LEABHARLANN CHOLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH | TRINITY COLLEGE LIBRARY DUBLIN
Ollscoil Átha Cliath | The University of Dublin

Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

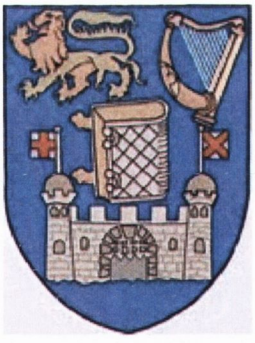
I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

The Population Biology of *Acanthocephalus clavula* in the European Eel *Anguilla anguilla* L., Brown Trout *Salmo trutta* L. and *Asellus meridianus* Rac., with Special Reference to the Suitability of Brown Trout as a Definitive Host

A dissertation submitted to the University of Dublin for the degree of Doctor of Philosophy

Emma Jane Dooley

Trinity College Dublin, 21st December 2009

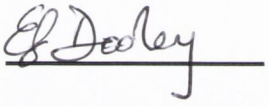


Department of Zoology
Trinity College Dublin

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other University and that it is entirely my own work. I agree that the library may lend or copy this thesis on request.

Signed,



Emma Jane Dooley

December 2009

TRINITY COLLEGE
10 APR 2012
LIBRARY DUBLIN
9416

SUMMARY

Acanthocephalus clavula is an acanthocephalan parasite of freshwater fishes which is distributed across Ireland, Great Britain and continental Europe. In Ireland, the intermediate host of *A. clavula* is the isopod *Asellus meridianus*. The preferred definitive host of *A. clavula* across its distribution is the European eel *Anguilla anguilla*. *A. clavula* has been recorded in brown trout *Salmo trutta* in Ireland on several occasions, however in other locations the occurrence of this host-parasite combination is rare.

Previous work on trout helminth communities in the west of Ireland has shown that *A. clavula* does not reach reproductive maturity in trout. A host shift of the parasite was postulated and, to this end, a primary aim of the present study was to revisit this topic and further explore the suitability of trout as a definitive host for *A. clavula*.

If a host shift had taken place, it was hypothesised that the prevalence and abundance of *A. clavula* would decrease from the almost epidemic levels found previously, and that the proportion of females reaching full reproductive maturity would increase. The results obtained from the present study suggest that no host shift of *A. clavula* has taken place and that the eel remains the preferred definitive host of this species. It is proposed that brown trout be re-designated as either an accidental or additional host of *A. clavula*.

The present study also explored the population biology of *A. clavula* in all three hosts in three lakes in the west of Ireland: Bunaveela Lake, Clogher Lough and Lough Feeagh. Previous studies have reported linked cycles in population dynamics between intermediate and definitive hosts, although this was not the case for *A. clavula* in eels, trout and *A. meridianus* in Clogher Lough and Lough Feeagh. While seasonal cycles in infection parameters were observed in both isopod and fish hosts, they were not correlated and in isopods even differed between sample years. The population biology of *A. clavula* was also explored in seven regional sites in order to extend the observations from a local to a regional scale.

The lack of consistency in seasonal cycles of *A. clavula* in *A. meridianus* between years, as well as the absence of a linked cycle between the parasite and its intermediate and definitive hosts suggests that the occurrence of *A. clavula* in its hosts is stochastic, unregulated, and solely dependent on intermediate host abundance, which in turn is dependent on

environmental factors, particularly water temperature and abundance of vegetation and detritus.

ACKNOWLEDGEMENTS

Thank you to my wonderful supervisor, Professor Celia Holland. There are no words, but I'll try anyway. Thank you so much for being an amazing, inspiring, understanding and immensely patient supervisor and friend for the past three years.

To Dr Russell Poole, my collaborator, for being so helpful with every aspect of my fieldwork, for finally finding where my *Asellus* were hiding and for being so enthusiastic about my work.

To Joe Cooney and Davy Sweeney – the best fieldwork buddies a girl could ask for! This project literally could not have been done without you. Thank you both for being so obliging, for teaching me so much and for being such great craic!
Joe: The hills are alive….
Davy: Work and talk!!!

Many thanks to my Steering Committee, Professor Jim Wilson, Dr Mark Brown, and Dr Ken Irvine for guiding me in the right direction and being so helpful with the planning of my project.

Thank you to the Zoology technicians Peter Stafford, Richard Hollinshead, Martyn Linnie and especially Alison Boyce – you are a living legend – for socks, maps, driving me to Kerry, singing to me, driving me to Mayo, stopwatches, fish tanks, photographs and on and on and on….

Thank you to Lughaidh O'Neill for the words of wisdom and encouragement, for keeping me on track and for being so great on the Eel Tour 2008.

Thanks to Mark Kavanagh of the Centre for the Environment for solving my weighing nightmare and being so helpful.

Thanks to all the staff of the Marine Institute, Newport, for making me feel so welcome, and very special thanks to Ciar O' Toole, my 'Mayo Mammy', for looking after me so well. Cheers Ted!

Thanks to my office mates Chris, Joe, Jason, Sile, Mike and Paddy for putting up with me.

Thank you to the undergraduates who did their projects with me: Sean, Claire, Naiara and Killian. Thank you all for being such good workers and for being so much fun in the lab and in the field.

Thanks to all who helped me with the thesis and its construction: Erna King, Katie Archer, Megan O'Brien, Alison Boyce, Paul Dooley, Jason Devoy-Keegan, Elaine McGoff, Blaithin NiAinin, Lynsey Stuart, Michelle Giltrap and Louise Donohue.

Very special thanks and appreciation to Emma Louise McGinley for feeding me, listening to me, taking me for coffee, providing me with television and being generally fabulous.

Thank you to Erna King for everything: the hot chocolates, the movie nights, the cocktails, for being such a good stress-buster and for pulling the final all-nighter of my Ph.D. – I couldn't have done it without you.

Thank you to my family, Majella, Paul and Jeff. Without your unending support, encouragement and hugs, I would never have gotten this far - I would have gotten much, much further!

Finally, thank you to my parasite, the endlessly fascinating and confusing *Acanthocephalus clavula*.

This thesis is dedicated to the memory of
Eileen O'Brien

'*Omnia meae debeo familiae*'

TABLE OF CONTENTS

Chapter 1: Introduction

1.1 Parasitism and free-living communities 1

1.2 Parasite community ecology 2

1.3 The Acanthocephala 3

1.4 Hosts of Acanthocephalans 5

1.5 Post-Cyclic Transmission 8

1.6 The Acanthocephala in Ireland 9

1.7 Irish Acanthocephalans and Host Specificity 10

1.8 *Acanthocephalus clavula* 11

1.9 *A. clavula* Hosts 12

1.9.1 The European Eel (*Anguilla anguilla*) 12

1.9.2 Eel Life Cycle 13

1.9.3 Eel Decline 13

1.9.4 The Brown Trout (*Salmo trutta*) 14

1.9.5 Trout Life Cycle 15

1.9.6 *Asellus meridianus* 15

1.9.7 *A. meridianus* Diet 16

1.10 The Life Cycle of *A. clavula* 16

1.11 The Taxonomy of *A. clavula* 18

1.12 The Geographical Distribution of *A. clavula* 19

1.13 *A. clavula*: Definitive Hosts 19

1.14 *A. clavula:* Intermediate Hosts 20

1.15 The Role of *A. clavula* in the Intestinal Helminth Community 22

1.16 Acanthocephalan Population Dynamics 22

1.16.1 Population Dynamics in Definitive Hosts 22

1.16.2 Population Dynamics in Intermediate Hosts 25

1.17 The Parasite Niche 28

1.18 Interspecific Interactions 30

1.19 Epizootics 32

1.20 Host shifts 34
1.21 Aims 34

Chapter 2: *A. clavula* in the Intermediate Host, *A. meridianus*
2.1 Introduction 35
2.2 Materials and Methods 36
2.2.1 Primary sample site descriptions 36
2.2.2 Burrishoole Catchment 36
2.2.3 Bunaveela Lake 39
2.2.4 Clogher Lough 39
2.2.5 Lough Feeagh 41
2.2.6 Data Analysis 43
2.2.7 *Asellus* Sampling 45
2.2.8 *Asellus* collection and processing 46
2.2.9 Behavioural alteration experiments 48
2.2.9.1 *Asellus* Sampling and Processing 48
2.2.9.2 Light/Dark Preference Experiment 48
2.2.9.3 Activity experiment 49
2.3 Results 50
2.3.1 *Asellus* sampling 50
2.3.2 Prevalence, abundance and intensity of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh 51
2.3.3 Multiple infections of *A. meridianus* by *A. clavula* in Clogher Lough and Lough Feeagh 56
2.3.4 Prevalence and abundance and *Asellus* size 56
2.3.5 Sex breakdown of the population of *A. meridianus* in Clogher Lough and Lough Feeagh 59
2.3.6 Factors affecting size of *A. meridianus* in Clogher Lough and Lough Feeagh 61
2.3.7 Reproductive cycle of *A. meridianus* in Clogher Lough and Lough Feeagh 79

2.3.8 Behavioural changes of *A. clavula*-infected *A. meridianus* 82
2.3.8.1 Light/dark preference experiment 82
2.3.8.2 Activity Experiment 84
2.4 Discussion 85

Chapter 3: *A. clavula* in the Eel Definitive Host
3.1 Introduction 96
3.2 Materials and Methods 99
3.2.1 Fish capture 99
3.2.2 Post-catching procedures 100
3.2.3 Fish dissection protocol 100
3.2.4 Treatment of parasites 101
3.2.5 Identification of parasites 101
3.2.6 Weighing, measuring and reproductive status of acanthocephalans 105
3.2.7 Infection intensity 107
3.2.8 Data Analysis 107
3.3 Results 108
3.3.1 Eel sample sizes 108
3.3.2 Eel length and mass 109
3.3.3 *Acanthocephalus clavula* from eels from Clogher Lough and Lough Feeagh 110
3.3.4 Overall prevalence, abundance and intensity of *A. clavula* 111
3.3.5 Seasonal patterns in prevalence, abundance and intensity of *A. clavula* in eels in Clogher Lough and Lough Feeagh 112
3.3.6 Aggregation 115
3.3.7 *A. clavula* length and mass in Clogher Lough and Lough Feeagh 117
3.3.8 *A. clavula* reproductive status 130
3.3.9 A. *clavula* niche 133
3.3.10 *Pomphorhynchus laevis* and other acanthocephalan 145

species in eels in Clogher Lough and Lough Feeagh
3.4 Discussion 146

Chapter 4: *A. clavula* in the Trout Definitive Host
4.1 Introduction 157
4.2 Materials and Methods 159
4.2.1 Fish Dissection Protocol 159
4.3 Results 160
4.3.1 Trout sample sizes 160
4.3.2 Trout length and mass 161
4.3.3 *Acanthocephalus clavula* from trout from Clogher Lough and Lough Feeagh 162
4.3.4 Overall prevalence, abundance and intensity of *A. clavula* 162
4.3.5 Seasonal patterns in prevalence, abundance and intensity of *A. clavula* in trout in Clogher Lough and Lough Feeagh 163
4.3.6 *A. clavula* length and mass in Clogher Lough and Lough Feeagh 169
4.3.7 *A. clavula* Reproductive Status 173
4.3.8 The *A. clavula* Niche 175
4.3.9 *Pomphorhynchus laevis* and other acanthocephalan species in trout from Clogher Lough and Lough Feeagh 188
4.4 Comparative analysis of *A. clavula* in eels and trout 191
4.4.1 Abundance of *A. clavula* in eels and trout 191
4.4.2 *A. clavula* Mass 192
4.4.3 *A. clavula* reproductive status 194
4.5 Discussion 195

Chapter 5: *A. clavula* in Eels from Regional Sites
5.1 Introduction 201
5.2 Materials and Methods 204
5.2.1 Regional sampling 204
5.2.2 Sample site descriptions 204

5.3 Results 206
5.3.1 Overall prevalence, abundance and intensity of *A. clavula* 207
5.3.2 *A. clavula* reproductive status 207
5.3.3 *A. clavula* niche 207
5.4 Discussion 208

Chapter 6: General Discussion 212

References 225

Appendix 254

TABLE OF TABLES

Table	Title	Page Number
1.1	Details of the acanthocephalan species found in Ireland	10
1.2	Status of fish and invertebrates as definitive and intermediate hosts for acanthocephalans in Ireland and Great Britain (modified from Lyndon & Kennedy, 2001)	11
1.3	The intermediate hosts of *A. clavula* across its range (modified and updated from Schmidt, 1985)	21
2.1	Overall prevalence and abundance of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh	51
2.2	Prevalence and abundance of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh by month	52
2.3	Results of χ^2 analysis on prevalence of infection within years in Clogher Lough and Lough Feeagh	53
2.4	Seasonal changes in infection of *A. meridianus* by *A. clavula* in Clogher Lough and Lough Feeagh	55
2.5	Abundance of *A. clavula* cystacanths in *A. meridianus* in Lough Feeagh from February 2007 to September 2008	56
2.6	Abundance of *A. clavula* cystacanths in *A. meridianus* in Lough Feeagh from February 2007 to September 2008	57
2.7	Distribution of multiple infections of *A. clavula* among *A. meridianus* in Lough Feeagh	58
2.8	Results of χ^2 analysis on sex of *Asellus* and season in Clogher Lough and Lough Feeagh in 2007 and 2008	60
2.9	Results of Kolmogorov-Smirnov tests investigating factors affecting *A. meridianus* length.	62
2.10	Number of *A. meridianus* individuals in various length categories in Clogher Lough and Lough Feeagh.	62
2.11	Mean lengths of male and female *A. meridianus* in Clogher Lough and Lough Feeagh in 2007 and 2008.	65
2.12	Mean lengths of infected and uninfected *A. meridianus* in Clogher Lough in 2007 and 2008.	72
2.13	Mean lengths of infected and uninfected *A. meridianus* in Lough Feeagh in 2007 and 2008	75
2.14	Results of Kolmogorov-Smirnov tests investigating the effect of sample month on *A. meridianus* length	76
2.15	Mean length of male and female *A. meridianus* in Clogher Lough and Lough Feeagh in all sample months	79

2.16	Results of χ^2 analysis on *Asellus* brooding and sample month in Clogher Lough and Lough Feeagh.	80
2.17	Results of χ^2 analysis on *Asellus* brooding and infection status in Clogher Lough and Lough Feeagh.	80
2.18	Mean amount of time spent in the light half of the tank by infected and uninfected *A. meridianus*	83
2.19	GLM table of the effect of various factors on the time spent by test individuals in the light half of the tank	83
2.20	GLM table of the effect of various factors on the number of boundary crossing made by test individuals	83
2.21	Mean number of squares crossed by infected and uninfected *Asellus*	84
2.22	GLM table of the effect of sex and infection status on *Asellus* activity	84
2.23	Prevalence values of *Acanthocephalus* sp. in isopod intermediate hosts	85
2.24	Summary of data on size of isopods and maximum number of acanthocephalan larvae harboured	87
2.25	Behavioural and pigmentation changes of acanthocephalan-infected isopod species	95
3.1	Population parameters of *A. clavula* in eels from various European locations	97, 98
3.2	Eel sample sizes from Clogher Lough and Lough Feeagh in 2007 and 2008	109
3.3	The sex of eel samples from Clogher Lough and Lough Feeagh	109
3.4	Mean length of eels sampled from Clogher Lough and Lough Feeagh	110
3.5	Mean mass of eels sampled from Clogher Lough and Lough Feeagh	110
3.6	Overall prevalence, mean abundance and mean intensity of *A. clavula* from eels from Clogher Lough and Lough Feeagh	112
3.7	Prevalence (± 95% C.I.), abundance (± S.E.) and intensity (± S.E.) of *A. clavula* in eels from Clogher Lough and Lough Feeagh by month	113
3.8	GLM table of the effect of lake and month on the abundance of *A. clavula* in eels in Clogher Lough and Lough Feeagh	114
3.9	Seasonal changes in infection of eels by *A. clavula* in Clogher Lough and Lough Feeagh. C denotes Clogher Lough; F denotes Lough Feeagh	116

