Thesis
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ASPECTS OF THE BIOLOGY OF THE CESTODE *PROTEOCEPHALUS FILICOLLIS* (RUDOLPHI) FROM *GASTEROSTEUS ACULEATUS* L.

A thesis presented for the degree of Doctor of Philosophy

to the University of Stirling.

by

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DECLARATION

I hereby declare that this thesis has been composed by myself and is the result of my Own investigations. It has neither been accepted, nor submitted for any other degrees. All sources of information have been duly acknowledged.

DEDICATION

To my loving sons

Daniyal Jalal ud- Din
Wishal Jalal ud-Din

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ABSTRACT

The present study investigated aspects of the biology of the cestode, *Proteocephalus filicollis* from the three-spined stickleback *Gasterosteus aculeatus* from Airthrey Loch, Scotland. The population biology study demonstrated that the parasite has an annual cycle of recruitment, which occur mostly in late summer and early autumn. The cestode did not show preference for any sex of the host. Maturation of the cestode also showed a seasonal cycle with the majority of worms maturing in late spring and early summer, but this period may be extended in different generations.

Proteocephalus filicollis was overdispersed throughout the year in all sizes of fish, moreover variance to mean ratio always exceeded unity. No severe pathology was observed due to attachment of the worm to the intestine of the fish. The worm population in different sections of the intestine varied according to season and maturity stage. The *P. filicollis* migrate from the rectum to the anterior intestine as they mature and it is suggested that growth and maturation of the worm is a major stimulus for this migration.

Proteocephalus filicollis has a high fecundity as indicated by the higher number of eggs per mm of gravid portion of the strobila and high fertility. Infrapopulation size did not show any relationship with length of worm, percentage gravid portion, number of gravid segments or mean length of gravid segments. Numbers of eggs are correlated to length of the worm, but not to infrapopulation size. Numbers of eggs per mm of the gravid portion are not correlated to length of worm or infrapopulation size.

Acanthocyclops robustus was used as an experimental intermediate host. 15-16° C was the optimum experimental temperature for growth and a fully developed larva was formed in 23-27 days at this temperature. No growth was observed at 4° C, growth was slow at 10° C, but

rapid at 21-22° C. The eggs are infective for 25 days at 4° C, 10° C and 15-16° C, but for only 15 days at 21-22° C. Prevalence and mortality of copepods are significantly correlated to their exposure time to parasite eggs, but mean intensity of infection did not show any relationship to the exposure time to the eggs.

Ultrastructural studies demonstrated that a mature egg is surrounded by at least four embryonic envelopes, the capsule, the outer envelope, the inner envelope, and the oncospheral membrane. All these envelopes originate differently and undergo definite changes during their development.

CONTENTS

	Page
Acknowledgement	**i**
Abstract	**ii**
Index	**v**
List of Tables	**xiii**
List of figures and Plates	**xvii**

INDEX

1: GENERAL INTRODUCTION		**PAGE**
1.1	General Introduction	1
1.2	Classification of cestode	1
1.3	Economic significance of cestode in fish	4
1.4	List of *Proteocephalus* species in British Isles	10
1.5	Taxonomy and biology of Proteocephalids	10
1.5.1	Order Proteocephalidea	10
1.5.2	Family Proteocephalidae	10
1.5.3	Subfamily Proteocephalinae	11
1.5.4	Genus *Proteocephalus*	12
1.5.5	Taxonomy of Genus *Proteocephalus*	12
1.5.6	Morphology of Genus *Proteocephalus*	12
1.6	Life cycle of Genus *Proteocephalus*	12
1.7	Taxonomy of *Proteocephalus filicollis*	16
1.8	Classification of *Proteocephalus filicollis*	16
1.9	Morphology of *Proteocephalus filicollis*	17
1.9.1	Scolex	17
1.9.2	Strobila	17
1.9.3	Life cycle of *Protoecephalus filicollis*	17
1.10	Geographical distribution of *Proteocephalus filicollis*	22
1.11	Host specificity of *Proteocephalus filicollis*	22
1.12	Aims of the present study	23

2: FEATURES OF AIRTHREY LOCH

2.1 History, site and structure of the loch 25

2.2 Climate 28

2.3 Water supply 30

2.4 Water level 30

2.5 Water temperature and dissolved oxygen 32

2.5 Water sampling and analysis 34

2.6.1 pH 34

2.6.2 Conductivity 34

2.6.3 TDS 36

2.7 Turbidity 36

2.8 Biotic factors 36

2.9 Aquatic plants 37

2.10 Discussion 37

3: POPULATION BIOLOGY OF *PROTECOCEPHALUS FILICOLLIS*

3.1 Introduction 40

3.1.1 Seasonal cycle of occurrence and maturation of *Proteocephalus filicollis* 40

3.1.2 Seasonal cycle of occurrence and maturation in other members of genus *Proteocephalus* 42

3.1.3 Seasonal cycle of occurrence and maturation in other grops of cestodes 44

3.2 Distribution of parasites within the host population 47

3.3 Size of the host and infection 48

3.4 Aims 49

3.5 Materials and methods 49

3.5.1 Fish sampling 49

3.5.2	Transport of fish	50
3.5.3	Weight and length of fish	50
3.5.4	Sex and maturity of fish	52
3.5.5	Examination of fish for parasites	52
3.5.6	Staining and mounting of parasites	53
3.5.7	Measurement of parasites	53
3.5.8	Maturity of parasites	53
3.5.9	Statistical methods	54
3.5.10	Ecological terms	54
3.6	Results	56
3.6.1	Prevalence and abundance of *Proteocephalus filicollis* in relation to the sex of the host	56
3.6.2	Monthly prevalence and abundance of *Proteocephalus*	
3.6.3	*filicollis* in *G. aculeatus*	61
3.6.3	Maturation of *Proteocephalus filicollis*	66
3.6.4	Recruitment	69
3.6.5	Infection and host length	69
3.6.6	Frequency distribution of *Proteocephalus filicollis*	73
3.6.7	Variance to mean ratio	73
3.6.8	Parasite size	85
3.6.9	Parasite size and maturation	85
3.6.9.1	Immature worms	85
3.6.9.2	Maturing worms	87
3.6.9.3	Mature worms	87
3.6.9.4	Gravid worms	89
3.6.10	Parasite length in relation to infrapopulation size	89
3.7	Discussion	93
3.7.1	Prevalence and abundance in relation to the sex of the host	93
3.7.2	Monthly prevalence and abundance of *Proteocephalus filicollis* in *G. aculeatus*	93

3.7.3	Maturation of *Proteocephalus filicollis*	95
3.7.4	Recruitment of *Proteocephalus filicollis* in host population	100
3.7.5	Infection and length of host	102
3.7.6	Distribution of parasite in the host population	103
3.7.7	Parasite length	104

4: HISTOPATHOLOGY, LOCATION AND MIGRATION OF *PROTEOCEPHALUS FILICOLLIS* IN THE INTESTINE OF *GASTEROSTEUS ACULEATUS*

4.1	Introduction	107
4.1.1.	Pathology	107
4.1.2	Location and migration of parasite in the intestine of the final host	111
4.2	Aims	114
4.3	Materials and methods	114
4. 3.1	Fish sampling	114
4.3.2	Histology	114
4.3.3	Location of parasite in the host intestine	115
4.4	Results	115
4.4.1	Gross pathology	115
4.4.2	Histopathology	116
4.4.3	Location of *Proteocephalus filicollis* in the host intestine	120
4.4.4	Monthly change in location of *Proteocephalus filicollis* in the host intestine	120
4.4.5	Seasonal change in location of *Proteocephalus filicollis* in the host intestine	120
4.5.6	Location of *Proteocephalus filicollis* in the host intestine in relation to maturity stages	125
4.4.7	Seasonal occurrence of different maturity stages of	

	Proteocephalus filicollis in sections of the host intestine	126
4.4.7.1	Immature worms	127
4.4.7.2	Maturing worms	127
4.4.7.3	Mature worms	127
4.4.7.4	Gravid worms	128
4.4.8	Migration of *Proteocephalus filicollis* in the intestine of the fish	133
4.5	Discussion	135
4.5.1	Histpathology	135
4.5.2	Location and migration of *Proteocephalus filicollis* in the intestine of the host	135

5: SIZE AND FECUNDITY OF *PROTEOCEPHALUS FILICOLLIS*

5.1	Introduction	139
5.2	Aims	143
5.3	Material and methods	144
5.3.1	Fish sampling, dissection, staining and measurement of worms	144
5.3.2	Estimation of fecundity	144
5.3.2.1	Procuremnet of *Proteocephalus filicollis* eggs	144
5.3.2.2	Estimation of egg numbers and measurement of egg and hexacanth	144
5.3.2.3	Percentage of gravid portion	145
5.3.2.4	Number of gravid proglottids	145
5.3.2.5	The mean length of gravid proglottids	145
5.4	Data analysis	145
5.5	Result	146
5.5.1	Occurrence and length of gravid worms	146
5.5.2	Percentage gravid portion	153
5.5.3	Number of gravid proglottids	153

5.5.4	Mean length of gravid proglottids	153
5.5.5	Seasonal variations in diameter of *Proteocephalus filicollis* eggs	159
5.5.6	Mean diameter of hexacanth in *Proteocephalus filicollis* eggs	163
5.5.7	Egg count and production	163
5.5.8	Estimation of fecundity of *Proteocephalus filicollis*	170
5.6	Discussion	175

6: DEVELOPMENT OF PROTEOCEPHALUS FILICOLLIS IN THE COPEPOD INTERMEDIATE HOST UNDER EXPERIMENTAL CONDITIONS.

6.1	Introduction	181
6.1.1	Life cycle of *Proteocephalus filicollis*	181
6.1.2	Infectivity period of oncosphere	185
6.1.3	Infection dynamics in the first intermediate host	185
6.2	Aims	186
6.3.	Materaials and methods	186
6.3.1	Collection of eggs	186
6.3.2	Estimation of egg numbers	187
6.3.3	Copepod culture	187
6.3.4	Infection of copepods	188
6.3.5	Morphological observations	188
6.3.6	Infectivity period of oncospheres	189
6.3.7	Infection dynamics of the *Proteocephalus filicollis* in the first intermediate host	189
6.3.7.1	Influence of exposure time of eggs on the infection of copepods	189
6.4	Statistical methods	190
6.5	Results	191
6.5.1	Morphology of *Proteocephalus filicollis* eggs	191

6.5.2	Hatching of the eggs and penetration of the intermediate host intestine by the hexacanth	194
6.5.3	Development of *Proteocephalus filicollis* larvae in the intermediate host	196
6.5.4	Effect of temperatureon larval development of *Proteocephalus filicollis*	203
6.5.5	Infectivity period of the oncosphere	206
6.5.6	Infectivity of winter eggs	206
6.5.7	Localization of *Proteocephalus filicollis* in the intermediate host	204
6.5.8	Infection of intermediate host in relations to the length of contact with parasite eggs	216
6.6	Discussion	214

7: ULTRASTRUCTURE OF THE EMBRYONIC ENVELOPES OF *PROTEOCEPHALUS FILICOLLIS*

7.1	Introduction	222
7.2	Aims	229
7.3	Materials and method	229
7.3.1	Histology	229
7.3.2	Scanning electron microscopy	230
7.3.3	Transmission electron microscopy	231
7.4	Results	232
7.4.1	Histology of the uterus of gravid *Proteocephalus filicollis*	232
7.4.2	SEM of eggs of *Proteocephalus filicollis*	232
7.4.2	Ultrastructure of embryonic envelopes of *Protepcephalus filicollis*	232
7.4.3.1	The capsule	235
7.4.3.2	The outer envelope	235
7.4.3.3	The inner envelope	239

7.4.3.4 The oncospheral membrane 243
7.5 Discussion 247

8: CONCLUSION AND SUGGESTIONS FOR FURTHER WORK 255
9: REFERENCES 263
10:Appendix

LIST OF TABLES PAGE

Chapter 1

Table 1.1 Classification of cestodes 3

1.2 Features of the life cycle of a range of *Proteocephalus* Species 15

1.3 Morphometric characteristics of *Proteocephalus filicollis* 20

Chapter 2

2.1 Morphometric data of Airthrey Loch 25

Climatological data of Parkhead area University of Stirling from January 1993 to June 1995 29

2.2 Total rainfall (mm)in Parkhead area, January 1993 to June 1995 28

2.3 Air temperature range (° C) in Parkhead area, from March 1993 to June 1995 30

2.4 Monthly range of water level at gauge at Airthrey Loch From January 1993 to June 1995 31

2.5 Range of water temperature, pH and conductivity in Airthrey Loch from April 1993 to June 1995 35

Chapter 3

3.1 Monthly samples of three-spined stickleback from Airthrey Loch from April 1993 to July 1995 in length classes (cm) (LC-1, < 2.0; LC-2, 2.1-3.0; LC-3, 3.1-4.1; LC-4, 4.1> 51

3.2 Length classes of fish sampled 52

3.3 Prevalence and abundance of *Proteocephalus filicollis* in all female, male and undetermined sex *G. aculeatus* from Airthrey Loch from April 1993 to July 1995 58

3.4 Seasonal prevalence in male, female fish and male to female ratio of *G. aculeatus* from Airthrey Loch from April 1993 to July 1995 59

3.5 Monthly change in prevalence and abundance of *Proteocephalus filicollis* in all examined *G. aculeatus* in Airtjhrey Loch from April 1993 to July 1995 63

3.6 Seasonal changes in prevalence and abundance of *Proteocephalus filicollis* in *G. aculeatus* in Airthrey Loch April 1993 to July 1995 64

3.7 Monthly percentage of different maturity stages of *Proteocephalus filicollis* in all *G. aculeatus* from Airthrey Loch from April 1993 to July 1995 67

3.8a Monthly prevalence and abundance of *Proteocephalus filicolis* in different length classes of *G. aculeatus* in Airthrey from April 1993 to July 1995 71

3.8 Prevalence and abundance of *Proteocephalus filicollis* in different length classes of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995 72

3.9 Variance to mean ratio of *Proteocephalus filicollis* in different length classes of *G. aculeatus* from Airthrey Loch from April 1993 to July 1995 83

3.10 Seasonal change in prevalence and variance / mean ratio of *Proteocephalus filicollis* in *G. aculeatus* in different seasons in Airthrey Loc from April 1993 to July 1995 84

3.11 Monthly mean length of different maturation stages of *Proteocephalus filicollis* from *G. aculeatus* from Airthrey Loch from April 1993 to July 1995 88

Chapter 4

4.1 Location of *Proteocephalus filicollis* in various sections of the intestine of all *G. aculeatus* examined from Airthrey Loch 122

4.2 Occurrence of various maturity stages of *Proteocephalus filicollis* as a percentage of the total number of worms in each sections of the intestine of *G. aculeatus* from April 1993 to July 1995 125

4.3 The percentage of the overall number of each maturity Stage of *Proteocephalus filicollis* found in each section of the intestine of *G. aculeatus* 126

4.4 Immature worms as percentage of the total number of *Proteocephalus filicollis* in each section of the intestine of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995 129

4.5 Maturing worms as a percentage of the total number of *Proteocephalus filicollis* in each section of the intestine of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995 130

4.6 Mature worms as a percentage of the total number of *Proteocephalus filicollis* in each section of the intestine of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995 131

4.7 Gravid worms as a percentage of the total number of *Proteocephalus filicollis* in each section of the intestine of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995 132

Chapter 5

5.1 Production of *Proteocephalus filicollis* eggs in gravid worms of different length sampled from mid May to mid June 1995 166

5.2 Eggs production of *Proteocephalus filicollis* in relation to infrapopulation size. These worms were collected from mid May to mid June 1995 169

5.3 Estimation of fecundity of *Proteocephalus filicollis* from *G. aculeatus* in Airthrey Loch during the year 1993-94 and 1994-95 173

5.4 Estimated egg production of *Proteocephalus filicollis* in 1993-94 and 1994-95 173

5.5 Ratio of egg production to worms recruited and recovery of Gravid *Proteocephalus filicollis* in 1993-94 and 1994-95. 174

5.6 Ratio of gravid *Proteocephalus filicollis* to egg production in1993-94 and 1994-95 from *G. aculeatus* from Airthrey Loch 174

Chapter 6

6.1 Infectivity period of oncosphere of *Proteocephalus filicollis* stored at different temperatures 207

6.2 Percenatge localization of all *Proteocephalus filicollis* recovered from different segments of *Acanthocyclops robustus* larvae 209

6.3 Infection of *Acanthocyclops robustus* with *Proteocephalus filicollis* eggs in relation to length of contact 212

Chapter 7

7.1 Origin and composition of the eggs shell layers of Cestodes. 227

List of Figures and Plates

	Title of figures and Plates	Page
1.1	Diagrammatic representation of the life cycle of *Proteocephalus filicollis*	21
2.1	Location of sampling site	26
2.2	Bathymetric map of Airthrey Loch	27
2.3	Monthly mean temperature (° C) of the Airthrey Loch from April 1993 to June 1995	33
3.1.	Seasonal prevalence (a) and abundance (b) of *Proteocephalus filicollis* in all female *G. aculeatus* examined in Airthrey Loch from April 1993 to July 1995	57
3.2.	Seasonal prevalence (a) and abundance (b) of *Proteocephalus filicollis* in all male *G. aculeatus* examined in Airthrey Loch from April 1993 to July 1995.	60
3.3.	Monthly prevalence (a) and abundance (b) of *Proteocephalus filicollis* in *G. aculeatus* from Airthrey Loch from April 1993 to July 1995	62
3.4.	Seasonal prevalence and abundance of *Proteocephsus filicollis* in *G. aculeatus* from Airthrey Loch from April 1993 to July 1995	65
3.4a	Monthly (from April 1993 to July 1995) percentage of all maturity stages of *Proteocephalus filicollis* from *G. aculeatus*	68
3.5	Percentage of plerocerciform of *Proteocephalus filicollis* (length of worm 0.32 to < 1 mm) in the total worm population in *G. aculeatus* in Airthrey Loch from April 1993 to July 1995	70
3.6	Truncated negative binomial distribution of *Proteocephalus filicollis* in *G. aculeatus* in Airthrey Loch from April 1993 to July 1995	74

	3.7	Frequency distribution of *Proteocephalus filicollis* in various length classes of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995	75
	3.8a-j	Frequency distribution of *Proteocephalus filicollis* in different Seasons in *G. aculeatus* in Airthrey Loch from April 1993 to July 1995	77
	3.9	Monthly variance to mean ratio *of Proteocephalus filicollis* in Airthrey Loch from April 1993 to July 1995	82
	3.10	Monthly mean length of *Proteocephalus filicollis* from *G. Aculeatus* in Airthrey Loch from April 193 to June 1995	86
	3.11	Relationship between length of immature *Proteocephalus filicollis* and infrapopulation size	91
	3.12	Relationship between length of maturing *Proteocephalus filicollis* and infrapopulation size	91
	3.13	Relationship between length of mature *Proteocephsalus filicollis* and infrapopulation size	92
	3.14	Relationship between length of gravid *Proteocephalus filicollis* and infrapopulation size	92
	4.1	Monthly mean length of uninfected and parasitised *G. aculeatus*	117
Plate	4.1	A transverse section of *G. aculeatus* intestine partially blocked by two *Proteocephalus filicollis* plerocercoids	118
Plate	4.2	A transverse section of *G. aculeatus* intestine: a sucker (S) of *Proteocephalus filicollis* has grasped a portion of the intestine	119
Plate	4.3	A transvers section of *G. aculeatus* intestine; a sucker (S) of *filicollis* is holding epithelium and portion of epithelium is erodeed around the scolex.	119
Fig	4.2	Overall location of *Proteocephalus filicollis* in the sections of intestine of *G. aculeatus*	121

4.3 Seasonal location of *Proteocephalus filicollis* in various sections of the intestine of *G. aculeatus* from Airthrey Loch from April 1993 to July 1995. 123

4.4 Monthly mean position of attachment of *Proteocephalus filicollis* in the intestine of *G. aculeatus* 134

5.1. Monthly mean number of gravid *Proteocephalus filicollis* per infected *G. aculeatus* 148

5.2. Monthly mean length of gravid *Proteocephalus filicollis* 149

5.3 Relationship between length of *Proteocephalus filicollis* and water temperature 150

5.4 Relationship between length of gravid *Proteocephalus filicollis* and length of stickleback (cm) 150

5.5 Number of gravid *Proteocephalus filicollis* in different length classes of *G. aculeatus* 151

5.6 Relationship between length of gravid *Proteocephalus filicollis* and infrapopulation szie. 152

5.7 Monthly mean percentage gravid portion of *Proteocephaus filicollis* 154

5.8 Relationship between length of *Proteocephalus filicollis* and percentage gravid portion of the worm 155

5.9 Relationship between infrapopulation size and percentage gravid portion of *Proteocephalus filicollis* 155

5.10 Monthly mean number of gravid proglottids of *Proteocephalus filicollis* 156

5.11 Relationship between length of *Proteocephalus filicollis* and number of gravid proglottids 157

5.12 Relationship between infrapopulation size of *Proteocephalus filicollis* and number gravid proglottids 157

5.13 Monthly mean length of gravid proglottids of *Proteocephalus filicollis* 158

5.14	Relationship between length of *Proteocephalus filicollis* and mean length of gravid proglottids	160
5.15	Relationship between infrapopulation size of *Proteocephalus filicollis* and mean length of gravid proglottids	160
5.16	Mean diameter of *Proteocephalus filicollis* egg and hexacanth in different months	161
5.17	Relationship between length of *Proteocephalus filicollis* and diameter of egg	162
5.18	Relationship between infrapopulation size and diameter of *Proteocephalus filicollis* egg	162
5.19	Relationship between diameter of *Proteocephalus filicollis* egg and daimeter of hexacanth	164
5.20	Number of eggs in *Protoecephalus filicollis* of different lengths	165
5.21	Relationship between length of *Proteocephalus filicollis* and number of eggs	167
5.22	Relationship between length of *Proteocephalus filicollis* and number eggs per mm gravid portion	167
5.23a	Number of *Proteocephalus filicollis* eggs in relation to infrapopulation size	168
5.23	Relationship between infrapopulation size and number of eggs of *Proteocephalus filicollis*	172
5.24	Relationship between infrapopulationsize and number of eggs per mm gravid portion	172
Plate 6.1	A micrograph of the fully developed egg of *Proteocephalus filicollis*	192
Plate 6.2	Eggs of *Proteocephalus filicollis*	193
Plate 6.3	Eggs of *Proteocephalus filicollis* showing hexacanth embryo released from the granular layer	194
Plate 6.4	Hexacanth embryo of *Proteocephalus filicollis* removed from the intestine of *A. robustus*	194

Fig. 6.1	Diagram of the sequence of the hook movemnets of *Proteocephalus filicollis* larvae during penetration of the copepod intestinal wall	195
Plate 6.5	A hexacanth embryo of *Proteocephalus filicollis* taken from the haemocel of *A. robustus*	197
Plate 6.6.	A 6 days old larvae of *Protoecephalus filicollis* from *A. robustus* maintained at 15-16 C	197
Plate 6.7	A 12 day old larva of *Proteocephalus filicollis* from *A .robustus* maintained at 15-16° C	198
Plate 6.8	Two 14 days old larvae of *Proteocephalus filicollis* from *A. robustus* maintaied at 15-16° C	198
Plate 6.9	A 12 days old larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16° C	200
Plate 6.10	A 15 days old larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16° C	200
Plate 6.11	A 16 days larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16° C	201
Plate 6.12	A 21 days old larva of *Proteocephalus filicollis* from *A. robusts* maintained at 15-16°C	201
Plate 6.13	A 22 days larva of *Proteocephalus filicollis* from *A. robustus* maintained at15-16° C	202
Fig. 6.2	Drawing of 27 days old larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16° C.	202
Fig. 6.3	Development of *Proteocephalus filicollis* in *A. robustus* at different temperature	204
Fig. 6.4	Development of individual *Proteocephalus filicollis* in *A. robustus* at 15-16° C	205
Plate 6.14	Four *Proteocephalus filicollis* larvae in the haemocoel of *A. robustus* 10 days infection	208
Plate 6.15	*Proteocephalus filicollis* larva in antennule of *A. robustus* 1 day post- infection	208

Fig. 6.5	Prevalence, mortality and mean intensity of infection of *Proteocephalus filicollis* in relation to exposure time of copepod to the eggs	213
Fig. 6.6	The effect of *.P filicollis* eggs density on the survival of *A. robustus*	213
Plate 7.1	A transverse section of gravid proglottid of *Protoecephalus filicollis* showing eggs	233
Plate 7.2	SEM micrograph of egg of *Proteocephalus filiocllis*	233
Plate 7.3	SEM micrograph of egg of *Proteocephalus filicollis* showing external Surface with irregularly contoured appearance	234
Plate 7.4	SEM micrograph of egg of *Proteocepphaalus filicollis* showing different surface sculpturing on opposite side of the embryo	234
Plate 7.5	TEM micrograph of preoncosphere stage of *Proteocephalus filicollis*	236
Plate 7.6	TEM micrograph of oncosphere of *Proteocephalus filicollis* during early development	236
Plate 7.7	TEM micrograph of oncosphere of *Proteocephalus filicollis* in later Stages of development	237
Plate 7.8	TEM micrograph of *Proteocephalus filicollis* oncosphere in later stages of development	237
Plate 7.9	TEM micrograph of oncosphere stage of *Proteocephalus filicollis*. Formation of outer envelope	238
Plate 7.10	TEM micrograph of outer envelope formation of oncosphere of *Proteocephalus filicollis*	238
Plate 7.11	TEM micrograph of oncosphere of *Proteocephalus filicollis* in later developmental stage	240
Plate 7.12	TEM micrograph of oncosphere of *Proteocephalus filicollis*, inner envelope of oncosphere.	240
Plate 7.13	TEM micrograph of the inner envelope of *Proteocephalus filicollis* oncosphere in early pahse of development	241
Plate 7.14	TEM micrograph of the inner envelope of *Proteocephalus filicollis* oncosphere	241

Plate 7.15	TEM micrograph of the outer part of the inner envelope of *Proteocephalus filicollis* oncosphere	242
Plate 7.16	TEM micrograph of the inner envelope of *Proteocephalus filicollis* Oncosphere covered with a thin membrane	243
Plate 7.17	TEM micrograph of the inner envelope of *Proteocephalus filicollis* oncosphere	244
Plate 7.18	TEM micrograph of *Proteocephalus filicollis* oncosphere;	245
Plate 7.19	TEM micrograph of *P. filicollis* oncosphere oncospheral membrane appearing	245
Plate 7.20	A high micrograph of *Proteocephalus filicollis* oncosphere with four embryonic envelopes surrounding the oncosphere	247
Fig. 8.1	Seasonal flow diagram of *Proteocephalus filicollis* in *G. aculeatus* in Airthrey Loch	256

CHAPTER 1
GENERAL INTRODUCTION

1. GENERAL INTRODUCTION.

1.1 General introduction

The cestodes or tapeworms are all endoparasitic, usually living as adults in the alimentary canal of the vertebrate host. However, some members of the order Caryophyllidea are found as adults in invertebrates and many have a neotenic form, e.g. genus *Archigetes,* where the procercoid stage reaches maturity in the body cavity of a tubificid annelid (Mackiewicz, 1994)

Adult cestodes are elongated and dorso-ventrally flattened. The anterior most region is the scolex, which serves as an organ of attachment by the parasite to the host intestinal mucosa. Posterior to the scolex is the undifferentiated neck area. The region posterior to the scolex and neck is the strobila. Cestodes lack a digestive system in both the larval and adult stages, the exchange of nutrients and waste products taking place through the body wall or tegument. The body of the cestode is usually divided into segments or proglottids, except in caryophyllaeids, gyrocotylids and amphilinids. Adult worms are hermaphrodite with a complete set of both male and female reproductive organs occurring in each proglottid (Schmidt, 1986).

1.2 Classification of cestodes.

Wardle & MCleod (1952) divided tapeworms into two classes, the Cestoda and Cestodaria. They included 11 orders in the class Cestoda and three orders in the class Cestodaria (Table 1.1).

Subsequently Wardle, MCleod & Radinovsky (1974) redefined this classification into two classes, Cotyloda and Eucestoda. The class Cotyloda includes six orders

(Gyrocotylidea, Amphilinidea, Caryophyllidea, Spathebothridea, Pseudophyllidea, Diphyllidea) which are found in marine and freshwater fish. Members of this class are monozoic or polyzoic, the holdfast is simple or lacking and the segments weak or absent. Two orders, the Caryophyllidea and Spathebothridea are not polyzoic; have multiple genitalia but not segments. An embryonated egg is ingested by an invertebrate in which a procercoid with cercomer is formed. The adult worms are found in vertebrate hosts.

Members of the class Eucestoda are almost all polyzoic, with one or two sets of reproductive organs per proglottid, a scolex is usually present and the embryo has six hooks and is surrounded by a shell. These are parasites of fish, amphibians, birds and mammals. This class includes 15 orders of which 5 (Proteocephalidea, Tetraphyllidea, Litobothridea, Lecanicephalidea, Trypanorhyncha) are found in fish (Wardle *et al.* 1974).

Schmidt (1986) has given a modified classification of the tapeworms, listing two subclasses in the class Cestoidea as the Cestodaria and Eucestoda. Schmidt further divided the subclass Cestodaria into two orders (Gyrocotylidea and Amphilinidea), and included 13 orders in the subclass Eucestoda, of which 12 are found in fish, and one order (Cyclophyllidea) has mostly terrestrial final hosts (Table1.1).

Recently Khalil *et al.* (1994) has divided the class cestoda into 14 orders (Table 1.1).

Table 1.1. Classification of cestodes.

Reference	Class	Subclass	order
A. Wardle & Mcleod (1952)	1- Cestoda		Proteocephala, Tetraphyllidea Disculicepitidea, Lecanicephala,Trypanorhynicha, Cyclophyllidea, Aporidea, Nippotaeniidea, Caryophyllidea, Spathebothridea, Pseudophyllidea.
	2-Cestodaria		Amphilinidea, Gyrocotylidea, Biporophyllidea.
B. Wardle *et al.* (1974)	1-Cotyloda		Gyrocotylidea, Amphilinidea, Caryophyllidea, Spathebothridea, Pseudophyllidea, Diphyllidea,
	2-Eucestoda		Proteocephalidea, Tetraphyllidea, Litobothridea, Lecanicephalidea, Trypanorhyncha, Mesocestoididea, Tetrabothriidea, Nematotaeniidea, Taeniidea. Davaineidea, Anoplocephalidea, Hymenolepididea, Dilepididea, Cyclophyllidea, Aporia.
C. Schmidt (1986)	Cestodea	1-Eucestoda	Caryophyllidea, Spathebothridea, Trypanorhyncha, Pseudophyllidea, Lecanicephalidea, Aporidea Diphyllidea, Tetraphyllidea, Diorectaeniidea, Litobothridea, Nippotaeniidea, Proteocephalidea, Cyclophyllidea.
		2-Cestodaria	Gyrocotylidea,Amphilinidea

D. Khalil *et al.* (1994)	Cestoda	Amphilinidea, Gyrocotylidea, Sapthebothriidea, Caryophyllidea, Diphyllidea, Trypanorhyncha, Tetraphyllidea, Lecanicephalidea, Pseudophyllidea, Haplobothriidea, Nippotaeniidea, Proteocephalidea, Tetrabothriidea, Cyclophyllidea

1.3 Economic significance of cestodes in fish.

Cestodes in both their adult and larval forms may have harmful physiological, behavioural and pathological effects on their intermediate or final host. In fish, members of the families Caryophyllidae, Triaenophoridae, Cyathocephalidae, Bothriocephalidae, Ligulidae and Proteocephalidae are pathogenic to their host.

There are many reports concerning epizootics in cultured fish (Bauer, 1961; 1962), although most of them deal with one parasite species responsible for a paraticular out break of disease. A number of cases of fish mortalities have been reported in wild and farmed fish populations due to infection with intestinal cestodes. *Caryophyllaeus fimbriceps*, infects one and two year old carp (*Cyprinus carpio* L.) in pond culture and may cause weight loss and retarded growth, and mortalities (Bauer *et al.*,1973).

Karanis & Taraschewski (1993) reported pathological effects of *Caryophyllaeus laticeps* in bream (*Abramis brama* L.), chub (*Leuciscus cephalus* L.) and roach (*Rutilus rutilus* L.). In these fish scolices of the worm caused local compression of the intestinal epithelium. The intestinal epithelium cells were vacuolized and their brush border ruptured. Pathological effects were more pronounced in bream which showed conspicuous granulomas in the tunica propria adjacent to the scolices.

Bothriocephalus acheilognathi a parasite of silver carp (*Hypophthalmichthys molitrix* Valenciennes), grass carp (*Ctenopharyngodon idella* Valenciennes) and other cyprinids in China, has now spread to Europe and North America. In the south eastern United States this parasite has been reported as pathogenic to grass carp and fathead minnow (*Pimephales promelas* Rafinesque) and golden shiner (*Notemigonus crysoleucas* Mitchill) (Scott & Grizzle, 1979). In European pond farms *B. acheilognathi* may be responsible for 100 % mortalities in carp fry (*Cyprinus carpio*) (Körting, 1984a). Kurashvili (1984) reported that bothriocephalosis causes great damage to fish breeding. Carp underyearlings show a decrease in weight and length and a loss of activity of digestive enzymes, when infected by *B. acheilognathi* (Kurovskaya, 1984) (in Williams & Jones, 1994).

Khawia sinensis was considered to be epizootiologically important in carp by Skomorokhova & Kashkovski (1979). Infection of farmed carp (*Cyprinus carpio*) with *K. sinensis* in Germany was thought to cause little loss among fish, although it delayed growth (Weirowski, 1979) (in Williams & Jones, 1994). Morley & Hoole (1995) reported that infection of carp (*Cyprinus carpio*) with *K. sinensis* induced a loss of the microvillus border of the gut. Separation of enterocytes and loss of gut epithelium occurred. Lymphocytes and macrophages were observed within the gut adjacent to the worm surface.

Larvae of *Diphyllobothrium*, *Ligula* and *Triaenophorus* present in the body cavity or muscles can cause significant pathology in fish (Williams, 1967). Wootten & Smith (1979) reported that large numbers of *Diphyllobothrium* capsules may cause viscera to become fused together into a solid mass in trout. Older fish can be made sterile by the presence of the cysts within the gonads.

Weiland & Meyers (1989) found that plerocercoids of *Diphyllobothrium ditremum* infecting coho salmon, (*Oncorhynchus kisutch* Walbaum) in two Alaskan lakes caused severe ascites, abdominal distension with loss of equilibrium, visceral adhesion, haemorrhaging and discolouration of the liver, oedema, congestion and damage to hepatic tissue. Mortality was suggested to be due to liver dysfunction, blood loss and osmotic imbalance.

Rodger (1991) found that the plerocercoid larvae of *Diphyllobothrium ditremum* were found encysted and free living in the visceral organs, musculature and pericardial cavity of Atlantic salmon (*Salmo salar* L.). An inflammatory response was observed with fibrosis and collagenous tissue formation in pancreatic tissue and pyloric caeca, and necrosis, oedema and fibrosis in liver parenchyma. A high parasite prevalence and the severity of the lesions were suggested to be significant factors in mortalities observed in freshwater cage systems.

Revenga (1993) found no evidence that either *Diphyllobothrium ditremum* or *Diphyllobothrium latum* are harmful to rainbow trout (*Oncorhynchus mykiss* Walbaum). A lack of variation in liver weight as related to intensity of infection strongly suggested that competition for energy is not an important aspect of the host pathology.

Burrough & Kennedy (1979) reported that substantial mortalities among the roach (*Rutilus rutilus*) population from a small lake in England were induced as a result of parasitization by the pseudophyllidean cestode *Ligula intestinalis*. Ghosh (1987) found *Ligula* spp in pond reared two year old Indian major carp (*Catla catla* Hamilton), the parasite making up 3.6 % of the body weight of the fish.

Taylor & Hoole (1989) also reported the effects of plerocercoids of *Ligula intestinalis* on the major lymphoid organs, the spleen and pronephros of roach. The spleen of infected fish showed a significant decrease in weight and in the differential cell count. It

was suggested the latter was due to a reduction in erythrocytes, despite significant increases in macrophages and vacuolated granulocytes.

The plerocercoids of *Triaenophorus nodulosus* caused mortality in farmed trout in the former U.S.S.R by infecting the liver (Bauer *et al.* 1973). De-Maeseneer (1979) suggested that *Triaenophorus* may cause poor growth, weakness and death in fish and tapeworm populations increased in water polluted with organic matter.

Pronina & Pronina (1982) observed significant histopathological disturbances in the digestive tract of pike (*Esox lucius* L.), infected with *Triaenophorus nodulosus.* Mirle *et al*; (1985) found that larval *T. nodulosus* infecting rainbow trout caused about a 6 % decrease in weight.

Cyathocephalus truncatus caused general exhaustion, discolouration of muscles and inflammation of the pyloric caeca in farmed trout (Vik, 1958). Hermanns & Körting (1987) also reported lesions induced by *C. truncatus* in the intestine of rainbow trout.

Boyce (1979) found that *Eubothrium salvelini* had deleterious effects on the growth, survival and swimming performance of the sockeye salmon (*Oncorhynchus nerka* Walbaum).

Garnick & Margolis (1990) reported that *Eubothrium salvelini* influences the orientation of the seaward migration of the sockeye, which could in turn have important implications for smolt survival. Parasitic infection may account for the considerable variability observed in the migratory behaviour of smolts.

Bristow & Berland (1991) reported that low level *Eubothrium* spp infection in farmed salmon in Norway has significant effects on the loss of potential growth in both female and male fish. The direct loss of growth was approximately 10 % in fish of market size.

Proteocephalosis is an epizootiolgically and economically important disease of rainbow trout (Zitnan & Hanzelova, 1987). *Proteocephalus exiguus* infects *Coregonus peled*

(Gmelin), and under high stocking density, parasite burden increases (up to 1800 per fish) resulting in exhaustion, anaemia, decrease in fat content and in some cases mortality of the host (Bauer, Egusa & Hoffman, 1981). *Proteocephalus exiguus* from indigenous coregonid fish, *Coregonus* spp have been reported to infect rainbow trout in Germany (Priemer & Goltz, 1986).

The epizootiological importance of proteocephalosis in North America was reported by Becker & Brunson (1968) who found that *Proteocephalus ambloplitis* plerocercoids concentrated in the reproductive organs of largemouth bass (*Micropterus salmoides* L.), resulting in parasite castration.

Joy & Madan (1989) found that *P. ambloplitis* in the liver of largemouth bass and spotted bass (*Micropterus punctulatus* Rafinesque), caused an extensive area of pressure necrosis, with macrophage infiltration, adjacent to the parasite tegument. In large bass (> 305 mm for large mouth > 254 mm for spotted) parasitized livers showed considerable damage with blood vessel congestion, bile stasis and extensive fibrosis.

Ingham & Arme (1973) suggested that under culture conditions the presence of *Eubothrium* and *Proteocephalus* in rainbow trout has little effect on nutrition absorption or on fish growth. They showed that extensive erosion of the villi occurred in infected caeca, but no histopathological changes were observed in other regions of the intestine infected by *Eubothrium* and *Proteocephalus*.

Pathology and damage is most severe with species which penetrate the intestinal wall, however this does not necessarily seem to lead to mortalities. The most pathogenic species are those which lack specialised attachment organs (Williams & Jones, 1994). Hayunga (1979) found that in white sucker (*Catostomus commersoni*) infected with the caryophyllidean *Hunterella nodulosa* there was a loss of intestinal epithelium and lamina propria of the submucosal tissue.

The proteocephalid *Jauella glandicephalus*, perforates all the layers of the intestine of *Paulica lutkeni* causing severe haemorrhage and intensive necrosis. An intensive host reaction results in hyperplasia of connective tissue and formation of nodules surrounding the scolex of the parasite (Rego & Pavenelli, 1985; Eiras *et al.*, 1986).

1.4 List of *Proteocephalus* species in the British Isles.

Chubb *et al.* (1987) listed following species of *Proteocephalus* in British and Irish freshwater fish:

P. filicollis; P. macrocephalus; P. neglectus; P. parallacticus; P. pollanicolla; *P. torulosus; P. exiguus; P. sagittus; P. ocellatus; P. ambiguus; Proteocephalus* species?. These authors have pointed out that gravid *Proteocephalus* from *Gymnocephalus cernua* should be critically examined for *P. cernua.*

1.5 Taxonomy and Biology of Proteocephalids.

1.5.1 Order Proteocephalidea Mola, 1928.

Members of this order have a scolex with four suckers which is clearly distinguished from the strobila, there may be a fifth apical sucker on the tip of the scolex. Segmentation of the strobila is distinct. The ovary is bilobed, vitellaria extend into the lateral margins of proglottids or surround it on all sides. The egg is generally embryonated. Adult worms are parasitic in the intestine of freshwater fish, sharks, amphibians and reptiles. This order includes two families, the Proteocephalidae and Monticellidae (Schmidt, 1986).

1.5.2 Family Proteocephalidae La Rue, 1911.

The members of this family have scolex of varied forms, ovary and uterus medullary, testes medullary. Vitelline foliccles medullary. Parasites of fish, amphibian and reptiles. In North and South America, Africa, Asia, Australia (Khalil *et al.* 1994)

Schmidt (1986) included seven sub-families in the family Proteocephalidae (Gangesiinae in silurid fish in Pakistan and India; Prosbothriinae in sharks in Japan, North America and Europe; Sandonellinae in freshwater teleosts in Africa; Corallobothriinae in silurid fisn in Paraguay; Acanthotaeniinae in lizards in Australia; Proteocephalinae in freshwater fish, amphibians and reptiles; and Marsipocephalinae in silurid fish in Africa).

Khalil *et al.* (1994) included six sub families in the family Proteocephalidae. These are; the Corallobothriinae, Marsyphalinae, Sandonellinae, Proteocephalinae, Gangesiinae, Acanthotaniiae. In the view of Rego (1994) the sub family Prosbothriinae from marine elasmobranchs (as discussed by Schmidt, 1986) within the order Proteocephalidea is more closely related to the tetraphyllidea. Moreover, proteocephalids of fish are found in hosts living in freshwater environments, none are found in marine waters.

1.5.3 Sub-family Proteocephalinae Mola, 1929.

According to Khalil *et al.* (1994) this sub family has the following diagnostic features. Scolex with four suckers of normal type, fifth or apical sucker may be present. Reproductive organs and vitellarium medullary or lateral. In fish, amphibians and reptiles.

The sub family has eight genera, i.e. *Travassiella* (in South American siluroid fish), *Proteocephalus* and *Ophiotaenia* (in fish, amphibians and reptiles), *Crepidobothrium* (in South American snakes), *Macrobothriotaenia* (in Indian snakes), *Brayela* (in South American siluroid fish), *Tejidotaenia* and *Deblocktaenia* (in colubrid snakes from Madagascar).

1.5.4 Genus *Proteocephalus* Weinland, 1858.

Members of this genus have a scolex with four normal suckers. A fifth apical sucker or apical organ may be present. Reproductive organs and vitellarium medullary or lateral (Khalil *et al.* 1994). The members of this genus are found in fish, amphibians, reptiles and are cosmopolitan.

1.5.5 Taxonomy of the Genus Proteocephalus.

The taxonomy of the genus *Proteocephalus* was discussed by Wardle & MCleod (1952), Yamaguti (1959), Freze (1965), Brooks (1978), Schmidt (1986) and recently by Khalil *et al.* (1994).

1.5.6 Morphology of Genus *Proteocephalus.*

Freze (1965) described the morphology of the genus *Proteocephalus.* The scolex is rounded or quadrilobate with four suckers, which may be covered with a dense network of spines. The apex of the scolex is the site of an apical sucker, a large glandular organ, or a rudimentary apical organ. The testes lie in one or several layers occupying the entire space between the vitellaria. The vagina opens into the genital atrium anteriorally, dorsally, or posteriorly to the cirrus pouch. The vitellaria are lateral, paired, and may form two bands on either side of the proglottid.

1.6 Life cycle of the Genus *Proteocephalus.*

Eggs of *Proteocephalus* are shed into water. The eggs contain a hexacanth oncosphere which is surrounded by three to five membranes. The egg is ingested by a copepod, the intermediate host. Specificity with respect to intermediate host has been observed under

experimental conditions. The egg hatches within the intestine of the copepod. The precise hatching mechanism of *Proteocephalus* is not known. Wootten (1974) suggested that hatching may initiated by :

1. The mechanical activity of the embryo.

2. The action of host enzymes stimulating the embryo and perhaps dissolving the embryonic membranes.

3. Mechanical action by the intermediate host on the embryonic envelopes.

The hexacanth penetrates through the copepod intestinal wall and passes into the body cavity, the time taken for this migration varies from species to species. Freeman (1964) and Wootten (1974) described similar hook movements for *Proteocephalus parallacticus*, and *Proteocephalus percae* during penetration which appeared to cause intestinal cell displacement rather than cell rupture. These authors observed the median hooks pushing apart the cells of the intestinal wall. The role of the hexacanth penetration glands in the migration of *Proteocephalus fluviatilis* through the intestinal wall has been described by Fischer (1968) and this hypothesis was supported by Wootten (1974).

In the copepod the hexacanth develops into the procercoid stage in the haemocoel. The life cycle continues when the infected copepod is ingested by a fish in which further development take place (Wardle & MCleod, 1952; Freze, 1965; Chubb, 1982).

Scholz (1991) reported that a cercomere appears only 5 days after infection of the copepod host in *Proteocephalus neglectus*; the suckers appear almost at the same time. On the other hand Wagner (1954) reported that sucker development commences before the cercomer is formed in *Proteocephalus tumidocollis*. In other species cercomer formation begins after 10-20 days. The larvae are fully developed to the plerocerciform stage from 18-27 days depending on species. Scholz (1993) found larvae infective to

fish after 9-12 days in *Proteocephalus torulosus* under experimental conditions at 20-22^0 C. The stage after formation of the suckers has been termed as plerocercoid 1 or cercoscolex by different authors (Fischer, 1968; Priemer, 1980).

Different *Proteocephalus* species have varied optimum temperatures for development, but this optimum temperature may depend on the copepod host species. Development rate is also influenced by the number of the larvae in individual copepods. In general the latter only contain 1 or 2 developed plerocercoids In most species the plerocercoid is ingested by a suitable fish host in the intestine of which the adult parasite develops. In some species, e.g. *Proteocephalus ambloplitis* the plerocercoid, after ingestion by the fish, first migrates to a parenteral site and only subsequently migrates to the intestine of the same fish and matures (Fischer & Freeman, 1969). Plerocercoids from the intestine may be transferred to other individuals of the same host species by cannibalism or individuals of other species by predation (Hopkins, 1959; Freze, 1965). Thus fish may serve as definitive hosts, obligate or facultative intermediate hosts (Hopkins, 1959; Freze, 1965). Proteocephalid plerocercoids are commonly found as transient members of parasite communities in the intestine of fish in which they cannot mature (Chubb *et al.* 1987). Some proteocephalids have a three host cycle involving two fish hosts. *Proteocephalus ambloplitis*, a parasite of smallmouth bass (*Micropterus dolomieui* Lacepede), yellowfin perch (*Perca flavescens* L.) and other fish, may use fry of bass or pumpkinseed (*Lepomis gibbosus* L.) as second intermediate hosts. Table.1.2 gives some features of the life cycle of the genus *Proteocephalus*.

Table 1.2 Features of the life cycle of a range of *Proteocephalus* species.

Species	Egg diameter µm	Egg layers	First intermediate host	Migration time	Cercomere appearance time	Suckers Appearance time	Age of infective larva	Experimental temperature (° C)	Oncosphere diameter	References
P. amblopliitis	26-60 µm	3	*C.albidus* *C. prasinus*	4-5 h	-	16 days	16-19 days	-	20 µ	Hunter,1928
P. pingius	45-52 µm	3	*C. vulgaris* *E. agilis*		-	6 days		-	20 µ	Hunter, 1929
P. tumidocollius	-	3	*C. vernalis* *E. agilis*	27 min	15-19 days	13 days	15-19 days	20° C	24-27 µm	Wagner, 1954
P. parallacticus	120-200 µm	3	*C.bicuspid -atus* *C. vernalis*	12 h	13-20 days	20 days	13-20 days	16° C	35 x 32 to 42 x 50 µm	Freeman, 1964
P. fluviatilis	57x 97 µm	5	*C.bicuspida-tus* *C. vernalis*	12 h	9 days	10 days	23 days	18° C	-	Fischer,1968
P. percae	47-82.6 µm	5	*M.leuckartus.* *E. agilis*	35 min	10-20 days	23-27 days	27 days	14° C	23-47µm x 22.1µm	Wootten, 1974
P. exiguus	40-45 µm	2-3	*C.sternuus.*	5 days	10 days	14 days	18 days	14-15° C	19-27 µm	Priemer,1987
P. neglectus	100 µm	3	*C. sternuus*	-	5 days	6-7 days	21-27 days	21-22° C	23-32 µm	Scholz,1991
P. torulosus	41-51µm	3	*C. sternuus*	-	7 days	7-8	9-12 days	20-22° C	0.23-0.32µm	Scholz,1993

1.7 Taxonomy of *Proteocephalus filicollis.*

La Rue (1914) published a detailed description of *Proteocephalus filicollis* which still stands. La Rue considered *I. filicollis* as identical with *Proteocephalus ambiguus* and synonymised *Ichthyotaenia* with *Proteocephalus.* However, Willemse (1968) reported *Proteocephalus filicollis* from *G. aculeatus* and *Proteocephalus ambiguus* from *Pungitius pungitius* as separate species. Willemse (1968) reported small morphometric differences in these two species, but found a vestige of a fifth apical sucker in worms from both hosts and claimed that a high degree of host specificity exists in worms from the two hosts. Freze (1965) stated that an apical organ is present in *P. filicollis,* and Rodland (1983) in his TEM study reported the presence of an apical organ in the scolex of adult *P. filicollis,* and its absence on *P. ambiguus.* Rodland stated that presence of an apical organ in *P. filicollis* and its absence in *P. ambiguus* is itself sufficent to distinguish these two species.

It is not clear whether the presence or absence of an apical organ or frontal pit in the larval stage (if present) can be used to differentiate *P. filicollis* and *P. ambiguus.* The point needs to be clarified by the use of the scanning electron microscope (SEM).

1.8 Classification of *Proteocephalus filicollis* Rudolphi, 1802.

According to Schmidt (1986):

Class	Eucestoda	Southwall, 1930.
Order	Proteocephalidea	Mola, 1928.
Family	Proteocephalidae	La Rue, 1911.
Sub-family	Proteocephalinae	Mola, 1929.
Genus	*Proteocephalus*	Weinland, 1858

Species *Proteocephalus filicollis* Rudolphi, 1802.

1.9 Morphology of *Proteocephalus filicollis.*

1.9.1 Scolex.

According to Rodland (1983), the scolex is round or dorsoventrally flattened and has four suckers, 37-70 µm in diameter. A distinct apical organ is always present on the scolex. The scolex measures 132-195 µm in length by 65-117 µm in width. A neck constriction is not visible on contracted specimens, but appears when the worm is relaxed.

1.9.2. Strobila.

Gravid worms vary in length from 10-55 mm by 0.63-1.12 mm in width. The total number of segments varies from 64 to 145 each. Of 100 segments counted 12 to 21 were immature, 23-43 mature and 21-100 gravid. Each mature segment has 46-68 testes lying in an irregular layer anterior to the ovary and medial to the vitellaria. The length of the cirrus sac/ width of segment ratio is 1:4-5. The cirrus and vagina terminate in a genital atrium. The cirrus sac measure 117-195 x 47-62 µm. There are between 5 to 10 diverticula on each side of the gravid uterus (Rodland, 1983). The morphometric characteristics are given in Table 1.3.

1.9.3 Life cycle of *Proteocephalus filicollis.*

Meggitt (1914) was the first author who experimentally studied the life cycle of *P. filicollis* (Fig. 1.1). The eggs are released by the gravid worms in the intestine of the fish. The egg is round in shape and contains an oncosphere which is surrounded by

a delicate membrane, a second membrane surrounds the latter. The outermost third layer is called the transparent membrane. The oncosphere bears three pairs of embryonic hooks (Meggitt, 1914).

Cyclops varius was found to be a suitable first intermediate host species by Meggitt (1914). After ingestion by the copepod the egg passes into the intestine where it hatches. The hatching mechanism is not described in detail. Meggitt (1914) stated that it takes usually one week for the oncosphere to break through the wall of the intestine into the dorsal sinus. It seems unlikely that the hexacanth takes so long to penetrate the intestinal wall, as variable penetration times for different species of *Porteocephalus* have been observed by different authors (Table1.2). In the haemocoel the hexacanth changes its shape, from round to oval and then become elongated and shows contracting and relaxing movements.

The cercomer was not observed by Meggitt (1914) but it may have been overlooked as it has been reported subsequently by many authors in *Proteocephalus* species (Table1.2). The hooks gradually disappear and highly refractive granules appear in the body. These granules are isolated or present as clusters.

The larva becomes infective to the final host within three weeks according to Meggitt (1914). At this stage there is no scolex or neck but at the anterior end are four suckers, there is no trace of a fifth sucker. The infective larva is now termed a plerocercoid and if ingested by a stickleback becomes established in the alimentary canal, particularly in the rectum (Hopkins, 1959). The reason for its initial attachment in the rectum is not clear.

In the intestine of the final host the worm grows in size and becomes mature and gravid. The worm migrates from the posterior part of the intestine to the anterior part as it become gravid (Hopkins, 1959). When the contents of the gravid

proglottids are ready to be discharged the worm migrates through the intestine, probably by the contractions of the latter and hangs out of the anus. After the eggs have been expelled the empty proglottids degenerate and eventually the whole worm is discharged with faeces (Meggitt, 1914).

Proteocephalus filicollis, like other *Proteocephalus* species, exhibits a seasonal cycle of occurrence and maturation. Recruitment is at its maximum in summer and autumn and gravid worms appear in spring and summer (Hopkins, 1959). Chappell (1969a) found that recruitment occurred throughout the year and gravid worms are available in all seasons.

Table 1.3 Morphometric characteristics of *Proteocephalus filicollis*.

Diameter egg µm	Egg layers	Diameter oncosphere µm	Diameter scolex µm	Neck length mm	Length gravid worm mm	No of segments	No of testes	Intermediate host	Apical sucker	Width of worm mm	References
50-75 µm	2	27 µm	5--6 to 6-7µm	-	4-35 mm	-	40-90	-	-	1-2 mm	**La Rue, 1914**
58 µm	3	23 µm	4-7 µm	0.25 mm	24-33 mm	24-33	40	*C.varius*	-	1.0-1.2 mm	**Meggitt, 1914**
-	-	-	-	-	14-30 mm	-	-	*C.vernalis*	-	-	**Hopkins, 1959**
50-75µm	-	27µm	-	-	35 mm	-	75-90	-	-	0.8 mm	**Bykhovskaya Pavlovskaya *et al.* 1964**
13µm	-	7-10 µm		-	-	-	-	-	present	-	**Freze, 1965**
-	-	20µm	4.5-9 µm	-	38 mm	-	45	-	-	-	**Willemse, 1968**
			-	-	5-25 mm	-	-	-	-	-	**Chappell, 1969a**
--	-	-	-	-	20-50 mm	-	40-70	-	-	-	**Andersen, 1979**
-	-	-	13-19µm	1.03-2.0 mm	10-65 mm	65-145	-	-	present	0.63-1.12 mm	**Rodland,1983**

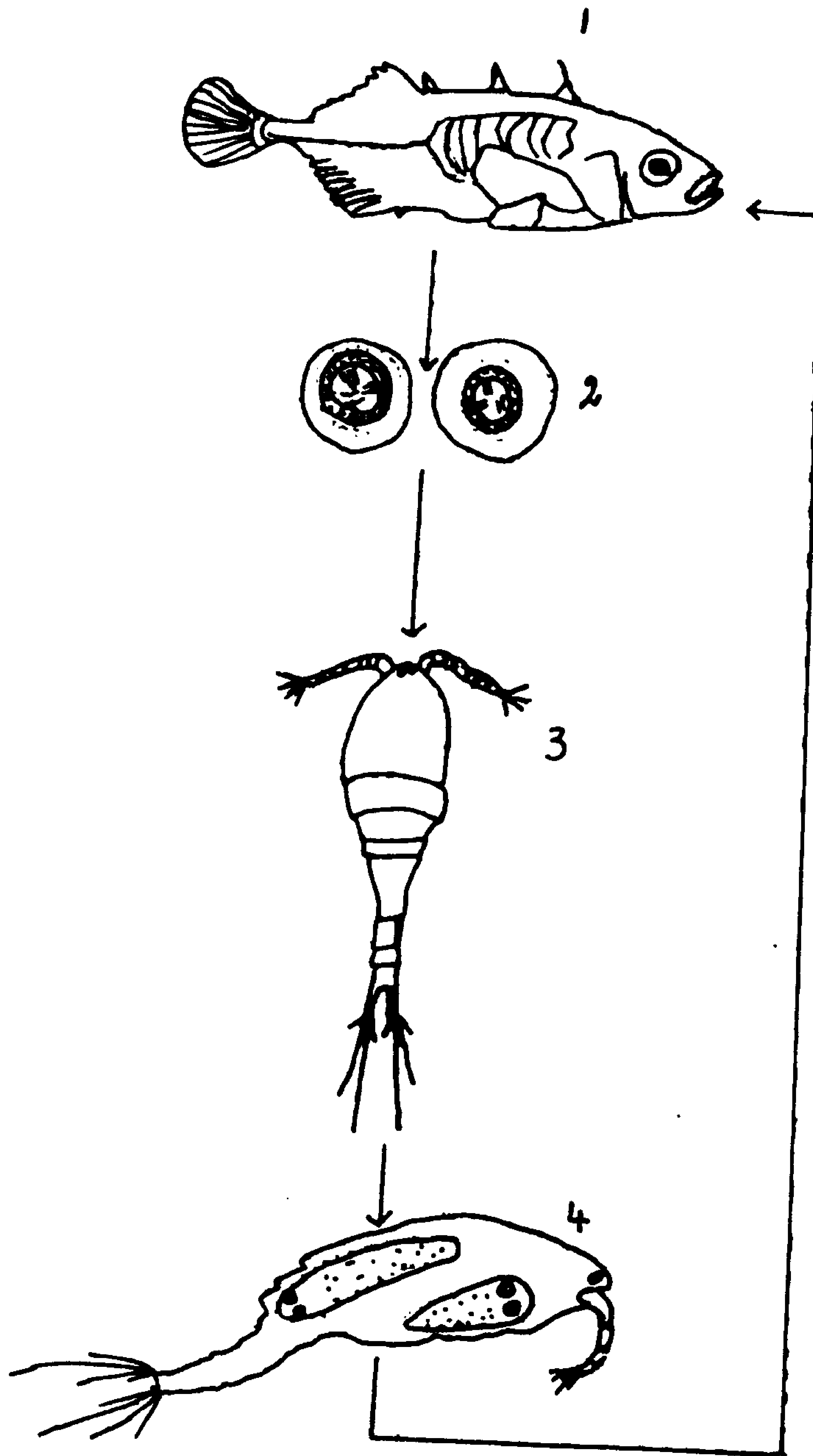


Fig 1.1. Diagrammatic representation of the life cycle of *Proteocephalus filicollis.*
1- *Gasterosteus aculeatus* final/definite host, 2-mature eggs with oncosphere,
3- *Cyclops* intermediate host, 4- *Cyclops* with fully developed procercoids
(drawings not to scale).

1.10 Geographical distribution of *Proteocephalus filicollis*.

Proteocephalus filicollis is distributed throughout Europe, North Asia and North America and from the former U.S.S.R in the basin of the Baltic Sea and in Kamchatka (Freze, 1965). In Britain this species has been reported from many localities including Scotland (Kennedy, 1974) and has also been recorded from Ireland (Conneelly & McCarthy, 1984), Norway (Andersen 1979; Rodland, 1979), Poland (Kuczkowski,1925), Netherlands (Willemese & Veltman,1962) former U.S.S.R (Banina & Isakov ,1972) and Finland (Andersen & Valtonen, 1990).

1.11 Host specificity of *Proteocephalus filicollis.*

Willemse (1968) experimentally demonstrated a high degree of host specificity in *P. filicollis* from *G. aculeatus* and *P. ambiguus* from nine-spined stickleback (*Pungitius pungitius*). He infected *P. pungitius* by feeding them *Cyclops strenuus* infected with *Proteocephalus ambiguus* eggs, but could not infect *G. aculeatus* by this route It also proved impossible to transfer adult worms from one species of stickleback to the other, whereas control transfers between the same stickleback species always proved successful.

Dartnall (1973) and Conneelly & McCarthy (1984) reported *P. filicollis* from *P. pungitius*. These were probably accidental infections as worms were found to occur in only a small number of fish. There is also the possibility of misidentification.

Sysoev (1985) reported the presence of *P. filicollis* from nine-spined stickleback from a small lake in Medvezhiegorsky region of the Kareline U.S.S.R. The nine - spined stickleback was reported to be the only fish species found in this lake and procercoids of *P. filicollis* were also found in *Mesocyclops oithonides* (Sars) under natural conditions. This indicates the completion of the life cycle of *P. filicollis* using

different hosts than the normal three-spined stickleback and throws doubt on the host specificity of this cestode.

Moreover, recently Hanzelova *et al.* (1996) reported that *P. exiguus* can survive in numerous fish hosts (rainbow trout, brown trout, brook trout and perch) even of different families under altered ecosystems. *Proteocephalus exiguus* modifies its seasonality in an unusual fish host (perch) which indicates a broader host specificity of this cestode.

Further investigation are required on the host specificity of *P. filicollis*.

1.12. Aims of the present study.

The aims of this study were:

1- To study the population biology of *Proteocephalus filicollis* from a wild population of three-spined stickleback, *Gasterosteus aculeatus*, from a freshwater Scottish loch and to compare this with previous studies from Britain by Hopkins (1959) and Chappell (1969) which demonstrated some contradiction.

2- To determine pathological effects of attachment of the worm on the intestine, location and migration of the parasite in the intestine of the fish.

3- To investigate egg production by *Proteocephalus filicollis* from three-spined stickleback and determine factors affecting egg production and fecundity of the parasite.

4- To provide information on the development of *Proteocephalus filicollis* in the intermediate copepod host with special reference to development at different temperatures, development of individual cestodes, infectivity period of the oncosphere, infection dynamics of *P. filicollis* in the intermediate host; infection

of copepods in relation to exposure time to parasite eggs

5- To investigate the ultrastructure of the embryonic envelopes of *P. filicollis* by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and to compare their structure with that of other cestode groups.

CHAPTER 2
FEATURES OF AIRTHREY LOCH

2. Features of Airthrey Loch

2.1 History, site and structure of the loch.

Airthrey Loch was constructed in 1930 and is situated within the grounds of the University of Stirling (Grid Reference NS 806965) (Fig 2.1). The area of the loch was originally about 9.7 ha and depth varies from 0.5 to more than 5.0 m (Wood, 1974). In the 1970's a road was built on the eastern side which resulted in the isolation of a small section of the loch. The bottom of the loch mainly consists of rotting leaves and mud. The western embankment of the loch is protected by a low dam; a boat house is built on this side.

Smith (1995) reported the area of the loch as approximately 6.9 ha and the volume as 127 400 m of water. The loch has three sections. The largest section is at the western end and is the deepest part, with a maximum depth of 4.5 m. The second section is channel like with a maximum depth of 2.5 m and connects the western and eastern sections of the loch, the latter has a maximum depth of 2.0 m (Fig 2.2). The morphometric data of the loch as given by Smith (1995) is shown in Table 2.1.

Table 2.1 Morphometric data of Airthrey Loch.

Maximum length	890 m
Mean width	140 m
Maximum depth	4.5 m
Shoreline length	2325 m
Surface area	69039 m
Volume	127 400 m^3

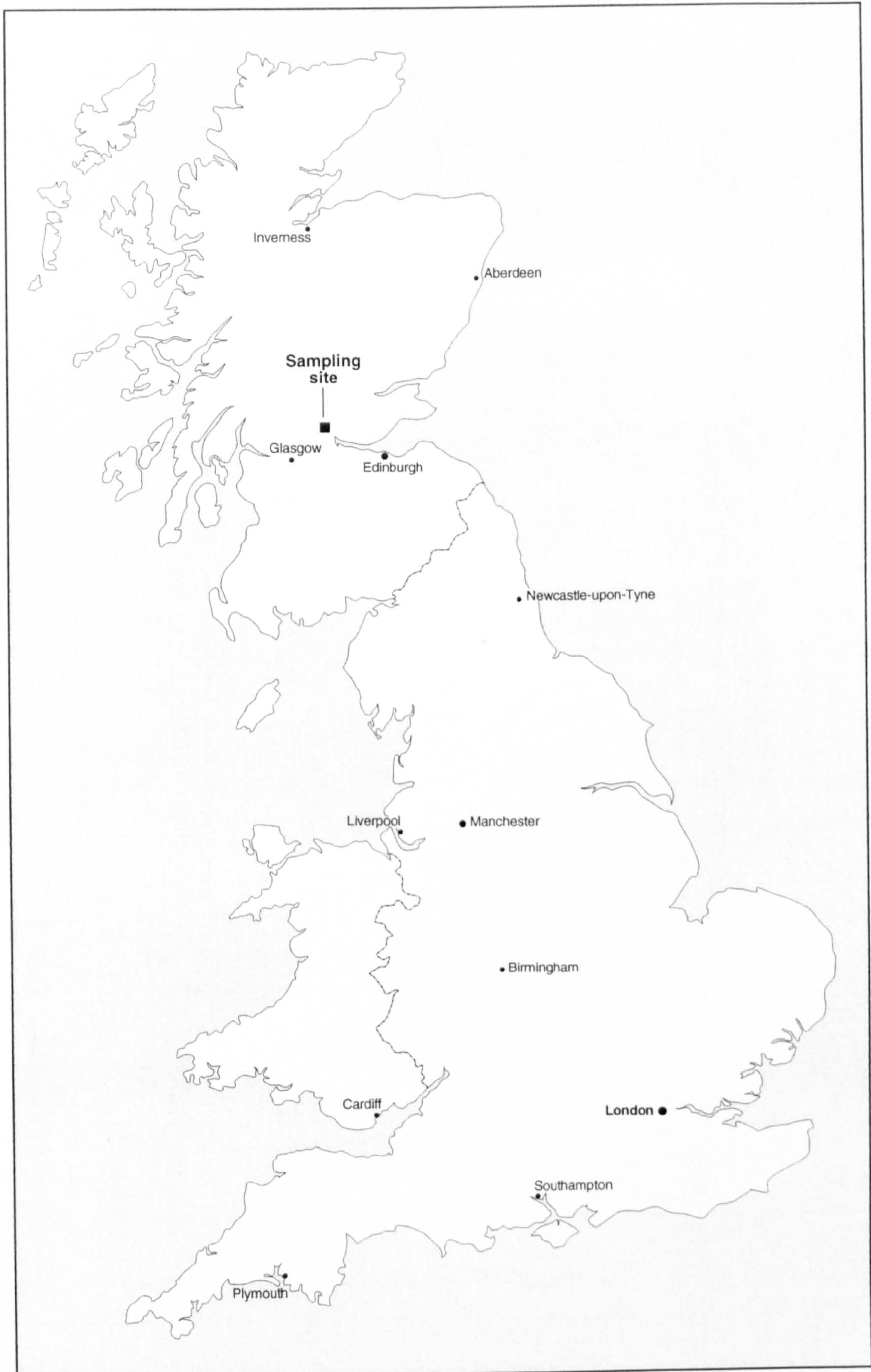


Fig 2.1. Location of sampling site.

Fig 2.2 Bathymetric map of Airthrey Loch (depth contours are in meters).
The sampling stations (*), inflow and outflow are indicated.

2. 2 Climate

Parkhead weather station within the grounds of Stirling University (Grid Reference NS 814968) records climatological data which is given in Table 2.2. A total of 952.2 mm of rainfall was recorded in 1993. During 1994 total rainfall was 1089.2 mm which was 72.6 mm (7.13%) more than the previous year. The total rainfall in the first six months of 1995 was 368.7 mm which is 83.0 mm (33.72%) less than in the first six months of 1993 and 141.6 mm (27.67%) less than first six months of 1994 (Table 2.3). The spring of 1993 was warmer than those of 1994 and 1995, whereas the summer of 1995 was the warmest of the three years. The autumns of 1994 and 1993 were similar, whilst the winter of 1993-94 was colder than 1994-95 (Table 2.4).

Table 2.3 Total rainfall (mm) in Parkhead area, January 1993 to June 1995.

Years	January-June	July-December	Total rain fall (mm)
1993	552.5	399.7	952.2
1994	510.7	578.5	1089.2
1995	368.7		

Table 2.2. Climatological data of Parkhead area University of Stirling from January 1993 to June 1995.

Months	Rainfall (mm)	Air temperature (°C)	
		Maximum	Minimum
January 1993	248.9	12.5	-1.5
February	5.6	13.0	-4.0
March	61.9	14.1	-4.6
April	77.0	20.0	0.0
May	100.9	25.8	1.0
June	58.2	24.3	5.8
July	53.3	20.3	3.4
August	38.1	23.4	3.5
September	55.8	21.3	0.0
October	57.2	16.0	-8.5
November	58.6	13.8	-5.7
December	136.7	14.0	-9.0
January 1994	129.8	10.8	-2.5
February	54.6	8.3	-9.5
March	183.5	11.6	-1.8
April	73.4	16.0	-1.5
May	20.3	20.1	-1.4
June	49.1	25.0	1.0
July	68.1	25.2	7.8
August	90.0	23.2	0.6
September	38.5	21.1	0.2
October	70.0	16.0	-0.7
November	133.5	14.4	-0.2
December	178.4	15.7	-5.0
January 1995	109.6	17.0	-7.2
February	113.5	10.7	-4.7
March	48.3	14.2	-4.0
April	40.6	18.6	-2.0
May	59.3	21.3	-1.2
June	19.7	28.5	1.2

Table 2.4. Air temperature range (°C) in Parkhead area from March 1993 to June 1995.

Months	1993	1994	1995
March, April, May	-4.6-25.8	-1.8-20.1	-4.0-21.3
June, July, August	3.4-24.3	0.6-25.2	1.2-28.5 (June)
September,October, November	-8.5-21.3	-0.7-21.1	
December,January, February	-9.5-14.0	-7.2-17.0	

2.3 Water supply.

Airthrey Loch is mostly fed by water from the Wharry Burn catchment, which flows into the western end of the loch. Water is released to the loch through Bridge of Allan Reservoir. A minimum flow of 70,000 gallons a day is allotted to the loch (Wood, 1974) (Fig.2.2).

2.4 Water level.

The water level of the loch fluctuates throughout the year. In summer the water supply from all sources decreases and evaporation from the large surface area of the loch exceeds the inflow and results in a drop in water level. The water level is recorded weekly by means of a gauge fixed on a jetty at the boat house. The outflow weir maintains a minimum level of water in the loch (Table 2.5). The depth of water at the gauge is almost 1.5 m. The lowest level (1.48 m) was recorded in August 1994, whereas the highest level (1.53 m) was recorded during January 1993 which

Table 2.5. Monthly range of water level at gauge at Airthrey Loch from January 1993 to June 1995.

Month	Range of water level(m)
January 1993	1.507—1.530
February	1.498—1.509
March	1.496—1.508
April	1.500—1.507
May	1.499—1.508
June	1.497—1.503
July	1.492—1.495
August	1.491—1.494
September	1.483—1.499
October	1.490—1.500
November	1.492—1.499
December	1.504—1.510
January 1994	1.055—1.509
February	1.500—1.505
March	1.512—1.520
April	1.500—1.511
May	1.493—1.500
June	1.486—1.490.
July	1.481—1.484
August	1.481—1.484
September	----------
October	----------
November	----------
December	----------
January 1995	----------
February	----------
March	----------
April	----------
May	1.500—1.502
June	1.498—1.504

--------Gauge under repair so no reading taken.

corresponds to the maximum rainfall (248.9 mm) occurring in that month. In most months the water level remained within 1 cm of 1.5 m.

2.5. Water temperature and dissolved oxygen.

The water temperature was recorded at a depth of 0.5 m at the western end from the boat house and sampling station 2 along the embankment of the loch with a mercury thermometer. The temperature was recorded twice a month. Figure 2.3 gives the range of temperature in each month. There is a wide range of temperature with the coldest from November 1993 to February 1994 when the loch surface was frozen at intervals. During this period the water temperature was between 0^0C- 1^0C. The temperature started rising with the onset of spring. Summer temperatures were similar with a maximum temperature of 21^0C in June 1993, 18^0 C in August 1994 and 19^0C in June 1995. The lowest temperature was recorded during winter 1993 (Dec. 1993 to Feb. 1994) whereas the winter of 1994 was mild compared to winter 1993. The autumn of 1994 was comparatively warmer than the previous year. The summer of 1993 was warmer than the summers of 1994 and 1995 (up to June).

Dissolved oxygen was measured twice a month in the loch along the embankment at the boat house and at sampling station 2, using a YSI Model 57 oxygen meter. Dissolved oxygen values varied seasonally. The variation in dissolved oxygen concentration is related to changes in temperature of the loch. The highest dissolved oxygen level was observed during winter and ranged from 12.10- 13.25 mg/l and the lowest values were observed in summer and ranged from 9.40-10.35 mg/l.

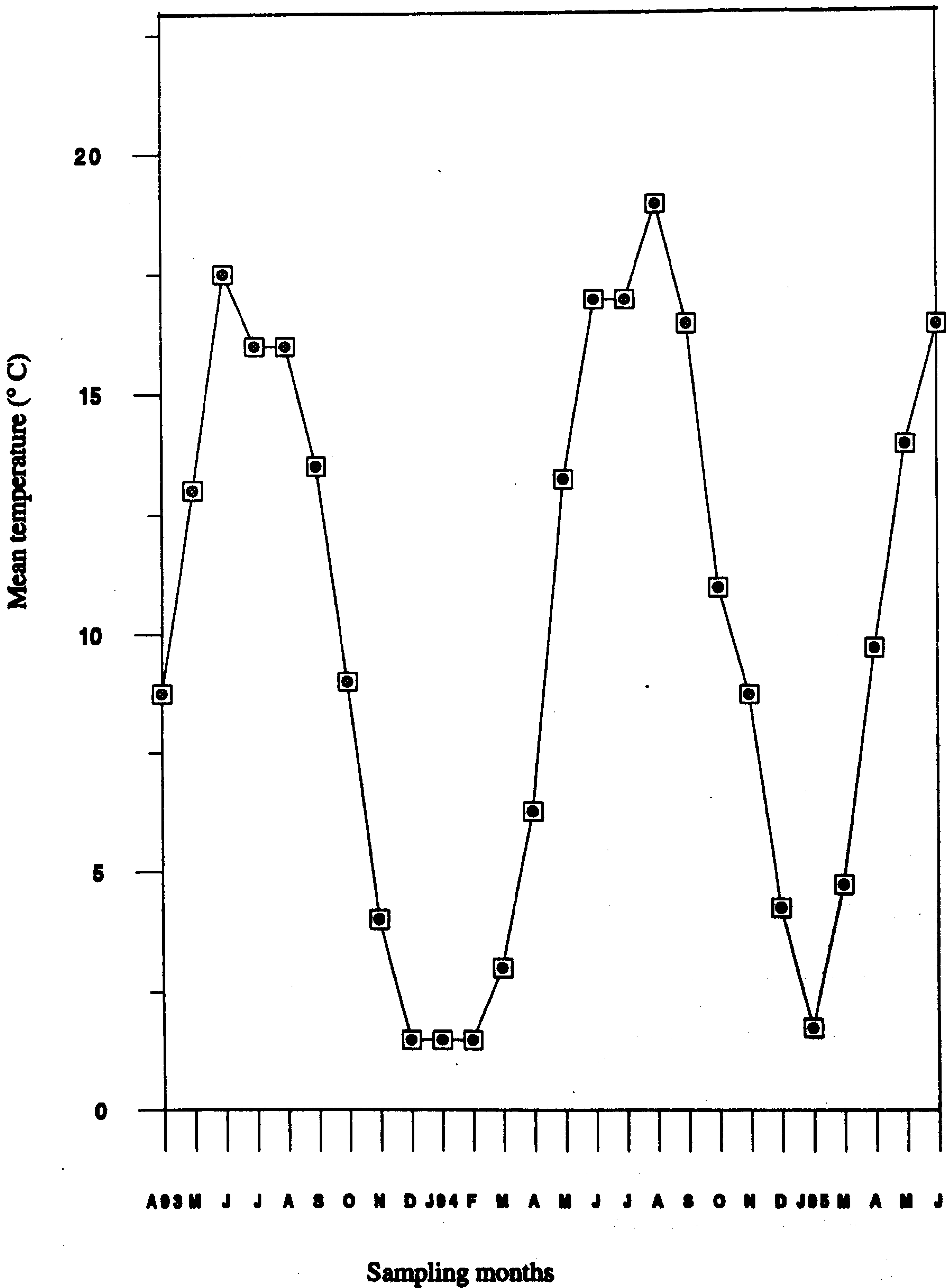


Fig.2.3. Monthly mean water temperature (° C) of Airthrey Loch from April 1993 to June 1995. The temperature was taken at 0.5 m depth at sampling stations 1 & 2.

2.6 Water sampling

Water samples were taken along the embankment of the loch at the boat house and sampling station 2 twice a month in one litre glass screw cap bottles. These bottles were washed and rinsed with distilled water prior to sampling. Sample bottles were rinsed at the site before taking samples. Water samples were analysed for the following parameters.

1. Hydrogen ion concentration (pH).
2. Conductivity.
3. Total dissolved solids (TDS).

Water analysis was carried out according to standard laboratory procedures of the water quality laboratory within the Institute of Aquaculture.

2.6.1 pH

pH was recorded with a Phillips PW 9409 digital pH meter which was calibrated prior to use. An unfiltered water sample was taken in a 50 ml beaker (the water sample was taken as discussed in section 2.6) and the pH electrode was placed in the sample and the reading taken. The electrode was rinsed thoroughly with distilled water after use. The range of pH values for both sites are given in Table2.6. There was a wide variation in the pH from 6.40 to 9.56, but it was usually between 7.0 and 8.0. Most variation was observed in summer months, i.e. 7.25 to 9.56 in summer 1993 and 7.33 to 9.49 during summer 1994 and also in spring 1994.

2.6.2 Conductivity

The conductivity of the loch water was measured using a CAMLAB HACH digital Conductivity / TDS meter model 44600. The unfiltered sampled water was

Table 2.6. Range of water temperature, pH and conductivity in Airthrey Loch from April 1993 to June 1995.

Month	Temperature. ⁰C (Range)	pH (Range)	Conductivity (Range)
April1993	07.5—10.0	7.20—7.80	230—282
May	12.0—14.0	7.16—7.84	228—258
June	14.0—21.0	7.60—7.96	208—252
July	14.0—18.0	7.56—7.81	199—233
August	14.0—18.0	7.25—9.04	175—215
September	13.0—14.0	7.08—7.30	168—204
October	07.0—11.0	7.64—7.70	140—212
November	01.0—07.0	7.50—7.68	138—192
December	01.0—02.0	7.45—7.52	188—212
January 1994	01.0—02.0	6.95—7.05	230—240
February	01.0—02,0	7.30—7.48	272—276
March	02.0—04.0	7.22—7.48	249—275
April	04.5—08.0	8.09—9.03	220—286
May	12.0—14.5	7.56—8.52	225—265
June	16.0—18.0	7.33—9.42	210—250
July	16.0—18.0	8..07—8.64	190—234
August	18.0—20.0	8.47—9.49	180—210
September	15.0—18.0	7.43—7.60	165—215
October	10.0—12.0	7.20—7.36	150—205
November	07.5—10.0	7.06—7.26	140—200
December	02.5—06.0	6.79—7.08	186—210
January 1995	02.0—02.5	6.79—7.08	208—240
February	01.5—02.0	6.40—6.66	268—274
March	04.5—05.0	7.20—7.60	240—272
April	08.5—11.0	7.58—8.64	222—280
May	12.0—16.0	6.77—7.05	218—270
June	15.0—18.0	7.30—8.35	185—255

poured in to a 50 ml clean beaker, the electrode of the conductivity meter was immersed in the water and the reading taken, after it became stabilised. Results are given in Table 2.6. The conductivity varied from 185-286 S/ cm during the year and the highest value of 220-286 S/ cm (Table 2.6) was recorded during spring 1994.

2.6.3 TDS

TDS was also measured using a CAMLAB HACH digital Conductivity / TDS meter model 44600. TDS values varied from 0.09 to 0.131 g/l during the year and was highest during spring at 0.123 to 0.131 g/l.

2.7 Turbidity

Turbidity of the loch was measured using a Secchi disc at the boat house jetty where the depth varied from 1.0 - 1.5 m. The Secchi disc reading in summer varied from 0.45-0.65 m, but during rest of the year loch water remained comparatively clear and readings varied from 1.0 to 1.25 m.

2.8 Biotic factors

Samples of zooplankton were taken using a hand held plankton net (mesh size 50 µm) from the littoral areas of the loch during June and July 1993. *Diaptomus* spp, *Cyclops* spp and *Daphnia* spp were commonly found in the loch. The *Cyclops* species identified according to Harding & Smith (1973) were *Cyclops agilis* (Koch, Sars), *Cyclops viridus* (Jurine), *Cyclops* spp. *Acanthocyclops robustus* (Sars) and *Acanthocyclops* spp. The identifications were confirmed by Dr Geoff Boxshall of the Natural History Museum, London. No quantitative estimates of zooplankton numbers were made, but the *A. robustus* population was apparently higher than other

Cyclops species in the sample. Hence this species was cultured in the laboratory for later experimental infection with *P. filicollis* eggs.

Wood (1974) mentioned the presence of the following species of fish in Airthrey loch.

Family **Salmonidae**

rainbow trout, *Oncorhynchus mykiss* (Walbaum).

brown trout, *Salmo trutta* (L.)

Family **Cyprinidae**

minnow *Phoxinus phoxinus* (L.)

Family **Anguillidae**

eel *Anguilla anguilla* (L).

Family **Gasterosteidae**

three-spined stickleback *Gasterosteus aculeatus* (L.)

2.9 Aquatic plants.

There is a thick plantation, consisting mainly of *Salix* sp (willow) around the loch. The littoral zone of the loch is dominated by *Iris pseudocorus* (yellow iris), *Typha* spp (bulrushes), *Phragmites* spp (common reed), and in open water, *Nuphar* spp (water lilies), *Potamogeton* spp (pond weed) and *Lemna trisulea* (duck weed) are abundant.

2.10 Discussion.

Airthrey Loch is a productive water body rich in organic and inorganic nutrients. The loch has become eutrophic as result of excessive input of phosphorus, and is

classified as a eutrophic hard water lake (Smith, 1995). Water bodies become more productive through the increased input of organic nutrients. In temperate ecosystems phosphorus is the nutrient controlling eutrophication (OECD,1992) (from Smith ,1995). Eutrophication is a complex process and has many effects on the quality of lake water. Problems which arise as a result of eutrophication include algal blooms which culminate in growth of toxic blue green algae, the growth of filamentous algae in shallow areas, increased macrophyte growth, deoxygenation of water and changes in the animal populations. Water from those lakes and reservoirs used for drinking supply has an unpleasant taste and odour due to dead and decaying algal cells.

Nutrient sources responsible for eutrophication may either be diffused, e.g. agricultural and urban runoff, or originate from a definite point such as sewage or industrial outfall. Eutrophication of fresh water in pristine areas has also been reported and attributed to afforestation (Harriman,1978).

According to Smith (1995) Airthrey Loch is capable of generating a significant proportion of annual phosphorus input through internal loading from sediments, and release of phosphorus does not take place throughout year, but depends on pH, temperature, dissolved oxygen and degree of mixing.

Jaceby *et al.* (1982) observed that phosphorus release was greater at higher water pH levels (up to 10) in shallow eutrophic lakes (as cited by Smith,1995). pH levels up to 9.5 were recorded from Airthrey Loch during summer, facilitating phosphorus release which is the major cause of eutrophication.

A large proportion of the area of the Airthrey Loch is shallow, and probably enables greater interactions between intermediate and definite hosts of parasites and may result in a higher infection level of these parasites in the host population. Black & Fraser (1984) from their study on dynamics of prevalence of *Ligula intestinalis* in

Catostomus commersoni from four lakes (surface area 6.3 ha to 27.1 ha and mean depth 4.3 m to 7.3 m) in Canada suggested that morphology of a lake basin probably influences the level about which the prevalence of many parasites fluctuates. This feature dictates the distribution of microhabitats in a lake and thus determines the quantity and quality of areas suitable for transmission of the parasites present.

Water level fluctuation, although not very pronounced in Airthrey Loch may affect the development stages of invertebrates, especially in littoral zones. The rise and fall in water level in dams and reservoirs has been suggested to control the parasite infection of fish by interrupting the developmental stages of parasites (Izyumova, 1988).

The temperature profile of Airthrey Loch ranges from 1-21 ^{0}C. This is typical of a temperate water body. The parasitic infection of freshwater fish has been studied in detail in temperate climates, and it has been shown that parasitic infections are heavily influenced by seasonal temperature. Temperature in these conditions is the chief factor causing seasonal cycles in parasites. Chubb (1977, 1979, 1980, 1982) has discussed in detail the occurrence of seasonal cycles in helminths from freshwater fish.

Although the water sampling carried out in this study was very limited it does indicate the enriched nature of the loch and also the relatively stable physico-chemical profile and productivity. In relation to the abundance of ***Proteocephalus filicollis*** the apparent abundance and diversity of the zooplankton population is obviously important. The high productivity of the loch also suggests a large stickleback population, although it was impossible to quantify the size of the latter. The loch would seem to be a favourable environment for the cycling of a parasite with a plankton bound life cycle such as ***P. filicollis***.

