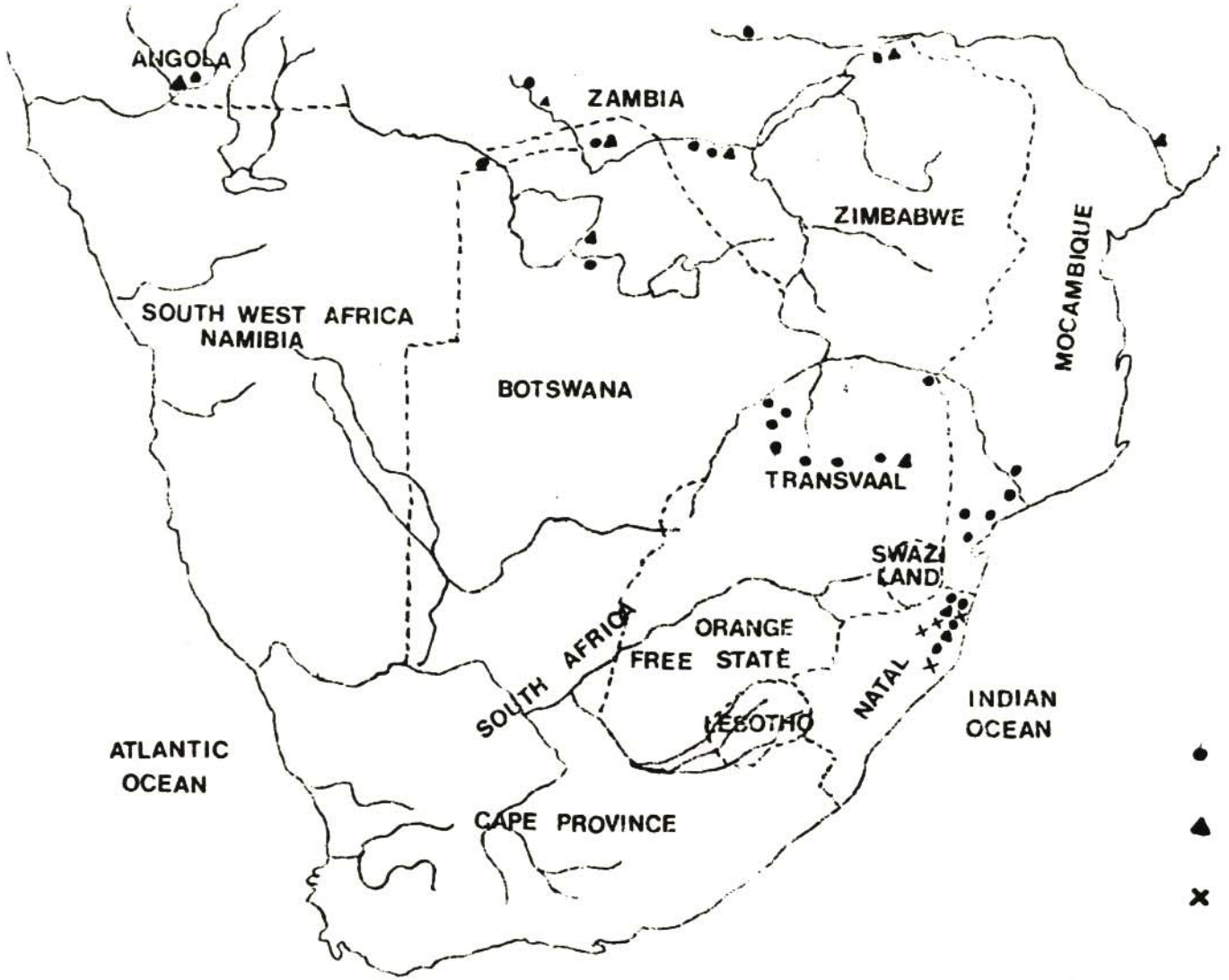


Fig. 3. The Distribution of *Aplocheilichthys spp.* in Southern Africa.



CHAPTER 3

PRELIMINARY LABORATORY EXPERIMENTS AND OBSERVATIONS

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AIM To investigate the possibility of using indigenous Cyprinodontidae for mosquito control by establishing
a) feeding preferences
b) the suitability of members of these genera for large scale breeding under controlled conditions.

3.1 NOTHOBRANCHIUS SPP

3.1.1 MATERIALS AND METHODS

Nothobranchius rachovii and *N. orthonotus* adults were collected from the Pembe pan, Kruger National Park. In addition *N. rachovii* (Beira strain) eggs were obtained from Holland via a local aquarist.

Adult fish were placed in glass aquaria 0,45mx0,45m x0,45m. The bottoms of the tanks were either covered with approximately 2,5 cm of peat or the tanks had a small plastic box half filled with peat placed in them. The lids of the boxes had holes cut in them to allow free entry and exit of adult fish.

Peat was used as a substrate since the fish laid in it readily. Also the pH of the water became acidic - comparable with that of the natural habitat in the Kruger National Park (pH 6,7). The use of peat was recommended by a local aquarist (Vermaak pers. comm.)

The peat was removed from the aquaria at fortnightly intervals and stored in plastic bags for three months to allow development of the embryos within the eggs which had been deposited in the peat. The peat was then immersed in water and the hatching response recorded.

Various substrates were initially tested for their suitability for spawning and storage of the eggs (viz.washed river sand, red clay soil and black clay soil from the malarious areas). Most of the rivers in the Transvaal Lowveld have a coarse sand bed but it was found that this sand was too coarse for the fish. Females soon showed fin damage and did not readily deposit their eggs or spawn in this substrate.

The two most common types of soil encountered in the Lowveld areas are a fine red clay-type and a heavy black loam. These are the soil types that the fish would normally encounter should they be released for biological control in the malarious areas.

When the fish attempted to spawn in both soil substrates a cloud of fine particles was caused. The thick, black clay soil ("black cotton soil") displayed problems in the drying process in that it was difficult to achieve even drying throughout. The surface tended to dry and crack while leaving the bottom still moist. This was conducive to either dessication, (if the mud was too dry,) or to fungal infections, (if too moist) during the storage period. With careful drying this problem was overcome.

3.1.2 RESULTS OF LABORATORY EXPERIMENTS

1. Breeding Experiments

Both species could be induced to spawn readily in the various substrates but the initial hatching rate obtained was a rather unsatisfactory 6 per cent. Experimental breeding trials were redesigned with a view to enhancing hatching success. For each trial 50 eggs were used. It was found that a

90% hatching rate could be achieved under the following conditions:

- peat was used as a substrate to maintain an acidic pH of the water and to minimise fungal infections.
- the peat was removed from the tank and eggs collected using a dissecting microscope.
- the eggs were then placed with a small quantity of wet peat and dried in a petri-dish to "a damp tobacco" feel.
- the eggs, with the peat, were placed in small plastic bags, labelled, and stored in a dark place at $\pm$ 20°C.
- after three months the eggs plus peat were put in a small plastic bowl and water at 4°C was added to immerse the peat to a depth of 2,5 cm. The water was then allowed to rise to room temperature.

Difficulty in obtaining reasonable hatching rates was experienced until, on the recommendation of a local aquarist, (Mr. J. Vermaak, who has succesfully bred most *Nothobranchius spp.*) cold water was initially used.

Once the eggs had hatched air was pumped through the water and more water, at room temperature, was added.

An important facet to consider when breeding *Nothobranchius* under captive conditions appeared to be the male - female ratio per tank. If a ratio of 1:1 was used it was found that the male spent most of the time pursuing the female who was then not so successful in laying eggs. If there were two males in a tank with various numbers of females the males expended a lot of energy and time in displays and aggression.

Visual stimuli appear to be very important in bringing the female into a ripe-running condition. If an opaque barrier was placed between the male and female fish the female would not spawn. When a transparent screen was placed in the tank to separate the same pair of fish, ova developed. When the female was swollen with ova the screen was removed and spawning took place. After spawning the fish were again separated.

The experiment with transparent screens was repeated a number of times using both the original pair of fish as well as other pairs.

The aggressiveness of males causes difficulties in trying to breed large numbers of fish since many small tanks would be necessary in preference to larger tanks.

Although an excellent hatching rate was achieved it was found that commercial fish fry food was not successful for rearing and a 90% mortality in the young fish was experienced. Turner and Pafenyk (1967) recommend rotifers or *Artemia nauplii* for feeding fry. It was found that pool water containing *Daphnia*, rotifers and filamentous algae with a supplement of dried and finely ground lettuce leaves and dog yeast biscuits (to encourage the production of micro-organisms) resulted in minimal mortality. The water in the bowls where the fish were developing was changed every second day to decrease the risk of fungal development. Under laboratory conditions both eggs and adults were prone to fungal infections. Aquarists store the *Nothobranchius* eggs in peat during embryonic development and the low pH(4,5-5) of the peat inhibits fungal development.

2. FEEDING EXPERIMENTS

N. rachovii and *N. orthonotus* are primarily substrate feeders. The younger fish spend more time near the water surface while *N. rachovii*, from approximately 2 cm. length, will spend more time near the substrate. The mouths of *Nothobranchius* are not as upturned as that of other Cyprinodontids and are therefore not suitably adapted for feeding at the infraneuston. Since it is at the infraneuston that mosquito larvae are found feeding and resting the fish will not feed preferentially on mosquito larvae. Both *N. rachovii* and *N. orthonotus* are attracted to the movement of their prey but size is also important. When mosquito larvae were just hanging from the infraneuston they were often ignored by the fish, but as soon as a larva moved it was actively pursued and consumed. They prefer to eat mosquito pupae rather than larvae. In the presence of other food e.g. Odonata larvae, Ephemeroptera larvae, Notonectidae and *Daphnia spp.*, the mosquito larvae were eaten only in preference to *Daphnia spp.*

Small anuran tadpoles, mainly *Rana angolensis*, were consumed by *Nothobranchius* spp. whereas they were not preyed upon by *Aplocheilichthys* spp.

Since *Nothobranchius spp.* occupy such ephemeral habitats many possible food sources will necessarily be precluded from their menu and mosquito larvae such as *Anopheles arabiensis*, which develop in temporary waters, may, in addition, become an obligative food source. It is this obligative feeding habit which led to the choice of using *Nothobranchius* for malaria mosquito control. During World War II *N. taeniopygus* was released by Vanderplank with reported success in malaria control in seasonal swamps in Tanganyika, Kenya, Malawi and Zambia. (Jubb, 1981).

Bransby-Williams (pers. comm.) reports that *N. taeniopygus* is still found in Zambia in widely separated areas. The original release of *N. taeniopygus* by Vanderplank was only in the vicinity of military airfields and this could possibly account for their present intermittent distribution in this area.

3.1.3 CONCLUSION

The possibility of using either *N. rachovii* or *N. orthonotus* on a wide scale in southern Africa for mosquito control is limited for the following reasons:

- difficulty in both mass breeding and mass rearing of both species.
- Both species tend to be more substrate than surface feeders.
- *Nothobranchius* prefer acidic waters, whereas most Lowveld waters are slightly basic. (Schutte, pers. comm., Jooste, unpublished data).

3.2 APLOCHEILICHTHYS SP.

3.2.1 MATERIALS AND METHODS

Aplocheilichthys katangae adult fish were collected from the Fisheries Research Institute, Lydenburg (25°6'S, 30° 29'E). *A. johnstonii* were collected from the Nyl River near Potgietersrus (24°12'S, 28°59'E). These two species were chosen for further studies since they are both indigenous to the Transvaal. Each species was placed in glass aquaria 0,9m x 0,45m x 0,45m. Air was bubbled through the water and *Valesneria sp* planted in soil on the bottom of the aquaria. The aquaria were kept in a room maintained at 25^{+} 2°C and relative humidity 70-80% throughout the year. The water in the aquaria stabilised with the following characteristics.

temperature	20^{+}_{-} 1°C
pH	5
dissolved Co_2	75 mg/ℓ
hardness	136 mg/ℓ
dissolved O_2	5 mg/ℓ
free acidity	0 mg/ℓ

Readings were done using a Hach Water Ecology kit AL36B.

For the breeding experiments sexually mature fish were placed in breeding tanks with neither soil substrate nor *Vallesneria sp.* Initially the *Vallesneria sp.* was left in but it was observed that eggs were laid on this vegetation and were eaten by the adult fish. The artificial spawning mop technique of Turner and Pafenyk (1967) was modified. Spawning mops of 100% nylon were hung in the aquaria with a maximum of three mops per aquarium (Photo 1). The spawning mops were checked twice daily, at 08h00 and again in the early afternoon. The "spawning mops" are further described in chapter 5.

The fish were fed on a diet of *Daphnia sp.* commercial fish flakes and mosquito larvae *(Aedes aegypti).* Snails *(Physa sp. and Heliosoma sp.)* were introduced into the aquaria to consume any surplus fish flakes which sank to the bottom of the tank. As a result the tanks were kept relatively clean.

Although *A. katangae* is reported as only having a very limited distribution in the Transvaal (Kleynhans, 1984), it may be found to be more widely distributed as more collections are done.

3.2.2 RESULTS

1) BREEDING EXPERIMENTS

Using similar methods as those used for *A. johnstonii* it was relatively easy to establish a laboratory colony of *A. katangae* (c.f. chapter 5).

The breeding season of *A. katangae*, in the laboratory of prevailing room temperatures, was from September to March, This was observed for three successive years (1982-84). These laboratory observations are similar to the field observations of Bruton (1979) and Van der Waal (1976). The eggs of *A. katangae* were laid singly and the major details of courtship appeared similar to that of *A. johnstonii* (chapter 5). Haas (1984) reported *Aplocheilichthys* as expelling 10-12 eggs in a cluster, rather than laying one at a time. This has never been observed during the four year duration of the present investigation for either *A. katangae* or *A. johnstonii*.

TABLE 2 DEVELOPMENT OF *A. KATANGAE* EGGS IN THE LABORATORY UNDER CONSTANT TEMPERATURE CONDITIONS ($25 \pm 2°C$)

DATE OF SPAWNING	NUMBER OF EGGS	NUMBER OF EGGS HATCHED	AVERAGE NUMBER OF DAYS TO HATCHING
28/01/82-18/03/82	14	10	15
09/06/82-26/07/82	77	61	23
27/07/82-08/10/82	60	58	18
20/10/82-14/12/82	52	51	15
18/03/82-05/06/82	Mosquito larvae as food only, no eggs laid.		

The above laboratory observations were made on sexually mature fish in breeding tanks kept under prevailing room temperatures. However, if mature fish were kept in constant temperature rooms

(25 ± 2°C) eggs could be collected throughout the year. Development of the eggs, even under conditions of constant temperature, was slower in the winter months than in the summer months (Fig. 4).

If all plants and "mops" were removed from the tanks for about eight weeks and then "mops" were re-introduced, spawning once again occurred. It was also found that if mosquito larvae were the only food available then no spawning took place. The number of eggs produced during spawning, number hatched and average days to hatching are summarised in Table 2. The number of eggs collected from the "mops" depended, to a certain extent, on the number of adults in the tank (Fig. 5). The decrease in the number of eggs with a relatively high density of adult fish could be due to either overcrowding or increased predation of the eggs by the adult fish.