3.10	Mean body length (± S.E) and mass (± S.E.) of male and female *A. clavula* from Clogher Lough and Lough Feeagh	117
3.11	GLM table of the effect of various factors on length of *A. clavula* in the intestine of eels in Clogher Lough and Lough Feeagh	118
3.12	Mean length (mm) ± S.E. of male and female *A. clavula* in infections of varying intensity from eels in Clogher Lough and Lough Feeagh	125
3.13	GLM table of the effect of various factors on mass of *A. clavula* in the intestine of eels in Clogher Lough and Lough Feeagh	127
3.14	Reproductive status of *A. clavula* from eels sampled from Clogher Lough and Lough Feeagh	131
3.15	Results of G-test on the pattern of occurrence of *A. clavula* reproductive stages in Clogher Lough and Lough Feeagh	133
3.16a	Details of factors involved in Kolmogorov-Smirnov tests of *A. clavula* niche	136
3.16b	Results of Kolmogorov-Smirnov tests investigating factors affecting *A. clavula* percentage position in the intestine of eels from Clogher Lough and Lough Feeagh.	136
3.17	Mean *A. clavula* percentage position in the intestine of eels from Clogher Lough and Lough Feeagh	136
3.18	Mean percentage position of male and female *A. clavula* in eels from Clogher Lough and Lough Feeagh	137
3.19	Percentage of male and female *A. clavula* in the posterior region of the eel intestine in Clogher Lough and Lough Feeagh	138
3.20	Summary statistics for the distribution of *A. clavula* in the intestine of eels in Clogher Lough by intensity level	140
3.21	Summary statistics for the distribution of *A. clavula* in the intestine of eels in Lough Feeagh by intensity level	142
3.22	Results of Kolmogorov-Smirnov tests investigating the effect of sample month on *A. clavula* percentage location in eels from Clogher Lough and Lough Feeagh	142
3.23	Summary statistics for the distribution of *A. clavula* in the intestine of eels in Clogher Lough for each sample month	143
3.24	Summary statistics for the distribution of *A. clavula* in the intestine of eels in Lough Feeagh for each sample month	145

3.25	Population parameters of *P. laevis* from eels from Lough Feeagh	145
4.1	Population parameters of *A. clavula* in trout from various European locations	158
4.2	Trout sample sizes from Clogher Lough and Lough Feeagh in 2007 and 2008	160
4.3	The sex of trout samples from Clogher Lough and Lough Feeagh	161
4.4	Mean length of trout sampled from Clogher Lough and Lough Feeagh	161
4.5	Mean mass of trout sampled from Clogher Lough and Lough Feeagh	161
4.6	Overall prevalence, mean abundance and mean intensity of *A. clavula* from trout from Clogher Lough and Lough Feeagh	163
4.7	Prevalence, abundance and intensity of *A. clavula* in trout from Clogher Lough and Lough Feeagh by month	165
4.8	GLM table of the effect of various factors on the abundance of *A. clavula* infecting trout from Clogher Lough and Lough Feeagh	167
4.9	Seasonal changes in infection of trout by *A. clavula* in Clogher Lough and Lough Feeagh. C denotes Clogher Lough; F denotes Lough Feeagh	168
4.10	Mean body length (± S.E) and mass (± S.E.) of male and female *A. clavula* from trout from Clogher Lough and Lough Feeagh	169
4.11	GLM table of the effect of various factors on length of *A. clavula* in the intestine of trout in Clogher Lough and Lough Feeagh	170
4.12	GLM table of the effect of various factors on mass of *A. clavula* in the intestine of trout in Clogher Lough	172
4.13	GLM table of the effect of various factors on mass of *A. clavula* in the intestine of trout in Lough Feeagh	173
4.14	Reproductive status of *A. clavula* from trout sampled from Clogher Lough and Lough Feeagh	174
4.15	Results of G-test on the pattern of occurrence of A. clavula reproductive stages in Clogher Lough and Lough Feeagh	176
4.16a	Details of factors involved in Kolmogorov-Smirnov tests of *A. clavula* niche	179
4.16b	Results of Kolmogorov-Smirnov tests investigating factors affecting *A. clavula* percentage position in the intestine of eels from Clogher Lough and Lough Feeagh	179

4.17	Mean *A. clavula* percentage position in the intestine of eels from Clogher Lough and Lough Feeagh	179
4.18a	Summary statistics for the distribution of *A. clavula* in the intestine of trout in Clogher Lough by intensity level	183
4.18b	Summary statistics for the distribution of *A. clavula* in the intestine of trout in Lough Feeagh by intensity level	183
4.19	Results of Kolmogorov-Smirnov tests investigating the effect of sample month on *A. clavula* percentage location in trout from Clogher Lough and Lough Feeagh	185
4.20a	Summary statistics for the distribution of *A. clavula* in the intestine of trout in Clogher Lough for each sample month	185
4.20b	Summary statistics for the distribution of *A. clavula* in the intestine of trout in Lough Feeagh for each sample month	187
4.21	Population parameters of *P. laevis* from trout from Lough Feeagh	188
4.22	Prevalence (± 95% C.I.), abundance (± S.E.) and intensity (± S.E.) of *P. laevis* in trout from Lough Feeagh by month	190
4.23	GLM table of the effect of lake, season and fish species on abundance of *A. clavula*	192
4.24	Mean abundance (± S.E.) of *A. clavula* in eel and trout in Clogher Lough and Lough Feeagh	192
4.25	GLM table of the effect of various factors on mass of female *A. clavula* in the intestine of eels and trout in Clogher Lough and Lough Feeagh	193
4.26	Mass of female *A. clavula* from eel and trout (data from Clogher Lough and Lough Feeagh combined)	194
4.27	Reproductive status of *A. clavula* from eel and trout (data for Clogher Lough and Lough Feeagh combined)	194
5.1	Population parameters of *A. clavula* in eels from various Irish locations	201
5.2	Summary of data on *Acanthocephalus* spp. in Ireland (Data from C.R. Kennedy, pers. comm.)	202
5.3	Summary of data on the sporadic occurrence of *A. clavula* in Irish locations	203
5.4	Descriptive parameters for regional sample sites	205
5.5	Population parameters of *A. clavula* from Lough Conn	207
6.1	Prevalence, abundance and size and reproductive status of *A. clavula* from various locations	216

TABLE OF FIGURES

Figure	Title	Page Number
1.1	The life cycle of *Acanthocephalus clavula*	18
2.1	Map showing the three primary sample sites: Bunaveela Lake, Clogher Lough and Lough Feeagh (Ordnance Survey, Ireland)	37
2.2	The Burrishoole Catchment (modified from Byrne, 2000)	38
2.3	Clogher Lough: (modified from Byrne, 2000)	40
2.4	Lough Feeagh: (modified from Byrne, 2000)	42
2.5	Ventral view of the (A) female and (B) male A. meridianus abdomen	47
2.6	Example of grid used underneath tank for activity experiment	49
2.7	Numbers of *Asellus* sampled in Clogher Lough and Lough Feeagh in 2007	50
2.8	Numbers of *Asellus* sampled in Clogher Lough and Lough Feeagh in 2008	51
2.9	Prevalence ± 95% C.I. of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh from February 2007 to September 2008	54
2.10	Mean abundance ± S.E. of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh from February 2007 to September 2008	54
2.11	Size distribution of uninfected *A. meridianus* in Clogher Lough and Lough Feeagh	57
2.12	Size distribution of infected *A. meridianus* in Clogher Lough and Lough Feeagh	58
2.13	Distribution of multiple infections of *A. meridianus* by *A. clavula* in Lough Feeagh among size categories of *A. meridianusA. meridianus*	59
2.14	Sex breakdown of the population of *A. meridianus* from Clogher Lough from February 2007 to September 2008	60
2.15	Sex breakdown of the population of *A. meridianus* from Lough Feeagh from February 2007 to September 2008	61
2.16	Length distributions of *A. meridianus* in Clogher Lough by sample year.	63
2.17	Length distributions of *A. meridianus* in Lough Feeagh by sample year.	64
2.18	Length distributions of male and female *A. meridianus* in Clogher Lough in 2007	66

2.19	Length distributions of male and female *A. meridianus* in Clogher Lough in 2008	67
2.20	Length distributions of male and female *A. meridianus* in Lough Feeagh in 2007	68
2.21	Length distributions of male and female *A. meridianus* in Lough Feeagh in 2008	69
2.22	Length distributions of infected and uninfected *A. meridianus* in Clogher Lough in 2007	70
2.23	Length distributions of infected and uninfected *A. meridianus* in Clogher Lough in 2008	71
2.24	Length distributions of infected and uninfected *A. meridianus* in Lough Feeagh in 2007.	73
2.25	Length distributions of infected and uninfected *A. meridianus* in Lough Feeagh in 2008.	74
2.26	Length distributions of *A. meridianus* in Clogher Lough by sample month	77
2.27	Length distributions of *A. meridianus* in Lough Feeagh by sample month	78
2.28	Seasonal changes in the reproductive cycle of *A. meridianus* in Clogher Lough from February 2007 to September 2008	81
2.29	Seasonal changes in the reproductive cycle of *A. meridianus* in Lough Feeagh from February 2007 to September 2008	82
3.1	*Acanthocephalus clavula* [images modified from Grabda-Kazubska and Chubb, 1968)]	102
3.2	Male *Acanthocephalus clavula*. Note the presence of two large, oval-shaped testes, leading to three pairs of cement glands (Image: Naiara O' Mahony)	103
3.3	Female *Acanthocephalus clavula*. (Image: Naiara O' Mahony)	104
3.4	Female reproductive status, Stage 1: Ovarian balls only	106
3.5	Female reproductive status, Stage 2: Ovarian balls and immature acanthors	106
3.6	Female reproductive status, Stage 3: Ovarian balls, immature acanthors, and mature shelled acanthors	107
3.7	Numbers of *A. clavula* recovered from eels from Clogher Lough and Lough Feeagh	111
3.8	Mean abundance (± S.E.) of *A. clavula* in Clogher Lough and Lough Feeagh	114
3.9	Mean intensity (± S.E.) of *A. clavula* in Clogher Lough and Lough Feeagh	115

3.10	Mean length of male and female *A. clavula* in Clogher Lough	119
3.11	Mean length of male and female *A. clavula* in Lough Feeagh	120
3.12	Length of female *A. clavula* from low, medium and high intensity infections in Clogher Lough	121
3.13	Length of male *A. clavula* from low, medium and high intensity infections in Clogher Lough	122
3.14	Length of female *A. clavula* from low, medium and high intensity infections in Lough Feeagh	123
3.15	Length of female *A. clavula* from low, medium and high intensity infections in Lough Feeagh	124
3.16	Seasonal variations in *A. clavula* body length in Clogher Lough and Lough Feeagh	126
3.17	Seasonal variations in *A. clavula* mass in Clogher Lough and Lough Feeagh	128
3.18	Mass of male and female *A. clavula* in Clogher Lough	129
3.19	Mass of male and female *A. clavula* in Lough Feeagh	130
3.20	Reproductive status of female *A. clavula* (± 95% C.I.) from eels sampled from Clogher Lough	132
3.21	Reproductive status of female *A. clavula* (± 95% C.I.) from eels sampled from Lough Feeagh	132
3.22	The fundamental niche of *A. clavula* along the intestine of eels in Clogher Lough	134
3.23	The fundamental niche of *A. clavula* along the intestine of eels in Lough Feeagh	135
3.24	Distribution of male and female *A. clavula* in the intestine of eels from Clogher Lough	137
3.25	Distribution of male and female *A. clavula* in the intestine of eels from Lough Feeagh	138
3.26	Distribution of *A. clavula* according to intensity level along the intestine of eels from Clogher Lough	139
3.27	Distribution of *A. clavula* according to intensity level along the intestine of eels from Lough Feeagh	141
3.28	Distribution of *A. clavula* in the eel intestine by sample month in Clogher Lough	143
3.29	Distribution of *A. clavula* in the eel intestine by sample month in Lough Feeagh	144
4.1	Numbers of *A. clavula* recovered from trout in Clogher Lough and Lough Feeagh	162
4.2	Prevalence of *A. clavula* (± 95% C.I.) in Clogher Lough and Lough Feeagh	164

4.3	Mean abundance (± S.E.) of *A. clavula* in trout in Clogher Lough and Lough Feeagh	166
4.4	Mean intensity (± S.E.) of *A. clavula* in trout in Clogher Lough and Lough Feeagh	166
4.5	Mean length (± S.E.) of male and female *A. clavula* in trout from Clogher Lough	171
4.6	Mean length (± S.E.) of male and female *A. clavula* in trout from Lough Feeagh	171
4.7	Mean mass (± S.E.) of male and female *A. clavula* in trout from Clogher Lough.	173
4.8	Reproductive status of female *A. clavula* (± 95% C.I.) from trout sampled from Clogher Lough	175
4.9	Reproductive status of female *A. clavula* (± 95% C.I.) from trout sampled from Lough Feeagh	175
4.10	The fundamental niche of *A. clavula* along the intestine of eels in Clogher Lough	177
4.11	The fundamental niche of *A. clavula* along the intestine of trout in Lough Feeagh	178
4.12	Distribution of male and female *A. clavula* in the intestine of trout from Clogher Lough	180
4.13	Distribution of male and female *A. clavula* in the intestine of trout from Lough Feeagh	181
4.14	Distribution of *A. clavula* according to intensity level along the intestine of trout from Clogher Lough	182
4.15	Distribution of *A. clavula* according to intensity level along the intestine of trout from Lough Feeagh	184
4.16	Distribution of *A. clavula* in the trout intestine by sample month in Clogher Lough	186
4.17	Distribution of *A. clavula* in the trout intestine by sample month in Lough Feeagh	187
4.18	Numbers of *P. laevis* recovered from trout from Lough Feeagh	188
4.19	Prevalence (± 95% C.I.) of *P. laevis* from trout in Lough Feeagh	189
4.20	Mean abundance (± S.E.) of *P. laevis* in trout from Lough Feeagh	190
4.21	Mean intensity (± S.E.) of *P. laevis* in trout from Lough Feeagh	191
5.1	Map of Ireland showing regional eel sampling sites	206

Chapter 1

Introduction

1.1 Parasitism and free-living communities

Parasitism is a ubiquitous condition, with virtually every free-living organism playing host to at least one, and usually several, species of parasite (Combes, 2001). Recently, the extent of the impact parasites can have on communities of free-living animals has become clear. A number of workers have explored the various structuring roles parasites can play in free-living communities (Freeland, 1983; Minchella & Scott, 1991; Mouritsen & Poulin, 2002). In order to assess the role of parasites in a community, it is first necessary to assess the importance of a particular parasite species for the community (Poulin, 1999). To do this, we can use the concept of "functional importance", as proposed by Hurlbert (1997). The functional importance of a species can be described as the sum, over all species in the community, of the changes in abundance or productivity which would occur in the event of the removal of that particular species from the community (Hurlbert, 1997). While it is difficult to measure accurately the functional importance of a parasitic species, it is often possible to estimate roughly the significance of one species of parasite to other species in the community, both free-living and parasitic (Poulin, 1999).

There are three principal ways in which parasite species can affect the community of free-living organisms among which they live. First, parasites may depress the abundance of some host species more than others, owing to varying levels of susceptibility. Second, parasites may directly decrease the functional importance of the host species in the community of free-living animals via pathological effects. Third, parasites may indirectly increase the functional importance of the host through some parasite-induced alteration in host phenotype, and thus act as ecosystem engineers (Poulin, 1999). Host and parasite biodiversity are therefore linked and should be studied in tandem, not independently, as has been the case in the past (Combes, 2001).

1.2 Parasite community ecology

In addition to being a part of the ecosystem as a whole, parasites also form communities among themselves inside their hosts (Esch *et al.*, 1990). The structure of helminth communities, their dynamics and diversity have always interested parasite ecologists. The origin of modern parasite ecology is accredited to V.A. Dogiel for his work with rats in the 1930s (Dogiel, 1964), although it was not until Holmes (1961, 1962) published his seminal works on the interaction between rat acanthocephalans and tapeworms that the quantitative study of parasite community dynamics became established in the literature.

Since then, significant advances have been made in this area. Many studies investigating various aspects of parasite community ecology have been published (e.g. Sousa, 1994; Bush *et al.*, 1990; Poulin & Mouillot, 2005) and it has become clear that parasite populations and communities are organised into a series of hierarchical levels. The lowest level is that of the infrapopulation, which includes all individuals of a parasite species in an individual host at a particular time (Bush *et al.*, 1997). Moving up, we reach the component population, which includes all parasite individuals of a particular life history phase at a particular place and time, while the suprapopulation includes all developmental stages of a species at a particular place and time (Bush *et al.*,1997). Parasite communities are also nested into hierarchical levels. The infracommunity denotes the community of infrapopulations in a single host, while the component community refers to all infrapopulations of parasites associated with some subset of a host species or a collection of free-living phases associated with some subset of the abiotic environment. Finally, the supracommunity comprises all parasite suprapopulations (Bush *et al.*, 1997). Different processes operate at these different levels, and a quantitative understanding of the hierarchies is essential for an understanding of parasite communities at the individual host level and at the host population level (Esch *et al.*, 1990).