CHAPTER 3
POPULATION BIOLOGY OF PROTEOCEPHALUS FILICOLLIS IN GASTEROSTEUS ACULEATUS

3. Population biology of *Proteocephalus filicollis* in *Gasterosteus aculeatus*.

3.1 Introduction.

Temporal variation in the infection and maturation of temperate freshwater fish parasitic helminths is diverse. Some species exhibit a definite cycle of occurrence or maturation, whilst others show a variable pattern (Kennedy, 1975).

3.1.1 Seasonal cycle of occurrence and maturation of *Proteocephalus filicollis*.

Seasonal cycles of maturation, growth and recruitment have commonly been observed in species of *Proteocephalus* (Kennedy, 1977). Meggitt (1914) reported that during autumn most threespined stickleback from a population in Birmingham, England were infected with *P. filicollis* whereas in winter there were considerably fewer infected fish. Adult worms comprised 75 % of the worm population in autumn in three-spined stickleback, whereas in winter adult worms were rare, but in spring their numbers again increased.

A marked seasonal cycle of occurrence of *P. filicollis* in stickleback was described by Hopkins (1959); a high prevalence rate of up to 50 % was observed from July to November, with a gradual decrease from February to July. There was little seasonal variation in the intensity of infection which was highest in August and September. A clear seasonal cycle of maturation was also observed by Hopkins (1959) with young worms appearing in fish from July to November and (a few) gravid worms present in June and July.

Willemse & Veltman (1962) found a very low prevalence (1.06 %) of *P. filicollis* in a population of three-spined stickleback in the Netherlands but a high prevalence (15.95 %)

in nine-spined stickleback. The monthly intensity index of infection (total number of worms / number of infected fish) fluctuated from 1.0 to 1.41 but was mostly 1.0. However, an annual cycle of maturation was not found by Willemse & Veltman (1962) in *P. filicollis* parasitising nine-spined stickleback, in which all stages of development occurred throughout the year.

Willemse (1968) reported that *P. filicollis* in three-spined stickleback from the Netherlands had a 27.58 % prevalence in April and 21.95 % in May in one locality, but in another freshwater locality the prevalence was 40.24 % in September. He also reported small numbers of mature worms in February, March and April from an anadromous population of three-spined stickleback, whilst in a freshwater population mature worms appeared in April and gravid worms in May. In another freshwater locality he observed all stages of development of *P. filicollis* in September.

On the other hand, Chappell (1969a) did not find a seasonal pattern of occurrence of *P. filicollis* in three-spined stickleback from Yorkshire, England. Prevalence rate was high in June-July (41 %), low in August (2.1 %) and higher again in September (40 %) and November (41 %). Intensity of infection fluctuated from 1.3 to 4.5 with lowest values in August and highest in May and June. In this population adult worms without eggs were present in May to August and gravid worms from late summer to early autumn. Interestingly, Chappell found all maturity stages throughout the year including gravid worms.

Dartnall (1973) reported an overall prevalence of 14.77 % of *P. filicollis* in nine-spined stickleback from four localities in England. The mean intensity of infection varied from 1.0 to 3.35. Dartnall (1972) found a high prevalence (66.71 %) of *P. filicollis* in threespined stickleback from Hadleigh Marsh, in March which gradually decreased until October-November. The mean number of worms per infected fish showed a similar trend.

Identifications of *P. filicollis* from the nine-spined stickleback are questionable, as most of authors have considered this parasite to be host specific to the three-spined stickleback. Rodland (1979) reported a very low prevalence (5 %) throughout the year in an anadromous population of three-spined stickleback from a low salinity (2.6 %) site in Norway. Intensity of infection was very low. He reported the appearance of gravid worms from early to mid summer (May to August). There is some contradiction between authors as to whether *P. filicollis* shows a seasonal cycle. Some of these contradictions may be due to small sample sizes or to misidentification of *P. filicollis.*

3.1.2 Seasonal cycle of occurrence and maturation in other members of the genus *Proteocephalus*.

Connor (1953) found a seasonal cycle of prevalence in *Proteocephalus stizostethi* parasitising yellow pikeperch (*Stizostedion vitreum vitreum*) in the U.S.A. He observed small immature worms in late September and large mature worms in autumn and winter. No infection was recorded in August & September. He found worms bearing eggs from April to June.

Fischer & Freeman (1969) found a seasonal prevalence cycle of *P. amblopiitis* in small-mouth bass in the U.S.A with the highest values in spring, a decline in summer and disappearance of the parasites in late autumn. Fischer & Freeman (1969) found that growth and maturation continued throughout the summer. Eure (1976), on the other hand, from a thermally elevated site in the U.S.A found that prevalence of *P. amblopiitis* in largemouth bass was low from July to September, higher in October-December and declined again from February to May. Moreover, in summer adult worms were rare and the highest number of adult worms (16 %) occurred from December through August, but at a low density.

Kennedy & Hine (1969) found a clear seasonal cycle in *P. torulosus* in dace (*Leuciscus leuciscus* L.) from England, with recruitment in winter and prevalence and mean worm burden rising until spring and then dropping in May and June. They reported a seasonal cycle of maturation with gravid worms in spring (April & May), although the worms were few in number. Scholz (1989) reported a seasonal cycle in the occurrence of *P. torulosus* in chub (*Leuciscus cephalus* L.), prevalence increased from autumn to spring attaining a peak in April and then fell in summer. Mean intensity of infection also showed a similar pattern. Young worms occurred in June and November when they comprised 100 % of the population. From winter to early spring (December to April) maturing worms comprised 67 % of the population. Gravid worms were found in April and May.

Willemse (1969) reported that an annual cycle existed in *Proteocephalus tetrastomus* in smelt, *Osmerus eperlanus* (L.) In winter and spring prevalence was high but low in summer. Intensity of infection fluctuated similarly. Willemse (1969) attributed low winter temperature to be the main reason for the annual cycle.

Willemse (1969) also briefly described that *Proteocephalus ocellatus* from perch showed a marked annual cycle similar to that of *P. filicollis*. He found that infection begins in July. From December to March (temperature 0-3^0 C) the worms were unsegmented and differentiation occured in spring when temperatures rose and egg bearing worms were seen in spring and early summer.

Wootten (1974) reported that *P. percae* in perch showed an annual cycle of occurrence. Infection commenced in June and continued to rise until November and gravid worms were lost from the fish in May. A high growth rate of the cestodes was observed in June and July, from October to November there was little increase in mean length but a further increase in mean length was observed in November and December. In spring mean length was highest as the worm matured and in May 92 % of the *P. percae* were gravid.

Ieshko & Anikieva (1992) found that *Proteocephalus percae* from perch had an annual cycle of occurrence and development in Lake Rendozero in the former USSR. *Proteocephalus percae* infected fish from summer to autumn, maturation took place in the spring with gravid worms in June and July and was followed by elimination from the host. Pertierra & Nunez (1990) found a cyclic and yearly occurrence of *Proteoceophalus jandia* in a catfish, *Rhamdia sapo* in Brazil. Highest prevalence was in autumn, winter and early spring. Infection was found to be a continuous process. This parasite also exhibited a seasonal maturation pattern with the highest percentage of mature worms (bearing eggs) appearing from May to August, although mature worms occurred throughout the year. However, this study was carried out in Brazil where seasonal conditions vary from those in USSR.

A seasonal cycle of invasion of *Proteocephalus neglectus* in rainbow trout is reported by Hanzelova *et al* (1990). The scoleces (recruitment stage) were found from June to October and adult worms occurred during the same period, but were higher in number in August. Nie & Kennedy (1991) did not fin a clear seasonal pattern in prevalence and abundance in *Proteocephalus macrocephalus* in the European eel, but growth and maturation of this parasite showed marked seasonality as both occurred mainly in early summer. Although there is some conflict concerning the seasonality of other members of *Proteocephalus* most authors in temperate waters observed worms maturing and shedding eggs in spring and summer and recruitment in summer to autumn. This has also been reported in South American species like *P. jandia* (Pertierra & Nunex, 1990).

3.1.3 Seasonal cycle of occurrence and maturation in other groups of cestodes.

Other groups of cestodes in fish also show a seasonal pattern of occurrence and development.

Chubb (1963) found a seasonal cycle of occurrence and maturation of *Triaenophorus nodulosus* in pike. Infective plerocercoids were found throughout the year and it was considered that invasion of the pike occurred throughout the year. Pike harboured plerocercoids from June to September, from October to December genital development occurred and eggs were observed in December. Chubb (1963) suggested that a dynamic equilibrium existed between gain of plerocercoids and loss of worms from the pike intestine at all times. The dynamic equilibrium concept suggested by Chubb (1963) for *T. nodulosus* had already been forwarded by Hopkins (1959) for *P. filicollis*, according to which, gain and loss of worms occur throughout the life of the fish, irrespective of availability of worms for infection.

Kennedy (1969) observed a clear seasonal cycle of occurrence of *Caryophyllaeus laticeps* in dace. Infection occurred between December and March and then declined in summer (from August to November). Gravid worms were present from January to July, but were most prevalent in April, May and June at a time of rising water temperature.

Wootten (1972) reported that *Eubothrium crassum* in brown trout and rainbow trout from Hanningfield Reservoir, Essex did not show a seasonal cycle of occurrence or maturation.

Granath & Esch (1983) found that *Bothriocephalus acheilognathi* in mosquito fish (*Gambusia affinis*) exhibited a seasonal cycle of recruitment, prevalence and density. Recruitment began in summer (June) and continued up to mid autumn (October) with prevalence showing an associated increase. Mean density was lowest in summer with a peak in early winter. However, in a thermally altered site, peak prevalence occurred during June & July before declining to the lowest level in mid to late summer, followed by an increase in autumn and a high level in winter. Mean densities followed a similar pattern. However, recruitment at the thermally altered site began about 2 weeks sooner, lasted about 2 weeks longer and was interrupted for several weeks in late summer when water

temperature exceeded 35 °C. Granath & Esch (1983a) found that prevalence within three size classes of fish increased steadily during summer months and early autumn.

Marcogliese & Esch (1989) found that prevalence and occurrence of *Bothriocephalus acheilognathi* in mosquito fish was high from April to July but declined sharply in August and September. There was a second peak of prevalence from September to October but this was not as high as earlier in the year. Recruitment of nonsegmented worms peaked in spring and summer and subsequently declined in late summer, but again increased slightly in autumn before a further decline in winter.

Fish cestodes from tropical climates and North America are also reported to show a pattern of occurrence and maturation similar to their counterparts in temperate climates. Chauhan (1988) reported that *Polyonchobothrium armatii* in *Mastacembelus armatus* (Lacepede); *Senga nayari* in *Mastacembelus armatus* and *Mastacembelus pancalus* (Hamilton) and *Bothriocephalus teleostei* from *Barilius bendeliris* (Hamilton), *Barrilius bola* (Hamilton) and *Schizothorax richardsonii* exhibited a definite seasonal cycle of prevalence in Himalayian riverine ecosystems. Chauhan suggested that seasonal rhythms in fish cestodes from Indian waters show close resemblance to earlier reports of Dogiel (1958), Awachie (1966), Kennedy (1969) and Amin & Mackiewicz (1977) (Chauhan, 1988). Egg production and maturation of cestodes appeared to be water temperature associated phenomena. 16-27 °C was a suitable temperature for egg production. However, there was a significant difference in optimum water temperature for parasitic infection in fish in tropical and temperate regions (Chauha & Malhotra, 1984) (as cited by Chauhan, 1988). In tropical Malaysia Leong (1986) found a different pattern of prevalence of *B. acheilognathi* in *Puntius binotatus*. Prevalence and mean density showed a gradual increase from October to December and there was no infection from March to September.

He also reported that both prevalence (60 %) and mean density (9.0) of infection were similar with the highest infection in the smallest size groups of fish (< 7 cm).

Bothriocephalus claviceps from European eel, on the other hand, did not show a significant seasonality in prevalence and abundance in contrast to growth and reproduction as reported by Nie & Kennedy (1992). They suggested that growth and development may cease until the following spring or continue at a slow rate over winter and that parasite numbers decrease as they reach maturity.

3.2 Distribution of parasites within the host population.

The distribution of parasites within the host population can be described by frequency distributions. Considerable attention has been paid to this aspect of parasite ecology (Crofton,1971a,b; Penncuick,1971; Boxshall, 1974; Anderson & May, 1978; May & Anderson, 1978). As stated by Anderson & Gordon (1982), three fundamental distribution pattern of parasites within the host population exist, i.e underdispersed, random and overdispersed. These patterns are described by three probability distributions

1. Positive binomial for underdispersed patterns of parasite distribution.
2. Poisson for random patterns of parasite distribution.
3. Negative binomial for overdispersed patterns of parasite distribution.

In nature, regular and random distributions of parasites within the host population rarely occurs. Generally parasite populations are overdispersed within the host population (Crofton, 1971b). Crofton (1971b) pointed out that the death of heavily infected hosts in overdispersed populations remove a large proportion of parasites from the overall population thus regulating the size of the parasite population. Principle factors responsible for the generation of overdispersion in parasite populations include heterogeneity between

hosts in their exposure to infection, susceptibility to infection, or defensive capabilities (Anderson & Gordon, 1982).

A simple measure of degree of dispersion that has been widely used is the variance to mean ratio of parasites. If this ratio is greater than unity the parasite is said to be overdispersed within the host population (Anderson & Gordon, 1982).

Hopkins (1959) fitted a Poisson distribution to a population of *P. filicollis* from stickleback and suggested that this arose because infected copepod intermediate hosts mostly contained only a single *P. filicollis* larva.

A number of other species of *Proteocephalus* have been shown to have overdispersed distributions. e.g. *P. torulosus* in dace (Kennedy & Hine, 1969), *P. percae* in perch (Wootten, 1974), and *P. neglectus* in trout (Hanzelova *et al.* 1990).

3.3 Size of the host and infection.

Individual species of parasite show a tendency for the prevalence and mean intensity of infection to vary with the age of the host. The difference in prevalence and mean intensity of infection may be due to feeding habits of the fish and other physiological processes. Hopkins (1959) found that 2-3 cm long (0 + group) sticklebacks were heavily infected with *P. filicollis*, but that the chance of a worm surviving to maturity was independent of the size of the host in which it occurred. Willemse & Veltman (1962) found that fish < 4.0 cm in length were more heavily infected. Chappell (1969b) reported that *P. filicollis* showed no significant variation in prevalence with fish size, but that the intensity of infection decreased in large fish and Dartnall (1972) reported a significant decrease in both prevalence and intensity of infection of *P. filicollis* with fish size. However, Rodland (1979) found a maximum prevalence of *P. filicollis* in fish from 5-6 cm in length and the lowest rate in fish up to 3.0 cm long.

Kennedy & Hine (1969) and Scholz (1989) found that the degree of infection with *P. torulosus* increased with the size of the fish, whilst Wootten (1974) reported that mean intensity of infection with *P. percae* generally increased with length of the host. Pertierra & Nunez (1990) found that adult catfish showed a higher prevalence rate of *P. jandia* than juvenile catfish.

3.4 Aims

The aim of this study was to investigate in detail features of the population biology of *Proteocephalus filicollis* from a wild population of three-spined stickleback from Airthrey Loch and to compare this with previous studies done in Britain.

3.5 Material and Methods.

3.5.1 Fish sampling.

Samples of fish were taken twice monthly from three sampling stations (350 m apart) in the littoral zone of Airthrey Loch from April 1993 to June 1995 (Fig 2.1). Fish were caught with a hand net (mesh size 3.0 x 3.5 mm). Sample size ranged from 20 to 85 per month (Table 3.1). Fish were most plentiful in late summer and autumn. Every winter the Loch was often at least partly frozen which made fishing difficult. Fish were also not abundant in spring and early summer, possibly due to changes in behaviour associated with breeding, and also because of mortality of the previous year class. Samples taken in any calendar month were combined for analyses. Additional samples were also taken after June 1995 for experimental purposes. For some analyses samples were grouped into seasons as follows:

April 1993 -May 1993	Spring 1993
June 1993 - August 1993	Summer 1993
September 1993-November 1993	Autumn 1993
December 1993 - February 1994	Winter 1993
March 1994-May 1994	Spring 1994
June 1994 -August 1994	Summer 1994
September 1994- November 1994	Autumn 1994
December 1994- February 1995	Spring 1994
June 1995-July 1995	Summer 1995

3.5.2 Transport of fish.

Fish were transported live to the laboratory in 20 litre plastic buckets filled with Loch water. In the laboratory fish were maintained in aerated Loch water at ambient temperature until examination and dissection within 12 hours after capture.

3.5.3 Weight and length of fish.

Individual fish were weighed on a Mettler Pm 2000 electronic balance to an accuracy of 0.01 gm. The length of each fish was recorded from the premaxilla to the tip of the caudal fin to an accuracy of 1 mm. The fish were grouped into 1.0 cm length groups for analysis of results. The length classes used are given in Table 3.2.

Table 3.1 Monthly samples of three-spined stickleback from Airthrey Loch from April 1993 to July 1995 in length classes in cm (LC-1, < 2.0; LC-2, 2.1-3.0; LC-3, 3.1-4.0; LC-4, 4.1 >).

Months	LC-1	LC-2	LC-3	LC-4	Total
April 93		10	20	32	62
May		2	17	26	45
June	14	14	1	11	40
July	15	25	12	2	54
August	2	53	18	4	77
September	-	34	39	12	85
October	-	19	37	9	65
November	-	23	22	5	50
December	-	24	19	5	48
January 94	-	31	25	5	61
February	-	13	19	2	34
March	-	35	35	3	73
April	-	21	28	14	63
May	-	8	15	31	54
June	-	1	16	23	40
July	37	23	-	-	60
August	21	16	2	1	40
September	10	28	12	3	53
October	-	10	10	-	20
November	1	25	20	5	51
December	-	9	12	3	24
January 95	-	7	11	2	20
February	-	10	14	4	28
March	-	13	13	6	32
April	-	3	7	10	20
May	-	3	20	24	47
June	2	9	7	37	55
July	2	14	10	24	50
Total	104	483	462	302	1351
% length class	7.62 %	35.75 %	34.19 %	22.35 %	

Table 3.2 Length classes of fish sampled.

Length of fish (cm).	Length class.
< 2.0	LC-1
2.1-3.0	LC-2
3.1-4.0	LC-3
4.1 >	LC-4

3.5.4 Sex and maturity of fish.

The gonads of all fish were examined microscopically and sex was determined where possible.

3.5.5 Examination of fish for parasites.

Fish were killed by pithing and then opened ventrally from mouth to cloaca. Any parasites present in the body cavity or encysted on the viscera were noted. The intestine from the stomach to rectum was removed intact and placed in a Petri dish containing tap water. The intestine was divided into four sections as follow:

1. Anterior intestine: S-1
2. Mid intestine : S-2
3. Posterior intestine: S-3
4. Rectum: S-4

The gut was opened from posterior to anterior and then examined under the dissecting microscope using transmitted light at x 10 and x 40 magnification. The site of attachment of any worms present was measured from the posterior of the rectum using a scale divided

into millimetres. Numbers of parasites were counted and they were preserved for further examination.

3.5.6 Staining and mounting of parasites.

For verification of maturity and to determine length, parasites were relaxed in tap water / distilled water at 4 or 10^0 C for one hour or till movement of worm stopped. Both adult and larval worms were treated in the same way. Parasites were fixed in 5 % buffered formalin.

In staining, two techniques (Meyer's paracarmine; Methylene blue (Schnur,1969) were used. The fixed specimen were stained with Mayer's paracarmine and differentiated in 50 % acid alcohol. Stained parasites were dehydrated in a graded series of alcohol and cleared in Cedar wood oil and mounted in Canada balsam.The staining procedure is given in Appendix 1.

3.5.7 Measurement of parasites.

Measurement of worms were taken from stained Mayers' paracarmine mounted specimens using an eye piece graticule fitted in a compound microscope. Readings were taken in µm and converted to millimeters.

3.5.8 Maturity of parasites.

Each specimen was allocated to one of the five groups according to their state of maturation:

1- Plerocerciforms: Newly recruited worms having no genital development or strobilization.

2- Immature worms: Worms which have started segmentation.

3- Maturing worms: Worms with developing genital structures.

4-Mature worms: Worms with developed genital organs.

5-Gravid worms: Worms which liberate eggs when placed in water, worms have some empty uteri in posterior part of strobila.

For data analysis maturity stage 1 & 2 are combined and are represented in result as immature worms except in section 3.6.4 on recruitment where plerocerciform worms are represented as a separate stage.

3.5.9 Statistical methods.

Observed parasite distributions were fitted to the theoretical negative binomial (Elliot, 1977) and variance to mean ratio was calculated (Gordon & Anderson, 1980). Significance level were determined by the Chi-square test (Elliot, 1977). A probability of $P < 0.05$ was considered significant. The prevalence rate between two sexes was compared by a 2 x 2 contingency table. All tests were performed using a computor package (Mintab and Quatro pro Version 1.00).

3.5.10 Ecological terms.

Prevalence, abundance, mean intensity and infrapopulation size are defined as in Margolis, Esch, Holmes, Kuris & Schad (1982).

Prevalence:

Number of individuals of a host species infected with a particular parasite species + Number of hosts examined x 100.

Abundance:

Total number of individuals of a particular parasite species in a sample of hosts + Total number of individuals of the host species (infected + uninfected)in the sample (= mean number of individuals of a particular parasite species per host examined).

Mean intensity :

Total number of individuals of a particular parasite species in a sample of a host species + Number of infected individuals of the host species in the sample (= mean number of individuals of a particular parasite species per infected host in a sample).

Infrapopulation size:

All individuals of a species of parasite occurring in an individual host.

3.6 Results.

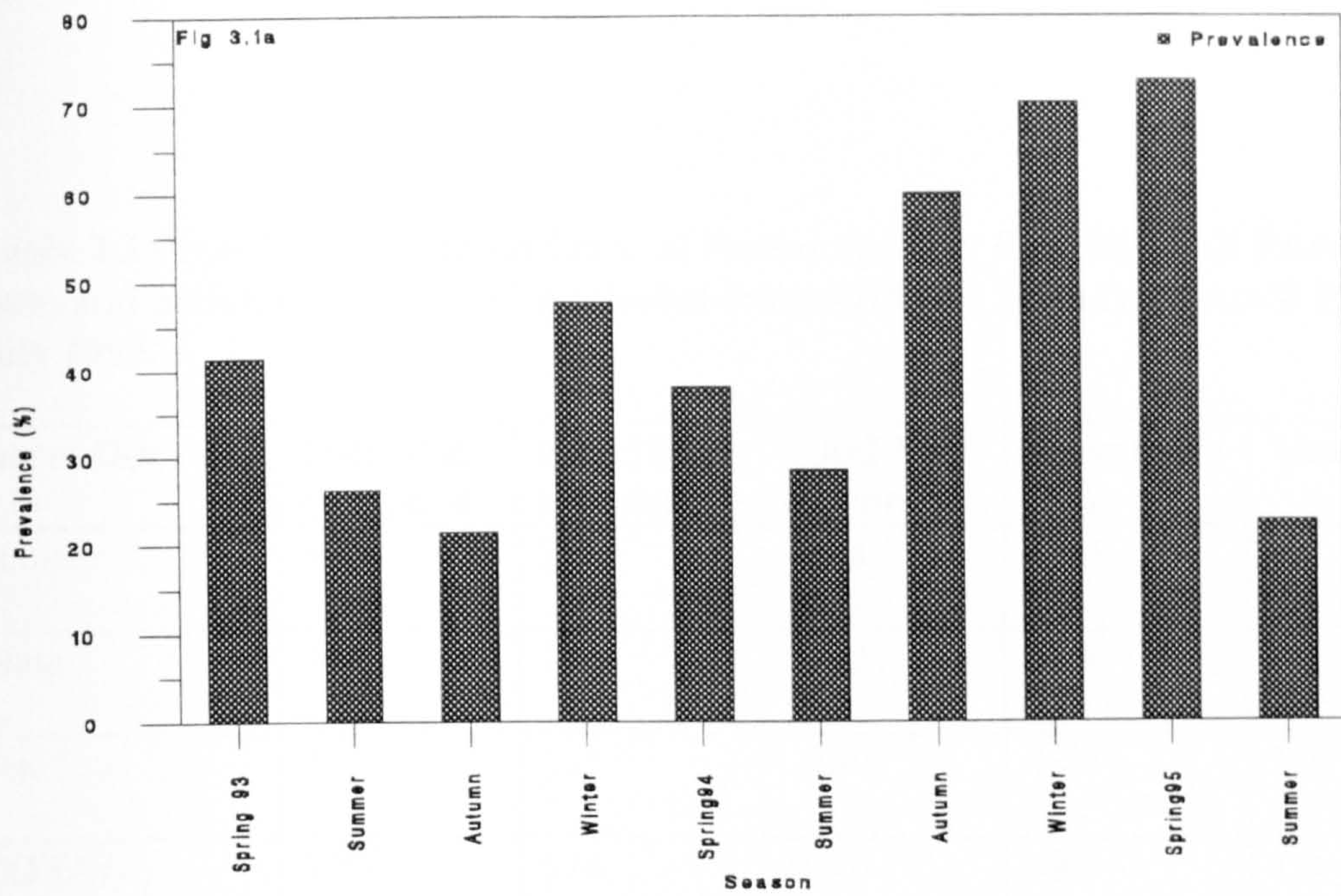
A total of 1351 three-spined sticklebacks were dissected and 516 were found to be infected with 1976 *Proteocephalus filicollis* giving an overall prevalence of 38.2 % and abundance of 1.46 (Table 3.3). *Proteocephalus filicollis* were found in the rectum and various sections of the intestine of the fish.

3.6.1 Prevalence and abundance of *Proteocephalus filicollis* in relation to the sex of the host.

Of the 516 infected fish, 280 were female, 119 male and in 117 fish sex was undetermined. The overall prevalence rate in females was 42 %, in males 37.9 % and in fish of undetermined sex 31.5 % (Table 3.3). There was no significant difference in prevalence between male and female fish (P = 0.75 =1.323 (1d.f): P > 0.75 N.S.D). Abundance in female fish was 1.70, in males it was 1.31 and in fish of undetermined sex 1.16. There was no significant difference in the abundance in both sexes (P = 0.75 = 0.575, P > 0.90).

In female fish prevalence was high in spring 1993 (41.3 %), but it was lower in the following summer and autumn. Prevalence was highest in winter months (47.6 %) after which there was a gradual decline from spring to summer 1994 (38 % to 28 .5 %). Prevalence was generally higher in 1994-95 in all seasons than in the previous year. Prevalence was 60 % in autumn and than rose again in winter and spring before declining in summer 1995 (Fig. 3.1a).

Abundance dropped from 2.2 in spring 1993 to 0.92 in summer. From autumn 1993 to winter 1993 it increased from 0.75 to 1.55 and then declined in spring and summer 1994. In autumn 1994 abundance was high (2.86) and it then rose again to reach a still higher



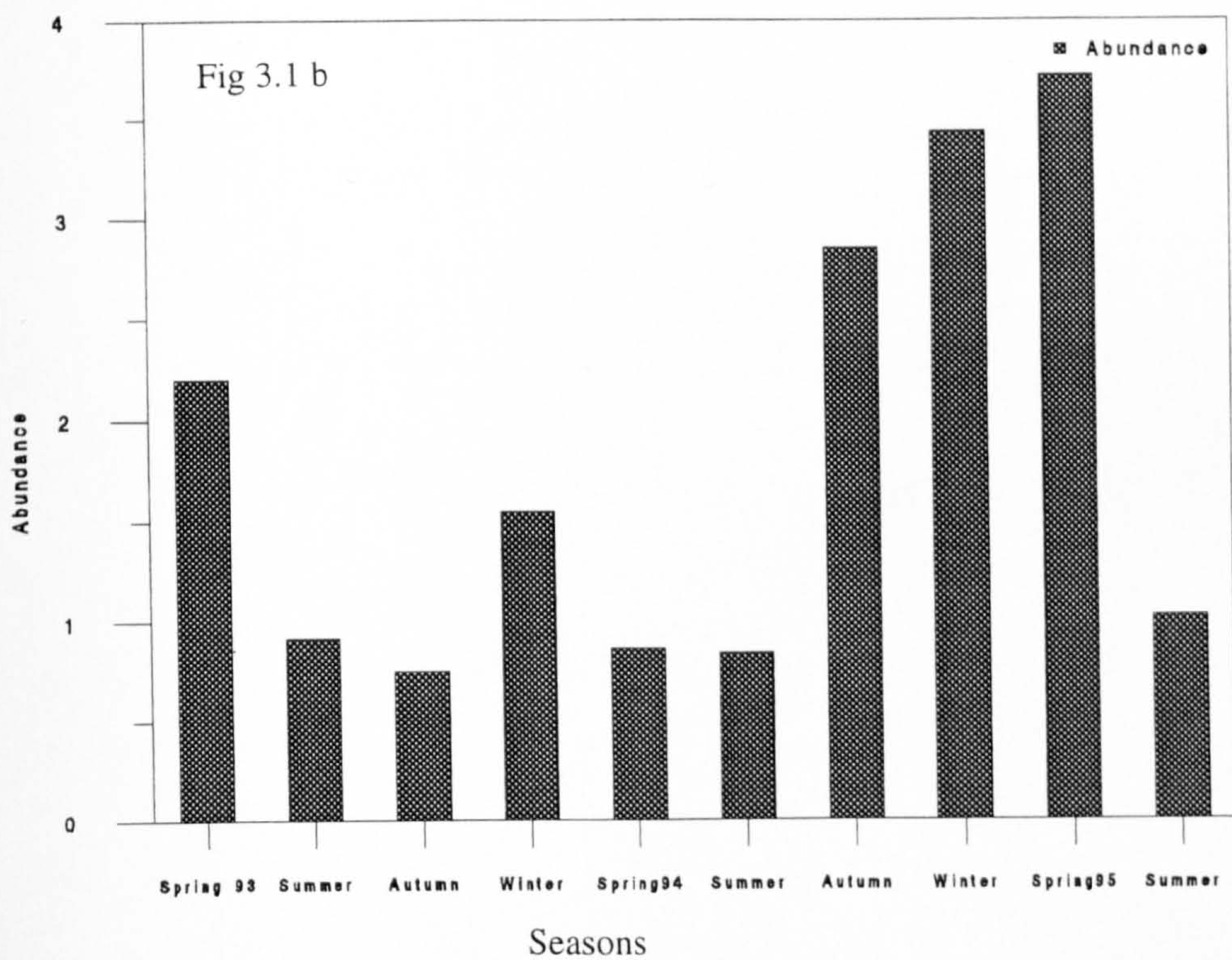


Fig 3.1. Seasonal prevalence (a) and abundance (b) of *Proteocephalus filicollis* in all female *G. aculeatus* examined in Airthrey Loch from April 1993 to July 1995.

Table 3.3 Prevalence and abundance of *Proteocephalus filicollis* in all female, male and undetermined sex *G. aculeatus* from Airthrey Loch from April 1993 to July 1995.

Sex of fish	Total fish examined	No of fish infected	Total No of worm	Prevalence (%)	Abundance
Female	666	280	1138	42.0	1.7
Male	314	119	352	37.9	1.1
Sex ?	371	117	486	31.5	1.3
TOTAL	1351	516	1976	38.1	1.4

level of 3.72 in spring before declining to 1.03 in summer 1995 (Fig. 3.1b). In general abundance was higher in spring and winter.

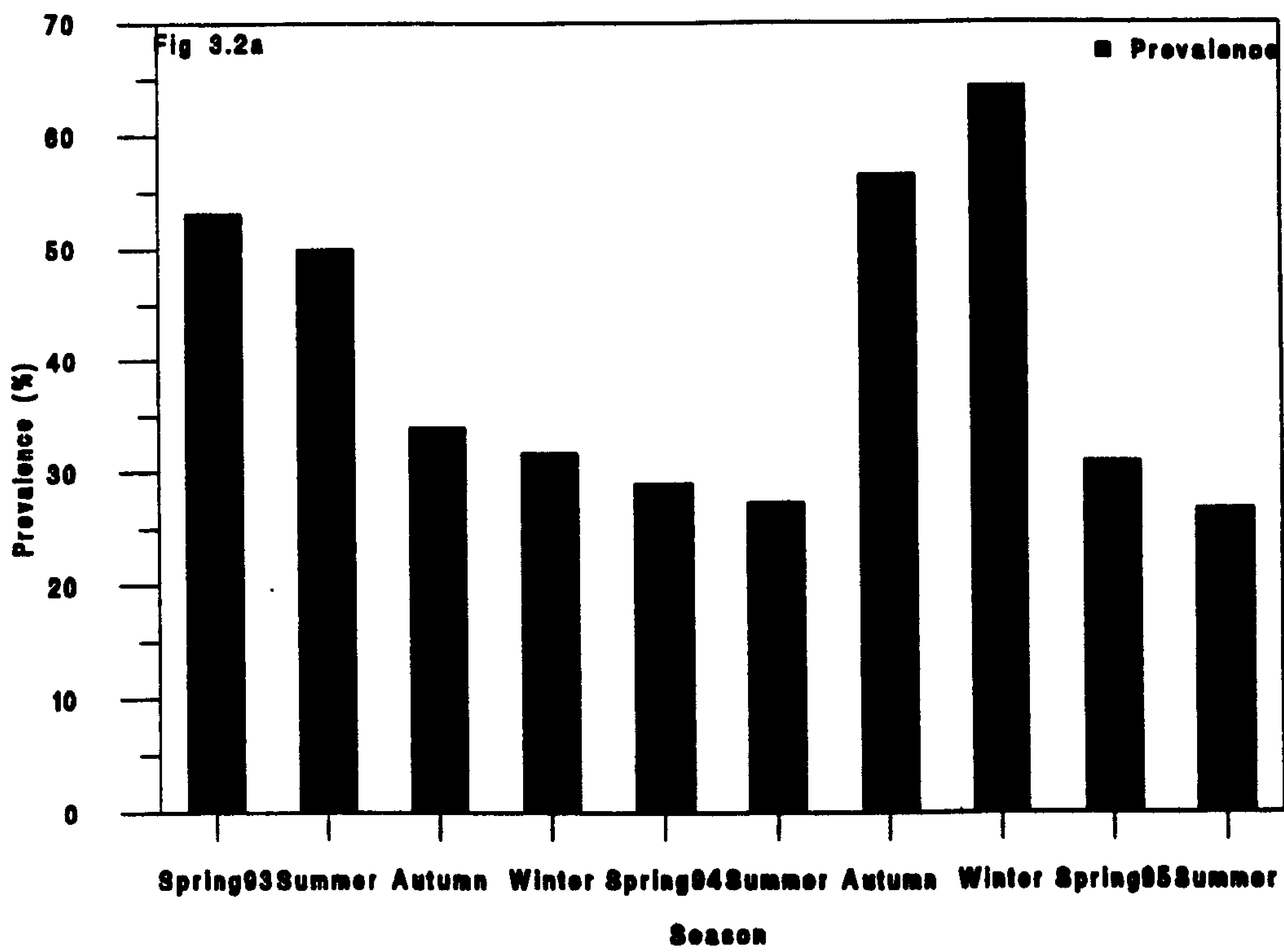
In male fish prevalence was high in spring and summer 1993, but was lower and declined slightly from autumn 1993 to summer 1994 (33.9 % to 27.2 %). During 1994-95 male fish showed a higher prevalence than previously. Prevalence was 56.6 % and 64.3 % in autumn and winter respectively followed by a decline from spring to summer 1995 (30.9 % to 26.8 %) (Fig. 3.2a & 3.2b).

Abundance increased from spring to summer 1993 but then declined and was much lower from autumn 1993 to summer 1994. Abundance was much higher in autumn 1994 and winter 1995 before falling in spring and summer 1995 (Fig. 3.2b).

In general female fish dominated the stickleback population of Airthery Loch (Table3.4).

Table 3.4 Seasonal prevalence, in male, female fish and male to female ratio of *G. aculeatus* population from Airthrey Loch from April 1993 to July 1995.

Seasons	Sex	Fish examined	Fish infected	Prevalence (%)	M:F ratio (infected)
Summer	M	47	15	31.9	1 :3.12
	F	165	48	29.1	
Autumn	M	79	32	40.5	1 :1.59
	F	148	51	34.4	
Winter	M	55	22	40.0	1 :2.9
	F	112	64	57.1	
Spring	M	133	50	37.6	1 :2.34
	F	241	117	48.5	



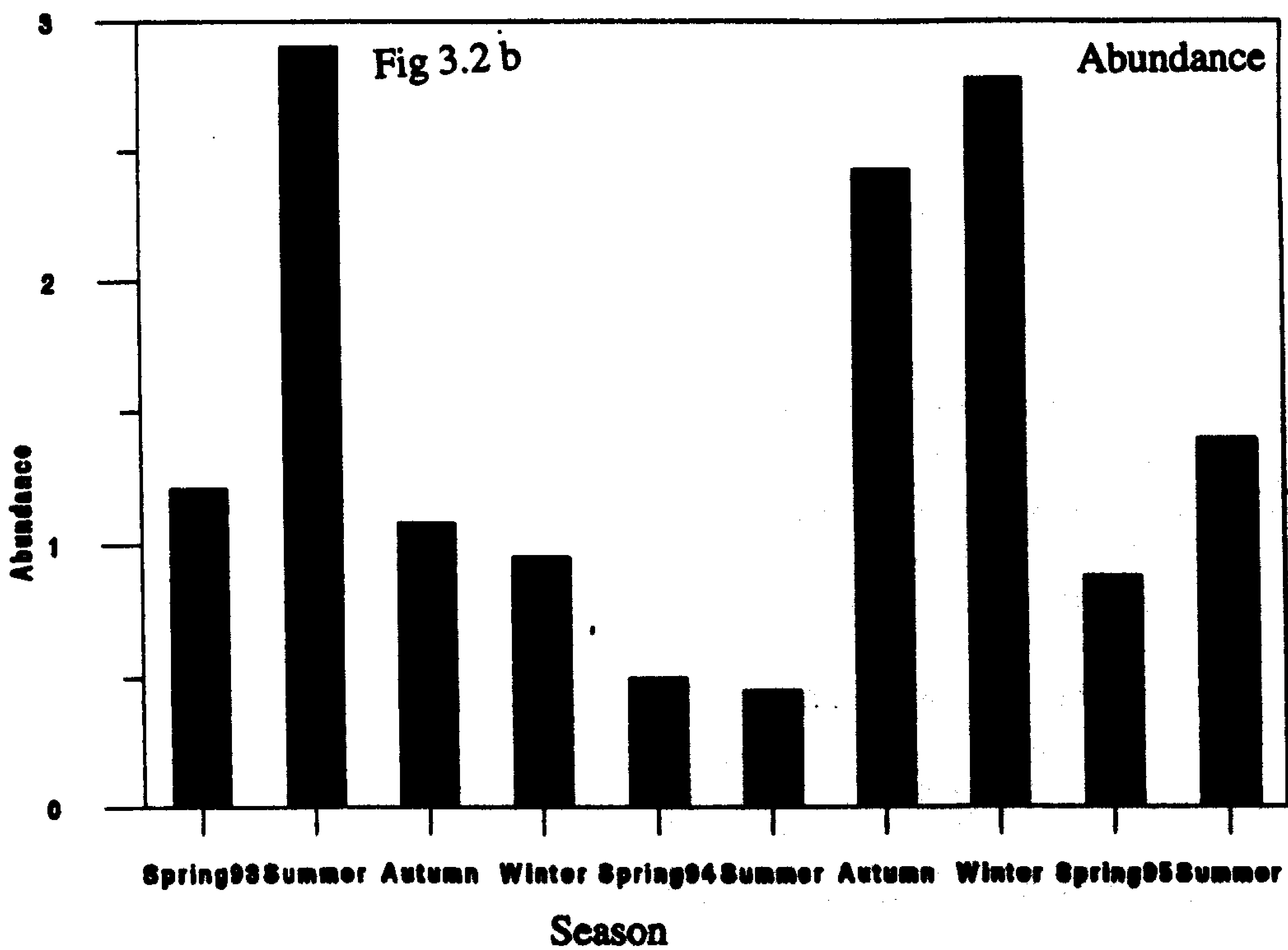


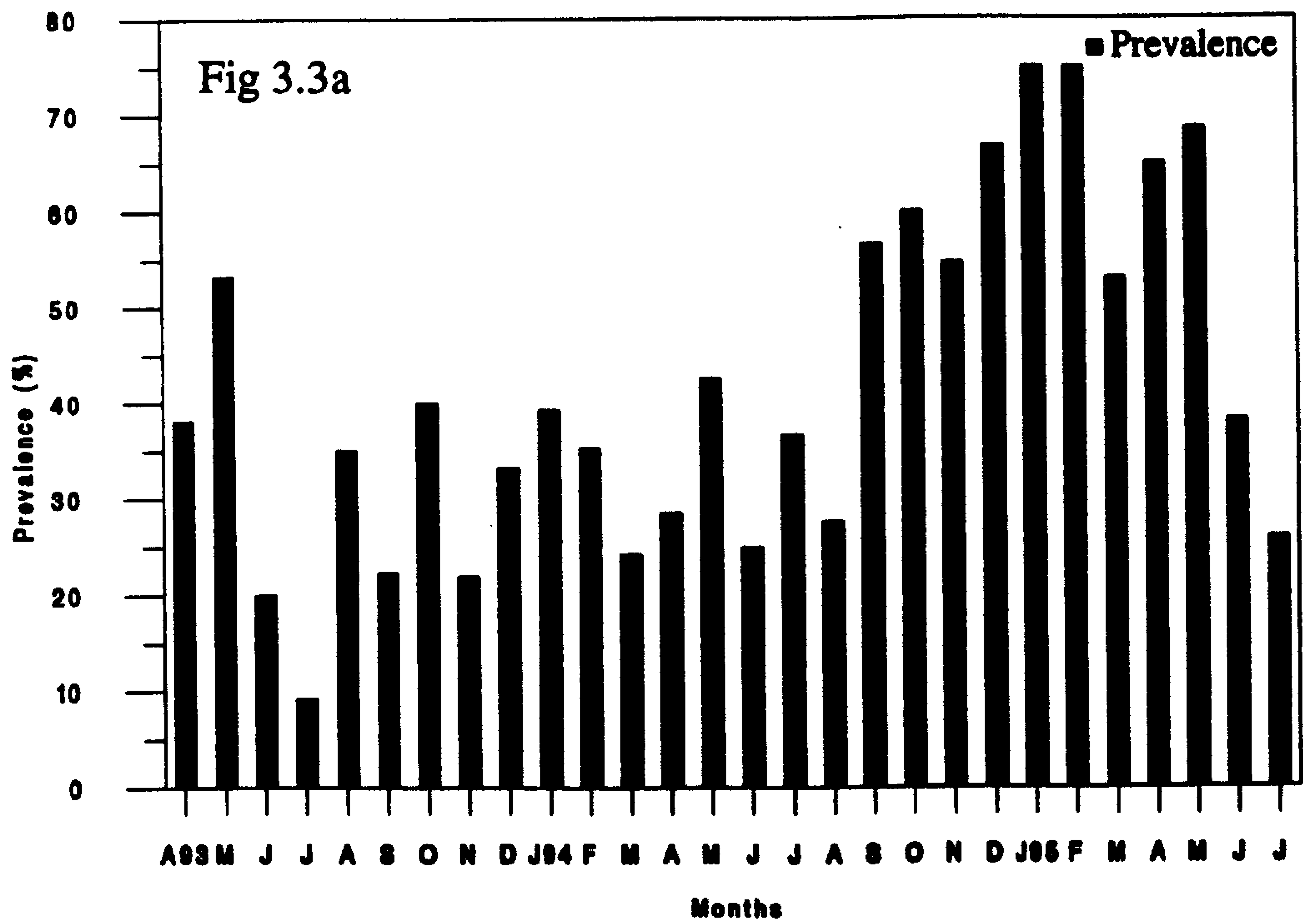
Fig 3.2 Seasonal prevalence (a) and abundance (b) of *Proteocephalus filicollis* from all male *G. aculeatus* examined in Airthrey Loch from April 1993 to July 1995.

3.6.2 Monthly prevalence and abundance of *Proteocephalus filicollis* in *G. aculeatus*.

The monthly prevalence and abundance of *Proteocephalus filicollis* in the stickleback population is shown in Fig. 3.3a & 3.3b. Both prevalence and abundance fluctuated irregularly over the period of study. Prevalence dropped from 53.3 % in May 1993 to < 20 % in June and July. Prevalence then ranged between 27.5 % and 35 % from August 1993 to August 1994. Prevalence was at much higher level from May 1994 to February 1995 (53 % to 75 %) after which it declined in June / July to 26 % in the latter month.

In April 1993 and May 1993 abundance was relatively high. It fluctuated considerably between July 1993 to June 1994, but with no obvious pattern. Abundance rose from July to February and then decreased from March until June. This trend is observed in both years with some fluctuation, although abundance was much higher in September 1994 to May 1995 than previously. The rise and fall in abundance almost followed the same pattern as the prevalence (Fig. 3.3a & 3.3b) (Table 3.5).

If the prevalence of *P. filicollis* in stickleback is examined by season a clear pattern emerges. Prevalence drops from spring 1993 (44.8 %) to summer 1993 (23.4 %). It then increases in autumn (28 %) followed by a further rise in winter (36.4 %) and then a drop in spring 1994 (31 %). A similar trend was repeated during 1994-95; prevalence was 30.7 % in summer rising to 56.6 % in autumn and 72.2 % in winter before declining to 62.2 % in spring. Abundance followed the same trend, rising from summer to winter and falling in spring (Fig 3.4) (Table 3.6).



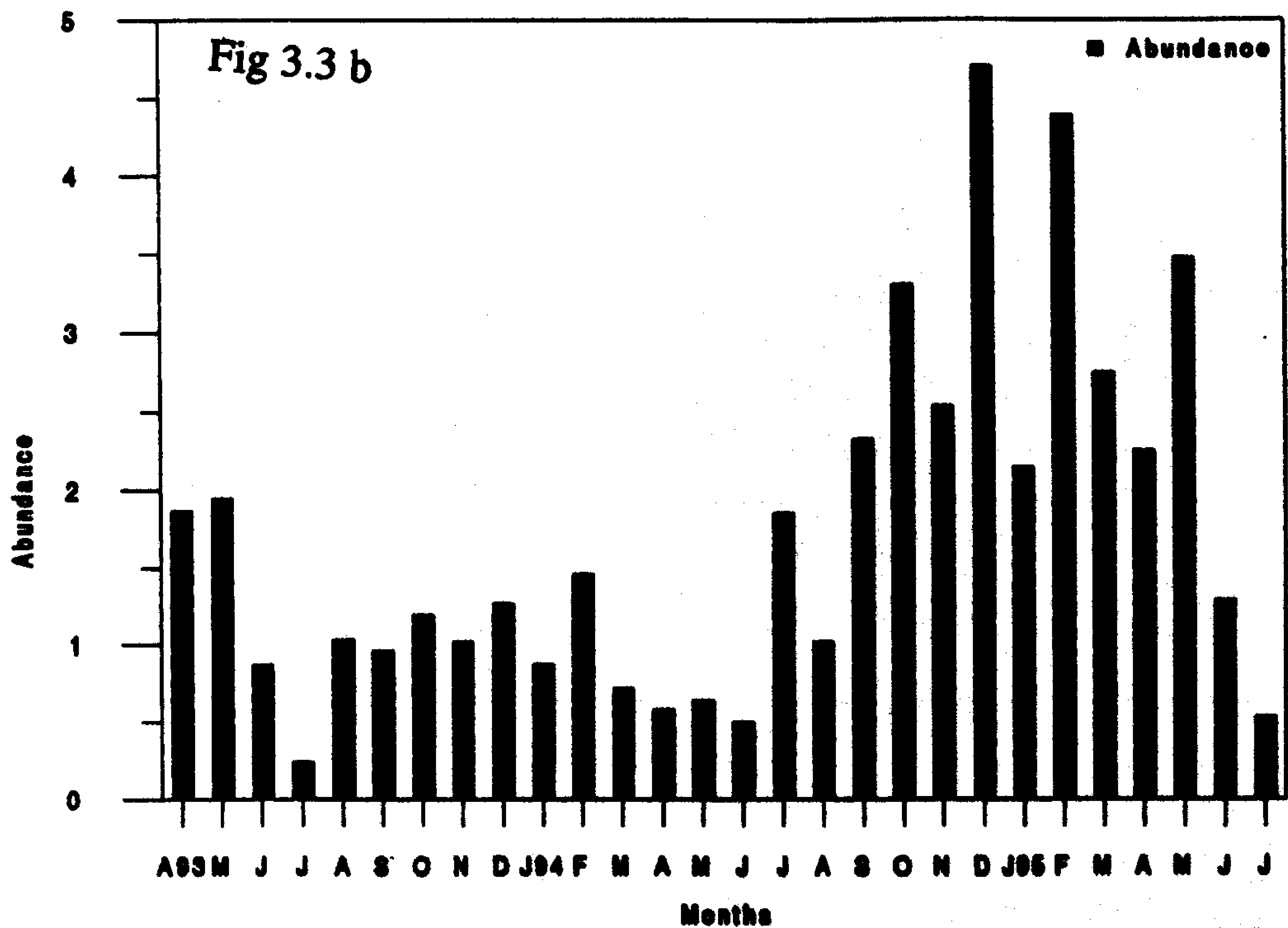


Fig 3.3. Monthly prevalence (a) and abundance (b) of ***Proteocephalus filicollis*** in all *G. aculeatus* examined from Airthrey Loch from April 1993 to July 1995.

Table 3.5. Monthly change in prevalence and abundance of *Proteocephalus filicollis* in all examined *G. aculeatus* from Airthrey Loch from April 1993 to July 1995.

Months	No of fish		Prevalence	Total no of worms	Maximum no of worms per fish	Abundance
	Exam	Infect				
April1993	62	24	38.1	116	35	1.8
May	45	24	53.3	88	23	1.9
June	40	8	20.0	35	15	0.8
July	54	5	9.3	13	6	0.2
August	77	27	35.1	80	16	1.0
September	85	19	22.4	82	18	0.9
October	65	26	40.0	78	14	1.2
November	50	11	22.0	51	20	1.0
December	48	16	33.3	61	22	1.2
January1994	61	24	39.3	54	09	0.8
February	34	12	35.3	50	07	1.4
March	73	18	24.3	53	12	0.7
April	63	18	28.6	37	07	0.5
May	54	23	42.6	35	04	0.6
June	40	10	25.0	20	06	0.5
July	60	22	36.6	112	46	1.8
August	40	11	27.6	41	08	1.0
September	53	30	56.6	123	11	2.3
October	20	12	60.0	66	14	3.3
November	51	28	54.7	120	16	2.3
December	24	16	66.6	113	30	4.7
January1995	20	15	75.0	43	10	2.1
February	28	21	75.0	123	27	4.3
March	32	17	53.1	75	16	2.7
April	20	13	65.0	45	8	2.2
May	47	32	68.9	164	25	3.4
June	55	21	38.2	72	8	1.3
July	50	13	26.0	27	6	0.5
TOTAL	1351	516	38.2	1976	46	1.4

Table 3.6. **Seasonal changes in prevalence and abundance of *Proteocephalus filicollis in G. aculeatus* in Airthrey Loch from April 1993-July 1995.**

Season	**No of fish Exam**	**Infected**	**Prevalence**	**Total no of worms**	**Maximum no of worms**	**Abundance**
Spring 1993	107	48	44.8	204	35	1.9
Summer 93	171	40	23.3	128	16	0.7
Autumn 93	200	56	28.0	211	20	1.0
Winter 93	143	52	36.3	165	22	1.1
Spring 94	190	59	31.0	125	12	0.6
Summer 94	140	43	30.7	173	46	1.2
Autumn 94	124	70	56.4	309	16	2.4
Winter 94	72	52	72.2	279	30	3.8
Spring 95	99	62	62.6	284	25	2.8
Summer 95	105	34	32.3	99	8	0.9
Total	1351	516	38.1	1976	46	1.4

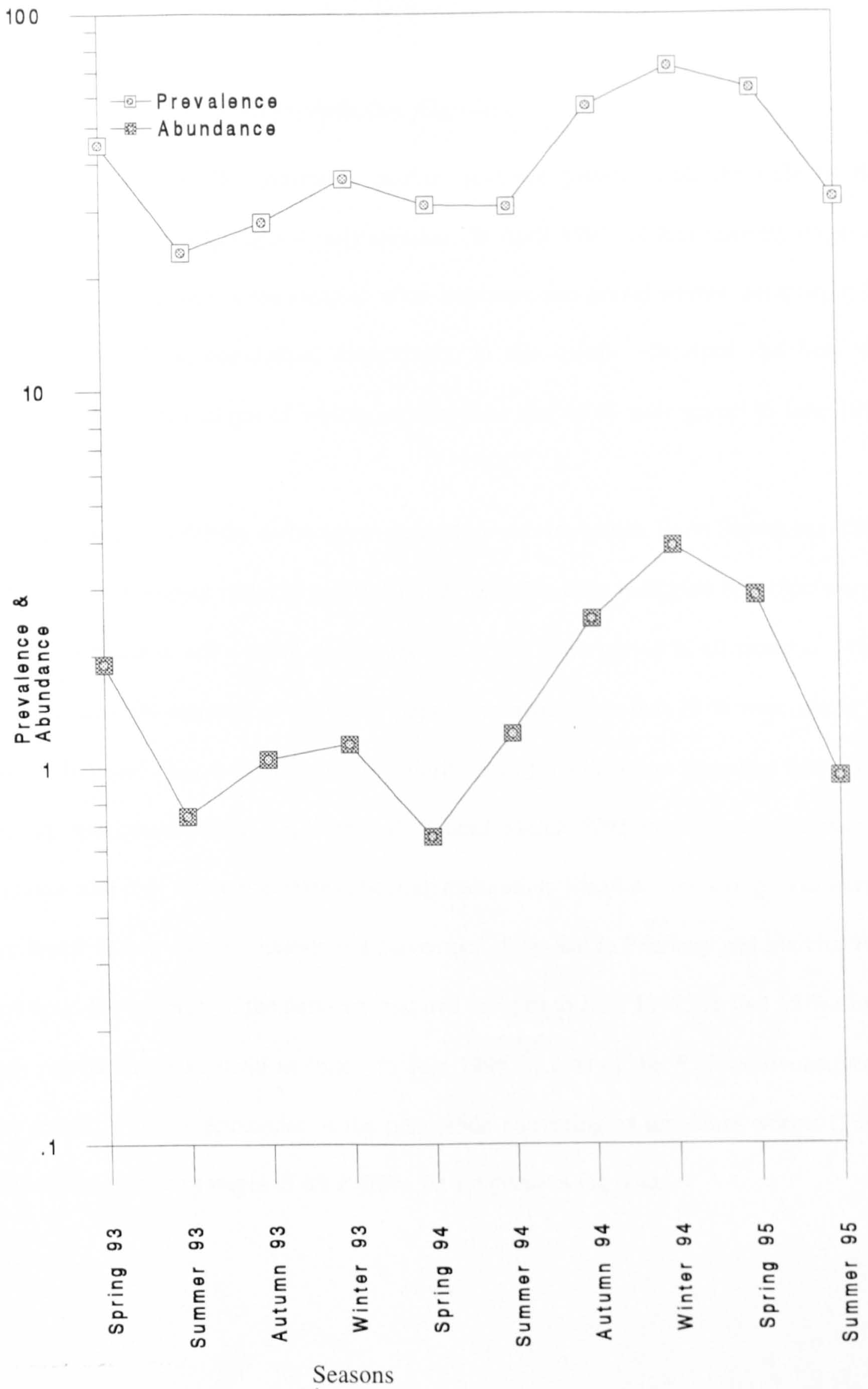


Fig 3.4 Seasonal prevalence and abundance of *Proteocephalus filicollis* in all *G. aculeatus* from Airthrey Loch from April 1993 to July 1995.

3 .6.3 Maturation of *Proteocephalus filicollis*.

Maturation of *P. filicollis* showed a marked seasonal pattern, with the bulk of the population maturing in spring and early summer. In April 1993, all four maturity stages of *P. filicollis* were present in the sample, with immature and gravid worms comprising 36 % and 19 % of the population, respectively, in this month. In April and May the proportion of mature and gravid worms increased, so that 46 % were gravid in June 1993 (Table 3.7).

In July and August 1993 the entire worm population was immature. From September 1993 to March 1994 the great majority (> 80 %) of *P. filicollis* were immature but some worms showed maturation and a small percentage (< 8 %) were gravid in all months. From April to June 1994 majority of the worm population matured so that 75 % were gravid by June. In July and August 1994 the great majority of *P. filicollis* were immature with a few maturing specimens. From September 1994 until March 1995 most parasites remained immature with only a few specimens showing maturation. Small numbers of gravid worms were found in September, October and November 1994, and in February and March 1995. Once again the majority of the parasites matured in April to June 1995, so that 65 % of the worm population was gravid in June. In July 1995 14.8 % of the *P. filicollis* recovered were gravid, with the remainder of the population consisting of immature worms (Table 3.7). Monthly maturity stages of all *P. filicollis* are given in Fig 3.4a.

Table.3.7. Monthly percentage of different maturity stages of *Proteocephalus filicollis* in all *G. aculeatus* from Airthrey Loch from April 1993 to July 1995.

Months	Immature worm	Maturing worm	Mature worm	Gravid worm
April 1993	36.3	18.9	25.9	18.9
May	18.2	-	31.8	50.0
June	5.7	-	48.6	45.7
July	100.0	-	-	-
August	100.0	-	-	-
September	97.6		-	2.4
October	90.0	1.3	2.6	5.1
November	96.1	-	-	3.9
December	95.1	3.3	-	1.6
January 1994	81.5	1.9	9.2	7.4
February	90.0	2.0	4.0	4.0
March	83.0	7.5	1.9	7.6
April	70.3	18.9	2.7	8.1
May	17.1	14.3	5.7	62.9
June	-	20.0	5.0	75.0
July	94.6	5.4	-	-
August	100.0	-	-	-
September	92.7	1.6	-	5.7
October	95.5	3.0	-	1.5
November	99.0	-	-	1.0
December	96.5	3.5	-	-
January 1995	95.4	2.3	2.3	-
February	89.4	4.9	1.6	4.1
March	77.3	10.7	2.7	9.3
April	21.7	44.4	6.7	26.7
May	25.0	32.9	11.0	31.1
June	15.3	2.8	16.7	65.2
July	74.1	11.1	-	14.8

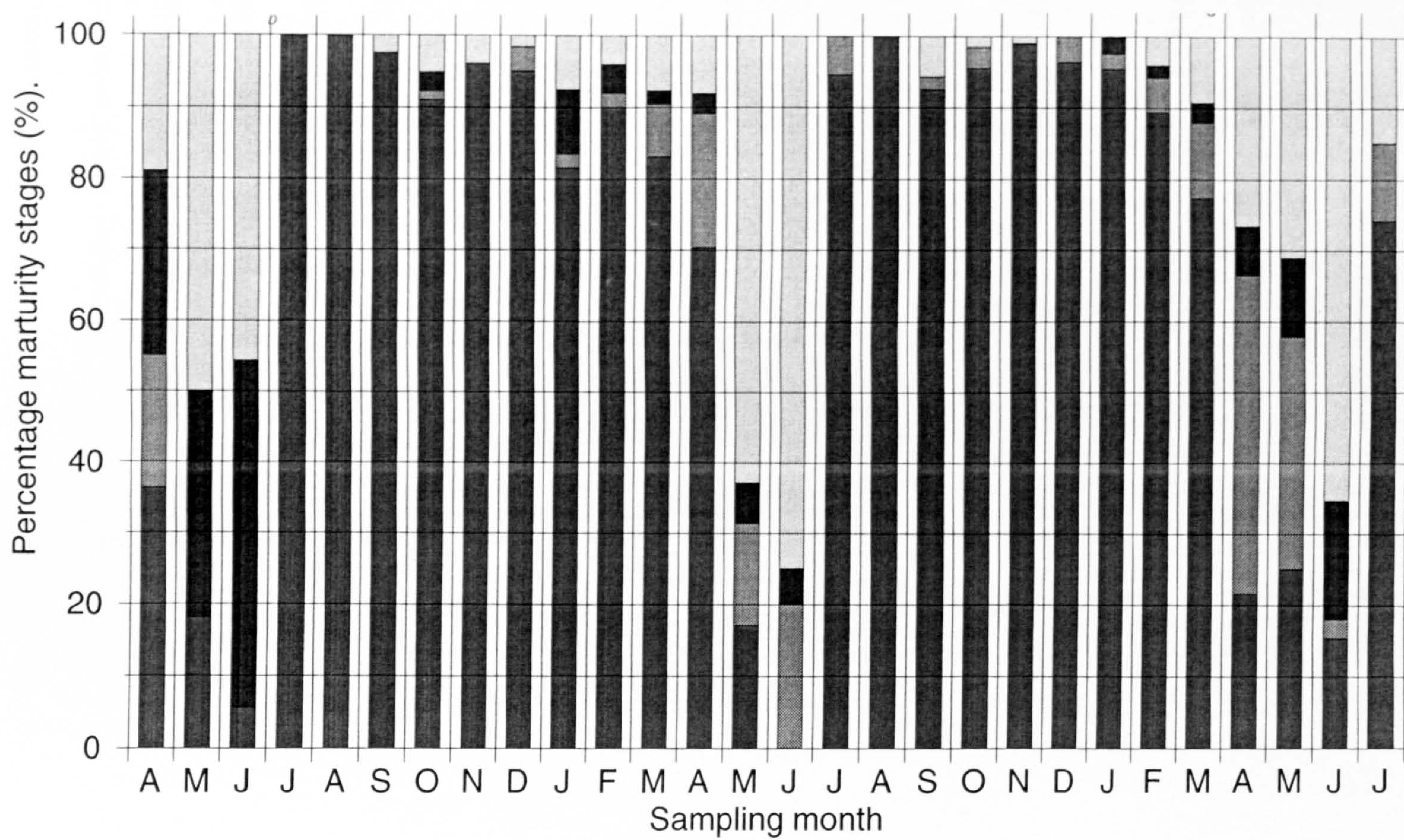


Fig 3.4 a. Monthly (from April 1993 to July 1995) percentage of all maturity stages of *Proteocephalus filicollis* from *G. aculeatus* (stage1-immature worms, stage2-maturing worms, stage3-mature worms, stage4-gravid worms).

3.6.4 Recruitment.

Plerocerciform worms (0.32 - <1 mm) were found throughout the year. The maximum size of plerocercoid larva obtained experimentally in copepods was 0.61mm. In April and May 1993, these plerocerciform parasites formed 7 % and 18 % of the worm population, respectively. In July the entire population consisted of presumably newly recruited plerocerciform worms and the recruitment continued during August and September. In October and November the proportion of small plerocerciform worms was <14 %. From December 1993 to April 1994 a small number of these parasites occurred but were not present in May and June 1994. In 1994-95 *P. filicollis* exhibited a different cycle of recruitment. Recruitment was maximum in July and August and it continued in September. Small plerocerciform worms were present every month from October to May 1995 even though maturation of 1994-95 generation occurred at this time. In July 1995 recruitment of the next generation started. Thus in 1993 and 1994 recruitment of the new generation of *P. filicollis* occurred within one month of the loss of the previous generation, but in 1995 there was some overlap of the two generations (Fig. 3.5).

3.6.5. Infection and host length.

The monthly prevalence and abundance of *P. filicollis* in each length class of stickleback is given in Table 3.8 a.

The prevalence rate of *P. filicollis* in each length class of fish is shown in Table 3.8. Parasites were found in all length classes of fish, but the prevalence rate was highest in LC-3 and lowest in LC-1. There is no significant difference in prevalence rate between all four length classes of fish (Chi-square, $P = 0.50 = 2.366$, $P \geq 0.50$).

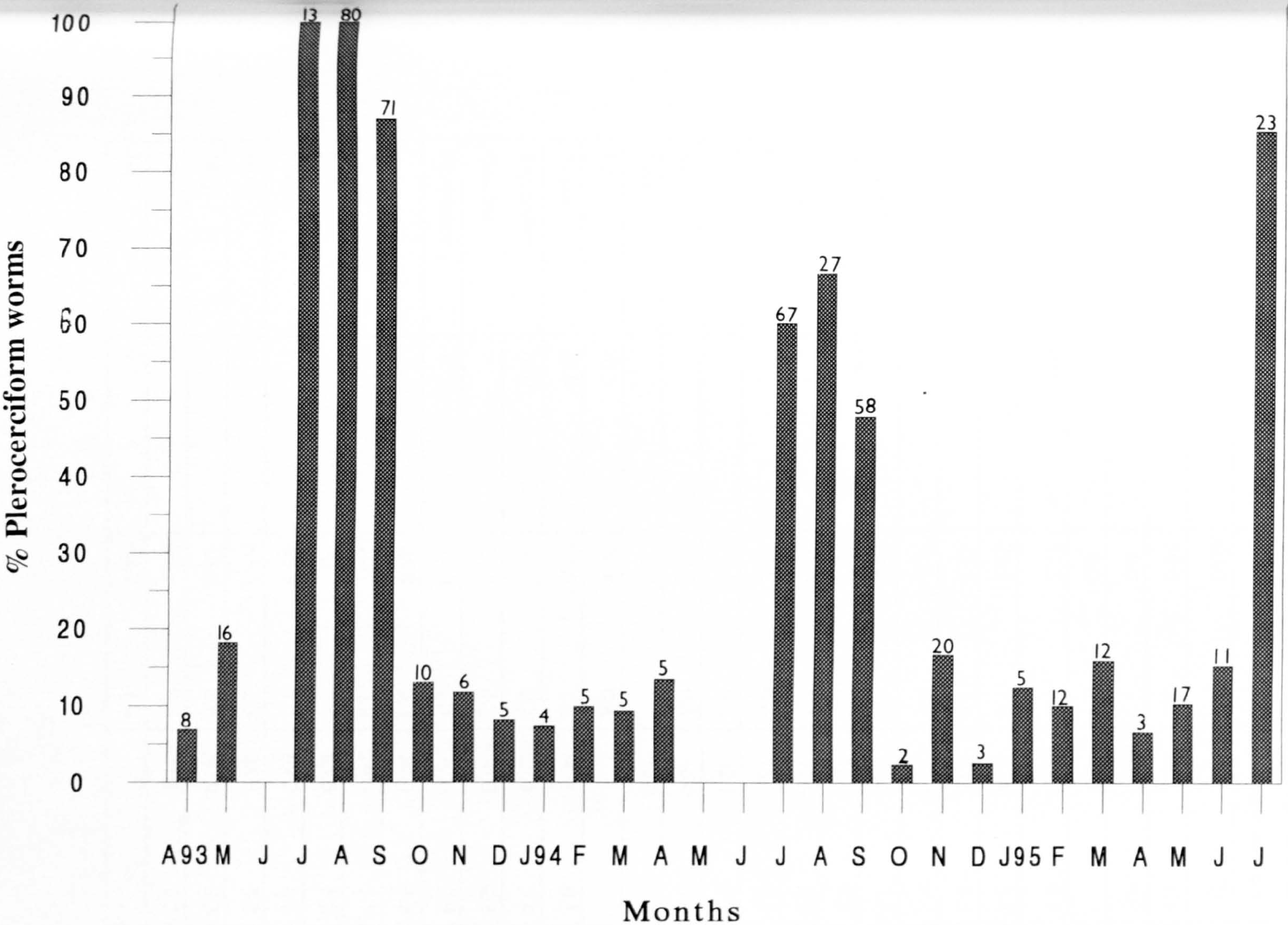


Fig 3.5. Percentage of plerocerciform *Proteocephalus filicollis* (length of worm 0.32 to < 1mm) in the total worm population in *G. aculeatus* in Airthrey Loch from April 1993 to July 1995. Number on bars are numbers of plerocercoid recorded each month.

Table 3.8 a. Monthly prevalence and abundance of *Proteocephalus filicollis* in different length classes of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995.

Month	LC-1 (< 2.0 cm)		LC-2 (2.1-3.0 cm)		LC-3 (3.1-4.0 cm)		LC-4 (4.1 > cm)	
	Prevalence	Abundance	Prevalence	Abundance	Prevalence	Abundance	Prevalence	Abundance
April 1993			60.0	3.2	40.0	2.9	21.8	0.8
May			50.0	3.0	35.3	1.1	53.8	2.5
June	0		0.0		100.0	4.0	63.6	2.8
July	20.0	0.2	8.0	0.4	0.0		0	
August	100.0	1.0	37.7	1.4	27.7	0.2	0	
September			29.4	1.0	17.9	1.1	16.6	0.1
October			42.1	1.5	43.2	1.3	22.2	0.2
November			8.6	0.1	30.4	2.1	40.0	0.4
December			33.5	1.0	31.5	1.6	40.0	1.4
January 1994			32.2	0.7	52.0	1.2	0.0	
February			23.0	0.9	47.3	2.0	0.0	
March			22.8	0.6	25.7	0.8	33.3	0.3
April			19.0	0.2	32.1	0.9	35.7	0.3
May			37.5	0.8	46.0	0.8	41.0	0.5
June			0.0		18.7	0.3	30.0	0.6
July	40.5	2.5	30.4	0.8				
August	23.8	0.7	25.0	1.0	50.0	0.5	100	7.0
September	70.0	2.5	64.2	2.7	25.0	0.4	66.6	5.0
October			50.0	4.3	70.0	2.3		
November	0		48.0	2.3	70.0	2.9	40.0	0.4
			88.8	5.7	50.0	4.3	66.6	3.0
January 1995			100	3.2	54.5	1.5	100	1.5
February			70	4.5	85.0	5.2	50.0	1.2
March			53.8	1.3	69.2	4.3	16.6	0.3
April			66.6	2.3	28.5	1.2	90.0	2.9
May			66.6	3.6	80.0	3.3	58.3	1.9
June	0		11.1	0.1	57.1	1.5	43.2	1.6
July	0		64.2	1.6			16.6	0.1

Table.3.8 **Prevalence and abundance of *Proteocephalus filicollis* in different length classes of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995.**

Length class	No of fish exam	infect	Prevalence (%)	Total no of worms	Maximum no of worms	Abundance
LC-1 < 2.0 cm	104	32	30.7	144	46	1.3
LC-2 2.1-3.0 cm	483	174	36.0	649	20	1.3
LC-3 3.1-4.0 cm	462	192	41.5	859	35	1.8
LC-4 4.0 > cm	302	118	39.0	324	23	1.0
Total	1351	516	38.1	1976	46	1.4

The highest abundance was found in LC-3 and lowest in LC-4. In each year class of stickleback examined there was no significant difference in prevalence rate and intensity of infection between the different length classes but there was a general tendency for the parasite population to fall in the largest fish. The mean intensity was also not significant between length classes (Chi-square P= 0.90 = 0.584, P > 0.90).

3. 6.6 Frequency distribution of *Proteocephalus filicollis.*

Proteocephalus filicollis was overdispersed within the stickleback population in Airthrey Loch and the distribution of the cestode fitted the truncated negative binomial distribution (Fig. 3.6).

The distribution of *P. filicollis* in different length classes of fish is given in Fig. 3.7a & 3.7b. In all length classes the distribution fitted the negative binomial.

The observed distribution of *P. filicollis* also fitted the truncated negative binomial in each season (Fig. 3.8a, 3.8b, 3.8c, 3.8d, 3.8e, 3.8f, 3.8g, 3.8h, 3.8I, 3.8j).

3.6.7. Variance to mean ratio.

Monthly changes in the variance to mean ratio are shown in Fig. 3.9. Variance to mean ratio was always found to be greater than unity which indicates an overdispersion of the parasite in the host population. Overdispersion peaked in LC-1 and declined in the larger hosts (Table 3.9).

The variance to mean ratio in each season is shown in Table 3.10. The ratio was greater than unity in each season but was highest in spring 1993, summer 1994 and lowest in spring 1994 and summer 1995.

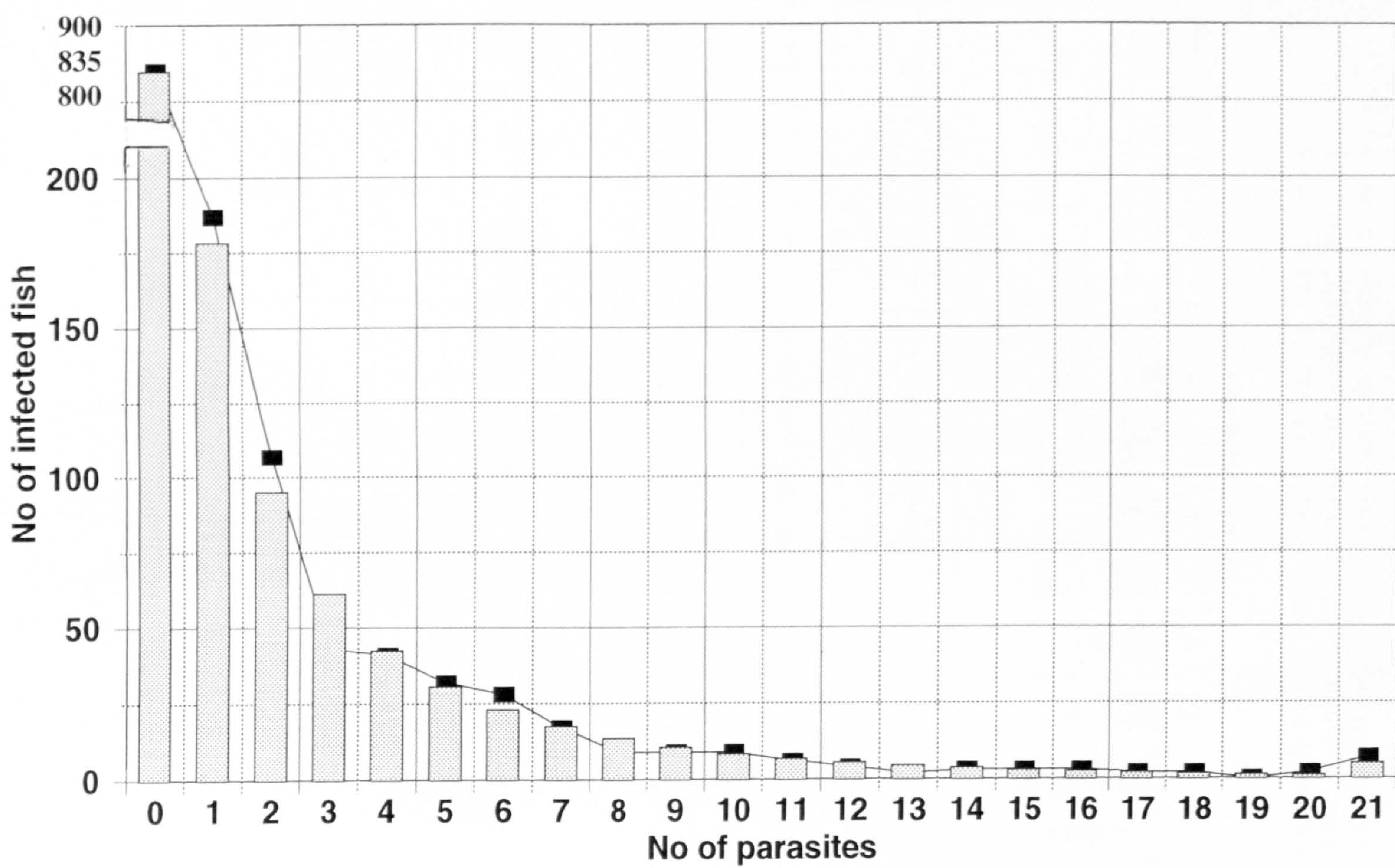
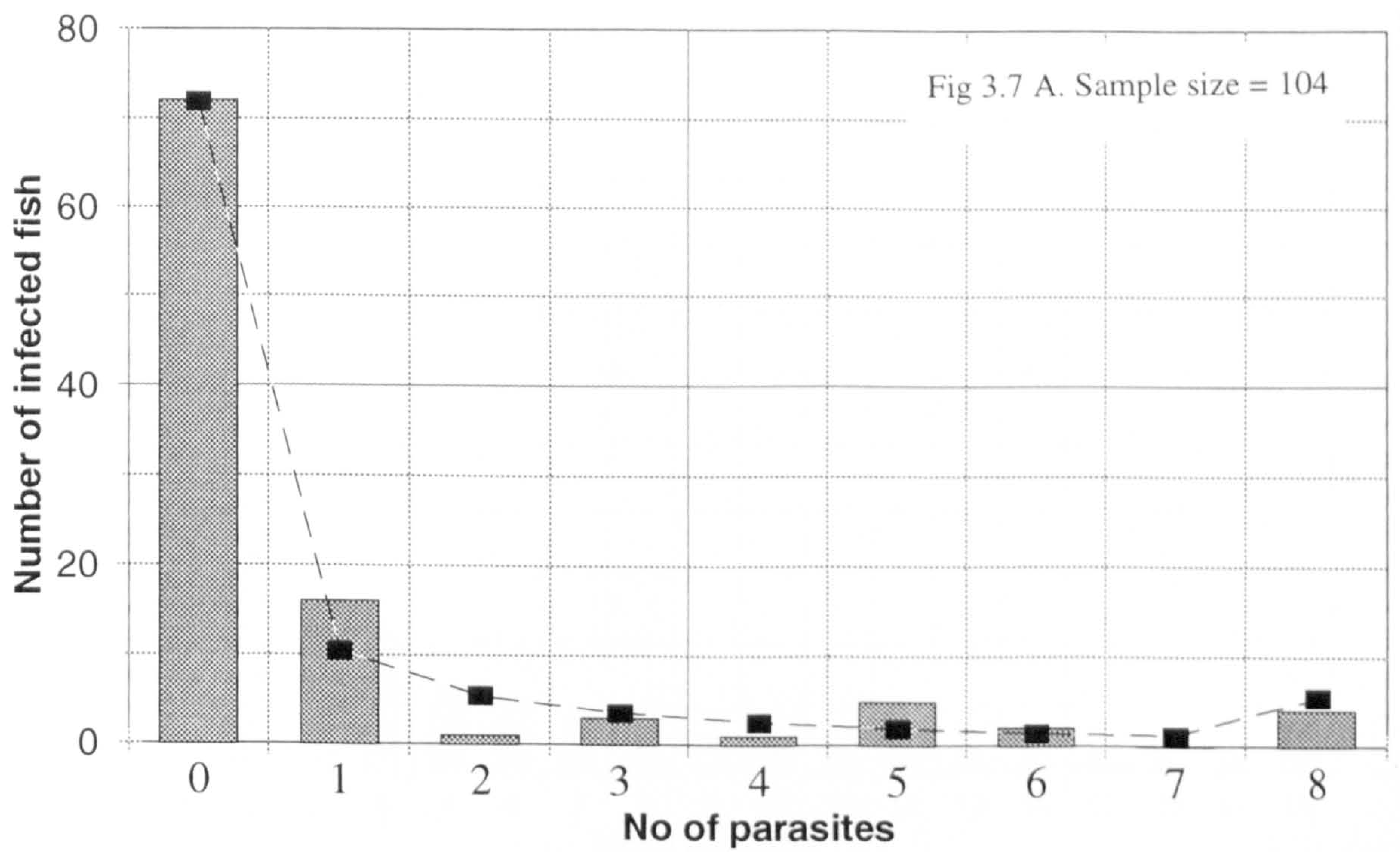


Fig 3.6 Truncated negative binomial distribution of *Proteocephalus filicollis* in all *G. aculeatus* from Airthrey Loch from April 1993 to July 1995. Bars and black squares represent observed and expected frequencies respectively.



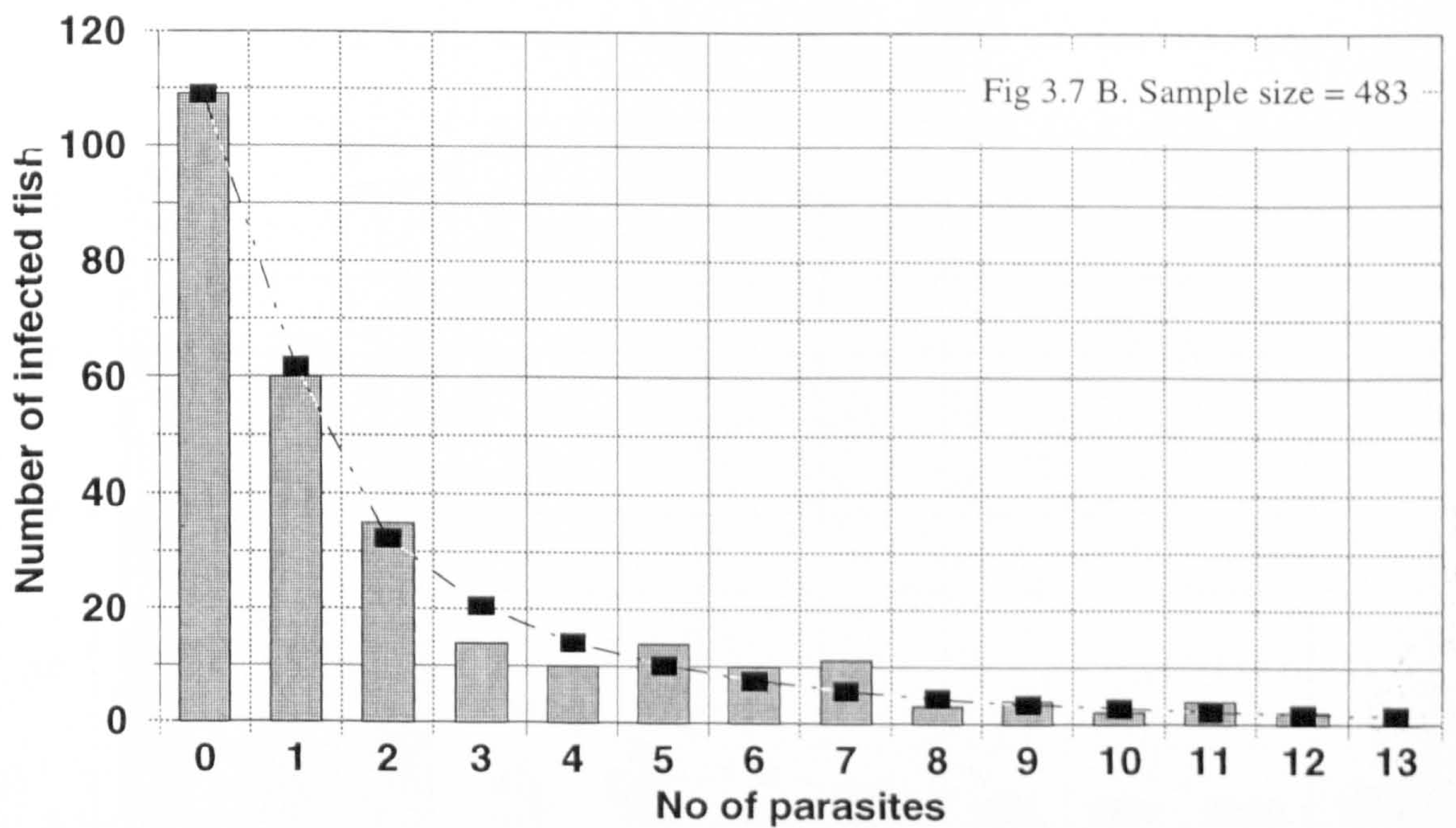


Fig 3.7 Frequency distribution of *Proteocephalus filicollis* in various length classes of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995. A = LC-1 (< 2.0 cm), B—LC-2 (2.1-3.0 cm), C—LC-3 (3.1-4.0 cm), D—LC-4 (4.1 > cm).