2) INTERACTION OF *A. KATANGAE* AND *A. JOHNSTONII.*

If young *A. katangae* and young *A. johnstonii* were placed in an aquarium only the *A. johnstonii* reached maturity. No dead *A. katangae* were found on the bottom of the aquarium so presumably the fish had been consumed. If a juvenile *A. katangae* (± 1,5cm) was placed in a tank with several adult *A. johnstonii* the *A. katangae* would spend most of the time under observation behind shelters or amongst the vegetation and if forced into more open waters it was actively pursued by the *A. johnstonii* until it again reached shelter. The *A. katangae* did not appear to secure a particular territory in an aquarium in which *A. johnstonii* was present.

When mosquito larvae were introduced into the aquaria containing both species of fish the *A. johnstonii* were more active and pursued, mos-

quito larvae with greater enthusiasm.

3.2.3 DISCUSSION AND CONCLUSION

Due to the similar habitats of *A. katangae* and *A. johnstonii* there is a possibility that, if the two species occurred sympatrically, the dominance of *A. johnstonii* could lead to the destruction of *A. katangae* in that locality.

Therefore due to its limited distribution and lack of competitiveness compared with *A. johnstonii* it was decided that *A. johnstonii* would be more successful for mosquito control in South Africa.

From this rationale a decision was made to undertake an indepth study of *A. johnstonii*. In an attempt to ascertain whether *A. johnstonii* would establish viable populations in the Transvaal Lowveld, fish were released into isolated pools at Skukuza and Satara in the Kruger National Park. The fish have become established in these pools and have provided breeding nuclei for releasing elsewhere in the K.N.P. (Pienaar pers. comm.). *A. johnstonii* has also been released into a pond at Tzaneen where a viable population has become established.

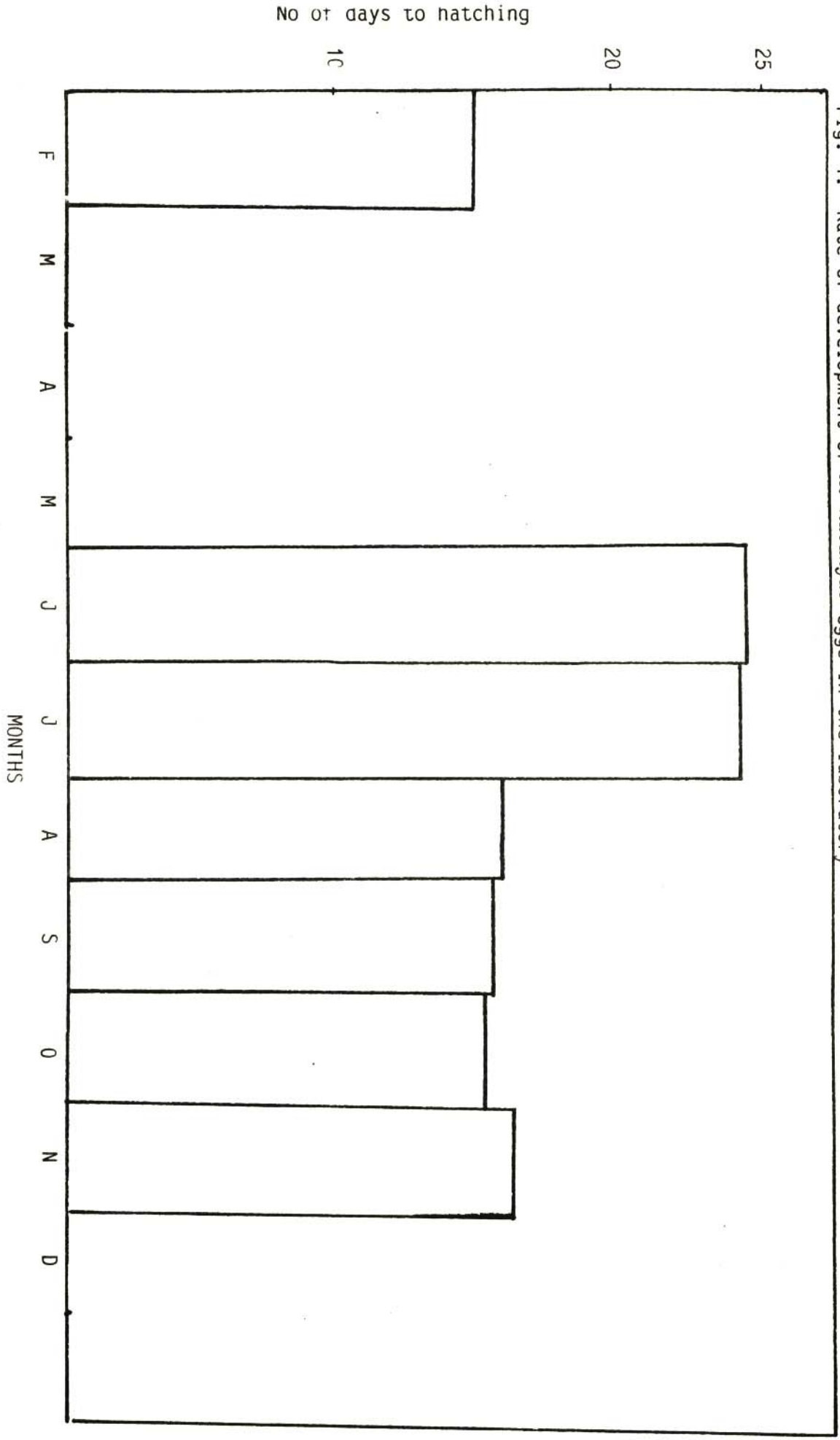


Fig. 4. Rate of development of *A. katangae* eggs in the laboratory

Fig. 5. No of eggs collected from *A. katangae* in a constant environment in relation to adult fish present (approximately 1M:1F)

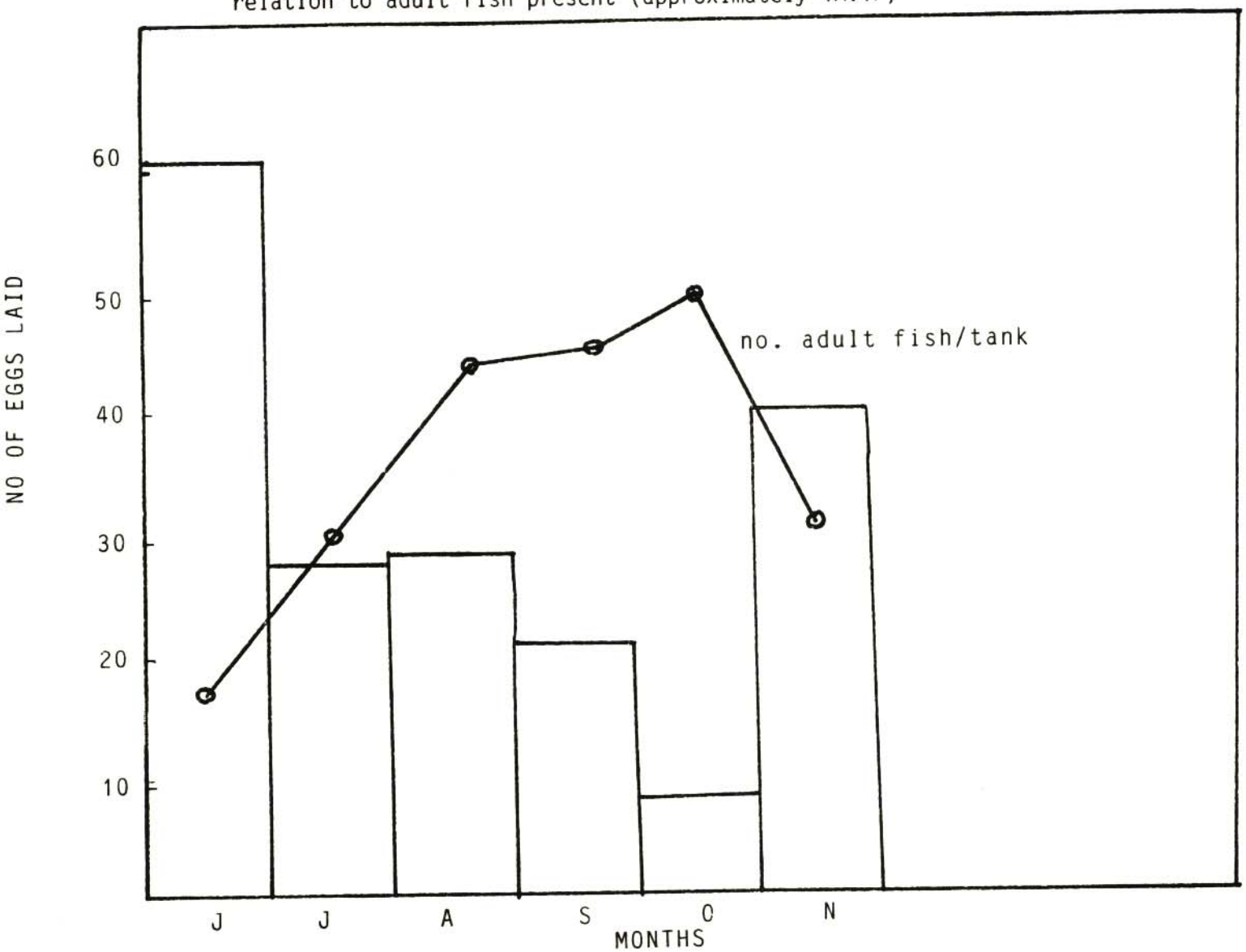


PHOTO 1. A "SPAWNING MOP" IN AN AQUARIUM

PHOTO 1. A "SPAWNING MOP" IN AN AQUARIUM

CHAPTER 4

HABITAT AND DISTRIBUTION OF A. JOHNSTONII IN SOUTHERN AFRICA

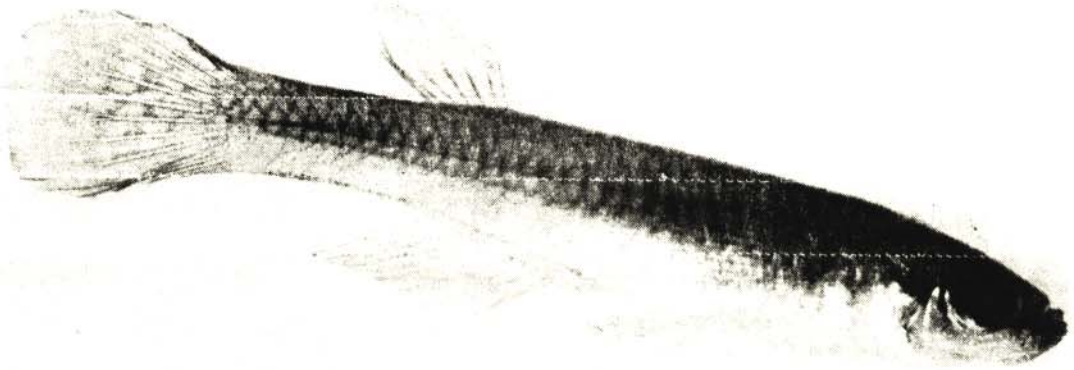


PHOTO 2. *APLOCHEILICHTHYS JOHNSTONII* (GÜNTHER, 1893)

CHAPTER 4

HABITAT AND DISTRIBUTION OF *A. JOHNSTONII* IN SOUTHERN AFRICA

4.1 DISTRIBUTION OF *A. JOHNSTONII* IN SOUTHERN AFRICA

Aplocheilichthys johnstonii has a wide distribution from Zambia, Zimbabwe, Caprivi, Kavango River, Botswana and Northern Transvaal to Northern Zululad (Fig. 3). The Transvaal lowveld is represented by one specimen collected from the Levubu River (Pienaar 1978) whereas the species is common in the Limpopo- and Incomati Rivers in Mocambique (Bell-cross 1976) as well as in the Pongola System in Northern Natal (Bruton and Kok, 1980). The latter authors report Maputaland as the southern distribution range of *A. johnstonii*. Despite fairly intensive collecting in the Eastern Transvaal lowveld, apart from the one specimen from the Levubu River, it does not appear to occur naturally in this region. Polling et al.(1983) did not find it in the Levubu subsystem and the present author has not collected it during malarial entomological surveys in the Eastern Transvaal Lowveld. Kleynhans (1984) has reported *A. johnstonii* from the Mogol, Polala and Marico Rivers in the North-western Transvaal (Fig.6) It is common in the Nyl River near Potgietersrus (24°12'S 28°59'E). Collections by the Lydenburg Fisheries Institute have been fairly extensive and together with collections done by the Zoology Dept, University of the North (Hecht and Mashego 1981, Hecht and Scholtz 1983, and Polling et al. 1983) would indicate that *A. johnstonii* is naturally restricted to the few river systems mentioned above. Where it does occur it is very abundant. Most of the rivers in the Eastern Transvaal Lowveld cease flowing during the dry months, but pools normally remain. With the increase in agricultural activities there has been a large increase in the number of dams in this area. Many of these dams provide

a suitable habitat for *A. johnstonii*. Altitude would not appear to be a limiting factor since it is found in the Kavango and also in the Lower Zambezi system at altitudes of 1 500 m in the former to 100m in the latter compared with the Transvaal lowveld attitudes of 300m to 600m. The Nyl River pans lie at an altitude of 850m.

4.2 HABITAT OF *A. JOHNSTONII* IN SOUTHERN AFRICA

The natural habitat is in shallow waters with associated aquatic vegetation e.g. *Phragmites sp.*, water grasses, both submerged and above surface.

At the Nyl Pans near Potgietersrus the depth of the water in which the fish were found varied according to the time of day. The majority of fish collected were in water of a depth between 10cm and 1,5m. Sampling was done at various depths and at various times of the day. Occasional fish were collected during the night at the surface of deeper water, but even then the majority was in shallow water. This supports the observations of Bruton (1979) in Maputoland. In the mornings from approximately 06h00 to 11h00 the fish were amongst the vegetation and not readily visible. The depth at which fish were caught (n=23) was between 10cm and 1,0m. From 11h00 to 17h00 the majority of *A. johnstonii* caught was at the periphery of the vegetation and was more readily visible (n=45). The depth of the water was between 1-1,5m. (Photos 3 and 4).

From 17h00 to 23h00 fish were mainly at the periphery of the vegetation but some fish were in open water with a depth greater than 2,0 metres. The fish collected in the open water (n=12/36) were all found just below the surface. The majority of fish caught during these hours (n=24/36) was in shallow water 10-20cm deep and this period seemed

to be that of maximal feeding.

During collecting expeditions in Kavango (Poppa, Kavango River 18°C3'S 20°30'E) and Botswana (Boteti River 20°10'S, 23°53'E) a similar depth distribution pattern was observed.

Water analysis was done at the Nyl Pans using a Hach water Ecology Kit (AL36B) for the chemical analysis and a Hach DrEl 4 for the physical analysis. The following results were obtained from three analyses during one year.