In recent years, a number of theories have emerged which attempt to explain how parasite communities are structured, including the interactive/isolationist continuum concept (Holmes & Price, 1986; Goater *et al.*, 1987), which attempts to describe the

varying levels of saturation and competition in parasite communities, as well as the concept of 'core' and 'satellite' species (Caswell, 1978; Hanski, 1982; Bush & Holmes, 1986), which endeavours to classify parasite species on the basis of their abundance and frequency in communities. The role of interactions between species, i.e. competition, has also been investigated, and several examples of competition in parasite communities have been documented in the literature (e.g. Holmes, 1973; Dobson, 1985; Stock & Holmes, 1987). Despite this, there is still considerable debate about the contribution of competition to the structure of parasite communities (Poulin, 2001; Dobson, 2009).

1.3 The Acanthocephala

The Acanthocephala are a small phylum, containing approximately 1100 described species, all of which are obligate intestinal parasites in vertebrates, particularly freshwater teleost fish (Crompton & Nickol, 1985). The name Acanthocephala (from the Greek, *acanthias*, "prickly"; *cephalo*, "head") derives from the presence of recurved hooks on an eversible proboscis at the anterior end (Brusca & Brusca, 2002). The Acanthocephala were thought to be a very old and monophyletic phylum (Kennedy, 2006), and a possible fossil acanthocephalan has been reported (Conway-Morris & Crompton, 1982). However, it has now been accepted that the Acanthocephala are not in fact a phylum, but a group within the phylum Rotifera (Sorenson & Giribet, 2006). Adult acanthocephalans are usually white to cream in colour, and individuals of most species do not exceed 10mm, although some species can reach up to 700mm in length (Crompton & Nickol, 1985). All studied species have the same fundamental life cycle and developmental stages. Adults mate sexually in the alimentary tract of their definitive hosts, with males moving down the alimentary canal, fertilising females as they do so, before being selectively lost (Crompton & Walters, 1972; Crompton, 1985). The ovaries of the female separate into ovarian balls, where eggs develop to maturity. The uterine bell of the female then acts as a sorting device prior to egg release, releasing mature, shelled acanthors and retaining immature acanthors (Whitfield, 1970). Eggs released from the female emerge with the host faeces. The shelled acanthors then reside in the environment for a period of time, which varies by species, from weeks to months

(Rojanapaibul, 1977), until eaten by an appropriate intermediate host. When ingested, the acanthor hatches and the acanthella larva moves from the host intestine into the haemocoel. There, the acanthella develops into a cystacanth, which is the final larval stage and is infective to definitive hosts. When the infected arthropod is eaten by an appropriate definitive host, the cystacanth emerges from the haemocoel and activates. The proboscis is everted and the worm embeds it into the intestinal wall of the host, where maturation is completed (Rojanapaibul, 1977).

Acanthocephala may cause some local damage in the intestine of their vertebrate definitive hosts (Bullock, 1963; Hine & Kennedy, 1974), for example inflammation of the region of infection and/or production of fibrous tissue in and around the proboscis. Alternatively, they may have no affect at all on some definitive hosts, even when heavily infected (Byrne *et al.*, 2004). The situation in intermediate hosts is quite different, however, with alterations in host behaviour and/or pathogenic effects recorded in almost every host-parasite system studied (Rumpus & Kennedy, 1974; Connors & Nickol, 1994; Franceschi *et al.*, 2008). The modification of intermediate host behaviour by cystacanths is thought by many to be adaptive, making the arthropod more vulnerable to predation by a potential definitive host (Moore, 1984), however the debate on whether parasite-altered host behaviour is truly adaptive is still very much in progress (Poulin, 2010)

The Acanthocephala show great uniformity in morphology and larval stages and exhibit a relative lack of pathogenicity in their vertebrate definitive hosts. However, it has recently been shown that acanthocephalans can exhibit diversity at the molecular level (O' Mahony *et al.*, 2004). They can have a major impact on intermediate hosts at both individual and population levels by altering their behaviour, and can therefore also affect the communities (Poulin, 1999) and food webs (Marcogliese & Cone, 1997) of free-living organisms. Acanthocephalan species have also been shown to play a role as bioindicators of heavy metal pollution (Sures *et al.*, 1999b). It is therefore important to explore the ecology of acanthocephalan species in both parasite communities and free-living communities, and particularly free-living communities where one or more species may be in decline (Loreau *et al.*, 2005).

1.4 Hosts of Acanthocephalans

The uniformity of the acanthocephalan life cycle suggests that there is little flexibility in development or host use (Kennedy, 2006). In reality however, a high degree of variability in host use is observed for many species (Kennedy, 2006). In addition, acanthocephalans may utilise paratenic (resting) hosts or undergo post-cyclic transmission (see Section 1.7.3), thus overcoming the rigidity and potential limitations of a two-host life cycle. All acanthocephalan species require a vertebrate definitive host to complete their life cycle. For the majority of species a freshwater, teleost fish is used as a definitive host, although birds and terrestrial and aquatic mammals may also be utilised. Acanthocephalans reach maturity and reproduce in the intestinal tract of their definitive hosts. The longevity of acanthocephalan species in definitive hosts is very variable and ranges from just days (Nickol & Heard, 1973; Rojanapaibul, 1977) to over a year (Kates, 1942, 1944). This variation in lifespan is thought to be positively correlated with the life history of the host (Kennedy, 2006).

Lyndon & Kennedy (2001) have defined a number of definitive host categories for acanthocephalans of freshwater fish. The categorisation is based on the size reached by adult parasites and the reproductive maturity of females. The definitions of the various host categories are explained below.

The **preferred definitive host** of a particular acanthocephalan species is defined as that in which the parasite matures and reaches maximum size (and, therefore, fecundity) and most females reach Stage 3 of reproductive maturity. A **suitable definitive host** is defined as one in which the parasite reaches a smaller adult size but in which many females reach Stage 3 of reproductive maturity. An **additional host** is one in which the parasite makes some growth and few females reach Stage 3 reproductive maturity, while an **accidental host** is defined as one in which the parasite makes little or no growth and females seldom, if ever, reach Stage 3. A **colonisation host** is defined as a migratory fish species (e.g. eel) which can act as either a suitable or preferred definitive host, thus facilitating the movement of reproducing parasites into new localities.

In order to determine the success of an acanthocephalan species in a particular host, it is necessary to assess the reproductive status of females within each host individual. Bates & Kennedy (1990) have developed a three stage classification system for assessment of the maturity status of female acanthocephalans: at Stage 1 females contain ovarian balls only, at Stage 2 ovarian balls and immature acanthors and at Stage 3 ovarian balls, immature acanthors and mature, shelled acanthors. The proportions of females at each stage in a particular host may then be used to determine whether a particular definitive host species is a preferred or suitable host for the parasite (Lyndon & Kennedy, 2001).

Holmes *et al.* (1977) have described definitive host use in an alternative, but complementary, manner. In an exploration of the regulation of parasite populations, they proposed that regulation may occur by one of three mechanisms: (1) by transmission, (2) by regulation at the population level through the immune response and (3) by regulation at the individual host level. Regulation of a parasite population at the individual host level may be achieved either by modification of the number of parasites which can establish (i.e. there is a "ceiling level"), or by a limit on the reproductive output of the parasites in each host (i.e. there is no limit on the number of parasites which may establish, but fecundity of those that do is reduced). However, when the population of a particular parasite species is spread throughout several host species, regulation becomes extremely complex. The flow of the parasite through each host species, as well as its reproductive output (the percentage of gravid females) must then be calculated in order to clarify the importance of each host species (Holmes *et al.*, 1977). The host species through which the majority of reproductively mature parasites flow is then termed the **primary host** or donor. The flow of parasites through this primary host may then maintain and regulate the entire population of all parasites of that species in all definitive host species. These other host species are termed **secondary hosts** or recipients. Reproduction may or may not occur in these secondary hosts. For example, in Cold Lake, Canada, the acanthocephalan *Metechinorhynchus salmonis* infects all fish species (including four species of salmonids) in the lake and represents 75% of all parasite individuals recovered, although reproduction occurs in the salmonid species only, and the

majority of parasites flow through just one fish species, the whitefish *Coregonus clupeaformus*. The whitefish is thus the primary host of *M. salmonis* in Cold Lake, and all other species are secondary hosts (Holmes *et al.*, 1977).

Both systems of categorising hosts are useful; however, the system of Holmes *et al.* (1977) pertains only to studies where all host species are examined. As the majority of parasite population dynamics studies (including the present one) tend to focus on just one or two host species from any one location, the use of the Lyndon & Kennedy (2001) system is often more appropriate when categorising hosts, and also allows for comparison between studies.

All acanthocephalan species require an arthropod intermediate host, in whose haemocoel the acanthella develops into the infective cystacanth stage. In order for the acanthocephalan life cycle to be completed the infected intermediate host must be ingested by an appropriate definitive host. While each acanthocephalan species infects just one species of intermediate host in any given area, that intermediate host may be infected with several acanthocephalan species. For example, in Ireland, both *A. anguillae* and *A. lucii* utilise the aquatic isopod *Asellus aquaticus* as their intermediate host (Kennedy & Moriarty, 1987). It has been proposed by Bush *et al.* (1993) that the use of a single intermediate host species by multiple acanthocephalan species results in the provision of a source community to definitive hosts, not simply a source population of one species.

Some acanthocephalan species may increase the flexibility of their life cycle by utilising a paratenic host. A paratenic (also known as a transport, or resting host) host is one in which the parasite can survive, but does not grow or reproduce further (Kennedy, 2006). If the paratenic host is ingested by an appropriate definitive host, the parasite may then continue development.

A paratenic host is a facultative one, and its use may or may not result in successful transmission to the definitive host. For acanthocephalans, paratenic host use occurs only

between the intermediate and definitive host stages (Kennedy, 2006). Paratenic host use has been documented for several acanthocephalan species (Schmidt, 1985), although not for *A. clavula* (the species which I shall be investigating in this thesis; see below). For example, *Corynosoma* species use marine amphipods as intermediate hosts, but adults are found in marine mammals, such as seals, which are primarily piscivorous. In order to overcome the rigidity of this two-host life cycle, *Corynosoma* species use several species of fish as paratenic hosts (Valtonen & Crompton, 1990). Over time, it is possible that the parasite may adapt to the conditions in one or more of these paratenic hosts, so that the parasite can mature and reproduce, thus extending definitive host possibilities and increasing the likelihood of parasite success.

1.5 Post-Cyclic Transmission

Another method by which acanthocephalans may escape the rigidity of their two-host life cycle is by the utilisation of post-cyclic transmission. Post-cyclic transmission has been defined by Nickol (1985) as 'when ingested as adults within their definitive hosts some acanthocephalans can survive and parasitise the predator'. The occurrence of post-cyclic transmission has now been confirmed for eleven acanthocephalan species (Nickol, 1985, 2003; McCormick & Nickol, 2004). The ease with which post-cyclic transmission takes place under experimental conditions suggests that it may be a widespread phenomenon in nature (Kennedy, 2006); however transferred parasites must be able to mature and reproduce if transmission is to be successful. Recent studies show that this is in fact possible (Kennedy, 1999; Rauque *et al.*, 2002 and McCormick & Nickol, 2004), although when acanthocephalan attachment to the intestinal wall involves encapsulation of the proboscis bulb (e.g. *Pomphorhynchus laevis*) successful post-cyclic transmission occurs only in less mature specimens, where the bulb has not yet fully developed (Kennedy, 1999).

Post-cyclic transmission may also explain how predators can harbour heavy acanthocephalan infections without intermediate or paratenic hosts comprising a significant portion of their diets. Indeed, in a study carried out by McCormick and Nickol

(2004), it was found that infection levels of *Paulisentis missouriensis* increased with size of creek chub, but the increase was due not to increased intermediate host consumption, but to cannibalism. As creek chub grew older, they consumed smaller individuals of their own species and thus harboured heavy infections of *P. missouriensis*.

Post-cyclic transmission has also been demonstrated, although not confirmed, for *A. clavula*. Chubb (1964) recovered gravid female *A. clavula* from pike in Llyn Tegid, Wales. The pike were of a size at which their diet is comprised entirely of fish; therefore the *A. clavula* individuals were most likely transferred from other fish species by means of a predator-prey interaction.

1.6 The Acanthocephala in Ireland

Six acanthocephalan species are present in Ireland, all of which have a number of possible definitive hosts. Each acanthocephalan species uses just one species of intermediate host (Table 1.1).

Table 1.1 Details of the acanthocephalan species found in Ireland

Species	**Intermediate host***	**Definitive hosts****
Acanthocephalus anguillae	*Asellus aquaticus*	Bream, brown trout, charr, eel, pike, rudd, 3-spined stickleback, 10-spined stickleback
Acanthocephalus clavula	*Asellus meridiaanus*	Brown trout, charr, dace, eel, perch, pike, roach, rudd, 3-spined stickleback, 9-spined stickleback
Acanthocephalus lucii	*Asellus aquaticus*	Bream, brown trout, charr, eel, perch, pike, roach, rudd, rudd/bream hybrid, stone loach, tench
Echinorhynchus truttae	*Gammarus pulex*	Brown trout, minnow, pike, rainbow trout, salmon, 3-spined stickleback, stone loach
Neoechinorhynchus rutili	Ostracods	Brown trout, pike, roach
Pomphorhynchus laevis	*Gammarus duebeni*	Brown trout, bream, charr, eel, minnow, perch pike, rainbow trout, roach, rudd/bream hybrid, rudd, salmon, 3-spined stickleback, 10-spined stickleback, stone loach, salmon, tench

* = data on intermediate hosts from Brown *et al.* (1986)

** = data on definitive hosts from Holland & Kennedy (1997)

1.7 Irish Acanthocephalans and host specificity

Reference to checklists of the parasites of freshwater fish of Britain and Ireland (Kennedy, 1974; Holland & Kennedy, 1997) suggests that the six acanthocephalan species present show very little specificity to their definitive hosts. Lyndon & Kennedy (2001) have suggested otherwise by showing that, in fact, each species has a number of possible definitive hosts. Using adult female size as a criterion, they demonstrated that

each acanthocephalan species has a preferred definitive host (in which the parasite matures and reaches maximum size and fecundity) as well as a suitable host (in which the parasite can grow to a smaller adult size but in which many females reach full maturity - defined as the stage when gravid females contain fully developed, shelled acanthors) (Lyndon & Kennedy, 2001). Each species may also have a colonisation host, usually a migratory fish species, which can act as either a preferred or suitable host, and facilitates movement of parasites into new localities (Lyndon & Kennedy, 2001). In contrast to the wider specificity of acanthocephalans to their definitive hosts, each species infects just one species of intermediate host, although the identity of this host may change between localities (Lyndon & Kennedy, 2001). The intermediate host is generally an arthropod species which is widespread and common (Table 1.2).

Table 1.2 Status of fish and invertebrates as definitive and intermediate hosts for acanthocephalans in Ireland and Great Britain (modified from Lyndon & Kennedy, 2001)

Acanthocephalan Species	**Preferred Host**	**Intermediate Host**	**Eel: Host status**	**Trout: Host status**
Neoechinorhynchus rutili	Brown trout	*Ostracoda*	Poor	Preferred
Echinorhynchus truttae	Brown trout	*Gammarus pulex*	Suitable	Preferred
Pomphorhynchus laevis	Chub, Barbel	*Gammarus pulex/ G. duebeni*	Accidental	Suitable
Acanthocephalus clavula	Eel	*Asellus meridianus*	Preferred	Suitable
Acanthocephalus lucii	Perch	*Asellus aquaticus*	Suitable	Suitable
Acanthocephalus anguillae	Chub	*Asellus aquaticus*	Suitable	Unrecorded

1.8 *Acanthocephalus clavula*

Acanthocephalus clavula is a dioecious pseudocoelomate intestinal parasite of freshwater fish, without an alimentary tract at any stage of development. The parasite is usually white in colour and is unsegmented and is composed of the presoma (proboscis and leminisci) and the metasoma (rest of the body). The mature male worm is smaller in size

than the mature female worm. The proboscis is equipped with sharp hooks arranged in rows, with 16-18 rows of hooks and 12-14 hooks in each row (Rojanapaibul, 1977).

The proboscis may be withdrawn into the proboscis sac by contraction of the proboscis retractor muscles and is everted by a hydrostatic system. The metasoma may be moved by the contraction of the longitudinal and circular muscles of the body wall. The nervous system is composed of a ganglion which is located posterior to the proboscis receptacle. Ten nerves project from the ganglion (Rojanapaibul, 1977).

