THE DIVERSITY AND ABUNDANCE OF PARASITES ASSOCIATED WITH *Xenopus laevis* (DAUDIN, 1803) IN SELECTED HABITATS

by

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A thesis submitted in fulfilment of the requirements for the degree of

MAGISTER SCIENTIAE IN ZOOLOGY

in the

DEPARTMENT OF ZOOLOGY AND ENTOMOLOGY
FACULTY OF NATURAL SCIENCES
of the
UNIVERSITY OF THE ORANGE FREE STATE
BLOEMFONTEIN
SOUTH AFRICA

March 1999

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The African clawed frog, *Xenopus laevis*.

ACKNOWLEDGEMENTS

Undeniable honour and appreciation to my Creator for giving me the strength and ability to successfully complete this study.

Sincere gratitude to Dr. L.H. Du Preez for his constant support and encouragement through all the years under his supervision. His inexhaustible willingness to share knowledge and render guidance proved invaluable. I would like to thank him mostly for his extensive constructive comments, particularly on the drafting of this thesis, while still giving me the freedom to work independently and pursue my own ideas.

The following persons and institutions are thanked:

- The Department of Zoology and Entomology for the use of facilities and equipment.
- The Foundation for Research Development for financial assistance.
- Mr. C.R. Newberry for allowing me to use a dam on his property for part of my research.
- Mr. M.T. Seaman for advice during various parts of the study.
- Prof. L. Basson for commenting on parts of the thesis.
- The South African Weather Bureau for climatological data.

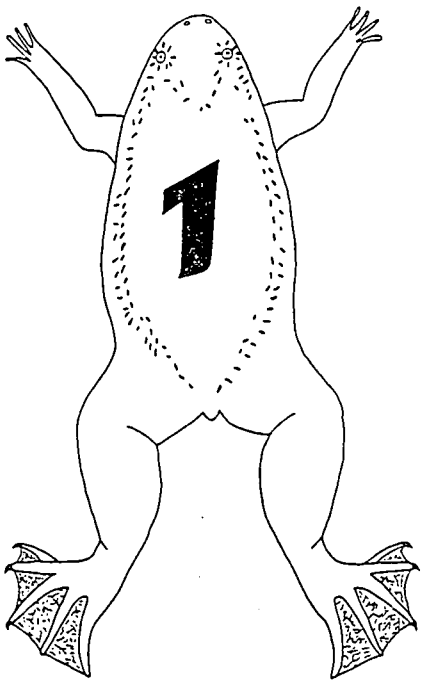
Special thanks to:

- My parents and sister for believing in me, their encouragement, and financial and emotional support through all my years of studying.
- All my friends for the important part they played in the non-academic part of my life.

I would finally like to express my profound gratitude to my loving wife Laetitia, who was a constant source of support and encouragement. Her motivation played an indispensable role in the completion of this study. All the sacrifices and understanding from her side are sincerely appreciated.

To my loving wife and family

CONTENTS



General Introduction & Literature Review

Price (1980) estimated that more than 50% of all plant and animal species are parasitic at some point in their life cycle, with Esch and Fernández (1993) adding that the number of plants and animals which are parasitised at some point in their lives definitely approaches 100%.

Parasitologists have debated the definition of parasitism for many years. Esch and Fernández (1993) rightly states that the extent of parasitism is debatable depending on how one defines the term. This poses a problem, as some authors would not see the symbiotic organisms associated with *Xenopus* as parasites according to their own definition. A few basic principles of parasitism will therefore be discussed.

The term 'symbiosis' describes organisms that live together in a broad sense, with no reference to the length or outcome of the association. Symbiosis covers several relationships which may exist between organisms, the most common being mutualism, commensialism and parasitism. The classical definition for parasitism describes an intimate relationship between two organisms in which one lives on, off or at the expense of the other. The key element of the definition, the implication of benefit to one and harm to the other poses the biggest problem, as harm is a relative term and not quantifiable (Esch & Fernández, 1993).

Barnard and Behnke (1990) stated that the investment of time and effort by one organism (producer) in procuring a resource provides an adaptive shortcut for selection in another (scrounger) which steals or usurps the resource. These strategies of usurpation are regarded as parasitic. Although the term 'parasite' is usually restricted to

organisms like tapeworms or fleas, these organisms only represent the extreme end of 'scrounger' strategies, in which the organism is totally dependent on the host for survival. In any form of parasitic relationship, there is a cost to the host and therefore counter-adaptive measures are likely to be favoured by selection to reduce the impact of exploitation. Parasites also have important effects on host behaviour, in terms of both pathological and other physiological changes, and in selecting for behavioural counter-responses to exploitation.

Crofton (1971) defined parasitism as an ecological relationship between two organisms, one being the parasite and the other the host. He identified four essential features of this relationship:

1. Physiological dependence of the parasite on the host,
2. heavily infected hosts will be killed by their parasites,
3. an overdispersed frequency distribution of parasites within the host population, and
4. the reproductive potential of the parasites exceed that of the host.

The last three characteristics are diagnostic of parasitism, as the physiological dependence of one organism on another is not restricted to parasitism. Features two and three ensures that more parasites than hosts die, and together with the higher reproductive potential of parasites, the size of both host and parasite populations are regulated. Lastly is the ability of the parasite to harm or kill the host the feature that distinguishes parasitism and commensialism.

Smyth (1994) recognised the importance of seeing the host-parasite relationship as an ecological one. He stated that the definition is relative depending on the emphasis being

put on certain aspects. These factors include the intimacy of the relationship, its pathogenic effect, metabolical and physical dependence, whether or not the parasite is recognised as 'foreign' and the ability of the parasite to 'recognise' the host as a suitable ecological niche. The second factor, the pathogenic effect of the parasite on the host, is proving to be the most complicating in defining parasitology, as some authors insist that a parasite must necessarily be harmful to a host to the point of killing it or causing serious physical harm. The problem is that the negative effects a parasite has on its host, is relative and not always visible or quantifiable. Crofton (1971) states that the term parasitism should be restricted which are potentially capable of killing their host. The emphasis here is on the potential of the host being killed. In all host-parasite relationships the host will be killed if parasite numbers are not regulated. However, parasites typically do not kill their hosts (Esch & Fernández, 1993) and mechanisms exist which control infection levels (Crofton, 1971), as the death of the final host is detrimental to the majority of parasites.

The problems encountered in defining parasitism can largely be ascribed to a failure in seeing the term as having a relative meaning. Using the metabolic dependence of a parasite on its host as criterion, a free-living organism shows zero dependence, whereas a totally parasitic organism, e.g. the blood-dwelling protozoan *Plasmodium*, is 100% dependent. All degrees of dependence between these two extremes are encountered (Smyth, 1994). The definition of parasitism by MacInnis (1976), also indicates the varying degrees of dependence, and puts no emphasis on harm. He stated that parasitism is an association in which "one partner, the parasite, of a pair of interacting species, is

dependent upon a minimum of one gene or its products from the other interacting species, defined as the host, for survival".

The evolution of certain host-parasite associations implicates adaptation of the parasite and host, so that the host ultimately accommodates the parasite, while the parasite does not severely harm the host. It is generally accepted that amphibians have a tolerance for the detrimental effects of parasites, and that these effects are seldom obvious (Prudhoe & Bray, 1982). The parasites of *Xenopus* are also completely dependent on the frog for their survival, without necessarily causing harm. Records of the detrimental effects caused by parasites are few and at best inconclusive.

The genus *Xenopus* is characterised by a rich parasite diversity, which is related to the fact that the frog is primarily water living which facilitates parasite transfer. No less than 27 parasite genera are known from the African clawed frog, *Xenopus laevis* (Daudin, 1803), and parasites infect virtually all organ systems of the frog (Table 1.1). This diverse assembly of parasites representing seven major invertabrate groups, makes *Xenopus* an ideal host to study and to use as material in presenting parasitology courses. Included in the array of parasites are nine species of Protozoa that did not form part of this study and will not be discussed further.

The origins and relationships of *Xenopus* parasites demonstrate two aspects of the host. Firstly, the specialisation of pipids for fully aquatic life and therefore being virtually the only anurans feeding underwater. This contributes to the ecological isolation of *Xenopus* from other anurans and its overlap with fish. The distinctiveness of the parasites

secondly suggests the phylogenetic isolation of clawed frogs. There is much evidence that the highly distinct parasite assemblage of *Xenopus* today is a product of prolonged phylogenetic and ecological isolation of the host (Tinsley, 1981).

The majority of *Xenopus* parasites is morphologically and taxonomically distinct from their nearest relatives, and is strictly host specific to *Xenopus*. The parasite fauna can be divided into two groups on the basis of their systematic relationships with other parasites. Some are related to forms occurring on fish, reflecting the ecological link between the host groups that share an aquatic habitat and diet. However, these parasites of *Xenopus* are not recent transfers from fish. They are morphologically distinct and taxonomically isolated, which reflects a long association with the clawed frog. Other parasites of *Xenopus* are related to parasites of other anurans, reflecting a common ancestry of some parasites that infected early anurans and evolved with respective host groups (Tinsley, 1981 & 1996a).

Table 1.1 The parasites of *Xenopus laevis* (modified from Tinsley, 1996a).

Parasite	Infection site
MONOGENEA	
*Gyrdicotylus gallieni**	Mouth, nostrils
*Protopolystoma xenopodis**	Urinary bladder, kidneys
DIGENEA	
Adults	
*Dollfuschella rodhaini**	Stomach
Oligolecithus elianae	Intestine
Xenopodistomum xenopodis	Gall bladder
Progonimodiscus doyeri	Rectum
Metacercaria	
*Tylodelphys xenopi**	Pericardium, body cavity
Echinostomum xenopodis	Eyelids, lateral line
Cercaria xenopodis	Eyelids, lateral line
Opisthioglyphe xenopodis	Dermis
Neascus sp.	Lateral line
Clinostomum sp.	Body cavity
CESTODA	
*Cephalochlamys namaquensis**	Intestine
NEMATODA	
*Camallanus kaapstaadi**	Oesophagus
Camallanus xenopodis	Intestine
*Batrachocamallanus slomei**	Stomach
Pseudocapillaroides xenopodis	Epidermis
Microfilariae	Blood
ACARI	
Xenopacarus africanus	Nostrils, eustachian passages
HIRUDINEA	
*Marsupiobdella africana**	External skin
PROTOZOA	
Balantidium xenopodis	Rectum
Nyctotherus sp.	Rectum
Protoopalina xenopodus	Rectum
Hexamita intestinalis	Rectum
Chilomastix caulleryi	Rectum
Entamoeba sp.	Intestine
Trichodina xenopodos	Urinary bladder
Trypanosoma sp.	Blood
Cryptobia sp.	Blood

(*Parasites found during the current study)

Many extensive publications exist on the parasites of *Xenopus*, the most important by Tinsley (1996a) on the diversity, life cycle patterns, pathology and population biology of the parasites, and Tinsley (1996b) on evolutionary deductions from host and parasite co-speciation. Other major publications on the parasites of *Xenopus* include Vercammen-Grandjean (1960) on the trematodes of southern Lake Kivu, Thurston (1970) on some protozoan and helminth parasites of *Xenopus*, Macnae, Rock and Makowski (1973) on the platyhelminth parasites of *X. laevis*, and Cosgrove and Jared (1974) on diseases and parasites of *Xenopus*. Several other authors gave accounts of studies on more than one of the parasites of *Xenopus*. These include Southwell and Kirshner (1937), Porter (1938), Elkan (1960), Pritchard (1964) and Tinsley and Whitear (1980).

The gyrodactylid monogenean ***Gyrdicotylus gallieni***, first described by Vercammen-Grandjean in 1960 from *X. laevis victorianus*, is closely related to the monogenean *Gyrodactylus*, which typically infects teleost fish. A form of viviparity unique to the Gyrodactylidae enables *in situ* reproduction. *G. gallieni* differs from other gyrodactylids in the haptor being modified for suctorial development, and in the structure of the excretory system and penis. The parasite seems to have been distinct and isolated since the first appearance of *Xenopus* (Harris & Tinsley, 1987). Thurston (1970), and Cosgrove and Jared (1974) first mentioned infection levels of *G. gallieni*. Extensive publications on the biology (Harris & Tinsley, 1987) and infrapopulation dynamics (Jackson & Tinsley, 1994) of the parasite included results of infection levels in larger samples taken during different times of the year, and population growth studies. Other publications dealt with sclerite growth and morphometric variation (Jackson & Tinsley, 1995a) and speciation and host specificity (Tinsley, Harris & Jackson, 1993).

The most extensively studied parasite of *Xenopus* is ***Protopolystoma xenopodis*** (Monogenea: Polystomatidae). It was first described from *X. laevis* by Price (1943) as *Polystoma xenopi*, but the genus was later changed to *Protopolystoma* (Bychowsky, 1957). Vercammen-Grandjean (1960) described a new subspecies *P. x. victoriani* from *X. l. victorianus*, but Pritchard (1964) concluded that the species were in fact the same, and also gave the first account of the infection levels of the parasite. In 1964, Thurston published an extensive article dealing with the morphology and life cycle of the parasite, mentioning infection levels, rate of egg-production and the presence of juveniles in the kidneys. Publications by Thurston (1970), Macnae *et al.* (1973), Tinsley (1972) and Cosgrove and Jared (1974) all dealt with infection levels of the *P. xenopodis*. Tinsley (1972) also gave one of the few existing accounts of seasonal variation of parasite burdens. Tinsley and Owen in 1975 investigated the correlation between life cycle and host ecology, physiology and behaviour. Jackson (1982) dealt with success of reproduction and infection levels, and Jackson and Tinsley (1988a&b) investigated the reproduction of *P. xenopodis*, and specifically the egg production and influence of environmental factors thereupon. The population biology of polystomatid monogeneans was discussed by Tinsley (1993 & 1996a). Recent publications on *Protopolystoma* focuses the correlation of speciation and specificity with host evolutionary relationships (Tinsley & Jackson, 1998a), speciation of *Protopolystoma* (Tinsley & Jackson, 1998b) and the incompatibility of *P. xenopodis* with an octoploid *Xenopus* species from southern Rwanda (Jackson & Tinsley, 1998a). Tinsley and Jackson (1998b) reviewed previous reports of *P. xenopodis*, particularly Tinsley (1973), and described five new species of *Protopolystoma* of which three were previously reported as *P. xenopi*.

The adult digenean ***Dollfuschella rodhaini*** Vercammen-Grandjean, 1960 (Digenea: Halipeginae) was first described from *X. l. victorianus*, but later as *Halipegus rhodesiensis* from *X. l. laevis* (see Beverley-Burton, 1963). These two publications, as well as Thurston (1970) mentioned the infection levels of the parasite. The parasite was identified as *Halipegus rodhaini* by Maeder (1969), but Macnae *et al.* (1973) stated that all were synonyms, and recorded a very low prevalence of the parasite. Jackson and Tinsley (1997) reviewed the taxonomy, host range and geographical distribution of *Dollfuschella*, redescribed the parasite and assigned it its original name.

Adult ***Oligolecithus elianae*** Vercammen-Grandlean, 1960 (Digenea: Telorchiidae) was first described from *X. l. victorianus*. Pritchard (1964) described *O. jonkershoekensis* from the intestine of *X. laevis*. Thurston (1970) indicated that the parasites were related, but Macnae *et al.* (1973) stated that the two were in fact the same. Unidentified digeneans found in the intestine of *X. laevis* by Cosgrove and Jared (1974) were probably *O. elianae*. Tinsley and Jackson (1995) reviewed the taxonomy, host range and geographical distribution of *Oligolecithus* Vercammen-Grandjean, 1960.

Grobbelaar (1922), Weinbrenn (1925), Chait (1938) and Elkan (1960) reported the presence of an unidentified adult digenean in the gall bladder of *X. laevis*. The parasite was identified as ***Xenopodistomum xenopodis*** sp. nov. by Macnae *et al.* (1973). Tinsley and Owen (1979) reported on the morphology, biology and infection levels of the parasite.

Progonimodiscus doyeri (Ortlepp, 1926) (Digenea: Paramphistomidae) was first described by Goeze (1787) as *Planaria subclavata*, but after discovery of the parasite in the rectum of *X. laevis*, Grobbelaar (1922) named the parasite *Diplodiscus subclavatus* according to the genus created by Diesing (1835). In 1926, Ortlepp described the parasite as a new species naming it *Diplodiscus doyeri*, and in 1960 it was described as a new species *Progonimodiscus doyeri victoriani* from *X. l. victorianus* by Vercammen-Grandjean. Pritchard (1964) finally named the parasite *P. doyeri*, and later publications also confirmed all the species to be synonymous (Macnae *et al.*, 1973; Bourgat, Roure & Kulo, 1996). Jackson and Tinsley (1998b) discussed the taxonomy, host-specificity and biogeography of the parasite.

The presence and description of ***Tylodelphys xenopi*** (Nigrelli & Maraventano, 1944) (Trematoda: Diplostomidae), a strigeid metacercaria occurring freely in the pericardial sac of *X. laevis*, was first recorded by Southwell and Kirshner (1937), who also commented on the parasite's considerable longevity. It was redescribed and named *Diplostomulum xenopi* by Nigrelli and Maraventano (1944) who also reported on the infection levels and pathogenity of the parasite. Vercammen-Grandjean (1960) described the parasite from *X. l. victorianus* as *Diplostomulum victorianus* and reported a high prevalence. Macnae *et al.* (1973) concluded that all previous accounts referred to the same parasite, and also reported very high infection levels possibly causing parasites to spread to the body cavity. Tinsley and Sweeting (1974) reported a slightly lower prevalence but very high numbers, and after studying the biology and taxonomy of the parasite they renamed it *Diplostomulum (Tylodelphylus) xenopodis*. The publication also mentioned seasonal variance in infection levels, differences in male and female burdens

and parasite longevity and pathology. The population structure of the parasite was discussed by Tinsley (1996a). In 1997, King and Van As described the life cycle of the parasite, and on the grounds of the adult morphology concluded that the parasite does indeed possess tylodelphid characters and proposed the species as *Tylodelphys xenopi* n. comb. (Trematoda: Diplostomidae).

Limited information is avaialable on the rest of the digenean metacercaria infecting *X. laevis*. Porter (1938) described ***Echinostomum xenopodis*** and ***Cercaria xenopodis*** from the eyes and lateral line, and ***Opisthioglyphe xenopodis*** from under the skin of *X. laevis* tadpoles. ***Neascus* sp.** was reported to cause deaths among *X. laevis* in captivity by encysting in the dermis below the lateral line organs (Elkan & Murray, 1952). Macnae *et al.* (1973) reported on the presence of all four metacercaria, as well as ***Clinostomum* sp.** found in the intermuscular lymph cavities, behind the peritoneal membrane and on the surface of the lungs.

The only adult cestode known to infect *X. laevis* is the pseudophyllidean ***Cephalochlamys namaquensis*** (Cohn, 1906) Blanchard, 1908. The parasite was first described as *Chlamydocephalus namaquensis* by Cohn (1906) who reported high burdens, and in 1926 as *Dibothriocephalus xenopi* n. sp. by Ortlepp. Southwell and Kirshner (1937) found the parasite in almost all toads examined, and confirmed it to be *Cephalochlamys namaquensis* according to the genus created by Blanchard (1908), all the other names being synonymous. Mettrick (1960 & 1963) reviewed other synonyms and the history of the species. Publications by Elkan (1960), Pritchard (1964) and Thurston (1970) all included information on the infection levels of the parasite. Thurston

(1967) discussed the morphology and life cycle of *C. namaquensis* infections in *X. laevis* and *X. muelleri*, including population structure and infection levels in hosts of different sizes. The infection levels of the parasite were also included in publications by Macnae *et al.* (1973) and Cosgrove and Jared (1974). Ferguson and Appleton (1988) and Tinsley (1996a) discussed some aspects of the population structure.

Camallanus kaapstaadi Southwell & Kirshner, 1937 (Nematoda: Camallaninae) was described after being found in the stomach from *X. laevis*. In 1970, Thurston reported on the presence of a camallanid *Camallanus johni* Yeh, 1960. Cosgrove and Jared (1974) found the nematode in the stomach and oesophagus. Jackson and Tinsley (1995b) gave an overview of the genus *Camallanus* and sited the oesophagus as only infection site. A new species, ***Camallanus xenopodis***, was also described from the intestine of *X. laevis* by the same authors. They also discussed the possibility of *C. johni* being the same as *C. kaapstaadi*, but it is currently considered *species inquirenda*.

In 1937, Southwell and Kirshner described a new nematode from the stomach as *Procamallanus slomei*. Thurston (1970) reported the presence of *Spirocamallanus xenopodis* (Baylis, 1929) Olsen, 1952 in the stomach of *Xenopus* sp. Both these species were put in a newly created genus, *Batrachocamallanus* (Jackson & Tinsley, 1995c) as the same species, ***Batrachocamallanus slomei*** (Nematoda: Procamallaninae).

Cosgrove and Jared (1974) first reported the presence of a species of *Capillaria* in the skin of 32% of frogs examined (n = 435). In 1982, Moravec and Cosgrove described and named the parasite ***Pseudocapillaroides xenopi*** gen. et sp. nov. (Nematoda: Capillariidae). In the same year Wade (1982) named the species *Capillaria xenopodis* (Nematoda: Trichuroidea). In subsequent publications, the parasite was referred to as *Capillaria xenopodis* (Cohen, Effridge, Parsons, Rollins-Smith, Nagata & Albright, 1984) and *Pseudocapillaroides xenopodis* (Tinsley, 1996a).

Thurston (1970) gave the only account of **microfilariae** found in the blood vessels of a single *Xenopus* sp. The parasites were never identified and not encountered again.

The only acarinid parasite of *X. laevis* is the mite ***Xenopacarus africanus*** Fain, Baker & Tinsley, 1969 (Ereynetidae: Trombidiformes) found in the nostrils and eustachian passages. Cosgrove and Jared (1974) reported a low prevalence of 2.5%. Fain and Tinsley (1993) discussed the evolutionary relationships and differences between the three *Xenopacarus* species.

To date, the only leech known to infect *Xenopus* was ***Marsupiobdella africana*** Goddard & Malan, 1912 (Hirudinea: Glossiphoniidae). It is unique in the presence of a brood pouch in which eggs and young are protected (Goddard & Malan, 1912 & 1913). Subsequent publications gave better descriptions of the parasite morphology and behaviour (Moore, 1958; Dick, 1959; Soós, 1969; Sawyer, 1971). The most extensive publication on the leech included studies on its anatomy, life history and behaviour (Van der Lande & Tinsley, 1976).

Ecologists have the past few years put an emphasis on the worth of biodiversity and the preservation thereof. Investigating the diversity of parasites associated with *Xenopus* therefore formed an important part of this study. This paid off, as a new parasite was found despite the extensive studies done on *Xenopus* previously. Cyclophyllidean plerocercoid cestode larvae infected the bile ducts of some hosts. It was first found by Kok[1] (*pers. comm.*) in 1988, and never again encountered until now. It was identified as *Valipora campylancristrota* (Wedl, 1955) (Cestoda: Dilepididae). A leech found on the external surface of *X. laevis* was initially thought to be a new species, but was preliminary identified as the juvenile form of *Marsupiobdella africana*.

Prudhoe and Bray (1982) stated that even though frogs and toads have been extensively used as study material for parasitological research, no comprehensive study exists of the relationship between parasite infection levels and the ages, habitat and habits of the host. Esch and Fernández (1993) did comprehensive work on the array of variables that affect the numbers and kinds of parasites present in an individual host, host population or community. The main aim of their study was to gain a fundamental understanding of the functional biology of parasites. Although the study did not include much in respect of anuran parasitology, very important and interesting factors that affect parasite-host interaction were identified and discussed. Tinsley (1990) gave a general account on the influence of seasonal temperature changes on helminth egg production, and Tinsley (1995) discussed some factors regulating infection levels of parasites in *X. laevis*. The reproductive output of the monogenean *Pseudodiplorchis americanus* from

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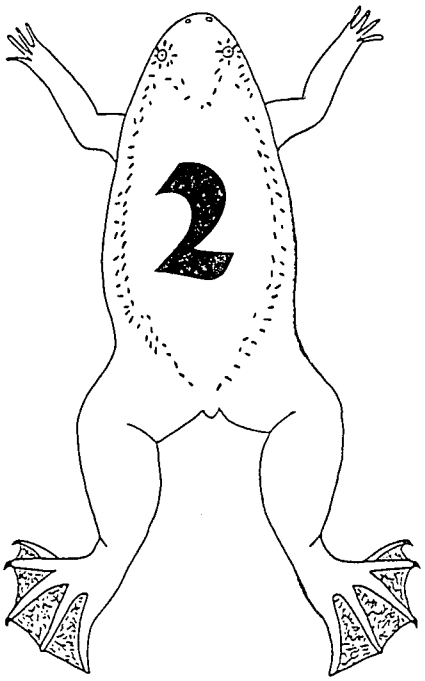
Scaphiopus couchii and the effect of temperature cycles thereupon were discussed by Tocque and Tinsley (1991a&b). Tinsley (1993) identified several factors influencing the population biology of polystomatid monogeneans.

The main aim of the current study was to determine whether the diversity and population dynamics of parasites associated with *X. laevis* are influenced by natural variables, with emphasis on climate, ecology and host-size, which also indicates the age of the host. In spite of the fact that *Xenopus* and its parasites have been studied for decades, very little information is available on parasite population dynamics under natural conditions. In previous studies, frogs were sometimes dissected after a relatively long time in captivity, where other factors such as diet, time and crowding may have influenced infection levels.

It was hypothesised that parasite infection levels and diversity in *Xenopus laevis* are influenced by:

1. The specific habitat in which the host occurs.
2. Seasonal climatic changes.
3. The size, and therefore age of the host.
4. The sex of the host.



The Host

Xenopus laevis

The African clawed frog, *Xenopus laevis* (Anura: Pipidae) and other species in the genus, have been extensively utilised in the past for a variety of research projects. These include physiological, biochemical, endocrinological, parasitological and developmental biology studies, of which much were initially based exclusively on *Xenopus laevis*. The frog is perhaps best known for its use in pregnancy assays in humans (Shapiro & Zwarenstein, 1934) until about 50 years ago. Modern research on *Xenopus* includes biomedical and genetic studies, for which it is particularly useful because of its relatively short life cycle. Parasitological research has been and still is one of the facets of science for which *Xenopus* has proven extremely useful as study material. Undergraduate studies in biology usually include a course on parasitology for which *Xenopus* is often used because of its rich parasite diversity. *Xenopus* has also proven to be a successful laboratory animal because of its relative ease of collection, resistance to disease and infection and its ability to successfully breed in captivity.

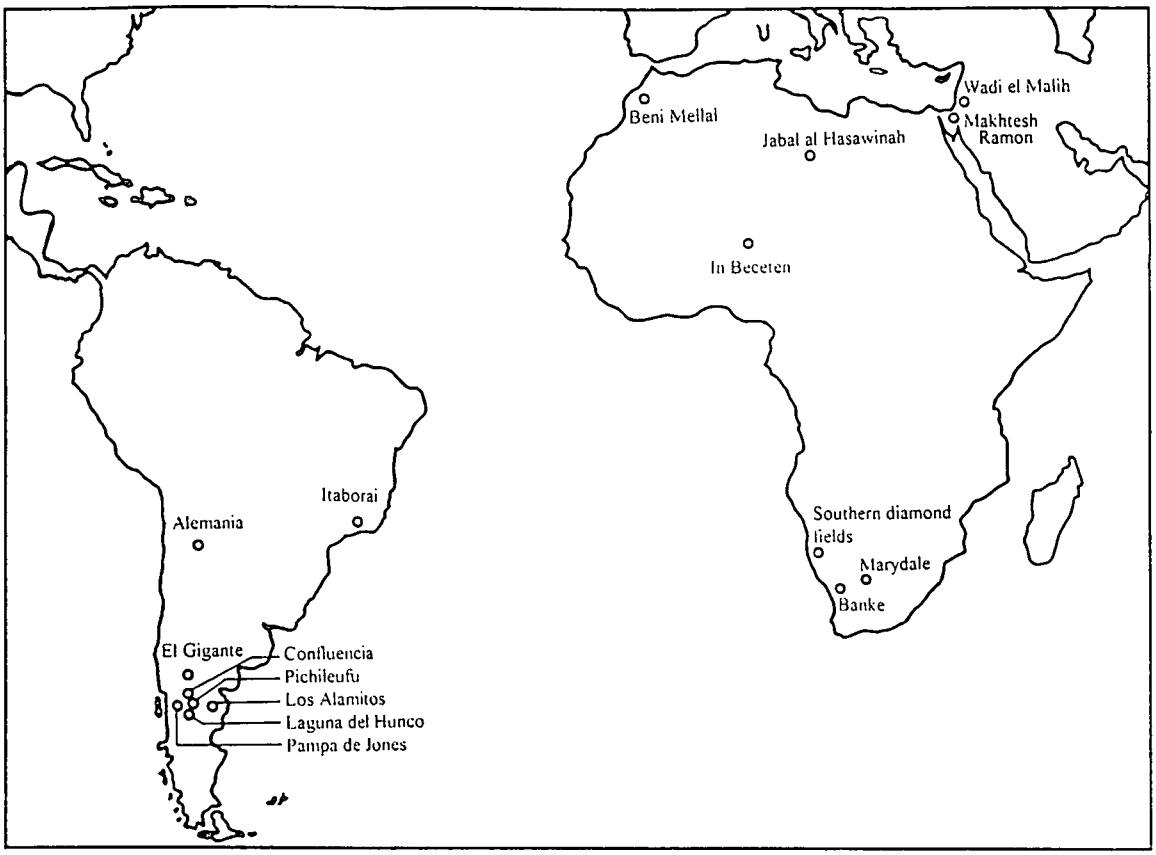
Pipids are archaic and not aligned with advanced families, representing an early specialised offshoot in Anuran evolution (Tinsley, 1981). Anuran fossil records are generally considered poor compared to those of other vertebrates, but pipids are one of the families with the best paleontological record available. The fossil record of primitive pipoids is from the Early Cretaceous (120 Ma) on the Arabian Peninsula of the Near East, and falls within the tropical belt which existed at that time. All other fossils of the Pipidae have been found in either Africa or South America (Fig. 2.1), where they still occur today. These continents formed part of Western Gondwanaland, but when the oldest pipids existed the supercontinent had already begun to split up, with only a small link existing between Brazil and western Africa as early as 110 Ma (Fig. 2.2).

Figure 2.1

Pipid fossil sites (modified from Baéz, 1996).

Figure 2.2

Cretaceous and Palaeogene pipid fossil sites (modified from Baéz, 1996).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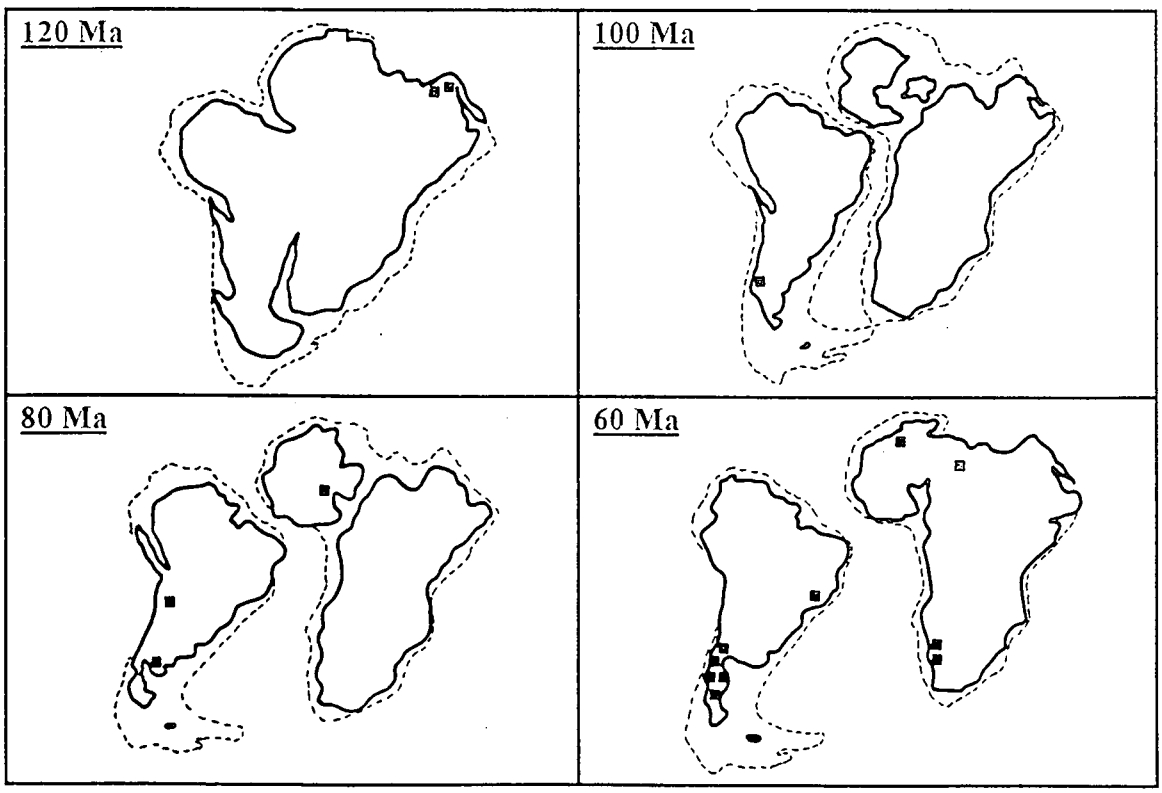
Makhtesh Ramon
Jabal al Hasawinah
In Beceten
Itaborai
Alemania
Southern diamond fields
Marydale
Banke
El Gigante
Confluencia
Pichileufu
Los Alamitos
Laguna del Hunco
Pampa de Jones


120 Ma
100 Ma
80 Ma
60 Ma

A pattern of vicariance and subsequent endemism is therefore expected (Báez, 1996), and the subfamily Xenopodinae, containing only the genus *Xenopus* is indeed endemic to Africa.

According to Kobel, Loumont and Tinsley (1996), the genus *Xenopus* currently consists of 22 species and subspecies (Table 2.1), the number of known species having trebled in the past 20 years due to sampling in more remote parts of Africa and detailed analysis of live specimens. A remarkable number of polyploid species can be found within the genus, representing ploidy levels of 2, 4, 8 and 12 based on two chromosome sets of 10 and 18. This forms the basis of defining different species, together with other criteria such as mating calls and experimental hybridisation. By utilising additional morphological and biochemical qualities, the genus *Xenopus* can be divided into two distinct groups - the *Silurana* subgenus (consisting of *X. (Silurana) tropicalis* (Gray, 1864) 2n=20 and *X. (Silurana) epitropicalis* Fischberg, Colombelli & Picard, 1982 2n=4X=40) and *Xenopus* (2n=4X=36, 8X=72 and 12X=108). The latter can be divided into five more groups:

1. The *laevis*-subgroup (A in Table 2.1).
2. The *muelleri*-subgroup (B in table 2.1).

   Species in groups 1 and 2 are all tetraploid.
3. The *fraseri*-like subgroup (C in Table 2.1).
4. Two closely related octoploid species (D in Table 2.1).
5. The dodecaploid *X. longipes* (E in Table 2.1).

Table 2.1 The extant *Xenopus* species and some interspecific differences (modified from Kobel *et al.*, 1996).

	Species	2n	Female size (mm) [Max]	Lateral-line organs (Dorsal)	Subocular tentacle length	Proportion of eye covered by lower eyelid
	Silurana					
	X. (s.) tropicalis	X=20	43 [55]	18-23	Medium	< 1/3
	X. (s.) epitropicalis	4X=20	64 [72]	18-23	Medium	< 1/3
	Xenopus					
A	*X. laevis*					
	X. l. laevis	2X=36	110 [130]	25-34	Short	3/4
	X. l. petersi	2X=36	65 [66]	20-25	Medium	1/2
	X. l. poweri	2X=36	70 [85]	19-24	Medium	1/2
	X. l. victorianus	2X=36	62 [78]	19-25	Short	< 3/4
	X. l. sudanensis	2X=36	62 [64]	18-24	Short	1/2
	X. gilli	2X=36	55 [60]	20-24	Absent	1/2
	X. largeni	2X=36	50 [55]	18-19	Absent	< 1/3
B	*X. meulleri* (East)	2X=36	65 [75]	22-27	Very long	3/4
	X. muelleri (West)	2X=36	53 [90]	19-25	Long	3/4
	X. borealis	2X=36	73 [95]	23-30	Medium	3/4
	X. clivii	2X=36	70 [82]	23-28	Medium	3/4
C	*X. fraseri*	2X=36	42 [51]	18-21	Long	< 3/4
	X. pygmaeus	2X=36	35 [44]	15-20	Long	1/2
	X. amieti	4X=72	53 [57]	14-23	Medium	1/2
	X. andrei	4X=72	40 [45]	14-22	Long	1/2
	X. boumbaensis	4X=72	46 [54]	17-21	Medium	3/4
	X. ruwenzoriensis	6X=108	55 [57]	17-21	Medium	1/2
D	*X. vestitus*	4X=72	47 [55]	18-28	Medium	1/3
	X. wittei	4X=72	46 [61]	18-25	Medium	1/2
E	*X. longipes*	6X=108	34 [36]	15-24	Medium	1/3

The validity of the taxonomic status of some species are still under investigation, particularly the justification of the six *X. laevis* subspecies. The species used in this study is in fact one of the subspecies, *X. l. laevis* (Daudin, 1803), but will be referred to as it is more commonly known - *X. laevis*. It is the most distinct subspecies and also the largest, with the average female snout-vent length being 110 mm up to a maximum of 130 mm, and the male about 83 mm up to a maximum of 98 mm. The frog has a dorsal colour pattern ranging from finely spotted to marbled or with larger or irregular spots in tints of yellowish to dark tan. Ventrally *X. laevis* is immaculate white-yellowish to densely spotted. The tibia is significantly shorter than the fifth toe (Kobel *et al.*, 1996).

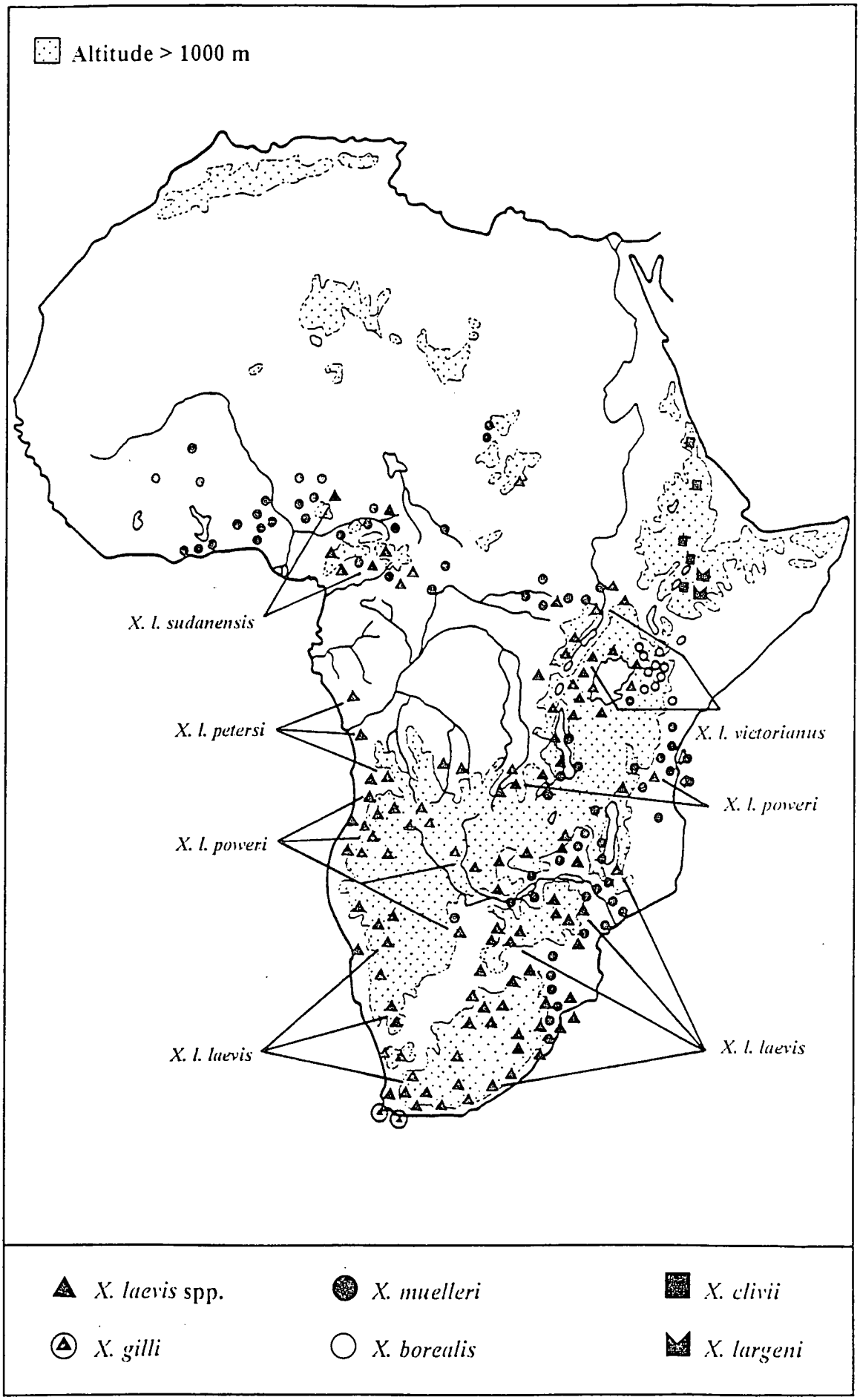
Xenopus is primarily water-living and it occupies almost every kind of water body in sub-Saharan Africa, including swamps, dams, man-made irrigation ditches, wells and reservoirs, and even fast-flowing rivers (Kobel *et al.*, 1996). Figure 2.3 shows the distribution of some *Xenopus* species in Africa. *X. laevis* is distributed over a large area in southern Africa, corresponding to the relatively cooler highland areas, but is excluded from much of the hotter eastern parts because of low tolerance to high temperatures. *X. laevis* does however have the ability to survive extreme temperatures by hibernation or aestivation. Significant variation exists in the chemical composition of the aquatic habitats utilised, with *Xenopus* being able to tolerate salinity and pH irregularities in varying degrees. *X. laevis* for example, can survive 40% seawater for several days, and *X. vestitus* is able to tolerate pH between 5.6 and 8.7. *X. gilli* is found in the acidic black-waters of the fynbos biome in the Cape, South Africa, surviving pH as low as 3.4 (Tinsley, Loumont & Kobel, 1996).

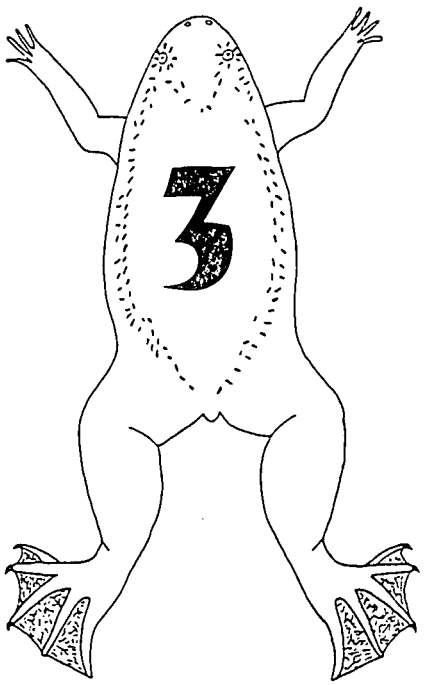
Aquatic invertebrates form the principal part of the *Xenopus* diet, but it will eat almost anything. *Xenopus* is more or less a non-selective predator, but cannibalism and scavenging also occur. The frog catches its prey with toothed jaws, and uses its forelimbs to fork the food into its mouth. Because *Xenopus* lacks a tongue it is less bound by the size of the prey, and uses its clawed hindlimbs to shred its prey. In accordance with the frog's ability to hibernate and aestivate, it also has a remarkable ability to tolerate starvation. Predators of *Xenopus* include fish, birds and otters. It is also used by man as a food source and for aphrodisiac and fertility medicines (Kobel *et al.*, 1996). In southern Africa, *Xenopus* is extensively used as bait by anglers fishing for catfish.

X. laevis reaches sexual maturity at approximately eight months in favourable conditions, and its natural life expectancy is about nine years, although records exist of 15 and 20 years survival in captivity (Kobel *et al.*, 1996). The success of *Xenopus* as a laboratory animal, its wide distribution and its ability to tolerate unfavourable conditions, emphasises its evolutionary success.

Figure 2.3

The geographical distribution of *Xenopus* species in savanna habitats (modified from Tinsley, Loumont & Kobel, 1996).