1. CHEMICAL ANALYSIS

pH	8,5
CO_2	15 mg/ℓ
hardness	156,5 mg/ℓ
dissolved O_2	7 mg/ℓ
temperature	24°C

2. PHYSICAL ANALYSIS

NO_3	11,0 mg/ℓ
NO_2	0,0132 mg/ℓ
NH_3	0,03 mg/ℓ
PO_4	0,38 mg/ℓ
SO_4	5,0 mg/ℓ
Cℓ	250 mg/ℓ

4.3 TOLERANCES OF *A. JOHNSTONII* TO ENVIRONMENTAL CONDITIONS

The pH tolerance of *A. johnstonii* has not been determined but this species would appear to tolerate the range to which it would be exposed in the Transvaal lowveld. Schutte and Frank (unpubl. data) sampled the Eland/Crocodile, White Crocodile, Sabie, Kaap-, Eastern Crocodile, Lemati and Komati Systems and found a pH range of 6,2-8,58. Most of the systems were between 6,5 and 8,0. Pienaar

(1978) found the pH of the K.N.P. waters to range between 7,6-8,3 except in the Sandveld areas where the water had a pH of 6,7. Jooste (unpublished report, 1982) reported a pH range of 6,52-8,3 in dams in the Northern Transvaal (Lebowa and Venda). The fish can tolerate a pH range of at least 7,0-8,5 and most fish were caught in waters with a pH of 8,0-8,5.

Aplocheilichthys johnstonii would also appear to tolerate a wide temperature range. Fish for this study were collected in waters with a temperature ranging from 21,5°C to 32,5°C. The higher temperatures have also been observed by Van der Waal in Caprivi (pers. comm.) whereas lower temperatures have been observed by Kleynhans (pers comm.) at Lydenburg where the surface temperature in winter drops below 0°C.

In Tzaneen it was discovered that most of the fish in an out-door plastic reservoir did not survive during the winter months. In laboratory experiments below 23°C the feeding of *A. johnstonii* decreased remarkably although to 21,5°C the adult fish tested (n=26) were not visibly affected other than the decreased feeding could play a role in the egg production capabilities of this species, since in the temperature controlled rooms (t=24$\pm$2°C) egg production was maintained throughout the year if food was available.

4.4 DISCUSSION OF DISTRIBUTION

The scattered distribution of *A. johnstonii* in the Transvaal cannot be attributed to pH, temperature or attitude. Although Pienaar (1978) remarks that *A. johnstonii* is only found at an altitude less than 300 metres it is certainly common also at

higher altitudes e.g. at Nyl Pan Potgietersrus (850 m) and the Kavango River, Kavango (altitude 1 500m) and has adapted to conditions at Lydenburg (1 373 m).

Since it has been possible to have and maintain a viable population of this species in the Transvaal Lowveld i.e. Tzaneen; Satara and Skukuza in the Kruger National Park, the natural distribution of this species to restricted localities may be due to geographical barriers.

Bell-Cross (1976) reported *A. johnstonii* from the Upper, Middle and Lower Zambezi system, although the Victoria Falls, Kariba Dam Wall and the Cabora Bassa Gorge provide barriers for this species. No such major barrier restricts its distribution in the Limpopo System and therefore other factors must be responsible. Two factors that may have a role is that, firstly, in the Eastern Lowveld only a few rivers are permanent throughout the year i.e. the Mutale-Levubu, Blyde-Olifants, Sabie and Crocodile Rivers. All the other rivers, including the Limpopo, dry up during the dry months. The Letaba River flows most years but during droughts it also ceases to flow. The second factor is that of salinity. During the dry months the few remaining pools in the Limpopo River become very saline and salt crystals precipitate out as the water evaporates. This is especially noticeable in the area where the Mogalakwena River joins the Limpopo River. *A. johnstonii* is not found in the latter stretch of the Mogalakwena River. The Mutale River can also reach high salinity levels during the dry months near its junction with the Levubu River. The distribution of *A. johnstonii* in the Transvaal is shown in Fig. 6.

4.5 PREDATORS OF *A. JOHNSTONII*

During collecting trips predation was observed, especially on young fish. Predators consisted of Ephemeroptera and Odonata larvae, *A. johnstonii* spp. in addition to larger fish of the same species. *Xenopus laevis* (Anura), *Serranochromis macrocephalus* (Van der Waal, 1976), *Glossogobius giuris*, *Ctenopoma multispinis*, *Clarias gariepinus*, *Haplochromis philander* as well as the spider *Thalassius spenceri* have all been found to prey on *Aplocheilichthys* in Northern Zululand (Bruton and Kok, 1980)

During the present investigation birds were not observed preying on *Aplocheilichthys* although it is reasonable to presume that birds such as *Ardea spp.*, *Scopus umbretta*, *Phalacrocorax* and the Alcedinidae would feed on the fish.

In the laboratory Ephemeroptera larvae were notable predators on fry, as was *Oreochromis mossambicus*. Pienaar (1978) does not consider *O. mossambicus* as a natural predator of small fish, being primarily an algal feeder, but laboratory observations revealed that it can be considered, at least, an incidental predator of *A. johnstonii*.

Hydra sp. did not consume small fish but the nematocysts killed small fish which then sank to the bottom of the aquaria. They did not appear to affect older fish.

Endoparasites of wild caught specimens were not studied although some specimens were observed with cestodan infections. However the fish did not appear to be unduly affected by these infections.

Dytiscidae beetles were placed in a tank with *A. johnstonii* juveniles and adults but no deleterious effects on either organisms were observed. It was

expected that the Dytiscidae would prey on immature fish.

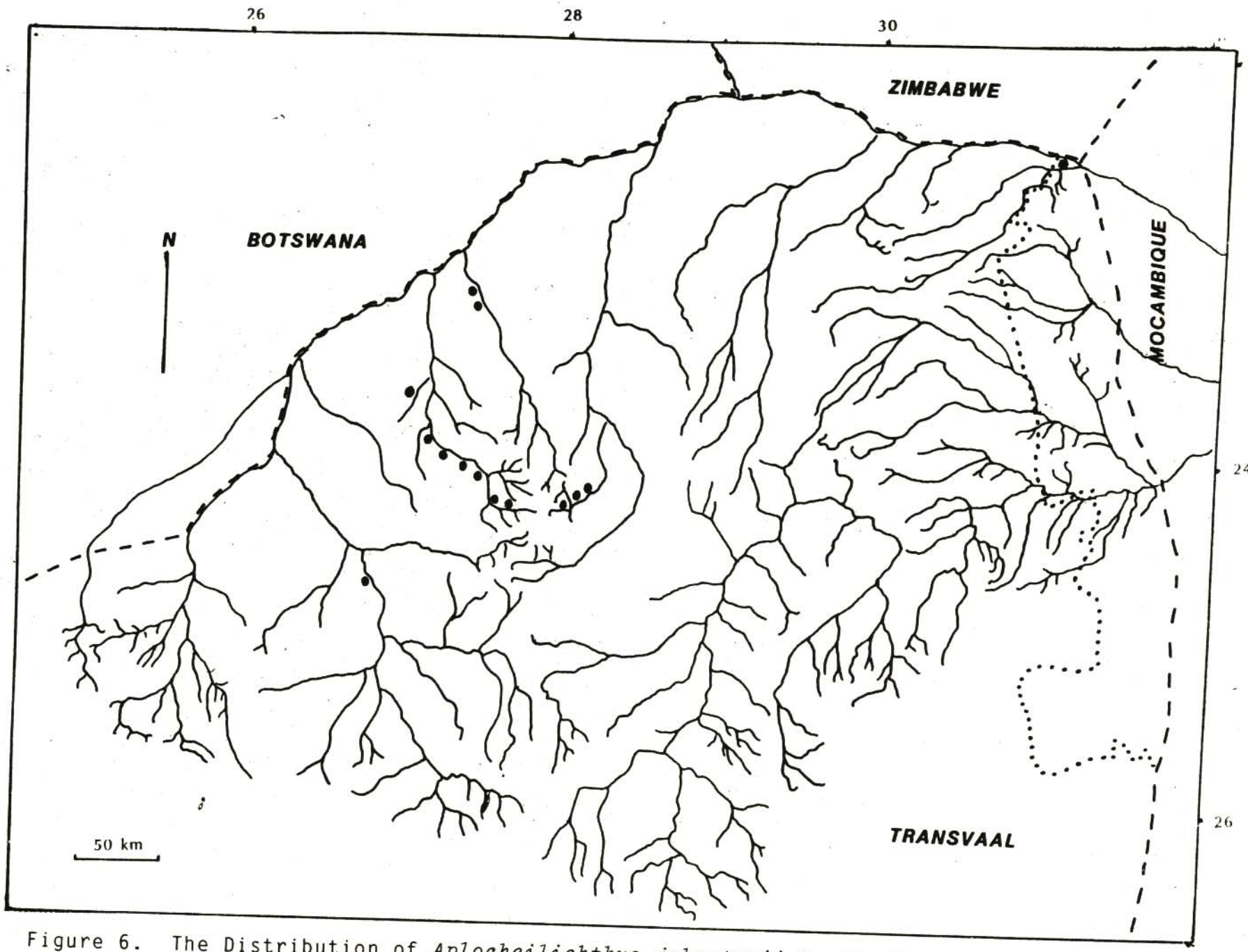


Figure 6. The Distribution of *Aplocheilichthys johnstonii* in the Transvaal

PHOTO 3. AN *A. JOHNSTONII* HABITAT

PHOTO 4. AN *A. JOHNSTONII* HABITAT IN KAVANGO, NAMIBIA

CHAPTER 5

BREEDING OF A. JOHNSTONII IN THE LABORATORY

CHAPTER 5

BREEDING OF *A. JOHNSTONII* IN THE LABORATORY

5.1 METHODS OF MASS BREEDING

As mentioned by Wright (1975) for *Aplocheilichthys katangae* it was found that *A. johnstonii* produced the best results if kept in small shoals in a moderate size aquarium (0.9 x 0.45 x 0.45m). Results are shown in Fig. 7. In an aquarium of this size the optimal stocking density appears to be of the order of 74 adult fish per cubic metre, with more females than males. Eggs laid in the early afternoon were eaten by adult fish if they were left on the mops. Eggs were laid singly in the thickest part of the mops and a mop with fifteen strands produced better results than a mop with eight strands. The colour of the mops did not appear to have a significant effect except for red. Green, brown, yellow, orange and red were tested and only on the red mops were no eggs laid. If the mops were suspended the green mops had the most eggs while when the mops were lying on the substrate the orange mops gave the best results. Up to 112 eggs were collected from the orange mop during one 60 hour period while 68 eggs were collected from a green mop during the same time. Two green mops were hung in the aquarium and one orange mop was allowed to lie on the substrate. It was important to keep the mops, especially the orange mop, clean and once a week the mops were washed with tap water to get rid of any algal growth or dirt particles stuck to them.

The eggs were removed from the mop strands by hand. Each egg had gentle pressure applied to it and some collapsed under this pressure. These eggs were probably infertile (Polling pers. comm.).

The remaining eggs were placed in a small enamel bowl. Each day the eggs were checked and any eggs whitish in colour were discarded. If unfertilised eggs were not removed from the bowl then within two to three days all the remaining eggs became whitish and none hatched. The average time for the eggs to hatch was 13.3 days (n=356) with a range from 10-18 days. (Table 3). The percentage of eggs hatched was 89.4% of those laid. (Photo 5).

Everyday the water in the bowls was agitated and when necessary topped up with aquarium water. The newly hatched fry were kept in the enamel bowls for three to four days before being transferred to an aquarium. (Photo 6). Very few "belly-sliders" were found.

It was essential to ensure that young fish were not placed in an aquarium with adult fish otherwise they were eaten. The newly hatched fish were fed on finely crushed dog pellets and "Tetramin" tropical fish food until they were big enough to feed on other foodstuffs. West's dog biscuits were used since it had been found that other Commercial dog pellets (Epol and Dogmor) had a higher oil content and an oily film formed on the surface of the water. The use of the West's product avoided this. The composition of the various brands of dog food is shown in Table 4.

There was a tendency to overfeed the fry, the excess food sinking to the bottom of the aquarium. This led to a fungal growth and the young fry succumbed. The problem was overcome by adding *Physa sp.* snails to the aquarium. The snails ate the excess food on the bottom of the tank.

The aquaria for the newly hatched fry were filled with borehole water which was then left for 24

hours before the fry were added. Pond water was not used since *Hydra sp.* were present in this water and these had been observed to kill small fry.

TABLE 3

DEVELOPMENT OF *A. JOHNSTONII* EGGS IN THE LABORATORY

DATE DEPOSITED	NUMBER OF EGGS DEPOSITED	NUMBER OF EGGS HATCHED	AVERAGE NUMBER OF DAYS TO HATCHING
10/83	175	167	12,2
11/83	83	74	12,8
12/83	22	8	15
01/84	34	30	14,8
02/84	53	51	15,5
03/84	6	4	14,8
25/08/84-01/01/85	25	22	16,0
	398	356	

5.2 THE BREEDING BIOLOGY OF *A. JOHNSTONII*

Fish would start laying eggs from the end of September/beginning of October and continue laying until early April in aquaria at ambient temperatures. This is slightly later than that observed for *A. katangae*. If the fish were fed solely on mosquito larvae egg laying ceased. It also appeared that if the fish were only fed on "Lopis" tropical fish flakes that egg laying was not maximal. The best success was achieved by feeding the fish on mosquito larvae together with *Daphnia sp.* and commercial fish flakes. Snails (*Physa sp.* and *Heliosoma sp.*)

TABLE 4 : THE DIETARY COMPOSITION OF VARIOUS BRANDS OF COMMERCIAL DOGFOOD.

	DOGMOR 1 KG	WESTS 1 KG	EPOL 1 KG
Protein mm	210g	200g	260g
Fat mm	50g	25g	80g
Linoleic acid	33g		20g
Fibre max.	50g	50g	50g
Moisture max.	100g	100g	100g
Calcium:			
Phosphorous min.	1,2:1	1,1:1	1,1:1
max.	1,5:1	1,5:1	1,5:1
Calcium	12g	12g	-
Phosphorous min.	10g	8g	9g
Potassium	6g	6g	-
Sodium chloride	10mg	10mg	-
Magnesium	500mg	500mg	-
Iron	60mg	60mg	60mg
Copper	6,5mg	6,5mg	6,5mg
Manganese	500mg	5mg	5mg
Zinc	50mg	50mg	75mg
Iodine	1,5mg	1mg	2mg
Selenium	0,1mg	0,1mg	0,1mg
Vitamin A	6000i.u.	6000i.u.	6000i.u.
Vitamin D	600 i.u.	600 i.u.	600 i.u.
Vitamin E	60 i.u.	60 i.u.	60 i.u.
Thiamine (B1)	2mg	2mg	1mg
Riboflavin (B2)	5mg	5mg	3mg
Pantothenic Acid	12mg	12mg	10mg
Niacin	15mg	15mg	15mg
Pyridoxine (B6)	1,3mg	1,3mg	1,0mg
Folic Acid	0,3mg	0,3	0,18mg
Biotin	0,1mg	0,1mg	0,1mg
Vit B12	0,03mg	0,03mg	0,022mg
Choline	1100,0mg	1100,0mg	1200mg
Cobalt -	0,5mg		

were introduced into the aquaria to consume any excess food left by the fish. As a result the tanks were maintained relatively clean.