The male reproductive system is composed of two testes which are located in the middle part of the body. Posterior to the testes, there are three pairs of cement glands. The *vas deferens* from the testes and the six cement ducts open onto the anterior portion of the seminal vesicle. Posterior to the seminal vesicle, there is a penis with two pouches opening into each side of the copulatory pouch. The copulatory bursa is located at the posterior end of the body and will evert when the worm copulates (Rojanapaibul, 1977). The female reproductive system is composed of ovaries, uterus, vagina and uterine bell. The uterus runs to the posterior of the body and joins with the vagina. The posterior part of the uterus and the vagina are closed by sphincter muscles. The uterine bell is located anterior to the uterus and its function is to sort the mature acanthors from the immature acanthors prior to the release of the former (Rojanapaibul, 1977).

1.9. *A. clavula* Hosts

1.9.1 The European Eel (*Anguilla anguilla*)

The European eel, *Anguilla anguilla* (L.) is widespread throughout Europe, being found from Iceland and North Cape in the north, to the coasts of Morocco in the south, and the Black Sea in the east and is found in fresh, brackish and coastal waters. The eel has a highly distinctive, elongate, snake-like brown body with yellow flanks. Minute scales are deeply embedded in the skin. The lower jaw protrudes and the eel has several rows of small, blunt teeth. The dorsal, caudal and anal fins form a continuous fringe; pelvic fins

are absent. The European eel is currently classified as 'critically endangered' by the IUCN (Freyhof and Kottelat, 2008).

1.9.2 Eel Life Cycle

The life cycle of the eel is complex and has not yet been fully elucidated. The European eel is thought to mate in the Sargasso Sea area, where the smallest larvae have been found (Schmidt, 1922, 1923; 1925; 1935, cited in van Ginneken & Maes, 2005). Larvae (*leptocephali*) of progressively larger size have been found between the Sargasso Sea and European continental shelf waters. At the shelf edge, the laterally flattened, leaf-shaped *Leptocephalus* transforms into a rounded glass eel, of the same shape as an adult eel, but unpigmented. In Ireland, glass eel migrate into coastal waters and estuaries between October and April. They then transform into pigmented elvers and migrate further into rivers, and eventually lakes and streams, between May and September. Following immigration into continental waters, the prolonged yellow/brown eel stage begins, which lasts for upwards of 20 years. During this stage, eels remain in freshwater or inshore marine or brackish areas, where they grow, feeding on a wide range of insects, worms, molluscs, crustaceans and fish. Little food is taken in winter. Sexual differentiation occurs when the eels are partly grown. The mechanism of sexual differentiation is not yet fully understood, although it is thought to depend on local stock density. At the end of the continental growing period, the eels mature and return to the Atlantic Ocean. This stage is known as the silver eel. Female silver eels grow much larger and may be twice as old as males. The biology of the returning silver eels in ocean waters is almost completely unknown, although current research being carried out by the EELIAD programme, involving tagged and monitored silver eels is expanding our knowledge rapidly.

1.9.3 Eel decline

Eel populations are in sharp decline throughout Europe (Dekker, 2003; Feunteun, 2002; Briand *et al.*, 2003; Knights, 2003). Wirth & Bernatchez (2003) highlighted the

sensitivity of eels to climate change, presenting evidence of a significant decline in numbers in recent historical times, due to climate changes that affected the Gulf Stream, an essential component of the transatlantic migration of eel larvae. As well as this, evidence of a very recent decline in glass eel recruitment – since the early 1980s - has been presented from all over Europe, for example in the Loire Valley catchment and the Vilaine estuary in France (Feunteun, 2002; Briand *et al.*, 2003), in Lough Neagh and Lough Derg in Ireland (Rosell *et al.*, 2005; Moriarty, 1996), in Lake Ijsselmeer in the Netherlands (Dekker, 2004) as well as all over England and Wales (Knights, 2003). Dekker (2003) estimated the decline after the early 1980s to be up to 90% on a pan-European basis, and also presented evidence that the decline in recruitment was preceded by a decline in eel landings approximately two to three decades earlier. This time delay between recruitment failure and the decline of the fishery is a result of the long life cycle and relatively low mortality rate of the European eel (Feunteun, 2002). The reasons for the current low level of recruitment are not very well known, however, a variety of possibilities have been put forward. These include: over-exploitation, changes in oceanographic conditions, possibly linked to climate change, reductions in accessible freshwater habitat, degradation of available freshwater habitat, pollution, and parasitism by the introduced swimbladder parasite *Anguillicola crassus* (Starkie, 2003).

1.9.4 The Brown Trout (*Salmo trutta*)

The brown trout (*Salmo trutta* L.) is a species of salmonid with a facultative migratory character. This species has a vast capacity for adaptation to different environments, and this capacity has resulted in a high level of polymorphism, which in turn has led to the classification of the brown trout under several different scientific names, however the existence of a single species remains the most likely suggestion (Bagliniere & Maisse, 1991).

The original distribution of the brown trout was pan-European, however following many successful introductions, the species is now present in many additional locations, including Australia, New Zealand, India, South Africa and the United States. The brown trout is an economically important species and is exploited throughout its distribution.

The freshwater form is fished extensively by anglers, while the sea trout is fished commercially. In recent decades, the brown trout has also been utilised in aquaculture (Bagliniere, 1999). The diet of the brown trout varies with age and habitat, but the most common food items consumed are aquatic invertebrates. Terrestrial invertebrates have been found to be consumed either rarely (O' Grady, 1983), or primarily by older individuals (Kelly-Quinn & Bracken, 1989). Older individuals are piscivorous (Hyvärinen & Huusko, 2006). In Irish lakes, *Gammarus* and *Asellus* were found to be the dominant food items in the diet of brown trout, but gastropods, trichopteran larvae, zooplankton and hemipterans were also important (O' Grady, 1983).

1.9.5 Trout Life Cycle

Brown trout reach maturity at 3-4 years of age, and between October and December they migrate upstream to mate. The female locates a bed of suitable gravel in fast flowing water where she excavates a hollow, or redd. She then gradually releases batches of ova into the redd where they are fertilised by a male releasing milt (a white milky liquid). As each batch is laid the female excavates the gravel upstream, thus covering the ova and creating a fresh hollow where a further batch of ova is deposited. The female lays between 800 and 1200 ova per kilogram of body weight. After three months, the ova hatch, releasing the alevins. Although all were spawned at the same time, hatching takes place over a period of approximately two weeks. The alevins survive on their yolk sac for 4-6 weeks, before beginning to feed on zooplankton. The alevins are now known as fry and live in stony areas with fast flowing water, feeding on insect larvae, zooplankton, snails and insects near or on the water surface. The trout gradually move to deeper water, where they live until mature and upstream migration takes place.

1.9.6 *Asellus meridianus*

A. meridianus is an aquatic, detritivorous isopod which is widespread throughout Britain and Ireland, and has also been found in France, Germany, Belgium and Holland (Williams, 1960).

Until 1919, however, all specimens of the genus *Asellus* in Britain and Ireland were ascribed to just two species: *Asellus aquaticus* and *A. cavaticus*. However, following re-examination by Racovitza (1919), it was concluded that '*A. aquaticus*' was in fact made up of two species: these were designated *A. aquaticus* L. and *A. meridianus* Racovitza. *A. aquaticus* and *A. meridianus* are very similar in terms of ecology and physiology (Chambers, 1977). It has been proposed that there are competitive interactions between the two species (Hynes, 1965; Williams, 1963; Wolff, 1973), although the nature of the competition has not been elucidated. It is also thought that *A. aquaticus* is gradually replacing *A. meridianus* in Western Europe owing to the greater tolerance of pollution by the former (Moon, 1968).

1.9.7 *A. meridianus* Diet

Williams (1960) investigated the diet and feeding mechanisms of *A. aquaticus* and *A. meridianus* and found that both species have a similar diet with respect to composition and method of feeding, although *A. aquaticus* was found to be slightly more omnivorous than *A. meridianus*.

Asellus spp. feed by scraping food material from leaf surfaces (Graça *et al.*, 1993). Their diet consists of a wide variety of food items which are abundant in sediments and vegetation (Moore, 1975; Marcus & Willoughby, 1978). Most often diatoms, filamentous algae, dead or fresh fragments of aquatic plants, detritus and decaying tree leaves are eaten (Petridis, 1990). Fungi have also been shown to comprise an important part of the *Asellus* diet (Graça *et al.*, 1993). It has also been shown that adult *Asellus* faeces are critical in the nutrition of juveniles (Rossi & Vitagliano–Tadini, 1978).

1.10 The Life Cycle of *A. clavula*

In appropriate definitive hosts, adult parasites mature and shelled acanthors are released from females and pass out of the fish with faeces. The shelled acanthors then reside in the environment until ingested by an appropriate intermediate host. If acanthors are ingested by *A. meridianus* they hatch in the middle or hindgut of the arthropod and the

motile acanthor larvae is released. The acanthor then penetrates the intestinal wall, where it remains for a period of time within the tissue of the wall of the intestine. Later, the acanthor migrates to the haemocoel while still attached to the intestinal wall, where development proceeds through the acanthella stage to the final, infective, cystacanth stage. Cystacanths are white and elongate and the sexes are easily distinguishable with the aid of a microscope, as the internal reproductive organs are clearly visible through the body wall. Cystacanths of *A. clavula* are precocious in their development. In both male and female *A. clavula* cystacanths, the reproductive system may be observed (Rojanapaibul, 1977).

When infected isopods are ingested by an appropriate definitive host, the cystacanths are activated, the proboscis everts and the parasite attaches to the intestinal mucosa of the host by proboscis penetration. Copulation may then occur. After fertilisation, the ovarian balls in the female worm will develop; when shelled acanthors are mature they are released from the fish with the faeces (Figure 1.1).

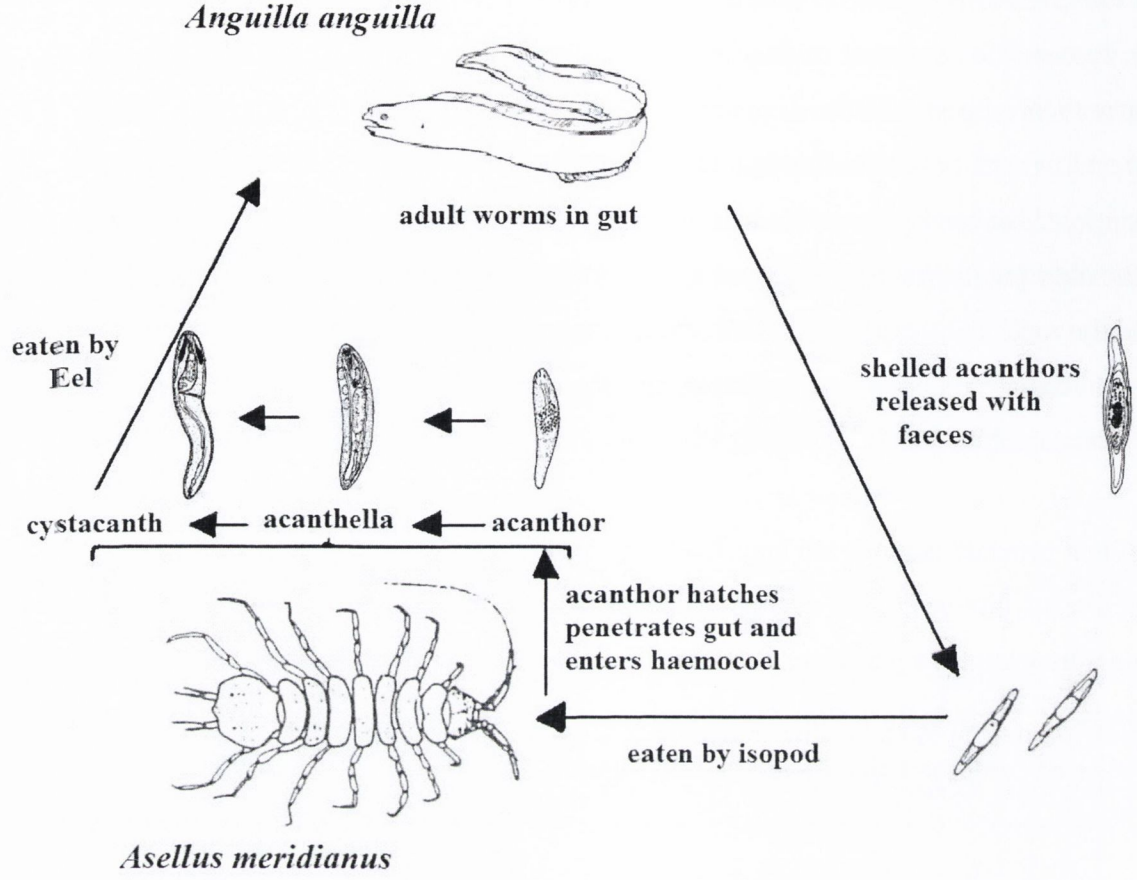


Figure 1.1 The life cycle of *Acanthocephalus clavula* (Naiara O'Mahony, 2009)

1.11 The Taxonomy of *A. clavula*

Upon initial discovery, *A. clavula* was placed in the genus *Echinorhynchus* by Dujardin (1845), however no drawings of the species were supplied with the original description. Luhe (1911), when describing acanthocephalan specimens from Poland, published a description and drawings of the worms under the name *Echinorhynchus clavula* Dujardin and this description was accepted by all later authors and was used as the basis for identification of *E. clavula*. However, when specimens of *E. clavula* from Wales (Chubb, 1964) were compared with *E. clavula* specimens from Poland (Luhe, 1911) it was discovered that two species (from two different genera) were in fact present. Further studies showed that only the Welsh specimens corresponded to the species *E. clavula*, while the Polish specimens did not and were renamed *Echinorhynchus borealis* Linstow

1901. It was also shown that the Welsh specimens belonged in the genus *Acanthocephalus*, and so *E. clavula* was renamed *Acanthocephalus clavula* (Grabda-Kazubska & Chubb, 1968).

1.12 The Geographical Distribution of *A. clavula*

Since 1968, *A. clavula* has been reported from many fish and invertebrate species from Ireland, Great Britain and continental Europe (Kennedy, 2006). While *A. clavula* tends to utilise just one species of arthropod per location as its intermediate host (Schmidt, 1985), it generally infects several fish species (Lyndon & Kennedy, 2001), however its ability to mature varies considerably according to the host species in which it is found (Rojanapaibul, 1977; Lyndon & Kennedy, 2001).

1.13 *A. clavula*: Definitive Hosts

A. clavula has been recorded from more than 25 species of freshwater fish across its range (Kennedy, 2006), 10 of which are found in Ireland (Table 1.2) (Holland & Kennedy, 1997). Eight of these species (charr, dace, perch, pike, roach, rudd, 3-spined stickleback and 9-spined stickleback) may be thought of as accidental hosts, owing to the fact that *A. clavula* was recovered only sporadically, and at extremely low levels of prevalence and abundance. Therefore, only eel and brown trout may be thought of as appropriate definitive hosts for *A. clavula* in Ireland.

A. clavula has been recorded in eels from Ireland (Kennedy & Moriarty, 1987; Conneely & McCarthy, 1986; Kane, 1966; Conneely & McCarthy, 1988; Byrne *et al.*, 2004), Great Britain (Kennedy, 1984) and continental Europe (Kennedy *et al.*, 1998; Borgsteede *et al.*, 1999) on a regular basis. The majority of female *A. clavula* in eels reach full reproductive maturity (Lyndon & Kennedy, 2001; Byrne *et al.*, 2004). For these reasons, the eel is designated as the preferred definitive host of *A. clavula* across its geographical distribution (Lyndon & Kennedy, 2001).

The situation in brown trout is quite different. While brown trout have been designated as a suitable definitive host for *A. clavula* (Lyndon & Kennedy, 2001), this host-parasite

combination has rarely been reported outside Ireland. Indeed, Kennedy & Hartvigsen (2000) reported it in only three of 72 localities sampled for trout in Britain and Norway. In northern Italy, *A. clavula* was recorded in trout in only one of three streams, and then only at a very low prevalence (Dezfuli *et al.*, 2001). In Ireland however, *A. clavula* has been reported from trout on several occasions (Conneely & McCarthy, 1984, 1988; Molloy *et al.*, 1993; Byrne *et al.*, 2002b).