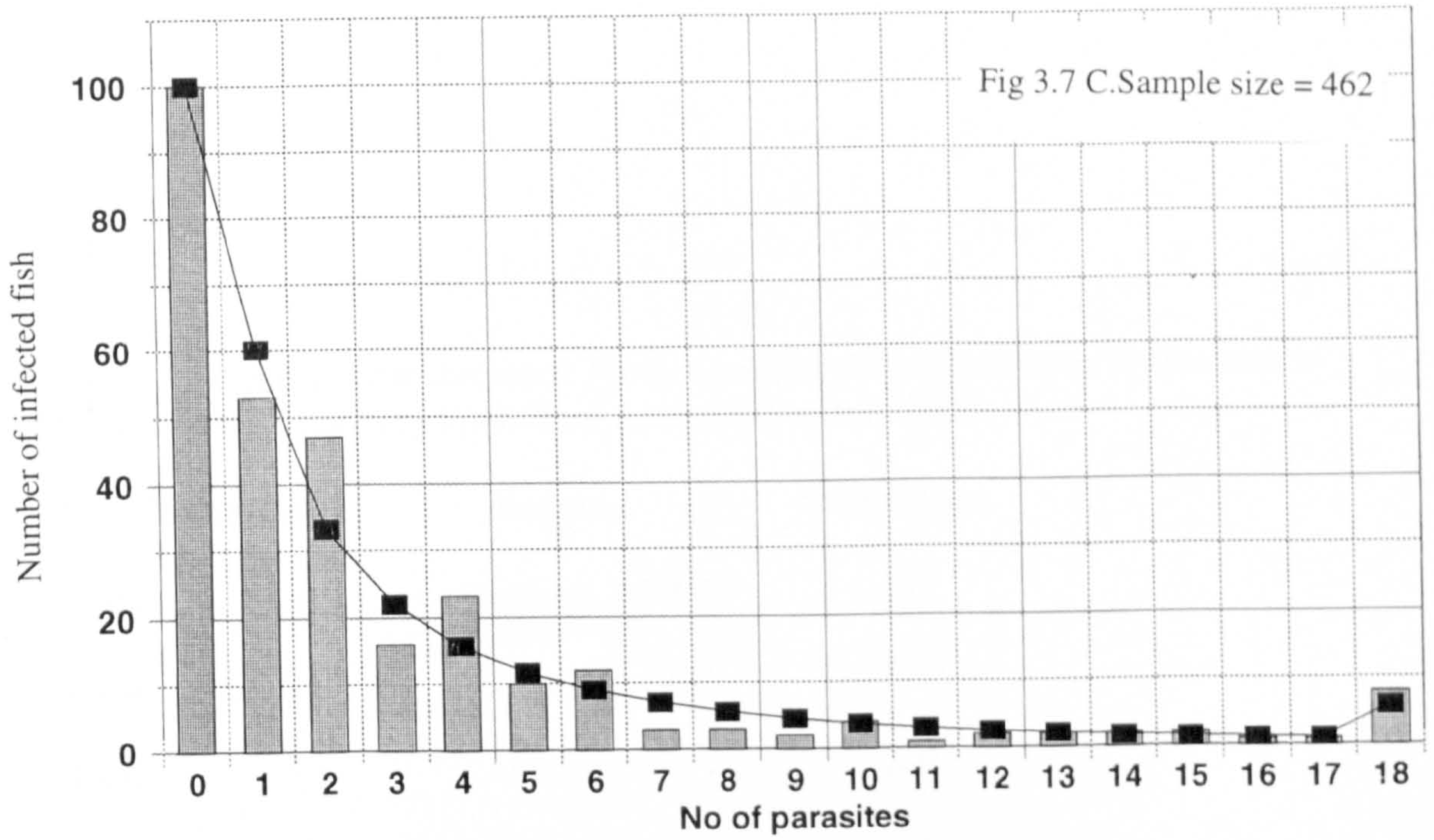


Fig 3.7 C.Sample size = 462
Number of infected fish
No of parasites
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

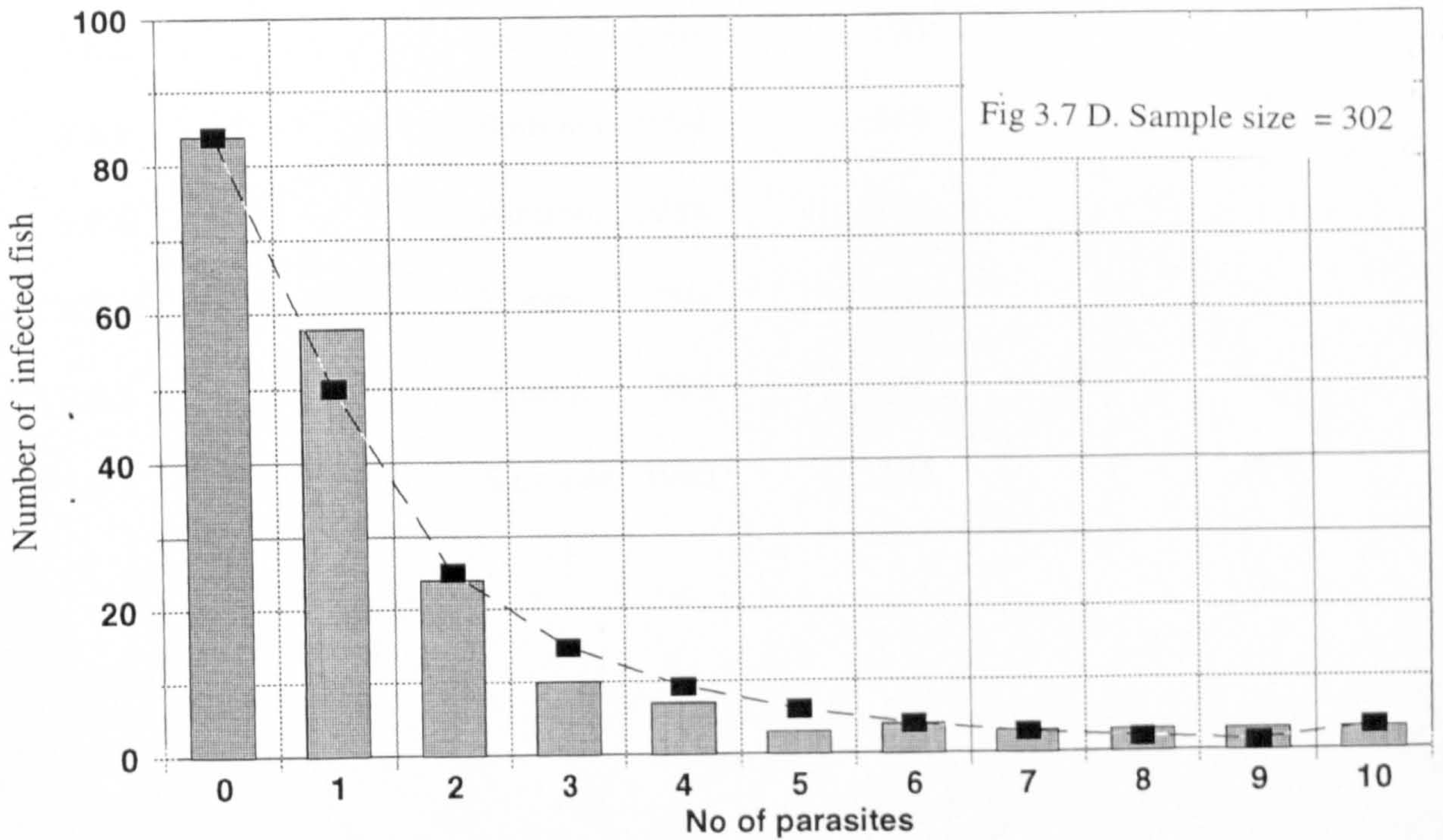
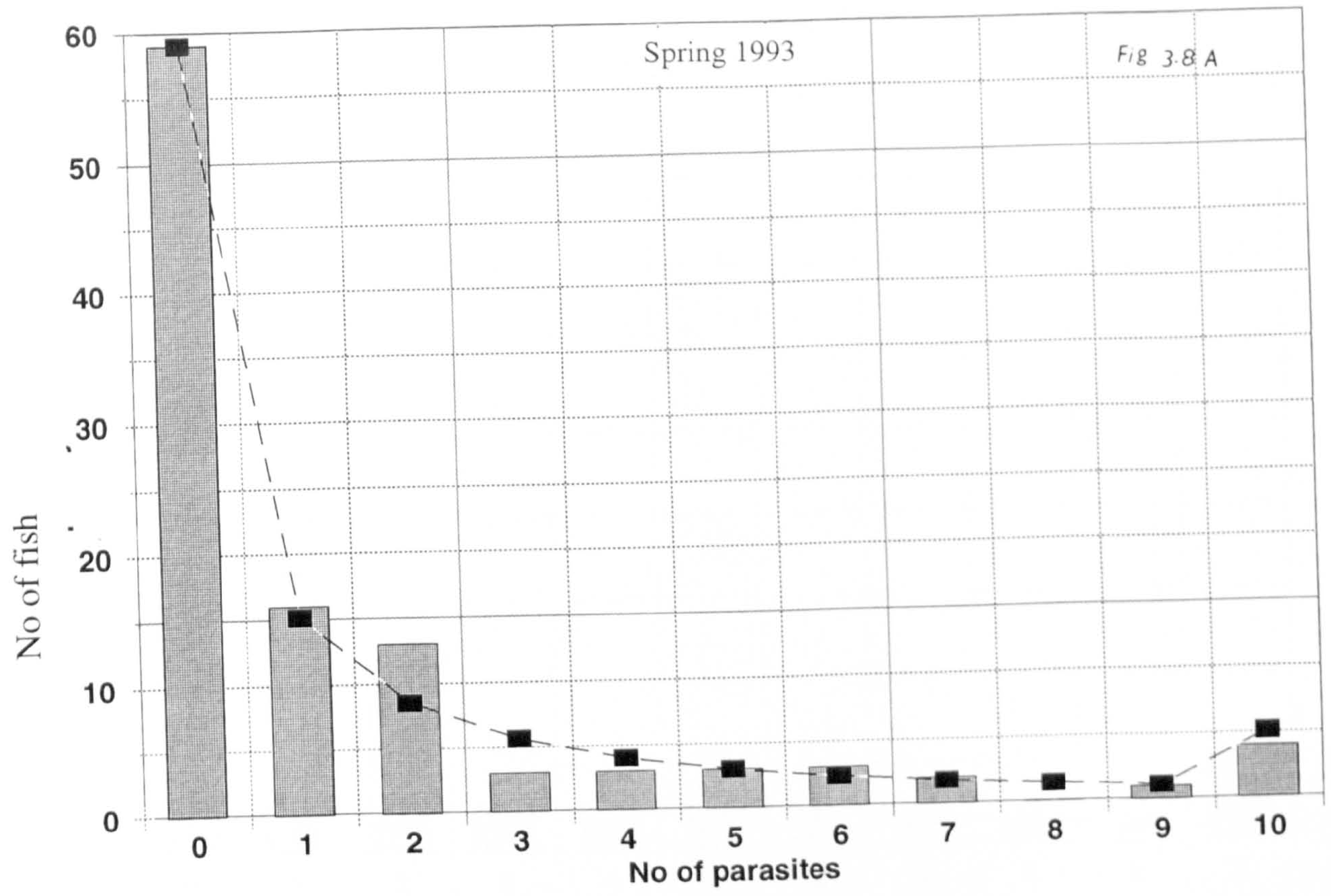
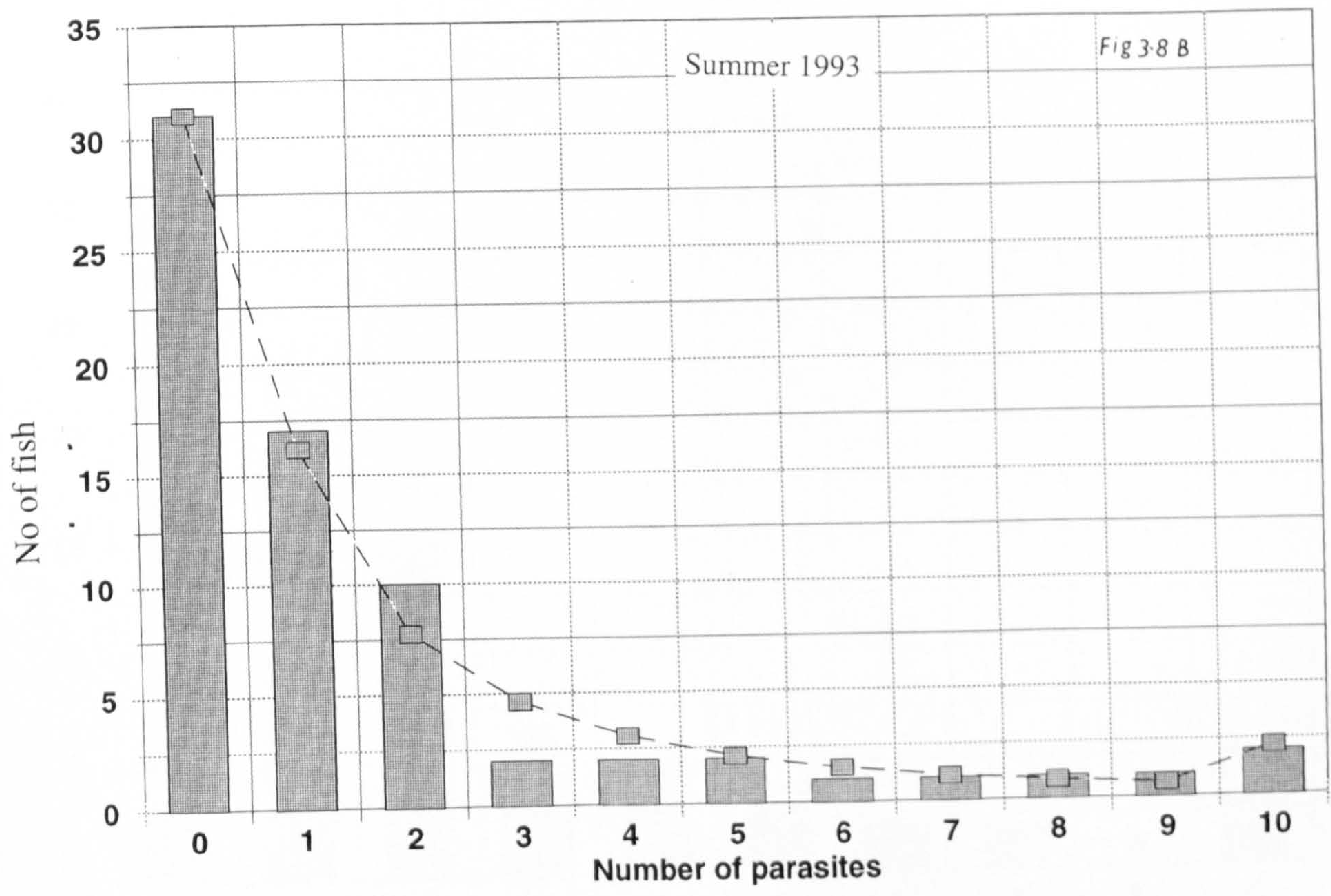


Fig 3.7 D. Sample size = 302
Number of infected fish
No of parasites
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10

Fig 3.8. Frequency distributionof *Proteocephalus filicollis* in different seasons in *G. aculeatus* in Airthrey Loch from April 1993 to July 1995.

Figure Number	**Season**	**Sample size**
3.8 A	Spring 1993	107
3.8 B	Summer 1993	171
3.8 C	Autumn 1993	200
3.8 D	Winter 1993	143
3.8 E	Spring 1994	190
3.8 F	Summer 1994	140
3.8 G	Autumn 1994	124
3.8 H	Winter 1994	72
3.8 I	Spring 1995	99
3.8 J	Summer 1995	155


Spring 1993
Fig 3.8 A
No of fish
No of parasites
0
10
20
30
40
50
60
0
1
2
3
4
5
6
7
8
9
10


Summer 1993
Fig 3.8 B
No of fish
Number of parasites
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6
7
8
9
10

Autumn 1993

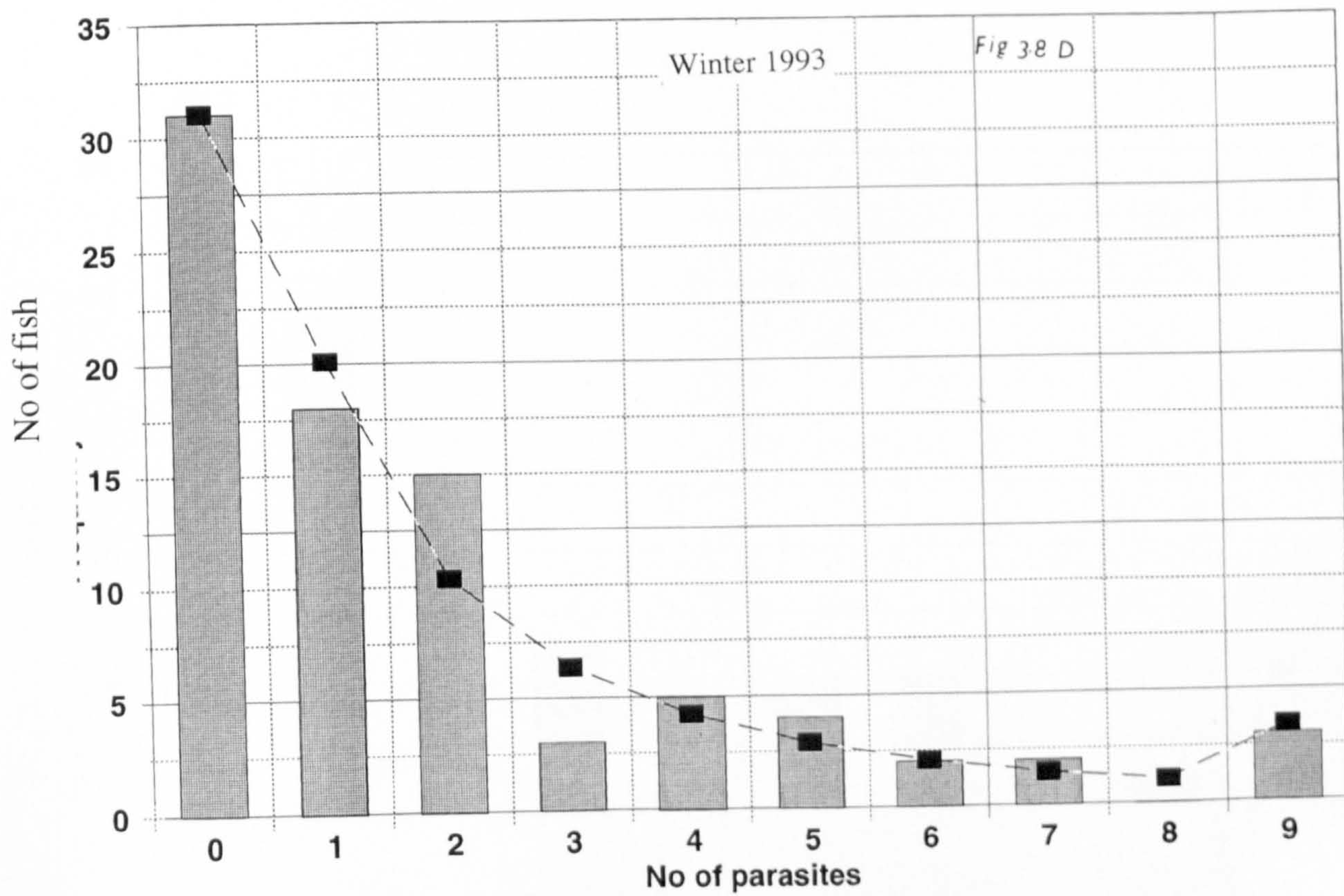
Fig 3.8C

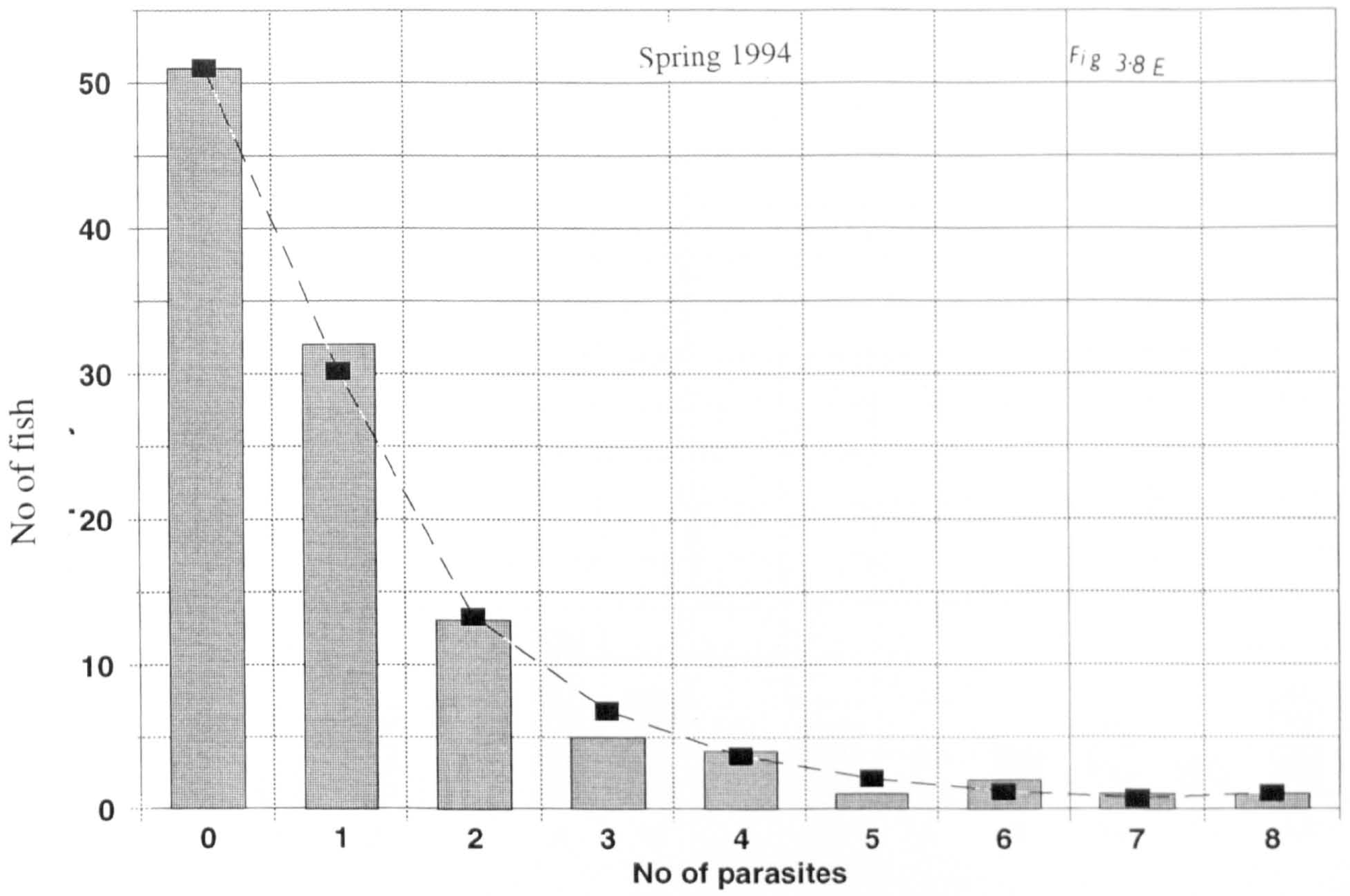
No of fish

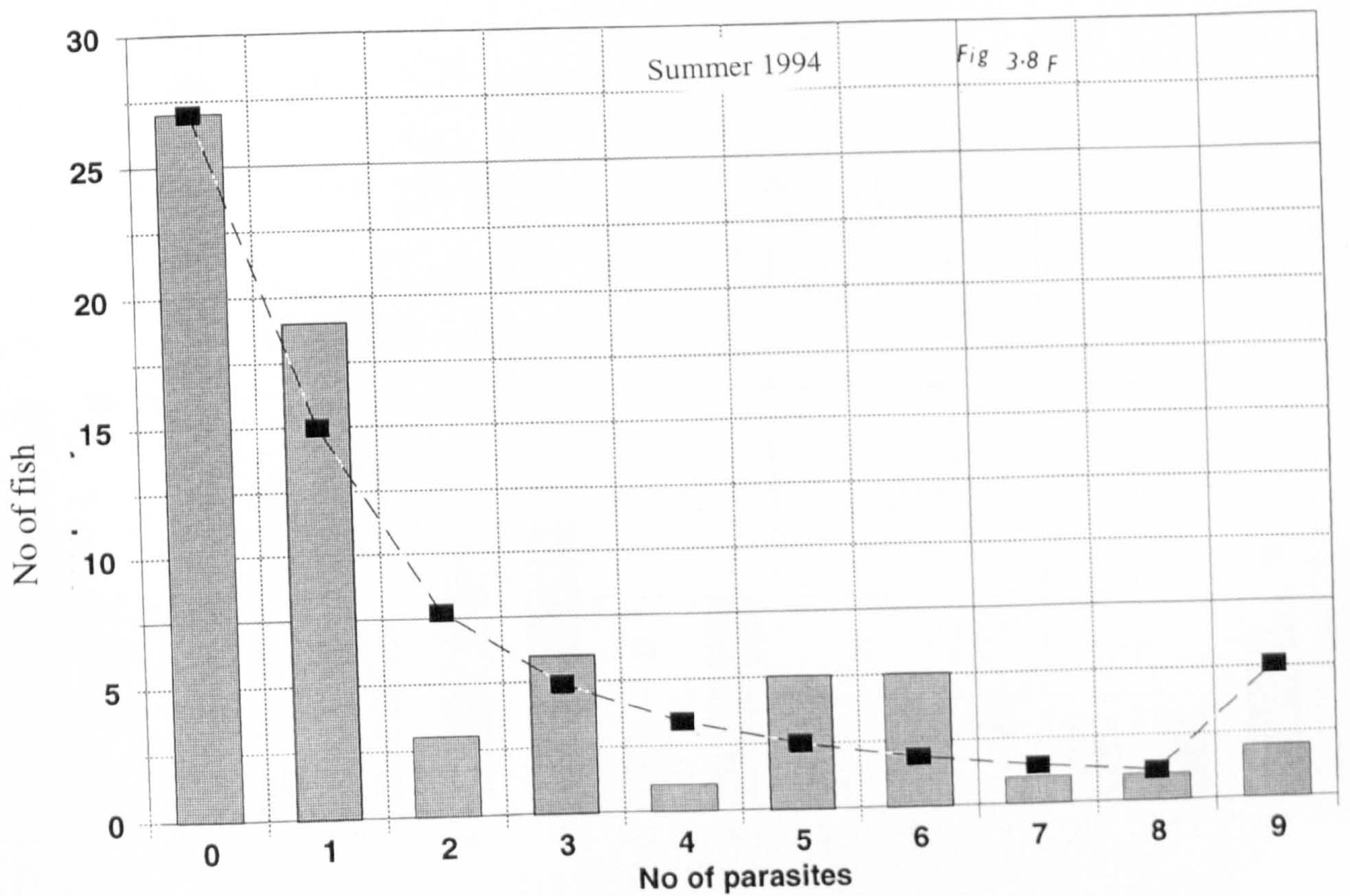
50
40
30
20
10
0

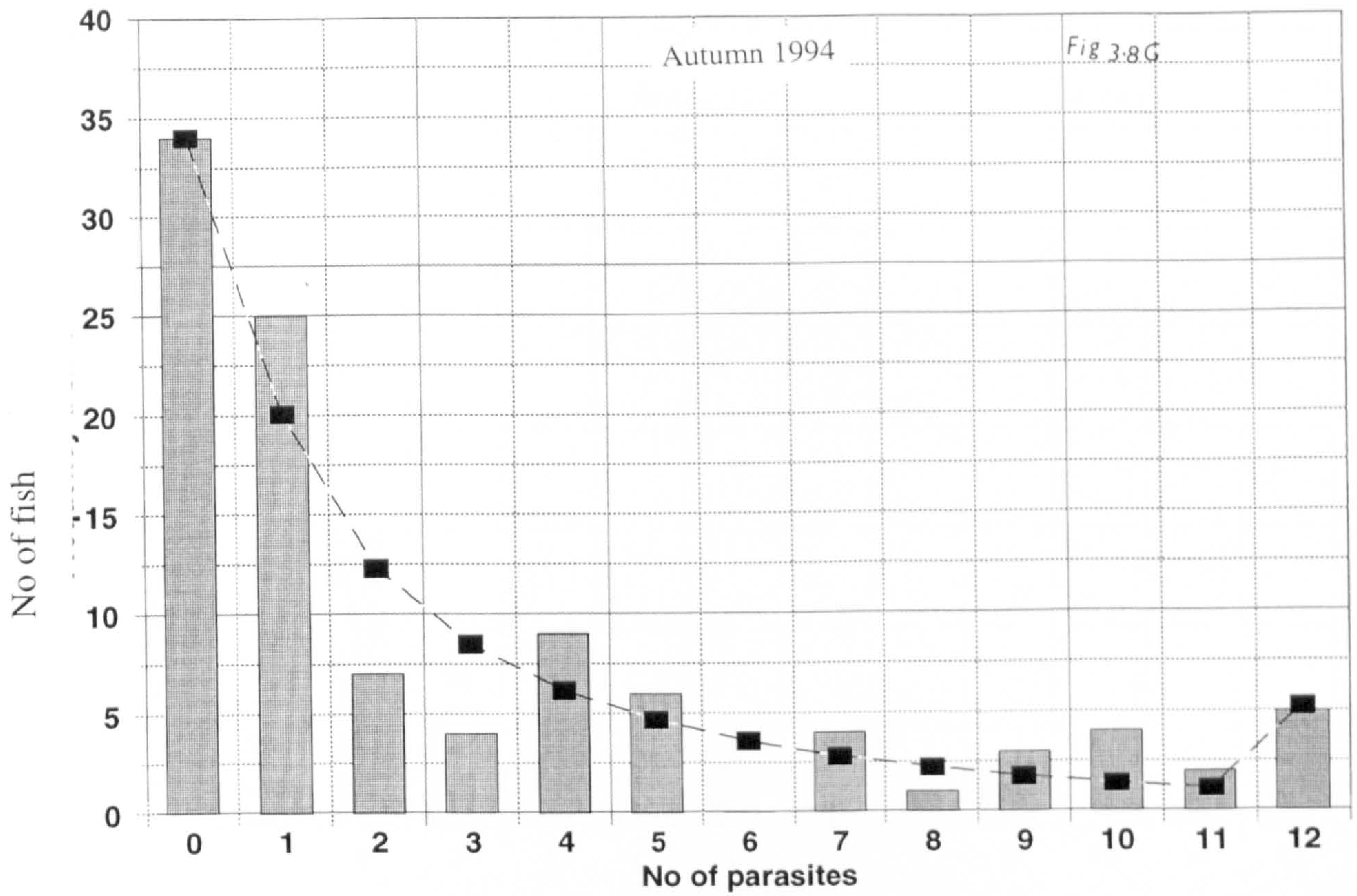
0 1 2 3 4 5 6 7 8 9 10

No of parasites




Spring 1994
Fig 3.8 E
50
40
30
20
10
0
No of fish
0
1
2
3
4
5
6
7
8
No of parasites


Summer 1994
Fig 3.8 F
30
25
20
15
10
5
0
No of fish
0
1
2
3
4
5
6
7
8
9
No of parasites



Winter 1994

Fig 3 8 H

No of fish

20

15

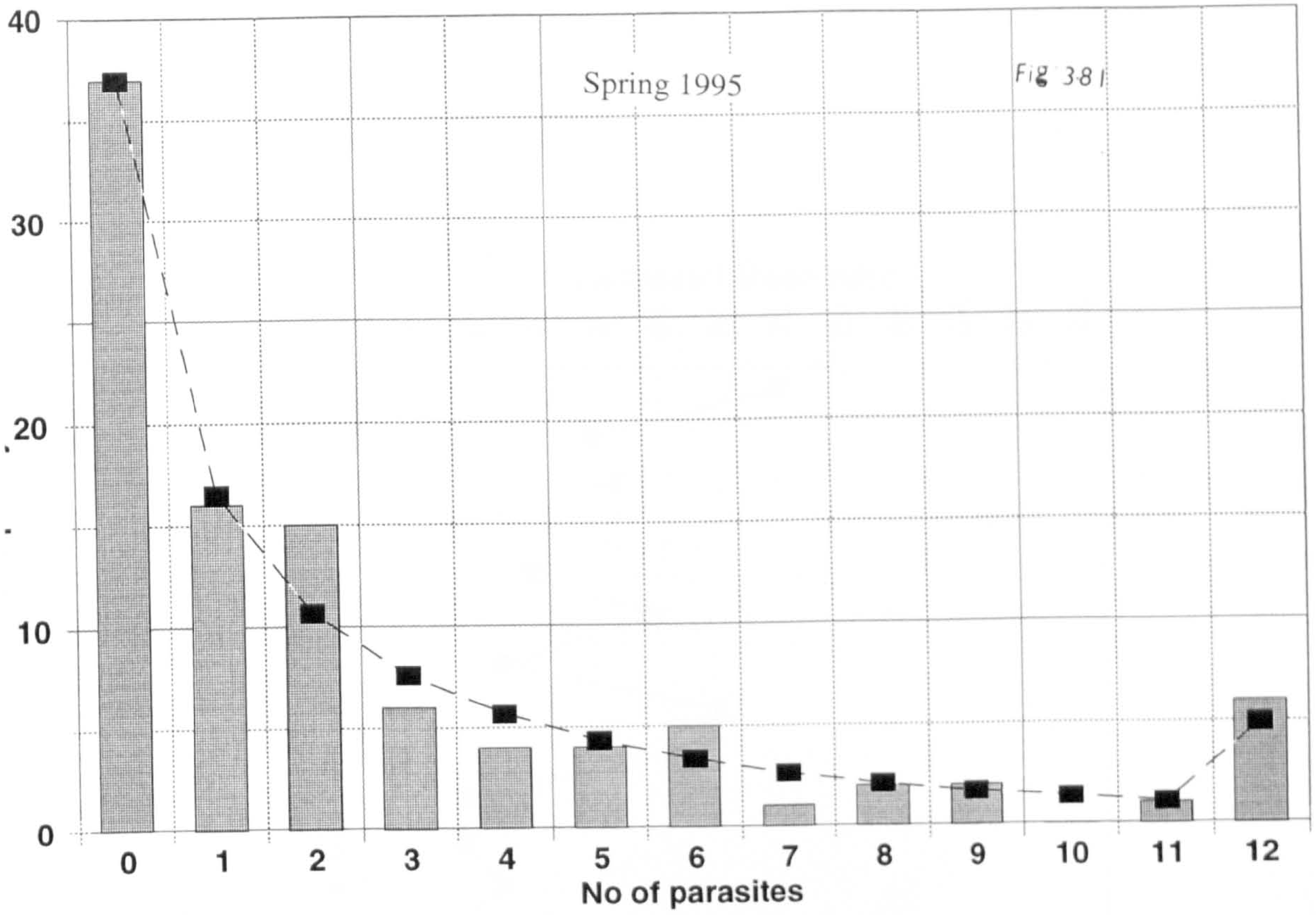
10

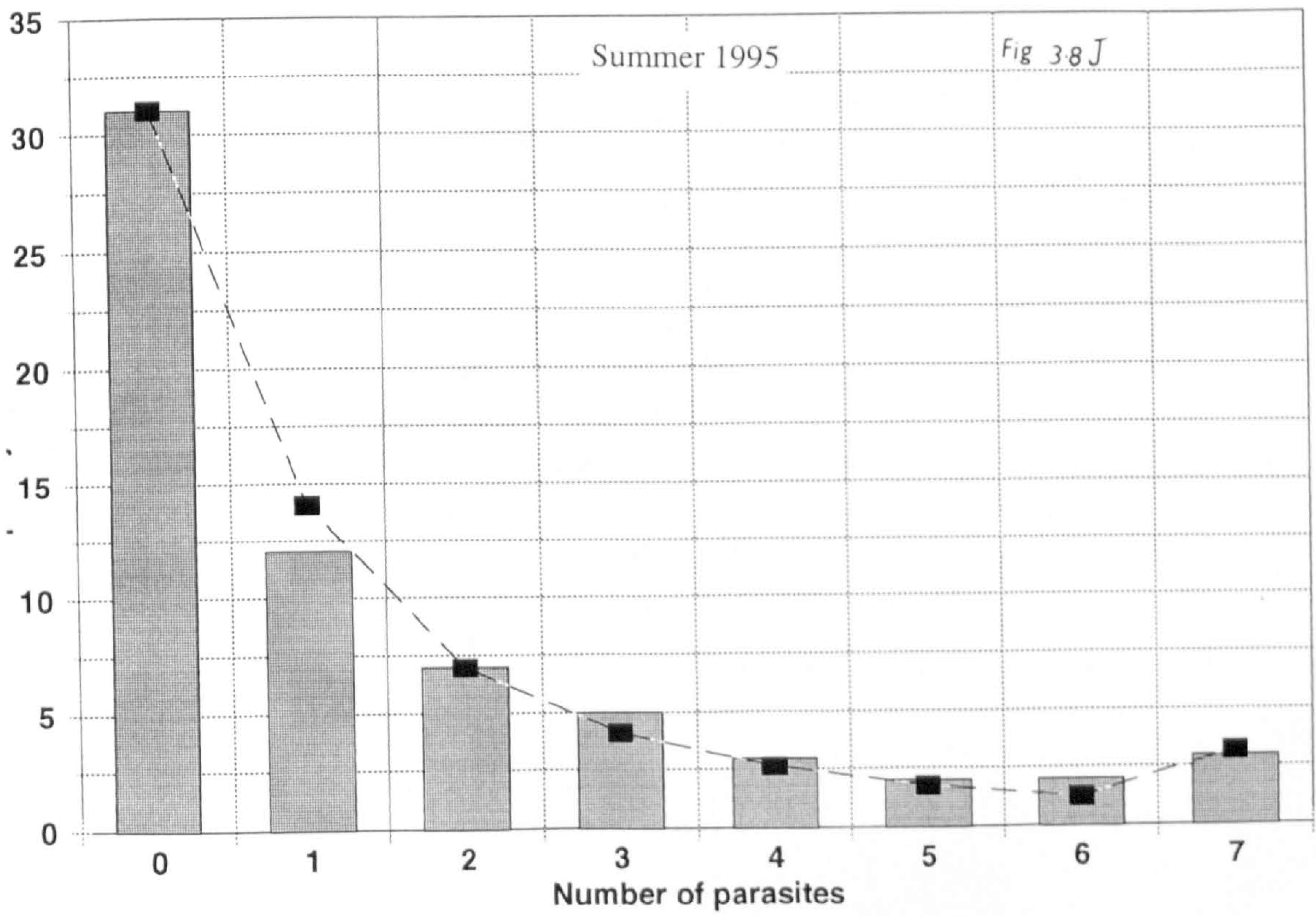
5

0

0 1 2 3 4 5 6 7 8 9 10 11 12

No of parasites


Spring 1995
Fig 3.8 I
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
11
12
No of parasites


Summer 1995
Fig 3.8 J
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
Number of parasites

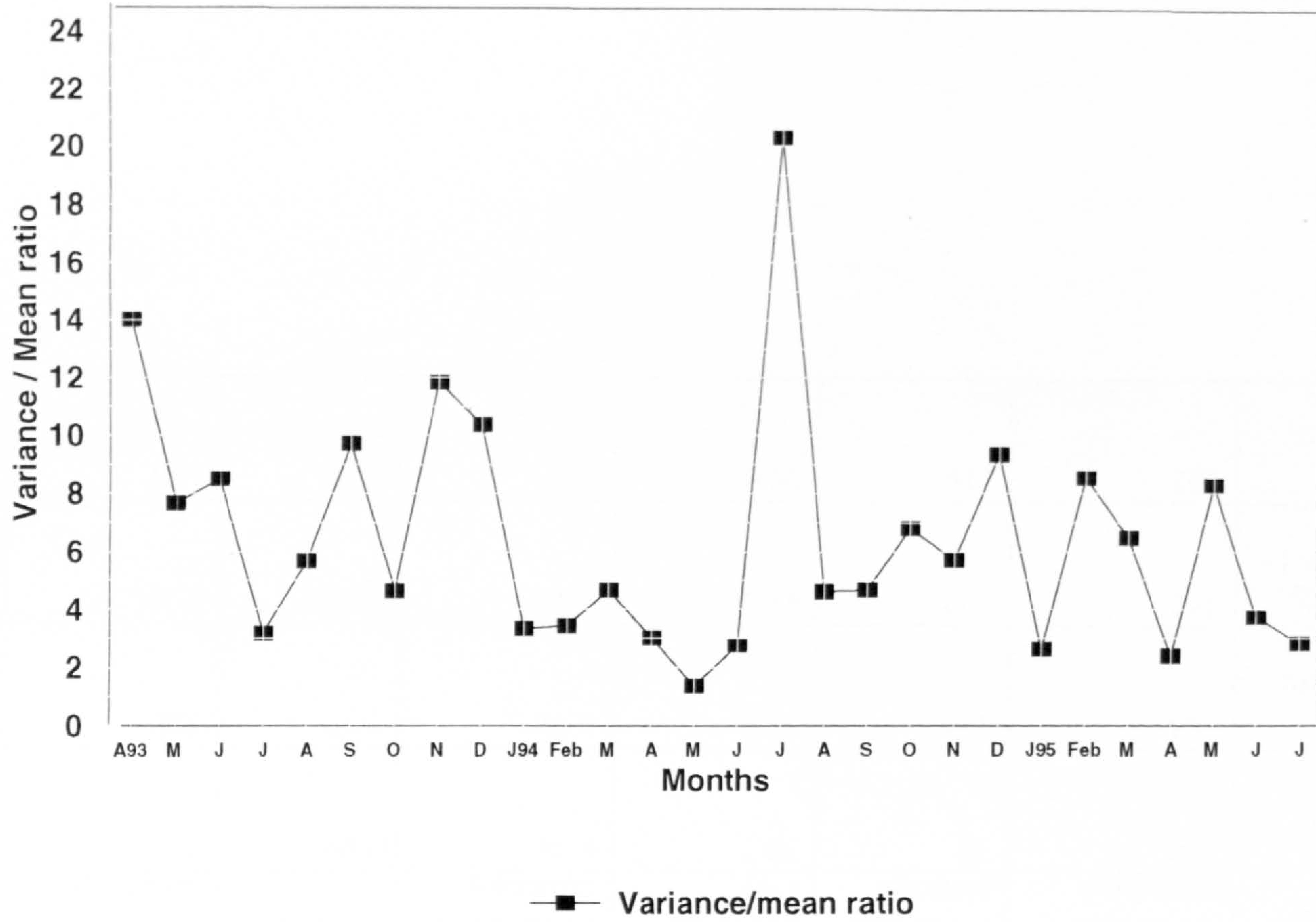


Fig 3.9 Monthly variance to mean ration of *Proteocephalus filicollis* in *Gasterosteus aculeatus* in Airthrey Loch from April 1993 to July 1995.

Table. 3.9 Variance / mean ratio of *Proteocephalus filicollis* in different length classes of *G. aculeatus* from Airthrey Loch from April 1993 to July 1995.

Length class	No of fish examined	No of fish infected	Prevalence (%)	Mean no of parasites	Variance	Variance/ mean ratio
LC-1 (< 2.0 cm)	104	32	30.7	1.38	24.04	17.36
LC-2 (2.1-3.0 cm)	483	174	36.0	1.34	7.77	5.77
LC-3 (3.1-4.0 cm)	462	192	41.5	1.85	17.10	9.19
LC-4 (4.1 cm >)	302	118	39.0	1.07	5.59	5.21

Table 3.10. Seasonal change in prevalence and variance / mean ratio of *Proteocephalus filicollis* in *G. aculeatus* in different seasons in Airthrey Loch from April 1993 to July 1995.

Season	No of fish examined	No of fish infected	Prevalence (%)	Mean no of parasites	Variance	Variance / mean ratio
Spring 1993	107	48	44.8	1.90	21.31	11.17
Summer1993	171	40	23.3	0.74	4.75	6.35
Autumn 1993	200	56	28.0	1.05	8.73	8.28
Winter 1993	143	52	36.3	1.15	6.69	5.80
Spring 1994	190	59	31.0	0.65	2.12	3.24
Summer1994	140	43	30.7	1.23	18.13	14.67
Autumn 1994	124	70	56.4	2.49	13.55	5.43
Winter 1994	72	52	72.2	3.83	13.15	8.03
Spring 1995	99	62	62.6	2.86	19.82	6.91
Summer1995	105	34	32.3	0.94	3.41	2.62

3.6.8 Parasite size.

The variation in monthly mean length of *P. filicollis* is shown in Fig. 3.10, and shows a very closely consistent pattern in growth between generations. After initial invasion of the fish in summer worms increased in length over the autumn and winter. There was a rapid increase in length in May and June as worms matured, so that mean length was 11.19 mm in May and 15.60 mm in June. A similar pattern occurred in 1994-95 although increase in length was slower over the winter so that mean length was 3.67 mm in March 1995. However, subsequent increase in length was rapid so that the mean length of *P. filicollis* in June 1995 was 14.90 mm.

3.6.9. Parasite size and maturation.

A wide variation in the size of the different maturity stages of *P. filicollis* was observed in the present study. Although mean length increased with maturation of the cestode there was a very wide overlap in the length of worms of different maturation stages (Table 3.11).

3.6.9.1 Immature worms.

Immature *P. filicollis* include all plerocercoid and early segmented worms which are found in most months. Because they form the bulk of the parasite population (Table 3.5) their mean length strongly influences that of the worm population as a whole (Table 3.9). Thus, in July and August 1993 and in July to September 1994 the mean length is between 0.61mm - 1.10 mm) and 0.96 mm - 1.46 mm and the length range is quite

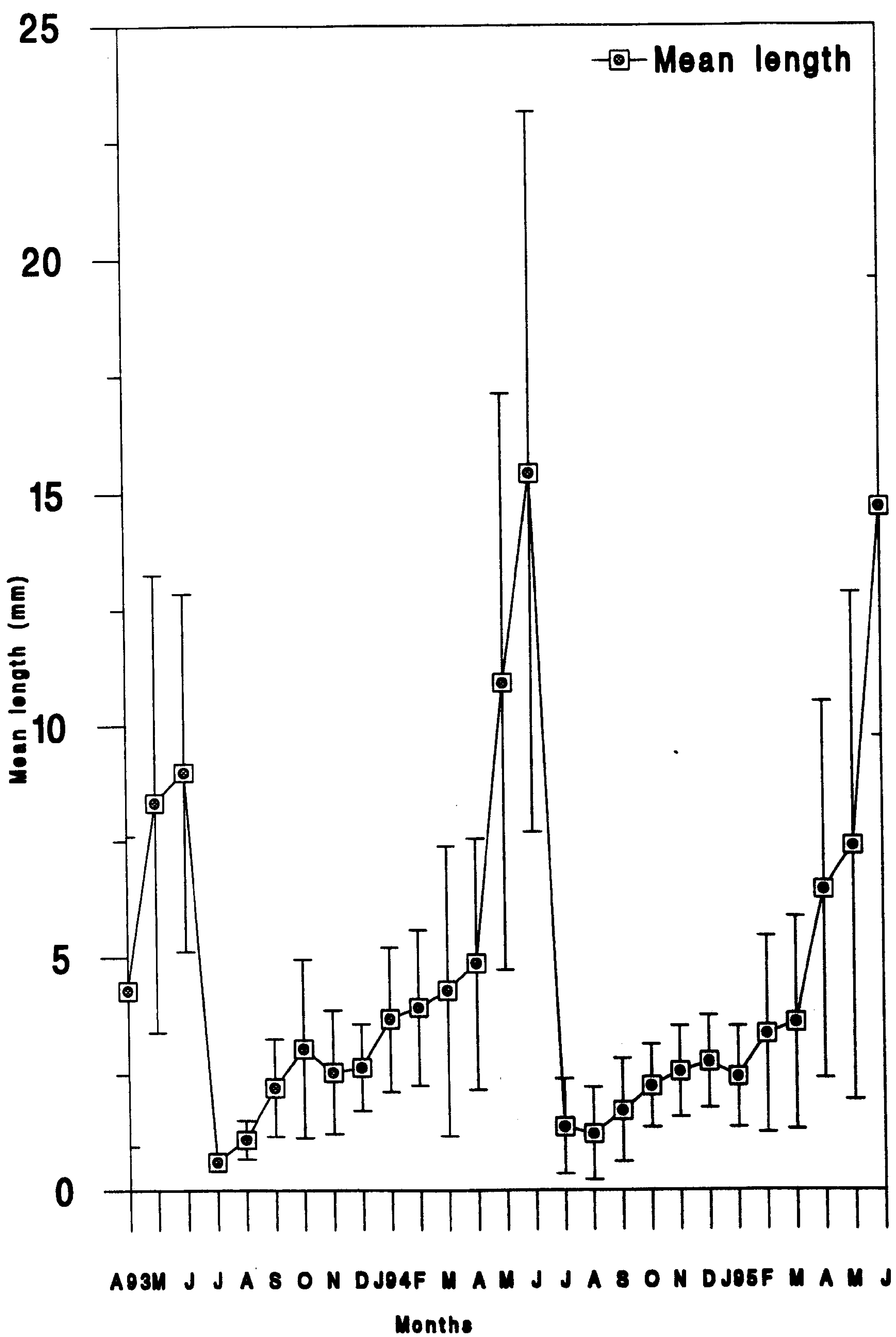


Fig 3.10 Monthly mean length of *Proteocephalus filicollis* from *Gasterosteus aculeatus* in Airthrey Loch from April 1993 to June 1995. Bars represent standard deviations.

small. Subsequently, there is an increase in mean length throughout the life of the parasite generation, but the length range is very wide and immature worms up to 6.79 mm in length were found

3.6.9.2 Maturing worms.

These worms comprise only a small proportion of the worm population and were not found in all samples (Table 3.7). In general this maturity stage of parasites also showed an increase in length with the general increase of the length of the whole *P. filicollis* population. A very small number of small maturing *P. filicollis* were found in summer and autumn in 1993 and 1994 with mean length < 3 mm and with little variation in length. From February to May each year maturing worms were longer in size and mean length varied from 5.40 - 8.55 mm (Feb. - May 1994) and 4.35 - 6.74 mm (Feb. - May 1995) (Table 3.11).

3.6.9.3 Mature worms.

Mature worms were found in April, May and June 1993, with mean lengths of 5.0 mm, 9.24 mm, 6.84 mm, respectively. In the 1993 worm population, one 5.06 mm long mature worm was recorded in October. From January 1994 to June 1994 mature worms appeared but their number was very small. Their lengths varied from 4.0 mm in January, 8.1 mm in February, 5.2 mm in March, 7.8 mm in April, 11.39 mm in May and 13.86 mm in June 1994 (Table 3.11).

In the 1994 population, no mature worms appeared from July to December 1994. In January, February and March 1995 mature worms appeared with mean lengths of 9.89 mm, 9.99 mm and 7.98 mm, respectively. An increase in the mean length of the mature

Table 3.11. Monthly mean length of different maturation stages of *Proteocephalus filicollis* from *G. aculeatus* from Airthrey Loch from April 1993 to June 1995.

Month	Immature worm		Maturing worm		Mature worm		Gravid worm	
	Mean length (mm)	Sd	Mean length (mm)	Sd	Mean length (mm)	Sd	Mean length (mm)	Sd
April 1993	2.6	± 1.85	3.2	± 0.52	5.0	± 0.95	11.6	± 2.39
May	2.1	± 0.77	-		9.2	± 1.72	10.6	± 4.66
June	3.4	± 1.49	-		6.8	± 1.23	12.4	± 4.29
July	0.6	± 0.10	-		-		-	
August	1.1	± 0.47	-		-		-	
September	2.4	± 1.10	-		-		4.0	
October	2.7	± 0.89	2.4		5.0		8.2	± 4.79
November	2.4	± 0.91	-		-		6.2	± 0.06
December	2.4	± 0.86	3.3	± 0.12			4.8	
January 94	3.3	± 1.28	-		4.0	± 0.17	6.0	± 1.12
February	4.4	± 0.25	5.7		8.1		8.0	
March	3.3	± 1.28	8.5	± 4.71	5.2		10.9	± 4.7
April	3.6	± 1.60	5.4	± 1.33	7.8		9.3	± 2.7
May	3.9	± 1.70	6.2	± 0.76	11.4	± 4.60	13.8	± 5.38
June	-		6.2	± 0.76	13.8		15.5	± 8.06
July	0.9	± 0.37	3.3		-		-	
August	1.0	± 0.55			-		-	
September	1.4	± 0.69	2.4		-		5.0	± 1.59
October	2.3	± 0.82	2.8	± 0.49	-		3.6	
November	2.5	± 1.04	-		-		4.0	
December	2.6	± 0.90	3.9	± 1.25	-		-	
January 95	2.59	± 1.28	3.2		9.8		-	
February	2.79	± 1.12	5.6	± 1.36	10.0	± 8.32	9.1	± 2.32
March	2.69	± 1.25	4.3	± 1.34	7.9	± 0.93	8.6	± 0.85
April	2.76	± 1.40	6.7	± 2.13	6.7	± 3.99	9.9	± 4.05
May	2.45	± 1.46	6.1	± 2.66	12.0	± 5.39	11.3	± 6.17
June	1.91	± 1.05	8.0	± 2.92	12.7	± 1.70	15.0	± 4.56

worms was observed from April to June with mean lengths of 6.78 mm in April, 12.02 mm in May and 12.75 mm in June.

3.6.9.4. Gravid worms.

Gravid *P. filicollis* were found throughout the year. In April, May and June 1993 mean lengths of gravid worms were 11.6 mm, 10.6 mm and 12.4 mm, respectively with an overall length range of 4.7 - 22.5 mm. From September 1993 to April 1994 the mean length of gravid worms ranged from 4.0 -10.9 mm with a range of 4.05 - 14.09 mm, but in May and June 1994 the whole parasite population increased in length as it matured and became gravid and mean length of gravid *P. filicollis* increased to 13.8 mm and 15.5 mm, respectively, with a range of 6.06 - 28.7 mm. A similar pattern of increase in length was repeated in the 1994-95 generation of *P. filicollis,* with the few gravid parasites found in September, October and November having mean lengths of 5 mm, 3.6 mm and 4.1 mm, respectively. From February to June there was an increase in the mean length from 9.1 mm in February to 15.0 mm in June. The increase was again prominent between April and June as the majority of the parasite population matured and the length range was also greatest (4.52 - 25.68 mm) during this period.

3.6.10. Parasite length in relation to infrapopulation size.

Regression analysis did not show any relationship between length of different maturity classes of worms and infrapopulation size. This is shown in following expressions respectively:

Immature worms, $r^2 = 0.001$; $P = 0.347$; $F = 0.88$; $F_{(CV)\ 0.005}\ (1)\ 691 = 3.84$ (Fig 3.11). Maturing worms, $r^2 = 0.001$; $P = 0.757$; $F = 0.10$; $F_{CV\ (0.05)}(1)_{71} = 3.98$ (Fig.3.12). Mature

worms, $r^2 = 0.055$; $P = 0.112$; $F=2.62$; $F_{CV\,(0.05)\,(1)\,46} = 4.06$ (Fig 3.13). Gravid worms, $r^2 = 0.013$; $r = -0.114$; $P = 0.185$; $F = 1.17$; $F_{c\,v\,(0.05)\,133} = 3.91$(Fig 3.14).

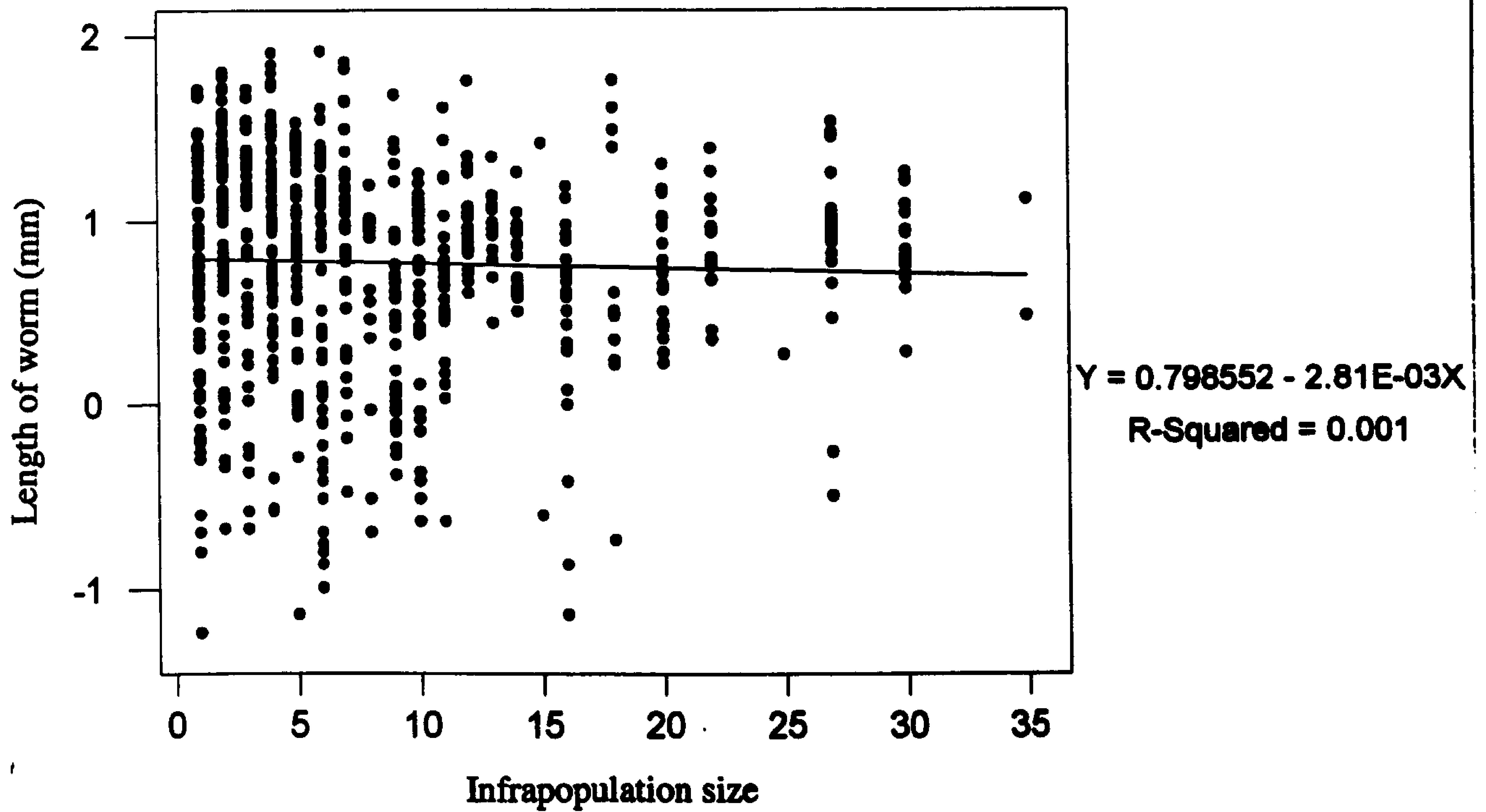


Fig 3.11 Relationship between length of immature ***Proteocephalus filicollis*** and infrapopulation size.

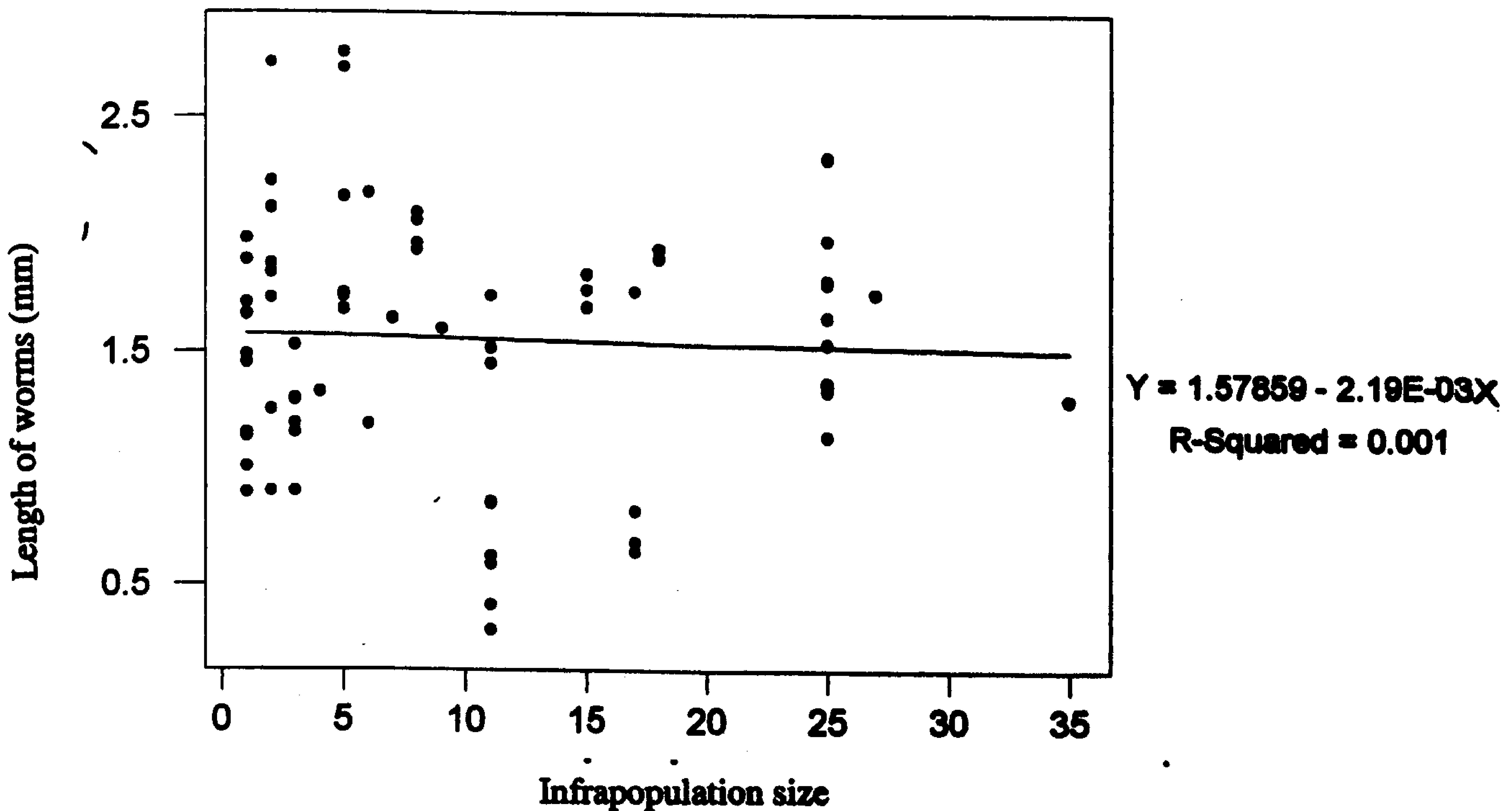


Fig 3.12 Relationship between length of maturing ***Proteocephalus filicollis*** and infrapopulation size.

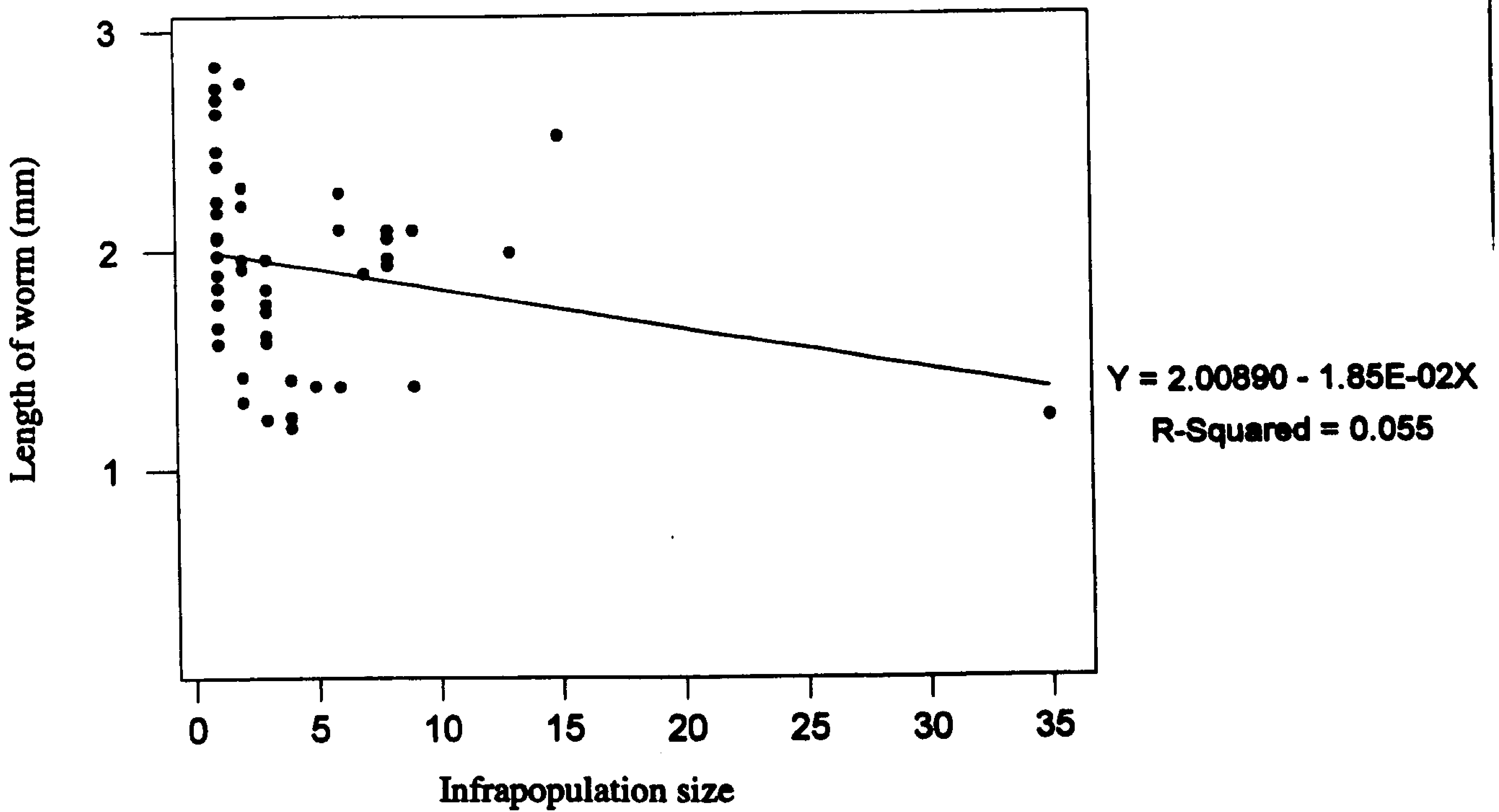


Fig 3.13 Relationship between length of mature *Proteocephalus filicollis* and infrapopulation size.

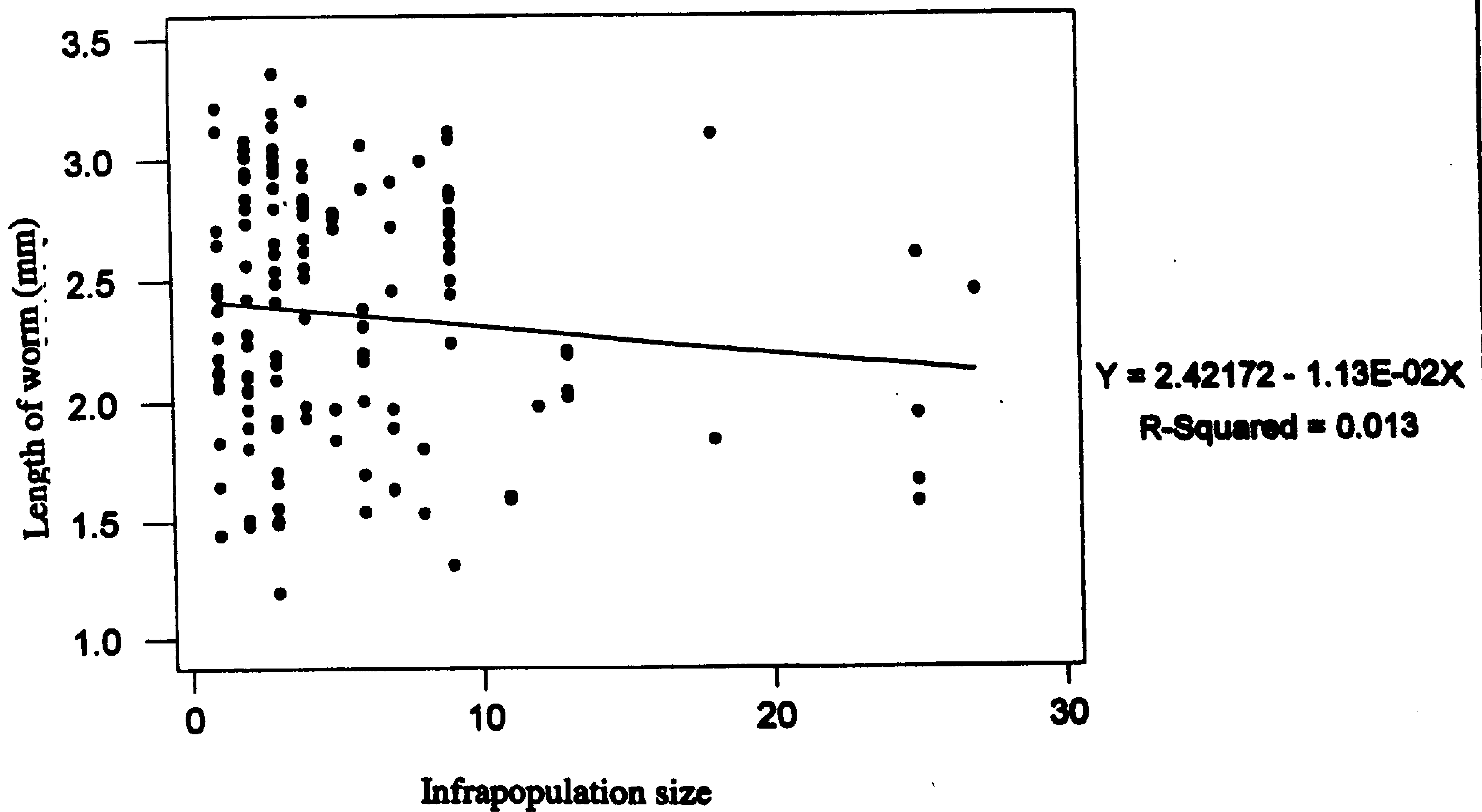


Fig 3.14 Relationship between length of gravid *Proteocephalus filicollis* and infrapopulation size.

3.7 Discussion.

3.7.1 Prevalence and abundance in relation to sex of host.

More female than male sticklebacks were caught from Airthrey Loch at all times of the year. No significant difference in overall prevalence rate between male and female fish was observed or in any particular season. Similarly the abundance in male and female sticklebacks was not significantly different. No sexual preference by *P. filicollis* was recorded in previous studies from sticklebacks (Hopkins, 1959; Chappell, 1969b; Dartnall, 1972). Similarly Fischer & Freeman (1969) reported no significant difference in the prevalence and mean intensity of penetrating plerocercoids of *P. ambloplitis* in male and female smallmouth bass from a single sample of 24 fish

3.7.2 Monthly prevalence and abundance of *Proteocephalus filicollis* in *G. aculeatus*.

The prevalence rate (38.3 %) of *P. filicollis* in sticklebacks from Airthrey Loch was high compared to that recorded in earlier investigations on this cestode from other freshwater geographical localities, e.g. 22.8 % in *G. aculeatus* from Scotland (Hopkins, 1959), 16 % from ten-spined sticklebacks in the Netherlands (Willemse & Veltman, 1962), 31.9 % from *G. aculeatus* in England (Chappell, 1969a), 5 % from *G. aculeatus* in Norway (Rodland, 1979). A high prevalence rate (40.2 %) was recorded from single sample of 82 fish (Willemse, 1969) in the Netherlands and 41 % prevalence rate from three samples (87 fish) by Dartnall (1972) in England.

Much higher prevalence rates have been reported for other species of *Proteocephalus* in their definitive host, 93.8 % for *P. stizostethi* in yellow pike perch in the U.S.A (Connor, 1953),

96.5 % for *P. torulosus* in dace in England (Kennedy & Hine, 1969), 100 % for *P. tetrastomus* in smelt, *Osmerus eperlamus* (Willemse,1969), 53.6 % for *P. percae* in perch in England (Wootten, 1973), about 60 % for larval *P. ambloplitis* in largemouth bass (Eure, 1976), 88 % for *P. torulosus* in chub (Scholz, 1990), 61 % for *P. neglectus* from rainbow trout, (Hanzelova, Zitonan & Sysoev,1990) and 88.8 % for *P. jandia* in catfish, (Pertierra & Nunez, 1990). On the other hand a low prevalence level of < 10 % is reported for *P. macrocephalus* in the European eel (Nie & Kennedy, 1991). The eutrophic nature of Airthrey Loch which increases the abundance and diversity of the zooplankton and large stickleback population may have influenced the higher transmission rate of *P. filicollis* population into the final host and resulted in higher prevalence and abundance of *P. filicollis* than reported by other authors.

The population of the 1994-95 generation of *P. filicollis* in *G. aculeatus* from Airthrey Loch was much higher than the 1993-94 generation. The reasons for this are not clear but may be associated with a warm late summer and autumn 1994 compared with 1993 (Fig.2.3).

The higher temperatures of the summer and autumn 1994 may have operated in three ways i) by enhancing the feeding rate of sticklebacks, ii) by favouring the establishment of worms in the stickleback and iii) producing higher biomass of plankton thus resulting in higher population of larval worms.

However, this data must be treated with caution since water temperature sampling was very restricted. It seems quite likely that a higher zooplankton population and also stickleback population in the summer and autumn of 1994 may have enhanced parasite transmission and thus led to larger parasite population. Parasite population may fluctuate on a year to year basis as reported by Kennedy (1996) who found there was a clear difference in timing of

transmission from year to year and subsequent infection level in *E. crassum* infecting brown trout.

Kennedy (1977) stated that the transmission rate of a parasite may determine the size of the parasite population, and many biological and environmental factors may in turn influence the transmission process. On the other hand Nie & Kennedy (1991) found that *P. macrocephalus* had lower prevalence and abundance in eels and they suggested that it merely reflected the low transmission rate of the plerocercoids to eels.

The composition and abundance of suitable intermediate hosts in a locality may contribute to the distribution and infection level of a parasite. This view has been supported by number of laboratory studies on *Proteocephalus* sp indicating that more than one species of *cyclops* sp may act as intermediate host in these cestodes (Wagner, 1954; Freeman, 1964; Fischer,1968; Wootten, 1974).

3.7.3 Maturation of *Proteocephalus filicollis*.

Proteocephalus filicollis, like many other cestode parasites of freshwater fish in temperate climates, shows an annual maturation and growth cycle. In the present study an increase in length of the worms, which may be considered as an indicator of growth was found to be a continuous process.

Hopkins (1959) found gravid *P. filicollis* only in June and July, whilst Chappell (1969a) on the other hand reported that gravid parasites made up 31-48 % of the *P. filicollis* population throughout the year. The results of the present study are rather intermediate between these two extremes. Gravid worms appeared from September 1993 to June 1994, comprising < 9 % of the total population from September to April but 63 % & 75 % in May and June

respectively. In the 1994-95 generation of *P. filicollis* a small proportion of gravid worms (< 10 %) were found in September, October, November and from February to March 1995, but they comprised 26–65 % of worm population from April to June 1995 and dropped to < 15 in July. Thus reproduction does exhibit seasonality with egg production taking place mainly in the spring and early summer. This type of reproductive cycle may have adaptive significance for parasites requiring an intermediate copepod host as it ensures that most eggs are released at the time of maximum copepod population (mid spring to early summer as observed in the present study for *P. filicollis*) when large numbers of susceptible copepods are available for infection.

In Airthrey Loch the *P. filicollis* population increased in length throughout its period in the stickleback, although the rate of length increase, after an initial rapid period following the recruitment of the new parasite generation slowed over the autumn before rising in the spring as the worm population matures. Hopkins (1959) suggested that growth and development of *P. filicollis* is checked at the plerocercoid stage during the period of low temperature from September until following spring. Chappell (1969a) on the other hand, did not find a definite seasonal pattern in maturation and growth in *P. filicollis* in England. Although the annual cycle of growth and maturation in these three studies overlapped, the majority of the worm population became mature and gravid in spring and early summer. There may be genetic differences in three populations of *P. filicollis* which accounts for the range of the maturation cycle at the three sites.

Although the spring increase in temperature appears to be a major factor influencing the final growth and maturation of *P. filicollis*, there is also a fall in the mean intensity of infection during this period. The latter may also reduce competition within the parasite infrapopulation

at a time when the metabolic requirement of individual cestodes associated with growth and egg production is presumably increasing. The cestodes are expelled from the stickleback after egg production.

What mechanism is responsible for this apparent loss of worms is not very clear, although a number of hypotheses have been put forward (Granath & Esch, 1983b); i.e. temperature dependent rejection, originally proposed by Kennedy (1969); immune response; crowding effect, such as intraspecific competition. The latter may be important in the loss of *P. filicollis* in the spring. Temperature related rejection has been suggested to decrease prevalence and abundance in some other fish and amphibian cestodes when they become mature (Kennedy, 1969; Jorrol, 1979). Selective mortality of heavily infected hosts would also reduce overall parasite population size although there is no evidence that this occurred in the present situation, although it has been demonstrated with more pathogenic cestodes such as *B. acheilognathi* (Granth & Esch, 1983c; Korting, 1984) and in *P. exiguus* infection of *C. peled* (Bauer *et al.*, 1981).

Host immune response also does not appear a likely explanation of worm loss, as immune responses are considered to be only secondary regulators of adult cestode populations (Holmes *et al.*, 1981). Therefore, temperature related rejection appears to be a possible cause of the parasite mortality When water temperature rises most of the parasite are lost from the host.

The reason for the apparent discrepancy between the results of the present study and that of Chappell (1969a) and Willemse & Veltman (1962) who all found gravid worms through out most of the year, compared with the results of Hopkins (1959) who observed a very restricted reproductive season, is unclear. It may lie in environmental differences between the

habitat studied, unfortunately Hopkins (1959) did not provide any information on water temperature. Nevertheless it seems clear that *P. filicollis* has a definite period of maximum reproduction in Spring and early summer coinciding with a period of rising water temperature. Temperature has been proposed as an explanation for the seasonal maturation of *Proteocephalus* sp in their hosts (Kennedy, 1977). Willemse (1969) recorded a rapid differentiation of immature *P. percae* to adult worms in perch after an increase in temperature of the experimental water.

The temperature hypothesis of maturation has been questioned by a numbers of authors (Kennedy & Hine, 1969; Eure, 1976). Wootten (1974) observed that *P. percae* were maturing as the environmental temperature was falling. Chubb (1963) also found that growth in *Triaenophorous nodulosus* was correlated with decreasing temperature. An increase in temperature alone did not cause *C. laticeps* to mature (Kennedy, 1969). Eure (1976) reported that the appearance of adult *P. ambloplitis* coincided with a decrease in water temperature. These results may be explained if parasite maturation was related to the hormonal state of the host. Kennedy (1969) suggested that maturation of *C. laticeps* in dace appear to be governed by changes in fish hormones. The period of maturation and egg production could easily be influenced by many other factors, e.g. the physiological state of the host (Halvorsen, 1972). Wootten (1974) suggested that factors other than water temperature are involved in controlling the onset and continuation of maturation of *P. percae.* Host maturation has also been considered to have some influence on the growth of *P. filicollis* (Rodland, 1983).

Forbes *et al.* (1989) in experimental studies with *B. luciopercae* from *P. fluviatilis* used host gonadotrophin to initiate and stimulate the early phase of gametogenesis of the parasite. The gonadotrophin may activate the parasites' own endocrine system, which then assumes control

of subsequent spermatogenesis and oogenesis. This mechanism provides a potential explanation for seasonal maturation of helminths of the fish in polar and tropical zones of the world, as suggested by these authors. Hanzelova *et al.* (1990) suggested that destrobilation of *P. neglectus* was caused by the poor physiological state of trout during spawning and the immune response of the host to preceding invasions. In the same paper these authors mentioned Malakhove & Anikieva s' (1976) findings that long term high temperature accelerated the maturation of *P. exiguus* in *Coregonus* sp. Pertierra & Nunez (1990) found that in *P. jandia* the maturation cycle is not triggered by annual temperature changes but rather that it is related to the reproductive cycle of the fish and thus governed by host hormonal level.

The fish endocrine system is similar to that of all vertebrates and is governed by the hypothalmic-pituitary axis. Environmental factors act as triggers for gonadal development and for reproduction in many species of fish (Lam, 1983).

In many fish reproducing in spring and early summer, gonadal development is stimulated by photoperiod, particularly in association with raised temperature. Maturation of stickleback at long photperiods is accelerated at high temperature (20° C) but maturation will occur under long photoperiod at temperature down to 10° C (Baggerman, 1957, 1980). The majority of adult *P. filicollis* were recorded in spring and summer during the present study when water temperatures rose and photoperiod was increasing, which would have a direct effect on the host in stimulating gonadal development, which in turn may influence the maturation of *P. filicollis*.

3.7.4 Recruitment of *Proteocephalus filicollis* in the host population.

The peak period of recruitment was July and August when all worms were plerocercoiforms. Hopkins (1959) reported only unsegmented plerocercoids in August and September. Chappell (1969a) found the highest percentage of plerocercoids of *P. filicollis* between November and January. The peak period of recruitment observed in July and August 1993 was repeated in the same months during 1994. Plerocercoids were found to occur in all months, except in May and June 1994. The plerocercoid population remained < 17 % of the total parasite population from October to June throughout the study period. The population of immature worms dropped gradually from July to the following early summer. A similar trend in the population of immature worms was reported by Hopkins (1959), although Chappell (1969) observed an increase in plerocercoids to mid summer.

Recruitment has been reported to occur for various lengths of time during the year in different species of *Proteocephalus* and other fish cestodes. Connor (1953) found immature *P. stizostethi* from yellow pike perch for a very short time in mid September only, whereas Kennedy & Hine (1969) reported continuous recruitment of *P. torulosus* in dace from November to January. In contrast Scholz (1989) found recruitment of *P. torulosus* in chub from end of autumn to the beginning of summer. Fischer & Freeman (1969) observed peak recruitment period of *P. ambloplitus* in smallmouth bass in May which dropped gradually as summer progressed. On the other hand, occurrence of larval *P. ambloplitis* in large mouth bass throughout the year was reported by Eure (1976). Wootten (1974) found that infestation of perch by *P. percae* took place for a limited period of time from June onward or not longer than two or three months. Ieshko & Anikeva (1992) found that *P. percae* in perch showed recruitment from summer to autumn.

Pertierea & Nunez (1989) reported that invasion of catfish with *P. jandia* was a continuous process. Hanzelova *et al.* (1990) found invasion of rainbow trout with *P. neglectus* from mid June to November. Nie & Kennedy (1992) found that recruitment of *P. macrocephalus* in European eel from June onwards in the River Clyst and August onwards in the River Otter in England.

The period of recruitment also varies considerably in other types of fish cestodes. Chubb (1963) stated that the infective plerocercoids of *T. nodulosus* are found throughout the year, but in July and August the entire population of the worm was plerocerciform. Kennedy (1969) found that infection of dace with *C. laticeps* started in December and built up to a peak in February and the size of population decreased until August when infection disappeared. Granath & Esch (1983a) reported the recruitment of *B. acheilognathi* in ambient and thermally altered areas of North Carolina reservoirs from early June to October and from May to October / November. Leong (1986) reported the occurrence of *B. acheilognathi* in *P. inotatus* from October to February and no infection of fish between March & September. Marcogliese & Esch (1989) reported the continuous recruitment of *B. acheilognathi* in mosquito fish in a North Carolina cooling reservoir.

In 1993 & 1994 recruitment of *P. filicollis* occurred one month after the loss of the previous generation, but in 1995 overlap of two generations was also observed. Meggitt (1914) did not clearly describe recruitment of *P. filicollis* in stickleback, but pointed out that every fish in autumn was infected, whereas Hopkins (1959) found that recruitment of *P. filicollis* in stickleback was at its peak from July to September and it continued until November. Willemse (1968) reported recruitment of *P. filicollis* in autumn. Rodland (1979) reported a high recruitment of *P. filicollis* in stickleback in July and August, when 100 % of the worm

population was plerocercoid. In contrast, Chappell (1969a) reported peak period of recruitment in November and January. Moreover, Chappell (1969a) observed plerocercoids throughout the year.

Although small plerocerciform *P filicollis* were found throughout most of the year it is not clear whether those worms found outside the peak Summer recruitment period were new arrivals or whether they were individuals which had merely failed to develop. The occurrence of gravid worms over the autumn and winter months and the fact that their eggs were infective (Chapter 6) may indicate that some limited recruitment can occur in this period. On the other hand heavy initial infection / recruitment by helminths followed by heavy mortality, has been reported in experimental and field studies for example *Pomphoryhnchus laevis* in rainbow trout (Bates & Kennedy, 1991), in *P. percae* and *P. torulosus* (Scholz, 1986; Moravec & Scholz, 1994) and *Bothriocephalus claviceps* (Scholz, 1986; Nie & Kennedy, 1992) *Bothriocephalus acheilognathi* (Riggs & Esch, 1987) and *E. crassum* (Wootten, 1972; Kennedy, 1996).

3.7.5 Infection and length of the host.

A rising trend in prevalence of *P. filicollis* with increasing host length was observed, but this did not show significant variation in all four length classes of fish. Abundance was high in the lowest length class and lowest in the highest length class. The drop in mean number of parasite in larger fish may reflect dietary and behavioural changes in older fish. No significant variation in the prevalence of *P. filicollis* with increasing fish size was observed in this study. The result of this study agrees with those of earlier studies on *P. filicollis*. Hopkins (1959) found that 2-3 cm (0 + year class) fish were more heavily infected than 1+ year class.

Chappell (1969) showed a rising mean intensity with length although this fell in the largest fish. A significant decrease in prevalence with fish size, and reduction in intensity was observed in *P. filicollis* from the Chew River in England (Dartnall, 1972). The degree of infection increased with fish size in *P. torulosus* in dace (Kennedy & Hine, 1969) and mean intensity of infection of *P. perace* tended to rise with increase in length of perch (Wootten,1972). Prevalence and mean intensity of *P. neglectus* also rose with increasing length of chub (Scholz,1989), reaching a maximum 56 % and 6.7 in the largest fish. In contrast to this, *Caryophyllaeus laticeps* in dace was spread throughout all the size and age groups of fish (Kennedy, 1969). Significant variation in infrapopulation density of *B. acheilognathei* occurred in different length classes of the mosquito fish (Granath & Esch, 1983). They stated that the largest size class of mosquito fish had a lower density of *B. acheilognathi* than the other two size classes.

The prevalence and mean density of infestation of *B. acheilognathi* in *Puntius binotatus* was highest in the smallest size group of fish (Leong, 1986). No significant effect of length of European eel on the abundance of *B. claviceps* was observed (Nie & Kennedy, 1992).

3.7.6 Distribution of parasite in the host population.

The population of *P. filicollis* in stickleback from Airthrey Loch was overdispersed throughout the study period. The overdispersion of parasite populations in natural hosts populations is well described by many authors (Crofton, 1971a,b; Pennycuick, 1971; Anderson, 1974; Gordon & Rau 1982). Anderson & Gordon (1982) suggested that the overdispersed pattern of parasite numbers per host in natural habitats acts to increase density dependent regulation of both host and parasite abundance. These authors stated that the

mechanism of "environmental stochasticity" implies the rate processes which govern the population growth of parasite species. These rate processes are birth, death, immigration and emigration which are not constant for a given species and depends on environmental factors like climate, host susceptibility and host behaviour. Anderson & Gordon (1982) suggested that for parasites in which a host is the main environment for population growth, differences in host behaviour or host susceptibility are the major factors generating overdispersion in the distribution of the parasite within the host population.