Altitude > 1000 m
X. l. sudanensis
X. l. petersi
X. l. poweri
X. l. victorianus
X. l. poweri
X. l. laevis
X. l. laevis
X. laevis spp.
X. muelleri
X. clivii
X. gilli
X. borealis
X. largeni



Study Area, General Materials & Methods

CONTENTS

3.1 STUDY AREA

For the purpose of the study, two earth-walled dams were selected on the outskirts of Bloemfontein in the Free State Province, South Africa (Fig. 3.1). Bloemfontein falls within the highveld, a characteristic grassland ecosystem. Although the water level of both dams decreased significantly at times, neither had dried up completely during the course of the study. This did however influence the availability of hosts at some point.

3.1.1 LOCALITY A

The one dam (locality A), was just outside the urban area at co-ordinates 29° 04' 20" S and 26° 14' 38" E and altitude 1441 m (Fig. 3.2A). The dam had no permanent source of water, and surrounding vegetation was typical of the dry sandy highveld biome (Low & Rebelo, 1996), with a variety of grasses dominating and very few trees. The watergrass, *Potamogeton thunbergii* was growing abundantly in the dam. The closest houses were only 400 m away, and human activity around the dam was evident. The grass around the dam was burned during both winters during the study period. Evidence existed of people using the dam for recreation, for example fishing, and hobos stayed in the bushes next to the dam. The dam was however in a relative good condition, with duck breeding in the water during spring and summer. However, bigger birds such as herons were not observed as often as at locality B, but no scientific evidence exists of a higher abundance at locality B.

3.1.2 LOCALITY B

The other dam (locality B) at co-ordinates 29° 05' 20" S and 26° 10' 28" E and altitude 1456 m (Fig. 3.2B&C), was situated on a farm approximately 1.5 km from the urban area. It formed part of a series of dams in a valley known as the "Valley of seven dams". The habitat was much less disturbed than locality A, the water much clearer, and no human interference was evident. The dam was surrounded by hills with lush vegetation and an abundance of trees and birds, including big waterbirds. The dam was larger and much deeper than locality A, and the surface was completely covered by the red waterfern, *Azolla filiculoides*, most of the time. The dam also had no permanent source, and was mainly fed by water from storm water drains in the northern suburbs of Bloemfontein.

3.1.3 CLIMATE

The climate was typical of the highveld region. Air temperatures at the study localities were relatively high in the summer months, and low during the winter (Fig. 3.3). Average temperatures ranged between a maximum of 33.4° C and a minimum of -3.1° C. During the study period, the highest temperature recorded was 39.4° C in December 1997, and the lowest -8.8° C in June 1996. The rainfall patterns corresponded to that of a summer rainfall region, with little or no rain during the winter months (Fig. 3.4). A maximum rainfall of 220.6 mm was recorded in January 1998 and a minimum of zero in June 1996.

Figure 3.1

Aerial photograph of the two study localities (18/8/1992).

Scale bar = 1 km.

Abbreviations: A, locality A; B, locality B.


N
B
A

Figure 3.2

Photographs of the two study localities.

A) Locality A.

B) Locality B.

C) Locality B covered with *Azolla filiculoides*.

A

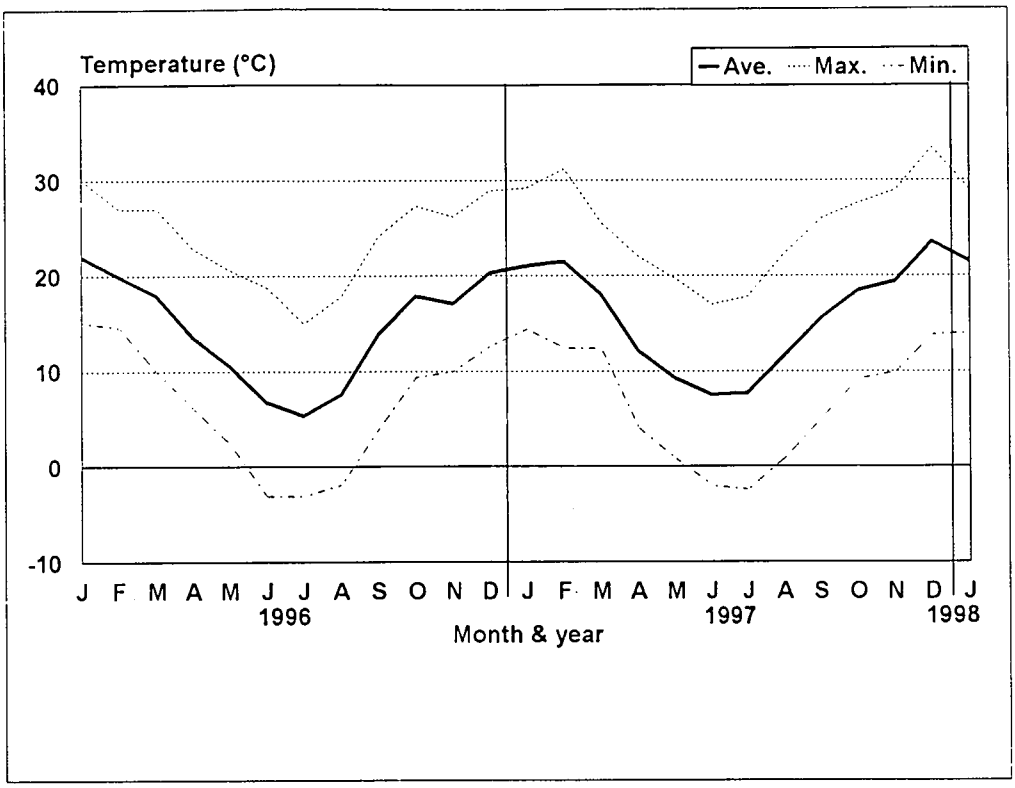
B

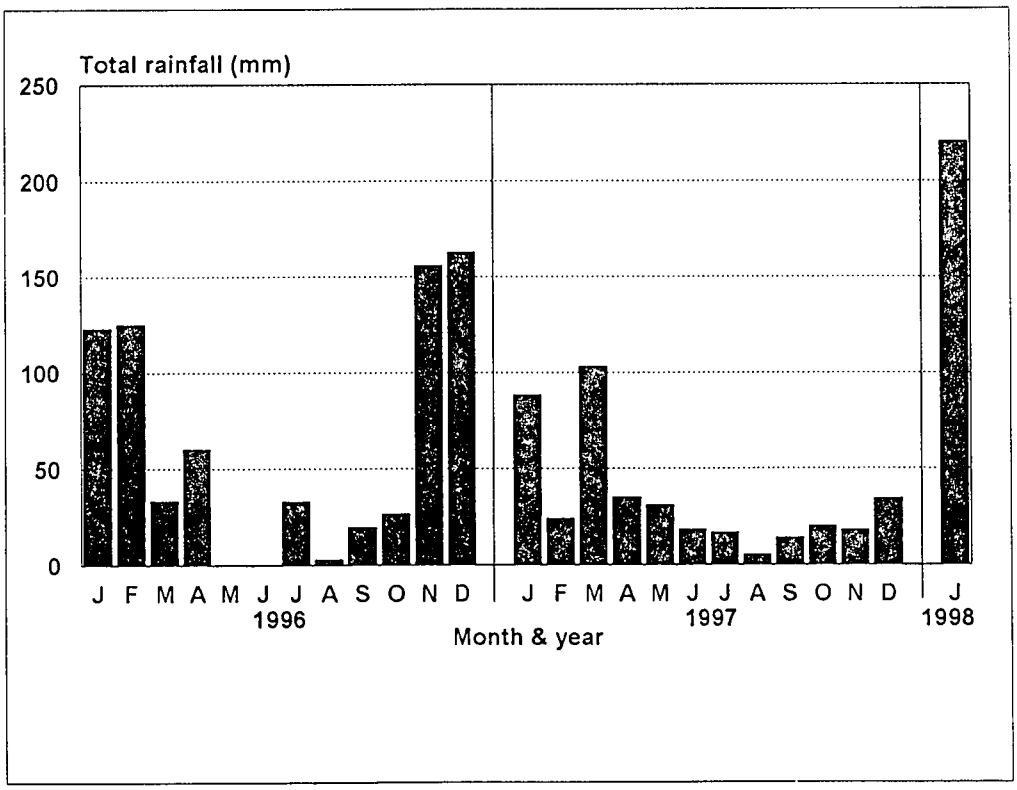
Figure 3.3

Line graph showing the average monthly temperatures for the study localities.

Figure 3.4

Bar graph showing the monthly rainfall for the study localities.


Temperature (°C)
Ave.
Max.
Min.
40
30
20
10
0
-10
J F M A M J J A S O N D J F M A M J J A S O N D J
1996
1997
1998
Month & year


Total rainfall (mm)
250
200
150
100
50
0
J F M A M J J A S O N D J F M A M J J A S O N D J
1996
1997
1998
Month & year

3.2 COLLECTION OF HOSTS

Specimens of *X. laevis* were collected by means of home-made traps (Figs. 3.5). They consisted of 20 litre plastic buckets with a 160 mm hole cut in the side. A cone-shaped funnel, made from galvanised sheeting which was painted black, was fitted in the hole with the narrow end of the cone inside the bucket and slightly pointing upwards. The funnel was approximately 220 mm long, and the small opening inside the bucket 50 mm in diameter. A few air holes were drilled into the bottom of the bucket. Soup bones were put inside the trap as bait, the lid of the bucket firmly closed, and the trap put into the water in an inverted position for 24 hours. Approximately seven traps were put out at a time. The buckets were put upside down in water shallow enough to let the bottom with the holes stick out above the water surface. This allowed specimens caught in the traps free access to air. Traps were covered with vegetation to prevent the water inside from reaching a lethal temperature during the daytime.

X. laevis were collected from the two sites during different seasons over a two year period. Table 3.1 contains the dates collections were made, the sample size, and the average snout-urostyle length of hosts in each sample. In total, 12 samples were taken from site A, and 11 from site B. The collections were made in such a way to have data for every month after a two year period. Unfortunately, the water-loss from locality B at the end of 1997 caused that only four specimens could be collected for November, and none during December. In June 1997, only eight frogs were collected from site A.

Figure 3.5

Home-made trap used to collect hosts.

A) Top-view photograph of trap with *Xenopus laevis* inside. Abbreviation: f, funnel.

B) Author setting trap at locality B.

C) Trap set at locality B.

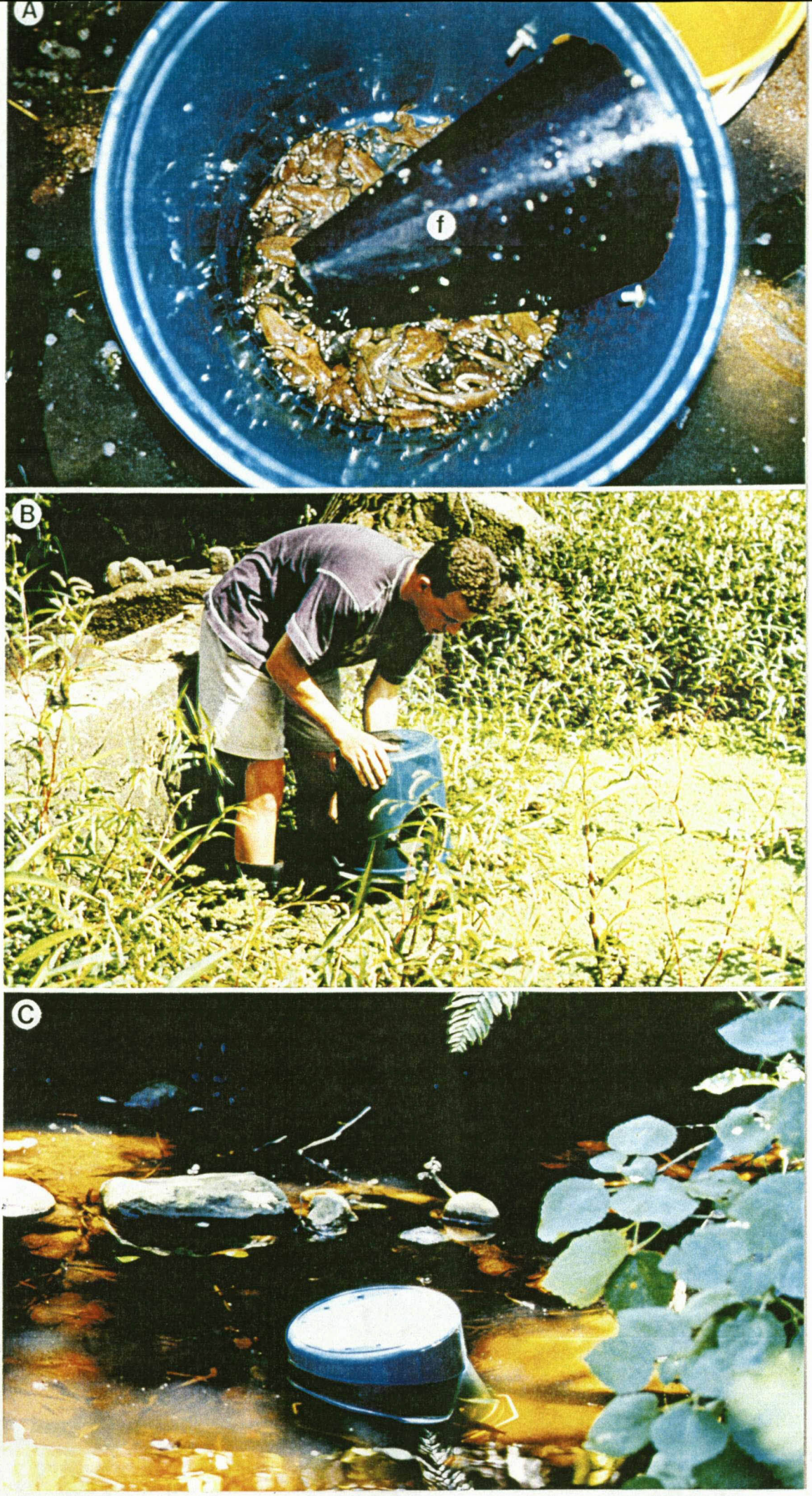

A
f
B
C

Table 3.1 Summary of the monthly data of hosts collected.

Month and Year	Locality A		Locality B	
	Sample size (n)	Average snout-urostyle length (mm)	Sample size (n)	Average snout-urostyle length (mm)
February 1996	10	62.9		
March 1996			10	81.7
April 1996	10	74.2	10	58.3
May 1996	10	78.4		
June 1996			10 (22*)	76.8 (79.2*)
July 1996	10	80.5		
August 1996			10	93.6
October 1996	10	78.5	10	82.4
January 1997	10	58.5		
February 1997			10	62.8
March 1997	10	74.4		
May 1997			10	72
June 1997	8	41.9		
July 1997			10	53.8
August 1997	10	70.1		
September 1997	10	74.6	10	59.2
November 1997	10	38.4	4	51.1
December 1997	10	53.1	0	0
January 1998			10	51.7
TOTAL	118		104 (116*)	
$\overline{X}$		65.8		68.5 (69.8*)

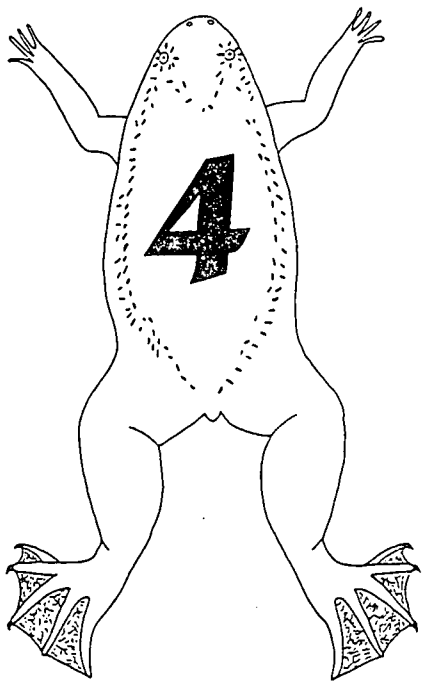
(* 12 additional frogs were examined for only two parasites - *Protopolystoma xenopodis* and *Valipora campylancristrota*.)

3.3 DISSECTION OF HOSTS

Specimens collected from the traps were transported to the laboratory in a bucket containing dam-water, in which they were also kept until dissected within days. After being anaesthetised with Benzocaine or MS 222 (Sandoz), the snout-urostyle length, head width and mass were determined. The frogs were examined for the presence of external parasites, and then dissected completely to determine the diversity and number of parasites in the body cavity and organs. All the information was transcribed on a data sheet (Appendix 1). The host tissue was continuously kept moist with, and dissections done in a 0.6% Amphibian Saline solution.

3.4 PARASITE INFECTION LEVELS

To quantify the infection levels of parasites infecting *X. laevis*, the prevalence and mean intensity were determined for each parasite species in each sample taken in a specific month (chapter 5), and also for the total samples taken from locality A and B respectively (chapter 4). Prevalence is defined as the percentage of hosts in a sample infected with a particular parasite, and mean intensity is the mean number of individuals of a specific parasite species per infected host in a sample (Margolis, Esch, Holmes, Kuris & Schad, 1982). Another term used to quantify infection levels in chapter 4 is abundance, which is defined as the mean number of individuals of a particular parasite species per host examined. Abundance therefore equals the total number of a particular parasite species in a sample of hosts, divided by the total number of hosts (infected and uninfected) in the sample. Although this concept causes a problem with respect to its terminology, often being used as a more general term with no quantitative meaning (Margolis *et al.*, 1982), it proved useful in the statistical analysis of infection levels where a mean was required.



Aspects of the Morphology & Biology of *Valipora campylancristrota* & *Marsupiobdella africana*

CONTENTS

4.1 INTRODUCTION

Despite extensive research that has been carried out on *Xenopus* and its parasites, a parasite not previously known to be associated with *Xenopus laevis* was found. The parasite was first noticed by Kok[1] (*pers. comm.*), but this is the first time its occurrence in *X. laevis* is documented. A cyclophyllidean plerocercoid was found in the bile ducts, and identified as *Valipora campylancristrota* (Wedl, 1855) (Cestoda: Dilepididae). An unknown leech was found on the external surface of *X. laevis*, and was preliminarily identified as the juvenile form of *Marsupiobdella africana*.

Compared to representatives of the Monogenea and Digenea, cestodes are rarely found in amphibians. To date, only one adult cestode is known to infect *Xenopus*. The pseudophyllidean, *Cephalochlamys namaquensis*, is found relatively frequently in the intestine of the frog (Tinsley, 1996a; see also Chapter 5). Only two reports on the occurrence of larval cestodes exist. Thurston (1970) found encysted plerocercoids on the intestine of *Xenopus* sp., with one heavily infected host bearing 62 cysts. Encysted cyclophyllidean cyticerci were found on the gut and mesenteries of 80% of *X. laevis* examined by Macnae *et al.* (1973).

The only leech known to infect *Xenopus* is *Marsupiobdella africana*. It has a low prevalence, and is found concentrated around the cloaca and upper hind limbs. The leech protects its eggs and young in a unique brood pouch on its ventral surface.

---

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4.2 MATERIALS & METHODS

4.2.1 PREPARATION OF MATERIAL FOR MORPHOLOGICAL STUDIES

a) Light microscopy

i. *Fixation*

To prepare permanent and temporary mounts, parasites were first flat fixed under coverslip pressure in 70% ethanol (EtOH) or 10% neutral buffered formalin (NBF). Additional pressure was applied by lead weights (± 13.5 g each) when fixing leeches. For histological sectioning specimens were fixed in Bouin's fixative, and then dehydrated to 70% EtOH.

ii. *Permanent mounts*

Before staining with Alum Carmine, specimens fixed in 70% EtOH were hydrated to 30% EtOH. Specimens fixed in 10% NBF were first transferred to water and then dehydrated to 30% EtOH. Specimens were stained in Alum Carmine for 12 hours and if necessary destained with 3N HCl, after which they were dehydrated in an ethanol series. After dehydration, specimens were transferred to a 50:50 solution of xylene and absolute ethanol and then cleared in xylene (2 X 20 min). The cleared specimens were finally mounted in Eukitt or Canada Balsam.

iii. *Temporary mounts*

To study scleritised parts, specimens were partially cleared in lactophenol (Humason, 1962) or ammonium picrate solution (adapted from Malmberg, 1956). Live parasites and specimens fixed in either 70% EtOH or 10% NBF were used for ammonium picrate or lactophenol temporary mounts. Ammonium picrate solution was prepared by mixing nine parts 10% NBF with one part glycerine. One drop of picric acid was added for every 10 ml of the solution. The coverslip was kept in position using clear nail varnish.

iv. *Histological sections*

Fixed material imbedded in paraffin wax was sectioned at 9µm on a Reichert Yung motorised microtome. Sections were stained with Mayer hematoxylin and eosin (Humason, 1962).

v. *Photography*

All specimens were examined on a Nikon Alphaphot compound microscope. Micrographs were taken on a Nikon Eclipse E800 compound microscope fitted with a HIII Nikon 35 mm camera using ISO 100 Fujichrome colourfilm or ISO 50 black and white film.

b) Scanning electron microscopy

Parasites were fixed in warm or cold Flemming's solution (Van Niekerk, Els & Krecek, 1987), 70% EtOH or warm 10% NBF, and cleaned in an OMO detergent solution or phosphate buffer in an ultrasonic bath. Specimens were dehydrated in an ethanol series and dried in a Polaron E3000 critical point drier. Dried material was mounted with epoxy resin (Pratley Clear) on 12 mm aluminium stubs or custom made conical brass stubs and gold-coated in a Polaron E5000 sputter coater. The specimens were finally examined in a JEOL 6400 scanning-electron microscope at 5 or 10 kV. Photographs were taken using ISO 50 or ISO 100 black and white film.

4.2.2 INFECTION SITE AND LIFE CYCLE OF THE CESTODE LARVA

To determine whether the parasite had any preference for certain parts of the bile duct system, it was necessary to establish the configuration of the system. This was achieved by injecting liquid latex rubber (Boscotex) into the bile duct system of a dissected frog.

To study the life cycle and determine the possible final host of the cestode, two juvenile black-headed herons, *Ardea melanocephala*, were removed from their nests in the wild and raised in captivity (Fig. 4.1A). The birds were initially force-fed (Fig. 4.1B), but started taking food after about one week. *A. melanocephala* were used because the Ardeidae are known final hosts of *Valipora* (Bona, 1993). *X. laevis*, infected with the cestode larvae, were fed to the birds on a regular basis. The rest of their diet consisted of fish, which was first frozen to prevent the transmission of unwanted parasites.

The faeces of the birds were screened for the presence of proglottids. An attempt to determine the first intermediate host by feeding oncospheres to copepods was unsuccessful, as the establishment of a copepod culture in the laboratory failed. The birds were dissected after approximately four months and all cestodes removed from the intestine and fixed for light- and scanning electron microscopy.

4.2.3 IDENTIFICATION OF CESTODES

As the rostellar hooks of cestodes have an important taxonomic value, the size and shape of the hooks of larval cestodes from *X. laevis* and adult cestodes from the herons were determined. In total, 20 large and 20 small hooks of both larvae and adults were measured using an eyepiece micrometer. Three measurements were taken from each hook (Fig. 4.2). Measurement L, the length of the hook, was taken from the tip of the blade to the extremity of the handle. Measurement G was taken from the tip of the blade to the tip of the guard, and H from the tip of the guard to the extremity of the handle.

4.2.4 SITE OF ATTACHMENT OF LEECHES

To determine the preferred site of attachment of the leech on *X. laevis*, four frogs were experimentally infected with one, three or four leeches. The frogs were kept in separate 4 litre containers with approximately 2 litres of water. The movements of the leeches were monitored until all had fallen off.

Figure 4.1

Keeping of two black-headed herons, *Ardea melanocephala*.

A) Photograph of *A. melanocephala* in cage.

B) Photograph showing initial force-feeding of *A. melanocephala*.

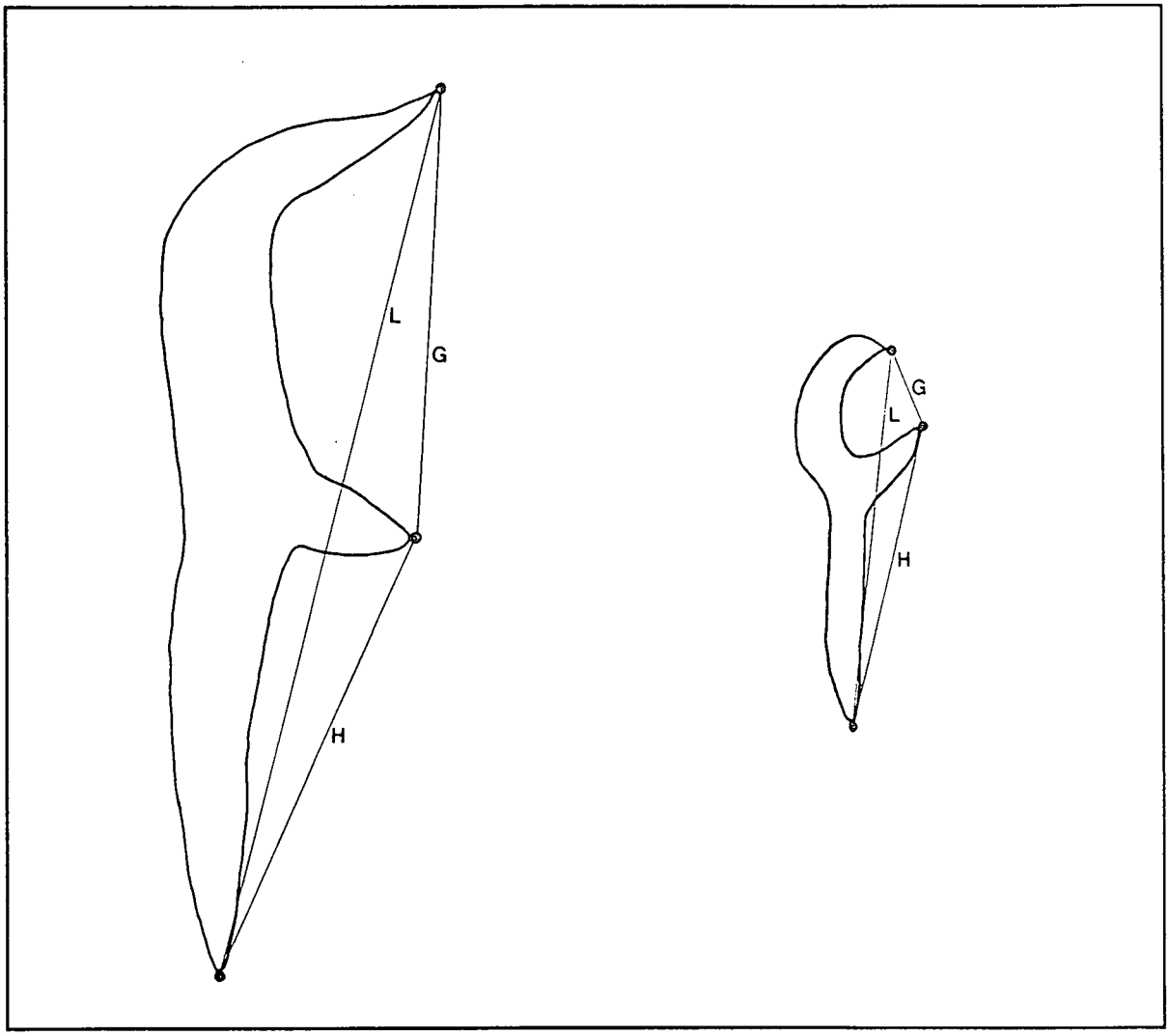

A


B

Figure 4.2

Measurements taken from large and small rostellar hooks.

(Hooks not drawn according to shape or to specific scale.)


L
G
H
G
L
H

4.3 RESULTS

4.3.1 *Valipora campylancristrota*

a) Infection site

A cestode larva was found in the bile ducts of *X. laevis*, one of the few sites in the frog not previously known to be utilised by parasites. The parasite had a relatively low prevalence, but sometimes occurred in high numbers in individual hosts (see Chapter 5). These plerocercoids were not encysted like most metacestodes, but were able to move freely within the bile ducts. Where more than one parasite occurred close together, a thickening of the duct was often caused. The parasite was not encountered in the gall bladder or intestine.

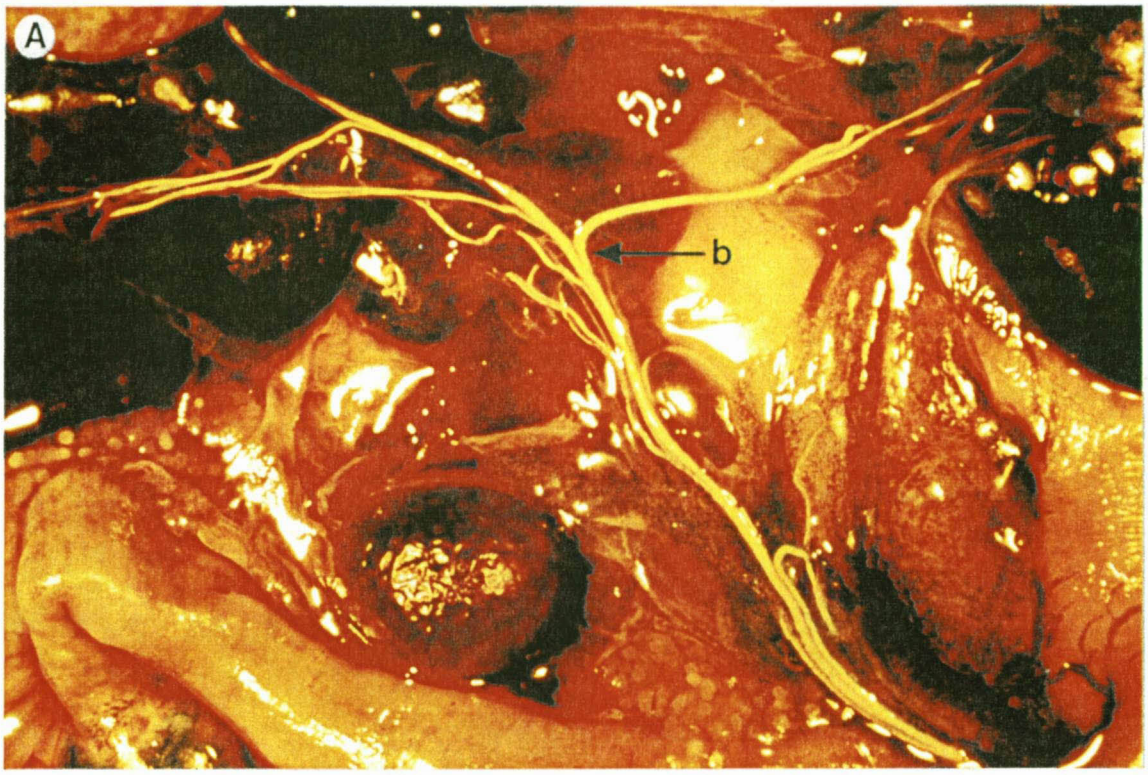
Within the bile duct system (Fig. 4.3A), the plerocerci had an interesting pattern of distribution. The percentage occurrence of the parasites in each of the ducts of 38 infected *X. laevis* from locality B (Fig. 4.3B), showed that most of the parasites occurred in the bile ducts from the right (36%) and left (36%) liver lobes.

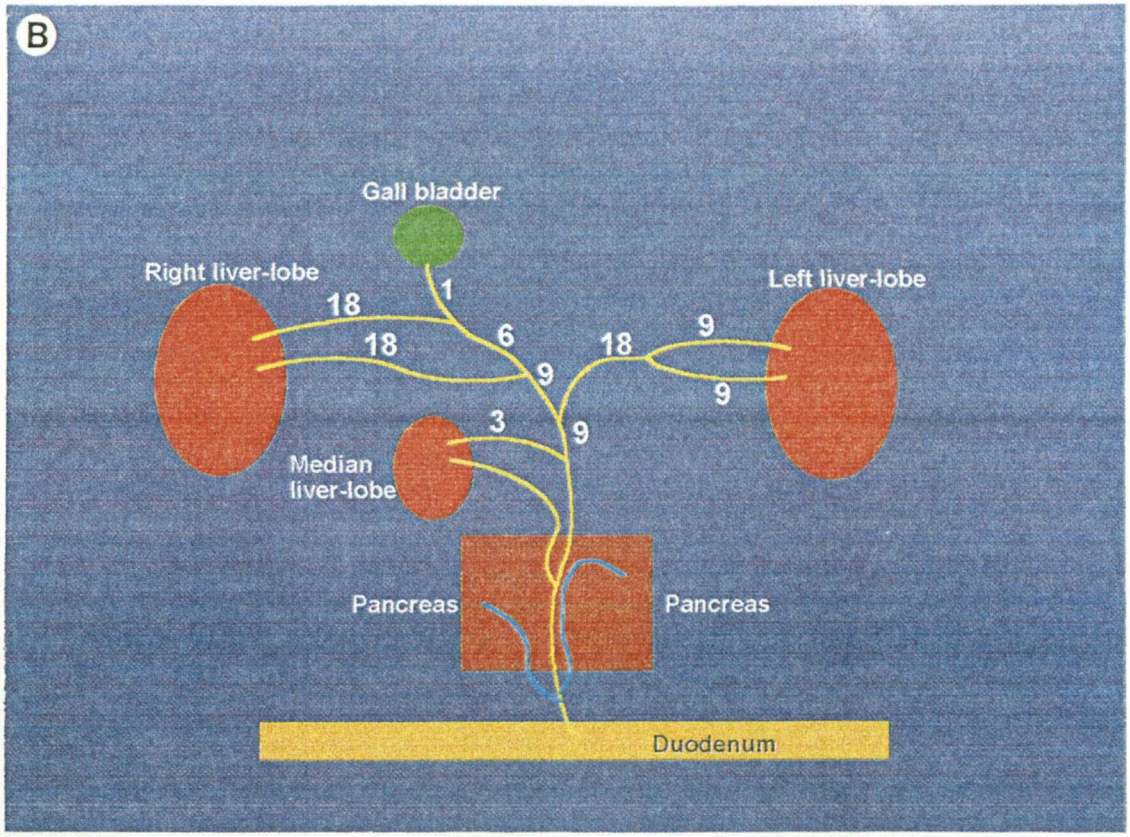
Figure 4.3

Occurrence of *Valipora campylancristrota* in the bile duct system.

A) Micrograph of the bile duct system injected with latex. Abbreviation: b, bile duct.

B) Schematic representation of the bile duct system showing the percentage occurrence of *V. campylancristrota* in each duct of hosts from locality B.


A
b


B
Gall bladder
Right liver-lobe
Left liver-lobe
18
1
18
6
18
9
9
9
3
9
Median
liver-lobe
Pancreas
Pancreas
Duodenum

b) Description

The plerocercoid, which is approximately 500 μm in length, has a cylindrical, oval hind body with a wavy surface pattern. The body ends rounded posteriorly and carries no appendages (Fig. 4.4A & 4.5A). The body contains corpuscles of granular shape (Fig. 4.6A). The scolex bears an invaginated rostellum, as well as four acetabula or suckers, approximately 60 μm in diameter (Fig. 4.4B&C), which are used for attachment and movement within the bile ducts (Fig. 4.5B&C). A capsule is completely lacking, and the epithelial lining of the duct is sucked into the acetabula when the parasite attaches itself (Fig. 4.5C). Opposite pairs of acetabula work together, and by moving up and down the scolex enable the parasite to move within the bile duct system. The whole scolex is lined with large, thick villi-like microtriches (Fig. 4.4D) which play a role in respiration. It may also assist in maintaining position in the ducts (Smyth, 1994).

The invaginated rostellum (Fig. 4.4E) carries two circles of hooks which differ significantly in shape and size (Fig. 4.6B&C). The first row consists of 10 large hooks, while the second has 10 smaller hooks. The blades of the hooks are strongly curved and sharply pointed, and the guards are short and rounded. Blades of the first circle of hooks are large and about the same length as the handles, while the blades of the small hooks are approximately half the length of the handles (Appendix 2). Larger hooks measure approximately 26.8 μm from the tip of the blade to the extremity of the handle, and smaller hooks 12.1 μm (Table 4.1; Appendix 3.1).

Table 4.1 Measurements of the rostellar hooks (as indicated in Fig. 4.2) of *Valipora campylancristrota*.

Measurement		n	Mean (µm)	Range (µm)	Coefficient of variation (%)
Large hooks	L	20	26.76	25.0 - 28.4	4.01
	G	20	12.43	11.3 - 14.2	7.44
	H	20	14.70	13.7 - 16.7	5.41
Small hooks	L	20	12.14	10.8 - 13.7	5.77
	G	20	4.53	3.9 - 5.4	9.40
	H	20	8.34	7.4 - 10.8	10.53

Using mainly the size and shape of the rostellar hooks, the parasite was identified as *Valipora campylancristrota* (Wedl, 1855), using the key by Khalil, Jones and Bray (1994). The eucestode belongs to the order Cyclophyllidea Van Beneden in Braun, 1900 and the family Dilepididae Ralliet and Henry, 1909. The identification was verified by Bona[2] (*pers. comm.*) and publications by Jarecka (1970), Kozicka (1971) and Priemer and Scholz (1989). In *X. laevis*, the parasite is in its II° larval stage in the form of a plerocercoid larva Bona[2] (*pers. comm.*).

[2] Prof. F.V. Bona, Dipartemento di Biologia Animale e Dell'uomo, Università Degli Studi di Torino. Via Accademia Albertina 17, Torino, Italy, 10123.

Figure 4.4

Scanning electron micrographs of the *Valipora campylancristrota* plerocercoid.

A) Total parasite. Scale bar = 100µm.

Abbreviations: hb, hind body; sl, scolex.

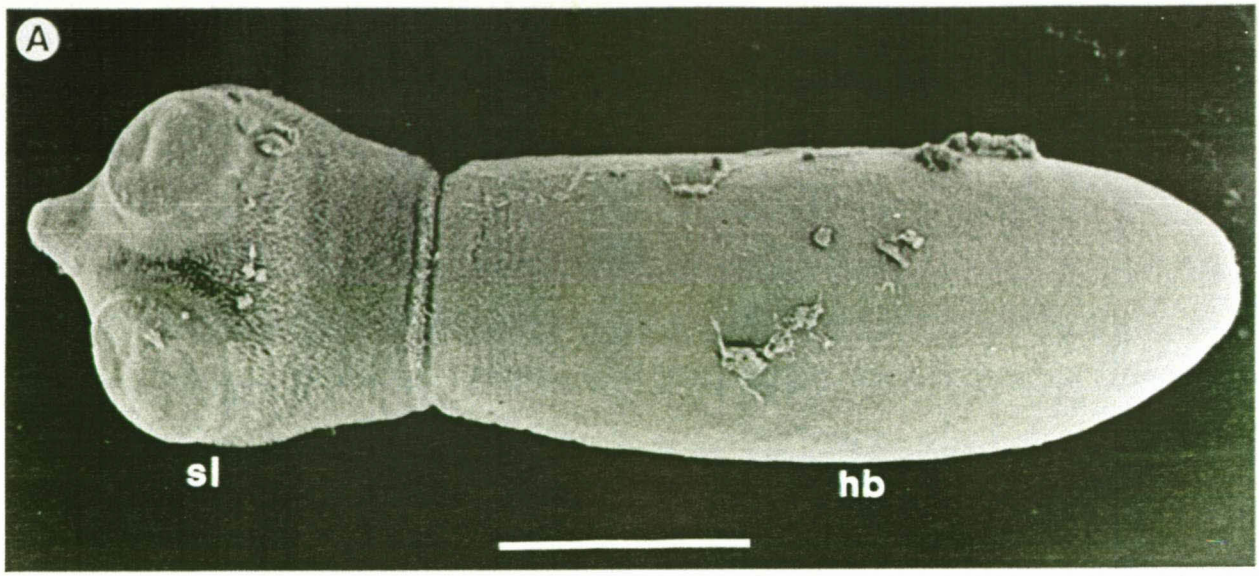
B) Scolex. Scale bar = 20µm.

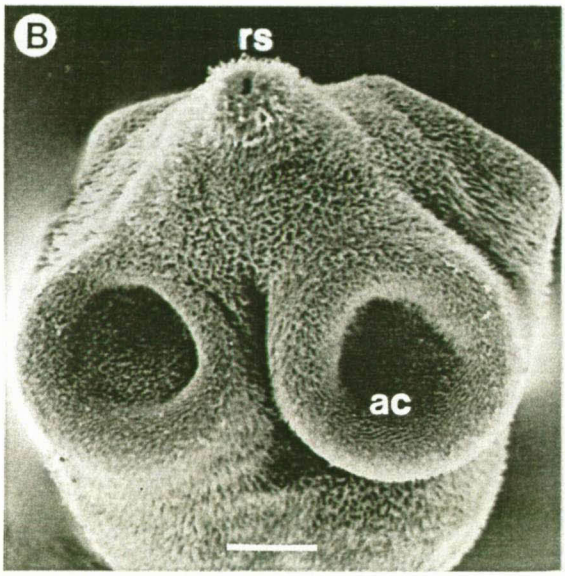
Abbreviations: ac, acetabulum; rs, invaginated rostellum.

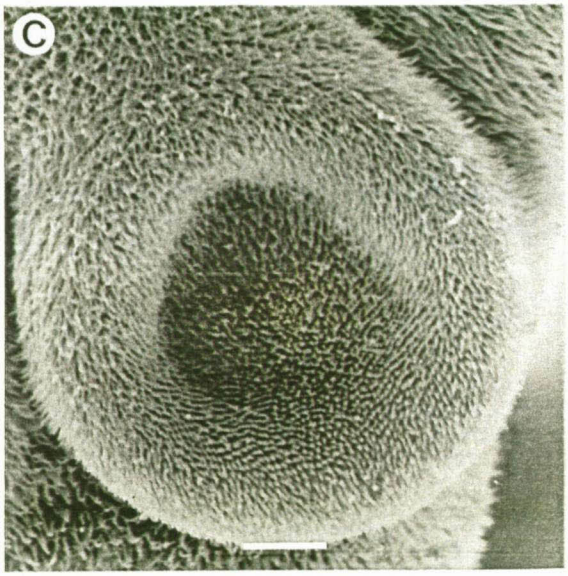
C) Acetabulum. Scale bar = 10µm.

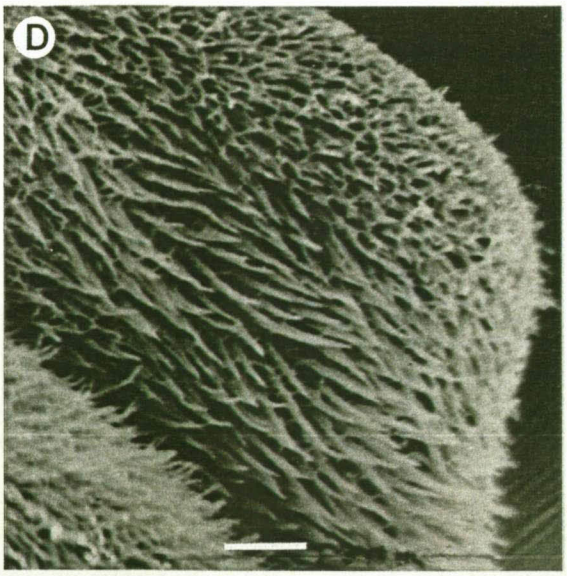
D) Microtriches. Scale bar = 5µm.

E) Invaginated scolex. Scale bar = 10µm.


A
sl
hb


B
rs
ac


C


D

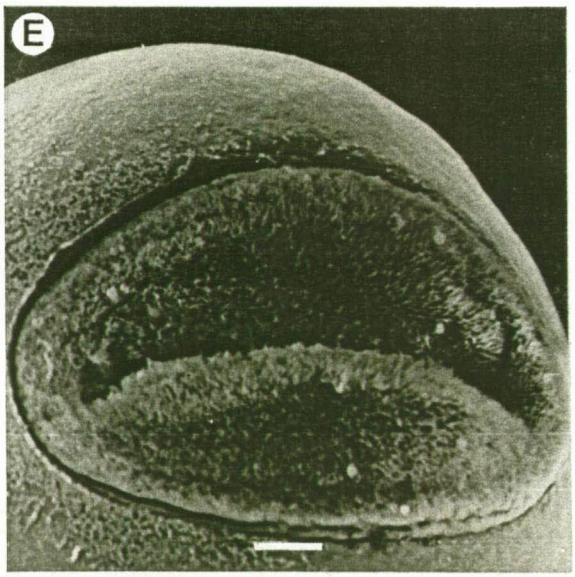

E

Figure 4.5

Light micrographs of the *Valipora campylancristrota* plerocercoid.

A) Whole parasite. Scale bar = 800µm.

Abbreviation: er, evaginated rostellum.

B) Histological section through a plerocercoid attached inside a bile duct. Scale bar = 80µm.

