The population dynamics of *Acanthocephalus lucii* (Müller 1776) (Acanthocephala: Echinorhynchidae), an intestinal parasite of freshwater fishes

Thesis submitted in accordance with the requirement of the University of Liverpool for the degree of Doctor of Philosophy

by

John Brattey

Department of Zoology,

University of Liverpool.

January, 1982

CONTENTS

Acknowledgements

I would like to thank Professor C.J. Duncan and Dr. R.G Pearson in whose department this work was undertaken.

I am extremely grateful to Mr. B. Lewis and Mr. R. Stammers for photography, Mr. P.J. Alden for technical assistance, Mr. S.M. Spelling and Drs. M. Begon, D.J. Thompson and J.O. Young for advice on computing and statistics, Miss Sue Scott for typing and to Mr. R. Doughty for providing chemical and biological data concerning the Forth and Clyde canal.

I would also like to thank Liverpool City Council, Department of Recreation and Open Spaces, for permission to sample Liverpool Park Lakes, the British Waterways Board for permission to sample the Forth and Clyde canal and the Science Research Council for the provision of a grant.

Many friends and colleagues assisted with sampling, to all of whom I would like to express my thanks, but I am especially grateful to Mr. D. Bowen, Miss Sally Cowley, Mr. J. Gittins, Mr. P.G. McMenamin and Mr. C.B. Shedden.

Finally, I would like to thank my supervisor Dr. J.C. Chubb for his advice, encouragement and guidance throughout this study.

ABSTRACT

The population dynamics of Acanthocephalus lucii (Muller, 1776) (Acanthocephala: Echinorhynchidae), an intestinal parasite of freshwater fishes, by John Brattey, Department of Zoology, University of Liverpool.

The population dynamics of Acanthocephalus lucii in the Forth and Clyde canal at Temple, Glasgow were investigated over a period of 22 months. A holistic approach was adopted and regular samples of both intermediate (Asellus aquaticus) and definitive (Perca fluviatilus) hosts were collected and examined for adult and larval parasites, respectively.

Laboratory infection experiments were also undertaken. These provided information on the effects of temperature on larval parasite growth, the influence of larval parasites on intermediate host survival, reproduction and susceptibility to predation. Experimental infections of definitive hosts provided information on the establishment, survival, growth, rate of maturation and intestinal distribution of adult worms at various temperatures.

Field studies indicated that output of parasite shelled acanthors (= eggs) was maximal in early summer and this coincided with the period of maximum intermediate host population density. Recruitment of acanthors into the intermediate host population commenced in summer and continued through autumn with maximum levels of infection in mid winter. Infection levels declined gradually through spring principally owing to turnover of the isopod population. A slight seasonal cycle in the size of the larval parasite population was observed. The

distribution of larval parasites within the intermediate host population was overdispersed and adequately described by the negative binomial model.

Stomach content analysis of perch indicated some recruitment of larval parasites into the definitive host population occurred throughout the year but with a maximum in spring. A seasonal cycle in incidence (= prevalence) and intensity of infection and in maturation was observed. Maximum levels of infection occurred in May with a gradual decline to minimum levels late the following winter. The sex ratio of adult parasites was close to 1:1 during summer but markedly in favour of females in winter. Frequency distribution analysis revealed an overdispersed pattern although the degree of over-dispersion decreased from a maximum in April-May to a minimum the following February-March.

During experimental infections, all sizes of isopod were susceptible to infection and isopods were not resistant to heavy infections, which reduced isopod survival. Infected isopods exhibited an increased susceptibility to predation by the definitive host. Female isopods harbouring cystacanths were sterile, whereas males could inseminate females normally. Infected isopods displayed a highly conspicuous pigmentation change whereby the pleopods on the ventral surface of the abdomen became darkened. The rate of development of larval parasites was markedly influenced by temperature.

Experimental infections of perch indicated that temperature did not markedly influence parasite establishment, but warm temperatures

increased the rate of growth, maturation and mortality of adult worms. Copulation between worms commenced immediately after establishment and was not inhibited by cold temperatures. Female worms underwent multiple inseminations and typically survived longer than males.

During this study no conclusive evidence was obtained to suggest that density dependent regulatory processes were operating on any stage in the parasites life cycle. Regulation did not appear to be achieved through intraspecific competition, partial immunity or by an aggregation-mortality mechanism. The observed seasonal changes in infection levels appeared to be solely due to the operation of density independent factors such as temperature. Temperature was shown to influence parasite recruitment via its influence on both intermediate and definitive host feeding intensity and parasite mortality and natality by its influence on the rate of maturation of adult parasites.

CHAPTER 1

INTRODUCTION

Chapter 1

General Introduction

Interest in the ecology of parasites of freshwater fish has risen considerably in recent years, especially since the publication, in English, of the extensive reviews of Russian works by Dogiel et al. (1961), Dogiel (1964) and Bauer (1962). In Britain an increasing interest in both fish farming and angling has emphasised the need for more detailed studies on the ecology of parasites of freshwater fish, particularly with a view to establishing which species are potentially pathogenic, such that effective control measures can be developed and the introduction of these species to new habitats prevented.

Many of the earlier studies on freshwater fish parasites were primarily concerned with problems of life cycles (Meggitt, 1914; Brown, 1927), checklists, or records of distribution (Ritchie, 1915; Nicoll, 1924; Baylis, 1928, 1939; Kane, 1966) or pathological effects (Rushton, 1937; Hickey and Harris, 1947; Kerr, 1948; Arme and Owen, 1967, 1968). More recently, detailed studies of fish parasite population dynamics have appeared (Awachie, 1965; Paling, 1965; Walkey, 1967; Kennedy, 1968; Kennedy and Hine, 1969; Hine and Kennedy, 1974a; Anderson, 1974a) and it is with this particular aspect of the study of freshwater fish parasites that the current study is primarily concerned.

Chubb (1977, 1979, 1980, 1982) has recently reviewed the extensive literature which is available on the seasonal dynamics of helminth

parasites of freshwater fish. Although sampling difficulties and problems associated with complex life cycles has resulted in much of the available information being too sketchy to be of value to the biologist interested in population dynamics, a number of very detailed field and experimental studies have now been published (Kennedy, 1968, 1969, 1971, 1972, 1974b; Kennedy and Hine, 1969; Kennedy and Rumpus, 1977; Hine and Kennedy, 1974b; Pennycuik, 1971a, 1971b, 1971c, 1971d; Anderson, 1974a, 1974b; Leong, 1975; Holmes, Hobbs, Leong, 1977; Anderson, Whitfield and Mills, 1977; Mills, Anderson and Whitfield, 1979; Mills, 1980) and it is becoming increasingly apparent that work on freshwater fish parasite population dynamics may well have a significant part to play in formulating modern concepts in the field of population biology as a whole.

Within this field, in recent years, a considerable amount of specialised jargon and terminology has appeared, but definition of the various terms and a discussion of current theories and concepts pertaining to this subject has been left until the final chapter of this thesis.

Briefly, the aim of the current study was to undertake a detailed investigation into the population dynamics of a single species of fish parasite by a combination of field and experimental work. Essentially the study involved estimating, by means of regular sampling throughout the year, the numbers of all the stages in the life cycle of the parasite. Seasonal changes in the numbers of each stage were detected and these changes related, if possible,

to environmental variables using both field and experimental data.

Of the approximately 100 different helminth parasites recorded from freshwater fish in Britain (see Kennedy, 1974a) very few are suitable for such a detailed study, since a variety of technical difficulties often make it impossible to examine more than a single stage in the parasite life cycle. However, careful consideration of the problems experienced by other workers in this field led to the decision that one of the acanthocephalan parasites might prove suitable for study, since they have a simple life-cycle. Initially work began on Acanthocephalus clavula Dujardin, 1845 in perch (Perca fluviatilus L.) in Bala Lake, Wales, but this was ceased when it became apparent that perch were auxiliary rather than preferred hosts of A. clavula, since in this fish species the parasite rarely attains sexual maturity. Work then commenced on Acanthocephalus lucii (Müller, 1776) (synonym Echinorhynchus angustatus Rudolphi, 1802) in perch in the Forth and Clyde canal at Temple, Glasgow and this parasite species proved to be particularly suitable for a detailed study.

The Acanthocephala

Since the Acanthocephala comprise one of the lesser known, or minor pseudocoelomate phyla, some mention of the biology of this group seems justified. Detailed accounts are available in the works of Hyman (1951), Petrochenko (1971), Nicholas (1967, 1973), Crompton (1970), 1975) and Parshad and Crompton (1981). Suitable works for the identification of the various species of Acanthocephala

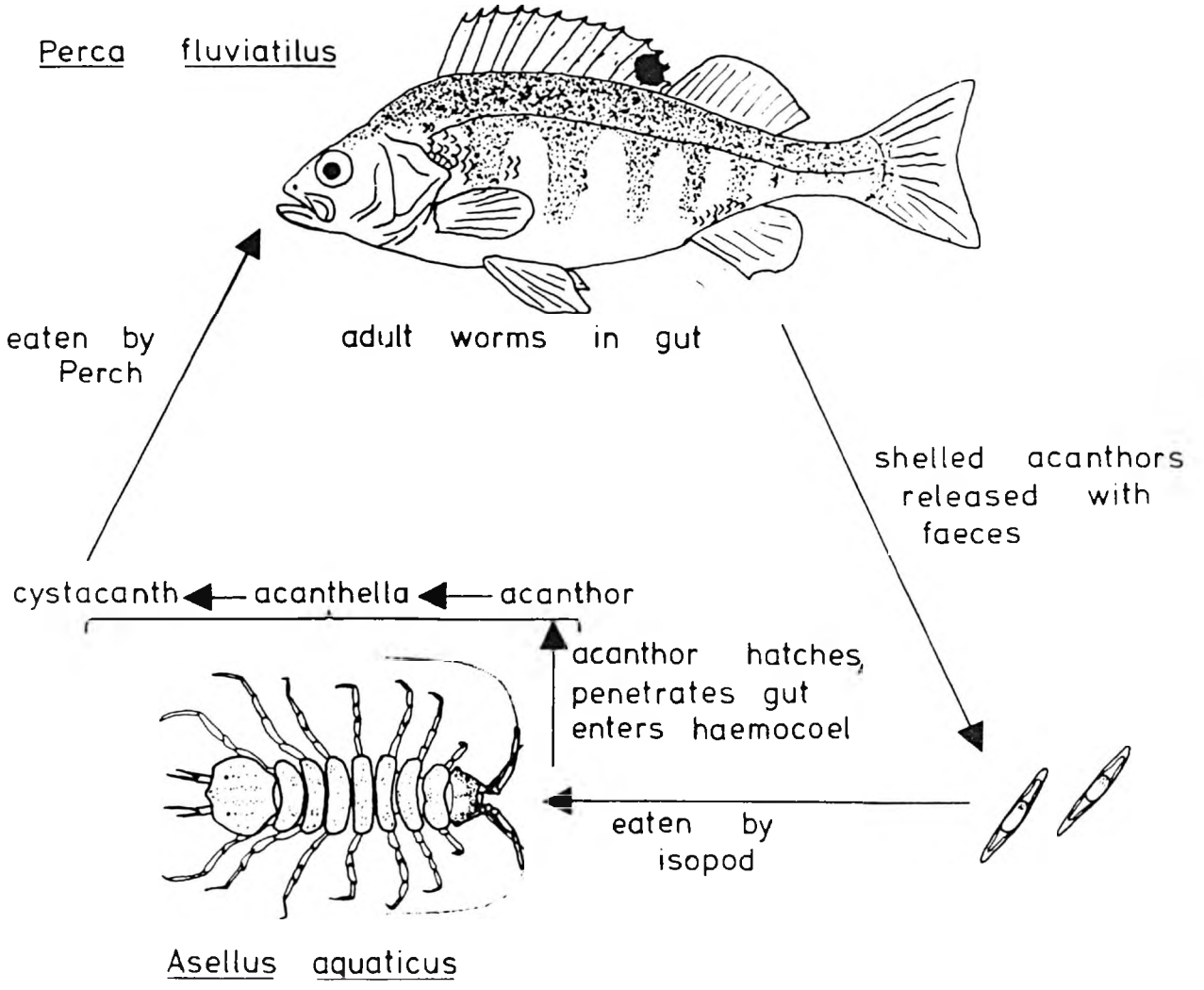
include Lühe (1911), Petrochenko (1971), Yamaguti (1963) and Golvan (1969). Only a brief account of the biology of the group will be given here and the reader should refer to the aforementioned works for further details. Throughout the thesis the terminology of Van Cleave (1947) has been adopted for the description of acanthocephalan larval stages (see Chapters 3 and 5).

The Acanthocephala are characterized by the possession of a spiny, retractable proboscis. The sexes are separate and have no alimentary tract at any stage during development, nutrients being absorbed through the body wall. The life cycle involves two hosts. Adult worms are found in the intestine of vertebrates and larval stages occur in the haemocoel of arthropods. The adult worms possess a unique reproductive apparatus, the details of which are described by Whitfield (1968), Crompton and Whitfield (1974) and Parshad and Crompton (1981).

The life cycle of Acanthocephalus lucii

The life cycle of Acanthocephalus lucii is illustrated in Figure 1.1. Perch (Perca fluviatilus L.) and the isopod crustacean Asellus aquaticus (L.) are generally considered to be the principal definitive and intermediate hosts, respectively. Although Petrochenko (1971) records adult A. lucii from the intestine of some 36 different species of fish, the status of many of these species with respect to parasite maturation remains to be determined. The explanation for this apparent low host specificity of the adult parasites lies

Figure 1.1. The life cycle of Acanthocephalus lucii



in the fact that they appear to be able to survive for long periods in the intestine of unsuitable fish without undergoing appreciable growth or maturation. In suitable hosts adult parasites will mature, shelled acanthors (= eggs) are released and pass out of the fish with faeces. If ingested by the isopod Asellus aquaticus they hatch, releasing the motile acanthor larvae which penetrate the isopod intestine and migrate into the haemocoele, where development proceeds through a series of recognizable stages until the final infective cystacanth stage is reached. A more detailed account of the development of larval A. lucii is given in Chapter 5. Cystacanths of A. lucii are precocious in their development. In males active sperm are present and in females the ovary is fragmented (Brattey, 1980). They are invariably white, elongate and the sexes are easily distinguishable with the aid of a microscope, since the internal reproductive organs are readily visible through the body wall. When infected isopods are eaten by the appropriate definitive host the cystacanths are activated, the proboscis everts and the parasite attaches to the host intestinal mucosa. Young adult A. lucii commence copulatory activity as soon as they establish (Brattey, 1980). Male parasites evert the copulatory bursa, which clasps the hind end of a female worm and sperm is transferred. Following copulation males leave a small copulatory cap on female worms. This cap falls off after a short period and female worms probably require multiple inseminations for their full reproductive

potential to be realized. The body cavity of female worms is filled with oval shaped structures termed ovarian balls. Each ovarian ball is effectively a single ovary and the functional organization of these structures has been examined in detail by Crompton and Whitfield (1974). Sperm fuse with mature oocytes on the surface of the ovarian ball and the resulting zygotes elongate, forming elliptical structures which break away from the ovarian ball into the surrounding fluid in the pseudocoelom. These elliptical structures are now termed immature acanthors. A number of envelopes or shell layers form round the developing acanthor until the characteristic 'rolling pin' shape of the mature shelled acanthor appears. On examination, a female worm can thus be classified according to the stage of development of the acanthors. Three stages can be recognised: firstly those with ovarian balls, secondly those with a mixture of ovarian balls and immature acanthors and thirdly those with a mixture of ovarian balls, immature and mature shelled acanthors. When sufficient mature shelled acanthors have developed they are released, thus completing the life cycle.

The geographic distribution of Acanthocephalus lucii

Acanthocephalus lucii apparently has a fairly widespread distribution, having been recorded from a number of habitats throughout much of the Palaearctic region. There are numerous records of A. lucii from the British Isles, including Ireland, many of which are unpublished. All records known to the author are indicated in

Table 1.1 and Figure 1.2. Figure 1.2 does not represent an accurate map of the distribution of A. lucii in Britain. The absence of A. lucii from certain regions on Figure 1.2 sometimes reflects the fact that the appropriate area has not been surveyed rather than indicating that the parasite does not exist there. Nonetheless, personal communications with the various water authorities does suggest that A. lucii is absent or extremely rare in certain regions, including Cornwall (Kennedy, pers. comm.), the Greensands area south of the Thames, numerous gravel pit lakes in the south and south east of England (Sweeting, pers. comm.) and from the north west of Scotland (personal observations).

On the continent Acanthocephalus lucii has been recorded from France (Golvan, 1969; Van Maren, 1979), Germany (Priemer, 1979), Switzerland (André, 1921), Poland (Grabda, 1971), Norway (Halvorsen, 1972; Andersen, 1978), Finland, Sweden, Rumania, Italy (Petrochenko, 1971) and from many parts of Russia including Lake Oneda, Lake Ladoga, lakes of Karelia, Gulf of Finland, Neva Bay, lakes and rivers of Leningrad region, Novgorod region, Dnieper Basin, Dneister, Kuban Delta, Volga, Kama and Klyazna river. The parasite is apparently absent from the Iberian peninsula, from the Aral and Amur districts, from Siberia (Petrochenko, 1971) and is extremely rare in the Caspian Sea (Dogiel and Bychowsky, 1939), which is in accord with the distribution of the intermediate host Asellus aquaticus as described by Williams (1960). Broadly speaking, A. lucii is a parasite of the Western Palaearctic region.

Figure 1.2. Summary of records of Acanthocephalus lucii from the British Isles (localities are listed in Table 1.1).

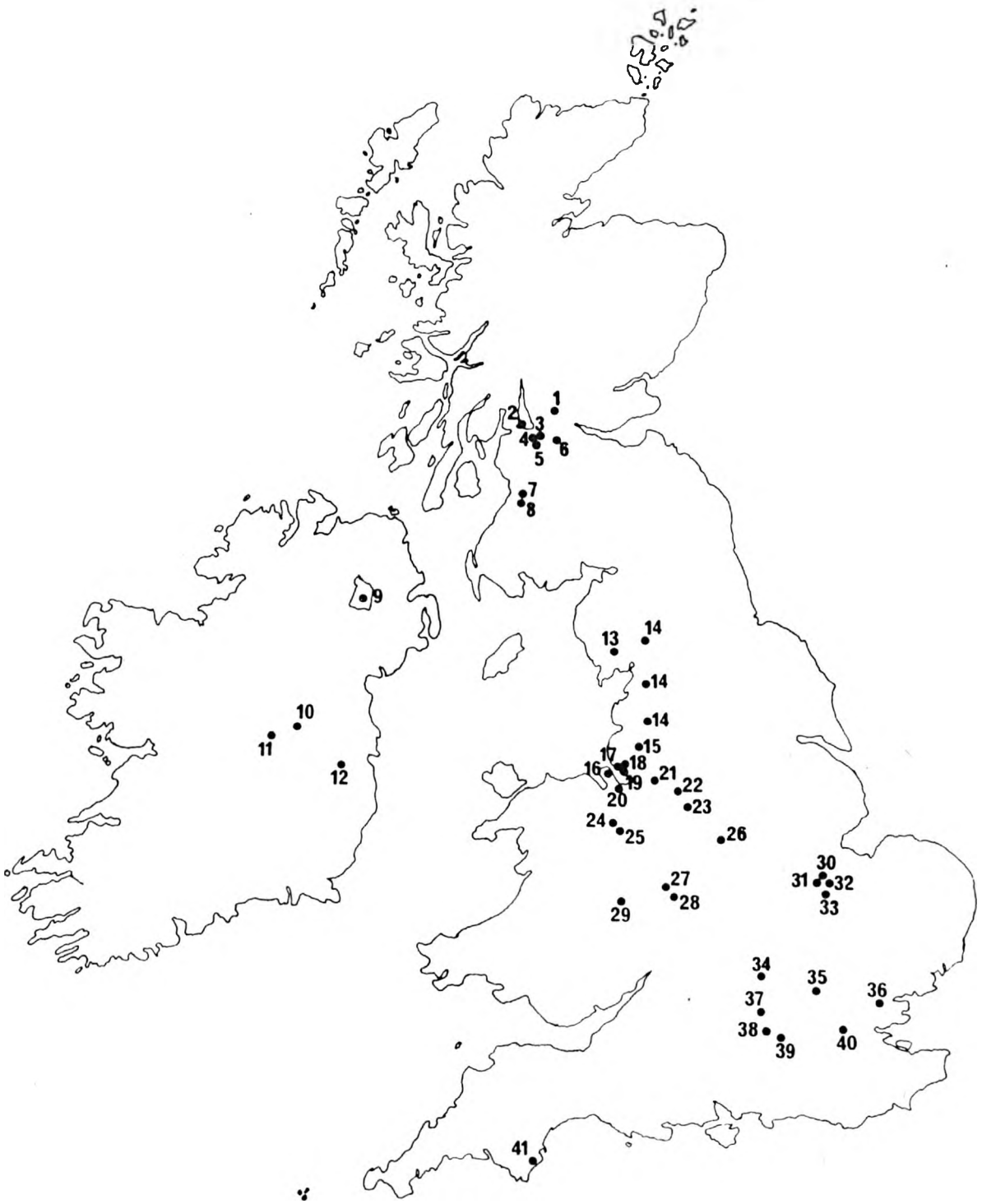


Table 1.1. Summary of records of adult *Acanthocephalus lucii* from the British Isles (see Figure 1.2)

Locality	Host species	Reference
Scotland		
1. Lake of Menteith, Central region	brown trout, rainbow trout, eels, pike	Campbell (pers. comm.)
2. Loch Lomond and Old Fruin, Strathclyde	pike, perch, powan, roach flounder	Copland (1956)
3. Tannoch Loch, Milngavie Glasgow	perch	Doughty (pers. comm.)
4. River Kelvin, Garscube estate, Glasgow	three-spined stickleback	pers. obs.
5. Forth and Clyde canal Temple, Glasgow	pike, perch, roach	Brattey (1979)
6. Johnstone Loch, Gartcosh, Glasgow	perch	Doughty (pers. comm.)
7. Glazert Burn, Dunlop Ayrshire	brown trout	Doughty (pers. comm.)
8. Beith, Ayrshire	brown trout	Ritchie (1915)
Northern Ireland and Eire		
9. Lough Neagh, N. Ireland	eel	Chubb (pers. comm.)
10. Lough Glore, C. Westmeath	perch	Healy (1954)
11. Lough Rea, Co. Westmeath	perch	Healy (1954)
12. Grand canal, Co. Durham	-	Kennedy (pers. comm.)

Table 1.1 (contd.)

Locality	Host species	Reference
England		
13. Lake Windermere Cumbria	perch	Rawson (1952) Bagenal (pers. comm.
14. Lancaster canal, Lancashire and Cumbria (from Preston-Kendal)	perch, pike	Cragg-Hine (pers. comm.)
15. Leeds-Liverpool canal, Lancashire (Halsall-Burscough area)	perch, roach	Cragg-Hine (pers. comm.)
16. Birkenhead Park Lake, Merseyside	perch	personal observation
17. Princes Park Lake Merseyside	perch	personal observation
18. Calderstones Park Lake, Merseyside	perch	personal observation
19. Sefton Park Lake, Merseyside	pike	personal observation
20. Shropshire Union Canal, Backford, Cheshire	perch, pike roach, eels	Mishra (1978)
21. Grey Mist Mere, Warrington, Cheshire	-	Linfield (pers. comm.)
22. Rostherne Mere, Cheshire	pike, perch, roach	Rizvi (1964)
23. Tatton Mere, Cheshire	perch	Goldspink (pers. comm.)
24. Ellesmere, Shropshire	perch	Okorie (1971)
25. Blakemere, Shropshire	perch	Cowley (pers. comm.)
26. Staunton Hall Pond, Melbourne, Derbys.	perch	Pocock (pers. comm.)

Table 1.1 (contd.)

27.	Hay Barns Pool, Enville Estate, Bridgenorth, Shropshire	perch	Pocock (pers. comm.)
28.	Lickey Hills Pool, Rubery, Birmingham	perch	Pocock (pers. comm.)
29.	River Lugg, Herefordshire	chub, dace, roach pike	Davies (1967)
30.	Deeping St. James Lincolnshire	perch	Gregory (pers. comm. via Chubb)
31.	Maxey New Cut, Cambs.	gudgeon	Gregory (pers. comm. via Chubb)
32.	Abandoned brick pit, Thorney, Cambs.	perch	Gregory (pers. comm. via Chubb)
33.	Moretons Leam, Whittlesay, Cambs.	perch	Gregory (pers. comm. via Chubb)
34.	Blenheim Lake, Oxfordshire	pike	Sweeting (pers. comm.)
35.	Lake at Denham, Bucks.	pike	Sweeting (pers. comm.)
36.	Ardleigh Reservoir, Essex	pike	Sweeting (pers. comm.)
37.	River Thames, Clifton, Hampden Reach, Oxfords.	pike	Sweeting (pers. comm.)
38.	River Kennet, Thatcham Berkshire	pike	Sweeting (pers. comm.)
39.	River Loddon, Stratfield Saye, Hampshire	pike	Sweeting (pers. comm.)
40.	Serpentine, Hyde Park, London	perch, ruffe	Lee (1980)
41.	Slapton Ley, Devon	eel	Canning et al. (1973)

The sampling site

The site chosen for this particular study was a short stretch of the Forth and Clyde canal at Temple, Glasgow (NGR NS 550 694). A preliminary study, undertaken while the author was an undergraduate, indicated that Acanthocephalus lucii was particularly abundant at this site (Brattey, 1979).

The Forth and Clyde canal extends from Bowling in the Clyde estuary in the west, to Grangemouth on the estuary of the Forth in the east and has been disused since 1963. Within the Glasgow area there are considerable differences in water quality between certain parts of the canal (Doughty, pers. comm.). Many stretches are organically polluted with an associated reduction in the diversity of the invertebrate fauna, although Asellus aquaticus, being pollution tolerant, appears to be particularly abundant in almost all parts. Extensive chemical and biological sampling of the canal has been undertaken by the Clyde River Purification Board and the results of their water chemistry analysis and preliminary biological survey for the Temple site are indicated in Tables 1.2 and 1.3, respectively. The results indicate that, at Temple, the water quality is extremely good and this part of the canal supports a rich and diverse fauna and flora. The canal water temperatures taken either at the time of sampling, or provided by the C.R.P.B., are indicated in Figure 1.3.

Table 1.2. Water chemistry analysis for the Forth and Clyde canal at Temple, Glasgow in 1979 (based on 12 monthly samples

	Mean	Minimum	Maximum
Suspended solids (mg/l)	8.25	2.00	25.00
B.O.D. (5-day, mg/l)	3.15	1.40	7.10
4-hr permanganate value (mg/l)	4.13	2.80	5.40
Dissolved oxygen (mg/l)	10.58	8.20	12.60
Dissolved oxygen (% air saturation value)	88.08	82.00	92.00
Ammonia nitrogen (mg N/l)	0.34	0.03	2.14
Total oxidized nitrogen (mg N/l)	0.89	0.01	1.75
Phosphate phosphorus (mg P/l)	0.09	0.01	0.17
Alkalinity (mg/l as $CaCO_3$)	116.92	99.00	146.00
Chloride (mg/l Cl^-)	59.50	32.00	90.00
Total hardness (mg/l as $CaCO_3$)	175.75	150.00	204.00
Nitrite nitrogen (mg N/l)	0.03	0.001	0.15
Nitrate nitrogen (mg N/l)	0.86	0.01	1.75
Conductivity (μS)	513.50	432.00	660.00
pH	7.79	7.40	8.70

Table 1.3. The fauna and flora of the Forth and Clyde canal at Temple, Glasgow (based on 2 samples taken in August 1979 and 1980)*

Flora

Macrophytes

Lemna minor L.
Potamogeton sp.
Callitriche intermedia L.
Alisma lanceolatum With.
Glyceria maxima (Hartm.) Holmberg

Rorippa nasturtium-aquaticum (L.)
Elodea sp.

Fauna

Macroinvertebrates

Tricladida: *Bdellocephala punctata* (Pallas)
Polycelis tenuis Ijima
Planaria torva (Müller)
Dendrocoelom lacteum (Müller)

Oligochaeta: *Stylaria lacustris* (L.)
Tubificidae

Hirudinea: *Glossiphonia complanata* (L.)
Helobdella stagnalis (L.)
Hemiclepsis marginata (Müller)
Erpobdella octoculata (L.)
Haemopis sanguisuga (L.)

Gastropoda: *Physa fontinalis* (L.)
Bithynia tentaculata (L.)

* from personal observations and data supplied by C.R.P.B.

Table 1.3 (contd.)

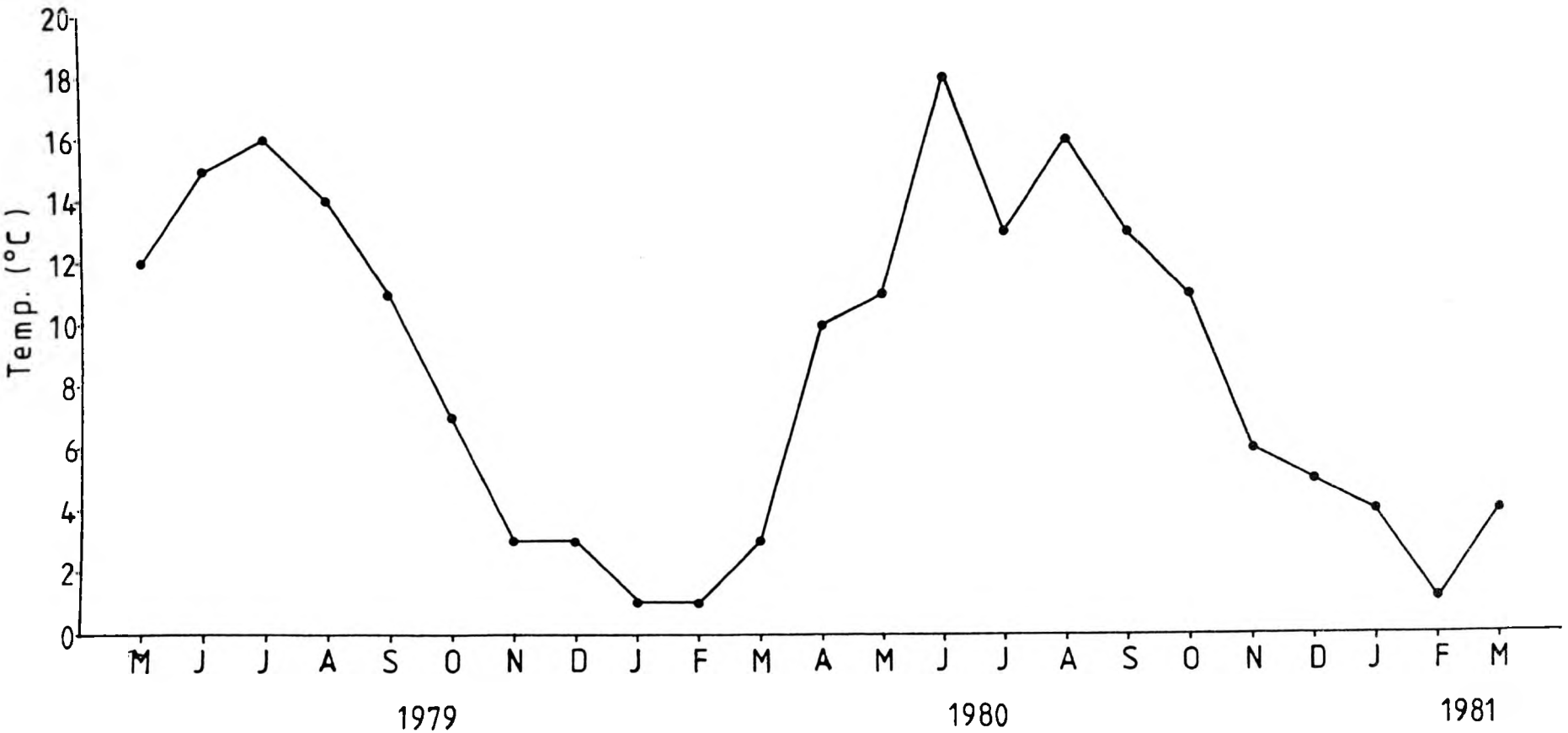
	Bithynia leachii (Sheppard)
	Valvata macrostoma Steenbuch
	Amnicola taylori (Smith)
	Planorbis carinatus (Müller)
	Limnaea pereger (Müller)
Bivalvia:	Pisidium sp.
	Sphaerium corneum (L.)
Crustacea:	Asellus aquaticus (L.)
Trichoptera:	Cyrnus flavidus McLachlan
	Halesus sp.
	Agraylea sp.
	Agapetus sp.
	Leptocerus sp.
Megaloptera:	Sialis lutaria (L.)
Hemiptera:	Sigara dorsalis Leach
	Sigara falleni (Fieb)
Coleoptera:	Haliplus sp.
	Dytiscidae
Odonata:	Enallagma cyathigirum Charpentier
	Ischneura elegans van der Linden
Chironomidae:	Tanypodinae
	Orthocladinae
	Chironominae
Microcrustacea	
Cladocera*:	Ceriodaphnia pulchella Sars
	Ceriodaphnia reticulata (Jurine)

*Based on 6 samples (August-November 1978)

Table 1.3 (contd.)

	Daphnia longispina Müller
	Scapholeberis mucronata (Müller)
	Simocephalus expinosus (Koch)
	Simocephalus vetulus (Müller)
	Acroperus harpae Baird
	Alona affinis Leydig
	Alona costata Sars
	Alona guttata Sars
	Alona weltneri Keilhack
	Camptocercus rectirostris Schodler
	Chydorus sphaericus Müller
	Eurycercus lamellatus (Müller)
	Graptoleberis testudinaria (Fischer)
	Peracantha truncata (Müller)
	Pleuroxus laevis Sars
	Pleuroxus trigonellus Müller
	Polyphemus pediculus (L.)
Copepoda:	Cyclops agilis (Koch, Sars)
	Cyclops albidus (Jurine)
	Cyclops viridus (Jurine)
Vertebrates	
Teleostei	
	Carassius auratus (L.)
	Esox luscius L.
	Perca fluviatilus L.
	Rutilus rutilus (L.)
	Gasterosteus aculeatus (L.)
Amphibia	
	Bufo bufo (L.)

Figure 1.3. Seasonal changes in the water temperature of the Forth and Clyde canal at Temple, Glasgow (taken at the time of sampling or provided by C.R.P.B.).



CHAPTER 2

ASPECTS OF THE BIOLOGY OF PERCH

Chapter 2

Introduction

Perch (Perca fluviatilus L.) are undoubtedly one of the most common and widespread fishes of the Palaearctic region. Consequently they have become the subject of considerable scientific investigation and a wealth of information on various aspects of the biology of this species is available in the literature. Previous studies include those of Allen (1935), Smyly (1952), Healy (1954), Banks (1968), McCormack (1970), Ali (1973), Craig (1974, 1978) and Thorpe (1977a) on feeding habits. Le Cren (1947, 1955, 1958), Le Cren et al. (1977). Goldspink and Goodwin (1979) have examined growth and population structure and Le Cren (1951), Treasurer and Holliday (1981) and Treasurer (1981) have studied seasonal changes in the development of the gonads of perch. Data on the biology of perch has recently been reviewed and summarized by Thorpe (1977b, 1977c).

Any study of the ecology of a parasitic animal requires detailed knowledge of the biology of the host species. However, the purpose of this chapter will not be to provide a comprehensive account of the biology of perch since this is already available in the literature. Only a selection of particularly relevant aspects concerning the biology of the perch population in the Forth and Clyde canal will be dealt with here. The relevance of each of the following sections, from a parasitological point of view, will be illustrated fully in Chapters 4 and 6, where previous relevant parasitological

work on perch will be discussed.

Materials and methods

Field methods

The nature of the habitat chosen for study imposed certain restrictions in the sampling methods available. The steep sides of the canal bank and numerous underwater obstructions made seine netting virtually impossible. The distance from Liverpool to the sampling site ruled out the possibility of using Windermere traps since these have to be examined frequently and only one sampling trip could be made each month. Only two methods proved reasonably successful at catching perch. These were the use of gill nets and a beam trawl.

Gill nets are highly selective for fish of certain sizes and this subject has been discussed in some detail by both Banks (1968) and Haram(1968). Initially a whole range of sizes of gill net were used (9, 19, 22, 24, 27, 32 and 40mm mesh, knot to knot). However, only the 19 and 22mm nets caught perch. The nets of larger mesh size caught roach (Rutilus rutilus L.) and pike (Esox luscius L.) but never perch, so their use was discontinued. A variable number of 19 and 22mm nets, each approximately 30 metres long were set diagonally across the canal in the evening and removed the next morning. Nets left throughout the day were regularly stolen or damaged.

A small beam trawl (approximately 2m beam) proved particularly

useful during the winter months when gill net catches were low. The trawl was pulled manually across the canal in diagonal fashion for a distance of about 40m. This was repeated a number of times. The trawl is fairly heavily weighted and tended to fish very deep, readily digging into the substrate if pulled too slowly.

The fish were removed from the nets, killed and placed in polythene bags labelled with the date, site and method of capture. All fish were transported back to Liverpool in an insulated cooling box and then deep frozen.

Laboratory methods

After thawing the fork length (mm) and weight (gms) of each fish was recorded and a single apercular bone removed, placed in boiling water, cleaned and sealed in an envelope for later age determination. Each fish was opened by a mid ventral incision extending from the anus anteriorly to between the gills. The cut was then extended dorsally to the lateral line, posteriorly along the lateral line and ventrally back down to the anus. Thus, virtually the whole of one side of the body musculature was removed, taking care not to damage the underlying viscera which were now fully exposed. The sex of the fish was noted and the stage of maturity of the gonads assessed and assigned to one of 4 categories based on a modification of the scale devised by Nikolsky (1963) (see Appendix I). The entire digestive tract was then removed, by cutting at the anus and just anterior to the stomach. This was placed in saline and the attached fat and viscera removed. The intestine was straightened out, the

stomach removed and placed in a labelled dish containing saline. The fullness of the stomach was estimated visually on a points system as used by Craig (1978) where 0 points = empty stomach, 1 point = some food in the pyloric region, 2 = some food in pyloric and cardiac region, 3 points = pyloric region full and some food in the cardiac, 4 points = full stomach. All food items in the stomach were identified (not necessarily to species). Special note was made of the presence of the isopod Asellus aquaticus in perch stomachs. The number of isopods present was noted and each isopod carefully dissected to see if it harboured any cystacanths of A. lucii. The remaining five sections of the gut were carefully examined with a binocular microscope for specimens of adult A. lucii.

Results and Discussion

Sample sizes

A total of 525 perch were caught and examined from 18 samples collected over a period of 22 months (May 1979 until March 1981). The numbers of males, females and fish of unknown sex in each sample are given in Table 2.1. Although the numbers of male and female fish often differed considerably in individual samples, overall approximately equal numbers of males and females were caught. The fish of unknown sex generally consisted of small immature individuals, although the occasional large fish with apparently no gonadal tissue was found. Only a limited number of samples were collected in 1979 and the early part of 1980. Considerable difficulty was experienced

Table 2.1. The number and sex of perch in each sample from the Forth and Clyde canal, Scotland

Date of sample	Males	Females	Unsexed*	Total examined
May 1979	10	12	1	23
July	8	15	1	24
September	14	10	-	24
October	12	14	1	27
Jan-March 1980	8	6	-	14
April	6	13	-	19
May	7	12	1	20
June	13	15	1	29
July	16	22	-	38
August	18	23	-	41
September	13	28	-	41
October	18	12	-	30
November	20	13	-	33
December	29	26	-	55
January 1981	36	23	-	59
February	14	15	-	29
March	8	11	-	19
Totals	250	270	5	525

* sex not distinguishable to naked eye

in collecting samples in the period January - March 1980 owing to ice on the canal. The data for this period, when a total of only 14 fish were caught, have been pooled. From April 1980 until March 1981 samples were collected monthly. Gill nets caught very few fish in winter and so samples were supplemented with fish caught with the beam trawl. No size difference was apparent in fish caught by either sampling method so the samples were pooled.

Size distribution of the perch samples

An analysis of the distribution of sizes of perch in each sample is given in Figure 2.1, where lengths are assigned to 1cm length groups. It can be seen that the majority of fish caught were within a fairly restricted size range. Most were within the range 10 - 15cms, which is not surprising in view of the highly size selective sampling methods used. Very few larger perch were caught in spite of considerable effort at the beginning of the study, which suggests that the larger size groups are poorly represented in the canal.

Age distribution of the perch samples

Each perch was aged by an examination of a single opercular bone using both reflected light on a dark background and transmitted light with polarisors as described by Le Cren (1947). The 'birthday' of perch was taken as the 1st June. The majority of operculars were fairly easy to read and the length frequency distributions

of each age group are given in Table 2.2, where fish are shown in 1cm length groups. Only five age groups were found but more than 90% of all perch caught were either two or three years old. The perch ranged in size from 7.0 to 24.0cms. The wide size range represented in each age class shows that individual fish varied quite considerably in their growth rate (e.g. 3 yr old fish varied in length from 9.0 to 18.0cm). However part of the wide variation can be explained by the fact that Table 2.2 represents data pooled for a 2 year sampling period and therefore each age group may contain fish from two different year classes. Furthermore, fish which were caught shortly after their 'birthday' are pooled with fish caught shortly before their next 'birthday', therefore there may be 11 months difference in age and hence growth, between individuals assigned to the same age group.

Table 2.3 illustrates the age distribution of the individual perch samples. From July 1979 until May 1980 most of the fishes caught were 2 or 3 years old. These would represent fishes from the 1977 and 1976 year classes, respectively. By June 1980 these perch would be 3 and 4 years old, respectively, and yet 4 year old fish (1976 year class) were very poorly represented in the samples from then onwards. This suggests that they had either disappeared, perhaps owing to a post-spawning mortality or, alternatively, had grown too large to be caught by the highly size selective sampling methods. By June 1980 the 1977 year class would be 3 yrs old and this year class was very well represented in the samples from June onwards. It is also interesting to note that very few 2 year old

Figure 2.1. Size frequency distribution histograms for the perch samples from the Forth and Clyde canal, Scotland.

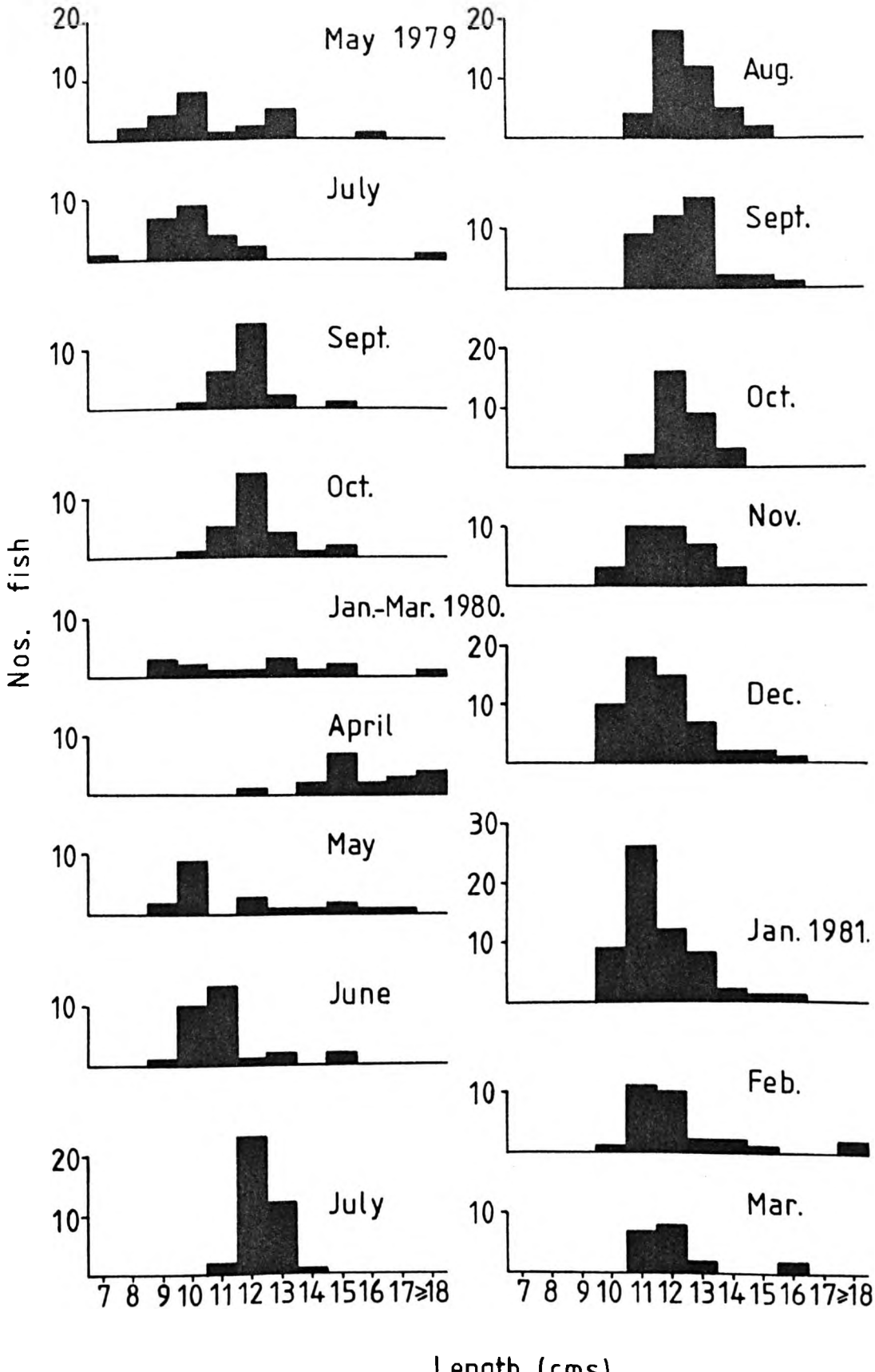


Table 2.2. Length frequency distribution in relation to age for all perch (Perca fluviatilus) caught in the Forth and Clyde canal, Scotland, during the current study

Length group (cms)	Age 1+	2+	3+	4+	5+	Total number
7.0-7.9	1	-	-	-	-	1
8.0-8.9	2	-	-	-	-	2
9.0-9.9	5	11	1	-	-	17
10.0-10.9	9	29	24	-	-	62
11.0-11.9	-	11	108	-	-	119
12.0-12.9	-	24	139	-	-	163
13.0-13.9	-	7	81	3	-	91
14.0-14.9	-	2	22	2	-	26
15.0-15.9	-	6	15	2	-	23
16.0-16.9	-	2	4	1	-	7
17.0-17.9	-	-	3	1	-	4
18.0-18.9	-	-	3	3	-	6
19.0-19.9	-	-	-	2	-	2
20.0-20.9	-	-	-	-	-	-
21.0-21.9	-	-	-	-	-	-
22.0-22.9	-	-	-	-	-	-
23.0-23.9	-	-	-	-	-	-
24.0-24.9	-	-	-	-	2	2
Totals	17	92	400	14	2	525

Table 2.3. Age distribution of the perch samples from the Forth and Clyde canal, Scotland

Date of sample	Age 1+	2+	3+	4+	5+
May 1979	14	8	1	-	-
July	1	21	1	-	1
September	-	6	18	-	-
October	-	15	12	-	-
Jan-Mar 1980	2	6	5	1	-
April	-	10	7	1	1
May	-	15	4	1	-
June	-	-	27	2	-
July	-	-	38	-	-
August	-	-	40	1	-
September	-	-	40	1	-
October	-	-	30	-	-
November	-	1	31	1	-
December	-	5	48	2	-
January 1981	-	3	54	2	-
February	-	2	25	2	-
March	-	-	19	-	-
Totals	17	92	400	14	2

fish were caught from June 1980 onwards which suggests that the 1978 year class was either a very poor one or, alternatively, a very slow growing one which had not reached a size which rendered them liable to capture by the methods used. It should be noted that Table 2.2 cannot really be used to estimate growth rates or year class strengths of the perch population since the sampling methods were too selective, although as discussed above the data in Table 2.3 suggests that the 1976 and 1977 year classes were both fairly strong.

Feeding habits

Previous literature concerning the feeding habits of perch is extensive. They are ideally suited for such studies since they possess a 'true' stomach and swallow food items whole. A variety of methods have been used to analyse the stomach contents of fishes and these methods have been reviewed by Hynes (1950) who concluded that for fish with a generalized diet, provided a large number of fishes were examined, all methods gave substantially the same result. However, as Thorpe (1977a) has shown, a number of important points have not been taken into account in many previous studies. Firstly almost all studies have been based on static samples, i.e. samples taken irrespective of the time of day and so factors such as diurnal feeding rhythms have not been taken into account. Secondly, sampling methods such as gill netting, or the use of traps tend to underestimate feeding intensity since the stomach of the fish begins to empty soon after capture and

continues to do so until the nets or traps are removed, which may be some hours after the fish has initially been captured. Thirdly, temperature has considerable effects on the rate of gastric evacuation in fishes. From personal observations of fish kept in the laboratory at various temperatures, it was found for perch kept at 19°C, a full stomach will be completely empty in less than 24 hrs. However for fish kept at 5°C there will still be food in the stomach after 48 hrs. Consequently results from the field study concerning the feeding intensity of the perch population may tend to over-estimate results in winter and underestimate them in summer.

For the purposes of the current study three important questions concerning the feeding habits of perch in the canal need to be answered. Firstly, does the overall feeding intensity of perch change with season? Secondly, does the rate of ingestion of Asellus aquaticus change with season? Thirdly, how does the importance of A. aquaticus in the diet of perch change with fish size?

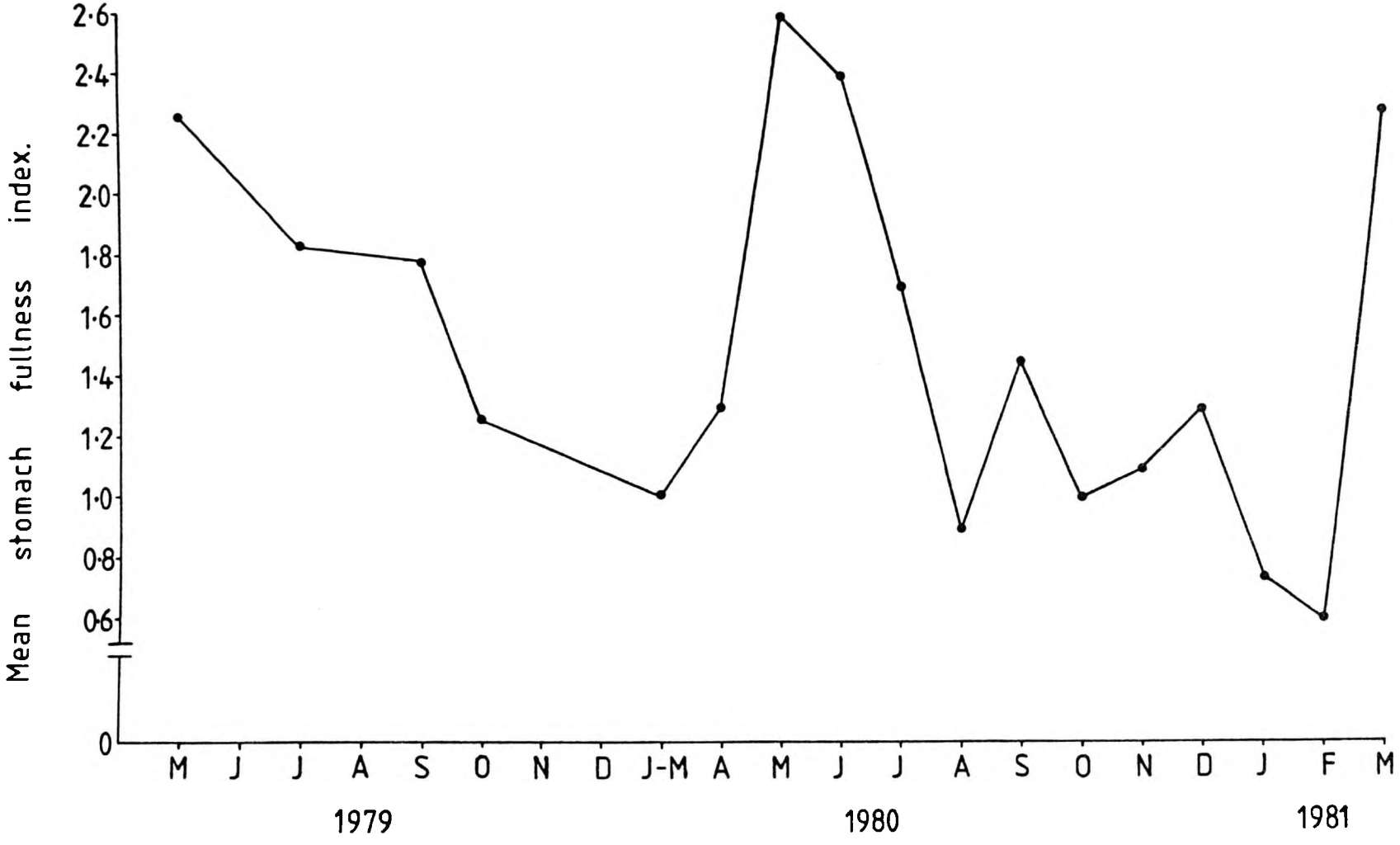
Seasonal changes in perch feeding intensity

Seasonal changes in the feeding intensity of perch have been examined in a number of localities. Brofeldt (1922) noted that the quantity of food present in perch stomachs in the Müggelsee (Germany) was less in winter than in summer. Allen (1935) found that perch in Windermere had empty stomachs at all times of the year, but more frequently in winter. Healy (1954) noticed a gradual decline in feeding intensity in autumn and winter in perch

in some Irish lakes. Rizvi (1964) observed that perch fed throughout the year in Rostherne Mere, although he examined very few fish during the winter months. Banks (1968) also working at Rostherne Mere, noticed a gradual decline in feeding intensity of perch during autumn and winter. Cragg-Hine (1965) did not find any seasonal trends in the feeding of perch in Willow Brook, although again he examined very few fish during the winter months. At Llyn Tegid in Wales, Chubb (1961), Ali (1973) and Andrews (1977) all observed a distinct fall in feeding intensity of perch during autumn and winter. Hartmann (1974) found an increase in the percentage of empty stomachs in perch in the Bodensee (Germany) in winter. Craig (1978) observed that perch in Windermere eat the bulk of their food in summer. Skorping (1980b) observed that perch in Lake Lille Åklungen (Norway) fed throughout the year but with a peak in May.