The higher variance to mean ratio is generated by the continued acquisition of parasites by the fish host. The degree of dispersion of *P. filicollis* as measured by variance to mean ratio was variable in different months. Higher values corresponded to higher numbers of parasite in fewer hosts. There was a general trend of rising variance to mean ratio from mid-summer to early winter (the time of peak recruitment), with a fall from mid-winter to early spring to summer. A peak observed in July 1994 was due to one heavily infected fish in that month. Overdispersion of *Proteocephalus* species has been reported by other authors (Kennedy & Hine, 1969; Wootten, 1974; Hanzelova *et al.* 1990) and in *B. claviceps* (Nie & Kennedy, 1992).

3.7.7 Parasite length.

Variation in the mean length of *P. filicollis* indicated that the parasite grows throughout the year. The variation in the mean length is greatest in spring and summer indicating the influence of water temperature on growth. The length of different maturity stages of *P. filicollis* is overlapping, indicating a different rate of development of individual worms and or repeated or continuous infection in the same host. The segmentation starts between 2-3 mm,

genital structures normally appear at 4-7 mm and size of gravid worms in spring and summer ranges from < 4 mm but the majority exceed 9 mm.

Hopkins (1959) stated that the development of *P. filicollis* is an annual cycle with a few gravid 1-year old worms overlapping the new seasons' population. Worms start segmentation at 5-6 mm in length and genital rudiments appear at 6 and 8 mm whereas gravid worms exceed 10 mm. Willemse (1968) found that at 3 mm proglottids start appearing and genital complex was visible when worms were 5 mm in length and eggs were present in 10 mm long *P. filicollis* worms. On the other hand Chappell (1969) found that there is no seasonal cycle in *P. filicollis* and the length of the worms in various stages of maturity were very clearly overlapping with each other. The length range of *P. filicollis* in different maturity stages observed in present study is quite comparable to the previous studies. A small proportion of gravid worms recovered in the present study were smaller in length than those reported in above mentioned studies which may be due to early infection of the fish with worms which matured prior to the peak period of maturation i.e.spring and early summer.

The pattern of increase in length seen in *P. filicollis* in the present study has been observed in other species of cestodes from temperate waters. Kennedy & Hine (1969) stated that the period of greatest length increase in *P. torulosus* always coincided with the period of rising temperature in spring. Wootten (1972) reported that the increase in the mean length of *P. percae* was high between June and September, with little increase in October, November and December and a considerable increase in the length in March, April and May. Nie & Kennedy (1991) also found that although there was an increase in the length throughout the year mean length of *P. macrocephalus* increased most rapidly from April to June when most of the gravid worms were found.

Although the length of all maturity stages of worms has not shown any significant relationship with infrapopulation size, it was observed that length of worms increased with season.

CHAPTER 4
HISTOPATHOLOGY, LOCATION AND MIGRATION OF PROTEOCEPHALUS FILICOLLIS IN THE INTESTINE OF GASTEROSTEUS ACULEATUS

4. Histopathology, location and migration of *Proteocephalus filicollis* in the intestine of *Gasterosteus aculeatus*.

4.1 Introduction.

4.1.1 Pathology.

Williams (1967) reviewed the literature on the helminth diseases of fish and concluded that fish parasites may harm their hosts in a variety of ways, i.e. (1) by causing mechanical injury such as irritation, injury or atrophy of tissues and occlusion of the alimentary canal, blood vessels or other ducts; (2) introducing toxic metabolic byproducts able to produce changes in the blood, enzymes, vitamin and /or hormone activity of the host; (3) depriving the fish of food; (4) acting as carrier or vectors of other pathogens. The host reaction may be expressed as tissue proliferation, degeneration and inflammation and probably, the development of immunity.

Williams & Jones (1994) reviewed the pathogenic effects of helminths on their fish hosts. Cestodes are reported to cause mechanical obstruction, displacement and flattening of the villi, mechanical injury by hooks, focal pressure necrosis, loss of epithelium at or near the point of attachment, hyperplasia of mucosa, haemorrhage and destruction of mucosa. All these effects result in formation of lesions, ulcers, nodules or diverticula, calcareous, collagenous or fibrous collars or capsules around parasites penetrating within the intestinal wall, fibrosis and the development of granuloma. Despite the abundance of cestodes in all groups of fish there are relatively few well documented cases of their causing significant harm.

A number of cases of fish mortalities have been reported in wild and farmed fish populations due to infection with intestinal cestodes. *Caryophyllaeus fimbriceps* infects

one and two year carp in ponds and causes mortality (Bauer *et al.,* 1973). Similarly, *Bothriocephalus acheilognathi* has been reported to cause 100 % mortality in carp fry in European ponds (Korting, 1984). *Proteocephalus exiguus* infects *Coregonus peled* and in some cases causes mortality (Bauer *et al.*, 1981).

Damage is most severe with species which penetrate the intestinal wall, however, this does not necessarily lead to mortalities. The most pathogenic species are those which lack specialized attachment organs (Williams & Jones, 1994).

Hayunga (1979) found that in white sucker (*Catostomus commersoni*) infected with the caryophyllidean, *Hunterella nodulosa* there was a loss of intestinal epithelium and lamina propria and a chronic inflammatory response, with infiltration of lymphocytes and extensive hyperplasia of the submucosal tissue. *H. nodulosa* produces a nodule with chronic inflammation. The parasite is separated from host tissue by an amorphous layer of electron lucent material, which acts as an adhesive.

Jauella glandicephalus, a proteocephalid, perforates all layers of the intestine of *Paulicea lutkeni*, a freshwater fish from Brazil, causing severe haemorrhage and intensive necrosis. An intense host reaction results in hyperplasia of connective tissue and the formation of nodules surrounding the scolex of the parasites and lymphocyte infiltration (Rego & Pavenelli, 1985; Eiras *et al.*, 1986).

Wakbuke-bunoti (1980) reported that the pseudophyllidean *Polyonchobothrium clarias* was abundant in the gall bladder of *Clarias mossambicus* where they caused nodules. These nodules ranged from one to nine per infected fish and each nodule carried one to five worms. The bothria of *P. clarias* penetrate the intestinal mucosa, resulting in inflammation of the epithelium.

Some species of cestodes do not penetrate the intestinal wall but are nevertheless pathogenic to their fish host. McVicar (1972) investigated the fine structure of the parasite-

host interface of three tetraphyllidean tapeworms, *Acanthobothrium quadripartitum*, *Phyllobothrium piriei*, and *Echeneibothrium* sp in *Raja naevus* Muller & Henle. He found that the bothridia of *Echeneibothrium* and *A. quadripartitum* damage the host intestine wall and the bothridia of both left a distinct imprint on the villi. The microvillous border of mucosal cells was compressed inside the rim of the bothridium but was undamaged. *Phyllobothrium piriei* caused considerable damage to the cells of the gut mucosa. Microvilli were stunted, abnormal and even completely absent from the epithelial cells.

Glaridacris catostomi and *Glaridacris laruei* infect white suckers. *Glaridacris laruei* causes a minor but distinct distortion in the gut topography, whereas *G. catostomi* with its weak attachment organs and tapered scolex causes compression of the intestinal folds (Hayunga, 1979).

Scott & Grizzle (1979) studied the pathology of *Bothriocephalus acheilognathi* in three cyprinid fish, the grass carp, shiner and fathead minnow. The attachment of the scolex of the parasite to the tissue of the intestine resulted in some mechanical damage. Focal pressure necrosis of the mucosa occurred where the edges of the bothria pressed against the lining of the intestine. Lymphocyte infiltration into the mucosa and submucosa was high and there was occasional haemorrhage.

Megathylacus brooki, a proteocephalid found in the intestine of *P. lutkeni*, causes necrosis and destruction of epithelium, haemorrhage and lymphocyte infiltration in the area of attachment of the suckers, even though the intestine is not perforated (Rego & Pavenelli, 1985; Eiras *et al.*, 1986).

Pronina & Pronin (1982) reported that infection of *Triaenophorus nodulosus* in the intestine of pike causes acute inflammation and structural rearrangement in the intestine. In some cases severe erosions occurred in the mucosa. In chronic cases intestinal folds were

stretched and thinned and partial atrophy of the folds occurred at high intensity of infestation.

The host-parasite interface of *Caryophyllaeus laticeps* in bream, chub and roach was studied by Karanis & Taraschewski (1993) using electron microscopy. At the attachment site of scolices of medium to large size worms the intestinal epithelial cells were vacuolated and their brush border was ruptured. The pathology was more pronounced in bream, which also showed conspicuous granulomas in the tunica propria adjacent to the scolices. In chub and roach, at the site of attachment of the worm, heavy infiltration of eosinophilic granulocytes was noted. Chronic infection was associated with the formation of granulomas in bream.

Infection of carp with *Khawia sinensis* induced loss of microvilli, compression of enterocytes and shedding of host material into the gut (Morley & Hoole,1995).

Intestinal cestodes may provoke changes in the blood and metabolism of their fish host in addition to histopathological effects. Inappetance, reduction in growth and emaciation, sometimes culminating in mortalities, are clinical signs of infection. Parasitized fish may also show behavioural changes, which make them vulnerable to predation and reduces their resistance to stress.

Reduction in haemoglobin level, in erythrocytes, monocytes, polymorphocytes and neutrophils is caused by *Khawia sinensis* infecting *C. carpio*. Infection of *C. carpio* with 35-45 *K . sinensis* were found to be fatal. Similarly, haemoglobin level is reduced in *Clarias batrachus* due to infection by *Lytocestus indicus* (Willaims & Jones, 1994).

The infection of *C. carpio* with *B. acheilognathi* resulted in a reduction in haemoglobin and total blood volume and higher numbers of leucocytes and phagocytes (Sapazhnikov, 1969; Kudryashova,1970 as cited by Williams & Jones, 1994). *Bothriocephalus acheilognathi*

caused a fall in body weight and fat content in carp (Musselius *et al.* 1963; Kurovskaya, 1984; cited by Williams & Jones, 1994).

Hoffman *et.al.* (1986) reported that *Eubothrium salvelini* caused blood changes in charr, *Salvelinus alpinus,* similar to those described for *K. sinensis* in *C. carpio.* They found that a significant correlation existed between the degree of haemosiderosis in the spleen and intensity of infection.

Heavy infection of sockeye salmon with *E. salvelini* resulted in inferior growth, poor swimming performance or aberrant behavior which might increase host mortality due to other causes (Smith, 1973; Boyce, 1979).

Bristow & Berland (1991) reported that low level *Eubothrium* spp infection in farmed salmon in Norway has significant effects on the loss of potential growth in both female and male fish. The direct loss of growth was approximately 10 % in fish of market size.

4.1.2 Location and migration of parasite in the intestine of the final host.

The position of *P. filicollis* in the intestine of the stickleback has been reported to vary as the worm matures. Meggitt (1914) found worms only in the intestine of three-spined stickleback and not in rectum. Hopkins (1959) reported *P. filicollis* from the rectum and intestine of the fish. Hopkins described the migration of *P. filicollis.* Over 80 % of the worms less than 5 mm in length were found in the rectum whereas those over 5 mm long had scolices attached in the posterior part of the small intestine and gravid worms were attached close to the pyloric valve.

Willemse & Veltman (1962) found the majority of *P. filicollis* in nine-spined stickleback in the intestine. Willemse (1968) found plerocercoids in the rectum and mature worms in the intestine of stickleback. However, according to Chubb (1982) identification of P. filicollis in nine-spined stickleback is doubtful.Dartnall (1972) found adults of *P. filicollis*

in the intestine of three-spined stickleback. Rodland (1979) also reported the presence of both adults as well as plerocercoids of *P. filicollis* in the intestine of an anadromous population of three-spined stickleback.

Chappell (1969c) found adult and plerocercoid *P. filicollis* in the intestine and less frequently in the rectum of the three-spined stickleback. Chappell (1969c) reported that in concurrent infection of *P. filicollis* and *Neoechinorhynchus rutili* in three-spined stickleback, the distribution of each species in the gut was significantly different from when these species occurred alone. Both adults and plerocercoids of *P. filicollis* attached more frequently in the anterior intestine while individuals of *N. rutili* attached more frquently in the rectum. In single species infection individuals of both species were distributed more widely throughout the gut. Chappell concluded that possibly the adverse effects of each species on the environment of the other result in competitive exclusion.

Proteocephalus neglectus from rainbow trout were always found in the first third of the pyloric caeca of the fish intestine, immediately behind the stomach (Hanzelova *et al.* 1990). On the other hand *P. jandia* in catfish were located in the midgut, extending to the anterior and posterior portions during the months of greater infection level, regardless of the amount of procercoids present in the water and its temperature (Pertierra & Nunez, 1990).

It is well known that the parasites actively select specific sites in their hosts. Some parasites respond to changing conditions within the host by making diel or other short term migrations, which may be modified by the presence of other parasites (Holmes, 1973). A number of studies have revealed that helminths are capable of undergoing various types of migrations within the intestine. Migration may be ontogenic, occurring during growth and maturation of a parasite, or daily as a result of a stimulus such as ingestion of a meal by the host.

Read & Kilejian (1969) reported that under specific experimental conditions, *Hymenolepis diminuta* exhibits a longitudinal circadian migration in the small intestine of the rat. Moreover the timing of the migration was changed by altering the feeding pattern of the host. However, they could not point out any specific stimulus for migration.

Mackenize & Gibson (1970) reported that a trematode, *Podocotyle* sp, and the nematodes *Cucullanous heterochronus*, *Cucullanus minutus* and *Contracaecum aduncum* migrated within the intestine of flounder (*Platichthys flesus,* L.) when food was withheld from the host. William *et al* (1970) found similar evidence of migration in cod (*Gadus morhua,* L.) infected with the trematode *Otodistomum* sp and the cestode *Grillotia* sp.

Bailey (1971) reported that mature *H. diminuta* in rats show two basic behavioural patterns, changes in body length and movement along the small intestine, both exhibiting circadian rhythms. He stated that it is probable that these patterns are adapted to and dependent upon, feeding behaviour of the rats since they are not exhibited by worms in starved rats.

Holmes (1973) concluded that sites selected by parasites, and even short-term movement of some parasites, are modified by the presence of related parasites (or their ecological equivalents). Parasites respond to the regular presence of competitors by niche specialization in essentially the same way as free-living organisms, and segregation is an important aspect of that niche specialization.

Grey (1977) reported that *Raillietina cesticillus* in fowl, undergoes diurnal migration in both multiple and single worm infections of the fowl. Migration involved movement of both scolices and strobilae and occurred in an anterior direction when the intestine was empty of food and in a posterior direction when the intestine contained food. Grey suggested that anterior migration is due to the response of the worm to stimuli provided by

a hungry host, moreover the worm activity may well increase in an empty intestine, being a simple kinetic response resulting in overall movement up the intestine.

Shostak & Dick (1989) reported that the position of scolices and strobilae of the cestode *Triaenophorus nodulosus*, *Triaenophorus crassus* and *Proteocephalus pinguis* did not vary with respect to host stomach content, but in contrast to this the position of *Raphidascaris acus* a nematode, was anterior when the stomach contained partially digested items, posterior when the stomach was empty, and in an intermediate position when the stomach contained only intact items. They suggested that migration of *R. acus*, but not of *T. crassus*, *T. nodulosus* and *P. pingius* is in response to the feeding activity of the host. Both adult and larval *R. acus* migrated, but the extent of migration was reduced in host harbouring *T. crassus*, more so for larval than adult *R. acus*.

4.2 Aims.

The aim of this study was to determine the effect of *P. filicollis* on the stickleback by a histological examination of the intestine of the parasitised fish; and to investigate the location and migration of the parasite in the intestine of fish.

4.3 Materials and methods.

4.3.1 Fish sampling.

The procedure for fish sampling and dissection have been given in detail in chapter three.

4.3.1 Histology.

Proteocephalus filicollis and the intestine at the point of attachment of the worm were fixed in 10 % neutral buffered formalin for at least 24 hours at room temperature. The fixed material was trimmed to an appropriate size and automatically processed in a Histokinette

2000 (see Appendix 2 for schedule). Sections of wax embedded material were cut at 5-6 µm. The cut sections were spread and floated on a water bath at 50° C, placed on a clean glass slide, and the slide placed face down on a hot plate to prevent dust contamination. Slides were dried in an oven at 60^0 C for at least one hour prior to staining. Sections were stained with haematoxylin and eosin (staining schedule is given in Appendix 3).

4.3.3 Location of parasite in the host intestine.

The gut of the dissected fish was divided into four sections as follows:

1. Anterior intestine: S-1
2. Mid intestine: S-2
3. Posterior intestine: S-3
4. Rectum: S-4

The gut was opened from posterior to anterior and then examined under the dissecting microscope using transmitted light at x10 and x 40 magnification. The site of attachment of any worms present was measured from the posterior of the rectum using a scale divided into millimeters. Posterior of the rectum was considered as the lowest position of attachment and anterior intestine as the highest attachment position. The mean position of attachment of worms was calculated for each month to represent results. Number of parasites were counted from each section of the intestine and their maturity was also recorded for later analysis. All four sections of the intestine almost comprised of the same length.

4.4 Results.

4.4.1 Gross pathology.

There were no external clinical signs in the fish infected with *P. filicollis*.

Plerocercoids of the cestode, *Schistocephalus solidus* (Muller,1776) were found in the body cavity of the stickleback (overall prevalence 30.6%, mean intensity 3.3. Up to 66 larval worms were recorded in a single fish). The parasite index (P.I) (Arme & Owen, 1967) from April to June 1993, April to June 1994 and April to July 1995 ranged from12.28 to 61.15 respectively. This infection obscured the relationship of body weight to infection with *P. filicollis* and hence this factor(weight of fish) was not taken into account in respect of fish infected with *P. filicollis.*

The body length of healthy sticklebacks and fish infected with *P. filicollis* throughout the sampling period is given in Fig. 4.1, which shows that there is little consistent difference in the body length of infected and healthy fish.

4.4.2. Histopathology.

There was distention of the intestine in some cases where several *P. filicollis* were attached. The number of parasites varied from 1- 35 per fish and appeared to block the intestine to at least some extent. At most times of the year worms of all maturity stages were observed to be entangled into a mass along the length of the intestine.

Histological examination showed that the intestinal wall was stretched or thinned in areas where worms were present (Plate 4.1 & 4.2). In infections where worms were large and maturing the intestinal folds were eroded and compressed. The intestinal epithelium was lost or was very thin. At the site of attachment the suckers of the parasite grasped the intestinal epithelium (Plate 4.3). There was no detectable host response to the attachment of the parasite.

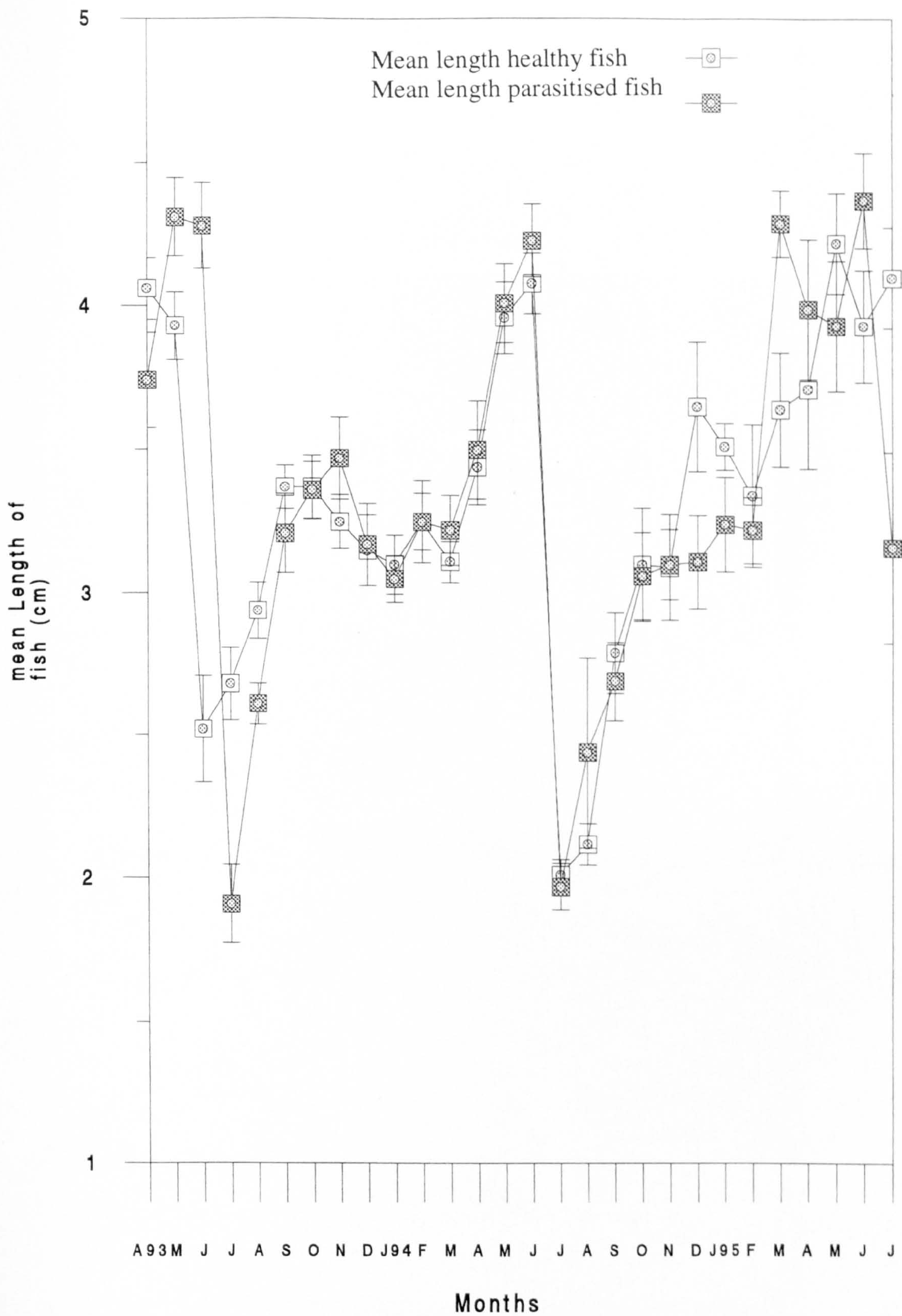


Fig 4.1 Monthly mean length of uninfected and parasitised *Gasterosteus aculeatus*. The bars represent standard errors.

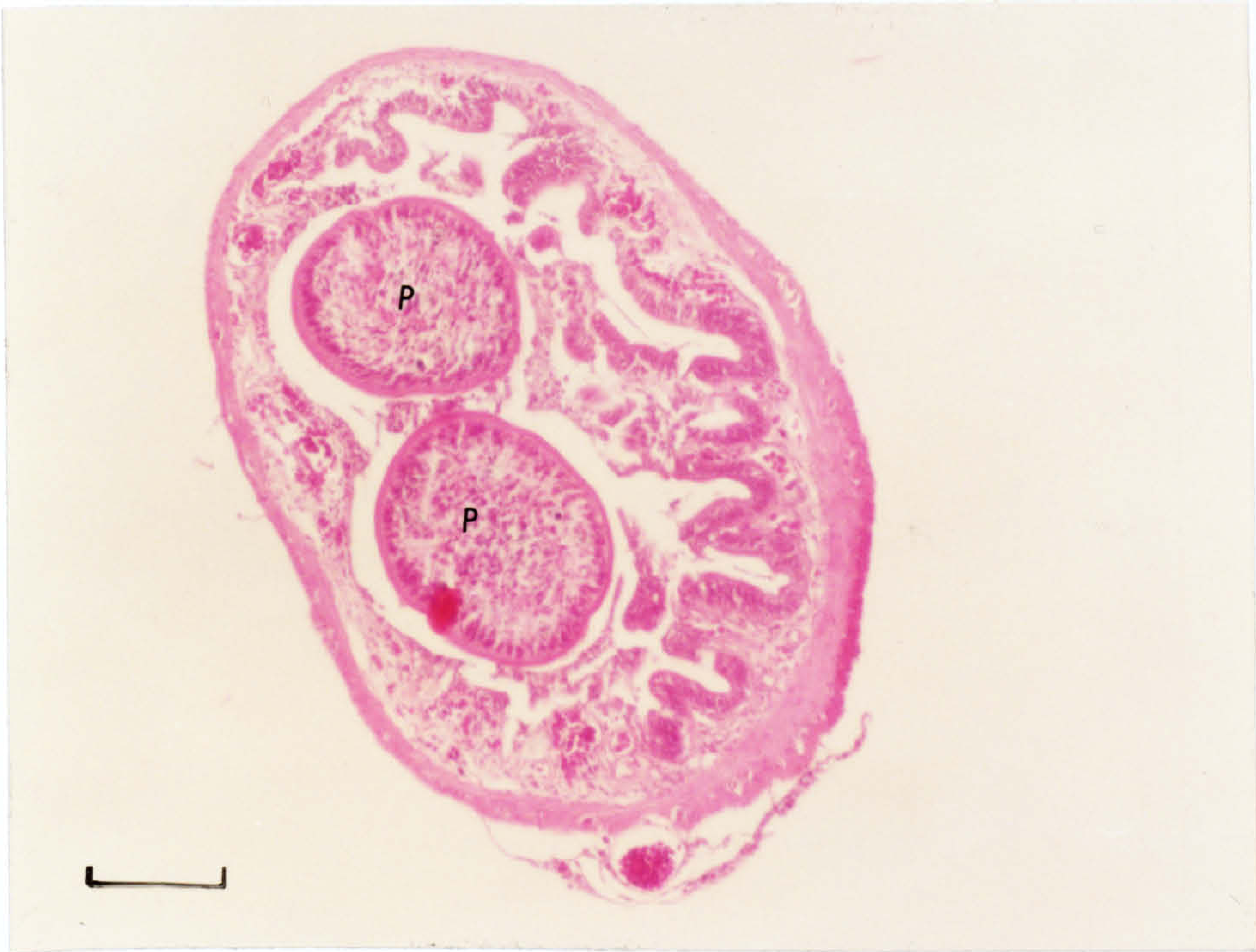


Plate 4.1. A transverse section of *G. aculeatus* intestine partly blocked by two *Proteocephalus filicollis* plerocercoid (P) (H & E) (Scale bar = 100 μ m).

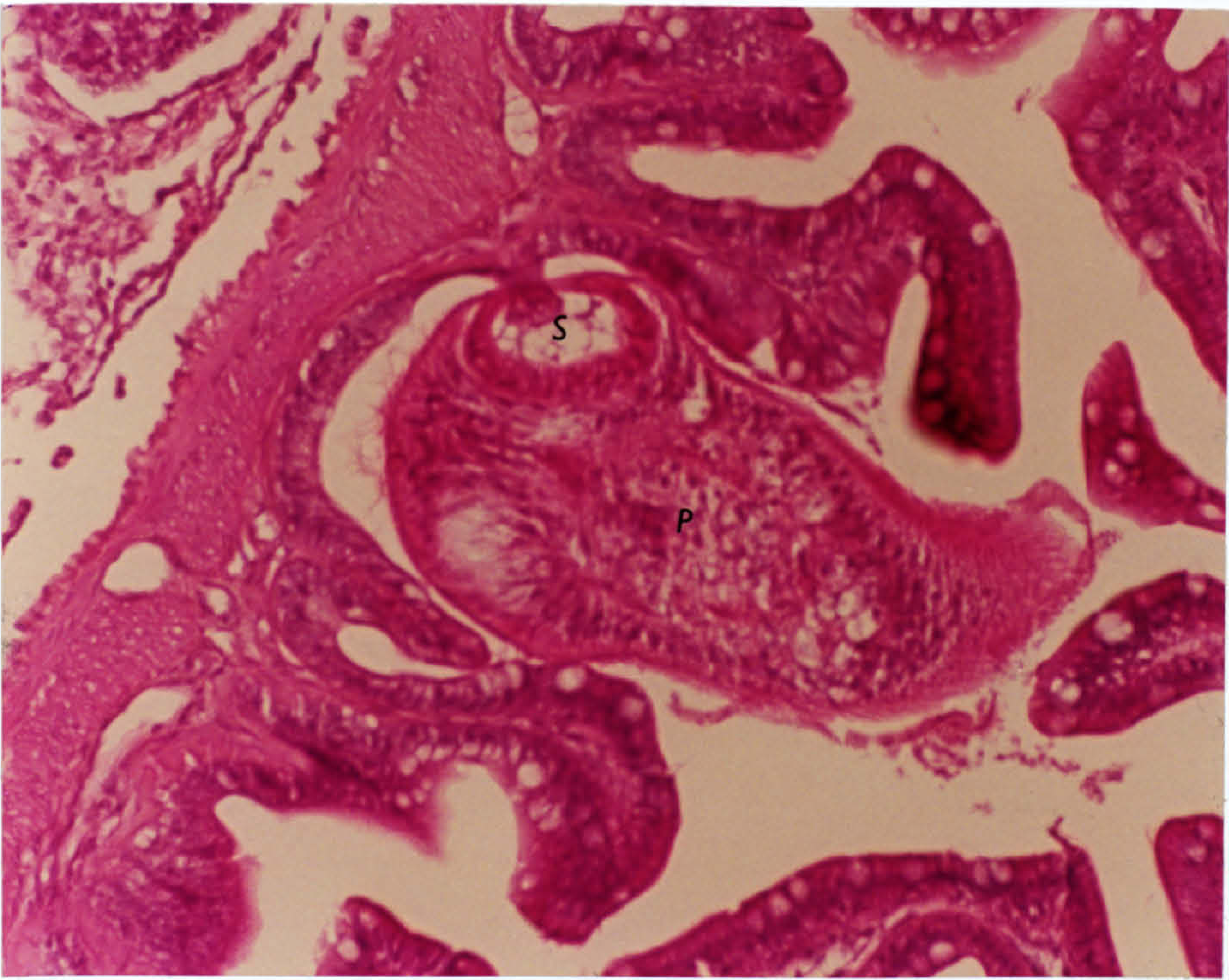


Plate 4.2. A transverse section of *G. aculeatus* intestine; a sucker (S) of *Proteocephalus filicollis* (P) has grasped a portion of epithelium. The area around the scolex is not showing much erosion (H & E) (Scale bar = 100 μm).

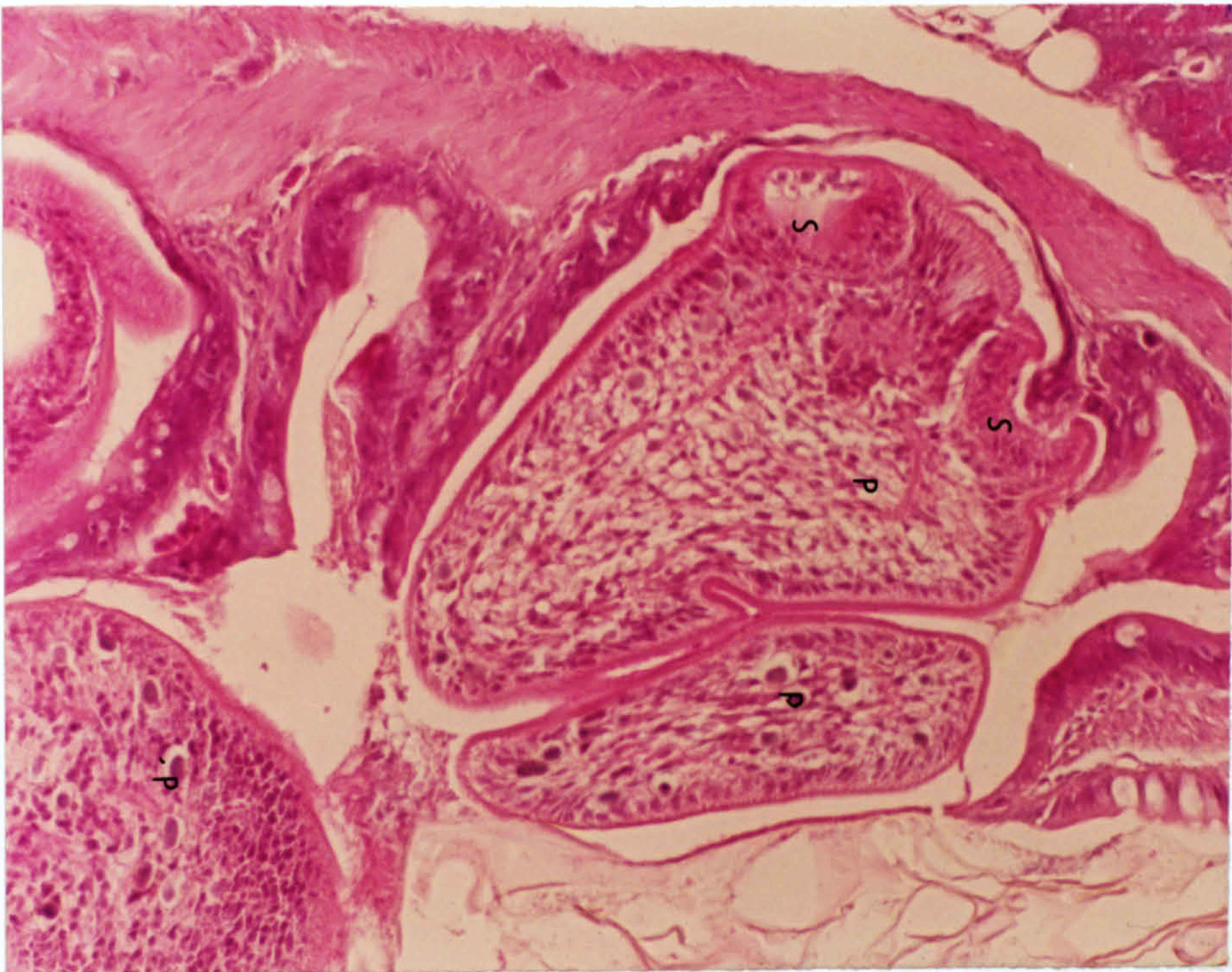


Plate 4.3. A transverse section of *G. aculeatus* intestine; a sucker (S) of *Proteocephalus filicollis* plerocercoid is holding epithelium and a portion of the epithelium is eroded around the scolex (H & E) (Scale bar = 100 μm).

4.4.3 Location of *Proteocephalus filicollis* in the host intestine.

Scolices of *P. filicollis* were found attached in all sections of the intestine (Fig. 4.2). Figure 4.2 shows that half of the entire population (50.4 %) of the worm was recovered from the rectum / section S-4. The second highest worm population (23.12 %) was found in the posterior intestine / section S-3, whereas mid intestine / section S-2 and anterior intestine / section S-1 harboured 17.8 % and < 9 % of the worm population respectively.

4.4.4 Monthly changes in location of *Proteocephalus filicollis* in the host intestine.

The monthly occurrence of *P. filicollis* (number and percentage) in different sections of the intestine of the stickleback is given in Table 4.1. It is clear from Table 4.1 that section S-4 contains the highest proportion of the worm population from August to February. From March to July worms occur mostly in the more anterior sections of the intestine.

4.4.5 Seasonal changes in location of *Proteocephalus filicollis* in the host intestine.

During spring 1993, the highest proportion (46.6 %) of worms were located in the posterior intestine (S-3) and the rectum (S-4) contained 24 % of the worm population. The mid and anterior intestine (S-2 & S-1) accommodated 29 % of the worms. In summer the worm population in the rectum increased to 54.6 %, with the recruitment of the new generation, whereas in the posterior intestine the worm population dropped to 18.7 %. The mid and anterior intestine contained 26.5 % of the parasite population (Fig. 4.3).

During autumn 1993, section S-4 contained 63 % of the worm population and S-3 and S-2 together 35 % of the parasites. In the following winter the worm population in S-4

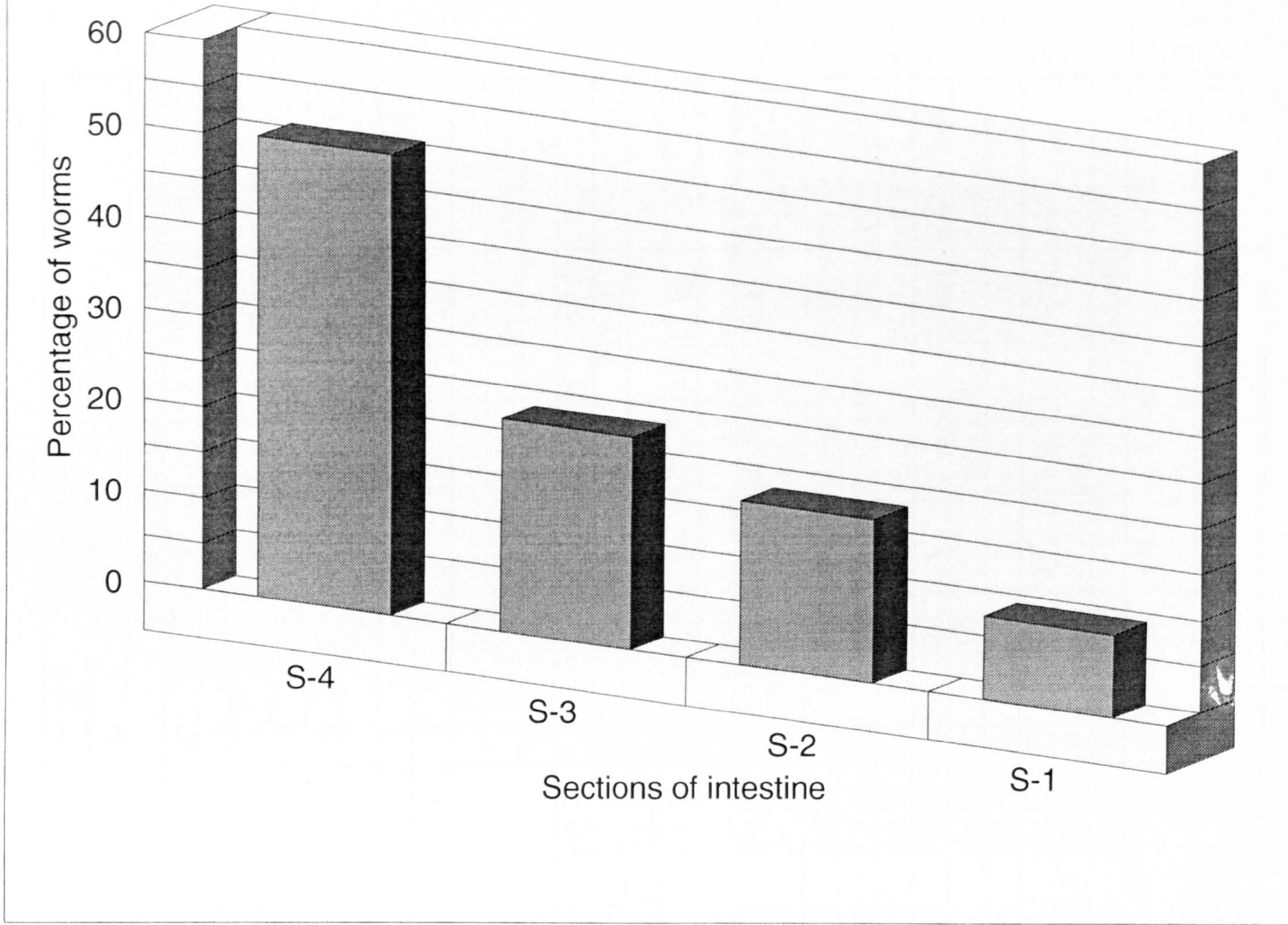


Fig 4.2. Overall location of *Proteocephalus filicollis* in the sections of intestine of *G. aculeatus*.

Table 4.1 Location of *Proteocepalus filicollis* in various sections of the intestine of all *G. aculeatus* examined from Airthrey Loch from April 1993 to July 1995

Months	S-1 (anterior intestine) No %	S-2 (mid intestine) No %	S-3 (posterior intestine) No %	S-4 (rectum) No %	Total
April 1993	15 (12.9)	-	60 (51.7)	41 (35.3)	116
May	25 (28.4)	20 (22.7)	35 (39.7)	8 (09.0)	88
June	2 05.7)	18 (51.4)	15 (42.8)	-	35
July	1 (07.6)	4 (30.7)	6 (46.1)	2 (15.3)	13
August	-	9 (11.2)	3 (03.7)	68 (85.0)	80
September	-	28 (34.1)	14 (17.0)	40 (48.7)	-
October	3 (03.8)	11 (14.1)	15 (19.2)	49 (62.8)	78
November	-	5 (09.8)	2 (03.9)	44 (86.2)	51
December	3 (04.9)	6 (09.8)	30 (49.1)	22 (36.0)	61
January	-	13 (25.4)	6 (11.7)	35 (68.6)	51
February	-	6 (12.0)	11 (22.0)	33 (66.0)	50
March	-	9 (16.9)	26 (49.0)	18 (33.9)	53
April	1 (02.7)	3 (08.1)	22 (59.4)	11 (29.7)	37
May	4 (11.4)	15 (42.8)	15 (42.8)	1 (02.8)	35
June	11 (55.0)	4 (20.0)	5 (25.0)	-	20
July	2 (04.7)	10 (08.9)	52 (46.4)	48 (42.8)	112
August	-	5 (12.1)	8 (19.5)	28 (54.9)	41
September	1 (00.8)	3 (02.4)	30 (24.3)	89 (72.3)	123
October	1 (01.5)	-	2 (03.0)	63 (95.4)	66
November	1 (00.8)	4 (03.3)	8 (06.6)	107 (89.1)	120
December	6 (05.3)	11 (09.7)	9 (07.9)	87 (76.9)	113
January	1 (02.3)	8 (18.6)	7 (16.2)	27 (62.7)	43
February	7 (05.6)	18 (14.6)	14 (11.3)	84 (68.2)	123
March	3 (04.0)	15 (20.0)	27 (36.0)	30 (40.0)	75
April	2 (04.4)	23 (51.1)	2 (04.4)	18 (40.0)	45
May	37 (22.5)	79 (48.1)	27 (16.4)	21 (12.8)	164
June	43 (59.7)	23 (31.9)	6 (08.3)	-	72
July	1 (03.7)	3 (11.1)	-	23 (85.0)	27
Total	170	352	457	997	1976
% of worm	8.60	17.86	23.12	50.45	

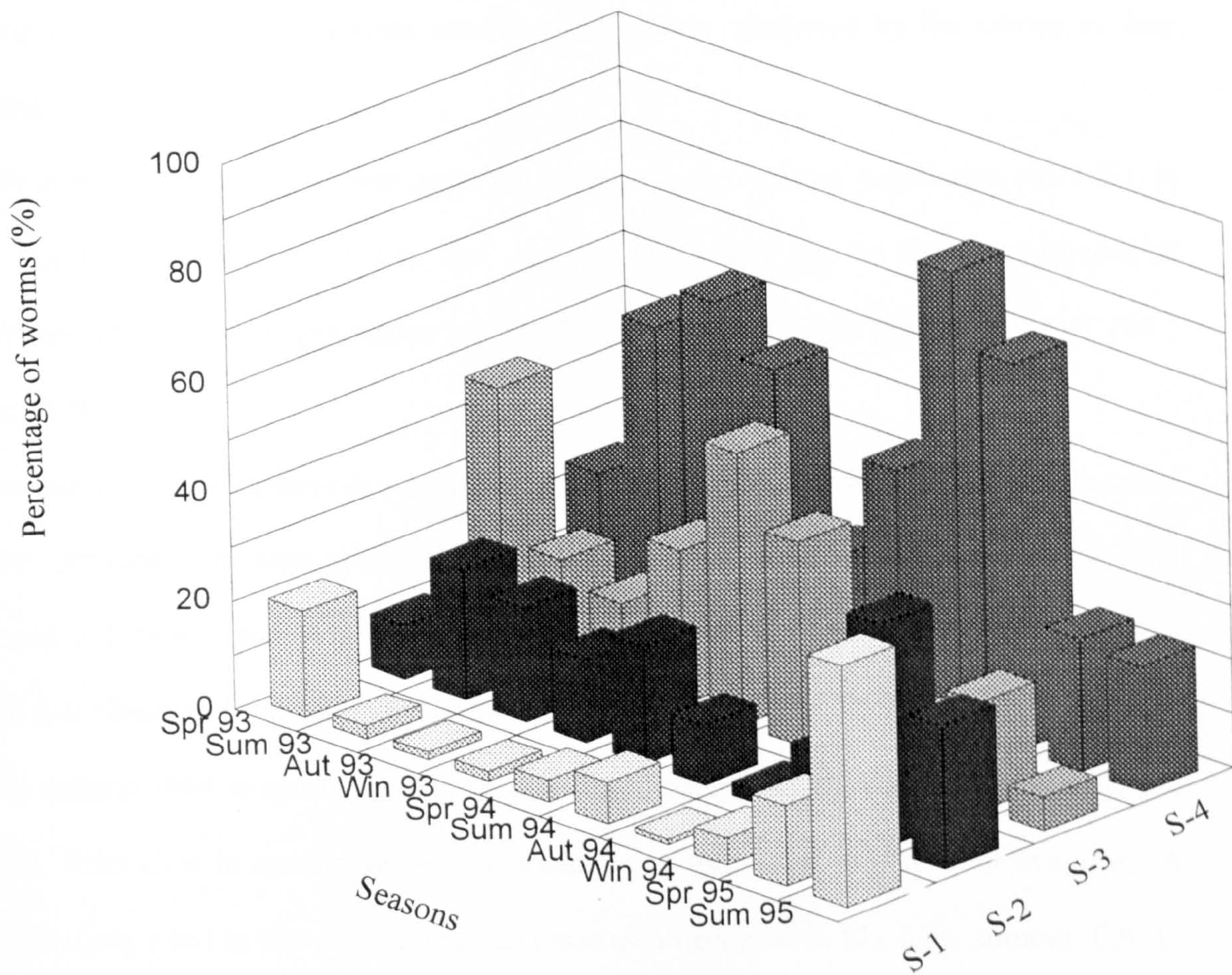


Fig 4.3 Seasonal location of *Proteocephalus filicollis* in various sections of the intestine of *Gasterosteus aculeatus* from Airthrey Loch from April 1993 to July 1995.

dropped to 54.6 % but there was a considerable increase in the worm population in S-3 & S-2 (43 %). In spring 1994 the worm population decreased by more than half (24 %) in S-4, but nearly doubled in S-3 & S-2 (72 %). Four percent of the worms were located in S-1 during this season. The high percentage of the parasite population in S-3 & S-2 during spring indicates that these are the sites in the intestine preferred by the worms as they mature.

In the summer of 1994 there was again an increase in the worms population (43.9 %) in S-4 and this continued to rise in the autumn (83.8 %) due to fresh recruitment of plerocercoids of the next generation and an elimination of parasites from S-3 & S-2 (48.5 % to 15 %).

From autumn to spring there is a clear pattern of occurrence of worms in different sections of the intestine. The high percentage (83.8 %) of worms observed in S-4 in autumn 1994 showed a decrease from winter (70.9 %) to spring 1995 (24.2 %). A similar decreasing trend was observed from summer 1993 to spring 1994.

From autumn 1994 to spring 1995 there is a clear increase in the worm population in S-3 & S-2, from 15 % in autumn to 24 % in winter and a further increase to 61 % in spring. A similar rising trend in the worm population was also observed in S-1 from autumn (0.9 %) to winter (5 %) to spring (14.7 %). A similar pattern of increasing worm population was also observed from autumn 1993 to spring 1994 in S-3 and S-2 and S-1.

In June and July 1995, S-4 contained 23 % of the parasites, whereas S-3 and S-2 had 32 % of the population, but the highest percentage of the worms was found in S-1 (44.4 %). This was due to the large number of gravid worms recovered in June and July.

4.4.6. Location of *Proteocephalus filicollis* in the host intestine in relation to maturity stages.

The overall location of worms according to their maturity stages is given in Table 4.2.

Table 4.2. Occurrence of various maturity stages of *Proteocephalus filicollis* as a percentage of the total number of worms in each section of the intestine of *G. aculeatus* from April 1993-July 1995.

Maturity stage of worm	S-4	S-3	S-2	S-1
Immature	96.4	64.1	43.3	8.2
Maturing	2.8	10.7	17.2	8.8
Mature	-	17.9	7.9	12.4
Gravid	0.8	7.3	31.6	70.6

Table 4.2 indicates that all four maturity stages of *P. filicollis* have a preferred site of attachment in the intestine. The population of immature worms decreases from S-4 to S-1 i.e. 96 % of the worms found in S-4 and only 8 % in S-1; maturing worms were largely found in S-2, but also in S-1 and S-3; mature worms have a preference for S-3, but also occurred in S-2 and S-1; gravid worms show increasing numbers from S-4 to S-1.

The percentage recovery of worms according to their maturity stage in various sections in the intestine of the fish is given in Table 4.3.

Table 4.3 The percentage of the overall number of each maturity stage of *Proteocephalus filicollis* found in each section of the intestine of *G. aculeatus*.

Maturity stage of worm	Immature	Maturing	Mature	Gravid
S-4	67.7	18.3	-	2.9
S-3	20.7	32.0	62.5	12.1
S-2	10.7	39.9	21.4	40.8
S-1	0.9	9.8	16.1	44.2

Table 4.3 shows that 67.6 % of all immature worms were recovered from S-4, and a small proportion of these worms were found in S-3 to S-1. Over 18 % of maturing worms occurred in S-4, but their number increased in S-3 and S-2 but dropped in S-1 indicating anterior migration of these worms. Mature worms were found mostly in S-3 (62.6 %) and were over 21 % and 16 % in S-2 and S-1 respectivily. The distribution of gravid worms is exactly in contrast to immature worms as the highest proportion of the gravid worms were recovered from S-1 (44.1 %) and the lowest percentage (2.9 %) in S-4.

4.4.7 Seasonal occurrence of different maturity stage of *Proteocephalus filicollis* in sections of the host intestine.

Each maturity stage of *P. filicollis* occurring seasonally in every section of the intestine of stickleback is given in Table 4.4 through Table 4.7. It should be noted that the sum for each box from Table 4.4 to Table 4.7 represents 100 %. For example box 1 x 1 (Row No 1 & Column No 1) represent spring 1993 and section of intestine, S-1 (anterior intestine). Hence the sum of all boxes 1x1 in Tables 4.4, 4.5, 4.6, 4.7 represent 100 %. In this

example for box 1x1 3.34 % worms are mature (Table 4.6) and 96.66 % worms are gravid (Table 4.7).

4.4.7.1 Immature worm.

A high proportion of the immature worms population was found in S-4 from summer 1993 to spring 1994 (100- 96.6 %). This trend was repeated in the 1994-95 generation from summer 1994 to winter 1994 (100 – 98.4 %) followed by a drop in spring 1995 and a rise again in summer 1995 (Table 4.4). The immature worm population in S-3 generally increased from summer to autumn and fell from winter to spring (Table 4.4).

The immature worm population in S-2 increased from summer to autumn and fell from winter to spring. The pattern of occurrence of immature worms in S-2 is similar to S-3. Immature worms were also found in S-1, but mostly in winter.

4.4.7.2. Maturing worms.

Maturing worms were occasionally found in S-4 and S-1 with the highest proportion in spring. Such worms were consistently found in S-3 in spring and as a small proportion during summer, autumn and winter (< 7 %). In S-2, the maturing worm population increases from winter to summer and fell from summer to winter before rising again in spring. Table 4.5 indicates that maturing worms mostly occur in all sections of the intestine in spring.

4.4.7.3. Mature worms.

Table 4.6 shows the very inconsistent pattern in the occurrence of mature worms in S-1 to S-3. Mature worms are mostly found in spring and summer in S-3, S-2 and S-1. During winter these worms are also found in S-2 and S-3.

4.4.7.4 Gravid worms.

Gravid worms were occasionally found in S-4. Gravid worms were found in S-3 in winter and spring with a higher proportion in spring than in winter. In S-2, gravid worms were found throughout the year, but the proportion of gravid worms increases from autumn to spring. This pattern was repeated in both years. Gravid worms were found in S-1 throughout the year and in most seasons were the dominant stage in this section of the

Table 4.4 Immature worms as percentage of the total number of *Proteocephalus filicollis* in each section of the intestine of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995 (Number in bracket is actual number of worms recovered from that section).

Intestinal section / seasons	S-1	S-2	S-3	S-4
Spring 1993	-	-	10.41 % (10)	100 % (49)
Summer 1993	33.33 % (1)	41.93 % (13)	45.85 % (11)	110 % (70)
Autumn 1993	-	84.09 % (37)	96.77 % (30)	100 % (133)
Winter 1993	66.66 % (2)	68 % (17)	85.10 % (40)	97.77 % (88)
Spring 1994	-	18.51 % (5)	66.66 % (42)	96.66 % (29)
Summer 1994	-	57.89 % (11)	92.30 % (60)	100 % (76)
Autumn 1994	-	71.42 % (5)	90 % (36)	98.82 % (256)
Winter 1994	78.57 % (11)	13.51 % (30)	80 % (24)	98.48 % (195)
Spring 1995	-	25.64 % (30)	60.71 % (34)	65.21 % (45)
Summer 1995	-	19.23 % (5)	100 % (6)	86.96 % (20)

Table 4.5 Maturing worms as a percentage of the total number of *Proteocephalus filicollis* in each section of the intestine of *G. aculeatus* from April 1993 to July 1995 (Number in bracket is actual number of maturing worms recovered from that section).

Intestinal sections / Seasons	S-1	S-2	S-3	S-4
Spring 1993	-	-	22.10 % (21)	-
Summer 1993	-	-	-	-
Autumn 1993	-	-	3.33 % (1)	-
Winter 1993	-	8 % (2)	-	2.23 % (2)
Spring 1994	-	14.81% (4)	19.04 % (12)	-
Summer 1994	100 % (2)	21.05 % (4)	6.15 % (4)	-
Autumn 1994	-	14.28 % (1)	5 % (2)	-
Winter 1994	7.14 % (11)	13.51 % (42)	6.66 % (7)	1.52 % (22)
Spring 1995	26.19 % (1)	35.89 % (3)	12.5 % (7)	31.88 % (1)
Summer 1995	2.27 % (1)	11.53 % (3)	-	4.34 % (1)

Table 4.6. Mature worms as a percentage of the total number of *Proteocephalus filicollis* in each section of the intestine of *G. aculeatus* from April 1993 to July 1995 (No in bracket is actual number of mature worms recovered from that section).

Intestinal sections / Seasons	S-1	S-2	S-3	S-4
Spring 1993	3.33 % (1)	-	60 % (57)	-
Summer 1993	-	12.90 %(4)	54.16 % (13)	-
Autumn 1993	33.33 % (1)	2.27 % (1)	-	-
Winter 1993	-	8 % (2)	10.63 % (5)	-
Spring 1994	-	7.40 % (3)	1.58 % (1)	-
Summer 1994	-	-	1.53 % (1)	-
Autumn 1994	-	-	-	-
Winter 1994	-	2.70 % (1)	6.66 % (2)	-
Spring 1995	21.42 % (1)	9.40 % (11)	5.35 % (3)	-
Summer 1995	13.63 (14)	23.07 % (6)	-	-

Table 4.7 Gravid worms as a percentage of total the number of *Proteocephalus filicollis* in each section of the intestine of *G. aculeatus* in Airthrey Loch from April 1993 to July 1995(No in bracket are gravid worms recovered from that section).

Intestinal sections / Seasons	S-1	S-2	S-3	S-4
Spring 1993	96.66 %(39)	89.47 %(20)	7.36 %(7)	-
Summer 1993	66.66 %(2)	-	-	-
Autumn 1993	66.66 %(2)	13.63 %(6)	-	-
Winter 1993	33.33 % (1)	16 %(4)	4.25 %(2)	-
Spring 1994	100 % (5)	55.55 %(15)	12.69 %(8)	3.37 %(1)
Summer 1994	84.61 % (11)	21.10 %(4)	-	-
Autumn 1994	100 % (3)	14.28 %(1)	5 %(2)	1.17 %(3)
Winter 1994	14.28 %(2)	2.70 %(1)	6.66 %(2)	-
Spring 1995	52.38 %(22)	29.05 %(34)	21.42 %(12)	1.44 %(2)
Summer 1995	84.09 %(37)	46.15 %(12)	-	8.69 %(2)

intestine. In general the proportion of gravid worms in S-1 increased from winter to summer as the population matured (Table 4.7).

4.4.8 Migration of *Proteocephalus filicollis* in the intestine of the fish.

The monthly mean attachment position of *P. filicollis* in the intestine is shown in Fig. 4.4. Analysis of variance demonstrated that a significant variation existed in the mean position of the worms between the months ($F= 4.46$; $P < 0.001$; $F_{0.05\,(1)\,26} = 1.66$).

Figure 4.4 shows that from September 1993 to February 1994 the mean attachment position of worms was 35-41 % along the intestine, and in the same period during 1994-95 the mean position of worms was 30-50 %. The highest mean attachment position of 59 % was in December 1993. From March to June 1994 the mean attachment position varied from 43- 79 %; in March to June 1995 it was 47-83 % and from April to June 1993 60-70 %.

Thus in autumn and winter the parasites are mainly found in the rectum and posterior intestine and in spring and early summer in the mid to anterior intestine. Figure 4.4 also shows that worms were never found in the posterior 15 % of the intestine and were exceptionally found in the anterior 5 % of the intestine.

The parasite occupied an anterior mean position in July 1993 (from only 13 worms) and in July 1994 and August 1994. The worms were widely spread within the intestine in this period. From September to November the mean attachment position of the worm population moved posteriorly and then moved anteriorly again. From February to June in each year of the sampling there was a clear further anterior movement in the mean attachment position.

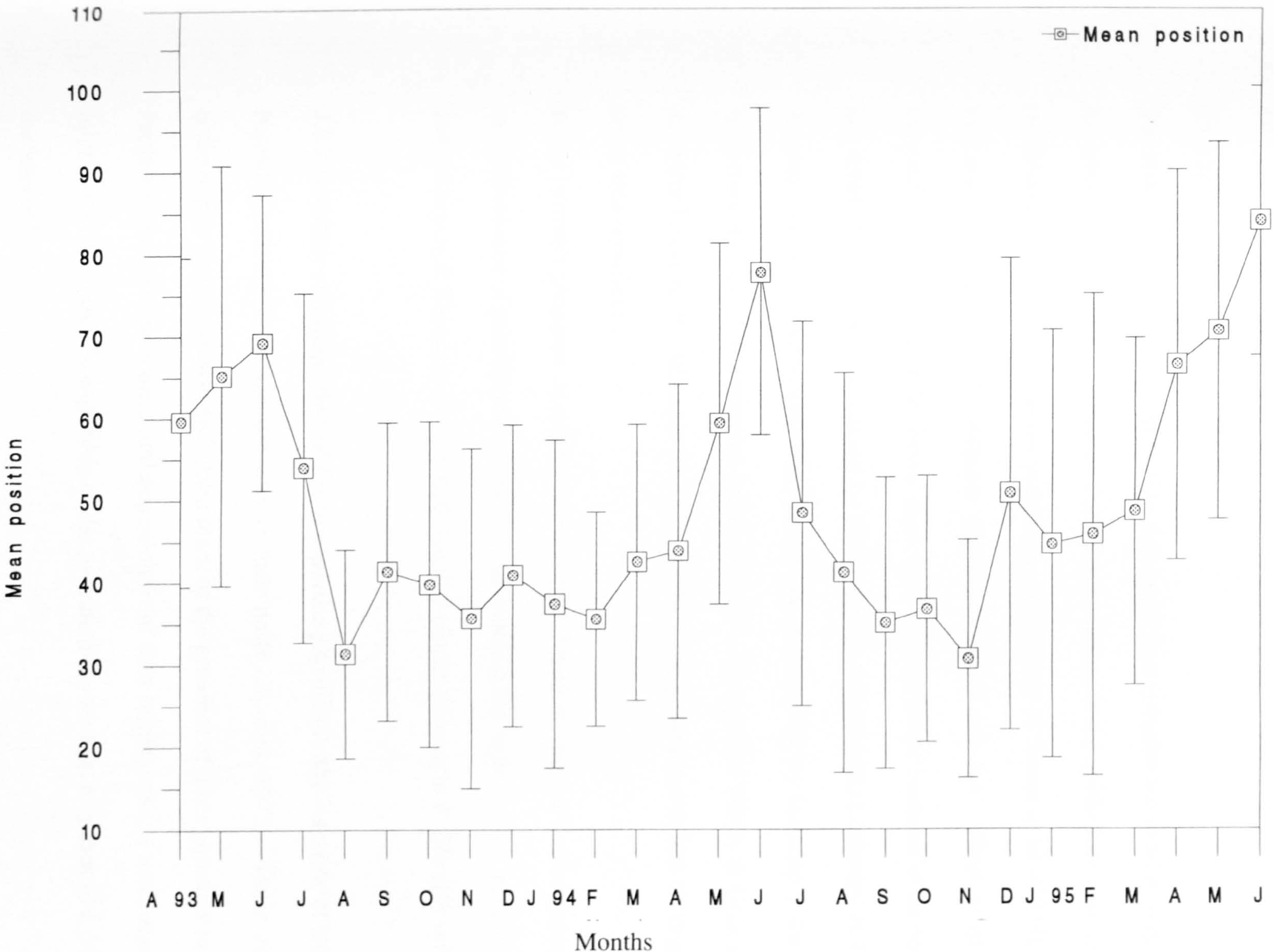


Fig 4.4. Monthly mean position of attachment of *Proteocephalus filicollis* in the intestine of *Gasterosteus aculeatus* (lower to higher mean position indicates section S-4 to S-1) (bars represent standard deviations).

4.5 Discussion.

4.5.1 Histopathology

The histopathological effects of *P. filicollis* on the host intestine are similar to those described for other fish cestodes. In spite of the desequamation of the epithelium seen at the point of attachment of *P. filicollis* severe pathology, as reported by Bauer *et al.* (1973) for cestodes, was not observed, possibly because parasite intensity was low. Eiras *et al.* (1986) also reported that the scolex of *M. brooki* does little damage to the intestine of its host. A similar low level of pathology was caused by *K. sinensis* in carp intestine (Morley & Hoole, 1995). A more significant pathological factor might be blockage of the intestine if the worms were numerous, as seen in *P. promelas* infected by *B. acheilognathi* (Scott & Grizzle, 1979), but infection levels of *P. filicollis* in sticklebacks were generally too low to suggest that this effect was significant.

When cestodes penetrate or perforate the intestinal wall an intense host response may result in the formation of granulomatous nodules surrounding the scolex of the parasite (Eiras *et al.*, 1986; Karanis & Tarasachewski, 1993), but this was never seen in *P. filicollis* infection.

4.5.2 Location and migration of *Proteocephalus filicollis* in the intestine of the host.

Parasites actively select specific sites in their hosts (Holmes, 1973). These sites may vary according to season, growth and maturation, or the presence of other parasites in the intestine. Parasites can show movement and migration from their original site of attachment and these migrations may result in expulsion of the parasite from the host or a shift to a different site in the intestine.

Proteocephalus filicollis were found to attach in various sections of the intestine, with 50 % of the overall worm population in the rectum and a decreasing proportion in more anterior sections of the intestine. The rectum always contained a high proportion of the worm population during summer and autumn although this decreased towards winter and spring. This corresponds to the higher immature worm populations in these seasons and suggests that the rectum is the initial site of infection of newly arrived parasites. Hopkins (1959) also reported over 80 % or more of the worm population in rectum in July to November and in March and stated that small immature worms are found in rectum (August to December).

The results show that the rectum (S-4) was the most favoured site of attachment for immature worms, the posterior intestine (S-3) was preferred by mature worms, the mid intestine (S-2) was the most favoured site for maturing worms, and the anterior intestine (S-1) was where the majority of gravid worms were attached. *Proteocephalus filicollis* obviously undergoes an anterior migration as it matures, so that by spring most gravid worms are attached in the anterior intestine.

A number of workers have reported migrations of worms in the intestine of fish and suggested different explanations for this effect. Mackenzie & Gibson (1970) found that a trematode, *Podocotyle* sp, and the nematodes *C. heterochronus*, *C. minutus* and *C. aduncum* migrated within the intestine of flounder when the fish was starved. A similar evidence of migration was found by Williams *et al.* (1970) in cod infected with *C. aduncum* and rays infected with a trematode, *Otodistomum* sp and a cestode *Grillotia* sp. Migration of the cestodes *T. nodulosus*, *T. crassus and P. pingius* in the host intestine was not in response to the feeding activity of the host, but this did occur in a nematode, *R. acus* (Shostak & Dick,

1989). Bates & Kennedy (1991) reported that the distribution of *P. laevus* in rainbow trout did not alter under feeding conditions.

Shostak & Dick (1989) pointed out that little is known about the factors that promote or inhibit migration in natural helminth communities. The cestodes and nematodes feed differently. Cestodes belong to the " absorber" guild, (absorb nutrients across the body surface) and nematode belongs to the "engulfer" guild (ingest host tissue or intestinal content) (Bush & Holmes, 1986; Stock & Holmes, 1988). From their study Shostak & Dick (1989) suggested that it is difficult to predict the migration of absorber (*T. nodulosus, T. carassus* and *P. pingius*). They suggested that, the tendency for a species to migrate is probably influenced by its ability to move, its specific nutrients and their rate of supply. They stated that a number of these factors may have inhibited the migration of the cestodes of pike.

There is no noticeable difference in the microscopic anatomy of the intestine anterior and posterior to the ilo-rectal valve in stickleback although the epithelial folds in the rectum are usually shorter than those present in the intestine (Hale, 1965). It seems reasonable to conclude that migration of *P. filicollis* from rectum to the anterior parts of the intestine in spring and early summer is under the stimulus of growth and maturation of the cestodes and even possibly the rise of water temperature.

As *P. filicollis* mature and increase in length they may migrate towards anterior simply to obtain more space for their considerably elongated strobila. This movement, combined with the apparent loss of a proportion of the cestode infrapopulation at the same time may permit the successful egg production of the survivors without excess demands on the hosts physical and metabolic resources. The anterior movement of the worms may also allow them to better exploit the available nutrients within the hosts intestine.

The monthly mean position of *P. filicollis* in the intestine of the stickleback as observed in the present study may be treated with care because of the variations in the host sample size, different length classes of the host, monthly mean intensity of infection, occurrence of various maturity stages of the worm simultaneously in same month. This study has demonstrated that the position of *P. filicollis* in the intestine of its final host stickleback is shifted during the life span of the worm. Further experimental investigations may explain better this concept of migration of fish cestode.

CHAPTER 5
SIZE AND FECUNDITY OF PROTEOCEPHALUS FILICOLLIS

5. Size and fecundity of *Proteocephalus filicollis.*

5.1 Introduction.

High fecundity is regarded as a characteristic feature of the cestodes, and egg output is often quoted in support of this (Kennedy, 1983). Examples of egg production per cestode per day, include *Hymenolepis diminuta* 250,000 (Keymer, 1980), *Taenia saginata* 720,000 (Pawlowski & Schultz, 1972;), *Taenia solium* 300,000 (Gemmell & Johnstone,1977, as cited by Moore,1981) and *Taenia hydatigena*, 60,000 (Featherstone,1969, as cited by Moore, 1981). Daily egg production depends on the size of the cestode, and there exists a positive correlation between the size of an adult cestode and its fecundity (Read *et al.* 1958).

Kennedy (1983) has pointed out that it is extremely difficult to find any reliable estimates of fecundity or of quantitative effects of the factors influencing it. There is an inverse relationship between cestode population density and parasite size and fecundity and thus the mean daily egg output per cestode declines as the parasite burden increases. This is referred to as the "crowding effect". Many authors have reported a crowding effect in different species of cestodes including *H. diminuta.*

Roberts & Mong (1968) found that, in addition to stunting due to crowding, the weight of individual worms from secondary infections is inversely proportional to the number of worms from a primary infection still present.

Befus (1975) reported that in *H. diminuta* infecting mice, are stunted due to crowding, which is generally attributed tointer-worm competition, and may be in part immunologically mediated.

Hesselberg & Andreassen (1975) found that the number of eggs of *H. diminuta* produced per worm, and even per infected rat, decreased with increasing population density. In *Hymenolepis nana* from mice, crowding decreased the linear dimensions of eggs, number of eggs per proglottid and rate of proglottid production, but the shape of the egg was not affected (Ghazal & Avery, 1974).

Denser populations of *Diphyllobothrium dendriticum* in golden hamster (*Mesocritcetus auratus*) had the smallest individual worms and some contained worms with primary strobilae at the start of egg production (Halvorsen & Andersen,1974).

Wootten (1972) reported that loss of *Eubothrium crassum* from older brown trout may be due to some form of immune response by the fish against the parasite, but possibly intraspecific competition, perhaps for available food material between a large number of growing cestodes, may be the cause. Wootten (1972) suggested that such competition appears to cause a 'crowding effect' in cestode infection.

It is generally agreed that a crowding effect is widespread in cestodes and inevitably results in the decreased fecundity of individuals, but the relationship between total egg output per host and parasite number is more complex.

Ghazal & Avery (1974) have shown that crowding of *H. nana* in mice resulted in a decrease in number of eggs per proglottid and a reduced rate of proglottid production; however egg production per host rose to a peak, declined and then levelled off to a constant rate with increasing parasite density.

In contrast to this pattern, Jones & Tan (1971) found that total egg output per day per host (mice) of *Hymenolepis microstoma* increased with increasing parasite density. They also reported that with increase in worms per host, there was decrease in the length of worms, a reduction in daily egg production, a progressive reduction in number of eggs per gravid proglottid and a decrease in the rate of proglottid formation.

Roberts & Kennedy (1983) pointed out that it is impossible to generalise concerning the effect of crowding upon fecundity of the total parasite population within a host. The variation in the effects of crowding at the population level may be due to any or all of the following factors.

(1) An optimum parasite density may have deleterious effects on the host. Halvorsen & Andersen (1974) reported that heaviest infections of 8-9 *D. dendriticum* or more appeared to block the intestine of the hamsters. Hosts with such infections looked unhealthy and some of them died. This situation they classified as overcrowded. These authors indicated that there is an optimal situation for worms when the dose of infection is between 15 and 8 plerocercoids.

(2) Heavy infections may result in a small proportion of the cestode population growing to a normal size and a large proportion remaining small and stunted, rather than all the cestodes being stunted to a similar extent (Ghazal & Avery,1974; Hesselberg & Andreassen, 1975).

(3) The crowding effect can result from an interaction of physiological, nutritional and immunological processes. The growth of cestodes in crowded conditions and in hosts maintained on diets deficient in carbohydrate has led many workers to believe that the crowding effect is due to competition for a limited carbohydrate resource (Robert & Davis 1983). Read (1959) reported that the crowding effect may be interpreted in terms of competition for utilised carbohydrates by the individual worms in the population. Roberts (1966) reported that similarities in developmental characteristics of worms from crowded infections and those from hosts with suboptimal carbohydrate diets provided evidence that competition for host dietary carbohydrates is involved in the crowding effect in cestodes.

It has also been determined that cestodes can provoke an immune response in their host at high densities and that the effect of this response is to reduce growth and fecundity (Befus,

1975; Henderson, 1977). Ghazal & Avery (1974) suggested that changes in fecundity in cestodes at high densities could be due to larger cestodes being more successful at evading the host immune response, or in competing for nutrients with smaller worms, or both. Excretory and secretory products (such as succinate, acetate, D-glucoseaminic acid and cyclic GMP) released by *H. diminuta* in vitro inhibit DNA synthesis in other worms of the same species (Insler & Roberts 1980; Zavras & Roberts 1984,1985). Perfusion of the hosts intestine with appropriate concentrations of these substances can stunt the growth of *H. diminuta* (Cook & Roberts, 1991).

There are very few documented reports on the fecundity of fish cestodes. Davydov (1978) reported that variation in potential fecundity was connected with conditions in the fish intestine, including parasite size, population density and temperature and food availability. Davydov (1978) showed that, other conditions being equal, egg output is lower when helminth population density is high rather than vice versa. He found that the maximum number of eggs from a single worm infection of ***Bothriocephalus acheilognathi*** (=*B. gowkongensis*) (from a worm 8 cm long and 240 mg in weight) was 60500, in a fish fed daily. In fish with two worm infections egg output varied from 35400 - 41280 over 12 days (mean length and weight of worms 7.7 cm & 184 mg respectively). Similarly, when density increased to 3 & 5 worms per fish respectively, the fecundity decreased to 15987 ± 1340 and 4020 ± 860 eggs. Davydov (1978) suggested that *B. acheilognathi* in grass carp is capable of producing between 60- 100,000 or more eggs and such variations in potential fecundity are connected with conditions in the fish gut.

The environmental conditions in which the host lives may also effect fecundity. Riggs *et al.* (1987) found that *B. acheilognathi* in fathead minnow (*Pimephales promelas*) at an unpolluted site produced 209 ± 17.8 eggs per proglottid, whereas at a polluted site, parasites produced 125 ±16.9 eggs per proglottid. In red shiner (*Notropis lutrensis*) *B.*

acheilognathi produced 152.0 ± 17.6 eggs at the unpolluted site which was reduced to 85.5 ± 9.2 at a polluted site.

The only published account of fecundity in proteocephalids is by Ieshko & Anikieva (1992). They found that the number of eggs produced by *Proteocephalus percae* was dependent on the length of the strobila. One group of *P. percae* was characterised by the relatively small size at which it reached maturity with a high fecundity, while another group matured at a larger size with a lower fecundity. Initial growth and maturation of the cestodes was rapid, but then an increase in the length of the parasites occurred against the background of the excretion of mature eggs that resulted in the apparent decrease in fertility. They determined the mean number of eggs per cm of strobila to be 4900 in *P. percae*. The period of intensive egg emergence was found to be fairly short (14 days). They suggested that the population of *P. percae* in perch in Lake Rendozero (former U.S.S.R) should increase 8000 fold in every generation if all eggs produced mature worms and estimated that at a fertility of x 8070, the 3,100,000 mature worms in Lake Rendozero are able to excrete 25,000,000,000 eggs per breeding season.

No details are so far available on the fecundity or egg production of *P. filicollis*.

5.2. Aims.

The objective of this study was to investigate egg production by *Proteocephalus filicollis* from a wild population of three-spined stickleback and to determine some factors affecting egg production and fecundity in the parasite population and estimate the fecundity of *P. filicollis* in Airthrey Loch.

5.3 Materials and methods.

5.3.1 Fish sampling, dissection, staining and measurement of worms.

The procedure for fish sampling, dissection of fish and staining and measurement of worms have been given in detail in chapter three.

5.3.2 Estimation of fecundity.

5.3.2.1 Procurement of *Proteocephalus filicollis* eggs.

To study the fecundity of the parasite, gravid worms taken from the intestine of stickleback were kept individually in separate Petri dishes containing warm water (20-25°C). On touching water the worms started shedding a stream of eggs. It was noticed that worms released eggs more quickly when placed in warm water than in ambient tap water. The worms were left in the petri dish for 1-2 hours to obtain the maximum quantity of eggs. The eggs were kept in a refrigerator at 4 ^{0}C for later use.

5.3.2.2 Estimation of egg numbers and measurement of egg and hexacanth.

Eggs obtained from worms were put in Bijou (capacity 10 ml) and tap water added to make a 5ml volume. The Bijou was closed by screw cap and the contents were shaken to obtain a uniform suspension. Using a fine Digital pipette one millilitre of suspension was placed on a Rafter cell S 50, taking care to ensure that no bubbles were formed and the suspension was uniformly distributed over the cell. The cell was then covered. The eggs were counted under a compound microscope using the grid of the Rafter cell and the number of the eggs ml $^{-1}$ recorded. The total number of eggs in the suspension were counted in the same way. The eggs were placed on a slide in water and measured under the compound microscope using an eye piece gratiule. Readings both for eggs and hexacanth were taken in µm.

5.3.2.3 Percentage gravid portion.

The gravid portion of each worm was measured with eye piece graticule fitted in the microscope. The length of the gravid portion was divided by the total length of that worm and multiplied by 100 to obtain the percentage gravid portion of that worm. This figure was subsequently used for analysis of results.

5.3.2.4 Number of gravid proglottids.

The number of gravid proglottids (segments containing eggs as well those segments which shed eggs) of each worm were recorded.

5.3.2.5 The mean length of gravid progottids.

The length of all gravid proglottids in a worm was measured. The mean length of gravid segments for each worm was calculated .

5.4. Data analysis.

Data was analysed using 'Minitab'. Linear regressions were calculated to determine the relationship between length of worm and water temperature and infrapopulation size and length of the host. The relationship between length of worm and infrapopulation size and parameters of fecundity, including percentage gravid portion, number of gravid segments, and the mean length of gravid segments was determined. In the same way the relationship between number of eggs and diameter of egg and hexacanth was calculated. The data for length of the worm, size of the egg and number of eggs was not normal and was transformed prior to calculation. Graphs were plotted using 'Cricket Graph'.

5.5. RESULTS.

5.5.1 Occurrence and length of gravid worms.

Gravid worms occurred mostly in spring and early summer (April, May and June), but some gravid individuals were found throughout the year (Fig. 5.1). The monthly mean number of gravid worms per infected stickleback is shown in Fig. 5.1. The mean number of gravid worms was 1 in September, October, November, December 1993; February, March, April, October, November 1994 ; and February 1995. In September, November 1993; February, November 1994 only one gravid worm was recorded, whereas in other months gravid worms occurred more than once.

The mean number of gravid worms remained between 1 and 2 in most months of the year, but increased slightly above 2 in June 1993, June 1994 and in March and May 1995, whereas the highest mean number of gravid worms per infected fish was recorded in June 1995. Figure 5.1 shows that higher numbers of gravid worms occurred in early summer (June) every year.

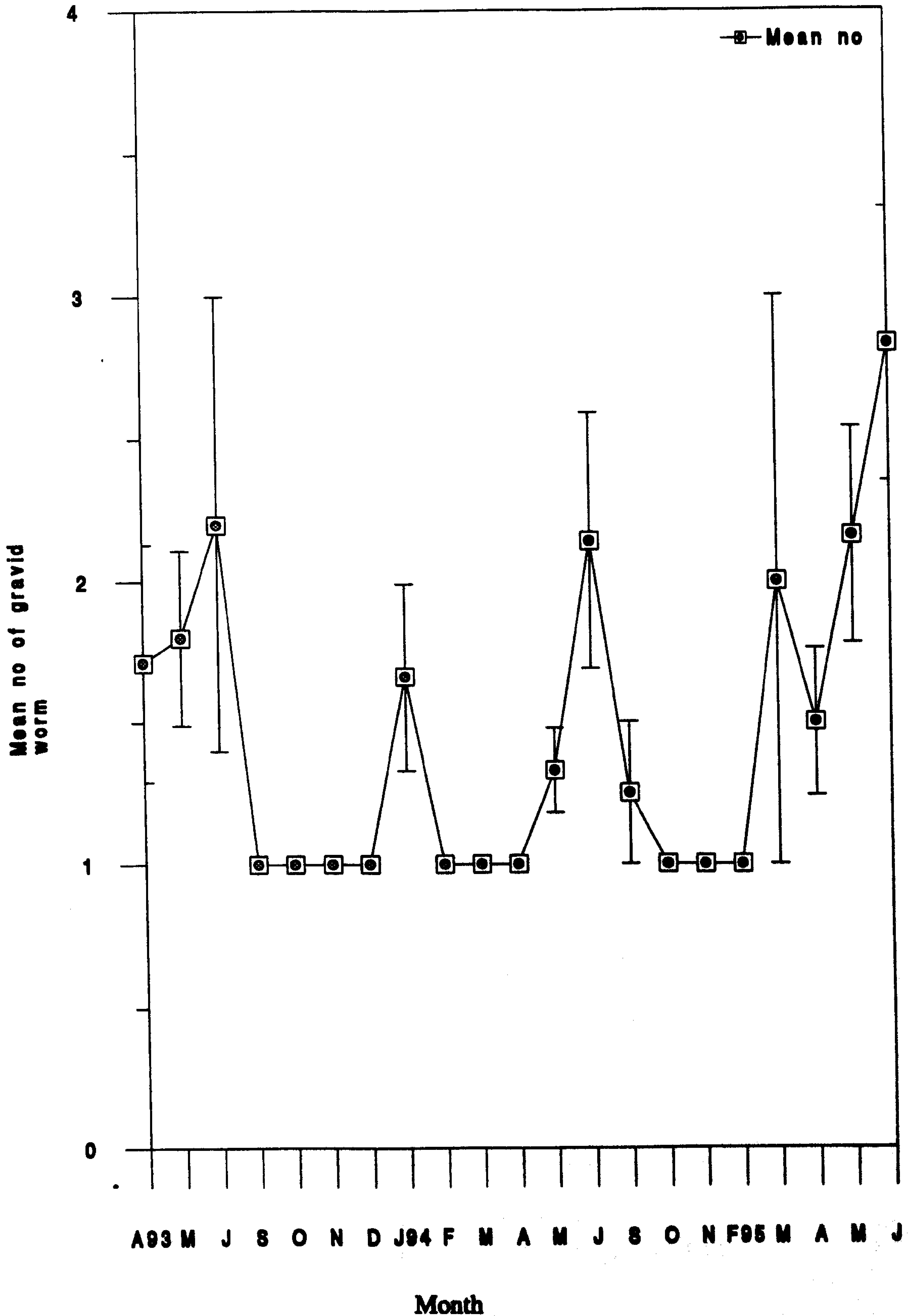
The variation in mean length of the gravid worms in different months is shown in Fig. 5.2. Analysis of variance showed that a significant variation in length of gravid worms occurred over the sampling period ($F = 9.70$, $P < 0.001$).

In the year1993-94 and 1994-95 generations of *P. filicollis* the minimum length of gravid worms were in September 1993 and October 1994, respectively. However, these are based on single worms. Worm length increased until maximum values were obtained in June 1994 (28.72 mm) and June 1995 (25.68 mm). During April - June mean length of gravid worms exceeded 10 mm, except in April 1994.

There was a statistically significant positive relationship between length of gravid parasites and water temperature (Fig 5.3) ($r^2 = 0.115$; $r = 0.339$; $P < 0.001$; $F = 17.10$; $F_{0.05\,(1)\,133} = 3.91$).

There is a statistically significant positive relationship between the length of gravid worms and the length of the host ($r^2 = 0.217$; $r = 0.466$; $P < 0.001$; $F = 36.37$; $F_{0.05\,(1)\,133} = 3.91$) (Fig 5.4). Figure 5.5 indicates that nearly 50 % of the gravid worms are found in fish above 4.1 cm in length.

Gravid worm length and infrapopulation size did not show any significant relationship ($r^2 = 0.013$; $r = -0.114$; $P \geq 0.185$; $F = 1.17$ $F_{0.05\,(1)\,133} = 3.91$) (Fig. 5.6).



Month

Fig 5.1 Monthly mean number of gravid *Proteocephalus filicollis* per infected *Gasterosteus aculeatus*. Bars represent standard deviations.

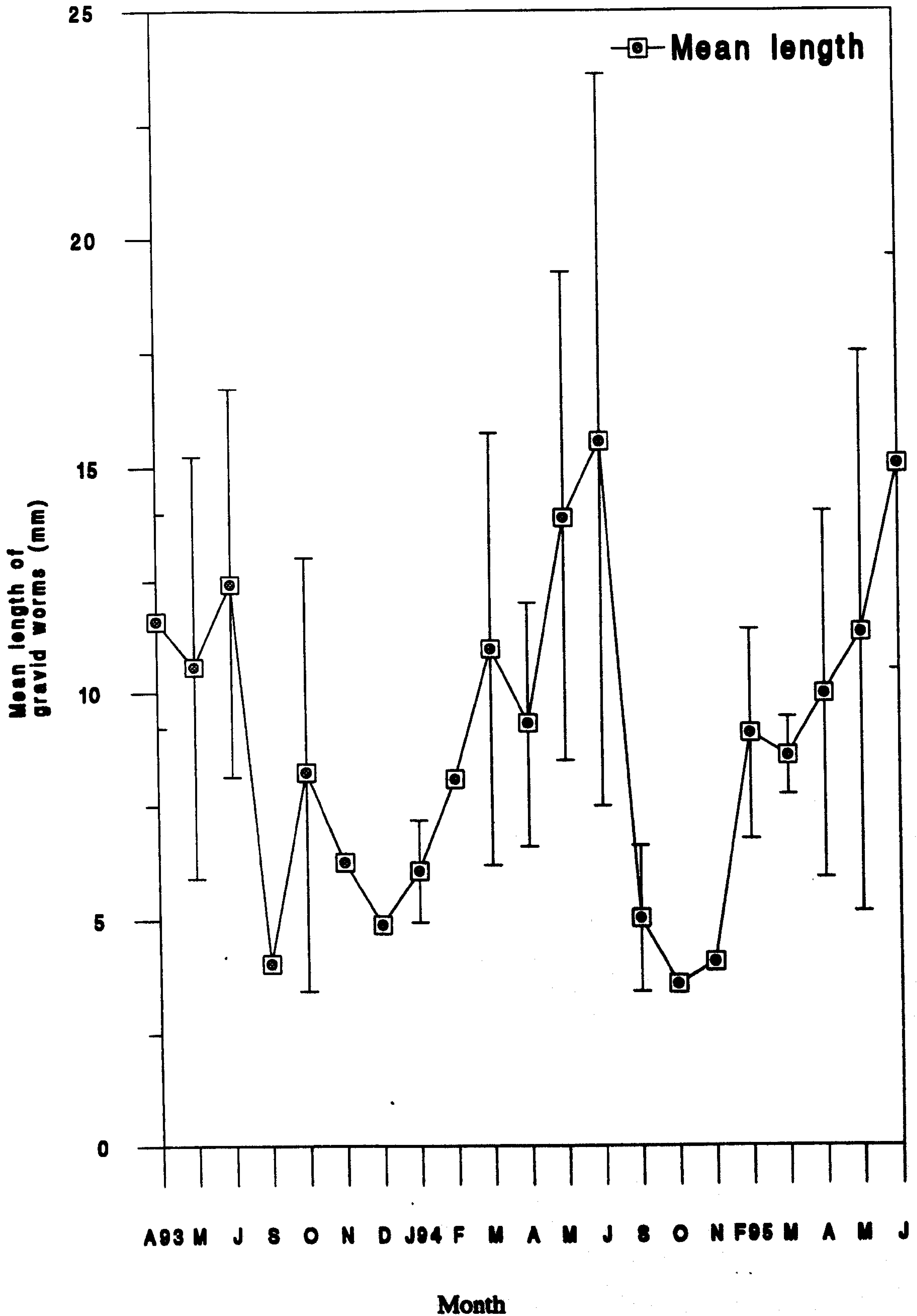


Fig 5.2 Monthly mean length of gravid *Proteocephalus filicollis*. Bars represent standard deviations.