Abbreviations: bw, bile duct wall; pc, plerocercoid.

C) Histological section through a plerocercoid attached inside a bile duct. Scale bar = 40µm.

Abbreviations: ac, acetabulum; el, epithelial lining.

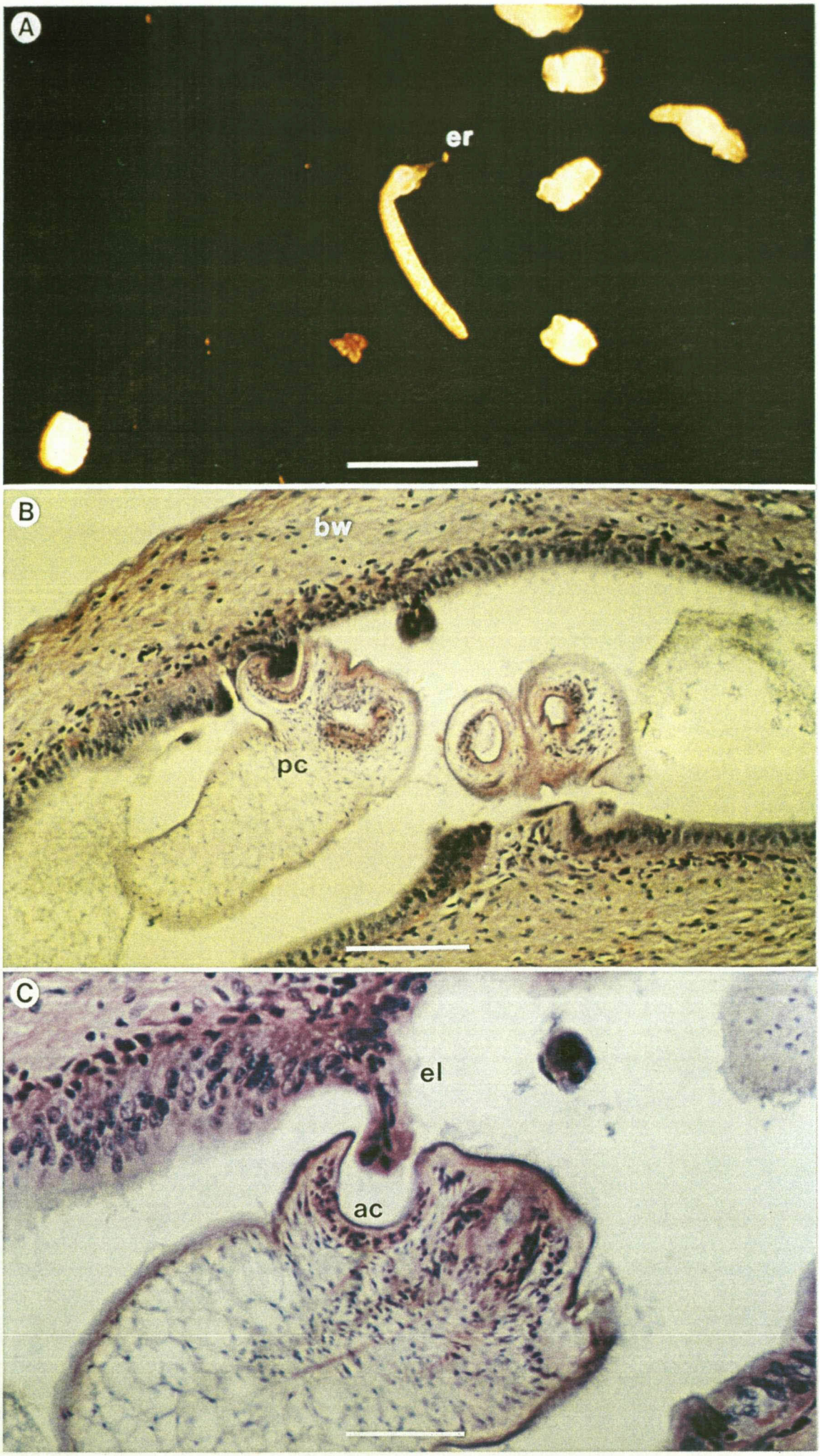

A
er
B
bw
pc
C
el
ac

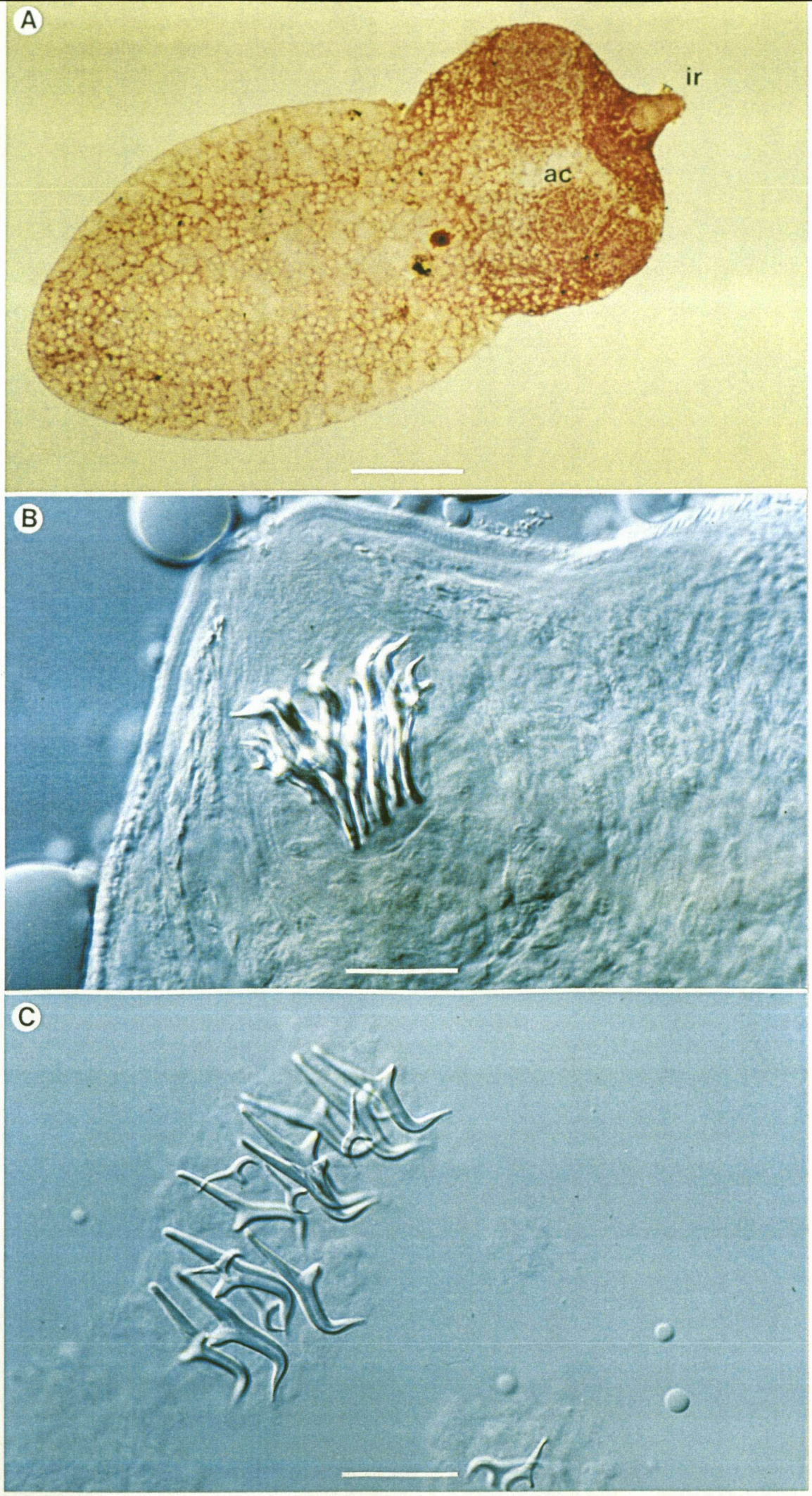
Figure 4.6

Light micrographs of the *Valipora campylancristrota* plerocercoid.

A) Alum carmine stained specimen. Scale bar = 100μm.
Abbreviations: ac, acetabulum; ir, invaginated rostellum.

B) Rostellar hooks of the plerocercoid. Differential interference contrast (DIC) on a lactophenol preparation.
Scale bar = 20μm.

C) Rostellar hooks of the plerocercoid. Differential interference contrast (DIC) on a squashed lactophenol preparation.
Scale bar = 20μm.


A
ir
ac
B
C

c) Life cycle studies

The oncospheres (Fig. 4.7A) removed from the faeces of experimentally infected herons, contained a hexacanth larva (Fig. 4.7B). The proglottids were approximately 500 µm in diameter, and the oval-shaped oncospheres were approximately 63 µm long and 43 µm wide. The larval hooklets were approximately 27 µm in length.

Bona[2] (*pers. comm.*) identified the adult cestodes removed from *A. melanocephala* as the cyclophyllidean *Neogryporhynchus cheilancristrotus* (Wedl, 1855) Baer & Bona, 1960 (Cestoda: Dilepididae). The scolex bears an evaginated rostellum and four acetabula (Fig. 4.8A&B & 4.9A&B). The suckers are approximately 48 µm in diameter (Fig. 4.8C). The rostellum carries two rows of hooks with similar shape but different sizes (Fig. 4.9C). Larger hooks measure approximately 69.0 µm from the tip of the blade to the extremity of the handle, and smaller hooks 41.0 µm (Table 4.2; Appendix 3.2). Both have comparatively long handles. The hooks are much larger than those of *V. campylancristrota*, and their shape differ significantly.

The mature proglottids of *N. cheilancristrotus* (Fig. 4.10) have very large, complex genital atria with spines. The cirrus pouch is massive and armed, with a tuft of spines emerging from its tip. The posteriourly extended ovary has few, large lobes. Four testes are found, and the posterior ones are partly dorsal to the ovary (Schmidt, 1986; Khalil *et al.*, 1994).

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2 Prof. F.V. Bona, Dipartemento di Biologia Animale e Dell'uomo, Università Degli Studi di Torino. Via Accademia Albertina 17, Torino, Italy, 10123.

Table 4.2 Measurements of the rostellar hooks (as indicated in Fig. 4.2) of *Neogryporhynchus cheilancristrotus*.

Measurement		n	Mean (µm)	Range (µm)	Coefficient of variation (%)
Large hooks	L	20	68.99	66.8 - 71.7	2.73
	G	20	36.48	34.2 - 38.3	3.34
	H	20	34.22	32.6 - 35.9	3.11
Small hooks	L	20	41.03	39.1 - 42.4	2.33
	G	20	22.28	17.9 - 24.5	6.69
	H	20	19.72	16.3 - 22.8	7.28

Figure 4.7

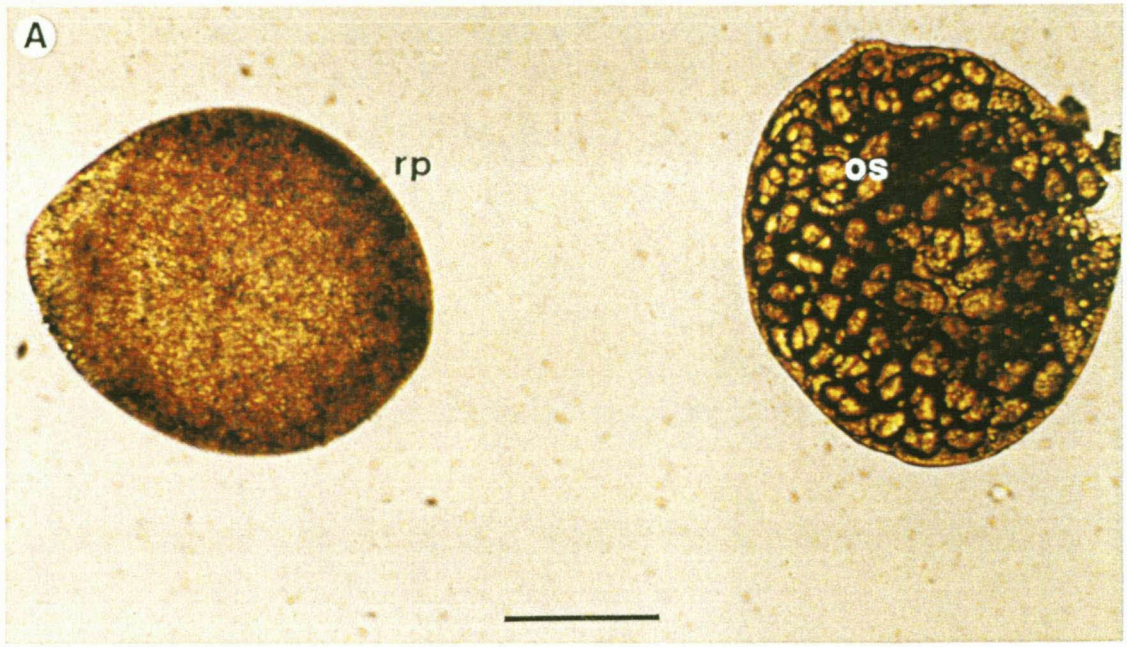
Light micrographs of the ripe proglottids and an oncosphere removed from the faeces of *Ardea melanocephala*.

A) Ripe proglottids. Scale bar = 200µm.

Abbreviations: os, oncosphere; rp, ripe proglottid.

B) Oncosphere containing hexacanth larva. Scale bar = 30µm.

Abbreviation: lh, larval hooklets.


A
rp
os

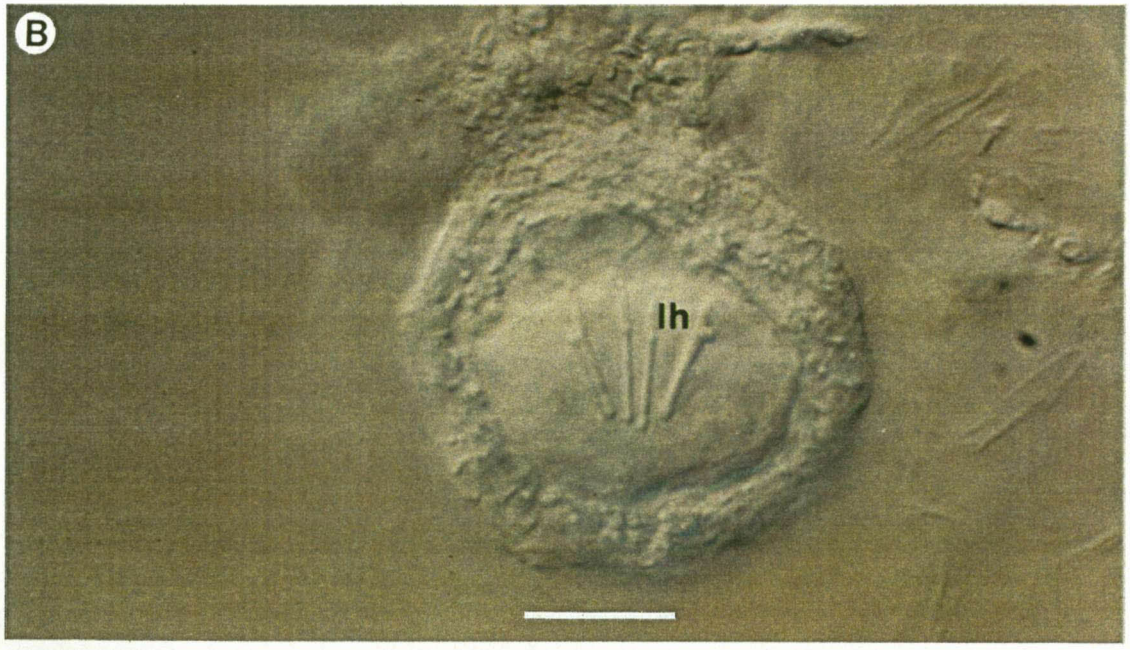

B
lh

Figure 4.8

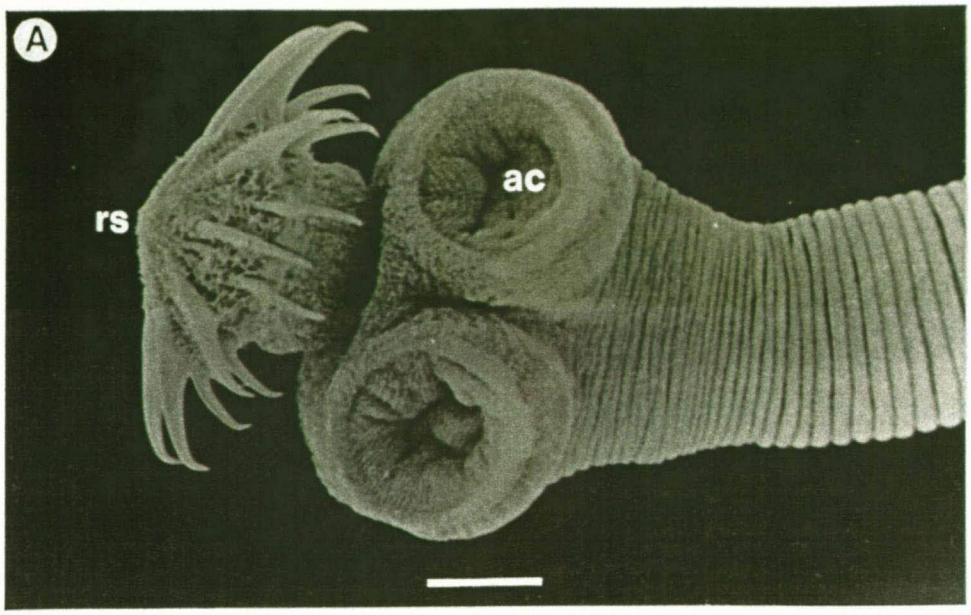
Scanning electron micrographs of adult *Neogryporhynchus cheilancristrotus* from *Ardea melanocephala*.

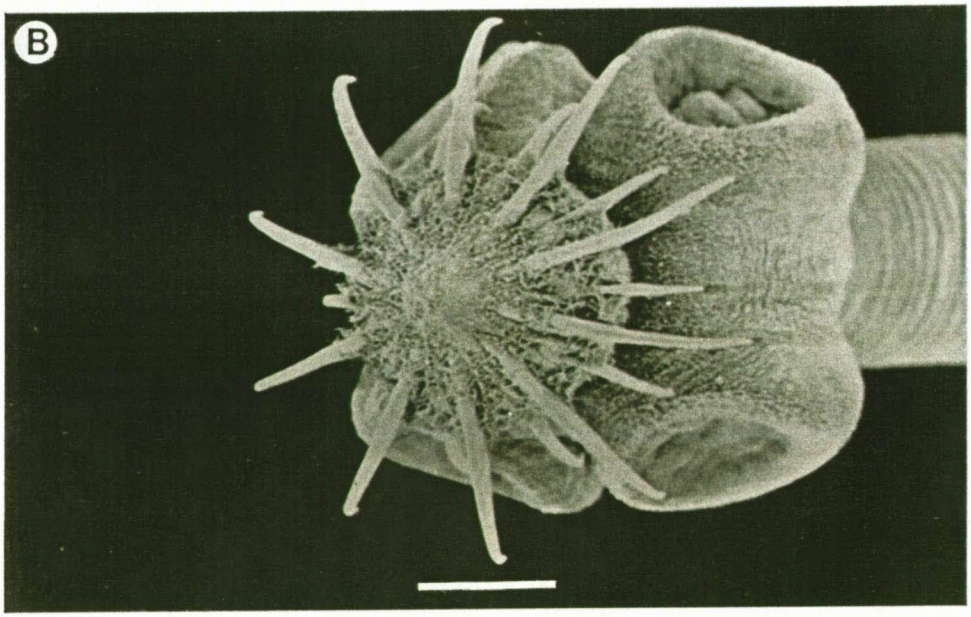
A) Scolex and neck region. Scale bar = 30μm.

Abbreviations: ac, acetabulum; rs, rostellum.

B) Scolex. Scale bar = 30μm.

C) Acetabulum. Scale bar = 10μm.


A
rs
ac


B

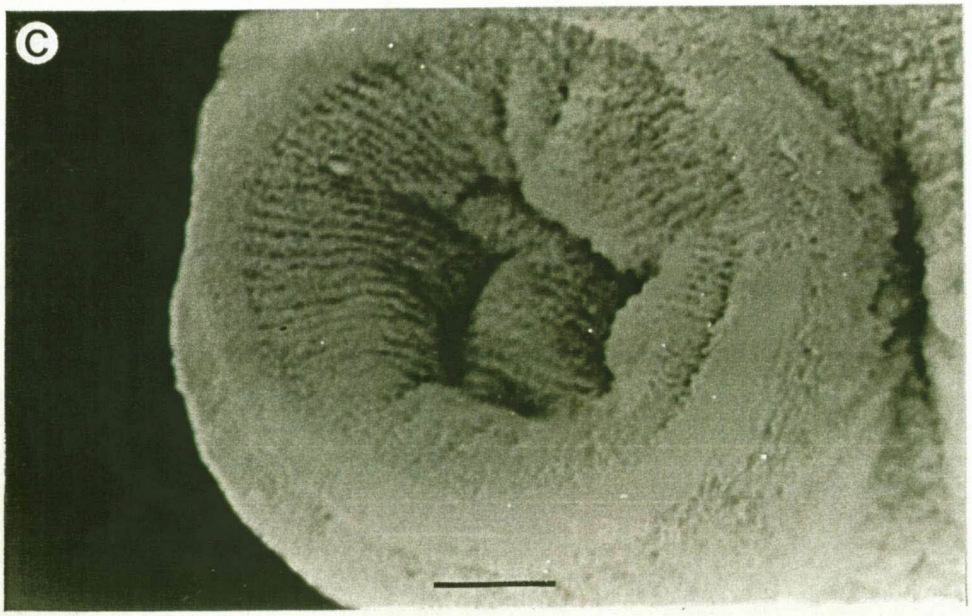

C

Figure 4.9

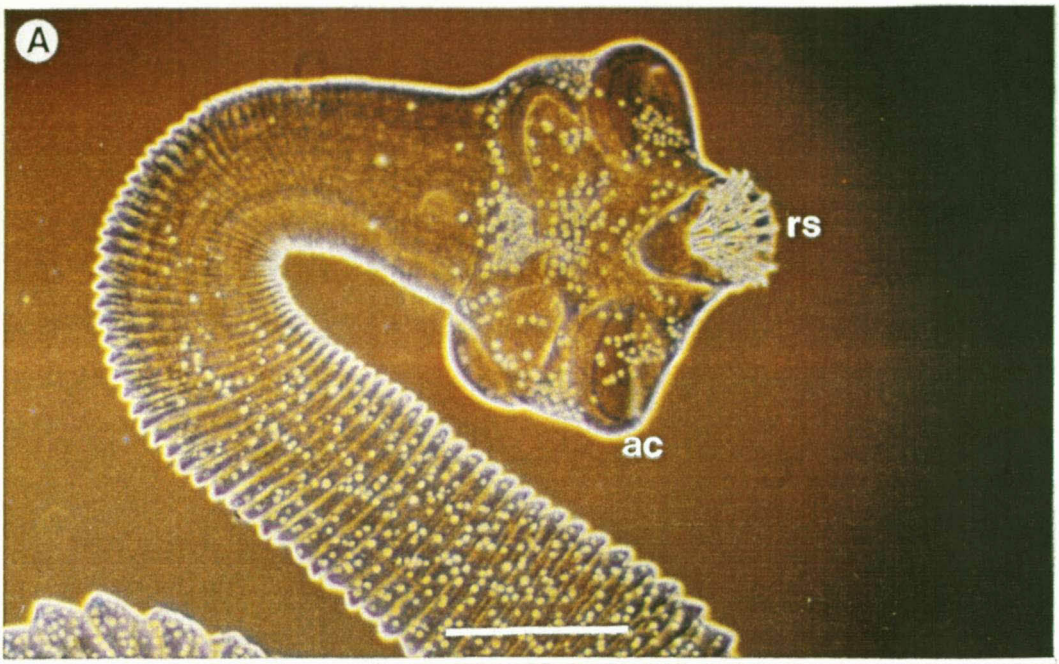
Light micrographs of adult *Neogryporhynchus cheilancristrotus* from *Ardea melanocephala.*

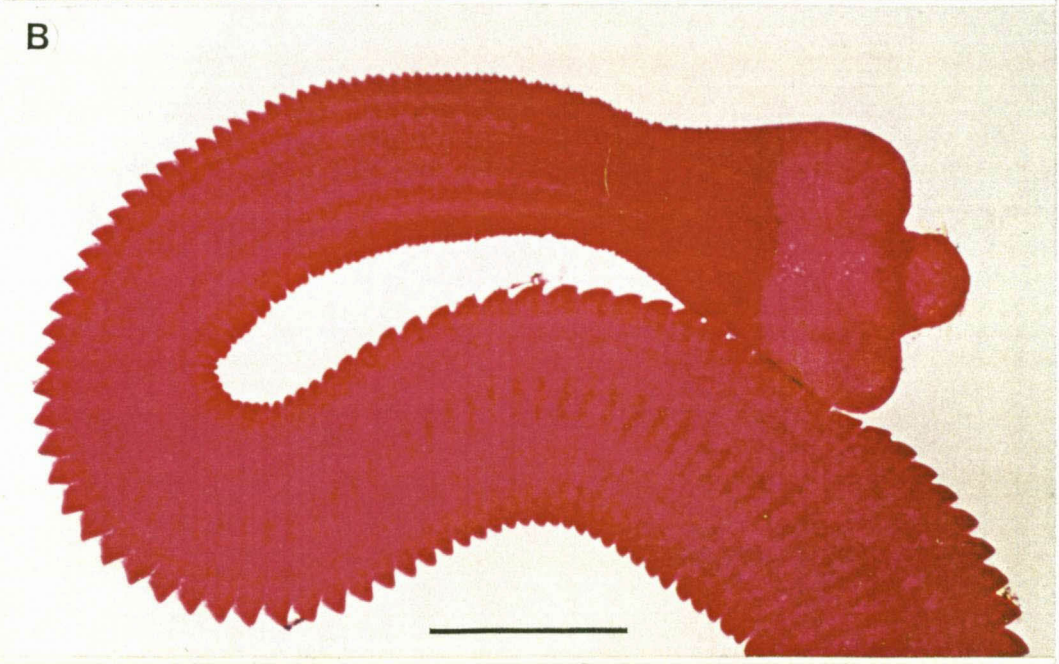
A) Ammonium picrate preparation of specimen using phase contrast. Scale bar = 200µm.

Abbreviations: ac, acetabulum; rs, rostellum.

B) Alum carmine stained specimen. Scale bar = 200µm.

C) Rostellum and hooks of alum carmine stained specimen. Scale bar = 40µm.


A
rs
ac


B

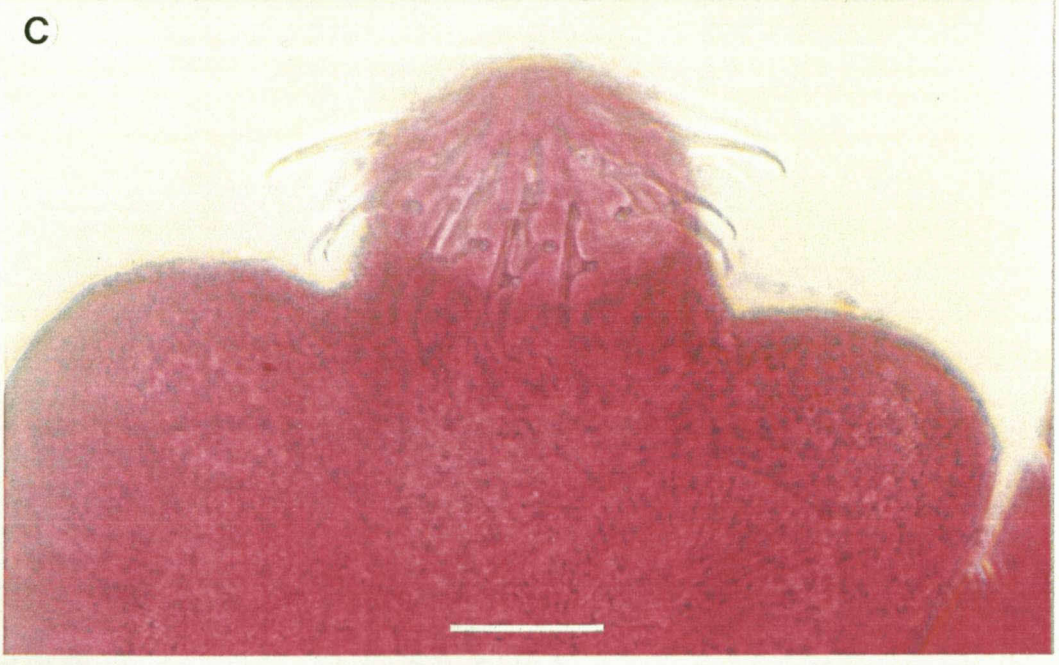
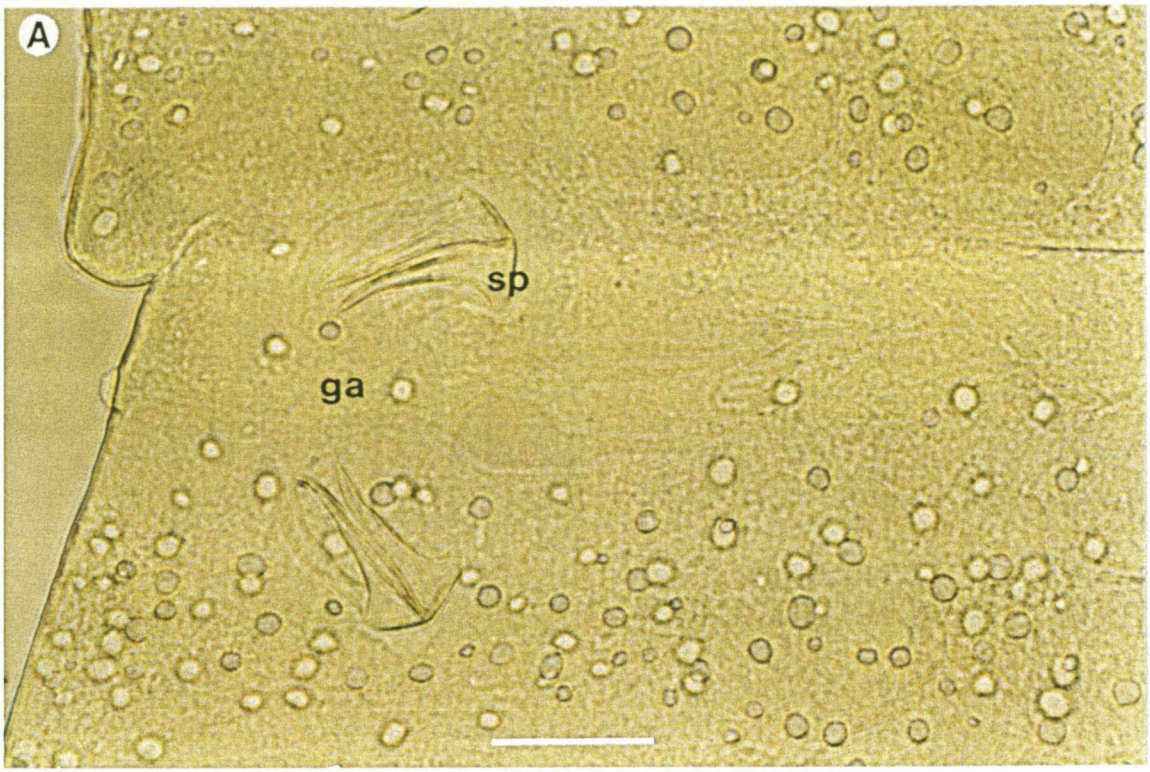

C

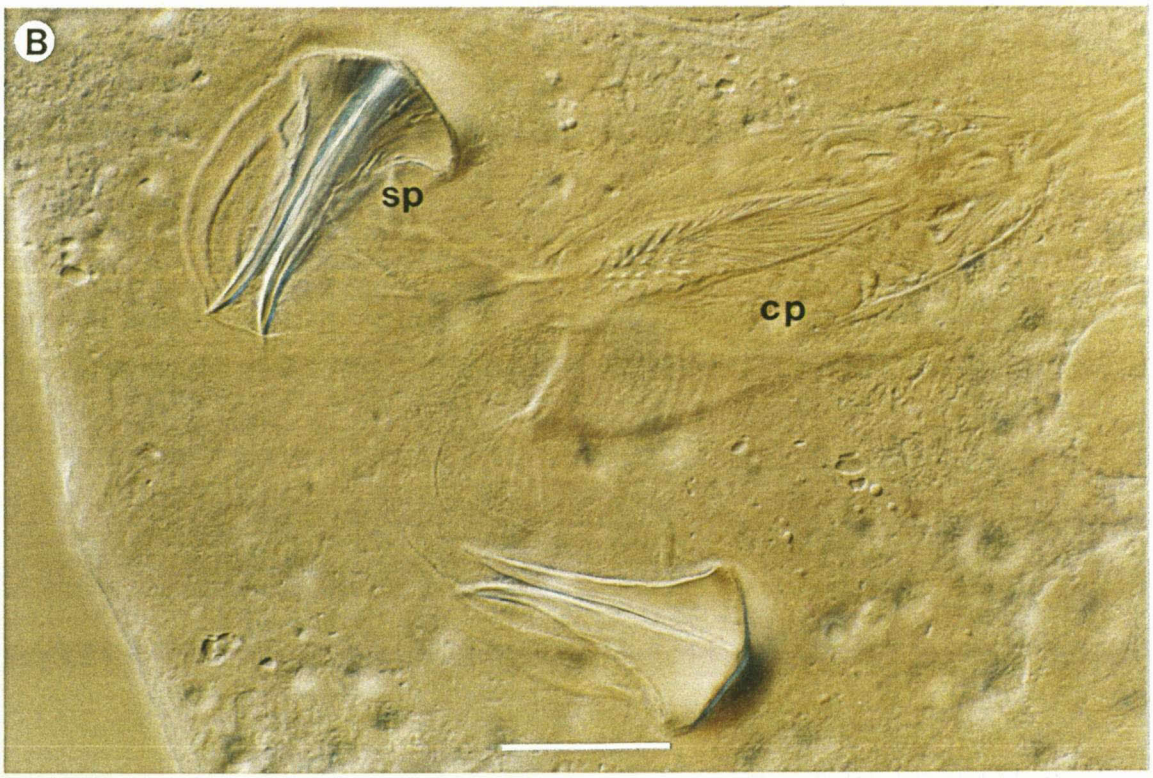
Figure 4.10

Light micrographs of mature *Neogryporhynchus cheilancristrotus* proglottids.

A) Lactophenol preparation of a proglottid. Scale bar = 40μm.
Abbreviations: ga, genital atrium; sp, spine.

B) Genital atrium and cirrus pouch. Differential interference contrast (DIC) on a lactophenol preparation.
Scale bar = 30μm.
Abbreviations: cp, cirrus pouch; sp, spine.


A
sp
ga


B
sp
cp

4.3.2 *Marsupiobdella africana*

a) Site of attachment

Juvenile *M. africana* was found externally on *X. laevis*, and preferred attachment sites seemed to be the dorsal trunk and feet (Table 4.3). Of 33 leeches removed from 10 different hosts, 14 parasites were found on the trunk dorsally and 12 on the feet. Only two were removed from the ventral side of the trunk, and five from the hind limbs.

b) Morphology

Leeches removed from *X. laevis* measured approximately 5mm long and 1 mm at its widest point (Fig. 4.11A&B). The crop and intestine of the leeches were green or red, the red colouration indicating that the parasites feed on the frog. The male gonopore is anterior to the female gonopore and six pairs of testes are present posterior to the genital atrium (Fig. 4.11C). No brood pouch was present. Siddall[3] (*pers. comm.*) stated that the leech was a juvenile, but was unable to identify the species. According to the description by Van der Lande and Tinsley (1976), the leeches were preliminarily identified as immature *M. africana*.

[3] Prof. M.E. Siddall, Museum of Zoology, University of Michigan, Ann Arbor, MI, 48109.

Table 4.3 Site of attachment of juvenile *M. africana* on experimentally infected *X. laevis*.

Experiment & number of parasites attached	Hours after attachment	Number of parasites in each body region										Number detached
		Forelimbs		Anterior trunk		Posterior trunk		Hind limbs		Feet		
		Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Left	Right	
A - 1	0			1								
	17	1										
	19.5					1						
	25					1						
	42						1					
	65						1					
	89							1				
	113											1
B - 3	0			3								
	35					1					1	1
	42					1					1	1
	59					1					1	1
	87					1						2
	135					1						2
	159											3
C - 3	0			2	1							
	35				1	1				1		
	42				1	1				1		
	59					1	1			1		
	87					1	1			1		
	135					1						2
	159					1						2
	180					1						2
	206											3
D - 4	0			1		2		1				
	35			1	1	1					1	
	42				1	2					1	
	59				1	2					1	
	87			1			1			1		1
	135			1						1		2
	159			1						1		2
	180									1	1	2
	206									1		3
	234											4

Figure 4.11

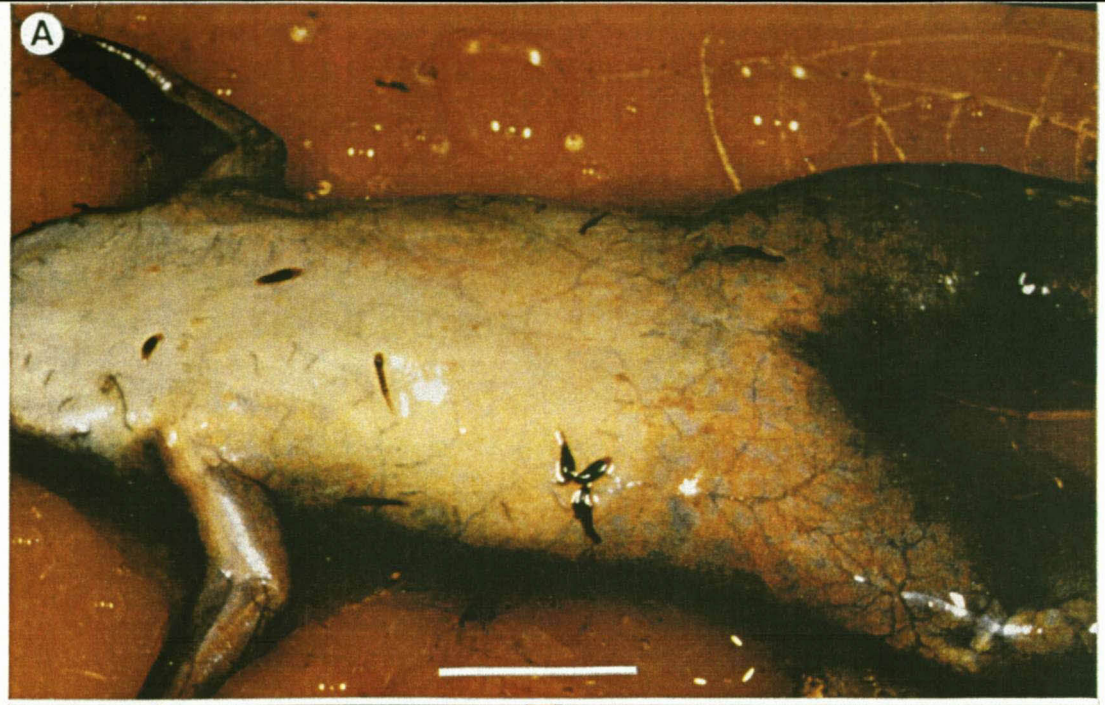
Micrographs of juvenile *Marsupiobdella africana*.

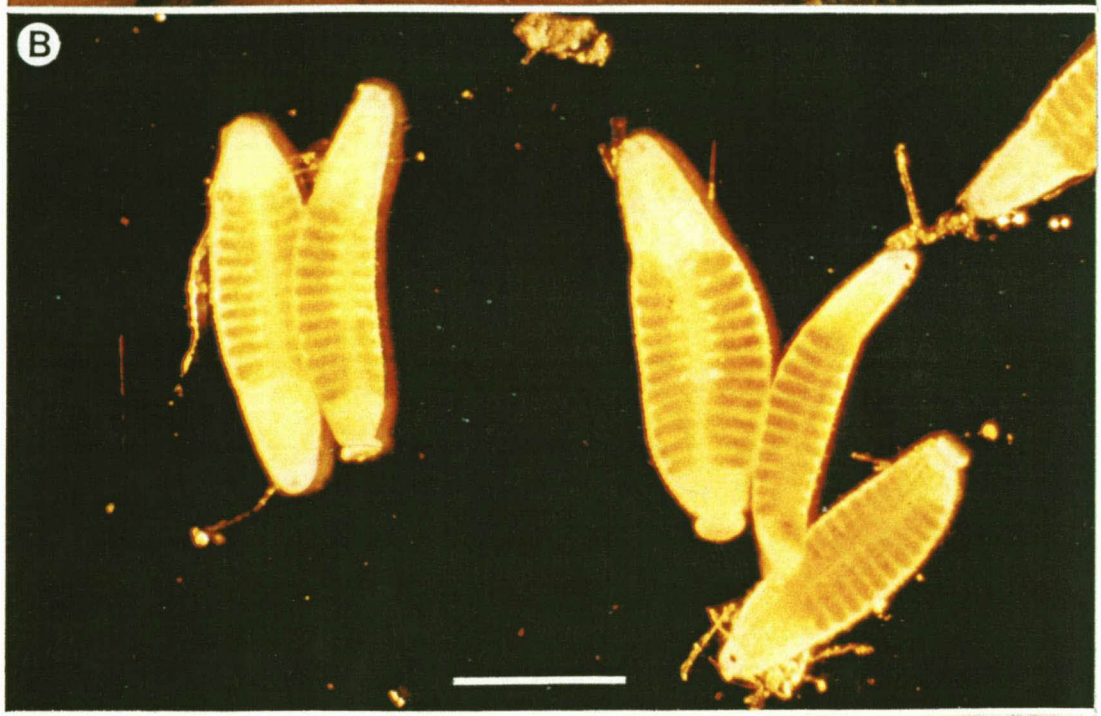
A) Leeches on *Xenopus laevis*. Scale bar = 20 mm.

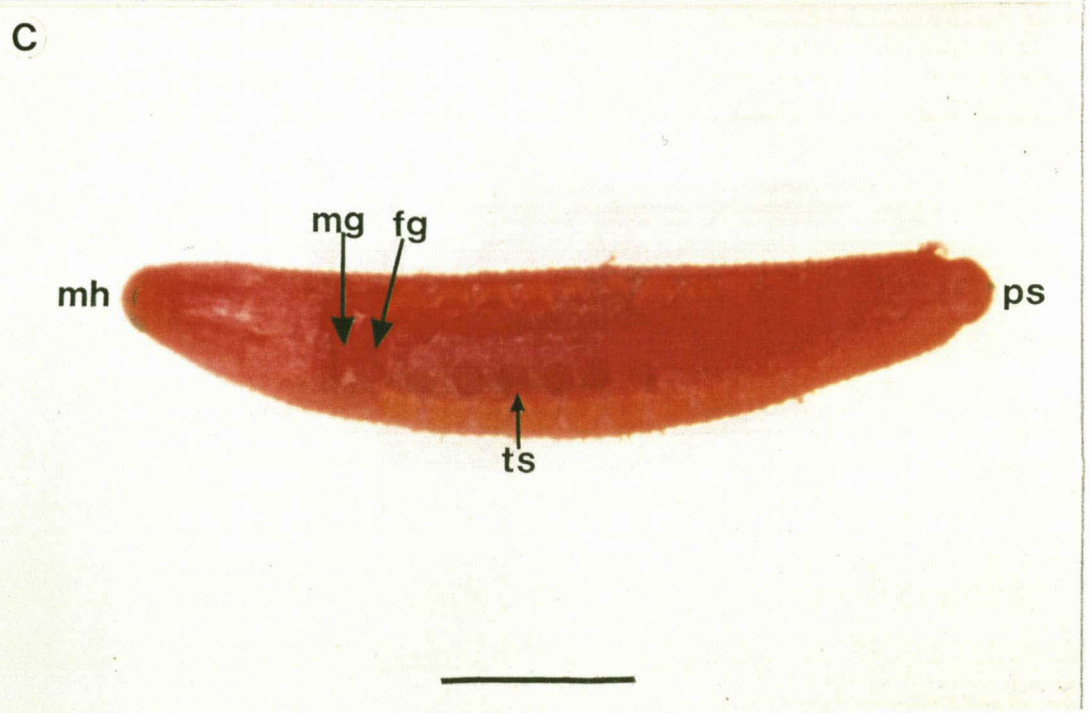
B) Leeches in a petri dish. Scale bar = 2 mm.

C) Light micrograph of alum carmine stained specimen. Scale bar = 1 mm.