In the course of a larger study on the parasite community structure of wild and stocked brown trout, Byrne *et al.* (2002b) found large numbers of *A. clavula* infecting trout in Clogher Lake in the west of Ireland, however, just 2% of the females had reached full maturity.

1.14 *A. clavula*: Intermediate Hosts

While the intermediate host of *A. clavula* in Britain and Ireland is the isopod *Asellus meridianus* (Chubb, 1964; Rojanapaibul, 1977; Brown *et al.*, 1986), the parasite has been found in ten other intermediate hosts across its range (Table 1.3). It is highly likely, however, that the parasite was misidentified in the studies which took place in Russia, due to possible taxonomic confusion (see Section 1.4.3).

Table 1.3 The intermediate hosts of *A. clavula* across its range (modified and updated from Schmidt, 1985)

Intermediate host	Host type	Location	Reference
Asellus meridianus	Isopod	Clogher Lough, Lough Feeagh, Ireland	Present study
Echinogammarus stammeri	Amphipod	River Brenta, Italy	Dezfuli *et al.* (1999)
Echinogammarus pungens	Amphipod	River Adige, Italy	Dezfuli *et al.* (1991)
Chaetogammarus ischnus	Amphipod	Kremenchug Reservoir, Russia	Kurandina (1975)
Dikerogammarus haemobaphes	Amphipod	Danube delta, Romania/Ukraine; Kremenchug Reservoir, Russia	Komarova (1969); Kurandina (1975)
Gammarus balcanicus	Amphipod	Carpathian mountain streams, Russia	Ivashkin (1972)
Gammarus (Rivulogammarus) balcanicus	Amphipod	Russia	Yalynskaya (1980)
Gammarus (Rivulogammarus) kischeneffensis	Amphipod	Russia	Yalynskaya (1980)
Pallasea quadrispinosa	Amphipod	Karelia, Finland/ Russia	Shtein (1959)
Pontogammarus obesus	Amphipod	Kremenchug Reservoir, Russia	Kurandina (1975)
Proasellus coxalis	Isopod	Central Italy	Fresi (1967)

1.15 The Role of *A. clavula* in the Intestinal Helminth Community

A. clavula tends to play one of two roles in the helminth communities (of appropriate definitive hosts) in which it is found: it is either the dominant acanthocephalan species (Kennedy, 1984; Kennedy, 1993; Kennedy *et al.*, 1998; Borgsteede *et al.*, 1999, Byrne *et al.*, 2004) or it occurs at extremely low levels of prevalence and abundance (Kennedy, 1984; Kennedy, 1993; Kennedy & Moriarty, 2002; Outeiral *et al.*, 2002; Aguilar *et al.*, 2005). While most of the above studies took place over short time periods, a small number were long-term (Kennedy, 1984; Kennedy, 1993; Kennedy & Moriarty, 2002), with samples taken over a number of years.

Kennedy & Moriarty (2002), in an 18-year study (1983 – 2001) of eel helminth communities in Lough Derg, reported *A. clavula* in only two sample years, at extremely low levels of prevalence and abundance . In contrast, Kennedy (1984), in a 4.5 year study (1979 – 1984) of *A. clavula* in eels in the River Clyst, found that *A. clavula* declined continuously over the period of study. At the beginning of the study, *A. clavula* was present in eels at high levels of prevalence and abundance; however the parasite underwent a steady and continuous decline so that by the end of the sampling period it was no longer detectable. Kennedy (1993), in a 19-year (1979 – 1992, overlapping with Kennedy, 1984) study of eel helminth communities, again in the River Clyst, found that *A. clavula* reappeared in the river from 1987 onwards, albeit at extremely low levels of prevalence and abundance.

1.16 Acanthocephalan population dynamics

1.16.1 Population Dynamics in Definitive hosts

In recent decades, studies of the population dynamics of individual parasite species have become common. The population dynamics of a species may be elucidated at several levels, from dynamics in a single host (Dezfuli *et al.*, 1991; Taraschewski, 2000), to the dynamics of the parasite in a population of hosts (Awachie, 1965). Population dynamics may also be studied over different time periods, from short-term, seasonal studies (Brattey, 1986, 1988) to studies investigating the stability of parasite populations in the

long-term (Kennedy & Rumpus, 1977). When investigating the population dynamics of parasite species, long-term studies taking place over a number of consecutive years are preferable, as they provide a realistic view of the situation, without the need for extrapolation of data or presumption of repetitive cycles. However, long-term studies are rarely feasible, due to logistical and financial demands. For this reason, the vast majority of studies investigating acanthocephalan population dynamics are short-term studies exploring seasonal changes in population parameters such as prevalence, abundance, intensity and aggregation.

Seasonal cycles in prevalence and abundance of acanthocephalan populations have been found on all continents and in all biomes (Chubb, 1982). Often, seasonal cycles are correlated with changes in extrinsic factors, particularly water temperature, and intrinsic factors, particularly host diet (Kennedy, 2006).

In the majority of studies of acanthocephalan parasites in fish definitive hosts, prevalence, mean abundance and intensity reach their highest levels in spring and summer, corresponding to increases in water temperature, which in turn correspond to increased feeding by fish (Awachie, 1965; Camp & Huizinga, 1980; Moravec, 1985; Brattey, 1988; Molloy *et al.*, 1995a). Ohtaka *et al.* (2002) reported no significant difference in mean intensity of *Acanthocephalus* sp. in rainbow trout *Onchorhynchus mykiss* in Japan from August to January, however the proportion of gravid females was greatest in summer and autumn, which suggests that parasites are recruited in winter and spring. Borgsteede *et al.* (1999), in an investigation of eel helminths in the Netherlands, found that the mean intensity of *A. clavula* was always highest in winter. While the reasons for this finding were not explored, the population dynamics of *A. meridianus* in the lakes more than likely play a role. Muzzall & Rabalais (1975a) found an unusual situation in their examination of seasonal periodicity in the occurrence of *A. jacksoni* in the creek chub *Semotilus atromaculatus* in Ohio, USA: *A. jacksoni* was completely absent from its definitive host from August to November. This was suggested to be a result of a lack of availability of its intermediate host, *Lirceus lineatus* from the environment during this period, thus making the completion of the parasite life cycle an

impossibility. The absence of *L. lineatus* was correlated with decreased water levels as well as death of vegetation (Muzzall & Rabalais, 1975b).

Several studies exist which explore the population dynamics of acanthocephalans in eels over long time periods (Kennedy, 1984; Kennedy, 1993; Kennedy *et al.*, 1998; Kennedy & Moriarty, 2002; Schabuss *et al.*, 2005). To my knowledge, however, there has not yet been a long-term investigation of acanthocephalan populations in brown trout. Kennedy & Moriarty (2002) explored eel helminth communities over an eighteen-year period in Lough Derg, Ireland and found that the community was dominated in all years by *A. lucii*, although the prevalence and abundance of this species fluctuated throughout the study period. *A. anguillae* was present in 16 of the 18 sample years, always at lower levels of prevalence and abundance than *A. lucii*. By the end of the study, *A. anguillae* had declined so as to be undetectable in the eel population. Kennedy (1993) investigated eel helminth communities in the River Clyst in Great Britain over a 13-year period (incorporating nine sample years) and reported that the community was heavily dominated by *A. clavula* in the first five sample year, however by the end of the sampling period *A. clavula* had declined to extremely low levels of prevalence and abundance. Eel helminth communities in the River Tiber in Italy were examined by Kennedy *et al.* (1998) in 1980 and again in 1996. *A. clavula* dominated the community, and occurred at similar levels of prevalence and abundance in both sample years.

Schabuss *et al.* (2005) explored eel helminth communities at two sites in Neusiedler See in Austria over an eight-year period and found that in one site *A. anguillae* dominated helminth communities in all years, whereas in the other site, *A. lucii* initially dominated. This is a highly unusual situation, as helminth communities in any one locality at a particular time are generally dominated by single species of acanthocephalan (Kennedy, 2006). However, by the end of the study period, both *A. lucii* and *A. anguillae* were present at high levels of prevalence and abundance.

The results of the studies detailed above show that eel helminth communities are often dominated by a single species of acanthocephalan, although the identity of this species

may change, both from location to location as well as over time in the same location. While the structure of some eel helminth communities remains stable over long periods of time (e.g. Kennedy & Moriarty, 2002) others vary widely in terms of composition and community dominance (e.g. Schabuss *et al.*, 2005). While the causes of these changes have yet to be elucidated, changes in intermediate host populations (Kennedy, 1984) and/or the operation of regulatory mechanisms (Brown, 1986) are likely to play a role.

1.16.2 Population Dynamics in Isopod Intermediate Hosts

Much work on various aspects of the population biology of *Acanthocephalus* species in isopod intermediate hosts has been carried out in recent years (e.g. Brattey, 1986; Oetinger & Nickol, 1982; Kakizaki *et al.*, 2003). The occurrence of seasonal cycles in infection of isopods by acanthocephalans has been investigated in Europe (Styczynska, 1958; Rojanapaibul, 1977; Brattey, 1986), the USA (Seidenberg, 1973; Amin *et al.*, 1980; Camp & Huizinga, 1980) and Japan (Ohtaka *et al.*, 2002).

In Japan, in Lake Wakitsubo-no-ike, *Acanthocephalus* sp. infection of the isopod *Asellus hilgendorfi* showed clear seasonal periodicity, despite the fact that water temperature remained constant throughout the year. The highest levels of infection were recorded in spring (March & April, >40%) followed by a sharp decline in May. Prevalence then remained relatively low (<20%) in the summer and winter months (Ohtaka *et al.*, 2002). In this case, feeding patterns of *A. hilgendorfi* were thought to account for the observed seasonal cycle (Ohtaka *et al.*, 2002).

In the USA, in Sugar Creek, Illinois, Camp & Huizinga (1980) investigated the seasonal cycle in infection of isopod *Asellus intermedius* by *A. dirus*. In this system, prevalence of *A. dirus* fluctuated significantly throughout the year, with a low in May (2.6%) followed by a sharp increase during the summer months (July & August, 50-60%). Prevalence was maintained at lower levels for the remainder of the year (20-40%). The seasonal changes in infection levels were attributed to three factors: (a) availability of acanthors in the environment, (b) the recruitment of young isopods into the population (resulting in a

decrease in prevalence) and (c) the death of older infected isopods. Seidenberg (1973), observed a similar cycle in *A. dirus* infection of isopods *A. intermedius* in Mud Creek, Illinois, USA.

Amin *et al.* (1980) found that the seasonal cycle of *Acanthocephalus parksidei* in the isopod *Caecidotea militaris* in the Pike River, Wisconsin, USA, was closely correlated with the life cycle of the host. In this case, infection levels were lowest in May and June, reached peaks in August and December and declined in the interims. The beginning of the increase in prevalence immediately followed the *C. militaris* breeding season, while the second peak in prevalence (in December) parallels a seasonal increase in size of isopods. It was suggested that the trough in prevalence of *A. parksidei* observed in May and June corresponded with the death of an isopod generation, particularly heavily infected, older isopods (Amin *et al.*, 1980).

Brattey (1986) investigated the seasonality in infection of the isopod *Asellus aquaticus* by *A. lucii* in the Forth and Clyde Canal in Glasgow, Scotland, and found that, while prevalence remained low throughout the study (1.5-8.3%), a slight seasonal cycle in infection was observed. The lowest prevalences were observed in the summer months (July & August), during the turnover in isopod generations (Brattey, 1986). In contrast, Styczynska (1958) found that *A. lucii*-infected isopods were more common in the summer months than in spring or autumn in Lake Druzno, Poland. Rojanapaibul (1977) examined the life history of *A. clavula* in the isopod *A. meridianus* in Bala Lake, Wales, however no evidence of a seasonal cycle in infection was found.

It is clear from the studies outlined above that larval *Acanthocephalus* species in North America and Japan show more pronounced seasonal cycles than those observed in Europe, although the reasons for this are not yet known. It is also interesting to note the occurrence of geographic variation in the life history of *Acanthocephalus* species (e.g. Brattey, 1986; Styczynska, 1958).

Numerous works also explore the effect of *Acanthocephalus* species on the size (Kakizaki *et al.*, 2003; Hasu *et al.*, 2007), reproduction (Brattey, 1983; Oetinger, 1987; Kakizaki *et al.*, 2003), pigmentation (Camp & Huizinga, 1979; Oetinger & Nickol, 1981, 1982; Brattey, 1983; Pilecka-Rapacz, 1986) and behaviour (Muzzall & Rabalais, 1975c; Camp & Huizinga, 1979; Brattey, 1983; Pilecka-Rapacz, 1986; Hechtel *et al.*, 1993; Lyndon, 1996) of their intermediate hosts.

Both experimental (Hasu *et al.*, 2007) and field (Oetinger & Nickol, 1981; Kakizaki *et al.*, 2003) studies have shown correlations between increased isopod size and infection with acanthocephalan parasites, however while Hasu *et al.* (2007) found that infected individuals of both sexes were significantly larger than uninfected individuals, Kakizaki *et al.* (2003) and Oetinger & Nickol (1981) found that only female isopods were affected.

Infection with acanthocephalans has also been shown to affect the reproductive capabilities of isopods (Brattey, 1983; Oetinger, 1987; Kakizaki *et al.*, 2003). The ovaries of infected females either do not develop (Oetinger, 1987) or are observed in an atrophied condition (Brattey, 1983). Dezfuli *et al.* (1994) reported shortened oostegites and fewer eggs in *A. anguillae*-infected *A. aquaticus* in an Italian river. No visible effects of acanthocephalan infection on the reproduction of male isopods have yet been detected (Brattey, 1983; Oetinger, 1987; Dezfuli *et al.*, 1994; Kakizaki *et al.*, 2003). The evidence for increases in the size of infected female isopods combined with the reduction in their reproductive capacity suggests that acanthocephalans can interfere with the resource allocation of isopods, causing them to reach greater sizes at the cost of reproductive success (Kakizaki *et al.*, 2003).

Acanthocephalans are known to alter the colouration of intermediate hosts. This may be done in one of two ways: pigment may be produced or normal host pigmentation may be altered. In both cases, intermediate hosts are rendered more conspicuous to potential predators (Brattey, 1983). In Europe, infected isopods tend to be darker than uninfected conspecifics, i.e. pigment is produced (Munro, 1953; Balesdent, 1965; Fresi, 1967; Needham, 1974; Brattey, 1983, Pilecka-Rapacz, 1986; Lyndon, 1996), while in North

America, infected isopods are generally lighter in colour than uninfected individuals, i.e. pigmentation is altered (Seidenberg, 1973; Muzzall & Rabalais, 1975b; Amin *et al.*, 1980; Camp & Huizinga, 1980, Oetinger & Nickol, 1981). The lighter colour of infected isopods in North America is thought to result when young, incompletely pigmented isopods become infected and then fail to develop further pigmentation. This process has been termed 'pigmentation dystrophy' (Oetinger & Nickol, 1981). The reasons for the difference in pigmentation changes between locations are, as yet, unknown.

Larval acanthocephalans are known to alter the behaviour of isopod intermediate hosts (Camp & Huizinga, 1979; Brattey, 1983; Pilecka-Rapacz, 1986; Hechtel *et al.*, 1993; Lyndon, 1996). Infected isopods have been reported to be hyperactive (Muzzall & Rabalais, 1975c; Camp & Huizinga, 1979; Pilecka-Rapacz, 1986) and spend more time near the water surface (Pilecka-Rapacz, 1986). Normal hiding and predator avoidance behaviours of isopods have also been shown to be reversed by acanthocephalan infection (Hechtel *et al.*, 1993). Lyndon (1996) investigated behavioural changes in *A. aquaticus* infected with *A. anguillae* and *A. lucii* and found that *A. anguillae*-infected isopods were markedly more photophilic in comparison with their uninfected conspecifics, and were attracted to water disturbances. Melanisation of the whole body was also observed in *A. anguillae*-infected isopods. *A. lucii*-infected isopods showed no apparent alterations in behaviour, however melanisation of the respiratory opercula (as described by Brattey, 1983) was observed. The different effects on infected *A. aquaticus* by *A. anguillae* and *A. lucii* were related to the feeding methods of their respective fish hosts (Lyndon, 1996).