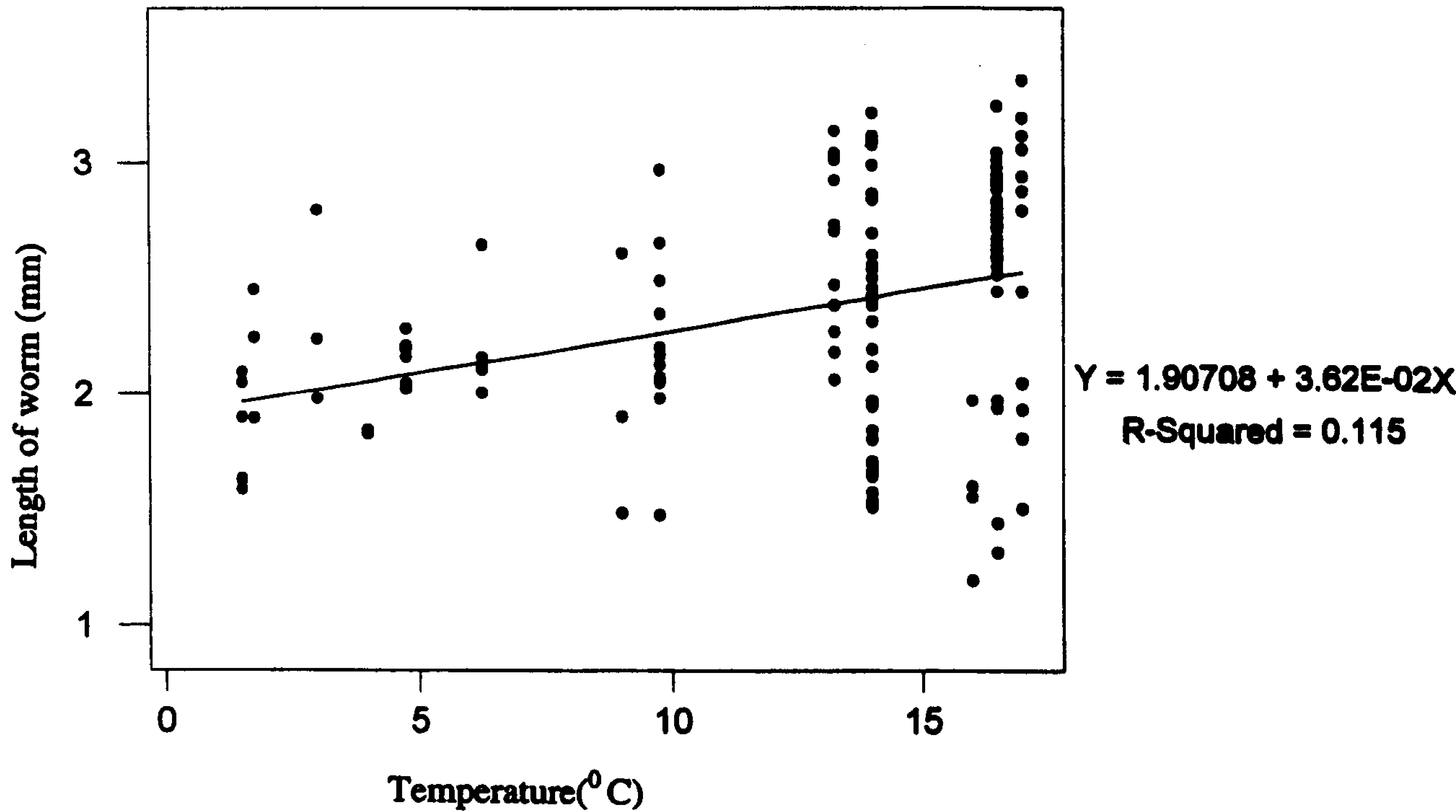


Fig 5.3 Relationship between length of gravid *Proteocephalus filicollis* (mm) and water temperature (° C).

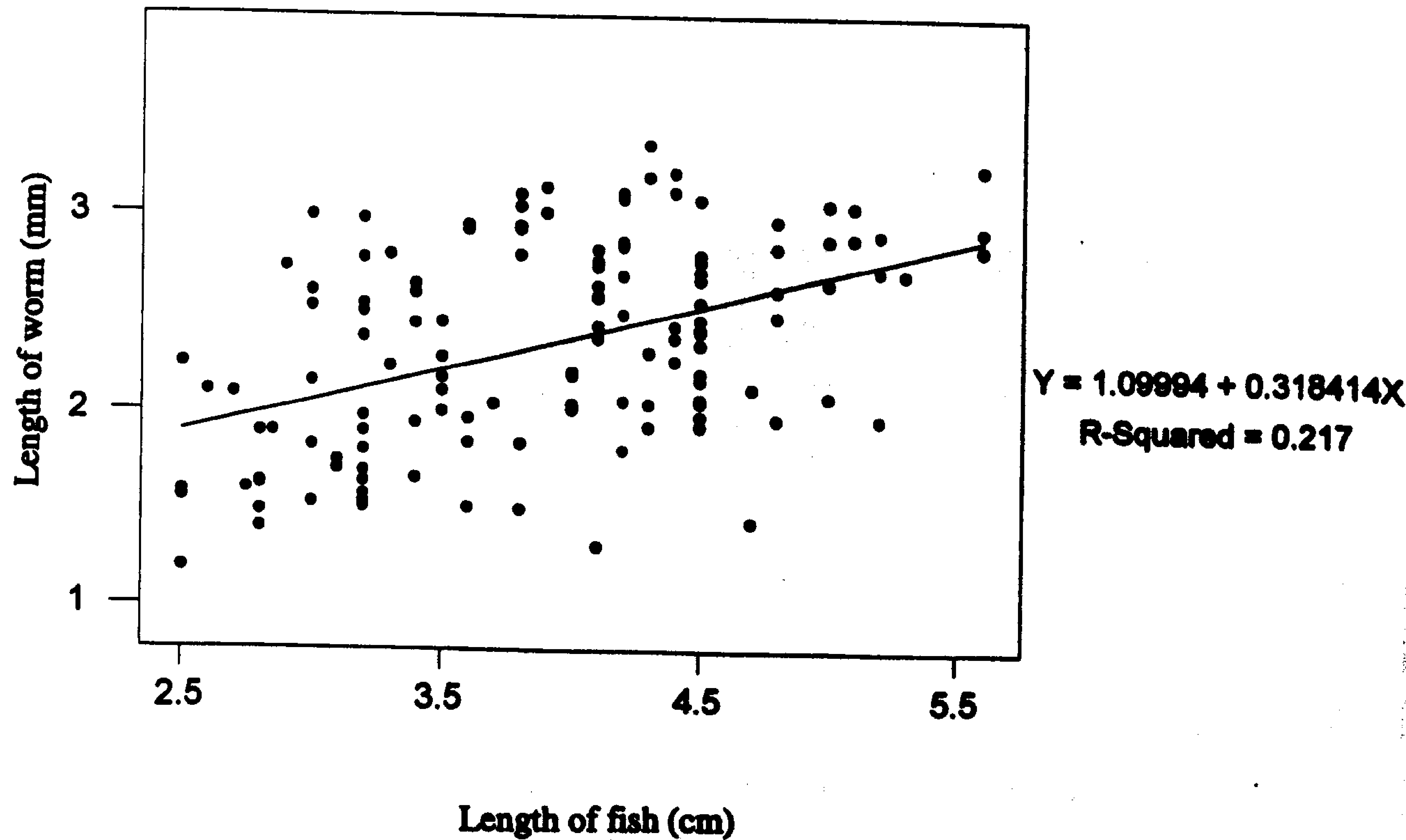


Fig 5.4 Relationship between length of gravid *Proteocephalus filicollis* (mm) and length of stickleback (cm).

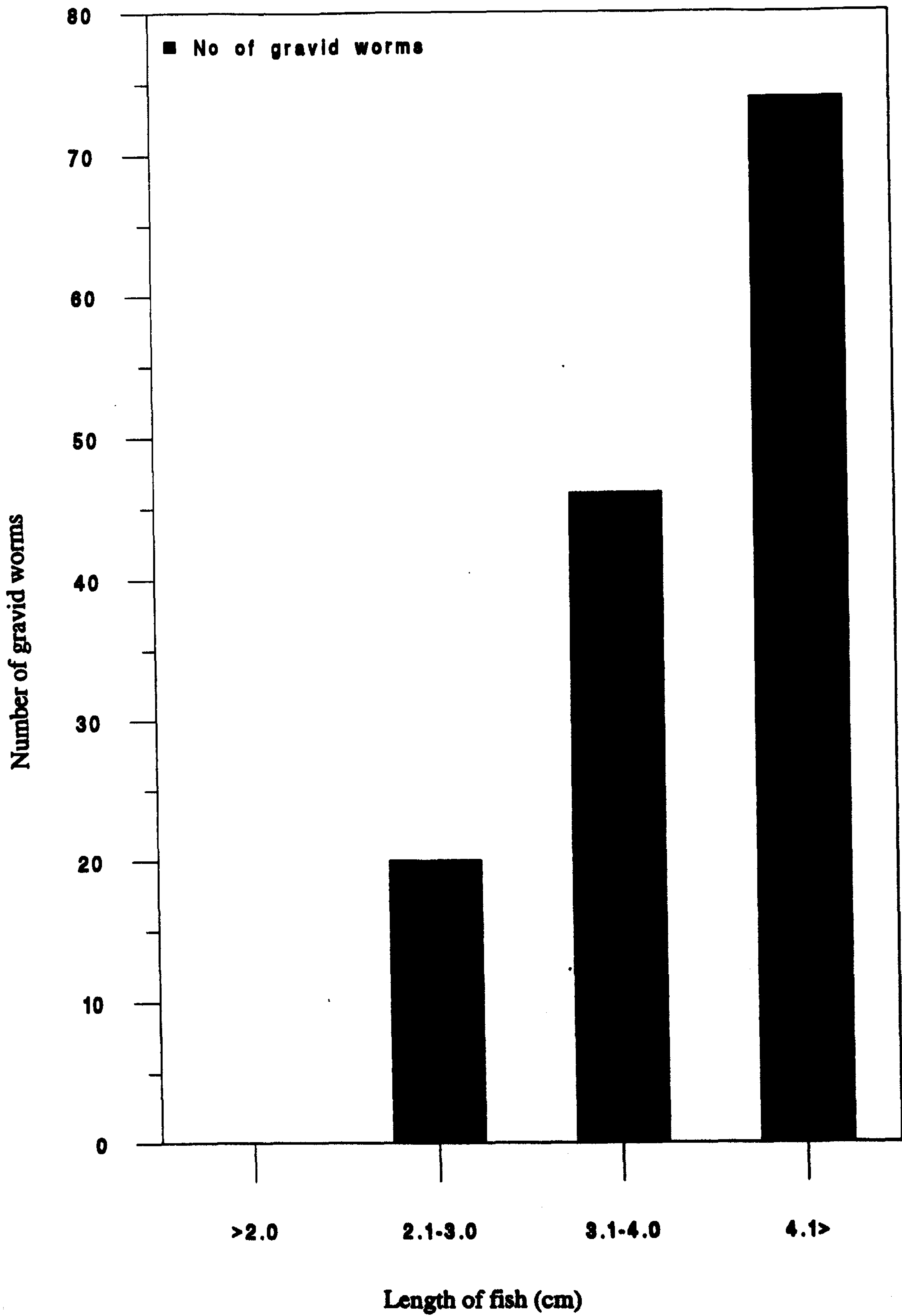


Fig 5.5 Number of gravid *Proteocephalus filicollis* in different length classes of *Gasterosteus aculeatus*.

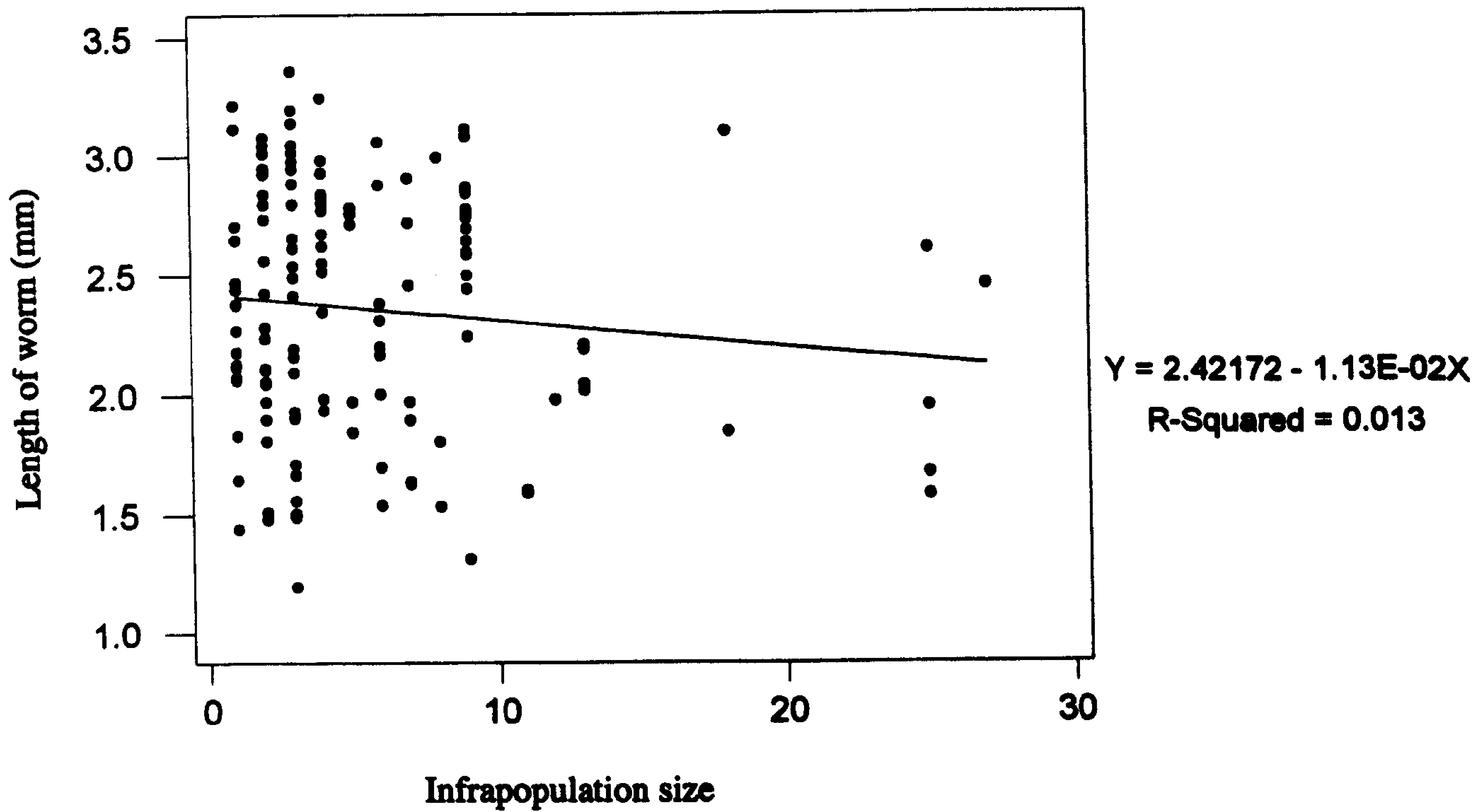


Fig 5.6 Relationship between length of gravid *Proteocephalus filicollis* (mm) and infrapopulation size.

5.5.2 Percentage gravid portion.

Maximum mean percentage gravid portion per worm was observed in May 1994 (48.2 %) and May 1995 (43.8 %) (Fig. 5.7).

The percentage gravid portion of worms was significantly positively correlated to the length of the worm as shown in Fig. 5.8 ($r^2 = 0.097$; $r = 0.311$; $F = 14.04$; $P < 0.001$; $F_{0.05\,(1)\,133} = 3.91$).

There was no significant relationship between percentage gravid portion and infrapopulation size ($r^2 = 0.015$ %; $r = -0.123$; $P \geq 0.116$; $F = 1.94$; $F_{0.05\,(1)\,133} = 3.91$) (Fig. 5.9).

5.5.3 Number of gravid proglottids.

The mean number of gravid proglottids per worm per month is given in Fig. 5.10. Maximum number of gravid segments in an individual worm were observed in June 1994 (30 segments) and in June 1995 (25).

Numbers of gravid segments are significantly positively correlated to the length of the worm ($r^2 = 0.563$; $r = 0.77$; $P < 0.001$; $F = 169.02$; $F_{0.05\,(1)\,133} = 3.91$) (Fig. 5.11).

The number of gravid proglottids per worm did not show any relationship to infrapopulation size ($r^2 = 0.009$; $r = -0.095$; $P \geq 0.272$; $F = 1.22$; $F_{0.05\,(1)\,133} = 3.91$) (Fig. 5.12).

5.5.4 Mean length of gravid proglottids

Monthly mean length of gravid proglottids of *P. filicollis* is shown in Fig. 5.13.

The high value observed in February 1994 is based on measurements from a single worm.

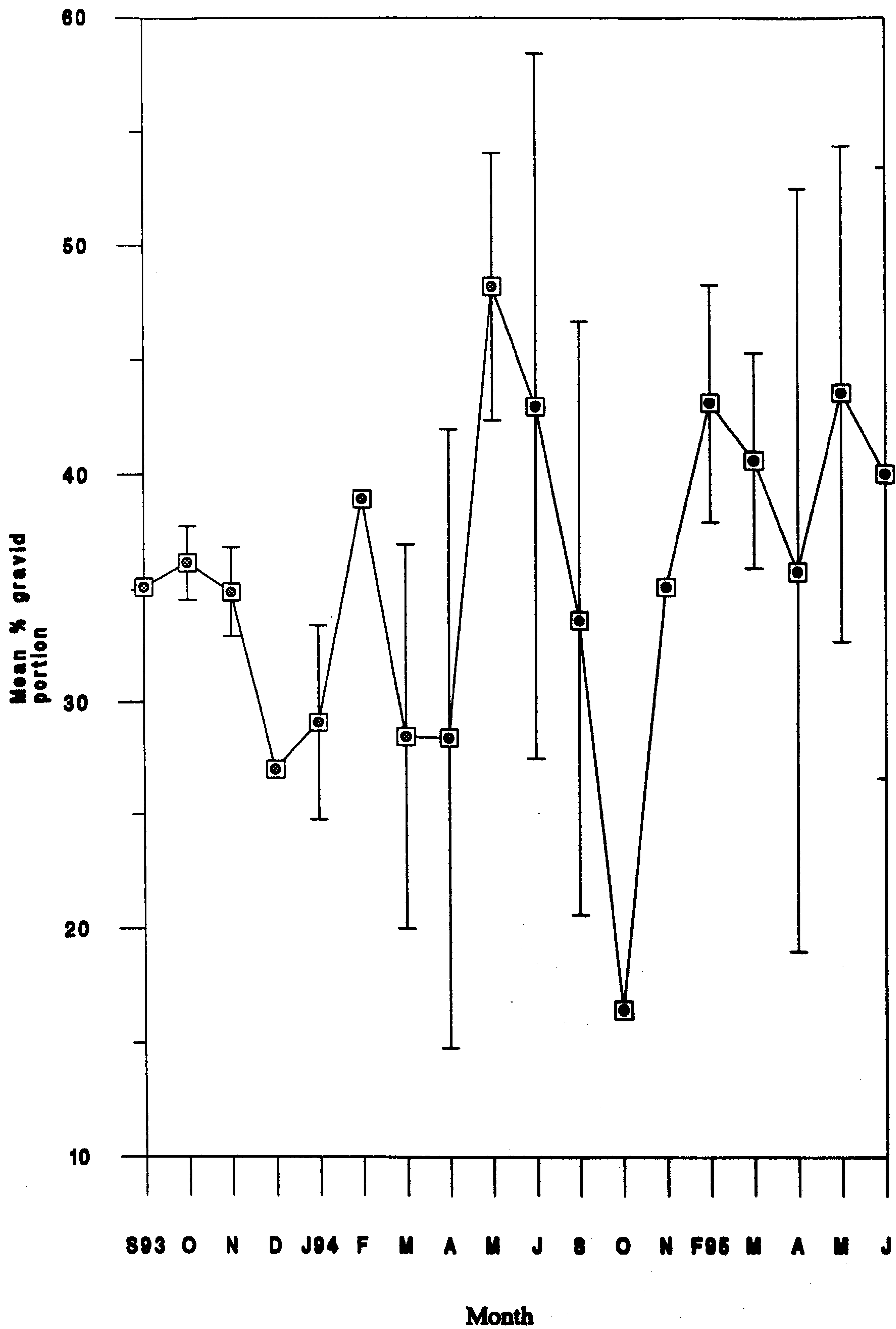


Fig 5.7 Monthly mean percentage gravid portion of *Proteocephalus filicollis*. Bars represent standard deviations.

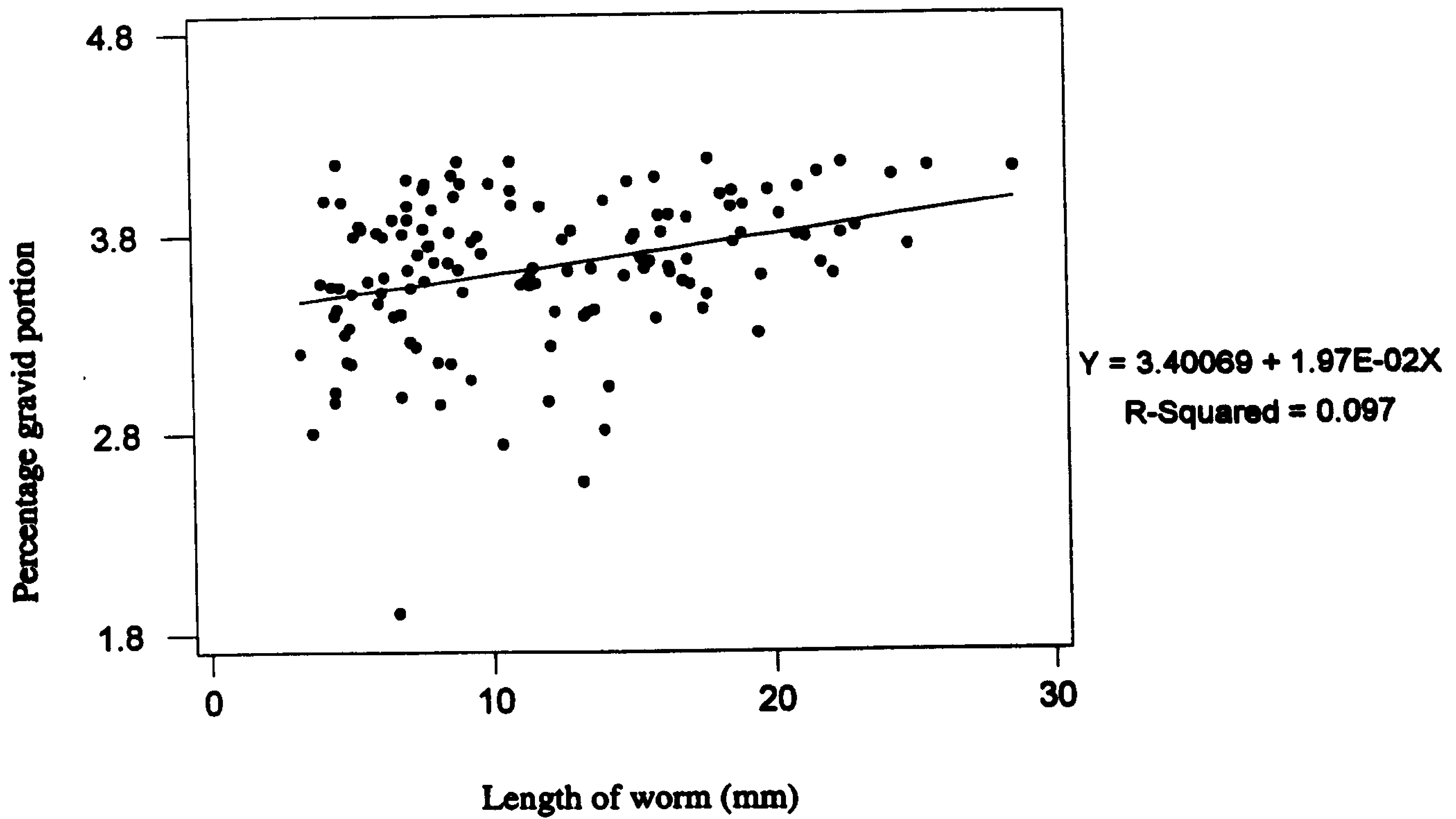


Fig 5.8 Re: lationship between length of *Proteocephalus filicollis* (mm) and percentage gravid portion of the worm.

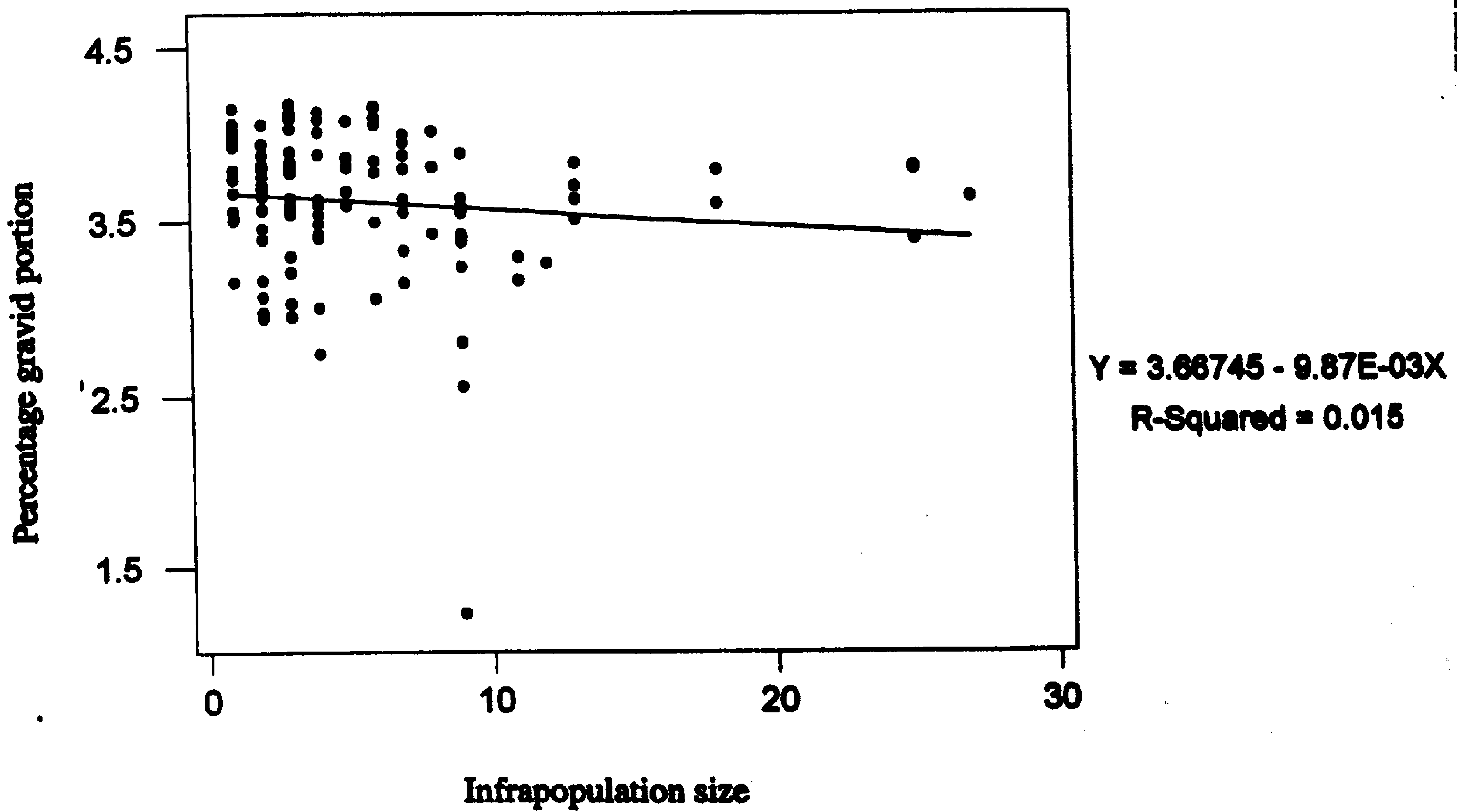


Fig 5.9 Relationship between infrapopulation size and percentage gravid portion of *Proteocephalus filicollis*.

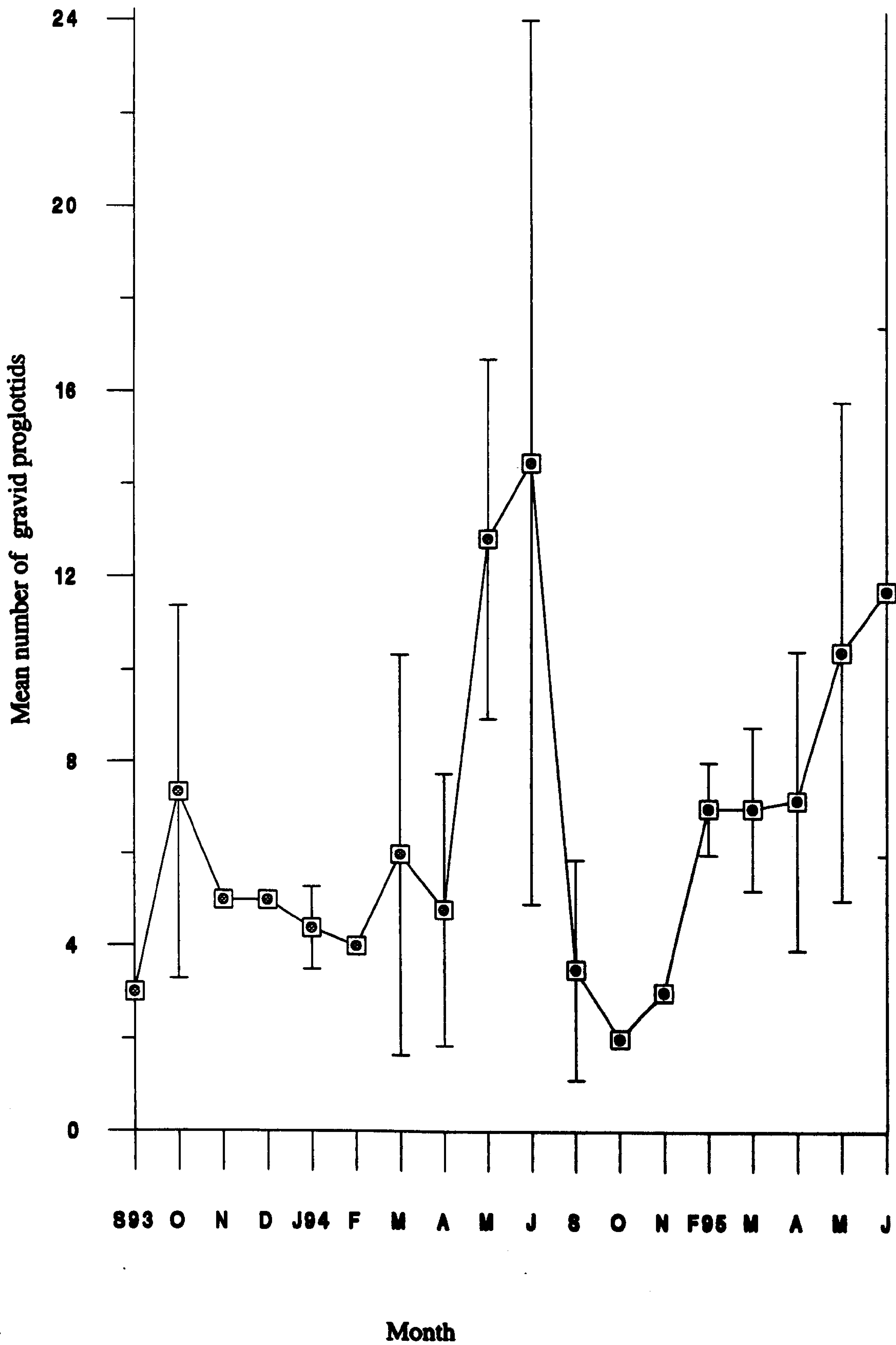


Fig 5.10 Monthly mean number of gravid proglottids of *Proteocephalus filicollis*. Bars represent standard deviations.

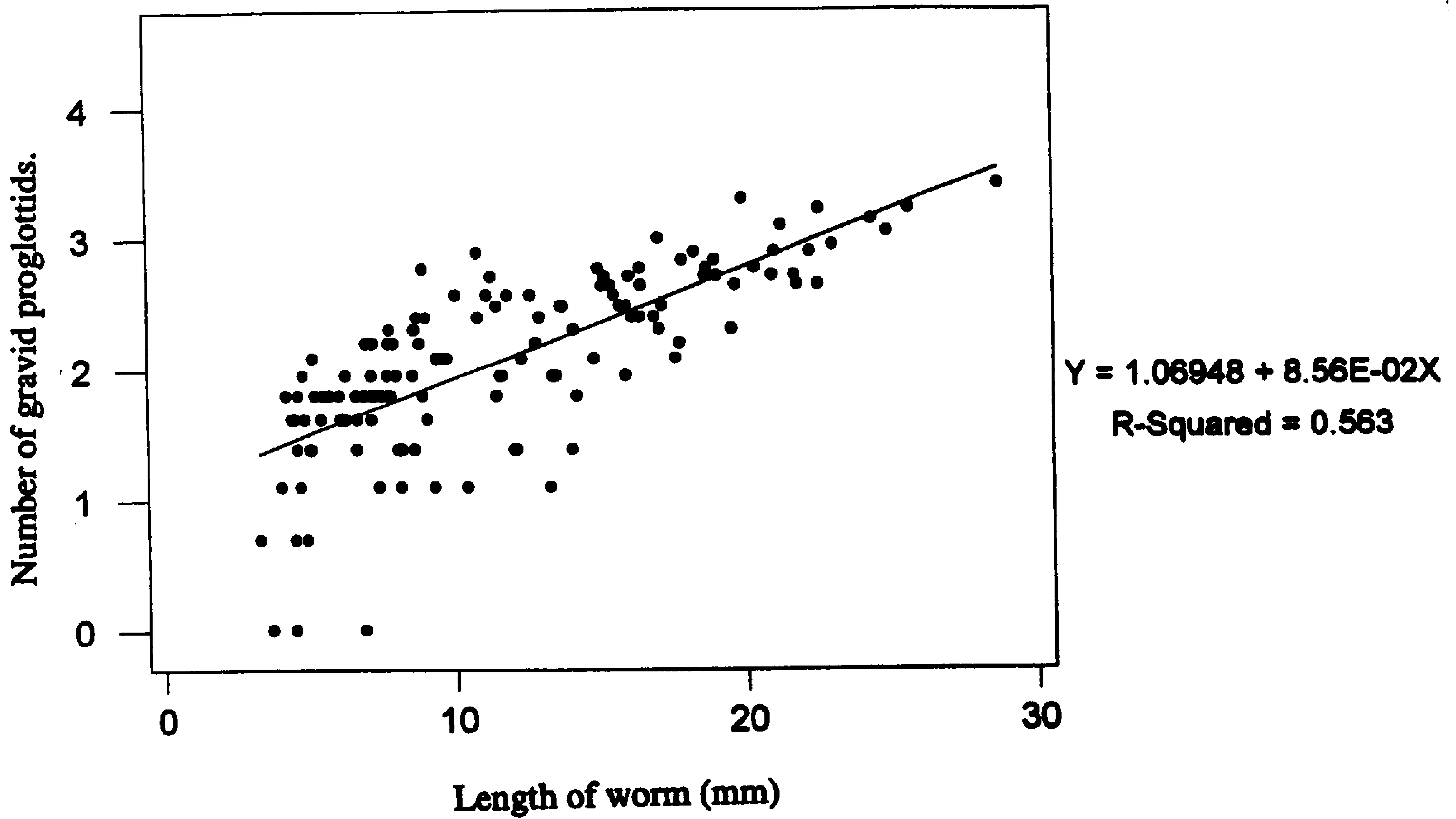


Fig 5.11 Relationship between length of *Proteocephalus filicollis* (mm) and number of gravid proglottids.

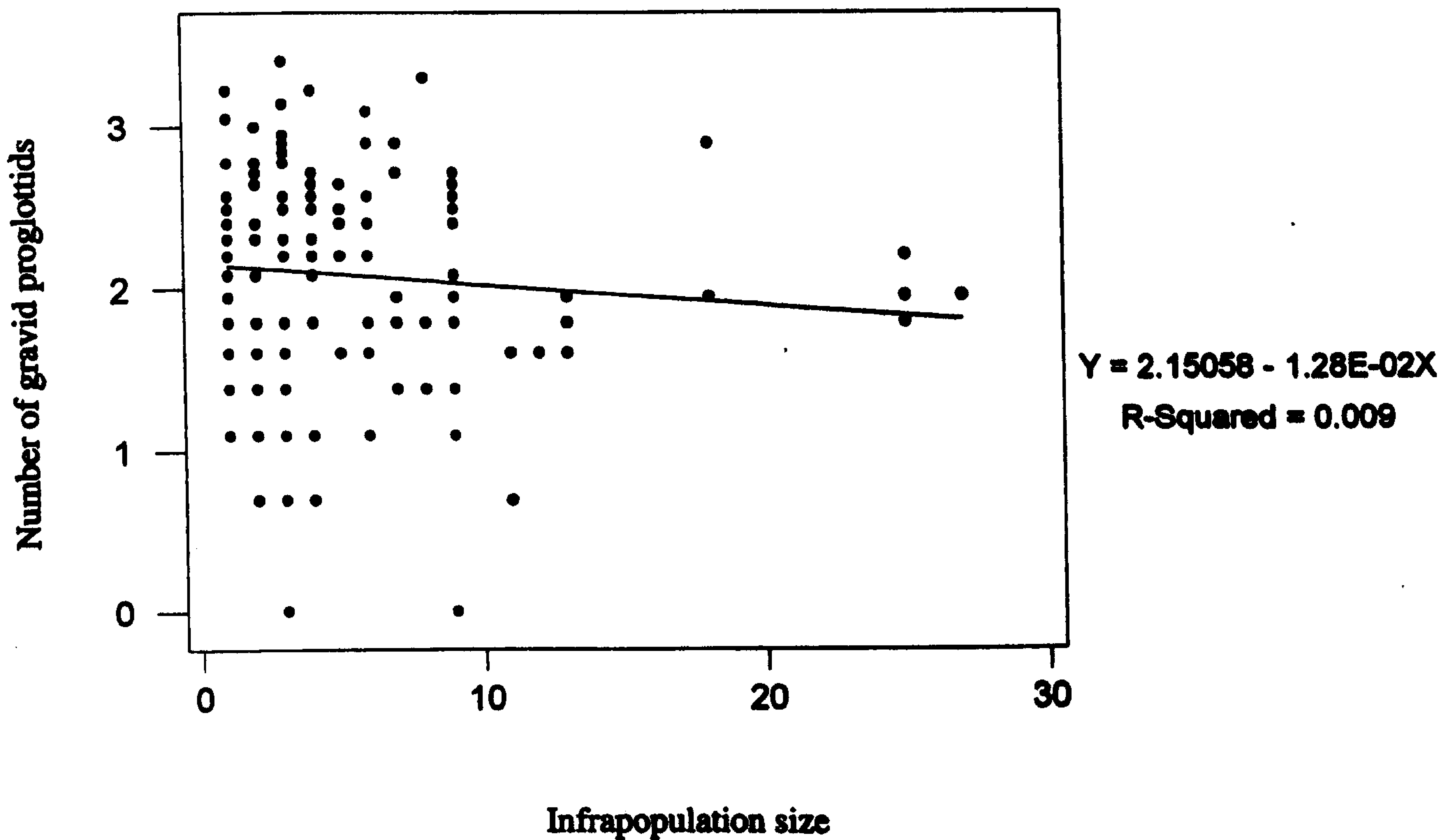


Fig 5.12 Relationship between infrapopulation size of *Proteocephalus filicollis* and number of gravid proglottids.

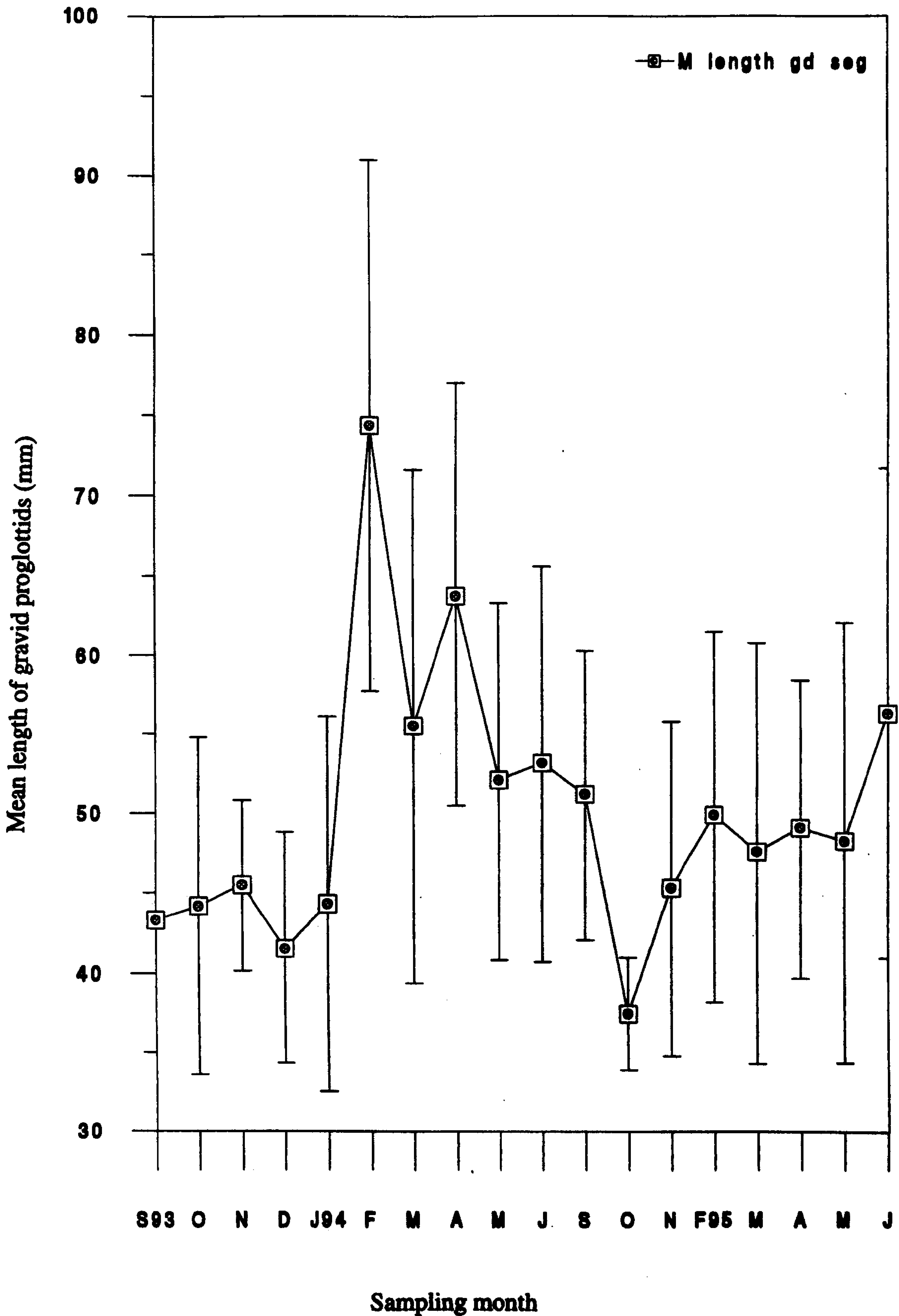


Fig 5.13 Monthly mean length of gravid proglottids of *Proteocephalus filicollis*. Bars represent standard deviations.

The mean length of gravid proglottids showed a significant positive relationship with worm length ($r^2 = 0.194$; $r = 0.044$; $P < 0.001$; $F= 31.60$; $F_{0.05\,(1)\,133} = 3.91$) (Fig. 5.14) but not show any relationship with infrapopulation size ($r^2 = 0.004$ %; $r = -0.06$; $P \geq 0.456$; $F = 0.56$; $F_{0.05\,(1)\,133} = 3.91$) (Fig. 5.15).

5.5.5 Seasonal variation in diameter of *Proteocephalus filicollis* eggs.

The mean diameter of *P. filicollis* eggs, recorded from September 1994 to July 1995 is shown in Fig 5.16. A total of 150 eggs was measured from 16 different worms;each sample included 8-10 eggs. Maximum mean size (40 μm) was observed in September and minimum mean size (16.5 μm) in the last week of June 1995.

Analysis of variance showed a significant variation in the diameter of eggs throughout the sampling period ($F = 135.52$; $P < 0.001$; $F_{0.05\,(1)\,149} = 3.90$). The eggs showed two distinct diameter ranges. Egg diameter ranged from 25-40 μm in September 1994, February, April and the first two weeks of May 1995. The mean egg diameter was reduced and the range narrowed to 16-21 μm from the second week of May to the second week of July 1995 (Fig. 5.16).

There was a significant negative relationship between egg diameter and worm length ($r^2 = 0.363$; $r = -0.605$; $P < 0.001$; $F = 85.33$; $F_{0.05\,(1)\,149} = 3.90$) (Fig. 5.17), and the range of egg diameter was greater in smaller worms.

A weak positive significant relationship was observed between egg diameter and infrapopulation size ($r^2 = 0.259$; $r = 0.509$; $F = 51.83$; $P < 0.001$; $F_{0.05\,(1)\,149} = 3.90$. This relationship may be due to the presence of egg samples from two infrapopulation size ranges (1 to 11 and 25 to 27) (Fig. 5.18).

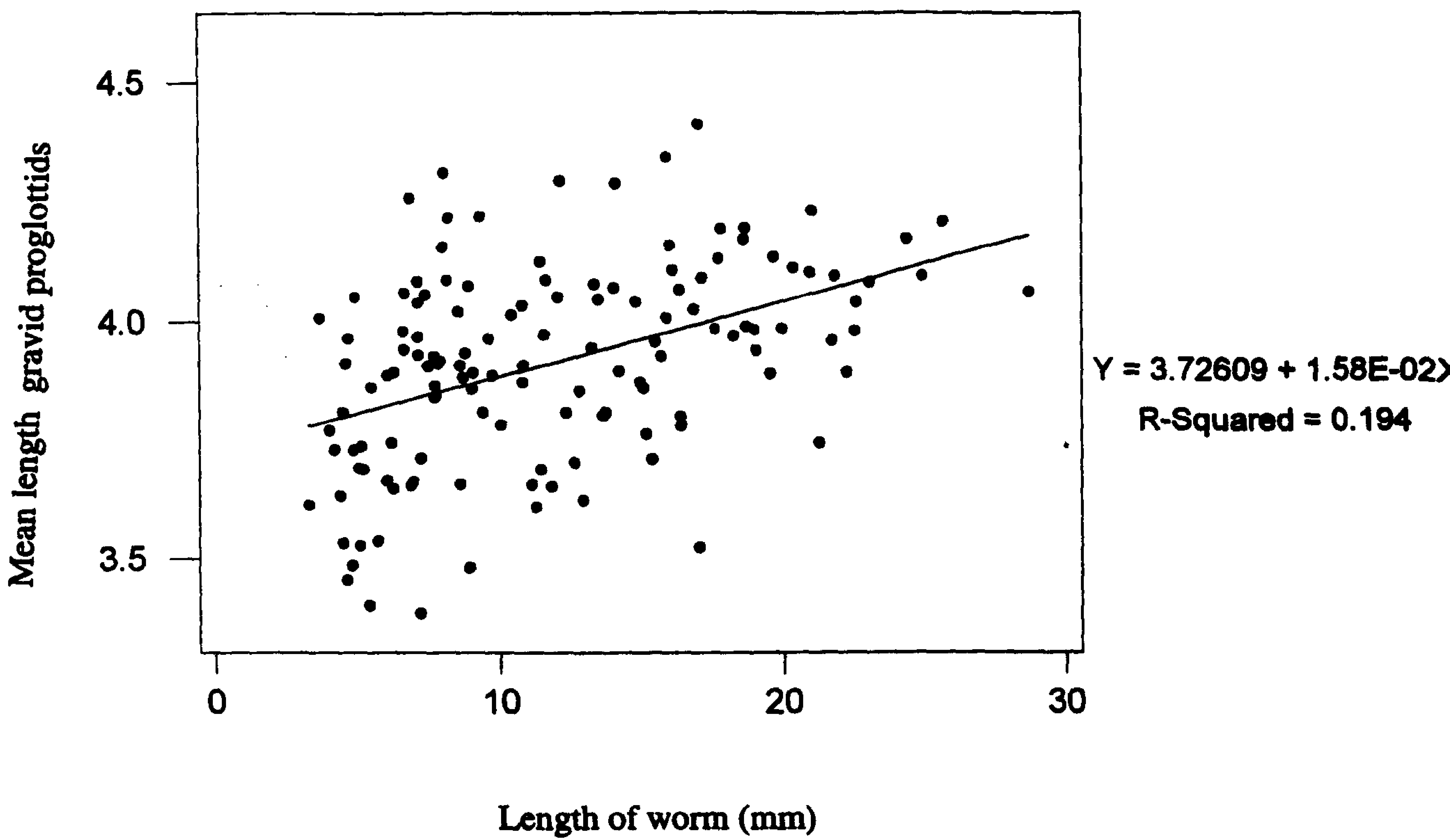


Fig 5.14 Relationship between length of *Proteocephalus filicollis* (mm) and mean length of gravid proglottids.

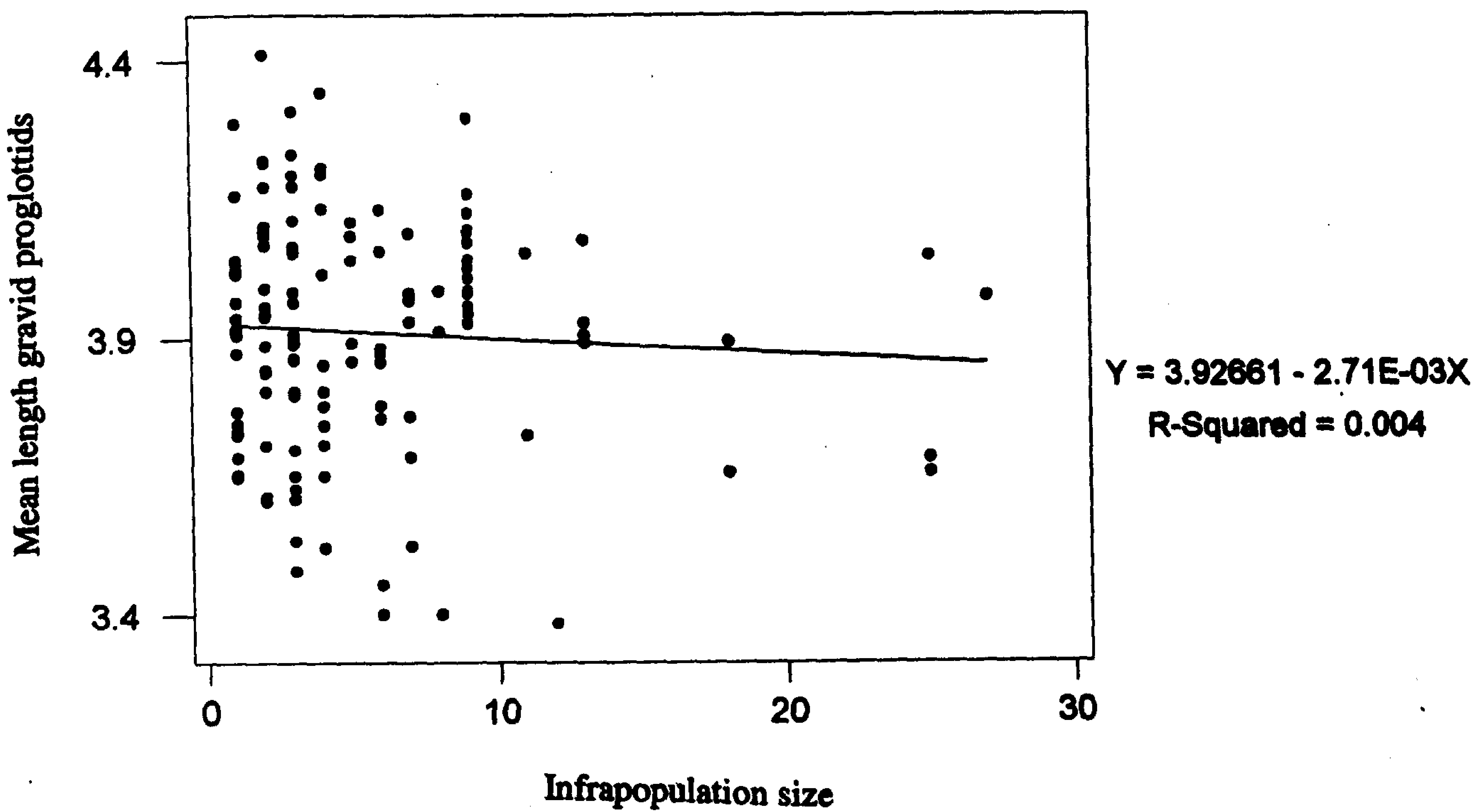


Fig 5.15 Relationship between infrapopulation size of *Proteocephalus filicollis* and mean length of gravid proglottids.

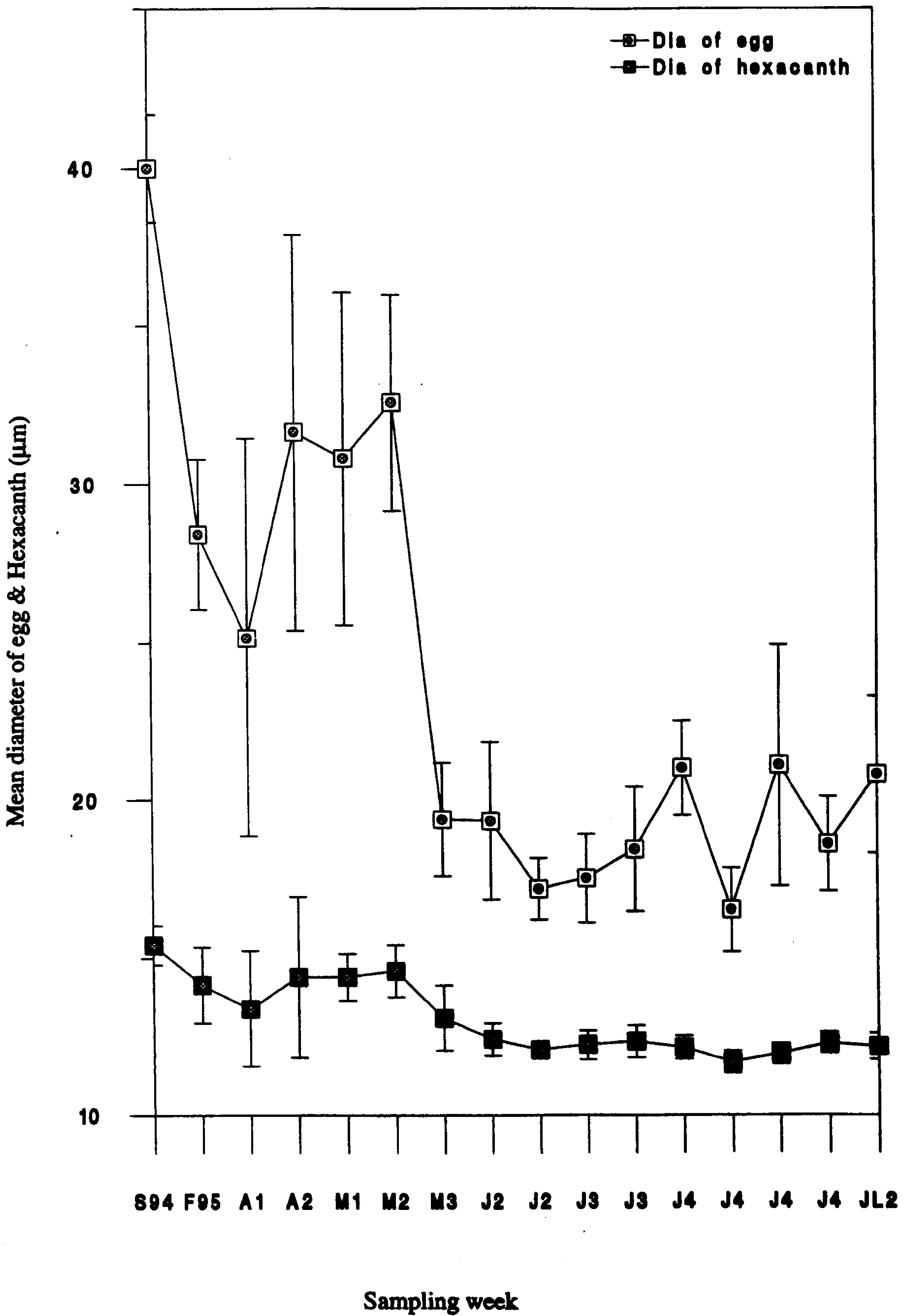


Fig 5.16 Mean diameter of *Proteocephalus filicollis* egg and hexacanth in different months. The letters S, F, A, M, J, JL stand for September, February, April, May, June, July and the number on the right of the letter indicates year and sampling week. Bars represent standard deviations.

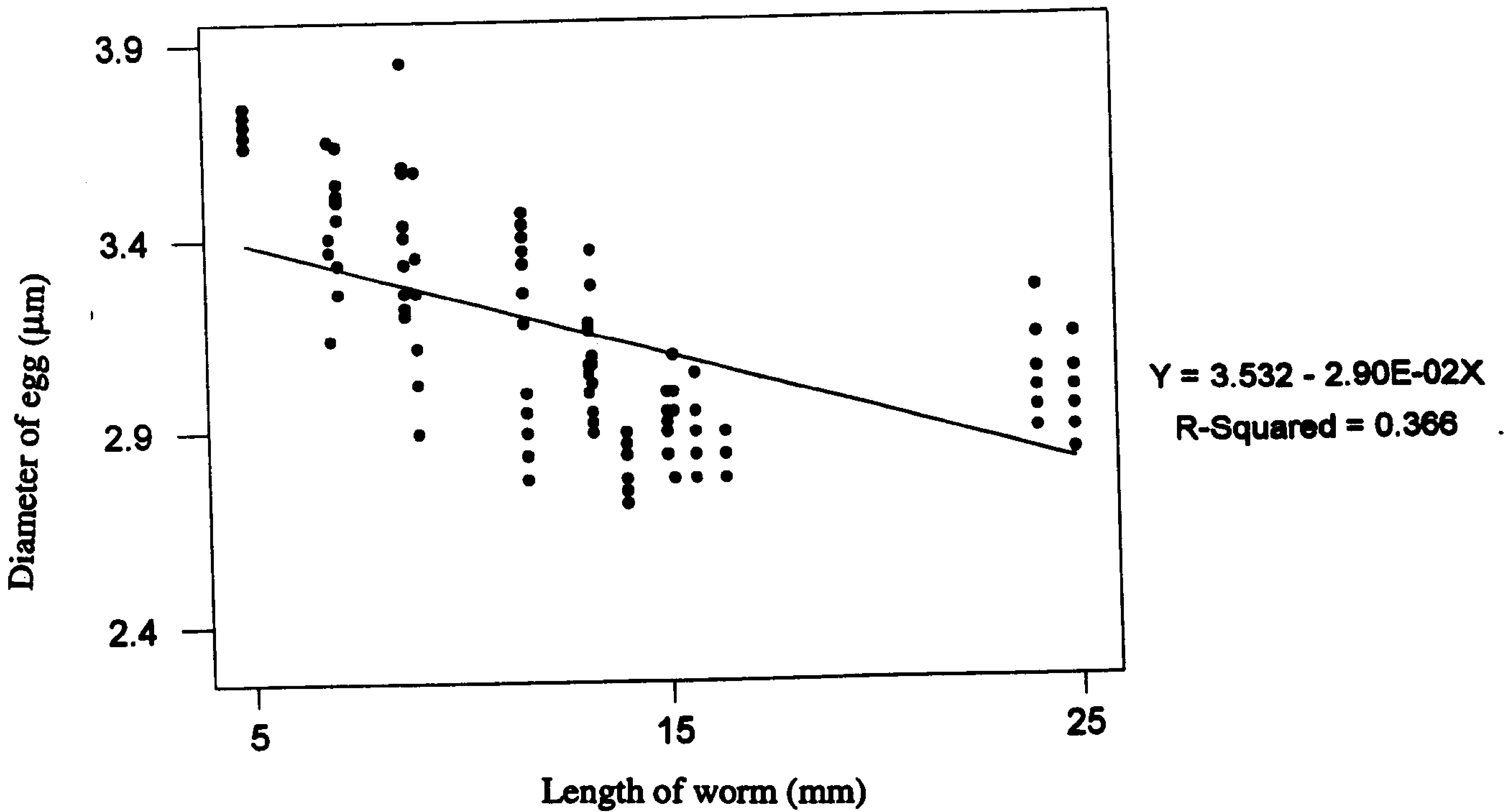


Fig 5.17 Relationship between length of *Proteocephalus filicollis* (mm) and diameter of egg (µm). These eggs were sampled in September 1994, February, April, May, June and July 1995.

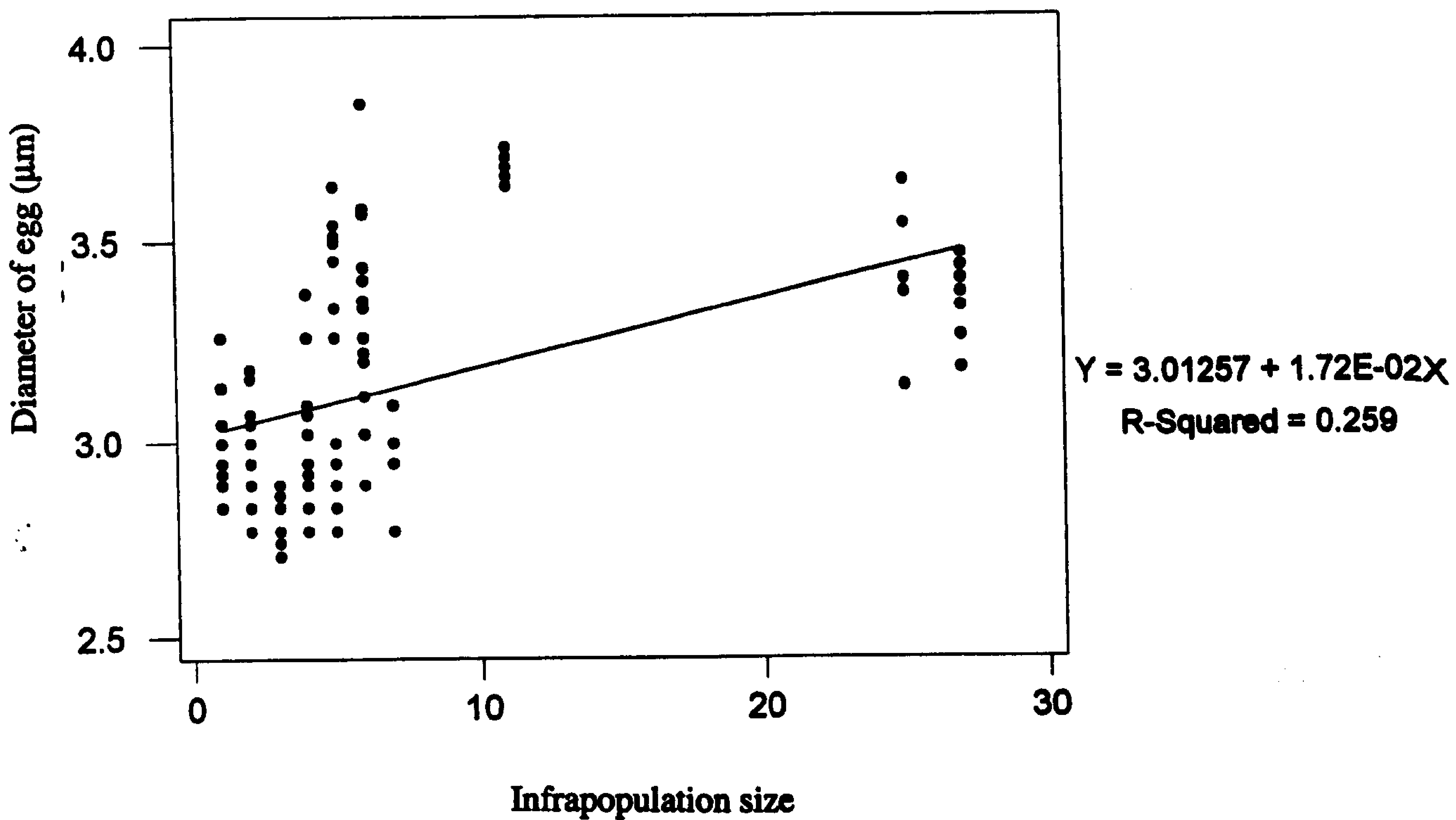


Fig 5.18 Relationship between infrapopulation size and diameter of *Proteocephalus filicollis* eggs (µm). These eggs were sampled in September 1994, February, April, May, June and July 1995.

5.5.6 Mean diameter of hexacanth in *Proteocephalus filicollis* eggs.

The mean diameter of *P. filicollis* hexacanths from September 1994 to July 1995 is shown in Fig. 5.16. The maximum mean diameter (15.4 µm) of hexacanths was observed in September 1994 and the smallest (7.5 µm) in the second week of June 1995. Analysis of variance demonstrated a significant variation in the diameter of hexacanths over the sampling period ($F = 121.97$; $P < 0.001$; $F_{0.05\,(1)\,149} = 3.90$). Mean diameter of the hexacanth showed a wide range (13-15 µm) from September 1994 to May 1995 and a narrow range (11.5 - 12.5 µm) from the second week of June 1995 to second week of July 1995 (Fig. 5.16).

The diameter of the hexacanth was significantly positively correlated to egg diameter ($r^2 = 0.646$; $r = 0.80$; $P < 0.001$; $F = 270.53$; $F_{0.05\,(1)\,149} = 3.90$) (Fig. 5.19).

5.5.7 Egg count and production.

A total of 200,439 *P. filicollis* eggs were released from 15 worms obtained from 12 different fish and were counted. The fish were sampled between the second half of May 1995 to mid-June 1995. The numbers of eggs obtained from different worms is shown in Fig. 5.20. A great variation in egg numbers was observed between worms (Table 5.1) (Fig 5.20).

Regression analysis showed that the number of eggs was positively correlated to worm length ($r^2 = 0.596$; $r = 0.772$; $P < 0.001$; $F = 19.07$; $F_{0.05\,(1)\,14} = 4.60$ (Fig. 5.21). There is no relationship between the number of eggs per mm of gravid portion and the length of worm ($r = 0.093$; $r = 0.305$; $P \geq 0.269$; $F = 1.34$; $F = 4.60$) (Fig. 5.22).

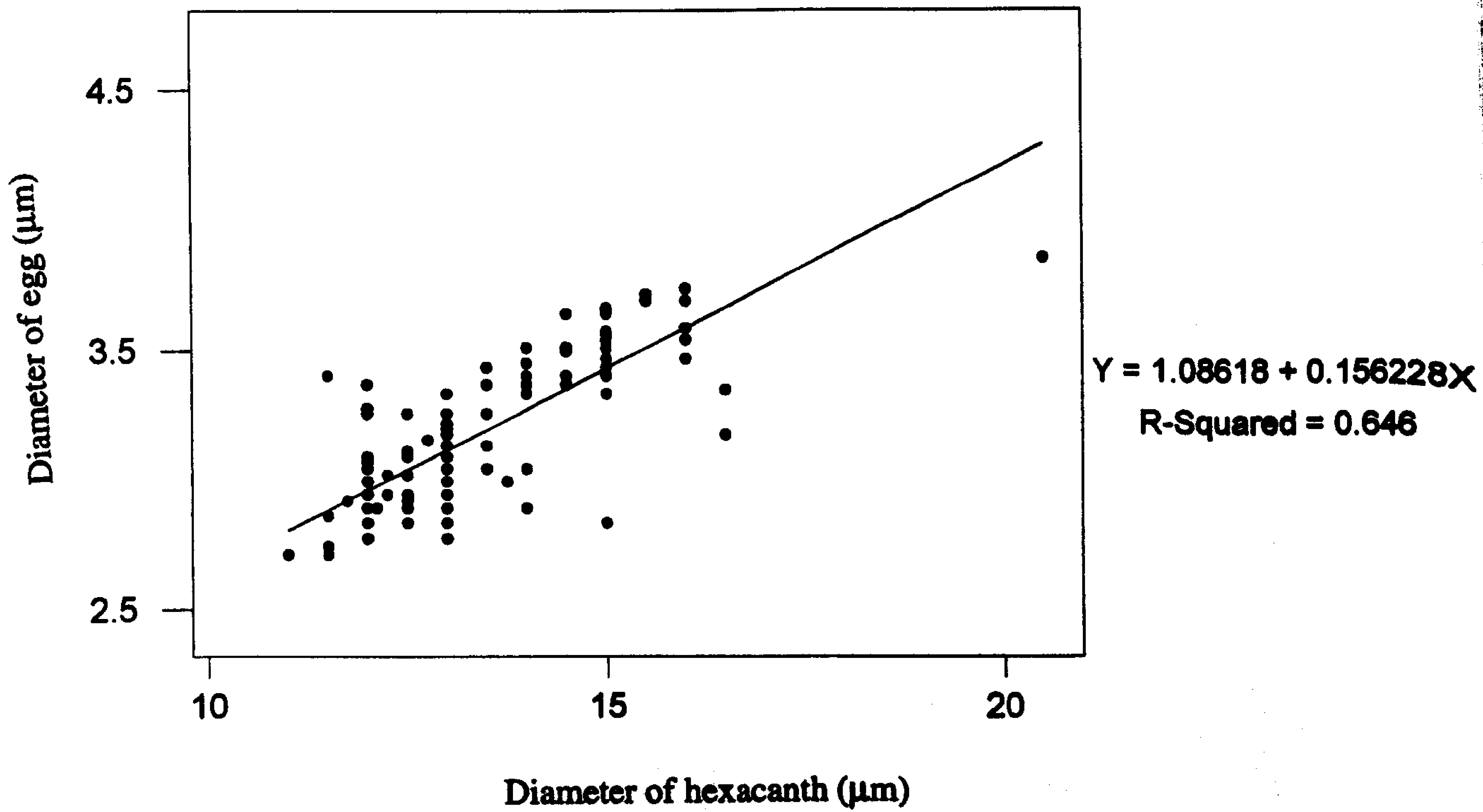


Fig 5.19 Relationship between diameter of ***Proteocephalus filicollis*** egg (μm) and diameter of hexacanth (μm). These eggs were sampled in September 1994, February, April, May, June, July 1995.

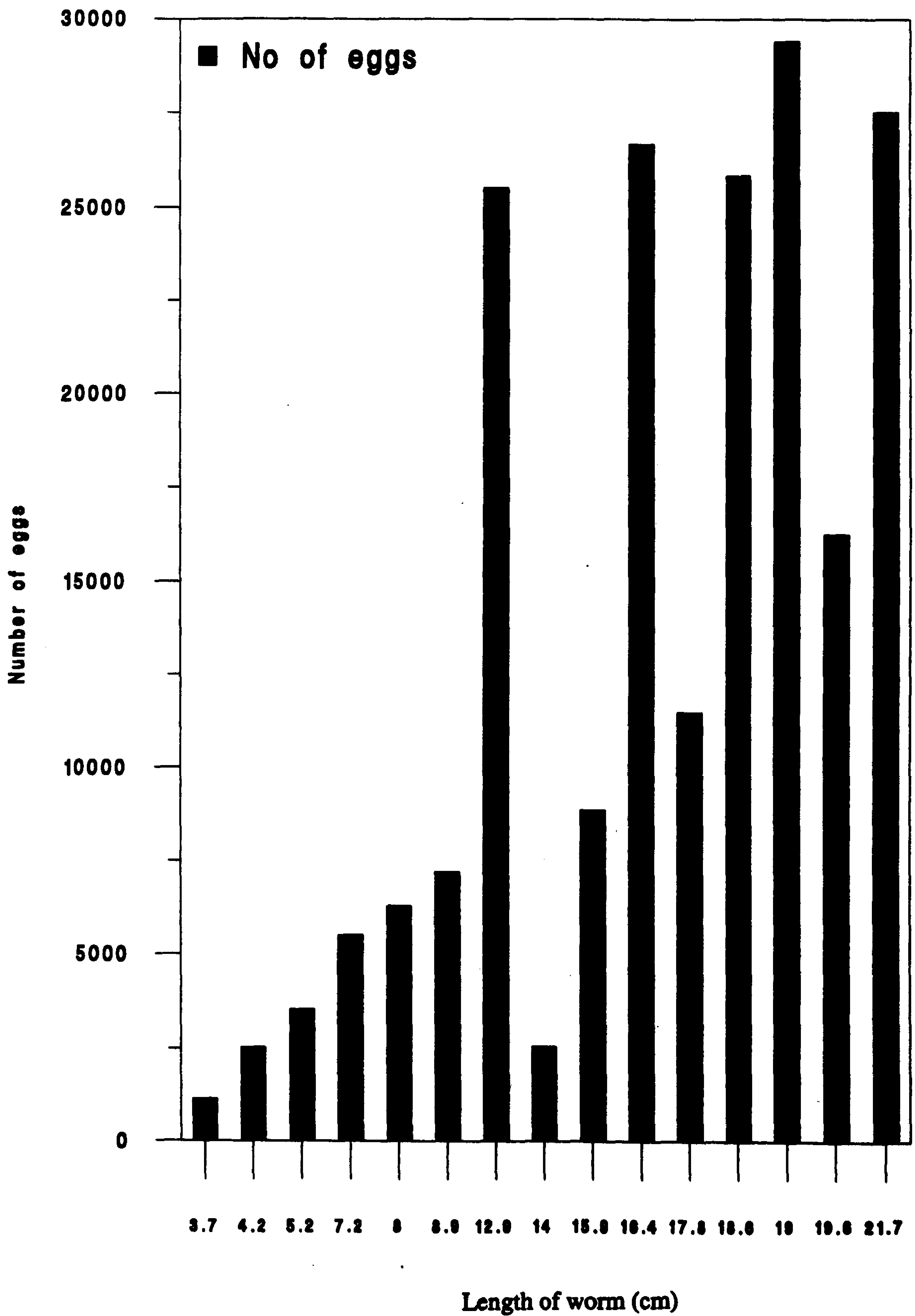


Fig 5.20 Number of eggs in *Proteocephalus filicollis* of different lengths. These worms were collected from mid May to mid June 1995.

Table 5.1. Production of *Proteocephalus filicollis* eggs in gravid worms of different length sampled from mid May to mid June 1995.

Length of worm (mm)	Number of eggs	% gravid portion	Eggs per mm gravid portion	Number of gravid proglottids	Eggs per gravid proglottids
3.71	1133	16.4	1857	1	1133
4.22	2565	50.4	1204	6	428
5.16	3546	33.3	2050	8	443
7.16	5508	48.2	1597	5	1102
8.03	6300	50.6	1560	4	1575
8.94	7200	64.2	1261	16	450
12.91	25549	45.6	4338	11	2323
14.01	2565	16.6	1101	4	641
15.89	8864	29.2	1906	7	1266
16.41	26674	36.4	4387	14	1905
17.84	11508	64.6	997	17	677
18.61	25825	50.9	2227	15	1722
19.06	29397	51.5	2994	15	1960
19.66	16278	36.1	2289	14	1163
21.71	27527	60.3	2101	15	1835

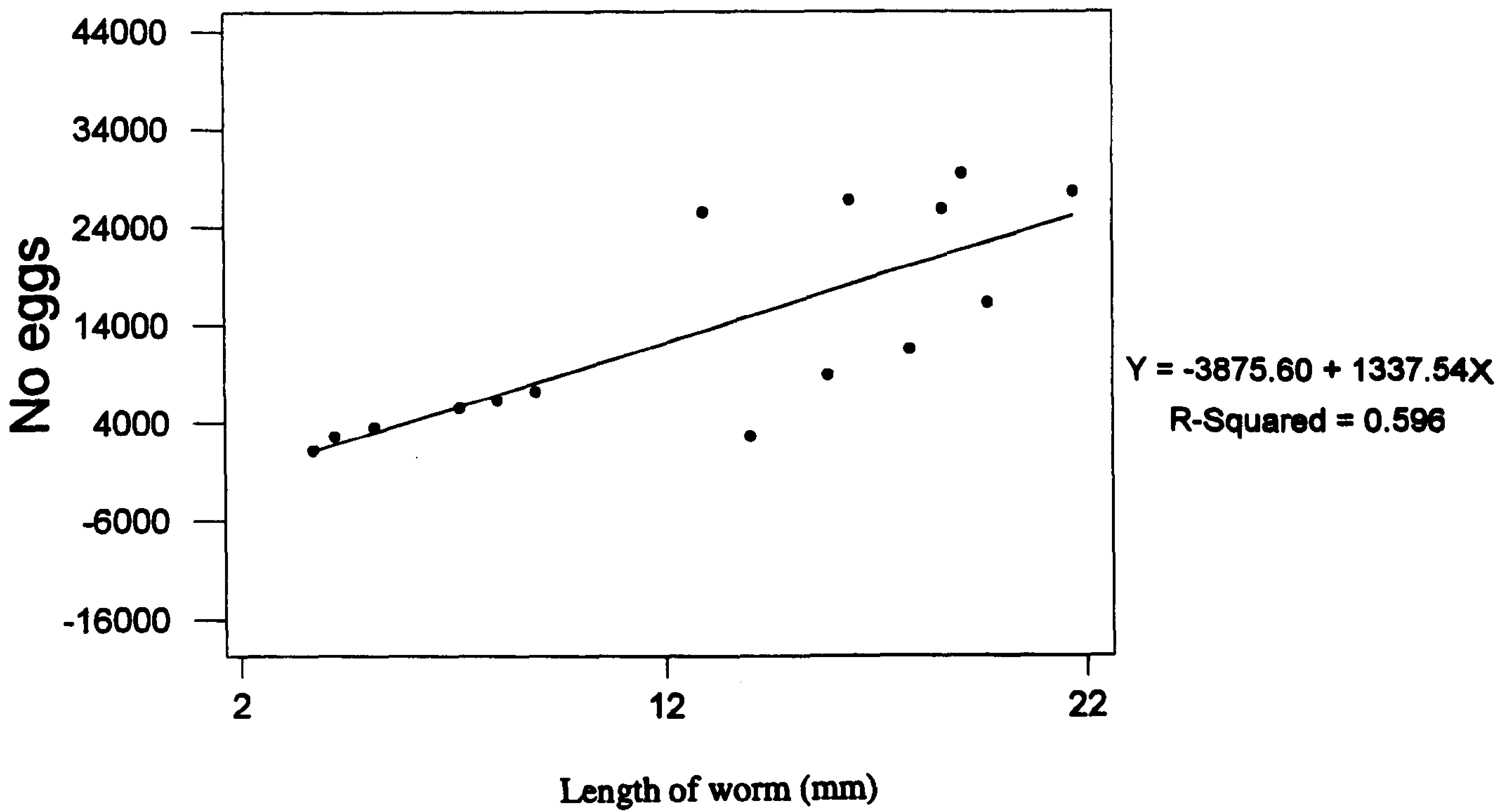


Fig 5.21 Relationship between length of *Proteocephalus filicollis* (mm) and number of eggs. These gravid worms were collected from mid May to mid June 1995.

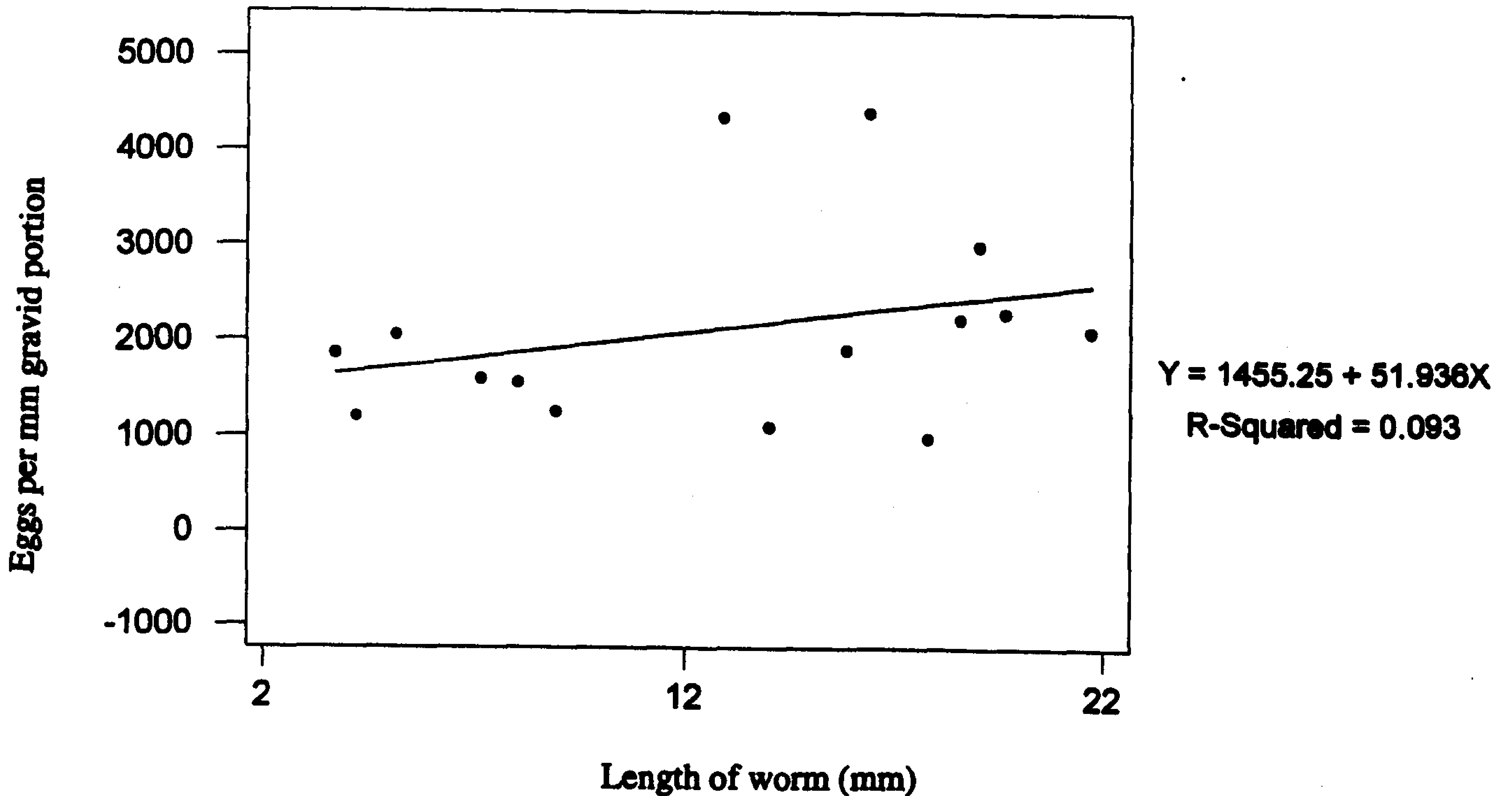


Fig 5.22 Relationship between length of *Proteocephalus filicollis* (mm) and number of eggs per mm gravid portion. These gravid worms were collected from mid May to mid June 1995.