Abbreviations: fg, female gonopore; mg, male gonopore; mh, mouth; ps, posterior sucker; ts, third pair of testes.


A


B


C
mg
fg
mh
ps
ts

4.4 DISCUSSION

4.4.1 *Valipora campylancristrota*

The discovery of yet another parasite infecting *X. laevis* emphasises the frog's suitability as host for a variety of parasites. *Valipora campylancristrota* is the first identified cestode larva, and only the second cestode to be identified from *Xenopus*. The parasite's utilisation of the bile duct system is also unique, and leaves only the lungs of *Xenopus* as largely unexploited by parasites (Tinsley, 1996a).

The higher incidence proximal to the gall bladder in ducts from the left and right liver lobes may be due to differences in pH. The larger diameter of the ducts in this part of the bile duct system could also play a role.

The plerocercoid belongs to the genus *Valipora*, and the species is most probably *V. campylancristrota*. This was verified by Bona[2] (*pers. comm.*). The length of the large hooks in previous reports of *V. campylancristrota* ranged between 24 µm and 30 µm, and the smaller hooks between 10 µm and 15 µm (Table 4.4). The length of between 25 µm and 28 µm of the large hooks and between 11 µm and 14 µm of the smaller hooks in the current study corresponds with previous reports.

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[2] Prof. F.V. Bona, Dipartemento di Biologia Animale e Dell'uomo, Università Degli Studi di Torino, Via Accademia Albertina 17, Torino, Italy, 10123.

Table 4.4 Comparison of the length of the rostellar hooks of *Valipora campylancristrota* from natural and experimental infections.

References	Length from tip of blade to extremity of handle (µm)	
	Large hooks	Small hooks
Jarecka (1970). experimental	24 - 26	10 - 12
Wedl (1855)	24 - 25	12
Kozicka (1971)	26 - 27	13 - 14
Bona (1975)	24 - 28	11 - 14
Priemer & Scholz (1989)	26 - 30	10 - 14
Crous (current study)	25 - 28	11 - 14
RANGE	24 - 30	10 - 14

According to Baer and Bona (1960), the plerocercoid of *V. campylancristrota* was originally recorded by Aubert (1857) and Krabbe (1869) in the gall bladder of the tench, *Tinca tinca* (Cypriniformes: Cyprinidae). Jarecka (1970) investigated the life cycle of the parasite which was known to utilise the grey heron, *Ardea cinerea* as final host. He found that the copepod *Eudiaptomus graciloides* was the first intermediate host, and transferring infected copepods to fish he established the carp, *Cyprinus carpio* to be the second intermediate host, carrying the plerocercoid in the gall bladder. The plerocercoid is also found in the intestine of fish (Jara & Olech, 1964). The parasite is currently known to usually infect cyprinids (Bona[2], *pers. comm.*), and is known from large parts of Europe and Asia (Ryzhikov & Ryšavý, 1985).

T. tinca and *C. carpio* are, together with several other cyprinids, introduced species in southern Africa. The tench was introduced in 1910 from England, and the carp as early as the 1700s (Skelton, 1993). *Xenopus* is known for its ecological overlap with fish in terms of diet and habitat (Tinsley, 1981). It is therefore possible that *V.*

[2] Prof. F.V. Bona, Dipartemento di Biologia Animale e Dell'uomo, Università Degli Studi di Torino. Via Accademia Albertina 17, Torino, Italy, 10123.

campylancristrota transferred from introduced species are utilising *X. laevis* as alternative second intermediate host.

Various authors have documented the pathological effects of *V. campylancristrota* plerocerci in fish. The parasite is known to negatively influence the health (Jara & Olech, 1964), decrease the growth rate and cause pathological changes in the gall bladders and intestines of infected fish (Sapoznikov, Skvorcova & Laduchen, 1974). Studnicka, Stankiewicz and Siwicki (1983) reported a 'rather slow' growth and 60 % mortality of infected fish in Polish carp farming. Haemorrhage, necrosis and cellular reactions in the intestines of carps and tenches have also been reported (Körting, 1984). During the current study, no pathological effects were observed in infected *X. laevis*. It is possible that the frog is not subjected to the serious pathological effects the parasite causes in fish, making it a suitable alternative second intermediate host to *V. campylancristrota*.

The fact that *X. laevis* can serve as host for the parasite has a number of possible implications. Infected frogs could spread the parasite to fish endemic to southern Africa, which might not be able to survive infection by the plerocercoid. This will have a detrimental effect on the already limited freshwater fish diversity in South Africa. As *X. laevis* is exported for research and feral populations of *Xenopus* outside Africa are already existent (Tinsley & McCoid, 1996), the parasite could also be spread to other parts of the world. *X. laevis* could also spread *V. campylancristrota* to different *Xenopus* species which might be less tolerant of the parasite, and have a detrimental effect on population numbers of already endangered species such as *X. gilli*.

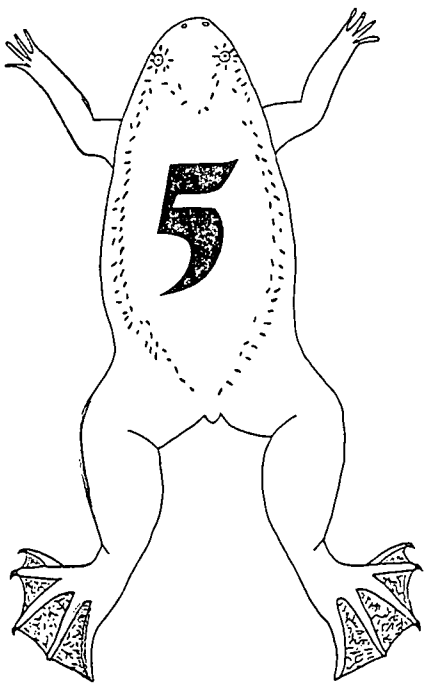
As only *Neogryporhynchus cheilancristrotus* were removed from experimentally infected *Ardea melanocephala*, it is possible, but unlikely, that *V. campylancristrota* is unable to complete its life cycle when utilising *X. laevis* as second intermediate host instead of a fish. The herons must have been already infected with *N. cheilancristrotus* when caught, and repetition of the experiment would require treatment with a deworming agent. The invasion success of *C. campylancristrota* may also be low when the frog is already infected with another cestode.

It is possible that *A. melanocephala* may not be a suitable final host even though the parasite has been found in other Ardeidae such as *Ardea cinerea* (Jarecka, 1970). *V. campylancristrota* is known to also infect Ciconiiformes, and future studies will require investigating the possibility of a stork being the final host. In the context of South African fauna and *X. laevis* being the second intermediate host, other possible final hosts include cormorants or darters.

4.4.2 *Marsupiobdella africana*

The juvenile *M. africana* showed a strong preference for attachment to the dorsal side of the hind trunk. Van der Lande and Tinsley (1976) recorded similar behaviour. Preferred sites of attachment included the upper hind limbs and the area around the cloaca. In experimentally infected toads, the foot webs and pelvis region were favoured. Several factors may influence the choice of attachment site, the most imported seemingly to avoid being removed by the host, which make frequent attempts to do so using its fore- and hind limbs (Van der Lande & Tinsley, 1976).

Although the leeches found on *X. laevis* strongly resembled immature *M. africana*, its identity is still under investigation. Future research would include culturing the leeches to acquire adults from which conclusive identification would be possible. Previous life cycle experiments by Van der Lande and Tinsley (1976) on *M. africana* were successful, with leeches breeding for up to three generations.



Parasite Diversity & Infection Levels at Two Localities

CONTENTS

5.1 INTRODUCTION

The genus *Xenopus* is characterised by a rich assembly of parasites representing seven major invertebrate groups. Eight of the 29 known parasites of *Xenopus laevis* were found during the current study. A ninth parasite, *Valipora campylancristrota*, has never been recorded from *Xenopus* before. The objectives of this part of the study were to determine which parasites of *X. laevis* are found in the particular geographical area, how the infection levels of different species vary and whether the abundance and diversity of parasites differ at two different localities. Additionally, the frequency distribution of each parasite within the host population was determined.

A major part of the life-history strategies employed within the field of parasitology are represented by the parasites of *X. laevis*. Parasites with indirect life cycles utilise *Xenopus* as intermediate or final host, while others have single-host life cycles. The transmission mechanisms of the parasites are also very diverse. The different strategies employed by parasites, together with other factors such as parasite longevity (Tinsley, 1996a), contribute to the different infection levels of parasites.

The helminth parasites of *Xenopus* can be divided into two categories according to the dynamics of their infection levels (Tinsley, 1995). Some interactions represent a closed system, with exposure to invasion more or less continuous, potentially giving rise to very high parasite densities. Conversely a balance between recruitment and loss determines the burden of other parasites.

Due to the complexity of parasite life cycles and transmission mechanisms, various ecological factors may influence the abundance of parasites, leading to different infection levels and parasite diversity at different localities. Esch and Fernández (1993) identified several external and internal environmental factors affecting parasite populations. External factors, of which host diet is the most basic, include host-finding capability in a certain environment, ecological succession, pollution, habitat stability and zoogeographical factors. Internal factors include parasite population density, host immunity and behaviour and competition. Several host related factors influence helminth parasite communities (Table 5.1).

Table 5.1 Impact of some host related factors on helminth parasite diversity and abundance (modified from Esch & Fernández, 1993).

Host-related factors	Number of parasite species	Number of individual parasites
Host abundance (Intermediate or final host)	higher host density = more parasite species	higher host density = higher number of parasites
Biotic interaction		
competition	often reduces numbers	often reduces numbers
immunity	may reduce numbers	may reduce numbers
Breadth of diet	if wide, then usually more species	no influence
Life style		
aquatic	highly variable	variable; potentially high
terrestrial	variable; often low	variable; often low

Concentrating on *Protopolystoma xenopodis*, Jackson and Tinsley (1988b) discussed the influence of host environment and parasite burden and age on parasite egg production. In a more extensive publication by Tinsley (1993) on the population biology of monogeneans, regulatory factors such as host immunity, continuity of parasite invasion, pattern and efficiency of recruitment and subsequent survival of the surviving

parasite population and the effect of intraspecific competition, the so-called 'crowding effect' were discussed. He also emphasised the important effect of parasite longevity.

5.2 MATERIALS & METHODS

5.2.1 STATISTICAL ANALYSIS OF INFECTION LEVELS

Due to the non-parametric nature of the data collected, a Mann-Whitney U-test was done to determine the significance of differences in parasite infection levels at locality A and B. The test compares the medians of two samples. A P-value was rendered by the test that indicated the significance of any differences. If $P \leq 0.05$, the difference in the infection levels was significant with 95% or more certainty.

5.3 RESULTS

During the current study, nine parasite species were found infecting *X. laevis*. This included a new parasite never before recorded. A plerocercoid cestode larva, *Valipora campylancristrota* was found in the bile ducts of *Xenopus laevis*. The prevalence and mean intensity of different parasites varied considerably (Table 5.2). Five of the parasites were found to have significantly different infection levels at the two localities (Table 5.3).

Table 5.2 Diversity and infection levels of parasites infecting *Xenopus laevis* from the two study localities.

Parasites	Infection site	Locality A		Locality B	
		Prevalence (%)	Mean intensity	Prevalence (%)	Mean intensity
PLATYHELMINTHS					
Monogenea					
Gyrdicotylus gallieni	buccal cavity	12.7	7.4	16.3	2.6
Protopolystoma xenopodis	urinary bladder	39.8	1.3	63.8	1.9
	kidneys	0	0	1.9	3.0
Digenea					
Dollfuschella rodhaini	stomach	1.7	1.0	0	0
Tylodelphys xenopi	pericardium	93.2	75.9	98.1	125.8
	body cavity	61.0	18.8	70.1	35.5
Cestoda					
Cephalochlamys namaquensis	intestine	72.9	5.6	76.9	5.2
Valipora campylancristrota	bile ducts	5.1	1.5	32.8	7.3
NEMATODA					
Camallanus kaapstaadi	oesophagus	68.6	5.2	76.0	4.5
Batrachocamallanus slomei	stomach	17.8	8.5	4.8	2.0
HIRUDINEA					
Marsupiobdella africana	external skin	5.1	3.8	20.2	5.6

Table 5.3 Statistical analysis of the infection levels of parasites at locality A and B.

Parasite	Sample size (n)		Mean ($\bar{x}$) (Abundance)		Standard error		Variance (s^2)		P
	A	B	A	B	A	B	A	B	
G. gallieni	118	104	0.94	0.42	0.35	0.13	14.62	1.78	0.703
P. xenopodis bladder	118	116	0.5	1.22	0.06	0.13	0.49	2	0.00004
T. xenopi pericardium	118	104	70.73	123.39	6.67	16.26	5252.78	27484.28	0.021
body cavity	118	104	11.46	24.95	1.6	3.8	301.93	1504.65	0.008
C. namaquensis	118	104	4.06	3.98	0.43	0.52	22.26	28.21	0.948
V. campylancristrota	118	116	0.08	2.38	0.03	0.61	0.12	42.66	0.0001
C. kaapstaadi	118	104	3.59	3.41	0.45	0.35	23.66	12.96	0.377
B. slomei	118	104	1.52	0.1	0.96	0.06	109.45	0.38	0.085
M. africana	118	104	0.19	1.13	0.12	0.33	1.78	11.42	0.049

5.3.1 *Gyrdicotylus gallieni*

G. gallieni was found in the buccal cavity of *X. laevis*. The infection intensity of the parasite was relatively low, with a prevalence of 12.7% at locality A, and 16.3% at locality B. The mean intensity was 7.4 (n = 118) and 2.6 (n = 104) at locality A and B respectively, and the maximum number of parasites found in one host was 30 at locality A and eight at locality B (Table 5.2).

The parasite exhibited a negative binomial distribution within the host population, with 87.4% and 83.4% of hosts uninfected at locality A and B respectively (Fig. 5.1A). The Mann-Whitney U-test (Table 5.3) indicated that no significant difference existed in the infection levels of *G. gallieni* at the two localities (P = 0.703).

5.3.2 *Protopolystoma xenopodis*

Adult *P. xenopodis* were commonly found in the urinary bladder of *X. laevis*. At locality B, a prevalence of 63.8% was recorded and at locality A a significantly lower prevalence of 39.8%. A mean intensity of 1.3 and 1.9 was recorded at locality A and B respectively. The number of parasites per host usually ranged between one and three, with a maximum of three at locality A (n = 118), but seven at locality B (n = 116).

Very few juvenile forms of *P. xenopodis* were found in the kidneys. Juveniles were in fact only found in two hosts from locality B (n=104), with one and five parasites in each frog respectively (Table 5.2). No juveniles were found in hosts from locality A.

The distribution of adult *P. xenopodis* in the host population was overdispersed. At locality A 60.2% of hosts were uninfected. At locality B only 36.2% of hosts were uninfected, but 34.5% carried only a single parasite giving a markedly skewed distribution (Fig. 5.1B).

A significant difference existed in the infection levels of the parasite at the two localities (P = 0.00004). Infection levels were significantly higher at locality B (Table 5.3).

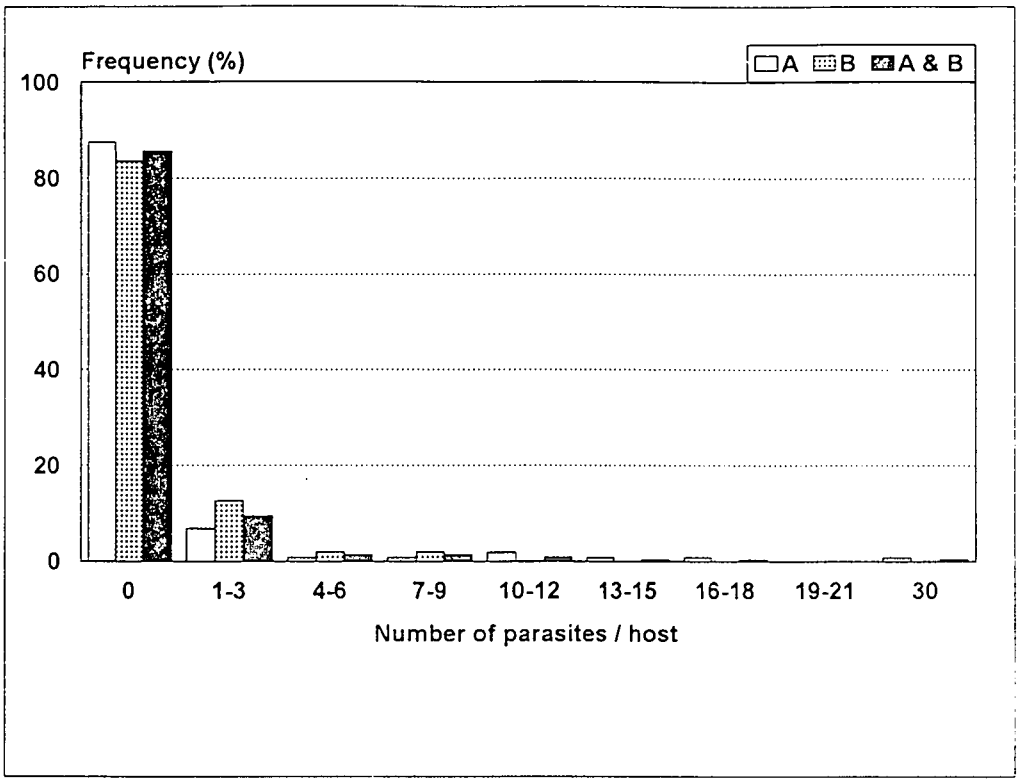
Figure 5.1

Bar graph illustrating the frequency distribution of parasite infection levels at locality A, B and for data from both localities combined.

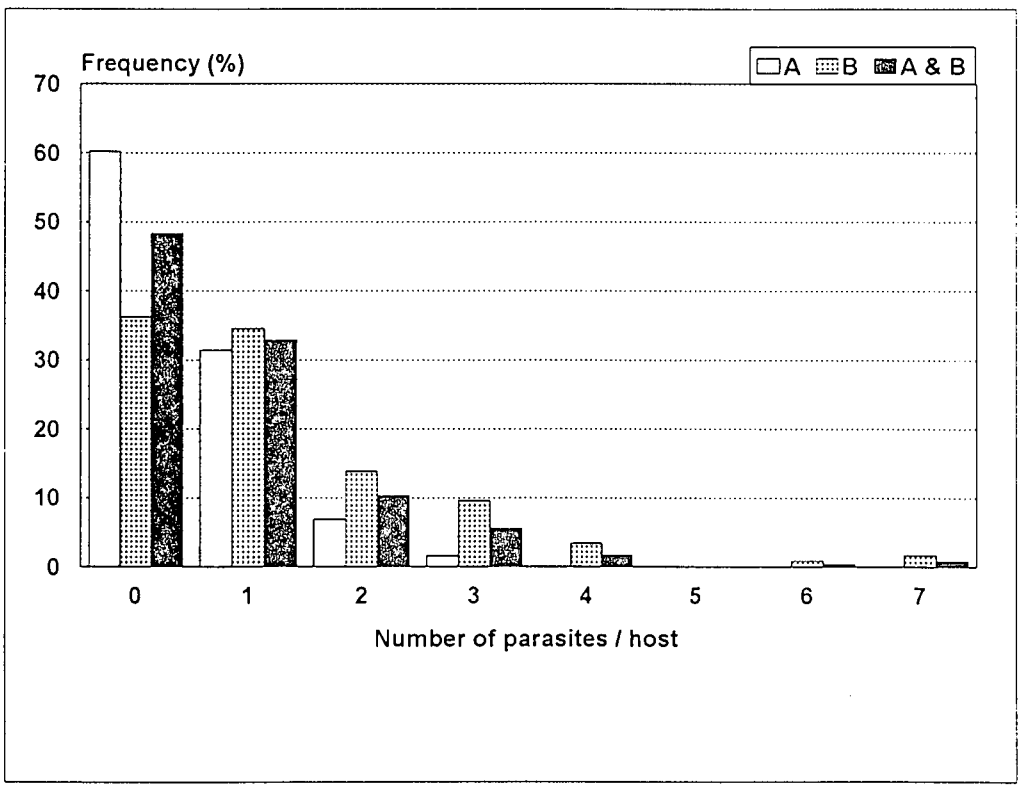
A) *Gyrdicotylus gallieni.*

B) Adult *Protopolystoma xenopodis.*

A


Frequency (%)
A
B
A & B
100
80
60
40
20
0
0
1-3
4-6
7-9
10-12
13-15
16-18
19-21
30
Number of parasites / host

B


Frequency (%)
A
B
A & B
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Number of parasites / host

5.3.3 *Dollfuschella rodhaini*

The adult trematode, *D. rodhaini* was found in the stomach of only two hosts from locality A (n=118), and each contained only a single parasite (Table 5.2).

5.3.4 *Tylodelphys xenopi*

The metacercaria, *T. xenopi* was the most common parasite found infecting *X. laevis*. The parasite occurred in high numbers in the pericardium and body cavity (Table 5.2). The prevalence of *T. xenopi* in the pericardium was very high, with 93.2% and 98.1% of hosts infected at localities A and B respectively. The mean intensity was 75.9 at locality A and 125.8 at locality B. The maximum number of parasites found in the pericardium of a single host was 380 at locality A and 950 at B.

The prevalence of *T. xenopi* in the general body cavity and on the surface of organs was also relatively high, occuring in 61% of hosts from locality A and 70.1% of hosts from B. The mean intensity was 18.8 at locality A with a maximum of 110 parasites per host, and 35.5 at locality B with up to 190 metacercaria in a single host.

The pericardium and body cavity of 118 hosts from locality A and 104 from locality B were examined for the presence *T. xenopi*. Only 6.8% of hosts at locality A and 1.9% at locality B were not infected with *T. xenopi*. However, at locality A 43.2% and at locality B 40.4% carried relatively low burdens in the pericardium of between one and 50 parasites per host (Fig. 5.2A). A similar pattern of distribution was found for *T. xenopi*

infections in the body cavity. At locality A 39% of hosts were uninfected and at locality B 29.8%. The percentage of hosts carrying low burdens of between one and 20 parasites were 44.9% and 35.6% at locality A and B respectively (Fig. 5.2B).

The infection levels of *T. xenopi* in both the pericardium and body cavity were significantly different at the two localities (Table 5.3). The Mann-Whitney U-test indicated that the parasite was significantly more abundant in the pericardium ($P = 0.021$) and body cavity ($P = 0.008$) at locality B.

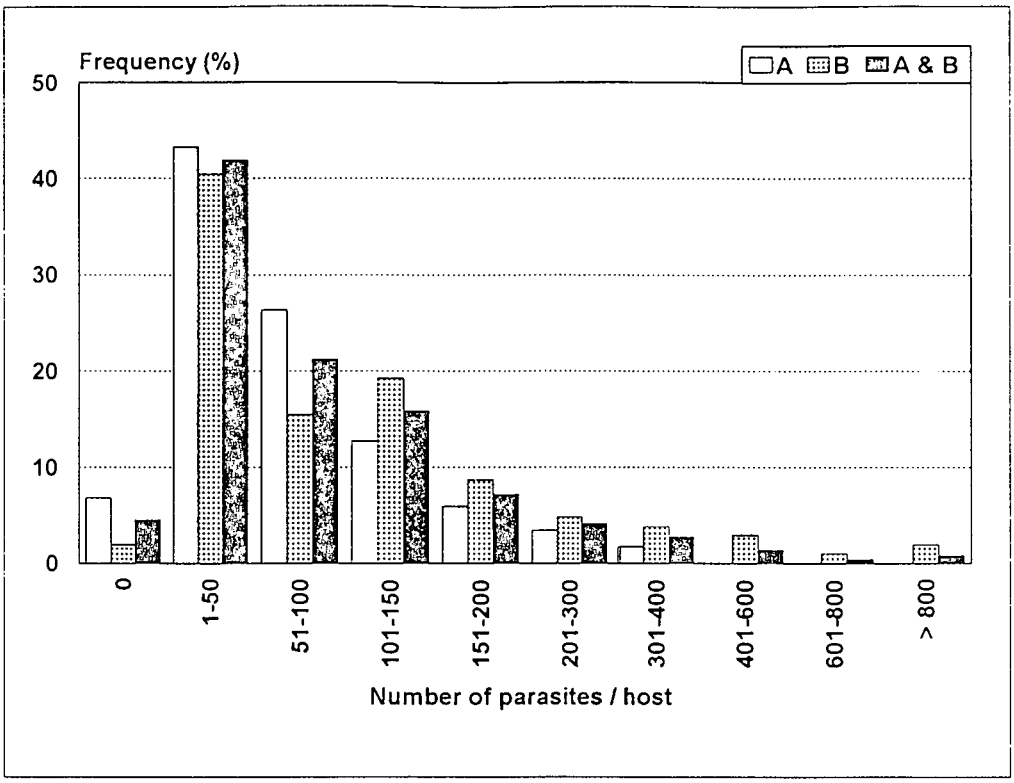
Figure 5.2

Bar graph illustrating the frequency distribution of parasite infection levels at locality A, B and for data from both localities combined.

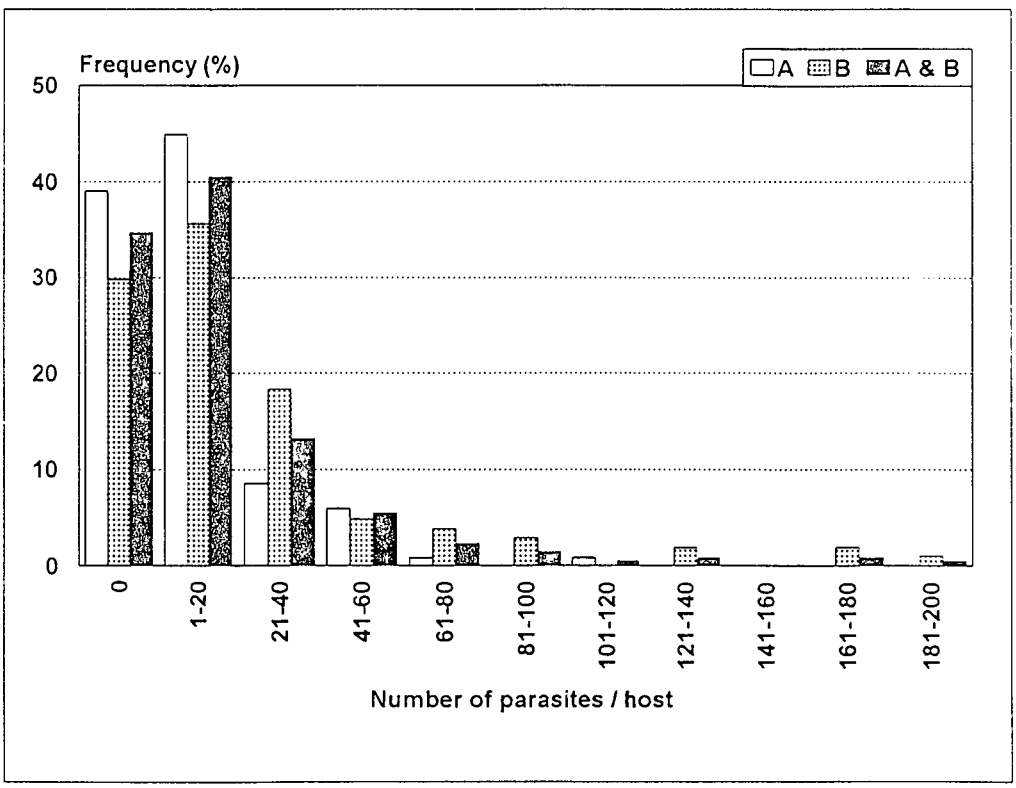
A) *Tylodelphys xenopi* (pericardium).

B) *Tylodelphys xenopi* (body cavity).

A


Frequency (%)
A
B
A & B
50
40
30
20
10
0
0
1-50
51-100
101-150
151-200
201-300
301-400
401-600
601-800
> 800
Number of parasites / host

B


Frequency (%)
A
B
A & B
50
40
30
20
10
0
0
1-20
21-40
41-60
61-80
81-100
101-120
121-140
141-160
161-180
181-200
Number of parasites / host

5.3.5 *Cephalochlamys namaquensis*

C. namaquensis, an adult tapeworm infecting the intestine of *Xenopus*, had the second highest prevalence of all parasites found (Table 5.2). The cestode was present in 72.9% of hosts collected from locality A (n = 118), and in 76.9% of hosts from locality B (n = 104). The maximum number of parasites per host was 21 with a mean intensity of 5.6 at locality A, and a maximum and mean intensity of 36 and 5.2 respectively at locality B.

At locality A 31.4% and at locality B 35.6% of hosts carried between one and three parasites (Fig. 5.3A). No significant difference (P = 0.948) existed in the infection levels of the parasite at the two localities (Table 5.3).

5.3.6 *Valipora campylancristrota*

The plerocercoid cestode larva was found in the bile ducts of *X. laevis*. The parasite was more prevalent at locality B with 32.8% of hosts infected (n = 116), while only 5.1% of hosts from locality A (n = 118) carried the plerocercoid. The mean intensity was 1.5 at locality A with a maximum of two parasites per host (Table 5.2). At locality B, a higher mean intensity of 7.3 was recorded with up to 52 parasites infecting a single host. The rest of the infected *X. laevis* from locality B contained less than 28 parasites, but most infections ranged between one and eight parasites per host.

V. campylancristrota exhibited a negative binomial distribution within the host populations from both locality A and B. At locality B 71.1% of infected hosts carried

low burdens of between one and six parasites (Fig. 5.3B). The Mann-Whitney U-test (Table 5.3) confirmed that the infection levels of the parasite was significantly higher at locality B ($P = 0.0001$).

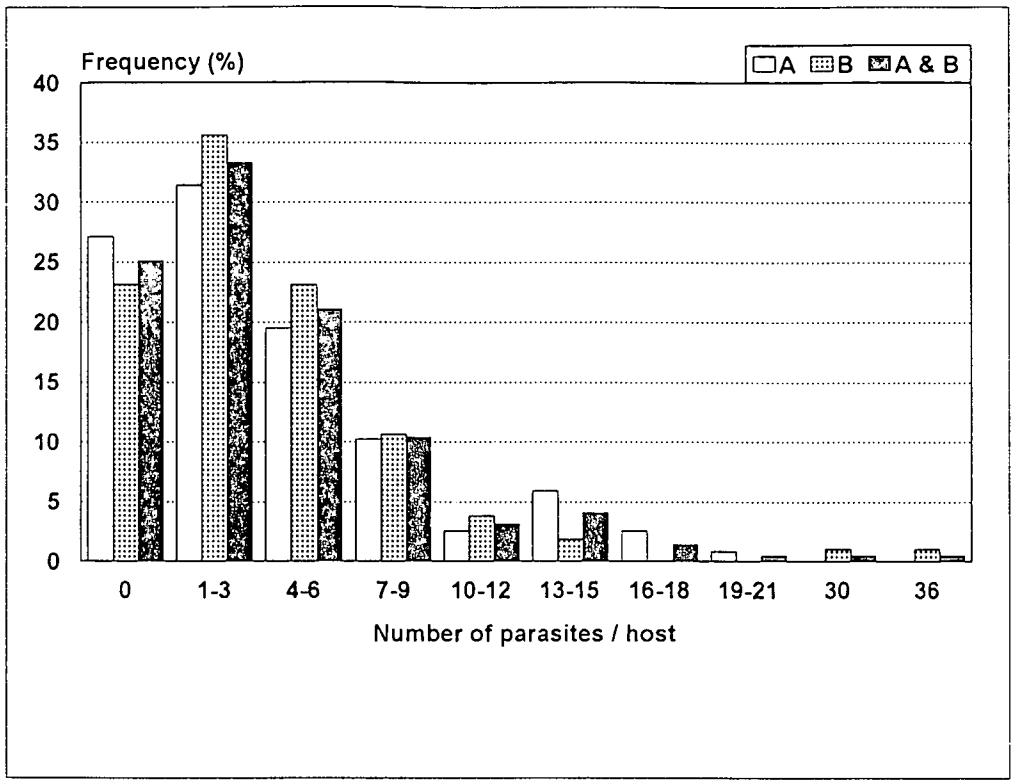
Figure 5.3

Bar graph illustrating the frequency distribution of parasite infection levels at locality A, B and for data from both localities combined.

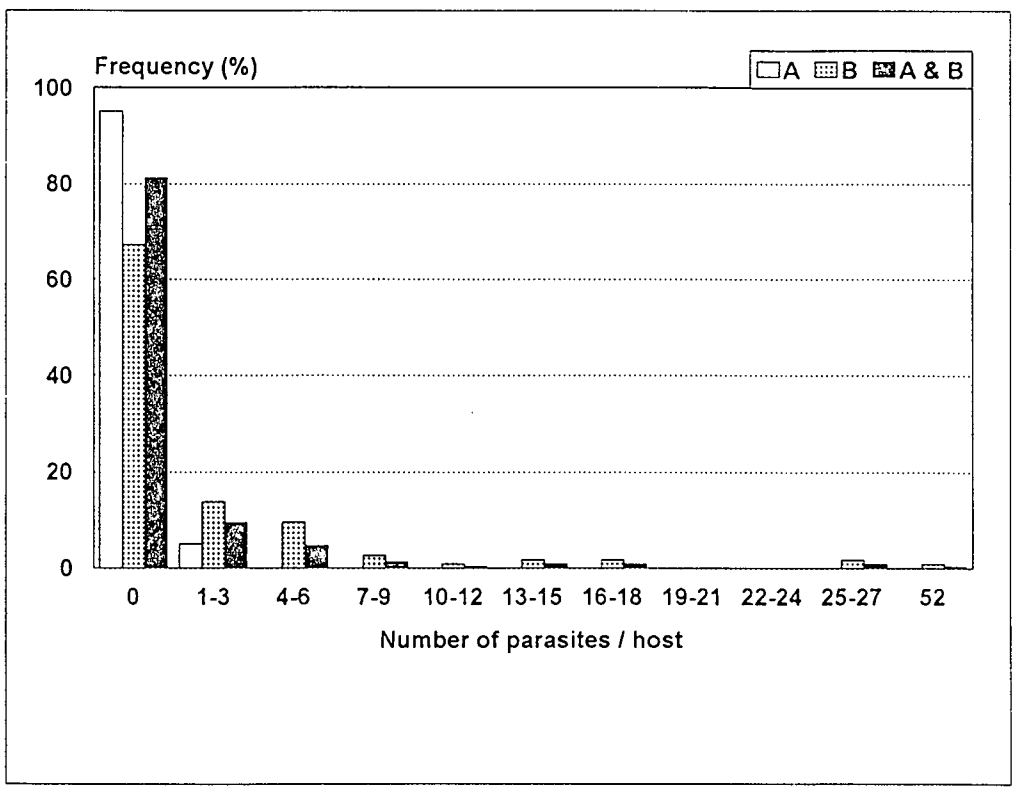
A) *Cephalochlamys namaquensis.*

B) *Valipora campylancristrota.*

A


Frequency (%)
A
B
A & B
40
35
30
25
20
15
10
5
0
0
1-3
4-6
7-9
10-12
13-15
16-18
19-21
30
36
Number of parasites / host

B


Frequency (%)
A
B
A & B
100
80
60
40
20
0
0
1-3
4-6
7-9
10-12
13-15
16-18
19-21
22-24
25-27
52
Number of parasites / host

5.3.7 *Camallanus kaapstaadi*

The adult roundworm, *C. kaapstaadi* is found in the oesophagus of *X. laevis*, and was the third most common parasite infecting the frog. A prevalence of 68.6% was recorded at locality A (n = 118) and 76% at locality B (n = 104). The mean intensity was 5.2 and 4.5 at locality A and B respectively (Table 5.2), with a maximum of 21 parasites infecting a host from locality A, and 17 infecting a host from locality B.

The highest percentage of hosts, 38.1% at locality A and 39.4% at locality B, carried between one and three parasites (Fig 5.4A). No significant difference (P = 0.377) was found in the infection levels of the parasite at the two localities (Table 5.3).

4.3.8 *Batrachocamallanus slomei*

B. slomei infects the stomach of *X. laevis* as adults. The prevalence of the parasite was relatively low, with only 17.8% of hosts (n = 118) from locality A and 4.8% of hosts (n = 104) from locality B infected (Table 5.2). The mean intensity was 8.5 at locality A, with a maximum of 112 worms found in a single host. This was, however, an exceptional case, as the rest of the infections were below 20 per host. A maximum of six parasites was recovered from a single host from locality B, with the mean intensity being two.

B. slomei exhibits a definite negative binomial distribution within the host population (Fig. 5.4B). The infection levels of the parasite was not significantly different (P = 0.085) at the two localities (Table 5.3).

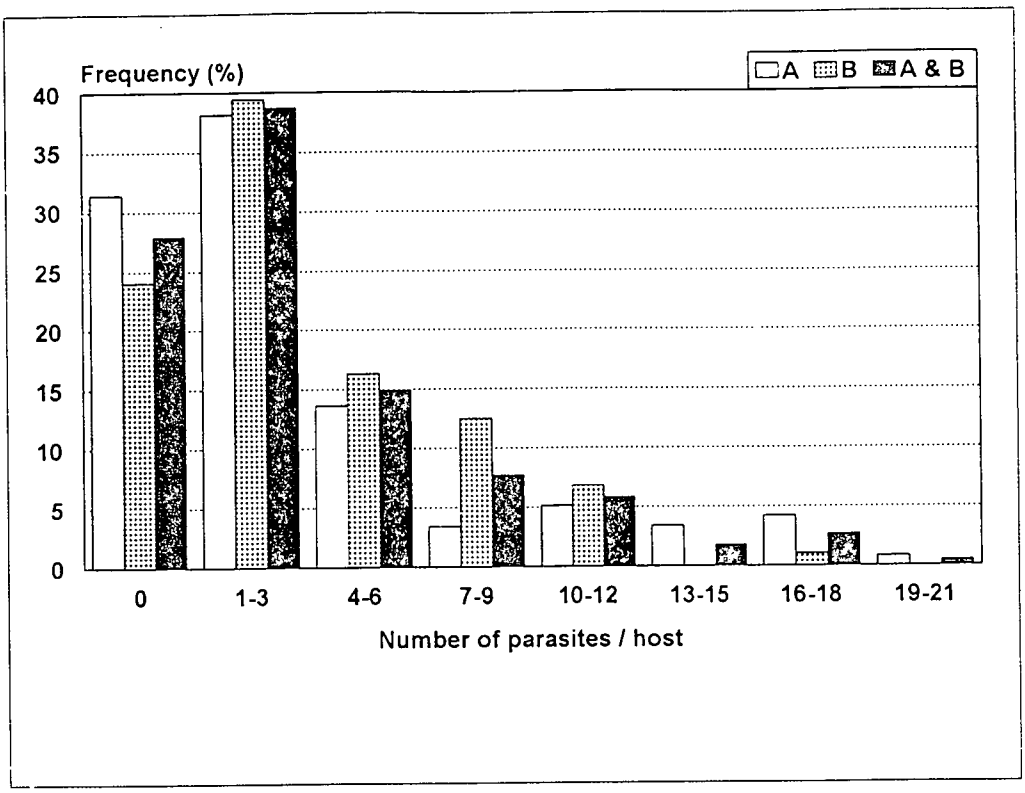
Figure 5.4

Bar graph illustrating the frequency distribution of parasite infection levels at locality A, B and for data from both localities combined.

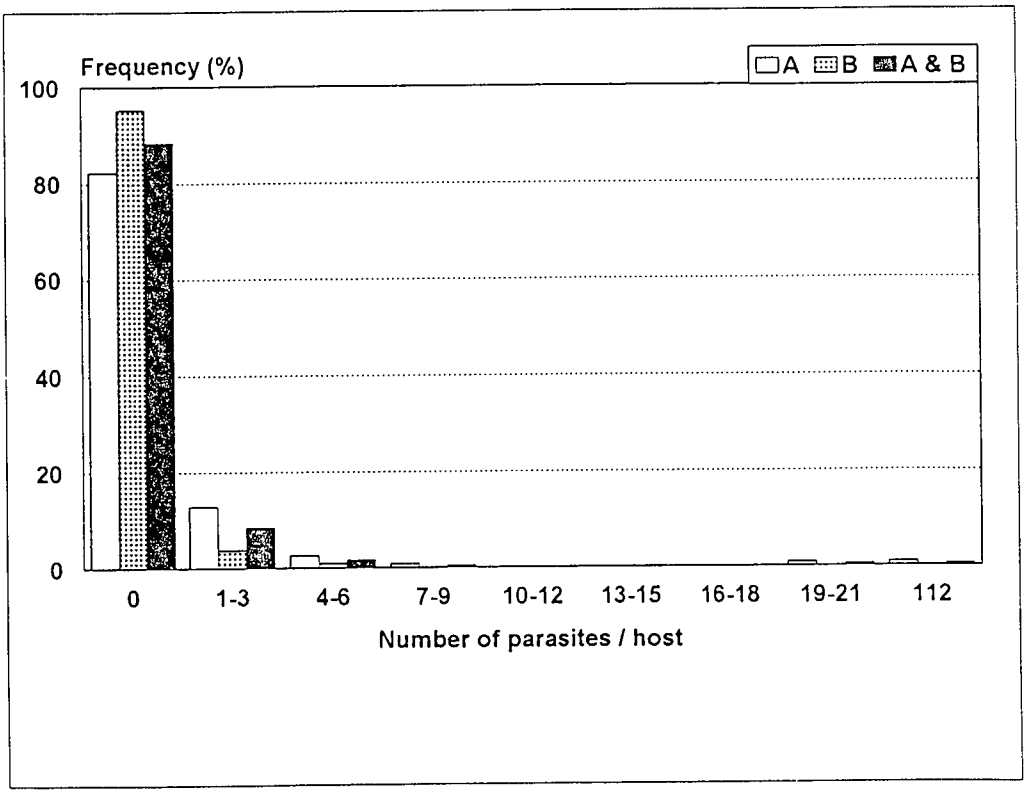
A) *Camallanus kaapstaadi.*

B) *Batrachocamallanus slomei.*

A


Frequency (%)
A
B
A & B
40
35
30
25
20
15
10
5
0
0
1-3
4-6
7-9
10-12
13-15
16-18
19-21
Number of parasites / host

B


Frequency (%)
A
B
A & B
100
80
60
40
20
0
0
1-3
4-6
7-9
10-12
13-15
16-18
19-21
112
Number of parasites / host

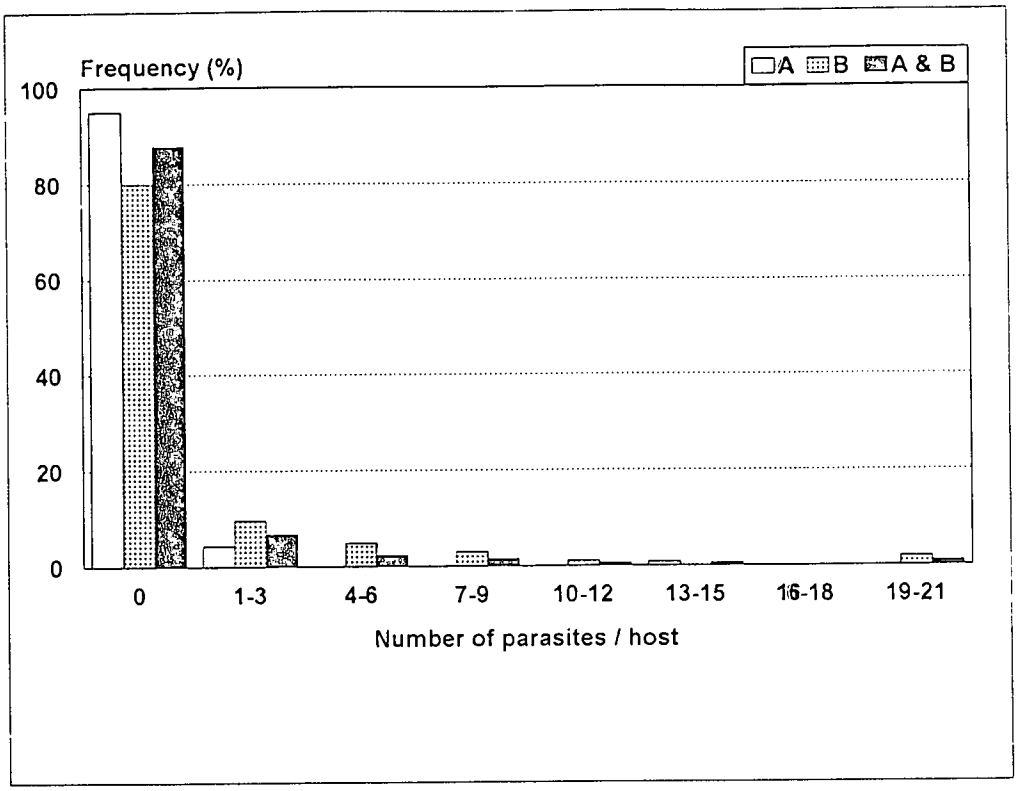
5.3.9 *Marsupiobdella africana*

A very low prevalence of 5.1% was recorded at locality A (n = 118), and 20.2% at locality B (n = 104). A mean intensity of 3.8 was recorded at locality A, with a maximum of 14 immature *M. africana* found on one host. At locality B a maximum of 21 parasites per host was recorded, with a mean intensity of 5.6 (Table 5.2).