1.17 The Parasite Niche

In order to explore population dynamics both within and between parasite communities, the parasite niche is investigated. While the concept of the niche is a complex one, for the purposes of this study, the definition of the niche of an organism is defined as its range in ecological rather than geographical space (Whitfield, 1979). Parasitic helminths do not occur randomly or uniformly along the alimentary tract of vertebrates (Crompton, 1973). Each species shows a preference for a particular region of the tract. The abundance of

parasite individuals is highest in this area, and declines in anterior and posterior directions (Kennedy et al., 1976). It has long been accepted that the site selection of each helminth species corresponds to its niche (Holmes, 1973). The niche of any helminth parasite thus represents the optimal concentration of the essential environmental resources that the species requires (Kennedy, 2006). The primary resources required by parasites are nutrients and space. While much work has been carried out on nutrient requirements and absorption of acanthocephalans, most of the studies have been limited to just three species, *Moniliformis moniliformis*, *Macracanthorhynchus hirudinaceus* and *Polymorphus minutus* (e.g. Edmonds, 1965; Crompton & Lockwood, 1968; Crompton *et al.*, 1982, 1983), and thus there remain large gaps in our knowledge of the nutrient requirements of acanthocephalans (Starling, 1985). However, it is eminently possible to describe their use of space. If the parasite habitat can be defined along a linear axis, such as the vertebrate alimentary canal, then a measure of the niche may be taken as the mean or median position of individual parasites. The niche itself would be the region encompassed by the range of their positions (Poulin, 2007a); however a distinction must be made between the fundamental and the realised niche. The fundamental niche is defined as the potential distribution of the parasite in the host, that is, the range of sites in which the parasites of one species can develop. The realised niche is a subset of the fundamental niche and consists of the sites actually occupied by the parasite in the host (Poulin, 2007a). The realised niche may represent either the optimal sites for the parasite, in the absence of interspecific interactions, or the portion of the fundamental niche available where competitive interactions with other species take place.

Much work on the site selection of acanthocephalans has taken place over recent decades, with an initial concentration on the exploration of site selection in relation to nutrition (Starling, 1985). For example, developmental emigrations of *Moniliformis moniliformis* were noted by Burlingame & Chandler (1941) and Holmes (1961) in the weeks post-infection, and were interpreted as site-finding behaviour carried out in order to optimise the positioning of helminth feeding surfaces in relation to nutrient gradients or other physiological conditions in the gut. Crompton (1973) noted that helminths do not occur randomly or uniformly throughout the alimentary tract of vertebrates.

Generally, each species shows a preference for a particular region of the tract. Parasite numbers are most concentrated in this region and then decline in anterior and posterior directions (e.g. Kennedy *et al.*, 1976; Kennedy & Lord, 1982). Since the seminal work of Holmes (1973) it has become accepted that the site selection of a parasite species does correspond to its niche (Kennedy, 2006).

The study of the parasite niche has also been used to explore interactions between parasite species. Kennedy (1985) explored the distribution of four acanthocephalan species (*A. anguillae, A. clavula, A. lucii* and *Pomphorhynchus laevis*) in the alimentary tract of eels, and found that the mean position of each species was significantly different, despite large overlaps in species distribution, i.e. each species occupied a different niche, although the niches did overlap to a certain extent.

However, when a species occurs alone in the alimentary canal, it still shows a preference for a certain region of the gut. For example, Kennedy & Lord (1982) examined the niche of *A. clavula* in the alimentary canal of eels and found that a preference was shown for the mid-region of the gut, and that the range increased with increasing infection intensity. It is thought that niche preferences in the absence of other species result from adaptations of a species to a set of resources, as no species can adapt to all possible niches (Kennedy, 2006). It has also been proposed that the restriction of a species to a definite niche will increase the probability of finding a suitable partner for mating (Rohde, 1994).

1.18 Interspecific Interactions

In recent times, there has been much debate around the possible role of interspecific interactions in structuring helminth communities in fish hosts (Poulin, 2001). Two different approaches have been undertaken. First, parasite responses have been measured in concurrent laboratory infections (Bates & Kennedy, 1990, 1991). Any change in numbers of parasite individuals or in their use of niche space, compared with what is observed in single infections, provides solid evidence that the species are interacting (Poulin, 2001). Second, infracommunity patterns of helminth species have been

compared in wild hosts (Kennedy, 1992; Byrne *et al.*, 2003), however field data are extremely difficult to interpret and alternative explanations are usually as plausible as invoking the effect of interactions among helminth species (Poulin, 2001).

It may be predicted that competitive interspecific interactions between acanthocephalans of freshwater fish would be a rare occurrence for several reasons. First, acanthocephalan communities are often dominated by a single species (e.g. Kennedy & Moriarty, 2002), so the opportunities for interactions between species are few. Second, as the degree of aggregation of parasite populations increases, the probability of two species occurring in sufficient numbers in the same host individual is very low (Dobson, 1985). Third, some acanthocephalan species (for example freshwater species in Britain and Ireland) exhibit resource partitioning, such that no species shares both intermediate and definitive hosts (Lyndon & Kennedy, 2001), which again limits the possibility of interspecific interactions. Fourth, each species has its own niche preference (Kennedy, 1985b), so that even if two acanthocephalan species occur in the same host individual, they may be present in different regions of the intestine, thus preventing potential interactions.

Interspecific interactions may be more frequent in Ireland than in other locations due to the depauperate nature of its fish fauna (Griffiths, 1997), which results in the forced sharing of definitive hosts by acanthocephalan species in the absence of their preferred definitive hosts from this island. For example, the extremely rare combination of *A. clavula* and *P. laevis* has been observed in Irish brown trout (Kennedy & Hartvigsen, 2000; Byrne *et al.*, 2003). In the absence of chub and barbel (the preferred definitive hosts of *P. laevis* in other locations), the preferred definitve host of *P. laevis* in Ireland is the brown trout. *A. clavula* has also been found in brown trout in Ireland, despite the availability of its definitive host, the eel.

Competitive interactions have now been demonstrated for four of the six freshwater acanthocephalan species present in Britain and Ireland (Bates & Kennedy, 1990; Bates & Kennedy, 1991; Kennedy & Moriarty, 1987; Byrne *et al.*, 2003). Two of the competitive pairings (*A. lucii* and *A. anguillae*, and *A. clavula* and *P. laevis*) have been demonstrated

from Ireland (Kennedy & Moriarty, 1987; Byrne *et al.*, 2003). In addition, it has been suggested that a competitive interaction between the remaining two species, *N. rutili* and *E. truttae*, is likely, owing to the fact that both utilise brown trout as a preferred definitive host (Lyndon & Kennedy, 2001).

Competitive interactions between acanthocephalan species may be examined through exploration of niche width changes in the presence of other species (e.g. Byrne *et al.*, 2003). Such a niche shift (a functional response) is generally accepted as evidence of competition between two species. The regular absence of one species in the presence of another or an inverse relationship between the density of one species and the density of another in the same host species (numerical responses) are also accepted as evidence of competition (e.g. Kennedy & Moriarty, 1987).

Bates & Kennedy (1990) reported interference competition between *A. anguillae* and *P. laevis* in rainbow trout *Onchorhynchus mykiss*, with *A. anguillae* being the inferior competitor. Kennedy & Moriarty (1987) postulated the occurrence of exploitation competition between *A. lucii* and *A. anguillae* in eels from Lough Derg, with *A. lucii* the dominant species. Byrne *et al.* (2003) investigated interactions between *P. laevis* and *A. clavula* in brown trout in the west of Ireland and found that *P. laevis* appeared to be the inferior competitor, as the niche width of *P. laevis* decreased markedly in concurrent infections with *A. clavula* in wild trout, suggesting the operation of competitive exclusion. However, as trout are a preferred definitive host for *P. laevis* in Ireland, but not for *A. clavula*, it would be expected that the superior competitor in this interaction would in fact be *P. laevis*, and that *P. laevis* would affect the the niche of *A. clavula* in this host. Laboratory infections of both species in brown trout are necessary in order to further explore possible interactions between them.

1.19 Epizootics

While the majority of acanthocephalan infections do not result in damage to or death of definitive hosts, isolated instances of morbidity and mortality have been recorded, for

example *Plagiorhynchus cylindraceus* has been linked with paralysis of robins and starlings (Holloway, 1966), as well as death of bluebirds (Thompson-Cowley *et al.*, 1979). The link between acanthocephalan infection and mortality in these cases is tenuous and difficult to interpret for two reasons. First, the evidence in these studies was not quantitative and second, acanthocephalan densities are often similar in dead animals supposedly killed by the infection as in conspecifics that showed no adverse effects. These cases more than likely represent situations where infection with acanthocephalans simply exacerbated the effects of other stresses, rather than being a true reflection of sudden increases in parasite prevalence in the host population (Nickol, 1985).

Sudden local increases in prevalence and abundance of acanthocephalans accompanied by pathogenic effects on hosts do occasionally occur, however (e.g. Bullock, 1963; Schmidt *et al.*, 1974; Itamies *et al.*, 1980). Infection with extremely high numbers of *Acanthocephalus dirus* caused an apparent epizootic in mottled sculpin, *Cottus bairdi*, in a creek in Illinois, USA (Schmidt *et al.*, 1974) which lasted from 1966 to 1970. After this period, mottled sculpin could no longer be found in the creek, their absence possibly caused by the *A. dirus* epizootic. It is thought that the physical characteristics of habitats may induce epizootics, for example, in a shallow and narrow portion of the River Merloux in France, the prevalence of *Polymorphus minutus* in amphipods was recorded at 48% (Van Maren, 1979), whereas further downstream, where the river was wider and deeper, prevalence of *P. minutus* was just 2%. Heavy infections of acanthocephalans do not always result in epizootics, however. Byrne *et al.* (2004) reported heavy burdens of *A. clavula* in trout from Clogher Lough in Co. Mayo but noted no pathological effects of infection.

While pathological effects of acanthocephalan infection clearly could result in increased mortality of invertebrate hosts, no acanthocephalan-induced 'die-offs' have yet been recorded from the field (Nickol, 1985), although they have been observed in experimental infections. For example, *Macroacanthorhynchus hirudinaceus* in June beetle larvae (Miller, 1943), *Neoechinorhynchus cylindratus* in ostracods (Ward, 1940) and *Neoechinorhynchus rutili* in ostracods (Merritt & Pratt, 1964) have been found to

cause mass death of their invertebrate hosts in the laboratory, most likely caused by penetration of the haemocoel by artificially high numbers of acanthors (eggs) (Schmidt, 1985).

1.20 Host Shifts

While no host shift of an acanthocephalan species has yet been documented, host shifts have occurred in other parasite groups. For example, the swimbladder nematode *Anguillicola crassus*, a parasite of the Japanese eel *Anguilla japonica* has shifted host to to the European eel *Anguilla anguilla*. *A. crassus* was introduced to the European continent in approximately 1980, most likely as a result of eel exports from south east Asia. The parasite quickly spread throughout Europe and had reached Great Britain and Ireland by the late 1980s (Norton, 2005). As the eel is an economically important species the shift of this parasite to a new host and its subsequent spread were closely monitored and thus the widespread ecological and environmental damage caused by this host shift has been well-documented (for example Starkie, 2003; Tesch, 2003). Recent work (Byrne, 2003) suggested that *A. clavula* was undergoing a shift in host from eel to trout. The current project was then designed to explore the possibility of the occurrence of the postulated shift.

1.21 Aims

The primary aims of the present study were (1) to explore whether the postulated host shift of *A. clavula* from eel to trout had occurred, (2) to investigate the suitability of trout as a definitive host for *A. clavula*, (3) to explore the population biology of *A. clavula* in eel, trout and *A. meridianus* in three lakes in the west of Ireland: Bunaveela Lake, Clogher Lough and Lough Feeagh and (4) to extend the observations from a local to a regional scale by exploring the population biology of A. clavula in seven further sites around Ireland.

Chapter 2

A. clavula in the Intermediate Host, *A. meridianus*

2.1 Introduction

The characteristic intermediate host of *A. clavula* in Great Britain and Ireland is *A. meridianus* (Racovitza), although nine other species of aquatic crustacean are known to function as intermediate hosts for *A. clavula* in other parts of Europe (Schmidt, 1985) (see Table 1.3). *A. meridianus* becomes infected with larval *A. clavula* by ingesting a shelled acanthor during feeding.

While many studies exploring various aspects of isopod-acanthocephalan systems have been carried out (e.g. Seidenberg, 1973, Muzzall & Rabalais, 1975a, b, c; Camp & Huizinga, 1980; Brattey, 1986), very few of these have investigated the *A. meridianus* -*A. clavula* relationship. Rojanapaibul (1977), investigated the life-cycle of *A. clavula* in *A. meridianus* in the laboratory by means of experimental infections, however no observations from the field were obtained (See Section 1.X). The other published studies on *A. clavula* in its intermediate host have been carried out in Italy: Dezfuli *et al.* (1994) explored the occurrence of *A. clavula* in the amphipod *Echinogammarus pungens*, while Fresi (1967) examined the occurrence of *A. clavula* in the isopod *Asellus coxalis*. However, no detailed ecological studies of an *A.clavula* – intermediate host relationship have been published to-date.

The aim of this chapter is to investigate the population dynamics of *A. clavula* in its intermediate host, *A. meridianus*, in Clogher Lough, Lough Feeagh and Bunaveela Lake. This will be achieved through an exploration of (1) seasonal cycles in *A. clavula* prevalence and abundance, (2) the size and sex breakdown of the *A. meridianus* population, (3) the reproductive cycle of the *A. meridianus* population, (4) the impact of *A. clavula* on *A. meridianus* behaviour and (5) the comparative occurrence of *A. meridianus* in the diet of eel and trout.

2.2 Materials and Methods

2.2.1 Primary sample site descriptions

The primary sites for investigation were three lakes in Co. Mayo (Clogher Lough, Lough Feeagh and Bunaveela Lake, where vast amounts of parasitological data have been gathered over a long period (Figure 2.1) (Byrne *et al.* 2002a, b, 2003, 2004; Molloy *et al.*, 1995a, b).

2.2.2 Burrishoole Catchment

Two of the primary study sites, Lough Feeagh and Bunaveela Lake, are in the Burrishoole catchment (Figure 2.2). The Burrishoole valley lies in a north-south direction in the Nephin Beg mountain range in Co. Mayo (9°55' W 53°55'N). The freshwater catchment has a total area of 8,949ha and contains three main lakes, Lough Furnace, Lough Feeagh and Bunaveela Lake, as well as a number of smaller lakes. The catchment is drained by approximately 45km of small, shallow streams (Poole, 1994). The western side of the catchment is composed primarily of Dalradian schists, gneiss and quartzite (Whittow, 1974), leading to poorly buffered, generally acidic, runoff. The northern and eastern sides of the catchment have small outcrops of limestone and sandstone. Forestry and mountain sheep farming are the main land uses. Overgrazing by sheep has led to severe hillside erosion, resulting in increased peat siltation and acidification of streams, particularly in the western part of the catchment (Whelan, 1995).