Fish would start laying eggs at about ten weeks old and continue for up to two years. Egg production of very young fish and older fish was not as high as for young mature adults. The actual age at which fish would start laying could not be ascertained since it depended on the numbers of fish present in the tank as well as the male to female ratio. Certain of the older fish were recognisable by physical characteristics and it was possible to estimate the ages of these individuals.

Courtship was observed on ten separate occasions. No references in the literature survey could be found to the courtship display. Each time a male would pursue a female and present to her. The display appeared to consist of a lateral display with the body curved towards the female. The female never accepted the male immediately but invariably swam away. The male would pursue her, overtake her and present again. On the third to fifth presentation the female would approach a mop and the male would force her into the thickest part of the mop where one egg was laid. Both fish would then depart. On no occasion did the male show interest in another female during the display or immediately after a successful egg laying. No one male appeared to be dominant and several males were successful in their mating attempts to different females in the tank.

On two occasions males were rejected by females and on both occasions another female was solicited - again unsuccessfully.

On two occasions there appeared to be an attempted spawning in the muddy substrate of the aquarium, although no eggs were seen and these efforts were presumed to be unsuccessful. Once, courtship was successful and one egg was laid on a rock in the aquarium. This was the only time an egg was found on the rock. It appeared that once eggs were laid in a mop there would be a general increase in egg laying. It was not possible to verify this hypothesis due to such factors as:

1) The consumption of eggs by adult fish.
2) colour variation of mops i.e. were certain mops more "attractive"?
3) positioning of mops in the tanks
4) effect of diet available. etc.

If too many males were present then there was conspecific rivalry and the males would display aggressively to each other. If a male ceased to display to a female in order to chase away a younger male which had come close to the courting male the courtship was never successfully resumed, even if a full courtship display was again initiated rather than a resumption of the interrupted courtship. Tinbergen (1969) refers to this type of courtship as a "reaction chain" where each reaction is dependent on the preceding action. The stimuli may be visual, tactile or chemical. As in the three-spined stickleback *(Gasterosteus aculeatus)* the stimuli would appear to be firstly visual followed by a tactile stimulus for spawning.

5.3 REARING OF YOUNG FISH

Once young fish were large enough to escape being eaten by adult fish they were placed in aquaria with adult fish until 30 fish/aquarium were reached.

This was done to achieve a reasonable male to female ratio and to preclude the occurence of too many males.

Originally once the eggs had hatched the fry were transferred to a small aquarium. But a mortality of up to 50% was experienced in some hatches. Later the fry were kept in small enamel bowls for one week before being transferred to the aquaria and a much higher survival rate was achieved. The feeding of the fish fry presented a problem and the finely ground preparation was not entirely satisfactory. Better results were achieved by adding natural pool water once a week to the tanks - usually two litres. Especial care had to be taken that no *Hydra spp.* or other possible predators were accidentally introduced. With these methods a survival rate of 80% was achieved.

It was also important to give more of the finely ground preparation than was necessary for *A. katangae*. Thus it can be assumed that young *A. johnstonii* feed more than *A. katangae*. *A. johnstonii* also had a more rapid growth rate and at six weeks the first adult colouration started appearing, although no eggs were laid. It was important to remove fish from the aquaria at this stage as they would consume any young fish introduced to the tank. Not all the fish developed at the same rate - some would only start assuming adult colouration at 10-12 weeks.

The younger fish would stay close to protective vegetation and nearer the surface when compared with larger juveniles. Also the smaller fish tended to feed by darting from the shelter to consume their prey - whereas the larger fish tended to remain longer in the unprotected areas

of the aquarium. This behaviour was also observed in the Kavango River flood plain in Kavango/Namibia. *A. johnstonii* were always found close to vegetation and would only occasionally dart across open stretches of water to reach the next clump of vegetation. If disturbed they hid in the vegetation, while if they felt secure they would be found on the finge of the patches of vegetation. The Kavango River tends to flood its banks in March/April and during two visits to the northern area at this time many young were seen and caught in the vegetation.

Fig. 7. No of Eggs Collected from *A. johnstonii* in a constant environment in relation to adult fish present (approximately IM:IF)

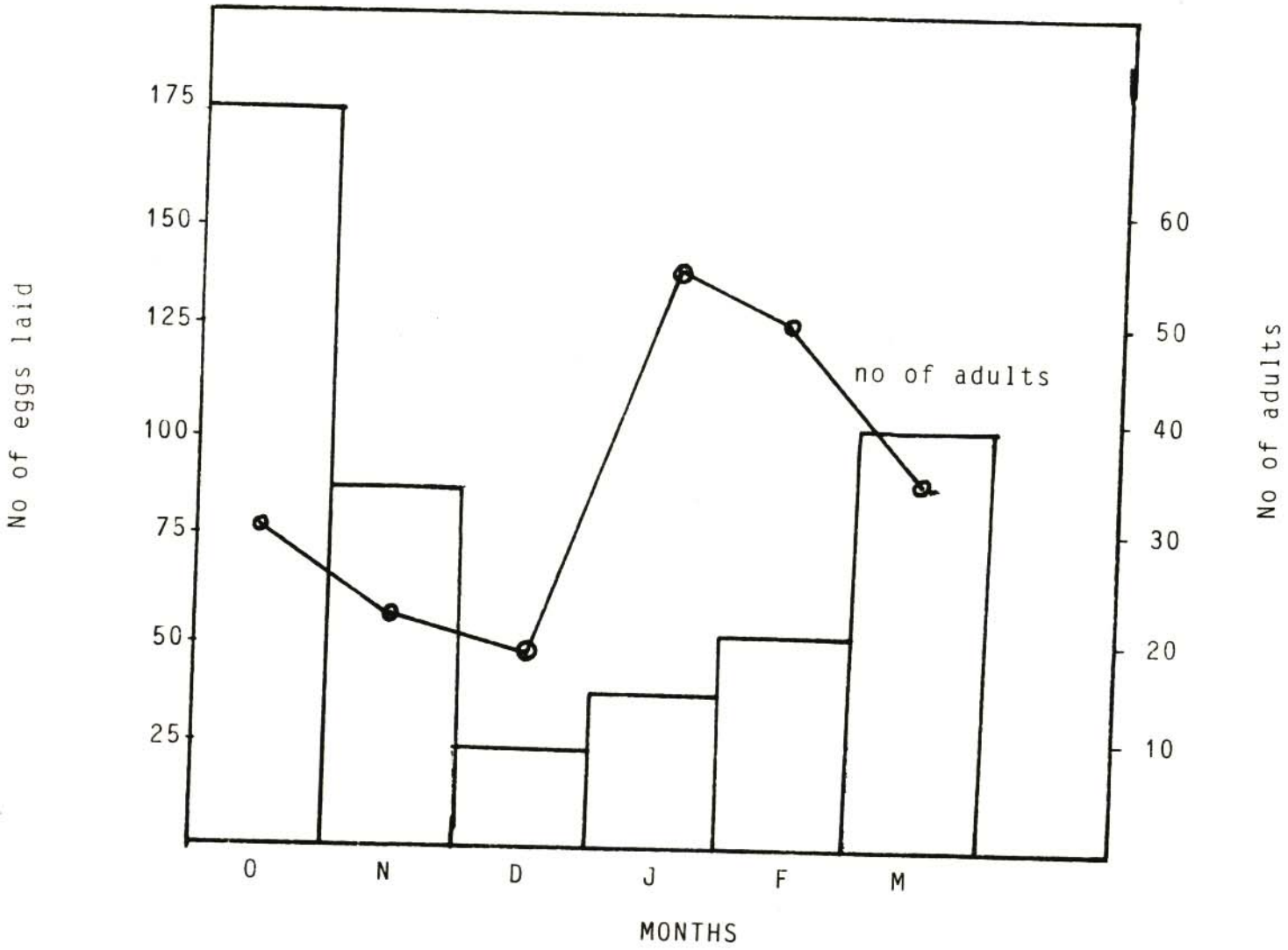


PHOTO 5. ENAMEL BOWLS USED FOR EGG HATCHING OF *A. JOHNSTONII*.

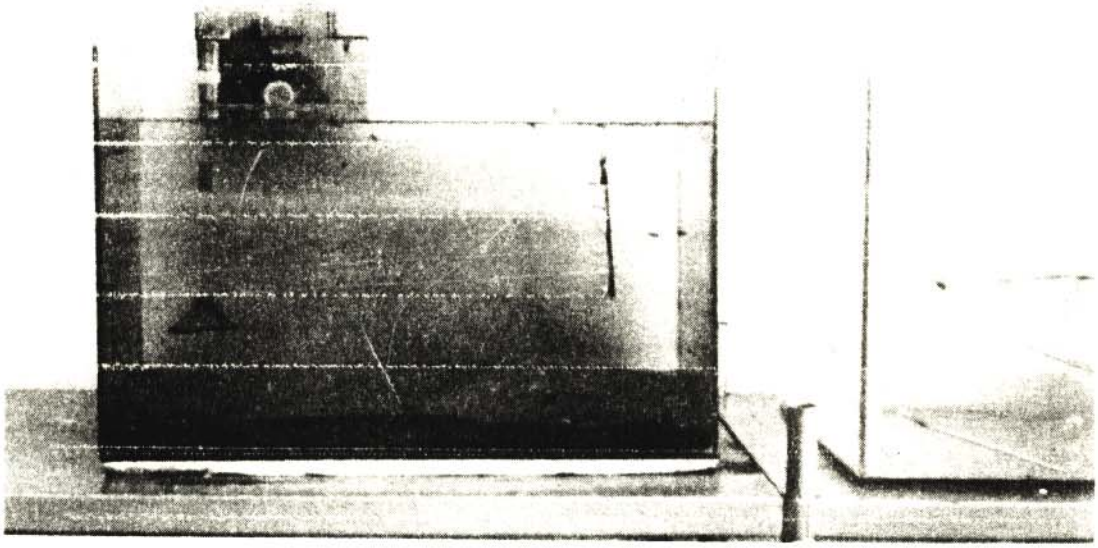


PHOTO 6. AQUARIUM USED FOR *A. JOHNSTONII* HATCHLINGS

CHAPTER 6

FEEDING PREFERENCES OF A. JOHNSTONII IN THE LABORATORY

CHAPTER 6

FEEDING PREFERENCES OF *APLOCHEILICHTHYS JOHNSTONII* IN THE LABORATORY

6.1 INTRODUCTION

In several books *A. johnstonii* is referred to as feeding on mosquito larvae (Jubb, 1967, Bell-Cross 1976, de V. Pienaar 1978 and Bruton, Jackson and Skelton 1982). However a comprehensive literature survey revealed that there are virtually no references to the diet of members of the genus *Aplocheilichthys*, let alone to *A. johnstonii*(Gerberich and Laird 1966, Gerberich 1985, Gerberich and Laird 1985). *A. pumilus* will feed on mosquito larvae in the laboratory (Wildekamp, 1980).Bruton (1979) reported that *A. myaposae* feeds on aquatic larvae and the Cladocera, *Bosmina longirostris*. It would appear that it has been assumed that since *Aplocheilichthys* are Cyprinodontidae they have similar diets to other members of the family eg. *Aphanius*, *Aplocheilus* and especially the Poeciliidae *Gambusia affinis* and *Poecilia reticulata*. Kleynhans (pers. comm.) as well as Kruger (pers. comm.) observed *Aplocheilichthys katangae* and *A. johnstonii* voraciously feeding on culicine mosquito larvae in an aquarium at the Lydenburg Fisheries Station. Because of these particular observations it was decided to investigate the feeding preferences of *A. johnstonii* and also to endeavour to establish whether a population of fish could be maintained in the complete absence of mosquito larvae as a food item.

6.2 SIZE AND AGE OF FISH VS. SIZE AND INSTAR OF MOSQUITO LARVAE TAKEN.

Young fish, less than 1.5cm in length were not

observed to eat any larvae. They were fed on *Daphnia spp.*, *Hydracarina* and finely ground "Tetramin" Tropical fish food. When they reached 1.5cm the fish began to feed on mosquito larvae. The younger fish ate first to third instars but did not eat fourth instar or pupae. Pupae were observed to be chased but were too large to be swallowed.

3cm. fish were eating all stages of larvae but were not eating the pupae. However they did not eat many fourth instar larvae.

Of the larvae and pupae tested the anopheline pupae (mainly *An. arabiensis)* were smaller than the culicine larvae *(Cx. tigripes, Cx. theilleri, Cx.quinquefasciatus)* and of the same size as *Ae. aegypti* pupae. The *An. arabiensis* larvae were smaller than *An. coustani* larvae and most of the culicine larvae tested.

From 3.5cm. the fish were feeding on all stages of larvae and also on pupae, although they were choosing larvae in preference to pupae.

It was noted that all fish would investigate culicine egg rafts and were observed to feed on newly hatched mosquito larvae. Although this type of behaviour would be effective in reducing rapidlyaculicine population it would have little effect on the anopheline population whose eggs are laid singly on the surface of the water.

Occasionally in the laboratory *Ae. aegypti* larvae are seen to congregate and form a "swarm" - this has not been seen in *An. arabiensis* larvae in the laboratory or *An. quadriannulatus* in the field. If such a behaviour had been observed it would have made the larvae more liable to be devoured

All stages of fish were feeding on the surface. Young fish (less than 3.5cm.) tended to feed almost exclusively on the surface and to stay closer to vegetation. This behaviour has also been seen in the Kavango River where the smaller fish stay close to vegetation. Adult fish, although primarily surface feeders, would pursue a diving larva and consume it. Feeding on the substrate was seen but was uncommon. Due to the position of the mouth of *Aplocheilichthys* the fish must be almost vertical to the substrate to feed on the larvae.

6.3 GENERA AND SPECIES OF MOSQUITO LARVAE TAKEN

For the feeding trials two well-established laboratory colonies of mosquitoes were available from the National Institute for Tropical Diseases, Tzaneen. These are *An. arabiensis* and *Ae. aegypti*. Thus, they were used principally for feeding trials. Several outdoor snail tanks were regularly checked for mosquito larvae. These were seeded with *Daphnia sp.* which provided the source of food and also several species of mosquito larvae eg. *An. coustani, Culex tigripes, Cx. theilleri* and *Cx. quinquefasciatus*. The latter species were from a heavily polluted tank and were also obtained from sedimentation tanks at the Nkowankowa sewage works. *An. pretoriensis, An. listeri* and *An. quadriannulatus* larvae which had been collected in the field were also used.

Using the wild caught larve it was possible to study preferences by the fish for the above species but the larval instars could only by investigated using the laboratory colonies.

6.3.1 METHODS

Before any larvae were introduced for a feeding

trial the fish to be tested were first fed with "Tetramin" fish food and *Ae. aegypti* larvae. After two hours the food source to be tested was introduced. It was attempted to introduce 100 larvae of each species or instar, although it was difficult to catch this precise number of mosquito larvae. The food to be tested was given to the fish a maximum of twice a week. Twenty replications of most experiments were done. However it was not possible to do twenty replications using *An. coustani* and *An. listeri*.

Originally the fish were tested both in the morning and in the afternoon but in the laboratory no difference in feeding behaviour was seen and therefore the trials were standardised on the morning schedule for convenience.