Seasonal changes in the mean stomach fullness index of perch from the Forth and Clyde canal are illustrated in Figure 2.2. Clearly perch fed throughout the year. The highest stomach fullness index was recorded in May in both 1979 and 1980 and the lowest in January-March 1980 and February in 1981. Although fairly wide fluctuations are apparent between adjacent months the overall pattern suggests that perch stomachs contained most food in late spring and summer and least in mid-winter, which, broadly speaking, agrees with the findings of most previous workers.

Figure 2.2. Seasonal changes in the mean stomach fullness index of perch from the Forth and Clyde canal, Scotland.



Seasonal changes in the occurrence of Asellus aquaticus in the diet of perch

Obviously Asellus aquaticus must constitute a significant part of the diet of perch to have provided sufficient evolutionary pressure for the host-parasite relationship to develop, but with respect to the seasonal dynamics of the parasite it is crucial to establish how the rate of ingestion of Asellus aquaticus by perch changes throughout the year.

The occurrence of members of the genus Asellus in the diet of perch has been examined in many habitats. Note that no separation is made here between Asellus aquaticus and Asellus meridianus Racovitza. Both isopods have very similar habits, although the latter species does not act as an intermediate host for A. lucii. At Llyn Tegid, Chubb (1964), Ali (1973) and Andrews (1977) all stressed the importance of A. meridianus in the diet of perch. Asellus meridianus were eaten throughout the year but with a predominant peak, in terms of percentage occurrence, in spring. Healy (1954) noted that chironomids, Asellus aquaticus and Gammarus sp. constituted the bulk of the diet in winter in Lough Glore (Ireland). Rizvi (1964) working at Rostherne Mere, found Asellus aquaticus in perch stomachs in all months except August and October. The percentage occurrence was low (1 - 4%), but from January to May and in November and December A. aquaticus was the dominant food item in terms of percentage volume. It should be noted that Rizvi's sample sizes were small in the winter

period. Banks (1968), also working at Rostherne, found A. aquaticus in perch stomachs in all months except October, with percentage occurrence being lowest during the late summer months. Goldspink and Goodwin (1979) examined the stomachs of perch from Rostherne Mere in 1973 and 1974 and from Tatton Mere in 1975 and 1978. In each instance they found A. aquaticus in perch stomachs in late spring and summer but not in winter or early spring, a result which differs quite markedly from the earlier studies of Rizvi (1964) and Banks (1968) at Rostherne. McCormack (1970) examined perch from Windermere from April until September and found Asellus sp. in all months, although the percentage occurrence declined somewhat in mid-summer. Thorpe (1977a) showed that perch in Loch Leven (Scotland) ate A. aquaticus throughout the summer, from June to September. Craig (1978), also working at Windermere, found A. aquaticus in perch stomachs throughout the year but with a very pronounced spring peak in percentage occurrence. Skorping (1980b) found A. aquaticus in the stomachs of perch in Lake Lille Åklungen (Norway) in all months of the year except August. In terms of percentage occurrence apparently many perch stomachs contained A. aquaticus during the winter months, but only a few in mid-summer.

From an examination of the previous literature it is clear that there is definitely no common seasonal pattern in the ingestion of Asellus aquaticus or A. meridianus by perch in the various habitats examined and in fact the pattern may even vary quite markedly in the same habitat in different years (e.g. in Rostherne Mere, the results

of Rizvi (1964) and Banks (1968) differ quite markedly from those of Goldspink and Goodwin (1979)). In some habitats Asellus sp. were eaten by many perch in winter and spring, but by very few in summer (Ali 1973, Andrews 1977, Craig 1974, 1978, Skorping 1980b). In others Asellus sp. were eaten by many fish in summer (McCormack, 1970; Thorpe, 1977a) and by few or none in winter and early spring (Goldspink and Goodwin, 1979).

Table 2.4 shows the seasonal changes in the percentage occurrence of various food items in the stomachs of perch from the Forth and Clyde canal. Clearly perch ate a wide variety of food organisms but in terms of percentage occurrence five groups were dominant. These were: chironomid larvae and pupae, planktonic microcrustacea, Sialis lutaria larvae, Trichoptera larvae and pupae and Asellus aquaticus. Seasonal changes in the percentage occurrence of A. aquaticus are illustrated separately in Figure 2.3. It appears that perch fed on A. aquaticus throughout the entire sampling period. Their absence only in September 1979 and April 1980 might easily be attributed to a sampling deficiency. The data for 1979 were too limited to suggest any seasonal trends in A. aquaticus ingestion during this period,but from April 1980 onwards,when samples were larger and more frequent,it appears that more perch were feeding on A. aquaticus in summer than in winter. A gradual decline from mid summer to winter was apparent. Furthermore, as was pointed out in the introduction to perch feeding habits, the effect of temperature on the

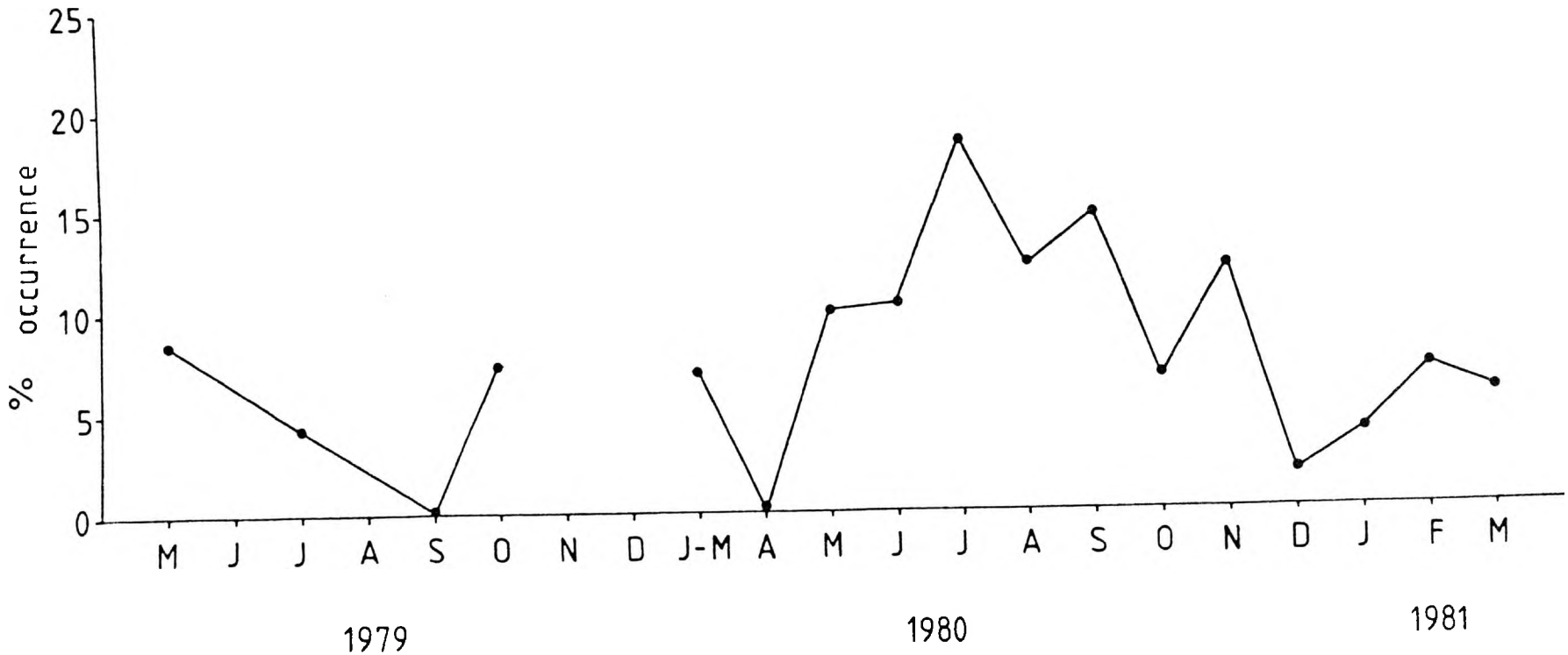
Table 2.4. Seasonal changes in the percentage occurrence of various food items in the stomachs of perch from the Forth and Clyde canal, Scotland

Food item	Date of sample: 1979 May	1979 July	1979 Sept	1979 Oct	1979 Jan-Mar	1980 April	1980 May	1980 June	1980 July	1980 Aug	1980 Sept	1980 Oct	1980 Nov	1980 Dec	1981 Jan	1981 Feb	1981 Mar	Total % occurrence
Oligochaeta	-	8.3	4.2	3.7	-	-	5	-	-	-	4.9	-	-	1.8	-	-	5.3	1.7
Argulus sp.	-	4.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2
Asellus aquaticus	8.7	4.2	-	7.4	7.1	-	15	10.3	18.4	9.8	14.6	6.6	12.1	1.8	3.4	6.9	5.3	7.8
Microcrustacea	30.4	50	50	33.5	7.1	-	35	17.2	28.9	9.5	31.7	3.3	18.2	16.4	1.7	-	15.8	20
Hirudinea	4.3	-	-	-	-	-	-	-	-	-	4.9	-	-	3.6	-	-	-	0.95
Chironomidae*	69.6	79.2	41.7	-	7.1	15.8	40	51.7	63.2	22	34.1	3.3	18.2	16.4	-	-	-	25.7
Coleoptera	13	4.2	4.2	-	-	20	5.3	5	10.3	-	2.4	4.9	3.3	-	-	-	-	2.9
Diptera	13	-	-	3.7	7.1	5.3	20	20.7	2.6	-	2.4	3.3	-	1.8	-	3.4	-	3.6
Ephemeroptera+	4.3	-	-	-	-	-	5	3.4	-	-	-	-	-	-	-	-	-	0.6
Lepidoptera	-	-	-	-	-	-	-	-	-	-	-	-	-	1.8	-	-	-	0.2
Odonata+	13	-	-	-	-	-	-	3.4	2.6	-	-	-	-	-	1.7	-	-	1.1
Sialis lutaria	-	25	8.3	18.5	28.6	26.3	5	17.2	7.9	4.9	14.6	10	12.1	18.2	10.2	13.8	47.4	14.3
Trichoptera*	26.1	12.5	16.7	7.4	7.1	31.6	65	51.7	34.2	7.3	9.8	16.7	3.0	1.8	8.5	6.9	15.8	16.6
Mollusca	8.7	-	-	3.7	-	-	10	3.4	-	-	4.9	3.3	-	1.8	-	-	-	1.9
Platyhelminths	-	-	-	3.7	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2
Fishes	-	-	16.7	3.7	-	-	-	-	-	-	-	-	-	-	-	-	-	1.1
Plant material	4.3	4.2	-	7.4	14.3	-	-	-	5.3	4.9	12.2	-	-	5.4	1.7	-	-	3.6
Detritus	-	-	-	-	-	-	-	-	-	-	2.4	-	-	3.6	3.4	-	-	1.1

* larvae and pupae

\+ nymphs only

Figure 2.3. Seasonal changes in the percentage occurrence of Asellus aquaticus in the stomach of perch.



rate of gastric evacuation will tend to minimize by underestimation, this difference between winter and summer.

It is important to note that the term percentage occurrence only gives an indication of the proportion of the perch population which are feeding on Asellus aquaticus; it gives no indication of the actual numbers of isopods ingested. So, in effect, all that one can reasonably conclude from Figure 2.3 is that a higher proportion of perch ate A. aquaticus in summer than in winter. Unfortunately, the number of perch stomachs containing A. aquaticus was too few to accurately assess the seasonal changes in actual numbers of these isopods eaten (although one might predict that this would follow a similar pattern to that of percentage occurrence). Consequently the second question which was asked at the beginning of the section on perch feeding habits (how does the rate of ingestion of A. aquaticus by perch vary with season?) cannot be answered fully from the data available. This is unfortunate, since had it been possible, an accurate assessment of the seasonal changes in the rate of recruitment of larval parasites into the fish population might have been possible. This will be discussed further in Chapter 4.

Changes in diet with size of perch

Many authors have reported a change in diet as perch get larger. Alm (1922) found that small perch in Scandinavian waters ate planktonic Crustacea, medium sized perch ate insect larvae and the largest perch ate fish. Allen (1935) found that Windermere perch <16.5cm in length

ate planktonic organisms, those from 11.5cm to 19cms ate benthos and those >16.5cms ate fish. Roper (1936) noted that small perch in waters in northern Germany ate zooplankton, those up to 15cms ate insect larvae and older perch fed on larger animals and fish. Swynnerton and Worthington (1940) working on perch from Haweswater, found results very similar to those of Allen (1935), as did Hartley (1947), who examined perch from East Anglian waters. Antosiak (1963) examined perch from Polish lakes and noted that fishes >13cms in length became predatory. At this size invertebrates were still eaten but accounted for a lower proportion of the food as the fish got older. Banks (1968) found that all sizes of perch which he examined ate plankton, benthos and fish, but the importance of fish increased as the perch got larger. McCormack (1970) found that perch of all sizes in Windermere ate plankton and benthos, but the number of perch eating A. aquaticus increased with fish length. She noted that the divisions between different groups were not nearly so clear as those found by Allen (1935). Ali (1973), working at Llyn Tegid, found that for perch from 2 - 9cms in length, zooplankton was by far the most important food item. Perch from 12 to 17.9cms ate zooplankton, Asellus meridianus and chironomids and those from 18 to 32cms fed on fish, A. meridianus, insect nymphs and dipteran larvae and pupae. The importance of fish in the diet increased as the perch got larger. Craig (1974) working at Slapton Ley, noticed that perch from 3 to 8.9cms ate plankton and chironomids, those from 9.0 to 13.9cms took plankton, Asellus sp, chironomids and insect nymphs, as did perch

>14cms in length, although fishes were also found in the stomachs of perch of the largest size groups.

In summary it appears that perch diet does indeed vary with size. Broadly speaking, small perch are almost exclusively plankton feeders, occasionally also eating chironomids. As the perch increase in size benthic organisms become increasingly important, with the largest perch also feeding on fishes. The actual sizes at which perch change their feeding habits to each of these food groups varies with habitat and the change appears to be a gradual one with considerable overlap between each group. Antosiak (1963), Banks (1968), Ali (1973) and Thorpe (1977a) all concluded that although perch diet did vary with fish size the seasonal availability of food organisms had a more pronounced effect on the diet. Perch apparently ate any available prey of a size smaller than the gape of their jaws.

With respect to the current study it is important to establish what sizes of perch eat Asellus aquaticus. From a consideration of the data available in the literature it appears that, broadly speaking, all but the smallest size groups of perch eat Asellus species. The relative importance of these isopods in the diet of the remaining size groups is, however, extremely difficult to determine from the published data available. Even if one assumed that the relative importance of Asellus in the diet remained the same as the fish got bigger, larger fish eat greater total amounts of food and so would probably eat greater total numbers of Asellus. This would have obvious effects on the level of infection in fish of different sizes.

Consequently, it is essential to ensure that the complete size range of perch are represented equally in each sample, or alternatively, a restricted but consistent size range is represented. The analysis of the length frequency distribution of the samples (Figure 2.1) indicates that the latter alternative was necessary in the current study.

Maturation and spawning of perch

The size at which perch attains sexual maturity appears to vary markedly with habitat. Thorpe (1977b) summarized results from previous studies in 11 different habitats and found considerable variation in the results, although male fish consistently reached sexual maturity at a smaller size than females. The actual sizes at which maturity was reached ranged from 5.7cms for males and 8.7cms for females in localities in East Anglia (Hartley 1947), to 16.0cms for males and 18 - 20cms for females in the Gr. Ploner See (Laskar, 1943 in Thorpe, 1977b).

From Figure 2.1 it can be seen that most perch examined in the current study were >10cms in length. By the time they had reached this size the majority were already sexually mature.

An analysis of the relationship between length and age (Table 2.2) showed that nearly all fish greater than 10cms in length were 2 or 3 years old and were sexually mature, whereas the four fish which were 10cms long or less and only 1 year old were usually

immature. Thus it appears that most perch in the Forth and Clyde canal first spawned on their second 'birthday'.

Perch spawn in spring during a period of accelerating temperature increase, which is usually in April or May in Britain. Many workers have studied seasonal changes in the development of the gonads of perch and in each instance a very similar pattern was found. Le Cren (1951) examined perch from Windermere and expressed gonad weight as a percentage of body weight. He found that in male fishes, after spawning, the gonads remained at a low resting level throughout the summer until August. Thereafter the gonads grew very rapidly and by October had reached maximum size (8% of body weight), which was maintained through autumn and winter until spawning the following spring. In females the single (left) ovary remained at a low resting level through summer and, as in males, began to develop in August. In females the growth was more gradual and proceeded through autumn, winter and spring until spawning the following year. Essentially similar patterns have subsequently been described by Craig (1974) for perch in Slapton Ley and recently by Treasurer and Holliday (1981) for perch in Lochs Kinord and Davan (Scotland).

The seasonal changes in the development of the gonads of perch are undoubtedly brought about by seasonally variable environmental factors such as water temperature and photoperiod stimulating hormonal changes in the perch. Swift and Pickford (1965) examined seasonal changes in the hormone content of the pituitary of perch. They found

Table 2.5. Seasonal changes in the development of the gonads of male perch from the Forth and Clyde canal, Scotland

Date of sample	Percentage at each stage*			
	I	II	III	IV
May 1979	20	-	-	80
July	37.5	62.5	-	-
September	-	-	57.1	42.9
October	-	-	-	100
Jan.-March 1980	-	-	-	100
April	-	16.7	-	83.3
May	-	100	-	-
June	-	92.3	-	7.7
July	-	100	-	-
August	-	5.5	89	5.5
September	-	-	61.5	38.5
October	-	-	16.6	83.4
November	-	-	-	100
December	6.9	-	6.9	86.2
January 1981	5.5	-	-	94.5
February	-	-	-	100
March	12.5	-	-	87.5

* stages modified after Nikolsky (1963), see Appendix I

Table 2.6. Seasonal changes in the development of the gonads of female perch from the Forth and Clyde canal, Scotland

Date of sample	Percentage at each stage*			
	I	II	III	IV
May 1979	-	-	-	100
July	20	80	-	-
September	-	30	70	-
October	-	57.1	35.7	7.2
Jan.-March 1980	-	33.4	16.6	50
April	-	7.7	-	92.3
May	-	91.7	-	8.3
June	-	100	-	-
July	-	100	-	-
August	-	34.8	65.2	-
September	-	14.3	85.7	-
October	8.3	-	91.7	-
November	-	7.7	92.3	-
December	11.5	7.7	80.8	-
January 1981	4.3	-	-	95.7
February	20	-	6.7	73.3
March	-	-	-	100

* stages modified after Nikolsky (1963), see Appendix I

that the pituitary contained the greatest amount of growth hormone, gonadotrophins, thyrotrophin and corticotrophins during spring and early summer. The hormones were almost depleted by July, exhausted in August and then returned to a resting winter level. Over the winter there was a gradual increase in the gonadotrophic content of the pituitary which coincided with the maturation of the gonads in the following spring.

The seasonal changes in the development of the gonads of male and female Forth and Clyde canal perch are given in Tables 2.5 and 2.6, respectively. The 4 developmental stages recognized are described in Appendix I. Essentially the same pattern described by Le Cren (1951) is evident, with a resting phase (Stage 2) in both males and females in summer and development (Stage 3) occurring from August onwards. Development was rapid in the gonads of male fish, with fully developed (Stage 4) testes in all males by November, but more gradual in female fish with a fully developed ovary in females by January. Spawning occurred in April or May in 1980.

The significance of the aforementioned details, with respect to the seasonal dynamics of Acanthocephalus lucii, will be discussed fully in Chapters 4 and 6.

CHAPTER 3

FIELD STUDIES ON LARVAL *ACANTHOCEPHALUS LUCII* IN THE ISOPOD *ASELLUS AQUATICUS*

Chapter 3

Introduction

'The numerical size of an animal population can be influenced by four kinds of population processes, namely birth, death, immigration and emigration. Adult populations of helminth parasites in their definitive host may be more simply controlled by the two processes immigration and death, since no births occur within the final or definitive host which directly increase the adult population of parasites and since also, by definition, no emigration of adult parasites from the host can occur without resulting in death' (Anderson, 1974a). Populations of larval parasites in the intermediate host are controlled in a similar way. The isopod Asellus aquaticus becomes infected with larval Acanthocephalus lucii by ingesting a shelled acanthor while feeding. The size of the larval parasite population within an isopod can be influenced by these same two processes, recruitment (= immigration) and mortality (death). Further recruitment can take place when an isopod ingests more shelled acanthors. Mortality can occur when the larval parasites are killed by the host (or by other parasites in the same host), or when the host dies. Emigration is not possible since the haemocoel of the isopod is effectively a closed environment and in any event if emigration were possible this would, as with adult parasites, by definition, result in death. Similarly

natality cannot take place since larval parasites do not reproduce.

Seasonal changes in the size of a population of free-living animals are often measured in terms of changes in the number of individuals per unit area. With helminth parasites seasonal changes in parasite population size are typically described in terms of changes in the percentage of host individuals infected (incidence or prevalence) and the number of parasites per host (intensity). However, these terms can be misleading and should only be used as such if the size of the host population remains constant throughout the year (which seldom occurs), or if a simultaneous assessment is made of seasonal changes in the size of the host population. Consider a hypothetical host population in which incidence is 50%, intensity is 10 parasites per host and incidence and intensity are similar in both summer and winter. If the host population consists of 10,000 individuals in summer but only 1000 in winter then the size of the parasite population will have changed quite dramatically, although incidence and intensity have remained constant.

If one considers the life cycle of Acanthocephalus lucii essentially three populations exist; a population of shelled acanthors free in the environment, a population of larval stages in the intermediate host (Asellus aquaticus) and a population of adult worms in the definitie host(s) (perch and other species of fish). The size of the population of shelled

acanthors could, theoretically at least, be measured and expressed in terms of numbers per metre^2 etc. The size of the population of larval stages would typically be expressed in terms of incidence and intensity of infection of the Asellus aquaticus population. For the reasons discussed previously seasonal changes in the size of the larval parasite population will also require a simultaneous detailed study of the dynamics of the intermediate host population. This should comprise an integral part of any study concerned with parasite population dynamics, but more especially when the host population exhibits large seasonal fluctuations in numbers as in many invertebrate intermediate host populations.

The abundance and widespread distribution of Asellus aquaticus is perhaps best manifest in the vast number of previous publications which deal with various aspects of the biology of this species. An appreciation of the information available can be obtained from the works of Williams (1960, 1979) and Holland (1976) on distribution, Williams (1962, 1963) on ecology, Prus (1971) and Adcock (1979) on energetics, Marcus and Willoughby (1978), Marcus, Sutcliffe and Willoughby (1978), Rossi and Vitagliano-Tadini (1978), Rossi and Fano (1979), Willoughby and Marcus (1979) on feeding, Steel (1961) and Andersson (1969) on life history, Lockwood (1959) and Sutcliffe (1974) on osmotic and ionic regulation, Needham (1970) and Needham and Brunet (1957)

on pigmentation and Manning (1975) and Ridley and Thompson (1979) on reproductive behaviour.

The sequence of events involved in the reproductive cycle of A. aquaticus are fairly well documented and a brief review of previous works on this topic is provided by Ridley and Thompson (1979). The reproductive cycle commences when a female isopod is seized by a larger male, who then clasps her with his 4th pair of legs and carries her dorsal side uppermost between these legs. This condition is termed 'precopula' (Maercks,1930) or mate guarding (Parker, 1974). The male guards the female because she will only be available for insemination during the short period of moulting which occurs in two phases. The first phase involves the shedding of the posterior half of the old cuticle which reveals the open vaginae; copulation then follows and a short time afterwards the male and female separate. The female undergoes the second phase of her moult which involves shedding the anterior half of the old cuticle. This releases the large oostegites which form a brood pouch beneath the anterior part of the thorax. Fertilization is internal and the eggs are released into the brood pouch shortly after the second phase of the female moult has been completed. Females which are incubating eggs are termed 'ovigerous' or 'brooding'. The eggs develop in the brood pouch into juveniles which are released when they are about 1mm in length. After

release of juveniles the females undergo another moult whereby the brood pouch is lost, the large oostegites being replaced by small club shaped lamellae. Each female can produce a number of broods in the breeding season. In the non-breeding season successive moults occur without the production of a brood pouch. Following their release from the brood pouch the juveniles disperse and begin feeding. The rate of growth is temperature dependent and males grow faster and reach a larger size than females. The maximum size to which A. aquaticus grows varies considerably with habitat. In Britain a 15mm long male and 13mm female would be considered very large. Separate sexes are distinguishable at 3mm but breeding does not commence until they are somewhat larger.

The isopod Asellus aquaticus is considered to be the characteristic intermediate host for Acanthocephalus lucii. Although Linstow (1872), Meyer (1932) and Komarova (1950) mention Gammarus pulex (L.) as an alternative intermediate host, subsequent studies have not confirmed this possibility. Gammarus sp. are in any event absent from the Forth and Clyde canal. Many previous studies have revealed larval stages of A. lucii in A. aquaticus (Copland, 1956; Styczynska, 1958; Andryuk, 1974; Brattey, 1979; Moravec, 1979) but none have examined the host-parasite relationship in any detail. Apparently no detailed ecological studies on larval stages of A. lucii in A. aquaticus have been published. However, a number of relevant publications

concerning other species of Acanthocephala with piscine definitive hosts exist in the literature. Awachie (1965) examined the occurrence of larval Echinorhynchus truttae Schrank in Gammarus pulex (L.), Rumpus (1973) and Hine and Kennedy (1974b)studied larval Pomphorhynchus laevis Müller in Gammarus pulex. Seidenberg (1973) and Camp and Huizinga (1979) investigated the ecology of Acanthocephalus dirus Van Cleave in Asellus intermedius Forbes. Muzzall and Rabalais (1975a) examined the host parasite relationships of larval Acanthocephalus jacksoni Bullock in Lirceus lineatus (Say), Muzzall (1978) examined the host parasite relationships and seasonal occurrence of Fessisentis friedi (Nickol) in the isopod Caecidotea communis (Say), Amin, Burns and Redlin (1980) studied the ecology of Acanthocephalus parksidei Amin in the isopod Caecidotea militaris (Hay). Ecological studies on species with avian definitive hosts include those of Hynes and Nicholas (1963) and Spencer (1974) on Polymorphus minutus Goeze in Gammarus pulex and Gammarus lacustris Sars, respectively.

Previous work by Brattey (1979, 1980) showed that the Asellus aquaticus population in the Forth and Clyde canal at Temple, Glasgow,harboured larval stages of Acanthocephalus lucii in reasonable numbers, so a detailed study of the dynamics of the host and larval parasite populations was undertaken using material from this site.

Materials and Methods

Large numbers of A. aquaticus were collected at approximately monthly intervals from the shallow margins at the sides of the Forth and Clyde canal. Due to technical difficulties no samples were available from the deeper central parts of the canal. The samples were collected with a fine meshed pond net (0.3mm mesh). The method of collection of isopods was not quantitative and so did not permit an accurate assessment of the density or spatial distribution of isopods. The pond net was worked vigorously along the mud and silt substrate and through the dense beds of Elodea sp. and Cladophora sp. along a distance of about 20 yds of the canal bank. Net-fulls of debris and invertebrates were placed in a large 10 gal capacity container until this was approximately half full. It was then topped up with canal water and transported back to the laboratory. Each net full could contain several hundred isopods, so the total number collected in each sample was probably of the order of several thousand. At the laboratory the entire sample was placed in a 20 gal. plastic tank , aerated and maintained at the appropriate ambient temperature. Sub-samples were then taken until it was ascertained that at least 500 isopods had been removed. Every attempt was made to ensure that a representative sample of each of the various sizes of isopod was obtained in the subsamples to make certain that a reasonably accurate

picture of the size structure of the isopod population present at the time of sampling could be constructed. Isopods were removed from the debris by hand sorting, placed in clean tap water and anaesthetized by the addition of a few mls. of 2-Phenoxy-ethanol. The length of each isopod (to the nearest mm) was measured from the anterior margin of the cephalothorax to the posterior margin of the abdomen. Specimens were placed ventral side uppermost in a petri dish containing 0.8% saline then viewed through a binocular microscope which had 1mm graph paper cellotaped onto the viewing stage. Only specimens > 3mm in length were examined. The sex of each isopod was noted based on the morphology of the 2nd pleopods (Gledhill, Sutcliffe and Williams, 1976). In female isopods the presence or absence of a brood pouch was noted (irrespective of whether it was empty, contained eggs or embryos). Each isopod was carefully teased open with dissecting needles and the number, stage of development and where possible, sex of each larval stage noted. Larval stages were assigned to one of three categories. The acanthor, acanthella or cystacanth.

Further infected isopods were removed from the remainder of each sample and divided into 1mm length groups (range 4-10mm). The wet weight of each (to the nearest 0.1mgs) was determined after removing excess water with absorbent filter paper. The contained cystacanth stages were then dissected out and wet weights (to nearest 0.1mg) determined after removing excess

water as before. Since cystacanths are large relative to the size of the host some indication of the effect of the parasite on the host was obtained by calculating a parasitization index (PI) where

$$PI = \frac{\text{Weight of cystacanth(s)}}{\left(\begin{array}{l}\text{Weight of Asellus} \\ \text{+ cystacanth(s)}\end{array}\right) - \text{Weight of cystacanth(s)}} \times 100$$

The data collected from isopods used in the determination of the parasitization index were also used to examine the intensity of infection in isopods of different sizes, since insufficient numbers of infected isopods were recovered from the individual monthly samples to assess this relationship accurately.

Results

Figure 3.1 illustrates the size structure of the A. aquaticus population in the Forth and Clyde canal in samples collected from January 1980 until March 1981. Male and female isopods are represented separately in the upper and lower parts of each histogram, respectively. No sample was taken in February 1980. At least 500 isopods were examined in each sample. Only isopods $\geqslant$ 3mm in length were examined. Figure 3.2 illustrates the seasonal changes in the proportion of female isopods $\geqslant$ 6mm in length with a brood pouch. Only females $\geqslant$ 6mm in length bred in the canal (see Figure 3.1), so this gives an approximation of

the intensity of breeding at different times of the year. From Figures 3.1 and 3.2 it is evident that breeding females were first observed in March in 1980. Breeding continued from March until September 1980, ceased between October and December and recommenced in January in 1981. Figure 3.2 indicates that breeding was not of equal intensity throughout the year. A single peak in breeding occurred in April in 1980, with a gradual decline thereafter. Figure 3.1 indicates that the largest females bred first in March and April, whereas the smaller individuals present at this time grew first then bred somewhat later in May and June. From April until July there was a gradual decline in the average size of isopods. This appeared to be due to a gradual disappearance of the entire overwintered isopod population. The largest males and females bred first then died. Smaller individuals grew somewhat before breeding then died later. Although brooding females occurred as early as March the appearance of the new generation in the samples did not occur until June, thereafter small isopods comprised a fairly high proportion of the population. The new generation of isopods grew very rapidly during summer and by September the average size of isopods was about 7mm. From October onwards there was evidence of a distinct bimodality in the histograms. The larger individuals (8-10mm) represented isopods born in spring, whereas the smallest sizes were probably the offspring

Figure 3.1. Size frequency distributions for Asellus aquaticus from the Forth and Clyde canal, Scotland, from January 1980 until March 1981. See text for details.

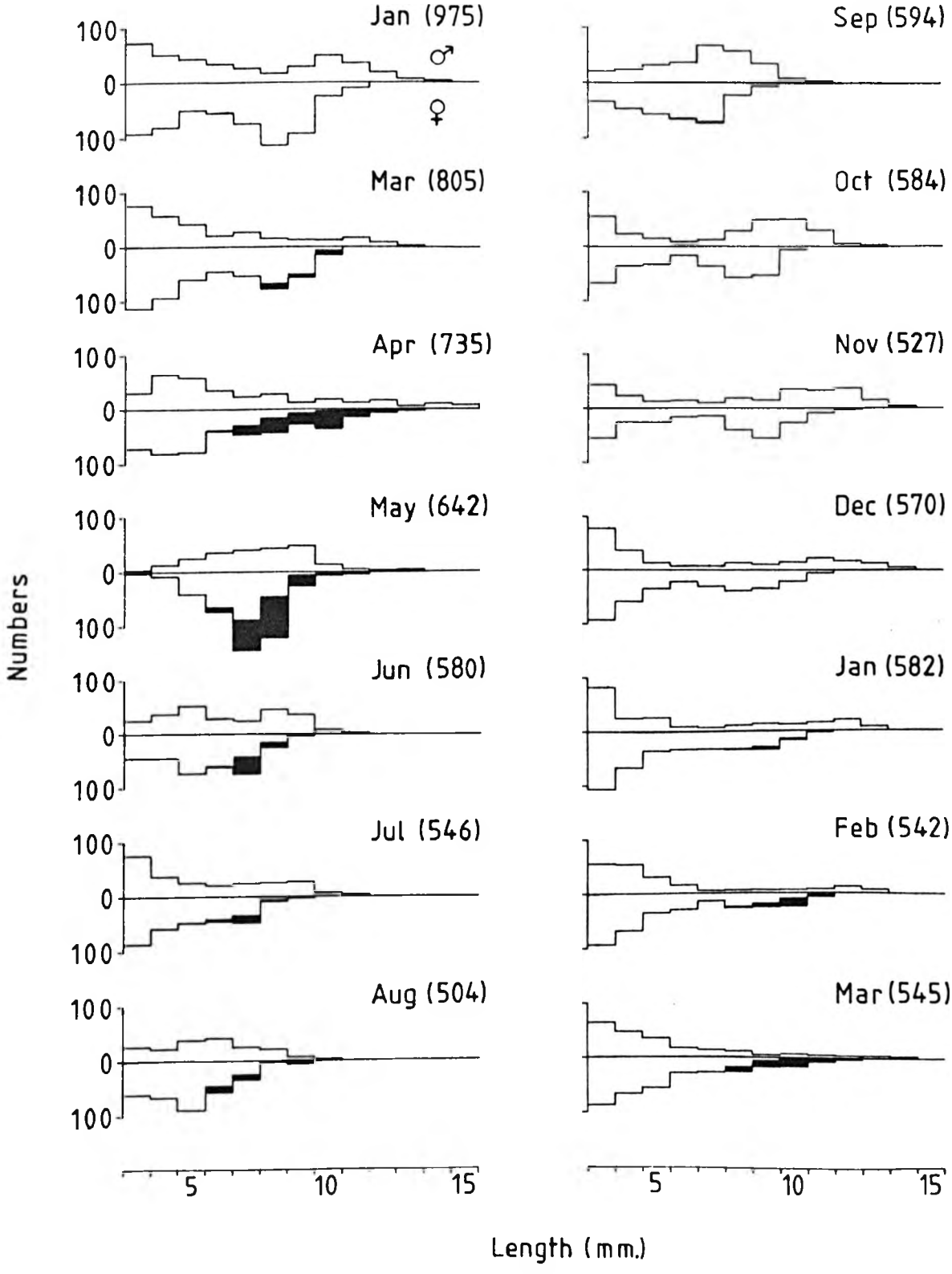
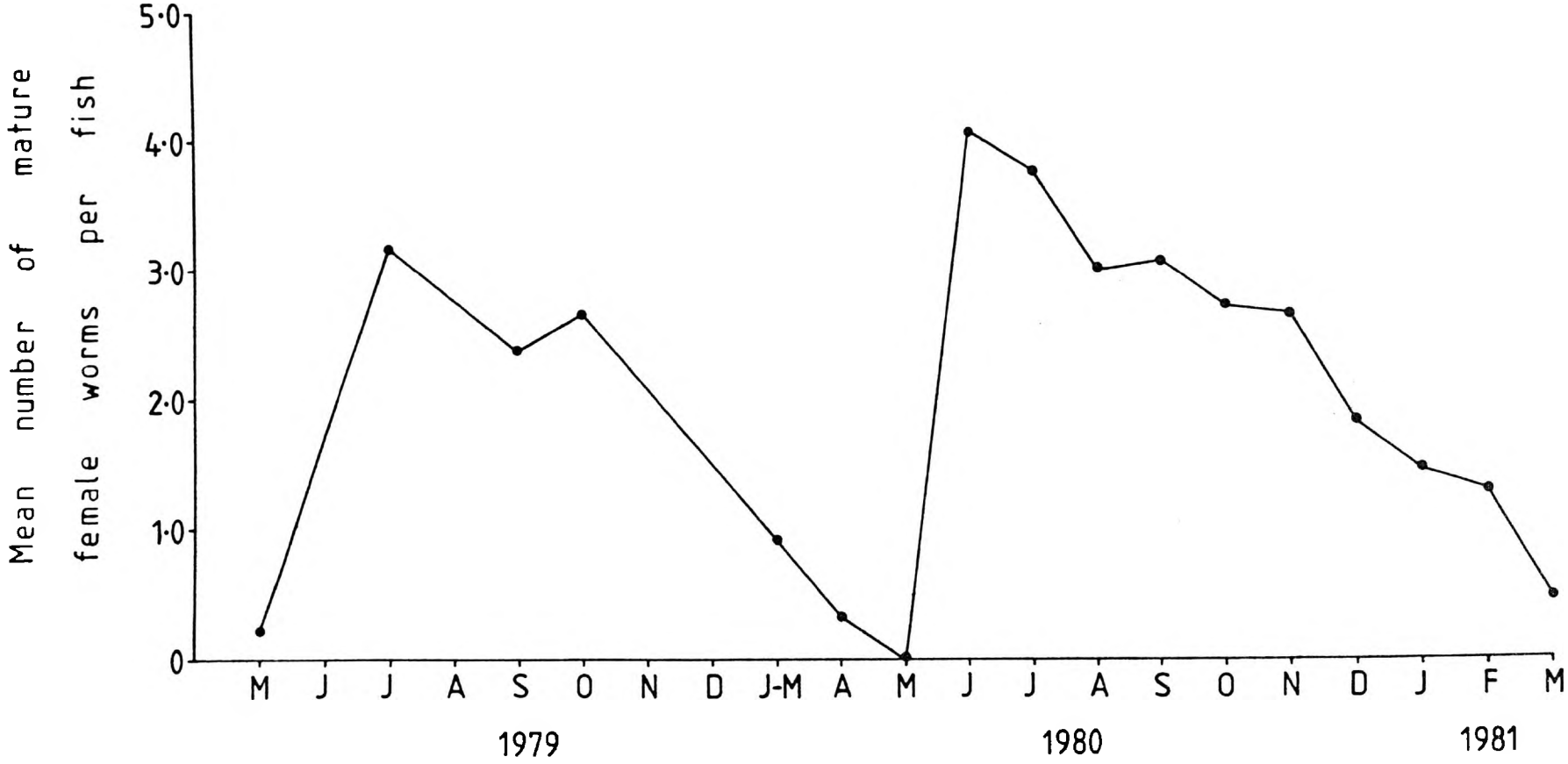


Figure 3.2. Seasonal changes in breeding intensity of Asellus aquaticus in the Forth and Clyde canal, Scotland (expressed as the percentage of females ⩾ 6mm in length with a brood pouch).

of these larger individuals, some of which produced broods in late summer and early autumn before breeding ceased in October. From November until January growth apparently ceased and the size structure of the population remained essentially the same. Breeding recommenced in January in 1981 and again the larger females bred first. The histograms indicate that isopods can take part in either one or two breeding seasons depending on when they were born. Individuals born in early spring could produce some broods in late summer and then again the following spring before dying. Individuals born in mid-summer or later did not breed until late the following spring then disappeared. The maximum life span of A. aquaticus in the Forth and Clyde canal appeared to be about 1 year.

Figure 3.3 illustrates the seasonal changes in the mean number of mature (i.e. containing shelled acanthors) female Acanthocephalus lucii in perch from the Forth and Clyde canal. It is important to note that Figure 3.3 only indicates the net change in the number of mature worms between months. Infection is a dynamic process and the infection level is a result of the balance between recruitment of new parasites into the fish and the loss of old ones. Figure 3.3 does not tell us anything about the rate of recruitment of newly matured parasites or the rate loss of old ones. Both these processes are extremely difficult to measure in natural populations. Figure 3.3 gives only a limited idea of the rate of shelled

Figure 3.3. Seasonal changes in the mean number of mature female Acanthocephalus lucii recovered from perch (P. fluviatilus) from the Forth and Clyde canal, Scotland.



acanthor output, which appeared to occur principally by loss of whole gravid worms from fishes (see discussion). In May almost all worms were immature and shelled acanthor output was virtually nil. By June the majority of worms were mature and shelled acanthor output commenced. Since the mean number of mature female worms declined from June onwards output of shelled acanthors must have occurred. It is important to note that stomach content analysis of perch during summer (Chapter 2) indicated that recruitment of new parasites into the fish population occurred right through summer in spite of the overall net loss of mature female worms. Since warmer temperatures increased the rate of maturation and decreased the survival of adult worms (see Chapter 6), it seems likely that in summer both recruitment and mortality rates were high and so loss of gravid female worms (i.e. shelled acanthor output) from fish was at a maximum. Through autumn and winter recruitment, maturation and mortality rates declined and so, therefore, did shelled acanthor output. In summary, shelled acanthor output was maximum in mid summer and declined gradually through autumn and winter to minimal level the following spring.

Seasonal changes in the incidence of infection and proportions of the various larval stages of A. lucii in A. aquaticus are given for all samples in Table 3.1. Some larval stages of the parasite were recovered in every month. Incidence ranged from a minimum of 1.5% in July to 8.3% in June. Intensity remained fairly

Table 3.1. Seasonal changes in the incidence of infection and proportions of the various larval stages of *Acanthocephalus lucii* in the isopod *Asellus aquaticus*

	1980											1981		
	Jan	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Number of *Asellus* examined	975	805	735	642	580	546	504	594	584	527	570	582	542	545
Number of *Asellus* infected (%)	76(7.8)	41(5.1)	57(7.8)	38(5.9)	48(8.3)	8(1.5)	8(1.6)	36(6.1)	31(5.3)	29(5.5)	37(6.5)	47(8.1)	40(7.4)	16(2.9)
Total parasites recovered	99	49	91	43	59	9	8	53	39	37	54	58	54	18
Mean no. parasites/ infected isopod	1.30	1.20	1.60	1.13	1.23	1.13	1.0	1.47	1.26	1.28	1.46	1.23	1.35	1.13
Number at acanthor stage (%)	1(1.0)	4(8.0)	14(15.4)	1(2.0)	1(1.7)	-	-	2(3.8)	3(7.7)	-	1(1.9)	3(5.2)	10(18.5)	1(5.6)
Number at acanthella stage (%)	40(40)	21(43)	36(39.6)	21(49)	22(39.3)	2(22.2)	5(62.5)	28(52.8)	11(28.2)	10(27)	15(27.8)	17(29.3)	31(57.4)	5(27.8)
Number at cystacanth stage (%)	58(58)	24(49)	41(45)	21(49)	36(61)	7(77.8)	3(37.5)	23(43.4)	25(64.1)	27(73)	38(70.4)	38(65.5)	13(24.1)	12(66.7)

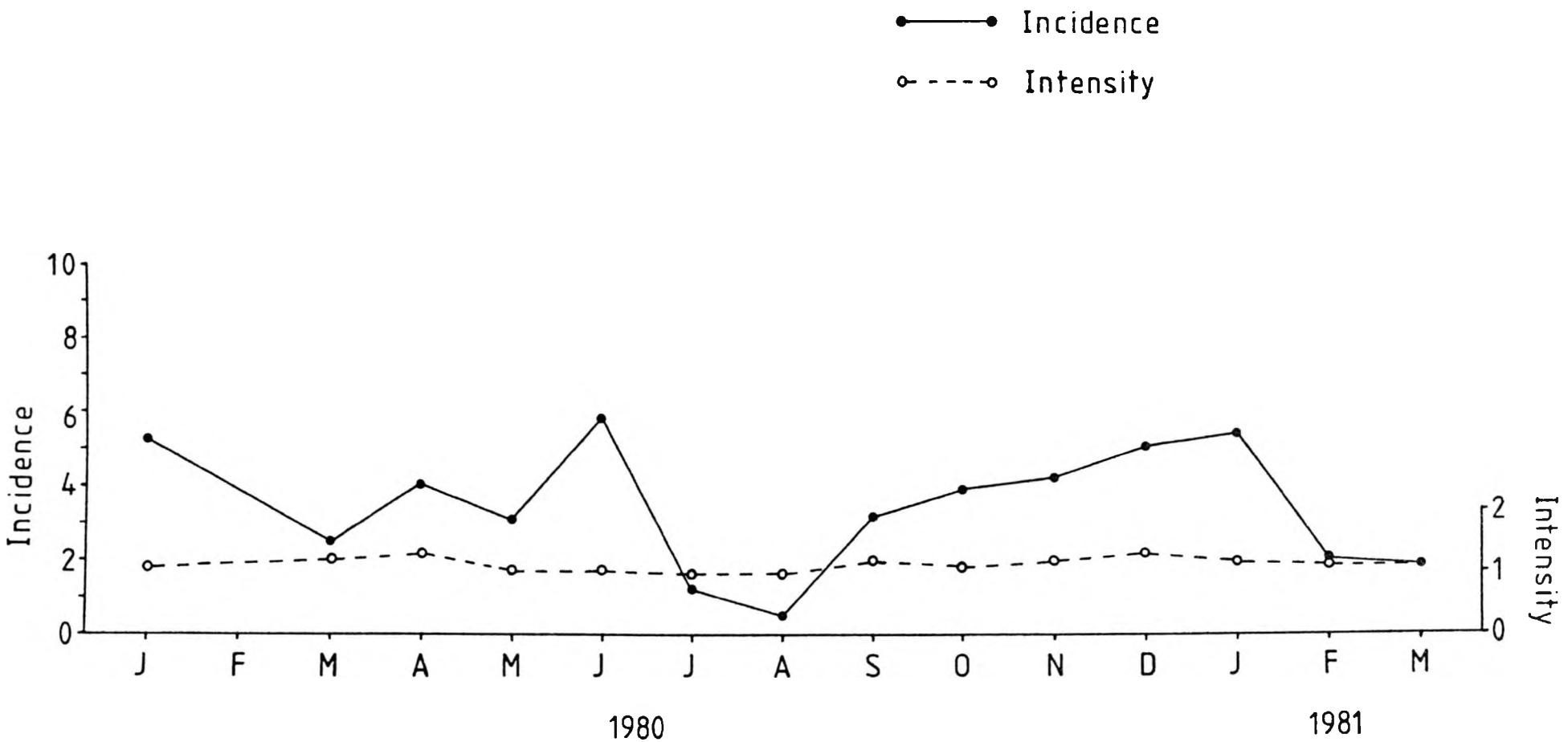
constant ranging from 1.0 larval stage per infected isopod in August to 1.6 in April. Levels of infection showed slight indications of a decrease through spring to minimum levels in mid-summer, followed by a gradual increase through autumn to maximum levels in mid winter. The proportions of the various larval stages recovered in each sample suggests that infection of the isopods occurred through many months of the year. The smallest acanthor stage was recovered in nearly all samples except in mid summer, but it should be borne in mind that the acanthor stage is extremely small and difficult to locate. The cystacanth stage comprised a large proportion of the total larvae in all months which is probably a reflection of its longer duration in isopods.

The incidence of infection of the A. aquaticus population with the infective cystacanth stage of A. lucii is given separately in Table 3.2 and illustrated in Figure 3.4. The infective cystacanth stage was recovered throughout the entire sampling period. Incidence ranged from 0.6% in August to 5.5% in January 1981. Intensity (per infected isopod) remained fairly constant between 1.0 in July and August and 1.3 in April 1980. The data suggested a slight seasonal trend in levels of infection. Over 5% of isopods harboured cystacanths in January 1980. Through spring and early summer there was evidence of a gradual decline in the incidence to a minimum in August. Thereafter incidence rose consistently through autumn and early winter to a maximum

Table 3.2. Seasonal changes in the incidence of infection of *Asellus aquaticus* with cystacanths of *Acanthocephalus lucii*.

	1980											1981		
	Jan	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Number of *Asellus* examined	975	805	735	642	580	546	504	594	584	527	570	582	542	545
Number of *Asellus* infected (%)	52(5.3)	20(2.5)	31(4.2)	20(3.1)	34(5.9)	7(1.3)	3(0.6)	19(3.2)	23(3.9)	23(4.4)	29(5.1)	32(5.5)	11(2.0)	10(1.8)
Total no. cystacanths	58	24	41	21	36	7	3	23	25	27	38	38	13	12
Mean no. cystacanths/ infected isopod	1.12	1.20	1.32	1.05	1.06	1.00	1.00	1.21	1.09	1.17	1.31	1.18	1.18	1.2

Figure 3.4. Seasonal changes in the incidence and intensity of infection of Asellus aquaticus with cystacanths of Acanthocephalus lucii.



of 5.5% in January 1981 before declining in February and March. The overall sex ratio of cystacanths was found to be close to 1:1.

The frequency distribution for all stages of larval parasite among isopods is given separately for each sample in Table 3.3b Up to 5 larval parasites were recorded from a single isopod. There was no evidence of pronounced seasonal changes in the frequency distribution except in the mid summer months of July and August when infection levels were at their lowest. Of the few isopods which were infected at this time most harboured only a single larval stage.

The frequency distribution of the infective cystacanth stage among isopods is given separately for each sample in Table 3.3a. A maximum of 3 cystacanths were found in a single isopod. Again there was no evidence of pronounced seasonal changes in the frequency distribution, except in the months of July and August when the few isopods which were infected harboured single cystacanths.

In both Table 3.3a and 3.3b the mean number of parasites per host was calculated for each sample together with, where possible, the variance and variance to mean ratio. In all cases the variance exceeded the mean, indicating overdispersion, the ratios ranging from 1.1437 to 2.1099 (Table 3.3a) and from 1.0503 to 1.4615 (Table 3.3b). Hence larval A. lucii are not randomly

Table 3.3a. Seasonal changes in the frequency distribution (of cystacanths) of Acanthocephalus lucii in the isopod Asellus aquaticus

		1980											1981	
		Jan	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Number of cystacanths	0	923	785	704	622	546	539	501	575	561	504	541	550	531	535
	1	47	16	21	19	32	7	3	15	21	19	21	27	10	8
	2	4	4	10	1	2	-	-	4	2	4	7	4	-	2
	3	1	-	-	-	-	-	-	-	-	-	1	1	1	-
Number of hosts		975	804	735	642	580	546	504	594	584	527	570	582	542	545
Number of cystacanths		58	24	41	21	36	7	3	23	25	27	38	38	13	12
Mean no. cystacanths/host ($\bar{x}$)		0.0595	0.0298	0.0558	0.0327	0.0621	0.0128	0.0059	0.0387	0.0427	0.0512	0.0667	0.0652	0.0240	0.0220
variance (s^2)		0.0704	0.0389	0.0799	0.0348	0.0652	-	-	0.0508	0.0479	0.0639	0.0975	0.0852	0.0345	0.0289
variance/mean ratio (s^2: $\bar{x}$)		1.1832	1.3054	1.4335	1.0642	1.0503	-	-	1.3128	1.1197	1.2483	1.4615	1.3052	1.4375	1.3148

Table 3.3b. Seasonal changes in the frequency distribution (of all larval stages) of Acanthocephalus lucii in the isopod Asellus aquaticus

		1980											1981	
		Jan	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Number of larval parasites	0	899	764	678	604	532	538	496	558	553	498	533	535	502	529
	1	61	33	36	33	38	7	8	24	25	22	23	38	32	14
	2	9	8	14	5	9	1	-	7	4	6	11	7	4	2
	3	5	-	4	-	1	-	-	5	2	1	3	2	2	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	2	-
	5	1	-	3	-	-	-	-	-	-	-	-	-	-	-
Number of hosts		975	805	735	642	580	546	504	594	584	527	570	582	542	545
Number of larval parasites		99	49	91	43	59	9	8	53	39	37	54	58	54	18
Mean no. parasites/host ($\bar{x}$)		0.1015	0.0609	0.1238	0.0670	0.1017	0.0165	0.0159	0.0892	0.0668	0.0702	0.0947	0.0997	0.0996	0.0330
variance (s^2)		0.1611	0.0771	0.2612	0.0766	0.1330	0.0199	-	0.1552	0.0967	0.0996	0.1562	0.1346	0.1878	0.0393
variance/mean ratio (s^2: $\bar{x}$)		1.5876	1.2666	2.1099	1.1437	1.3076	1.2067	-	1.7444	1.4480	1.4191	1.6500	1.3501	1.8855	1.1909

Table 3.4. The observed frequency distribution of larval *Acanthocephalus lucii* in *Asellus aquaticus* compared with the frequency distribution predicted by the Negative Binomial model

All larval stages

	No parasites per isopod	0	1	2	3	4.5
	Observed	899	61	9	5	1
Jan. 1980	Expected	899.15	59.01	12.37	3.19	1.16

χ^2 = 2.04 ($p > 0.05$), 2 D.F., k = 0.19

	Observed	678	36	14	4	3
Apr. 1980	Expected	677.9	37.75	11.44	4.41	2.73

χ^2 = 0.72 ($p > 0.05$), 2 D.F., k = 0.10

	Observed	502	32	4	2	2
Feb. 1981	Expected	502.21	29.06	7.23	2.26	1.002

χ^2 = 2.60 ($p > 0.05$), 2 D.F., k = 0.13

Cystacanth stage only

	Observed	541	21	7	1
Dec. 1980	Expected	540.90	22.68	4.68	1.22

χ^2 = 1.31 ($p > 0.05$) 1 D.F., k = 0.11

distributed amongst their hosts (Poisson distribution), the distribution is aggregated or clumped suggesting that some alternative distribution might describe the distribution of larval stages amongst their hosts more accurately. However, in spite of the large numbers of isopods examined in each sample, the low level of infection, small numbers of infected isopods recovered, and the small number of classes in the frequency distribution make it impossible to attempt to fit data from each individual sample to frequency distribution models due to insufficient degrees of freedom. In certain months data were suitable for attempting to fit theoretical distribution models. In each instance the negative binomial distribution was fitted to the data of the original distribution using the maximum likelihood method to estimate the parameter k (Bliss and Fisher, 1953). Agreement between the data from the original distribution and the negative binomial was fitted by χ^2 (goodness of fit). The results are indicated in Table 3.4. In each sample agreement between the negative binomial and the original distribution was satisfactory ($p > 0.05$). Attempts to fit the data to the Poisson model proved unsatisfactory ($p < 0.05$).