At locality A and B, most of the hosts were not infected with the leech, corresponding to a negative binomial distribution within the host population (Fig. 5.5). A significant difference in the infection levels of *M. africana* was found between the two localities (P=0.049). The parasite was more abundant at locality B (Table 5.3).

Figure 5.5

Bar graph illustrating the frequency distribution of *Marsupiobdella africana* infection levels at locality A, B and for data from both localities combined.


Frequency (%)
A
B
A & B
100
80
60
40
20
0
0
1-3
4-6
7-9
10-12
13-15
16-18
19-21
Number of parasites / host

5.4 DISCUSSION

5.4.1 *Gyrdicotylus gallieni*

In the original description of *G. gallieni* by Vercammen-Grandjean (1960), the site of infection was wrongly given as the stomach and intestine. This was however rectified by Thurston (1970) who reported the infection site to be the buccal cavity. Harris and Tinsley (1987) indicated that previous records of *G. gallieni* occurring in the digestive system were probably because the parasites were swallowed by the host.

Thurston (1970) reported the first infection levels for the parasite, finding 77 specimens in the only infected frog in a sample of 'approximately' 26, but adding that the host had been in an aquarium tank for 6 weeks during which time reinfection probably took place. In 1987, Harris and Tinsley reported finding between one and seven parasites per host, with an overall mean intensity of 1.91 (n = 439). The current study's findings of a maximum of 30 parasites found in a single host, and four hosts infected with between 10 and 18 parasites seem to be an exception, as the rest of the hosts examined (n = 217) all had between zero and eight parasites, with an overall mean intensity of 7.4 at locality A and 2.6 at locality B.

Various authors have confirmed the relatively low prevalence of the parasite. Cosgrove and Jared (1974) reported a prevalence of 8.7% (n = 435), and Harris and Tinsley (1987) a prevalence between 0% and 30%, with the overall prevalence in a sample of 483 frogs being 3.1%. They also reported the prevalence of the parasite in

smaller samples that didn't form part of their study. They found five out of a sample of 19 frogs infected (26%) in one sample, and in an additional sample of 94 a total of 18 frogs (19%) were infected. The current study's overall prevalence of 14.4% (n = 222) confirms the low prevalence of previous reports.

G. gallieni possesses distinctive gyrodactylid characters, including a unique form of viviparity where the parent may contain *in utero* offspring, which in turn may have further offspring in the uterus and so on. In this way, the adult may contain up to four generations which are born in quick succession and also start reproducing. The parasite therefore has the potential capability of an enormous population growth (Tinsley & Whitear, 1980). However, the population growth is slow due to a slow rate of reproduction (Harris & Tinsley, 1987). Laboratory studies on the infrapopulation dynamics of the parasite showed that numbers increased exponentially, but at a slow rate, up to 50 days after infection to reach numbers of more than 200 worms per host, after which established infections became extinct within two to five months due to some form of host reaction. The slow infrapopulation growth may elicit the host response more slowly, extending the time for dispersal (Jackson & Tinsley, 1994). The current study's findings confirm that, despite potentially large population numbers, the prevalence and mean intensity of *G. gallieni* is low. Although infections of up to 30 worms per host were recorded, and Harris and Tinsley (1987) found up to 55 parasites detached from a single host, a possible host response usually keeps numbers within a limit of approximately eight parasites per host. The negative binomial distribution of *G. gallieni* within the host population is a product of its slow population growth and possible host responses.

G. gallieni is sensitive to water currents and is transmitted directly (Harris & Tinsley, 1987). Possible differences between the localities such as host abundance and diet (Esch & Fernández, 1993) did not influence the parasite or its transmission, and therefore no significant difference in the infection levels of the parasite at the two localities were found. The only factor that could influence the infection levels therefore seemed to be host responses that would not differ at the two localities.

5.4.2 *Protopolystoma xenopodis*

The study confirmed the presence of adult *P. xenopodis* in the urinary bladder and juveniles in the kidneys. Larvae of the parasite enter the cloaca of the frog and migrate to the kidneys where they mature, and finally move down the urinary ducts and settle in the bladder (Thurston, 1964). The very low prevalence (1.9%; n = 104) of the parasite in the kidneys is a reflection of the relatively short time the parasite spends in the kidneys and urinary ducts as immature worms. A maximum of five juveniles was found in the kidneys, but Thurston (1964) retrieved 12 individuals from a single experimentally infected host. Thurston (1970) studied the incidence of parasites in an unknown *Xenopus* species in numerous relatively small samples from different localities. She found immature *P. xenopodis* in the kidneys of between 0% and 22% of hosts, and a mean intensity ranging from 1.0 to 7.5 with up to 11 parasites per host.

Thurston (1964) reported a prevalence of adult *P. xenopodis* in *X. l. victorianus* (see Tinsley, 1973) of between 40% and 50% throughout the year, with the number of parasites per host usually between two and three with a maximum of eight. Thurston

(1970) found the adult parasite in between 0% and 65% of hosts examined, with a mean intensity range of 1.0 to 3.1. Macnae *et al.* (1973) reported a burden of up to nine worms per host and a high infection rate of more than 60%. A considerably lower prevalence of 25% (n = 435) were reported by Cosgrove & Jared (1974). Jackson (1982) did, however, also find a higher prevalence of 40% (n = 1200). The prevalence of *P. xenopodis* at locality A (39.8%) and B (63.8%) corresponds to the previous records. The mean intensity at locality A (1.3) and B (1.9) were slightly lower than previously recorded, and very high infection levels were not encountered. The infection intensity determined by this study seems to be the normal expected level.

The maximum of seven adult *P. xenopodis* per host found in the current study gives an indication of the restricted range of infection levels. Although as many as eight (Thurston, 1964 & 1970), nine (Macnae *et al.*, 1973), 12 (Jackson & Tinsley, 1988a) and 15 (Jackson & Tinsley, 1988b) adult worms have been found per host, Jackson and Tinsley (1998b) confirmed a normal maximum of six worms per host. In natural *Xenopus* populations, at least 50% of hosts are uninfected, and 60% of infected hosts only carry a single parasite (Tinsley, 1996a). The infection levels found at locality A corresponds with this with 60.2% of hosts carrying no adult *P. xenopodis*, and 78.7% of infected hosts carrying one worm. At locality B, however, only 36.2% of hosts were not infected, but 54% of infected individuals carried one adult parasite and 43.7% between two and four worms. This limited range of infection levels suggests that strong measures exist which regulate population numbers of a parasite capable of reaching high burdens through highly efficient transmission, continuous re-infection and longevity of up to two years (Tinsley, 1996a). Circumstantial evidence exists of host immunity mediating regulation

by elimination of established parasites. The egg production of *P. xenopodis* also has an extreme sensitivity to so-called crowding effects, which means that egg output per parasite is lower in burdens of two to four worms per host than in single worm infections (Jackson & Tinsley, 1988b).

The infection levels of *P. xenopodis* differed significantly at the two localities. The parasite was found in 24% more hosts from locality B than A, and burdens were also higher at locality B. The lower infection levels at locality A is a mystery. Higher burdens were expected because the dam is shallower and the resultant water temperature higher than at locality B. This should have enhanced egg output and increased infection levels. It is also possible that the temperature reached an above optimum level, which could have inhibited egg production. The lower infection levels could also be an indication of a smaller *X. laevis* population that would have had a negative effect on transmission success.

5.4.3 *Dollfuschella rodhaini*

Vercammen-Grandjean (1960) recorded a very high prevalence of *Dollfuschella rodhaini* in *X. l. victorianus*. He found 85.0% of hosts infected with up to 30 parasites. A much lower prevalence of 7.4% (n = 27) in *X. laevis* was reported by Beverley-Burton (1963). Thurston (1970) found a prevalence of up to 33.0% with a maximum of seven parasites in *Xenopus* sp. Macnae *et al.* (1973) reported that the parasite was only found in a small proportion of *X. laevis* examined, but mentioned unpublished data by Badenhorst where a prevalence of 73,3% was recorded. During the current study, *D.*

rodhaini was the only adult digenean found to infect *X. laevis*. The parasite was only recorded from locality A, with a very low prevalence of 1.7% and only one parasite found per host. The miracidia of *D. rodhaini* are known to infect the snail *Lymnaea natalensis* (see Vercammen-Grandjean, 1960), but the nature of transmission of cercariae to the final host is still unknown, although it is probably by an insect larva or copepod (Macnae *et al.*, 1973). Low numbers, or absence, of one of the intermediate hosts at localities A and B may explain the low prevalence of the parasite.

5.4.4 *Tylodelphys xenopi*

The high infection levels of *T. xenopi* found in the pericardium during the current study correspond with those in previous reports. Vercammen-Grandjean (1960) reported a very high prevalence of 96.0% (n = 50) of *T. xenopi* in *X. l. victorianus*. Nigrelli and Maraventano (1944) found the parasite in the pericardium of 78.2% (n = 55) *X. laevis* examined, with infected hosts carrying up to 150 parasites. A prevalence of 100% (n = 100) was reported by Macnae *et al.* (1973), and a minimum of 10 parasites per host. Tinsley and Sweeting (1974) found 60.0% (n = 410) of *X. laevis* infected with up to 3000 parasites. Very high infection levels were recorded for the parasite during the current study. Combining the data from locality A and B, 95.5% (n = 222) of hosts were infected with up to 950 parasites. The parasite had a lower prevalence of 65.3% (n = 222) in the body cavity with a maximum of 190 found in a single host. This indicates that the pericardium is the primary infection site, and only when infection levels are high do the parasite utilise the body cavity as reported by Macnae *et al.* (1973) and Tinsley and

Sweeting (1974). The parasite was, in fact, never found exclusively in the body cavity, but in numbers correlated with those in the pericardium (see also 6.4.2c).

Although the percentage of hosts at locality A and B not carrying *T. xenopi* in the pericardium was much less than reported by Tinsley (1996a), the parasite was overdispersed within the host population, with most of the infected hosts carrying low burdens. At locality A 46.4% and at locality B 41.2% of infected hosts carried between one and 50 parasites. An overdispersed distribution was also found for *T. xenopi* occurring in the body cavity.

The very high prevalence and particularly mean intensity of *T. xenopi* is a function of its longevity and its interaction with *X. laevis*, which represents a closed system. The frog is more or less continuously exposed to invasion by *T. xenopi*, and because of a longevity exceeding three and a half years, no decline in infection levels are found in the absence of continuous infection (Tinsley & Sweeting, 1974). The frequency distribution of the parasite burdens therefore represents its accumulation in the whole lifetime of the frog. Older hosts are therefore expected to carry more parasites (Tinsley, 1996a).

A significant difference was found in the infection levels of *T. xenopi* at the two localities. The mean intensity especially was significantly higher at locality B. One explanation could be the possible higher occurrence of the first intermediate host, the snail *Bulinus tropicus*, at locality B and that it therefore forms a more prominent part of the host diet. Alternatively, at locality B the higher numbers of birds such as herons, which are possible final hosts of the parasite, could infect more snails.

5.4.5 *Cephalochlamys namaquensis*

The tapeworm, *C. namaquensis* was found regularly in the intestine of hosts examined. Although low prevalences were reported by Elkan (1960) (2.4%), Macnae *et al.* (1973) (10-15%) and Cosgrove and Jared (1974) (4.8%), other reports indicate a higher incidence. Thurston (1967 & 1970) found the parasite in between 22% and 100% *Xenopus* sp., and in 50% of *X. laevis* examined. In 1988, Ferguson and Appleton reported a prevalence of 68.9%. The prevalence of *C. namaquensis* at locality A (72.9%) and locality B (76.9%) was higher than most previous reports. The maximum of 21 and 36 parasites per host found at locality A and B respectively is, however, lower. Cohn (1906) and Thurston (1967) reported finding more than 100 parasites in a single host, and Ferguson and Appleton (1988) found a maximum of 88.

Although only approximately 25% of hosts were not infected with *C. namaquensis*, most of the infected hosts carried a low burden. At locality A, 69.8% and at locality B 76.3% of infected hosts carried between one and six parasites, the same range Thurston (1967) reported to be the norm. The skewed distribution of infection levels within the host population corresponds with previous records of Ferguson and Appleton (1988) where 45% of the population carried between one and two parasites. As in most infections where helminths occupy open organ systems, the infrapopulation size of *C. namaquensis* is determined by the balance between a continuous recruitment and loss (Tinsley, 1996a), keeping infection levels below 10 parasites per host most of the time. The fairly high prevalence of the parasite is ascribed to a longevity of more than one year (Tinsley, 1996a).

The difference in the infection levels of *C. namaquensis* at the two localities was not significant. Infective stages of the tapeworm is transmitted to *X. laevis* by copepods which form a major part of the host's diet (Thurston, 1970). As the abundance of the specific copepods could influence the infection levels of *C. namaquensis*, it must be assumed that it did not differ significantly at the two localities.

5.4.6 *Valipora campylancristrota*

The difference in infection levels of the plerocercoid at the two localities was highly significant. The higher infection levels of the parasite at locality B may be because of the presence of higher numbers of the copepod first intermediate host. Also likely is that the habitat surrounding the dam, which provides a more suitable environment for bird life, were conducive to numbers of the final host. Possible final hosts such as herons and cormorants were much more frequently noticed at locality B than at A.

5.4.7 *Camallanus kaapstaadi*

Limited information is available on the nematode *C. kaapstaadi*, and especially records on infection levels are lacking. Thurston (1970) found up to 32 *C. johni* in one *Xenopus* sp. with a mean burden of 9.4 and prevalence of 64%. In the rest of her records, no distinction was made between different nematode species, but the prevalence ranged between 22% and 100% in *Xenopus* sp., and a maximum of 28 parasites in *X. laevis*. Cosgrove and Jared (1974) found *C. kaapstaadi* in 50.1% of *X. laevis* examined.

During the current study, *C. kaapstaadi* was only found in the oesophagus of the frog, and not in the stomach or with the heads imbedded in the mucosa as in previous reports (Southwell & Kirshner, 1937; Thurston, 1970; Cosgrove & Jared, 1974). The parasite was found frequently at locality A (68.6%) and B (76%) with a maximum of 21 and 17 parasites per host respectively. These records are comparable with those of previous reports. Populations of *C. kaapstaadi* are also subjected to continuous recruitment and loss, therefore infection levels are limited.

The parasite's infection exhibited a negative binomial distribution, although the uninfected hosts were less than those infected with low burdens. At locality A 55.6% and at locality B 51.9% of infected hosts carried burdens of between one and three. Only 12.3% and 1.3% of infected hosts at locality A and B respectively carried burdens of more than 12. The infection levels of *C. kaapstaadi* did not differ significantly at the two localities, even though the prevalence was slightly higher at locality B.

5.4.8 *Batrachocamallanus slomei*

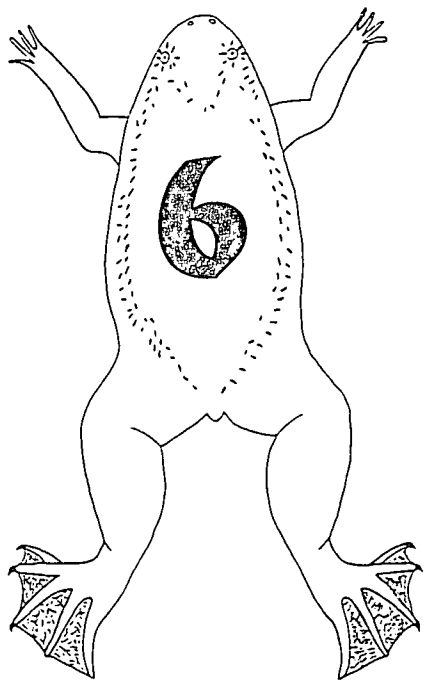
Southwell and Kirshner (1937) found the nematode in 100% (n = 10) of *X. laevis* examined. As Thurston (1970) did not distinguish between *Spirocamallanus xenopodis* and *Camallanus johni*, the infection levels mentioned (see 5.4.7) are the only other indication of the possible expected burdens of *B. slomei*.

Very low infection levels were recorded at both localities for the parasite occurring in the stomach. The heads were not imbedded in the stomach wall as reported previously

(Southwell & Kirshner, 1937). The parasite was only found in 4.8% of hosts from locality B, four of which were infected by a single parasite and one carrying six. The prevalence of the parasite was higher at locality A (17.8%) with one host carrying 112 *B. slomei*. However, 71.4% of infected hosts carried less than five parasites. The frequency distribution of parasite infection levels is thus strongly negative binomial. Although the infection levels of *B. slomei* were higher at locality A, the difference was not significant.

5.4.9 *Marsupiobdella africana*

Van der Lande and Tinsley (1976) found *X. laevis* carrying moderate burdens of *Marsupiobdella africana*. Infection levels ranged between one and 10 parasites per host with a mean of about three. A maximum of 21 unidentified leeches per host was found during the current study, with a mean intensity of 3.8 and 5.6 at locality A and B respectively. The prevalence was relatively low, especially at locality A (5.1%). The parasites were overdispersed in the host population. At locality A 53.3% and at locality B 76.5% of infected hosts carried less than three leeches. The explanation for significantly higher infection levels of *M. africana* at locality B remains to be investigated.



Influence of Climate, Host Size & Host Sex on Infection Levels

CONTENTS

6.1 INTRODUCTION

The aim of this part of the study was to determine to which extent parasite infection levels are influenced by seasonal climatic changes, as well as the size, or age, and sex of the host. Due to the very low prevalence of juvenile *Protopolystoma xenopodis* in the kidneys and *Dollfuschella rodhaini*, these parasites are not discussed.

Esch and Fernández (1993) indicated that climatic change, host age and sex are factors that could influence parasite burdens. In 1990, Tinsley stated that the infection levels of many helminths might be influenced by changes in temperature. Lower temperatures may inhibit egg production contributing to a seasonal decline in parasite transmission. Not only temperature, but also rainfall may influence infection levels by affecting host density (Tinsley, 1993). The control of parasite reproductive biology by host sex hormones has been speculated upon, but has not been experimentally confirmed (Tinsley, 1993).

6.2 MATERIALS & METHODS

6.2.1 SEASONAL VARIANCE IN INFECTION LEVELS

The prevalence and mean intensity were determined for each parasite in each sample from locality A and B respectively. To give a more holistic picture of changes in the infection levels according to season, the monthly results (Appendix 4) were arranged three-monthly to divide the years over which the study stretched into quarters corresponding to season.

6.2.2 CORRELATION BETWEEN HOST SIZE AND INFECTION LEVELS

To determine if any correlation exists between the size of the hosts and the infection levels of parasites, the hosts were grouped according to their snout-urostyle length (Table 6.1). The length was used as an indication of size, as the amount of stored fat varies through the year, which influences the weight of the host. The weight also gives no clear indication of the physical dimensions of the frog, which plays a role in the number of certain parasites it can accommodate.

Table 6.1 Grouping of hosts according to size. Sample size (n), average snout-urostyle length ($\bar{x}$).

Snout-urostyle length (mm)	Locality A		Locality B		Total sample (locality A+B)	
	n	$\bar{x}$ (mm)	n	$\bar{x}$ (mm)	n	$\bar{x}$ (mm)
31-45	23	36.75	5	39.56	28	37.25
46-60	26	50.97	40 (1*)	52.01	66 (1*)	51.61
61-75	25	67.14	20	68.96	45	67.95
76-90	25	81.84	23 (10*)	81.67	48 (10*)	81.63
91-105	14	94.69	13 (1*)	97.21	27 (1*)	95.95
106-120	5	109.82	3	111.77	8	110.55
Total (n)	118		104 (12*)		222 (12*)	

(*Additional frogs examined for *Protopolystoma xenopodis* and *Valipora campylancristrota*)

The prevalence and mean intensity of the parasites were determined for each of the 'size-groups'. This was done for locality A and B respectively, and also for the total sample. To conclude whether the infection levels were significantly related to the size of the host, the correlation coefficients (r) were compared to values defined in 1990 by Fowler and Cohen (Table 6.2).

Table 6.2 The strength of a correlation (modified from Fowler & Cohen, 1990)

Value of coefficient r	Meaning
± 0.00 to 0.19	Very weak correlation
± 0.20 to 0.39	Weak correlation
± 0.40 to 0.69	Modest correlation
± 0.70 to 0.89	Strong correlation
± 0.90 to 1.00	Very strong correlation

6.2.3 HOST SEX AND INFECTION LEVELS

To determine whether any differences exist between the infection levels of parasites in male and female frogs, the data from locality A and B were combined and the prevalence and mean intensity for each parasite determined for male and female hosts respectively.

Table 6.3 Male and female host data.

Sex	Sample size n	Snout-urostyle length $\bar{x}$ (mm)	Length range (mm)
Male	99 (4*)	59.72	39.5-85.3
Female	123 (8*)	75.54	31.3-114.9

(*Additional frogs examined for *Protopolystoma xenopodis* and *Valipora campylancristrota*)

6.3 RESULTS

6.3.1 *Gyrdicotylus gallieni*

During the study period, the prevalence of *G. gallieni* ranged between 0% and 40% in samples from locality A, and at locality B between 0% and 60%. The mean intensity at locality A ranged from 2.0 to 9.7, and between 1.0 and 4.0 at locality B. At locality A, the parasite was not recorded between December and August 1997, and at locality B from September 1996 to May 1997 (Fig. 6.1A&B).

The negative correlation between size of the host and prevalence of the parasite (Fig. 6.2A) was very strong when the data from both localities were combined ($r = -0.91$). The same correlation coefficient applies at locality B, but only a modest correlation exists at locality A ($r = -0.68$). A strong negative correlation between mean intensity and host size (Fig. 6.2B) was found when data were combined ($r = -0.86$). The correlation at localities A ($r = -0.6$) and B ($r = -0.59$) respectively was however only modest.

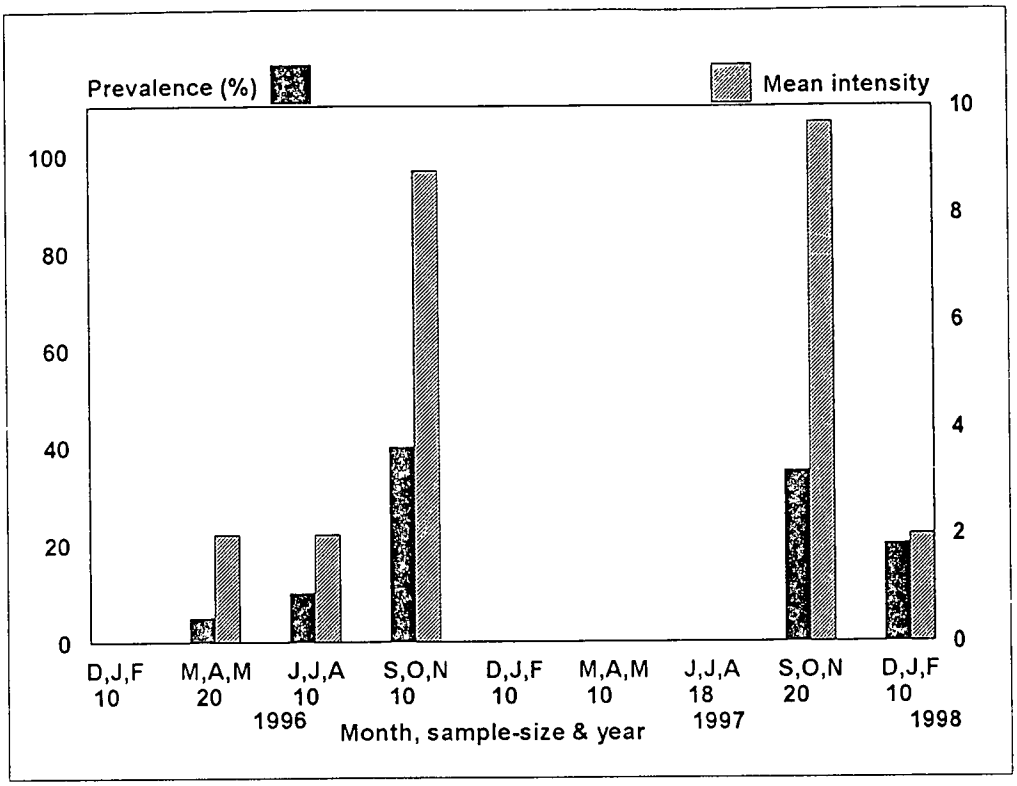
Figure 6.1

Bar graphs illustrating seasonal prevalence and mean intensity of *Gyrdicotylus gallieni*.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
10
8
6
4
2
0
D,J,F 10
M,A,M 20
J,J,A 10
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 18
S,O,N 20
D,J,F 10
1996
1997
1998
Month, sample-size & year

B

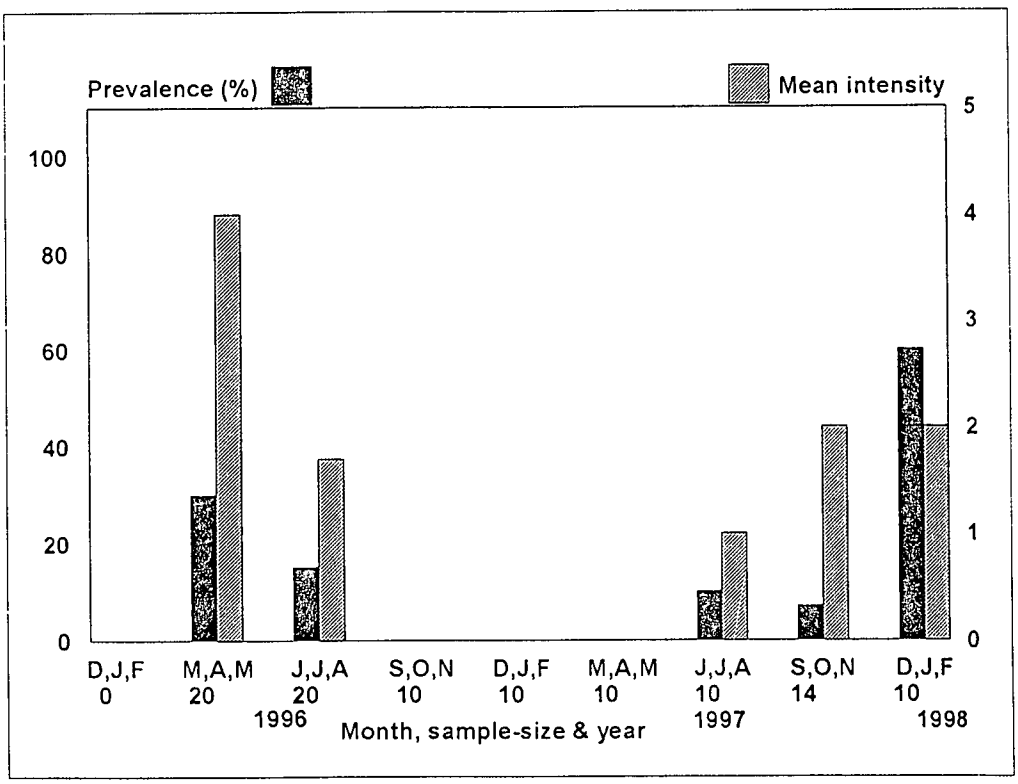

Prevalence (%)
Mean intensity
100
80
60
40
20
0
5
4
3
2
1
0
D,J,F 0
M,A,M 20
J,J,A 20
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 10
S,O,N 14
D,J,F 10
1996
1997
1998
Month, sample-size & year

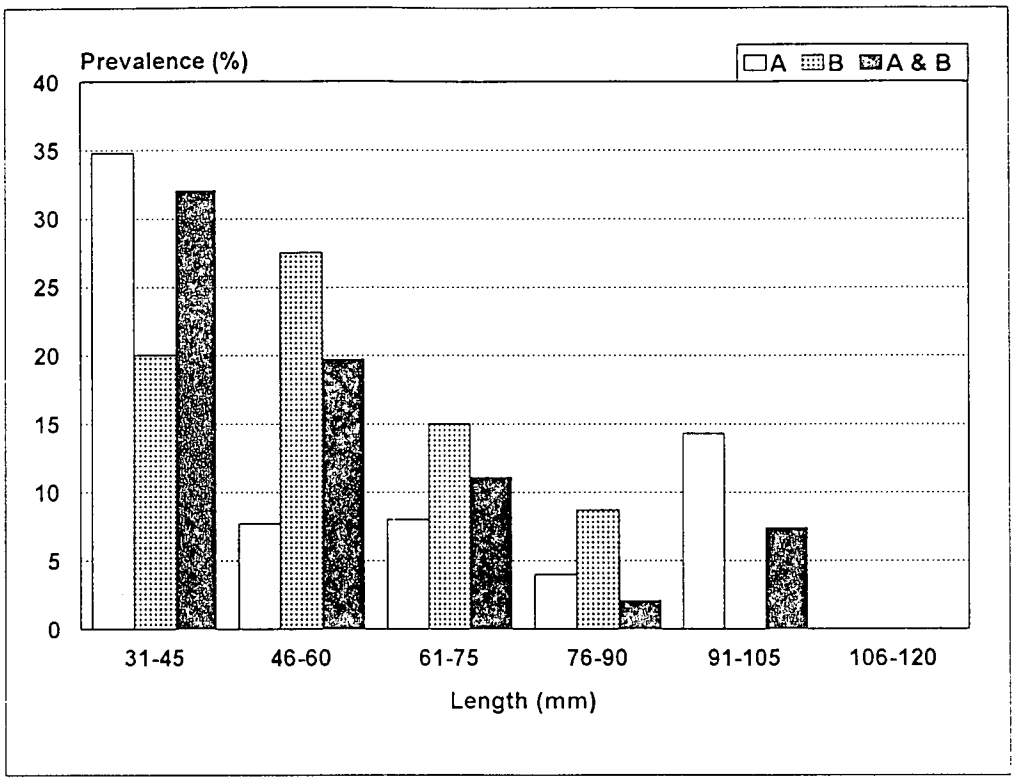
Figure 6.2

Bar graphs illustrating correlation between host size and infection levels of *Gyrdicotylus gallieni* at locality A, B and for data from both localities combined.

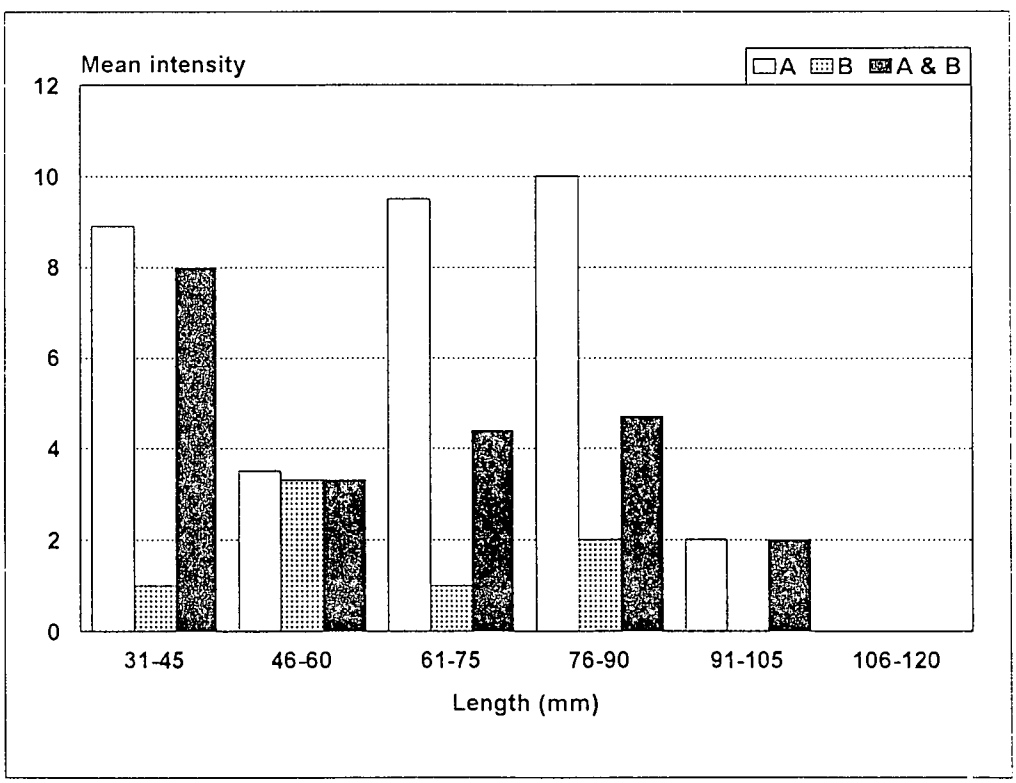
A) Prevalence.

B) Mean intensity.

A


Prevalence (%)
A
B
A & B
40
35
30
25
20
15
10
5
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

B


Mean intensity
A
B
A & B
12
10
8
6
4
2
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

6.3.2 *Protopolystoma xenopodis*

A prevalence of between 10% and 80% was recorded at locality A, and between 50% and 90% at locality B. The mean intensity of the parasite ranged between 1.0 and 1.8 at locality A, and from 1.3 to 2.6 at locality B (Fig. 6.3A&B).

At locality B ($r = 0.43$) and for the combined data ($r = 0.45$), only a modest positive correlation exists between host size and prevalence of *P. xenopodis* (Fig. 6.4A), and only a weak correlation at locality A ($r = 0.36$). Mean intensity (Fig. 6.4B) was however strongly correlated with the size of the host for the combined data ($r = 0.78$). The correlation was strongly positive at locality A ($r = 0.81$), but weak at locality B ($r = 0.25$).

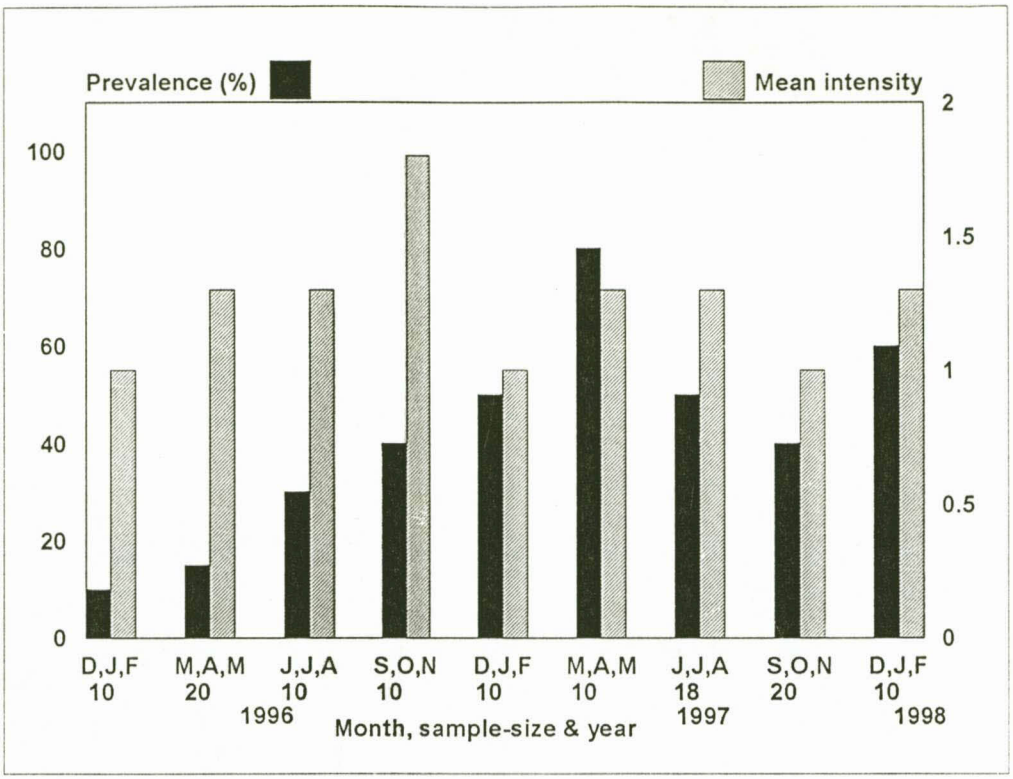
Figure 6.3

Bar graphs illustrating seasonal prevalence and mean intensity of *Protopolystoma xenopodis*.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
2
1.5
1
0.5
0
D,J,F 10
M,A,M 20
J,J,A 10
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 18
S,O,N 20
D,J,F 10
1996
1997
1998
Month, sample-size & year

B

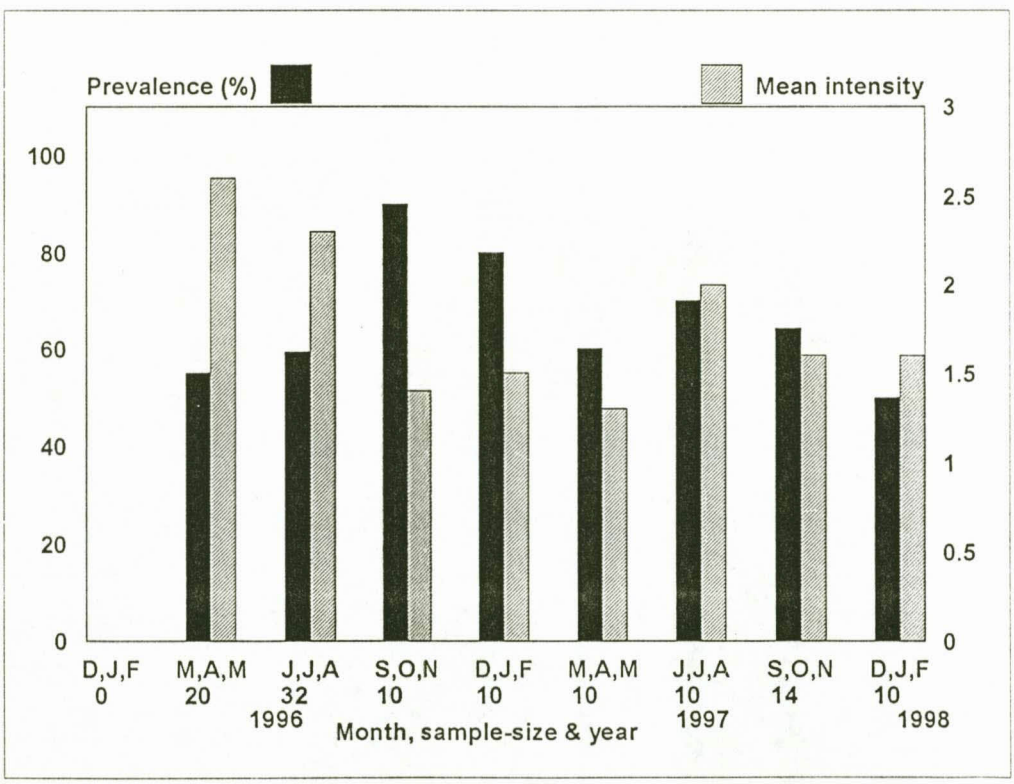

Prevalence (%)
Mean intensity
100
80
60
40
20
0
3
2.5
2
1.5
1
0.5
0
D,J,F 0
M,A,M 20
J,J,A 32
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 10
S,O,N 14
D,J,F 10
1996
1997
1998
Month, sample-size & year

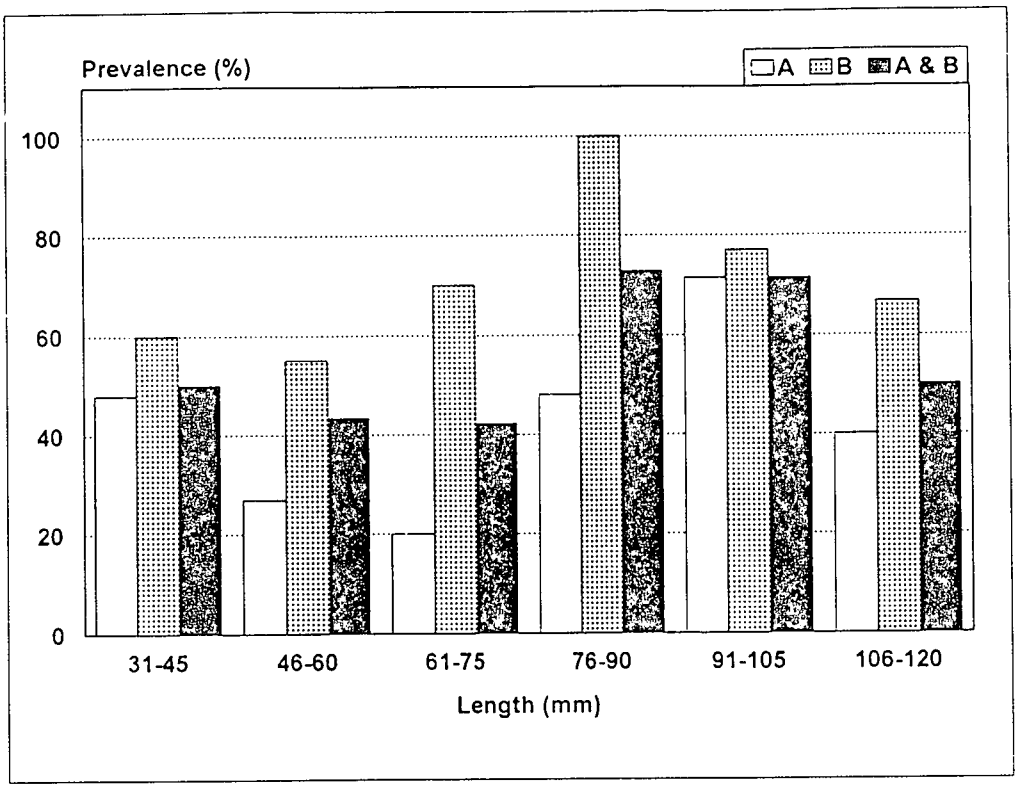
Figure 6.4

Bar graphs illustrating correlation between host size and infection levels of *Protopolystoma xenopodis* at locality A, B and for data from both localities combined.

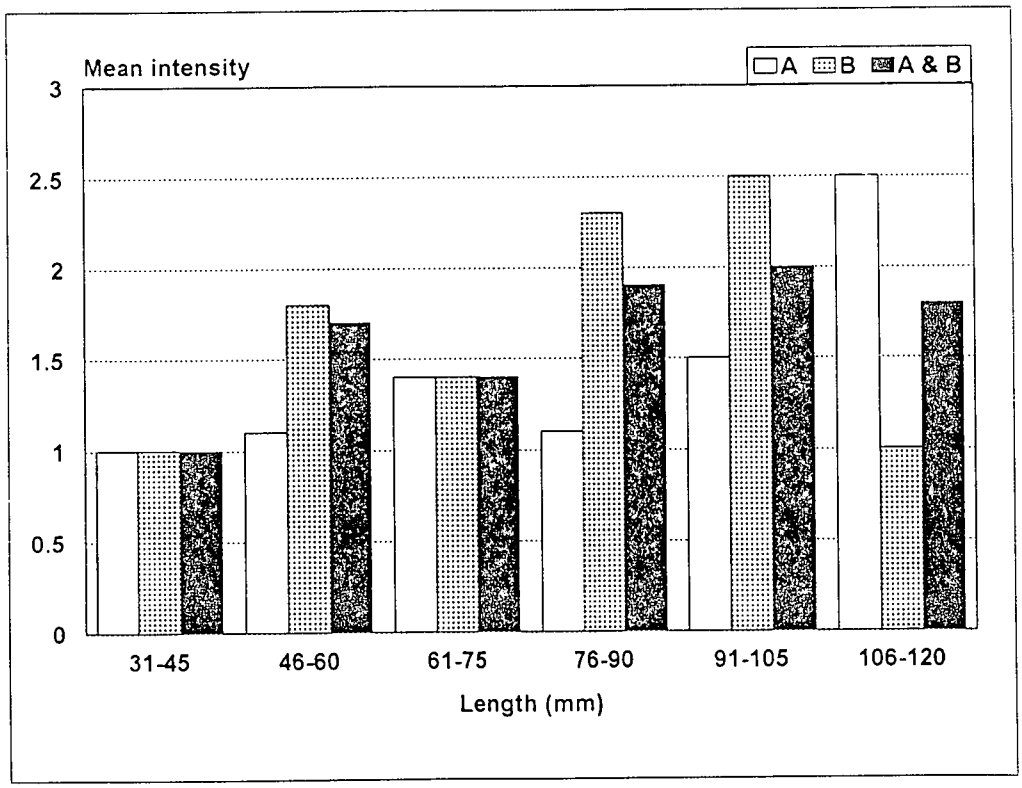
A) Prevalence.

B) Mean intensity.