Fish were left in one tank and the food source introduced to the tank. By this method, rather than introducing the fish to the food supply, it was hoped to eliminate any stress effects on the fish. While the fish were feeding the observer sat still approximately three feet from the aquaria. No one was allowed to enter the room during a trial to limit external influences on the trials. Eight adult fish were used for preferential trials. For the younger fish eight to twenty were used. This was due to a lack of aquaria.

6.3.2 RESULTS AND DISCUSSION

Eight adult fish consumed approximately 100 *Aedes aegypti* larvae during a twenty four hour period. Initially there was an increase in feeding when the larvae were added which then decreased after approximately thirty minutes. In the labo-

ratory feeding occurred throughout the twenty four hours. Fourth instar larvae took longer to consume than earlier instars.

Culicine larvae were observed to hang motionless from the infraneuston more so than the *Ae. aegypti* larvae. After the initial feeding the fish tended to ignore these motionless larvae and only be attracted to the movement of the larvae. It was obvious that *A. johnstonii* is attracted to the movement of prey. It could not be ascertained whether the fish were more attracted to specific types of movement e.g. the "wriggling" movement of mosquito larvae rather than the jerking movement of Crustacea etc. Chaoboridae (phantom midges) have almost transparent aquatic larvae and, when collected in the field, were added to the fish aquaria. They were not eaten, thus it was presumed that the primary motivation for prey searching was visual, since their movement is similar to the jerky movement of mosquito pupae.

Ae. aegypti larvae tended to be more active than anopheline larvae and were more readily eaten. The genera of mosquito larvae eaten were, in order of preference, *Ae. aegypti, Anopheles arabiensis, Culex spp.* Fig. 8 illustrates the average time for 12 separate experiments and lastly using 100 fourth instar larvae in each case. More than 50% of the larvae were consumed within the first four hours.

Of the Culicines tested *Culex (Lutzia) tigripes* was the least preyed upon. It was the most passive of the culicines and tends to lie more parallel to the surface of the water than other culicines. It is also a large larva and smaller fish would not consume a fourth instar larva.

There was no significant difference between *C. quinquefasciatus* and *C. theilleri*.

Sih (1986) has compared antipredator responses by *C. pipiens* and *Ae. aegypti* and has suggested that *C. pipiens* has evolved an antipredator response to *Notonecta undulata*. This response includes factors such as being less mobile, and changing the utilisation of a particular microhabitat. This phenomenon of being less mobile could be what was also observed when fish were present.

Thus the preference of *Aplocheilichthys johnstonii* for *Ae. aegypti* to anopheline larvae or culicine larvae could be due to the lack of a predator response by *Ae. aegypti* rather than a positive preference by the fish. This, as suggested by Sih, could be because in their natural environment the predator would occupy a different habitat from the prey. The anophelines tested occupy either permanent waters or, in the case of *An. arabiensis*, both temporary and permanent waters and are exposed to the natural predatory habit of fish. There was no difference between *An. arabiensis*, *An. coustani*, *An. listeri* and *An. pretoriensis* which was observable on the replicates done (*An. listeri* - 3 replicates, *An. pretoriensis* - 2 replicates).

Observations on feeding behaviour made during the present study compare closely with those of Romand (1985) for *Aplocheilichthys normani* from Senegal. An adult pair of fish averaged 103.8 ± 24.6 larvae per day and eight adult fish averaged 101 ± 32 larvae per day. Pupae were also not taken for preference (20.9%). Romand also reported that *Aedes* were preferred to *Culex* larvae (57.3% vs. 42.7%) and when anophe-

lines were included the proportions were *Aedes: Anopheles : Culex ::* 46.4 : 35.7 : 17.9. He also comments on *Aedes* being more active than either culicine or anopheline larvae, thus his results are similar to those observed for *A. johsntonii* during the present series of experiments.

6.4 OTHER INSECTS TAKEN

Other aquatic insects collected in the field were placed in the aquaria and were classified as either eaten or not eaten on visual observations only. Since the supply of these aquatic insects was erratic it was not possible to estimate total numbers eaten. All aquatic insects were placed in the aquaria at the same time as *Aedes aegypti* larvae. In no cases were the other insects eaten in preference to the mosquito larvae. Table 5 summarises the results of these observations.

TABLE 5 INSECTS TESTED FOR PREDATION BY *A. JOHNSTONII* IN THE LABORATORY

	EATEN	NOT EATEN
Ephemeroptera	Baetidae	
Odonata	Zygoptera	
	Anisoptera	
Hemiptera	Gerridae	Notonectidae
	Corixidae	Belastomatidae
Coleoptera	Dytiscidae larvae	Dytiscidae adult
	Gyrinidae	
Diptera	Ceratopogonidae	Chaoboridae
	Culicidae	
	Chironomidae	

Only small Anisoptera were eaten. Larger specimens were ignored and eventually adults emerged. It had been expected that Notonectidae would be eaten since this was observed in the field but did not occur in the laboratory.

On three occasions fish were seen attempting to catch adult female *Aedes aegypti* mosquitoes which were trying to lay eggs on the surface of the aquaria. Dead adult female mosquitoes were placed on the surface of the water of the aquaria and were consumed.

6.5 ORGANISMS OTHER THAN INSECTS CONSUMED

Other organisms collected in the field were brought back to the laboratory and, as for insects, added to the aquaria and were also classified on visual observations as either being eaten or not. *Aedes aegypti* larvae were always added prior to the other organisms. As for the insects, none of these were eaten in preference to mosquito larvae. The most readily eaten were Crustacea.

The following organisms were consumed by the fish in the laboratory:

Crustacea	: Branchiopoda	: Cladocera (*Daphnia*)
Crustacea	: Branchiopoda	: Anostraca (brine shrimps)
Crustacea	: Arachnida	: Acarina (Hydracarina)
	Gastropoda	: Pulmonata (eggs only)

The following were not eaten, some multiplied in the aquaria:

Cnidaria : Hydrozoa (*Hydra*)
Platyhelminthes : Turbellaria (flat worms)
Nematoda (wire worms)
Annelida : Hirudinea (leeches)

Amphibia : Anura (eggs only)

Of the organisms eaten the Cladocera were the most readily consumed. It was not possible to count directly the number of snail eggs since they were deposited on aquatic vegetation. Therefore a series of experiments, with controls, was set up to compare results.

In the control tanks in which snails had been placed a rapid population explosion occurred. In the tanks where fish were present the population increase was not so marked. The snails used were *Lymnaea, Heliosoma, Physopsis* and *Biomphalaria.* In no case was the snail population eradicated and on every occasion population increased with time. There was always surplus food available to the snails. Thus *A. johnstonii* although consuming snail eggs to a certain extent, does not do enough to be used as a means for the successful control of snail populations.

Brine shrimps had been suggested as an alternative food source (Vermaak pers. comm.) but great variability was experienced in the hatching success of various varieties. The eggs were readily eaten by the fish as were hatchlings.

Of the organisms not eaten the Hydrozoa increased in number and were capable even of killing small fish. The aquaria had to be checked periodically and any Hydrozoa present removed. The *Hydra* were brought in from an outside pond when the aquaria were being topped with fresh water.

Platyhelminthes were also occasionally found in the pool water and although they were added to aquaria it was not expected that they would be

eaten as their form and behaviour precluded ready consumption by the *A. johnstonii* as they remain benthic.

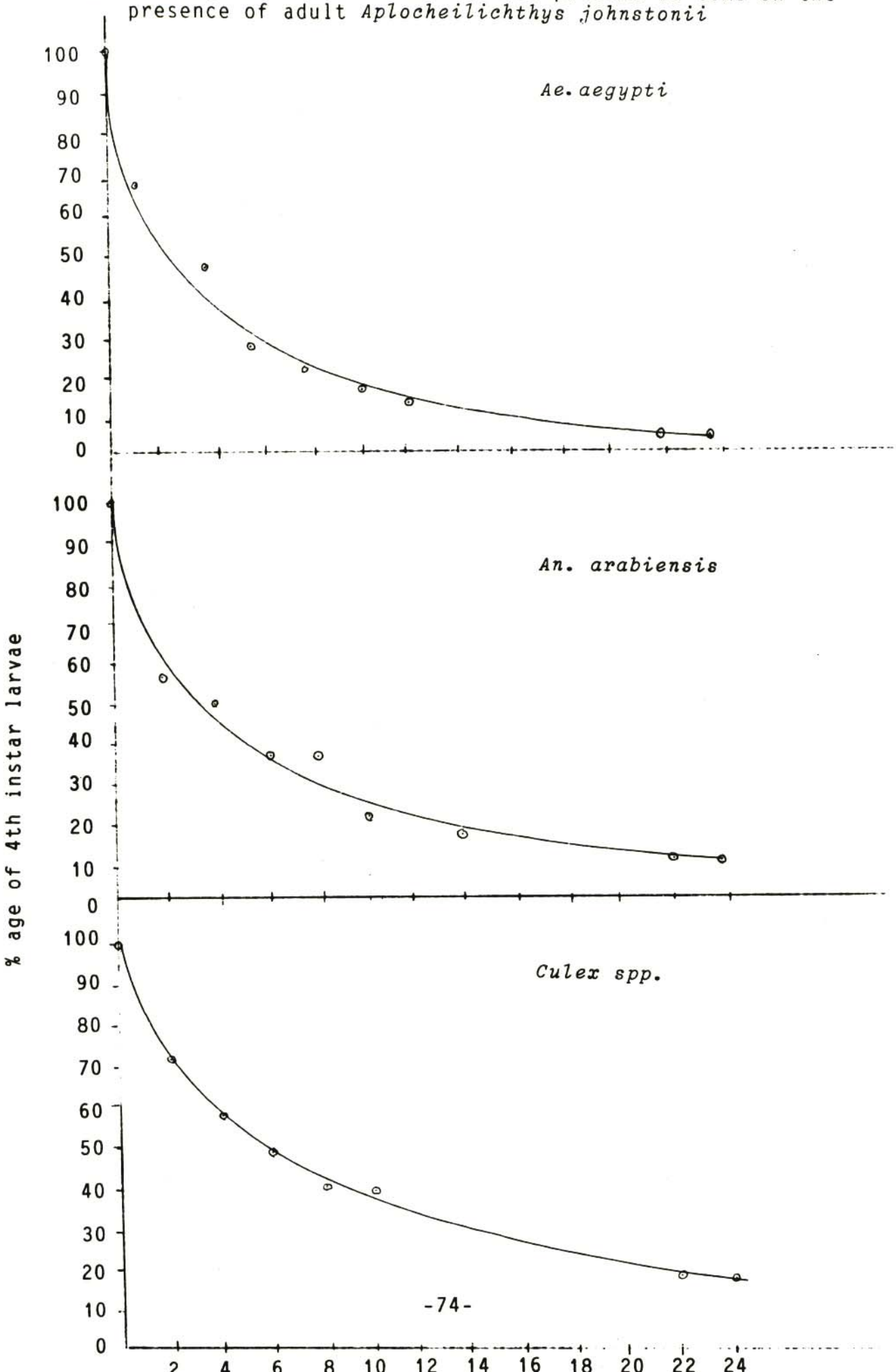
No Hirudinea were seen to be consumed although it had been expected that their movement would have elicited some response from the fish. The leeches tested came from *Daphnia* ponds seeded with guinea pig faeces. The leeches did not attach to the fish and were never observed to feed.

Freeliving nematodes are often found in local water bodies. These were added to the aquaria but did not attract the fish. Amphibian eggs of *Bufo gutteralis* and *Rana angolensis* were collected and put at the disposal of the fish. It was observed that these Amphibian eggs were occasionally taken into the buccal cavity. However the fish never attempted to swallow them but spat them out. All the eggs placed in the aquaria hatched and the tadpoles were not removed. They were never preyed upon by the fish.

A few of the fish which died naturally in the aquaria were dissected to observe gut contents. Algae were present in all the guts where contents could be identified. Mosquito larvae were also present. Of ten fish dissected six had organisms present but two were unidentifiable and four had empty guts.

Thus it can be concluded that in the laboratory *Aplocheilichthys johnstonii* prefers to eat mosquito larvae, an alternative food source would be Cladocera. Insects were the most common prey and other organisms which would be encountered in the shallow waters were not so readily, if at all, consumed.

FIG. 8 % age of fourth instar larvae present vs time in the presence of adult *Aplocheilichthys johnstonii*



CHAPTER 7

FEEDING PREFERENCES OF A. JOHNSTONII IN THE FIELD

CHAPTER 7

FEEDING PREFERENCES OF *APLOCHEILICHTHYS JOHNSTONII* IN THE FIELD

7.1 INTRODUCTION

With the experience gained from the observations in the feeding behaviour and feeding preferences of *A. johnstonii* in the laboratory, it was decided to investigate the diet of the fish in a natural environment by means of gut content analysis. It is probable that the feeding preference of an organism is totally dependent on the presence of a particular prey. Absence of the prey being studied may be due to two factors, either the habitat is not suitable due to chemical or physical reasons etc., or the predator has decimated the population. The only way the latter situation can be ascertained is by removing the predator from the environment, which in many cases is impossible.

Gut content analysis of fish from a natural environment has certain inherent problems and difficulties. These include the following:

1) The time of collecting the fish compared with the peak feeding activity.

2) A bias that occurs in the person analysing the gut content; for example an entomologist can easily identify Coleoptera or Hymenoptera on the presence of a leg only, whereas a botanist could identify plant seeds much more easily.

3) Insecta exoskeletons are not readily digested are therefore found more often in the intestine than soft-bodied prey which are more rapidly digested.

4) Since many insects are classified according to morphological characters in the exoskeleton the identification of this group of organisms is easier than for some other groups.

Bruton (1979) recorded the main food of both *Aplocheilichthys myaposae* and *A. katangae* at Lake Sibaya as *Bosmina longirostris* and for *A. myaposae* also "aquatic insect larvae". In the same table other fish species are reported as feeding on Chironomidae, Odonata etc. This is the only record of what *Aplocheilichthys* feeds on in the Lake Sibaya habitats. Polling (1982) reported that *A. johnstonii* in the Northern Transvaal near Potgietersrus was feeding on small plant seeds, Coleoptera and Diptera, especially "midge" larvae (presumably Chironomidae). Romand (1985) reported that *A. normani* from West Africa was feeding primarily on Cladocera, Chironomidae and plants. He also commented that there was a variation between the diets of two populations. Also the absence of Culicidae larvae was noted and it was shown that *A. normani* has similar feeding preferences in the laboratory as was found during the present study for *A. johnstonii* (Chapter VI).

7.2 MATERIALS AND METHODS

Gut content analyses were made of fish from the following places

a) Nyl Pan, Potgietersrus, Transvaal
b) Chinchimanic, Lake Liambezi, E. Caprivi.

9 specimens were examined from Chinchimanic of which 7 had identifiable gut contents. 14 specimens were examined from Nyl Pan of which 11 had identifiable gut contents. The occurrence of organisms in the gut of the fish was calculated

as well as the frequency of the particular item in the gut content.

Statistical analysis was carried out in the following manner:

a) Occurence was calculated by expressing the number of prey in a particular category as a percentage in relation to the total prey identified.

b) The frequency index was calculated by expressing the ratio between the number, n, of fish whose gut contents contained a particular prey, and the number, N, of intestines studied. This can be expressed by the formula
$f=n/N$

This numerical analysis was done so as to permit comparison of the results of this study with those of Romand (1985).