The number, sex and incidence of infection of isopods in each 1mm length group is given in Table 3.5. Separate categories are again given for infections including all larval stages and for infection with the cystacanth stage only. Isopods ranged in size from 3 to 15mm. Considering infection levels

Table 3.5. The incidence of infection of Asellus aquaticus with larval Acanthocephalus lucii in relation to the size and sex of the isopod

			Length of Asellus (mm)	3	4	5	6	7	8	9	10	11	12	13	14	15
All isopods			No. examined	1712	1321	1167	920	1018	961	740	428	238	142	63	20	1
	Inf.		all stages	16	48	92	100	99	92	41	16	6	2	0	0	0
			(%)	(0.9)	(3.6)	(7.9)	(10.9)	(9.7)	(9.6)	(5.5)	(3.7)	(2.5)	(1.4)	-	-	-
			cystacanths	0	17	46	61	71	74	30	10	4	1	-	-	-
			(%)	-	(1.3)	(3.9)	(6.6)	(7.0)	(7.7)	(4.1)	(2.3)	(1.7)	(0.7)	-	-	-
male isopods only			No. examined	705	513	450	329	321	336	312	243	184	133	62	20	1
	Inf.		all stages	4	12	38	36	45	49	26	13	5	2	0	0	0
			(%)	(0.6)	(2.3)	(8.4)	(10.9)	(14.0)	(14.6)	(8.3)	(5.4)	(2.7)	(1.5)	-	-	-
			cystacanths	0	4	21	21	32	37	17	8	3	1	-	-	-
			(%)	-	(0.8)	(4.7)	(6.4)	(9.9)	(11)	(5.4)	(3.3)	(1.6)	(0.8)	-	-	-
female isopods only			No. examined	1007	808	717	591	697	625	428	185	54	9	1	-	-
	Inf.		All stages	12	36	54	64	54	43	15	3	1	0	0	-	-
			(%)	(1.2)	(4.4)	(7.5)	(10.8)	(7.7)	(6.9)	(3.5)	(1.6)	(1.9)	-	-	-	-
			cystacanths	0	13	25	40	39	37	13	2	1	-	-	-	-
			(%)	-	(1.6)	(3.5)	(6.8)	(5.6)	(5.9)	(3.0)	(1.1)	(1.9)	-	-	-	-

Inf. = Infected

when all larval stages are included, it is apparent that even the smallest (3mm) isopods examined were infected. Incidence of infection rose with increasing size of isopods up to a maximum of 14.6% in 8mm long male isopods and 10.8% in 6mm long females. Incidence then declined with further increase in size of isopods. Male isopods > 12mm and females > 11mm in length were uninfected. The absence of infections in these largest size groups may in part be owing to the small sample sizes. Overall there was no significant difference in the level of infection of male and female isopods when all larval stages were included χ^2 = 2.88. 1 D.F. ($p > 0.05$). The incidence of infection with the cystacanth stage alone followed a similar pattern to that when all larval stages were included, although no cystacanths were recorded from male or female isopods in the 3mm length group. Thereafter infection levels rose with increasing size of isopod to a maximum of 11.0% in 8mm long males and 6.8% in 6mm long females. In larger isopods incidence fell. Males > 12mm and females > 11mm in length were not infected. Overall there was no significant difference in the level of infection of male and female isopods with cystacanths χ^2 = 2.75. 1 D.F. ($p > 0.05$).

Seasonal changes in the incidence of infection of each size group of A. aquaticus with cystacanths of A. lucii (expressed as a percentage of the numbers in each size group) for samples collected from January 1980 until March 1981 are given in Table 3.6. No cystacanths were recovered from isopods < 4mm

Table 3.6. Seasonal changes in the incidence of infection of *Asellus aquaticus* with cystacanths of *Acanthocephalus lucii* in isopods of different sizes

		Percentage infection													
							1980							1981	
		Jan	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Asellus size group (mm)	4	0.75	0.66	1.4	10	-	-	1.1	2.8	3.4	-	-	-	-	-
	5	8.5	3.9	3.6	11.9	2.4	1.3	0.8	3.3	4.2	10.5	5.2	1.1	0.8	0.9
	6	12.2	4.6	6.6	2.7	15.7	3.0	-	2.9	15.4	3.3	6.5	4.9	1.5	-
	7	14.7	7.6	8.8	1.1	9.2	-	-	5.6	6.3	15.4	13.8	16.7	4.2	4.3
	8	6.5	4.3	8.5	3.1	8.8	8.8	-	3.9	10.3	10.2	10.5	17.5	11.1	11.6
	9	5.7	1.4	7.5	1.4	5.2	3.3	9.1	-	2.0	8.5	17.6	20.9	12.1	5.6
	10	2.7	3.7	3.9	5.9	-	-	-	-	1.8	-	7.0	4.4	3.0	-
	11	-	-	10.5	-	-	-	-	-	-	2.3	2.8	7.1	-	-
	12	-	-	-	-	-	-	-	-	-	2.6	-	5.9	-	-
overall monthly incidence		5.3	2.5	4.2	3.1	5.9	1.3	0.6	3.2	3.9	4.4	5.1	5.5	2.0	1.8

or 12mm in length. Although the overall level of infection was low (< 6%) in all samples, clearly individual size groups of isopods had much higher levels of infection at certain times of the year. The highest level of infection recorded was 20.9% in isopods 8mm in length in January 1981. Broadly speaking the widest size range of isopods were infected and highest level of infection recorded in individual size groups in the winter months.

The intensity of infection (per infected isopod) of male, female and both sexes of A. aquaticus in each size group, with cystacanths of A. lucii, is given in Table 3.7. Only isopods $\geqslant$ 4mm and $\leqslant$ 10mm in length are included. The other size groups are excluded owing to there being too few isopods to assess the relationship accurately. Intensity was clearly low in all size groups, but a slight increase in intensity was evident with increasing size of isopods. The range and mean wet weight of cystacanths recovered from isopods of different sizes (4-10mm in length) is given in Table 3.8. Means and ranges are given separately for infections with male and female cystacanths. The relationship is illustrated in Figure 3.5, where means and standard deviations are given. Both the mean wet weight and the range appeared to increase with the size of the isopod. Female cystacanths are typically larger than males from isopods of the same length.

From the data in Table 3.8 frequency distribution histograms for wet weights of male and female cystacanths were constructed

Table 3.7. The intensity* of infection in different sizes of Asellus aquaticus infected with cystacanths of Acanthocephalus lucii

		length of isopod (mm)							
		4	5	6	7	8	9	10	Totals
male Asellus	no. examined	13	35	37	47	51	28	15	230
	no. cystacanths recovered	14	37	39	50	65	42	20	271
	Intensity	1.1	1.1	1.1	1.1	1.3	1.5	1.3	1.2
female Asellus	no. examined	23	41	56	56	54	31	5	267
	no. cystacanths recovered	24	44	62	65	68	39	9	312
	Intensity	1.0	1.1	1.1	1.2	1.3	1.3	1.8	1.2
Total Asellus	no. examined	36	76	93	103	105	59	20	497
	no. cystacanths	38	81	101	115	133	81	29	583
	Intensity	1.06	1.07	1.09	1.12	1.27	1.37	1.45	1.2

* per infected isopod

Table 3.8. The wet weights of cystacanths of Acanthocephalus lucii recovered from Asellus aquaticus of different sizes

		length of isopod (mm)						
		4	5	6	7	8	9	10
male cystacanths	Number of cystacanths	15	22	14	13	21	22	10
	Mean wet weight (mgs)	0.31	0.42	0.58	0.59	0.8	0.94	0.9
	Range	0.1–0.5	0.1–0.7	0.3–0.8	0.3–0.8	0.3–1.1	0.3–1.7	0.5–1.1
female cystacanths	Number of cystacanths	5	9	19	21	15	23	6
	Mean wet weight (mgs)	0.46	0.54	0.92	1.07	1.55	1.47	1.78
	Range	0.4–0.6	0.2–1.0	0.3–1.4	0.7–1.6	0.7–2.6	0.5–2.8	0.7–2.9

Figure 3.5. The mean wet weight (± s.d.) of male and female cystacanths of Acanthocephalus lucii in relation to the length of the isopod host

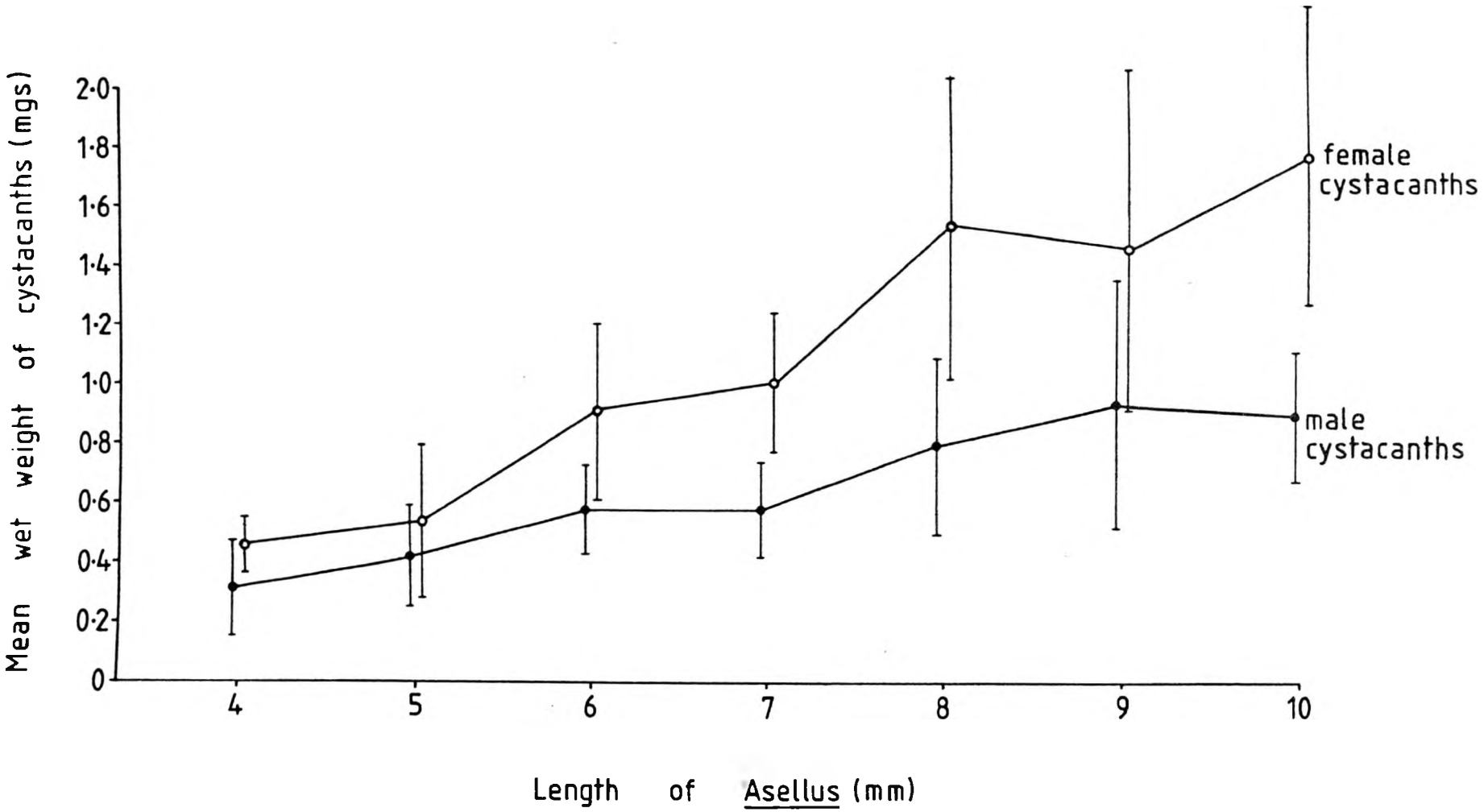
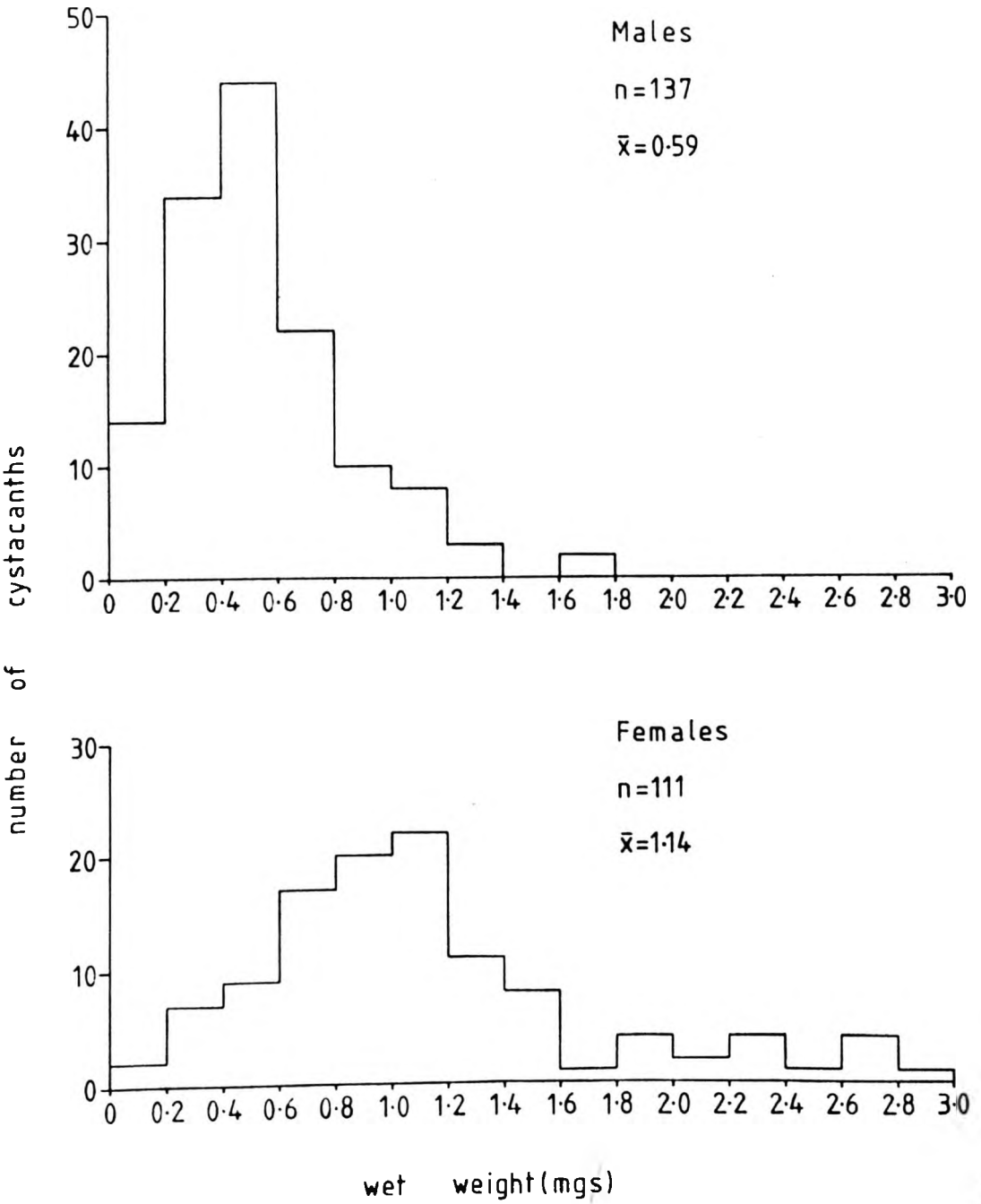


Figure 3.6. Frequency distribution histograms for wet weights of male and female cystacanths of Acanthocephalus lucii recovered from isopods of various sizes (see Table 3.8).



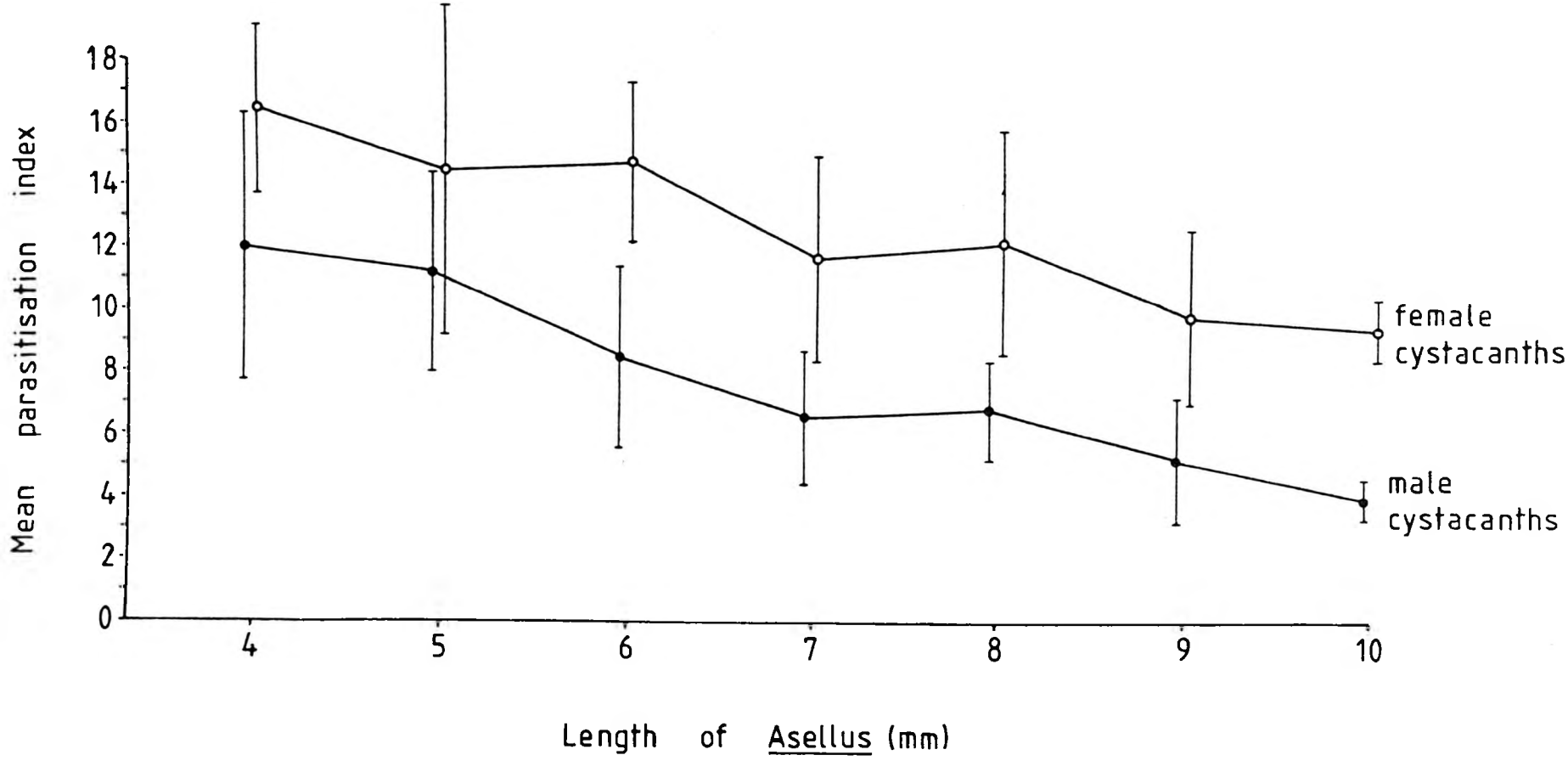
and these are indicated in Figure 3.6, where wet weights are assigned to 0.2mg groups. The range of sizes of both male and female cystacanths was considerable (from 0.1 - 1.7 mgs for males and 0.2 - 2.9 mgs for females), although female cystacanths were on average, approximately twice as large as males (0.59 mgs and 1.14 mgs, respectively).

Since cystacanths were large relative to the size of the isopod (Plate 3.1), cystacanth size increased with host length (Table 3.8 and Figure 3.5) and intensity of infection increased slightly with isopod size (Table 3.7), some indication of the burden imposed by larval parasites on isopods of different sizes was estimated by calculating a parasitization index (PI) (see Materials and Methods). The range and mean PI for isopods from 4 to 10mm in length is indicated in Table 3.9. Since female cystacanths were typically larger than males in isopods of the same length, separate PI's were calculated for infections with either sex of parasite. Both single and multiple infections were included (but mixed sex infections had to be excluded). Multiple infections were more common in the larger isopods so their exclusion would tend to underestimate the PI in these isopods. The wide range of PI's was at least partly owing to isopods having either 1, 2 or 3 cystacanths. The relationship between the PI and isopod length is illustrated in Figure 3.7, where means and standard deviations are given. The smallest

Table 3.9. The mean parasitization index for different sizes of Asellus aquaticus infected with cystacanths of Acanthocephalus lucii

		Length of isopod (mm)						
		4	5	6	7	8	9	10
Infection with male cystacanths	Number of isopods	11	21	14	9	13	11	6
	Mean P.I.	13.89	12.75	9.91	6.89	7.35	5.78	4.33
	Range	5.3 – 22.7	5.8 – 19.4	5.8 – 16.1	3.1 – 13.3	5.1 – 10.6	3.1 – 9.7	3.1 – 5.8
Infection with female cystacanths	Number of isopods	7	8	19	20	10	10	3
	Mean P.I.	17.80	15.46	16.73	13.18	13.56	13.70	10.53
	Range	9.5 – 26.1	6.1 – 25	10.4 – 23.7	5.5 – 21.6	5.6 – 20.6	5.9 – 31.2	9.2 – 11.9

Figure 3.7. The mean parasitisation index (PI) (± s.d.) for isopods infected with either male or female cystacanths of Acanthocephalus lucii, in relation to the length of the isopod host



isopods appeared to have the highest PI values and with increasing isopod (and hence cystacanth) size the PI appears to decrease linearly.

Discussion

Although levels of infection of Asellus aquaticus with larval stages of Acanthocephalus lucii remained fairly low throughout the year in the Forth and Clyde canal, the evidence presented suggests a slight seasonal cycle occurred in the size of the larval parasite population. Incidence and intensity do not necessarily give a true assessment of the size of the larval parasite population at various times of the year since they do not take into account fluctuations in host population density. Although this was not actually measured the details of the life cycle of the A. aquaticus population in the canal provided during the course of the study permit a reasonably accurate assessment of the relative changes in the size of the A. aquaticus population throughout the year.

Prior to the onset of breeding in spring the size of the isopod population was at a minimum. As breeding commenced density rose rapidly with the release of large numbers of small individuals from the brood pouch of large overwintered female isopods. Although a peak in brooding females was observed in April, the actual peak in isopod density probably did not occur until slightly later, with a time lag owing to the fact

that the embryos present in the brood pouch in April still had to develop somewhat and then be released before they actually contributed to isopod population size. In the current study this time lag was apparently further increased by the fact that only isopods $\geq$ 3mm in length were collected, whereas young isopods are actually released from the brood pouch when they are approximately 1mm in length. The new generation of isopods began to appear in the samples in June, when they had grown to a length of 3mm. Maximum isopod population density probably occurred around June - July. The smallest isopods probably suffered considerable losses due to predation so the peak in density was probably followed, at least initially, by quite a sharp decline. The decline may have been reduced by breeding of the larger members of the isopod population during mid-summer, but with cessation of breeding in autumn isopod density probably slowly declined through autumn and winter until breeding commenced the following spring.

It is interesting to note that the breeding cycle of A. aquaticus shows considerable geographic variation which appears to be correlated with climate (Williams, 1960). In sub-Arctic northern Scandinavia the breeding season is short and extends only from late spring to summer. In some populations individuals may take 2 years to mature (Andersson, 1969). In more temperate regions the breeding season extends earlier into

spring and later towards autumn (Williams, 1960 and current study). In the most temperate regions breeding may even occur throughout the year (Kaulberz,1913), although a seasonal peak still occurs. This geographic variation in breeding cycle will have considerable influence on the seasonal dynamics of A. lucii such that the parasite will probably exhibit a different cycle in different climatic regions throughout its range.

Shelled acanthor output by adult A. lucii in the definitive host occurred over many months of the year. Maximum output took placed in mid-summer with a gradual decline thereafter to minimum levels the following spring. Initial recruitment of shelled acanthors into the isopod population commenced in mid summer and at summer water temperatures of approximately 19°C experimental evidence (see Chapter 5) indicated that cystacanths should take about two months to develop to infectivity. A rise in the level of infection was noticeable from September onwards. Initial recruitment of acanthors into the isopod population in mid-summer was unfortunately not reflected in a high proportion of early acanthor and acanthella stages at this time, which is difficult to explain, but could partly be attributed to them being overlooked owing to their extremely small size. Through autumn (September - November) the level of infection continued to rise. The actual rate of recruitment of new parasites into the isopod population through autumn probably declined due to decreasing water temperatures reducing the feeding activity of

A. aquaticus. Similarly the rate of development of larval A. lucii was greatly reduced by low temperatures (see Chapter 5) and by mid-winter, when water temperatures were below 5°C, both development of existing larval stages and recruitment of new parasites into the isopod population will have virtually ceased. So it appears that A. lucii can overwinter either as a shelled acanthor, or inside an isopod as an acanthor, acanthella, or cystacanth. Clearly at least some isopods harbouring cystacanths survived right through the winter. Development of early larval stages recommenced the following spring when water temperatures increased. At this time the largest isopods began to breed then disappeared, and a further decline in the density of the isopod population occurred. The decline in isopod density in spring coincided with an abrupt rise in both incidence and intensity of infection of the definitive host (see Chapter 4) so there can be no doubt that at least part of this mortality (of both infected and uninfected isopods) is due to predation by perch. In May, although infection levels of the definitive host were at a maximum, almost all the worms were immature and so shelled acanthor production was at a minimum. Isopod population density was rising very rapidly at this time and highest density probably occurred in early summer. The adult parasites in the definitive host then matured very rapidly in June. Maximum shelled acanthor output therefore occurred when isopod population

density was at a maximum and so this synchronization maximized the probability of a shelled acanthor being eaten by an isopod. In spite of this a high incidence of infection did not occur. The mean number of worms per infected isopod also remained low throughout the year, and therewas no indication of any isopods becoming 'heavily' infected at any time of year. There is no indication of heavily infected isopods appearing then disappearing due to parasite induced host mortality as described by Seidenberg (1973) and Amin, Burns and Redlin (1980) for Acanthocephalus dirus and A. parksidei, respectively. Broadly speaking the seasonal changes in infection levels of Asellus aquaticus with larval A. lucii are similar to those described by Awachie (1965) for Echinorhynchus truttae and Hine and Kennedy (1974b) for Pomphorhynchus laevis, both of which occur in Gammarus pulex.

The evidence presented here indicated that the size of the larval parasite population rose from mid-summer through autumn to a maximum the following spring. Since host population density decreased during this period the parasite actually infected an increasing proportion of a decreasing host population. Asellus aquaticus in the Forth and Clyde canal survived for a maximum period of approximately one year, so larval parasites probably survived, at the very most, for a slightly shorter time period. The fact that larval parasites never disappeared completely from isopods at any time of year

was caused by a combination of factors principally involving the considerable overlap of generations owing to the extended breeding period of the intermediate host, together with the prolonged period of shelled acanthor output by adult worms in the definitive host.

Although incidence and intensity indicated the number of hosts infected and the average number of worms per host they did not give any indication of the way in which the parasites were distributed amongst their hosts. This can be achieved by calculating the frequency of hosts with different numbers of parasites. The resulting frequency distribution can then very often be fitted to a theoretical distribution model. Cassie (1962) explained the value of such models. "The frequency distribution model may be applied at two levels, empirical and fundamental. Empirically, it is desirable to condense the sample data, so that any given population may be described by a few parameters which are readily comparable with the corresponding parameters of another population. The fundamental model, on the other hand, is based on some hypotheses of real biological significance. If it fits the data better than other possible models, it provides some justification for the hypotheses concerned. The principle must, however, be used with some caution since the same distribution may often be generated by different and even contradictory sets of postulates. Ideally,

the empirical and fundamental models are the same". In recent years this subject has received considerable attention among parasitologists. With only a few exceptions (Hopkins, 1959) nearly all parasites have been shown to have a clumped or overdispersed distribution, with a small proportion of the host population harbouring a large proportion of the parasites (Crofton, 1971 a ; Pennycuick, 1971c; Schmid and Robinson, 1972 ; Anderson, 1974a; Boxshall, 1974; Burrough, 1978 ; Skorping, 1980a, b).

The negative binomial (Fisher, 1941) has proved to be a good empirical model of such distributions. It is defined by two parameters. A positive exponent (k) and the population mean (μ). k varies inversely with respect to the degree of aggregation of the parasites within the sampling units, which in this case are the hosts (i.e. as k gets smaller the degree of aggregation of parasites among their hosts gets larger). With caution, the value of k can be used as an index of dispersion. Ideally when comparing k values the sample sizes should be similar and there should be little variation in the mean number of parasites per host.

Crofton (1971a) stated that "the negative binomial distribution is a 'fundamental' model of parasitism in so far as it described the distribution of parasites amongst hosts. That it is a 'fundamental model' as well as an empirical one can be

seen by examining the mathematical basis of the distribution and considering the hypothetical situations in which it could arise. These situations are completely translatable into parasitological terms". Both Crofton (1971a) and Pennycuick (1971c) described a number of situations which could give rise to a negative binomial distribution.

The relationship between the mean numbers of parasites per host ($\bar{x}$) and variance (s^2) gives an indication of the dispersion pattern of parasites amongst their hosts. If $s^2 = \bar{x}$ the parasites are randomly distributed (Poisson distribution). If $s^2 > \bar{x}$ then overdispersion is indicated. In the current study, in all samples where analysis was possible $s^2 > \bar{x}$, indicating overdispersion. Only in certain months was the data suitable for attempting to fit theoretical distribution models, but in each instance the negative binomial fitted the data well. The mean number of parasites per host varied little between samples, although the sample sizes were somewhat larger in the first few months of the study, so possibly some caution should be used with regard to using k as a comparative index of dispersion between samples. The values of k obtained were all < 0.4 which is low and suggests that the distribution of larval parasites in the intermediate host population is highly overdispersed and in accord with the pattern shown by many other parasites (see Anderson, 1978).

The mechanisms responsible for the generation of the negative binomial distribution of larval A. lucii in A. aquaticus are extremely difficult to determine since little is known about the feeding behaviour of A. aquaticus with respect to the spatial distribution of shelled acanthors in the environment. Although it is technically impossible to measure the spatial distribution of shelled acanthors there is good reason to suggest that it will depart from random. The distribution of adult worms in fish is highly aggregated (see Chapter 4) and furthermore the deposition of shelled acanthors appears to occur, principally by loss of whole gravid worms from fish, since no 'spent' worms were ever recovered and gravid worms were often found trailing from the anus of infected fish. Muzzall and Rabalais (1975a) made similar observations regarding Acanthocephalus jacksoni in its definitive hosts. Disintegration of whole gravid worms will result in a highly clumped distribution of shelled acanthors in the environment, which over a period of time could give rise to an aggregated input of larval parasites into isopods.

The maximum number of parasites recorded in a single isopod was 5 if all larval stages are included, or 3 if cystacanths are considered alone. It seems pertinent to ask why were heavier infections not recorded? Two explanations seem likely. The first is simply that considering the overall level of

infection was so low throughout the year, the probability of any isopod having ingested sufficient shelled acanthors to generate an individual level of infection of > 5 larval parasites (or > 3 cystacanths) approached zero. The second explanation is that larval A. lucii are highly pathogenic to A. aquaticus and isopods which ingest sufficient shelled acanthors to generate higher infection levels than those recorded are killed by the parasite. This may well be true (see Crofton, 1971a) but if so, one might reasonably expect to come across isopods with large numbers of small early larval stages (i.e. acanthor stages), since the parasites are not likely to exert their lethal effect until they become quite large relative to the size of the host. However, no such infections were ever found in the current study. Seidenberg (1973) noticed the disappearance of Asellus intermedius which were heavily infected with Acanthocephalus dirus. He attributed their disappearance to 'mortality caused by growth of the numerous larvae present in the early part of the parasites life cycle'.

Many species of larval acanthocephalan are known to exert a variety of pathological effects on their hosts (see Munro, 1953; Hynes and Nicholas, 1957; Seidenberg, 1973; Muzzall and Rabalais 1975b, c; Camp and Huizinga, 1979) and larval Acanthocephalus lucii have proved to be no exception,

which is not altogether surprising in view of the size of the parasite relative to the isopod host (see Plate 3.1).

Examination of large numbers (> 200) of infected isopods of breeding size (i.e. ≥ 6mm in length) during spring has shown that female isopods which harbour even a single cystacanth of *A. lucii* are sterile. In population terms sterilization is equivalent to death measured on a different time scale (Crofton 1971a). Crofton (1971a) stressed the importance of quantifying the degree of harm a parasite inflicts on its hosts and suggested the definition of a 'lethal level' whereby a given parasite would typically kill its host if infection reaches a certain level. Anderson (1978), in a theoretical consideration of the regulation of host population growth by parasites, felt it was more appropriate to consider the 'degree of harm' in terms of the effect of the parasite on parameters such as the natural intrinsic growth rate of the host population and he considered that the severity of the parasites influence on such host population parameters is likely to depend, in the case of an individual host, on the number of parasites harboured. He considered that a more precise understanding of the influence of parasitic infection on the natural intrinsic growth rate of the host population can be obtained from information relating host reproduction or survival to the number of parasites harboured. Ideally one should consider the whole range of classes within

Plate 3.1. Dorsal view of an 8mm long Asellus aquaticus with a female cystacanth of Acanthocephalus lucii alongside.

a frequency distribution of the number of parasites per host. With reference to larval A. lucii infecting A. aquaticus this is apparently not necessary. A single cystacanth renders a female isopod sterile and sterilization is equivalent to death, so there are effectively only two classes to consider, infected and uninfected isopods. It is important to note that female isopods harbouring early larval stages (i.e. acanthor, acanthella) were observed with brood pouches containing embryos which indicates that the parasite probably did not exert the sterilizing effect until it reaches the largest cystacanth stage. Experimental evidence has suggested that male isopods harbouring cystacanths are apparently less affected by the parasites and can fertilize female isopods quite normally (see Chapter 5).

The incidence of infection of both male and female A. aquaticus was shown to rise with increasing length of the isopod up to a maximum in medium sized isopods (8mm for males, 6mm for females). Thereafter incidence declined with further increase in isopod size. In other species of Acanthocephala where the relationship between intermediate host size and incidence of infection has been examined in field populations, various patterns have emerged, although in many instances a decline in infection levels in the largest individuals was evident. Seidenberg (1973) examined larval Acanthocephalus dirus in

Asellus intermedius (size range 4 - 16mm). In female isopods incidence increased linearly with isopod size. In males incidence rose in isopods up to 8mm in length and thereafter remained relatively constant. The relationship for male isopods was well described by a quadratic regression. Camp and Huizinga (1979) also examined larval Acanthocephalus dirus in Asellus intermedius (size range 2 - 17mm). Larvae were recorded in all isopods except the 17mm class. Prevalence (= incidence) varied significantly between host length classes and was lowest in smallest and largest isopods. The overall infection levels in the latter study were much lower than those described by Seidenberg. Hine and Kennedy (1974b) found that the incidence of Pomphorhynchus laevis in Gammarus pulex was low in individuals < 5mm in length, increased to about 30% in 9mm long gammarids, then declined sharply in those over 9mm in length. Muzzall (1978) examined larval Fessisentis friedi in the isopod Caecidotea communis (size range 2-17mm). Isopods < 3.9mm and > 13.0mm were seldom infected. Prevalence increased in isopods over 4.0mm in length and was maximum in medium sized isopods (8 - 8.9mm).

The fact that incidence rises with host length (at least initially) is not surprising and can be attributed to the fact that as isopod size increases the total volume of food eaten will increase and so the probability of having ingested

a shelled acanthor will rise accordingly. With respect to larval A. lucii and F. friedi medium sized isopods had the highest incidence of infection and in larger isopods incidence declined. The reasons for this decline are unknown but a number of interesting explanations are possible. Firstly, it should be pointed out that in A. lucii, at least, the decline is not due to host induced parasite mortality in the larger isopods. In none of the many thousands of isopods examined was there any evidence of encapsulation and destruction of larval parasites in the haemocoel. If this does occur it must be when the larval stages are extremely small (i.e. acanthor or early acanthella stages). The most attractive hypothesis to explain the decline is that large infected isopods are selectively preyed upon by the definitive host. It has been shown experimentally, at least, that perch selectively ate infected isopods (Chapter 5). However, the experimental design was such that it could not be ascertained if the perch selectively ate large infected isopods. Field data also support the hypothesis that perch ate large infected isopods since the rapid rise in infection levels of the definitive host coincided with the period when the largest isopods disappeared from the population. Unfortunately stomach content analysis of perch revealed little information on this subject.

An alternative explanation for the decline may be related

to the possible effect of the parasite on the growth rate of isopods, as has been suggested by Hine and Kennedy (1974b) for G. pulex infected with P. laevis. Cystacanths are large relative to the size of the isopod and it is quite possible that infected isopods will show reduced growth rates. If this is so then infected isopods will never reach the largest sizes attained by uninfected individuals. The degree of retardation in growth will depend on the size of the isopod when it initially becomes infected. This will vary considerably, giving rise to a gradual decline in incidence in the larger size groups. However, whether or not larval A. lucii retards the growth of A. aquaticus remains to be determined experimentally.

Cystacanth size was shown to increase with the size of the isopod host with female cystacanths typically larger than males in isopods of the same size. Whether this increase represents growth of parasite larvae once they reach the infective cystacanth stage is not known. The fact that female cystacanths were larger than males in isopods of the same size suggests that the size of the cystacanth is not limited simply by the available space in the haemocoel. Thus an extremely wide range of sizes of cystacanth were recovered from isopods and experimental infection of definitive hosts has indicated that both small and large cystacanths are infective. This led to a very wide range of sizes of adult worms in fish, even if all

parasites enter the fish at the same time. The significance of this finding will be discussed further in relation to growth of adult parasites in Chapter 6.

Although both male and female cystacanths tended to increase in size in larger isopods, the parasitization index revealed that relative to the size of the isopod, cystacanths are bigger in the smaller isopods. Small isopods have to support a relatively greater volume of parasite tissue, especially if the parasite is female. Rumpus and Kennedy (1974) showed that cystacanths of P. laevis alter the rate of respiration of G. pulex. Since cystacanths of A. lucii can weigh approximately 12 - 16% of the weight of their host (in small isopods) it would not be surprising if A. lucii has considerable effects on the respiration of A. aquaticus.

Perhaps the most striking influence of larval Acanthocephalus lucii was on the pigmentation of the isopod host. Preliminary studies (Brattey, 1979, 1980) showed that infected isopods undergo a distinctive 'blackening' of the large pleopods on the ventral surface of the abdomen (see Plate 3.2). Towards the end of the development of the larval parasite in the haemocoel (i.e. as the cystacanth stage is reached) the pleopods begin to darken, presumably due to localized deposition of melanin. The 'darkening' sometimes affects adjacent areas including the abdominal appendages, the reproductive pleopods and even the edges of the posteriormost thoracic segments. Dorsally, the

Plate 3.2. Ventral view of two 6mm long male Asellus aquaticus. The posterior three pairs of legs have been removed from both specimens. The specimen on the right is a normal isopod, the specimen on the left is infected with cystacanth(s) of Acanthocephalus lucii. Note the 'blackening' of the abdominal pleopods on the infected specimen.

pigmentation is less affected and when viewed from above infected and uninfected isopods usually, although not always, are identical. In some infected isopods a reduction in pigmentation of the dorsal surface was evident and the black pleopods were visible through the now semi-transparent dorsal surface. Since pigment patterns in uninfected A. aquaticus show such enormous variation, this feature may not be related to the presence of the parasite. It is important to note that every single isopod which had black pleopods harboured at least one cystacanth of A. lucii.

The pleopods on the ventral surface of the abdomen periodically beat up and down to maximize water flow over the respiratory surfaces, so while climbing among water plants the feature may well increase the conspicuousness of infected isopods, thereby increasing the probability of ingestion by the definitive host and so continuing the parasites life cycle. Numerous instances of parasite induced changes in host pigmentation by larval Acanthocephala have been recorded. Munro (1953) noticed that over 90% of A. aquaticus infected with an unknown larval acanthocephalan were darker than normal. Hindsbo (1972) observed G. lacustris infected with Polymorphus minutus to be blue in colour whereas they are normally brown. A reduction in pigmentation of Asellus intermedius, Lirceus lineatus and Caecidotea communis was observed when infected with

Acanthocephalus dirus, A. jacksoni and Fessisentis friedi, respectively (Seidenberg, 1973; Muzzall and Rabalais, 1975a; Muzzall, 1978; respectively).

For many species of Acanthocephala, pigmentation changes and/or altered behaviour have been shown, in experiments, to increase the susceptibility of intermediate hosts to predation by the definitive host (Bethel and Holmes, 1973; Kennedy, Broughton and Hine, 1978; Muzzall and Rabalais, 1975c; Camp and Huizinga, 1979). With respect to the current study no critical experimental evaluation of possible behavioural changes was undertaken but observation of numerous specimens in the laboratory suggested that infected isopods are more active and spend more time wandering on the surface of the substrate, whereas uninfected isopods are less active and tend to remain hidden, which is similar to the observations of Muzzall and Rabalais (1975c) on Lirceus lineatus infected with Acanthocephalus jacksoni.

In addition to larval stages of Acanthocephalus lucii a few isopods harboured cystacanths of another acanthocephalan tentatively identified as Filicollis anatis (Schrank), which occurs as an adult worm in the intestine of ducks. Isopods harbouring cystacanths of this species were noticeably darker than normal. Concurrent infections of A. lucii and F. anatis (?) were also found.

Numerous unidentified metacercariae were also noticed on various parts of the haemocoel of A. aquaticus. Although numbers were not counted, at certain times of the year a considerable proportion ($\sim$15%) of the isopod population were infected.

CHAPTER 4

FIELD STUDIES ON ADULT *ACANTHOCEPHALUS LUCII* IN THE DEFINITIVE HOST(S)

Chapter 4

Introduction

In the previous chapter it was emphasised that the numerical size of a population of adult helminth parasites in a definitive host can be influenced by two processes, namely immigration (recruitment) and mortality. With respect to Acanthocephalus lucii the size of the adult worm population in the intestine of perch will be a result of the balance between gain of worms (recruitment) from the ingestion of infected Asellus aquaticus and loss of worms (mortality) from the intestine of infected fish. Chubb, Awachie and Kennedy (1964), while referring to intestinal fish parasites, stressed that the significant point in the host parasite relationship, usually overlooked, was that the incidence of the parasite in the host throughout the year was the result of a dynamic rather than a static process. Even when infection levels remained constant this did not necessarily indicate that recruitment and mortality were not occurring but rather they were equal and opposed one another so that the system was in a state of dynamic equilibrium.

Many, although not all fish parasites, undergo seasonal cyclical fluctuations in numbers. These fluctuations are brought about by differential changes in recruitment and

mortality and perhaps the most interesting aspect of this system is establishing which factors bring about these changes. Walkey (1967) pointed out that since fish are poikilothermic, temperature changes in the environment are experienced by both host and parasite. In temperate zones freshwaters undergo considerable seasonal fluctuations with both host and parasite being subjected to a wide range of temperatures. If recruitment and mortality are differentially affected (directly or indirectly) by temperature change then this may well result in a seasonal cycle in parasite numbers (seasonal incidence cycle).

Following recruitment and establishment in the definitive host most parasites undergo a period of development and maturation, prior to the release of eggs or infective stages. The rate of maturation of the adult parasite depends on the environmental conditions and since in temperate regions these vary seasonally the actual rate of maturation may do likewise. Although some species of parasite appear to develop and mature at all times of the year in others maturation is restricted to periods when conditions are favourable. Consequently seasonal maturation cycles arise.

Kennedy (1975) stressed the importance of distinguishing maturation cycles from incidence cycles since there is no consistent correlation between them. Species which show seasonal incidence

cycles invariably show seasonal maturation, but in species where incidence remains relatively steady throughout the year, maturation does not necessarily occur throughout the year. It should be noted that there is of course no sharp transition between seasonal and non seasonal incidence cycles, so that a whole range of intermediate patterns between the two extremes can occur.

Seasonal maturation cycles undoubtedly influence incidence cycles, but the resultant outcome of that influence appears to depend on the nature of the life cycle of the species of parasite in question. In species with a direct life cycle, such as most ectoparasites, a seasonal maturation cycle results in the synchronous appearance of a whole new generation of eggs or infective stages. This is often followed by mortality of the breeding population of adult worms. A time lag then occurs during which the eggs reach an infective stage, followed by recruitment of larval parasites into the definitive host population. Clearly in such instances a seasonal maturation cycle results in a seasonal incidence cycle. In species which have an intermediate host in the life cycle the situation is more complex. Although maturation may be seasonal the larval stages may persist in the intermediate host throughout the year, enabling recruitment to take place throughout the year, with

the result being the absence of a well defined seasonal incidence cycle. Again adult parasites die soon after breeding so clearly maturation cycles can influence seasonality of incidence by affecting both recruitment and mortality.

Seasonal changes in the incidence and maturation of a variety of adult helminth parasites from freshwater fishes has been described in the literature and these have recently been reviewed by Chubb (1977, 1979, 1980, 1982). In spite of the vast amount of information available much of it is of little value with respect to describing population changes in terms of seasonal changes in recruitment and mortality. Many publications describe levels of infection over restricted periods of the year, in others sample sizes are too small for firm conclusions to be made. The situation is further complicated by the fact that some parasites show different cycles of occurrence and maturation in different habitats. Nonetheless a considerable amount of useful information is available in the literature.

Seasonal studies on the occurrence of Acanthocephala in freshwater fishes began with the works of Van Cleave (1916) on Neoechinorhynchus (= Gracilisentis) gracilisentis (Van Cleave, 1913) and Tanaorhampus (= Neoechinorhynchus) longirostris (Van Cleave) in the gizzard shad (Dorosoma cepedianum (Le Sueur)). More recent studies include those

on Acanthocephalus clavula Dujardin, 1845 (Chubb 1964; Pennycuick, 1971a, b, c; Rojanapaibul, 1977; Andrews, 1977), on A. jacksoni (Bullock, 1962) (Muzzall and Rabalais, 1975a, b, c), on A. parksidei (Amin, 1975) (Amin, 1975), on Echinorhynchus salmonis Muller, 1784 (Bauer and Nikolskaya, 1957; Tedla and Fernando, 1969, 1970; Leong, 1975; Amin and Burrows, 1977; Holmes et al, 1977; and recently Valtonen, 1981), on E. truttae Schrank, 1788 (Awachie, 1963, 1965), on Fessisentis friedi Nickol, 1972 (Fried et al, 1964; Muzzall, 1978), on Leptorhynchoides thecatus (Linton, 1891), (Pearse, 1924; DeGuisti, 1949), on Neoechinorhynchus cylindratus (Van Cleave, 1913) (Ward, 1940; McDaniel and Bailey, 1974; Eure 1976; Eure and Esch, 1974), on N. rutili (Müller, 1780) (Steinstrasser, 1936; Merritt and Pratt, 1964: Walkey, 1967; Chappell, 1969; Bibby, 1972), on N. saginatus Van Cleave and Bangham, 1949 (Muzzall and Bullock, 1978), on Octospinifer macilisentis (Van Cleave, 1919) (Harms, 1963, 1965), on Pomphorhynchus bulbocolli Linkins, 1919 (Lawrence, 1970; Esch et al, 1976) and on P. laevis (Müller, 1776) (Hine, 1970; Hine and Kennedy 1974a, b; Kennedy and Rumpus, 1977; Kennedy et al, 1978). Further references are given by Chubb (1982).

With respect to Acanthocephalus lucii a number of seasonal studies have been published. Although recorded from the intestine of a variety of freshwater fishes perch (Perca fluviatilus) are considered to be the principle definitive host. Komarova

(1950) found a pronounced seasonal occurrence of A. lucii in the perciid fish of the river Dniepr, U.S.S.R. Adult A. lucii deposited shelled acanthors in summer then perished, with a gradual lowering of the incidence and intensity of infection from June to September. Through autumn (October - November) there was a virtual absence of adult worms. In winter (December - January) incidence rose sharply but the worms were immature. In March - April incidence and intensity increased, the worms grew, but were still immature. At the end of spring and summer A. lucii were mature, the female worms being filled with shelled acanthors. Komarova considered that infection of the intermediate host occurred in summer and autumn, and infected isopods were eaten by perch in winter. Izyumova (1958) examined perch from the Rybinsk Reservoir, U.S.S.R. A high incidence of A. lucii occurred throughout the year with maximum (66.6%) in the May - July period and minimum (40% in October - November). Malakhova (1961) observed the occurrence of A. lucii in perch in Lake Konche, Karelia U.S.S.R. Maximum levels of infection were found in summer (incidence 62.2%. intensity 6.5, range 1-38) and minimum levels in winter (36.3%, 3.7 and 1-12, respectively). Mishra (1978) found A. lucii throughout the year in the Shropshire Union Canal, Backford, Cheshire. Incidence was highest between January and June (fluctuating between 61 - 85%)

but possible seasonal trends are obscured by the small sample sizes at certain times of the year. Shelled acanthors were observed in almost every month throughout the year and where absent could easily be attributed to sampling deficiency. Rizvi (1964) found A. lucii in perch in Rostherne Mere, Cheshire. Again the parasite was present throughout the year but small sample sizes made accurate conclusions about seasonality of occurrence impossible. Unfortunately, different stages of maturation were not examined by Rizvi. Halvorsen (1972) found no evidence of seasonality of occurrence or maturation of A. lucii in perch in the river Glomma, Norway. Andersen (1978) found A. lucii in perch throughout the year in Lake Røyetjern, Norway. A slight seasonal incidence cycle was evident. Highest incidence was found in summer and autumn (June - October) with up to 65% of the perch infected. During winter and spring (November - May) incidence dropped to a minimum of 15.4%. Lowest intensity of infection was recorded in winter (January - April, 1.3 worms/fish). Immature worms were recovered in every month throughout the year, and worms containing shelled acanthors were present in all samples except December - January. Moravec (1979) examined the seasonal occurrence of A. lucii in pike (Esox luscius L.) from the Macha Lake fishpond system, Czechoslovakia, which were secondarily acquired from eating infected perch. Maximum incidence and intensity occurred in May - June with minimum

levels in July - August. Female worms containing shelled acanthors were present in every month except December. Moravec believed that the occurrence of A. lucii showed partial quantitative changes, these apparently being evoked by water temperature and the availability of the intermediate host. He stressed that a dynamic equilibrium between recruitment and loss of worms was taking place continuously throughout the year. Priemer (1979) found A. lucii in perch in lakes on the outskirts of Berlin (Germany) throughout the year. Incidence was low (< 23%) at all times of the year, especially between June and October. Lee (1980) examined the occurrence of A. lucii in perch in the Serpentine, London, but due to the extremely low infection levels (overall incidence 7.8%, intensity 0.2 worms per fish) seasonal changes in infection levels and maturation could not be assessed.