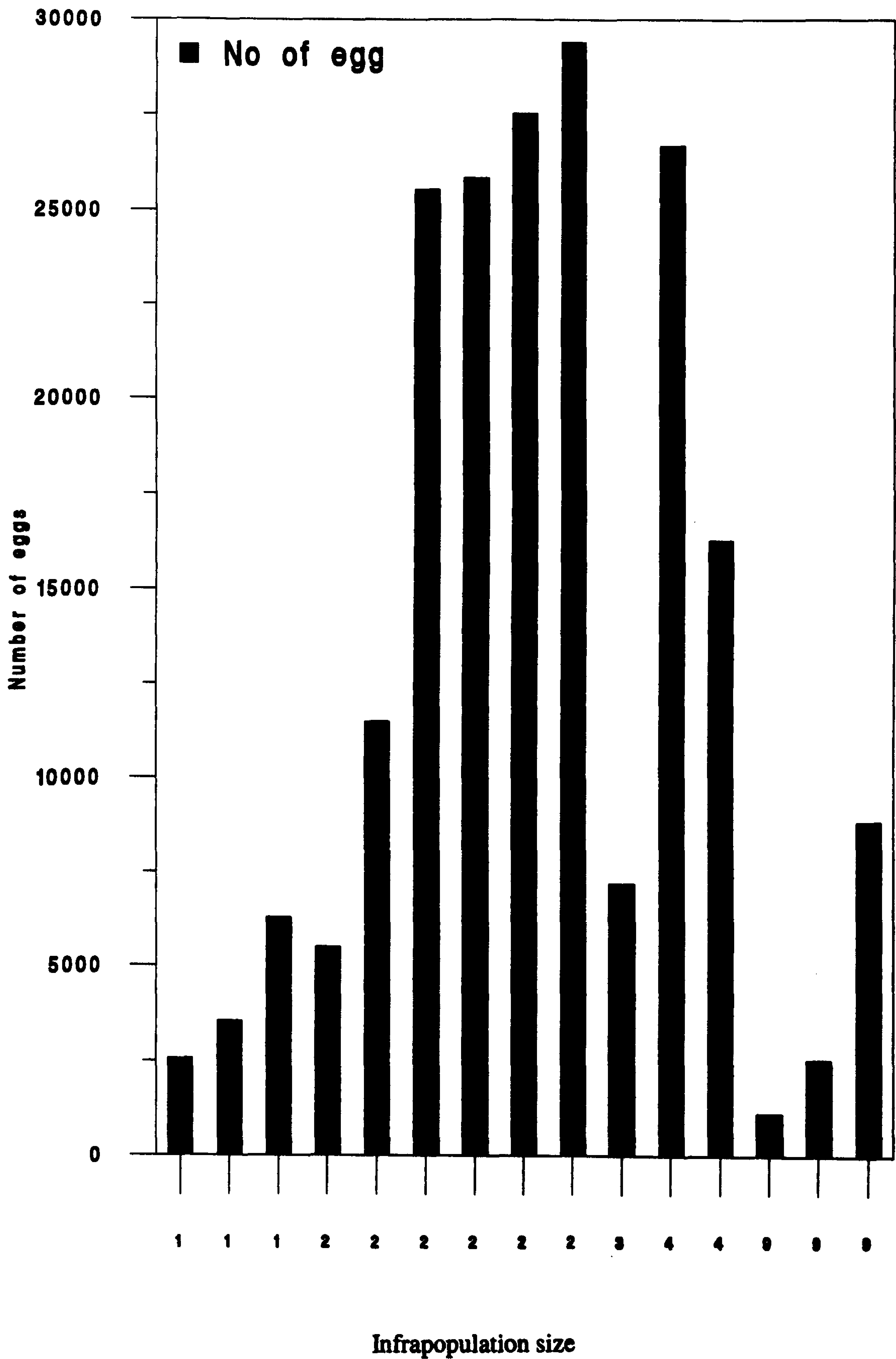


Fig 5.23a Number of *Proteocephalus filicollis* eggs in relation to infrapopulation size. These worms were collected from mid May to mid June 1995.

Table 5.2. Egg production of *Proteocephalus filicollis* in relation to infrapopulation size. These worms were collected from mid May to mid June 1995.

Infrapopulation size	Number of eggs per worm	Percenrage gravid portion	Eggs per mm gravid portion	Number of gravid proglottids	Eggs per gravid proglottids
1	2565	40.47	1204	6	428
1	3546	33.33	2050	8	443
1	6300	50.63	1560	4	1575
2	5508	48.23	1597	5	1102
2	25549	45.62	4338	11	2323
2	11508	64.63	997	17	677
2	25825	50.94	2227	15	1722
2	27527	60.33	2101	15	1835
2	29397	51.52	2994	15	1960
3	7200	64.2	1261	16	450
4	26674	36.44	4387	14	1905
4	16278	36.15	2289	14	1163
9	8864	29.26	1906	7	1166
9	2565	16.63	1101	4	641
9	1133	29.26	1857	1	1133

The number of eggs per worm in different infrapopulation sizes is shown in Fig. 5.23a. The highest number of eggs (29397) was observed in an infrapopulation of 2 worms and the lowest (1133) in an infrapopulation of 9 worms (Table 5.2).

Regression analysis demonstrated that the number of eggs in an individual worm was not significantly correlated to infrapopulation size ($r^2 = 0.159$; $r = -0.398$; $P \geq 0.14$; $F = 2.47$; $F_{0.05(1)14} = 4.6$) (Fig. 5.23).

The number of eggs per mm of gravid portion did not show a significant relationship with infrapopulation size ($r^2 = 0.059$; $r = -0.243$; $P \geq 0.384$; $F = 0.81$; $F_{0.05(1)14} = 4.6$) (Fig. 5.24).

5.5.8 Estimation of fecundity of *Proteocephalus filicollis*

In 1993-94, 614 worms were recovered from 209 stickleback (abundance = 0.87) of which 58 worms were gravid (abundance = 0.082). In 1994-95 from 228 fish 1097 worms were recovered (abundance = 2.41) and 115 worms were gravid (abundance = 0.25). The ratio of total worms to gravid worms recovered in the two years is given in Table 5.3. The ratio indicates that for every 10 worms recruited one worm reaches maturity. The mean number of egg per mm gravid portion is estimated to be 2124.0 ($\pm$ 1050.38 Sd, n= 15) (all worms sampled from 1994-95 population). The estimated number of eggs produced by the gravid worms is given in Table 5.4. The ratio of egg production to worms recruited and the recovery of gravid worms is given in Table 5.5. This Table indicates that for every 426 viable eggs produced one egg becomes an infective larva and invades the final host. However, one of every 10 larvae which infect fish only one will become gravid. Thus every mature egg has a 0.00024% chance of becoming an infective larva and every larva invading fish has an 8.75% chance of becoming a gravid worm. So the ratio of estimated egg production to gravid worms is

approximately 4064 :1. The ratio of total gravid worms to the estimated eggs production in the two years is given in Table 5.6.

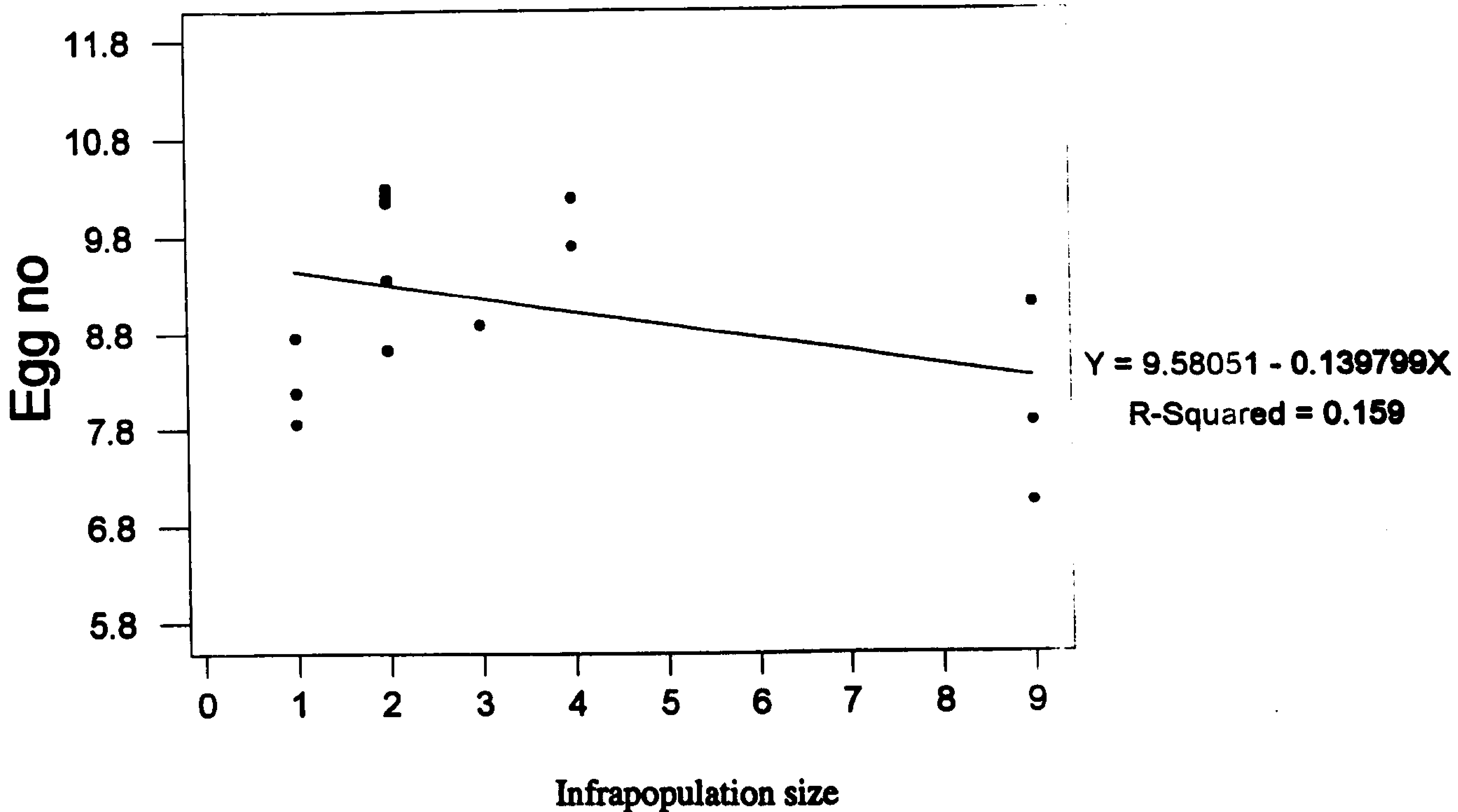


Fig 5.23 Relationship between infrapopulation size and number of eggs of *Proteocephalus filicollis* . These worms were collected from mid May to mid June 1995.

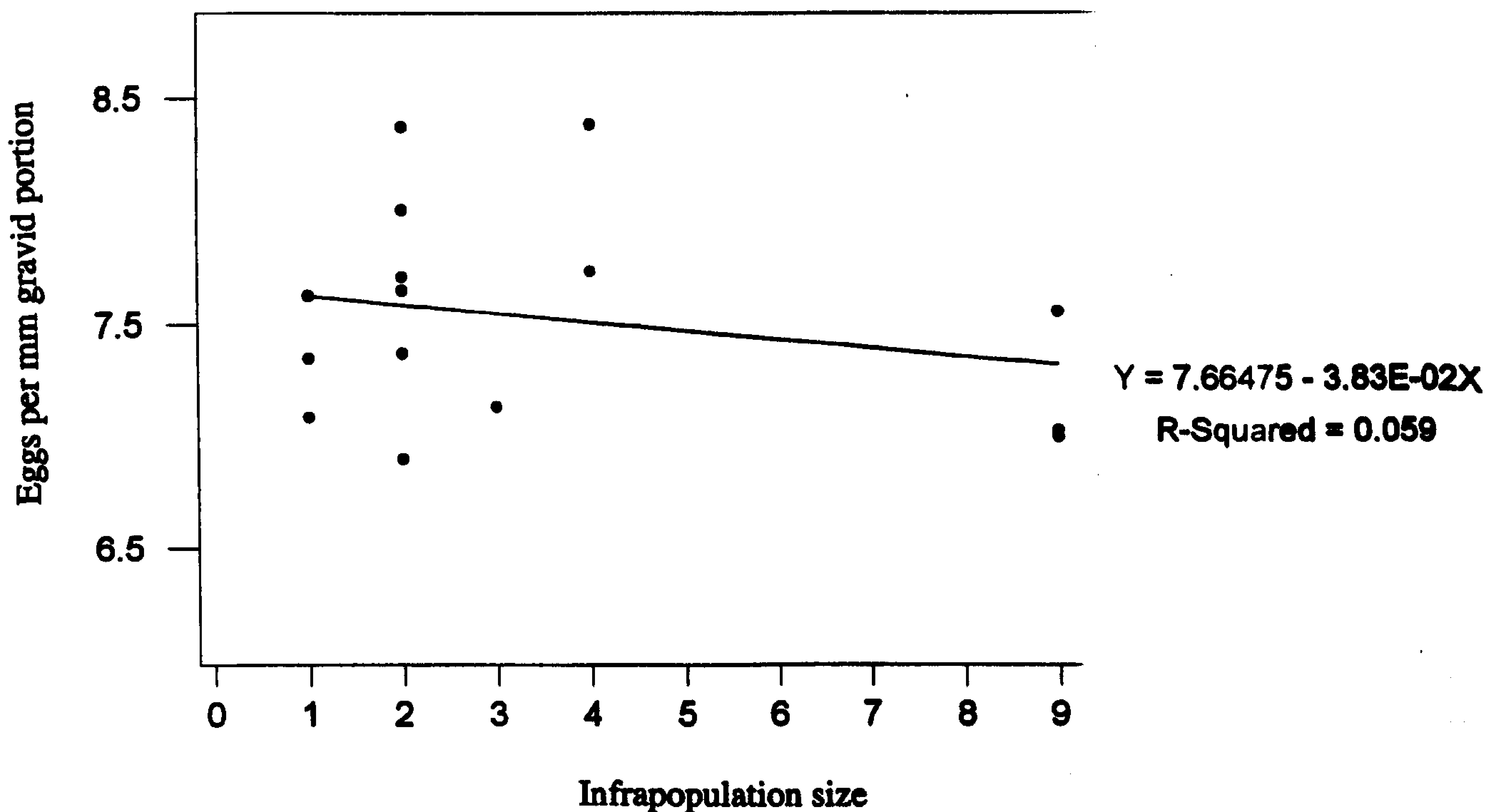


Fig 5.24 Relationship between infrapopulation size and number of eggs of *Proteocephalus filicollis* per mm gravid portion. These worms were collected from mid May to mid June 1995.

Table 5.3. Estimation of fecundity of *Proteocephalus filicollis* from *Gasterosteus aculeatus* in Airthrey Loch during the years 1993-94 and 1994-95.

Year	Total fish sampled	Infected fish	Total worm recovered	Abundance	Total gravid worm recovered	Abundance of gravid worm	Ratio total worm to gravid worm
1993-94	704	209	614	0.87	58	0.082	10.58 :1
1994-95	445	228	1097	2.46	115	0.258	9.53 :1
Total	1159	437	1711	3.91	173	0.149	9.89 :1

Table 5.4. Estimated egg production of Proteocephalus filicollis in 1993-94, 1994-95 from Airthrey Loch.

Year	Total worm Recruited	Total gravid worm measured	Length gravid portion (mm)	Eggs / mm gravid portion	Total estimated Egg production
1993 94	614	45	220.20	2124.6 *	467,837
1994-95	1097	96	454.36	2124.6	965,333

* Estimated from 1994-95 data.

Table 5.5 Ratio of egg production to worms recruited and recovery of gravid Proteocephalus filicollis in 1993-94 and 1994-95 from Airthrey Loch.

Year	Estimated egg Production	Worm recruited	Ratio column 2 & 3	Gravid worm recovered	Ratio column 3 & 5	Ratio column 2 & 5
1	2	3	4	5	6	
1993-94	467,837	1097	426.46:1			
1994-95		1097		115	9.53: 1	4068.1 : 1

Table 5.6 Ratio of gravid *Proteocephalus filicollis* to egg production In 1993-94 and 1994-95 from *G. aculetus* from Airthrey Loch.

Year	Gravid worms Measured	Estimated egg production	Ratio column 2 & 3
1	2	3	4
1993-94	45	467,837	1: 10396.3
1994-95	96	965,333	1: 10055.5
Total	141	1433,170	1:10164

5.6 DISCUSSION.

As pointed out by Kennedy (1983) it is extremely difficult to find any reliable estimates of fecundity of cestodes or of quantitative effects of the factors influencing it. The mean intensity of *P. filicollis* was observed to drop towards the start of summer in the present study, similarly Hopkins (1959) observed a reduction of mean intensity of *P. filicollis* at the beginning of summer. However, in this study the mean number of gravid worms per infected fish rose towards the beginning of the summer which is in contrast to Hopkins (1959) findings.

The length of gravid *Proteocephalus filicollis* was correlated to water temperature. Temperature has generally been considered a major factor influencing the development and maturation of the cestodes. The rise in temperature from spring towards summer was found to have an influence on the growth and development of *P. filicollis*. The longer days (photoperiod) coincide with the higher temperature of summer, hence it may be that photoperiod has some influence on the length of gravid worms. This influence would have to be exerted indirectly via the host.

The length of gravid *P. filicollis* was significantly positively correlated with the length of the host. The worm length showed a stronger relationship with the length of the host than water temperature. This probably reflect the fact that majority of *P. filicollis* infect juvenile stickleback over a limited period in summer. Parasite and fish then grow together over the winter before both mature in the spring. Gravid worms were found in all length classes of the host. This may be due to repeated or continuous recruitment of worm in the host population at random, different rates of development in different sizes of host, host behaviour or host habitat. Hopkins (1959) also pointed out that the chance of a worm surviving to maturity is independent of the size of the fish in which it occurs.

Although the length of gravid worms was not significantly correlated to infrapopulation size., observations from the present study have shown that worms grow and attain a greater length in infrapopulation sizes of 1-4 and beyond that there was a tendency for a decrease in length of worms. Gravid worms recovered from infrapopulations of 5 to 27individuals were in general shorter in length as compared to worms recovered from infrapopulations of 1- 4 parasites. Worms recovered from infrapopulations of 5 individuals and above were not all gravid, but these populations contained worms of different maturity stages, indicating repeated or continuous infection and / or different rates of development. It is suggested that there may be an optimum infrapopulation size above which the length of gravid worms is affected by the population density of the worms.

These findings support Ghazal & Avery (1974) and Hasselberg & Andereassen (1975) who stated that in heavy infections of *Hymenolepis nana* and *Hymenolepis diminuta*, respectively, a small proportion of the cestode population grows to a normal size and a larger proportion remain small and stunted. Dense populations of *Diphyllobothrium dendriticum* in golden hamster have been reported to have the smallest individuals (Halvorsen & Andersen, 1974). Davydov (1978) also stated that fecundity of the cestode *Bothriocephalus acheilognathi* depends on population density.

The percentage gravid portion of the strobila shows a positive relationship with length of the worm, but the percentage gravid portion did not demonstrate any relationship with infrapopulation size. This may reflect the wide variation in the percentage of gravid portion in the infrapopulation sizes observed in the present study.

This study has also indicated that one group of gravid worms, although small in size (4.22 mm, 7.16 mm, 8.03 mm, 8.94 mm) had a higher fecundity (% gravid portion, 50.47 %, 48.23 %, 50.63 % and 64.20 %, respectively), whereas another group of worms were longer in size (14.01 mm, 15.89 mm, 16.41 mm and 19.06 mm) but had a lower fecundity

(% gravid portion 16.63 %, 29.26 %, 36.44 % and 36.15 % respectively) during the peak period of egg production (Table 5.1). Ieshko & Anikieva (1992) also found that one group of *P. percae* reached higher fertility at a small size whilst an other group of worms were larger in size but had low fertility.

The number of gravid proglottids of *P. filicollis* per worm was correlated to the length of the worm, whereas gravid proglottids were lower in number, in worms from higher infrapopulation sizes as compared to lower infrapopulation size. Ghazal & Avery (1974) also reported that crowding decreased the rate of proglottid production in *H. nana*.

The mean length of gravid proglottids of *P. filicollis* was also correlated to the length of the worm. The mean length of gravid proglottids showed no relationship with infrapopulation size. Although the sample size of *P. filicollis* is reasonable it is difficult to ascertain the influence of infrapopulation size on the length of gravid proglottids. This may be because measurements were taken from different groups of gravid worms throughout the year, rather than in a particular season.

The results and findings of this study are perhaps not strictly comparable to those obtained for terrestrial tapeworms, especially *Hymenolepis* species where most fecundity studies are based on experimental infections and worms shed eggs in a few weeks post infection.

The variation observed in the diameter of *P. filicollis* eggs during this study indicates that winter eggs are larger than those obtained in spring and summer. In the same egg samples (September - July) the diameter of the hexacanth showed a similar trend compared to the egg diameter. The variations in egg diameter observed over three seasons may be an adaptation to different environmental conditions in those seasons. The variation in the diameter of the egg may be due to differences in the swelling of the outer float membrane in different seasons, since the diameter of the hexacanth did not show much variation.

It is difficult to explain the small eggs recorded in larger worms and larger eggs found in smaller worms. Smaller eggs were observed in lower infrapopulation sizes and larger eggs in higher infrapopulation sizes but the reason for this is unclear. It contrasts with the results of Ghazal & Avery (1974) who reported that crowding decreased the linear dimensions of *H. nana* eggs, although the shape of eggs was not affected.

The numbers of eggs released by worms are positively correlated to the length of worms. Although this relationship is strong it is suggested that there may be two groups of worms in the sample. 1) Those worms which have a moderate number of eggs, perhaps as a result of the release of a small number of eggs whilst retaining many eggs. 2) Those worms with a large number of eggs may be those which have not started releasing eggs. Ieshko & Anikieva (1992) also reported that numbers of eggs of *P. percae* are dependent on the length of strobila.

Number of eggs per cestode showed a tendency to be smaller in higher infrapopulation size. The highest number of eggs were recorded in infrapopulation sizes 2 & 4 and was lower in higher infrapopulation sizes. No data was available for infrapopulation sizes between 4 to 9 worms so it cannot be stated with certainity that the low numbers of eggs in infrapopulation size 9 was due to a crowding effect or to the length of the cestode.

One piece of evidence which supports the view that the numbers of eggs per cestode are fewer in higher infrapopulation sizes is the occurrence of a lower percentage of gravid portion of strobila in worms from higher infrapopulation sizes, compared to the higher percentage gravid portion in lower infrapopulation sizes.

The number of eggs per unit length of the gravid portion did not show any relationship with length of worm and infrapopulation in this study. Similarly, Ghazal & Avery (1974) demonstrated that crowding results in a decrease in the number of eggs per proglottid in *H. nana*.

Davydov (1978) pointed out that egg output is lower when helminth population density is high rather than vice versa. Although he reported a sharp decrease in egg output from single, two, three and five worm infections with *B. acheilognathi* this trend was not observed in our study, possibly because of the small sample size or the loss of eggs from worms prior to counting.

Proteocephalus filicollis has a higher fecundity than *B. acheilognathi* if egg output and worm size is taken into account (*B. acheilognathi*, 2 worm infection, worm size 7.7 cm, eggs output 35400 - 41280 (Davydov, 1978): *P. filicollis* 2 worm infection, worm size 7.16 - 21.71 mm, egg output 5508-29397, Table 5 .1).

The number of eggs per gravid segment in *P. filicollis* (423- 2323 eggs per gravid segment) suggests that this cestode also has a higher fecundity than *B. acheilognathi* from fathead minnow and red shiner (egg output per gravid segment 209 ± 17.8, and 152.0 ± 17.6 respectively) (Riggs *et al.*, 1987).

The mean egg output in *P. filicollis* is higher (mean number of eggs per mm = 2124.6, SD ± 1050.38, approximately 21246 eggs per cm of gravid portion) than *P. percae* (4900 per cm of gravid portion of strobila or 490 per mm) (Ieshko & Anikieva, 1992). The peak period of egg production in *P. filicollis* from Airthrey Loch in 1995 was 79 % longer (25 days, from 15.5.1995 to 8.6.1995) than the 14 days in *P. percae* from Lake Rendozero (Ieshko & Anikieva, 1992). According to Hopkins (1959), of every 200 *P. filicollis* which become attached to the intestine of *G. aculeatus* only one reached full maturity. In the present study it was estimated that one out of every 10 worms recruited becomes gravid and produces eggs. The reason for this higher rate of recovery of gravid worm from the population of stickleback from Airthrey Loch may be a larger population of infected

copepods in the loch, a higher rate of establishment and survival of worms in the sticklebacks with more reaching maturity.

Ieshko & Anikieva (1992) stated that the success of the survival of the parasite population is dependent on the survival of the mature parasites and their fertility, which is mainly regulated by the structure of the final host population. The number and distribution of many helminth species depends on the ratio between young and adult fish age groups in the population. Less than 1 % of adult sticklebacks survive to a second year in Scotland (Giles, 1987) and in Airthrey Loch individual sticklebacks mostly survive for only one year, which suggests that this situation may not apply in *P. filicollis*.

CHAPTER 6

DEVELOPMENT OF PROTEOCEPHALUS FILICOLLIS IN THE COPEPOD INTERMEDIATE HOST UNDER EXPERIMENTAL CONDITIONS.

6. Development of *Proteocephalus filicollis* in the copepod intermediate host under experimental conditions.

6.1 Introduction.

6.1.1 Life cycle of *Proteocephalus* species.

The members of the Proteocephalidae usually have a two host life cycle, in which the egg is ingested by a copepod intermediate host, and in which the larval parasites develop and grow to become infective to the definitive host.

Meggitt (1914) was the first to study the life cycle of *Proteocephalus filicollis* from *Gasterosteus aculeatus* and found that *Cyclops varius* could act as an intermediate host. He successfully infected *cyclops* with tapeworm eggs and also infected sticklebacks by feeding the infected copepods to the fish. Subsequently Kuczkowski (1925) studied the life cycle of a cestode he named *Proteocephalus percae*, the adult specimens of which were obtained from *G. aculeatus*. However, as suggested by Bauer (1962), Kuczkowski may have been dealing with *P. filicollis*.

Hunter (1928) studied the life cycle of *Proteocephalus ambloplitis* and found that *Cyclops prasinus* and *Cyclops albidus* were the first intermediate hosts. He infected these copepods and studied the development of procercoids up to day 16 post-infection. He did not observe the development of a cercomer in the procercoid. Hunter reported the transfer of *P. ambloplitis* from largemouth black bass fry to yearlings of the same species in the laboratory. Similarly, Hunter (1929) described the life cycle of *Proteocephalus pinguis*. Hunter found that *Eucyclops agilis* and *Cyclops vulgaris* acted as intermediate hosts for this parasite. Once again, he did not observe cercomer formation in the procercoid. *Perca flavescens* and *Notropis atherinoides* act as second

intermediate hosts and *Esox lucius* and *E. vermiculatus* as definitive hosts for *P. pinguis*.

In the last four decades there have been a considerable number of studies on the life cycle of the genus *Proteocephalus*.

Wagner (1954) studied the life history of *Proteocephalus tumidocollus* from rainbow trout and found that several species of copepod could serve as intermediate hosts for this parasite. Wagner found that the development of larvae in *Cyclops* was affected by temperature and that 20^0 C was the optimal temperature for growth, plerocercoids becoming infective to fish in only 9 days. No second intermediate host was required for completion of the life cycle but *P. tumidocollus* could transfer from fish to fish.

Freeman (1964) gave a detailed account of the biology of *Proteocephalus parallacticus* from lake trout and found that *Cyclops bicuspidatus*, *Cyclops vernalis* and *Cyclops scutifer* could act as intermediate hosts. Infective plerocercoids developed in 30 to 40 days at 16^0 C, and *C. bicuspidatus* was the most suitable copepod host. The maximum temperature for parasite growth was approximately 14^0 C in brook trout, and this temperature was higher than the maximum temperature for worm development in lake trout. The minimum temperature for growth of the parasite was less than 10^0 C but growth at such temperatures was very slow. Freeman (loc.cit) found that trout can be infected with plerocercoids directly from the copepods or with plerocercoids taken from the gut of other fish.of the same species.

Fischer (1968) studied the life cycle of *Proteocephalus fluviatilis* from smallmouth bass and reported that the plerocercoids developed in mature female *Cyclops bicuspidatus*, *Cyclops vernalis*, *Cyclops scutifer* and *Trophocyclops prasinus*. Development from egg to plerocercoid-1 required 18 to 21 days at 18^0 C. Plerocercoid-1 could infect the smallmouth bass fry directly from the copepod. Plerocercoid-1 could increase in size and mature in smallmouth bass. Fischer transferred segmented but not

gravid worms from one bass to another in which the worms continued to grow and differentiate. The plerocercoid could survive in the intestine for 6 days in fish other than bass.

Wootten (1974) investigated the life cycle of *Proteocephalus percae* from *Perca fluviatilis* and reported that development of larvae within the copepod intermediate host varies with the species of copepod, the temperature at which the latter are maintained and the intensity of infestation of the individual copepods. Fully developed larvae of *P. percae* were formed in *Acanthocyclops viridis* in 27 days at 14^0 C. Moreover, Wootten (loc.cit) found that in terms of prevalence, *Mesocyclops leuckarti* was the most successful host of *P. percae* with 62.9 % infection level, whereas *Eucyclops agilis* and *A. viridis* had prevalence rates of 52.5% and 16.1%, respectively. Multiple infection of copepods with larvae of *P. percae* was common.

Dupont & Gabrion (1987) studied the development of *Bothrocephalus claviceps* in the intermediate host, using five potential host copepod species in their experiments. They also found that growth and development of the plerocercoid depended on host species, sex and intensity of infection. The growth of plerocercoids was density dependent, whereas their development was independent of density.

Priemer (1987) studied the life cycle of *Proteocephalus exiguus* from rainbow trout and used *Cyclops strenuus* as an experimental intermediate host. He found *P. exiguus* plerocercoids in copepods up to 62 days post-infection. Priemer named plerocercoids (larvae) from copepods as cercoscolices (Cercoscolex, according to Jarecka, 1975). Rainbow trout and *Coregonus albula* can be infected if they are fed with copepods containg *P. exiguus* larvae, and in these two definitive hosts invasion took place with copepods 18 days post infection.

The development of *Proteocephalus neglectus* in the intermediate host, *Cyclops strenuus* and the early phase of development in the definitive host, rainbow trout, was

described by Scholz (1991). *Proteocephalus neglectus* plerocercoids are formed in *C. strenuus* in 8-10 days at 21-22^0 C, 18-21 days at 15^0 C and 24-28 days at 10^0 C. Most larvae are found in the first segment of the cephalothorax of the copepod and this location did not change during development. Scholz could not observe much development of the parasite in experimentally infected definitive hosts due to premature death of the fish, but by day 17 post-infection the parasite increased in size although neither segmentation nor genital primordia were observed. Moreover, he reported that infective *P. neglectus* larvae can survive briefly (2 days) in atypical fish hosts, for example fish of the families Cottidae and Cyprinidae which support the views of Willemse (1968) that infective larvae can survive in atypical hosts for a short time.

Kennedy *et al.*(1992) reported the larvae of *Proteocephalus* species in the intestine of *Sialis lutaria* (Insecta : Megaloptera) from a single locality in England. On comparison of scolex morphology of larvae obtained from *S. lutaria* with the adult *Proteocephalus* from sticklebacks from the same river, Kennedy *et al.* suggested that the most likely identity of the parasite is *P. filicollis*. This was supported by recovery of two parasites from sticklebacks experimentally fed infected *S. lutaria*. Hence, they suggested that the ability to use *S. lutaria* as an additional host may be associated particularly, though not exclusively, with parasites of sticklebacks.

Scholz (1993) studied the development of *P. torulosus* in the intermediate host. He used eight copepod species in his experiments but complete development was observed only in *Cyclops strenuus*. Scholz did not observe the cercomer stage in the development. He found that growth of larvae was complete in 9-12 days at 21-22^0 C and 4 weeks at 9-10^0 C. The infectivity of larvae from *C. strenuus* for the definitive hosts, cyprinid fish, was found to be very low.

6.1.2 Infectivity period of oncosphere.

Wagner (1954) reported that oncospheres of *P. tumidicollus* remain infective to copepods for one month at 0, 1, 5 and 10^0 C, but this is reduced to 19 days at 20 & 26^0 C and to 8 days at 32^0 C. Freeman (1968) reported that eggs of *P. parallacticus* degenerated within a week in physiological saline when placed in the refrigerator. Scholz (1991) found oncospheres of *P. neglectus* remain infective to copepods for 20-25 days if incubated at 5 or 10^0 C, whereas at $21\text{-}22^0$ C the infective period is only 10 days. Scholz (1993) also reported that the infective period of oncospheres of *P. torulosus* decreases as the temperature at which eggs are incubated rises. Thus eggs stored at $5\text{-}7^0$ C are infective to copepods for up to 35 days, whilst oncospheres kept at $10\text{-}12^0$ C can infect copepods up to 12 days. At $20\text{-}22^0$ C oncospheres are infective for 8 days. Thus infectivity is maintained at low temperatures.

6.1.3 Infection dynamics in the first intermediate host.

The rate of encounter between host and parasite is influenced principally by their respective densities and their spatial distribution (Crofton, 1971a; Anderson, 1978). These conclusions were later confirmed by Keymer (1980, 1981,1982) and Keymer & Anderson (1979) in their work on infection dynamics of *Hymenolepis diminuta* in *Tribolium confusum*. These authors demonstrated that transmission rates were strongly influenced by host feeding behaviour. Although a number of authors have studied infection of copepod intermediate hosts with larvae of *Proteocephalus* as described above, most of these studies have not focused on transmission dynamics. Meggitt (1914) observed that some of his infected copepods died and attributed this to heavy infections, as up to six larvae were found in one copepod. Similarly, Jarecka (1959) reported that the procercoids of *B. claviceps* developing in multiple infections are smaller than those in single infections. Scholz (1991) found that infection of the

intermediate host by *P.neglectus* depended on the length of time it was in contact with the parasite eggs. Similarly, in *P. torulosus,* Scholz (1993) found that the percentage of infected copepods increased with time of exposure, whereas intensity of infection did not markedly change with exposure time. Nie & Kennedy (1993) provided a comprehensive account of the infection dynamics of larval *Bothriocephalus claviceps* in the intermediate host *Cyclops vicinus* They found that the number of procercoids per copepod rose as egg density increased, while the number of copepods surviving decreased correspondingly. Moreover, copepods exposed to eggs survived less well than controls. The mean number of parasites / copepod and the mortality of copepods rose with increasing time of exposure to eggs even when egg density was constant.

6.2 Aims.

The objective of this study was to provide detailed information on the development of *Proteocephalus filicollis* in the intermediate host with special reference to development at different temperatures, development of individual cestodes, and infectivity period of oncospheres. Infection dynamics of *P. filicollis* in the intermediate host, i.e. infection of copepods in relation to exposure time to the parasite eggs was also studied.

6.3 Materials and methods.

6.3.1 Collection of Eggs.

To study the morphology of the egg of *P. filicollis* and to provide material for the experimental infection of copepods, the eggs were obtained from freshly killed sticklebacks from Airthrey Loch. When gravid worms were placed in warm water (25-30° C) they readily shed their eggs. The proglottids were also teased apart with dissecting needles to release eggs. The eggs were washed and stored in tap water at 4^0 C

for one to five days. There were no apparent adverse effects on the eggs after using warm water and storing them at 4° C. The morphology of eggs was studied using light and phase-contrast microscopy at up to X 40 magnification.

6.3.2 Estimation of Egg Numbers.

Eggs obtained from worms were put in to a Bijou and tapwater added to a volume of 5 ml. The Bijou was closed by a screw cap and the contents were shaken to obtain a uniform suspension. Using a fine pipette (Digital) one millilitre (1ml) of suspension was placed on a Sedgewick Rafter cell S 50, taking care to ensure that no bubbles were formed and the suspension was uniformly distributed over the cell. The cell was then covered. The eggs were counted under the microscope using the grid of a Rafter cell and the number of eggs per ml recorded. The total number of eggs in the suspension was then calculated.

6.3.3 Copepod Culture.

Copepod cultures were established in the laboratory as a source of experimental material. Copepods were caught from Airthrey Loch in May and June 1993 using a plankton net. The plankton sample was allowed to stand two to three hours in a beaker in the laboratory, during which time the non-copepod animals died and sank to the bottom of the container. The water containing copepods was then decanted and the copepods sorted and identified according to the key of Harding & Smith (1973). Copepods were maintained at room temperature (approximately 20^0 C to 22 ^{0}C) under normal light conditions (10 h light/ 14 h dark) in 3 litre plastic transparent tanks in tap water which had been left with aeration for three hours before use. Copepods were fed with algae (*Chlorella vulgaris*), porridge oats cooked in water and then dried and ground. Water level in the tanks was maintained by adding aged fresh water every third

day and feed was added every second day. A constant air supply was maintained in the copepod culture tanks.

6.3.4 Infection of Copepods.

To experimentally infect copepods, up to 30 individual females of selected species of copepod were placed in duplicate Petri dishes containing 1 cm depth of water and enough *P. filicollis* eggs to cover the base of the Petri dish in a single layer. The copepods were starved for 48 h prior to infection. The copepods and eggs were kept in contact for three hours (development experiments) during which time the copepods were seen feeding on the eggs. The copepods were then separated from the eggs, washed and placed in a fresh Petri dish. The experimental Petri dishes were kept at the required constant temperature in an incubator. Copepods were examined for the presence of larvae using a compound microscope. Some infected copepods were maintained separately in Petri dishes to observe the growth of individual larvae. Duplicates were combined for analysis.

6.3.5 Morphological observations.

In situ morphological study of the development of larvae of *Proteocephalus filicollis* was carried out by physical measurements on copepods on cavity slides and then manipulating them into any desired position by gently rolling them under the cover slip. All larvae present could be measured without damage to the copepod which could then be returned to its culture dish. Measurements of length and breath in μm of the cestode larvae were made using a standardised eye piece graticule fitted in the microscope. Live larvae in situ showed considerable extension and contraction, so measurements were taken at least twice on each larva. The mean of measurements was used to represent the size of the parasites. Because of the extension and contraction of larvae the

mean figure must be considered as indicating a trend rather than an absolute measurement. The larvae were photographed at various stages of development with the help of an automatic microphotographic camera "Olympus C.35 AD" fitted on Olympus BH.2 research microscope and drawings were made using Olympus BH.2 research microscope.

The growth of individual larvae in infected copepods maintained at 15 to 16^0 C and 21 to 22^0 C was studied. The copepods were examined regularly under the microscope according to the procedures described in the previous paragraph. The size and location of larvae were recorded so that individual larvae could be subsequently identified.

6.3.6 Infectivity period of oncospheres.

To study the infectivity period of oncospheres the eggs from 10 *P. filicollis* were mixed and divided into four batches. Each batch of eggs was further divided into four subgroups in Petri dishes and maintained at 4^0 C, 10^0 C, 15-16^0 C and 21-22^0 C. Fifteen gravid copepods were added to one of each subgroup on days 10, 15, 20 and 25. The copepods were maintained at 15 to 16^0 C after being exposed to eggs and fed regularly. The copepods were examined as described above in section 5.3.5 and the presence of any larvae in the copepods recorded.

6.3.7 Infection dynamics of *Proteocephalus filicollis* in the first intermediate host.

6.3.7.1 Influence of exposure time to eggs on the infection of copepods.

To study the influence of exposure time to eggs on the infection of copepods, 25 gravid copepods previously starved for 48 hours were exposed to eggs for 15, 30, 60, 90, 120, 150, or 180 minutes in Petri dishes in 15 ml of water. Copepods were separated then

from the eggs and washed in water and put into fresh dishes containing 15ml of water. These dishes were kept at 15-16^0 C. The copepods were examined from1 to 5 days post infection. The number of parasites in the copepods was observed and recorded.

6.4 Statistical methods.

Mean size of larvae was calculated in development experiments at various stages. Correlation coefficients were calculated between exposure time and the parasite mean intensity and the host mortality. The correlation coefficient were also calculated between egg densities and copepod mortality.

6.5 RESULTS.

6.5.1 Morphology of *Proteocephalus filicollis* eggs.

The description given in this section on the morphology of the egg of *Proteocephalus filicollis* is based on light microscope observations of fresh preparations. The dimensions of the eggs given are based on measurements of 150 individual eggs obtained from 16 adult specimens of *P. filicollis* from 16 fish. The adult worms were obtained in different months of the year (February, March, April, May, June, July 1995).

Eggs released into the water always contained a fully formed oncosphere. The structure of the egg of *P. filicollis* is shown in Plate 6.1. The outer envelope is collapsed when the egg is first discharged from the adult cestode but on contact with water it immediately swells and becomes turgid (Plate 6.1). In the expanded state the outermost envelope is transparent and between 37.5 - 118.7 μm in diameter (mean diameter 55.31μm). The remaining egg membranes and envelopes are more or less spherical in shape and between 27.5 - 52.5 μm in diameter. The oncosphere is usually not centred within the outer envelope.

The outermost membrane of the egg is very thin and hyaline (Plate 6.2) and surrounds a thicker, granular layer (inner envelope) which corresponds to the second oncosphere membrane of Meggitt (1914) or the embryophore of Rybicka (1972). The thickness of this granular layer ranges from 3.1 - 7.5 μm (mean thickness 5.7μm). Inside the thick granular layer there appears to be a further membrane. The oncosphere or hexacanth is enclosed in an oncospheral membrane which encircles it even after hatching from the remaining egg membranes in the gut of the copepod

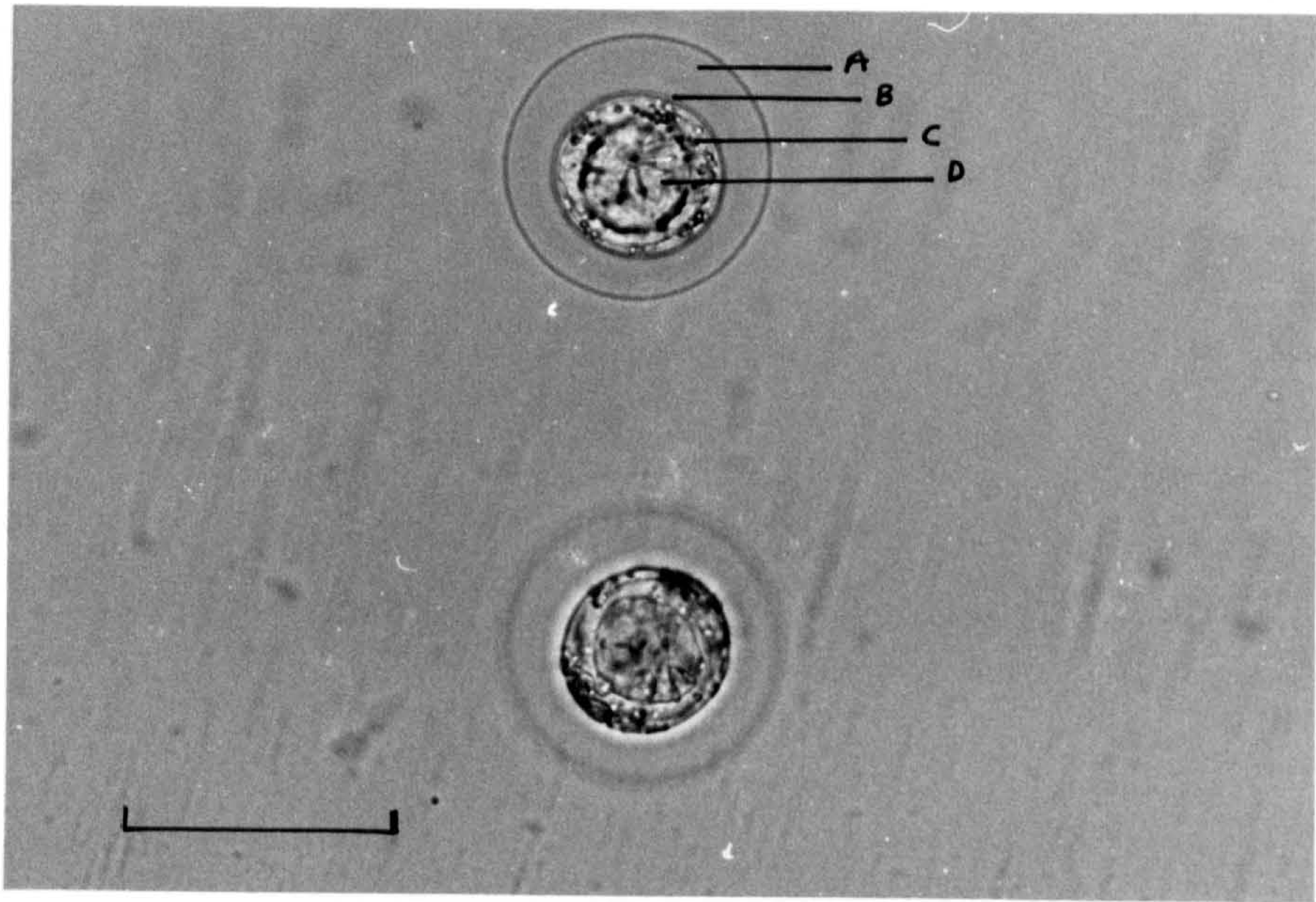


Plate 6.1 A micrograph of the fully developed egg of *Proteocephalus filicollis*.(A) Outer float membrane, (B) hyaline membrane, (C) granular layer (inner envelope, (D) hexacanth embryo (Scale bar =30 µm).

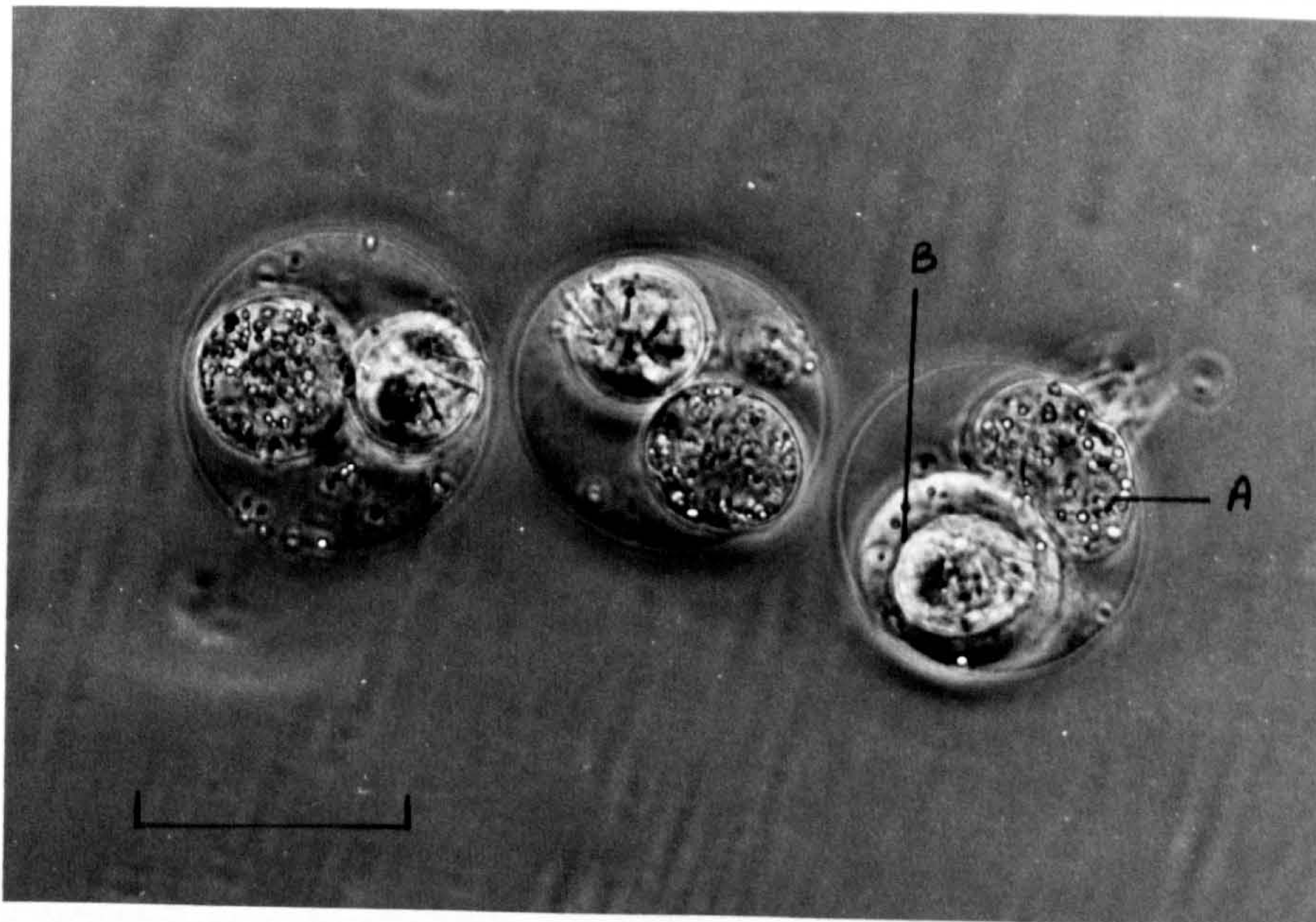


Plate 6.2. Egg of *Proteocephalus filicollis*. (A) Arrow indicates a thin hyaline membrane which surrounds the granular layer. (B) Arrow indicates a membrane on the inner surface of the granular layer surrounding the oncosphere (Scale bar = 30µm).

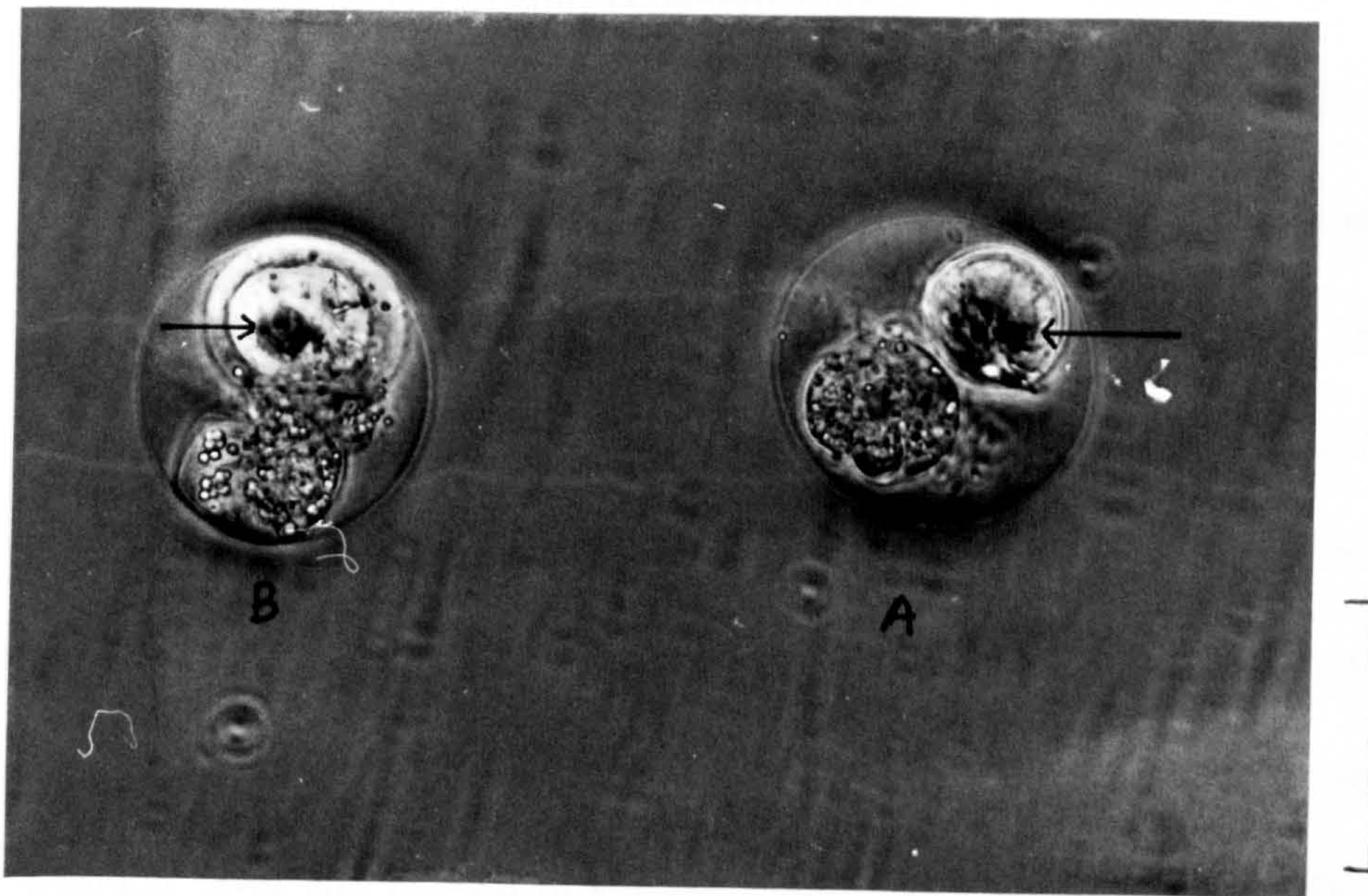


Plate 6.3. Eggs of *Proteocephalus filicollis* showing the hexacanth embryo released from the granular layer (A). In the hexacanth (B) embryo is still encircled by the granular layer. Arrow indicates the probable penetration glands in the posterior of the embryo (Scale bar = 30 µm).

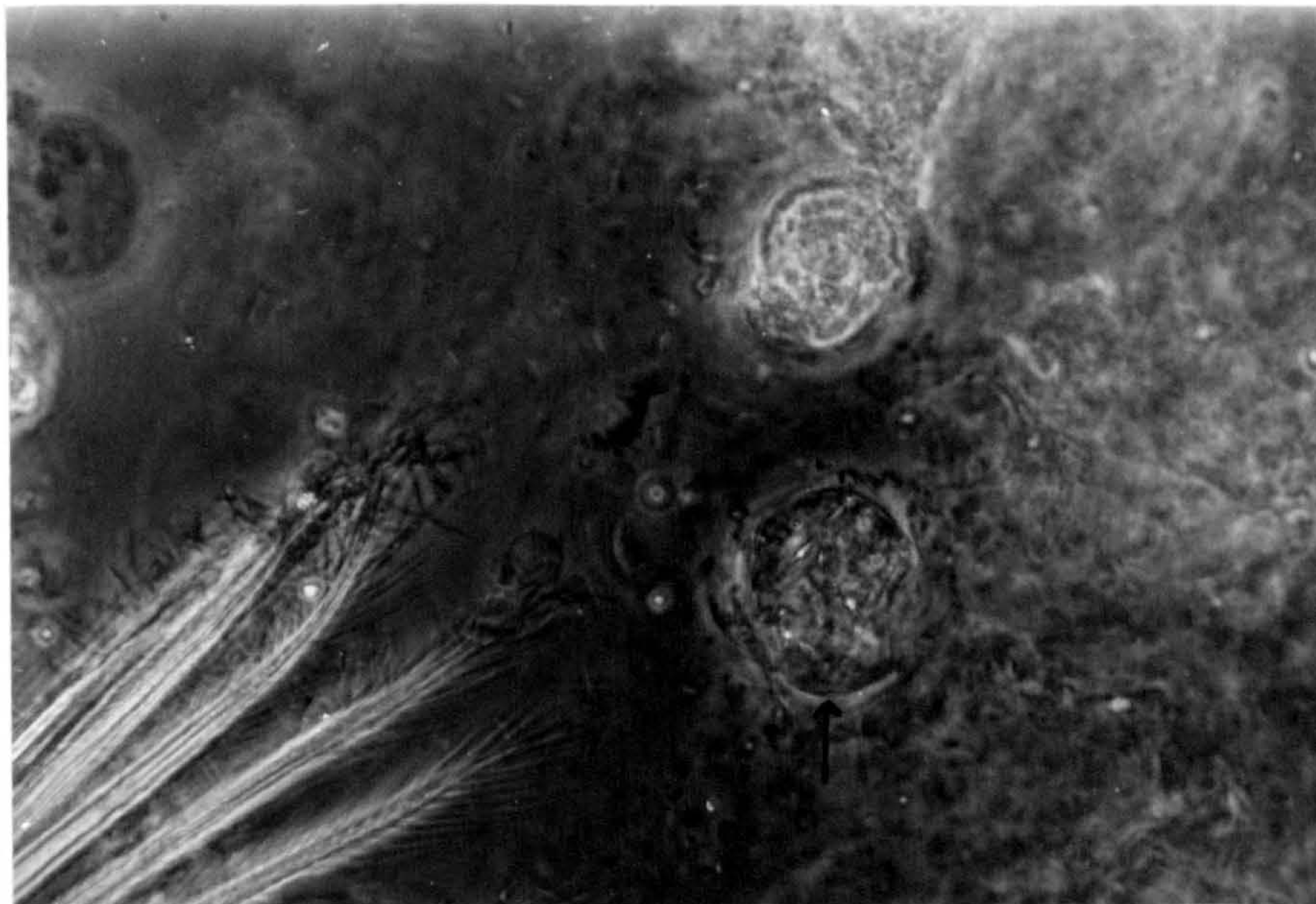


Plate 6.4. Hexacanth embryo of *Proteocephalus filicollis* removed from the intestine of *A. robustus.* Arrow indicates a thin oncospheral membrane surrounding the embryo (Scale bar = 10 µm).

(Plate 6.4). (The detailed structure of embryonic membranes is described in Chapter 7, on ultrastructure of the embryonic envelopes).

The hexacanth embryo is bilaterally symmetrical and measures 17.5 x 27.5 μm (range of 124 specimens 17.5 to 25 μm x 18.7 to 27.5 μm). The mean diameter of the embryo is 21.34 μm. Three pairs of embryonic hooks are present at the anterior end of the oncosphere, the median hooks (1 pair) are 11 -12.5 μm long, and the marginal hooks (two pairs) 11.2 -13.5 μm in length. The handle is straight, the blade narrow, slightly curved and sharply pointed. A darker area is obvious in the posterior end of the oncosphere. This becomes more apparent when the hexacanth emerges from the granular layer and is seen to contain darker spots. These spots may be penetration glands and are clearly seen in fresh preparations (Plate 6.3).

6.5.2 Hatching of the egg and penetration of the intermediate host intestine by the hexacanth.

The hatching of *P. filicollis* eggs was not studied experimentally. However, some observations were made on eggs which had been ingested by copepods which indicated the sequence of events during the hatching process. In the intact egg, the hexacanth embryo did not show any activity. The hatching of *P. filicollis* eggs in the intestine of copepods was a rapid process and the hatched hexacanths were seen moving very actively in the intestine less than 10 minutes after both eggs and copepods were in contact. Hexacanths were seen surrounded by the oncosphere membrane even after hatching in the intestine of the copepod (Plate 6.4). The hooks of the larvae showed very active movement in a regular cycle in the copepod gut (Fig 6.1). The median pairs of hooks moved forward and then backward in a breast stroke like movement. As the median hooks move back the marginal pairs of hooks

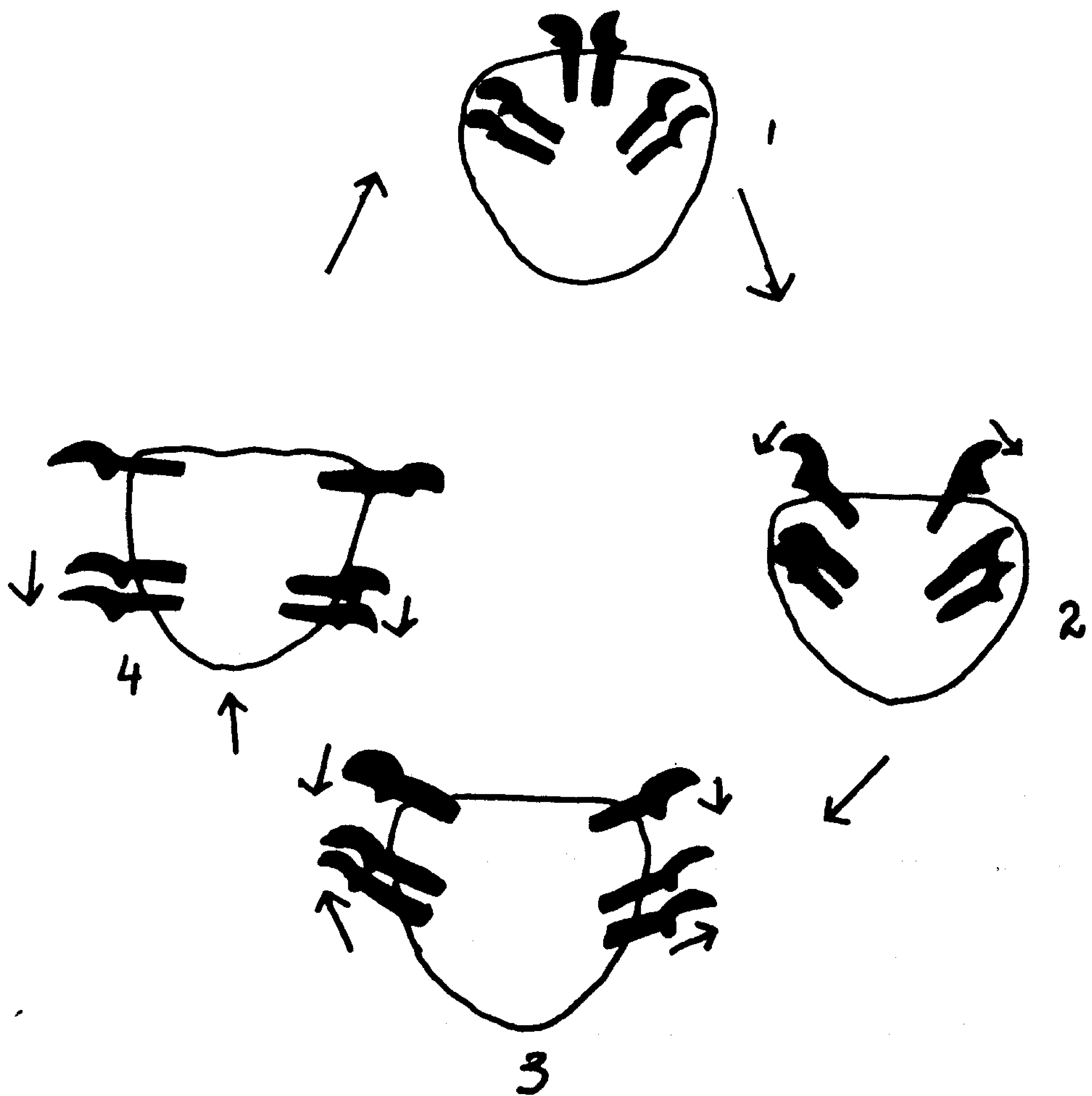


Fig 6.1 Diagrammatic representation of the sequence of the hook movements of *Proteocephalus filicollis* larvae during penetration of the copepod intestinal wall (not to scale).

also extend forward. As soon as the median hooks complete their backward movement the marginal hooks begins a similar backward movement and the larva is moved forward. Each cycle takes about two to three seconds to complete. Larvae were found in the body cavity of the copepod within 30 minutes of exposure to eggs.

6.5.3 Development of *Proteocephalus filicollis* larvae in the intermediate host.

The development of *P. filicollis* larvae in the copepod intermediate host depends on the species of copepod, the water temperature in which the latter are kept and the intensity of infestation of the individual copepod. The development of the larvae of *P. filicollis* in *Acanthocyclops robustus* maintained at 15-16^0 C is described here. The egg, after being ingested by the copepod, hatches in the latter's intestine. The larva does not remain static but moves about by contractile movements of the intestine of the copepod. Within the haemocoel of the copepod the larva continues its movement for several days until it begins to increase in size. The shape of the larva remains similar for about 6-8 days (Plate 6.6). During this time the movement of hooks remains quite coordinated. The larvae do grow in size during this period as the mean length increases from 35 µm to 75 µm. Twelve days after infection the larva has assumed an oval and subsequently an elongated shape (Plate 6.7). The mean length of the larvae is 120 µm on day 15. The anterior end of the larva becomes more active than the rest of the body. The three pairs of embryonic hooks are moved apart, possibly due to the growth of the larva and then cease coordinated movements.

The calcareous bodies first appear after day 15, almost at the time of appearance of the cercomer. The calcareous bodies are at first irregular in shape and measure from 5-12.5 µm in size. The mean length of larvae is 160 µm by day 20.

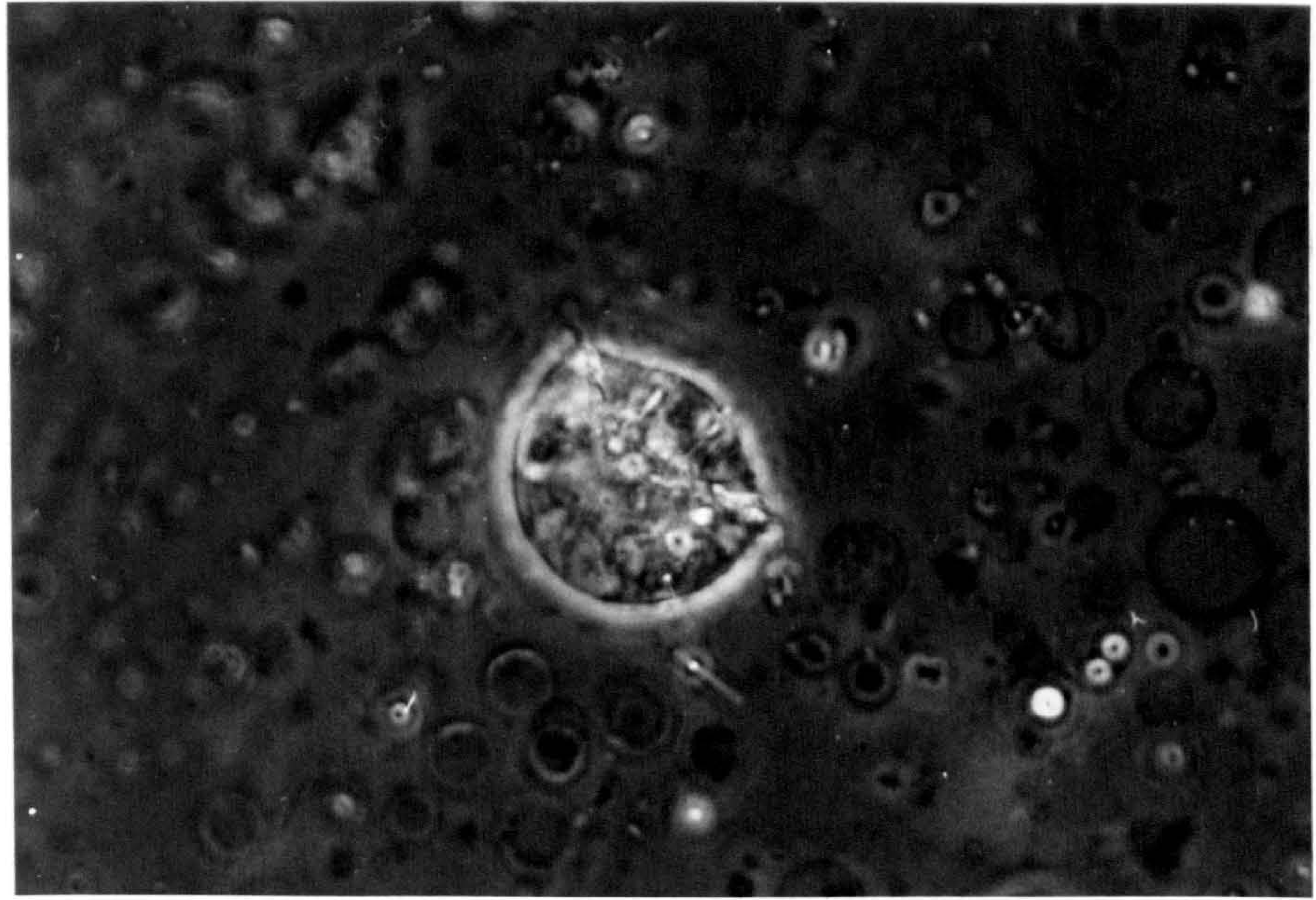


Plate 6.5. A hexacanth embryo of *Proteocephalus filicollis* taken from the haemocoel of *A. robustus*. The probable penetration gland is no longer visible (Scale bar = 10 μm).

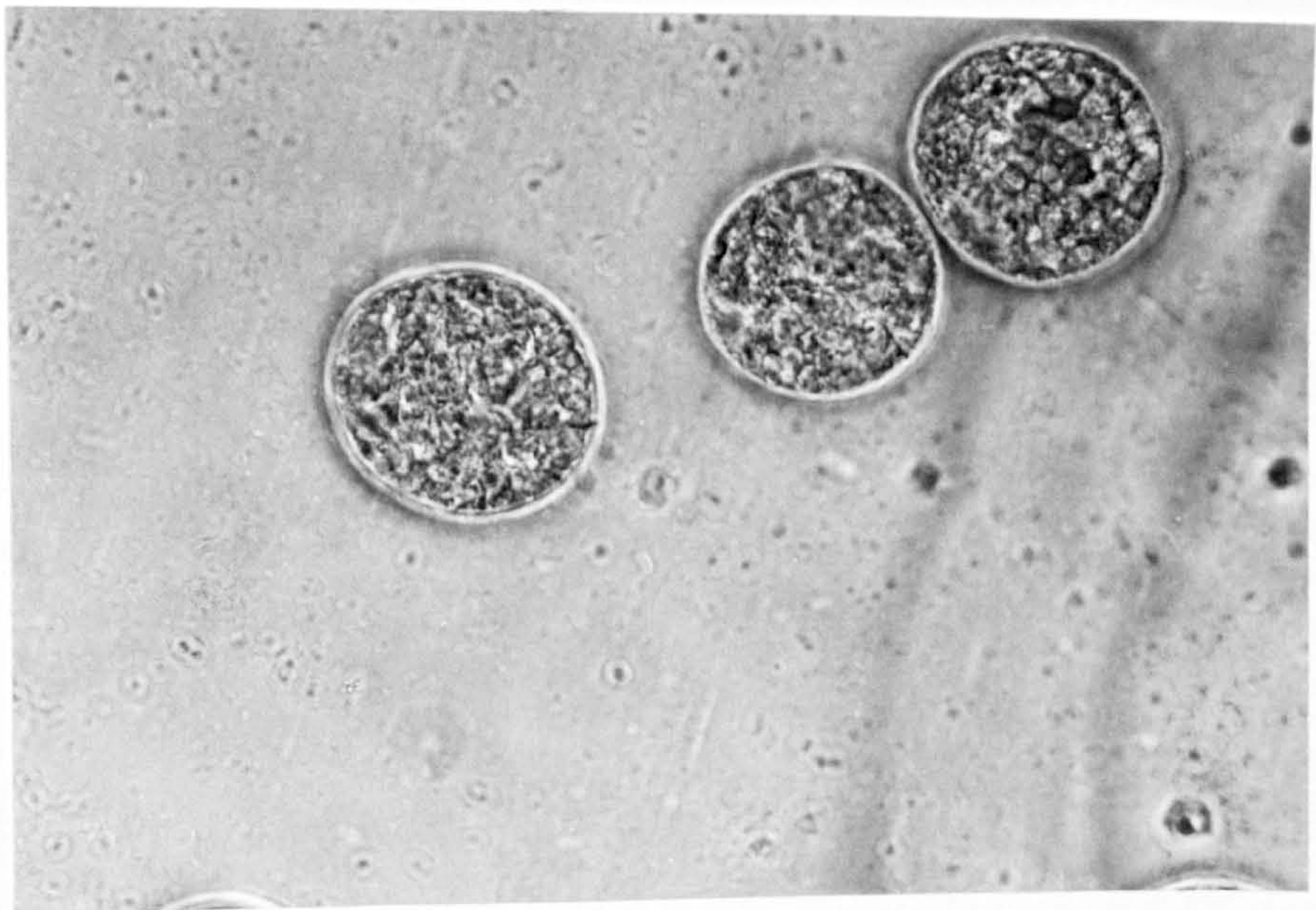


Plate 6.6. 6 day-old larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16° C (Scale bar = 50 μm).

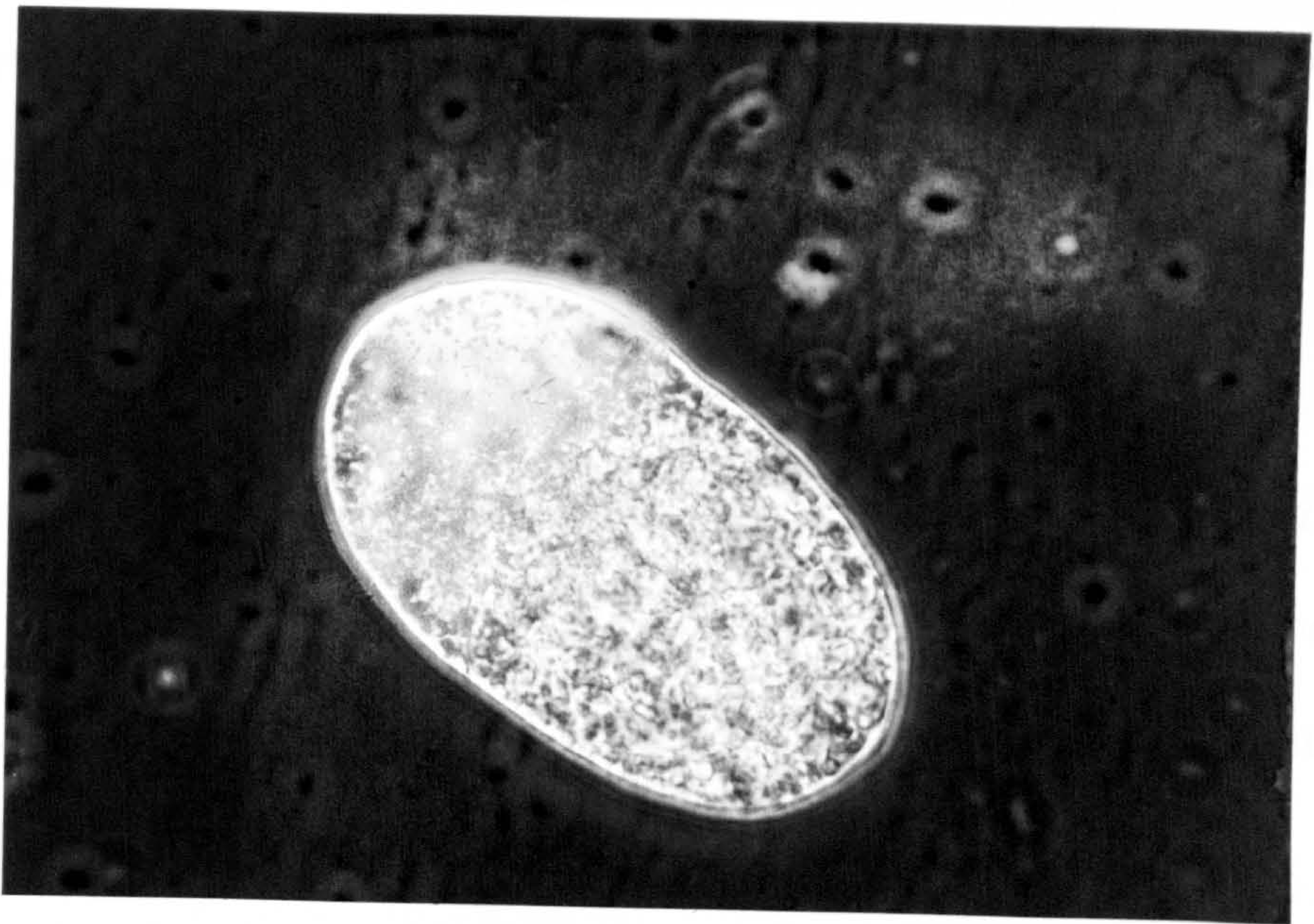
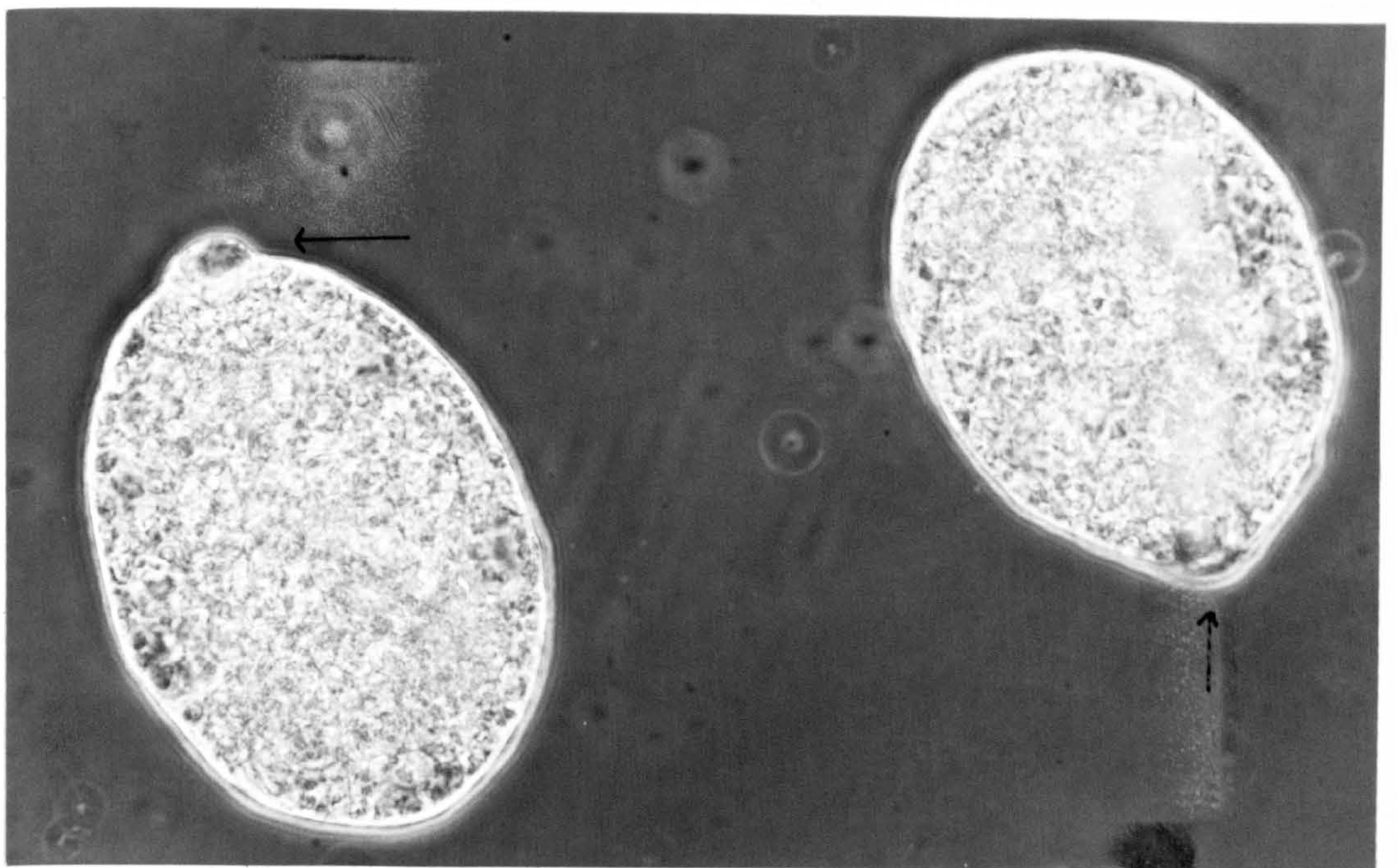


Plate.6.7. A 12 day larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16^0 C, elongated in shape (Scale bar = 50 μm).



Pl ate.6.8. Two 14 day old larvae of *P. filicollis* from *A. robustus* maintained at 15-16^0 Cercomer formation is in progress (arrow) (Scale bar = 50μm).

Cercomer formation occurs from day 12-23. The cercomer first appears as an out growth or a small bulge at the posterior end of the larva and later develops into a rounded structure, which is separated from the main body by a thin tissue connection. The phases in formation of the cercomer are shown in Plate 6.8 — 6.12. The size of the cercomer varies from 20 - 27.5 µm x 10 - 12.5 µm. The embryonic hooks were mostly seen in the main body of the larva, but very occasionally these hooks were visible in the cercomer (Plate 6.13). Cercomer formation seems a rapid process as very few larvae were seen with developing cercomers. The cercomer persists for three to five days before it disappears.

Up to four larvae with cercomers were seen in an individual copepod but normally one to two larvae with cercomers were seen per copepod. Two to three dark spots were visible in the developing as well as in fully formed cercomers (Plate 6.10 & Plate 6.11). The function and significance of these spots is not clear.

The lateral suckers also begin to form by day 23-29, approximately the time at which the cercomer is also still present. The frontal pit was not observed in the developing larva in light microscope observations. The lateral sucker diameter in fully developed larvae varies from 37.5 to 50 .0 µm. By day 27- 29 the larva is fully developed and measures from 250 to 610.2 µm in length (Fig.6.2). The number of calcareous bodies increases to a maximum of 50. The size of fully developed larvae and time taken to achieve this size was related to the intensity of infestation of individual copepods. In single larval infections, the fully developed larva ranged in length from 567 - 610.2 µm in 23 - 29 days, whereas in one case with three larvae per copepod, the length of developed larvae varied from 295.5 - 304.5 µm after 32 days.

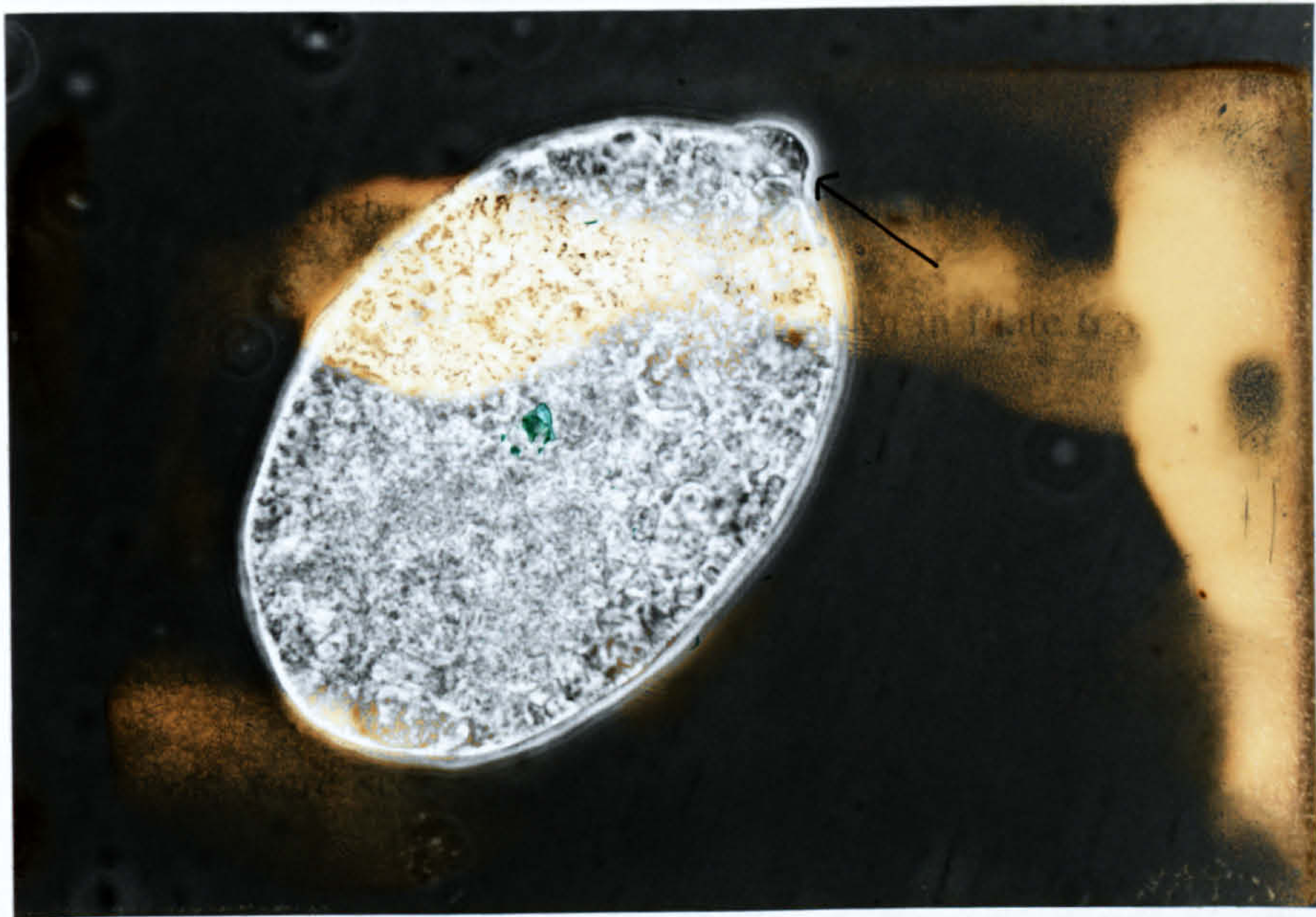


Plate 6.9. A 12 days old larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16^0 C. The cercomer (arrow) is in the formation stage (Scale bar = 50 µm).