Some fish were preserved in alcohol and some in 10% formalin. The guts of the fish preserved in formalin were brittle and the contents more liable to break up during dissection than those preserved in alcohol.

7.3 RESULTS

Organisms identified in the guts of the fish, the numbers caught, as well as the occurrence and frequency index are shown in Table 6. Pie diagrams of the diet composition of the two populations are shown in Figures 9 and 10.

No distinction was made between male and female fish. Fish from the Nyl River were col-

TABLE 6 DIET COMPOSITION OF TWO POPULATIONS OF *APLOCHEILICHTHYS JOHNSTONII*

POPULATION METHOD OF ANALYSIS	TOTAL NO.	OCCUR-RENCE	FRE-QUENCY	TOTAL NO.	OCCUR-RENCE	FREQUENCY
Chironomidae larvae	10	4,83	0,33	0	0	
adult	18	8,7	0,56	27	13,78	0,5
Culicidae larvae	78	37,68	0,44	1	0,51	0,07
adult	3	1,45	0,11	0	0	
Cyclorrhapha	0	0		2	1,02	0,14
Coleoptera spp larvae	0	0		1	0,51	0,07
adult	1	0,48	0,11	3	1,53	0,14
Odonata/ Ephemeroptera	3	1,45	0,22	1	0,51	0,07
Collembola	3	1,45	0,11	13	1,53	0,36
Hydracarina	1	0,48	0,11	4	2,04	0,21
Gerridae	2	0,97	0,11	0	0	
Gyrinidae	0	0		2	1,02	0,07
Hymenoptera (Formicidae)	2	0,97	0,22	5	2,55	0,14
Gryllidae	0	0		2	1,02	0,07
Cladocera	0	0		1	0,58	0,07
Notonectidae	1	0,48	0,11	1	0,51	0,07
Arachnida	1	0,48	0,11	0		
unidentified insect sp.	4	1,93	0,22	0		
filamentous algae	67	32,37	0,22	60	30,56	0,07
plant seeds	9	4,35	0,22	68	34,64	0,5
plant remnants	4	1,93	0,44	5	2,55	0,21
TOTAL	207			196		

lected at various times of the day whereas those from Chinchimanic were collected between 1400 and 1600 hours.

A t - test was calculated on those organisms common to the guts of the fish from both localities. The hypothesis was that there was a significant difference in the diet of the fish from the two localities. The following results were obtained:

degrees of freedom = 10
t - test = -0.0097
p 0.005

Thus there is no significant difference in the diet of the two populations.

7.4 DISCUSSION

The differences between the diet of the two populations may be explained by differences in food availability. Adult mcsquito collections were done at Chinchimanic and at the Nyl pans. These were achieved by means of ultra-violet light traps and man-baited trap nets. In one night at Chinchimanic 996 *Anopheles wellcomei* and 2 *An. arabiensis* as well as large numbers of *Mansonia africanus* and *M. uniformis* were caught. At the Nyl pans *M. africanus* and *M. uniformis* were also present in large numbers and the only anophelines caught were five specimens of *An. coustani*. *Culex spp.* were not found but this may have been due to the collection methods used. Since *Mansonia spp.* larvae insert the air siphon into plant stems and do not come to the surface to breathe and they look like rootlet hairs on the plant stems it is probable they are

not easily preyed upon by the fish.

The Chironomid adults consumed all had undeveloped wings. When the adults emerge from the pupae the wings must dry and during this time the Chironomid imago rests on the pupal skin on the surface of the water and thus is susceptible to attack by surface feeders.

In the Nyl pans there was more vegetation on the edge of the water than at Chinchimanic and this could explain the larger number of terrestrial organisms. If a terrestrial organism falls on the surface of the water the fish will possibly eat it thus explaining the presence of Hymenoptera, Gryllidae and vegetable matter such as seeds and parts of plants.

As mentioned in Chapter 2. fish were found in the shallow water and maximum feeding occurred between 1700-2300 hours. This is the time when imago mosquitoes and midges are emerging from pupae (personal observations) as when adult Nematocera lay their eggs on the surface of the water.

There is a large diversity in the diet of the fish examined and this is advantageous when considering *A. johnstonii* for biological control of mosquito larvae in that the fish are not dependent on particular food sources for survival and reproduction in natural habitats.

7.5 CONCLUSION

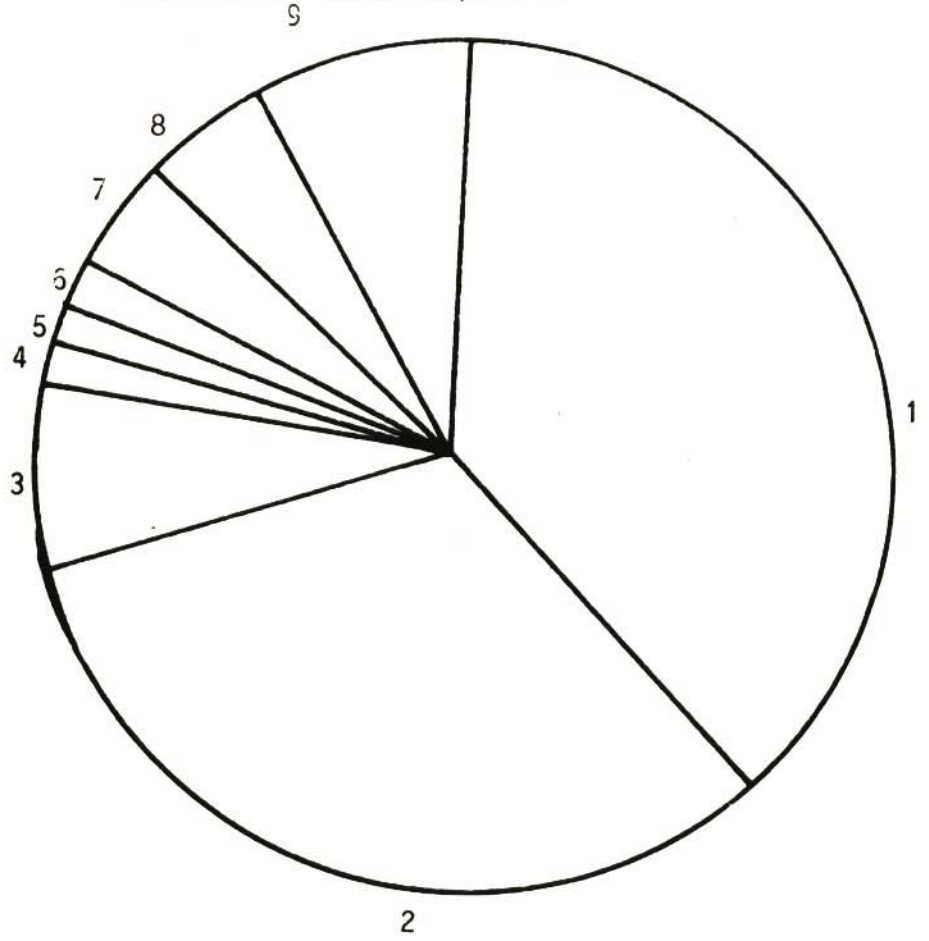
In the two populations studied a wide diversity of organisms was found in the gut contents. *A. johnstonii* appears to be an opportunistic

feeder since both terrestrial and aquatic were consumed.

Aplocheilichthys can survive in natural habitats in the absence of Culicidae larvae which is an important factor when considering them as biological control agents.

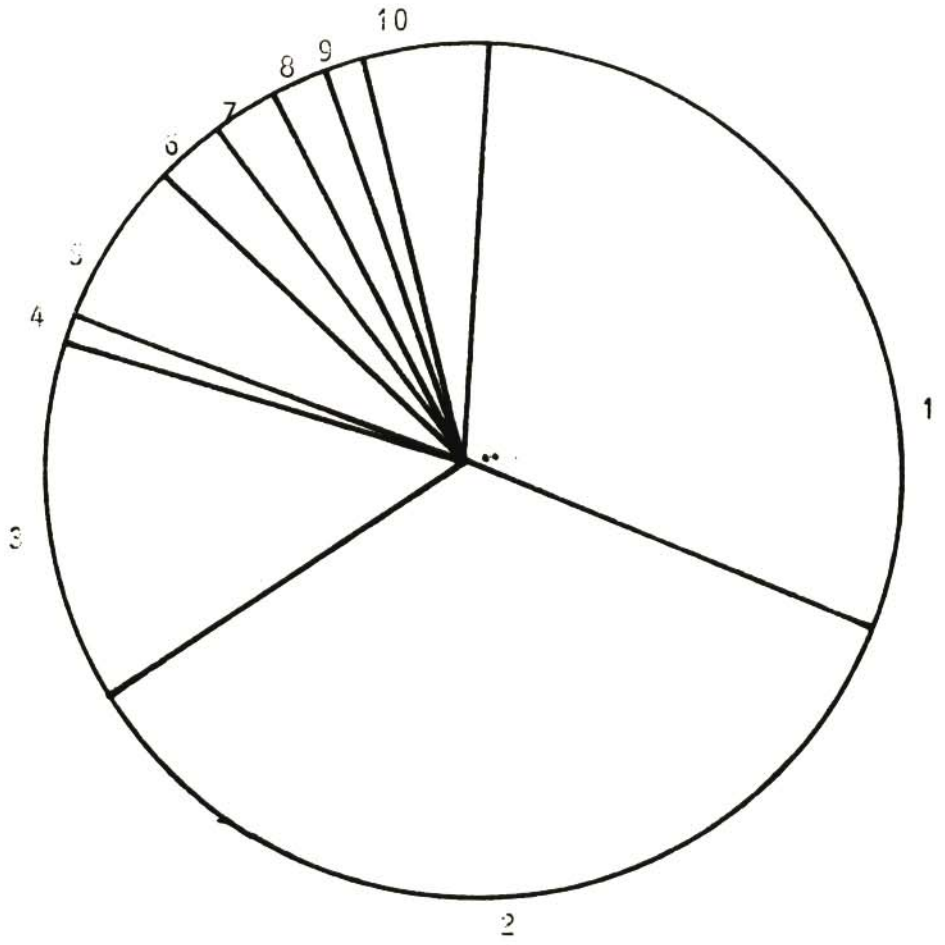
Since there was no statistically significant difference in the diet of the two populations studied from widely separated localities, it can be postulated that the diet of the species will also not vary in the populations of other localities provided the aquatic environments are comparable.

Fig. 9. Gut Content Analysis of *Ap. johnstonii* from *Chinchimanic*, East Caprivi.



1.	Culicidae larvae	47,68
2.	filamentous algae	32,37
3.	others	7,72
4.	Collembola	1,45
5.	Odonata	1,45
6.	Culicidae adults	1,45
7.	plant seeds	4,35
8.	Chironomidae larvae	4,83
9.	Chironomidae adults	8,70

Fig. 10. Gut content analysis of *Ap johnstonii* from the Nyl Riv near Potgietersurs, Northern Transvaal.



1.	Filamentous Algae	30,56
2.	plant seeds	34,64
3.	Chironomidae adults	13,78
4.	Culiadae larvae	1,84
5.	Collembola	6,63
6.	grass	2,55
7.	Hymenoptera	2,55
8.	Hydroacarina	2,04
9.	Coleoptera adults	1,53
10.	Others	3,88

CHAPTER 8

SENSITIVITY OF A. JOHNSTONI TO INSECTICIDES IN AN INTEGRATED CONTROL PROGRAMME

CHAPTER 8

THE SENSITIVITY OF *A. JOHNSTONII* TO INSECTICIDES IN AN INTEGRATED CONTROL PROGRAMME

8.1 INTRODUCTION

Malaria control in South Africa is primarily aimed at insecticidal control of vector mosquitoes. Adult mosquitoes are controlled by intra-domicillary spraying with residual insecticides. The insecticide used mainly is D.D.T., especially in the traditional houses of the black population. More westernised type structures are sprayed with alternative insecticides eg. Fenitrothion, Bendiocarb and Baythroid.

The formulations used for the insecticides depend on the surface to which they are applied. When possible, wettable powder formulations (w.p.) are used since these give the longest residual action. Thus D.D.T. 75% w.p., Bendiocarb 80% w.p. and Baythroid 50% w.p. are the formulations routinely used. A Fenitrothion 60% emulsifiable concentrate (e.c.) has been used by the Department of Health in the Transvaal but is being phased out in favour of newer, more acceptable insecticides such as those mentioned above.

Most insecticides are not readily soluble in water and solvents are utilized in the formulations to suspend or dissolve the active ingredients in the aqueous solution. These solvents may have an effect in contributing to the toxicity of the products being tested and should themselves be tested (Mulla et al., 1981) Toxicity trials normally involve disolving the insecticide active ingredient in ethanol (Rongsriyam et al., 1968, Mathis and Pant, 1972 and Shim & Self, 1972) or acetone

(Denison et al.1985). This does not necessarily give results applicable to practical usage but merely a comparison of the active ingredients under laboratory conditions.

The efficacy of an insecticide often varies considerably with different formulations eg. D.D.T. 75% w.p. applied to a mud surface at a dosage of 2 gm a.i./m^2 gives a residual effect of at least eighteen months whereas DDT 5% in Kerosene at a dosage of 4.86gm a.i./m2 was ineffective at one month against adult *Aedes aegypti* mosquitoes using the standard W.H.O. bio-assay technique authors (unpubl. data).

To a lesser extent in South Africa insecticides are applied as larvicides, normally in densely populated areas where intradomicillary spraying is logistically impractical. The recommended insecticide is temephos (Abate) with newer insecticides recently becoming available eg. methoprene, an insect growth regulator and *Bacillus thuringiensis var. israelensis* a microbial insecticide (Ware 1983). The latter insecticides have been applied overseas for larval control but are not yet in use in South Africa for this purpose.

Chemical insecticides can be grouped into four categories i.e.

1. Chlorinated hydrocarbons eg. DDT, HCH
2. Organophosphates eg. Fenitrothion, Malathion
3. Carbamates eg. Bendiocarb, Carbaryl
4. Pyrethroids eg. Permethrin, Cyfluthrin (Baythroid), Deltamethrin etc.

The chlorinated hydrocarbons tend to be long-lasting, relatively stable and have low mammalian

toxicity.

The organophosphates are mainly short-acting with a moderate to high toxicity. Fenitrothion and Abate are two exceptions to this generalisation.

The carbamates act very similarly to the organophosphates, i.e. they are cholinesterase inhibitors, but are more rapidly broken down in the body and thus have a lower toxicity to mammals.

The pyrethoids are a relatively new group of insecticides and generally have a lower mammalian toxicity than the organophosphates and carbamates. Some of this group of insecticides are, however, extremely toxic to aquatic organisms (Jolly et al. 1978). They are rapidly broken down by sunlight and heat so that a short-term but relatively drastic effect is seen.

Before an insecticide can be released for use toxicity trials must be undertaken and those used in Public Health control are more strictly regulated than those used in agriculture. Toxicity results for registration include rodent LD50 estimations. Thus an insecticide that may be safe for human use but lethal to fish could be classified as a "least dangerous" insecticide eg. permethrin (Ware 1983).