A


Prevalence (%)
A
B
A & B
100
80
60
40
20
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

B


Mean intensity
A
B
A & B
3
2.5
2
1.5
1
0.5
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

6.3.3 *Tylodelphys xenopi*

The prevalence of *T. xenopi* in the pericardium was constantly very high at both localities. It ranged between 80% and 100% at locality A and B (Fig. 6.5A&B). The prevalence of the parasite in the body cavity had a range between 0% and 90% at locality A, and between 50% and 90% at locality B (Fig. 6.6A&B). In the pericardium, a very high mean intensity of between 32 and 160 was recorded at locality A, and between 32.6 and 272.4 at locality B (Fig. 6.5A&B). The mean intensity of *T. xenopi* in the body cavity ranged between 11.4 and 43.3 at locality A, while at locality B a range of 3.6 to 58.2 was recorded (Fig. 6.6A&B).

At locality A, the correlation between host size and prevalence of the parasite in the pericardium ($r = 0.85$) and body cavity ($r = 0.89$) was strongly positive (Fig. 6.7A). The correlation at locality B (Fig. 6.7B) was however weak for occurrence in the pericardium ($r = 0.39$), and modest for the body cavity ($r = 0.58$). With the data combined, a strong correlation ($r = 0.84$) existed between the prevalence of *T. xenopi* in the pericardium and body cavity and the size of the host for both infection sites (Fig. 6.7C).

A very strong positive correlation existed between host size and the mean intensity of the parasite in the body cavity ($r = 0.97$) and pericardium ($r = 0.9$) at locality A (Fig. 6.8A). At locality B (Fig. 6.8B), the correlation coefficient for the pericardium was the same as at locality A, and the correlation was strong for the body cavity ($r = 0.88$). The combined data showed a very strong correlation between the mean intensity in the pericardium ($r = 0.99$) and body cavity ($r = 0.89$) and the size of the host (Fig. 6.8C).

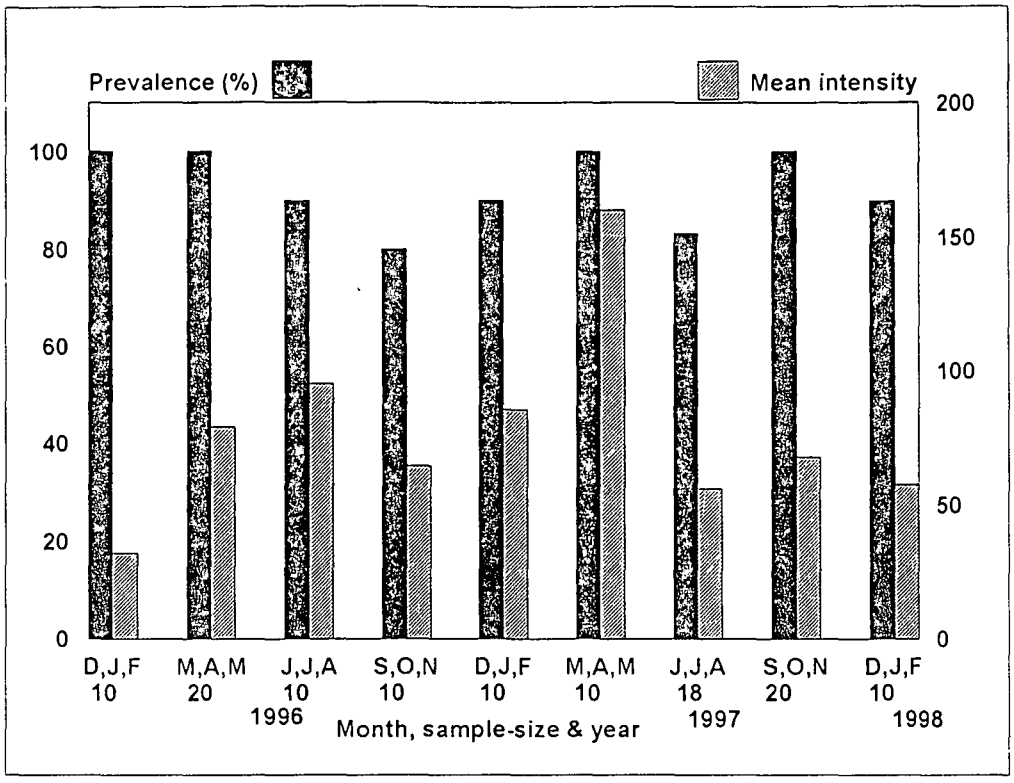
Figure 6.5

Bar graphs illustrating seasonal prevalence and mean intensity of *Tylodelphys xenopi* in the pericardium.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
200
150
100
50
0
D,J,F
10
M,A,M
20
J,J,A
10
S,O,N
10
D,J,F
10
M,A,M
10
J,J,A
18
S,O,N
20
D,J,F
10
1996
1997
1998
Month, sample-size & year

B

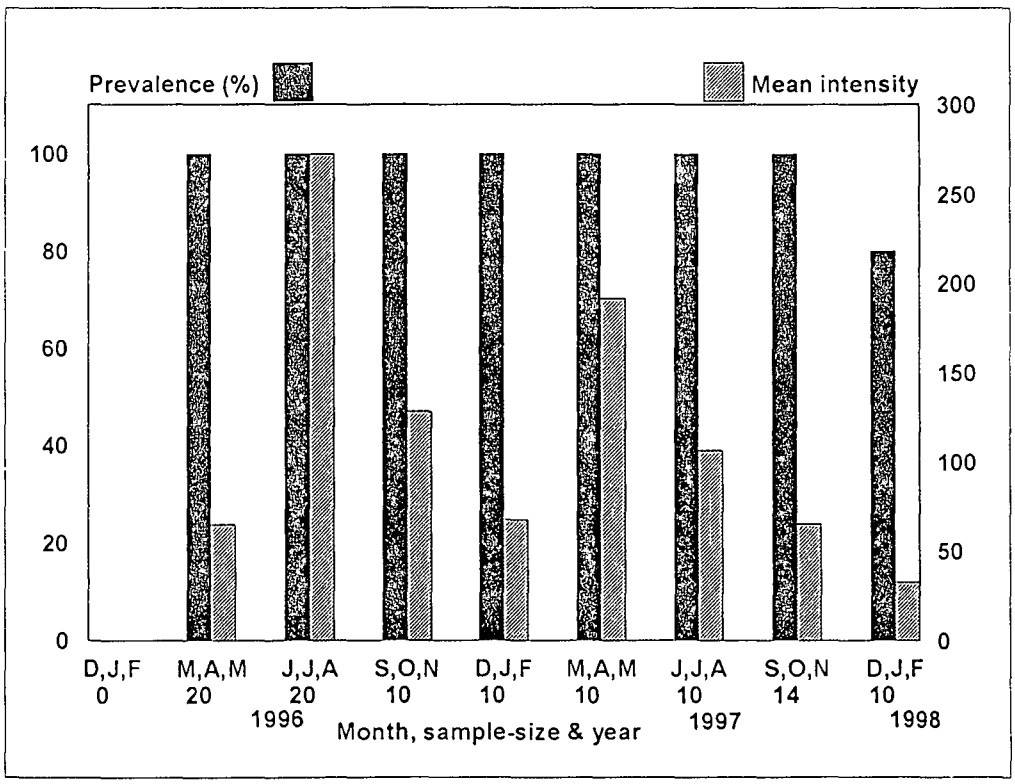

Prevalence (%)
Mean intensity
100
80
60
40
20
0
300
250
200
150
100
50
0
D,J,F
0
M,A,M
20
J,J,A
20
S,O,N
10
D,J,F
10
M,A,M
10
J,J,A
10
S,O,N
14
D,J,F
10
1996
1997
1998
Month, sample-size & year

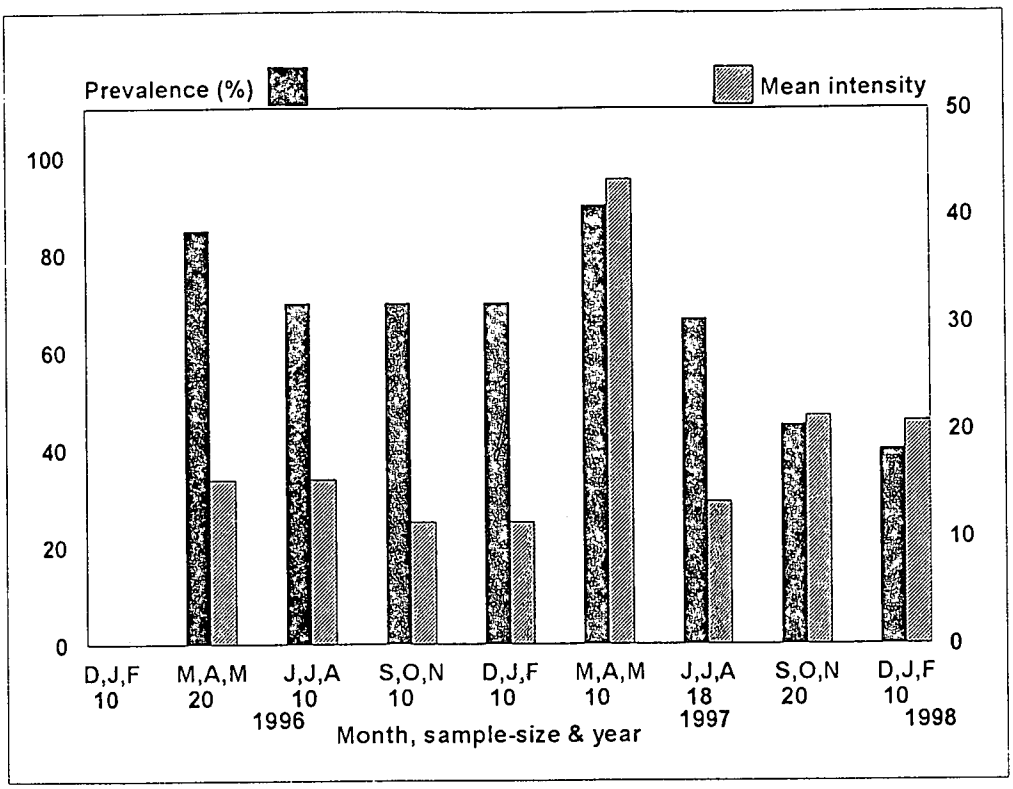
Figure 6.6

Bar graphs illustrating seasonal prevalence and mean intensity of *Tylodelphys xenopi* in the body cavity.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
50
40
30
20
10
0
D,J,F 10
M,A,M 20
J,J,A 10
1996
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 18
1997
S,O,N 20
D,J,F 10
1998
Month, sample-size & year

B

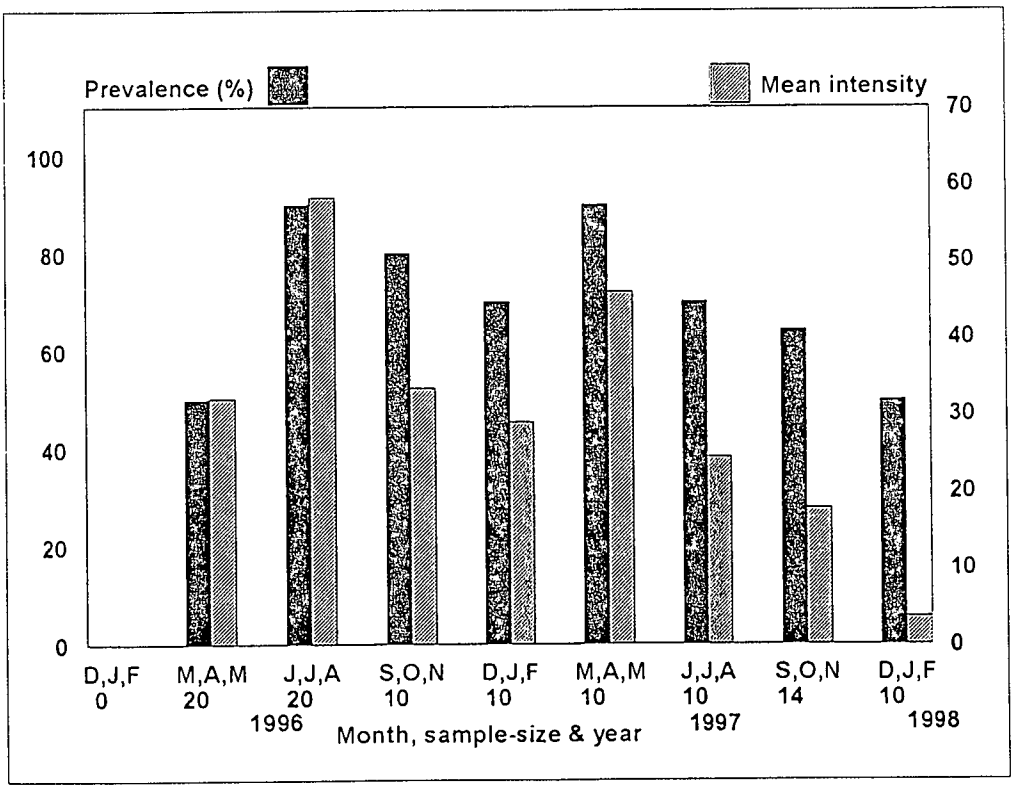

Prevalence (%)
Mean intensity
100
80
60
40
20
0
70
60
50
40
30
20
10
0
D,J,F 0
M,A,M 20
J,J,A 20
1996
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 10
1997
S,O,N 14
D,J,F 10
1998
Month, sample-size & year

Figure 6.7

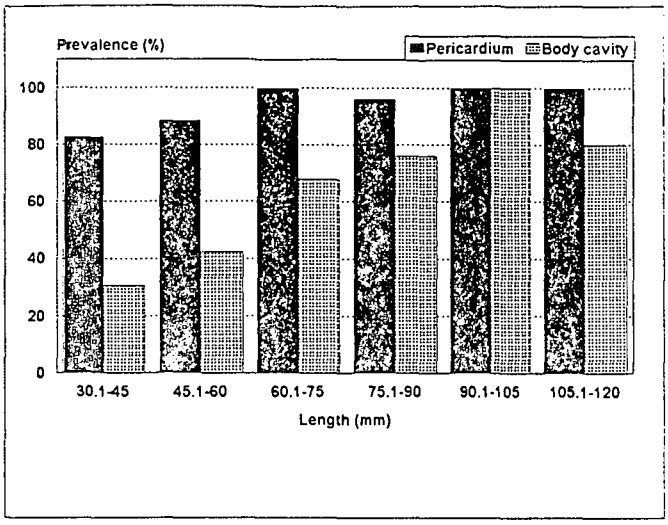
Bar graphs illustrating correlation between host size and prevalence of *Tylodelphys xenopi*.

A) Locality A.

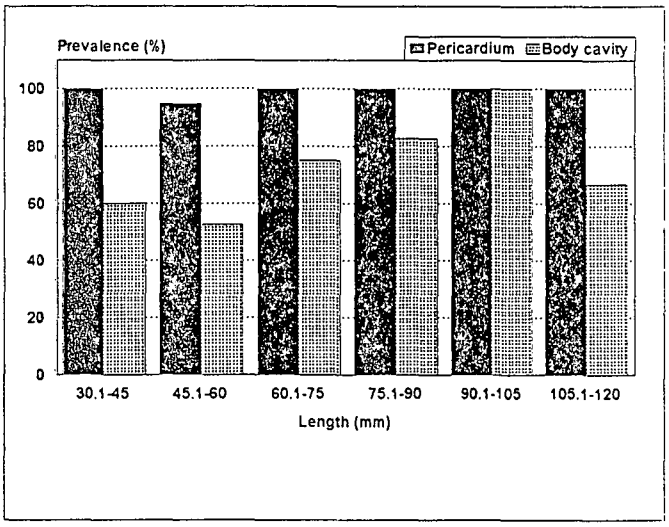
B) Locality B.

C) Locality A & B.

A


Prevalence (%)
Pericardium
Body cavity
100
80
60
40
20
0
30.1-45
45.1-60
60.1-75
75.1-90
90.1-105
105.1-120
Length (mm)

B


Prevalence (%)
Pericardium
Body cavity
100
80
60
40
20
0
30.1-45
45.1-60
60.1-75
75.1-90
90.1-105
105.1-120
Length (mm)

C

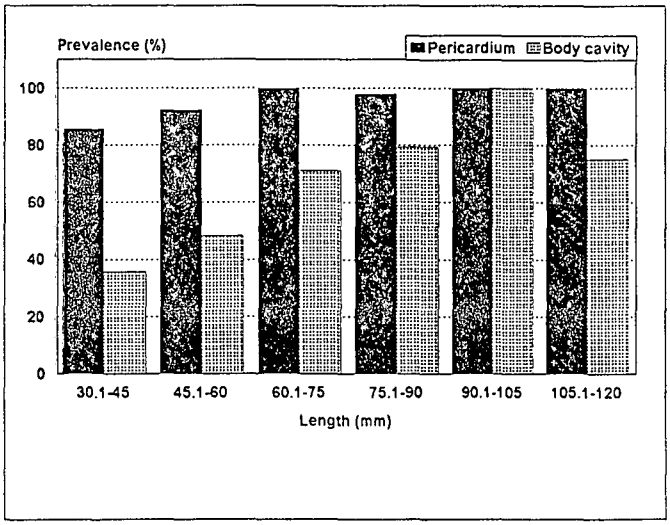

Prevalence (%)
Pericardium
Body cavity
100
80
60
40
20
0
30.1-45
45.1-60
60.1-75
75.1-90
90.1-105
105.1-120
Length (mm)

Figure 6.8

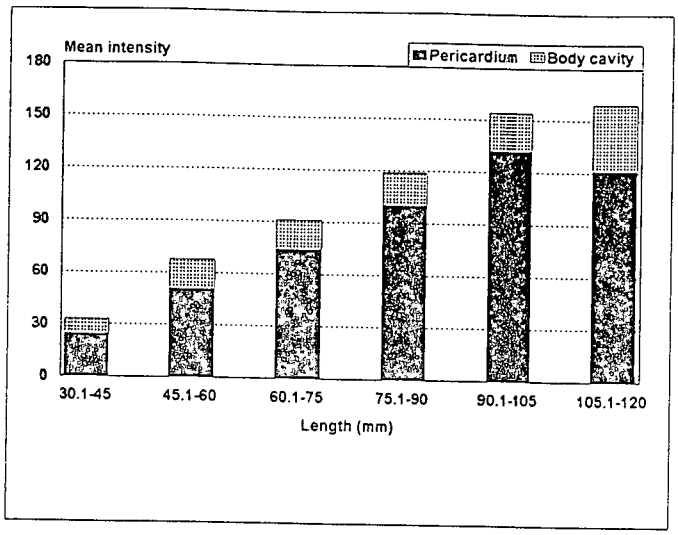
Bar graphs illustrating correlation between host size and mean intensity of *Tylodelphys xenopi*.

A) Locality A.

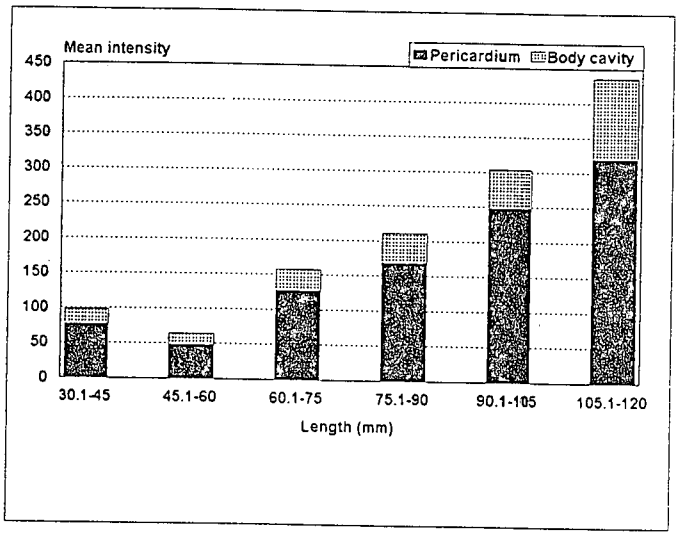
B) Locality B.

C) Locality A & B.

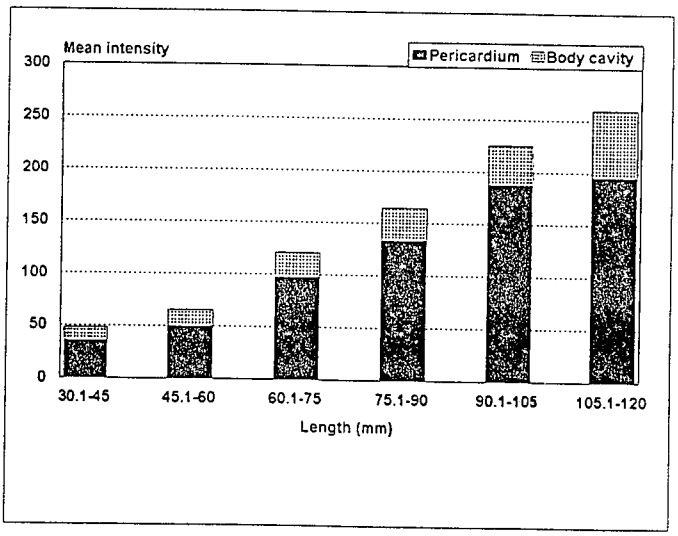
A


Mean intensity
Pericardium
Body cavity
180
150
120
90
60
30
0
30.1-45
45.1-60
60.1-75
75.1-90
90.1-105
105.1-120
Length (mm)

B


Mean intensity
Pericardium
Body cavity
450
400
350
300
250
200
150
100
50
0
30.1-45
45.1-60
60.1-75
75.1-90
90.1-105
105.1-120
Length (mm)

C


Mean intensity
Pericardium
Body cavity
300
250
200
150
100
50
0
30.1-45
45.1-60
60.1-75
75.1-90
90.1-105
105.1-120
Length (mm)

6.3.4 *Cephalochlamys namaquensis*

The prevalence of *C. namaquensis* was relatively high in most of the samples. It ranged between 40% and 100% at locality A, and from 10% to 100% at locality B. At locality A, a mean intensity of between 1.7 and 8.9 was recorded, and at locality B between 1.0 and 8.3 (Fig. 6.9A&B).

The correlation between prevalence of the parasite and host size was strong at locality A and B (Fig. 6.10A), the correlation coefficient in both cases being 0.88. The combined data did however show a very strong positive correlation ($r = 0.91$). The mean intensity (Fig. 6.10B) of *C. namaquensis* infections was also very strongly correlated with the size of the host at locality A ($r = 0.99$) and for the combined data ($r = 0.94$). At locality B, only a modest positive correlation existed ($r=0.67$).

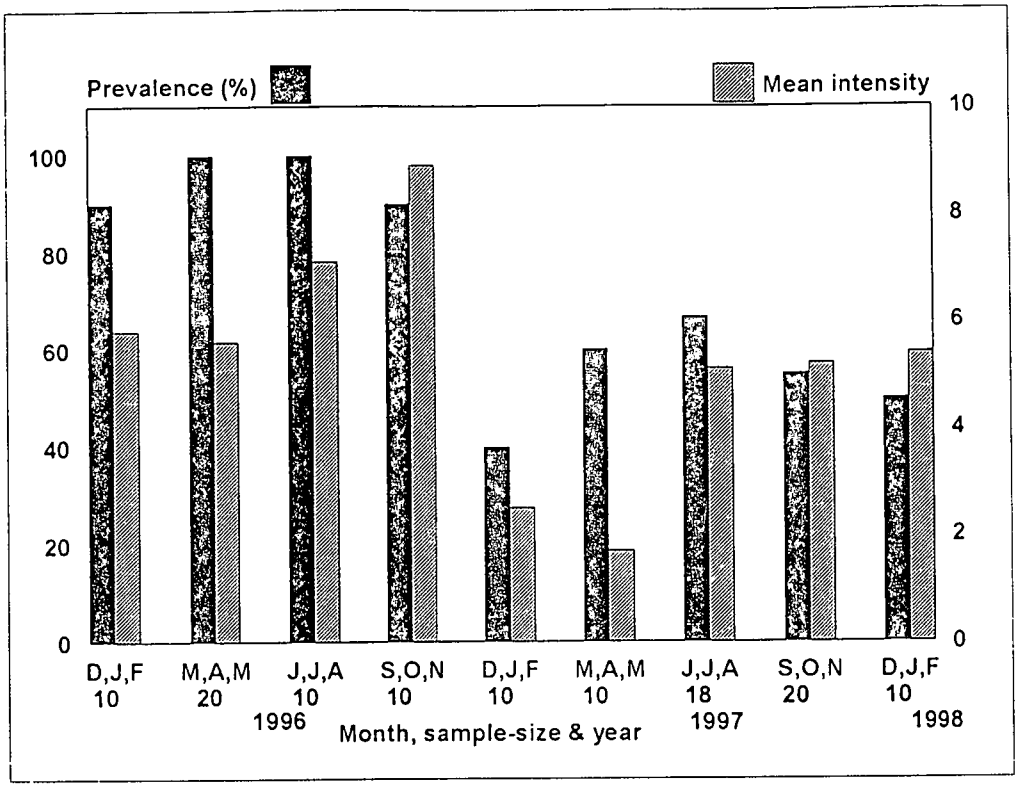
Figure 6.9

Bar graphs illustrating seasonal prevalence and mean intensity of *Cephalochlamys namaquensis*.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
10
8
6
4
2
0
D,J,F 10
M,A,M 20
J,J,A 10
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 18
S,O,N 20
D,J,F 10
1996
1997
1998
Month, sample-size & year

B

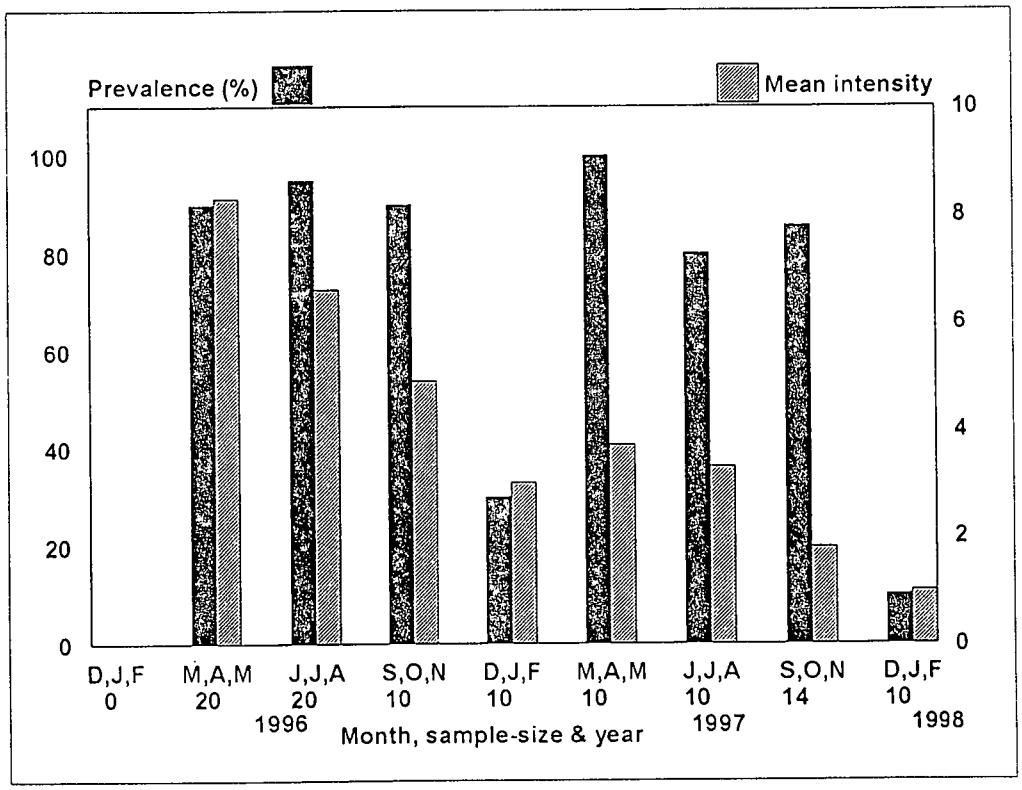

Prevalence (%)
Mean intensity
100
80
60
40
20
0
10
8
6
4
2
0
D,J,F 0
M,A,M 20
J,J,A 20
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 10
S,O,N 14
D,J,F 10
1996
1997
1998
Month, sample-size & year

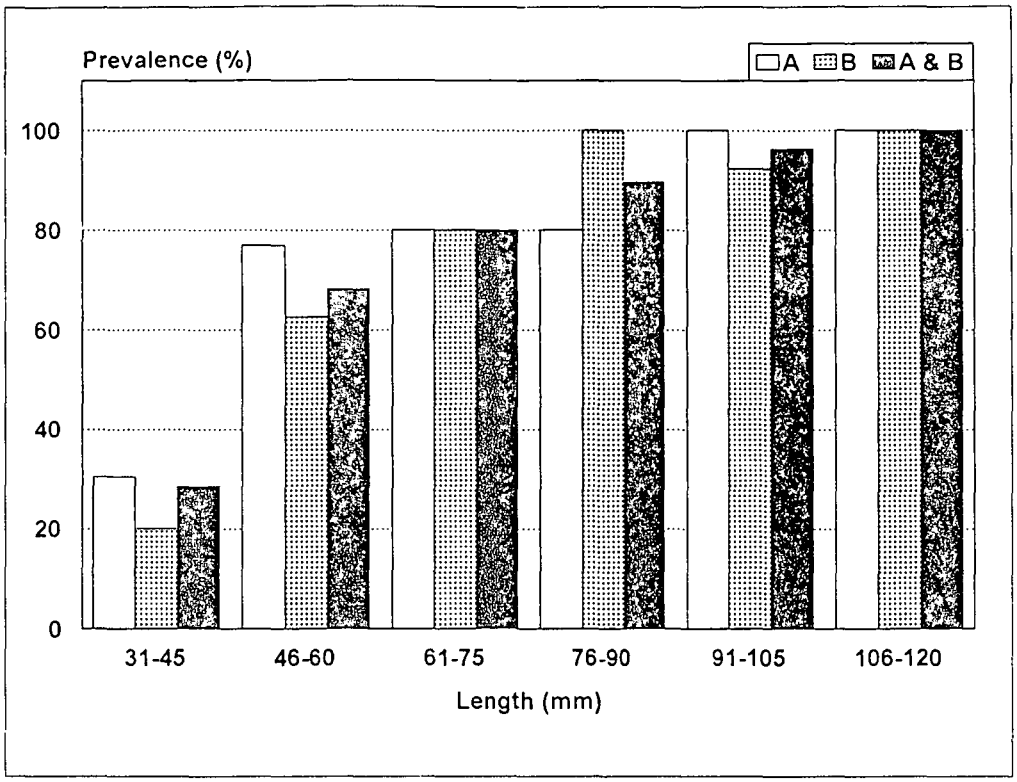
Figure 6.10

Bar graphs illustrating correlation between host size and infection levels of *Cephalochlamys namaquensis* at locality A, B and for data from both localities combined.

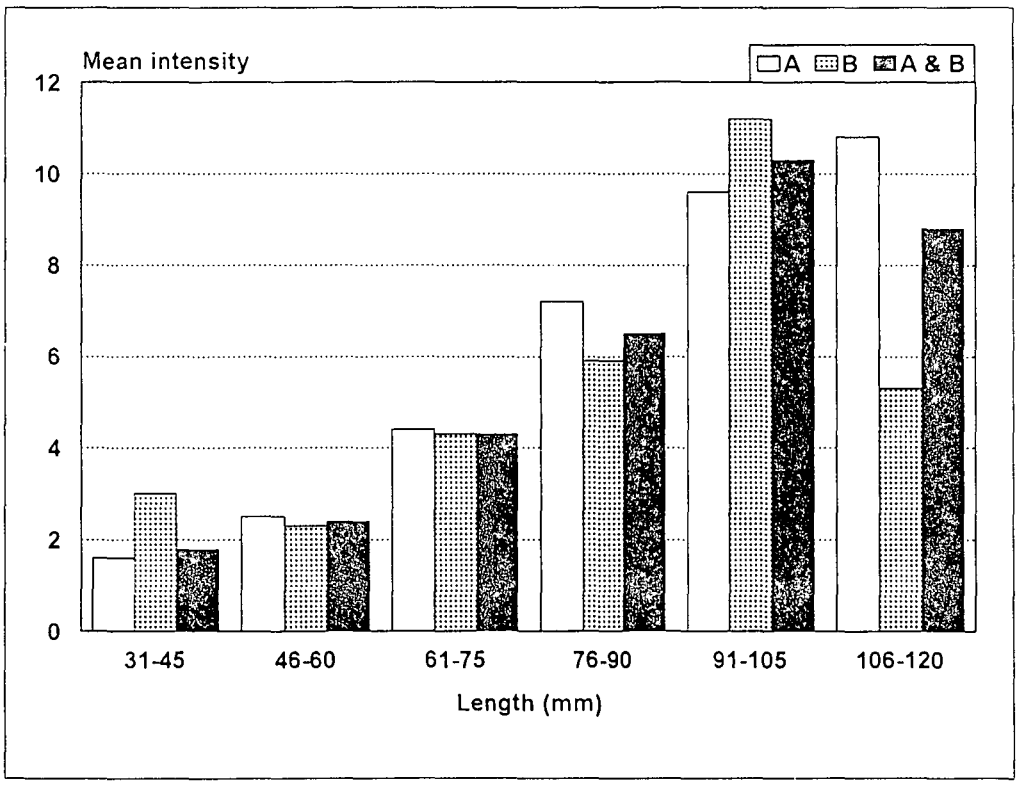
A) Prevalence.

B) Mean intensity.

A


Prevalence (%)
A
B
A & B
100
80
60
40
20
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

B


Mean intensity
A
B
A & B
12
10
8
6
4
2
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

6.3.5 *Valipora campylancristrota*

At locality A the prevalence of the plerocercoid was very low, ranging between 0% and 10%, while a higher prevalence of between 0% and 70% was recorded at locality B. The mean intensity differed similarly, ranging between 1 and 2 at locality A but from 2.4 to 24.2 at locality B (Fig. 6.11A&B).

The correlation between the prevalence of the plerocercoid and host size (Fig. 6.12A) was very strong at locality B ($r = 0.95$), but weak at locality A ($r = 0.27$). The combined data rendered a strong positive correlation ($r = 0.83$). At locality A ($r = 0$) and B ($r = -0.17$) and for the combined data ($r = -0.24$), a weak or very weak negative correlation between mean intensity and the size of the host were recorded (Fig. 6.12B). Not taking the relatively high mean intensity of the parasite in the smallest hosts into account (30.1-45.0 mm.), the correlation is modest at locality B ($r = 0.45$) and for the combined data ($r = 0.5$). At locality A however, the negative correlation is modest ($r = -0.57$).

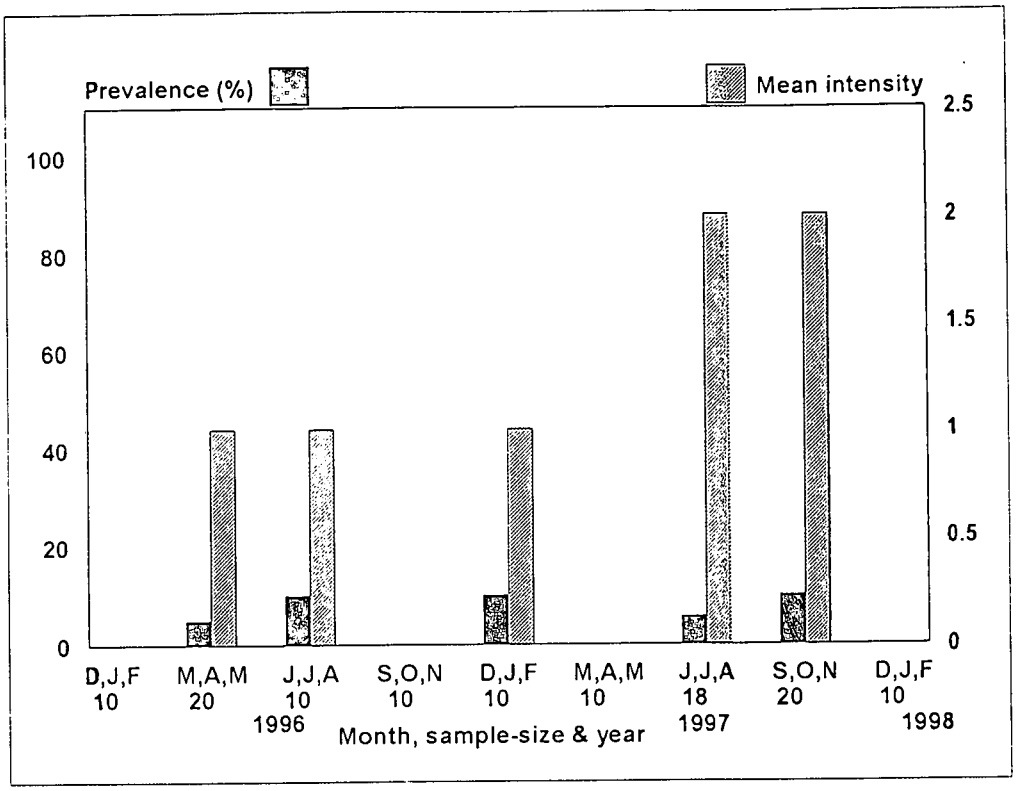
Figure 6.11

Bar graphs illustrating seasonal prevalence and mean intensity of *Valipora campylancristrota*.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
2.5
2
1.5
1
0.5
0
D,J,F 10
M,A,M 20
J,J,A 10
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 18
S,O,N 20
D,J,F 10
1996
1997
1998
Month, sample-size & year

B

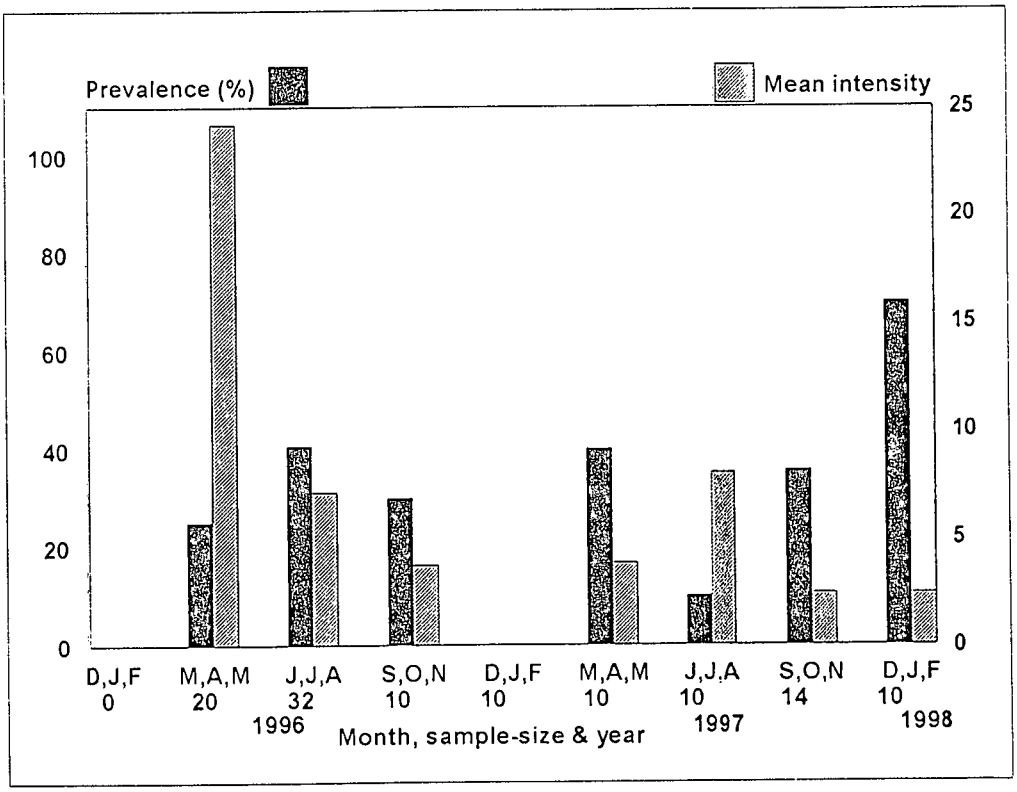

Prevalence (%)
Mean intensity
100
80
60
40
20
0
25
20
15
10
5
0
D,J,F 0
M,A,M 20
J,J,A 32
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 10
S,O,N 14
D,J,F 10
1996
1997
1998
Month, sample-size & year

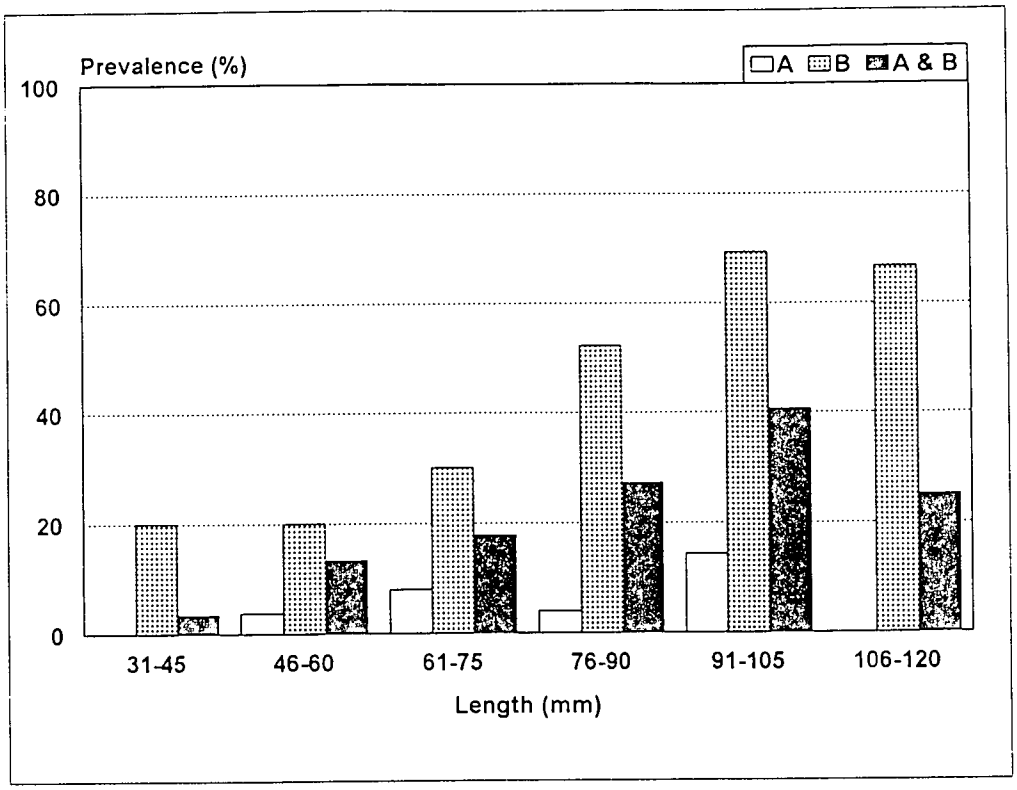
Figure 6.12

Bar graphs illustrating correlation between host size and infection levels of *Valipora campylancristrota* at locality A, B and for data from both localities combined.

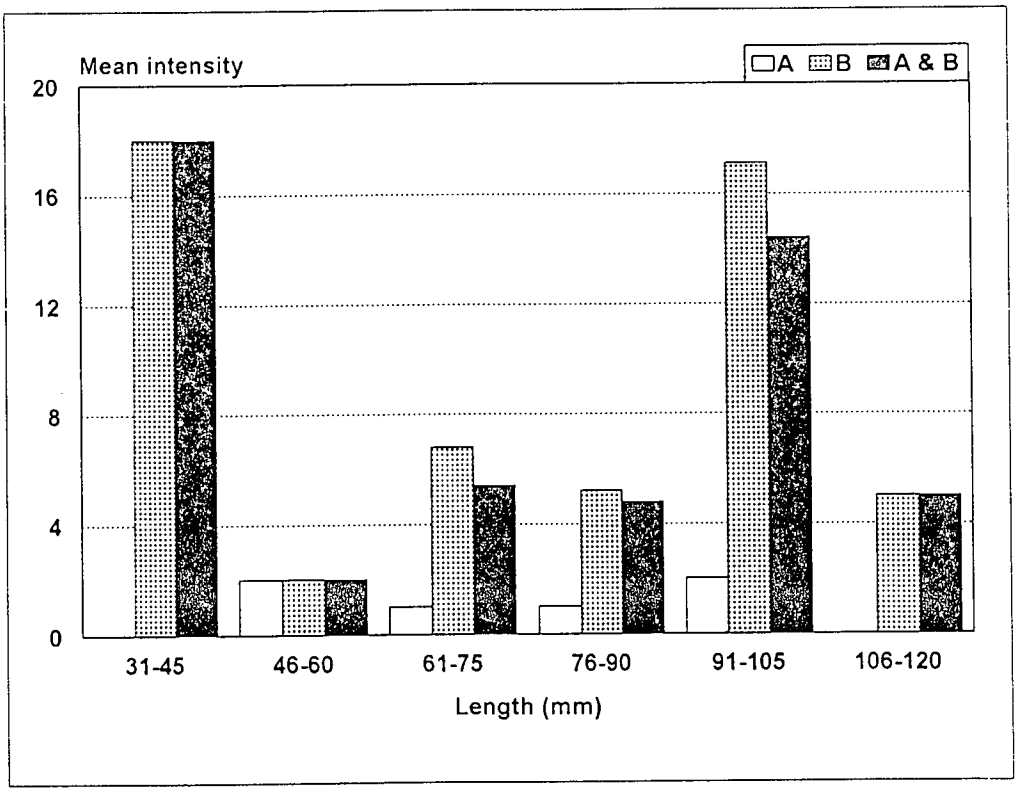
A) Prevalence.