Figure 2.1 Map showing the three primary sample sites: Bunaveela Lake, Clogher Lough and Lough Feeagh (Ordnance Survey, Ireland)

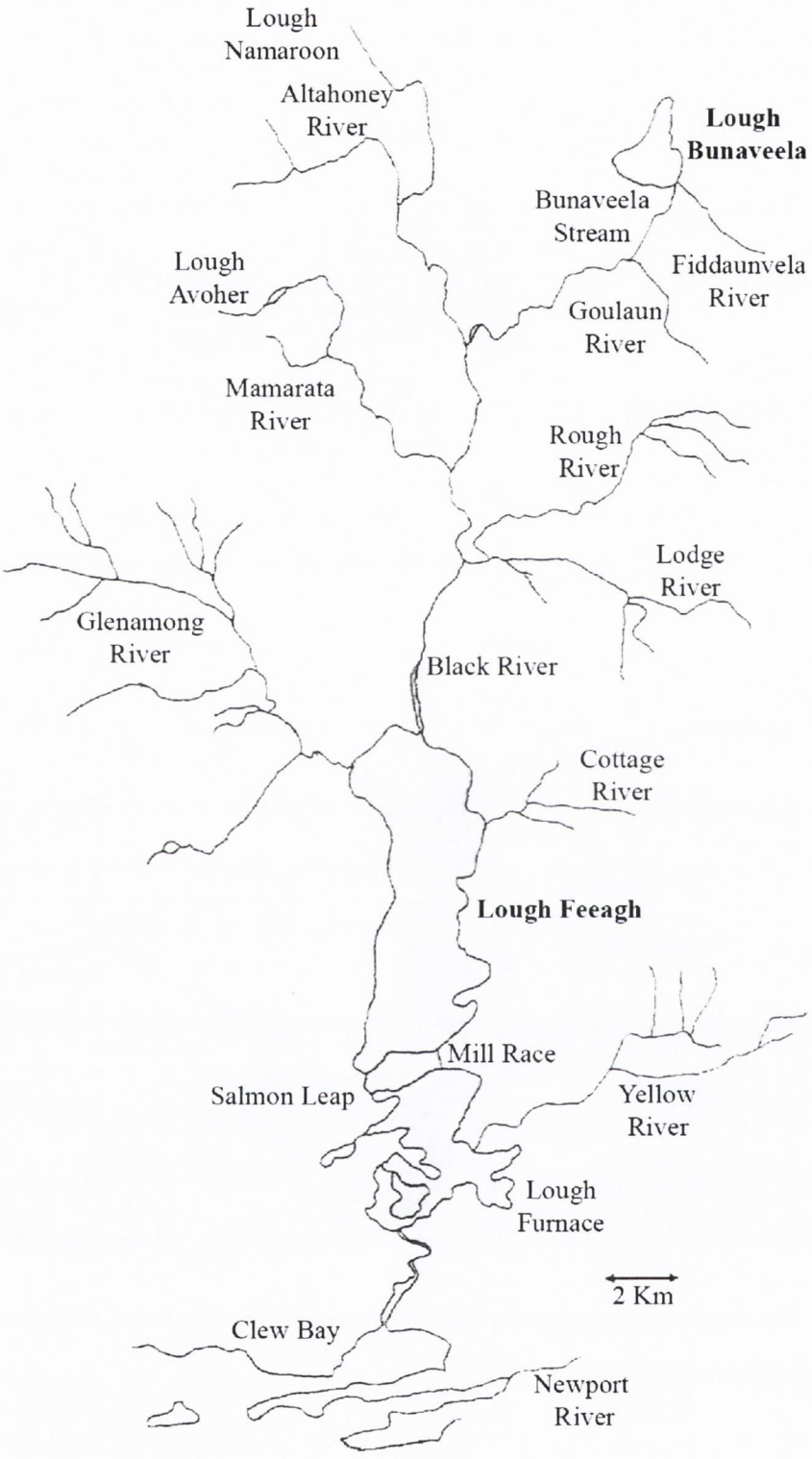


Figure 2.2 The Burrishoole Catchment (modified from Byrne, 2000)

2.2.3 Bunaveela Lake

Bunaveela Lake lies on the north-eastern side of the main catchment (Figure 2.2). It has an area of 42ha, a maximum depth of 23m and lies at an altitude of 150m above sea level. There is one main inflow, the Fiddaunveela River, and one main outflow, the Goulaun River. The lake is naturally divided by a promontory emerging from the eastern shore. The northern end of the lake is shallow, with an average depth of between two and eight metres. The southern portion of the lake is composed of three basins, the deepest of which is 23m.The geology of the lake is a combination of limestone and sandstone. The buffering effect of limestone and sandstone on the water boosts productivity and allows considerable numbers of molluscs and amphipods to inhabit the lake (Byrne, 2000).

2.2.4 Clogher Lough

Clogher Lough lies in a north to south facing valley approximately 8km from Westport, Co. Mayo and 6km from the Burrishoole catchment (Figure 2.3). The lake has an area of 60ha and lies at an altitude of 25m above sea level. Clogher Lough has one inflowing stream from the north and two out flowing streams to the south. It is surrounded by low-lying farmland and could probably be classified as a mesotrophic lake, although no environmental data are presently available to confirm this (Byrne, 2000). The lakeshore is composed of soft marl, and reed beds cover most of the shoreline. The reed beds are particularly dense on the southern and eastern shores.



Figure 2.3 Clogher Lough: — =fish sampling site, X = *Asellus* sampling site (modified from Byrne, 2000).

2.2.5 Lough Feeagh

Lough Feeagh is a low-lying, oligotrophic freshwater lake of glacial origin (Figure 2.4). The lake has an area of 410ha, a maximum depth of 43m and is 14m above sea level. Lough Feeagh is joined to Lough Furnace by two narrow channels, the Salmon Leap (natural) and the Mill Race (man-made). The lake is comprised of a series of basins; the largest and deepest occupies the northern portion of the lake. Another, shallower, basin occupies the south-west corner of the lake. The southern section of the lake is relatively shallow (15 – 18m) and has an undulating bottom. The water of Lough Feeagh is neutral to acidic and distinctly coloured, due to peat siltation (Byrne, 2000).

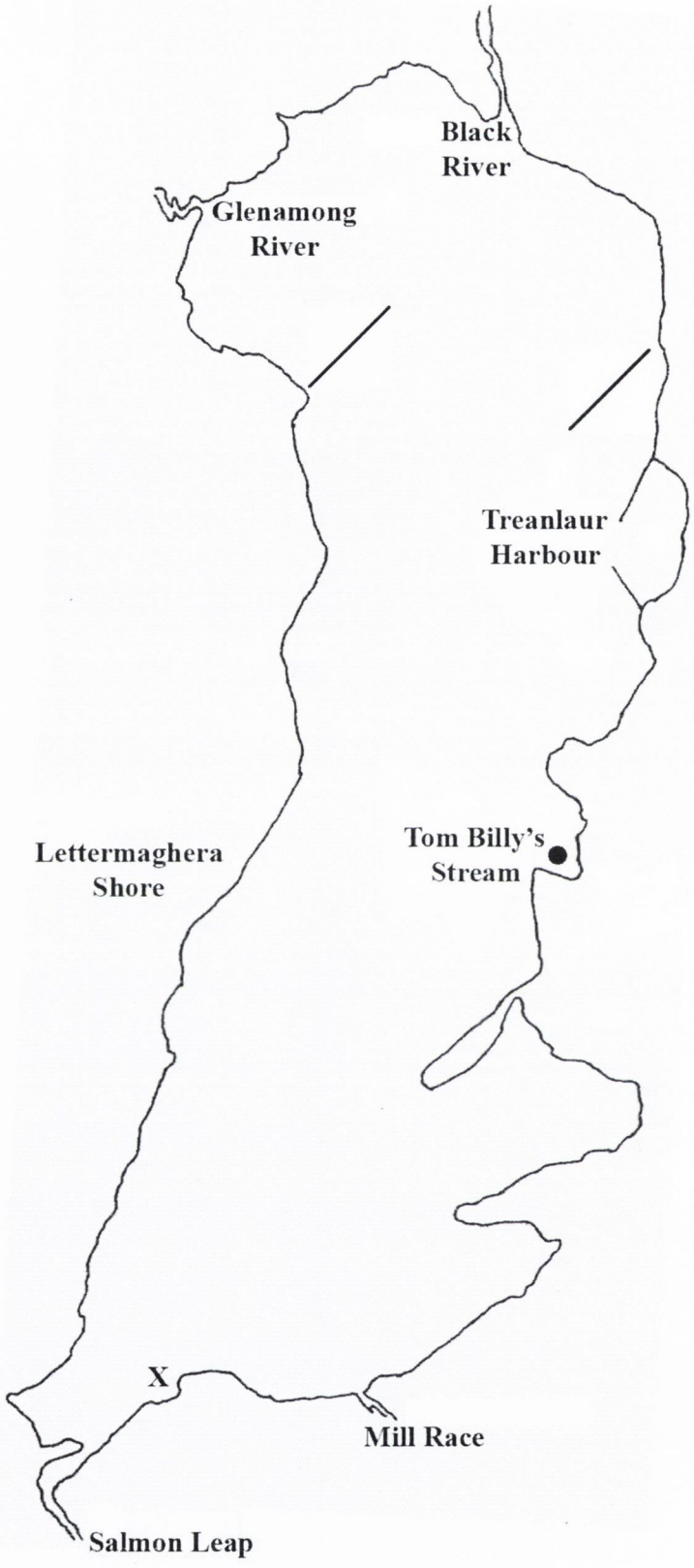


Figure 2.4 Lough Feeagh: — = fish sampling site, X = *Asellus* sampling site (modified from Byrne, 2000)

2.2.6 Data Analysis

Several descriptive terms and statistics were used to explore and analyse the parasite data presented in this thesis. All terms and statistical procedures used are described below, in sequential order. All descriptors of *A. clavula* infection are defined as per Bush *et al.* (1997). Where necessary, data which were not normally distributed (e.g. fish and parasite mass) were log (y) - transformed prior to analysis.

Range

The range is the difference between the minimum number of parasites recorded and the maximum number of parasites recorded.

Prevalence

Prevalence is the number of hosts infected with a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species. In this thesis, prevalence values are expressed as percentages ± 95% confidence intervals.

Mean abundance

Mean abundance is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including infected and uninfected hosts). It is thus the average abundance of a particular parasite species among all members of a particular host population. In this thesis, mean abundances are quoted in conjunction with the standard error.

Mean intensity

Mean intensity is the average intensity of a particular species of parasite among infected individuals of a particular host species. In other words, it is the total number of parasites

of a particular species found in a sample divided by the number of hosts infected with that parasite. In this thesis, mean intensities are quoted in conjunction with the standard error.

Variance to mean ratio

The variance to mean ratio is the simplest and most commonly used measure of parasite aggregation and is defined as the ratio of the variance to the mean number of parasites per host (Poulin, 2007b). The variance to mean ratio was employed in this study for ease of comparison with previous work. If parasites are distributed at random according to a Poisson distribution, the mean number of parasites per host would equal the variance. A variance to mean ratio greater than unity indicates a departure from randomness and a tendency toward aggregation, with aggregation levels increasing with the value of the ratio.

t-test

t-tests were used to make comparisons between two groups of unpaired data. The t-test assumes that the data are continuous, are approximately normally distributed and that the variances of the two datasets are homogeneous. The null hypothesis of the t-test is that the two sample means being compared come from a population with the same true mean, μ.

Chi-squared (χ^2) analysis

Chi-squared analysis was utilised to explore differences between categorical variables. The chi-squared test allows comparison of observed data with expected data, if following a Poisson distribution with the same mean. The null hypothesis of the chi-squared test is that the observed and expected frequencies are not different from each other.

Generalised linear models (GLMs)

GLMs were used to assess the significance of the contribution by a factor or factors to the difference in variance observed between the means of a number of samples. One-, two, three- and four-way analyses of variance were employed in this thesis. One-way GLM was used to assess the contribution of a single factor to the difference between the means of a number of samples, two-way GLM was used to assess the contribution of two factors, and so on. For two-, three- and four-way GLMs, the interactions between individual factors were also assessed. In all cases, only significant results are presented. The cut-off point for significance used is $p \leq 0.05$

Kolmogorov-Smirnov tests

The Kolmogorov-Smirnov test is a non-parametric test which is used to investigate whether two underlying one-dimensional probability distributions differ. The null hypothesis of the Kolmogorov-Smirnov test is that there is no difference between the distributions. The cut-off point for significance used is $p \leq 0.05$.

2.2.7 *Asellus* sampling

A. meridianus sampling was carried out in Bunaveela Lake, Clogher Lough and Lough Feeagh from February 2007 to September 2008 on a seasonal basis. A sample size of 600 individuals was chosen in accordance with the work of Brattey (1986) and also because it was found during preliminary work for the present study that the prevalence of *A. clavula* in *A. meridianus* changes very little above a sample size of 400 individuals. However, owing to sampling constraints, mainly weather, this sample size was not always achieved.

Bunaveela Lake was sampled in multiple locations (Figure 2.5) in February, May and July 2007, but *A. meridianus* was never found, so sampling ceased after the July 2007

sample. A total of 6760 *A. meridianus* were recovered in 2007 and 2008 from Clogher Lough and Lough Feeagh.

Overall, satisfactory *Asellus* samples were obtained in all sample months, with two notable exceptions. In February 2007, the occasion of the first sample, the sample of isopods obtained from Lough Feeagh was sub-optimal in size. This was due to difficulties in finding locations in the lake where *Asellus* were present. A large number of sites were sampled, however *Asellus* were found in very small numbers. On the next sampling occasion (May 2007) two sites were discovered where *Asellus* are present in relatively high numbers, however one of these sites was not suitable for regular sampling due to unsafe conditions (severe difficulty in accessing the site due to dense vegetation as well as relatively deep water, with a quicksand-like bottom at the site). Both sample sites where *Asellus* are present are small, sheltered bays with dense vegetation, overhanging trees and a boggy/sandy bottom. *Asellus* were found in the high numbers required for this study in water less than two metres deep.

In February 2008, an extremely small sample (43 individuals) was recovered from Clogher Lough. This low sample size was due to inclement weather. In the weeks prior to sampling, heavy rainfall was recorded and the lake flooded, which resulted in a lack of access to the regular sampling site.

Flooding was also encountered in Lough Feeagh in September 2007 and as a result, it was necessary to delay sampling until November 2007, when the flooding had dissipated and the water level had again reached a normal level.

2.2.8 *Asellus* collection and processing

A. meridianus were recovered by kick-sampling using a sweep net with a mesh size of 1mm and transported back to the laboratory where they were immediately placed in aerated containers. All collected *A. meridianus* were then scanned for infection by examining the abdomen of each individual under a dissection microscope in a watch

glass of lake water (when infected with *A. clavula*, the cystacanth/acanthella is visible through the ventral surface of *A. meridianus*). Uninfected individuals were preserved whole in 70% ethanol for later measurement and dissection. All uninfected isopods were dissected in order to ensure that no *A. clavula* infections were overlooked. Infected individuals were killed by pinching their heads with a fine forceps and were measured, sexed and dissected in turn. The length of each isopod was measured from the anterior margin of the cephalothorax to the posterior margin of the abdomen using digital vernier callipers. The sex of each isopod was determined based on the morphology of the pleopods. Males have two pairs of pleopods, while females lack the first pair (Gledhill *et al.*, 1976) (Figure 2.5).

Female: note one rounded pleopod (X)

Male: note copulatory style (Y) and pleopod 1 (Z)

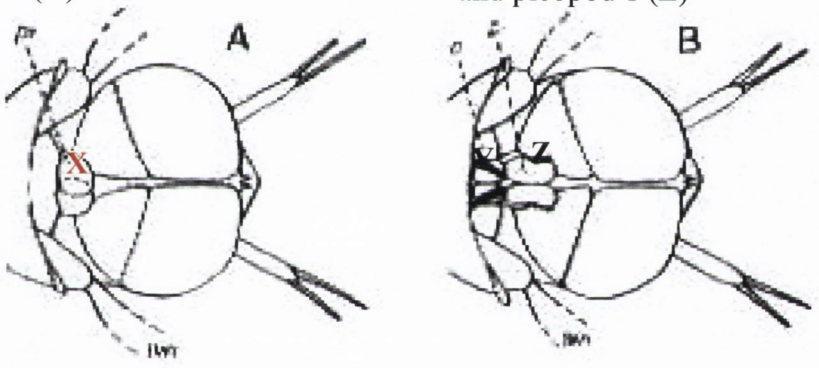


Figure 2.5 Ventral view of the (A) female and (B) male *A. meridianus* abdomen. The female has one pair of pleopods, which is equivalent to pleopod 2 in the male. The male has a copulatory style and two pairs of pleopods, one beneath the other (only pleopod 1 is visible)

After dissection, *A. clavula* cystacanths were activated in a bile salt solution (0.01 - 0.02% sodium taurocholate) at room temperature for 24 – 48 hours, then identified, measured and sexed. Activated cystacanths were fixed in 6% formalin for 24 – 48 hours and preserved in 70% ethanol with 4% glycerol for long-term storage.

Aggregation of the *A. clavula* population in *A. meridianus* was measured using variance to mean ratios. Generally in parasite populations, the variance-to-mean ratio is greater

than one, which indicates an overdispersed or aggregated population of parasites (Anderson & Gordon, 1982).

2.2.9 Behavioural alteration experiments

2.2.9.1 *Asellus* Sampling and Processing

The *A. meridianus* used for behavioural alteration experiments were collected in Lough Feeagh in early July 2008, separately from the *A. meridianus* collected for the population dynamics study. Once transported back to the laboratory, *A. meridianus* individuals were sorted from detritus and placed in a transparent basin, where their ventral surfaces were viewed from below. Five infected and five uninfected *A. meridianus* of similar size (4-6mm) were removed from the basin for each replicate of each experiment using a 3ml plastic Pasteur pipette. Each of the ten test individuals were maintained in separate containers of lake water until ready for testing. Individuals in each replicate were tested in random order.

2.2.9.2 Light/Dark Preference Experiment

A clear plastic tank (29x20x20cm) was split into light and dark halves by covering one half of the tank with black sugar paper. The tank was filled to a depth of 7cm with lake water. A fibre optic light (Olympus Highlight 2001) was shone directly above the uncovered portion of the tank. Experiments were carried out on five replicates of ten individuals (five infected and five uninfected). Individuals were tested separately. Within each replicate, individuals were processed in random order. The testing period consisted of fifteen minutes, comprising five minutes acclimatisation followed by ten minutes testing. At the beginning of the test period, isopods were placed at the division between the light and dark halves of the tank.