Due to the use of fish for malaria control in certain parts of the world some estimation of the effect of the insecticides has been made. The only reference to South African data is on *Aplocheilichthys myaposae* using an organo-phosphate, mercaptothion (Wager 1965). He reported that there was a 100% mortality at a dilution of

1 ppm. No concentration is given so these results are open to interpretation.

As a result of this lack of reliable data from South Africa it is necessary to extrapolate from relevant overseas data. In a review of the toxicity of DDT, malathion and carbanyl Jacob et al. (1982a) showed that the LD50 varied considerably between species eg. for these insecticides the following toxicities have been reported.

TABLE 7 The toxicity of certain Insecticides to *Cyprinodontidae*

Fish Species	DDT LD50 (ppm)	Malathion LD50	Carbaryl LD
Lepomis macrochirus	0,016	0,12	2,0
Aplocheilus lineatus	0,012	1,15	3,7
Gambusia affinis	0,5	-	-
Macropodus cupanus	2,28	4,6	13,91

In America DDT was sprayed from the air at a rate of "0,1 lbs DDT/acre/week" by the Tennessee Valley Authority. It concluded that DDT at this dosage "had no injurious effect upon the resident fish population". (Hess and Keener, 1947). It did not estimate the LD50 for the fish or the long term effect of the insecticide. Neither did the authority report on its effect on other aquatic organisms.

Other workers have compared the LD50 of more recent insecticides for malaria control (Rongsriyam et al. 1968; Shim & Self, 1972). Their results are shown in Table 8.

For this investigation insecticides used for malaria control and other insecticides which may possibly find future useage in South Africa were tested. The formulations used in the field, as well as concentrations to be applied, were investigated in an attempt to get realistic results.

8.2 MATERIALS AND METHODS

Plastic bowls with an 18cm diameter were used for the trials. After each trial the bowls were destroyed in case any insecticide had been absorbed into the plastic which could affect further experimentation.

Four litres of distilled water were placed in each bowl and air pumped into the water. Two sexually mature *Aplocheilichthys johnstonii* were placed in each bowl 24 hours before the trials were started as an attempt to limit stress on the fish. Three replicates were done for each concentration of the insecticide tested.

The trials were carried out in constant environment rooms (T = 25 $\pm$ 2 degrees C, R.H. = 70 - 80%)

Aedes aegypti larvae and commercial fish food were added to the water as a food source as well as to ascertain the efficacy on mosquito larvae of the particular concentration of the insecticide

The experiments were concluded 24 hours after the insecticide had been added.

Any fish surviving the experiments were returned to a new aquaria so that no fish were used in more than one trial. This was done to obviate any synergistic effect that may occur if the insecticides previously tested were stored in fat

bodies etc. and enhance the effect of another insecticide when the latter was being tested. This effect has been reported (Denison et al., 1981, 1985.)

8.3 RESULTS

The following insecticides were tested. All mosquito larvae were killed at the dosage applied.

TABLE 9 THE EFFECT OF CERTAIN INSECTICIDES ON *APLOCHEILICHTHYS JOHNSTONII* IN THE LABORATORY.

INSECTICIDE	(a.i.)	Fish mort. at 24hrs
DDT 75% w.p.	1.5mg/ℓ	0
DDT 75% w.p.	2.25mg/ℓ	0
Bendiocarb 80% w.p.	14.0mg/ℓ	100
Permethrin 25% w.p.	0.06mg/ℓ	100
Methoprene	Standard slow release formulation	0
B.t.i. w.p.	0.001 mg/ℓ	0
B.t.i. flowable concentrate	2 microl/ℓ	0
B.t.i. flowable concentrate	32 microl/ℓ	0

8.4 DISCUSSION

The majority of insecticides registered for mosquito larval control have already been tested for toxicity to other aquatic organisms in other countries, therefore (at the recommended doses), one would not expect them to be toxic to fish.

Permethrin was tested in America for toxicity to fish at an application rate of 0.025 and

0.125 lbs/acre with no observable effects (Mulla et al., 1981). The American Environmental Protection Agency (EPA) has restricted the use of permethrin because of its effect on the aquatic environment (Ware, 1983). The results reported here also indicate the toxicity of Permethrin to aquatic organisms (Table 6). The pyrethroids appear to be toxic to fish and the following toxicity results have been reported: Deltamethrin 1 microgram/1 LC50 for *Gambusia affinis* (Bocquet & L'Hotellier, 1985) and Cypermethrin 1 microgram/1 for *Salmo gairdneri* (Stephenson, 1985).

Bendiocarb is applied as an intradomicillary insecticide and when applied properly should not pollute the aquatic environment. If pollution does accidently occur fish mortality would be expected. Bendiocarb, being a carbamate, is rapidly broken down in the environment and there is no build up of sub-lethal doses in the organism since it is broken down within twenty four hours of being ingested.

Shim & Self (1972) reported the LD50 of DDT for *Aplocheilus latipes* as 0.21 p.p.m. The present investigation showed a zero mortality at 2.25 mg/ (mg/l = p.p.m.) This discrepancy is probably because Shim & Self used ethanol as the solvent for technical DDT so that it could be dissolved in water, whereas in the present investigation 75% w.p. was used. This formulation does not readily form a suspension in water and precipitates out within one hour. Thus, surface feedinc fish would not easily ingest a toxic dose. All the mosquito larvae were killed and sank to the bottom of the bowls and were not eaten by *A. johnstonii*. This phenomenon would also be observed in other aquatic organisms at the neuston

and therefore it is unlikely that *A. johnstonii* would pick up lethal doses from other aquatic organisms.

Methoprene is an insect juvenile hormone and would therefore not be expected to have any deleterious effect on other non-insect organisms in the aquatic environment.

Bacillus thuringiensis var. israelensis is unusual in that the toxins produced by the *Bacillus* are specific for mosquitoes and other closely related Nematocera eg. Simuliidae. It appears to be harmless to mammals and most other non-target organisms tested (Service, 1983; Stewart et al., 1983). It would appear to be an ideal control insecticide but is limited by the application rate (1 Kg/ha), high cost and because the formulations available are not suitable for anopheline larvae control. The formulations precipitate out and are not readily available for surface feeders. *B.t.i.* is also rapidly broken down by ultra-violet light and temperatures higher than 35 degrees C, and therefore requiring weekly applications for effective larval control.

It can be concluded that *A. johnstonii* can be used in an integrated control programme where the present insecticides are used as recommended. Pyrethoids are toxic to fish and should be avoided in control programmes where direct contamination of the aquatic environment may occur. Since pyrethoids are rapidly broken down by "biologically active substrates" they would only have an effect on the aquatic environment if they were directly applied to it. Therefore they may still have a role in intradomicillary spraying.

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The most studied species has been *Gambusia affinis* (Nakagawa, 1969; Tabibzadah et al., 1970; Pandian & Reddy, 1971; Bheema Rao, 1982; Gerberich, 1985; Gerberich and Laird, 1985) and this fish has been widely distributed in many countries. However with the advent of chemical pesticides the emphasis of mosquito control, and especially malaria control, changed to insecticidal control of adults.

In the last decade in many developing countries, especially in tropical Africa, there has been a decrease in malaria control activities, for a variety of reasons eg. financial, lack of suitable expertise, vectors becoming resistant to insecticides etc. Cheaper, equally or more effecive means of control must be investigated including the biological control of vectors. There has been increased interest in biological control methods, including fish (Pant and Rishikesh, 1981). *G. affinis* is still the most investigated but may have a detrimental effect on the environment into which it is released (Gerberich and Laird, 1985).

Indigenous species are being more intensively studied (Legner and Medved, 1973; Velmirovic and Clarke, 1975; Costa and Fernando, 1977; Singh et al., 1977; Ataur-Rahim, 1981; Jacob et al., 1982). There is a paucity of information on indigenous larvivores in Africa (Service, 1960; WHO, 1981; Haas and Pal, 1984; Alio et al., 1985; Romand, 1985).

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World-wide the fish which have been the most successful for mosquito control are the Cyprinodontiformes. In Southern

Africa two indigenous genera are found viz. *Aplocheilichthys* and *Nothobranchius*.

9.1 *NOTHOBRANCHIUS*

Two species occur in South Africa - *N. orthonotus* and *N. rachovii*. Both species have very limited distribution and would appear to have a limited habitat preference. They are not primarily surface feeders and therefore will not choose genera to feed on mosquito larvae.

Mass production of these species requires much patience and expertise. Eggs should be stored for at least three months before hatching. Preferably fish should be kept in small numbers in small tanks, rather than in one big tank, otherwise intraspecific aggression occurs.

The present investigation has indicated convincingly that *Nothobranchius* species are not suitable for mosquito control in South Africa. The principal reasons for this are:

1 a limited habitat which could be utilised because of very specific habitat preferences and environmental conditions,

2 the extreme difficulty in raising large populations under control conditions.

9.2 *APLOCHEILICHTHYS*

Three species occur in South Africa viz. *A. myaposae*, *A. katangae* and *A. johnstonii*.

A. myaposae is restricted to Northern Natal and Zululand and does not occur in the Transvaal.

A. katangae has a very limited distribution in the Transvaal (Kleynhans, 1964) although

it is common in Kwazulu, Zimbabwe and Namibia.

It can be readily bred in the laboratory and produces several generations per season.

A. katangae is a surface feeder and readily feeds on mosquito larvae.

It is only common in the Transvaal in the Matlapitsi and Groct Nyl rivers where it forms in excess of 20% of the total fish population. It does not appear able to compete with *A. johnstonii* in the Transvaal and where they were collected together *A. johnstonii* was found in greater numbers (38.2%) (Kleynhans, 1984). Laboratory experiments also showed a tendency for *A. johnstonii* to dominate *A. katangae*.

A. johnstonii is common in the Transvaal, Namibia (Kavango and E. Caprivi), Botete River, Botswana and in parts of Zimbabwe and Mocambique. Numerically this species is one of the more commonplace species in its habitat and is able to occupy floodplains rapidly during the breeding season (October - March).

Mass breeding techniques were developed using nylon "mops" and the fish easily adapted to aquaria. In the laboratory fish survived approximately two years and could be induced to spawn throughout the year.

A. johnstonii is a surface feeder and will readily feed on mosquito larvae, although it will also feed on other organisms. This ability to maintain a viable population in the absence of mosquito larvae is advantageous since it would prevent future mass breeding of mosquitoes in that habitat.

Like other Cyprinodontids *A. johnstonii* is relatively sensitive to insecticides but would not appear to be adversely affected by the dosage of insecticides currently used in the malaria control programme.

Maximal feeding occurs in the early evening (1700-2000 hours) which is the time when mosquito imagos emerge from the pupae and when adult females lay their eggs.

The World Health Organisation has drawn up several reports on the selection of indigenous fish for mosquito control (WHO, 1981; WHO, 1982; Ahmed et al., 1986). *Aplocheilichthys* has been proposed as a genus requiring further study in West Africa for the following reasons (WHO, 1982).

1. Fish Bionomics
   size: 2-6 cms
   food: carnivorous
   incubation period: 10-18 days
   size of fry: small
   rate of growth: rapid
   position of mouth:superior
   breeding period: year round
   habitat: slow streams, pools, ponds
   feeding: surface feeder

2. Chemistry of Water Tolerances
   pH: 5 -7.5
   dH:soft salinity: slightly tolerant
   organic pollution: not resistant

3. Temperature
   max. range: 16-30 deg. C
   min. for breeding: 20-25 deg. C

A. johnstonii also shows similar characteristics and therefore meets these criteria for selection as a candidate species.

Ahmed et al. (1986) have drawn up a rating system for candidate species (Appendix 1). With this system *A. johnstonii* receives a rating of 102 out of a possible 155. Thus it readily meets the requirements for a fish for mosquito control as has been emphasised also by the results of the previous investigation.

A. johnstonii was released into three ornamental ponds at Skukuza in the Kruger National Park. A viable population was soon established and no mosquito larvae were found in the pools (personal data).

At the plant propagation nursery at Skukuza a mosquito population was found in small dams. *A. johnstonii* was released into these ponds and within two weeks no mosquito larvae were found. The fish population continued to survive in the pools in the absence of mosquito larvae.

Fish were removed from this location and transferred to ornamental pools at the Satara tourist camp. *Anopheles coustani* and *Culex spp.* larvae had been present in these pools prior to the release of the fish. No mosquito larvae have been found since the establishment of fish in these ponds.

Following an increase in malaria cases amongst K.N.P. staff at Skukuza during December 1985 an investigation was conducted by officials of the Dept. of Health. *An. gambiae s.l.* were found breeding in seepage from a dam and it was recommended that the seepage be canalised and pools

constructed so that *A. johnstonii* can be released into these pools. This work was undertaken by K.N.P. officials (Pienaar, pers. comm.) and during a follow up survey no *An. gambiae s.l.* were found.

A. johnstonii was released into an isolated ornamental pond at the National Institute for Tropical Diseases, Tzaneen. The water was analysed with the following results:

pH	7.0
dissolved CO_2	15.0 mg/ℓ
hardness	85.5 mg/ℓ
dissolved O_2	5.0 mg/ℓ
temp.	22.0 deg. C.

Vallesnaria sp. was planted in the muddy substrate and other aquatic plants present included *Nymphaea sp.* and *Dracaena sp.*

An. coustani, An. rufipes and *Culex neavei* had been collected from this pond. After the release of the fish no mosquito larvae have been found.

Thus it can be concluded that *A. johnstonii* will establish itself in the Lowveld and form viable breeding populations. This species will effectively control mosquito larvae in the same habitat and therefore can be used in South Africa for mosquito control purposes in an integrated control programme.

SUMMARY

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Indigenous Cyprinodontidae from South Africa were investigated as possible candidates for biological control agents for mosquito larvae. Two genera, *Nothobranchius* and *Aplocheilichthys*, were investigated. *N. orthonotus* and *N. rachovii* were collected from a locality in the Kruger National Park and experiments conducted to ascertain their feasability as candidate species. Problems with mass breeding techniques, a restricted habitat preference, as well as this genus not being a preferential mosquito larvivore do not make it the preferential choice for mosquito larvae control.

Aplocheilichthys was also investigated and *A. johnstonii*, due to its widespread distribution including the Transvaal, was chosen for further experimentation rather than *A. katangae* or *A. myaposae*.

Mass-breeding techniques in the laboratory were developed. The maximum breeding involved the use of nylon "mops" in which the eggs were laid. In constant environment rooms spawning occurred throughout the year.

Fish reached sexual maturity at approximately six weeks and would continue spawning for at least two years.

Diet had an influence on spawning and Culicidae larvae, Crustacea and commercial tropical food was shown to give the best results. Eggs were removed from the mops and placed in enamel bowls where egg development occurred. Young fish were placed in aquaria and when large enough were transferred to aquaria with adult fish. The adult fish were cannabilistic on small fish.

In the laboratory *A. johnstonii* was shown to preferentially feed on mosquito larvae. Different species of anophelines and culicines as well as other aquatic organisms were tested for diet preferences. The size of fish versus the stages of larvae consumed were investigated. Due to an increase in

feeding with more than one individual present in an aquarium it was not possible to estimate the number of mosquito larvae consumed per fish.

Laboratory results were compared to field samples. A high proportion of Insecta were found in the gut of *Aplocheilichthys johnstonii* from field localities and filamentous algae was also fairly common. Feeding times in the field were also investigated and the peak feeding time of *A. johnstonii* was at dusk. This species was also shown to inhabit shallow waters, normally less than one metre in depth and to be found associated with aquatic vegetation.