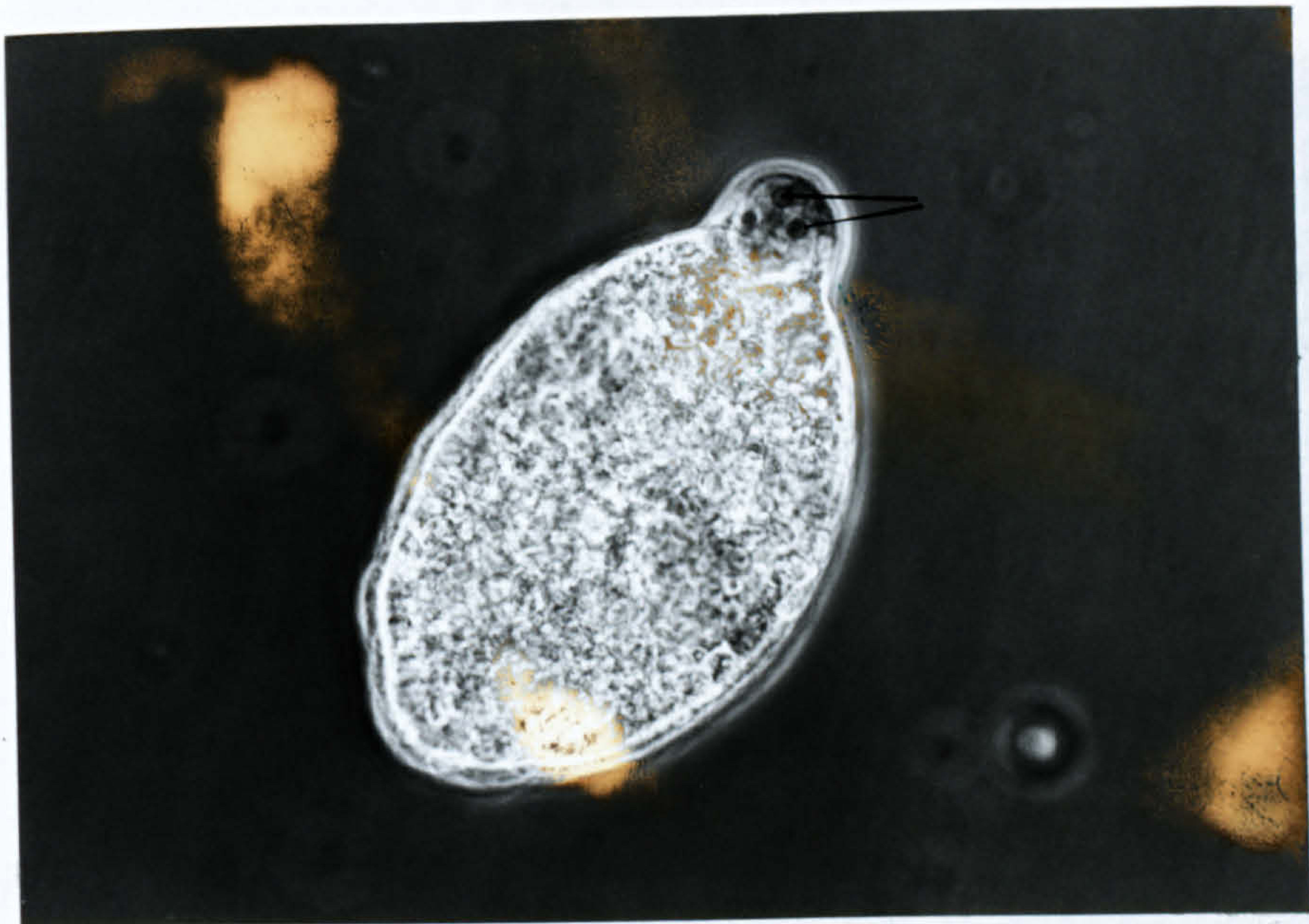


Plate 6.10 A 15 days old larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16^0 C. The cercomer is in the later stages of development. The dark spots in cercomer are clear (Scale bar = 50 µm).

Plate 6.11. A 16 days larva of *Proteocephalus filicollis* from *A. robustus* maintained at 15-16^0 C. The cercomer is fully formed. The dark spots in cerceomer are also very clear (arrow) (Scale bar = 50 μm).

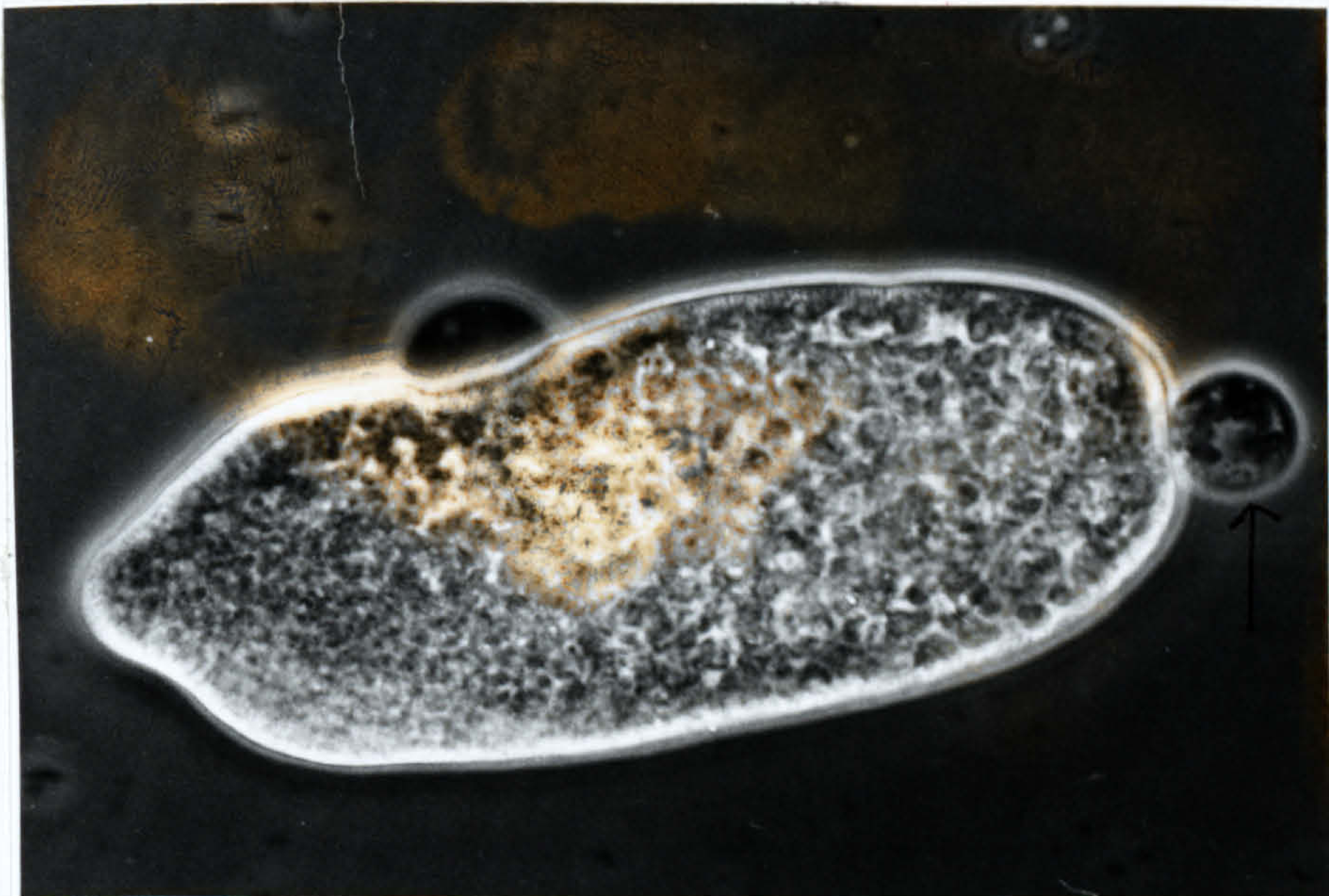


Plate 6.12 A 21 day old larva of *Proteocephalus filicollis* from *A.robustus* maintained at 15-16^0 C. The dark spots in the cercomer are not visible (Scale bar = 50μm

Plate 6.13 A 22 day old larva of *P. filicollis* from *A. robustus* maintained at $15\text{-}16^{0}$ C. Arrow indicates cercomer, embryonic hooks are visible in the cercomer. Calcareous corpuscles are also clear in the larva (A) (Scale bar = 50 µm).

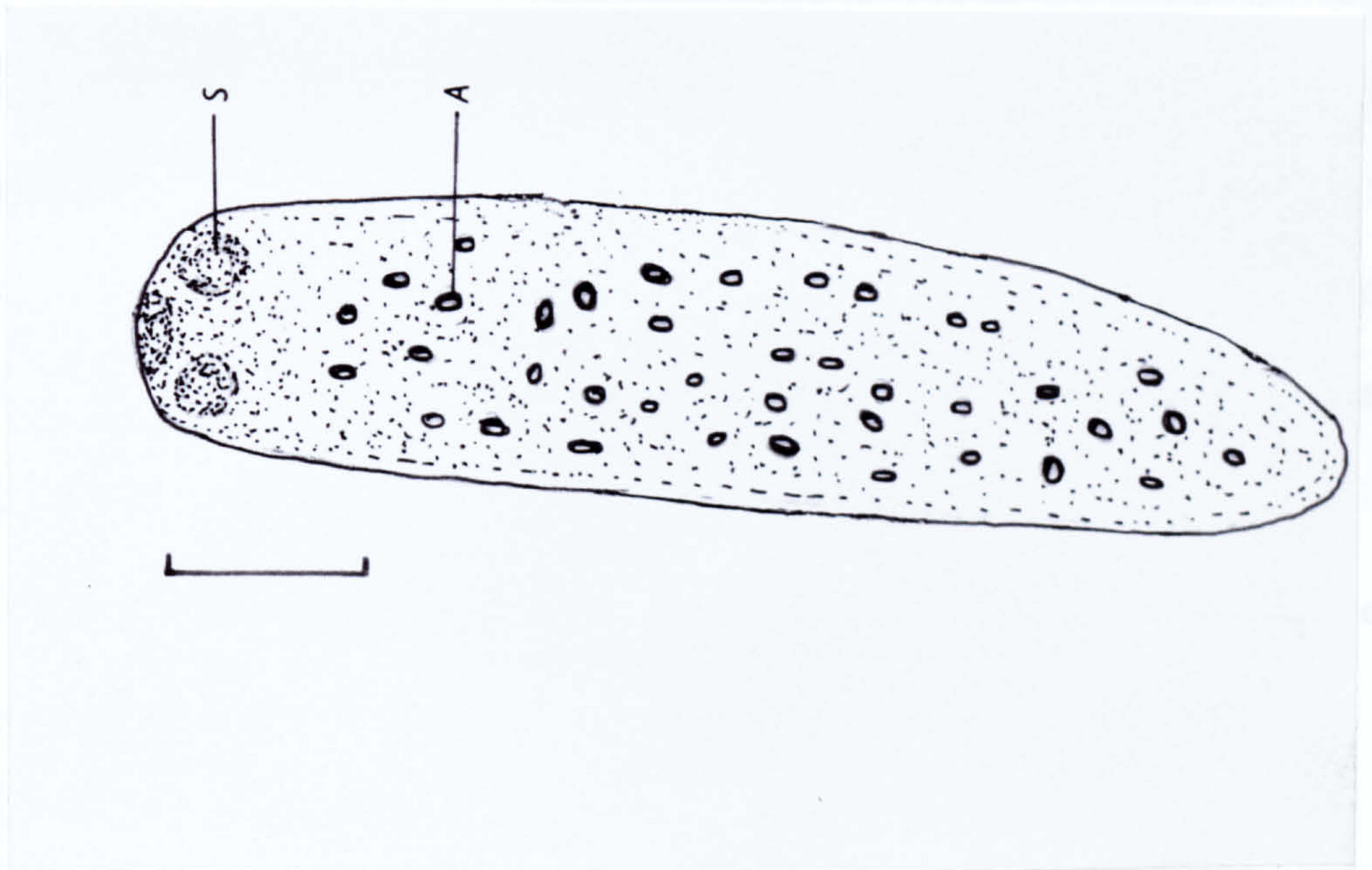


Fig 6.2 Drawing of a 27 day old larva of *Proteocephalus filicollis* from *A. robustus* maintained at $15\text{-}16^{0}$C. The lateral suckers (S) and calcareous corpuscles (A) are very clear (Scale bar = 100 µm).

6.5.4 Effect of temperature on the larval development of *Proteocephalus filicollis*.

The development of *P.filicollis* larvae at 4^0 C, 10^0 C, 15-16^0 C and 21-22^0 C was studied, using *A.robustus* as an intermediate host (Fig. 6.3). Full development occurred only in copepods maintained at 15-16^0 C and 10° C. By day 12 mean length of the parasite was 90 µm. After this there was a continued increase in length until day 32, when fully formed larvae were first found. There was no further increase in the mean length of larvae after day 32. The experiment was terminated on day 35.

At 21-22^0 C, development of larvae was faster than at 15-16^0 C. Larvae with cercomers were found from day 9 to 15. No development beyond the cercomer stage was seen in larvae kept at 21 - 22^0 C because of premature death of the copepods.

At 10^0 C *P. filicollis* larvae showed slower growth. Up to day 19 the larvae were still oval in shape although they showed some increase in length. Cercomers developed from day 29-34 and persisted for 5-6 days. Fully developed larvae were first observed on day 44 when the mean was 290 µm. Subsequently larvae further increased in length until the experiment was terminated on day 55 when mean length was 330 µm.

At 4^0 C no development of larvae occurred beyond the hexacanth embryo up to 23 days. Larvae observed on days 29, 36 and 45 were slightly larger in size, but still resembled hexacanth embryos. Development beyond 45 days was not followed.

The increase in length of individual larvae is shown in Fig. 6.4. A, B, C & D show the development of four larvae from four independent single infections of *A. robustus* maintained at 15 -16^0 C. All these larva developed normally with a longest increase in the length between day 8 & 17. Formation of the cercomer occurred from day 17 - 22. These larvae were fully developed by day 28.

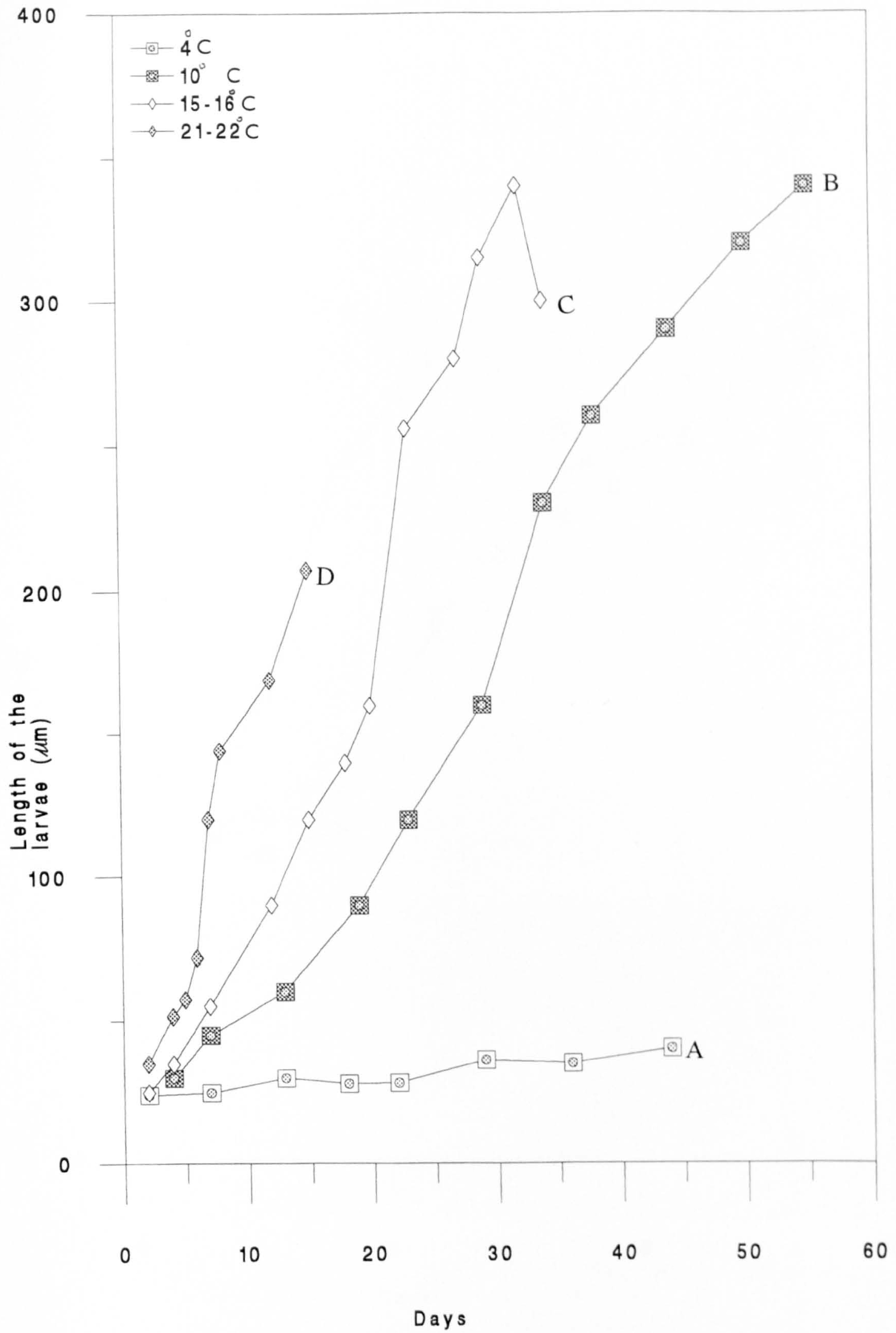


Fig.6.3 Development of *Proteocephalus filicollis* in *A. robustus* at differeent temperatures (°C), at 4° C (A), 10° C (B), 15-16° C (C) and 21-22° C (D).

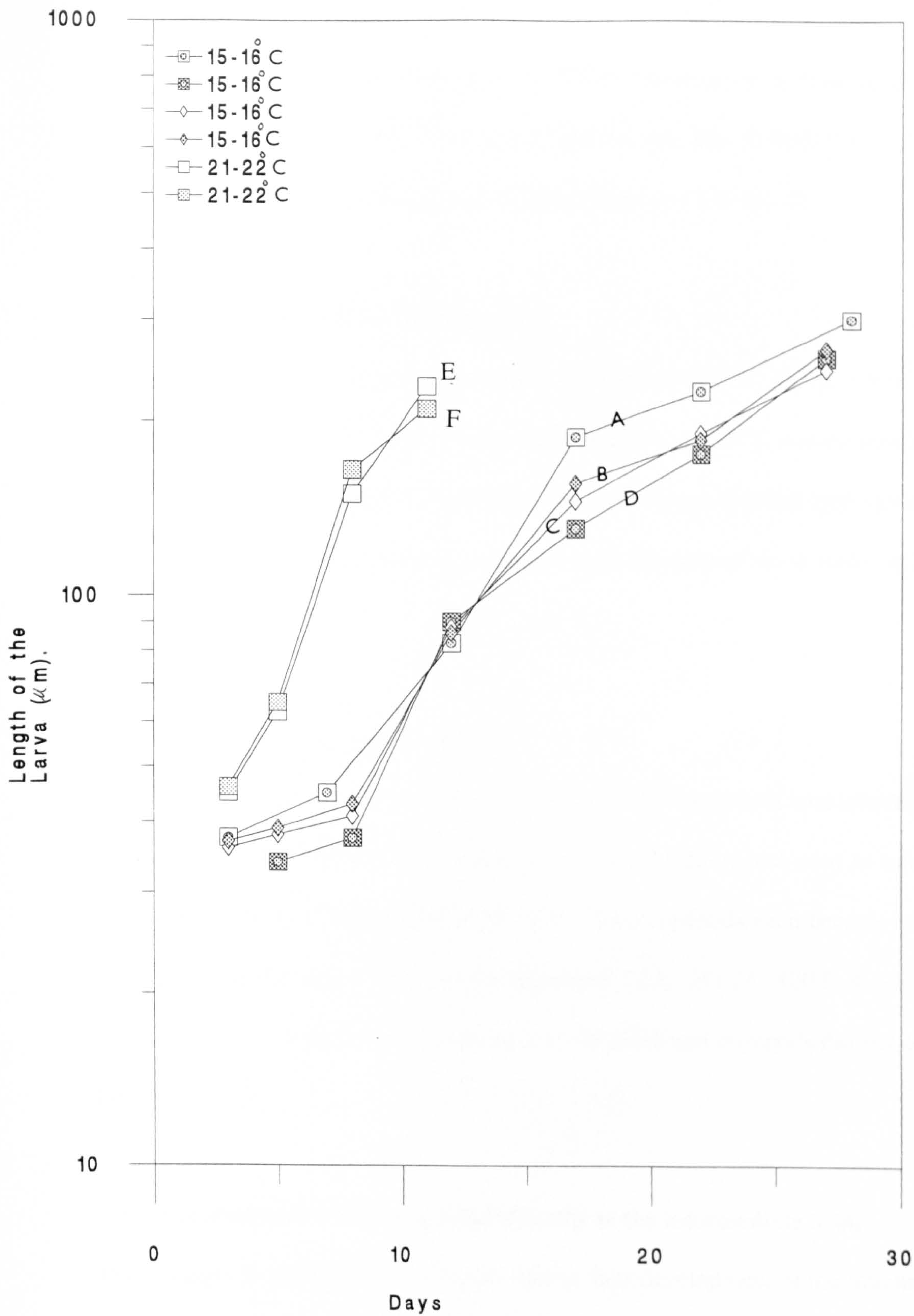


Fig.6.4 Development of individual *Proteocephalus filicollis* in *A. robustus* at 15-16° C and 21-22° C. A, B, C, D at 15-16° C and E, F, at 21-22° C.

Two individual larvae were studied at 21 - 22^0 C. These larvae developed up to the cercomer stage, which appeared on days 8 and 12. On day 13 both copepods died. The larvae showed continous increase in length from day 3 to day 12.

6.5.5 Infectivity period of oncosphere.

The results of this experiment showed that eggs stored at 4^0 C, 10^0 C, 15-16^0 C are infective at least up to 25 days, whereas eggs stored at 21-22^0 C remain infective up to 15 days. The results of this experiment clearly indicates that the eggs stored at 4^0 C, 10^0 C, 15-16^0 C remain infective almost double the time of those stored at 21-22^0 C (Table 6.1).

6.5.6. Infectivity of winter eggs.

In a February 1995 gravid worms were recovered from a sample of sticklebacks. Eggs obtained from these worms were stored at 4^0 C for 24 hours and used to infect *A. robustus*, which were maintained at 15-16^{0}C. Two copepods each became infected with 2 larvae. By day 13 the larvae measured 72.5, 141.25, 120.5 & 96.8 µm. Unfortunately no fully developed larvae were obtained and copepods did not survive beyond 13 days.

6.5.7 Localization of *Proteocephalus filicollis* in the intermediate host.

The changes in location of *P. filicollis* during their development in the intermediate host was investigated in *A.robustus*. A total of 269 larvae were observed from day 1 to 27 post-infection. The number of larvae in one host varied from 1 to 14. The majority of the larvae were found in the cephalothorax region (93.22 %) of which 62 % were in the first and second segments (Plate 6.14). In those copepods with a

Table 6.1 Infectivity period of oncosphere of *Proteocephalus filicollis* stored at different temperatures.

No	Temperature (° C)	Days eggs stored	No of copepod exposed to eggs	No of Copepod infected	No of larvae per copepod	Mean No of larvae per copepod
1	4° C	10	15	8	3,2,4,6,5, 1,2,4	3.37
		15	15	5	4,2,5,3,6	4.0
		20	15	7	1,4,2,6,3,5 ,1	3.14
		25	15	6	1,3,2,4,1,2	2.16
2	10° C	10	15	6	2,1,4,2,3,1	2.1
		15	15	3	2,3,1	2.0
		20	15	4	1,3,1,1	1.25
		25	15	6	2,2.4,3,1,1	2.16
3	15-16° C	10	15	4	5,1,2,1	2.25
		15	15	3	1,3.1	1.66
		20	15	5	1,2,2,1,1	1.44
		25	15	2	2,2	2.0
4	21-22° C	10	15	3	1,2,2	1.66
		15	15	2	1,1	1.0
		20	15	-		
		25	15	-		

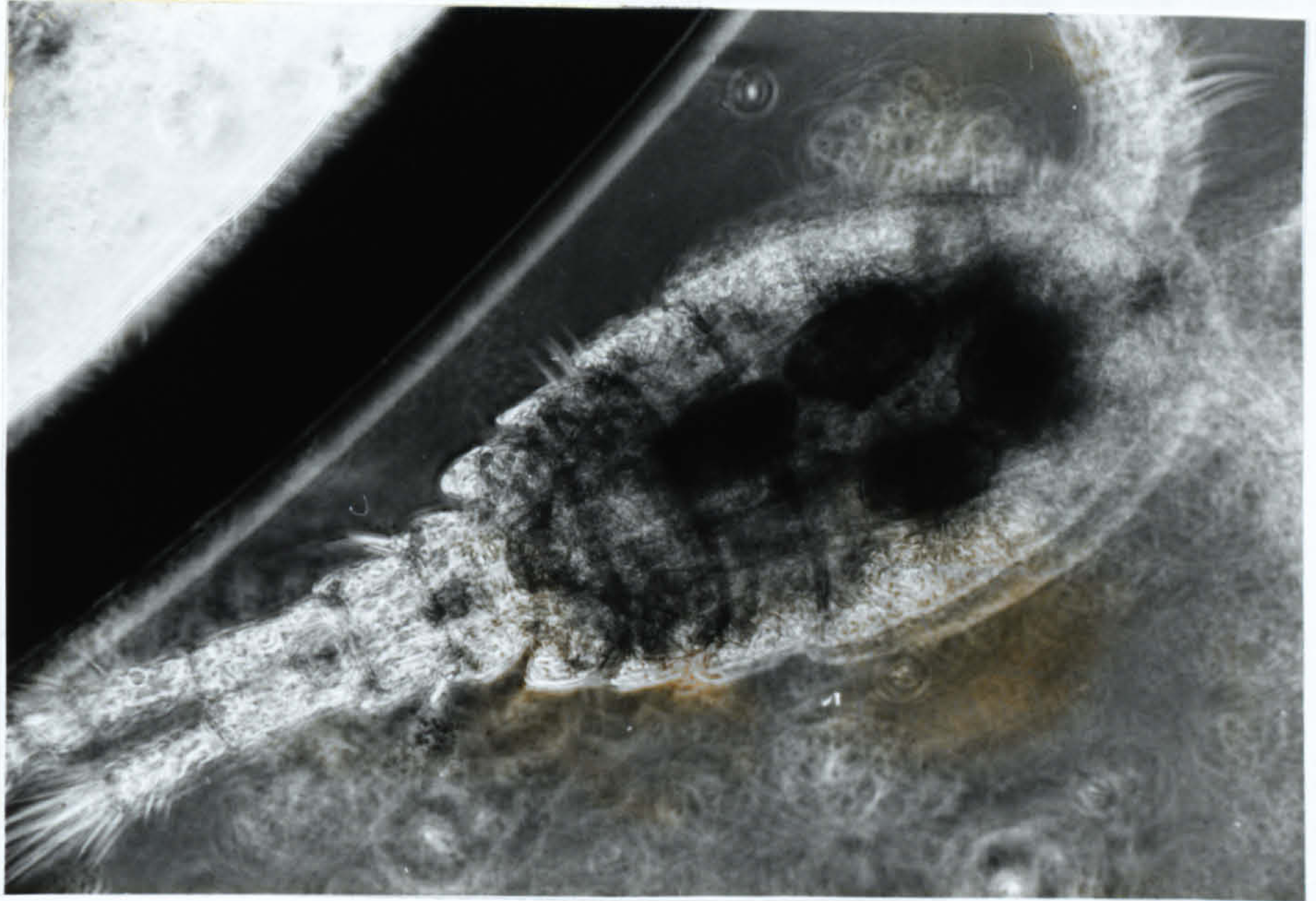


Plate.6.14. Four *Proteocephalus filicollis* larvae in the haemocoel of *A. robustus* 10 days post -infection (Scale bar = 250 μm).

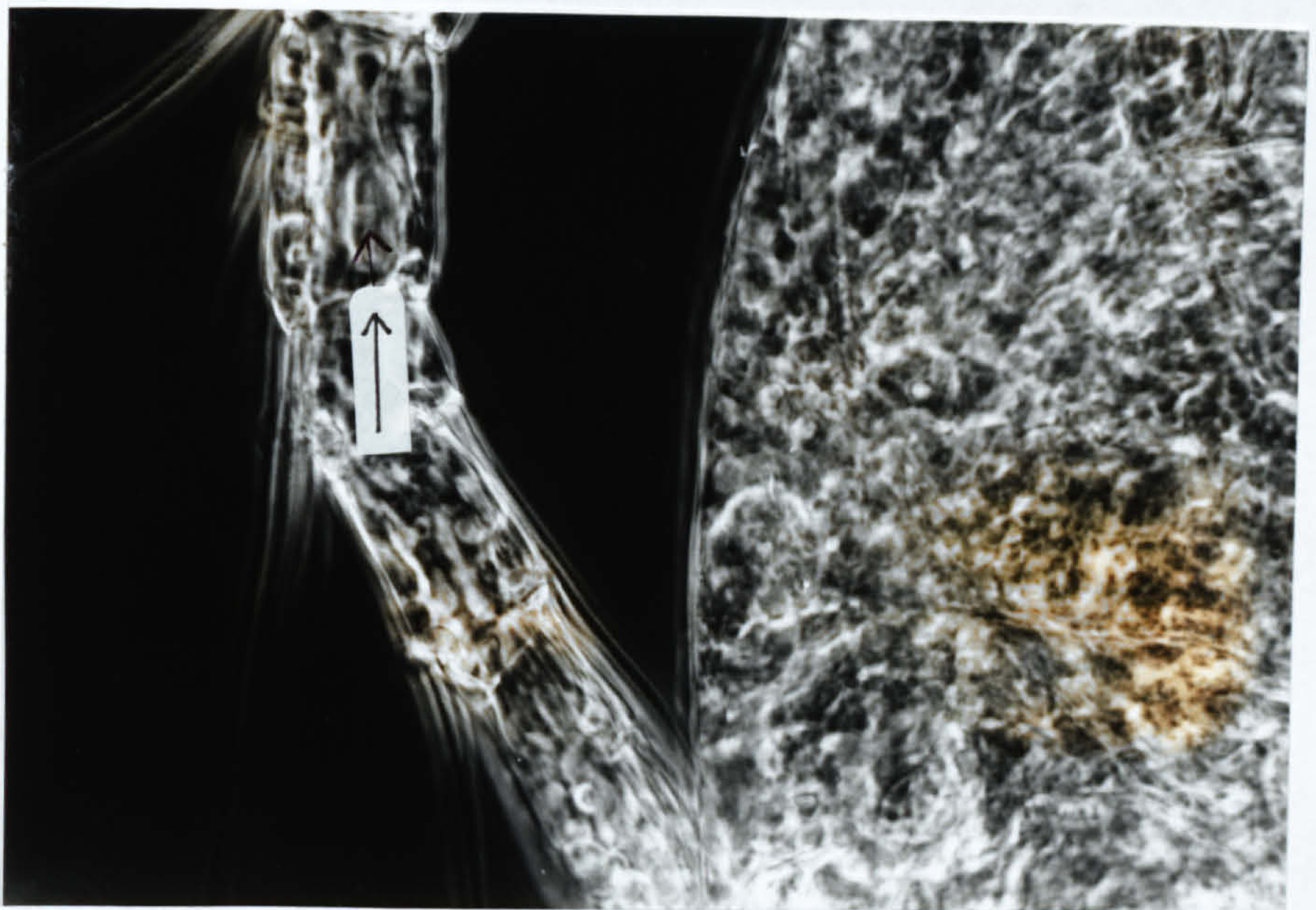


Plate.6.15. Arrow indicates a *Proteocephalus filicollis* larva in antennule of *A. robustus* 1 day post-infection (Scale bar = 100).

Table 6.2 **Percentage localization of all *Proteocephalus filicollis* larvae recovered from different segments of *Acanthocyclops robustus*. The figures given are percentage.**

	Cephalothorax segments									Abdominal segments					
Days Pi	**1**	**1-2**	**1-3**	**2**	**2-3**	**2-4**	**3**	**3-4**	**4**	**4-5**	**1-2**	**2**	**2-3**	**3**	**3-4**	**Total larvae**
1-5	41	1.4		31	1.4	0.7	12	0.7	2.4	2.4	1.4	3.4	0.6		1.4	143
6-11	29	7.8		13	6.1	4.3	4.3	1.5	11	11	12					66
13-18	49	3		15	15		9	3	6							33
21-27	45	11	11	3.7	7.2	3.7	11		7.4							27

Days post infection	**Development stages of larvae**
1-5	oval in shape
6-11	elongated shape
13-18	calcareous bodies and cercomer starts appearing
21-27	completion of of ceromer formation and appearance of lateral suckers.

higher number of larvae (7 - 14) the larvae were localized in up to 4-5 different sites in the cephalothorax and abdomen. 54.65 % of the larvae were localized in a single segement, 28 % in 2 segments and 11.62 % in three segments. Less than 3 % of the larvae were found in 4, 5 or 6 segments of the copepod. Larvae were found throughout the body cavity of the copepod from days 1 to 11 post-infection. From day 13 to 27 the number of larvae located in segment 1 increased (Table 6.2). The larvae were not normally found in antennules or furcae, but occasionally very young larvae (1day old) were recorded in the antennules (Plate 6.15).

6.5.8 Infection of intermediate host in relation to the length of contact with parasite eggs.

The results obtained from this experiment are shown in Table 6.3 & Fig.6.5. It is clear that infection of the copepod host depends on the length of time it is in contact with the parasite eggs. The percentage of infected copepods increased with increasing length of their contact with eggs and it reached a maximum (52 %) after 180 minutes. Regression analysis showed that prevalence of infection is significantly related to the time of contact (r^2 = 0.833; P = 0.004; r = 0.912; F = 24.89; P = 0.004; $F_{0.05\,(1)\,6}$ =-5.09).

The mean intensity of infection attained a maximum value (4.16) when the copepods were exposed for 150 minutes but was lower in copepods exposed for 180 minutes. The maximum number of larvae per copepod (9) was recorded after 60 minutes of exposure.

The mean intensity of infection did not demonstrate any relationship to the contact time between the egg and the copepod (r^2 = 0.504; $P \geq 0.074$; r = 0.502 ; F = 5.07; $F_{0.05\,(1)\,6}$ =- 5.09).

Mortality of copepods increased with the length of exposure time and was highest after 120 and 180 minutes exposure. A statistically significant relationship existed between the time of contact of eggs and copepod mortality ($r^2 = 0.818$; $P = 0.005$; $r = 0.90$; $F = 22.47$; $F_{0.05(1)6} = -5.09$).

Table 6.3 Infection of *Acanthocyclops robustus* with *Proteocephalus filicollis* eggs in relation to length of contact.

Time of contact (Minutes)	15	30	60	90	120	150	180	Total
No copepods examined	25	25	25	25	25	25	25	175
No of infected copepods	9	8	10	10	8	12	13	70
Prevalence (%)	36	32	40	44	40	48	52	40
No of larvae	23	29	34	23	18	31	21	179
Mean intensity of infestation	2.55	3.62	3.4	2.3	2.25	2.58	1.61	2.55
Maximum no of larvae	6	7	9	7	4	7	3	
Mortality	28	36	44	56	60	56	60	57.33

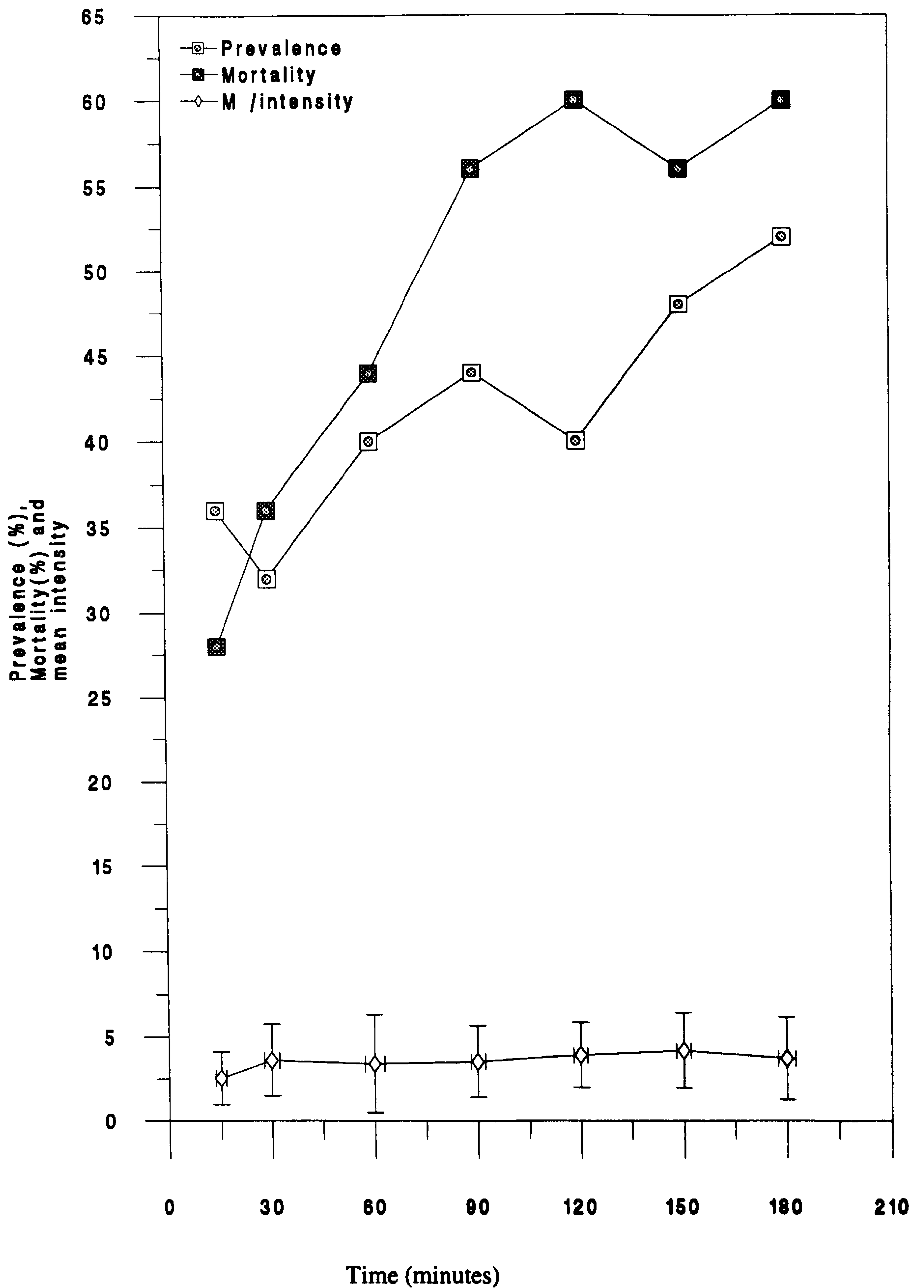


Fig.6.5 Prevalence, mortality and mean intensity of infection of *A. robustus* With *Proteocephalus filicollis* in relation to exposure time of copepods to the eggs. The bars represent standard deviations.

6.5. DISCUSSION.

Meggit (1914) described and figured three membranes in the egg of *P. filicollis*, as the first, second and third oncospheric membranes, which corresponds to the outer envelopes, embryophore and oncospheral membrane of Rybicka (1966). Studies by Freeman (1964) on *P. parallacticus*; Fischer (1968) on *P. fluviatilis*; Wootten (1974) on *P. percae*; Priemer (1987) on *P. exiguus* and Scholz (1991,1993) on *P. neglectus* and *P. torulosus* described two to five membranes and envelopes which surround the hexacanth embryo in the egg of these species. In addition to the outer envelope, embryophore and oncospheral membrane, Freeman (1964), Fischer (1968) and Wootten (1974) described a very thin membrane on the outer surface of the embryophore which corresponds to the thin hyaline membrane found in *P. filicollis*. These authors also described one other thin membrane on the inner surface of the embryophore. This inner membrane formed a fluid filled sac which discharged when the membrane was damaged. Freeman (1964) suggested that this membrane was not normally seen in the intact egg and was either closely applied to the inner surface of the embryophore or created de novo after the embryophore had ruptured. In the present study a possible membrane inside the granular layer of the *P. filicollis* egg was only visible in those eggs in which the granular layer had ruptured and the embryo escaped into the outer envelope. As shown in Plate 6.2, the inner membrane appears to have shrunk away from the inner surface of the embryophore, possibly due to the escape of fluid it contained. The exact nature of this membrane is unknown. The function of the granular layer seems to be protective until hatching / emergence of the egg and the provision of nutrients.

The eggs of *P. filicollis* described by Meggit (1914) are slightly larger than those measured in the present study, with an overall diameter of 58 µm compared with the mean diameter of 55.31 µm in eggs from Airthrey Loch. The inner capsule has a diameter of 35 µm according to Meggit (1914), as compared with a mean diameter of 33.31 µm in eggs from Airthrey Loch. The oncosphere diameter measured by Meggit (1914) was 23 µm as compared to the mean diameter of 21.34 µm in the present study. Thus, the size difference between the eggs of *P. filicollis* described by Meggitt and those from the present study is rather small.

The mechanism of hatching in proteocephalid eggs is not known. The release of the embryo may be due to a combination of the action of the mouth parts of the intermediate host, causing mechanical damage, and of the effects of the host digestive enzymes on the egg membranes (Wootten, 1974). The outer envelope may be broken during ingestion of the egg by the copepod. The granular layer may also be ruptured at this time, dissolved by the action of host digestive enzymes. The larva thus is left surrounded only by the oncospheral membrane, but is active within it and may escape by the action of its own hooks or membranes may be removed by digestive enzymes of the host. According to Freeman (1964) when water comes in contact with the oncospheral membrane of *P. parallacticus* the embryo becomes active and begins clawing movements. The oncospheral membrane becomes pliable and is easily ruptured by the hooks.

Hatching mechanisms have been studied in a number of Cyclophyllidea, and comprise four stages (Holmes & Fairweather, 1982):

Stage-1. Involves the mechanical breakage and removal of the shell and the outer cytoplasmic layer of the inner envelope.

Stage-2. The activation of the oncosphere and the swelling of the gelatinous layer of the inner envelope.

Stage-3. The digestion of and rupture of the embryophore. This is accomplished both by secretions from penetration glands and by the action of external digestive enzymes together with hook activity. Trypsin is more effective than amylase in digesting the embryophore.

Stage-4. Involves the enzymatic weakening of the gelatinous layer which helps the oncosphere in freeing itself with hooks. Amylase is more effective than trypsin in attacking the gelatinous layer. On emergence from the gelatinous layer the oncosphere is still enveloped by the "oncospheral membrane " although this covering is soon lost. Simi/ar stages to these may be involved in the hatching of *P. filicollis* eggs, but more detailed work on the hatching of *Proteocephalus* species eggs is required.

The development of *P. filicollis* under experimental conditions in the intermediate host, as observed in this study, is similar to that of other proteocephalids including *P. percae* (*P. filicollis*) (Kuczkowski,1925); *P. tumidocollus* (Wagner,1954); *P. parallacticus* (Freeman,1964); *P. fluviatilis* (Fischer, 1968); *P. percae* (Wootten,1974); *P. exiguus* (Primer,1987) and *P. neglectus* and *P. torulosus* (Scholz,1991,1993). In all these species the developing larva maintains its embryonic shape during the first four to ten days after ingestion by the copepod, and the size increases slowly during this period. Subsequently the larva increases in size more rapidly and the cercomer is formed between 6 and 38 days according to species and the temperature at which copepods are maintained, fully developed larvae are formed after 10-65 days.

The development of *P. filicollis* in the intermediate host was quickest at 15-16^0 C. Fifteen degrees Centigrade was reported to be optimal for the development of *P. parallacticus* (Freeman,1964), *P. percae* (Wootten,1974), *P. exiguus* (Priemer,1987) and *P. neglectus* (Scholz,1991). All these authors observed fully developed larvae from day 18 to 32 at these temperatures. The species of intermediate host used in these studies may also influence the development time of proteocephalid species.

At 10^0 C the development of *P. filicollis* was slower. Although Wootten (1974) suggested that *P. percae* would develop eventually at 10^0 C, the time required was two months. On the other hand Scholz (1991) reported a fully developed larvae of *P. neglectus* in 28 days at 10^0 C.

Proteocephalus filicollis larvae did not survive beyond 12 days at 21-22^0 C, and growth and development after cercomer formation could not be determined. In contrast to this Wagner (1954) recorded 20^0 C as the optimal temperature for growth of *P. tumidocollus* although Wootten (1974) did not observe any development beyond the cercomer stage in *P. percae* at 20^0 C. Scholz (1991) reported very rapid growth of *P. neglectus* at 21-22^{0}C and he found fully formed larvae in 8-10 days at this temperature. The reason for the apparent incomplete development of *P. filicollis* at 21-22^0 C may simply be because infected copepods did not survive beyond day 12-13 at this temperature in laboratory conditions. At 4^0 C no development of *P. filicollis* occurred. Wagner (1954) found that at temperatures between 1^0 C and 10^0 C development of *P. tumidocollus* did not occur, although larvae kept at 1^0 C for 40 days showed no development, but when moved to 20^0 C exhibited a normal growth and pattern of development. Wootten (1974) also reported no development of *P. percae* at 5^0 C. On the other hand, Scholz (1991) found fully developed larvae of *P. neglectus* in the intermediate host in nine weeks at 4^0 C.

Temperature is obviously an important factor in the growth and development of *Proteocephalus* spp. The optimum temperature for growth and development of *Proteocephalus* species may be expected to correspond to the environmental temperature at the time when in nature infection of the copepods occurs. In the case of *P. filicollis* from Airthrey Loch this would be between 12-16 C which corresponds to the optimum temperature of 15-16 C observed during experimental infections of *A. robustus*.

Temperature is also considered to be an important factor influencing growth of procercoids. In general low temperatures inhibit the growth and development of *Proteocephalus* species whilst faster growth and development of larvae occurs at high temperatures.

As the complete development of *P. filicollis* at 21-22° C could not be observed in this study no definite conclusions on the development of *P. filicollis* at high temperatures could be drawn.

The development of larvae of *P. filicollis* observed in this study is generally similar to that reported for other species of *Proteocephalus*, e.g, Kuczkowski (1925); Freeman (1964); Fischer (1968); Wootten (1974); Priemer (1987) & Scholz (1991). All these authors reported the formation of a cercomer in the larvae in the intermediate host as recorded in this study. Kuczkowski (1925) working with *P. filicollis* described cercomer formation during the larval development, although Meggitt (1914) failed to observe cercomer formation in the larvae he studied. Some other early authors also failed to observe a cercomer in *Proteocephalus* species, e.g *P. torulosus* (Wagner,1917) (as cited by Scholz 1993); *P. ambloplitis* and *P. pingius* (Hunter,1928,1929). It may be that these authors overlooked the cercomer as it appears for only short time during larval development.

Embryonic hooks of *P. filicollis* were usually seen in the main body of the larva, but occasionally hooks were seen in the cercomer. Wagner (1954) & Priemer (1980, 1987) also reported the occasional occurrence of embryonic hooks in the cercomer of *P. tumidocollus* and *P. neglectus* and *P. exiguus* respectively. The function of the round dark spots observed in the cercomer of *P. filicollis* in this study is not clear.

Wagner (1954) described the formation of suckers in *P. tumidocollus*, three or four days before the formation of the cercomer. In *P. filicollis*, lateral suckers were observed while the cercomer was still present.. In *P. parallacticus* (Freeman,1964) and *P. fluviatilis* (Fischer,1968) the suckers do not become fully differentiated until the cercomer is shed. Wootten (1974) reported a similar observation in the development of *P. percae*. Priemer (1987) reported the formation of suckers and the existence of cercomer at the same time in *P. exiguus* as did Scholz (1991) for *P. neglectus*.

Many factors, such as host species, host sex, density dependent processes and temperature may influence the growth of procercoids in cyclopoid copepods (Rosen & Dick 1983; Granath & Esch 1983a,b; Dupont & Gabrion 1987). Dupont & Gabrion (1987) reported that the growth of procercoid of the *Bothriocephalus claviceps* in copepods was density dependent, but development of the procercoid was density independent. However, other authors (Clark, 1954; Halvorsen, 1966; Rosen & Dick,1983) reported that growth and development of procercoids in copepods are related to the parasite burden, and environmental temperature (Granath & Esch 1983a). The reduced growth of larvae of *P. filicollis* in *A. robustus* with high numbers of parasites per copepod suggest that the growth of the larvae is density dependent and interspecific competition for space or food may have occurred, thus confirming the views of Rosen & Dick (1983). The relative importance of parasite

population density and temperature may differ in their influence on the growth and developmentof *Proteocephalus* larvae between host parasite system (Dupont & Gabrion, 1987).

The results of this study show that eggs of *P. filicollis* can survive for an extended period of at least 15 days at 21-22^0 C and 25 days at 4^0 C, 10^0 C & 15-16^0 C. Wagner (1954) reported a longer egg infectivity in *P. tumidocollus*, more than a month at 0, 1, 5 and 10^0 C, 19 days at 20 and 26^0 C, 13 days at 23-27^0 C and 8 days at 32^0 C. Scholz (1991) reported that eggs of *P.neglectus* can be infective for 10 days at 21-22^0 C, 25 days at 10^0 C and 20 days at 5^0 C. The length of infectivity of eggs of *P. filicollis* is not very different from other *Proteocephalus* spp. An ability to remain infective for reasonably long period will obviously increase the chance of eggs being ingested by suitable copepod hosts

The majority of the *P. filicollis* larvae observed in this study, both young and fully developed were located in cephalothorax segments of *A. robustus*, especially in the first segment. Similar results were reported for larvae of other species of *Proteocephalus*, e.g. *P. fluviatilis* (Fischer,1968), *P. percae* (Wootten,1974) and *P. neglectus* (Scholz,1991). The location of *P. filicollis* in the intermediate host did not change during development. Priemer (1987) found 5-day old larvae of *P. exigus* in the antennules of *C. strenuus*, and similarly Scholz (1991) found young larvae (less than 8 days old) in the antennules in *C. strenuus* males. Similarly, in the present study, young larvae (1-day old) were found in the antennules of the copepod. It is doubtful if there is sufficient space in this site to allow full development.

The experimental infection of *A. robustus* with *P. filicollis* eggs showed that the rate of infection is affected by the length of time that the intermediate host is in contact with the eggs of the parasite. Prevalence increased with increasing time of

contact, becoming maximum at the longest exposure time of three hours, whereas the mean intensity of infection was not much different in 15 min or 3 hour exposures. Scholz (1991) reported that the percentage of infected copepods increased with increasing length of their contact with the parasite eggs. However, he also found that the mean intensity of infection did not differ significantly in copepods exposed to *P. neglectus* eggs for 15 minutes or longer.

Copepods exposed to *P. filicollis* eggs for a longer period of time showed higher mortality than those exposed to parasite eggs for a short time. Similarly high mortality occurred in those copepods which were exposed to eggs for longer times, presumably caused by heavy infection of *P. neglectus* (Scholz, 1991).

Nie & Kennedy (1993) obtained similar results during their study on infection of larval *B. claviceps* in *C. vicinus*. A lethal level of parasite burden has been proposed for every host parasite system (Crofton, 1971). Parasite induced host mortality in several parasite-invertebrate host stystems has been described in laboratory experiments (Anderson & Crombie, 1984; Keymer, 1980, 1981). Significant mortality of *C. bicuspidatus thomasi*, infected with procercoids of *Triaenophorus crassus* has been reported by Rosen & Dick (1983). These authors suggested that mortality of *C. bicuspidatus thomasi* is related to the penetration process of the procercoid, the mechanical pressure of the procercoid on internal organs of copepods and the nutritional stress of the host. Higher parasite burden would thus result in higher host mortality and infected hosts would survive less well than uninfected ones, as observed during the present study.

CHAPTER 7
ULTRASTRUCTURE OF EMBRYONIC ENVELOPES OF PROTEOCEPHALUS FILICOLLIS

7.Ultrastructure of the embryonic envelopes of *Proteocephalus filicollis.*

7.1 Introduction.

A review of embryological studies in cestodes has indicated that embryonic envelopes are formed by a defined type of cells (macromeres) which arise during cleavage (Rybicka,1966a). Four envelopes are found in the embryonic development of cyclophyllidean eggs, the capsule, outer envelope, inner envelope and oncospheral membrane. The capsule forms as a thin membrane from the vitelline cell, while the outer envelope is formed by macromeres detached from the embryo. A coat is formed on the outer surface. The inner envelope is formed by cells detached from the preoncosphere and eventually forms the embryophore. The origin of the oncospheral membrane is not known (Rybicka, 1965). In *Hymenolepis diminuta* the capsule is formed by vitelline cells which surround the embryo. The outer envelope is formed by two macromeres and the shell is deposited on the surface of the outer envelope. The inner envelope is formed by three macromeres and the embryophore appears within it. The oncospheral membrane develops beneath the inner envelope (Rybicka,1966b).

There are relatively few ultrastructural studies on the embryonic envelopes of cestodes. Nieland (1968) in an electron microscopic study on hatched and unhatched eggs of *Taenia taeniaeformis* from cats, found that embryophoric blocks form beneath the plasma membrane of the embryophore cell. The oncospheral membrane is composed of two pairs of laminae separated by closely spaced vesicles which are formed on the inner surface of the embryophore cell. The oncospheral membrane later detaches from the embryophore and surrounds the oncosphere. The outer surface of the oncosphere is

composed of a thin layer of cytoplasmic folds that rest on the basal lamina. In mature oncospheres the cells are filled with inclusions that may represent penetration gland granules. These probably form a secretion that appears to be extruded through the surface of the hatched oncosphere.

Infective eggs of a cyclophyllidean, *Dipylidium caninum* from a dog were studied ultrastructually by Pence (1967). The vitelline material within the uterus and uterine capsule forms a layer at the surface of the outer capsule of the eggs. In the outer capsule a thin lamina separates the outer material from an inner homogeneous PAS-positive layer. Polysaccharide or glycoprotein is present in the outer capsule. The cytoplasmic layer beneath the outer capsule contains cell fragments, lipid droplets, mitochondria and alpha-glycogen. The embryophore is composed of two layers of rods at right angles to each other and an oncospheral membrane surrounds the hexacanth embryo. The oncosphere contains germinative and somatic cells, keratinaceous hooks with associated embryonic musculature, and penetration glands.

In a study on the ultrastructure and histochemistry of mature eggs of *Hymenolepis diminuta* from rats, Pence (1970) reported that the oncosphere is surrounded by several 'protective layers', the outer coat, the outer envelope, the inner envelope and the embryophore. The outer coat has a inner zone of electron dense material and an outer zone of granular material. The outer coat is mucopolysaccharide or mucoprotein. The outer envelope showed a clear space in some eggs, devoid of cellular or other elements. However, in some eggs electron opaque material was observed. The inner envelope and embryophore are a continuous morphological unit with a proteinaceous nature. The embryophore is composed of electron-dense homogeneous material. The oncosphere is surrounded by an outer syncytial layer with numerous cytoplasmic extrusions. As the

oncosphere reaches the infective stage, structural and liquefaction changes occur in the cytoplasm and granules of the penetration glands.

The developing embryo of *Catenotaenia pusilla*, a cyclophyllidean, has five main embryonic envelopes, the capsule, outer envelope, inner envelope, embryophore and oncospheral membrane (Swiderski,1968). The inner envelope is composed of two layers.

These early descriptions of the embryonic envelopes were confusing because of the non uniformity of the terminology and misinterpretation of particular structures of the embryonic envelopes. For example, confusion arose when the shell in *D. caninum*, defined as 'outer capsule', was compared to the sclerotin egg capsule in trematodes ana cestodes (Pence,1967). The 'lamina' observed by Pence (1967) within the shell of *D. caninum* appears to be a true capsule. Rybicka (1972) reported that capsule and shell have a different ultrastructure and originate independently.

Similarly, Pence (1970) suggested that the apparent absence of cellular material in the outer envelope of *H. diminuta* is actually a misinterpretation of the space between shell and envelope. A thin layer of the outer envelope is clearly seen covering a dense part of the inner envelope in his Figure 2. On the other hand, Lethbridge (1971) regarded the thickened outer plasma membrane of the inner envelope as a 'subshell membrane'.

Swiderski (1968) reported that the embryophore in *C. pusilla* does not arise within the inner envelope but is formed by a separate cellular layer. According to Rybicka (1972), however, none of Swiderskis' (1968) photographs justified this claim. Rybicka (1972) pointed out that, if as according to Swiderski (1968) a cellular layer separate from the two embryonic envelopes is detached from the embryo of *C. pusilla*, it must be given a different name and should not be confused with the embryophore which is a well-known derivative of the inner envelope.

Rybicka (1972) reviewed the literature on the embryogenesis of cestodes and proposed a uniform terminology of embryonic envelopes, that enables comparisons to be made between the structures described in various species. Rybickas' terminology is now widely accepted. Rybicka (1972) classified three envelopes and membranes and gave a simple classification allowing recognition of homologous structures in all cestode eggs. The three basic structures surrounding the cestode embryo are:

1) Capsule

2) Outer envelope.

3) Inner envelope.

According to Rybicka (1972) the derivatives of embryonic envelopes may differ morphologically and chemically in various species.

Light microscope studies by Freeman (1964) on *P. parallacticus*, Fischer (1968) on *P. fluviatilis* and Wootten (1974) on *P. percae* described five membranes and envelopes surrounding the hexacanth embryo in the egg of these species. Wootten (1974) diagrammatically described a fully developed egg of *P. percae* with five membranes surrounding it. These are 1) outer float membrane, 2) hyaline membrane (which covers the embryophore), 3) embryophore , 4) possible membrane on the inner surface of the embryophore, and 5) oncospheral membrane.

Lethbridge (1971) studied the chemical composition and properties of the egg layers in *Hymenolepis diminuta*, and demonstrated by various techniques that the egg shell has a high level of aromatic and heterocylitic acids, which act as cross-linking agents in the shell protein. The lipid containing subshell membrane protects the embryo against change in pH and osmolarity in the external environment. The cytoplasmic layer consists of a glucosamine-containing mucoprotein present in a dehydrated or

semidehydrated state and the embryophore is composed of sulphur rich protein that is weakened by some proteolytic enzymes.

Rybicka (1972) reported that there are three basic layers of independent origin in the egg of *Hymenolepis diminuta*. The capsule is secreted by vitelline cells surrounding the embryo. Beneath the capsule are two layers, the outer and inner envelopes which arise as syncytial cytoplasmic layers formed by two separate groups of macromeres. The oncospheral membrane delaminates from the inner envelope and becomes a separate membrane. Simultaneously with the formation of the oncospheral membrane the embryophore appears within the inner envelope. The embryophore is initally deposited beneath the outer plasma membrane of the envelope, and subsequently contracts and moves towards the embryo and becomes thicker.

Rybicka's (1973) study on *H. diminuta* indicates that the oncospheral membrane may delaminate from the surface epithelium of the developing embryo.

Since the publication of Rybicka' s (1972) paper on the ultrastructure of embryonic envelopes and their differentiation in *H. diminuta*, many more ultrastructural studies have been carried out on the eggs and reproductive structures of various species of cestodes.

Swiderski & Subilia (1978) reported the formation of four main embryonic envelopes, the capsule, the outer envelope, the inner envelope and the oncospheral membrane around the developing embryo of *Proteocephalus longicollis*.

Fairweather & Threadgold (1981) reported three layers surrounding the oncosphere of *Hymenolepis nana*. They described an additional layer, the polar filament layer, between the oncospheral membrane and the oncosphere. The shell material is secreted by the outer envelopes which degenerate once shell formation is complete. The uterus may also contribute to shell formation. These authors also studied the cell types present

in the oncosphere of *H. nana*. The cells in the oncosphere include penetration gland cells, oncoblast or hook forming cells, nerve cells, muscle cells and undifferentiated "stem" cells.

Wharton (1983) classified the egg shells of helminthsinto layers on the basis of their origin. Primary layers are formed by the oocyte either before or after fertilization. Secondary layers are formed by follicle cells or ovarian epithelial cells and the tertiary layers are formed by other parts of the reproductive system. This information was summarized by Wharton (1983) in a Table 7.1.

Table 7.1 Origin and composition of the egg shell layer of cestodes (adapted from Wharton (1983).

Shell layer	Origin	Composition	Classification	Reference
Capsule	Vitelline cell	Sclerotin	Tertiary	Rybicka (1972)
Shell	Outer envelope	Sclerotin	Primary	Fairweather & Threadgold (1981)
Outer envelope	Embryo	Cellular	Primary	
Inner envelope	Embryo	Cellullar	Primary	
Embryophore	Inner envelope	Keratin	Primary	
Oncospheral membrane	Inner envelope	?	Primary	

Conn *et al.* (1984) reported that the egg of *Mesocestoides lineatus* consisted of the oncosphere enclosed by a double unit oncospheral membrane and a syncytial

cytoplasmic envelope containing an embryophore. An outer envelope and a capsule were absent.

In his study on the porcupine tapeworm *Monoecocestus amesicanus,* Conn (1985) found that each egg included an oncosphere larvae surrounded by an oncosphere membrane, pyriform embryophore, inner and outer envelope, subshell membrane and embryonic capsule.

Arfin & Nizami (1986) reported that in *Moniezia expansa*, where vitelline glands are present, the egg shell is stabilized by -S-S linkages, whereas in *Avitellina lahorea* and *Stilesia globipunctata* the vitelline glands are absent and the double membrane fibrous capsule and parauterine organ contain mainly elastokeratin-type structural proteins. They concluded that no generalization is possible about egg shell formation and the chemical nature of the egg shell / capsule in cyclophyllidean cestodes.

Berrada-Rkhami & Gabrion (1990) studied the embryonic envelopes of eggs of two bothriocephalids, *Bothriocephalus gregarius* from turbot (*Psetta maxima*) and *Bothriocephalus barbatus* from brill (*Scophthalmus rhombus*), before and after hatching. They found that there are two embryonic envelopes between the egg shell and the oncosphere, the outer envelope and a ciliated inner envelope.

Chomicz & Czubay (1991) in their study on the ultrastructure of oncospheral envelopes of *Fimbriaria fasciolaris* from experimentally infected chickens, found three primary layers, a uterine, outer and inner envelope, but a capsule was not found. A thin folded shell is a derivative of the embryo, and the mother organism. The embryophore and oncospheral membranes originate intracellularly from the inner oncospheral envelopes. The secondary envelopes arise during embryo development and are formed in a way different from that described for other Hymenolepididae.

Chomicz & Walski (1991) described the oncospheral envelopes of *Diorchis elisea* (Hymenolepididae) from domestic duck. They found a thin folded envelope composed of moderately electron dense material in young and older larvae, similar to the shell in other species of cestodes. Under the shell lies a cytoplasmic envelope that contains fragments of granular endoplasmic reticulum and small mitochondria. This envelope is similar to that of the outer oncospheral envelope of other Hymenolepidae. The inner oncospheral envelope contains droplets of moderately and highly electron dense material and within the inner oncospheral envelope there is the embryophore. The oncosphere is surrounded by an oncospheral membrane which is often as thick as the embryophore.

Conn (1993) described the ultrastructure of the gravid uterus of *Hymenolepis diminuta*. He suggested that a high level of synthetic activity occurs within the epithelium, but the chemical products and functional significance of this activity is not known.

7.2 Aims.

There is little information available on the structure of embryonic envelopes of *Proteocephalus* species. The objective of this study was to investigate the ultrstructure of embryonic envelopes of *P. filicollis* by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

7.3 Materials and methods.

7.3.1 Histology.

The orientation of *P. filicollis* eggs in the uterus of the worm was studied histologically. Gravid worms were cut into very small pieces, and pieces from the posterior part of the

worm were fixed in 10 % buffered formalin for at least 24 h. The fixed material was automatically processed in a Histokinette 2000. Sections of embedded worms were cut at 5-6 μm. The cut sections were spread and floated on a water bath and then placed on a clean glass slide placed face down on a hot plate. Slides were dried in an oven at 60^0 C for at least one hour prior to staining. Sections were stained with haematoxylin and eosin. (Staining procedure is given in Appendix 3).

7.3.2 Scanning electron microscopy (SEM).

Proteocephalus filicollis eggs were collected in water in a syringe and deposited on a Sartorius polyamide filter with a pore diameter of 0.45 μm. The filter membrane with the eggs was put into a Petri dish and flooded with 1 % glutaraldehyde buffered with 0.1 M sodium cacodylate and left at 4^0 C for one hour, after which the solution was replaced with 3 % glutaraldehyde buffered with 0.1 M sodium cacodylate at 4^0 C, in which the specimen was kept for a further 2 days. The eggs were then washed well in Na cacodylate buffer and postfixed in 1 % osmium tetroxide in 0.1 M sodium cacodylate for two hours at room temperature. The specimens were dehydrated through an acetone series and then transferred to a mixture of 50 % Peldri (Ted Pella Inc, Redding, California) and 50 % acetone in the fume cupboard for one hour. This was replaced by full strength Peldri for one hour, after which the Petri dish was placed on ice to solidify the Peldri. The Peldri was sublimed off in the fume cupboard overnight. The filter was then mounted on an aluminium stub and sputter coated with gold in an Edwards 150 B sputter coater, before being examined in a Philips 500 scanning electron microscope at 6 Kv.

7.3.3 Transmission electron microscopy (TEM)

The gravid worms were cut into three equal parts for TEM processing.. The posterior part of each worm was further cut into smaller fragments of 1 proglottid each and placed in Karnovsky fixative (1.3 % paraformaldehyde, 1.65 % glutaraldehyde) for 4-6 h at 4^0 C. The fixed tissues were then washed briefly in cacodylate rinse, placed in fresh rinse and left overnight at 4^0 C. The tissue was postfixed in 1 % osmium tetroxide in cacodylate buffer for one hour and then dehydrated in a graded acetone series before being embedded in epoxy resin (Araldite CY 212). Semi-thin 0.5 µm sections, were cut and mounted on slides, then stained in toluidine blue in 1 % borax. Ultra thin sections at 90 nm were cut using glass and diamond knives in an ultra microtome Leica Ultracut E. Sections were picked up on a copper grid and double stained with uranyl acetate and lead citrate. These were then examined and photographed with a Philips 301 transmission electron microscope at 80 Kv.

7.4 RESULTS.

7.4.1 Histology of the uterus of gravid *Proteocephalus filicollis*.

The uterus of gravid *P. filicollis* consists of a number of diverticula containing eggs. Eggs are not tightly packed and are distributed throughout the diverticula (Plate 7.1).

7.4.2. SEM of eggs of *Proteocephalus filicollis*.

When viewed by SEM the outer float membrane of *P. filicollis* eggs is not swollen but is stretched as a sheath from the outer envelope. This is an artefact of SEM processing (Plate 7.2). The external surface of the outer envelope has an irregularly contoured appearance with small invaginations or pits. In a few cases the external surface of the outer envelope showed two forms of surface sculpturing on opposite sides of the embryo, one with broad invaginations and the other with a more wrinkled appearance (Plates 7.3 & 7.4).

7.4.3 Ultrastructure of embryonic envelopes of *Proteocephalus filicollis*.

TEM study has revealed that four main embryonic envelopes, the capsule, outer envelope, inner envelope and oncospheral membrane surround the embryo of *P. filicollis*.

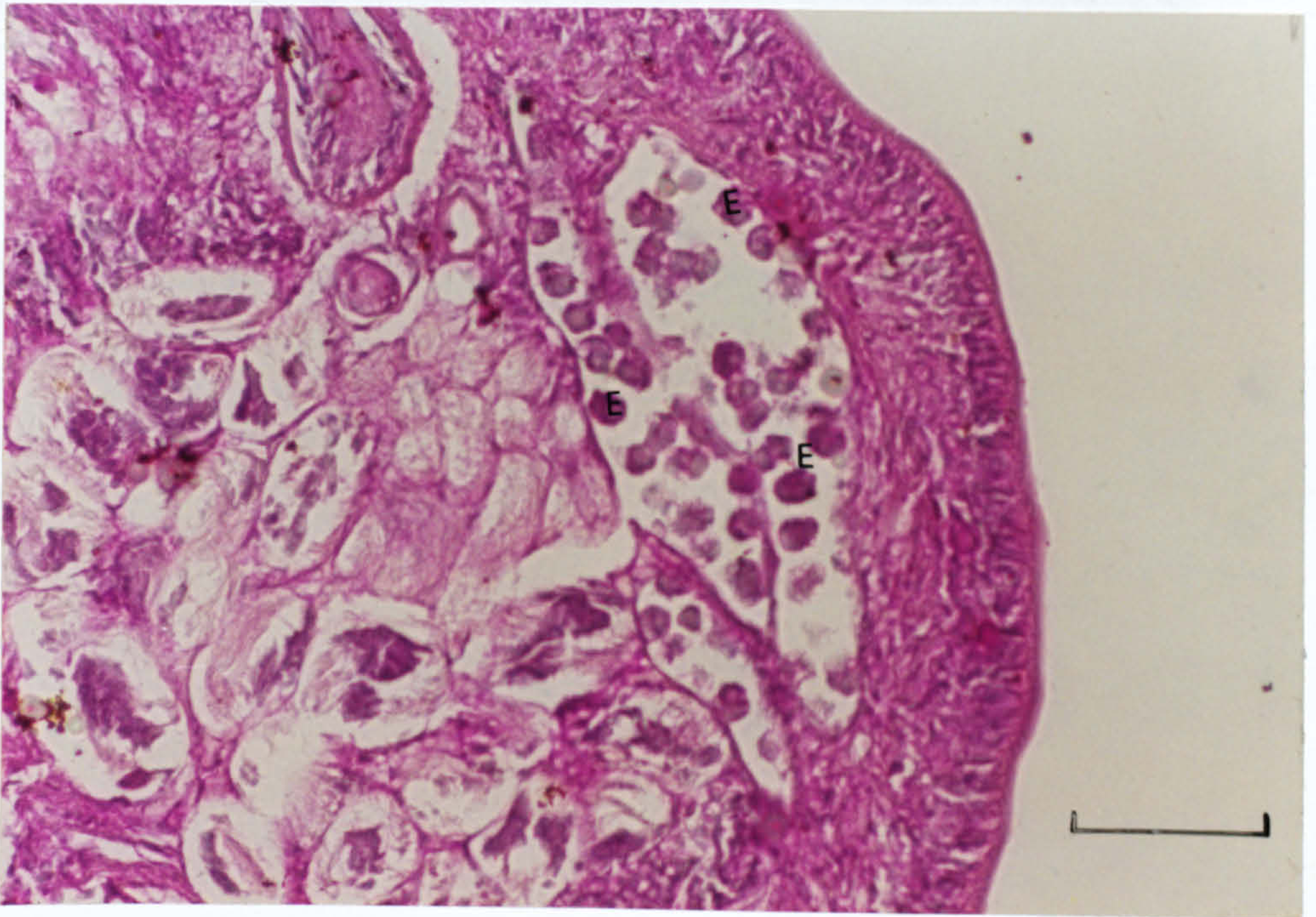


Plate 7.1 A transverse section of gravid proglottid of *Proteocephalus filicollis* showing eggs (E) in uterus (H & E, Scale bar = 100 µm).

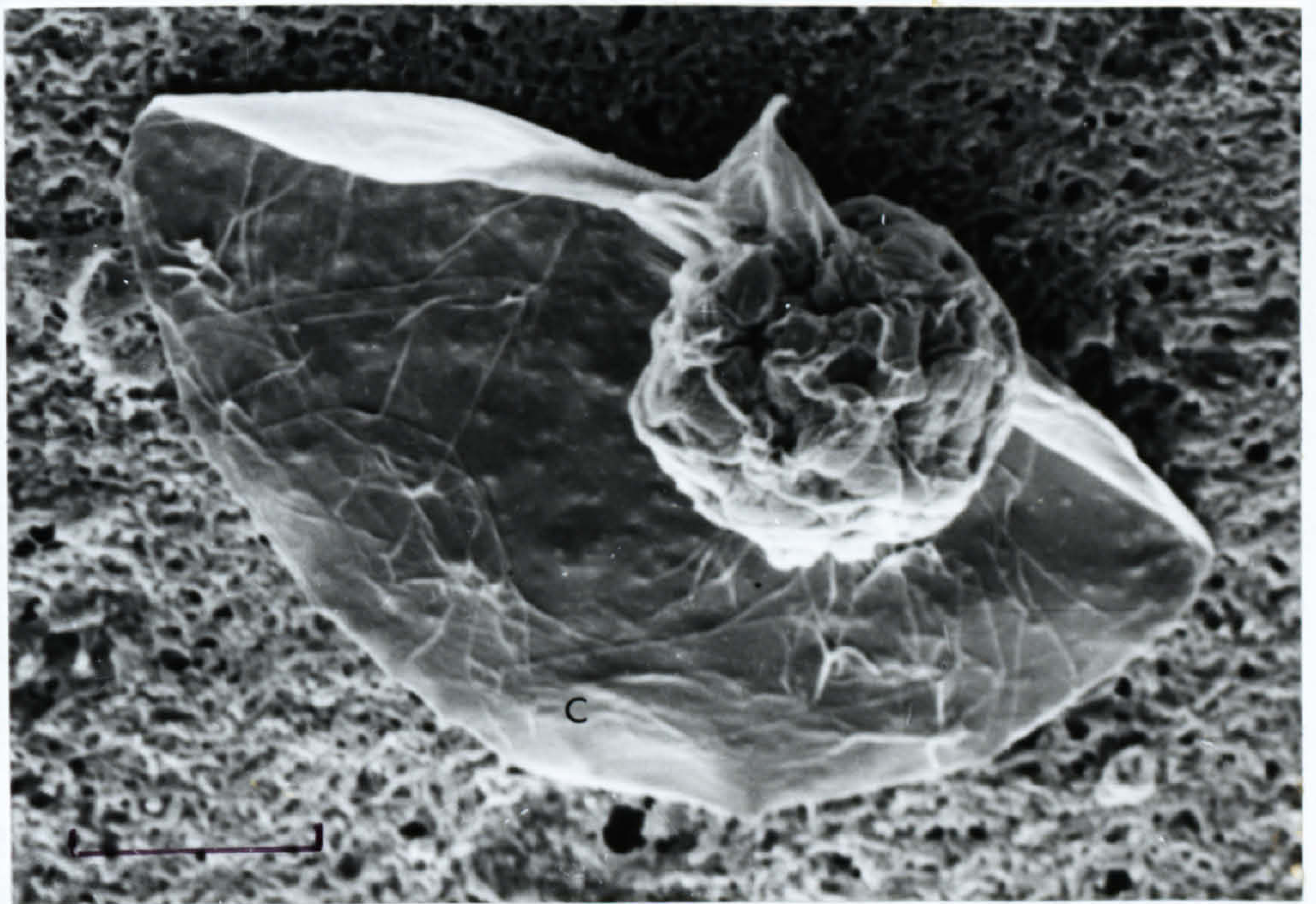


Plate 7.2. SEM micrograph of egg of *Proteocephalus filicollis*. The probable capsule (C) is seen stretched as a sheet (scale bar =10 µm).

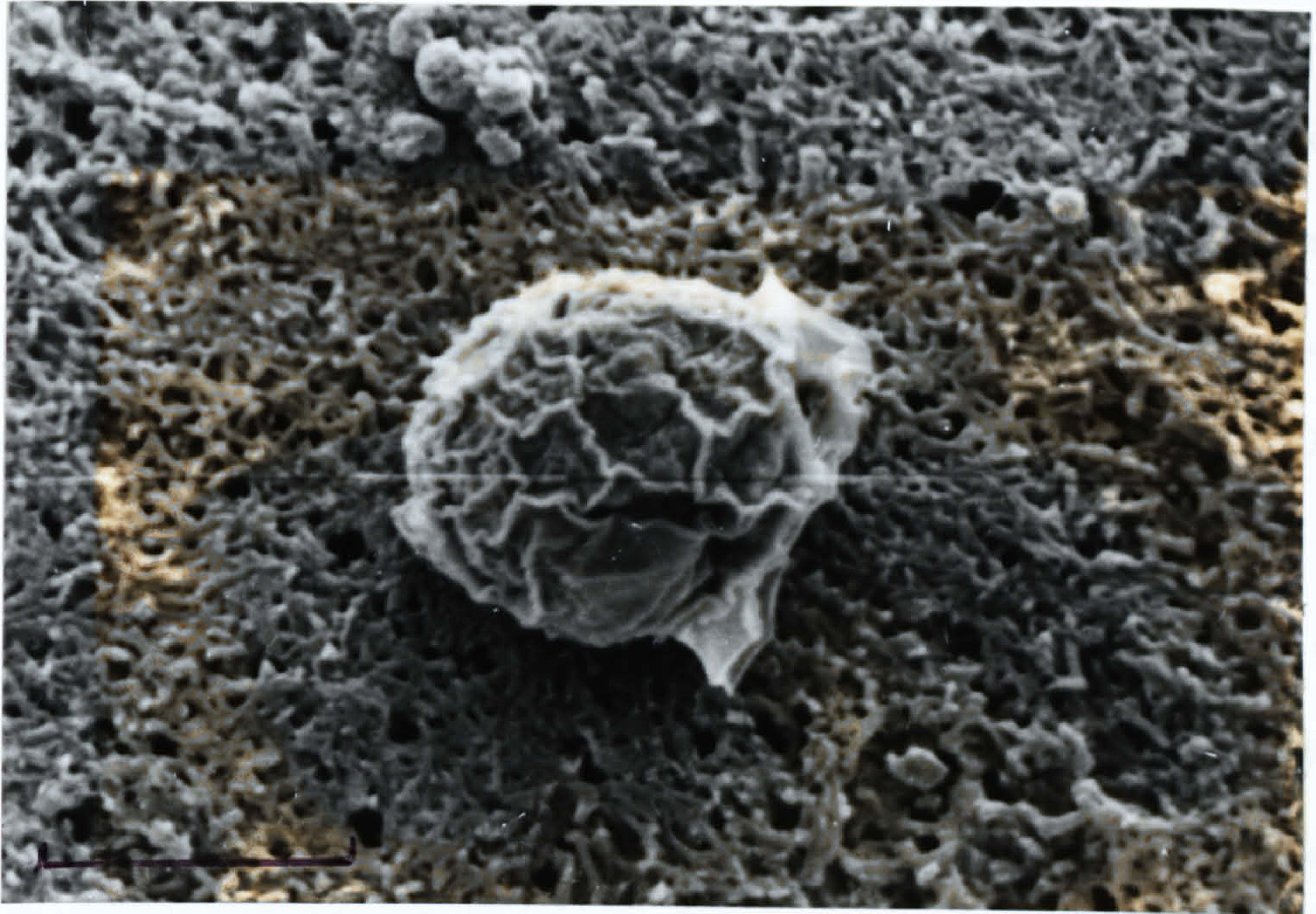


Plate 7.3. SEM micrograph of an egg of *Proteocephalus filicollis* showing external surface with irregularly contoured appearance (scale bar = 15 μm).

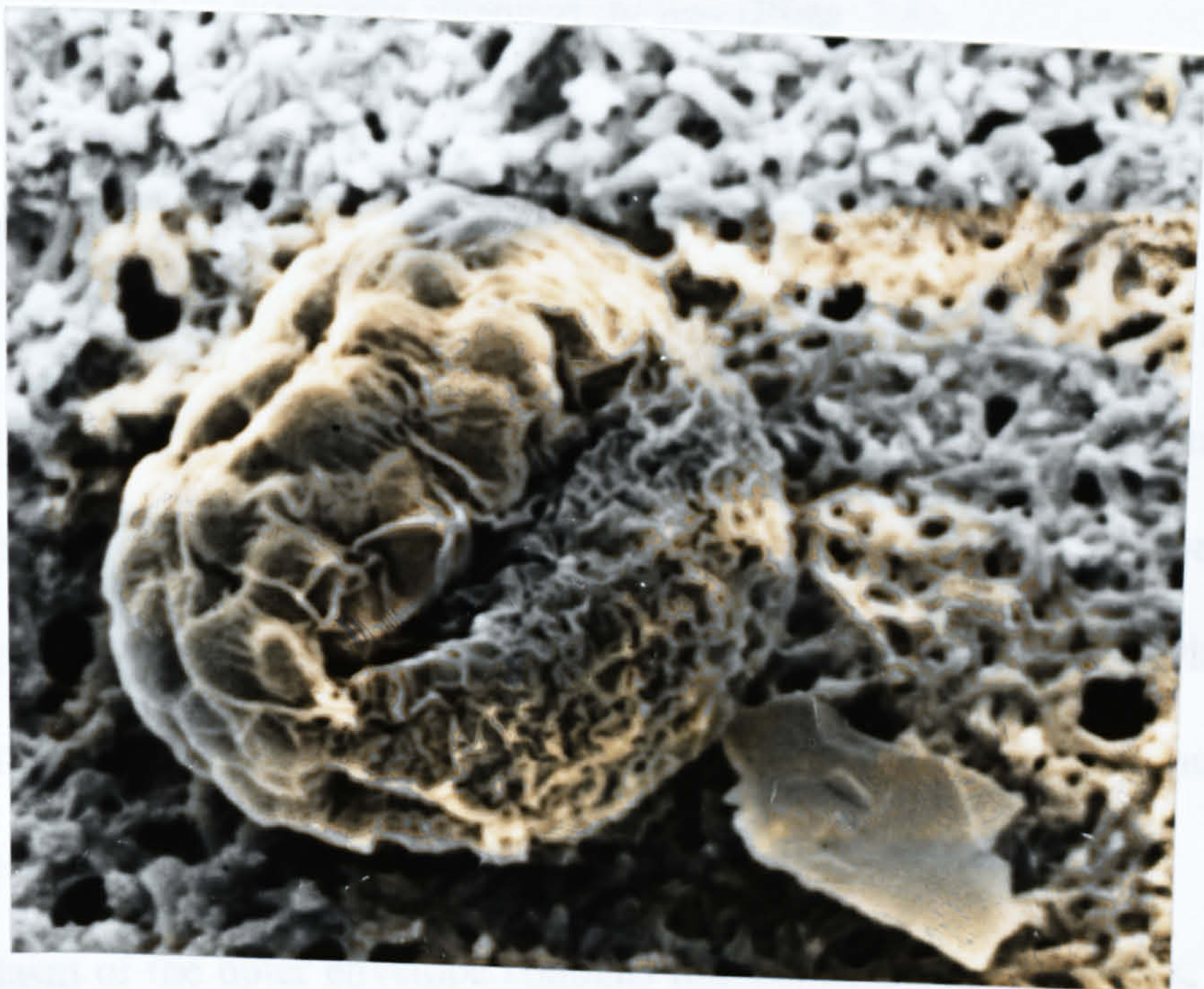


Plate 7.4. SEM micrograph of an egg of *Proteocephalus filicollis* showing different surface sculpturing on opposite sides of the embryo (scale bar = 10 μm).

7.4.3.1 The capsule:

The outermost envelope which surrounds the embryo is the capsule. This consists of two closely apposed membranes (Plate 7.5). The double membrane structure of the capsule is seen from the early stages of development (Plate 7.6). The capsule shows an uneven surface contour on either side in these early stages. It varies in thickness from 100-116 nm. In later stages of development it becomes smooth (Plate 7.7) and thickness varies from 76-171 nm. Vesicles of uniform size and shape are seen on the outside of the capsule (Plate 7.5). In the final stages of oncosphere development the capsule is moderately electron dense and thickened (357-500 nm) (Plate 7.7). Granular material is very clearly seen in the capsule and this becomes darker and compact as development proceeds (Plate 7.7). The capsule appears non- cellular. Multiple membraneous uterine folds with a vesicular appearance surround the egg (Plate 7.8).

7.4.3.2 The outer envelope:

The outer envelope is formed beneath the capsule around the oncosphere. It is not clear how many macromeres take part in the formation of the outer envelope in *P. filicollis,* but in Plate 7.6a two large macromeres are seen taking part in the formation of the outer envelope of an embryo. These macromeres are shown in more detail in Plate 7.9. The nuclei of the outer envelope seem to degenerate in the early stages of formation and the nucleus has a rather indefinite shape (Plate 7.9).

The cytoplasm of the outer envelope contains mitochondria, lipid droplets, cisternae of granular endoplasmic reticulum, polysomes and vesicles of

Plate 7.5 TEM micrograph of oncosphere of *Proteocephalus filicollis*; in early stage note the double membrane capsule (C) with vesicles (V) on the outer side (x 16700).

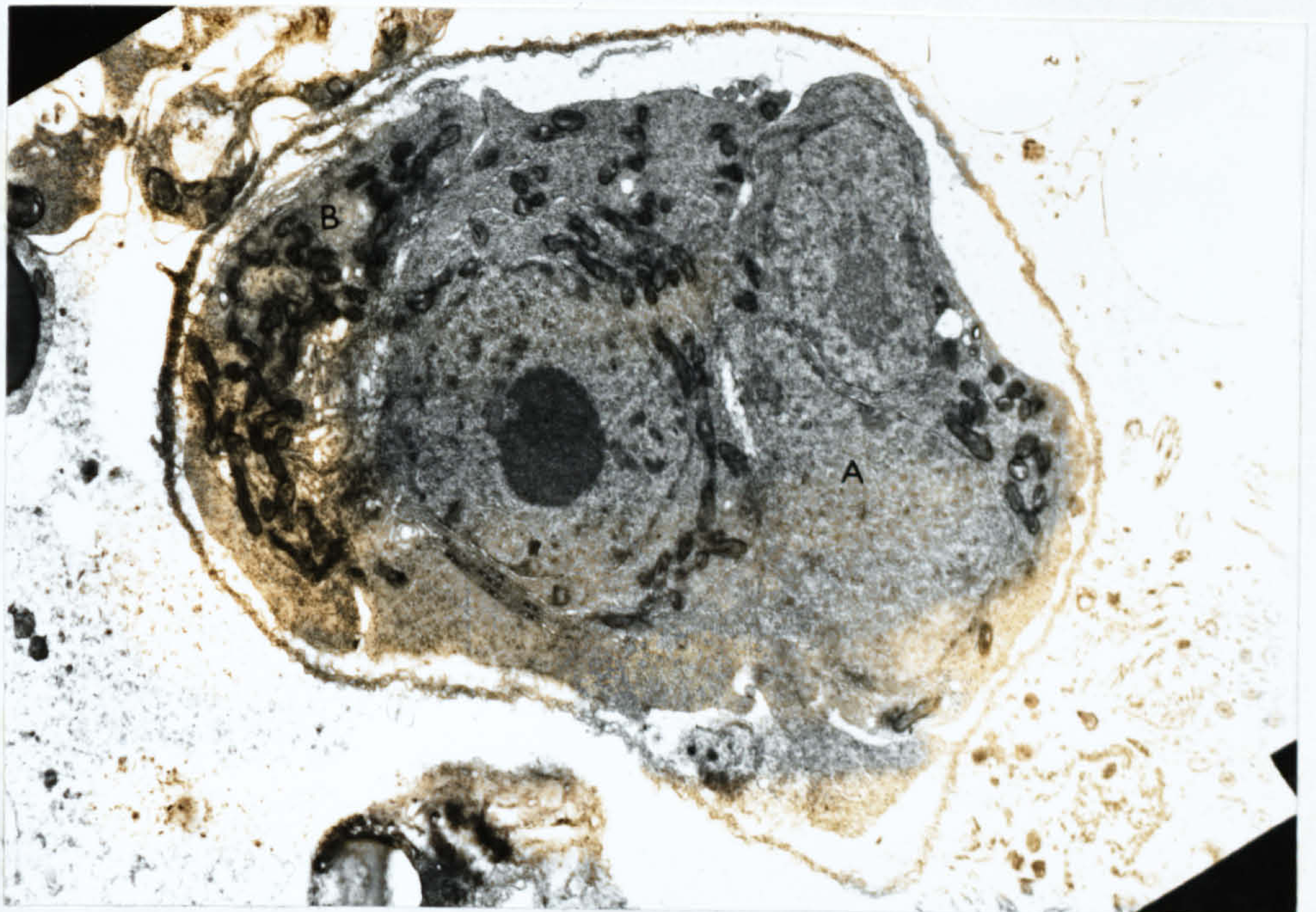


Plate 7.6 TEM micrograph of oncosphere of *P. filicollis* during early development. The outer envelope (A) and inner envelope (B) are also seen (x 9600).