B) Mean intensity.

A


Prevalence (%)
A B A & B
100
80
60
40
20
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

B


Mean intensity
A B A & B
20
16
12
8
4
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

6.3.6 *Camallanus kaapstaadi*

The prevalence of *C. kaapstaadi* was relatively high at both localities, ranging between 50% and 90% at locality A, while a prevalence of between 40% and 100% was recorded at locality B. At locality A, the mean intensity ranged between 2.7 and 8.4, and at locality B from 2.7 to 6.4 (Fig. 6.13A&B).

A very strong correlation existed between the prevalence of the parasite and host size (Fig. 6.14A) at locality A ($r = 0.98$) and for the combined data ($r = 0.96$). At locality B the correlation was strong ($r = 0.83$). The correlation between mean intensity of *C. kaapstaadi* and the size of the host (Fig. 6.14B) was also very strong at locality A ($r = 0.91$) and for the combined data ($r = 0.95$). At locality B however, $r = -0.02$, but with the data of the smallest frogs (30.1-45.0 mm.) not included, $r = 0.96$.

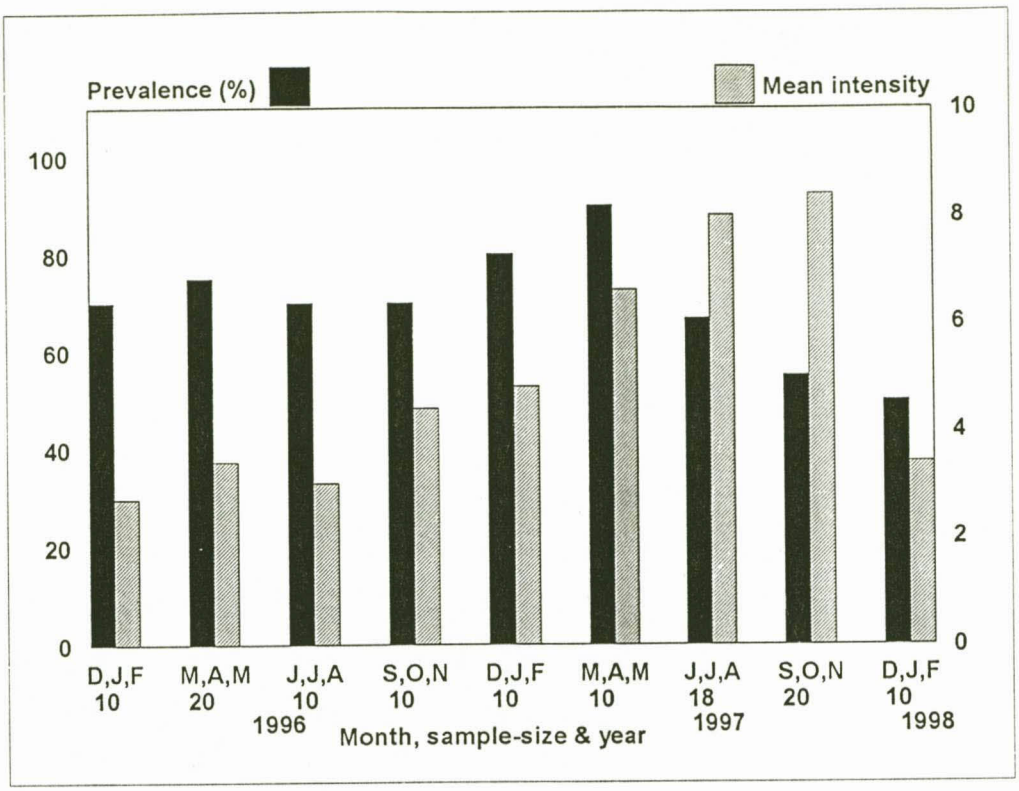
Figure 6.13

Bar graphs illustrating seasonal prevalence and mean intensity of *Camallanus kaapstaadi*.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
10
8
6
4
2
0
D,J,F 10
M,A,M 20
J,J,A 10
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 18
S,O,N 20
D,J,F 10
1996
1997
1998
Month, sample-size & year

B

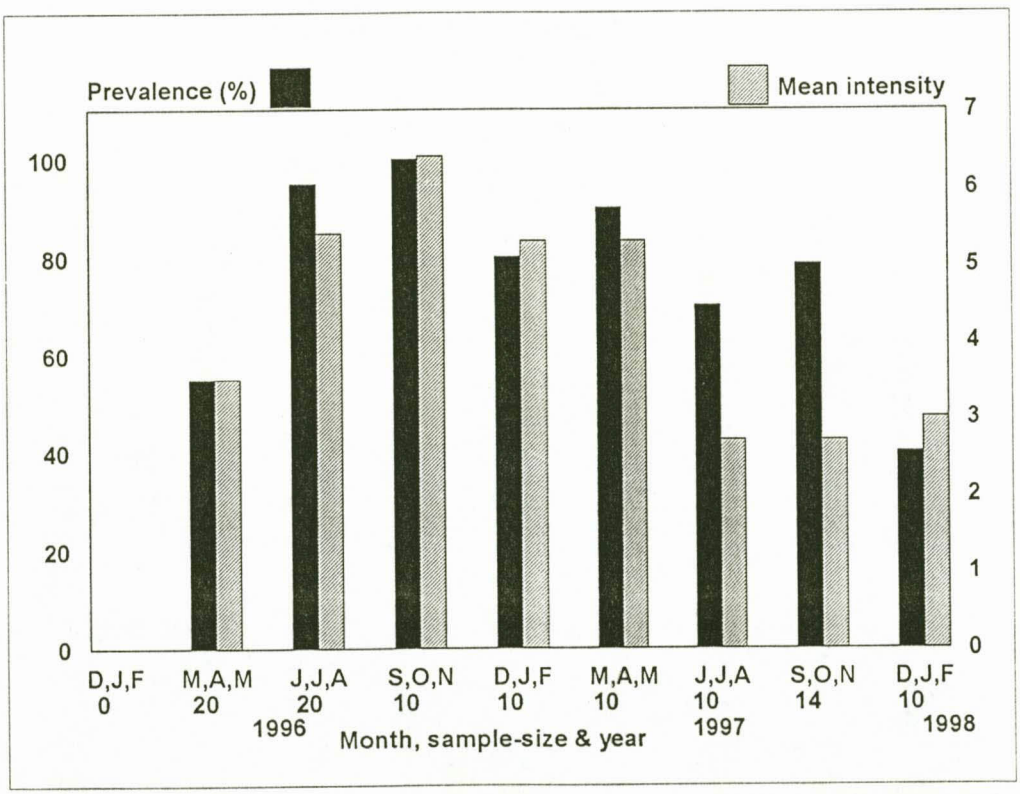

Prevalence (%)
Mean intensity
100
80
60
40
20
0
7
6
5
4
3
2
1
0
D,J,F 0
M,A,M 20
J,J,A 20
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 10
S,O,N 14
D,J,F 10
1996
1997
1998
Month, sample-size & year

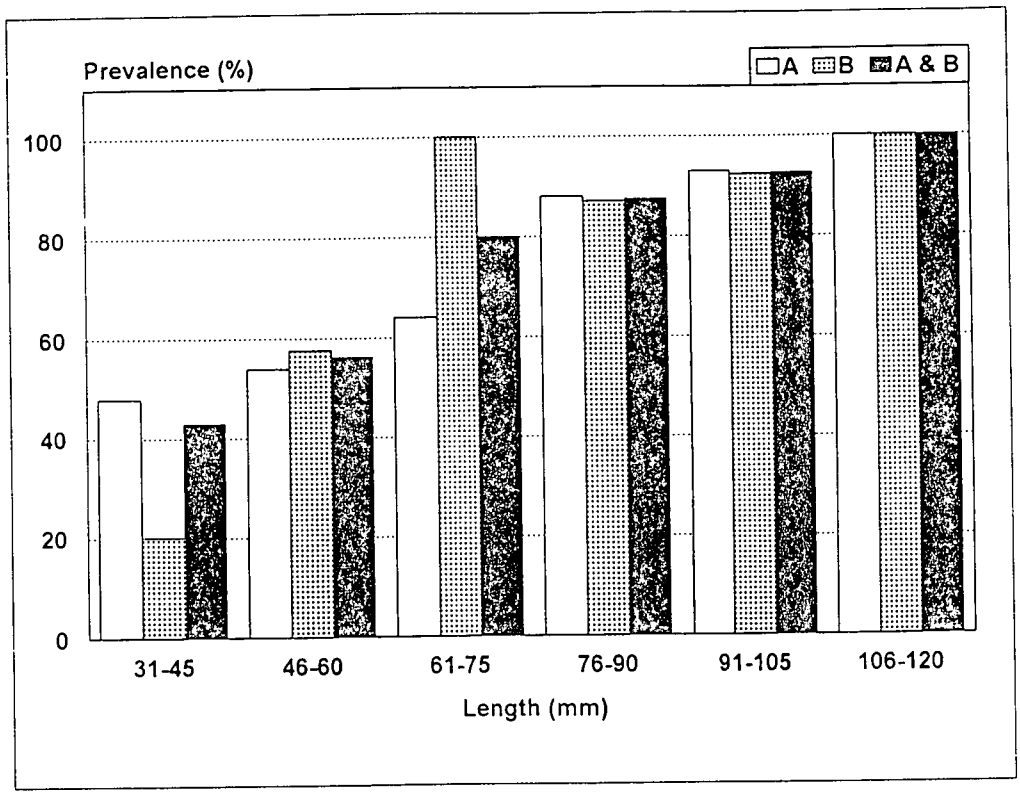
Figure 6.14

Bar graphs illustrating correlation between host size and infection levels of *Camallanus kaapstaadi* at locality A, B and for data from both localities combined.

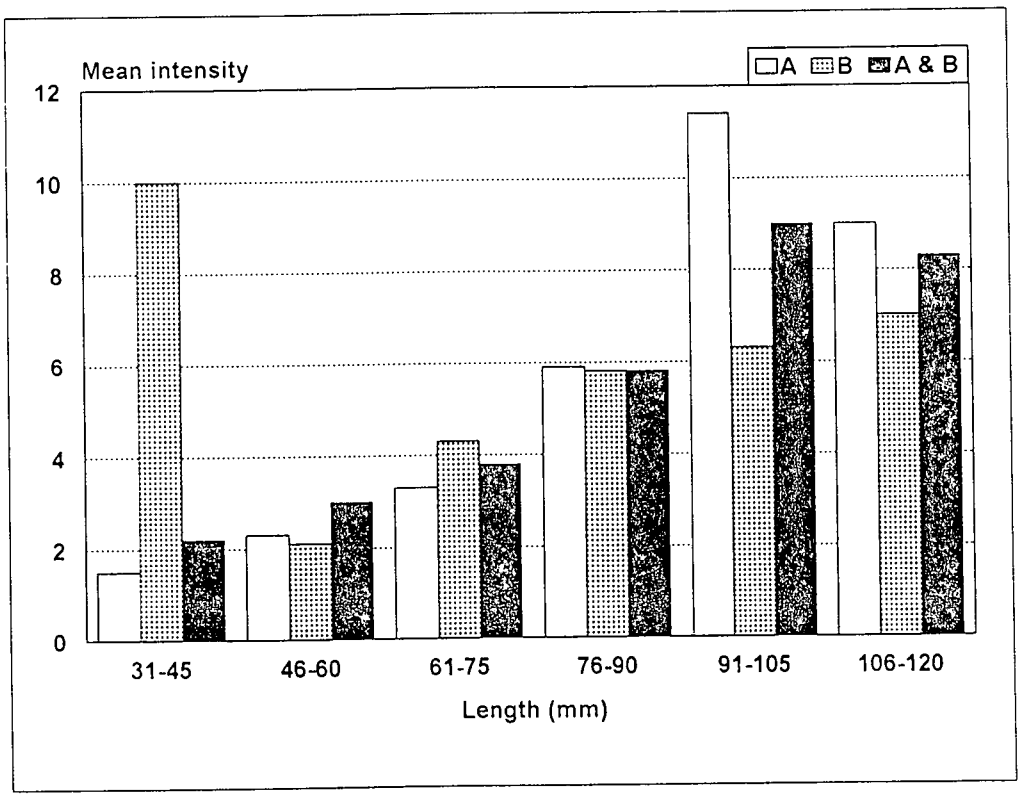
A) Prevalence.

B) Mean intensity.

A


Prevalence (%)
A
B
A & B
100
80
60
40
20
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

B


Mean intensity
A
B
A & B
12
10
8
6
4
2
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

6.3.7 *Batrachocamallanus slomei*

At locality B, the prevalence of the parasite was low, between 0% and 15%, but at locality A it ranged from 10% to 30% Mean intensity as high as 19.0 and 40.3 were recorded at locality A, but the normal range seemed to be from 1.3 to 5.0. At locality B mean intensity ranged from 1.0 to 2.7 (Fig. 6.15A&B).

The correlation between prevalence of *B. slomei* and host size (Fig. 6.16A) was very high for locality A ($r = 0.93$) and the combined data ($r = 0.95$). At locality B it was however only modest ($r = 0.44$). At locality A ($r = 0.17$) and for the combined data ($r = 0.18$), the correlation between mean intensity and the size of the host was very weak (Fig. 6.16B). The correlation was modest at locality B ($r = 0.44$).

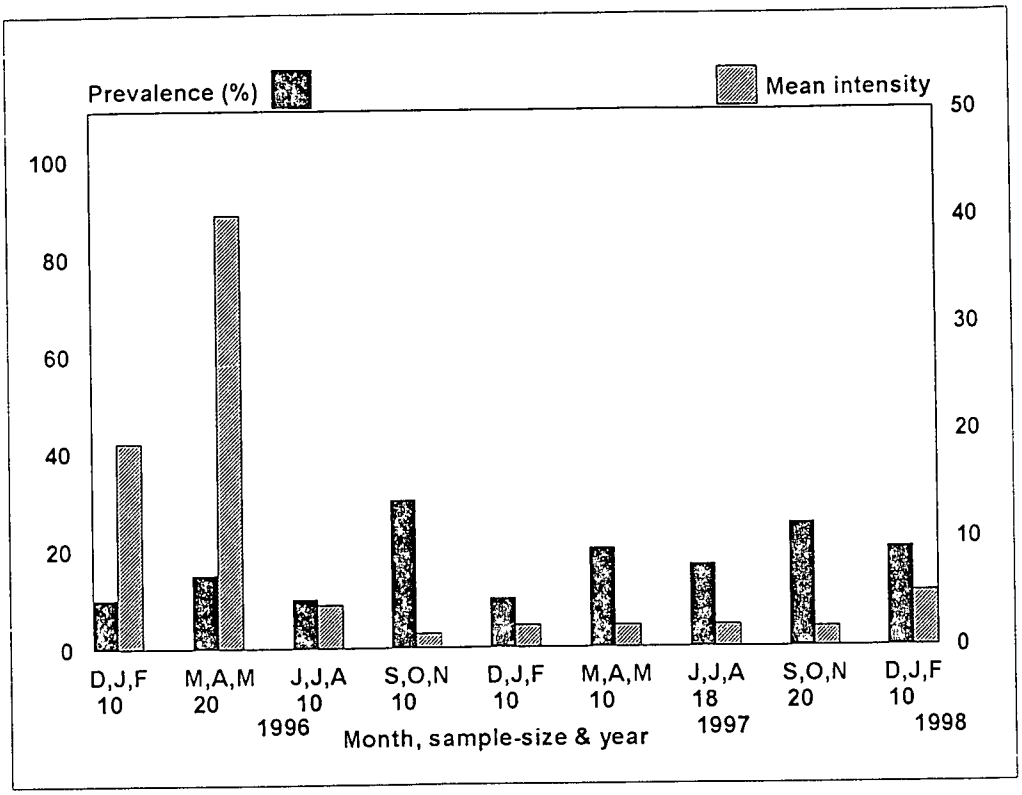
Figure 6.15

Bar graphs illustrating seasonal prevalence and mean intensity of *Batrachocamallanus slomei*.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
50
40
30
20
10
0
D,J,F 10
M,A,M 20
J,J,A 10
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 18
S,O,N 20
D,J,F 10
1996
1997
1998
Month, sample-size & year

B

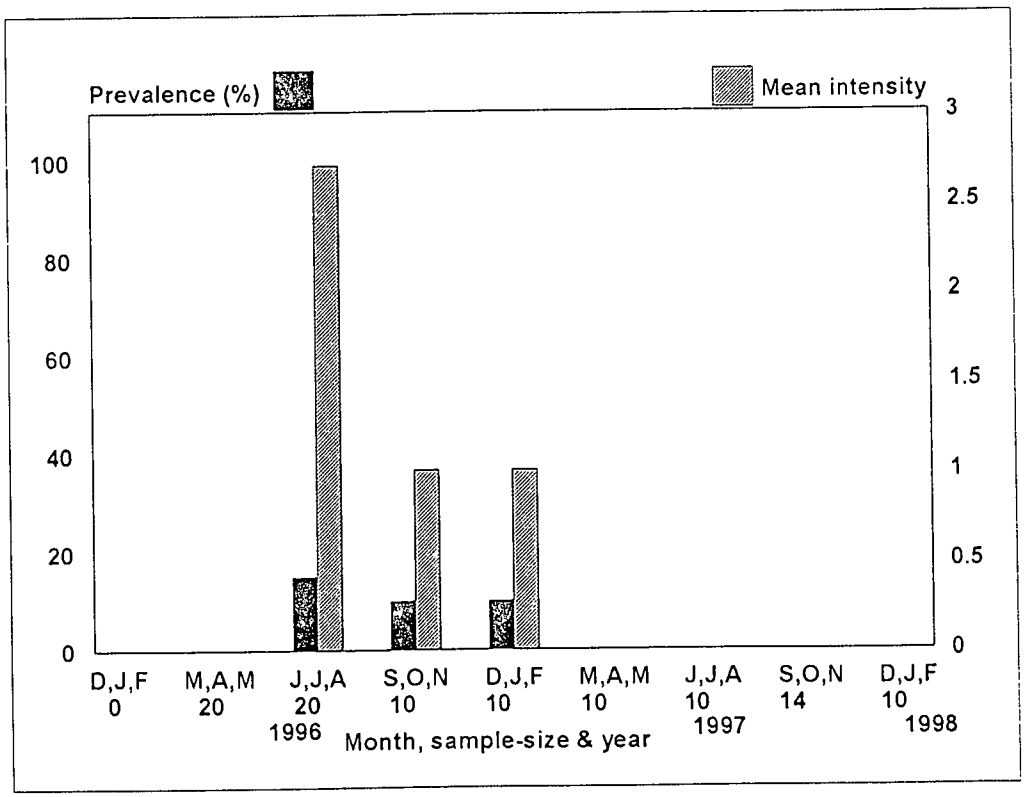

Prevalence (%)
Mean intensity
100
80
60
40
20
0
3
2.5
2
1.5
1
0.5
0
D,J,F 0
M,A,M 20
J,J,A 20
S,O,N 10
D,J,F 10
M,A,M 10
J,J,A 10
S,O,N 14
D,J,F 10
1996
1997
1998
Month, sample-size & year

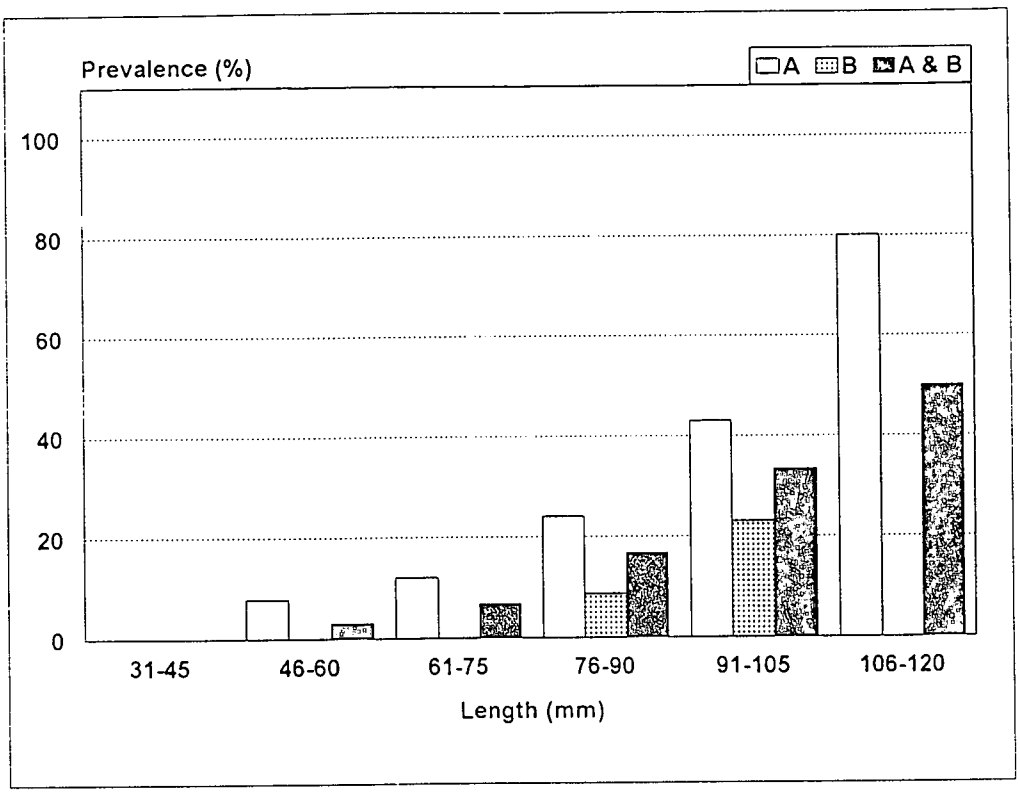
Figure 6.16

Bar graphs illustrating correlation between host size and infection levels of *Batrachocamallanus slomei* at locality A, B and for data from both localities combined.

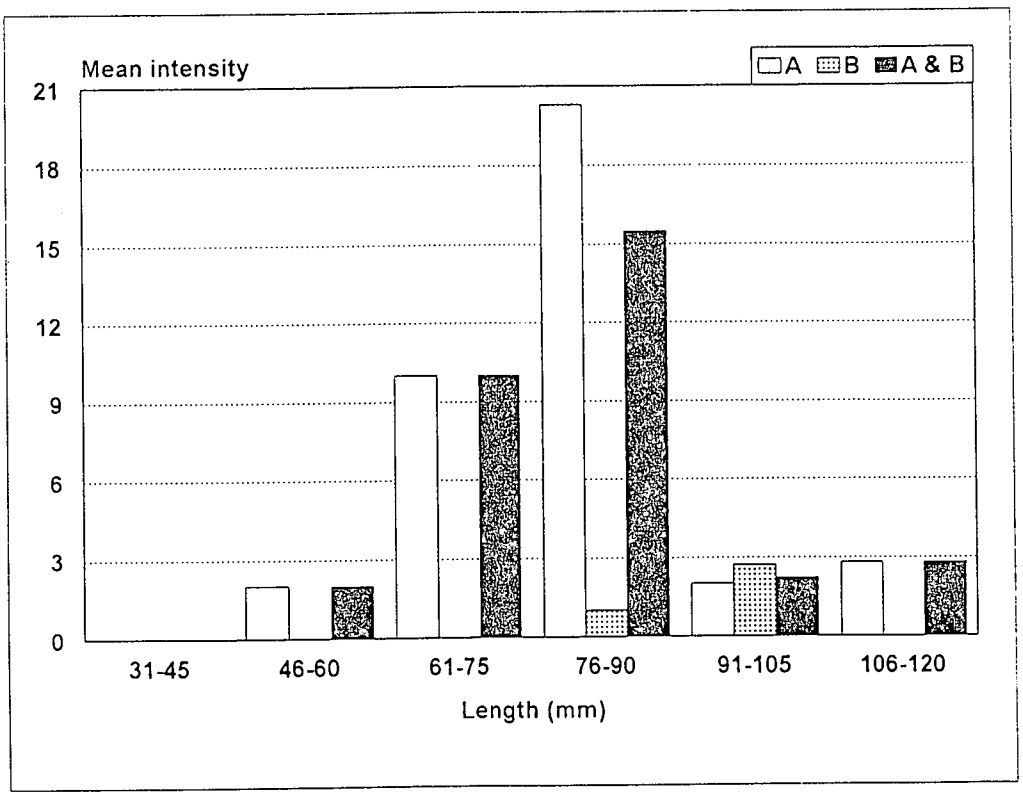
A) Prevalence.

B) Mean intensity.

A


Prevalence (%)
A
B
A & B
100
80
60
40
20
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

B


Mean intensity
A
B
A & B
21
18
15
12
9
6
3
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

6.3.8 *Marsupiobdella africana*

The prevalence of the leech was 0% most of the time at locality A, but as high as 50% was recorded. At locality B prevalence ranged between 0% and 64.3%. The mean intensity at locality A was between 1.8 and 14.0, while at locality B it ranged from 1.0 to 9.0 (Fig. 6.17A&B).

The correlation between host size and the prevalence of the leech (Fig 6.18A) was only modest at locality B ($r = 0.66$) and for the combined data ($r = 0.48$), but weak at locality A ($r = 0.27$). At locality B the correlation between mean intensity and the size of the host was weak ($r = 0.34$), but at locality A and for the combined data it was very weak with $r = 0.09$ in both cases (Fig. 6.18B).

6.3.9 HOST SEX AND INFECTION LEVELS

Apart from *T. xenopi* in the pericardium, the prevalence of all parasites was higher in female hosts (Fig. 6.19A). The mean intensity of the parasites was, apart from *G. gallieni* and *M. africana*, also higher in females (Fig. 6.19B).

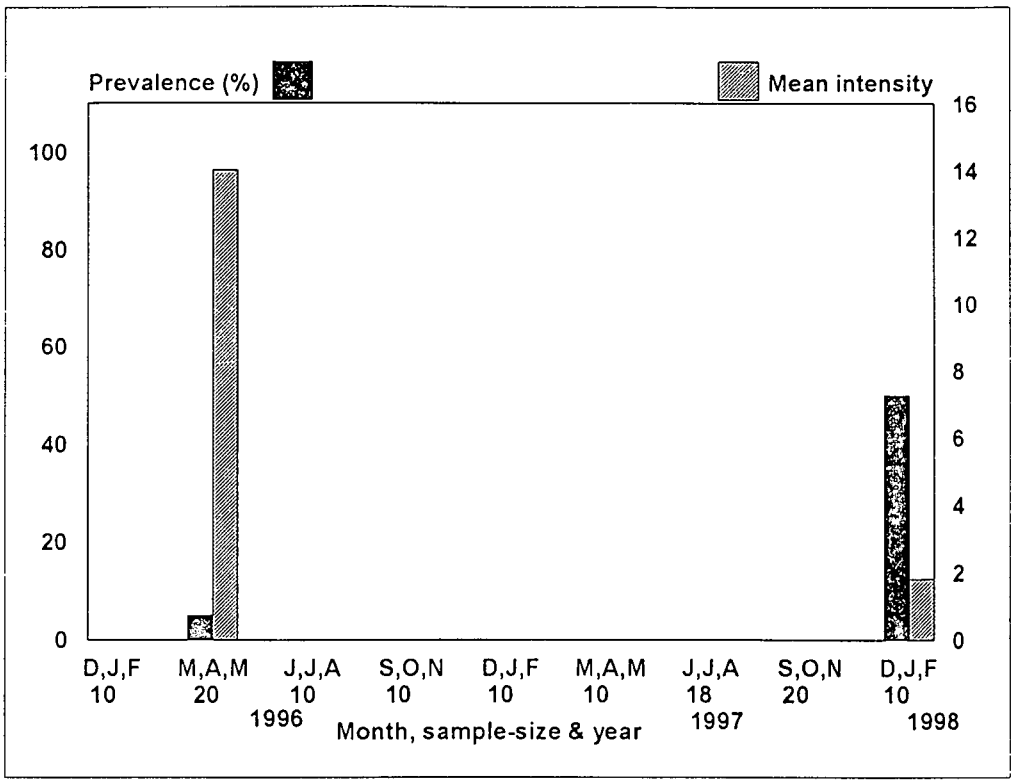
Figure 6.17

Bar graphs illustrating seasonal prevalence and mean intensity of *Marsupiobdella africana*.

A) Locality A.

B) Locality B.

A


Prevalence (%)
Mean intensity
100
80
60
40
20
0
16
14
12
10
8
6
4
2
0
D,J,F
10
M,A,M
20
J,J,A
10
S,O,N
10
D,J,F
10
M,A,M
10
J,J,A
18
S,O,N
20
D,J,F
10
1996
1997
1998
Month, sample-size & year

B

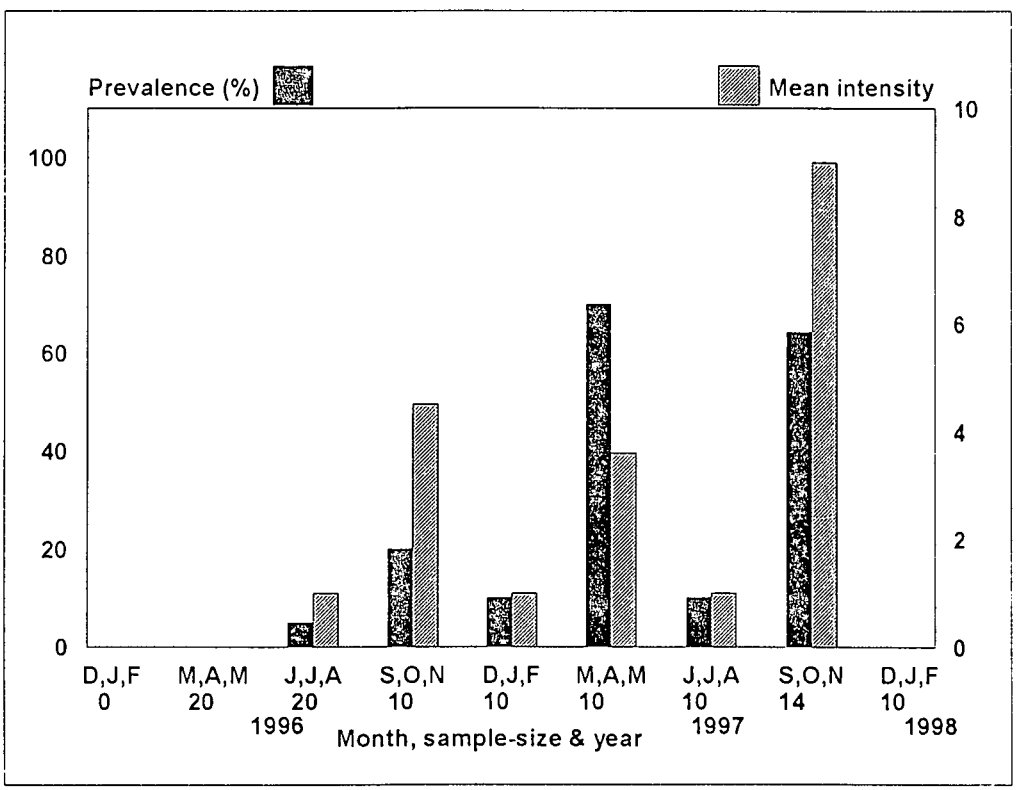

Prevalence (%)
Mean intensity
100
80
60
40
20
0
10
8
6
4
2
0
D,J,F
0
M,A,M
20
J,J,A
20
S,O,N
10
D,J,F
10
M,A,M
10
J,J,A
10
S,O,N
14
D,J,F
10
1996
1997
1998
Month, sample-size & year

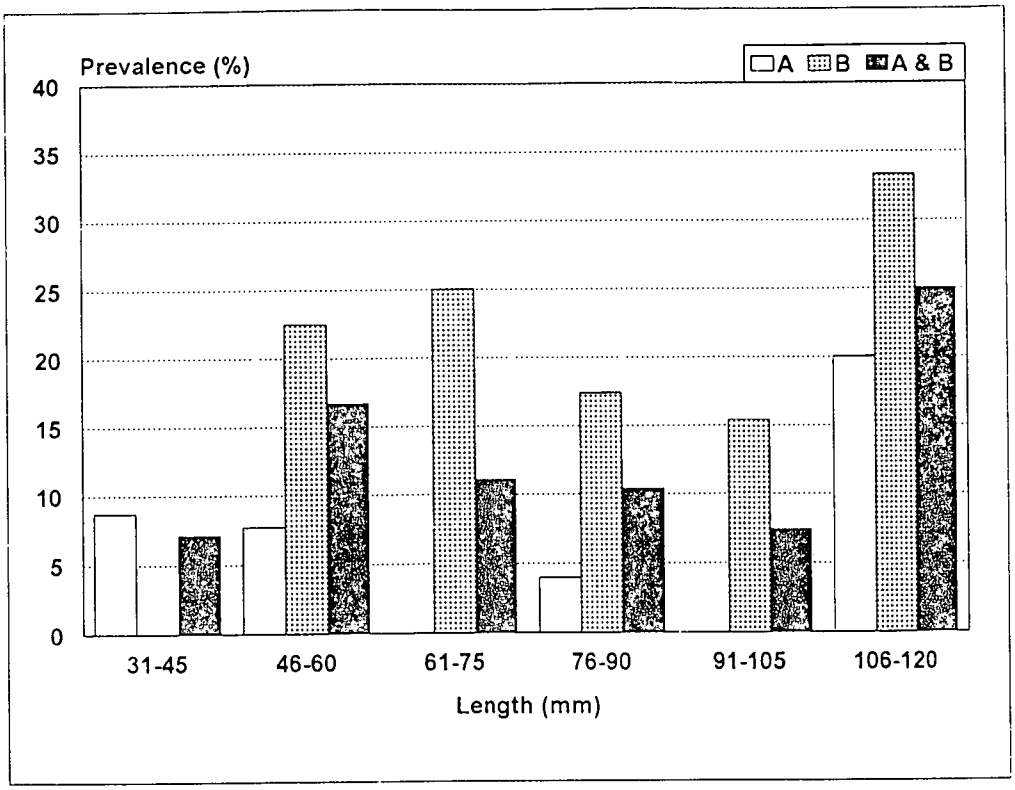
Figure 6.18

Bar graphs illustrating correlation between host size and infection levels of *Marsupiobdella africana* at locality A, B and for data from both localities combined.

A) Prevalence.

B) Mean intensity.

A


Prevalence (%)
A
B
A & B
40
35
30
25
20
15
10
5
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

B

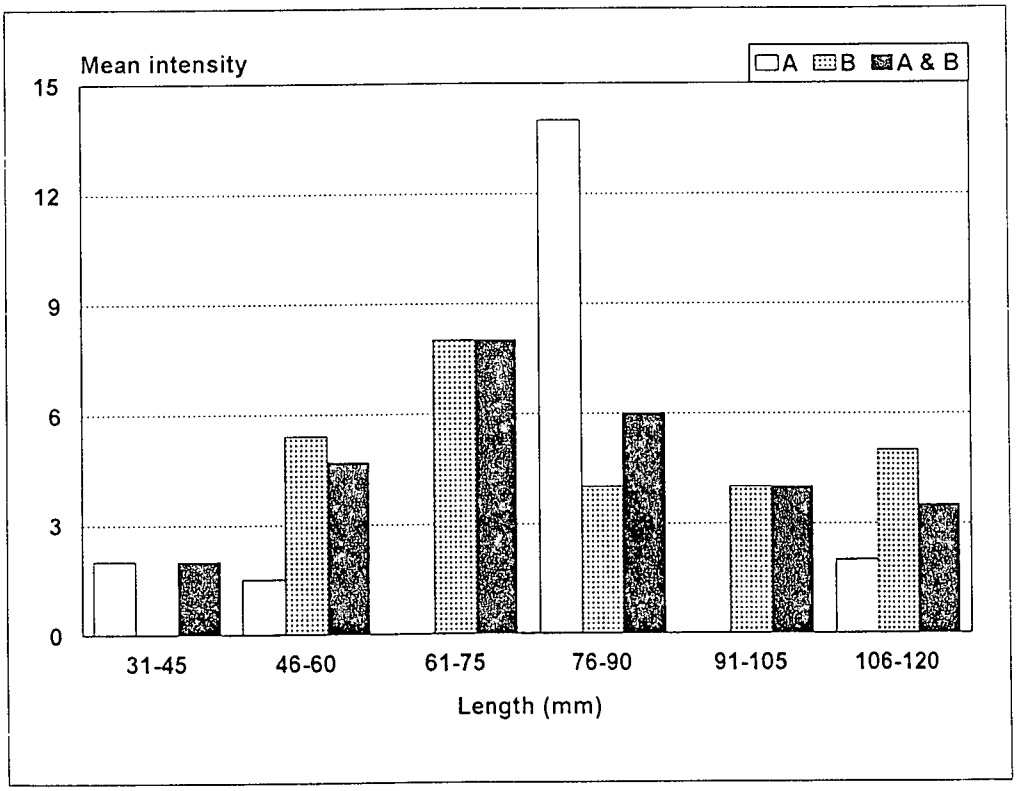

Mean intensity
A
B
A & B
15
12
9
6
3
0
31-45
46-60
61-75
76-90
91-105
106-120
Length (mm)

Figure 6.19

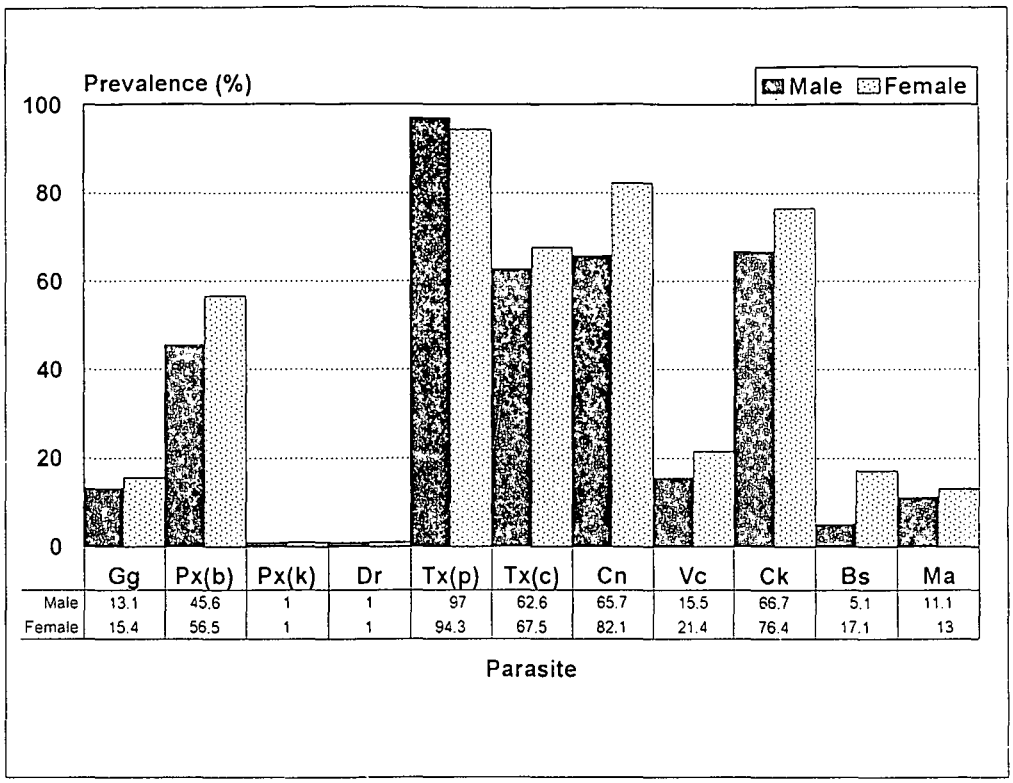
Bar graphs illustrating the relationship between host sex and infection levels for data from both localities combined.

A) Prevalence.

B) Mean intensity.

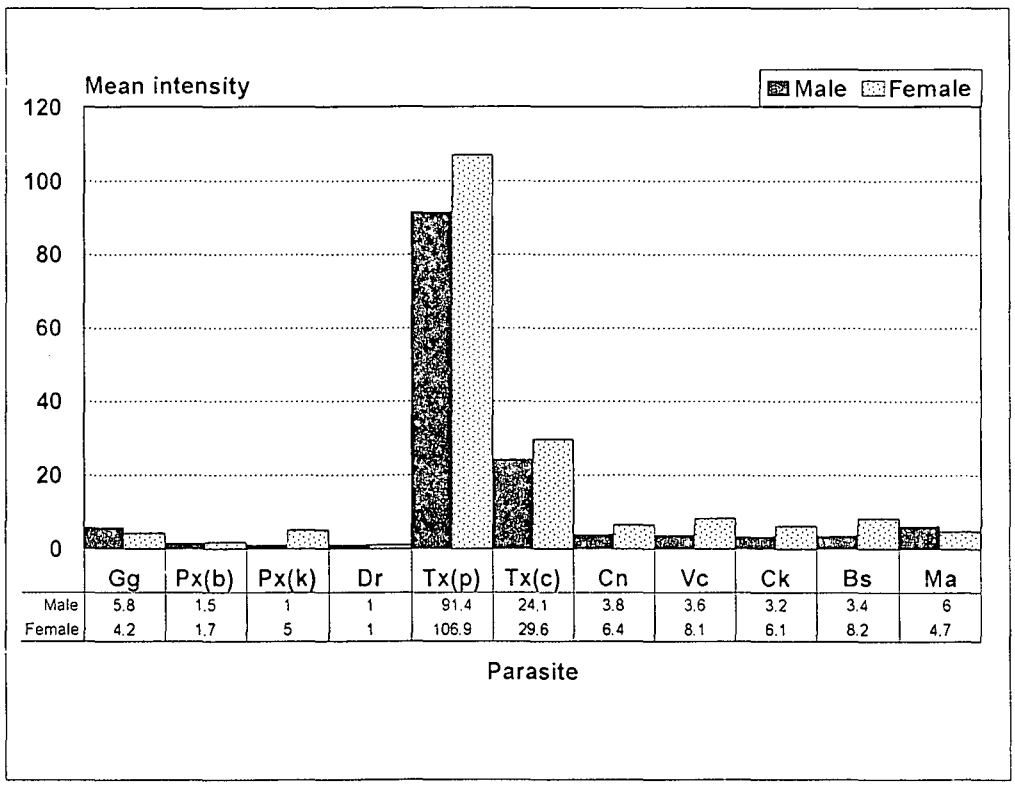
Abbreviations: Gg, *Gyrdicotylus gallieni*; Px(b), *Protopolystoma xenopodis* (bladder); Px(k) *Protopolystoma xenopodis* (kidneys); Dr, *Dollfuschella rodhaini*; Tx(p), *Tylodelphys xenopi* (pericardium); Tx(c), *Tylodelphys xenopi* (body cavity); Cn, *Cephalochlamys namaquensis*; Vc, *Valipora campylancristrota*; Ck, *Camallanus kaapstaadi*; Bs, *Batrachocamallanus slomei*; Ma, *Marsupiobdella africana*.

A



	Gg	Px(b)	Px(k)	Dr	Tx(p)	Tx(c)	Cn	Vc	Ck	Bs	Ma
Male	13.1	45.6	1	1	97	62.6	65.7	15.5	66.7	5.1	11.1
Female	15.4	56.5	1	1	94.3	67.5	82.1	21.4	76.4	17.1	13

B



	Gg	Px(b)	Px(k)	Dr	Tx(p)	Tx(c)	Cn	Vc	Ck	Bs	Ma
Male	5.8	1.5	1	1	91.4	24.1	3.8	3.6	3.2	3.4	6
Female	4.2	1.7	5	1	106.9	29.6	6.4	8.1	6.1	8.2	4.7

6.4 DISCUSSION

6.4.1 SEASONAL VARIANCE IN INFECTION LEVELS

Harris and Tinsley (1987) mentioned the specific months during which specimens of *X. laevis* were imported from the Cape, South Africa. The prevalence of ***Gyrdicotylus gallieni*** in these samples was the highest in November 1971 (12.1%) and April 1982 (29%). Prevalence of 0% was recorded in May 1971, October 1971 and February 1976. These records are however not useful to determine seasonal variance in infection levels. The frogs probably came from different populations and the time spent in captivity prior to exportation and actual dissection for every sample is unknown. The time spent in captivity could have influenced the population structure of *G. gallieni* (Thurston, 1970 and Jackson & Tinsley, 1994). The current study found no conclusive seasonal pattern in infection levels. Noteworthy though was the absence of the parasite in hosts from both localities during summer and autumn 1996. The particularly high rainfall prior to and during this period could have had an influence, as the dams were fuller and the host population consequently more dispersed. This would have caused reduced transmission rates and a subsequent decline in infection levels. Tinsley (1996a) stated that because the population structure of *G. gallieni* changes over time with exponential increases and subsequent host reaction, records of natural infection levels only provide a 'snapshot' of the various stages in the population cycle. The population being reduced by a host reaction could, therefore also explain the absence of the parasite at the two localities during the aforementioned months.