A number of explanations have been given for the observed seasonal patterns of occurrence and maturation of the various Acanthocephala mentioned previously. The principle factors involved appear to include temperature induced changes in host feeding intensity, changes in the level of infection of intermediate hosts, and hence availability of infective larvae, and seasonal changes in the susceptibility of hosts to infection. It is clear from the many previous studies that a proper

understanding of the factors responsible for the observed seasonal pattern of occurrence of the parasite requires an extremely detailed study of the relationship between the parasite and both intermediate and definitive hosts. Although the results of many previous studies on the seasonal occurrence of A. lucii have been published much of the information is of little value to the current study. None of the previous workers undertook a simultaneous assessment of the seasonal occurrence of the larval parasite in the intermediate host. Few examined the seasonal changes in the feeding habits of the definitive host and in many instances different stages of maturation of adult worms were not recognised. Furthermore since A. lucii is a geographically widely distributed parasite (see Chapter 1) and the various regions in which it is found have considerable differences in climate, the precise nature and timing of the factors responsible for seasonal changes in maturation and population size are likely to vary considerably between different habitats.

Materials and Methods

The details concerning the site, methods of capture and examination of perch are given in the materials and methods section of Chapter 2. Additional materials and methods were

as follows: Following removal from perch, the intestine was divided into five sections of equal length, the first including the 3 pyloric caecae. Each section was placed in a labelled petri dish containing saline, slit open and thoroughly searched for helminth parasites with the aid of a binocular microscope. The number, sex and position of each specimen of A. lucii was recorded. Female worms were teased open on microscopic slides to assess their stage of maturity. They were assigned to one of three stages. Stage 1 - immature worms with ovarian balls only. Stage 2 - fertilized worms with a mixture of ovarian balls and immature acanthors. Stage 3 - mature worms with shelled acanthors and (usually) a mixture of ovarian balls and immature acanthors. Male worms complete their development in the intermediate host, so different stages cannot be recognized. All additional intestinal helminth parasites were also removed and identified.

In addition to perch, some pike (Esox luscius L.) and roach (Rutilus rutilus (L.)) were captured. The method of examination was as for perch except the ages of the fish were not determined and the intestine was not divided into sections prior to removal of worms. Again all species of parasite were removed and identified.

Results

Incidence and intensity of infection

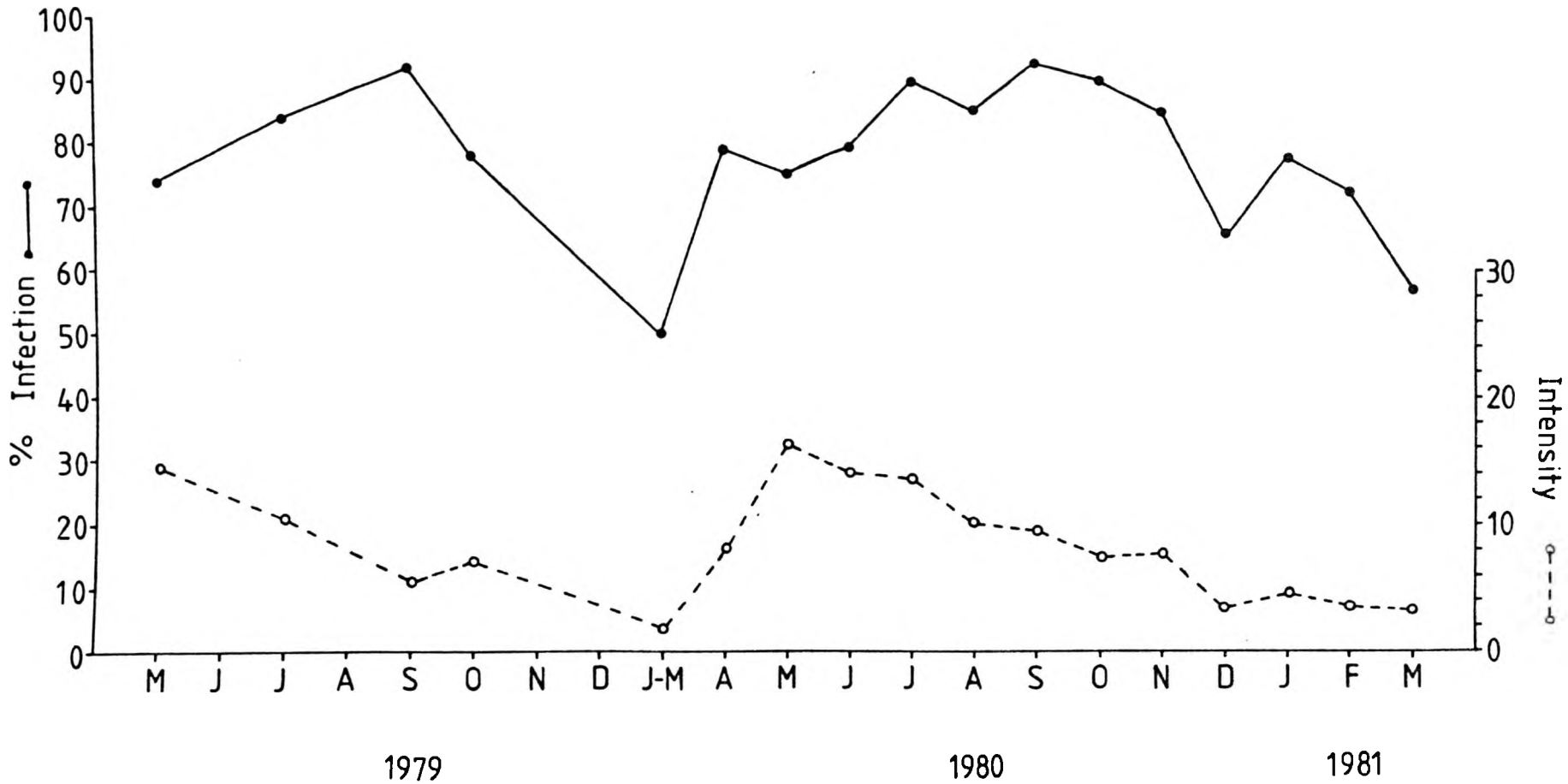
A total of 525 perch from 17 samples collected over a period of 22 months were examined during the course of the study. Overall 417 (79.4%) were infected and a total of 4251 specimens of A. lucii recovered. Average intensity was 8.1 worms/fish. 1896 male worms were recovered and 2355 females, which differs significantly from a 1:1 sex ratio $\chi^2 = 49.56$ ($p < 0.001$) with 1 D.F.

Sample sizes, together with seasonal changes in the incidence and intensity of infection over the approximately two year period are given in Table 4.1 and Figure 4.1. It should be noted that data for 1979 was limited to only 4 samples and data for the January - March period of 1980 was pooled owing to difficulties in obtaining sufficient fish. Nonetheless a clear repeating pattern was apparent in the results. Incidence was very high (73.9 - 91.7%) in May, July and September 1979 but declined somewhat by January - March 1980 (50%). By April 1980 incidence had risen again (78.9%) and remained high through summer until September - October when approximately 90% of the fish were infected. From November 1980 onwards there was evidence of a slight downward trend in incidence to a minimum

Table 4.1. The incidence and intensity of infection of perch (Perca fluviatilus) with Acanthocephalus lucii, in the Forth and Clyde canal, Scotland, from May 1979 until March 1981, together with the total numbers and sex of all the worms recovered from each sample

	1979					1980										1981	
	May	Jul	Sep	Oct	Jan-Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
Nos fish examined	23	24	24	27	14	19	20	29	38	41	41	30	33	55	59	29	19
Nos fish infected (%)	17 (73.9)	21 (87.5)	22 (91.7)	21 (77.8)	7 (50)	15 (78.9)	15 (75)	23 (79.3)	34 (89.5)	35 (85.4)	38 (92.7)	27 (90)	28 (84.9)	36 (64.5)	46 (77.9)	21 (72.4)	11 (57.9)
Total no. worms	344	251	140	190	28	150	324	405	515	414	382	224	261	182	269	108	64
Intensity (per fish)	14.9	10.5	5.8	7.0	2.0	7.9	16.2	14.0	13.6	10.1	9.3	7.5	7.9	3.3	4.6	3.7	3.4
Intensity (per infected fish)	20.2	12.0	6.4	9.0	4.0	10.0	21.6	17.6	15.2	11.8	10.1	8.3	9.3	5.1	5.9	5.1	5.8
Male worms	142	129	75	85	9	62	153	215	235	222	164	96	87	60	108	34	20
Female worms	202	122	65	105	19	88	171	190	280	192	218	128	174	122	161	74	44

Figure 4.1. Seasonal changes in the incidence and intensity of infection of perch with *Acanthocephalus lucii* in the Forth and Clyde canal, Scotland.



of 57.9% in March 1981. In summary incidence appeared to exhibit a slight seasonal cycle, remaining high from late spring right through summer and autumn then declining slightly in mid-late winter.

Intensity exhibited a much more pronounced seasonal cycle. In 1979 intensity was highest in May (14.5 worms/fish) decreased through July and September, and although a slight increase was evident in October, by January - March of 1980 intensity had declined to minimum levels of approximately 2 worms per fish. A similar cycle was evident in 1980-81 when samples of perch were more regular and more frequent. Maximum intensity was found in May (16.2 worms/fish) and thereafter declined steadily through summer and autumn to minimum levels of approximately 3 - 4 worms per fish in mid winter - early spring (December - March).

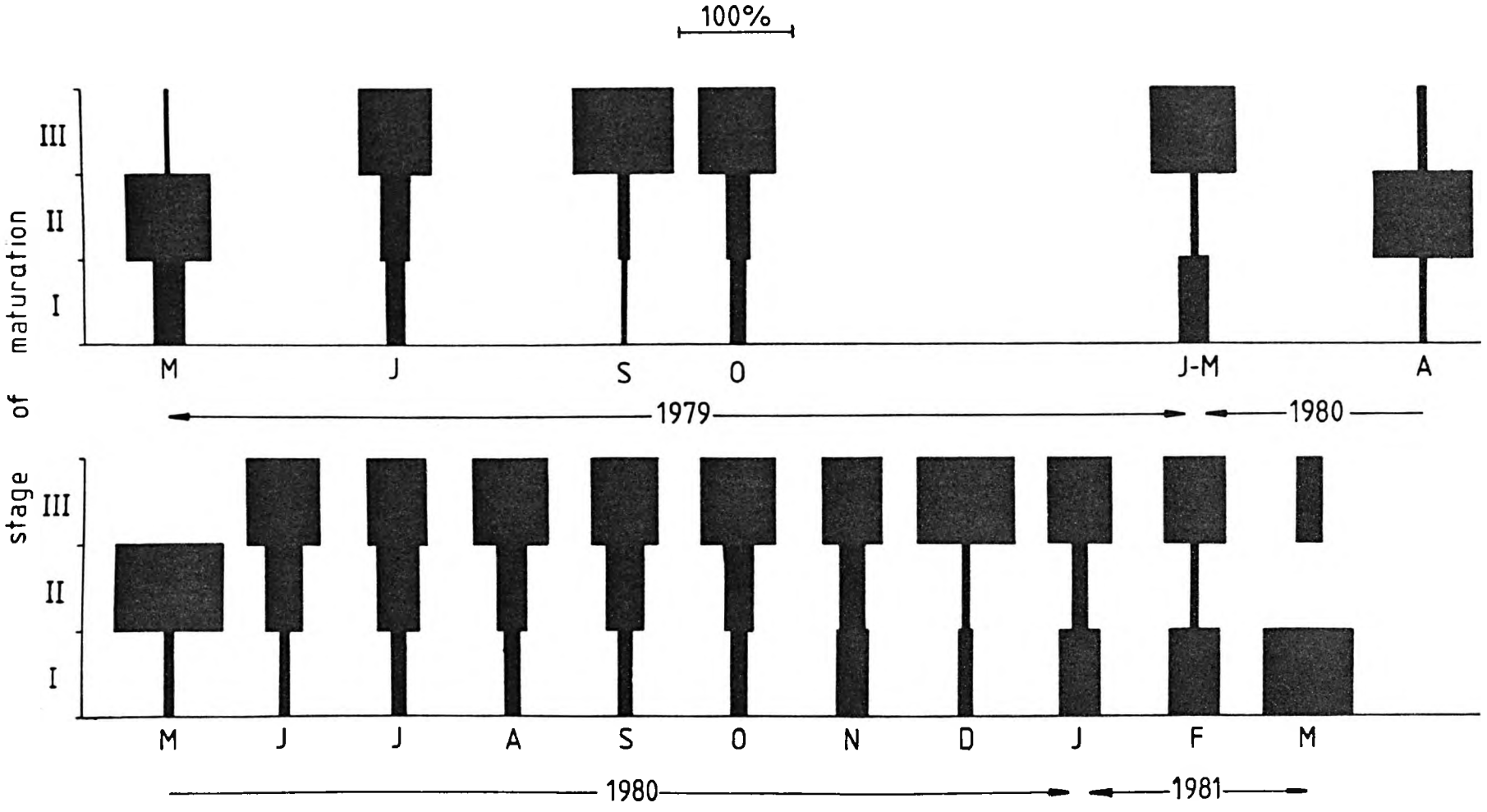
Seasonal changes in the maturation of female worms

Table 4.2 and Figure 4.2 indicate the seasonal changes in the stage of maturation of female worms. Results are expressed for each stage as a proportion of the total number of female worms recovered from each sample. Although data for 1979 were limited to only 4 samples the results for the respective months of 1980-81 were much the same indicating a similar

Table 4.2. Seasonal changes in the total number of female Acanthocephalus lucii recovered from each sample of perch from the Forth and Clyde canal, Scotland, together with the number and proportion of females at each stage of maturation

	1979					1980										1981	
	May	Jul	Sep	Oct	Jan-Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Number of fish examined	23	24	24	27	14	19	20	29	38	41	41	30	33	55	59	29	19
number of female worms recovered	202	122	65	105	19	88	171	190	280	192	218	128	174	122	161	74	44
Stage 1	50	17	2	12	5	3	12	14	32	23	23	16	48	13	56	32	35
(%)	(24.8)	(13.9)	(3.1)	(11.4)	(26.3)	(3.4)	(7.0)	(7.4)	(11.4)	(12.0)	(10.6)	(12.5)	(27.6)	(10.7)	(34.8)	(43.2)	(79.5)
Stage 2	147	29	6	21	1	79	159	58	105	46	68	30	38	8	18	4	0
(%)	(72.8)	(23.8)	(9.2)	(20)	(5.3)	(89.8)	(93)	(30.5)	(37.5)	(24.0)	(31.2)	(23.4)	(21.8)	(6.6)	(11.2)	(5.4)	(-)
Stage 3	5	76	57	72	13	6	0	118	143	123	127	82	88	101	87	38	9
(%)	(2.4)	(62.3)	(87.7)	(68.6)	(68.4)	(6.8)	(-)	(62.1)	(51.1)	(64.0)	(58.2)	(64.1)	(50.6)	(82.7)	(54)	(51.4)	(20.5)

Figure 4.2. Seasonal changes in the maturation of female Acanthocephalus lucii in perch from the Forth and Clyde canal, Scotland.



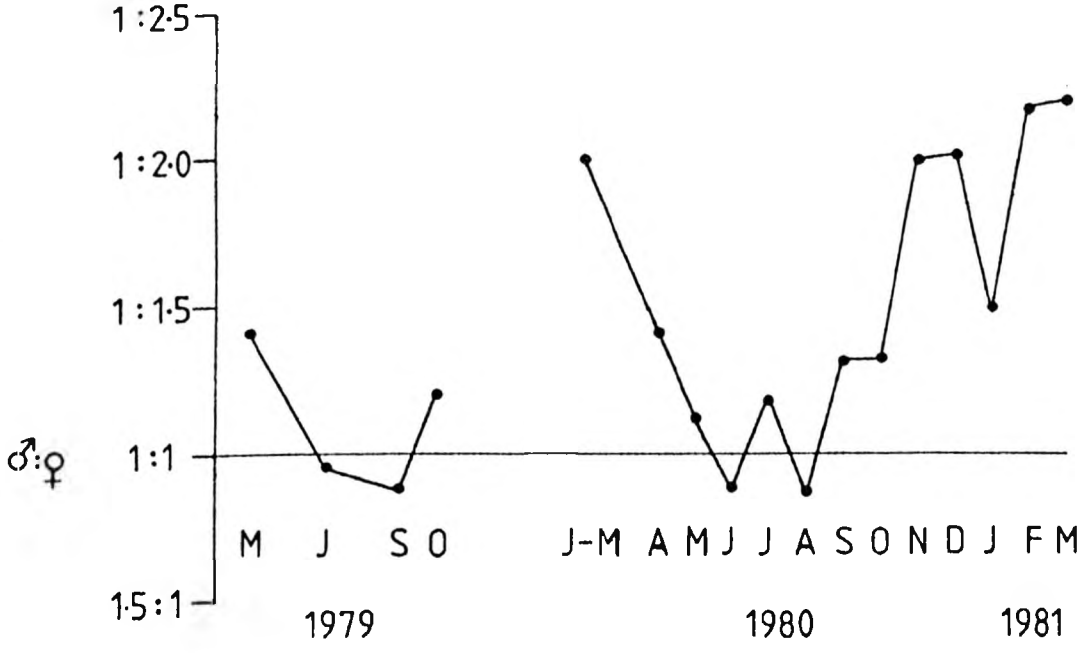
cycle in both years. Stage 1 worms were recorded in every sample collected, indicating that recruitment occurred throughout the year. Table 4.2 and Figure 4.2 indicate a gradual increase in both the mean number and proportion of stage 1 worms between December 1980 and March 1981. During this period both the number and proportion of stage 3 worms declined. Overall the level of infection declined at this time so the rate of mortality of female worms at stage 3 exceeded the rate of recruitment of those at stage 1. Stage 2 worms were recorded in every sample except in March 1981. A very high proportion of stage 2 worms were present in May 1979 and April and May 1981. This indicated that following fairly rapid recruitment of stage 1 worms, probably around April, further development of worms commenced very soon after they established. By July in 1979 and June in 1980 the majority of female worms had reached maturity, their body cavities being filled with mature shelled acanthors. A similar pattern of proportions of the three stages were present from early summer (June - July) right through autumn until mid-winter, but during this period the overall level of infection declined steadily. This suggests that although recruitment occurred throughout the year, from approximately June onwards mortality exceeded recruitment and a net loss of

worms occurred. The rapid rise in the level of infection which was apparent in May 1980 indicates that recruitment exceeded mortality only for a very short time, between approximately March and May.

Seasonal changes in sex ratio

Seasonal changes in the sex ratio of adult Acanthocephalus lucii are indicated in Figure 4.3. Whilst it is appreciated that no data were available for much of 1979 overall the results still indicate a seasonal change in the sex ratio. In the summer of both 1979 and 1980 the sex ratio was close to 1:1. Between May 1980 and August 1981 a total of 1658 parasites were recovered, 833 males and 825 females, which does not differ significantly from a 1:1 sex ratio. $\chi^2 = 0.04$ ($p > 0.5$). However in winter, as Figure 4.3 indicates, the sex ratio was distinctly in favour of female worms. Between November 1980 and February 1981 a total of 820 worms were recovered, 289 males and 531 females, which is highly significantly different from a 1:1 sex ratio. $\chi^2 = 71.42$ ($p < 0.001$). Since intensity fell through autumn until mid winter, the changes in sex ratio indicate that the decline was undoubtedly due, at least partly, to a proportionately greater mortality of male worms.

Figure 4.3. Seasonal changes in the sex-ratio of Acanthocephalus lucii in perch.



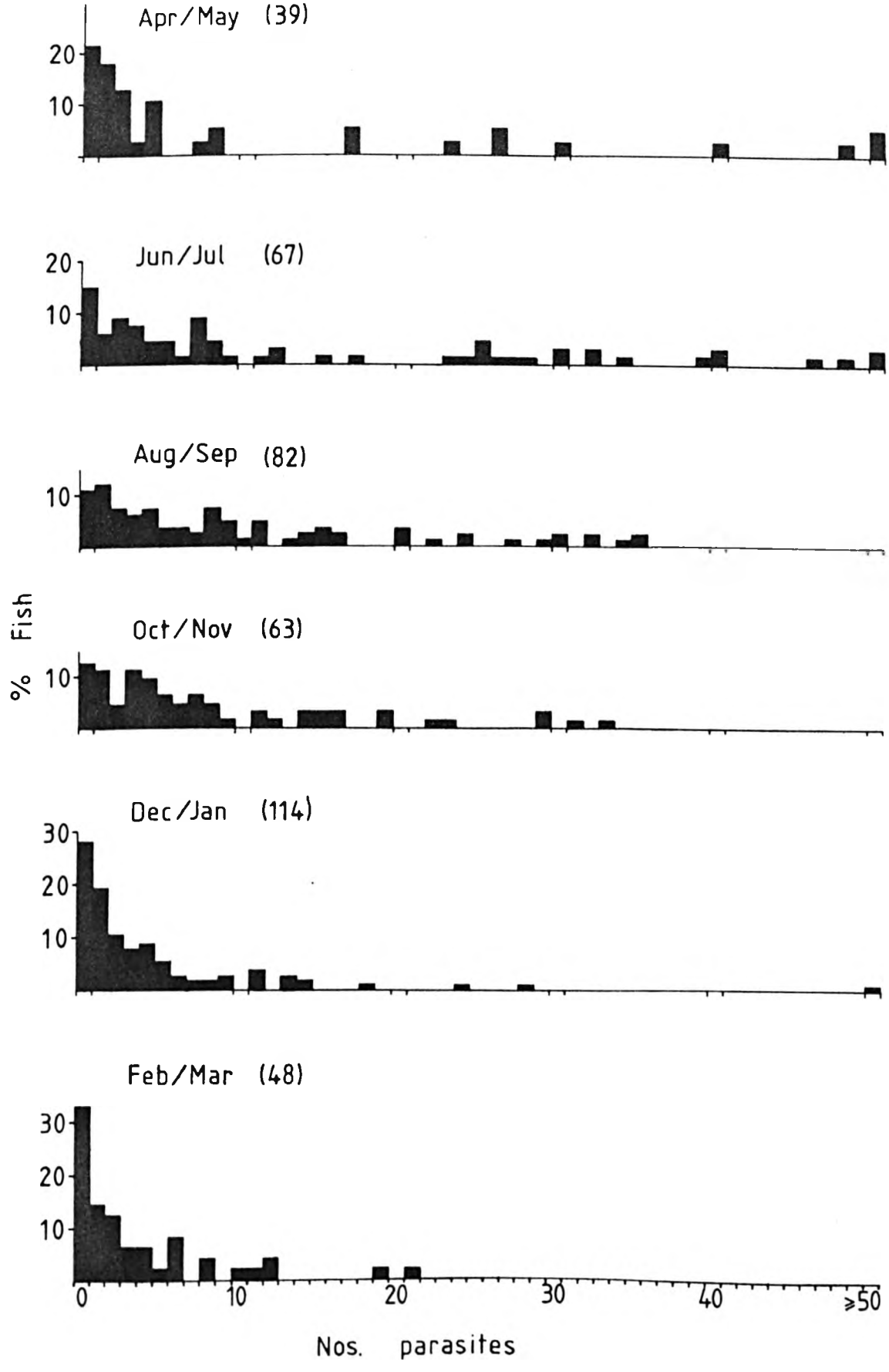
Seasonal changes in the frequency distribution of adult *Acanthocephalus lucii*

Seasonal changes in the frequency distribution of adult *A. lucii* in perch are indicated in Figure 4.4. Only samples from April 1980 until March 1981 are included in this analysis since the samples were larger and more regular during this period. The sample sizes in individual months were, however, still small relative to the large number of classes in the frequency distribution so results from individual months were paired as indicated.

In each of the samples the variance was much greater than the mean, indicating a very high degree of overdispersion. A clear seasonal change in the variance to mean ratio is evident. The variance to mean ratio was maximum in April - May (42.14) and thereafter decreased steadily to a minimum of (6.68) in February - March. Figure 4.4 indicates a gradual disappearance of the most heavily infected fish from the samples from April - May onwards, which explains the gradual decline in intensity described previously.

No attempt was made to fit theoretical distribution models to the data since the sample sizes were still too small in relation

Figure 4.4. Seasonal changes in the frequency distribution of Acanthocephalus lucii in perch (the figure in parentheses denotes the number of fish on which each histogram is based).



to the very high degree of overdispersion observed. (In each paired monthly sample > 20% of the classes in the frequency distribution had observed values of less than 5).

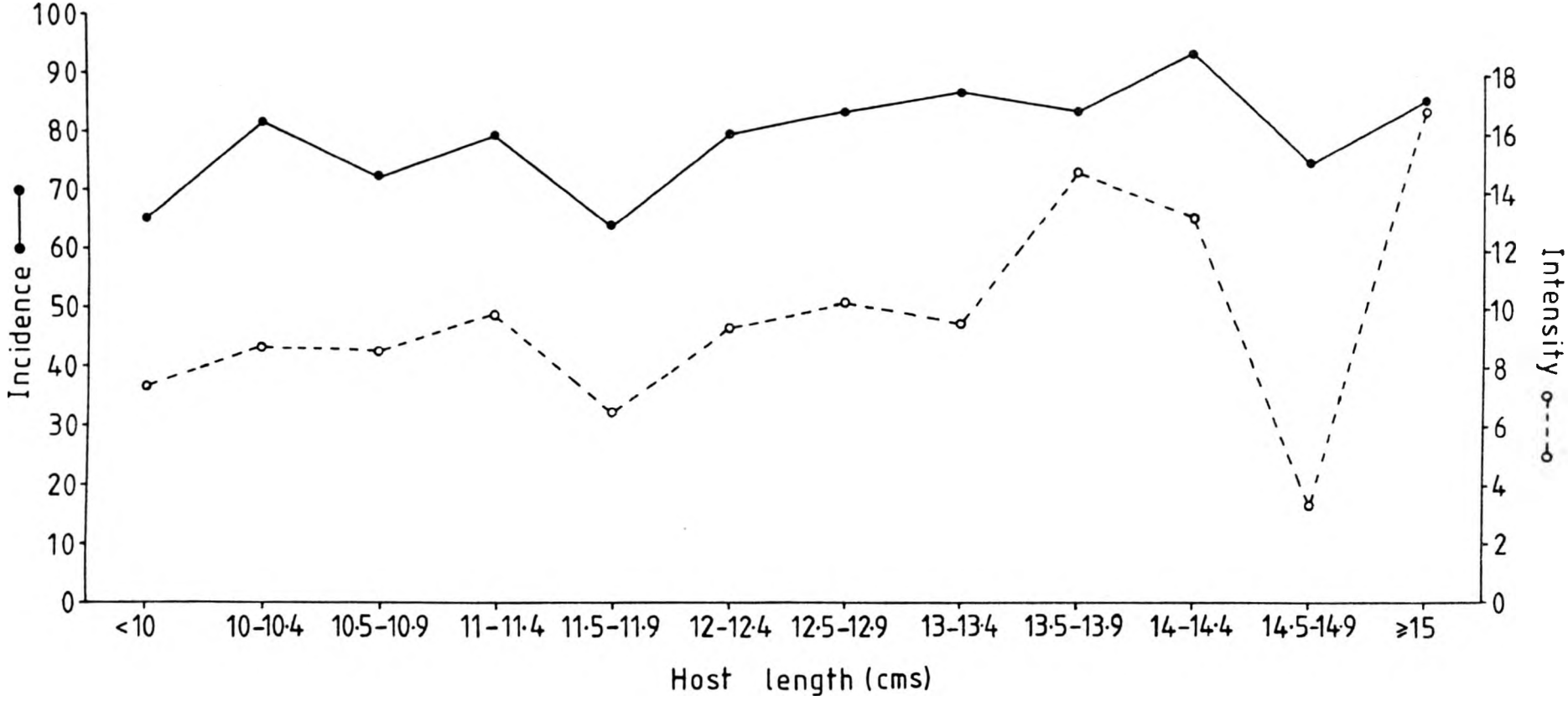
Levels of infection in perch of different sizes

The incidence and intensity of infection of perch in each 0.5cm length group is given in Table 4.4 and Figure 4.5. Only a limited size range of perch were captured (see Chapter 2). Consequently the relationship is only described for perch between 10 and 15cms in length. Few perch outside this size range were captured so data for perch $\geq$ 15cms and < 10cms in length were pooled. Table 4.4 indicates that incidence was high (64 - 93.8%) in all length groups and there was no evidence of any noticeable trend with increasing length of perch. Intensity showed evidence of a slight increase with increasing fish length but owing to the highly overdispersed nature of the distribution a single heavily infected fish in a given length group could considerably influence overall intensity. The extremely low intensity of infection of the 14.5 - 14.9cms length group can be attributed to the small sample size.

Table 4.4. Data for the relationship between incidence and intensity of infection and perch length (illustrated in Figure 4.5)

	Length group (cms)											
	<10	10-10.4	10.5-10.9	11.0-11.4	11.5-11.9	12.0-12.4	12.5-12.9	13.0-13.4	13.5-13.9	14.0-14.4	14.5-14.9	⩾15
Nos. fish examined	20	27	36	54	64	84	79	54	37	16	8	43
Infected (%)	13 (65)	22 (81.5)	26 (72.2)	43 (79.6)	41 (64.1)	67 (79.8)	66 (83.5)	47 (87)	31 (83.8)	15 (93.8)	6 (75)	37 (86.1)
Total worms	95	190	221	420	264	627	675	448	454	196	19	622
Intensity	7.3	8.6	8.5	9.8	6.4	9.4	10.2	9.5	14.7	13.1	3.2	16.8

Figure 4.5. The incidence and intensity of Acanthocephalus lucii in various sizes of perch (see Table 4.4).



The distribution of adult worms in the alimentary canal of perch

The distribution of adult male and female A. lucii in the alimentary canal of perch is given in Figure 4.6. The alimentary canal was divided into six regions these being the stomach plus five intestinal sections of equal length. Only data for the period April 1980 until March 1981 are given and samples were paired as in Table 4.3. The distribution of male and female worms is given separately in the upper and lower part of each histogram, respectively. Shading was used to represent female worms at different stages of maturation as in Figure 6.4. Worms recorded in the stomach were invariably cystacanths often still inside the undigested Asellus aquaticus. The presence of cystacanths in the stomach gives positive evidence of recruitment of larval parasites into the perch population (provided that mechanisms to prevent establishment do not exist). The absence of cystacanths from the stomach in certain months should not, of course, be taken to indicate that recruitment does not occur in that particular month.

Both male and female worms were recorded from all sections of the intestine and at almost all times of the year. There was no evidence of seasonal changes in the pattern of distribution of either male or female worms along the intestine. However,

Figure 4.6. Seasonal changes in the distribution of male and female Acanthocephalus lucii along the alimentary canal of perch.

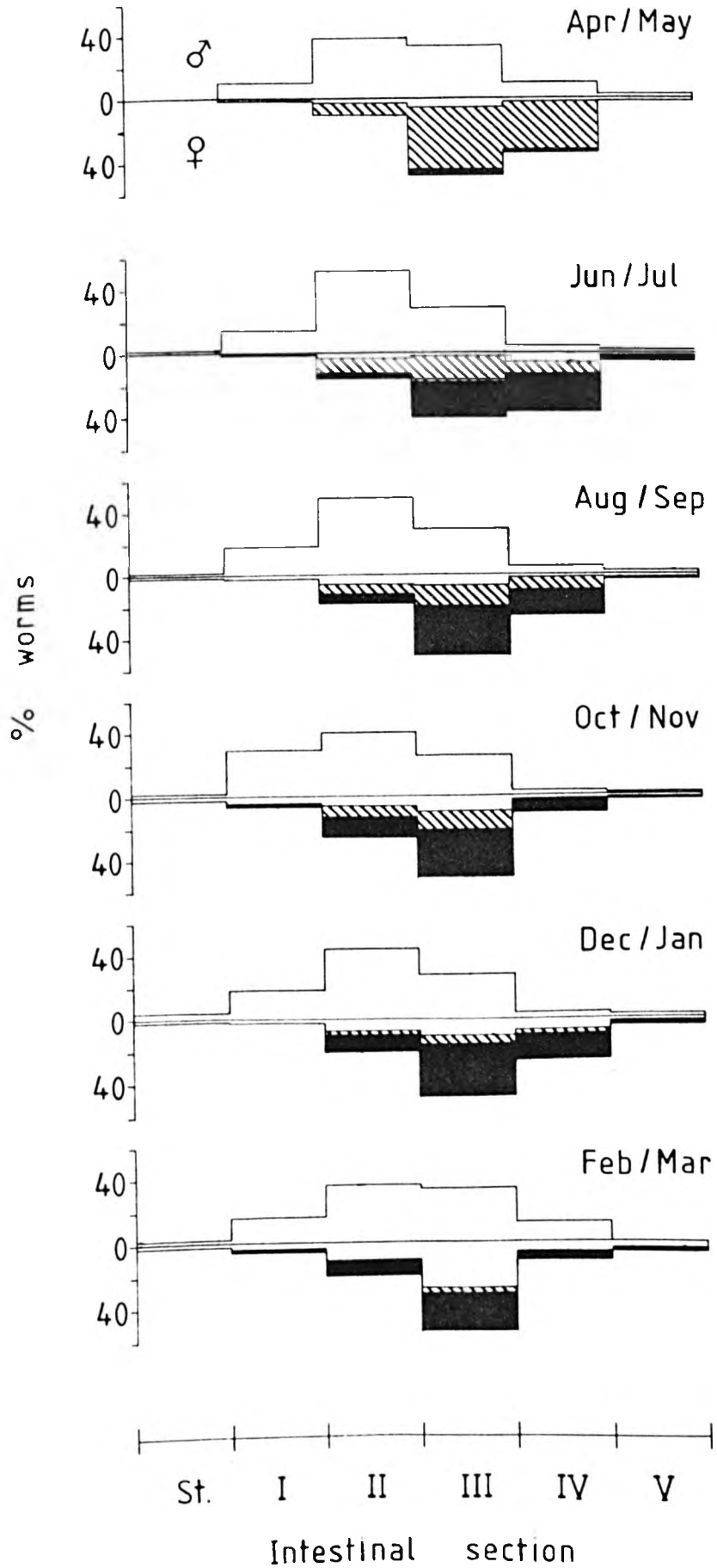


Table 4.5. Changes in the proportion of mature (stage 3) female *Acanthocephalus lucii* in different regions of the intestine of perch*

Intestinal+ regions	Total number of female worms	Total number of stage 3 female worms	Percentage stage 3 female worms
0-20%	38	11	28.9
21-40%	342	117	34.2
41-60%	864	452	52.3
61-80%	496	281	56.6
81-100%	88	61	69.3

* Data pooled for all female worms recovered from April 1980 until March 1981

+ expressed as % posterior from pylorus

worms were consistently more abundant in certain sections of the intestine. Considering male worms first, very few were found in sections 1 and 5, but in every sample males were most abundant in section 2. Female worms were also rare in sections 1 and 5, but were most abundant in section 3, indicating a difference in the distribution between the two sexes, males being more abundant in a more anterior position. No worms were ever found in the pyloric caeca. The total number of female worms in each region of the intestine, together with the proportion of mature (stage 3) female worms (data pooled for the entire sampling period April 1980 until March 1981) is given in Table 4.5. An increase in the proportion of stage 3 worms was evident in the more posterior regions of the intestine. This suggests a posterior migration of female worms with maturation.

Occurrence of Acanthocephalus lucii in other species of fishes

In addition to perch, small numbers of pike (Esox luscius) and roach (Rutilus rutilus) were also obtained from the Forth and Clyde canal. The results of the occurrence of A. lucii in these hosts is summarized in Table 4.6. Pike were quite

Table 4.6. The occurrence of *Acanthocephalus lucii* in pike (*Esox luscius*) and roach (*Rutilus rutilus*) from the Forth and Clyde canal, Scotland

	pike	roach
Number of fish examined	19	6
Infected (%)	12 (63.2)	2 (33.3)
Total no. worms recovered	196	8
Maximum intensity	68	6
Mean intensity	10.3	1.33
Male worms	77	3
Female worms	119	5
Stage 1 (%)	51 (42.9)	4 (80)
Stage 2 (%)	64 (53.8)	1 (20)
Stage 3 (%)	4 (3.4)	- -

heavily infected with A. lucii, the overall figure for intensity of 10.3 worms/fish was in fact higher than that for perch (8.1 worms/fish). Female worms were more abundant than males and the figures differ significantly from a 1:1 sex ratio $\chi^2 = 9$ ($p < 0.01$) with 1 D.F. Very few mature female worms were recorded from pike.

Although 2 of the roach from the canal were infected with A. lucii, too few fishes were examined for further conclusions to be drawn.

Discussion

The size of the population of adult Acanthocephalus lucii in perch in the Forth and Clyde canal clearly undergoes a seasonal cycling or periodicity. Previous studies (Van Cleave, 1916; Steinstrasser, 1936; De Guisti, 1949; Komarova, 1950; Tedla and Fernando, 1970; Amin, 1975; Muzzall and Rabalais, 1975a; Camp and Huizinga, 1979) have revealed that seasonal cycles occur in a number of species of Acanthocephala parasitizing freshwater fish, although the nature and timing of the cycles appears to vary between species and even within a single species in different habitats. During the course of the current study, although a considerable amount of detailed information has been collected concerning both host and parasite, it is

clear that a whole complex of factors are involved in the generation of the observed seasonal cycle and it should be appreciated that many interpretations of the data are possible. Field data alone cannot provide all the answers and whilst it is hoped that at least some parts of the following interpretations prove to be accurate, this can only be unequivocally demonstrated when a considerable amount of further field and experimental work has been undertaken.

Much of the information collected supports the suggestion that recruitment of larval parasites into the perch population occurred throughout the year. Infective cystacanths were present all year, stomach content analysis revealed the presence of the intermediate host in perch stomachs in almost every sample and when absent this could easily be attributed to a sampling deficiency. Furthermore immature (stage 1) female worms were also recorded from samples at all times of the year.

Seasonal changes in the actual rate of recruitment are more difficult to assess from the available data. It was unfortunate that sample sizes between the months of January and May were so low since this appears to be a critical period in the life cycle of the parasite. Nonetheless, a reasonable assessment of the seasonal changes in the recruitment rate would be as follows: from January until March water temperatures

in the canal were typically below 5°C and so feeding intensity of perch was probably very low. A few isopods were definitely eaten by perch at this time, some were infected and so recruitment occurred, albeit at a very slow rate. Between March and May water temperatures began to rise quite rapidly, stimulating perch feeding activity. Some isopods were infected, perch fed on them and rapid recruitment of larval parasites into the perch population took place. Some indication of the potential rate of recruitment is given by the fact that a single perch caught in May 1980 had 73 specimens of Asellus aquaticus in its stomach, 10 of which were infected, harbouring a total of 13 cystacanths of A. lucii. One can assume that all the A. aquaticus present in that stomach had been eaten within the previous 24 hrs so clearly extremely high recruitment rates are possible. In mid summer (July and August) perch continued to feed on A. aquaticus although very few isopods contained cystacanths at this time and so recruitment rates declined. Through autumn (September - November) declining water temperatures resulted in a reduction in perch feeding intensity, so although an increasing proportion of the isopod population became infected at the time, recruitment rates probably declined further to minimum levels in mid winter - early spring (December - March).

Evidence of mortality of worms from field data came

from two sources. Analysis of seasonal changes in the mean number of mature (stage 3) female worms per fish (see Figure 3.3, Chapter 3) indicated mortality of mature female worms from June 1980 until the following March. However, as was emphasized in Chapter 3, Figure 3.3 only gives an indication of seasonal changes in the net mortality of mature female worms.

Whilst Figure 3.3 indicates that mortality of mature female worms occurred from June until March it gives no indication of seasonal changes in the rate of flow of parasites through the perch population. The actual rate of flow (i.e. recruitment and mortality rates) is difficult to assess but using a combination of field and experimental data (from Chapter 6) it seems reasonable to assume that the rate of flow will be higher in late-spring and summer, since warmer water temperatures have been shown (see Chapter 6) to stimulate perch feeding activity and also increase both the rate of maturation and mortality of adult worms (i.e. high recruitment and mortality rates). In autumn and winter declining water temperatures have been shown to result in a reduction in perch feeding activity and in the rate of maturation and mortality of worms (low recruitment and mortality rates).

Further evidence for adult worm mortality is provided by an examination of the seasonal changes in sex ratio of

adult worms. The level of infection of perch declined steadily through autumn and winter. As indicated above the decline was at least partly owing to mortality of mature female worms. Since the sex ratio of the remaining population changed progressively in favour of females as winter approached this indicates a higher rate of mortality of male worms. An earlier elimination of male worms has been noted in many species of Acanthocephala including Moniliformis dubius (Burlinghame and Chandler, 1941; Crompton and Walters, 1972), Echinorhynchus truttae (Awachie, 1966), Acanthocephalus parksidei (Amin, 1975), Echinorhynchus salmonis (Amin and Burrows, 1977) and Acanthocephalus dirus (Camp and Huizinga, 1979). In summary, the only source of worm mortality for which there is positive evidence from the field data appears to be death of both male and female worms after reproduction.

In the interpretation of the data described above it was suggested that recruitment, although changing seasonally, probably occurred throughout the year. There was no doubt that at least some cystacanths are ingested by perch at all times of the year. However one important factor which has not yet been considered is that although they were undoubtedly ingested throughout the year, there might well be seasonal changes in the proportion which actually established. A number of

factors, such as water temperature or the presence of an existing infection could influence establishment. For example Kennedy (1972) has shown that warmer water temperatures reduced the proportion of Pomphorynchus laevis which established in goldfish (Carassius auratus). Experimental evidence (see Chapter 6) does not support this suggestion for A. lucii in perch.

The field data are consistent with the hypothesis that the presence of an existing infection reduced the establishment of subsequent infections although it is stressed that this remains to be proven. The various mechanisms which could be responsible are discussed in Chapter 7. This may explain the rather unusual situation whereby intensity declined through summer whereas incidence remained high or possibly showed a tendency to increase slightly. The field study has shown that by May approximately 75% of the perch population were infected with A. lucii and intensity was at a maximum of about 21 worms per infected fish. During summer the rate of ingestion of cystacanths by perch probably declined somewhat, owing to a reduction in the availability of infected isopods. From May onwards intensity declined steadily. Incidence, however, did not and in fact there was evidence of a slight increase and by September approximately 90% of the perch were infected. This indicates that previously uninfected perch acquired infection during

the summer months. As the frequency distribution analysis indicated, the decline in intensity was principally owing to a disappearance of the most heavily infected fish from the samples. Such fish were infected by May, but through summer loss of worms in these fish greatly exceeded gain (mortality exceeded recruitment). This could well have been due to the fact that the presence of an existing heavy infection reduced the establishment of subsequent infection during the summer months.

The field data provide two sources of evidence which indicate that the entire perch population (i.e. both infected and uninfected fish) does not become resistant to infection during the warmer summer months. Firstly, as discussed above, incidence appears to rise slightly through summer which indicates that new infections are acquired during this period. Secondly, seasonal changes in the proportion of female worms at each stage of maturation revealed the presence of immature (stage 1) worms throughout the summer months. Experimental studies have indicated that female worms mature very rapidly at warm summer temperatures suggesting that in summer immature worms represent recent acquisitions.

Although the data are entirely consistent with the hypothesis that the presence of an existing infection reduced

the proportion of subsequent infections which establish this remains to be unequivocably demonstrated. In fact, experimental studies (Chapter 6) suggest that it is not necessary to postulate the occurrence of such a mechanism to explain the observed seasonal changes in infection levels. Laboratory infection experiments have indicated that warmer water temperatures increased the rate of mortality of adult A. lucii. The full implication of this result, with respect to the seasonal dynamics of the adult worm, will be discussed in Chapters 6 and 7.

The seasonal pattern of occurrence exhibited by Acanthocephalus lucii in the Forth and Clyde canal is apparently very similar to that described by Andersen (1978) for A. lucii in perch in a small oligotrophic lake in Norway. The pattern is, however, somewhat different from not only all other studies on A. lucii (Izyumova, 1958; Rizvi, 1964; Mishra, 1966; Halvorsen, 1972; Priemer, 1979) but also to the patterns shown during studies on all other acanthocephalan parasites of freshwater fish where the seasonal occurrence has been examined in sufficient detail to permit comparison. Although a number of species show certain similarities to A. lucii in the Forth and Clyde canal, the overall pattern of occurrence differs. Recruitment of larval A. lucii into the perch population apparently occurred throughout the year. Incidence remained high through summer and autumn and declined in late winter - early spring.

Intensity was maximum in May and declined thereafter. Other species also showed recruitment throughout the year, but a different overall pattern of occurrence resulted. Awachie (1965) showed that recruitment of larval Echinorhynchus truttae into brown trout (Salmo trutta) occurred throughout the year in the Afon Terrig, North Wales. A seasonal incidence cycle was not observed and intensity was maximal in summer and minimal in winter. Hine and Kennedy (1974b) observed recruitment of larval Pomphorhynchus laevis into dace (Leuciscus leuciscus) throughout the year, but no seasonal cycle of incidence or intensity was observed.

In the many species of Acanthocephala which do exhibit seasonal cycles in both incidence and intensity of infection recruitment is restricted to a particular period of the year, typically during the cooler months of autumn and winter. Examples of species which exhibited such a pattern include Gracilisentis (= Neoechinorhynchus) gracilisentis (Van Cleave, 1916), Neoechinorhynchus rutili (Steinstrasser, 1936), Echinorhynchus salmonis (Tedla and Fernando, 1970), Neoechinorhynchus cylindratus (Eure, 1976), Acanthocephalus parksidei (Amin, 1975), A. jacksoni (Muzzall and Rabalais, 1975a) and A. dirus (Camp and Huizinga, 1979).

Although recruitment of larval A. lucii apparently occurred throughout the year, recruitment only exceeded mortality for a very restricted period of the year, during April and May. This was the only time when infection levels rose and during this brief period they did so quite dramatically. It is interesting to note that as well as coinciding with the time when water temperatures increased rapidly (and so stimulated perch feeding activity) this was also the period when perch spawned (see Chapter 2). It is tempting to suggest that perch were also more susceptible to infection at this time owing to hormonally induced physiological changes associated with spawning, but this remains to be proven. Following rapid recruitment in April and May mature parasites first appeared in June, which was approximately at the time the hosts spawned, suggesting there might be a relationship. However, mature parasites were recovered from immature perch and during experimental infections of adult perch mature parasites were recovered from fish each with gonads at different stages of development. Furthermore, during experimental infections of rainbow trout (Salmo gairdneri), mature parasites were again recovered, although the rainbow trout had undeveloped gonads. These results indicate that hormonally induced physiological changes

associated with spawning are not an essential prerequisite for maturation of Acanthocephalus lucii.

Where the relevant analysis has been carried out, nearly all intestinal fish parasites have been shown to have an over-dispersed distribution with a small proportion of the host population harbouring the majority of the parasites (Pennycuick, 1971c; Anderson, 1974a, b; Kennedy, 1968, 1969; Skorping, 1980a, b; Lee, 1980). The distribution of adult A. lucii in perch in the Forth and Clyde canal was in accord with this pattern. Furthermore the degree of overdispersion appeared to change seasonally, with a gradual disappearance of the most heavily infected fish from April - May onwards. The possible mechanisms whereby this seasonal change occurred have been discussed previously.

In many instances the negative binomial model has been shown to adequately describe the distribution of parasite counts in the fish population. A number of factors can give rise to this type of distribution (see Crofton, 1971a; Pennycuick, 1971c). Kennedy (1968) and Kennedy and Hine (1969) felt that the most likely explanation for the overdispersed distribution of the cestodes Proteocephalus torulosus (Batch) and Caryophyllaeus laticeps (Pallas) in dace (Leuciscus leuciscus) was that some

parasites had a greater chance of infecting fish than others owing to selective feeding by fish and or that some fish were physiologically more resistant to infection than others. Anderson (1974a) suggested two distinct generating processes which might have given rise to the observed (negative binomial) distribution of C. laticeps in bream (Abramis brama); one assumed random input of larval parasites and the other assumed contagious input. Without detailed experimental evidence concerning the feeding behaviour of fish and the distribution of encounters with infective stages of C. laticeps Anderson was unable to discriminate between the two suggested processes. Skorping (1980b) stated that the factors which gave rise to the observed negative binomial distribution of the nematode Camallanus lacustris in perch were possibly a combination of different events leading to a non random infection process. Genetic variation in the perch population might have influenced the infection pattern. Non random dispersion of infected intermediate hosts might also have been involved.

Although the data for the distribution of A. lucii among perch were unsuitable for attempting to fit theoretical distribution models the distribution was clearly highly overdispersed, especially in April - May, during the period of rapid recruitment of larval parasites into the fish population. This highly over-

dispersed pattern was undoubtedly at least partly owing to a contagious input of larval parasites. The distribution of infective cystacanth stages in the intermediate host was shown to be over-dispersed, so even assuming random feeding by perch and random spatial distribution of infected intermediate hosts contagious input of larval parasites could still occur. The spatial distribution of Asellus aquaticus has in fact been shown to be highly aggregated (Berglund, 1968; Andersson, 1969) and so, combined with the fact that perch feed selectively on infected isopods, this suggests that a highly contagious input of larval parasites into the perch population will occur. Differences in the resistance of individual fish to infection may also be important.

Many previous studies on acanthocephalan fish parasite systems have revealed a change in the level of infection in hosts of different sizes (ages) (Rizvi, 1964; Mishra, 1966; Walkey, 1967; Pennycuick, 1971a; Amin, 1975; Amin and Burrows, 1977; Muzzall and Bullock, 1978; Andersen, 1978; Lee, 1980). Such changes have been attributed to both qualitative and quantitative changes in feeding habits and age related changes in susceptibility to infection. Although Mishra (1966), Andersen (1978) and Lee (1980) all observed a marked increase

in the infection levels of perch with A. lucii, with increasing host length (or age), such a marked change was not evident in the current study. This can simply be attributed to the fact that only a restricted length/age group of perch were examined (see Chapter 2). The current results are highly significant in this respect, since they quite clearly indicated that the differences in infection levels at various times of the year were not simply owing to heterogeneity in the fish samples.

For many species of Acanthocephala parasitizing freshwater fish some information is available on the distribution of adult worms along the gut. However, much of the information has come from publications primarily concerned with describing the seasonal changes in infection levels with the result that only brief details were given concerning the distribution of worms in the gut. The details often consist of merely a brief mention of the region where worms were most abundant. A few more quantitative studies have been undertaken, including those by Uglem and Beck (1972), Amin (1975) and Kennedy, Broughton and Hine (1976). Studies on the intestinal distribution of Acanthocephala with non piscine definitive hosts include Crompton and Whitfield (1968), Crompton (1970) on Polymorphus minutus in ducks and Burlinghame and Chandler (1941) and Holmes (1962) on Moniliformis dubius in rats. Crompton (1973) has reviewed

the literature about the sites occupied by helminth parasites in the alimentary tract of vertebrates.

In the present study adult A. lucii were found throughout the intestine of perch, except in the pyloric caeca. This is in marked contrast to the observations of Lee (1980) who recovered A. lucii only from the third quarter of the intestine of perch, which corresponds to the region approximately mid way between the pyloric caeca and anus. However, the infection levels described by Lee were far lower than those in the current study, and as Lee pointed out, the distribution of A. lucii in the perch gut may well have been influenced by concurrent infections with the cestode Proteocephalus percae (Müller) and the nematode Camallanus lacustris (Zoega).

The fact that A. lucii was found throughout the intestine of perch indicates that it is capable of attachment and survival, at least for short periods, in all regions of the intestine. Other species of acanthocephalan have been found throughout the intestine of their hosts. Kennedy, Broughton and Hine (1976) found Pomphorynchus laevis throughout almost the entire intestine of dace (Leuciscus leuciscus), chub (Leuciscus cephalus) and barbel (Barbus barbus). Muzzall and Bullock (1978) found Neoechinorhynchus saginatus throughout the intestine of fallfish

(Semotilus corporalis (Mitchill)). Bibby (1972) found Neoechinorhynchus rutili throughout the intestine of minnow (Phoxinus phoxinus). Although recovered from all regions of the intestine, A. lucii and each of the three species mentioned above were typically more abundant in specific regions in the gut. Approximately 50% of all specimens of A. lucii recovered in the current study were found in intestinal sections 2 and 3 (20-60% posterior from the pylorus). Crompton (1973) felt that such preference might be owing to the parasite having very particular requirements in the way of food or physicochemical conditions that could only be satisfied in the preferred regions, or alternatively, it might be a consequence of the process of liberation, activation and establishment of the cystacanths such that the parasite established wherever it was liberated and space was available. Kennedy, Broughton and Hine (1976), based on detailed information from field and experimental studies, considered the latter alternative was important in determining the distribution of Pomphorhynchus laevis in the intestine of its fish hosts. These authors further suggested that the mechanism of site selection in P. laevis might be atypical compared to other acanthocephalans, since P. laevis was unable to move in the host intestine a short while after establishment, owing to host encapsulation of the proboscis bulb. For A. lucii,

the limited information available at present makes it virtually impossible to decide unequivocably which of the two mechanisms described previously are involved in determining the intestinal distribution of this species. Observations on specimens of A. lucii in the intestine of freshly killed perch indicated that the worms had considerable locomotory ability, continually withdrawing the proboscis from the intestinal mucosa and re-attaching at an adjacent site. There was no evidence of encapsulation of the proboscis preventing movement. So it appeared that adult A. lucii might have the ability to move up and down the perch intestine.