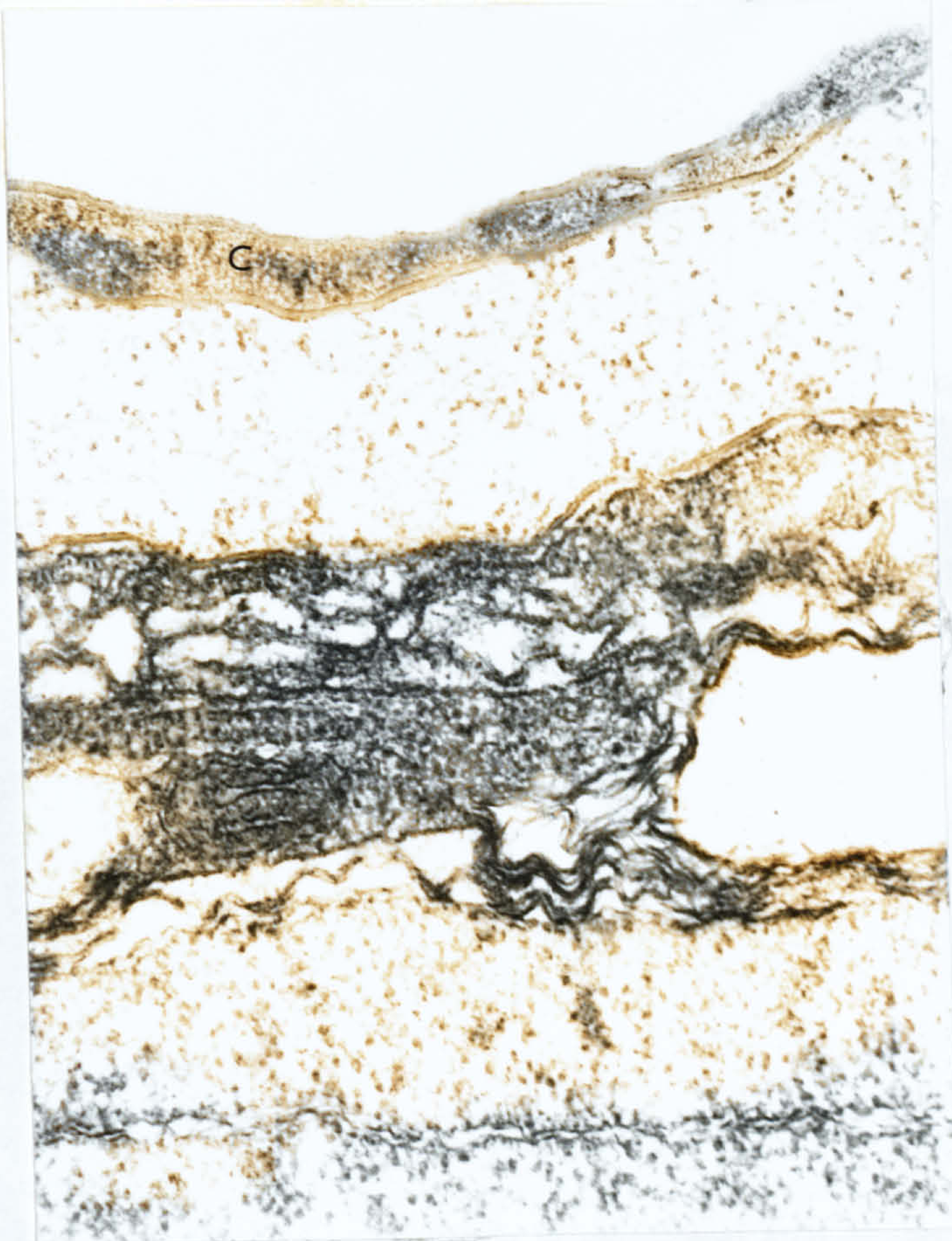


Plate 7.7. TEM micrograph of oncosphere of *Proteocephalus. filicollis* in later stages of development, showing thick dark capsule containing compact granular material (x 59700).

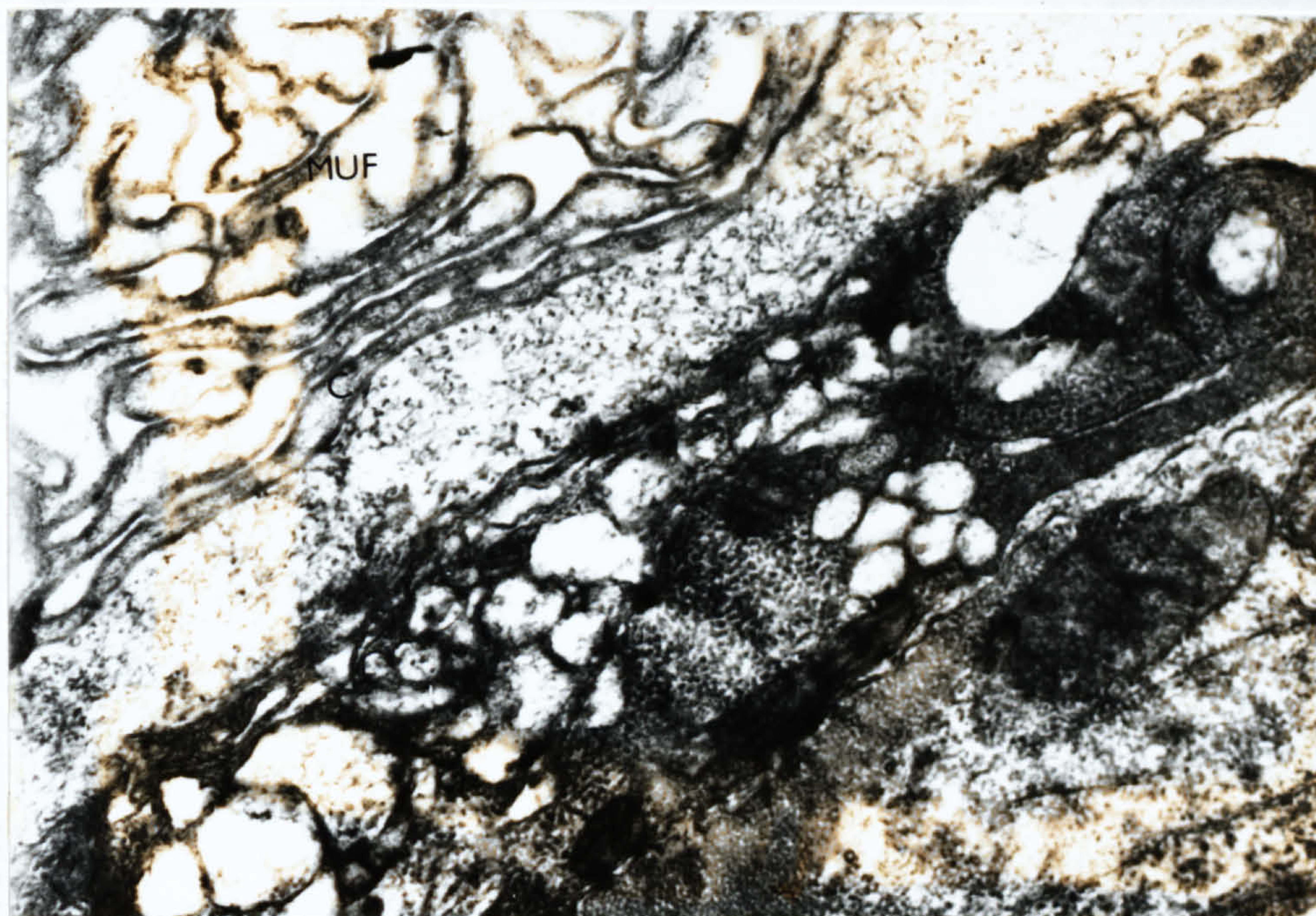


Plate 7.8. TEM micrograph of *P. filicollis* oncosphere in later stages of development. The capsule (C) is thick with a smooth surface. Multiple membraneous uterine folds (MUF) cover the capsule (x 29700).

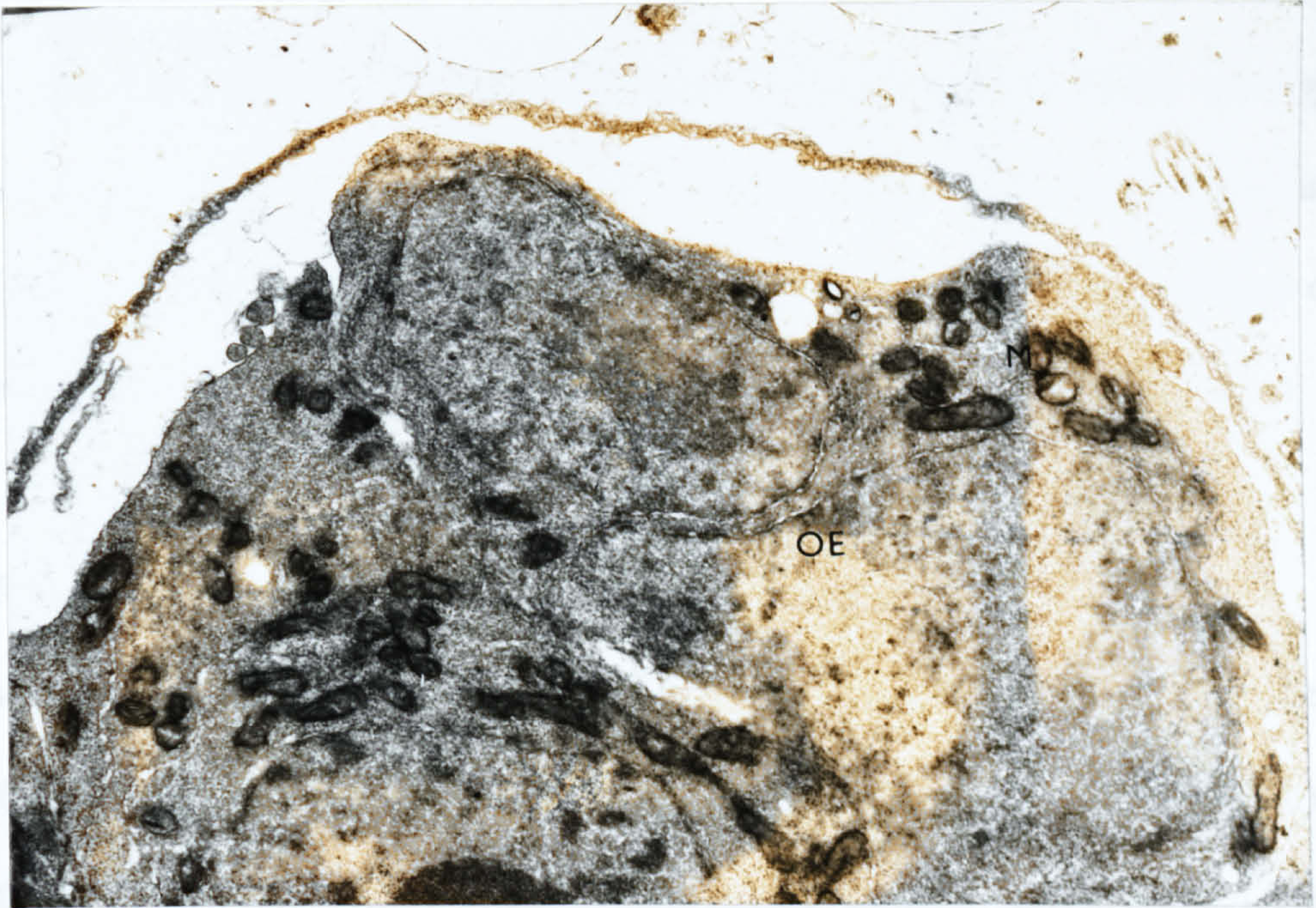


Plate 7.9. TEM micrograph of early oncosphere stage of *Proteocephalus filicollis* showing formation of outer envelope (OP).The macromere nuclei degenerate and have an indefinite shape, mitochondria in peripheral area of the outer envelope are also seen (x 15500).

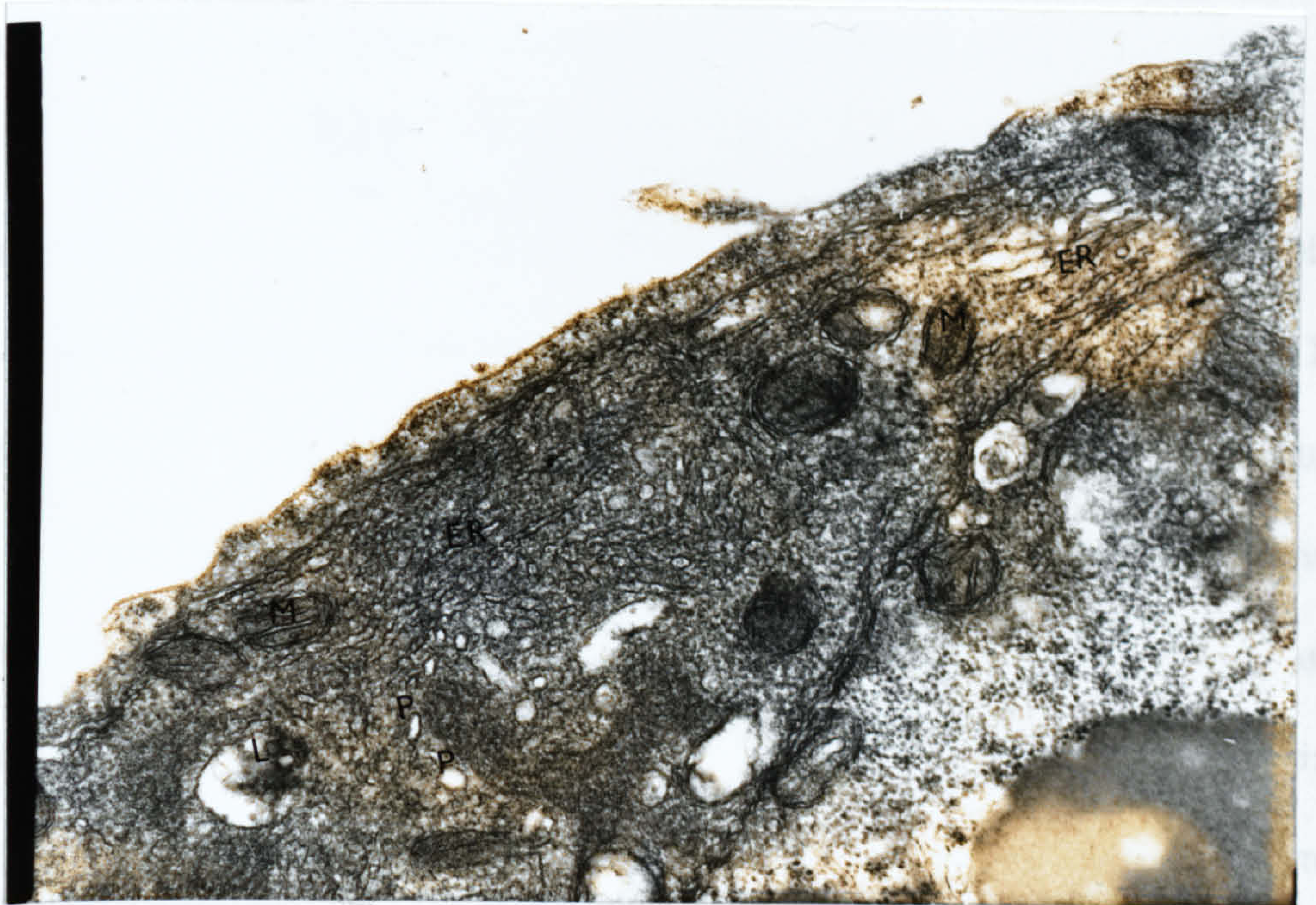


Plate 7.10. TEM micrograph of outer envelope formation of oncosphere of *Proteocephalus filicollis*, mitochondria (M), lipid droplets (L), cisternae of granular endoplasmic reticulum (ER), polysomes (P) and vesicles are visible (x 37100).

different sizes (Plate 7.10). The outer envelope in the preoncosphere stage is uneven in thickness varying from 364 -1767 nm. The outer and inner surfaces of the envelope are membrane bound, and the envelope is filled with dark electron dense granular material (Plate 7.11). It seems that cytoplasmic organelles disappear in the later stages of the development (Plate 7.11) and thickness decreases to around 500 nm.

7.4.3.3 The inner envelope:

The inner envelope appears at the beginning of the preoncosphere stage. During the early phase of formation numerous mitochondria and vesicles of various sizes and shapes are seen in the inner envelope. These vesicles seem to fuse to form larger vesicles, which in turn join to form intracellular spaces in the inner envelope (Plates 7.12, 7.13, 7.14). The inner envelope looks electron dense in the early phase of development and a membrane separating it into two parts seems to appear at this stage (Plates 7.12, 7.13, 7.14).

The inner envelope has two distinct areas. The outer part contains numerous cytoplasmic structures, mitochondria, vesicles, lipid droplets and secretory globules and is highly vacuolized (Plate 7.15) and varies in thickness from 551 - 672 nm. In some areas it appears to have a membraneous structure (Plate 7.16). There are also areas of annulate lamellae (Plate 7.17) of uneven thickness. The inner part is more homogeneous and contains electron dense granular material with only a small number of mitochondria and vesicles; it varies in thickness from 862-1034 nm (Plate 7.16). No definite membrane separating these two parts was observed in later stages of development (Plate

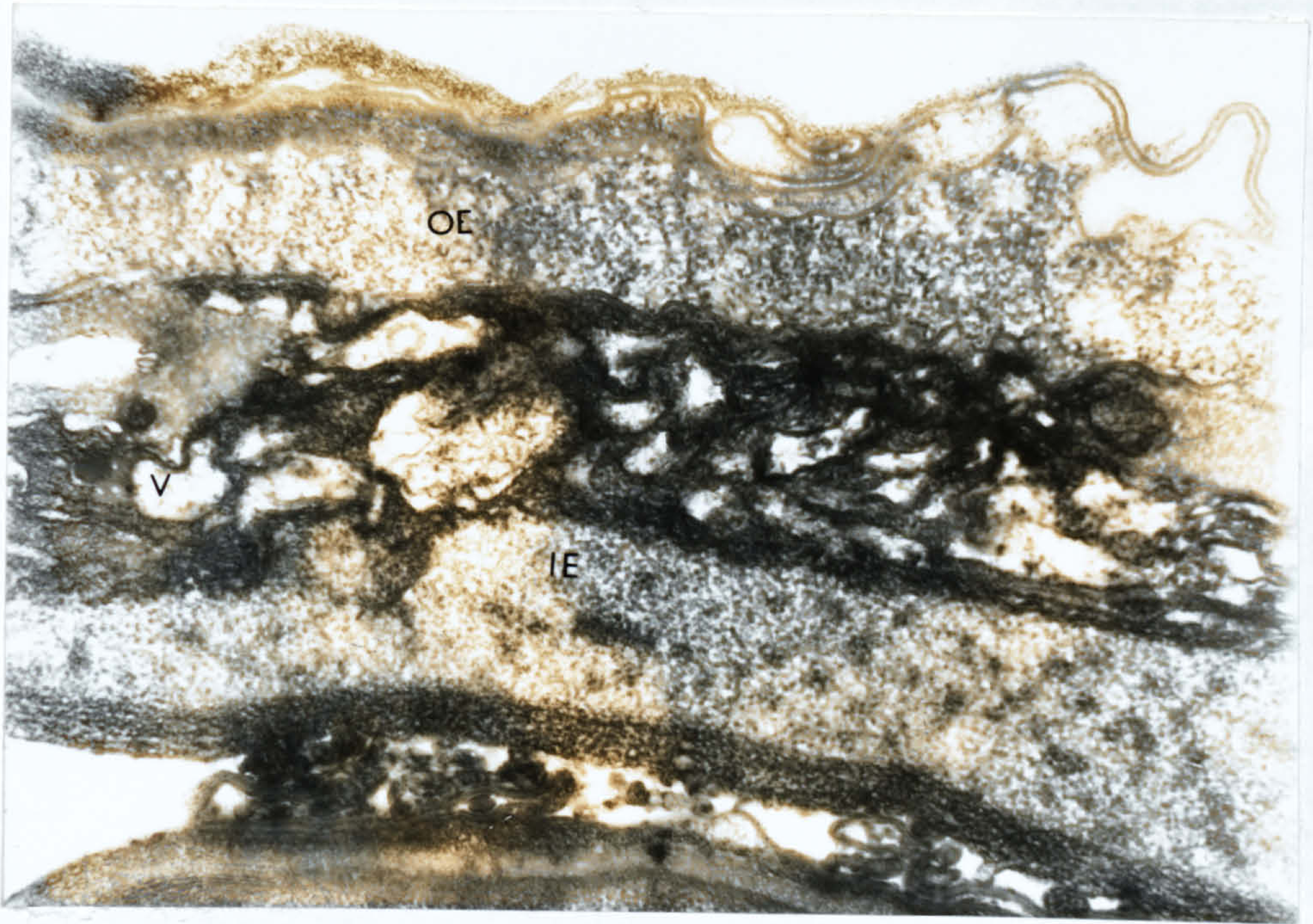


Plate 7.11. TEM micrograph of oncosphere of *Proteocephalus filicollis* in later developmental stage; the outer envelope is filled with electron dense material and cytoplasmic organelles have disappeared. Larger vesicles (V) are present in the inner envelope (x 35800).

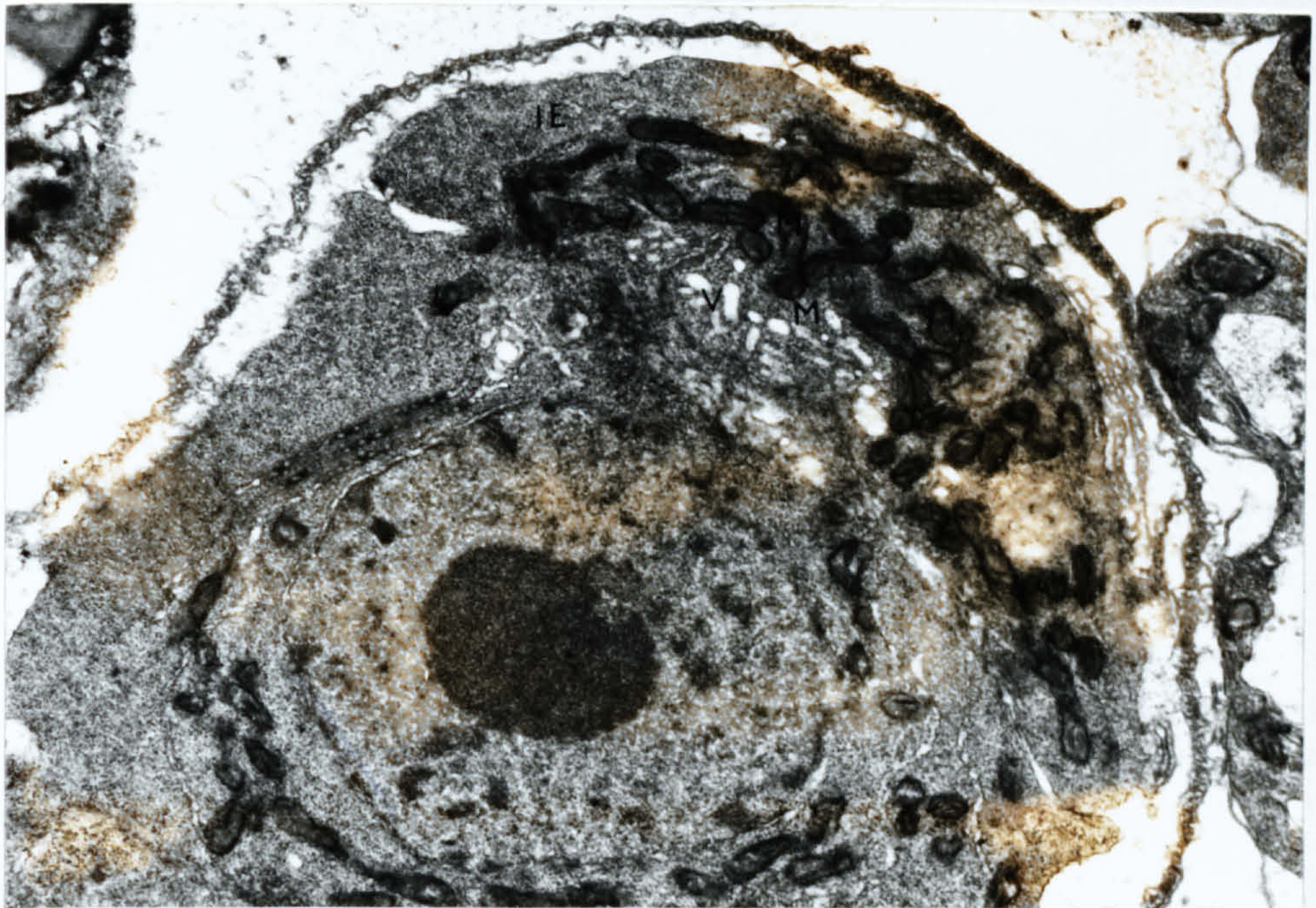


Plate 7.12. TEM micrograph of oncosphere of *Proteocephalus filicollis* showing inner envelope (IE) of early oncosphere, numerous mitochondria (M) and vesicles (V) are present (x 16900).

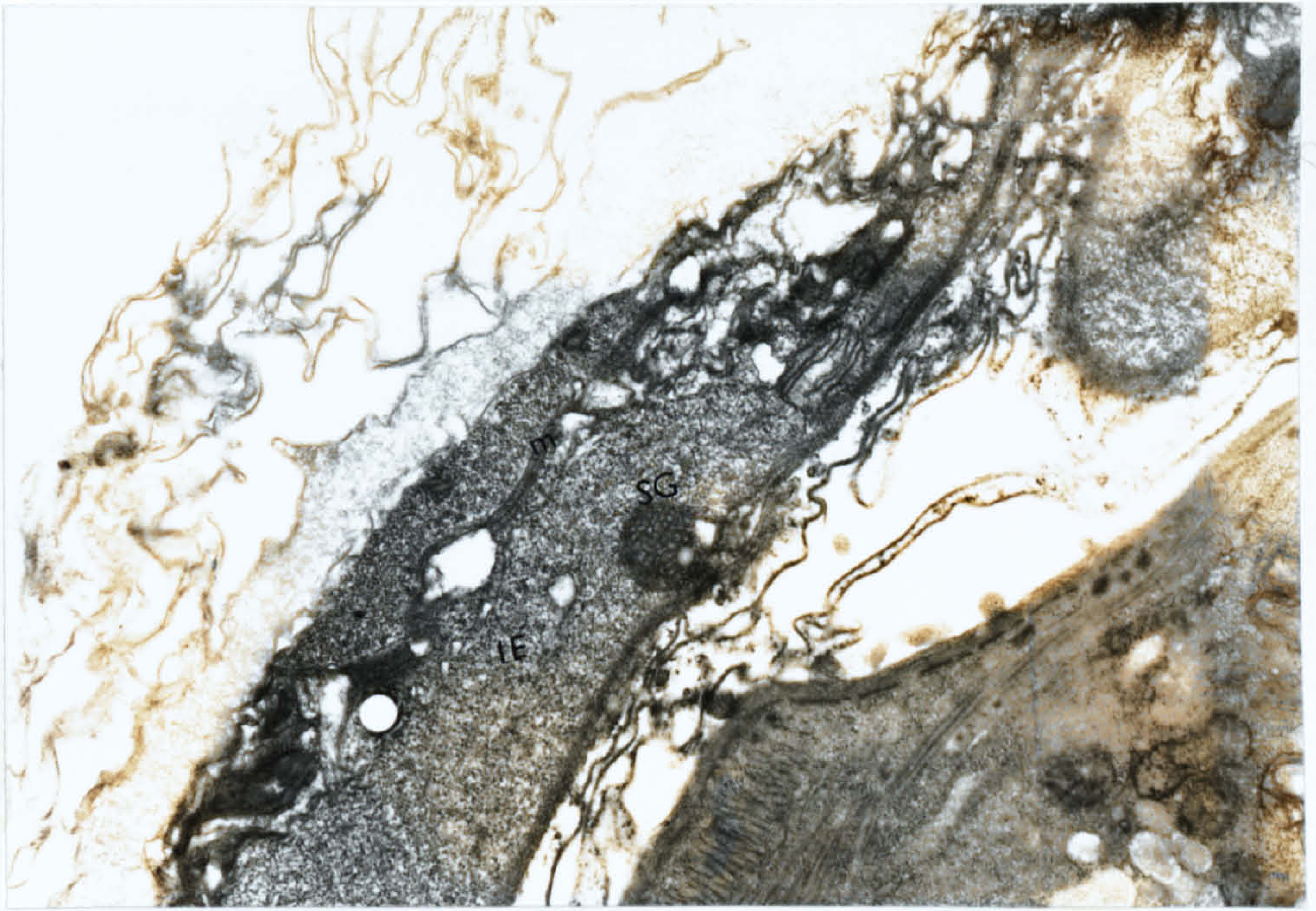


Plate.7.13. TEM micrograph of the inner envelope of *Proteocephalus filicollis* oncosphere in an early phase of development with electron dense granular material uniformly distributed. A membrane (m) appears to separate this layer into parts. A secretory globule(SG) is also evident (x 15740).

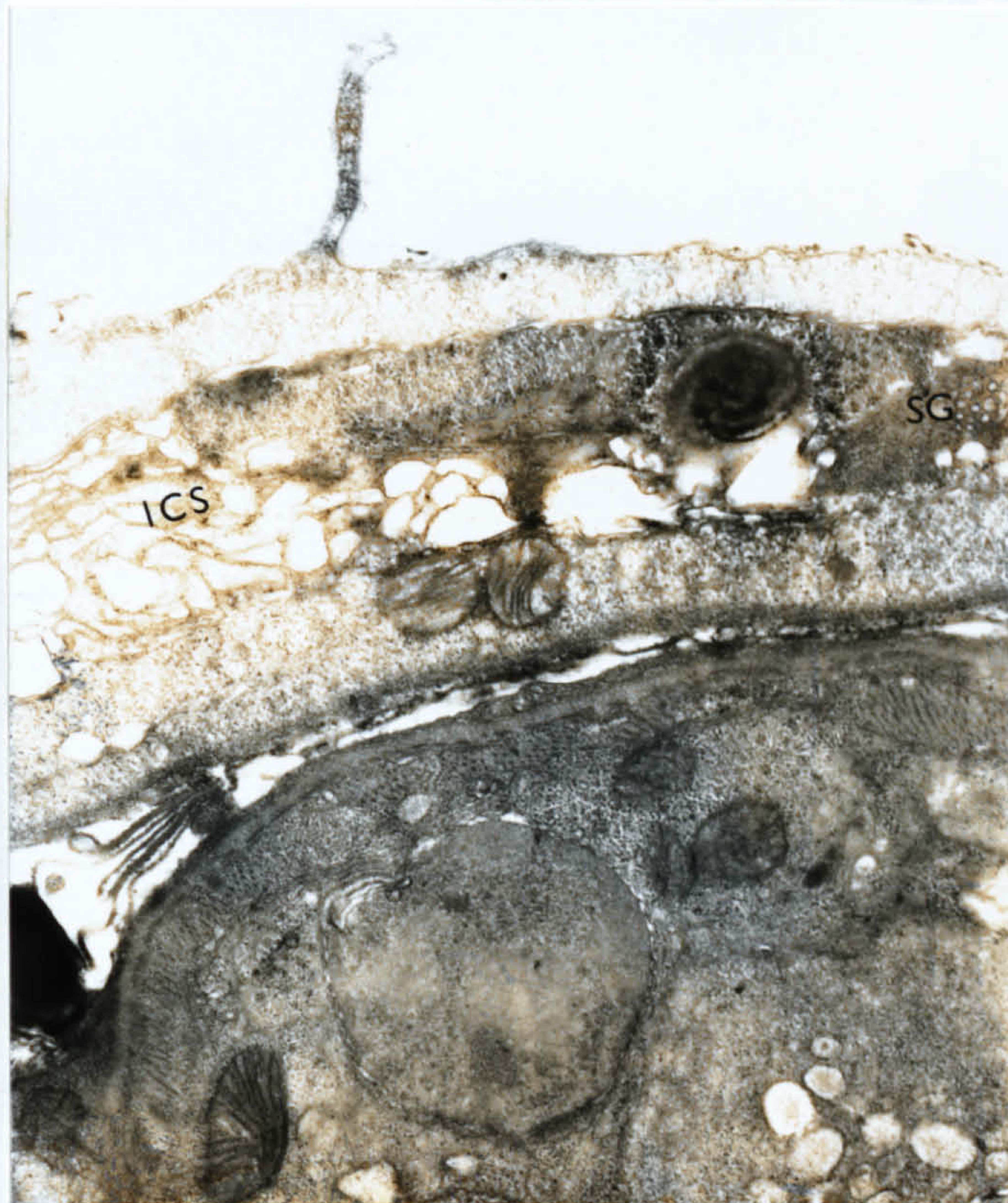


Plate 7.14. TEM micrograph of the inner envelope of *P. filicollis* oncosphere, numerous intracellular spaces (ICS) are clear. Two different electron densities of granular material are evident and a secretory globule (SG) is also present (x 15900).

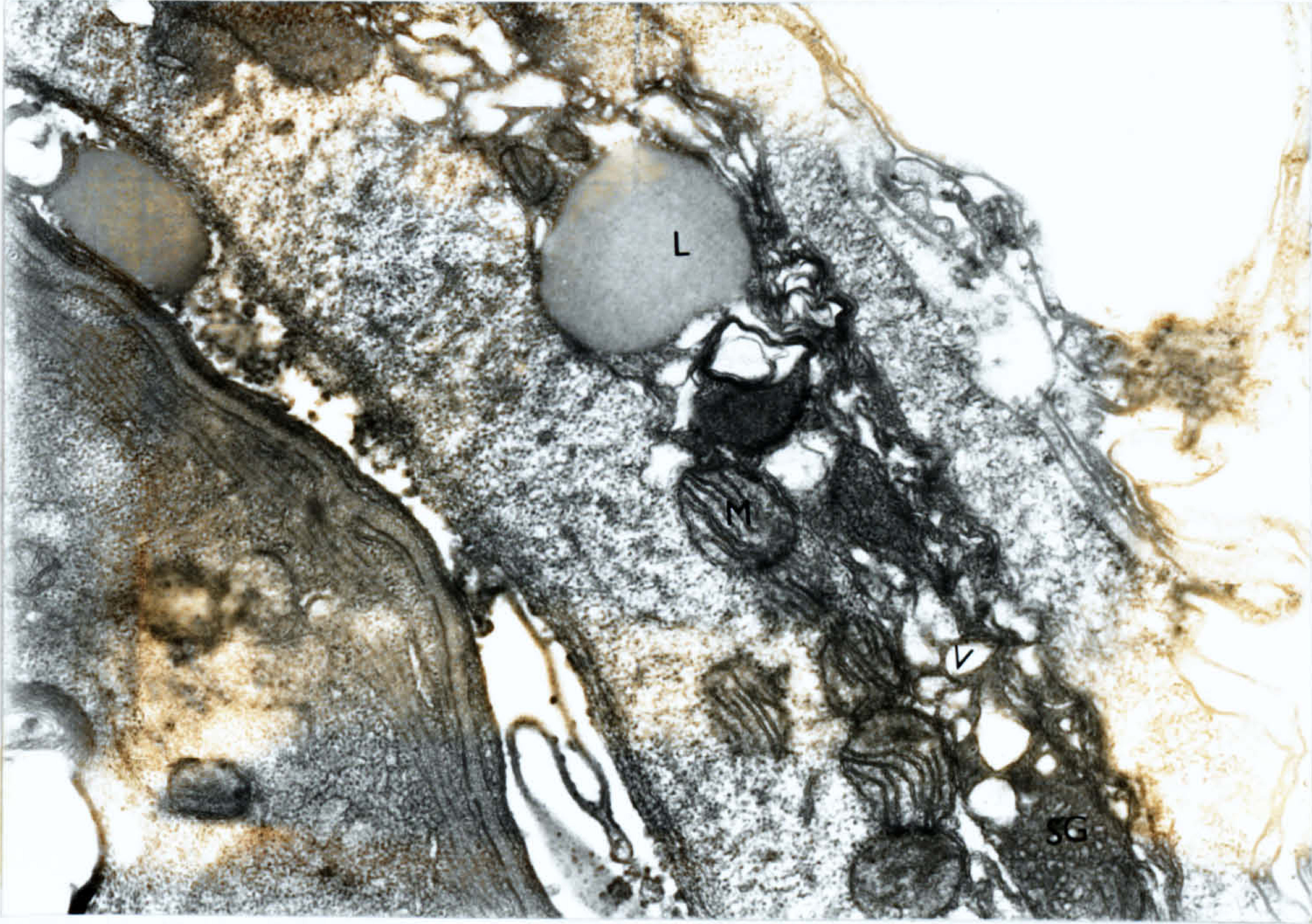


Plate 7.15. TEM micrograph of the outer part of the inner envelope of *Proteocephalus filicollis* oncosphere showing many mitochondria (M), lipid droplets (L), vesicles (V) and secretory globules (SG) (x 20800).

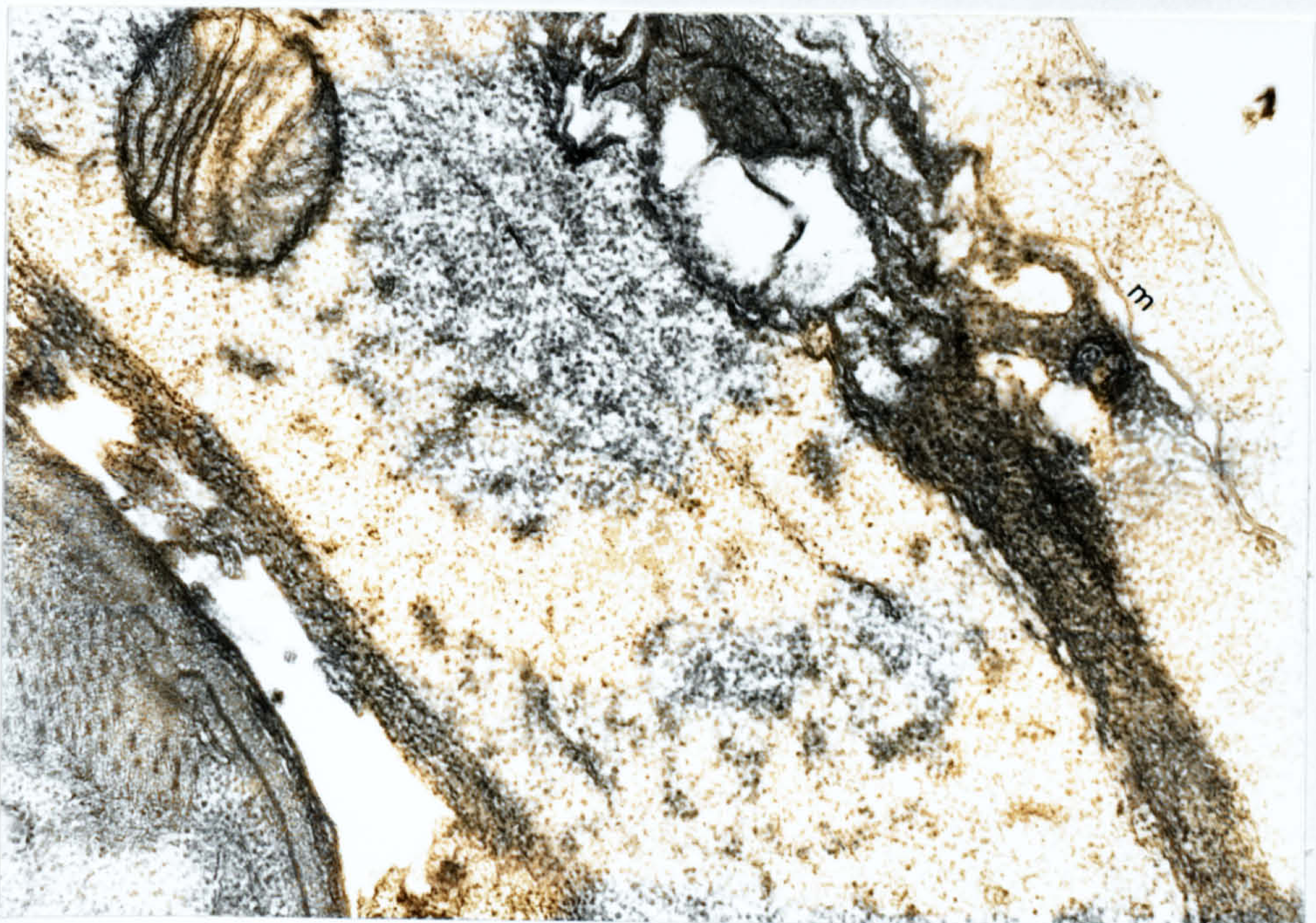


Plate 7.16 A TEM micrograph of the inner envelope of *Proteocephalus filicollis* oncosphere covered with a thin membrane (m) which separates it from the outer envelope. Randomly distributed electron dense granular material is clearly seen in the inner part of the inner envelope (x 35000).

7.15). The inner envelope is a much thicker layer than the outer envelope and is covered by a membrane which separates it from the outer envelope. This membrane is thin and less electron dense than the membrane which is adjacent to the oncospheral membrane (Plate 7.16).

7.4.3.4 The oncospheral membrane:

The oncospheral membrane is formed at the end of embryogenesis. It seems that the oncospheral membrane is formed by delamination from the outer surface of the oncosphere (Plate 7.18 & 7.19). This membrane seems to be produced in the form of electron dense blocks laid closely side by side horizontally. It seems probable that these blocks of electron dense material fuse to form a continuous membrane around the oncosphere (Plate 7.20). The oncospheral membrane has no cellular characteristics and has an electron dense and homogeneous structure.

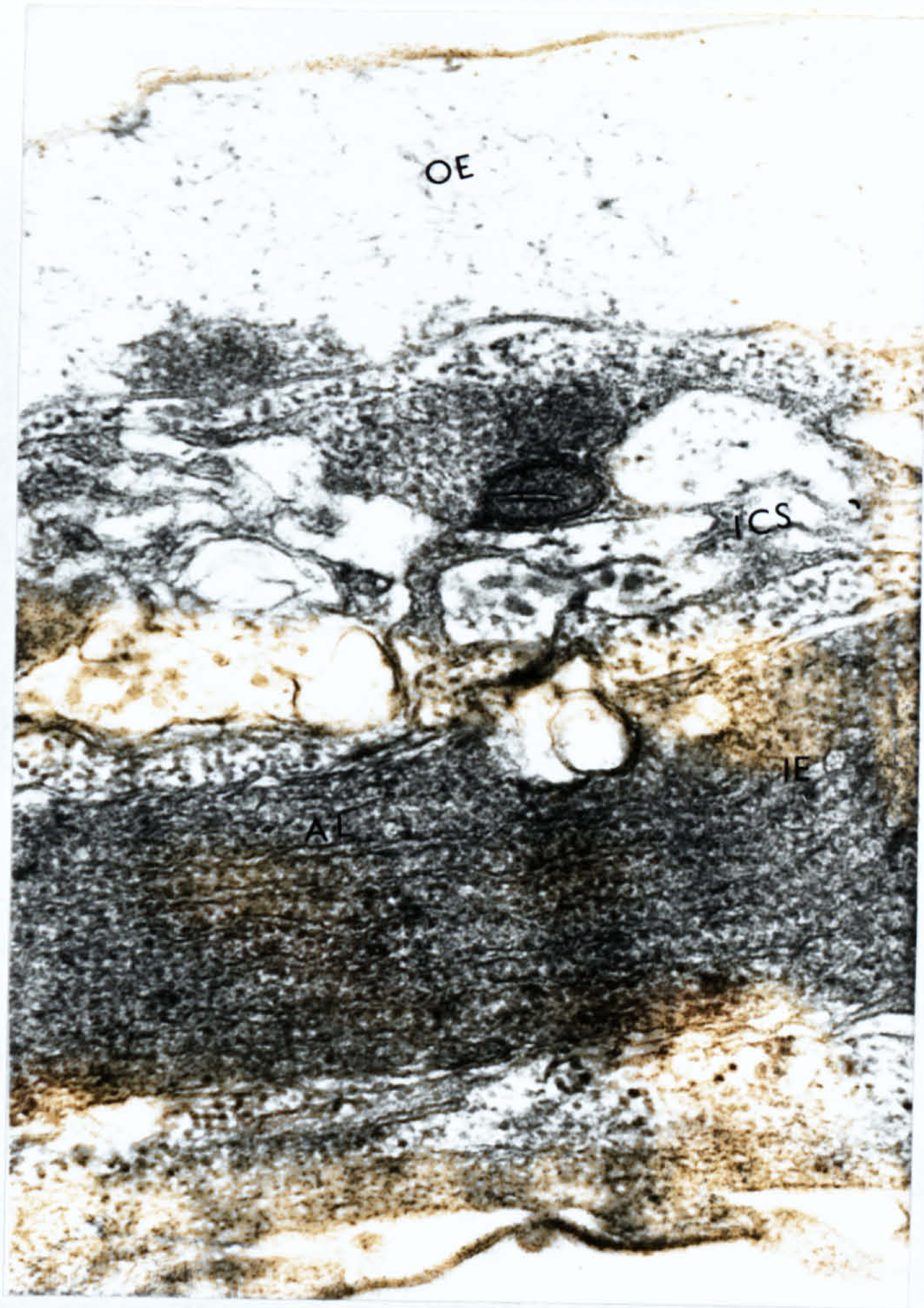


Plate. 7.17. TEM micrograph of the inner envelope of *Proteocephalus filicollis* oncosphere, intensive intracellular spaces (ICS) and annulate lamellae (AL) are very clear in the inner envelope. The outer envelope is not electron dense at this stage but its content has a fibrous appearance (x 59670).

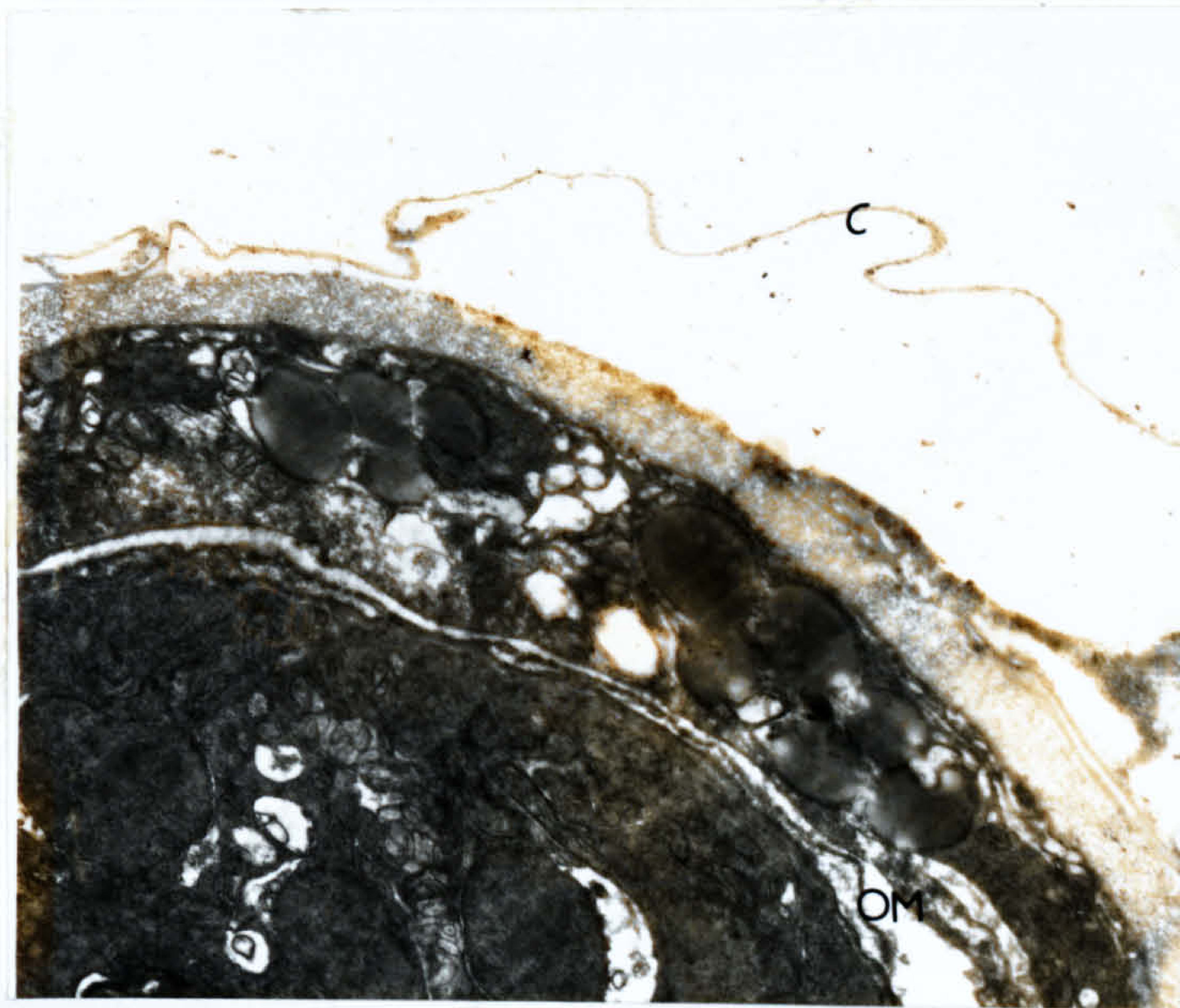


Plate 7.18. A TEM micrograph of *Proteocephalus filicollis* oncosphere; oncospheral membrane (OM) appears to be delaminating from outer surface of the oncosphere. The capsule (C) is stretched (x 7500).

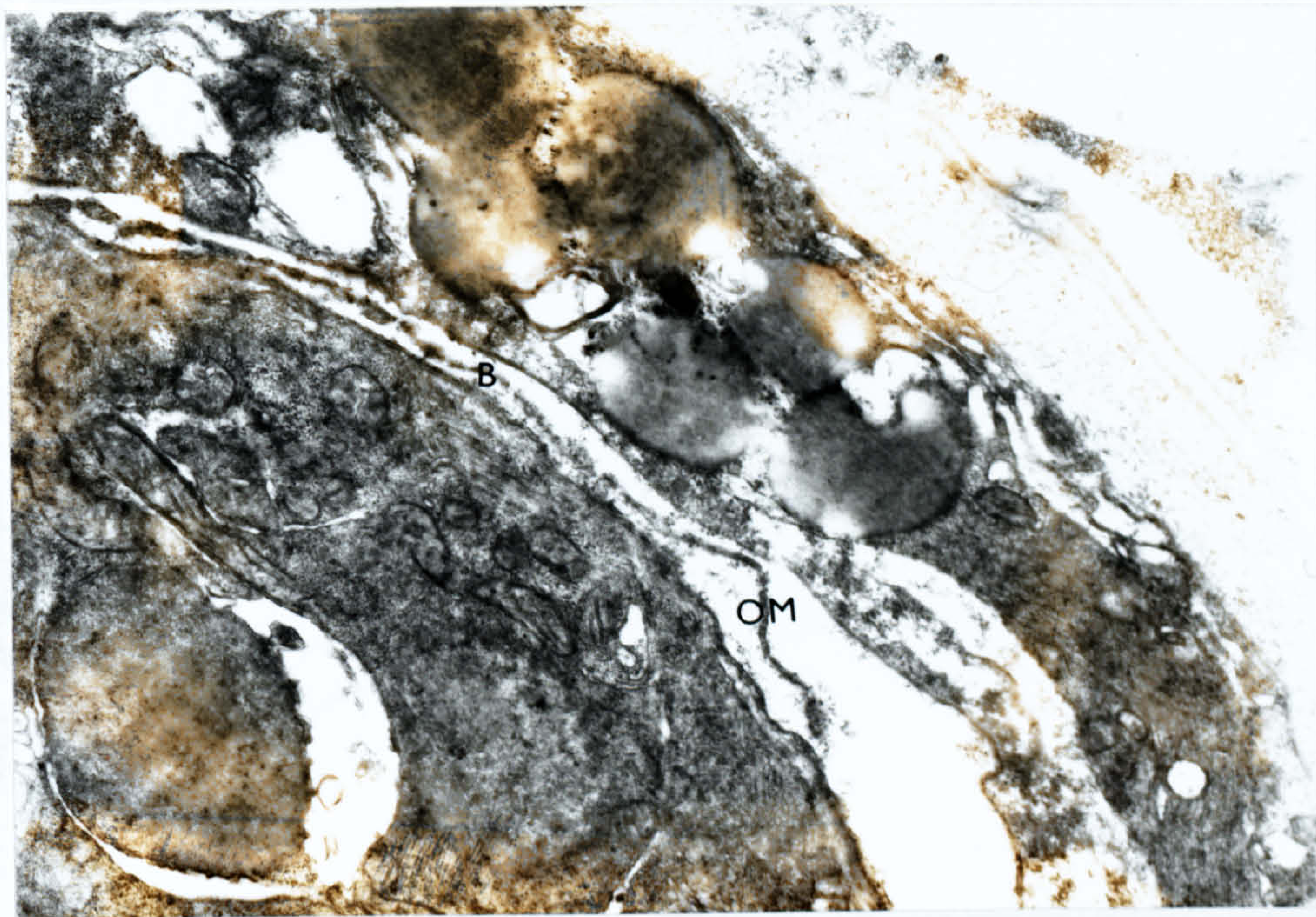


Plate.7.19 A TEM micrograph of *Proteocephalus filicollis* oncosphere; oncospheral membrane (OM) appearing in the form of electron dense blocks (B) laid side by side around the oncosphere (x 21000).

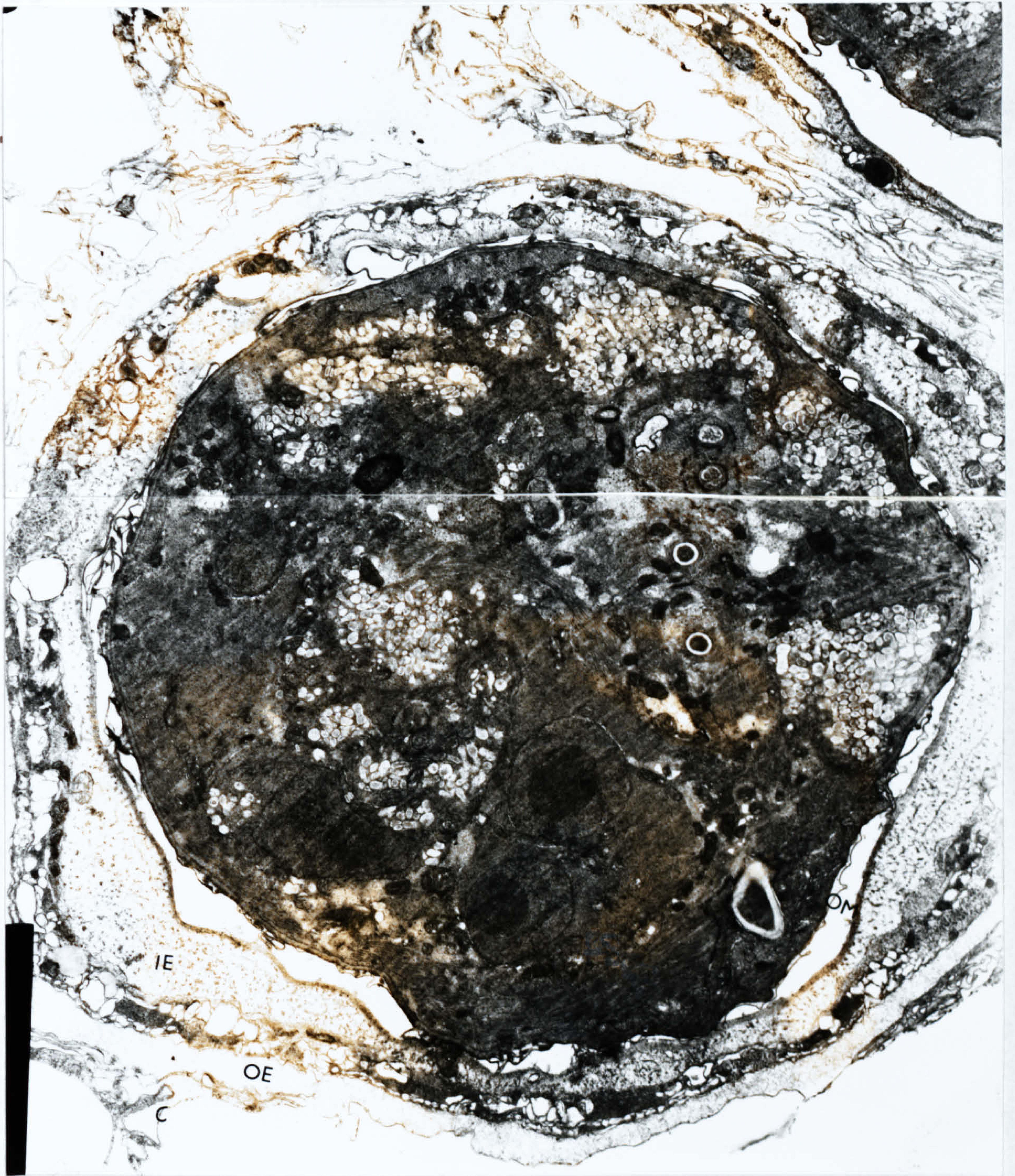


Plate 7.20 A higher power micrograph of *P. filicollis* oncosphere, four embryonic envelopes are surrounding the oncosphere. Oncospheral membrane (OM) is seen slightly folded, the inner envelope (IE) is wider than the outer envelope (OE) and the capsule (C) is next to the outer envelope (x 7800).

7.5 DISCUSSION.

Scanning electron microscopy of *Proteocephalus filicollis* eggs does not provide much information about the membranes surrounding the oncosphere. The stretched appearance of the membrane may represent the capsule, which would have obtained this shape during SEM processing. The membraneous and somewhat folded structure of the *P. filicollis* capsule allows it to swell to form the float membrane when it comes into contact with water. Coil (1977) reported that when the outer capsule of *Shipleya inermis* is fully formed it is folded much as a packed parachute, with a very large surface area contained in a small volume.

Explanation of the invaginations or pits observed on the outer surface of the oncosphere of *P. filicollis* is somewhat problematical. Hillhead (1972) reported that in cestodes having the aquatic phase of their life cycle in freshwater, the egg shell is only superficially pitted. Coil (1977) reported that in the eggs of *Shipleya inermis* the outer surface of the outer capsule is relatively smooth but has numerous pits. Similarly, Fairweather & Threadgold (1981) described the outer envelope of the oncosphere of *H. nana* as showing an irregular contoured appearance marked by numerous pits. They also reported that much wider and deeper crests are present in the outer envelope and suggested that this may indicate that the layer is undergoing some degree of degeneration. A similar explanation may account for the surface pits on the eggs of *P. filicollis*. The two types of surface sculpturing on opposite sides of the egg of *P. filicollis* seen in the present study are difficult to explain and they may represent a processing artefact.

The formation of the capsule in *P. filicollis* has not been demonstrated in the present study. Rybicka (1965) in her review of the embryonic envelopes in cyclophyllidean cestodes stated that the formation of the capsule is not fully understood. Pence (1967) found that the vitelline material within the uterus and uterine capsule forms a layer at the surface of the outer capsule of the egg of *Dipylidium caninum*. Swiderski (1968) did not describe the formation of the capsule in *Catenotaenia pusilla*. The earlier hypothesis that the capsule is secreted by the vitelline glands (Rybicka,1966a) is supported by Kings & Lumsden (1969) who showed that vitelline cells in *H. diminuta* contain large numbers of secretory granules. Rybicka (1972) stated that the capsule in *H . diminuta* is secreted by vitelline cells before the oocyte enters the uterus, but she did not observe secretory granules in the vitelline cells. Swiderski & Subilia (1978) found that the capsule is formed from shell-globule material of vitelline cells in *P. longicollis*. It is likely that the capsule in *P. filicollis* is secreted by the vitelline cells before the oocyte enters the uterus.

Rybicka (1964) stated that in cyclophyllideans it is probable that once a capsule is formed it remains around the embryo in the form of a coat. The capsule in *H. diminuta* remains around the embryo until the beginning of shell deposition and is later either replaced or covered by the shell (Rybicka, 1972). Swiderski & Subilia (1978) found that a capsule surrounds the embryo of *P. longicollis* in all development stages of the embryo. The present study supports this same view as electron micrographs clearly show the presence of a capsule surrounding the embryo of *P. filicollis* at all stages of embryonic development. Swiderski (1968) also found that a capsule was present around the embryo of *C. pusilla* throughout its development.

The capsule in *P. filicollis* is a nonfolded and smooth structure, although it appears to vary in thickness. Swiderski (1968) found that the capsule in *C. pusilla* is folded, and

Rybicka (1972) also reported that the capsule in *H. diminuta* is strongly folded in the early stage of shell development. She also stated that the growth of the shell may be connected with the stretching of the capsule. Swiderski & Subilia (1978) reported that the capsule in *P. longicollis* is unfolded and smooth.

The number of macromeres taking part in the formation of the outer envelope of *P. filicollis* was not precisely determined. It is now believed that two to three macromeres take part in the formation of the outer envelope in cestodes. Rybicka (1966b) showed that the outer envelope in *H. diminuta* is formed by two macromeres, although Swiderski (1968) could not state with certainty how many macromeres participate in the formation of the outer envelope in *C. pusilla*. In *S. inermis* the outer envelope was reported to form from the cytoplasm of two macromeres (Coil, 1977). Swiderski & Subilia (1978) stated that three macromeres fuse together to form a syncytial outer envelope in *P. longicollis*. The outer envelope of *C. variabilis* is reported to be formed from macromeres which cleave off early in development (Coil, 1979). In *H. nana* the outer envelope is cellular in origin and probably syncytial in nature (Fairweather & Threadgold, 1981).

A different idea about the origin of the outer envelope has been demonstrated and confirmed in some species of the cestodes. Berrada-Rkhami & Gabrion (1990) demonstrated two types of nuclei and their location in the outer envelope of *B. barbatus* and *B. gregarius*. They also confirmed the mixed origin of the outer envelope from vitelline cells and macromeres.

The outer envelope of *P. filicollis* observed in the present study initially contains cellular organelles, particularly mitochondria, lipid droplets, cisternae of endoplasmic reticulum and polysomes. These cytoplasmic organelles seem to disappear in later stages of egg development. Cytoplasmic organelles have also been found in the outer

envelope of other species of cestodes. In *D. caninum* lipid droplets, mitochondria and alpha-glycogen were reported (Pence, 1967). Similarly, Swiderski (1968) stated that the outer envelope is characterized by the presence of vital organelles, like mitochondria, in *C. pusilla.* In contrast to this, Pence (1970) stated that the outer envelope of *H. diminuta* is devoid of cellular material. However, Rybicka (1972) reported that the outer envelope of *H. diminuta* contains relatively small mitochondria. Numerous mitochondria, polysomes, cisternae of granular endoplasmic reticulum and lipid droplets are also reported in the outer envelope of *P. longicollis* (Swideski & Subilia, 1978).

Berrada-Rkhami & Gabrion (1990) reported that the syncytial cytoplasm of the outer envelope is disorganized and the electron dense granules remain visible during the maturation of the oncosphere in *B. barbatus* and *B. gregarius.*

The presence of cytoplasmic organelles in the outer envelope in *P. filicollis* and other species as described above indicates that the outer envelope is a very vital structure. Pence (1967) has also indicated the outer envelope to be a metabolically active area. The trophic function of the outer capsule (outer envelope) was evidenced by Johari (1957) and this was confirmed by Berrada-Rkhami & Gabrion (1991) in *B. barbatus* and *B. gregarius,* using a fluorescent lectin labelling technique, which showed an abundance of polysaccharides, particularly glycogen, in this layer.

The changes observed in the outer envelope of *P. filicollis* during the development of the egg are: 1) The cytoplasmic organelles disappear. 2) The thicknesss of the outer envelope decreases. 3) The outer envelope becomes filled by electron dense granular material.

The inner envelope of *P. filicollis* also contains cytoplasmic structures including mitochondria, vesicles, and lipid droplets. It is highly vacuolized and has two

morphologically distinct parts. Annulate lamellae are also seen in this layer. The cytoplasmic structures, including mitochondria, lipid droplets, Golgi complex, endoplasmic reticulum, polysomes and free ribosomes are also reported in the inner envelopes of other species of cestodes (Swiderski, 1968; Pence, 1970; Rybicka, 1972; Fairweather & Threadgold, 1981; Conn, *et al.*, 1984; Conn, 1985; Berrada-Rkhami & Gabrion, 1990; Chomicz & Czubay, 1991).

The number of macromeres taking part in the formation of the inner envelope was not determined. Swiderski (1968) stated that the inner envelope is formed from the so called 'third macromere' in the oncosphere of *C. pusilla* and is composed of two separate layers which originate independently. The inner envelope in *P. longicollis* is reported to form from three mesomeres which detach from the developing embryo (Swiderski & Subilia, 1978). Similarly, the inner envelope of *C. variabilis* develops from mesomeres (Coil, 1979). In *H. nana* two cells contribute to form the inner envelope, which is probably syncytial in nature (Fairweather & Threadgold, 1981).

Ten blastomeres are reported to take part in the formation of the inner envelope in *B. barbatus* and *B. gregarius* eggs. The inner envelope is a ciliated syncytium of constant thickness. The syncytium contains nuclei and many mitochondria, lipid droplets and Golgi apparatus (Rkhami & Gabrion, 1990).

Morphologically distinct outer and inner parts are observed in the inner envelope of *P. filicollis.* These resemble the two separate layers of the inner envelope reported by Swiderski (1968) in *C. pusilla,* and the distal and proximal portion of the inner envelope of *D . elisae* described by Chomicz & Walski (1991).

Cellular organelles remain in the inner envelope of *P. filicollis* until the later stages of development. The annulate lamellae become thicker and more electron dense in later stages of development of the oncosphere, and the overall thickness of the inner

envelope increases. The inner envelope seems to be a vital and metabolically active structure around the oncosphere, which probably plays an important role in providing nourishment and protection to the oncosphere prior to hatching.

The oncospheral membrane is the last layer formed around the oncosphere in *P. filicollis*, and it appears to form from electron dense blocks which seem to delaminate from the outer surface of the oncosphere, and which eventually fuse to form a continuous thin membrane around the oncosphere. In *T. taeniaeformis* (Neiland,1968), *H. diminuta* (Rybicka,1972) and *H. nana* (Fairweather & Threadgold, 1981) this layer has been reported to form by the delamination of the inner part of the inner envelope, from where it detaches as a thin separate layer which adheres to the oncosphere. In *P. longicollis*, the origin of the oncospheral membrane is similar to that of *T. taeniaeformis*, *H. diminuta* and *H. nana,* but it is composed of two closely apposed membranes (Swiderski,1978). Although the oncospheral membrane of *P. filiocollis* is similar in structure to that of *T. taeniaeformis* and *H. diminuta,* it does not delaminate as a separate layer from the inner envelope as in these species.

In contrast to the typical origin of the oncospheral membrane from the inner envelope, Berrada-Rkhami & Gabrion (1990) reported that the oncospheral membrane does not detach from the inner envelope in *B. barbatus* and *B. gregarius.*

In *F. fasciolaris*, the oncospheral membrane has been reported to be formed by numerous spherical or cylindrical vesicles of varying size. These vesicles are formed within the inner envelope near the proximal plasma membrane. These vesicles fuse to form elongated cisternae, which finally fuse to form the oncospheral membrane (Chomicz & Czubj, 1991). Hence, Berrada- Rkhami & Gabrion (1990), Chomicz & Czubj (1991) and the present study support Rybicka's (1973) suggestions that the

oncospheral membrane may delaminate from the surface epithelium of the developing embryo.

The origin of the oncospheral membrane of *O. filicollis* does not seem to be similar to *T. taeniaeformis*, *H. diminuta* and *P. longicollis*, but its formation shows some similarities to the structure of *B. barbatus* and *B. gregarius*.

The oncospheral membrane of *P. filicollis* is a very thin layer and resembles the oncospheral membrane of cyclophyllidean species. However, it may not be like that of *P. longicollis* which is reported to compose of two closely opposed membranes (Swiderski & Subilia, 1978).

Chomicz & Czubazc (1991) pointed out that the term oncospheral membrane is not entirely precise, as its structure is not like a cytoplasmic membrane, but the term is widely used for an analogous structure found in other Cyclophyllidea (Rybicka, 1972; Lethbridge, 1980; Ubelaker, 1980).

The capsule is double membrane structure, which remains around the oncosphere throughout the development. The thickness of the capsule increases in later stages and it becomes moderately electron dense. Probably two macromeres take part in the formation of the outer envelope. The cytoplasmic organelles appearing initially seem to disappear as the thickness of this envelope decreases. The inner envelope also contains cytoplasmic oranelles and vesicles which fuse to form larger vesicles. A membrane appears to separate this envelope into two distinct parts which become very clear in later stages of development. The outer part contains numerous cytoplasmic structures whereas the inner part is more homogenous and contains electron dense granular material. The oncospheral membrane seems to appear by delamination from the outer surface of the oncosphere and is not cellular in origin.

The overall ultrastructure of embryonic envelopes of *P. filicollis* resembles that of cyclophyllidean and pseudophyllidean cestodes, as suggested by Swiderski & Subilia (1978) for *P. longicollis.*

CHAPTER 8
CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

8. Conclusions and suggestions for further studies.

The present study investigated aspects of the biology of the cestode *Proteocephalus filicollis*, found as adults in the intestine of the three-spined stickleback *G. aculeatus* from Airthrey Loch, Scotland.

A study of the population biology of *P. filicollis* demonstrated that the parasite has a seasonal cycle of recruitment and maturation, each generation surviving for around 12 months in the stickleback (Fig 8.1).

Recruitment of each new generation showed a seasonal trend with most recruitment in late summer and early autumn, but recruitment was possibly a continuous process. This indicates that infective larvae are available to fish all the year around at least in small numbers, and this is supported by the recovery of copepods from the stomach of the fish throughout the year. A quantitative study of zooplankton throughout the year and determination of infection levels in natural copepod populations would help to verify this aspect of parasite biology.

The high prevalence and mean intensity of *P. filicollis* observed in the present study may be due to the eutrophic nature of the Airthrey Loch, with large zooplankton populations and a high availability of infective larvae and thus a high transmission rate of the parasite to the fish. Moreover there may be some additional intermediate hosts bearing procercoids which are available to fish at some times of the year as suggested by Kennedy *et al.*(1992). Maturation also showed a seasonal cycle with the majority of gravid worms found from late spring to early summer, although some gravid worms were recovered during the remainder of the year indicating an extended reproductive cycle. The proportion of maturing and mature worms found in the *P. filicollis* population observed in the present study was very small compared to immature and gravid worms. It may be that at this stage

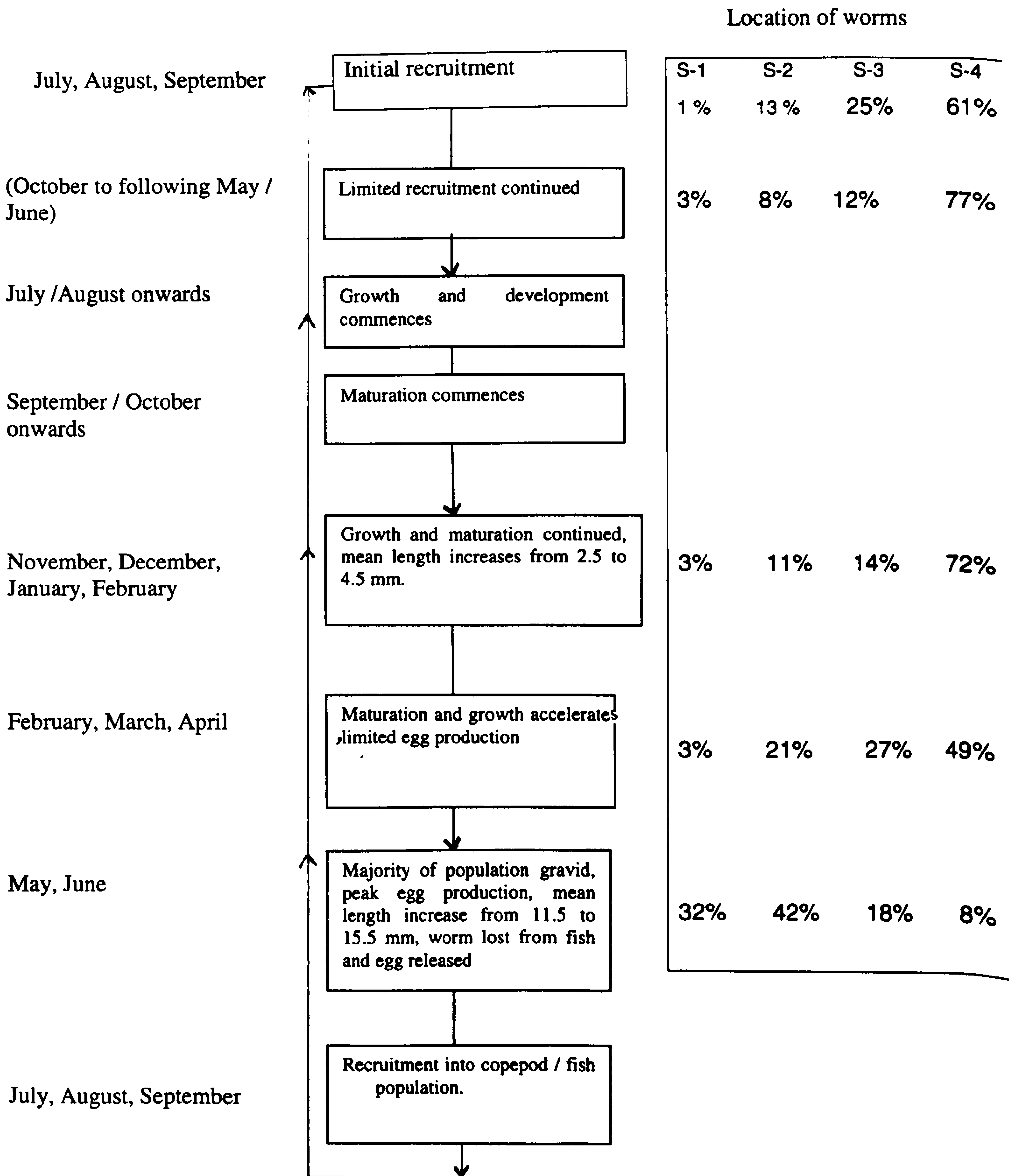


Fig.8.1 Seasonal flow diagram of *Proteocephalus filicollis* in *Gasterosteus aculeatus* in Airthrey Loch. Location of overall worm population in sections of the intestine of the fish.The percentage worm population shown corresponds to the respective months / month (data from April 1993 to July 1995).

of transformation many of the worms are lost from the host or alternatively this stage is passed through very quickly.

Proteocephalus filicollis for the first time is found to be overdispersed throughout the year, in both sexes of fish and in every season. The variance to mean ratio was found to be correlated to prevalence and abundance. The cause of this overdispersion is not clear, but may reflect variation in the susceptibility of individual fish, abundance of infected copepods and enhanced rate of feeding of sticklebacks. More work is necessary to determine which, if any of these mechanisms cause the overdispersion of *P. filicollis* in natural conditions.

In spite of the desquamation of the epithelium at the point of attachment of *P. filicolllis* no severe pathology is observed. Possibly the most significant pathological factor is the blockage of the intestine due to heavy infection. Experimental infection are needed to determine sub-lethal effects of the parasite on the fish.

Proteocephalus filicollis are attached in various sections of the intestine. The worm population in various sections of the intestine varies according to season and maturity stage. The rectum always contained a large population of immature worms from summer to autumn which decreased in the following winter and spring. A higher population of *P. filicollis* was found in the posterior intestine in spring compared to other seasons. Mature worms preferred this section of the intestine. The mid intestine had a higher population of worms in spring and summer as the cestodes matured, whereas gravid worms were mostly found in the anterior intestine. This is the first detailed account of the distribution of *P. filicollis* in the intestine of fish, according to their maturity stages and season. Over 50 % of worms are found to be located in rectum of fish as compared to over 80 % reported by Hopkins (1959).

Proteocephalus filicollis were found to migrate in the intestine of the fish. The worms migrated from the rectum towards the anterior intestine as they matured. It is suggested

that growth and maturation of the worms is a major stimulus for this migration, which might reflect competition for nutrients and space for the rapidly growing worms. Again, experimental studies might provide more detailed explanations for these observations.

Proteocephalus filicollis had a high fecundity. A statistically significant relationship existed between length of the gravid worms and water temperature. The percentage gravid portion of the worm, the number of gravid proglotids and the mean length of individual gravid proglottids are significantly positively related to the length of the gravid worm. Infrapopulation size did not shown any relationship with length of gravid worms, percentage gravid portion, number of gravid proglottids and mean length of the gravid segments

Parasite eggs were larger in winter and smaller in summer. There was a tendency for smaller worms to contain larger eggs and a smaller egg diameter was recorded in larger worms, the reason for this is not clear. The size of the hexacanth showed more consistency in its diameter and was positively related to the diameter of the eggs.

The number of eggs were positively correlated to the length of the worm, but infrapopulation size and number of eggs showed no relationship. The number of eggs per mm of gravid portion and length of worm, and infrapopulation size did not show any relationship. Further experimental work is needed to study the effect of infrapopulation size on the egg production.

It is estimated that for every 10 worms recruited, one reaches maturity and produces eggs. The number of eggs per mm of the gravid portion of the individual cestodes was estimated to be 2124, which is higher than *P. percae* (Ieshkov & Anikeeva, 1992) and *Bothriocephalus acheilognathi* (Riggs *et al.* 1987). Late spring (May) was the peak period of egg production.

The total number of eggs produced by gravid worms recovered were estimated, and the result of the present study indicated that for every 426 viable eggs produced one produced an infective larva and invaded the final host, and for every 12 larvae which infect fish only one becomes a gravid worm. This is the first detailed account on the fecundity and egg production of *P. filicollis* or any *Proteocephalus* species in Britain.

Acanthocyclops robustus was the most suitable experimental host and was used for the first time for infection of *P. filicollis*. *Proteocephalus filicollis* eggs are ingested by copepods, in the intestine of which they hatch. A series of processes involving the action of the mouth parts of the copepod, the action of digestive enzymes and even the role of the possible penetration glands are probably involved in the hatching of the eggs. The hexacanth penetrate intestinal wall of *A . robustus* in 10 minutes. The regular movement of the embryonic hooks is important in the penetration of the hexacanth through the intestinal wall of the copepod. The hatching mechanism needs to be investigated to understand this important phase in the life cycle of *P. filicollis*.

The optimum temperature for the development of *P. filicollis* in the copepod intermediate host is 15-16° C. Fully developed larvae are formed in 23-29 days at this temperature.

At 4° C there was no development of larvae even after 45 days, whereas at 10 C the development was slow and fully formed larvae were formed in 44 days. At 21-22° C development was rapid but could not be monitored due to premature death of the copepods after day 15 post-infection. Thus 21-22° C does not seems a suitable temperature for the development of *P. filicollis* in the intermediate host.

The eggs of *P. filicollis* are viable and infective for at least 25 days at 4° C, 10° C and 15-16° C but the infectivity period was reduced to 15 days when eggs were stored at 21-22° C. Cercomer formation is also reported in this study for the first time in *P. filicollis*.

Infection experiments indicated that eggs produced in winter are infective to copepods. Although larvae showed development up to day 13 no fully developed larvae were obtained due to premature death of the copepods. This is the first study on the infectivity of winter eggs in *P. filicollis*. The infectivity of winter egg needs to be further investigated and their contribution to the overall parasite population determined.

Over 90 % of the developing larvae were localized in the cephalothorax region of the copepod, of which 60 % were in the first and second segments. Larvae were found throughout the body cavity of the copepod from day 1 to11 post-infection but their number increased in the first segment from day 13 to day 27. In copepods with higher numbers of larvae, these were localized in 4-5 different sites in the cephalothorax and abdomen region. The majority of these larvae were found localized in a single site. This provides the first detailed account of the development of *P. filicollis* in the copepod intermediate host.

The prevalence of *P. filicollis* in the copepod host is significantly related to the time of contact of parasite eggs and the copepod, whereas the mean intensity of infection was not significantly related to the contact time between eggs and copepods. On the other hand mortality of infected copepods showed a significant relationship with contact time.

Histological study revealed that the uterus of *P. filicollis* consists of number of diverticula containing eggs. Scanning electron micrographs did not show any embryonic envelope around the egg as a discrete layer, but an irregular contoured appearance of the outer envelope with small invaginations and pits was clear. Two types of surface sculpturing on the opposite sides of the embryo were observed. These may be an artefact of SEM processing and needs further investigation.

This study is the most detailed so far on the ultrastructure of embryonic envelopes of proteocephalids. Transmission electron microscopy demonstrated that there are four embryonic envelopes around the mature egg. These are the capsule, the outer envelope, the

inner envelope and the oncospheral membrane. The capsule is the outermost envelope which surrounds the embryo and is composed of two closely apposed membranes. The capsule becomes electron dense and thickened in the final stages of development and dark granular material becomes evident. The capsule appears non-cellular.

The outer envelope probably originates from two macromeres. The cellular organelles like mitochondria, cisternae of endoplasmic reticulum, polysomes, vesicles of different sizes and lipid droplets are present in the outer envelope which is filled with electron dense granular material.

The inner envelope appears at the formation of the preoncosphere and contains mitchondria and vesicles. These vesicles fuse to form larger vesicles which join to form larger intracellular spaces. Two distinct parts appear in the inner envelope. The outer part contains numerous cytoplasmic structures, mitochondria, vesicles, lipid droplets, secretory granules and is vacuolized and has peculiar annulated lamellae. The inner part is homogeneous and contains electron dense granular material and a small number of mitochondria and vesicles. The inner envelope is much thicker than the outer envelope.

The oncospheral membrane is formed at the end of embryogenesis and it appears to rise by delamination from the outer surface of the oncosphere. This layer has no cellular characteristics and has an electron dense homogeneous structure.

The embryonic envelopes undergo the following changes during development: 1) The capsule become thicker and smooth and more electron dense. 2) The cytoplasmic organelles in the outer envelope disappear and the thickness of this layer decreases and the outer envelope is filled with electron dense granular fibrous material. 3) Morphologically distinct parts of the inner envelopes appear. The thickness of the inner envelope increaes but the cytoplasmic organelles remain in the inner envelope.

The histochemistry of these embryonic envelopes needs to be investigated and their nutritional role for the developed oncosphere determined.

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Appendix 1

Staining procedure using Mayer's Paracarmine.

Stain

Carminic acid	1 gram
Aluminium chloride	0.5 gram
Calcium chloride	4 gram
Alcohol 70 %	100 ml

(Dissolve the components in 70 % alcohol (warming gently if necessary), allow to settle and then filter.

Procedure for staining.

1 Wash the specimen in water for several time to remove the solution

2 Place specimen in 70 % alcohol for one hour

3	Stain with Mayer's Paracarmine	3-5 minutes
4	50 % acid alcohol	3-4 minutes
5	80 % alcohol	5-6 minutes
6	100 % alcohol	5-6 minutes
7	Clear in beechwood creosote	3-5 minutes
8	Mount in Canada balsam	

Note: The time given in staining procedure is approximate and depends on the size and thickness of the specimen and vary from specimen to specimen.

(Source: D.I Gibson, Parasitic Worms Section, British Museum (Natural History) London. July 1985.

Appendix 2

Processing schedule for histology sections.

The fixed tissue after placing in cassettes are loaded on to the processor for processing as under

1	50 % Methylated sprit	1 hr
2	80 % Methylated sprit	2 hrs
3	100 % Methylated sprit	2 hrs
4	100 % Methlated sprit	2 hrs
5	100 % Methylated sprit	2 hrs
6	100 % Ethanol	2 hrs
7	100% Ethanol	2 hrs
8	Chloroform	1 hr
9	Chloroform	1hr
10	Molten wax	2 hrs
11	Moltenwax	2 hrs
12	Molten wax	2 hrs

Appendix 3

Haematoxylin and Eosin staining.

No	Steps	Time (Minutes)
1	Xylene (dewaxing)	5
2	Absolute alcohol 1	2
3	Methylated spirit	1.5
4	Wash in tap water	
5	Haematoxylin	5
6	Wash in tap water	
7	Acid alcohol	3 quick dips
8	Wash in tap water	
9	Scott's tap water	1
10	Wash in tap water	
11	Eosin	5
12	Wash in tap water	
13	Methylated spirit	30 Sec
14	Absolute spirit 11	2 Min
15	Absolute alcohol 1	1.5
16	Xylene (clearing)	5
17	Xylene (cover slip)	