Gut contents of wild caught specimens as well as laboratory observations showed that *A. johnstonii* is not an obligatory mosquito larvivore and can thus maintain a reproductive population in the absence of mosquito larvae.

Problems of gut content analysis are discussed as well as the difference between the results of this study to those previously reported in the literature. These include the absence of species from certain localities and also the inherent bias of the investigator.

The toxicity of insecticides that are used in the malaria control programme as well as insecticides that may be used in the future was investigated. The formulations of the insecticides as well as doseage rates that are applied were tested and it was shown that *A. johnstonii* would tolerate the doseages that would be encountered in the field.

A. johnstonii was released in restricted localities in the Transvaal Lowveld and established viable populations and also controlled the mosquito larvae in these habitats.

A. johnstonii could be used in an integrated malaria control programme and would be as effective as other Cyprinodontidae in other parts of the world.

OPSOMMING

OPSOMMING

Daar is ondersoek ingestel na die moontlikheid om inheemse Suid-Afrikaanse Cyprinodontidae te gebruik vir die beheer van muskietlarwes. Twee genera, *Nothobranchius* en *Aplocheilichthys* is ondersoek. *N. orthonotus* en *N. rachovii* is versamel by 'n punt in die Nasionale Kruger-wildtuin, en eksperimente is uitgevoer om hul lewensvatbaarheid as kandidaatspesies te bepaal. Probleme in verband met massabroeitegnieke, 'n beperkte habitatvoorkeur, asook die feit dat hierdie genus nie by voorkeur 'n muskietlarwevreter is nie, het gelei tot die gevolgtrekking dat dit nie by uitstek geskik is vir muskietlarwebeheer nie.

Aplocheilichthys is ook ondersoek, en *A. johnstonii* - weens die spesie se wye verspreiding, wat die Transvaal insluit - is gekies vir verdere eksperimentering, eerder as *A. katangae* of *A. myaposae*.

Massabroeitegnieke is ontwikkel vir laboratoriumtoestande. Maksimale broei is behaal deur die gebruik van nylon - "dweile" waarin die eiers gelê is. Eiers is dwarsdeur die jaar gelê in kamers wat aan konstante omgewingsfaktore onderworpe was.

Die visse het geslagsrypheid bereik teen ongeveer ses weke, en sou kon voortgaan om eiers te lê vir minstens twee jaar.

Dieet het lêvermoë beïnvloed: Culicidaelarwes, Crustacea en kommersiële tropiese viskos het die beste resultate gelewer. Die eiers is van die "dweile" verwyder en in emaljebakke geplaas om verder te ontwikkel. Jongvis is vervolgens in akwaria geplaas en later, nadat hulle 'n geskikte grootte bereik het, is hulle oorgeplaas na akwaria met volwasse visse. Die volwasse visse het kleiner visse gekannibaliseer.

In die laboratorium het *A. johnstonii* duidelik muskietlarwes verkies. Dieetvoorkeurtoetse is met verskillende spesies Anophelinae en Culicinae, asook ander akwatiese organismes,

uitgevoer. Die verband tussen visgrootte en die ontwikkelingstadia van larwes gevreet is ondersoek. Weens die feit dat voeding vermeerder het met meer as een vis teenwoordig in 'n akwarium, was dit ongelukkig nie moontlik om die konsumpsie van muskietlarwes per vis te skat nie.

Laboratoriumtoetsresultate is vergelyk met veldmonsters. Die ingewande van *Aplocheilichthys johnstonii* het na verhouding baie Insecta bevat, terwyl draadalge ook taamlik algemeen voorgekom het. Daarbenewens is ook gevind dat die spesie vlak water (gewoonlik minder as een meter diep) bewoon, en in assosiasie met akwatiese plantegroei aangetref word.

Ingewandsinhoud van vrylewende vis wat versamel is sowel as waarnemings in die laboratorium het daarop gedui dat *A. johnstonii* nie uitsluitlik op muskietlarwes voer nie en dat dit dus 'n lewensvatbare bevolking sonder muskietlarwes sal kan onderhou.

Probleme in verband met die ontleding van ingewandsinhoude word bespreek. So ook verskille tussen die bevindings van hierdie studie en dié van bestaande literatuur, met inbegrip van die afwesigheid van spesies op sekere plekke en, les bes, die inherente vooroordeel van die navorser.

Die giftigheid van insekdoders wat tans vir malariabeheer gebruik word en wat moontlik in die toekoms gebruik mag word vir dié doel, is ondersoek. Proewe met huidige dosisformulerings en doseertempo's het getoon dat *A. johnstonii* die dosisse wat in die veld teëgekom kan word sou kon verduur.

A. johnstonii sal gebruik kan word in 'n geïntegreerde malariabeheerprogram, en sal netso doeltreffend kan wees as ander Cyprinodontidae elders ter wêreld.

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APPENDIX

WORLD HEALTH ORGANIZATION WHO/V
ORGANISATION MONDIALE DE LA SANTE ENCL

A RATING SYSTEM FOR THE SELECTION OF APPROPRIATE, INDIGENOUS FISH SPECIES FOR MOSQUITO CONTROL

by

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1. INTRODUCTION

Fish have been recognized as effective biological control agents of mosquito lar almost 100 years (Coykendall, 1980). During the twentieth century, the use of fish fo purpose has steadily grown. Because of its effectiveness, the mosquito fish, Gambusia affinis, has been introduced from the south-eastern United States of America to waters many countries around the world. However, many other fish species have been shown to effective in mosquito control. In an annotated bibliography published in 1946, Gerber listed 216 species of fish having potential for mosquito control. In the later versio this bibliography (Gerberich & Laird, 1966; Gerberich, 1985), references are listed t 1981, but concentrate on mosquito fish and the guppy, Lebistes reticulata. Mosquito-predatory fish are found in nearly all countries of the world, in both tempera tropical regions. They occur in fresh and brackish water in a wide variety of habitat wells and temporary pools to rivers, lakes and extensive swamps and salt marshes. Som been employed for the direct destruction of larvae, and others for modifying the habit render it unsuitable for mosquitos. Various indigenous species of fish are reported t highly effective in controlling mosquito larvae in their native habitat. However, few experiments have been conducted to demonstrate their potential for more intensive applications. Some species of fish have been effective larvivorous predators when transported beyond their native ranges. Two of these are the mosquito fish from south-eastern North America and the guppy from northern South America and Caribbean ar The purpose of this paper is to provide (a) operational mosquito control technicians a rating system to help select the proper fish species for control, and (b) basic inform on appropriate handling of fish.

In all cases when stocking fish, one must be aware of potential disruption to the aquatic community through the introduction of an exotic organism. Ecologically disast circumstances have resulted from well-intentioned introductions of exotic fish specie (Courtenay & Stauffer, 1984). Stocking of fish should be conducted within the natura boundries of the species concerned and according to national and international regulat governing the movement of live organisms. To facilitate the minimization of introducti exotic species and to provide the best possible control of mosquito larvae, the followi sample rating system is described below.

This rating system has been designed for operational mosquito control technicians select appropriate fish species as larvivorous predators. Although semi-qualitative i nature, its flexibility allows useful inclusion of both habitat and species variability eventual selection of suitable fish. The flexibility includes and excludes specific questions to tailor the rating system for specific habitats. Success of mosquito conti

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when using this rating (or other) system should always be evaluated with follow-up such as intense monitoring of mosquito larvae and fish populations. These evaluations should allow the rating system to be used to the best effect, including the modification of scores for categories which have primary importance in particular areas. A similar rating system has been applied to status determinations of threatened and endangered species of wildlife (Landry et al., 1979). The maximal usefulness of the fish selection rating system will come through modifications and additions provided by the mosquito control technicians as experience is gained in a variety of systems using fish.

2. COLLECTION AND TRANSPORT OF FISH

Fish used for mosquito control should be obtained from artificial breeding places or natural environments, transported and stocked in areas needing control. The fish are usually captured using seine-nets or traps. As fish are removed from the net or trap, great care should be used to minimize stress on the fish (Coykendall, 1980; Carmichael, 1984a & b). Fish should not be removed from water, if possible. If this is impossible, the amount of exposure time to air should be minimized. Fish can be held in "livecars" or net enclosures within the sample area or placed into containers such as buckets or earthenware pots. The water in these buckets should be renewed at appropriate intervals to maintain adequate dissolved oxygen levels, ensuring fish survival. For transport to the stocking site for mosquito control, fish should be contained in a large, well-aerated tank, if possible, filled with fresh water from the habitat where the fish were caught. If available, common salt (sodium chloride, i.e., NaCl) and nitrofurozone (Furacin®) should be added to the water in this tank at the concentrations of 0.5% and 20 mg/l (active ingredient) by weight, respectively. Addition of these compounds will minimize fish mortality from osmotic stress and external bacterial infections, respectively. Transport to the stocking area should take the minimum of time possible without unduly jostling the fish (Coykendall, 1980). Where transit times are lengthy, provisions may have to be made for reaerating or cooling the water in the transport tank. During the stocking procedure, fish should be quickly dip-netted from the transport tank and placed into buckets of water, minimizing stress as much as possible. Then the buckets should be gently floated onto the receiving waters and tilted so that some of the new water mixes with the water from the tank containing the fish. The temperature of the water where the fish will be stocked should approximate that in the transport tank. If the temperature of the receiving waters is much warmer or, especially, much cooler than the water where the fish were captured, more extensive temperature acclimatization in the transport (or other) tank is warranted.

3. A RATING SYSTEM FOR EVALUATING FISH FOR MOSQUITO CONTROL

Guidelines: All the questions do not have to be considered if they are not appropriate for the situation. Additional questions can be inserted and used as needed.

The letter "E" in answer to any of the questions eliminates a fish from further consideration.

First, eliminate any questions that are not relevant to the particular situation where the fish are to be used. For example, if the fish are to be used as direct predators on mosquito larvae, eliminate the question on consumption of aquatic weeds. If the fish are to be used in an area of strictly fresh water, eliminate the question on tolerance to salt water. In addition, remaining questions may need to be modified, based on local acclimatizing conditions for fish and seasonal effects which may influence exposure time to extreme temperatures, dissolved oxygen levels, or other significant parameters.

For each species of fish under consideration, answer the relevant questions and add the total score. The fish species with the highest score is the most likely candidate for the control application planned.

Rating System Questions

1. Ability to withstand low dissolved oxygen (D.O.) levels

   a. Can live in water with D.O. at or near 0 .....

   b. Can live in water with D.O. of 0 to 2 mg/l .....

   c. Can live in water with D.O. of 3-5 mg/l .....

   d. Can only live in water with D.O. >5 mg/l .....

2. Ability to withstand high temperatures

   a. Can live at temperatures >35°C .....

   b. Can live at temperatures of 30-35°C .....

   c. Can live at temperatures of 25-30°C .....

   d. Can only live at temperatures of <25°C .....

3. Feeding habits (when direct larval predation desired)

   a. Insectivorous, with strong preference for mosquito larvae .....

   b. Insectivorous, mid-water or surface feeder, lacking strong preference for mosquito larvae .....

   c. Insectivorous, mainly benthic feeder .....

   d. Omnivorous .....

   e. Herbivorous .....

4. Ability to penetrate vegetation

   a. Will penetrate thick stands of emergent/submergent vegetation .....

   b. Will penetrate open stands of vegetation .....

   c. Avoids vegetation .....

5. Reproductive rate in the field

   a. High, quickly produces large, self-sustaining population .....

   b. Moderate .....

   c. Low .....

   d. Reproduces only in a subset of the habitats .....

   e. Does not reproduce in mosquito habitats .....

6. Endemicity

   a. Native to area where control desired 10

   b. Native to zoogeographic region 5

   c. Not native to area 0

7. Susceptibility to mass culture

   a. Easy to culture in large numbers 5

   b. Culture possible, with some difficulty 3

   c. Culture probably possible but difficult 1

   d. Impossible to culture 0

8. Economics

   a. Cost low compared to other methods of control .....

   b. Cost comparable to other methods of control 3

   c. Cost high compared to other methods of control .....

9. Ease of transport

   a. Can survive extended periods of being crowded with minimal care .....

   b. Moderately easy to transport .....

   c. Difficult to transport far .....

10. Acceptability for use in an integrated control programme

   a. Highly acceptable, resistant to pesticides .....

   b. Moderate acceptability .....

   c. Difficult to use .....

11. Impact of the fish on native fish species

   a. Fish studied, no impact found .....

   b. No information available .....

   c. Suspected cases where caused extinctions .....

   d. Well-documented cases where caused extinctions .....

12. Impact of the fish on other predators of mosquito larvae

   a. No effect on invertebrate predators .....

   b. Minor impact on invertebrate predators .....

   c. Fish reduces populations of invertebrate predators .....

13. Number (density) of fish needed for control

    a. Low numbers effect good control .....

    b. Moderate numbers needed .....

    c. Very high numbers of fish needed .....

14. Likelihood of human interference with the fish

    a. Fish unlikely to be harvested .....

    b. Fish have high ornamental or food value .....

15. Potential for control during floods

    a. Fish will travel with flood waters, effect control .....

    b. Fish resist moving with flood waters .....

16. Ability to withstand low temperatures

    a. Can overwinter at temperatures of 0-5°C .....

    b. Can overwinter at temperatures of 5-10°C .....

    c. Can overwinter at temperatures of 10-15°C .....

    d. Can overwinter at temperatures of 15-20°C .....

    e. Can only live at temperatures of >20°C .....

17. Tolerance to salinity and other minerals

    a. Can live in sea water (33 g/l) .....

    b. Can live in strong brackish water (up to 20 g/l) .....

    c. Can live in slightly brackish water (5-10 g/l) .....

    d. Can only live in fresh water .....

18. Availability of the fish

    a. The fish can be collected in large numbers locally .....

    b. The fish can be collected in moderate numbers .....

    c. The fish must be imported .....

19. Ability to withstand physical disturbances in their environment

    a. Fish highly tolerant of disturbances .....

    b. Fish moderately tolerant of disturbances .....

    c. Fish intolerant of disturbances .....

20. Disease resistance

a. Fish highly resistant to disease in the area .....

b. Fish moderately resistant to local diseases .....

c. Fish susceptible to local diseases .....

21. Government regulations

a. There are no government regulations regarding use or importation of this fish ...

b. Following required procedures will take some time and effort .....

c. Use of this fish is prohibited .....

22. Ability to survive dry spells

a. Eggs resistant to desiccation (annual fish) .....

b. Species not able to survive desiccation .....

23. Behaviour of the fish in running water

a. The fish aggregate in the shallows and backwaters .....

b. The fish remain in the current .....

24. Ability to withstand pollution

a. Highly resistant to polluted water .....

b. Can withstand moderately polluted water .....

c. Requires clean water .....

25. Tolerance to low pH

a. Can live in very acidic water .....

b. Can tolerate moderately acidic water .....

c. Requires alkaline water .....

26. Larval control by elimination of aquatic weeds

a. Known to consume the target weed species .....

b. Known to consume other weed species .....

c. Herbivorous, specifics unknown .....

d. Will not eat aquatic weeds .....