Many species of Acanthocephala apparently undergo a posterior movement down the intestine during the course of infection. Awachie (1963) found that Echinorhynchus truttae moved from the upper intestine to the rectum of brown trout (Salmo trutta) and mature female worms were only recorded in the posterior part of the host intestine. Tedla and Fernando (1970) noted that in autumn Echinorhynchus salmonis were immature and occupied the upper part of the intestine of yellow perch (Perca fluviatilus = P. flavescens) but in May when the worms were mature most were recovered from the lower part of the alimentary canal. Amin (1975) noticed that

Acanthocephalus parksidei was located anteriorly in the midgut of Lepomis cyanellus, Semotilus atromaculatus and Catastomus commersoni in autumn. With progressive development, maturing worms clearly moved posteriorly to become considerably more abundant in the hindgut regions in spring in all three hosts. Uglem and Beck (1972) showed that sub-adult Neoechinorhynchus cristatus had a mean attachment point 60% down the intestine of Catastomus macrocheilus, whereas adults had a mean attachment position of 68%, suggesting a slight posterior migration. Uglem and Beck also demonstrated significant changes in amino-peptidase activity of N. cristatus. The loss of aminopeptidase activity in developing worms corresponded with population migration and increase in habitat specificity. The extent of aminopeptidase activity in N. cristatus was found to be inversely related to that of the intestinal habitat occupied by it. The posterior migrations described above might be at least partly related to changing biochemical requirements of worms associated with the maturation process.

With respect to A. lucii the overall distribution of the adult worm population did not appear to change seasonally. The distribution of female worms when they were immature (April - May), appeared to be identical to that when they were mostly mature (i.e. at all other times of year). However,

if one pools the data for the entire sampling period and analyses the proportion of mature female parasites in any one section of the intestine, the proportion appears to increase in a posterior direction, suggesting that a posterior migration associated with maturation did in fact occur. Amin and Burrows (1977) described a similar overall pattern concerning the distribution of Echinorhynchus salmonis in the intestine of rainbow smelt (Osmerus mordax).

Amin (1975) noticed a distinct anterior localization of male compared to female Acanthocephalus parksidei in the intestine of Catastomus commersoni during autumn. A pronounced anterior localization of male A. lucii was also noted during the course of the present study. Furthermore, the anterior localization appeared to persist throughout the year. A relatively simple hypothesis may explain why this takes place. In both male and female A. lucii complete development of the reproductive organs takes place in the intermediate host and copulation between adult worms commences as soon as they establish in the intestine of the definitive host (Brattey, 1980). Competition in the intestine, between male parasites for females with which to copulate is undoubtedly intense. The evolution of a 'cement plug' or 'copulatory cap' in Acanthocephala verifies this. Since copulation is possible as soon as worms reach the intestine

male worms in a slightly more anterior position will have a greater probability of encountering the most recently acquired and hence unfertilized females. Consequently, there will be considerable selective pressure for males to occupy a more anterior position. Since physicochemical conditions along the intestine will vary, it seems likely that there will be an optimal region of the intestine for acquisition of nutrients etc. Outside this region conditions may be less favourable, or even harmful to the parasite, so the distribution of male worms may well be the result of a balance between these two factors.

Further information on the distribution of adult A. lucii in the intestine of perch, based on results from experimental infections, will be given in Chapter 6.

Records of the occurrence of A. lucii in pike (Esox luscius) are numerous. Copland (1956) found 39% and 34% of pike from Loch Lomond and Old Friun, respectively, infected with A. lucii. Rizvi (1964) found 6.29% of pike in Rostherne Mere, Cheshire, infected with A. lucii. Mishra (1966) found 13.2% of pike from the Shropshire Union canal infected with A. lucii. Moravec (1979) examined the seasonal occurrence of A. lucii in pike in the Macha Lake fishpond system, Czechoslovakia.

Pike were infected throughout the year with maximum incidence and intensity in May - June and minimum July - August.

Nearly all previous workers have reasoned that large pike acquire infection secondarily from infected perch prey. In the current study eight of the nineteen pike examined had perch in their stomachs, which further supports this suggestion.

Very few mature female worms were recorded from pike. This appears to be a reflection of the fact that nearly all the pike examined were caught in April and at this time female worms in perch were predominantly immature.

The very small number of pike examined make it difficult to assess the importance of this host in maintaining the parasite population in the canal. The actual level of infection of pike may merely be a reflection of the extent to which pike feed on perch, together with the level of infection of perch at the appropriate time. It remains to be determined whether A. lucii can actually undergo any development in pike, or whether the parasite only establishes for a short time before being passed out of the intestine. However, even if A. lucii can develop in pike there is good evidence to suggest that the direct importance of pike as a host in maintaining the existence of the parasite population in the Forth and Clyde canal will be small. Pike are effectively the top carnivore in the canal

ecosystem. Consequently the size of the pike population will be many times smaller than that of perch. By far the greatest flow of parasites, and hence shelled acanthor output, will be through the much larger perch population. The major influence of pike on the parasite population will be an indirect one mediated through the influence of predation by pike on the population dynamics of perch.

Acanthocephalus lucii has also been recorded from roach (Rutilus rutilus) in a number of localities. Rizvi (1964) found 6.4% of roach in Rostherne mere infected with A. lucii. Mishra (1966) found 2.6% of roach in the Shropshire Union canal infected with A. lucii. Although large numbers of roach were examined by both these authors and in both localities perch were quite heavily infected with A. lucii, the level of infection of roach was extremely low in both Rostherne Mere and the Shropshire Union canal. This suggests that either roach do not eat many A. aquaticus, and/or, roach are poor hosts for Acanthocephalus lucii. Experimental evidence (see Chapter 6) supports the latter alternative.

In view of the very small numbers of roach examined an assessment of the importance of roach as hosts for A. lucii in the Forth and Clyde canal was not possible.

CHAPTER 5

EXPERIMENTAL STUDIES ON LARVAL *ACANTHOCEPHALUS LUCII* IN THE ISOPOD *ASELLUS AQUATICUS*

Chapter 5

Introduction

Although numerous experimental studies concerning larval acanthocephalans have been published, with the exception of the works of Andryuk (1979) very little experimental work has been undertaken on larval Acanthocephalus lucii in the isopod Asellus aquaticus. The results of a detailed field study on the population dynamics of this host-parasite system have been described previously (Chapter 3) and a considerable amount of information collected. However, certain aspects of the host parasite relationship remain to be investigated. Asellus aquaticus are readily available and keep well in captivity, so the opportunity to carry out some experiments was taken.

Previous studies on larval acanthocephalans have indicated that intermediate hosts are easily infected in the laboratory. This might well explain why the details of the morphological development of larval stages has been described for so many different species. Rojanapaibul (1977) lists 33 species whose larval development has been described. Species with piscine definitive hosts for which detailed descriptions are available include Neoechinorhynchus cylindratus (Ward, 1940), Leptorhynchoides thecatus (DeGuisti, 1949), Neoechinorhynchus rutili (Merritt and Pratt, 1964), Octospinifer macilisentis (Harms, 1965), Echinorhynchus truttae (Awachie, 1966),

Paulisentis fractus (Cable and Dill, 1967), Neoechinorhynchus saginatus (Uglem and Larson, 1969) and Acanthocephalus clavula (Rojanapaibul, 1977). The development of larval Acanthocephalus lucii was investigated by infecting Asellus aquaticus in the laboratory using a modification of the technique described by Hynes and Nicholas (1957). The results are compared with the species described above.

The field study (Chapters 3 and 4) has indicated that shelled acanthors of Acanthocephalus lucii are deposited by adult worms throughout most of the year. Since conditions in the natural habitat show marked seasonal variations, especially with respect to some factors such as water temperature, larval parasites present in summer experience quite different conditions from those present in winter. To investigate the effects of such conditions on the rate of development of larval parasites, groups of isopods were infected and maintained at constant temperatures over the whole range experienced by those in the natural habitat.

Temperature has been shown to exert considerable influence on the rate of development of many larval acanthocephalans. For example Kates (1943) studied the development of larval Macracanthorhynchus hirudinaceus in various species of beetle. At 24^{o}C cystacanths took between 60 and 90 days to develop. Higher and lower temperatures accelerated

and delayed development, respectively. At approximately 6°C development practically ceased. DeGuisti (1949) examined the development of Leptorhynchoides thecatus in the amphipod Hyallela azteca. After 2 months at 13 - 15°C the stage of development reached was similar to that after 8 days at 25°C. At 20 - 25°C infective cystacanths were recovered after 30 - 32 days. Awachie (1966) examined the rate of development of larval Echinorhynchus truttae in Gammarus pulex maintained at various temperatures. Cystacanths were recovered after 80 days at 13 - 15°C, 82 days at 10 - 21°C, 96 days at 10 - 14°C, 196 days at 3 - 14°C and 240 days at 2 - 10°C. Lackie (1972) studied the development of larval Moniliformis dubius in the cockroach Periplaneta americana. At 28°C cystacanths were recovered after 26 days. At 20°C development took 156 days and at 37°C all acanthors were encapsulated by host cells and did not develop further. Rojanapaibul (1977) examined larval Acanthocephalus clavula in the isopod Asellus meridianus at three experimental temperatures. At 5, 10 and 19°C cystacanths were recovered after 20, 16 and 7 - 8 weeks, respectively. Andryuk (1979) experimentally infected Asellus aquaticus with Acanthocephalus lucii. At temperatures of 25, 22, 19, 18, 15 - 16 and 15°C development took 19, 32, 51, 60, 72 and 89 days, respectively. In the current study the rate of development of larval A. lucii was investigated at 5, 9, 12, 19 and 22°C.

Many studies, both field and experimental, have indicated that larval acanthocephalans exert pathogenic effects on their hosts. These effects manifest themselves in a number of different ways. Seidenberg (1973) noticed a gradual disappearance of Asellus intermedius which were heavily infected with Acanthocephalus dirus. This disappearance was attributed to mortality caused by growth of the numerous larval A. dirus present in the haemocoel of such isopods. Similar observations were made by Amin et al. (1980) for the isopod Caecidotea militaris when heavily infected with Acanthocephalus parksidei. In experimental situations some authors have noticed a reduction in survival of hosts exposed to large numbers of shelled acanthors, death being attributed to the mass penetration of the intestinal wall by newly hatched acanthors (Hynes and Nicholas, 1957; Rojanapaibul, 1977). The survival of Asellus aquaticus after exposure to shelled acanthors of Acanthocephalus lucii was investigated experimentally and the survival compared with that of unexposed control groups.

In some instances the presence of larval acanthocephalans alters the normal behaviour of the hosts, often in a manner which renders them more susceptible to predation by definitive hosts. Hindsbo (1972) demonstrated that Gammarus lacustris infected with Polymorphus minutus showed greater positive

phototropism and were more easily detected in surface water than uninfected individuals. In feeding experiments infected amphipods were captured and eaten more easily by the domestic duckling. Bethel and Holmes (1973), in a detailed study, demonstrated behavioural changes in Gammarus lacustris infected with either Polymorphus marilis or P. paradoxus and in Hyallela azteca infected with Corynosoma constrictum. In feeding experiments infected individuals of both species of amphipod were selectively preyed upon by definitive hosts. Kennedy et al (1977) found that specimens of Gammarus pulex infected with cystacanths of Pomphorhynchus laevis spent more time in open water and moved more often towards the water surface than uninfected specimens. In feeding experiments infected G. pulex were selectively eaten by grayling (Thymallus thymallus) and dace (Leuciscus leuciscus). Muzzall and Rabalais (1975c) analysed the behaviour of the isopod Lirceus lineatus infected with cystacanths of Acanthocephalus jacksoni. Infected isopods spent significantly more time 'wandering' and on the surface of leaves rather than underneath them, compared with uninfected isopods. Infected isopods were also nonpigmented. Camp and Huizinga (1979) noticed that cystacanths of Acanthocephalus dirus induced pigmentation and behavioural changes in the isopod Asellus intermedius. Infected isopods were light coloured and

hyperactive, continuously moving over the top of the substrate. In feeding experiments significantly greater numbers of infected isopods were eaten by creek chubs (Semotilus atromaculatus). Holmes and Bethel (1972) have reviewed the literature concerning the modification of intermediate host behaviour by parasites.

Predation experiments, involving infected and uninfected Asellus aquaticus were carried out with the natural definitive host Perca fluviatilus. Unfortunately, lack of infected isopods prevented detailed experimental work on isopod behaviour.

Many, although not all species of acanthocephalan, are known to interfere with the sexual development and reproduction of their intermediate hosts. Le Roux (1931) and Hynes (1955) both noticed that larval Polymorphus minutus interfered with the reproductive activity of female Gammarus pulex and prevented them from reaching maturity. Male isopods were apparently unaffected. Hynes and Nicholas (1963) examined many thousands of infected Gammarus and found only six females carrying eggs, whereas normally 30 - 50% were breeding. Spaeth (1951) noticed that the amphipod Hyallela azteca infected with Leptorhynchoides thecatus could still reproduce, although there was some evidence of reduced oocyte production. Munro (1953) found an as yet unidentified acanthocephalan which interfered with the sexual development of the isopod Asellus aquaticus. Many intersex isopods were found, which appeared to be modified females, since

they possessed immature setae on the oostegites. Schmidt and Olsen (1964) found that the ovaries of terrestrial isopods infected with Prosthorhynchus formosus became vestigial or occasionally hypertrophied. Rumpus and Kennedy (1974) concluded that Pomphorhynchus laevis reduced the reproductive capacity of female but not male Gammarus pulex. Spencer (1974) noticed that many specimens of Gammarus lacustris infected with Polymorphus minutus had fewer eggs than non-parasitized individuals. Muzzall and Rabalais (1975b) never observed female Lirceus lineatus infected with Acanthocephalus jacksoni carrying eggs in the field or the laboratory. Amin et al (1980) found that gravid female isopods Caecidotea militaris were never infected with Acanthocephalus parksidei.

The field study has indicated the female Asellus aquaticus infected with cystacanths of Acanthocephalus lucii are sterile. However, it is not known if infected male isopods can inseminate females normally.

The sequence of events involved in the reproductive cycle of Asellus aquaticus have been described in detail in Chapter 3. Asellus aquaticus are easily maintained and breed readily in the laboratory, so the influence of Acanthocephalus lucii on the reproduction of A. aquaticus was investigated experimentally. Various combinations of infected and uninfected male and female

isopods were isolated and the reproductive condition of the female in each pair closely monitored.

Materials and Methods

The development of larval Acanthocephalus lucii

Experiments on the rate of development of larval Acanthocephalus lucii were carried out in constant environment rooms maintained at the appropriate temperature. All specimens of Asellus aquaticus were obtained from drainage ditches at Frodsham, Cheshire. Test batches of isopods (n > 200) were examined and no larval parasite recovered, indicating A. lucii was absent or extremely rare at this site. Mature female worms were obtained from experimental infections of perch. The body wall of each worm was ruptured using dissecting needles, releasing the shelled acanthors which were collected with a pipette, washed and stored in distilled water at 4°C.

Isopods were hand sorted from debris and maintained at the appropriate experimental temperature for at least one month prior to the start of the experiments. Groups of 300 - 500 isopods were kept in 3 gallon plastic aquaria containing aerated tap water with autumn shed sycamore leaves (Acer pseudoplatanus) and Elodea sp. for food. Five experimental temperatures were used: 5, 9, 12, 19 and 22°C.

At each temperature isopods were infected in a uniform

manner using a modification of the method described by Hynes and Nicholas (1957). A large enamel dish (30 x 35cms) was filled with matured tap water and approximately 20 thoroughly soaked autumn shed sycamore leaves added. A thin suspension of shelled acanthors was pipetted onto the leaves, 300 - 500 isopods added and these left to feed for 48 hrs. The isopods were then removed, washed to remove adhering shelled acanthors and returned to their original aquaria. At approximately weekly intervals, between 5 and 10 isopods, at each temperature, were removed, placed in 0.8% saline and dissected and examined for larval parasites with the aid of a binocular microscope. Since the body wall of larvae remained transparent, observations on the development of the internal organs were made on freshly dissected larvae mounted in saline. To test the infectivity of larval stages, groups of 10 isopods were force fed to uninfected perch and these dissected 3 days later. If parasites were found attached to the intestinal mucosa larvae were designated infective.

Survival of infected and uninfected isopods

In the experiments on survival of isopods exposed to shelled acanthors of A. lucii the source and method of infection of isopods was as described above. Following exposure to

shelled acanthors, isopods were isolated singly in small plastic vessels containing 150mls of matured tap water with an autumn shed sycamore leaf for food. Food and water were changed at fortnightly intervals. Throughout the experiments isopods were maintained at a constant temperature of 19^{o}C and photoperiod of 16 hours daylight, 8 hours darkness. Unexposed control isopods were maintained under identical conditions. A total of 290 isopods were used, 145 control and 145 experimental and both groups contained isopods of similar size range (5 - 12mm). All isopods were examined at intervals of between 2 and 9 days until the termination of the experiment, 59 days after exposure. If isopods failed to respond to repeated prodding with a dissecting needle they were assumed dead. Dead isopods were not examined for larval parasites. The start of the experiment was designated as the time when isopods were first exposed to shelled acanthors.

Predation by perch on infected and uninfected isopods

For the selective predation experiments all isopods were obtained from the Forth and Clyde canal at Temple, Glasgow. Although cystacanths cannot normally be seen through the exoskeleton, infected isopods were easily recognised by the black pleopods on the ventral surface of the abdomen. All perch

used were between 12 and 14cms in (fork) length and were obtained from a lake at Ruabon, Wales, where A. lucii did not occur. Both isopods and perch were maintained at the appropriate experimental temperature for at least 1 month prior to the start of the experiments. Isopods were fed on sycamore leaves and Elodea sp., perch were fed on Calliphora sp. and chironomid larvae.

The experiments were carried out in a small glass aquarium (65 x 30 x 35cms) with a substrate consisting of 4cms of coarse gravel with 20 strands of Elodea sp., each about 15cms in length, and 20 thoroughly soaked autumn shed sycamore leaves, lying on top of the gravel. These provided refuge for the isopods. The water in the tank was aerated gently, maintained at a constant temperature of 19^{o}C and photoperiod of 16 hours daylight, 8 hrs darkness. Equal numbers of infected and uninfected isopods of similar size range (4 - 10cms) were introduced to the tank and allowed to disperse for 1 hour. A single perch was then introduced to the tank and left to feed for up to 48 hrs. At the end of the experiment the perch was removed, the tank searched and the numbers of infected and uninfected isopods remaining counted. Preliminary experiments indicated that searching was 100% efficient so any isopods not recovered were assumed to have been eaten by the perch. Different isopods and perch were used in each experiment.

The effects of A. lucii on isopod reproduction

In the experiments concerned with examining the effects of the parasite on reproduction, all isopods were obtained from the Forth and Clyde canal at Temple, Glasgow. Only isopods of breeding size (> 6mm in length) were used. Four experiments were carried out, involving pairing various combinations of infected and uninfected male and female isopods as follows: uninfected male and uninfected female (control group), infected male and uninfected female, uninfected male and infected female and finally infected male and infected female. Ten pairs of isopods were used in each experiment and in each pair the male was slightly larger than the female. Each pair was maintained in a separate plastic vessel containing 150mls of aged tap water with an autumn shed sycamore leaf for food. A constant temperature of 19°C and photoperiod of 16 hours daylight, 8 hrs darkness was used. All female isopods were in the non-brooding condition at the start of the experiments. Each pair was examined twice per day (at approximately 8 hr intervals), and the reproductive condition of the female isopod in each pair noted and assigned to one of four categories; not guarded by male, guarded by male, with a brood pouch containing eggs/embryos, or with a brood pouch containing juvenile isopods. As soon as a brood pouch was observed on a female the respective male isopod was removed from the vessel to prevent intraspecific cannalabism.

Results

The development of larval *Acanthocephalus lucii*

The following description of the morphological development of larval *Acanthocephalus lucii* is based on observations on larvae recovered from isopods maintained at 19°C. The mature shelled acanthor was extremely elongate or spindle shaped and there were four membranes surrounding the acanthor. These were a thin outermost envelope, a thick inner shell and two thin inner membranes. The acanthor larva was essentially oval in shape with a dense centrally placed embryonic nuclear mass and a number of anterior spines. Once hatched, the acanthors were extremely motile and actively penetrated the intestinal tissue using these spines. Acanthors were never actually observed in isopod intestinal tissue in spite of extensive searching, but after approximately 2 weeks a small 'bud' appeared on the outer surface of the intestine, projecting into the haemocoel. This 'bud' was in fact the developing acanthor. Internally a number of giant nuclei and the embryonic nuclear mass were seen. The acanthor remained attached to the intestinal tissue and gradually enlarged. The anterior spines disappeared and the larva is now termed an acanthella. The acanthella, which was spherical at first, enlarged and began to elongate rapidly. Internally the embryonic nuclear mass divided and

differentiated into the various internal organs, lemnisci plus the ovary and uterine bell in females and the testis, cement glands and copulatory bursa in males. Initially the proboscis developed in the erect position but later retracted. At the early acanthella stage the outer surface of the parasite was covered in a thin layer of what appeared to be host cells, which tended to obscure the internal structures of the developing larva. This layer of cells eventually disappeared and the internal structures once more became visible and remained so even in the final cystacanth stage. From approximately the late acanthella stage onwards a thin transparent envelope was noticed around the developing larva. The envelope appeared to be attached to the outer surface of the isopod intestine and was filled with a yellowish fluid. Once the proboscis was retracted the developing larvae in many respects resembled an adult worm, except it was much smaller and the ovary was not fragmented in females and no active sperm were present in males. The acanthella further increased in size and then, in females, the ovary fragmented into a number of oval shaped structures termed ovarian balls. In males the six cement glands became more distinct and spermatogenesis commenced. The larva, which is now termed a cystacanth, was now infective to the definitive host. Cystacanths varied enormously in size depending on the size of the isopod in which they developed. Female cystacanths were

typically much larger than males and the sexes were readily distinguishable at low magnification.

The effects of temperature on the rate of development of larval Acanthocephalus lucii

During development six stages of larval parasite were recognised and these are indicated in Table 5.1. The times at which each larval stage was first observed, during experimental infections at each temperature are also indicated in Table 5.1. It is stressed that these figures are only approximate, since considerable variation was evident in the rate of development of individual parasites even in isopods maintained at the same temperature. Development appeared to be markedly influenced both by the size of the isopod and the number of larval parasites present.

At 5°C development was extremely slow. The spherical acanthella stage was first observed 21 weeks after exposure. None of the later stages were observed in any of the isopods examined and in fact very few larvae were found in isopods maintained at 5°C. This can probably be attributed to the marked reduction in feeding activity of isopods at low temperatures resulting in very few shelled acanthors being eaten. At 9°C the infective cystacanth stage was recovered

Table 5.1. The development of larval *Acanthocephalus lucii* in the isopod *Asellus aquaticus* at different temperatures

		Number of hours (hrs), days (d) or weeks (w) until larval stage was first observed				
	Temperature	5°C	9°C	12°C	19°C	22°C
Larval stages	Shelled acanthors hatching in gut	n. obs.	n.obs.	24 hrs	10 hrs	10 hrs
	Spherical acanthellae in haemocoel	21w	7w	5w	2w	10d
	Early elongate acanthellae (sex not distinguishable)	n. obs.	12w	7w	3w	2w
	Middle acanthellae (sex distinguishable, proboscis erect)	n. obs.	13w	9w	4w	3w
	Late acanthellae (proboscis retracted, ovary not fragmented (♀) no active sperm present (♂))	n. obs.	18w	12w	6w	4w
	Infective cystacanth (ovary fragmented (♀), active sperm present (♂))	n. obs.	22w	15w	7w	5w

* n. obs. = not observed

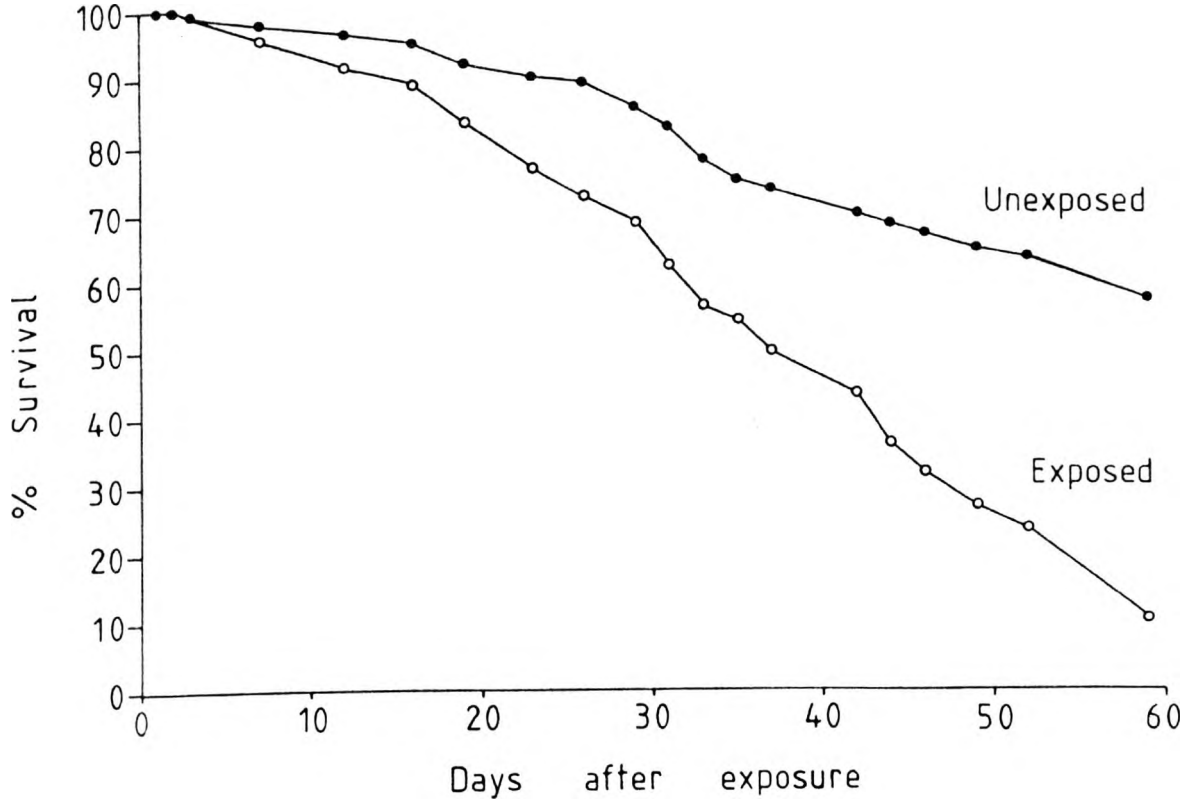
after 22 weeks. Development was progressively faster at higher temperatures and at 22°C, which is probably slightly warmer than the highest temperature to which parasites are normally exposed in the Forth and Clyde canal, cystacanths were recovered after 5 weeks.

Many of the isopods examined, especially from the experiments at 19 and 22°C, had multiple infections of larval stages with up to 15 larval parasites in a single isopod. This can probably be attributed to the higher feeding activity of isopods at warmer temperatures. In heavily infected isopods the rate of development was considerably retarded. Many abnormally shaped larvae were recovered. Some of the larvae in heavily infected isopods did develop to the infective cystacanth stage but they were typically much smaller than those recovered from naturally infected isopods. Similarly in small isopods (< 5mm in length) development was slower and the cystacanth stages smaller than in large isopods (> 10mm in length). Thus the actual number of larval parasites which could develop normally in an isopod appeared to depend on its size. Whilst a 12mm long isopod could contain 4 normal cystacanths a 5mm long one could contain only 1 or 2.

Survival of infected and uninfected isopods

Figure 5.1 gives the percentage survival of Asellus aquaticus after exposure to shelled acanthors of Acanthocephalus lucii, compared with the survival of unexposed control isopods.

Figure 5.1. The survival of Asellus aquaticus after exposure to shelled acanthors of Acanthocephalus lucii (open circles) compared with the survival of unexposed control isopods (closed circles)



No difference in survival was evident in the first few days after exposure, thereafter survival was considerably reduced in the exposed group. At the end of the experiment (59 days) over 60% of the unexposed control isopods were still alive, which indicates that the method of maintaining isopods in the laboratory was very favourable. In the exposed group only approximately 10% were still alive after 59 days. These surviving isopods, 15 in all, were dissected and examined for larval parasites at the end of the experiment. Eight were infected and a total of 62 larval parasites (acanthor, acanthella and cystacanth stages) were recovered. Up to 9 parasites were recovered from a single isopod (mean 7.8, range 2 - 9).

Predation by perch on infected and uninfected isopods

The selective predation experiments were repeated six times and the results are indicated in Table 5.2. In each instance perch ate more infected than uninfected isopods, although equal numbers of each were available. In five of the experiments the difference in the number of infected and uninfected isopods eaten was significant at the 5% probability level and overall the difference was significant at the 0.01% level, which clearly indicates that perch fed selectively on

Table 5.2. Predation by perch (Perca fluviatilus) on Asellus aquaticus either uninfected, or infected with cystacanth(s) of Acanthocephalus lucii

Fish no	Duration of experiment (hrs)	Number of A. aquaticus eaten		p*
		Infected	Uninfected	
1	48	17/20	9/20	<0.01
2	48	19/20	11/20	<0.01
3	48	16/20	4/20	<0.001
4	48	17/20	12/20	>0.05
5	48	19/20	2/20	<0.001
6	30	9/15	2/15	<0.01
Total		97/115	40/115	<0.001

*p = probability, by chi-squared test

infected isopods. The figures for the totals in Table 5.2 indicate that during the experiments, infected isopods were approximately 2.5 times more likely to be eaten by perch.

The results of the experiments concerned with the influence of the parasite on the reproduction of Asellus aquaticus are indicated in Table 5.3. In the first experiment (control group) all female isopods were observed being guarded by their respective male. Nine of these females produced a brood pouch (1 died). Of these 9 a further 5 died, but 4 survived to produce juvenile isopods. In the second experiment only 7 females were observed being guarded by their respective male but 9 produced a brood pouch (1 died). This suggests that in 2 of the females with a brood pouch the eggs were not fertilized. Alternatively, they were fertilized, but the duration of the guarding period was short and was simply not observed. Of these 9, a further 5 died. but 4 survived to produce juvenile isopods. These results indicate that at least some infected male isopods are fertile and can inseminate females normally. In the third and fourth experiments, which involved infected female isopods, guarding was observed intermittently in only a few pairs and no females produced a brood pouch. This supports the observation from the field study that female isopods infected with cystacanths are sterile.

Table 5.3. The effects of larval Acanthocephalus lucii on the reproduction of Asellus aquaticus

Isopod pair		Number of females from 10 pairs			
		Never guarded by male	Guarded by male	With a broad pouch	
Male	Female			containing eggs or embyros	containing juvenile isopods
Uninfected	Uninfected	0	10	9	4
Infected	Uninfected	3	7	9	4
Uninfected	Infected	8	2*	0	0
Infected	Infected	6	4*	0	0

* Guarding observed only for short periods (< 1 day)

Discussion

Although the description of the development of larval Acanthocephalus lucii was fairly brief, some comparison with the development of other species is possible.

The elongate shape of the shelled acanthor is considered to be a modification to permit passage through the gastric mill of the host, since all species whose intermediate hosts are small aquatic amphipod or isopod crustacea have shelled acanthors of similar shape (Hynes and Nicholas, 1957). As Hynes and Nicholas pointed out the shelled acanthors of species with ostracod intermediate hosts (such as Neoechinorhynchus saginatus, N. rutili and N. cylindratus) are not elongate, nor are they in the many terrestrial species whose intermediate hosts are typically species of Coleoptera.

The acanthors of Acanthocephalus lucii penetrated the intestinal tissue of Asellus aquaticus and remained there for a short period before development continued. They did not pass directly into the haemocoel, like the acanthors of Polymorphus minutus (Hynes and Nicholas, 1957), Echinorhynchus truttae (Awachie, 1966) and Neoechinorhynchus saginatus (Uglem and Larson, 1969). As development proceeded the acanthor of A. lucii remained attached to the outer surface of, usually, the posterior half of the isopod intestine. This initial phase of

development appeared to be rather slow. However, from the spherical acanthella stage onwards development proceeded much more rapidly and the basic architecture of the parasite was soon formed. Initially the proboscis of A. lucii developed in the erect position but later retracted. This is similar to many species including E. truttae (Awachie, 1966), Paulisentis fractus (Cable and Dill, 1967) and Acanthocephalus clavula (Rojanapaibul, 1977), but unlike Leptorhynchoides thecatus (DeGuisti, 1949) and Polymorphus minutus (Hynes and Nicholas, 1957) where the proboscis began development in the retracted position.

The thin transparent envelope which appeared around the acanthella of Acanthocephalus lucii during development has been observed in many species of larval acanthocephalan and considerable controversy exists regarding both its origin and function. Crompton (1975) has discussed this subject in some detail and it appears that this membrane is associated in some way with host resistance.

Before infectivity was reached, the ovary, in female A. lucii, fragmented into ovarian balls. Similar observations have been made for a number of species, including Prosthorhynchus formosus (Schmidt and Olsen, 1964), Neoechinorhynchus rutili (Merritt and Pratt, 1964), E. truttae (Awachie, 1966),

Fessisentis necturorum (Nickol and Heard, 1973), Acanthocephalus jacksoni (Muzzall and Rabalais, 1975b) and Fessisentis friedi (Muzzall, 1978). Active sperm was seen in male cystacanths and this has apparently not been observed previously in other species of larval acanthocephalan.

In the experiments on the rate of development of larval A. lucii the temperatures chosen were based upon those experienced by the parasite in the Forth and Clyde canal, although 22°C would be experienced only in a particularly warm summer. The results indicated that the rate of development was markedly influenced by temperature. Overall the rates appeared to be slightly faster than those described by Andryuk (1979) for A. lucii. At 5°C development was almost completely inhibited, so infective cystacanths present in the natural habitat during the winter and early spring months must presumably have developed earlier in the year, when water temperatures were higher. This suggests that isopods harbouring cystacanths can survive for many months, at least at cool temperatures. At warm summer temperatures of 19°C cystacanths of A. lucii took approximately 7 weeks to develop. This fairly long period necessary for development to infectivity might be attributed to the advanced stage of the development exhibited by cystacanths of A. lucii. Complete development, including spermatogenesis takes place

in the haemocoel of the intermediate host. In species such as most neoechinorhynchids, where development in the intermediate host takes only a few weeks, the parasites reproductive organs have to undergo considerable further development in the definitive host before copulation can take place. For example, larval Neoechinorhynchus saginatus develop to infectivity in 16 days at 25°C. However, even after 46 days in the intestine of the definitive host, the creek chub Semotilus atromaculatus, the parasites are unable to copulate since the ovaries have not yet fragmented (Uglem and Larson, 1969).

During the early stages of larval development some authors (Awachie, 1966; Lackie, 1972) observed encapsulation and destruction of some of the acanthor stages by host cells. Although these may have been overlooked, these so called melanized acanthors were never observed in the haemocoel of Asellus aquaticus, nor was there any evidence of successful host encapsulation and destruction of the larger acanthella or cystacanth stages.

In the experimental infections, if only a few larval parasites were found in the haemocoel of an isopod, development proceeded normally. If many larvae were present (> 5) then development was noticeably retarded. Many of the larvae appeared abnormal in shape and some did not develop beyond the

early acanthella stage even after 2 months at 19°C. It was, unfortunately, extremely difficult to control the number of shelled acanthors eaten by each isopod.

In the field study heavily infected isopods (> 5 larvae) were never observed. In Chapter 3 two explanations for this were offered. These being that isopods simply never ingest sufficient shelled acanthors to generate higher infection levels than those observed or, alternatively, the parasite is highly pathogenic and isopods which ingest large numbers of shelled acanthors are killed. The experimental results provide further support for the former hypothesis since isopods with many larvae (> 5) in the haemocoel were frequently encountered during the experiments, although they did show reduced survival.

The results of the experimental infections indicate that a wide size range of isopods are susceptible to infection and at none of the experimental temperatures were isopods completely resistant to infection. This suggests that isopods of all sizes are susceptible to infection at all times of the year.

During the experiments concerned with the survival of isopods exposed to shelled acanthors of Acanthocephalus lucii, no differences in survival were detected between experimental and control groups during the first few days after exposure.

Some workers (Hynes and Nicholas, 1957; Crook and Grundeman, 1964; Rojanapaibul, 1977) found that intermediate hosts were killed by mass penetration of the intestinal wall by acanthors under laboratory conditions where no control was exercised over the number of shelled acanthors eaten. This clearly did not occur in the current experiments. With increasing time after exposure a considerable reduction in survival of exposed isopods was evident. Although this indicates that some isopods are killed by the developing larval parasites, some caution is needed here in considering the significance of these results in relation to the situation in the field. Dissection of the isopods in the exposed group which survived until the end of the experiment indicated much higher infection levels than those observed in natural infections (see Chapter 3), so the field and experimental results are not readily comparable.

The results of the survival experiment are in contrast to those of Uznanski and Nickol (1980) who observed no differences in survival in the amphipod Hyallela azteca exposed to shelled acanthors of Leptorhynchoides thecatus, compared to unexposed control groups. In their experiments the infection levels were again much higher than those observed in natural populations of H. azteca (Esch et al, 1976). It is, however, extremely difficult to compare these results with those of the

current study, since larval L. thecatus are much smaller than larval A. lucii. Furthermore a direct comparison of the infection levels in both experiments would be required, but in neither instance were infection levels monitored throughout the experiments. Ideally such experiments should involve a comparison between the survival of unexposed and exposed groups over the whole range of infection levels experienced by naturally infected intermediate hosts.

Whilst survival of laboratory infected isopods was apparently reduced by large numbers of larvae developing simultaneously in the haemocoel, it was found that some naturally infected isopods, most of which contained only 1 or 2 cystacanths, could survive for up to six months in the laboratory. Although this requires experimental verification, it appears that isopods infected with small numbers of parasites may survive, at least in the laboratory, for as long as uninfected isopods.

In the feeding experiments perch ate statistically significantly greater numbers of infected isopods. Selective predation has been demonstrated in similar experiments involving many different species of Acanthocephala. Although it seems reasonable to assume that selective predation occurred in the natural host-parasite system, in none of the previous studies

was this confirmed by examination of the stomach contents of freshly caught definitive hosts. This was, of course, not always possible since many of these hosts chewed their food before it was swallowed. However, perch swallowed food items whole and infected and uninfected isopods were readily distinguishable, once removed from the stomach. Between January 1980 and March 1981 a total of 174 specimens of Asellus aquaticus were recovered from perch stomachs. Of these, 27 (15.5%) harboured cystacanths of Acanthocephalus lucii. The overall infection level of isopods (with cystacanths) during this period never exceeded 5.5% (see Chapter 3), so this strongly suggests that perch do indeed prey selectively on infected isopods. However, this assumes that perch feed randomly on all sizes of isopod. Table 3.6 indicates that, at certain times of year, some isopod size groups had much higher infection levels than the average figure for the entire population (in January 1981, 20.9% of 9mm long isopods harboured cystacanths). Consequently size selective predation alone (irrespective of whether or not isopods were infected) might account for the high level of infection of isopods in perch stomachs. Unfortunately an analysis of the sizes of isopod eaten by perch was not undertaken. It seems prudent to conclude that selective predation on infected isopods by perch in the wild, although a strong

possibility, remains to be unequivocably demonstrated.

The mechanism whereby larval acanthocephalans influence the susceptibility of intermediate hosts to predation appears to involve changes in behaviour and/or pigmentation, such that they become more conspicuous to visually orientating predatory definitive hosts. Pigmentation changes in Asellus aquaticus have been dealt with previously (Chapter 3). Unfortunately, lack of sufficient infected individuals prevented detailed experimental work on the behaviour of isopods.

The results from the current field and experimental study indicate that female Asellus aquaticus infected with cystacanths of Acanthocephalus lucii are sterile, whereas males can inseminate females normally. The mechanism whereby this sterilization takes place, is unknown. Hynes and Nicholas (1963) have suggested that the effect was not simply a mechanical one since female Gammarus infected with Polymorphus minutus were sterile, whereas those infected with cystacanths of Echinorhynchus truttae, which is much larger, were not.

In host-parasite systems where infection results in sterilization of the host, the parasite has the potential to exert considerable detrimental effects on the dynamics of the host population. The magnitude of this effect will depend

on the proportion of the host population of breeding size which are sterile. Hynes (1955), based on observations on populations of Gammarus sp. infected with Polymorphus minutus suggested that this parasite could be responsible for almost complete elimination of populations of Gammarus sp. from certain habitats. The infection levels observed in the Gammarus sp. - P. minutus system were, however, far higher than those observed in the current study. Of the female Asellus aquaticus of breeding size ($\geqslant$ 6mm) which were examined, overall 5.1% harboured cystacanths of Acanthocephalus lucii. Although this will undoubtedly reduce the reproductive potential of the isopod population the proportion of hosts infected was so low that the overall effect was probably rather small.

These experiments have indicated that larval Acanthocephalus lucii, like many species of acanthocephalan, exerts a variety of detrimental effects on its intermediate host. Infected isopods exhibit reduced survival (at least at high individual parasite burdens), altered pigmentation and susceptibility to predation and infected female isopods are sterile. Although the experiments have provided a considerable amount of useful information, more detailed quantitative experimental studies will only be possible when a method is found whereby isopods can be

infected, in the laboratory, with a known number of parasite larvae. King and Robinson (1967) working on Moniliformis dubius in the cockroach Periplaneta americana solved this problem by artificially hatching shelled acanthors in vitro, then introducing them directly into the host haemocoel with a micropipette. Further work might indicate if such a method would be suitable for the Asellus aquaticus - A. lucii system.

CHAPTER 6

EXPERIMENTAL STUDIES ON ADULT *ACANTHOCEPHALUS LUCII* IN THE DEFINITIVE HOST(S)

Chapter 6

Introduction

The literature concerning the seasonal occurrence of helminth parasites of freshwater fishes has recently been summarized in a series of reviews by Chubb (1977, 1979, 1980, 1982). Although the literature is extensive, the limited nature of many of the previous studies has resulted in much of the information being of little use with respect to the current study, since a proper understanding of fish parasite population dynamics ideally requires a simultaneous and detailed examination of all stages in the parasites life-cycle. Unfortunately, most previous works have concentrated solely on a single stage in the life cycle. This is particularly true for Acanthocephalus lucii, where all earlier studies have centered solely on adult worms in the definitive hosts.

Where sufficient details are available field studies have indicated that many species of intestinal fish parasite exhibit seasonal cycles in incidence and intensity of infection and in maturation. Such cycles have been described for acanthocephalans (DeGuisti, 1949; Muzzall and Rabalais, 1975a, b; Camp and Huizinga, 1979), for cestodes (Hopkins, 1959; Kennedy, 1969; Kennedy and Hine, 1969; Wootten, 1974), digeneans (Awachie, 1965; Skorping, 1980a) and nematodes (Stromberg and Crites,

1975). From the details of the current field study described in Chapter 4 it is apparent that the population of adult Acanthocephalus lucii, although present throughout the year in the Forth and Clyde canal, also undergoes pronounced seasonal fluctuations in numbers.

The results from various field studies have led to much speculation regarding the causes of seasonal cycles in fish-parasite systems. Unfortunately, in such natural conditions a whole complex of factors, both biotic and abiotic, are acting on the system simultaneously, such that it becomes virtually impossible to separate the factors which have direct or indirect causal effects from those which merely exhibit coincidental correlation.

Both Shulman (1979) and Chubb (1982) have suggested that temperature is a major factor influencing the seasonal dynamics of fish parasites in freshwater ecosystems. In poikilothermic hosts temperature change can influence the parasites either directly, or indirectly, via some response in the host. Increasing temperatures can influence parasite recruitment by stimulating host feeding activity. They can also influence parasite mortality by altering the physiological resistance of host to infection, such that a smaller proportion of ingested parasites establish, or by rejection of already established

parasites. Temperature also determines the length of time required for growth of many larval parasites in the intermediate host and this is particularly true of larval Acanthocephala (see Chapter 5 and references therein).

The habitat of a helminth parasite of a poikilotherm is such that the direct effects of some factors, such as temperature, on the parasite simply cannot be separated from the indirect effects mediated through some temperature sensitive change in host physiology. The classic example is where the general similarity in time of maturation of many species of fish tapeworm and the correlation with temperature has led to the suggestion that the relationship may be a causal one (Chubb, 1967). However, as Kennedy (1975) has pointed out this is not supported by any experimental evidence. The relationship may be merely coincidental, or it may be a direct or indirect causal one, since the maturation period coincides not only with the spring rise in water temperature, but also with an increased food intake by the host and with physiological changes associated with spawning. The direct causal factors responsible for maturation in such species of tapeworm remain to be determined by experimental work.

Both Kennedy (1977) and Chubb (1982) have stressed the need for more experimental work on fish parasite population

dynamics. Following field investigations many hypotheses have arisen to explain the observed pattern of occurrence of many species of fish parasite, but for only a few species has any attempt been made to test these hypotheses with detailed experimental investigations. This is not altogether surprising in view of the considerable technical difficulties involved in acquiring and maintaining sufficient numbers of suitable fishes for such work. Furthermore, the nature of the life cycle of many species of fish parasite is such that even when sufficient fishes are available, other factors such as the supply of enough parasite infective stages makes detailed experimental work virtually impossible.

One of the fundamental considerations involved in the design of the current research project was that a species of parasite suitable for laboratory study be chosen, such that hypotheses which arose from field work could be tested. The A. lucii - perch system was found to adequately fulfill the necessary requirements for laboratory infection experiments. The natural definitive host of the parasite was available in reasonable numbers from many sources around Liverpool, although the parasite proved to be so common that locating a source of uninfected fish initially proved quite difficult. Provided care was taken, perch adapted extremely well to

laboratory conditions and many were kept in the laboratory for more than a year. The fact that the laboratory infection experiments involved use of the parasites natural definitive host was an extremely important point, since the results were used to provide support for hypotheses from field data. Had an alternative laboratory host been chosen the results might not have been comparable, since the characteristics of the laboratory host - parasite system could be quite different from those of the natural host - parasite system.

The field study has provided a considerable amount of information about the population dynamics of A. lucii. Although adult parasites were recovered throughout the year a seasonal cycle in both incidence and intensity of infection was observed. The size of the adult parasite population was influenced by changes in both recruitment and mortality. The field study has provided evidence for the existance of factors affecting both these processes. However, the information collected from the field study had considerable limitations and certain fundamental questions concerning the life history of A. lucii remain unanswered. Recruitment, although varying seasonally, was shown to occur throughout the year. Since conditions in the intestine of perch show marked seasonal variations, at least with respect to temperature and availability of nutrients, parasites ingested in winter will experience quite

different conditions from those ingested in summer. Unfortunately the nature of the data from the field study was such that it was impossible to age individual parasites, and so determine what effects such conditions had on the establishment, survival, growth and rate of development of individual adult parasites. Consequently, the principle aim of the experimental work was to simulate the conditions experienced by parasites at specific times of year and to examine the effects of these conditions on various aspects of the biology of the adult parasite. The complete details of the experimental design are described in the following materials and methods section. Essentially the experiments involved following the course of a primary infection in immunologically naive perch maintained under one of three sets of experimental conditions. In terms of photoperiod, temperature and availability of nutrients these conditions approximated to those experienced by both host and parasite in either winter ($5^{o}C$), summer ($19^{o}C$), or spring and autumn ($12^{o}C$). Information on the establishment, survival, growth, rate of maturation and intestinal distribution of adult parasites was collected. The results are discussed in relation to the observed pattern of occurrence of the adult parasites in a natural ecosystem, the details of which have been discussed in Chapter 4.

During both the results and discussion sections of this chapter reference to any of the three experimental regimes utilized will be made by quoting the appropriate temperature only. This is merely for convenience and whilst temperature will undoubtedly be involved, this should not be taken to imply that temperature is the only direct causal factor involved in bringing about a particular result. As described previously, it is normally impossible to differentiate between direct and indirect effects of temperature in such host - parasite systems.

In addition to perch, experimental infections of small numbers of goldfish (Carassius auratus), rainbow trout (Salmo gairdneri), roach (Rutilus rutilus) and tilapia (Tilapia zilli) were attempted. The details are given in the following sections where appropriate.

Materials and methods

Infected intermediate hosts

All specimens of Asellus aquaticus were obtained from a population of naturally infected isopods in the Forth and Clyde canal at Temple, Glasgow. The method of collection of isopods was as described in the materials and methods section of Chapter 3. Infected isopods were easily recognized by

the presence of black pleopods in the ventral surface of the abdomen (see Figure 3.2). The infected isopods were maintained until required at the appropriate experimental temperature for at least 2 weeks in aerated glass tanks with Elodea sp. and autumn shed sycamore leaves (Acer pseudoplatanus L.) for food.

Definitive hosts

The perch used in the experiments were captured using a seine net, or small beam trawl, from ponds at Capenhurst, Wirral, or were obtained from a commercial supplier. Prior to experimentation large numbers (n > 50) from each source were examined for the presence of Acanthocephalus lucii. None were found and therefore it was assumed that none of the experimental fish had previously been infected with this parasite. All perch were within the size range 10 - 20cms with the majority around 15cms. Attempts to capture sufficient numbers of perch of identical size proved unsuccessful. Fishes were maintained in tap water in aerated glass tanks with undergravel filtration and acclimatized at the appropriate temperature and photoperiod for at least 2 months prior to infection.

Method of infection

A uniform infection procedure was adopted for all experiments. Since perch would not consistently and naturally ingest the

requisite number of infected isopods all fishes were force fed. Prior to infection food was witheld for 48 hrs. Each fish was anaesthetized using MS222 (Sandoz) (1:10,000 w:v in tap water), gently wrapped in a damp paper towel to reduce handling damage and 6 infected isopods administered using invertebrate forceps. Initially fishes were then isolated singly in small plastic tanks for 24 hrs to ensure that isopods were not regurgitated. This procedure was ceased when it became apparent that regurgitation did not occur. Fish were grouped in batches of 10 or 20 in glass tanks of 15 or 30 gallon capacity, respectively, each with under-gravel filtration and continuous aeration.

Great care was taken to ensure that each group of 6 infected isopods administered to each fish contained individuals of similar size range. Although it proved impossible to count the exact number of cystacanths contained within a single isopod, extensive field studies described in Chapter 3 had shown that over 90% of all infected isopods harboured a single cystacanth, with the mean number being 1.2 cystacanths per infected isopod. Consequently each fish was given an initial infection of, on average, 7.2 parasites.

Three experimental regimes were utilized and the details of temperature, photoperiod and feeding regime are summarized as follows:

Temperature (^{o}C)	Photoperiod	Food	
		Type	Frequency
5 ± 1	16 hrs dark 8 hrs light	Calliphora larvae, Chironomid larvae.	Twice/week
12 ± 1	12 hrs dark 12 hrs light	Calliphora larvae, Chironomid larvae.	Four times/week
19 ± 2	8 hrs dark 16 hrs light	Calliphora larvae, Chironomid larvae.	Daily

Two groups of 130 fishes, one maintained at 5^{o}C and one at 19^{o}C, were used. Fishes were sacrificed in batches of 10, the first 3 days post infection and then at weekly intervals thereafter. At 12^{o}C a total of only 30 fish were used. Six were sacrificed 3 days post infection and thereafter batches of 4 were sacrificed at weekly intervals until 24 days post infection, then at fortnightly intervals until 66 days post infection. Shortage of space prevented the use of larger numbers of fish in the 12^{o}C experiment.

All fish were sacrificed around mid-day. The length (mms), weight (gms), sex and gonad condition of each fish was noted, the entire digestive tract dissected out and the attached fat and viscera removed. The intestine was placed in a wax dissecting tray, slit open, straightened out and pinned down

in a relaxed position. The length of the intestine from the pylorus to the anus was noted (in mm). All specimens of A. lucii were counted and the exact point of attachment of the proboscis noted (in mm, posterior to the pylorus). Each specimen was removed, sexed and placed in a small plastic phial containing physiological saline. The presence or absence of a copulatory cap on female parasites was noted. Wet weights of parasites (to the nearest 0.1mgs) were determined using a torsion balance (Sauter), after excess water had been removed by gently blotting on filter paper. Female parasites were placed on a microscope slide and teased open to assess their stage of maturation. They were assigned to one of three stages as for parasites collected in the field study. These stages are described in the materials and methods section of Chapter 4.

For the experiments involving alternative definitive hosts goldfish and rainbow trout were obtained from a commercial supplier, tilapia were obtained from a 'natural' population in a thermally polluted stretch of the St. Helens canal and roach were obtained from ponds on Merseyside. Test batches indicated that none of these species harboured specimens of A. lucii. All these species were infected by allowing them to ingest infected isopods naturally, rather than by force feeding, and all were

fed on commercial trout pellets (Ewos T54). Various experimental temperatures and numbers of infected isopods were used, depending on the host species. These are described in the results section.

Results

Establishment and survival

Three days after infection and at weekly intervals thereafter, experimental fishes were dissected in batches of 10: one batch from the 5°C and one from the 19°C experiment. Establishment and survival at 12°C were not assessed owing to the small numbers of fishes involved. Table 6.1 gives the maximum, minimum and mean percentage recovery of A. lucii throughout the course of experimental infections at 5 and 19°C. Although fishes were given, on average, an initial infection of 7.2 parasites, slight variation in the numbers of cystacanths contained in a single Asellus aquaticus resulted in some fish receiving an initial infection of more than 7 parasites. For convenience all recoveries of > 7 worms from individual perch were considered to represent recoveries of 100% in Table 6.1.