Protopolystoma xenopodis is the only parasite for which formal records of seasonal changes in infection levels exist. Tinsley (1972) reported that *X. laevis* collected in a winter rainfall region showed an increase in infection levels during late summer and autumn. Habitats drying up causes the dense aggregation of hosts in available pools, and parasite transmission success is enhanced during autumn (prevalence 50%; mean intensity 2.3). Winter rainfall causes the dispersion of hosts and transmission is reduced and larval infection reaches a minimum.(prevalence 11%; mean intensity 2.0). Tinsley (1996a) added that the highest burdens (prevalence 53%; mean intensity 2.3) are found in spring when parasites, which invaded in autumn, reach maturity. Corresponding with the reduced invasion rates during the preceding winter, reduced recruitment into the adult population occurs in summer and autumn and results in a minimum adult parasite burden in winter (prevalence 11%; mean intensity 1.5). Maturation of the autumn invasions again contributes to increased adult burdens the following spring. Two environmental factors determine these seasonal cycles. Higher temperatures in summer increase the rates of egg production, egg development and maturation, and coincide with low rainfall and subsequent dense aggregation of hosts in confined habitats. During winter, the opposite situation exists. Peaks in the abundance of recently invaded larvae and therefore adults occur around 5-6 months during winter when lower temperatures reduce development rates and around 3-4 months during summer at highest temperatures. The seasonal cycles show that the peak in parasite burdens after maximum autumn invasion is however temporary. Parasite populations decline because of continuous worm losses until the next autumn's recruitment (Tinsley, 1996a).

The situation in the current study is different as it was done in a summer rainfall region. Maximum invasion and prevalence of larva is expected in winter and early spring when hosts are most densely aggregated, and a minimum prevalence when hosts are dispersed during summer rainfall. The parasites that invaded in winter and spring should reach maturity in autumn and cause a maximum prevalence of adults. Corresponding with reduced invasion rates during the preceding summer, there is a reduction in recruitment into the adult population during winter and spring. A minimum prevalence of adult parasites is therefore expected in summer. The adult infections are increased in autumn when the winter invasions mature. In a winter rainfall region, the rate of egg production, egg development and maturation would be increased by higher temperatures during summer and autumn when the situation for host invasion is optimal. These two environmental factors therefore function together to increase the success of larval invasion. In a summer rainfall region however, the time when hosts are most densely aggregated coincides with very low temperatures and thus a low rate of egg production and development when larval numbers low. It would seem then that invasion occurs mostly in spring when temperatures are higher but rainfall still low, giving rise to maximum adult parasite burdens the following autumn as described above.

At locality A, the prevalence of adult *P. xenopodis* increased constantly from 10% in the summer of 1996 up to 80% in autumn 1997. The mean intensity had a similar pattern of increase, but it was less prominent. At locality B the changes in the infection levels had no conclusive seasonal pattern.

The only existing record on possible seasonal variance in infection levels of ***Tylodelphys xenopi*** is inconclusive. Very high and low prevalence were recorded both during summer and winter, and the records rather reflected the differences in populations from which collections were made than true seasonal variation (Tinsley & Sweeting, 1974). During the current study, the prevalence of the parasite in the pericardium was continuously high and no conclusive seasonal pattern was found at either locality. The mean intensity did however fluctuate, and especially at locality B the number of parasites per infected host seem to be at a minimum during summer. The infection levels of *T. xenopi* in the body cavity reflected the patterns in the pericardium, with the prevalence and mean intensity of the parasite at both localities a minimum during summer. The reduced infection levels are possibly a result of lower rates of infestation during the previous winter months. Possible lower numbers of *Bulinus tropicus* and the final host, coupled with lower rates of development of the parasite in the snail and bird could all contribute to reduced invasion rates even though hosts populations are dense.

The infection levels of the parasite in both pericardium and body cavity were almost always at a maximum during autumn. In a pattern similar to that of *Protopolystoma xenopodis*, higher temperatures in spring coupled with the high density of host populations lead to higher infestation success and maximum parasite burdens the following autumn.

The infection levels of ***Cephalochlamys namaquensis*** reached a minimum at both localities during the summer of 1997 and 1998. At locality A, the prevalence dropped to 40% and 50%, and at locality B to 30% and 10% during the two respective summers.

The mean intensity at locality B decreased at a constant rate to reach minima of 3.0 and 1.0 during the summer months, but at locality A the decrease was not as obvious. *C. namaquensis* seemed to be most abundant around autumn of 1996 and 1997 after the decrease during summer. The dispersed host population during the rainfall season could have contributed to the low summer infection levels. Peaks during autumn could be ascribed to possible higher numbers of the copepod intermediate host.

At locality A, the prevalence of ***Valipora campylancristrota*** was low with no seasonal pattern and only a slightly higher mean intesity during winter and spring 1997. No seasonal variance in infection levels of the plerocercoid at locality B was found either.

No conclusive seasonal patterns were found in the infection levels of ***Camallanus kaapstaadi*** at either locality. Prevalence reached a maximum during autumn of 1997 and declined thereafter but mean intensity remained high at locality A. At locality B, the prevalence and mean intensity changed randomly during the study period.

The infection levels of ***Batrachocamallanus slomei*** showed no variation according to seasons. At locality A, prevalence and mean intensity were fairly constant during the study period, except in autumn 1996 when a single host was found infected with 112 parasites. At locality B, the parasite was only found during winter and spring 1996 and summer 1997.

Marsupiobdella africana was only found in autumn 1996 and summer 1998 at locality A. At locality B however, it was found from winter 1996 to spring 1997, but the infection levels did not show any conclusive trends with seasonal changes.

6.4.2 CORRELATION BETWEEN HOST SIZE AND INFECTION LEVELS

The infection levels of ***Gyrdicotylus gallieni*** were the only instance where a negative correlation existed between prevalence and mean intensity. The best correlation was found when the data of the two localities were combined. *X. laevis* is known to eliminate, or at least reduce numbers of, populations of *G. gallieni* by some host response (Harris & Tinsley, 1987, and Jackson & Tinsley, 1994). The fact that larger, and thus older frogs have the lowest parasite infection levels, indicates that the host response may be better developed in older individuals.

The infection levels of ***Protopolystoma xenopodis*** showed only a modest positive correlation with the size of the host. Establishing a definite correlation between host size and worm burdens proved difficult. At locality A for example, the correlation with prevalence was weak, but very strong for mean intensity. At locality B the correlation with prevalence was modest, but weak for mean intensity. Combining the data gave a modest and very strong correlation for prevalence and mean intensity respectively. Parasite burdens do therefore seem to increase as hosts get older. The increasing burdens could be a factor of the larger dimensions of the urinary bladder, but more likely the increasing time older hosts had been subjected to parasite invasion. Although the sample

size were small for the largest hosts, it does seem as if burdens don't increase indefinitely but reach a maximum due to a possible host reaction or crowding effects (see 5.4.2).

Tinsley (1996a) mentioned that a correlation between ***Tylodelphys xenopi*** burdens and host age is very likely. At both localities, the infection levels of the parasite were very strongly correlated with the size (age) of the hosts. The prevalence of *T. xenopi* in the pericardium was very high and reached a maximum in the largest hosts. In the body cavity however, the prevalence was lower in the oldest hosts than in the second last group. Relatively low sample sizes of five and three at locality A and B respectively probably caused this phenomenon. The mean intensity of *T. xenopi* in the pericardium and body cavity increased with host size, and the relationship between the burdens in the two infection sites are very clear. The number of parasites in the body cavity increased proportionally to higher burdens in the pericardium. As stated by Tinsley (1996a), the burdens of *T. xenopi* accumulate in hosts throughout their life without loss, explaining the correlation between the size of hosts and infection levels of the parasite. The bigger dimensions of the pericardium and body cavity in larger hosts could also make the accommodation of more parasites possible.

Ferguson and Appleton (1988) found no correlation between host size and tapeworm burden. The infection levels of ***Cephaloclamys namaquensis*** was however strongly correlated with the size of hosts collected from localities A and B. This is possibly a function of the relatively longer time older hosts had been subjected to infection, but also larger dimensions of the intestine in larger frogs and thus more space and resources to accommodate more parasites. The lower mean intensity in the largest group of frogs

gives an indication of the existence of certain measures that limits infection levels (see 5.4.5).

The mean intensity of ***Valipora campylancristrota*** had no correlation with host size. The prevalence however did seem to increase in larger individuals, probably a factor of older hosts having been exposed to infections for longer.

The infection levels of ***Camallanus kaapstaadi*** were found to be strongly correlated with the size of hosts. Prevalence was higher in larger individuals, probably as a result of a longer time being subjected to infections. The mean intensity also increased with host size as larger frogs can probably tolerate more parasites, but burdens seemed to to be regulated as a maximum level is reached in frogs above 90 mm in length (see 5.4.7).

The correlation between host size and prevalence of ***Batrachocamallanus slomei*** was very strong at locality A. The correlation with mean intensity was however very weak, with average hosts seemingly carrying the highest burdens. The results are however inconclusive, as the number of infected hosts were too little to make a decisive comparison between host size and infection levels.

Sample sizes to test correlation between host size and infection levels of the ***Marsupiobdella africana*** were small, and although average size hosts seemed to carry the highest burdens, the results are not conclusive.

6.4.3 HOST SEX AND INFECTION LEVELS

The difference in prevalence of ***Gyrdicotylus gallieni*** in male (13.1%; n = 99) and female (15.4%; n = 123) hosts were insignificant. The mean intensity was however less in females (4.2) than in males (5.8).

Jackson & Tinsley (1988b) found that the egg production of ***Protopoystoma xenopodis*** in male and female hosts do not differ significantly. In the current study, the difference in prevalence of *P. xenopi* in male (45.6%; n = 103) and female (56.5%; n = 131) hosts were different, but the mean intensity in males (1.5) and females (1.7) were very similar.

Previous records suggest that no differences exist in the infection levels of ***Tylodelphys xenopi*** in males and females (Tinsley & Sweeting, 1974). The current study's results supports this, and the slight difference in the mean intensity are probably because of the relatively larger average size of *X. laevis* females.

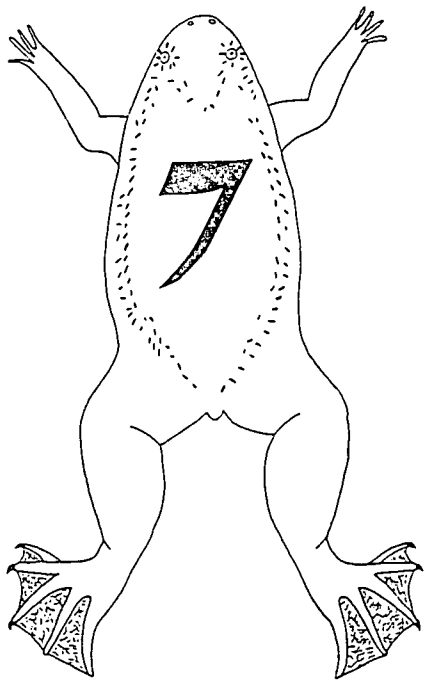
Ferguson and Appleton (1988) found no difference in the infection levels of ***Cephalochlamys namaquensis*** in male and female frogs. During the present study, the prevalence in male (65.7%) and female (82.1%) hosts did however differ markedly, with the mean intensity 3.8 and 6.4 respectively.

Infection levels of the ***Valipora campylancristrota*** were only slightly higher in females.

The prevalence of ***Camallanus kaapstaadi*** was approximately 10% higher in females than males, and the mean intensity with about three. The larger size of females probably caused the variation.

Batrachocamallanus slomei was more prevalent in females (17.1%) than males (5.1%), and the mean intensity was approximately 5.2 higher in females probably due to their larger size.

Marsupiobdella africana had a higher prevalence in females, but the mean intensity was higher in males. The differences were very small though, and there seems to be no significant difference in the infection levels of the leech in male and female hosts.



7

General Discussion

The African clawed frog, *Xenopus laevis*, hosts a large number of diverse parasites that represent all major invertebrate groups except the Acanthocephala. This unique parasite assemblage and the fact that the hosts are readily available makes *Xenopus* and its parasites the ideal study material in parasitology courses. Although the diversity and morphology of *Xenopus* parasites have been studied fairly well, little information is available on the parasite population dynamics under natural conditions. The present study was initiated against this background.

Results were presented and the significance thereof discussed in the foregoing chapters. This chapter serves only to provide a brief perspective of the findings with regard to the initial aims given in the introduction.

Parasites found in the present study represent six of the seven invertebrate groups known to infect *Xenopus*. Although the Protozoa did not form part of the study, almost all hosts dissected had some protozoans in the rectum. As the diverse parasite assembly of *Xenopus* is linked to its fully aquatic life, it was important to investigate the connection between the specific habitat of the host and the parasite diversity. The infection levels of five of the nine parasites found were significantly different at the two study localities, most probably due to different ecological influences on the life cycles of the parasites. All the parasites displayed a negative binomial, or overdispersed distribution within the respective host populations.

In spite of the fact that the parasites of *Xenopus* have been extensively studied, *Valipora campylancristrota*, a parasite usually associated with fish, was found infecting

the bile duct system of *Xenopus laevis*. This once again confirmed the suitability of the frog as host for a variety of parasites. The fact that the frog serves as suitable alternative host gives an indication of its ecological overlap with fish.

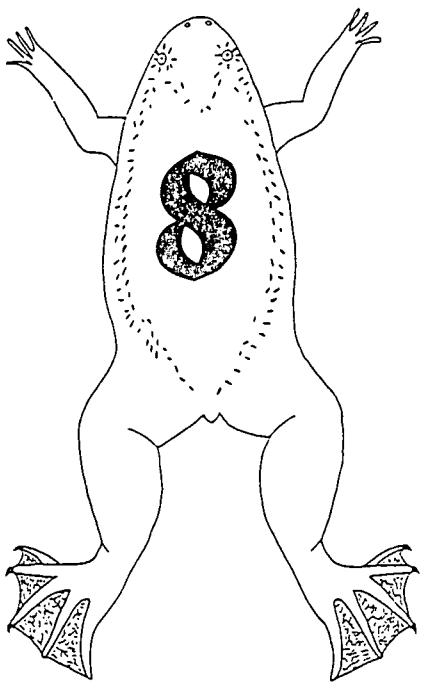
Although some form of seasonal variance in infection levels were found for four parasites, more research is necessary to conclusively identify and explain these patterns. The biggest problem with studies of this kind is the possibility of destructive sampling occurring due to the kill and count procedure required (Esch & Fernández, 1993), especially if the size of the host population is not known. As the density of the host population plays an important role in the success of parasite transmission, and therefore the infection levels, diminishing the host population would render inaccurate results. Another problem is that variation in infection levels could be natural fluctuations in parasite population which are not related to season.

More interesting results found in the current study were the correlation between parasite infection levels and host size or age. The numbers of four of the parasites were found to increase as hosts get older, while one occurred in decreasing numbers in older hosts. The main reasons for increasing infection levels in older hosts seem to be their having been exposed to infections for a longer time, and the larger sizes of their organs. The negative correlation found were probably due to a host response, which also explains the maximum levels reached in older hosts where parasite infection levels increased with host size.

The differences of parasite infection levels in male and female hosts were very small, and the significance thereof highly debatable. As the effects of hormonal differences on the parasites of *Xenopus* is unknown, and probably less important in *Xenopus* than in terrestrial frogs, the slight differences in the infection levels can only be ascribed to the fact that female *X. laevis* are larger than males.

As is the case with most natural science research projects, where every answer results in more questions, the current study identified several areas for future research:

1. The life cycle and biology of *Valipora campylancristrota*: The first intermediate host will probably be a copepod, and the final host a stork, heron or cormorant. Only a successful experimental completion of the life cycle will confirm the identity of other hosts. The transfer of the parasite from fish to *X. laevis* also needs investigating.
2. Conclusive identification of the juvenile leech: The leeches were only preliminarily identified as *Marsupiobdella africana*, and further research might prove the species to be different.
3. Influence of climate on *Xenopus* parasites: Further research is needed to determine the effects of temperature variations on parasite reproduction and behaviour. This will contribute to the explanation of the seasonal variation in infection levels exhibited by some parasites.
4. Influence of host hormones on *Xenopus* parasites: No conclusive evidence exists that host hormones affect parasites, but determining whether such influences exist will conclude if the different infection levels in males and females are due to hormonal differences, or simply difference in size.



Summary /

Opsomming

The African clawed frog, *Xenopus laevis*, has been extensively utilised over the years for a variety of research projects. The frog proved particularly useful as parasite study material because of its diverse parasite assemblage. The parasites of *Xenopus* represent seven major invertebrate groups, and no less than 29 parasite species are found associated with *X. laevis* utilising all organs except the lungs. The rich diversity of *Xenopus* parasites is related to the fact that the frog is primarily water living, which facilitates parasite transfer.

Despite the extensive research that has been done on the parasites of *Xenopus*, there is little information available on parasite ecology from field based studies. It is known, however, that parasite infection levels are determined by interaction of ecological factors and parasite and host characteristics. Due to the lack of information in this respect, a field based study was undertaken to determine what impact variations in climate, ecology, host size or age, and host sex have on the diversity and infection levels of parasites of *X. laevis*.

Two ecologically different localities were chosen for the purpose of the study. Using baited traps, *X. laevis* were collected during different months over a two year period, and the infection levels of the different parasites determined. The infection levels of parasites in hosts of different size and sex were also determined.

All the parasites found in the study occurred at both localities, but infection levels sometimes differed significantly. Although the infection levels varied through the time of the study, the seasonal patterns were not always clearly defined. Definite positive or

negative correlation between size and infection levels was found for most of the parasites. The relationship between host sex and infection levels was, however, inconclusive.

Although the parasites of *Xenopus* have been extensively studied, a new parasite was found in the bile ducts of the frog. The cyclophyllidean plerocercoid, *Valipora campylancristrota* (Wedl, 1955) (Cestoda: Dilepididae), was originally recorded from the gall bladder of the tench, *Tinca tinca*. The morphology and life cycle of the parasite was investigated. A juvenile leech found on the external surface of *X. laevis*, was preliminarily identified as *Marsupiobdella africana*, a leech known to infect *Xenopus*.

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Die platanna, *Xenopus laevis*, is deur die jare aangewend vir 'n verskeidenheid navorsingsprojekte. Die padda is veral geskik vir parasitologiese studies as gevolg van 'n diverse parasiet samestelling. Die parasiete van *Xenopus* verteenwoordig sewe invertebraat groepe, en nie minder as 29 parasiet spesies word geassosieer met *X. laevis*. Parasiete word in al die organe behalwe die longe van die platanna gevind. Die feit dat *Xenopus* waterlewend is dra by tot die ryk samestelling van parasiete omdat dit parasiet oordrag fasiliteer.

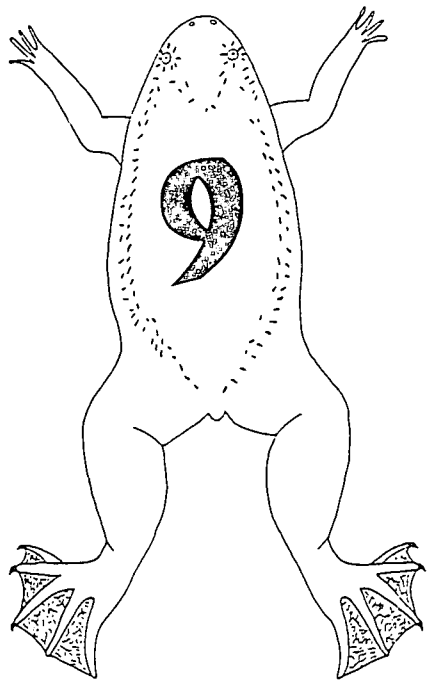
Ten spyte van die uitgebreide navorsing wat al op die parasiete van *Xenopus* gedoen is, is inligting ten opsigte van natuurlike parasiet ekologie beperk. Dit is egter bekend dat die infeksie vlakke van parasite beïnvloed word deur die interaksie van ekologiese faktore, en kenmerke van die parasiet en gasheer. As gevolg van die gebrek aan inligting

in die veld, is 'n studie gebasseer op veldwerk gedoen om te bepaal watter effek variasie in klimaat, ekologie, gasheer grootte of ouderdom, en die geslag van die gasheer op die diversiteit en infeksie vlakke van parasiete van *X. laevis* het.

Die parasiete van *X. laevis* in twee lokaliteite wat ekologies verskil is bestudeer. Platannas is versamel met behulp van fuike gedurende verskillende maande oor 'n periode van twee jaar, en die infeksie vlakke van verskillende parasiete bepaal. Die infeksie vlakke in gashere van verskillende grootte en geslag is ook bepaal.

Al die parasiete wat gedurende die huidige studie gevind is het in beide lokaliteite voorgekom, maar die infeksie vlakke het soms betekenisvol verskil. Alhoewel die infeksie vlakke gevarieer het deur die loop van die studie, was seisoenale patrone nie altyd duidelik nie. Betekenisvolle korrelasies tussen die grootte van die gasheer en infeksie vlakke is vir meeste parasiete gevind. Die verhouding tussen infeksie vlakke en die geslag van die gasheer was egter onbeslis.

Alhoewel die parasiete van *Xenopus* al deeglik bestudeer is, is 'n nuwe parasiet in die galbuise van die padda gevind. Die lintwurm larf, *Valipora campylancristrota* (Wedl, 1955) (Cestoda: Dilepididae) was oorspronklik gevind in die galblaas van die seelt, *Tinca tinca*. Die morfologie en lewensiklus van die parasiet is ondersoek. 'n Onvolwasse bloedsuier wat op die eksterne oppervlak van *X. laevis* gevind is, is voorlopig geïdentifiseer as *Marsupiobdella africana*, 'n bloedsuier wat bekend is van *Xenopus*.



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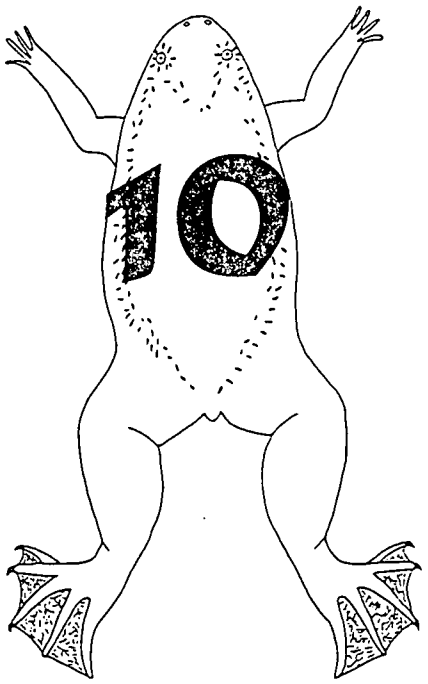


Appendices

Appendix 1

Data sheet used for recording parasite diversity and infection levels

PARASITE ACCOUNT: FROGS

General

Date colleccted: 19___ / ___ / ___ Date dissected 19___ / ___ / ___

Grid ____o____'____"S ____o____'____"E Locus _____

Locality: _____

Habitat: _____

Collector: _____

Host No: _____

Species: _____

Sex: _____ Mass: _____g

Snout-urostyle length _____mm Headwidth _____mm

Remarks: _____

Parasites (species, number, reference)

External surface: _____

Buccal cavity: _____

Nostrils: _____

Eyes: _____

Pericardium: _____

Blood: _____

Body cavity: _____

Surface of organs: _____

Excretory bladder: _____

_____

Esophagus: _____

Stomach _____

_____

Duedenum: _____

_____

Rectum: _____

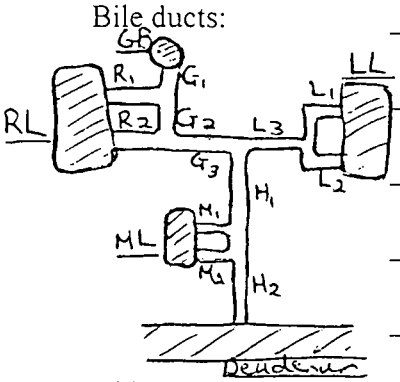
_____

Lungs: _____

Liver: _____

Gall bladder: _____

Bile ducts: _____



_____

_____

_____

_____

Kidneys: _____

Wolfian ducts: _____

Remarks: _____

_____

_____

_____

_____

Appendix 2

Du Preez, L.H., Crous, H.P. & Kok, D.J. (1996) Morphology of an unknown cyclophillidean cysticercoid found in the clawed frog *Xenopus laevis*. *Mic. Soc. S. Afr. Proc.* 26: 111.

MORPHOLOGY OF AN UNKNOWN CYCLOPHYLLIDEAN CYSTICERCOID FOUND IN THE CLAWED FROG *Xenopus laevis*

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The frog genus *Xenopus* is characterized by its extraordinary richness in parasite diversity. *X. laevis* serves as host for no less than 25 parasite genera representing seven major invertebrate groups[1].

Unlike the Monogenea and Digenia, cestodes are found comparatively rarely in amphibians, but both adult and larval stages do occur. Only one adult pseudophyllidean cestode, *Cephaloclamys namaquensis,* is known from *Xenopus* and is found in the intestine. Encysted larval stages of tapeworms have been documented to occur in the tissues of the gut wall as well as in the peritoneal membranes of *Xenopus*. Clawed frogs from Uganda contained plerocercoids and one heavily infected intestine bore 62 cysts[2]. Cyclophyllidean cysticerci were reported from the guts and mesenteries of *X. laevis* collected near Marble Hall[3]. No less than 80% of the host sample was infected with between 3 and 36 cysts. The larvae were observed to lay freely in the cyst. Each had four suckers but lacked both rostellum and hooks[3].

This paper reports the finding of a yet unknown cyclophyllidean cysticercoid from the bile ducts of *X. laevis*. The cysticercoids cause thickenings in the bile ducts and are found in both the thickenings and ducts . Up to four larvae were found in one thickening and up to 10 thickenings occurred per individual host. To date the parasite has only been recorded from three waterbodies. At one of the localities 25% of the clawed frogs collected were infected, with up to 52 parasites per host.

After removal from the bile ducts, parasites were fixed for 1 hour in Flemming's solution[4], cleaned in an OMO detergent solution in an ultrasonic bath, dehydrated in an ethanol series and critical point dried. Dried material was mounted with the aid of epoxy resin (Pratley Clear) on 12,5 mm aluminium stubs. Specimens were gold-coated in a sputter coater (Polaron E5000) and examined in a JEOL 6400 scanning-electron microscope at 5 KV.

The hind body is cylindrical (Fig. 1) and the posterior end is rounded with no appendages. The scolex bears four acetabula (Fig. 2) as well as an invaginated rostellum. The complete scolex is lined with villi-like microtriches (Fig. 3) which play a vital role in respiration. Two circles of hooks are present on the invaginated rostellum (Fig. 4). Blades are strongly curved and sharply pointed (Fig. 5). The guards are short and rounded. In the first circle the blades are large and about the same length as the handles, but the blades are smaller in the second row with the handles about twice as long as the blades.

Preliminary identification assigns this parasite to the family Dilepididae with a final host probably a heron or cormorant. In order to identify the parasite to generic level carefully controlled feeding experiments will have to be carried out by force feeding parasite-free potential hosts with *Xenopus* carcasses known to be infected.

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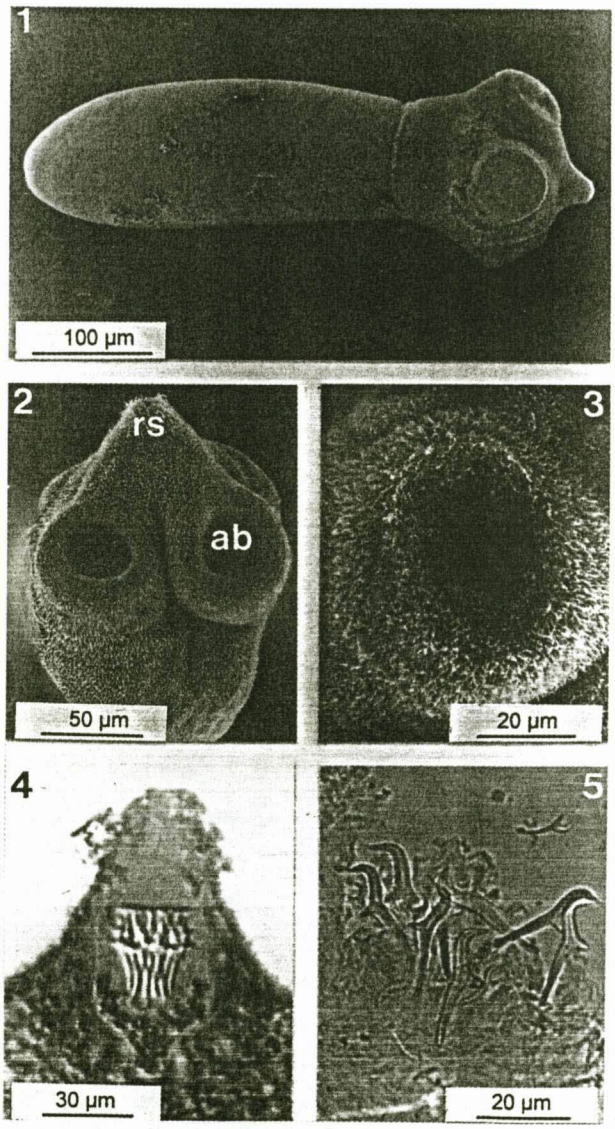

Fig. 1. Cysticercoid from bile duct of *X. laevis*.
Fig. 2. Scolex bearing four acetabula (ab) and an invaginated rostellum (rs).
Fig. 3. Acetabulum covered in microtriches.
Fig. 4. Invaginated rostellum with hooks.
Fig. 5. Squashed lactophenol preparation to reveal the shape of the hooks.

Appendix 3.1

Measurements of the rostellar hooks of *Valipora campylancristrota.*

Appendix 3.2

Measurements of the rostellar hooks of *Neogryporhynchus cheilancristrotis.*

HOOK	MEASUREMENT (μ)					
	Large hooks			Small hooks		
	L	G	H	L	G	H
1	27.4	14.2	14.7	11.8	4.9	7.8
2	25	11.6	13.7	12.3	4.9	8.8
3	25.5	11.8	13.7	12.7	4.9	8.8
4	28.4	12.3	14.7	11.8	4.9	7.8
5	27.4	13.2	13.7	10.8	4.4	7.4
6	27.9	13.2	14.7	11.8	4.4	7.4
7	25.5	11.8	13.7	11.8	4.4	7.4
8	27.4	13.7	14.7	12.7	4.9	8.8
9	25.5	11.8	13.7	13.2	5.4	10.8
10	26.5	11.8	15.7	11.3	3.9	7.8
11	27	11.8	14.7	11.8	4.4	7.8
12	27.4	11.8	14.7	12.3	4.4	8.8
13	28.4	13.2	16.7	12.7	4.4	8.8
14	26	11.8	14.7	12.7	4.4	8.8
15	27.9	13.7	14.7	11.3	3.9	7.8
16	25.5	11.3	14.7	11.8	4.4	7.8
17	27	11.8	14.7	11.8	3.9	7.8
18	26.5	13.7	15.7	11.8	3.9	7.8
19	27.4	12.7	15.7	13.7	4.9	9.8
20	25.5	11.3	14.7	12.7	4.9	8.8

HOOK	MEASUREMENT (μ)					
	Large hooks			Small hooks		
	L	G	H	L	G	H
1	66.8	35.9	33.4	40.8	23.1	19.6
2	66.8	35	34.2	40.8	22	19.6
3	68.5	36.7	34.2	40.8	17.9	17.9
4	67.6	35	34.2	40.8	22.8	19.6
5	68.5	36.7	34.2	40.8	22.8	19.6
6	66.8	34.2	32.6	39.9	21.7	18.7
7	68.5	36.7	34.2	41.6	23.6	19.6
8	66.8	35.9	32.6	41.2	22.1	19.6
9	70.1	38.3	34.2	41.2	22.5	21.6
10	68.5	36.7	34.2	42.4	24.5	22.8
11	71.7	36.7	35.9	42.4	24.5	19.6
12	70.1	35.9	35.9	42.4	22.8	21.2
13	71.7	37.5	34.2	41.6	23.6	21.2
14	70.1	37.5	34.2	42.4	23.6	21.2
15	71.7	38.3	35.9	39.1	22	16.3
16	70.1	37.5	35	40.8	21.2	17.9
17	70.1	37.5	35	40.8	21.2	19.6
18	71.7	37.5	35	40.8	21.2	19.5
19	66.8	35.9	32.6	39.1	21.2	19.6
20	66.8	34.2	32.6	40.8	21.2	19.6

Appendix 4

Monthly infection levels of parasites at the two localities.

Parasite	Locality	YYMM	Prevalence	Mean intensity	Abundance
Gyrdicotylus gallieni	A	9602	0.0%	0.0	0.0
		9604	10.0%	2.0	0.2
		9605	0.0%	0.0	0.0
		9607	10.0%	2.0	0.2
		9610	40.0%	8.8	3.5
		9701	0.0%	0.0	0.0
		9703	0.0%	0.0	0.0
		9706	0.0%	0.0	0.0
		9708	0.0%	0.0	0.0
		9709	0.0%	0.0	0.0
		9711	70.0%	9.7	6.8
		9712	20.0%	2.0	0.4
	B	9603	20.0%	1.0	0.2
		9604	40.0%	5.5	2.2
		9606	20.0%	2.0	0.4
		9608	10.0%	1.0	0.1
		9610	0.0%	0.0	0.0
		9702	0.0%	0.0	0.0
		9705	0.0%	0.0	0.0
		9707	10.0%	1.0	0.1
		9709	10.0%	2.0	0.2
		9711	0.0%	0.0	0.0
		9801	60.0%	2.0	1.2
Protopolystoma xenopodis (urinary bladder)	A	9602	10.0%	1.0	0.1
		9604	30.0%	1.3	0.4
		9605	0.0%	0.0	0.0
		9607	30.0%	1.3	0.4
		9610	40.0%	1.8	0.7
		9701	50.0%	1.0	0.5
		9703	80.0%	1.3	1.0
		9706	37.5%	1.0	0.4
		9708	60.0%	1.5	0.9
		9709	50.0%	1.0	0.5
		9711	30.0%	1.0	0.3
		9712	60.0%	1.3	0.8
	B	9603	80.0%	3.3	2.6
		9604	30.0%	1.0	0.3
		9606	59.1%	2.1	1.2
		9608	60.0%	2.7	1.6
		9610	90.0%	1.4	1.3
		9702	80.0%	1.5	1.2
		9705	60.0%	1.3	0.8
		9707	70.0%	2.0	1.4
		9709	70.0%	1.7	1.2
		9711	50.0%	1.0	0.5
		9801	50.0%	1.6	0.8

Parasite	Locality	YYMM	Prevalence	Mean intensity	Abundance
Protopolystoma xenopodis (kidneys)	A	9602	0.0%	0.0	0.0
		9604	0.0%	0.0	0.0
		9605	0.0%	0.0	0.0
		9607	0.0%	0.0	0.0
		9610	0.0%	0.0	0.0
		9701	0.0%	0.0	0.0
		9703	0.0%	0.0	0.0
		9706	0.0%	0.0	0.0
		9708	0.0%	0.0	0.0
		9709	0.0%	0.0	0.0
		9711	0.0%	0.0	0.0
		9712	0.0%	0.0	0.0
	B	9603	0.0%	0.0	0.0
		9604	0.0%	0.0	0.0
		9606	10.0%	5.0	0.5
		9608	0.0%	0.0	0.0
		9610	0.0%	0.0	0.0
		9702	0.0%	0.0	0.0
		9705	10.0%	1.0	0.1
		9707	0.0%	0.0	0.0
		9709	0.0%	0.0	0.0
		9711	0.0%	0.0	0.0
		9801	0.0%	0.0	0.0
Dolfuschella rodhaini	A	9602	0.0%	0.0	0.0
		9604	0.0%	0.0	0.0
		9605	0.0%	0.0	0.0
		9607	10.0%	1.0	0.1
		9610	0.0%	0.0	0.0
		9701	0.0%	0.0	0.0
		9703	10.0%	1.0	0.1
		9706	0.0%	0.0	0.0
		9708	0.0%	0.0	0.0
		9709	0.0%	0.0	0.0
		9711	0.0%	0.0	0.0
		9712	0.0%	0.0	0.0
	B	9603	0.0%	0.0	0.0
		9604	0.0%	0.0	0.0
		9606	0.0%	0.0	0.0
		9608	0.0%	0.0	0.0
		9610	0.0%	0.0	0.0
		9702	0.0%	0.0	0.0
		9705	0.0%	0.0	0.0
		9707	0.0%	0.0	0.0
		9709	0.0%	0.0	0.0
		9711	0.0%	0.0	0.0
		9801	0.0%	0.0	0.0

Parasite	Locality	YYMM	Prevalence	Mean intensity	Abundance
Tylodelphys xenopi (pericardium)	A	9602	100.0%	32.0	32.0
		9604	100.0%	51.2	51.2
		9605	100.0%	106.5	106.5
		9607	90.0%	95.2	85.7
		9610	80.0%	64.4	51.5
		9701	90.0%	85.3	76.8
		9703	100.0%	160.0	160.0
		9706	75.0%	46.5	34.9
		9708	90.0%	62.2	56.0
		9709	100.0%	115.3	115.3
		9711	100.0%	20.0	20.0
		9712	90.0%	57.4	51.7
	B	9603	100.0%	72.9	72.9
		9604	100.0%	56.1	56.1
		9606	100.0%	253.3	253.3
		9608	100.0%	291.4	291.4
		9610	100.0%	128.0	128.0
		9702	100.0%	67.3	67.3
		9705	100.0%	191.4	191.4
		9707	100.0%	106.0	106.0
		9709	100.0%	74.8	74.8
		9711	100.0%	40.0	40.0
		9801	80.0%	32.6	26.1
Tylodelphys xenopi (body cavity)	A	9602	0.0%	0.0	0.0
		9604	80.0%	8.5	6.8
		9605	90.0%	21.3	19.2
		9607	70.0%	15.4	10.8
		9610	70.0%	11.4	8.0
		9701	70.0%	11.4	8.0
		9703	90.0%	43.3	39.0
		9706	62.5%	7.6	4.8
		9708	70.0%	17.3	12.1
		9709	80.0%	21.5	17.2
		9711	10.0%	20.0	2.0
		9712	40.0%	20.8	8.3
	B	9603	60.0%	19.2	11.5
		9604	40.0%	51.3	20.5
		9606	90.0%	53.4	48.1
		9608	90.0%	62.9	56.6
		9610	80.0%	33.3	26.6
		9702	70.0%	28.9	20.2
		9705	90.0%	45.8	41.2
		9707	70.0%	24.4	17.1
		9709	60.0%	20.7	12.4
		9711	75.0%	11.7	8.8
		9801	50.0%	3.6	1.8

Parasite	Locality	YYMM	Prevalence	Mean intensity	Abundance
Cephaloclamys namaquensis	A	9602	90.0%	5.8	5.2
		9604	100.0%	3.9	3.9
		9605	100.0%	7.2	7.2
		9607	100.0%	7.1	7.1
		9610	90.0%	8.9	8.0
		9701	40.0%	2.5	1.0
		9703	60.0%	1.7	1.0
		9706	37.5%	5.7	2.1
		9708	90.0%	4.9	4.4
		9709	80.0%	6.3	5.0
		9711	30.0%	2.3	0.7
		9712	50.0%	5.4	2.7
	B	9603	100.0%	12.4	12.4
		9604	80.0%	3.3	2.6
		9606	100.0%	6.5	6.5
		9608	90.0%	6.7	6.0
		9610	90.0%	4.9	4.4
		9702	30.0%	3.0	0.9
		9705	100.0%	3.7	3.7
		9707	80.0%	3.3	2.6
		9709	80.0%	1.8	1.4
		9711	100.0%	2.0	2.0
		9801	10.0%	1.0	0.1
Valipora campylancristrota	A	9602	0.0%	0.0	0.0
		9604	10.0%	1.0	0.1
		9605	0.0%	0.0	0.0
		9607	10.0%	1.0	0.1
		9610	0.0%	0.0	0.0
		9701	10.0%	1.0	0.1
		9703	0.0%	0.0	0.0
		9706	0.0%	0.0	0.0
		9708	10.0%	2.0	0.2
		9709	10.0%	2.0	0.2
		9711	10.0%	2.0	0.2
		9712	0.0%	0.0	0.0
	B	9603	40.0%	17.3	6.9
		9604	10.0%	52.0	5.2
		9606	27.3%	8.0	2.2
		9608	70.0%	6.3	4.4
		9610	30.0%	3.7	1.1
		9702	0.0%	0.0	0.0
		9705	40.0%	3.8	1.5
		9707	10.0%	8.0	0.8
		9709	50.0%	2.4	1.2
		9711	0.0%	0.0	0.0
		9801	70.0%	2.4	1.7

Parasite	Locality	YYMM	Prevalence	Mean intensity	Abundance
Camallanus kaapstaadi	A	9602	70.0%	2.7	1.9
		9604	80.0%	3.5	2.8
		9605	70.0%	3.3	2.3
		9607	70.0%	3.0	2.1
		9610	70.0%	4.4	3.1
		9701	80.0%	4.8	3.8
		9703	90.0%	6.6	5.9
		9706	37.5%	4.0	1.5
		9708	90.0%	9.3	8.4
		9709	70.0%	12.1	8.5
		9711	40.0%	1.8	0.7
		9712	50.0%	3.4	1.7
	B	9603	70.0%	4.4	3.1
		9604	40.0%	1.8	0.7
		9606	90.0%	4.9	4.4
		9608	100.0%	5.8	5.8
		9610	100.0%	6.4	6.4
		9702	80.0%	5.3	4.2
		9705	90.0%	5.3	4.8
		9707	70.0%	2.7	1.9
		9709	80.0%	3.0	2.4
		9711	75.0%	2.0	1.5
		9801	40.0%	3.0	1.2
Batrachocamallanus slomei	A	9602	10.0%	19.0	1.9
		9604	20.0%	59.5	11.9
		9605	10.0%	2.0	0.2
		9607	10.0%	4.0	0.4
		9610	30.0%	1.3	0.4
		9701	10.0%	2.0	0.2
		9703	20.0%	2.0	0.4
		9706	0.0%	0.0	0.0
		9708	30.0%	2.0	0.6
		9709	40.0%	1.8	0.7
		9711	10.0%	2.0	0.2
		9712	20.0%	5.0	1.0
	B	9603	0.0%	0.0	0.0
		9604	0.0%	0.0	0.0
		9606	20.0%	1.0	0.2
		9608	10.0%	6.0	0.6
		9610	10.0%	1.0	0.1
		9702	10.0%	1.0	0.1
		9705	0.0%	0.0	0.0
		9707	0.0%	0.0	0.0
		9709	0.0%	0.0	0.0
		9711	0.0%	0.0	0.0
		9801	0.0%	0.0	0.0

Parasite	Locality	YYMM	Prevalence	Mean intensity	Abundance
Marsupiobdella africana	A	9602	0.0%	0.0	0.0
		9604	10.0%	14.0	1.4
		9605	0.0%	0.0	0.0
		9607	0.0%	0.0	0.0
		9610	0.0%	0.0	0.0
		9701	0.0%	0.0	0.0
		9703	0.0%	0.0	0.0
		9706	0.0%	0.0	0.0
		9708	0.0%	0.0	0.0
		9709	0.0%	0.0	0.0
		9711	0.0%	0.0	0.0
		9712	50.0%	1.8	0.9
	B	9603	0.0%	0.0	0.0
		9604	0.0%	0.0	0.0
		9606	0.0%	0.0	0.0
		9608	10.0%	1.0	0.1
		9610	20.0%	4.5	0.9
		9702	10.0%	1.0	0.1
		9705	70.0%	3.6	2.5
		9707	10.0%	1.0	0.1
		9709	90.0%	9.0	8.1
		9711	0.0%	0.0	0.0
		9801	0.0%	0.0	0.0