The maxima and minima indicate considerable variation in the percentage recovery of worms from individual fish maintained at either temperature. At 19°C percentage recovery remained high (> 97.2%) in some fish only until 45 days post

Table 6.1. The minimum, maximum and mean percentage recovery of Acanthocephalus lucii during experimental infections of perch at 5 and 19°C

		Days post infection												
	% recovery	3	10	17	24	31	38	45	52	59	66	73	80	87
5°C	Minimum	55.5	13.8	27.8	27.8	13.8	27.8	13.8	13.8	13.8	55.5	13.8	13.8	13.8
	Maximum	100	100	97.2	97.2	100	100	100	83.3	100	100	83.3	83.3	97.2
	Mean	93	59.7	61.1	63.9	83.3	70.8	52.8	51.4	65.2	83.3	54.1	48.6	51.3
19°C	Minimum	41.7	13.8	27.8	27.8	55.5	13.8	13.8	0	13.8	0	0	0	0
	Maximum	100	100	97.2	97.2	97.2	100	97.2	69.4	41.6	55.5	55.5	69.4	41.7
	Mean	83.3	68.1	56.9	63.9	69.4	56.9	59.7	38.8	34.7	25	12.5	27.7	15.3

infection, but at 5°C percentage recovery remained high (> 83.3%) in some fish throughout the experimental period. Alternatively percentage recovery could decline to very low levels (13.8%) in some fish only 10 days after infection at either experimental temperature. Overall, mean percentage recovery appeared to decline gradually during the course of the infection at both 5 and 19°C. For the first 45 days post infection the decline was similar at each temperature, but from approximately 52 days post infection onwards the percentage recovery was consistently lower in perch maintained at 19°C.

In summary, these results suggest that susceptibility to infection varied considerably between individual fish, no fish were completely resistant to infection at either expermental temperature and temperature appears to have no influence, at least initially, on the resistance of individual fish to infection. Differences in resistance between individual fish, slight variation in the initial number of cystacanths administered and the fairly small numbers of fish in each batch resulted in considerable fluctuation in the mean percentage recovery. Nonetheless an overall decline in percentage recovery was evident with increasing time after infection. The rate of decline appears to be more rapid at the higher temperature towards the end of the experimental infection period.

The mean numbers of A. lucii recovered (± standard error) from each batch of 10 perch during the course of the experimental infections at 5 and 19°C are given in Figure 6.1. Although there is considerable variation between adjacent means at each temperature overall there is a gradual decrease in mean survival with increasing time after infection at both temperatures. Initially the survival rate is similar at the two temperatures but from approximately 52 days post infection onwards survival was considerably lower at the higher experimental temperature.

Table 6.2 gives the mean number of worms recovered (mean survival ($\bar{x}$)) and the variance (s^2) for each batch of 10 fish examined at weekly intervals during the experimental infections at 5 and 19°C. Worm survival at the two experimental temperatures was compared each week using a non parametric Mann-Whitney test. The use of a two sample t-test was avoided, since only rarely did both samples have a normal distribution and equal variance. The Mann-Whitney test indicates no significant difference in survival from 3 until 52 days post infection, but from 66 days post infection until the end of the experimental period worm survival differed significantly at the two experimental temperatures.

Maturation of female parasites

During the experimental infections 3 maturation stages

Figure 6.1. The survival of Acanthocephalus lucii at 5 and 19°C during experimental infections of perch. Each mean (± standard error) is based on 10 fish.

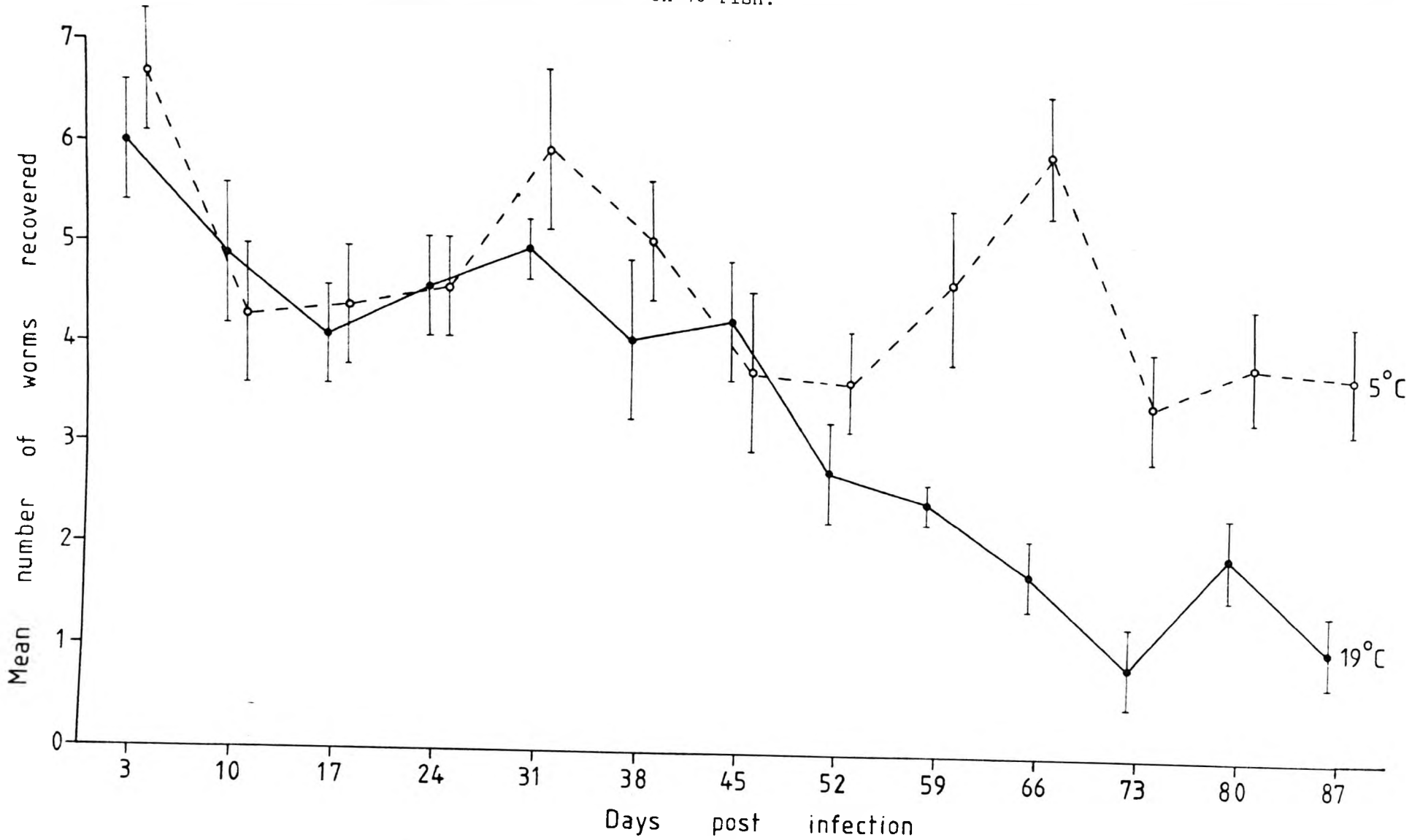


Table 6.2. The mean survival, variance and the significance of the difference in survival of *Acanthocephalus lucii* during experimental infections of perch at 5 and 19°C

		Days post infection												
		3	10	17	24	31	38	45	52	59	66	73	80	87
5°C	mean survival ($\bar{x}$)	6.7	4.3	4.4	4.6	6.0	5.1	3.8	3.7	4.7	6.0	3.5	3.9	3.7
	variance (s^2)	4.83	5.34	3.82	2.93	6.00	4.10	5.95	2.68	6.68	3.33	3.39	3.88	3.12
19°C	mean survival ($\bar{x}$)	6.0	4.9	4.1	4.6	5.0	4.1	4.3	2.8	2.5	1.8	0.9	2.0	1.1
	variance (s^2)	4.00	5.21	2.77	2.49	0.89	6.10	4.01	2.62	0.50	1.51	1.43	1.78	1.43
	Significance of difference in survival	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	*	**

n.s. = not significant

*, **, *** denote significant differences at the 5, 1 and 0.1% probability levels, respectively

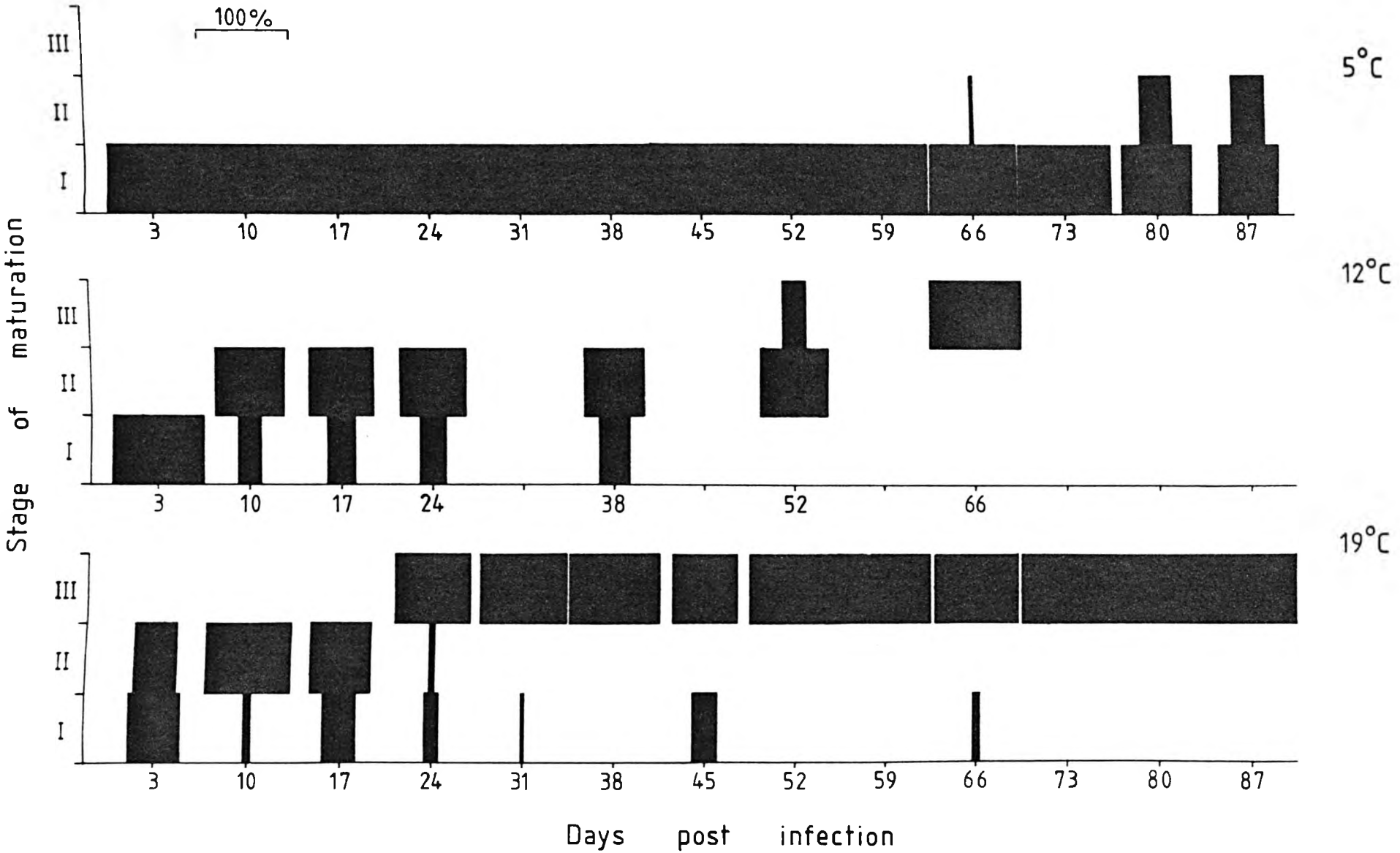
of female parasites were recognised. These are described in the materials and methods section of Chapter 4. The total number of female parasites recovered from each batch of fishes, and the proportions at each stage of maturation during experimental infections at 5, 12 and 19°C are given in Table 6.3. Only small numbers of fish and hence parasites were used in the 12°C experiment. These proportions are represented in the form of a series of histograms in Figure 6.2. Figure 6.2 indicates considerable differences in the rate of maturation of female parasites at the experimental temperatures. At 5°C no parasites with mature shelled acanthors (stage 3) were recovered even after 87 days, although a few parasites had immature shelled acanthors 66 days post infection. At 12°C mature parasites were recovered after 52 days, and a large proportion of parasites were at stage 2 by 10 days post infection. At 19°C the majority of female parasites had some mature shelled acanthors by 24 days post infection and a large proportion had immature shelled acanthors by 3 days post infection.

The results of the experiment at 19°C also indicate that some female parasites can persist in the intestine of perch for at least a further 63 days (or two months) after mature shelled acanthors first appear in the body cavity. The small numbers of stage 1 parasites recovered after 45 and 66 days

Table 6.3. The number of female Acanthocephalus lucii recovered and the number and percentage at each stage of maturation during experimental infections of perch at 5, 12 and 19°C

		Days post infection												
		3	10	17	24	31	38	45	52	59	66	73	80	87
5°C	Number of female worms recovered	39	17	17	24	34	22	19	15	28	24	17	18	19
	Stage 1 (%)	39(100)	17(100)	17(100)	24(100)	34(100)	22(100)	19(100)	15(100)	28(100)	23(96)	17(100)	14(78)	13(68)
	Stage 2 (%)	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	1(4)	0 -	4(22)	6(32)
	Stage 3 (%)	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -
12°C	Number of female worms recovered	11	4	10	7	-	6	-	4	-	4	-	-	-
	Stage 1 (%)	11(100)	1(25)	3(30)	2(29)		2(33)		0 -		0 -			
	Stage 2 (%)	0 -	3(75)	7(70)	5(71)		4(66)		3(75)		0 -			
	Stage 3 (%)	0 -	0 -	0 -	0 -		0 -		1(25)		4(100)			
19°C	Number of female worms recovered	34	22	22	21	31	21	21	16	17	11	8	10	5
	Stage 1(%)	18(53)	2(9)	8(36)	3(14)	1(3)	0 -	6(29)	0 -	0 -	1(9)	0 -	0 -	0 -
	Stage 2 (%)	16(47)	20(91)	14(64)	1(5)	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -
	Stage 3 (%)	0 -	0 -	0 -	17(81)	30(97)	21(100)	15(71)	16(100)	17(100)	10(91)	8(100)	10(100)	5(100)

Figure 6.2. The maturation of female Acanthocephalus lucii at 5, 12 and 19°C during experimental infections of perch (data in Table 6.3).



at 19°C were from fish in which apparently no male worms had established. This indicates that at 19°C female parasites can survive for a considerable time in the intestine of perch in the unfertilized condition.

In summary, it appears that the rate of maturation of female A. lucii is considerably increased at warm temperatures. Maturation, although not completely inhibited, was extremely slow at 5°C and no parasites containing mature shelled acanthors were recovered even after 87 days (almost 3 months). At 12°C worms containing mature shelled acanthors were recovered after 52 days and at 19°C maturation was extremely rapid with parasites containing mature shelled acanthors after only 24 days.

Growth of adult parasites

During the experimental infections at 5 and 19°C growth of adult worms was assessed. Growth was based on wet weights and so only an approximate assessment was possible. Female parasites were initially much larger than males and no comparison was made between the growth rates of the two sexes.

Growth of males

The wet weights of male parasites recovered during experimental infections of perch at 19 and 5°C are depicted in a series of

histograms in Figure 6.3. Male worms at 19 and 5°C are shown in the upper and lower part of each histogram, respectively. Wet weights are assigned to 0.2mg groups in Figure 6.3. Semi-log plots of the wet weights of male worms are also given (Figure 6.5).

The histograms in Figure 6.3 indicate that wet weights of male worms recovered each week during the infection period approximate to a normal distribution. Both Figure 6.3 and 6.5 indicate a considerable range of wet weights of male worms even after only 3 days in the intestine of perch. This correlates with the variable size of cystacanths as described in Chapter 3 and further indicates that both small and large cystacanths are infective. The wide range of sizes exhibited by male worms of the same age appears to be maintained throughout the experimental period (although the use of a log scale in Figure 6.5 gives the impression that it decreases).

The histograms in Figure 6.3 suggest that, at certain times during the experimental period, male parasites from infections at 19°C are somewhat larger than those from infections at 5°C and vice versa. However, there is no clear trend and this difference does not appear to be maintained for any length of time during the infection. Furthermore, the histograms do not give a clear indication of any increase or decrease in the

wet weight of worms with time at either temperature. The semi-log plots, however, suggest a gradual increase in wet weight of male worms with increasing time after infection at both temperatures.

Since the wet weights of male worms recovered at each week approximated to a normal distribution and the variance was independent of the mean, linear regression lines were calculated for the relationship between male parasite wet weight and days post infection. The equations were: at $5^{o}C$ ($n = 287$), $r^2 = 0.31$, $y = 0.003x + 0.72$. Significance test on slope $t = 5.51$ ($p < 0.001$), D.F. = 285. At $19^{o}C$ ($n = 190$), $r^2 = 0.51$, $y = 0.007x + 0.71$. Significance test on slope $t = 8.07$ ($p < 0.001$), D.F. = 188. Both regression coefficients were significant, the slope of each line was positive and differed significantly from zero, which indicates that the wet weights of male parasites increased with time after infection during experiments at both temperatures.

Mean wet weights were calculated for male parasites recovered each week during the experimental infections at 5 and $19^{o}C$. These means, together with the results of a t-test on the significance of the difference between respective means at each temperature, are given in Table 6.4. Initial F-tests revealed no significant difference between the variances within each pair of means ($p > 0.1$). Although means did differ significantly

at certain times during the infection period, no clear overall pattern emerges and the results are difficult to explain. Even only 3 days after infection the means differed significantly. This perhaps suggests that the initial size of the parasites (i.e. the size of the cystacanths) administered to fish at 5 and 19°C differed significantly and yet great care was taken to ensure that a similar range of size of isopod was given to each fish at the beginning of the infection. It is suggested that the most likely explanation for the significant differences only at certain times during the infection period lies in the fact that wet weights were used to measure growth. Short term fluctuations in parasite wet weight are quite likely to take place, owing to changes in the availability of nutrients and osmotic effects, these being associated with the presence or absence of food in the perch gut. Worms recovered from a recently fed perch might well exhibit a considerable difference in wet weight from those recovered from a perch which has not been fed for a few days. As indicated in the materials and methods section, perch maintained at 19°C were fed daily, whereas those at 5°C were fed only twice per week. It is suggested that such short term fluctuations as described above might also account, at least partly, for the wide range in wet weights of male parasites of the same age.

Growth of females

The wet weights of female parasites recovered during experimental infections of perch at 19 and 5°C are depicted in a series of histograms in Figure 6.4. Worms from perch maintained at 19 and 5°C are shown in the upper and lower part of each histogram, respectively. Wet weights are assigned to 0.5mg groups in Figure 6.4. Shading is used in Figure 6.4 to represent female worms in different stages of development as follows: unshaded - immature or stage 1 worms with ovarian balls, diagonal shading - stage 2 worms with a mixture of immature acanthors and ovarian balls, black shading - stage 3 worms with mature shelled acanthors and (usually) some immature acanthors and ovarian balls. Semi-log plots of the wet weights of female worms are given in Figure 6.6. It should be noted that Figures 6.4 and 6.6 do not include every female parasite which was recovered from perch during the experimental infections. Many female worms, especially the larger specimens, were accidentally damaged while opening the perch intestine. Consequently their wet weights could not be determined accurately and they are not included in Figure 6.4 and 6.6.

The histograms in Figure 6.4 indicate that wet weights of female worms also approximate to a normal distribution

although the variance tends to be somewhat larger than that described for male parasites, especially for female worms recovered from perch maintained at 19°C. Female parasites are also somewhat larger than males. Both Figure 6.4 and 6.6 indicate a considerable range of wet weights of female worms even after only 3 days in the intestine of perch. The factors responsible and the significance of this finding have been discussed previously (see section on growth of males). The wide range of sizes of female worms is maintained throughout the infection period at both experimental temperatures. Furthermore the shading reveals considerable difference in the size of the individual female parasites irrespective of their age or stage of development. Figure 6.4 indicates that immature (Stage 1) parasites ranged from 0 - 0.5mgs to 4 - 4.5mgs and mature (stage 3) parasites ranged from 0.5 - 1mgs to 7 - 7.5mgs wet weight. This considerable overlap indicates that the use of size alone is not a consistently accurate method of estimating the age or stage of development of individual parasites.

The histograms in Figure 6.4 suggest that the wet weight of female parasites increased during the course of the infection at both 5 and 19°C. Furthermore, the increase appeared to be greater at 19°C. The semi-log plot (Figure 6.6) suggests a similar trend, although both these Figures illustrate the

considerable overlap in wet weight between worms of the same age, but recovered from perch maintained at different temperatures.

Linear regression lines were calculated for the relationship between female parasite wet weight and days post infection. The equations were as follows: at $5^{o}C$ ($n = 259$), $r^2 = 0.32$, $y = 0.009x + 1.37$. Significance test on slope $t = 5.41$ ($p < 0.05$), D.F. = 257. At $19^{o}C$ ($n = 214$), $r^2 = 0.65$, $y = 0.036x + 1.48$. Signifiance test on slope $t = 12.50$ ($p < 0.001$), D.F. = 212. Both regression coefficients were significant, the slope of each line was positive and differed significantly from zero, which indicates that the wet weights of female parasites increased with time after infection during experiments at both temperatures.

Mean wet weights were calculated for female parasites recovered each week during the experimental infections at 5 and $19^{o}C$. These means, together with the results of t-tests on the significance of the difference between respective means at each temperature are given in Table 6.5. Initial F-tests revealed no significant differences between the variances within each pair of means ($p > 0.1$). With the exception of 3 and 17 days post infection, the means differed significantly throughout the infection period. Although the slopes of the two regression lines described above did not differ significantly, the

results of the t-tests support the suggestion that female worm wet weights increased more rapidly at the warmer temperature.

Sex ratio

Table 6.6 indicates the number of male and female parasites recovered and the sex ratio throughout the course of experimental infections of perch maintained at 5 and 19°C. The sex ratio fluctuates around 1:1 throughout the course of the infection at 5°C. However, at 19°C, towards the end of the infection period (from 52 days post infection onwards), there is some indication of a change in sex ratio in favour of female parasites. Since rather small numbers of parasites were recovered at specific weekly intervals during this period, data for the period 52-87 days post infection was pooled. At 19°C, 111 parasites were recovered, 44 males and 67 females, which differs significantly from a 1:1 sex ratio, χ^2 ($p < 0.05$) = 4.76, 1 D.F. For the respective period at 5°C, a total of 235 parasites were recovered, 114 males and 121 females, which does not differ significantly from a 1:1 sex ratio, $\chi^2 (p > 0.5) = 0.21$, 1 D.F. Previous results (Figure 6.1, Table 6.1) indicated a fairly rapid decline in survival of parasites from 52 days post infection onwards, in perch

Figure 6.3. Histograms comparing the wet weights of male Acanthocephalus lucii recovered from perch during experimental infections at 5°C (lower histograms) and 19°C (upper histograms).

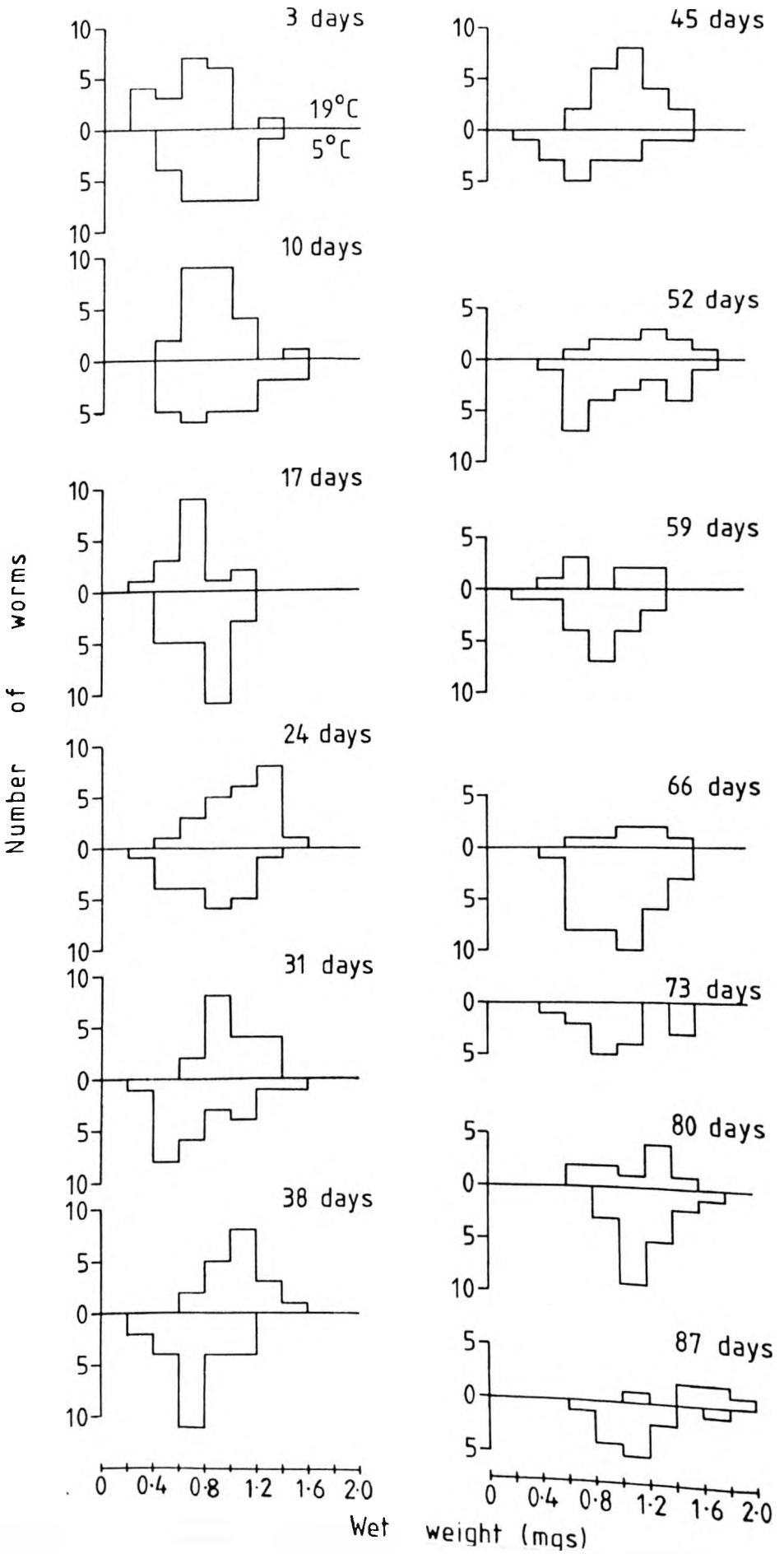


Figure 6.4. Histograms comparing the wet weights of female Acanthocephalus lucii recovered from perch during experimental infections at 5°C (lower histograms) and 19°C (upper histograms).

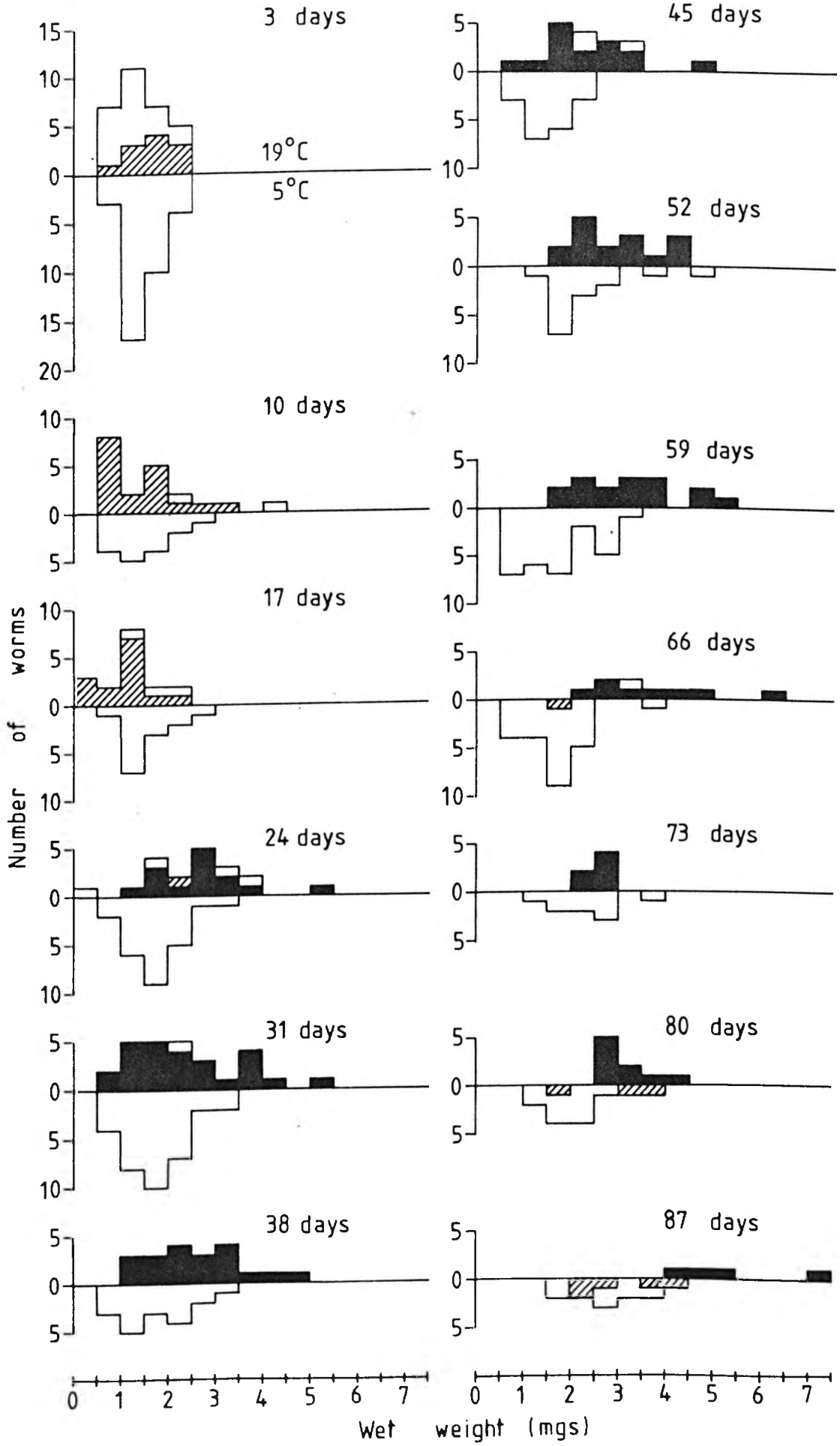


Figure 6.5. Semi-log plots of the wet weights of male Acanthocephalus lucii recovered from perch during experimental infections at 5°C (open circles) and 19°C (closed circles).

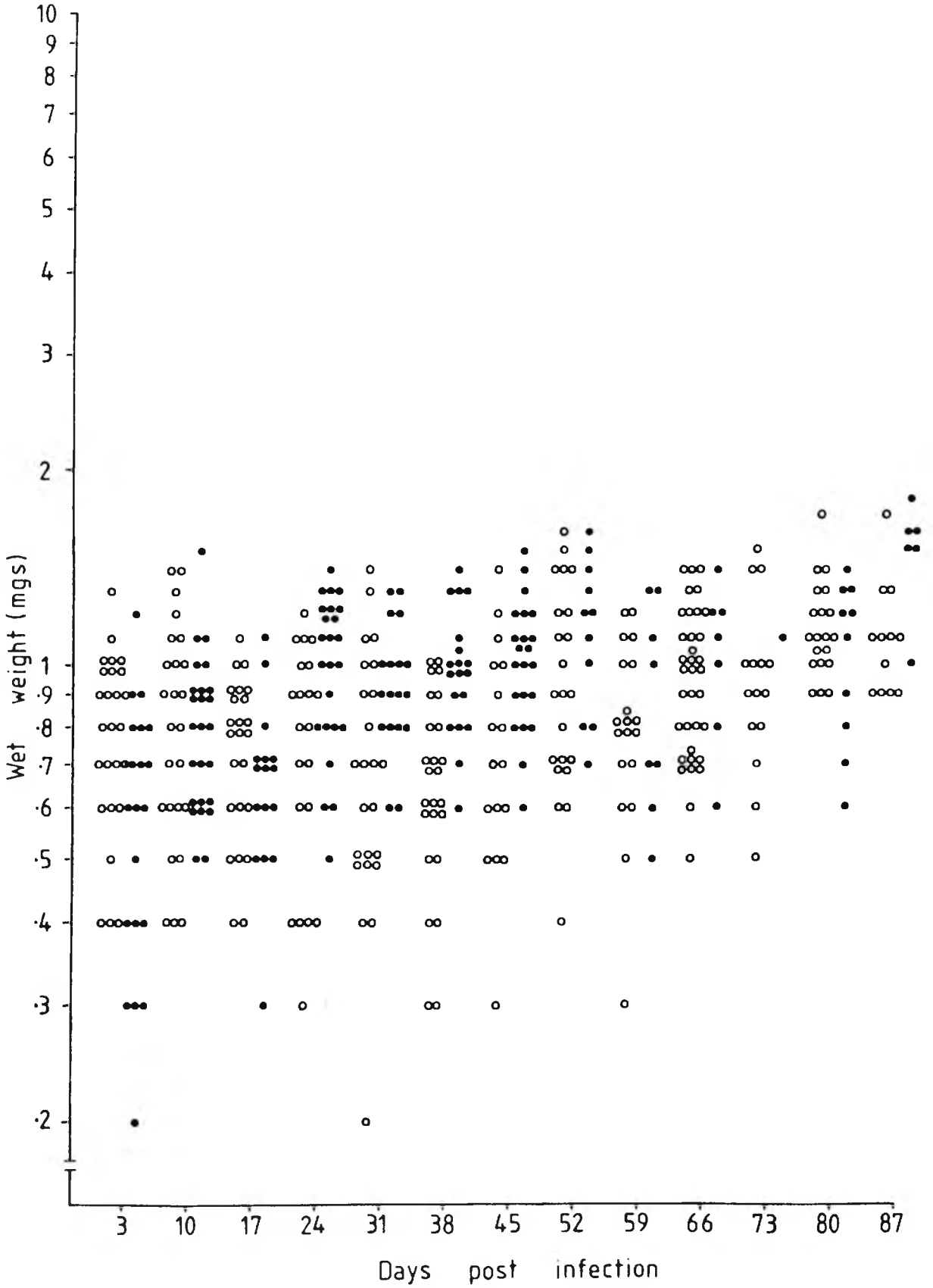


Figure 6.6. Semi-log plots of the wet weights of female Acanthocephalus lucii recovered from perch during experimental infections at 5°C (open circles) and 19°C (closed circles).

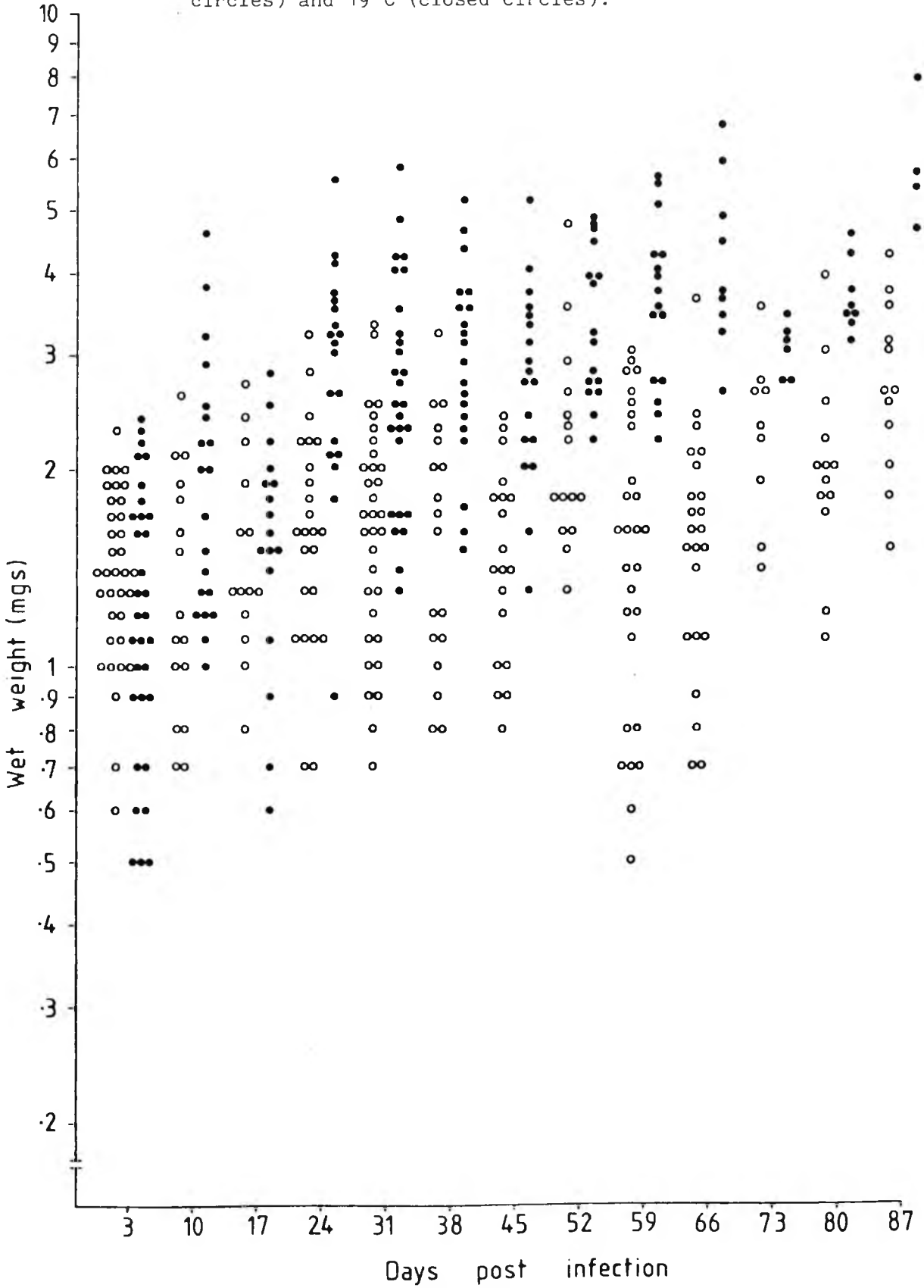


Table 6.4. The mean wet weights of male Acanthocephalus lucii recovered during experimental infections of perch at 5 and 19°C, together with the significance of the difference between respective means

		Days post infection												
		3	10	17	24	31	38	45	52	59	66	73	80	87
5°C	Number of male worms weighed	26	25	24	21	24	25	17	22	19	36	15	20	13
	Mean wet weight (mgs)	0.80	0.83	0.75	0.77	0.73	0.68	0.78	0.98	0.82	0.96	0.96	1.14	1.07
19°C	Number of male worms weighed	23	25	16	24	18	19	22	11	8	7	1	10	6
	Mean wet weight (mgs)	0.62	0.81	0.67	1.0	0.94	0.99	1.03	1.15	0.9	1.04	1.1	1.05	1.5
	Significance of difference between means	*	n.s.	n.s.	**	*	***	***	n.s.	n.s.	n.s.	n.t.	n.s.	**

Legend as for Table 6.2 (n.t. = significance not tested)

Table 6.5. The mean wet weights of female Acanthocephalus lucii recovered during experimental infections of perch at 5 and 19°C, together with the significance of the difference between respective means

		Days post infection												
		3	10	17	24	31	38	45	52	59	66	73	80	87
5°C	Number of female worms	35	16	14	24	33	18	19	15	28	23	9	13	12
	Mean wet weight (mgs)	1.43	1.38	1.53	1.68	1.70	1.66	1.51	2.25	1.63	1.61	2.30	2.08	2.73
19°C	Number of female worms	33	20	17	19	27	20	18	16	16	9	6	9	4
	Mean wet weights (mgs)	1.30	2.04	1.62	2.98	2.8	3.02	2.83	3.40	3.66	4.23	3.02	3.61	5.8
	Significance of difference between means	n.s.	*	n.s.	***	***	***	***	**	***	***	*	***	***

Legend as for Table 6.2

Table 6.6. Changes in the number of male and female Acanthocephalus lucii recovered and the sex ratio during experimental infections of perch at 5 and 19°C

		Days post infection												
		3	10	17	24	31	38	45	52	59	66	73	80	87
5°C	Number of male worms	28	26	27	22	26	29	19	22	19	36	18	21	18
	Number of female worms	39	17	17	24	34	22	19	15	28	24	17	18	19
	Sex ratio (♂ : ♀)	1.39	0.65	0.63	1.09	1.55	0.76	1	0.68	1.47	0.67	0.94	0.86	1.06
19°C	Number of male worms	26	27	19	25	19	20	22	12	8	7	1	10	6
	Number of female worms	34	22	22	21	31	21	21	16	17	11	8	10	5
	Sex ratio (♂ : ♀)	1.31	0.81	1.16	0.84	1.63	1.05	0.95	1.33	2.13	1.57	8.00	1	0.83

maintained at 19°C. Although this decline is due to mortality of both male and female parasites, these results indicate that a higher rate of mortality of male parasites takes place during this period.

Copulatory caps

Following copulation male parasites leave a small copulatory cap over the posterior end of females. Table 6.7 gives the number of female parasites recovered and the proportion with copulatory caps throughout the course of experimental infection at 5 and 19°C. At 5°C female parasites with copulatory caps were recovered throughout the experimental period. There was no evidence of a gradual increase in the proportion of females with caps during the infection and in fact the proportion never exceeded 33.3% at any time during the experimental period. This tends to suggest that copulatory caps remain on females for only a short time, or alternatively, only a small proportion of female parasites were inseminated during the experimental period at 5°C. The fact that copulatory caps were observed from only 3 days post infection indicates that copulation commenced as soon as parasites established in the intestine of perch. Although Figure 6.2 indicated that all female parasites recovered from perch at 5°C remained at stage 1 until at least 66 days post infection, these results indicate that this was

Table 6.7. The number of female Acanthocephalus lucii recovered and the number and percentage with copulatory caps during experimental infections of perch at 5 and 19°C

		Days post infection												
		3	10	17	24	31	38	45	52	59	66	73	80	87
5°C	Number of female worms recovered	39	17	17	24	34	22	19	15	28	24	17	18	19
	Number with copulatory cap (%)	5(12.8)	3(17.6)	5(29.4)	4(16.6)	2(5.9)	1(4.5)	1(5.3)	5(33.3)	1(3.6)	6(25)	1(5.9)	4(22.2)	1(5.2)
19°C	Number of female worms recovered	34	22	22	21	31	21	21	16	17	11	8	10	5
	Number with copulatory cap (%)	5(14.7)	4(18.2)	6(27.3)	4(19.1)	4(12.9)	2(9.5)	3(14.3)	2(12.5)	3(17.6)	0(-)	0(-)	0(-)	0(-)

not due to inhibition of copulatory activity by the cooler water temperature.

At 19°C some female parasites with copulatory caps were recovered from 3 until 59 days post infection. Again the presence of copulatory caps only 3 days after infection indicates that copulatory activity commenced as soon as the parasites established in the intestine. As Figure 6.2 shows, at 19°C, almost all female parasites recovered from 31 days post infection onward contained mature shelled acanthors, indicating that they had been inseminated previously. However, during the first 31 days of the infection, the proportion of females with copulatory caps never exceeded 27.3% This suggests that copulatory caps remained on females for relatively short periods. The presence of copulatory caps on females many weeks after mature shelled acanthors first appear suggests that each female parasite underwent multiple inseminations during the course of the infection. No copulatory caps were found on any female parasite recovered between 66 days post infection and the end of the infection period. This suggests that at 19°C no further copulations occurred from, at the latest, about 59 days post infection. Since it is not known exactly how long a copulatory cap remained on a female parasite at 19°C, it is impossible to say exactly when copulatory activity ceased. These results are in accord with the survival pattern of adult parasites at 19°C, since the cessation of copulatory activity appears to coincide

precisely with the period when the sex-ratio of the adult parasite (Table 6.6) changes in favour of females, indicating a higher mortality of male parasites.

Distribution along the host alimentary canal

The distribution of male and female parasites along the intestine of perch throughout the course of experimental infections at 5 and 19°C is shown in a series of histograms as follows. Figure 6.7 - male parasites at 5°C, Figure 6.8 - female parasites at 5°C. Figure 6.9 - male parasites at 19°C, Figure 6.10 - female parasites at 19°C. From these Figures it is apparent that there is considerable variation between the position of individual parasites which appears to be slightly greater for both males and females at 5°C. There is no evidence of marked differences between the distributions of male and female parasites at either temperature. Furthermore there is no evidence of a noticeable change in attachment position of either sex of parasite at either temperature during the course of the infection. The slightly more anterior position of both male and female parasites recovered 3 days post infection at 5°C can be attributed to the slow rate of digestion of the host at this temperature. These worms had probably spent about 2 of the first 3 days after ingestion in the host stomach

and so had simply not yet reached the more posterior attachment site occupied by older infections at this temperature.

The number of male and female parasites recovered and their respective mean attachment positions at each week during the course of the experimental infections are given in Table 6.8 ($5^{o}C$) and Table 6.9 ($19^{o}C$). Each Table also gives the results of a t-test on the significance of the difference between respective mean positions of male and female parasites at each temperature. Initial F-tests revealed no significant difference between the variances within each pair of means.

Examination of the means in Table 6.8 reveals that at most times during the experimental infections, at both temperatures, male parasites were located slightly anterior to females. Only at certain times were the differences statistically significant. The mean attachment positions of both male and female parasites at each temperature also show slight changes during the course of the infection. Although one-way analysis of variance revealed that these slight changes were statistically significant for both sexes of parasite at both temperatures ($p < 0.005$ for males at both 5 and $19^{o}C$ and for females at $5^{o}C$, and $p < 0.05$ for females at $19^{o}C$) there was no evidence of a mass movement of parasites either up or down the intestine during the course of the infection.

Table 6.9 gives the overall mean attachment positions for all parasites in a given category recovered throughout the infection period at each temperature. At both 5 and 19°C the overall mean position of males was slightly anterior to that of females and in both instances the difference was statistically significant ($p < 0.001$ at both temperatures). Within the female parasite population (at 19°C) stage 1 and stage 2 females were located approximately 4% nearer the pylorus than stage 3 females and again the differences were statistically significant ($p < 0.05$ in both instances), indicating a slightly posterior migration of mature female parasites.

The overall mean attachment position of male parasites differed significantly between 5 and 19°C ($p < 0.05$) but the overall mean position of female worms at the two temperatures did not. This suggests that temperature influenced the position of attachment of male but not female worms.

These results indicate that slight changes in the attachment positions of various members of the adult parasite population do take place. However, when one considers the size of adult parasites (see Plate 7.1), and the fact that the intestine of, say, a 15cm perch is typically about 7cms in length, then a movement down the intestine of 4% represents an actual distance of only about 3mm, so in reality there is very little difference

Figure 6.7. The distribution of male Acanthocephalus lucii in the intestine of perch during experimental infections at 5°C.

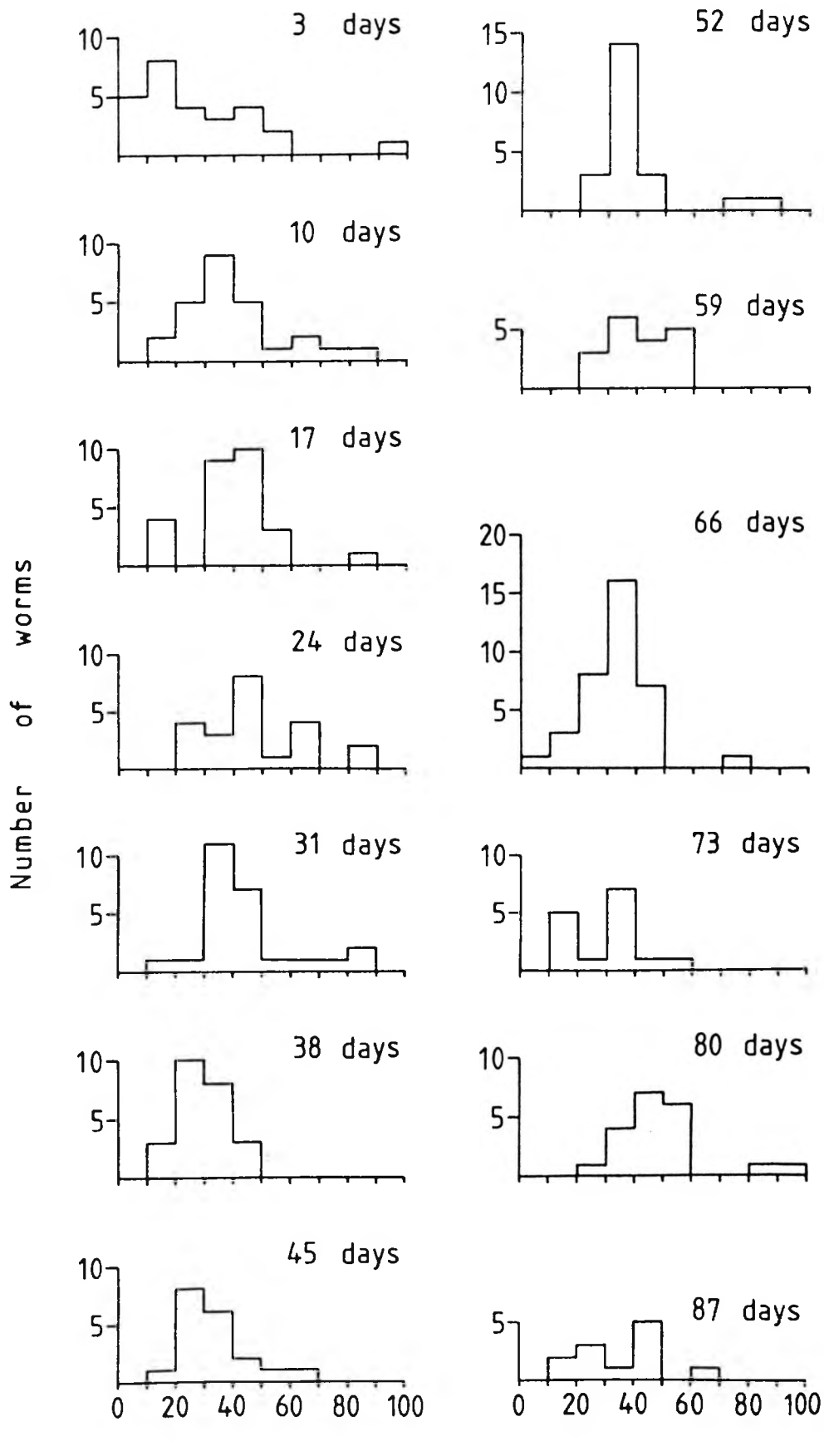


Figure 6.8. The distribution of female Acanthocephalus lucii in the intestine of perch during experimental infections at 5°C.

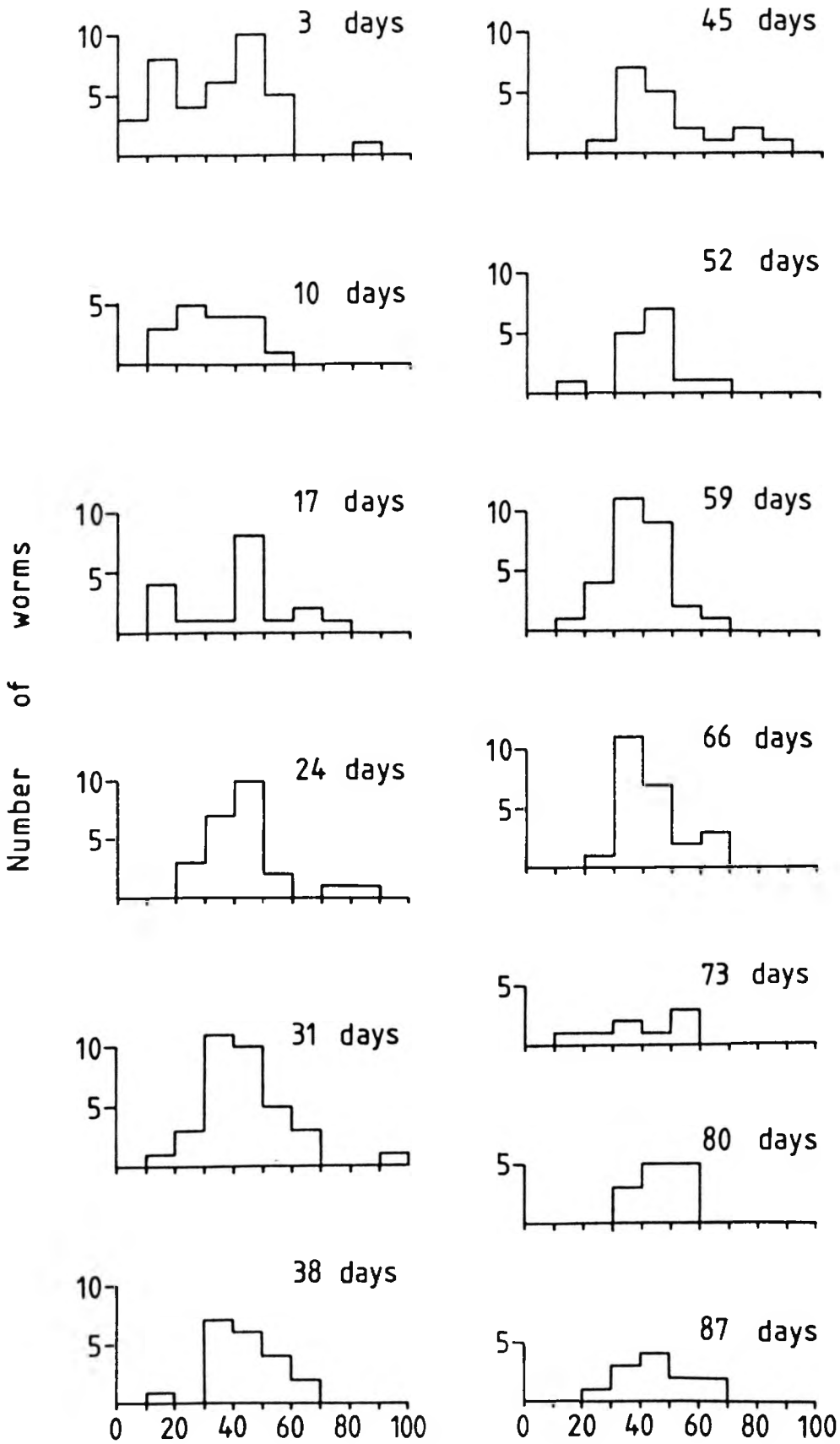


Figure 6.9. The distribution of male Acanthocephalus lucii in the intestine of perch during experimental infections at 19°C.

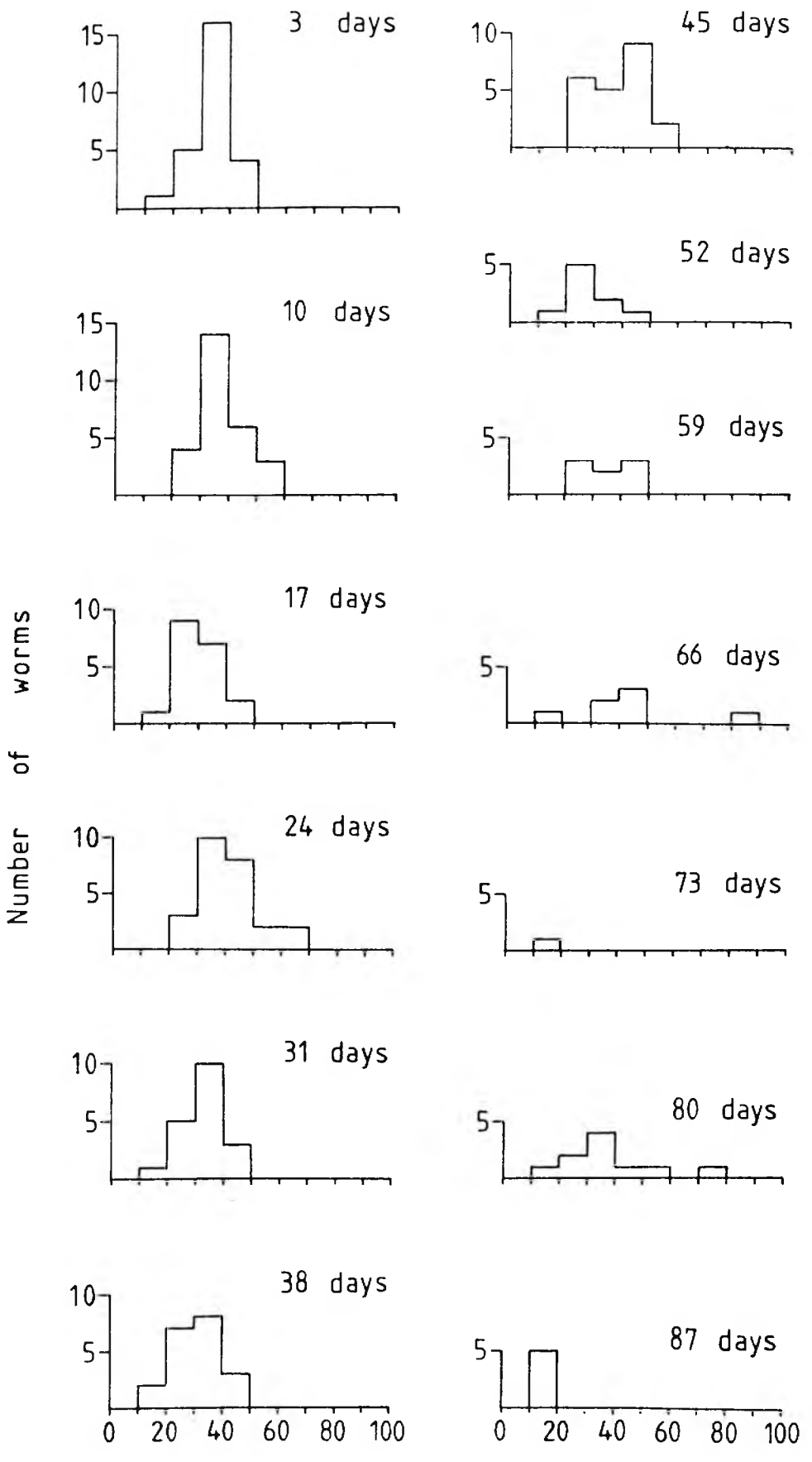


Figure 6.10. The distribution of female Acanthocephalus lucii in the intestine of perch during experimental infections at 19°C.

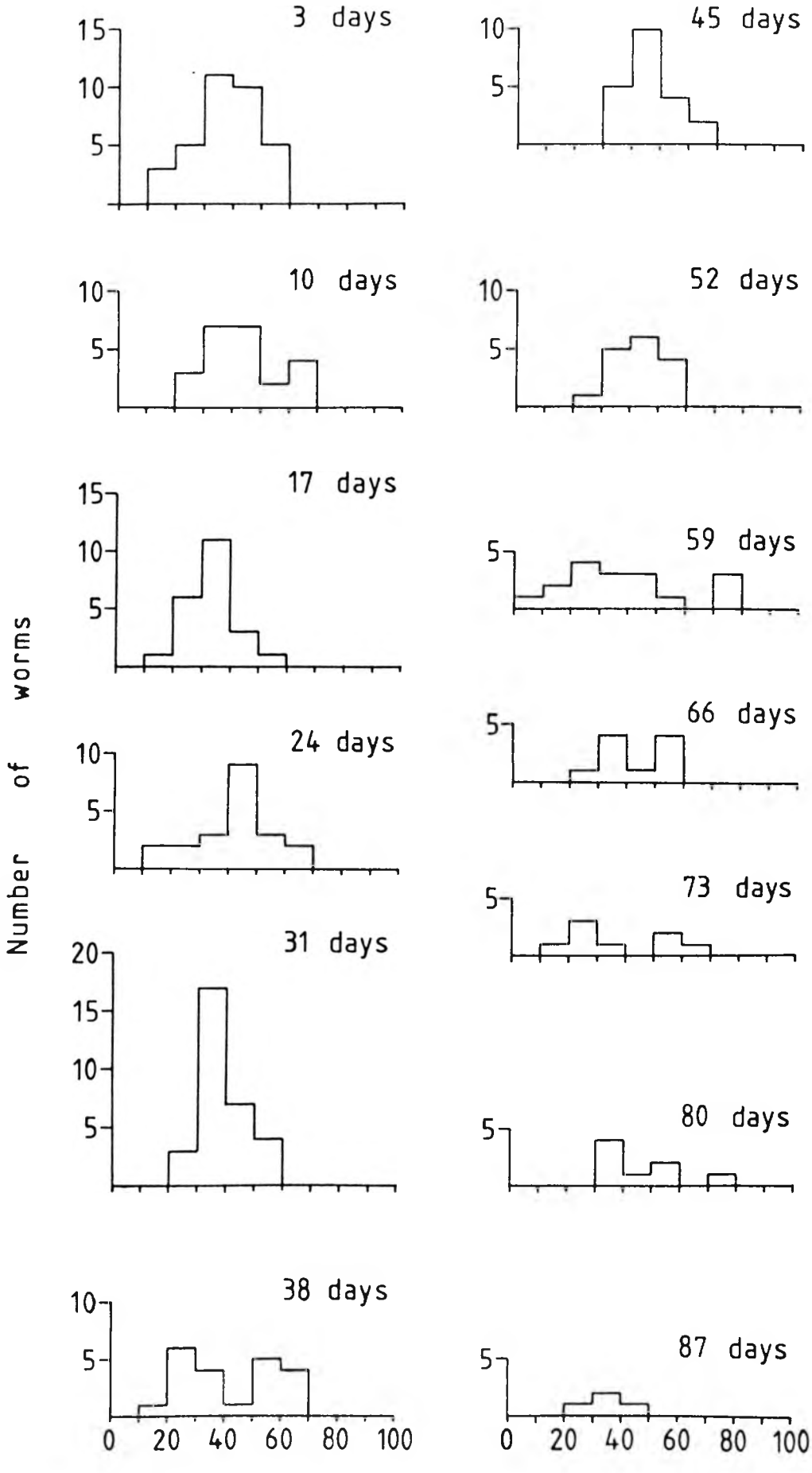


Table 6.8. The mean positions of attachment of male and female Acanthocephalus lucii in the intestine of perch during experimental infections at 5°C, together with the significance of the difference between respective means

	Days post infection												
	3	10	17	24	31	38	45	52	59	66	73	80	87
Number of male worms	27	26	27	22	26	25	19	22	19	36	15	20	13
Mean position	26.63	39.62	39.04	46.32	42.54	32.44	34.16	38.64	35.52	33.06	29.33	48.75	41.23
Number of female worms	37	17	17	24	34	20	19	15	28	24	9	13	14
Mean position	34.35	32.29	39.94	42.42	43.00	42.05	47.42	40.40	38.25	42.75	38.89	46.08	49.29
Significance of difference between means	n.s.	n.s.	n.s.	n.s.	n.s.	*	**	n.s.	n.s.	**	n.s.	n.s.	n.s.

Legend as for Table 6.2

Table 6.9. The mean positions of attachment of male and female Acanthocephalus lucii in the intestine of perch during experimental infections at 19°C, together with the significance of the difference between respective means

	Days post infection												
	3	10	17	24	31	38	45	52	59	66	73	80	87
Number of male worms	26	27	19	25	19	20	22	12	8	7	1	9	6
Mean position	32.92	37.67	30.06	40.16	32.26	30.10	35.09	31.25	33.75	42.14	16	32.44	22.67
Number of female worms	34	22	22	21	31	21	21	16	17	11	8	10	5
Mean position	36.97	42.09	31.73	41.95	38.90	40.52	45.19	42	37.88	41.45	37.75	47.89	36.8
Significance of difference between means	n.s.	n.s.	n.s.	n.s.	**	**	***	**	n.s.	n.s.	n.t.	*	N.S.

Legend as for Table 6.2 (n.t. = significance not tested)

Table 6.10. The mean attachment positions (expressed as percentage distance posterior from pylorus) of various groups of adult Acanthocephalus lucii recovered from experimental infections of perch at 5 and 19°C

	5°C		19°C	
	Total number of parasites	Mean position	Total number of parasites	Mean position
Males	297	37.19	199	34.05
Females	271	40.59	239	39.73
Stage 1 females only	-		39	37.15
Stage 2 females only	-		49	37.04
Stage 3 females only	-		151	41.71

between the mean attachment positions of the various groups of adult parasites with increasing time after infection at either temperature.

Experimental infections of alternative definitive hosts

Goldfish

Six goldfish, acclimatized to 12°C, were each given 6 infected isopods and dissected 24 hrs post infection. No specimens of A. lucii were recovered.

Roach

Seven roach, acclimatized to 19°C, were each given 6 infected isopods. One roach was dissected 2 hrs post infection. Eight specimens of A. lucii were recovered. Six were dead (4 females and 2 males) and all showed visible signs of mechanical damage. Two were alive (both males) and attached to the upper intestine. Six roach were dissected after 24 hrs. No specimens of A. lucii were recovered.

Rainbow trout

Twelve rainbow trout, acclimatized to 12°C, were each given 6 infected isopods and dissected in batches of 4 at weekly intervals post infection. On examination all trout

harboured infections of from 1 to 8 specimens of A. lucii. Many female worms were at stage 2 but no mature stage 3 females were recovered. Many females bore copulation caps. The parasites were distributed throughout the intestine posterior to the pyloric caeca.

One rainbow trout, acclimatized to 19°C, was given 15 infected isopods and dissected 31 days after infection. Ten specimens of A. lucii were recovered, 3 males and 7 females. All females recovered contained mature shelled acanthors and were of a size comparable to those from the appropriate experimental infection of perch.

Tilapia

Six tilapia, acclimatized to 24°C were each given 6 infected isopods and dissected 24 hrs post infection. No specimens of A. lucii were recovered.

Discussion

The results of these experiments suggest that similar proportions of Acanthocephalus lucii establish in perch regardless of whether the parasites are ingested in summer or in winter. At both 5 and 19°C a high proportion of parasites

established and although slight differences in recovery at the two temperatures were apparent, these differences were not statistically significant. This contrasts strongly with the results of experimental infections of Echinorhynchus truttae in brown trout (Salmo trutta) (Awachie, 1963, 1966) and Pomphorhynchus laevis in dace (Leuciscus leuciscus) (Hine, 1970) and goldfish (Carassius auratus) (Kennedy, 1972). In both E. truttae and P. laevis establishment was considerably reduced at higher temperatures.

Considerable differences in the proportion of A. lucii establishing in individual fish were apparent although at neither temperature was any fish completely resistant to infection. This agrees well with the findings of Kennedy (1972) for P. laevis in goldfish. This variation can be attributed to a number of factors. These include slight variation in the number of cystacanths administered, and in the size, age and so perhaps infectivity of individual cystacanths. Differences in the physicochemical conditions in the fish gut with respect to cystacanth eversion might also be involved as might differences in the resistance of individual fish to infection. As Kennedy (1974) has pointed out this wide variation in the proportion of parasites establishing in individual fish is important in indicating just how much

fish do differ in susceptibility to infection. Consequently it suggests a potential mechanism whereby the observed highly overdispersed distribution of adult parasites in the definitive host population could be generated.

Following establishment a gradual decline in parasite survival was evident with increasing time after infection at both temperatures. This is in agreement with the pattern exhibited by other acanthocephalans during experimental infections, including P. laevis in goldfish (Kennedy, 1974b), Polymorphus minutus in ducks (Nicholas and Hynes, 1958) and Moniliformis dubius in rats (Crompton and Walters, 1972). This gradual decline is important in that it indicates that not every parasite which established survived long enough to reach maturity. The factors responsible for this loss are unknown although Awachie (1963) suggested it was an accidental one owing to parasites lodging the proboscis in debris in the intestinal lumen, and so being passed out of the intestine by peristalsis. Although the decline in parasite survival was initially similar at both experimental temperatures, from approximately 59 days post infection onwards survival was consistently lower at the higher temperature. Kennedy (1972) found no evidence of reduced survival of P. laevis in goldfish at higher temperatures during experimental infections, although his

experiments were terminated after 49 days and P. laevis does not mature in goldfish. Anderson (1974a) found evidence for reduced survival of the cestode Caryophyllaeus laticeps (Pallas) in bream (Abramis brama (L.)) at higher summer temperatures. Mills (1980) found that temperature affected the survival of the ectoparasitic digenean Transversotrema patialense (Soparkar) on the zebra fish (Brachydanio rerio Hamilton-Buchanan), survival being reduced by any deviation from an optimum temperature of 23°C.

The fact that survival of A. lucii was reduced at the higher temperatures is extremely significant with regard to explaining the observed seasonal changes in infection levels of perch in the field described in Chapter 4. In both 1979 and 1980 intensity declined steadily from May onwards, in spite of the fact that recruitment of larval parasites into the perch population continued through the summer months. This indicates that mortality exceeded recruitment, which is consistent with the results from the experimental work, since the higher water temperatures in the warmer summer months would result in higher mortality of worms in perch in the field. In actual fact it is suggested that the level of infection declined through summer as a result of both a temperature dependent increase in mortality and a decline in recruitment

owing to a reduction in the availability of cystacanths in the intermediate host population.

Analysis of changes in the sex ratio during the course of the experimental infections indicated that initially equal proportions of each sex of parasite established in the intestine of perch. At 5°C such a pattern was maintained throughout the experimental period, but at 19°C, towards the end of the infection period there was a proportionately greater loss of male worms leading to a sex ratio in favour of females. The latter result is in agreement with experimental infections on Polymorphus minutus in ducks (Crompton and Whitfield, 1968; Nicholas and Hynes, 1958), Moniliformis dubius in rats (Burlinghame and Chandler, 1941; Crompton and Walters, 1972) but in contrast to those on Pomphorhynchus laevis in goldfish (Kennedy, 1972). Similarly a sex ratio in favour of female worms has been observed in a number of natural populations including Echinorhynchus truttae in brown trout (Awachie, 1965), Acanthocephalus parksidei in various definitive hosts (Amin, 1975), Acanthocephalus dirus in Semotilus atromaculatus (Camp and Huizinga, 1979), Echinorhynchus salmonis in various definitive hosts (Amin and Burrows, 1977) and A. lucii in the present field study (see Chapter 4).

The higher rate of mortality of male A. lucii towards the end of the infection period (at 19°C) coincided with the

time when copulatory activity of male worms ceased. Although an immunological response by the host cannot be ruled out, it appears that male worms were in fact 'spent' and the mortality appeared to be simply due to natural senescence.

The presence of copulatory caps on female worms only 3 days after infection at both 5 and 19°C indicated that copulatory activity of worms was not inhibited by cold temperatures and that it commenced as soon as worms established in the intestine. In fact female A. lucii with copulatory caps have been recovered only 24 hrs post infection at 20°C (Brattey, 1980). Active spermatozoa have been observed in smears of testes removed from cystacanths, which indicates that spermatogenesis commenced whilst the parasite was still in the haemocoel of the intermediate host. Also, the ovaries of female cystacanths were fragmented into ovarian balls. Precocious development of cystacanths has been observed in a number of acanthocephalans, including Prosthorhynchus formosus (Schmidt and Olsen, 1964), Echinorhynchus truttae (Awachie, 1963), Acanthocephalus jacksoni (Muzzall and Rabalais, 1975b), Fessisentis friedi (Muzzall, 1978), although only in a few instances (Awachie, 1963; Muzzall and Rabalais, 1975b) have experimental infections of the appropriate definitive host been attempted, indicating that copulation commenced immediately after establishment. In many species

further development of the parasite in the intestine of the definitive host is necesary before copulation can take place. Thus evidence of insemination was first noted in female Polymorphus minutus 5 and 8 days after infection by Crompton and Whitfield (1968) and Nicholas and Hynes (1958), respectively, and in Moniliformis dubius 16 days after infection (Crompton 1974). Uglem and Larson (1969) considered Neoechinorhynchus saginatus to be incapable of copulation even after 46 days in the intestine of chub (Semotilus atromaculatus).

Wet weights of parasites were used to estimate the growth of A. lucii during the course of the experimental infections. As Crompton (1970) has pointed out such a method is only approximate and should only be used when the parasites are weighed quickly when taken from normal hosts with access to food and water. Consequently the results from both the current and previous studies where wet weights have been used should be treated with caution. This point was clearly illustrated by Read and Rothmann (1958) who found that starvation of the host for 48 hrs resulted in the wet weight of female Moniliformis dubius decreasing from 190 to 84mgs.

Many previous studies have shown that adult female acanthocephalans are larger than males (Graff and Allen, 1963; Crompton and Whitfield, 1968; Crompton, 1972; Kennedy,

1974b; Amin et al, 1980), which is in agreement with the results on A. lucii. The current study has also demonstrated that male and female cystacanths of A. lucii can vary enormously in size and female cystacanths are typically much larger than males. Similar observations were made for cystacanths of Acanthocephalus dirus by Camp and Huizinga (1979). Thus even before growth commenced in the intestine of the definitive host female parasites were somewhat larger than males. Male parasites apparently underwent only slight growth in the intestine of the definitive host, which is in accord with the earlier observation that complete development, including spermatogenesis, is achieved in the haemocoel of the intermediate host. Females, on the other hand, underwent considerable growth to accommodate the large numbers of developing shelled acanthors. Growth of female A. lucii was considerably reduced, but not completely inhibited at 5°C, which is not altogether surprising in view of the reduced food intake and rate of digestion by the host at such low temperatures. At 19°C growth was rapid and mature shelled acanthors were present in female worms after 24 days. Shelled acanthors were, however, not easily detected in fish faeces (although this may be owing to the inefficiency of the methods employed - see Chapter 7). There was no evidence of a decrease in worm weight towards the end of the infection period which tends to suggest that many worms pass out of

the intestine intact. This is in accord with the results from the field study (Chapter 4).

The distribution of A. lucii in the intestine of perch during the course of the experimental infections is in agreement with the results from the field investigations described in Chapter 4. Both field and experimental investigations indicated a slightly more anterior localization of male worms but gave no evidence of a pronounced change in attachment site of either male or female worms during the course of the infection.

Kennedy et al (1976) provided evidence to suggest that the process of activation and liberation of the cystacanths is a major factor determining the distribution and site of Pomphorhynchus laevis in the intestine of its various definitive hosts. Soon after infection the parasite proboscis became encapsulated by host response tissue thereby preventing movement. These authors suggested that P. laevis may, atypically, be a passive animal, incapable of site recognition or location. The distribution of A. lucii in the intestine of perch shows many similarities to that of P. laevis. Both parasites are capable of surviving in all regions of the intestine, they show preference for a particular region of the intestine and appear to remain at the same site throughout the course of the infection. However, the proboscis of A. lucii is not

encapsulated by host response tissue and so movement is not restricted. Unfortunately the nature of the data from the field and experimental studies was such that it is impossible to ascertain the principle factors involved in determining the distribution of A. lucii in the intestine of perch. Although it is suggested that A. lucii has considerable mobility, as has Gracilisentis gracilisentis (Jilek, 1979), whether or not A. lucii is capable of both site recognition and location will probably only be determined when surgical transfer experiments of the type described by Braten and Hopkins (1969) and Alphey (1970) are attempted.

The results of the experimental infections indicate that the rate of maturation of shelled acanthors in female Acanthocephalus lucii correlates quite clearly with water temperature. Maturation was slowest at 5°C with no mature female parasites recovered even after 87 days. At 12°C mature females were recovered after 52 days and at 19°C after 24 days. Unfortunately there appears to be few similar experimental studies concerned with rate of maturation of female Acanthocephala in piscine definitive hosts maintained at different temperatures with which these results can be compared. DeGuisti (1949) studied the development of Leptorhynchoides thecatus in the rock bass (Ambloplites rupestris (Rafinesque)).

Female worms with mature shelled acanthors were recovered after 8 weeks, although the experimental temperature was not given. Awachie (1963, 1966) based on experimental studies, found that temperature influenced the rate of development of Echinorhynchus truttae in brown trout (Salmo trutta). At 15-20°C mature female parasites were recovered after 51 days and although complete details of additional experiments were not given he concluded "at the lowest temperature which trout has to endure naturally the development of the parasites proceeds at a slower rate". Rojanapaibul (1977) experimentally infected bullhead (Cottus gobio) with Acanthocephalus clavula (Dujardin, 1845). At 8-13°C female worms containing shelled acanthors were recovered 45 days post infection. Andryuk (1979) experimentally infected perch with Acanthocephalus lucii and found the parasite took 3 weeks to mature, although again the experimental temperature was not given. For Acanthocephala with homeothermic hosts Crompton (1974) recovered mature female Moniliformis dubius from the intestine of rats 35 days after infection. Nicholas and Hynes (1958) and Crompton and Whitfield (1968) recovered mature female Polymorphus minutus from the intestine of ducks 19 and 18 days post infection, respectively.

Analysis of the stages of maturation of female A. lucii removed from perch during the course of the field study indicated

that immature female parasites (stage 1) were present in every sample collected throughout the year. In Chapter 4 this was considered to provide evidence to support the suggestion that recruitment of larval parasites into the perch population continued throughout the year. However, as Walkey (1967) has pointed out, the validity of this assumption depends on the extent to which immature individuals are representative of recent acquisitions. The results from the 5°C experiment indicate that for A. lucii, at winter temperatures, this assumption may be invalid. After 87 days (almost 3 months) at 5°C, over 50% of all female parasites recovered were still immature (stage 1). Consequently immature parasites recovered from perch in winter may be at least 3 months old and so do not necessarily represent recently acquired individuals. However, since the rate of maturation of female A. lucii is greatly reduced at winter temperatures, if recruitment does take place at this time, then one would expect a gradual increase in the mean number of immature female worms in perch during the winter months. The data in Figure 4.2 and Table 4.2 indicate that this did occur. Between December 1980 and March 1981 there was a gradual increase in both the proportion and the mean number of immature female worms per fish indicating that some recruitment of parasites into the perch population takes place, even in the

coldest winter months. The overall infection level between December and March still declined, owing to the fact that mortality of old mature parasites exceeded recruitment of new immature ones. The results in Figure 4.2 further indicated that the worms ingested between December and March did not reach maturity until the following June.

Although rapid recruitment of larval parasites into the perch population takes place in spring in none of the samples was a large proportion of stage 1 parasites found, but a very large proportion of parasites at stage 2 were recovered in April and May. The results of the 12^{o}C experiment indicate that at this temperature the majority of worms reach stage 2 approximately 10 days after infection. Furthermore, most remain at this stage for a further 40 days. It might be tempting to predict that rapid recruitment in April and May would result, at least initially, in large numbers of stage 1 worms in perch. These experimental results suggest the fairly rapid development of stage 1 worms into stage 2, combined with the much longer duration of stage 2 make it more likely that a high proportion of stage 2 worms would result. The results in Figure 4.2 for May 1979, and April and May 1980 indicate that this did occur.

The field study revealed that mature (stage 3) female A. lucii were present throughout almost the entire sampling period (the exception being May 1980). Many authors (Chubb,

1964; Amin and Burrows, 1977; Muzzall and Bullock, 1978) have observed mature female acanthocephalans in fish throughout the year and have concluded from this, that maturation takes place throughout the year. The experimental results in the current study suggest, for A. lucii at least, this is not true. The fact that mature female A. lucii were recorded throughout almost the entire year does not necessarily indicate that the maturation process 'per se' takes place throughout the year. Although there was some development of female A. lucii at all experimental temperatures, the results suggest that parasites ingested in winter do not reach maturity until the warmer months in late spring - early summer. Consequently the mature female parasites present in perch in the winter months probably represent parasites which actually reached maturity (stage 3) earlier in the year when water temperatures were higher and have simply survived in the intestine of perch for many months in the mature condition. The results of the 19°C experiment indicate quite clearly that some female worms can survive in the intestine of perch for at least a further 2 months after mature shelled acanthors first appear in the body cavity.

Although the rate of maturation of female A. lucii appears to be greatly reduced at winter temperatures the results from a number of previous field studies suggest that in many species

of Acanthocephala with piscine definitive hosts maturation is not inhibited during the winter months. In many of the species which show seasonal cycles in both incidence and maturation, recruitment of larval parasites into the fish population takes place during the cooler months, typically during autumn. Close inspection of the data in such studies reveals that mature parasites first appear in early spring, but long before the spring rise in water temperatures takes place. Thus Steinstrasser (1936) observed immature Neoechinorhynchus rutili in rainbow trout (Salmo gairdneri (= irideus)) in November. The parasites grew slowly through the winter months and mature parasites were recovered in February. Tedla and Fernando (1970) described recruitment of Echinorhynchus salmonis into yellow perch (Perca fluviatilus) from October until February, by which time 75% were infected. Mature worms first appeared in January and by April over 50% of all female worms recovered were mature. Van Cleave (1916) observed recruitment of larval Neoechinorhynchus (= Gracilisentis) gracilisentis into gizzard shad Dorosoma cepedianum (Le Sueur) in October, when parasites were small and immature. By the latter part of November most individuals had reached full sexual maturity and thereafter infection levels declined until the parasites were completely absent in June. Amin (1975) observed recruitment of Acanthocephalus

parksidei into Semotilus atromaculatus and Catastomus commersoni during late summer. A gradual increase in the proportion of mature female worms occurred through the winter months. Muzzall and Rabalais (1975a) observed recruitment of A. jacksoni into its definitive host in autumn, with the first mature worms recovered in January. Similar results, where mature parasites first appear before the spring rise in water temperature, have been described for a number of other species of fish parasite including cestodes such as Triaenophorus nodulosus (Chubb, 1963), Caryophyllaeus laticeps (Kennedy, 1969), Proteocephalus torulosus (Kennedy and Hine, 1969), Proteocephalus percae (Wootten, 1974), and digeneans such as Sphaerostoma bramae (Davies, 1967), Crepidostomum farionis (Awachie, 1963) and Bunodera luciopercae (Skorping, 1980a) to name but a few examples. These results indicate that warm water temperatures are not essential for maturation in many species of fish parasite.

In many of the examples given above adult parasites are completely absent from fish at certain times of the year, typically during the warmer summer months when parasites die after breeding. Many factors can result in an absence of adult worms at specific times of the year. These include seasonal changes in host diet or level of infection of

intermediate hosts such that larval parasites are no longer ingested by fish and the existing adult parasite population gradually disappears. Seasonal changes in the resistance of fish to infection may also be involved such that larval parasites which are ingested do not establish, and established parasites are rejected. None of these factors apply to A. lucii. The field study indicated that larval parasites were present all the year round and they were ingested by perch throughout the year. The experimental studies indicate perch are susceptible to infection at all times of the year and as a result adult parasites are present in perch at all times of the year.

Since the results from the experimental study have been used to answer questions which arose from the field study it seems appropriate to consider to what extent the experimental results are applicable to the situation in the field. A number of points suggest the results are comparable. In the laboratory study the natural definitive host was used, the initial parasite population density (7.2 parasites per fish) was comparable to the overall density of A. lucii in perch in the field (8.1 parasites per fish). The parasites also grew to a size comparable to those in the field, they produced viable shelled acanthors and occupied a similar region in the host intestine. However,

in natural populations the situation is much more complex. The diet of the host is more varied, fish are continuously being reinfected and so harbour parasites of different ages and a considerable proportion of the host population experience much higher parasite densities than those used in the experimental infection. Some caution is needed therefore, when comparing the field and experimental results. The latter point is particularly important since ideally the experiments should have been repeated at the complete range of parasite population densities experienced by fish in the field. Unfortunately, lack of sufficient infected intermediate hosts prevented such experiments.

In the experiments involving alternative definitive hosts, although only small numbers of fish were involved the results indicate considerable differences between the species in their suitability as hosts for A. lucii. No parasites were recovered from tilapia or goldfish after only 24 hrs, suggesting the parasites had passed through the intestine or had been digested. Since no specimens of A. lucii were found in the faeces the latter alternative appears more likely.

The results from the experiment with roach are of considerable interest in that they quite clearly indicate that the masticatory action of the roach pharyngeal teeth is responsible for the

destruction of many of the cystacanths which are ingested. Although field data (Rizvi, 1964; Mishra, 1966) suggest that the roach intestine can provide suitable conditions for parasite growth and maturation, these results suggest that a high proportion of the parasites ingested do not establish.

Rainbow trout appear to be particularly favourable hosts for A. lucii. Although no mature female worms were recovered 21 days post infection at 12°C this can simply be attributed to a shortage of time. At 19°C the parasites grew normally and produced mature shelled acanthors. Campbell (pers. comm. 1981) has examined rainbow trout from the Lake of Menteith, Central Region, Scotland, and found over 700 specimens of A. lucii in a single intestine. Since the parasite matures in rainbow trout, and at least in some localities rainbow trout feed quite extensively on Asellus aquaticus, it would not be surprising if numerous records of the occurrence of A. lucii in rainbow trout appear. Many natural freshwaters in Britain have recently been stocked with rainbow trout and the parasite can clearly complete its life cycle using this species as a definitive host.

Rainbow trout might also prove to be a suitable alternative laboratory definitive host for further experimental work on A. lucii.

CHAPTER 7

CONCLUSIONS

Chapter 7

Although interest in fish parasite population dynamics has risen considerably in recent years the vast majority of previous studies have had two major shortcomings. Firstly, most have been concerned solely with a single stage in the life cycle of the parasite and secondly, in very few instances have hypotheses which have arisen from field studies been tested by experimental work. Such drawbacks are largely a consequence of both the complex nature of the life cycle of many parasites and the technical difficulties associated with maintaining the appropriate hosts in the laboratory. Consequently, a species with a relatively simple life cycle and hosts that are easily maintained in the laboratory has been chosen here and these shortcomings have, at least partly, been avoided. As detailed in the preceding chapters this has permitted a fairly detailed account of the population dynamics of all stages in the life cycle of Acanthocephalus lucii.

Previous workers in this field have realised that the term 'population' with respect to parasites is ambiguous and requires definition. Do all members of a given parasite species within an individual host constitute a population, or should all members of a species at all stages of development in all hosts within an ecosystem be considered a population? Esch,

Gibbons and Bourque (1975) solved this problem by referring to the former as an 'infrapopulation' and the latter as a 'suprapopulation' and this terminology has been adopted here. As Esch, Hazen and Aho (1977) have pointed out parasitological studies which concentrate on a single stage in the life cycle may provide information necessary for evaluating the dynamics of an infrapopulation, but all stages in the life cycle should be examined simultaneously for investigations into the dynamics of the suprapopulation.

The life cycle of Acanthocephalus lucii thus essentially consists of two infrapopulations, adult parasites in the definitive host, larval parasites in the intermediate host and in addition, a population of shelled acanthors free in the environment. When examining the dynamics of such a system it is often convenient to initially examine each stage in the life cycle separately. In adopting such an approach the size of the parasite population within a single host can be influenced by two population processes, namely recruitment (= immigration) and mortality. In the preceding Chapters seasonal changes in the size of both infrapopulations were detected and these related to factors causing changes in recruitment and mortality. However, previously little has been said about the way in which these factors operate on each infrapopulation, or indeed the

suprapopulation. It is with this particular aspect of the population dynamics of A. lucii that this final Chapter is concerned.

Population biologists have recognized that all populations exhibit certain fundamental characteristics. Firstly although a population may increase in size exponentially this increase does not continue indefinitely. Ultimately constraints will operate such that population size does not increase further. In natural systems populations tend to reach a balance or equilibrium level around which they fluctuate slightly. If disturbed from the equilibrium level they tend to return to the equilibrium level or to a new one. Populations which exhibit this characteristic are said to be regulated. Fluctuations in population size are brought about by changes in the basic population processes. Thus, at the infrapopulation level these would be recruitment and mortality, but at the suprapopulation level this would also include natality. These processes can be influenced by a number of factors which fall into two categories. Those which operate with equal influence as population density increases (density independent factors) or with increasing influence as population density increases (density dependent factors). Regulation of a population can only be achieved by the operation of factors of the latter type.

Such factors operate in a negative feedback manner. They tend to reduce the size of populations above the equilibrium level and increase the size of those below it.

The second fundamental characteristic of natural populations is that they vary in size from place to place. Some species are abundant in some areas, rare in others. Thus the equilibrium level may change both spacially and temporally due to the variable nature of the environment. This introduces the problem of recognizing what determines abundance, which is quite different from determining what factors regulate a population. The abundance of a population is determined by the combined effects of all factors (density dependent and independent) acting on the basic population processes. Regulation on the other hand can only occur as a result of density dependent factors or negative feedback controls. It is stressed that although only density dependent factors can regulate a population density independent factors can still have major effects on population size, as will be illustrated later in this Chapter.

Kennedy (1977) has further stressed that persistence of a population alone, even at fairly constant levels, should not be taken as evidence of stability. Under certain circumstances environmental factors operating in a density independent manner can maintain populations at fairly constant levels over periods

of time. However, populations subjected only to density independent factors are unregulated and the probability of such populations becoming extinct tends to increase with time.

With respect to parasite populations, as described previously, it is often convenient to consider each stage in the life cycle separately. The number of stages in the life cycle has significance with respect to regulation since the more stages in the life cycle the greater the number of opportunities for regulatory processes to act. However, this also introduces the opportunity for time lags owing to developmental processes and in general these tend to act in a destabilizing manner (May and Anderson, 1978). Although several regulatory processes may operate at various stages in the parasites life cycle the entire suprapopulation can be regulated by a single regulatory process operating at only one stage in the life cycle. Regulation at the infrapopulation level can lead to regulation at the supra-population level, which further emphasises the importance of examining all stages in the parasites life cycle.

Holmes, Hobbs and Leong (1977) have further demonstrated that regulation can be achieved not only by a single mechanism operating on a single stage in the parasites life cycle, but by a mechanism operating on only one of many hosts infected

with the same stage. Leong (1975) examined 10 species of fish from Cold Lake, Alberta (Canada) and found that all were infected with the acanthocephalan Metechinorhynchus salmonis (Müller). However, only the salmonids (lake whitefish Coregonus clupeaformis (Mitchell) and C. artedii (L.), lake trout, Salvelinus namaycush (Walbaum) and coho, Oncorhynchus kisutch (Walbaum)) appeared to be important hosts for this parasite, since in other species the parasite showed little or no gonadal development. The study revealed that regulation of the M. salmonis supra-population in Cold Lake could be achieved by a regulatory mechanism operating solely on the infrapopulation in lake whitefish. This was possible provided the combined flow of parasites through the other host species was insufficient to maintain the suprapopulation.

In the present study adult Acanthocephalus lucii were found in pike and roach, as well as perch. Although the data are sparse it appears that roach are auxiliary rather than principal hosts of A. lucii and pike acquire infections secondarily from predation on perch. Therefore, it seems reasonable to assume that if regulatory mechanisms do exist at the adult worm stage in the life cycle of A. lucii then they should operate on the infrapopulations in perch.

Regulation of the Acanthocephalus lucii suprapopulation could, therefore, be achieved by the operation of one or many density dependent factors acting on the infrapopulations in either perch or Asellus aquaticus, or both. It was decided that the simplest way to approach this potentially complex subject would be as follows: firstly to identify and describe more specifically some of the processes which can regulate parasite populations. Secondly, to illustrate these with examples from the literature (if possible by reference to species with a fish host at some stage in the life cycle). Finally to discuss the relevance (or otherwise) of each of these processes with regard to the regulation of each infrapopulation and hence the suprapopulation of A. lucii.

Parasites, by definition, inflict a degree of 'harm' upon their hosts. The degree of 'harm' is therefore likely to increase as the number of parasites harboured increases and ultimately, if the parasite burden reaches a high enough level the host should, theoretically at least, be killed by the parasites. By examining the frequency distribution of parasite burdens within a host population it should, therefore, be possible to assess the degree of 'harm' which the parasite inflicts on the entire host population, where 'harm' can be defined as the extent to which the parasite influences host reproduction

and survival. Previous studies (references in Chapter 3) have indicated that most parasites are not randomly distributed amongst their hosts, but rather the distribution is typically overdispersed with a large proportion of the parasite population harboured by only a small proportion of the hosts. Theoretical studies (Crofton, 1971b; Bradley, 1972; Anderson and May,1978; Anderson, 1978) have indicated that regulation of parasite (and host) populations can be achieved through a combination of over-dispersion and death of heavily infected hosts. Predictions from mathematical models suggest that this can only occur when the degree of aggregation and pathogenicity of the parasite both lie within certain limits. A more detailed discussion of this is given by Anderson (1978). The mechanism is density dependent since both the lethal level and the degree of over-dispersion depend upon the number of parasites.

Field studies suggest that such a mechanism is rare in natural populations and has only been found where parasites are particularly harmful to their hosts. For example Pennycuick (1971a, b, c) has suggested that populations of the cestode Schistocephalus solidus which utilizes the three-spined stickleback (Gasterosteus aculeatus L.) as an intermediate host could be regulated in this way. The plerocercoids of this tapeworm inhabit the body cavity of the stickleback and they exert a variety of pathological effects including reductions in

growth rate and fecundity and increased susceptibility to predation. The frequency distribution of the larval cestode in the stickleback population was highly overdispersed and changed seasonally in a manner which suggested death of the most heavily infected fish took place principally during the winter months, when the host experienced the most adverse environmental conditions. Populations of the eye-fluke Diplostomum gasterostei infecting the same population of sticklebacks appeared to be regulated in a similar way. Further examples of parasite populations which might be regulated in this manner are described by Kennedy (1977).

In the present study Acanthocephalus lucii was shown to have an overdispersed distribution in both intermediate and definitive host populations and the mechanisms whereby such a distribution was generated have been discussed previously (see Chapters 3 and 4). With respect to the intermediate host, larval parasites have been shown to exert a variety of pathological effects. Isopods harbouring cystacanths of A. lucii exhibited increased susceptibility to predation, altered pigmentation and infected female isopods were sterile. In the laboratory heavy infections reduced isopod survival. Thus it appears that the mechanism described above could be

involved in regulating the A. lucii population. However, the level of infection of the intermediate host population never exceeded 5.5%, no more than 5 larval parasites were found in a single isopod and over 90% of infected isopods harboured only 1 parasite. Experimental infections revealed that this was not simply owing to resistance and isopods could harbour much heavier infections (although host survival was reduced). Thus it appears that the overall degree of infection of the intermediate host population was probably kept well below a level at which such a mechanism would have significant regulatory influence by factors influencing the probability of contact between isopod and shelled acanthors. The observation that cystacanths can sterilize female isopods could have significant effects on the isopod population although this would depend upon the proportion of the host population which were infected. A single cystacanth appears to be sufficient to sterilize a female isopod so if incidence alone was high the parasite could drastically reduce the size of the intermediate host population, which in turn would reduce the probability of contact between isopod and shelled acanthor.

The frequency distribution of adult Acanthocephalus lucii was highly overdispersed and changed seasonally in a manner which could have been attributed to death of the most heavily

infected fish. However, A. lucii differs from Schistocephalus solidus as described previously, in that the disappearance of fish heavily infected with A. lucii could equally be attributed to loss of individual worms from the most heavily infected fish. The available evidence suggests this is exactly what happened. Even the most heavily infected fish appeared to be quite healthy. There was no evidence of either emaciation or much destruction of the intestinal mucosa. Although there was a gradual reduction in the degree of overdispersion from April - May onwards, this appeared to be simply owing to worms passing out of the intestine. It appears that during the current study the level of infection of perch by A. lucii was kept below the level at which the parasites induced host mortality, by some other mechanism. Regulation of A. lucii did not appear to be achieved by a mechanism involving aggregation and parasite induced death of heavily infected intermediate or definitive hosts.

Of the various mechanisms which can regulate animal populations some, such as the one just described, are unique to parasite populations. Another such mechanism involves host immune responses. The efficiency of host responses appears to depend primarily on the evolutionary status of the host and it is in the most advanced groups, the homeothermic vertebrates that the immune response is most highly developed. In such hosts the immune response can, in some instances, operate

in a density dependent manner and so regulate the parasite population. Once parasite burdens reach a certain level the host responds to the parasite by producing specific antibodies directed against it. Such responses can (although they do not always) impart a degree of partial immunity, preventing high levels of infection in individual hosts as long as the initial infection persists. Although fish appear to be capable of producing antibodies directed specifically against parasites, the available evidence (Molnar and Breczi, 1965; Harris, 1972) at least with respect to intestinal helminths, suggests that these antibodies are not effective in regulating parasite numbers. Although it has been shown that fish responses can, in some instances, operate in a density dependent manner (Nigrelli, 1935, 1937) it appears that fish can only respond in this way to ectoparasites and it has yet to be demonstrated unequivocably that such responses involve antibodies.

With respect to invertebrate intermediate hosts although host responses can and do occur these tend to be non-specific in action and do not prevent re-infection. Although in some instances, such as with larval Caryophyllaeus laticeps in the oligochaete Psammoryctides barbatus, an age resistance to infection does develop, this does not appear to be related to the presence of the existing larval parasites (Kennedy, 1969).

Generally speaking there is no barrier to re-infection and levels of infection tend to increase as the intermediate hosts age. If levels of infection become high then some hosts may be killed (this would be an example of regulation by the aggregation-mortality mechanism described previously) but it appears that in many intermediate host populations levels of infection are kept below those at which such a mechanism occurs to any significant extent.

In the current study there was no evidence to suggest that intermediate or definitive host responses played any part in regulating the Acanthocephalus lucii population. Although the appropriate experiments were not carried out it appears that concurrent infections are possible in both intermediate and definitive hosts. In the field study infected isopods containing all three stages of larval development (acanthor, acanthella and cystacanth) simultaneously were recovered, which strongly suggests that re-infection can occur. Laboratory infection experiments further suggested that the parasite burden imposed on an isopod appeared to depend solely on the number of shelled acanthors eaten. If large numbers were eaten then many acanthors would hatch and develop in the haemocoel until eventually the host was killed. With respect to adult parasites in the definitive host there appeared to

be no mechanism preventing high levels of infection. Up to 119 parasites were recovered from a single fish. Furthermore there was a distinct lack of evidence suggesting an immune response. There was no evidence of encapsulation of the proboscis or of gross cytological changes in the intestinal wall as described by Wurmbach (1957) and Hine and Kennedy (1974a), respectively, for Acanthocephalus anguillae and Pomphorhynchus laevis in the intestine of barbel. This is surprising in view of the mechanical damage which the proboscis hooks of A. lucii must inflict on the perch intestine. Thus regulation of the A. lucii populations does not appear to be achieved by mechanisms involving host responses.

In populations of both free-living and parasitic animals, part of the environment of each individual consists of members of the same species. Each individual can, therefore, affect and be affected by, other individuals. As population density increases certain essential resources such as space or nutrients may become limited in supply. Individuals then compete for these resources giving rise to intraspecific competition. This can act in a regulatory manner since it influences the basic population processes and is density dependent. The greater the number of individuals in the population the greater the adverse effects on survival and fecundity of each individual.

In parasite populations, as Holmes et al (1977) have

pointed out, intraspecific competition regulates at the level of the infrapopulation and can manifest itself in various different ways. The first is through a modification of the number of individuals in the infrapopulation. Once infrapopulation density reaches a certain limit, any additional parasites which enter the host simply do not establish. This is, in effect, very similar to the partial immunity described previously. Indeed nearly all the manifestations of intraspecific competition could equally be attributed to some form of host response. The second is through a modification of the proportion of reproducing adult parasites in the infrapopulation. Although there may be no precise limit to the number of individuals in the infrapopulation, the proportion which actually reproduce decreases as infrapopulation density increases. The third manifestation involves a modification of the number of eggs or infective stages produced by each mature parasite. As infrapopulation density rises the proportion of parasites which reproduce does not decrease, but growth rates are reduced, the average size of individuals is smaller and the number of eggs or infective stages produced decreases. These modifications described above are not all mutually exclusive and certain combinations may operate simultaneously. The significant point is that they all represent the effects of

a density dependent process, namely intraspecific competition, on the basic population processes of recruitment, mortality and natality and so can regulate the parasite infrapopulation and hence the suprapopulation.

There appear to be no conclusive examples of intraspecific competition resulting in a very precise limitation in the number of individuals in the infrapopulation which suggests that such a mechanism is rare, although numbers of Gyrocotyle, in various hosts, as described by Simmons and Laurie (1972) may be limited in this way. Their studies revealed that the majority of infections were limited to only two mature parasites per fish, with infections of a single parasite being next in frequency. However, as Kennedy (1977) has pointed out mechanisms other than intraspecific competition could have been responsible. In the present study there appeared to be no precise limit on the number of individuals in the infrapopulations in either intermediate or definitive hosts other than those imposed by the availability of infective stages and host feeding intensity. Therefore, the A. lucii population was not regulated in this way.

Regulation by a rather precise limit on the number of reproducing parasites per host individual (i.e. a decrease in the proportion of reproducing individuals as infrapopulation

density increases) has been described by Holmes et al (1977) for the acanthocephalan Metechinorhynchus salmonis in whitefish. These authors found that in spite of a linear almost five fold increase in the mean number of acanthocephalans per fish through whitefish age classes IV to IX, the mean number of gravid female worms per fish remained fairly constant. This constancy was maintained throughout the year in spite of wide seasonal fluctuations in the total number of acanthocephalans per fish (monthly means ranged from 83 - 348). Furthermore, there was a significant negative regression of the percentage of gravid females on the total numbers of acanthocephalans in individual fish. The suprapopulation of M. salmonis was regulated by intraspecific competition in whitefish resulting in density dependent reduction in natality at high infrapopulation densities.

In the present study the levels of infection of perch with Acanthocephalus lucii were far lower than those described for M. salmonis in whitefish. Thus, from the available evidence, it seems unlikely that there was a limit on the number of reproducing female worms in individual fish, at least over the range of infrapopulation densities recorded (1 - 119 worms per fish). It should be noted, however, that the data were difficult to analyse in the context, since a pronounced

maturation cycle was observed and maximum infrapopulation densities (in May) coincided with the period when almost all the parasites were immature. Furthermore, only a few fish with large numbers of worms were recorded, making it difficult to analyse the relationship over a wide range of parasite infrapopulation densities. Nonetheless up to 22 mature female A. lucii were recovered from a single fish, so it appears that the regulatory mechanism described for M. salmonis does not seem to operate on A. lucii, at least over the range of infrapopulation densities recorded during the current study.

An example of regulation by intraspecific competition resulting in a reduction in the mean size of parasites and hence output of infective stages is provided by Kennedy (1977) for the acanthocephalan Pomphorhynchus laevis, although quantitative data to support this suggestion are lacking. This parasite is found in many species of fish, although only in barbel (Barbus barbus) and chub (Leuciscus cephalus) does it mature to any significant extent. Experimental and field studies suggested that the parasite did not kill its host or induce an effective immune response and it appeared that the parasite continued to establish in fish as long as there was physically room for it. Regulation did not, therefore, appear to be a result of density dependent mortality so, according to Kennedy,

it had to be a result of density dependent natality which could only happen in barbel and chub. The parasite appeared to establish and survive in all regions of the alimentary tract of chub and barbel, but showed clear preference for a particular region in which it reached maximum size and once established did not change site. As individual infrapopulation densities increased and the preferred site became fully occupied the proportion of parasites establishing outside the preferred region increased. These parasites were smaller than those in the preferred site and so in crowded populations the mean size of parasites tended to be smaller and shelled acanthor production decreased.

Intraspecific competition was observed in the current study with respect to larval parasites in the intermediate host. This is not altogether surprising when one considers the relatively enormous size of a cystacanth in relation to the available space in the haemocoel. In experimental infections of Asellus aquaticus if large numbers of larval parasites were present in a single isopod reduced growth rates were observed and cystacanths were considerably smaller than those recovered from isopods containing only a single parasite. However, heavily infected isopods were not recovered in the field study, so although such a mechanism could be involved in regulation, it appears that,

again, infection levels were kept well below those at which such a mechanism operated to any significant extent.

With respect to adult parasites in the definitive host it is quite possible that at high infrapopulation densities reduced growth rates and hence decreased shelled acanthor output might have taken place. When one considers the size of adult worms relative to the available space in the perch intestine it would not be surprising if competition for space and/or nutrients arose at high infrapopulation densities. Plate 7.1 illustrates this point for an infection of about 30 worms (although 119 were recorded from a single fish). Even when only 30 worms were present much of the available space in the intestine was occupied. Acanthocephalus lucii also exhibited clear preference for a particular region of the intestine, so the regulatory mechanism described by Kennedy (1977) for P. laevis might well apply here. Unfortunately the manifestations of intraspecific competition of this type, reduced growth rates and reduced shelled acanthor output, are extremely difficult to measure quantitatively. To assess the former one really needs to determine and compare the mean sizes of worms from infected perch over a wide range of infrapopulation densities. Field data were of little value since recruitment occurred throughout the year and therefore perch contained worms of

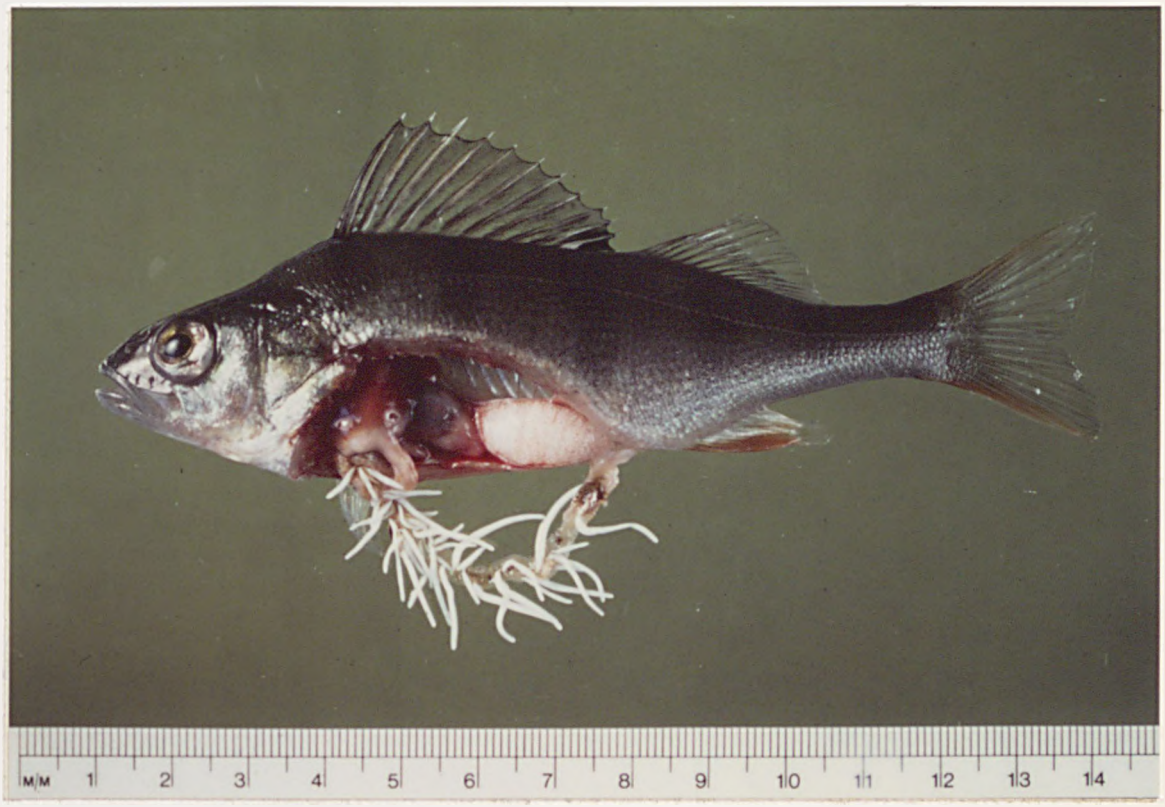


Plate 7.1. A perch (Perca fluviatilus) with the left lateral body wall removed and the intestine opened, illustrating an infection of approximately 30 specimens of Acanthocephalus lucii

different ages. Furthermore the size of individual cystacanths varied considerably, depending on the size of the isopod in which they were found. The presence of small parasites in some fish could be attributed to them having fed on smaller isopods rather than owing to intraspecific competition. An experimental investigation into this problem would have been quite feasible, had sufficient infected intermediate hosts been available.

A decrease in the mean size of parasites with increasing infrapopulation density is not, on its own, evidence of a regulatory mechanism. Only if there is a decrease in shelled acanthor output at high infrapopulation densities (density dependent natality) can regulation take place. Unfortunately attempts to measure shelled acanthor output proved unsuccessful. The method used involved keeping infected fish in a small plastic tank containing only clean water for a given time period. The fish was then removed and the water filtered through Whatmann 541 filter papers with a Millipore all-glass filter apparatus. The filter papers were removed and stained with ninhydrin. Although stained shelled acanthors were observed on filter papers, when a known number were passed through the apparatus the percentage recovery was very low. This was at least partly owing to the elongate shape of the shelled acanthor, since if orientated 'end-on' among the filter

paper fibres they could easily be overlooked. Thus, although it is tentatively suggested that regulation through intraspecific competition resulting in density dependent natality might have taken place, this could not be confirmed from the data collected during the current study.

During this study a seasonal cycle in the level of infection of both intermediate and definitive hosts was demonstrated. In the latter, although the data were limited, the cyclical pattern appeared to be repeated over a two year period. In the absence of conclusive evidence for the operation of density dependent regulatory processes at any stage in the life cycle, it appears that the observed cyclical pattern might have been entirely due to the operation of density independent factors. The results from both field and experimental work suggest that seasonal changes in water temperature, operating in a density independent manner, could have been responsible for the observed cyclical changes in infection levels. Temperature was shown to exert considerable influence on the basic population processes of recruitment, mortality and natality.

The field study has indicated that infective cystacanths were present all year round, although the level of infection showed slight seasonal changes with a maximum in early spring. Perch apparently ate isopods throughout the year, but in spring,

increasing water temperatures (and day length) stimulated perch feeding activity. The result was a rapid recruitment of parasites into the definitive host and a sudden rise in infection levels. During summer, although perch continued to feed on isopods, a decline in recruitment rates probably took place. This was principally owing to a turn over of the intermediate host population, the result being fewer infected isopods were available. However, a decline in recruitment rates alone could not explain the slight decline in infection levels through the summer months. Experimental studies revealed that warm temperatures increased the rate of mortality of adult worms. The mortality was not owing to a temperature dependent rejection response by the fish, since the experiments revealed that fish were equally susceptible to infection at summer and winter temperatures. Warm temperatures increased the rate of maturation of the worms the result being that the worms matured quickly, deposited their shelled acanthors and died, apparently from natural senescence. Thus in summer there was a more rapid output (mortality) of parasites than input. Warm temperatures increased the rate of mortality and natality. During autumn decreasing water temperatures resulted in a decrease in perch feeding activity and hence also a fall in recruitment. Infection levels declined further, since mortality of mature parasites

apparently continued through autumn and into winter, even when water temperatures were very low, which gives further support to the suggestion that mortality was simply owing to natural senescence rather than a host response. Minimum infection levels were recorded in early spring the following year.

Maturation of adult parasites correlated quite clearly with increasing water temperatures and it is suggested that the relationship is a causal one. Host hormonal changes associated with spawning do not appear to be involved, since the parasite matured in immature perch and in fish species other than the natural definitive host whose gonads were in an under-developed condition. Although maturation was seasonal some mature worms could persist in fish for many months, the result being that mature worms were recovered throughout almost the entire year. Nonetheless, maximum shelled acanthor output (natality) occurred in summer, when isopod population density was high. Combined with warm water temperatures stimulating isopod feeding activity, this would maximize the probability of isopods ingesting shelled acanthors and thus recruitment of larval parasites into the intermediate host population. The rate of development of larval parasites was also highest at warm summer temperatures and the level of infection of the intermediate host population rose gradually through autumn.

High levels of infection, however, were not recorded and it appeared that the probability of contact between isopods and shelled acanthors was still rather low. During autumn decreasing water temperatures reduced shelled acanthor output (natality) and isopod feeding activity, so recruitment rates probably declined to minimum levels in mid winter. Maximum infection levels were recorded in spring before increasing water temperatures and day length stimulated isopod breeding. Owing to a combination of natural senescence after breeding and selective predation by perch on infected isopods, infection levels declined to a minimum the following summer. Thus temperature appeared to influence parasite recruitment via its influence on both intermediate and definitive host feeding activity and mortality-natality by its influence on the rate of maturation.

In summary, although the observed seasonal changes in infection levels could have been brought about by the action of density independent factors such as temperature alone, the failure to demonstrate the existance of regulatory processes acting on any stage in the parasites life cycle could easily be attributed to the nature of the data collected, rather than to an actual absence of such processes. Equally their absence could be attributed to the short duration of the study, since such processes might only operate when infection levels

are far higher than those observed during the current study. It seems reasonable to suggest that there should be strong evolutionary pressure for regulatory mechanisms to exist, since the probability of extinction of unregulated populations should tend to increase with time. Equally, if parasite population growth continued exponentially then ultimately the parasite would cause extinction of the host and hence parasite populations. As Anderson (1978) has pointed out, population rate parameter values in parasite life cycles are very far from being a haphazard selection of all numerically possible values.

There can be little doubt that there is still considerable scope for further experimental (and field) work on A. lucii, especially with regard to infections of the definitive host over a wide range of infrapopulation densities. The single limiting factor in this context was the supply of infected intermediate hosts. Should a more reliable and abundant source be found it is hoped that the information presented in this thesis will provide both a sound background and a stimulus for further work.

References

Adcock, J.A. (1979). Energetics of a population of the isopod Asellus aquaticus: life history and production. Freshwat. Biol., **9**, 343-356.

Ali, S.S. (1973). "A study of perch (Perca fluviatilus L.) and roach (Rutilus rutilus L.) of Llyn Tegid, North Wales". Ph.D. Thesis, University of Liverpool.

Allen, K.R. (1935). The food and migration of the perch (Perca fluviatilus L.) in Windermere. J. Anim. Ecol., **4**, 264- 273.

Alm, G. (1922). Bollenfaunen och Fiskens Biologici Yxtasjon. Medd. K. Lanlbruksstyr., **236**, 1-186.

Alphey, T.J.W. (1970). Studies on the distribution and site location of Nippostrongylus brasiliensis within the small intestine of laboratory rats. Parasitology, **61**, 449-460.

Amin, O. (1975). Host and seasonal associations of Acanthocephalus parksidei Amin 1974 (Acanthocephala: Echinorhynchidae) in Wisconsin fishes. J. Parasitol., **61**, 318-329.

Amin, O. and Burrows, J.M. (1977). Host and seasonal associations of Echinorhynchus salmonis (Acanthocephala: Echinorhynchidae) in Lake Michigan Fishes. J. Fish Res. Board Can., **34**, 325-331.

Amin, O.M., Burns, L.A. and Redlin, M.J. (1980). The ecology of Acanthocephalus parksidei Amin, 1975 (Acanthocephala: Echinorhynchidae) in its isopod intermediate host. Proc. Helminth Soc. Wash., **47**, 37-46.

Andersen, K. (1978). The helminths in the gut of perch (Perca fluviatilus L.) in a small oligotrophic lake in Southern Norway. Z. Parasitenk ., **56**, 17-28.

Anderson, R.M. (1974a). Population dynamics of the cestode Caryophyllaeus laticeps (Pallas, 1781) in the bream (Abramis brama L.). J. Anim. Ecol. **43**, 305-321.

Anderson, R.M. (1974b). An analysis of the influence of host morphometric features upon the population dynamics of Diplozoon paradoxum (Nordman, 1832). J. Anim. Ecol., **43**, 873-887.

Anderson, R.M. (1978). The regulation of host population growth by parasitic species. Parasitology, **76**, 119-157.

Anderson, R.M. and May, R.M. (1978). Regulation and stability of host-parasite population interactions. I. Regulatory processes. J. Anim. Ecol., **47**, 219-247.

Anderson, R.M., Whitfield, P.J. and Mills, C.A. (1977). An experimental study of the population dynamics of an ectoparasitic digenean Transversotrema patialense: the cercarial and adult stages. J. Anim. Ecol., **46**, 555-580.

Andersson, E. (1969). Life cycle and growth of Asellus aquaticus with special reference to the effects of temperature. Rep. Inst. Fw. Res. Drottningholm., **49**, 5-26.

André, E. (1921). Catalogue des Invertébrates de la Suisse. Acanthocéphales. Mus. d'Hist. Nat. Genev., Fasc. **13**, 1-32.

Andrews, C.R. (1977). "The biology of the parasite fauna of perch, Perca fluviatilus L., from Llyn Tegid, North Wales". Ph.D. Thesis, University of Liverpool.

Andryuk, L.V. (1974). The infection rate of fish with acanthocephalans in the upper reaches of the Dnepr. Byulletin' Vesosoyuznogo Instituta Gel'mintologii im K.I. Skryabina, 13, 5-8. (In Russian) (English summary in Helminthological Abstracts)

Andryuk, L.V. (1979). [The life-cycle of Acanthocephalus lucii (Echinorhynchidae).] Parazitologiya, 13, 530-539. (In Russian) (English summary in Helminthological abstracts)

Antosiak, B. (1963). Udzial Ryb. W. Pokarmie Starszych Rockznikow Okonia (Perca fluviatilus) W. Niektorych Jeziorach okolic Wegorzewa. Roczn. Naukro. In. Seria B., 82, 274-294.

Arme, C. and Owen, R.W. (1967). Infections of the three-spined stickleback (Gasterosteus aculeatus L.) with the plerocercoid larvae of Schistocephalus salidus (Müller, 1776) with special reference to pathological effects. Parasitology, 57, 301-314.

Arme, C. and Owen, R.W. (1968). Occurrence and pathology of Ligula intestinalis infections in British fishes. J. Parasitol., 54, 272-280.

Awachie, J.B.E. (1963). "The ecology of intestinal helminth parasites of the fish of Afon Terrig, North Wales". Ph.D. Thesis, University of Liverpool.

Awachie, J.B.E. (1965). The ecology of Echinorhynchus truttae

Schrank, 1788 (Acanthocephala) in a trout stream in North Wales. Parasitology, **55**, 747-762.

Awachie, J.B.E. (1966). The development and life history of Echinorhynchus truttae Schrank 1788, (Acanthocephala). J. Helminthol., **40**, 11-32.

Banks, J.W. (1968). "Studies on pike (Esox lucius L.), perch (Perca fluviatilus L.) and roach (Rutilus rutilus (L.)) from three British waters". Ph.D. Thesis, University of Liverpool.

Bauer, O.N. (1962). The ecology of parasites of freshwater fish. In "Parasites of freshwater fish and the biological basis for their control". I.P.S.T. Jerusalem.

Bauer, O.N. and Nikolskaya, G.V. (1957). Dynamics of the parasite fauna of the whitefish Coregonus lavaretus from Lake Ladoga and its epizootic importance. Bull. All-Union Sci. Res. Inst. Freshwater Fish., **42**, 224-238. Translation N.S.F. Washington D.C.

Baylis, H.A. (1928). Records of some parasitic worms from British vertebrates. Ann. Mag. nat. Hist. Ser., **10**, 1, 329-343.

Baylis, H.A. (1939). Further records of parasitic worms from British vertebrates. Ann. Mag. nat. Hist. Ser., **11**, 4, 473-498.

Berglund, T. (1968). The influence of predation by brown trout on Asellus in a pond. Rep. Inst. Freshw. Res. Drottningholm, **48**, 77-101.

Bethel, W.M. and Holmes, J.C. (1973). Altered evasive behaviour and responses to light in amphipods harbouring acanthocephalan cystacanths. J. Parasitol., 5, 945-956.

Bibby, M.C. (1972). Population biology of the helminth parasites of Phoxinus phoxinus (L.) the minnow, in a Cardiganshire lake. J. Fish Biol., 4, 289-300.

Bliss, C.I. and Fisher, R.A. (1953). Fitting the negative binomial distribution to biological data. Biometrics., 176-200.

Boxshall, G.A. (1974). The population dynamics of Lepeophtheirus pectoralis (Müller): dispersion pattern. Parasitology 69, 373-390.

Bradley, D.J. (1972). Regulation of parasite populations: a general theory of the epidemiology and control of parasitic infections. Trans. Roy. Soc. Trop. Med.Hyg. 66, 697-708.

Bråten, T. and Hopkins, C.A. (1969). The migration of Hymenolepis diminuta in the rat's intestine during normal development and following surgical transplantation. Parasitology, 59, 891-905.

Brattey, J. (1979). Intestinal helminths of fishes in the Forth and Clyde canal at Temple, Glasgow. Glasg. Nat., 19, 475-479.

Brattey, J. (1980). Preliminary observation on the occurrence of

larval Acanthocephalus lucii (Acanthocephala: Echinorhynchidae) in the isopod Asellus aquaticus (L.). Parasitology, 81, xliv.

Brofeldt, P. (1922). Uber die Nahrung des Barches und Kaulbarsches im Winter. Z. Fisch., 21, 124-150.

Brown, F.J. (1927). On Crepidostomum farionis O.F. Mull. (Stephanophiala laureata Zeder) a distome parasite of trout and grayling. Parasitology, 19, 86-89.

Burlinghame, P.L. and Chandler, A.C. (1941). Host parasite relations of Moniliformis dubius (Acanthocephala) in albino rats; and the environmental nature of resistance to single and superimposed infections with the parasite. Amer. Jour. Hyg., 31, 1-21.

Burrough, R.J. (1978). The population biology of two species of eye-fluke, Diplostomum spathaceum and Tylodelphys clavata in roach and rudd. J. Fish. Biol., 13, 19-32.

Cable, R.M. and Dill, W.T. (1967). The morphology and life history of Paulisentis fractus Van Cleave and Bangham, 1949 (Acanthocephala: Neoechinorhynchidae). J. Parasitol., 53, 810-817.

Camp, J.W. and Huizinga, H.W. (1979). Seasonal population interactions of Acanthocephalus dirus in the creek chub Semotilus atromaculatus and the isopod Asellus intermedius. J. Parasitol., 66, 298-304.

Canning, E.U., Cox, F.E.G., Croll, N.A., Lyons, K.M. (1973). The

natural history of Slapton Ley nature reserve: VI. Studies on the parasites. Fld. Stud., 3, 681-718.

Cassie, R.M. (1962). Frequency distribution models in the ecology of plankton and other animals. J. Anim. Ecol., 31, 65-92.

Chappell, L.H. (1969). The parasites of the three-spined stickleback Gasterosteus aculeatus L. from a Yorkshire pond. I. Seasonal variation of parasite fauna. J. Fish Biol., 1, 137-152.

Chubb, J.C. (1961). "A preliminary investigation of the parasite fauna of the fish of Llyn Tegid (Bala Lake), Merionethshire". Ph.D. Thesis, University of Liverpool.

Chubb, J.C. (1963). Seasonal occurrence and maturation of Triaenophorus nodulosus (Pallas, 1781) (Cestoda: Pseudophyllidea) in the pike Esox lucius L. of Llyn Tegid. Parasitology, 53, 419-433.

Chubb, J.C. (1964). Occurrence of Echinorhynchus clavula (Dujardin, 1845) nec Hamann, 1892 (Acanthocephala) in the fish of Llyn Tegid (Bala Lake), Merionethshire. J. Parasitol., 50, 52-59.

Chubb, J.C. (1967). A review of seasonal occurrence and maturation of tapeworms in British freshwater fish. Parasitology, 53, 13P.

Chubb, J.C. (1977). Seasonal occurrence of helminths in freshwater fishes. Part I. Monogenea. In "Advances in Parasitology" (B. Dawes, ed.), 15, 133-199. Academic Press. London and New York.

Chubb, J.C. (1979). Seasonal occurrence of helminths in freshwater fishes. Part II. Trematoda. In "Advances in Parasitology" (W.H.R. Lumsden, R. Muller and J.R. Baker, eds.), **17**, 141-313. Academic Press, London and New York.

Chubb, J.C. (1980). Seasonal occurrence of helminths in freshwater fishes. Part III. Larval Cestoda and Nematoda. In "Advances in Parasitology" (W.H.R. Lumsden, R. Muller and J.R. Baker, eds.), **18**, 1-120. Academic Press, London and New York.

Chubb, J.C. (1982). Seasonal occurrence of helminths in freshwater fishes. Part IV. Adult Cestoda, Nematoda and Acanthocephala. In "Advances in Parasitology". (W.H.R. Lumsden, R. Muller, J.R. Baker, eds.), **20**, 1-292. Academic Press, London and New York.

Chubb, J.C., Awachie, J.B.E. and Kennedy, C.R. (1964). Evidence for a dynamic equilibrium in the incidence of Cestoda and Acanthocephala in the intestines of freshwater fish. Nature, Lond., **203**, 986-987.

Copland, W.O. (1956). Notes on the food and parasites of pike (Esox lucius) in Loch Lomond. Glasg. Nat., **17**, 230-235.

Cragg-Hine, D. (1965). "The biology of the coarse fish of Willow Brook, Northamptonshire". Ph.D. Thesis, University of Liverpool.

Craig, J.F. (1974). Population dynamics of perch (Perca fluviatilus L.) in Slapton Ley, Devon. I. Trapping behaviour, reproduction,

migration, population estimates, mortality and food. Freshwat. Biol., 4, 417-431.

Craig, J.F. (1978). A study of the food and feeding of perch, Perca fluviatilus L., in Windermere. Freshwat. Biol., 8, 59-68.

Crofton, H.D. (1971a). A quantitative approach to parasitism. Parasitology, 62, 179-193.

Crofton, H.D. (1971b). A model of host parasite relationships. Parasitology, 63, 343-364.

Crompton, D.W.T. (1970). An ecological approach to acanthocephalan physiology. Cambridge University Press, London.

Crompton, D.W.T. (1972). The growth of Moniliformis dubius (Acanthocephala) in the intestine of male rats. J. Exp. Biol., 56, 19-29.

Crompton, D.W.T. (1973). The sites occupied by some parasitic helminths in the alimentary tract of vertebrates. Biol. Rev., 47, 27-83.

Crompton, D.W.T. (1974). Experiments in insemination in Moniliformis dubius (Acanthocephala). Parasitology, 68, 229-238.

Crompton, D.W.T. (1975). Relationships between Acanthocephala and their hosts. Symp. R. Soc. Exp. Biol., XXIX. Cambridge University Press, London.

Crompton, D.W.T. and Walters, D.E. (1972). An analysis of the course of infection of Moniliformis dubius (Acanthocephala) in rats. Parasitology, 64, 517-823.

Crompton, D.W.T. and Whitfield, P.J. (1968). The course of infection and egg production of Polymorphus minutus (Acanthocephala) in domestic ducks. Parasitology, 58, 231-246.

Crompton, D.W.T. and Whitfield, P.J. (1974). Observations on the functional organization of the ovarian balls of Moniliformis and Polymorphus (Acanthocephala). Parasitology, 69, 429-443.

Crook, J.R. and Grundemann, A.W. (1964). The life history and larval development of Moniliformis clarki (Ward, 1917). J. Parasitol., 50, 689-693.

Davies, E.H. (1967). "Parasite fauna of the fish of the River Lugg, a tributary of the River Wye, Herefordshire". Ph.D. University of Liverpool.

DeGuisti, D.L. (1949). The life cycle of Leptorhynchoides thecatus (Linton), an acanthocephalan of fish. J. Parasitol., 35, 437-460.

Dogiel, V.A. (1964). General Parasitology (Translation Z. Kabata), 1-516, Oliver and Boyd, Edinburgh and London.

Dogiel, V.A. and Bychowsky, B.E. (1939). (Fish parasites in the Caspian Sea.) Caspian Sea Research Commission, Complex Studies of the Caspian Sea, 7, 1-149. (In Russian)

Dogiel, V.A., Petrushevski, G.K. and Polyanski, Yu. I. (1961). Parasitology of Fishes. Oliver and Boyd, London.

Esch, G.W., Gibbons, J.W. and Bourque, J.E. (1975). An analysis

of the relationship between stress and parasitism. Amer. Mid. Nat., 93, 339-353.

Esch, G.W., Campbell, G.C., Conners, R.E. and Coggins, J.R. (1976), Recruitment of helminth parasites by blue gills (Lepomis macrochirus) using a modified live box technique. Trans. Am. Fish. Soc., 105, 486-490.

Esch, G.W., Hazen, T.C. and Aho, J.M. (1977). Parasitism and r- and k-selection. In "Regulation of parasite populations" (G.W. Esch, ed.), 9-62. Academic Press, London and New York.

Eure, H. (1976). Seasonal abundance of Neoechinorhynchus cylindratus taken from largemouth bass (Micropterus salmoides) in a heated reservoir. Parasitology, 73, 355-370.

Eure, H.E. and Esch, G.W. (1974). Effect of thermal effluent on the population dynamics of helminths in largemouth bass. In "Thermal Ecology". (J.W. Gibbons and R.R. Sharitz, eds.) AFC Symposium Series (Conf. - 730505).

Fisher, R.A. (1941). The negative binomial distribution. Ann. Eugen., 11, 182-187.

Fried, B., Kitchen, J.G. and Koplin, R.S. (1964). An intestinal helminth study of Catastomus commersoni from the Bushkill Creek, Northampton county, Pennsylvania, with observations on seasonal distribution of Triganodistomum sp. (Trematoda) and Fessisentis sp. (Acanthocephala). Proc. Penn. Acad Sci., 38, 95-98.

Gledhill, T., Sutcliffe, D.W. and Williams, W.D. (1976). A key to British freshwater Crustacea: Malacostraca. F.B.A. publication No. 32.

Goldspink, C.R. and Goodwin, D. (1979). A note on the age, composition, growth rate and food of perch Perca fluviatilus (L.) in four eutrophic lakes, England. J. Fish Biol., **14**, 489-505.

Golvan, Y.J. (1969). Systematique des acanthocephales (Acanthocephala Rudolphi 1801). Premiere partie: l'ordre des Palaeacanthocephala Meyer 1931, premier fascicul: la super-famille des Echinorhynchoidea (Cobbold 1876) Golvan et Mouin 1963. Memoires du Museum National d'Histoire Naturelle. Paris. Serie A, Zoologie **57**, 1-373.

Grabda, J. (1971). Katalog fauny Polski Czesc X. Kolcogtowy

Graff, D.J. and Allen, K. (1963). Glycogen content in Moniliformis dubius (Acanthocephala). J. Parasitol., **49**, 204-208.

Halvorsen, O. (1972). Studies on the helminth fauna of Norway. XX: Seasonal cycles of fish parasites in the River Glomma. Norw. J. Zool., **20**, 9-18.

Haram, O.J. (1968). "A preliminary investigation of the biology of the gwyniad (Coregonus clupeoides pennantii Cuv. et Val.) of Llyn Tegid". Ph.D. Thesis, University of Liverpool.

Harms, C.E. (1963). The development and cultivation of the acanthocephalan, Octospinifer macilentis (macilentus) Van Cleave, 1919. Dissert. Abs., 23, 2632-2633.

Harms, C.E. (1965). The life cycle and larval development of Octospinifer macilentis (Acanthocephala: Neoechinorhynchidae). J. Parasitol., **51**, 286-293.

Harris, J.E. (1972). The immune response of a cyprinid fish to infections of the acanthocephalan Pomphorynchus laevis. Internat. J. Parasitol., 2, 459-469.

Hartley, P.H.T. (1947). The natural history of some British freshwater fishes. Proc. zool. Soc. Lond., **117**, 129-206.

Hartmann, J. (1974). Der Barsch (Perca fluviatilus) in eutrophierten Bodensee (MS) Langenargen. Staath. Inst. f. Seenfarschung, 27p.

Healy, A. (1954). Perch (Perca fluviatilus L.) in three Irish Lakes. Scient. Proc. R. Dubl. Soc., **26**, 397-407.

Hickey, M.D. and Harris, J.R. (1947). Progress of the Diphyllobothrium epizootic at Poulahouca Reservoir, Co. Wicklow, Ireland. J. Helminthol., **22**, 13-28.

Hindsbo, O. (1972). Effects of Polymorphus (Acanthocephala) on the colour and behaviour of Gammarus lacustris. Nature, London, **283**, 333.

Hine, P.M. (1970. "Studies on the parasites of some freshwater fish". Ph.D. Thesis, University of Exeter.

Hine, P.M. and Kennedy, C.R. (1974a). Observations on the distribution specificity and pathogenicity of the acanthocephalan Pomphorynchus laevis (Müller). J. Fish Biol., 6, 521-535.

Hine, P.M. and Kennedy, C.R. (1974b). The population biology of the acanthocephalan Pomphorhynchus laevis (Müller) in the River Avon. J. Fish Biol., **6**, 665-679.

Holland, D.G. (1976). The distribution of the freshwater Malacostraca in the area of the Mersey and Weaver River Authority. Fresh. Biol., **6**, 265-276.

Holmes, J.C. (1962). Effects of concurrent infections on Hymenolepis diminuta (Cestoda) and Moniliformis dubius (Acanthocephala) II. Growth. J. Parasitol., **48**, 87-96.

Holmes, J.C. and Bethel, W.M. (1972). Modification of intermediate host behaviour by parasites. In "Behavioural aspects of parasite transmission". (E.U. Canning and C.A. Wright, eds.). Zool. J. Linn. Soc., **51**, suppl. 1, 123-149.

Holmes, J.C., Hobbs, C.P. and Leong, T.S. (1977). Populations in perspective: community organization and regulation of parasite populations. In "Regulation of parasite populations" (G.W. Esch, ed.) 209-245. Academic Press, London and New York.

Hopkins, C.A. (1959). Seasonal variations in the incidence and development of the cestode Proteocephalus filicollis (Rud. 1810) in Gasterosteus aculeatus (L. 1766). Parasitology, **49**, 529-542.

Hyman, L.H. (1951). The Invertebrates. Vol. 3. Acanthocephala, Aschelminthes and Entoprocta. McGraw-Hill Book Co., New York.

Hynes, H.B.N. (1950). The food of freshwater sticklebacks (Gasterosteus aculeatus and Pygosteus pungitius), with a review of methods used in studies of the food of fishes. J. Anim. Ecol., **19**, 36-58.

Hynes, H.B.N. (1955). The reproductive cycle of some British freshwater Gammaridae. J. Anim. Ecol., **24**, 352-387.

Hynes, H.B.N. and Nicholas, W.L. (1957). The development of Polymorphus minutus (Goeze, 1782) (Acanthocephala) in the intermediate host. Ann. trop. Med. Parasit., **51**, 380-391.

Hynes, H.B.N. and Nicholas, W.L. (1963). The importance of the acanthocephalan Polymorphus minutus (Goeze, 1782) (Acanthocephala). Ann. trop. Med. Parasit., **52**, 376-383.

Izyumova, N.A. (1958). [Seasonal dynamics of the parasites of fish in the Rybinsk water reservoir.] Trudi Biologicheskoi Stantsii "Borok". Moscow, 3, 384-398 [In Russian].

Jilek, R. (1979). Histopathology due to the presence of Gracilisentis gracilisentis in Dorosoma cepedianum (Le Sueur). J. Fish Biol., **14**, 593-595.

Kane, M.B. (1966). Parasites of Irish fishes. Scient. Proc. R. Dubl. Soc. B **1**, 205-220.

Kates, K.C. (1943). Development of the swine thorn-headed worm, Macracanthorhynchus hirudinaceus in its intermediate host. Am. J. Vet. Res., **4**, 173-181.

Kaulberz, G.J. (1913). Biologische Beobachtungen an Asellus aquaticus. Zool. Jb., **33**, 287-360.

Kennedy, C.R. (1968). Population biology of the cestode Caryophyllaeus laticeps (Pallas, 1781) in dace, Leuciscus leuciscus L. of the river Avon. J. Parasitol., **54**, 538-543.

Kennedy, C.R. (1969). Seasonal incidence and development of the cestode Caryophyllaeus laticeps (Pallas) in the River Avon. Parasitology, **59**, 783-794.

Kennedy, C.R. (1971). The effects of temperature upon the establishment and survival of the cestode Caryophyllaeus laticeps in orfe, Leuciscus idus. Parasitology, **63**, 59-66.

Kennedy, C.R. (1972). The effect of temperature and other factors upon the establishment and survival of Pomphorhynchus laevis (Acanthocephala) in goldfish, Carassius auratus. Parasitology, **65**, 283-294.

Kennedy, C.R. (1974a). A checklist of British and Irish freshwater fish parasites with notes on their distribution. J. Fish Biol., **6**, 613-644.

Kennedy, C.R. (1974b). The importance of parasite mortality in regulating the population size of the acanthocephalan Pomphorhynchus laevis in goldfish. Parasitology, **68**, 93-101.

Kennedy, C.R. (1975). Ecological animal parasitology. 1-163. Blackwell Scientific Publications London.

Kennedy, C.R. (1977). The regulation of fish parasite populations. In "Regulation of parasite populations" (G.W. Esch, ed.), 63-109. Academic Press, London and New York.

Kennedy, C.R., Broughton, P.F. and Hine, P.M. (1976). The sites occupied by the acanthocephalan Pomphorynchus laevis in the alimentary tract of fish. Parasitology, **72**, 195-206.

Kennedy, C.R., Broughton, P.F. and Hine, P.M. (1978). The status of brown and rainbow trout, Salmo trutta and S. gairdneri as hosts of the acanthocephalan Pomphorhynchus laevis. J. Fish Biol., **13**, 265-275.

Kennedy, C.R. and Hine, P.M. (1969). Population biology of the cestode Proteocephalus torulosus (Batsch) in dace Leuciscus leuciscus (L.) of the River Avon. J. Fish Biol., **1**, 209-219.

Kennedy, C.R. and Rumpus, A. (1977). Long term changes in the size of the Pomphorhynchus laevis (Acanthocephala) population in the River Avon. J. Fish Biol. **10**, 35-42.

Kerr, T. (1948). The pituitary in normal and parasitised roach (Leuciscus rutilus Flemm.). Q. Jl. microsc. Soc., **89**, 129-137.

King, D. and Robinson, E.S. (1967). Aspects of the development of Moniliformis dubius. J. Parasitol., **53**, 142-149.

Komarova, M.S. (1950). K voprosy o zhiznennom tsikle skrebnya Acanthocephalus lucii Müll. Dokladi Akademii Nauk SSSR Novaya Seriya **70**, 359-360.

Lackie, J.M. (1972). The effect of temperature on the development of Moniliformis dubius (Acanthocephala) in the intermediate host Periplaneta americana. Parasitology, **65**, 371-377.

Lawrence, J.L. (1970). Effects of season, host age, and sex on endohelminths of Catastomus commersoni. J. Parasitol., 56, 567-571.

Le Cren, E.D. (1947). The determination of the age and growth of the perch (Perca fluviatilus) from the opercular bone. J. Anim. Ecol., 16, 188-204.

Le Cren, E.D. (1951). The length weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatilus L.). J. Anim. Ecol., 20, 201-219.

Le Cren, E.D. (1955). Year to year variation in the year class strength of Perca fluviatilus. Proc. int. Ass. Theor. appl. Limnol., 12, 187-192.

Le Cren, E.D. (1958). Observations on the growth of perch (Perca fluviatilus L.) over twenty-two years with special reference to the effects of temperature and changes in population density. J. Anim. Ecol., 27, 287-334.

Le Cren, E.D., Kipling, C. and McCormack, J.C. (1977). A study of the numbers, biomass and year class strengths of perch (Perca fluviatilus L.) in Windermere from 1941 to 1966. J. Anim. Ecol., 46, 281-307.

Lee, R.L.G. (1980). Ecology of Acanthocephalus lucii (Müller, 1776) in perch Perca fluviatilus L., in the Serpentine, London, U.K. J. Helminthol., 55, 149-154.

Leong, R. Tak Seng (1975). "Metazoan parasites of fishes from Cold Lake, Alberta: a community analysis". Ph.D. Thesis, University of Alberta.

Le Roux, M.L. (1931). Castration parasitaire et caracteres sexuels secondaires chez les Gammariens. C.r. Hebd. Seanc. Acad. Sci. Paris., **192**, 889-891.

Linstow, O.V. (1872). Zur Anatomie und Entwicklungsgeschichte des Echinorhynchus angustatus Rudolphi. Arch. Naturgesch., **38**, Jg., Bd., 1, 6-16.

Lockwood, A.P.M. (1959). The osmotic and ionic regulation of Asellus aquaticus (L.). J. Exp. Biol., 36, 546.

Lühe, M. (1911). Acanthocephalen. Brauer. Suswasserfaurra Deutschlands. **16**, 1-116.

Maercks, H.H. (1930). Sexualbiologische Studien an Asellus aquaticus L. Zool. Jb. Abt. J. Allgem Zool. Physiol. Tiere, **48**, 399-508.

Malakhova, R.P. (1961). (Seasonal changes in the parasitofauna of certain freshwater fishes from Karelian lakes (Lake Konche).) Trudy Karelskogo Filiala Akademiya Nauk SSSR, **30**, 55-78 (In Russian)

Mamer, B.E. (1978). The parasites of trout in northwest Washington. J. Parasitol., **64**, 314.

Manning, J.T. (1975). Male discrimination and investment in Asellus aquaticus (L.) and A. meridianus Racovitza (Crustacea: Isopoda). Behaviour, **55**, 1-14.

Marcus, J.H., Stucliffe, D.W. and Willoughby, L.G. (1978). Feeding and growth of Asellus aquaticus (Isopoda) on food items from the littoral of Windermere, including green leaves of Elodea canadensis. Freshwat. Biol., **8**, 505-519.

Marcus, J.H. and Willoughby, L.G. (1978). Fungi as food for the aquatic invertebrate Asellus aquaticus. Trans. Br. mycol. Soc., 70, 143-146.

May, R.M. and Anderson, R.M. (1978). Regulation and stability of host parasite population interactions: II. Destabilising processes. J. Anim. Ecol., 47, 249-267.

McCormack, J.C. (1970). Observations on the food of perch (Perca fluviatilus L.) in Lake Windermere. J. Anim. Ecol., 39, 255-267.

McDaniel, J.S. and Bailey, H.H. (1974). Seasonal population dynamics of some helminth parasites of centrarchid fishes. Southwest. Nat., 18, 403-416.

Meggitt, F.J. (1914). The structure and life history of a tapeworm (Ichthyotaenia filicollis Rud.) in the stickleback. Proc. zool. Soc., Lond., 1914, 113-138.

Merritt, S.V. and Pratt, I. (1964). The life history of Neoechinorhynchus rutili and its development in the intermediate host (Acanthocephala: Neoechinorhynchidae). J. Parasitol., 50, 394-400.

Meyer, A. (1932). Acanthocephala. In Bronn-Klassen und Ordnungen des Tierreichs., 4, (2). Lief 1, 1-332. Lief 2, 333-582.

Mills, C.A. (1980). Temperature-dependent survival and reproduction within populations of the ectoparasitic digenean Transversotrema patialense on the fish host. Parasitology, 81, 91-102.

Mills, C.A., Anderson, R.M. and Whitfield, P.J. (1979). Density-dependent survival and reproduction within populations of the ectoparasitic digenean Transversotrema patialense on the fish host. J. Anim. Ecol., **48**, 383-399.

Mishra, T.N. (1966). "The parasite fauna of the fish of the Shropshire Union Canal, Cheshire". Ph.D. Thesis, University of Liverpool.

Mishra, T.N. (1978). The occurrence of Acanthocephalus lucii in the fishes of the Shropshire Union Canal, Cheshire, England, U.K. Ann. Zool. (Agra)., **14**, 181-188.

Molnar, K. and Berczi, I. (1965). Demonstration of parasite specific antibodies in fish blood by agar gel diffusion precipitation test. Z. Immun. Allergic-Forsch., **129**, 263.

Moravec, F. (1979). Occurrence of the endo-parasitic helminths in pike (Esox lucius) from the Macha (Czechoslovakia) Lake fish pond system. Vestn. Cesk Spol. Zool., **43**, 174-193.

Munro, W.R. (1953). Intersexuality in Asellus aquaticus L. parasitized by a larval acanthocephalan. Nature, London, **172**, 313.

Muzzall, P.M. (1978). The host parasite relationships and seasonal occurrence of Fessisentis friedi (Acanthocephala: Fessisentidae) in the isopod Caecidotea communis. Proc. Helminth. Soc. Wash., **45**, 77-82.

Muzzall, P.M. and Rabalais, F.C. (1975a). Studies on Acanthocephalus

jacksoni Bullock, 1962 (Acanthocephala : Echinorhynchidae). 1. Seasonal periodicity and new host records. Proc. Helminthol. Soc. Wash., 42, 31-34.

Muzzall, P.M. and Rabalais, F.C. (1975b). Studies on Acanthocephalus jacksoni Bullock, 1962 (Acanthocephala: Echinorhynchidae) II. An analysis of the host-parasite relationship of larval Acanthocephalus jacksoni in Lirceus lineatus (Say). Proc. Helminthol. Soc. Wash., 42, 35-38.

Muzzall, P.M. and Rabalais, F.C. (1975c). Studies on Acanthocephalus jacksoni Bullock, 1962 (Acanthocephala: Echinorhynchidae) III. The altered behaviour of Lirceus lineatus (Say) infected with cystacanths of Acanthocephalus jacksoni. Proc. Helminthol. Soc. Wash., 42, 116-118.

Muzzall, P.M. and Bullock, W.J. (1978). Seasonal occurrence and host parasite relationships of Neoechinorhynchus saginatus Van Cleave and Bangham 1949 in the fallfish, Semotilus corporalis (Mitchill). J. Parasitol., 64, 860-865.

Needham, A.E. (1970). The integumental pigments of some isopod crustacea. Comp. Biochem. Physiol., 35, 509-539.

Needham, A.E. and Brunet, P.C.J. (1957). The integumental pigment of Asellus aquaticus. Experientia, 13, 207-209.

Nicholas, W.L. (1967). The biology of the Acanthocephala. In "Advances in Parasitology". (B. Dawes, ed.), 5, 205-246. Academic Press, London and New York.

Nicholas, W.L. (1973). The biology of the Acanthocephala. In

"Advances in Parasitology" (B. Dawes, ed.), **11**, 671-706. Academic Press, London and New York.

Nicholas, W.L. and Hynes, H.B.N. (1958). Studies on Polymorphus minutus Goeze 1782 (Acanthocephala) as a parasite of domestic ducks. Ann. Trop. Med. Parasit., **52**, 36-47.

Nickol, B.B. and Heard, R.W. (1973). Host-parasite relationships of Fessisentis necturorum (Acanthocephala: Fessisentidae). Proc. Helminthol. Soc. Wash., **40**, 204-208.

Nicoll, W. (1924). A reference list of the trematode parasites of British freshwater fishes. Parasitology, **16**, 127.

Nigrelli, R.F. (1935). On the effects of fish mucus on Epibdella melleri, a monogenetic trematode of marine fishes. J. Parasitol., **21**, 6-15.

Nigrelli, R.F. (1937). Further studies on the susceptibility and acquired immunity of marine fishes to Epibdella melleri, a monogenetic trematode. Zoologia, (N.Y.), **22**, 185-197.

Nikolsky, G.V. (1963). The ecology of fishes. Academic Press, London and New York.

Okorie, O.O. (1971). "Aspects of the ecology of the coarse fish population of Ellesmere Mere". Ph.D. Thesis, Liverpool Polytechnic.

Paling, J.F. (1965). The population dynamics of the monogenean gill parasite Discocotyle sagittata Leuckart on Windermere trout, Salmo trutta L. Parasitology, **55**, 661-694.

Parshad, V.R. and Crompton, D.W.T. (1981). Aspects of Acanthocephalan reproduction. In "Advances in Parasitology" (W.H.R. Lumsden, R. Muller, J.R. Baker, eds.), **20**, 73-139.

Parker, G.A. (1974). Courtship persistence and female guarding as male time investment strategies. Behaviour, **48**, 157-184.

Pearse, A.S. (1924). The parasites of lake fishes. Trans. Wisc. Acad. Sci., **21**, 161-194.

Pennycuick, L. (1971a). Differences in the parasite infections in three-spined sticklebacks (Gasterosteus aculeatus L.) of different sex age and size. Parasitology, **63**, 407-418.

Pennycuick, L. (1971b). Seasonal variations in the parasite infections in a population of three-spined sticklebacks, Gasterosteus aculeatus L. Parasitology, **63**, 373-388.

Pennycuick, L. (1971c). Frequency distributions of parasites in a population of three-spined sticklebacks, Gasterosteus aculeatus L., with particular reference to the negative binomial distribution. Parasitology, **63**, 389-406.

Pennycuick, L. (1971d). Quantitative effects of three species of parasites on a population of three-spined sticklebacks, Gasterosteus aculeatus L. J. Zool. (Lond.), **105**, 143-162.

Petrochenko, V.I. (1971). Acanthocephala of Domestic and Wild Animals. Vol. II. (K.I. Skrjabin, ed.) Translated from Russian, I.S.P.T. Jerusalem.

Priemer, J. (1979). [Gut helminths of perch, Perca fluviatilus L. and pope Acerina cernua (L.) (Pisces) from lakes on the outskirts of Berlin]. Darmhelminthen von Perca fluviatilus L. and Acerina cernua (L.) Pisces aus Gewassen des Berliner.

Zool. Anz., 203, 214-253.

Prus, T. (1971). The assimilation efficiency of Asellus aquaticus L. (Crustacea, Isopoda). Freshwat. Biol., 1, 287-305.

Rawson, D. (1952). The occurrence of parasitic worms in British freshwater fishes. Ann. Mag. nat. Hist. Ser. 12, 5, 877-888.

Read, C.P. and Rothman, A.H. (1958). The carbohydrate requirement of Moniliformis (Acanthocephala). Expl. Parasit., 7, 191-197.

Ridley, M. and Thompson, D.J. (1979). Size and mating in Asellus aquaticus (Crustacea: Isopoda). Z. Tierpsychol., 51, 380-397.

Ritchie, J. (1915). A contribution to the parasite fauna of the West of Scotland. Glasg. Nat., 7, 33-42.

Rizvi, S.S.H. (1964). "The parasite fauna of the fish of Rostherne Mere, Cheshire". Ph.D. Thesis, University of Liverpool.

Rojanapaibul, A. (1977). "The biology and life history of Acanthocephalus clavula Dujardin 1845 in Llyn Tegid (Bala Lake), North Wales". Ph.D. Thesis, University of Liverpool.

Roper, K.C. (1936). Ernahrung und Wachstum des Barches (Perca fluviatilus) in Gewassen Mecklenburgs und der Mark Brandenburg. Z. Fisch., 34, 507-638.

Rossi, L. and Fano, E.A. (1979). Role of fungi in the trophic niche of the congeneric detritivores Asellus aquaticus and

A. coxalis (Isopoda). Oikos, 32, 380-386.

Rossi, L. and Vitagliano-Tadini, G. (1978). Role of adult faeces in the nutrition of larvae of Asellus meridianus Racovitza (Crustacea: Isopoda). Oikos, 30, 109-113.

Rumpus, A. (1973). "The ecology of the parasites of Gammarus pulex in the river Avon, Hampshire". Ph.D. Thesis, University of Exeter.

Rumpus, A.E. and Kennedy, C.R. (1974). The effect of the acanthocephalan Pomphorynchus laevis upon the respiration of its intermediate host Gammarus pulex. Parasitology, 68, 271-284.

Rushton, W. (1937). Blindness in freshwater fish. Nature (Lond.), 141, 289.

Schmidt, G.D. and Olsen, O.W. (1964). Life cycle and development of Prosthorhynchus formosus (Van Cleave, 1918) Travassos, 1926, an acanthocephalan parasite of birds. J. Parasitol., 50, 721-730.

Schmid, W.D. and Robinson, E.J. (1972). The pattern of a host parasite distribution. J. Parasitol., 57, 907-910.

Seidenberg, A.J. (1973). Ecology of the acanthocephalan, Acanthocephalus dirus (Van Cleave 1931), in its intermediate host Asellus intermedius Forbes (Crustacea: Isopoda). J. Parasitol., 59, 957-962.

Shulman, R.E. (1979). (Dependence of the seasonal dynamics of fish parasites on some environmental factors) In "Ekologicheskaya i eksperimental'naya parazitologiya, Vypusk 2" (Yu. I. Lopyanskogo, ed.), 117-136. Izdatel'stvo Leningradskogo Universiteta, Leningrad.

Simmons, J.E. and Laurie, J.S. (1972). A study of Gyrocotyle in the San Juan Archipelago, Puget Sound, U.S.A. with observations on the host Hydrolagus colliei (Lay and Bennett). Internat. J. Parasitol., 2, 59-77.

Skorping, A. (1980a). Seasonal dynamics in abundance, development and pattern of infection of Bunodera luciopercae (Müller) in perch, Perca fluviatilus L. from an oligotrophic lake in Norway. J. Fish Biol., **18**, 401-410.

Skorping, A. (1980b). Population biology of the nematode Camallanus lacustris in perch, Perca fluviatilus L., from an oligotrophic lake in Norway. J. Fish Biol., **16**, 483-492.

Smyly, W.J.P. (1952). Observations on the food of the fry of perch (Perca fluviatilus L.) in Windermere. Proc. Zool. Soc. Lond., **122**, 407-416.

Spaeth, F.E. (1951). The influence of acanthocephalan parasites and radium emanations on the sexual characters of Hyallela (Crustacea: Amphipoda). J. Morph., **88**, 361-383.

Spencer, L.T. (1974). Parasitism of Gammarus lacustris (Crustacea: Amphipoda) by Polymorphus minutus (Acanthocephala) in Colorado. Am. Midl. Nat., **91**, 505-509.

Steel, E.A. (1961). Some observations on the life history of Asellus aquaticus (L.) and Asellus meridianus Racovitza. Proc. Zool. Soc., **137**, 71-87.

Steinstrasser, W. (1936). Acanthocephalen als Forellenparasiten.

Zeit. Fisch., 34, 177-212.

Stromberg, P.C. and Crites, J.L. (1975). Population biology of Camallanus oxycephalus Ward and Magath, 1916 (Nematoda: Camallanidae) in white bass in Western Lake Erie. J. Parasitol., 61, 123-132.

Styczynska, E. (1958). Acanthocephala at the biocenosis of Druzno Lake. (Parasitofauna of the biocenosis of Druzno Lake. Part VI). Acta Parasit. pol., 6, 195-211.

Sutcliffe, D.W. (1974). Sodium regulation and adaptation to freshwater in the isopod genus Asellus. J. Exp. Biol., 61, 719-736.

Swift, D.R. and Pickford, G.E. (1965). Seasonal variations in the hormone content of the pituitary gland of the perch, Perca fluviatilus L. Gen. Comp. Endocr., 5, 354-365.

Swynnerton, G.H. and Worthington, E.B. (1940). Note on the food of fish in Haweswater (Westmorland). J. Anim. Ecol., 9, 183-187.

Tedla, S. and Fernando, C.H. (1969). Observations on the seasonal changes of the parasite fauna of the yellow perch (Perca flavescens) from the Bay of Quinte, Lake Ontario. J. Fish. Res. Board Can., 26, 833-843.

Tedla, S. and Fernando, C.H. (1970). Some remarks on the ecology of Echinorhynchus salmonis Müller 1784. Can. J. Zool., 48, 317-321.

Thorpe, J.E. (1977a). Daily ration of adult perch, Perca fluviatilus L.

during summer in Loch Leven, Scotland. J. Fish Biol., **11**, 55-68.

Thorpe, J. (1977b). Synopsis of biological data on the perch, Perca fluviatilus L., 1758 and Perca flavescens Mitchell, 1814. F.A.O. publication. Synopsis No. 113.

Thorpe, J. (1977c). Morphology, physiology, behaviour and ecology of Perca fluviatilus L. and P. flavescens Mitchill. J. Fish. Res. Bd. Can., **34**, 1504-1514.

Treasurer, J.W. (1981). Some aspects of the reproductive biology of perch Perca fluviatilus L. fecundity, maturation and spawning behaviour. J. Fish Biol., **18**, 729-740.

Treasurer, J.W. and Holliday, F.G.T. (1981). Some aspects of the reproductive biology of perch (Perca fluviatilus L.) a histological description of the reproductive cycle. J. Fish Biol., **18**, 359-376.

Uglem, G.L. and Beck, S.M. (1972). Habitat specificity and correlated aminopeptidase activity in the acanthocephalans Neoechinorhynchus cristatus and N. crassus. J. Parasitol., **58**, 911-920.

Uglem, G.L. and Larson, O.R. (1969). The life history and larval development of Neoechinorhynchus saginatus Van Cleave and Bangham, 1949 (Acanthocephala: Neoechinorhynchidae). J. Parasitol., **55**, 1212-1217.

Uznanski, R.L. and Nickol, B.B. (1980). Parasite population regulation: lethal and sublethal effects of Leptorhynchoides thecatus (Acanthocephala: Rhadinorhynchidae) on Hyallela azteca.

J. Parasitol., 66, 121-126.

Valtonen, E.T. (1981). Metechinorhynchus salmonis (Müller, 1780) (Acanthocephala) as a parasite of the whitefish in the Bothnian Bay. I. Seasonal relationships between infection and fish size. Acta Parasit. Pol., 27, 293-200.

Van Cleave, H.J. (1916). Seasonal distribution of some Acanthocephala. J. Parasitol., 2, 106-110.

Van Cleave, H.J. (1947). A critical review of terminology for immature stages in acanthocephalan life histories. Ibid., 33, 118.

Van Maren, M.J. (1979). Structure and dynamics of the French upper Rhone Ecosystems. XII. An inventory of helminth fish parasites from the upper Rhone River (France). Bull. zool. Mus. Univ. Amsterdam, 6, 189-200.

Walkey, M. (1967). The ecology of Neoechinorhynchus rutili (Müller). J. Parasitol., 53, 795-804.

Ward, H. (1940). Studies on the life history of Neoechinorhynchus cylindratus (Van Cleave, 1913) (Acanthocephala). Trans. Am. microsc. Soc., 59, 327-347.

Whitfield, P.J. (1968). A histological description of the uterine bell of Polymorphus minutus (Acanthocephala). Parasitology, 58, 671-682.

Williams, W.D. (1960). "The ecology of Asellus aquaticus L. (1758) and A. meridianus Racovitza 1919". Ph.D. Thesis, University of Liverpool.

Williams, W.D. (1962). Notes on the ecological similarities of Asellus aquaticus (L.) and Asellus meridianus Rac. Hydrobiologia, 20, 1-30.

Williams, W.D. (1963). The ecological relationships of isopod crustaceans Asellus aquaticus (L.) and Asellus meridianus Rac. Proc. zool. Soc. Lond., 140, 661-679.

Williams, W.D. (1979). The distribution of Asellus aquaticus and Asellus meridianus (Crustacea, Isopoda) in Britain. Fresh. Biol., 9, 491-501.

Willoughby, L.G. and Marcus, J.H. (1979). Feeding and growth of the isopod Asellus aquaticus on actinomycetes, considered as model filamentous bacteria. Fresh. Biol., 9, 441-449.

Wootten, R. (1974). Studies on the life history and development of Proteocephalus percae (Müller) (Cestoda: Proteocephalidea). J. Helminthol., 48, 269-281.

Wurmbach, H. (1937). Zur krankheitserregenden Wirkung der Acanthocephalan. Die Kratzererkrankung der Barben in der Mosel. Z. Fisch., 35, 217-232.

Yamaguti, S. (1963). Systema Helminthum. V. Acanthocephala. John Wiley and Sons, New York and London.

Appendix 1

Stages in the development of the gonads of perch (Perca fluviatilus) (modified after Nikolsky (1963))

Stage 1 - Gonads have not yet started to develop or have just discharged. No gonadal tissue visible to naked eye. Post-spawning stage.

Stage 2 - Gonads of very small size. 2 thin greyish strands (testes) visible in males, single ovary visible in females. Individual eggs not yet visible to naked eye. 'Resting' stage.

Stage 3 - Gonads clearly visible. Increase in size very rapidly. Testes now white, ovary yellowish, individual eggs visible to naked eye. Developing stage.

Stage 4 - Gonads of maximum size. Sperm and eggs released on application of light pressure to sides of fish. Ripe stage.