The amount of time (in seconds) the individual spent in the light half of the tank was noted, as well as the number of times each individual crossed the boundary from the light

half to the dark half and vice versa. After the experimental period, each individual was dissected. Upon dissection, if an individual was found to be gravid or contain multiple *A. clavula* cystacanths, a replacement individual was tested, in order to avoid the possible introduction of confounding variables.

2.2.9.3 Activity experiment

The tank used, the test period, replicates, dissections and replacement protocols were as for the light/dark experiment. A $2cm^2$ grid was constructed and placed below a clear plastic tank (Figure 2.6). Test individuals were placed in the centre of the tank at a junction between squares at the beginning of the testing period. The number of times an *Asellus* moved from one square into another was noted.

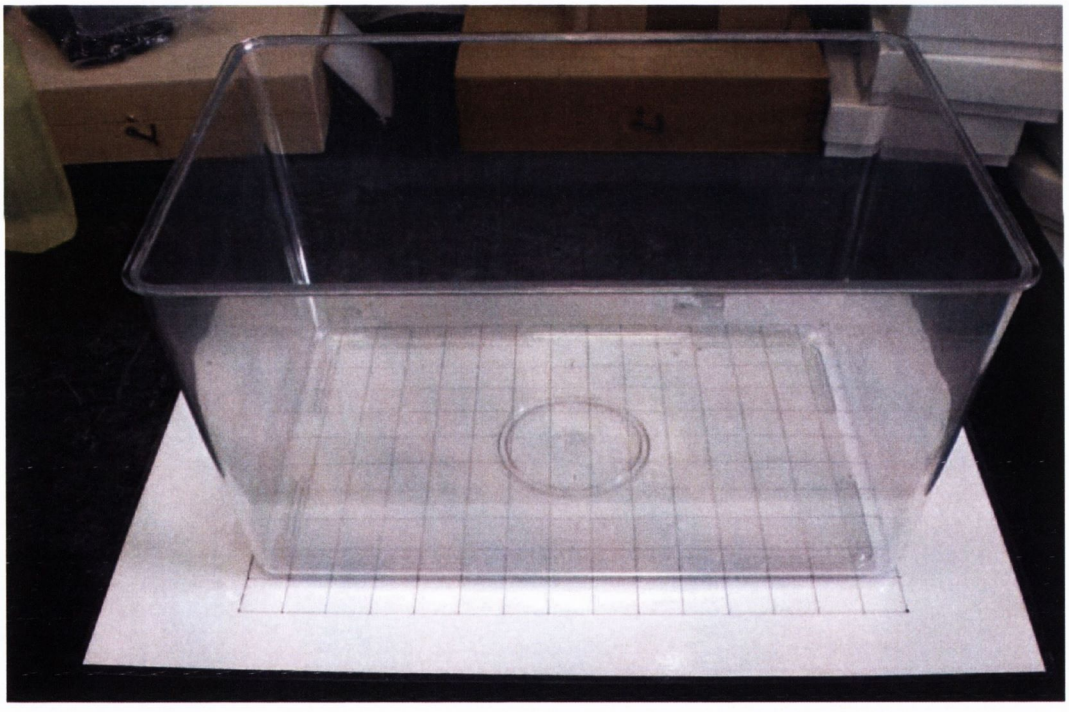


Figure 2.6 Example of grid used underneath tank for activity experiment

2.3 Results

2.3.1 *Asellus* sampling

Satisfactory samples of *Asellus* were obtained in all sample months in both lakes, with the exception of February 2007 in Lough Feeagh and February 2008 in Clogher Lough, the former due to difficulty in locating *Asellus* in the lake, the latter due to inclement weather (Figures 2.7 & 2.8).

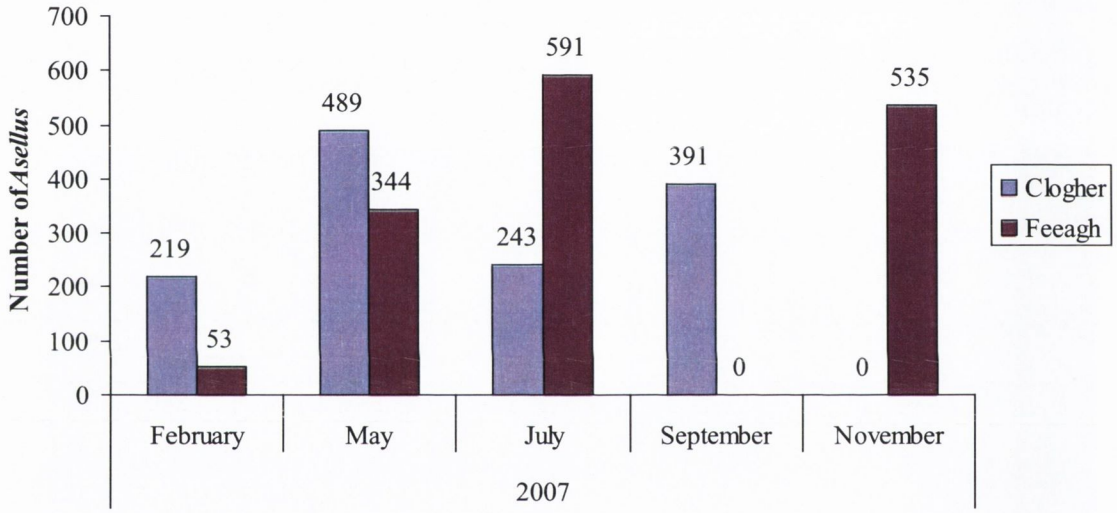


Figure 2.7 Numbers of *Asellus* sampled in Clogher Lough and Lough Feeagh in 2007

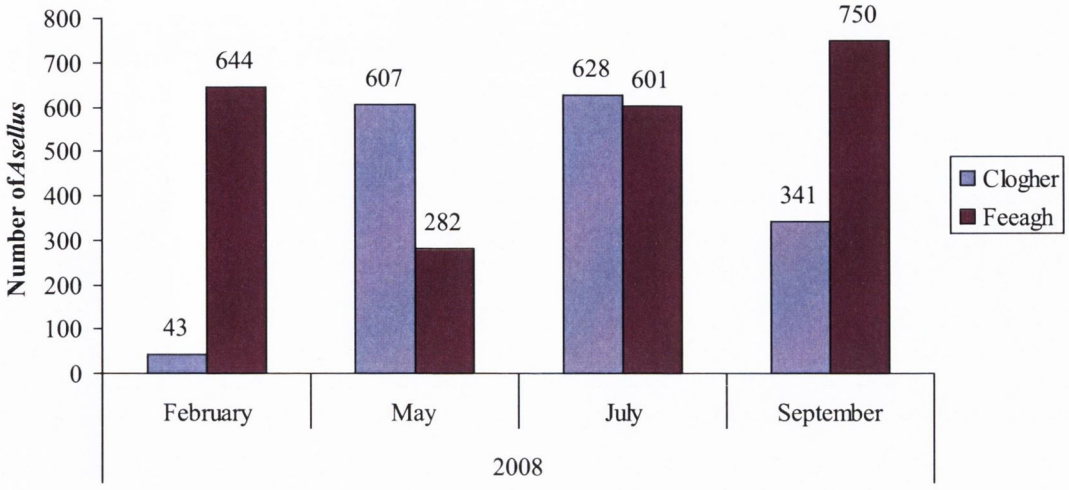


Figure 2.8 Numbers of *Asellus* sampled in Clogher Lough and Lough Feeagh in 2008

2.3.2 Prevalence, abundance and intensity of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh

Prevalence and abundance values differ greatly between the lakes; with Lough Feeagh showing much higher values than those of Clogher Lough in all months (Tables 2.1 & 2.2).

Table 2.1 Overall prevalence and abundance of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh

Lake	N	Prevalence (%)	Mean Abundance ± S.E.
Clogher	2961	1.9	0.02 ± 0.003
Feeagh	3799	11.4	0.14 ± 0.007

Table 2.2 Prevalence and abundance of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh by month

Month	Lake	N	No. Asellus infected	Prevalence (%)	Mean Abundance ± S.E.
2007					
February	Clogher	219	1	0.46	0.005 ± 0.005
	Feeagh	53	0	0	0
May	Clogher	489	10	2.0	0.020 ± 0.006
	Feeagh	344	45	13.1	0.151 ± 0.022
July	Clogher	243	19	7.8	0.078 ± 0.017
	Feeagh	591	78	13.2	0.173 ± 0.020
September	Clogher	391	6	1.5	0.021 ± 0.0
November	Feeagh	535	76	14.2	0.186 ± 0.022
2008					
February	Clogher	43	0	0	0
	Feeagh	644	36	5.6	0.059 ± 0.01
May	Clogher	607	2	0.33	0.003 ± 0.002
	Feeagh	282	71	25.2	0.298 ± 0.032
July	Clogher	628	11	1.8	0.019 ± 0.006
	Feeagh	601	85	14.1	0.171 ± 0.018
September	Clogher	341	8	2.4	0.032 ± 0.012
	Feeagh	750	40	5.3	0.066 ± 0.011

In both lakes, prevalence was significantly different between seasons within years (Table 2.3)

Table 2.3 Results of χ^2 analysis on prevalence of infection within years in Clogher Lough and Lough Feeagh

Lake	N	χ^2	df	p
Clogher				
2007	1342	31.45	3	≤ 0.0001
2008	1619	8.948	3	0.03
Feeagh				
2007	1523	8.586	3	0.0353
2008	2277	117.6	3	≤ 0.0001

The patterns of infection across seasons also differ between the lakes (Figure 2.9).

In Clogher Lough, a peak in prevalence of 7.8% was observed in July 2007. In all other months prevalence was extremely low, ranging from just 0.33% in May 2008 to 2.4% in September 2008.

In Lough Feeagh, a peak in prevalence of 25.2% was observed in May 2008. In February 2007, insufficient numbers of *Asellus* were recovered in Lough Feeagh and no infected individuals were found. The lowest prevalences occurred in February and September 2008 (5.6 and 5.3%, respectively). In all other months, a prevalence of infection of between 13 and 14% was maintained.

Mean abundance of infection follows an identical pattern to that of prevalence in both lakes (Figure 2.1).

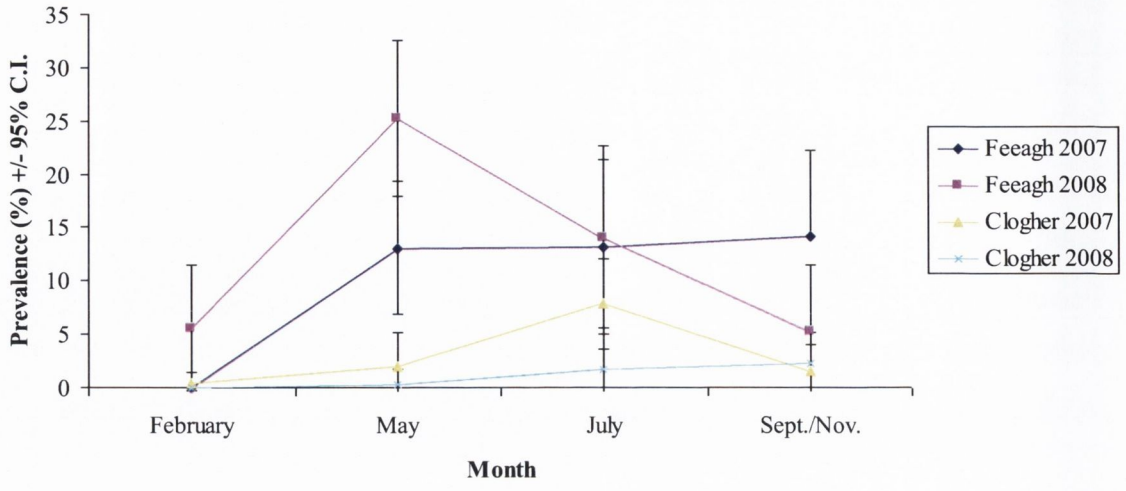


Figure 2.9 Prevalence ± 95% C.I. of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh from February 2007 to September 2008

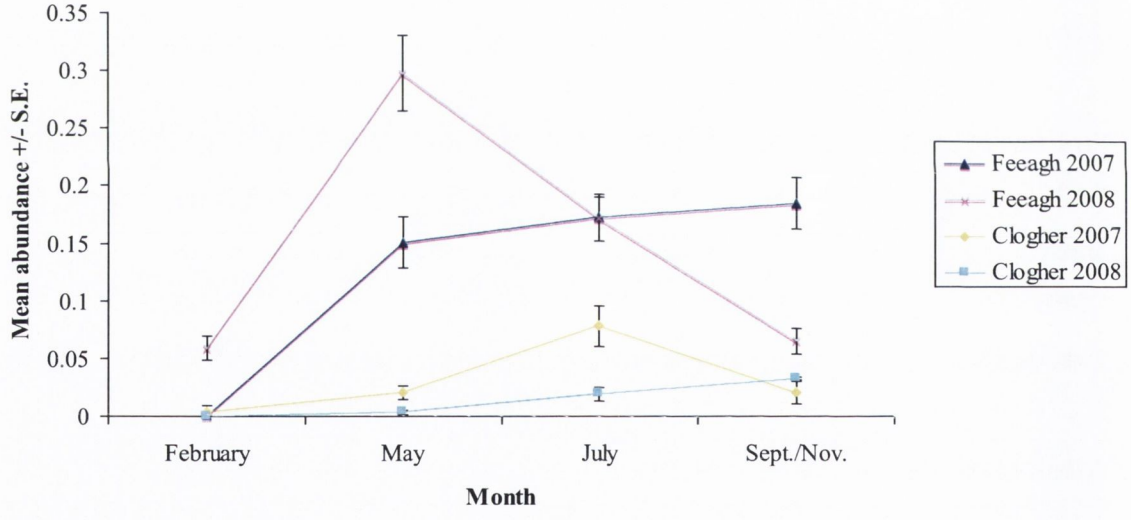


Figure 2.10 Mean abundance ± S.E. of *A. clavula* in *A. meridianus* in Clogher Lough and Lough Feeagh from February 2007 to September 2008

In Table 2.4, the mean abundance was calculated, along with the variance and variance-to-mean ratio. In Clogher Lough and Lough Feeagh however, a variety of results were found. In Clogher Lough the population was underdispersed (ratio <1) in May and July '07, overdispersed in September '07 and randomly dispersed (ratio=1) in February and May '08. In Lough Feeagh the population was marginally overdispersed in all months.

Table 2.4 Seasonal changes in infection of *A. meridianus* by *A. clavula* in Clogher Lough and Lough Feeagh. C denotes Clogher Lough; F denotes Lough Feeagh

Month	No. Examined		No. infected		Total no. of cysts		Mean abundance		Variance		Variance:mean ratio	
	C	F	C	F	C	F	C	F	C	F	C	F
2007												
Feb.	219	53	1	0	1	0	0.005	0	0.005	0	1	0
May	600	325	10	45	10	52	0.054	0.15	0.051	0.169	0.944	1.267
Jul.	601	390	19	71	19	92	0.078	0.31	0.072	0.379	0.923	1.223
Sept.	500	0	6	0	8	0	0.020	0	0.035	0	1.75	0
Nov.	0	600	0	75	0	108	0	0.31	0	0.417	0	1.345
2008												
Feb.	43	600	0	31	0	33	0	0.09	0	0.097	0	1.077
May	600	281	2	71	2	84	0.006	0.298	0.006	0.302	1	1.007

No dead or encysted larvae were found in isopods. The presence of dead or encysted larvae is widely used as an indicator of host response to parasite infection.

2.3.3 Multiple infections of *A. meridianus* by *A. clavula* in Clogher Lough and Lough Feeagh

The occurrence of multiple infections was also explored. In Clogher Lough, multiple infections were extremely rare (Table 2.4), indeed only five individuals were found to have a multiple infection. In Lough Feeagh however, infections of ≥ 2 *A. clavula* larvae were frequent (19%) and occurred in all months except February 2007 (Table 2.5).

Table 2.5 Abundance of *A. clavula* cystacanths in *A. meridianus* in Clogher Lough from February 2007 to September 2008

Month	**0**	**1**	**2**	**3**
February	218	1	0	0
May	479	10	0	0
July	224	19	0	0
September	385	5	0	1
February	43	0	0	0
May	605	2	0	0
July	617	10	1	0
September	333	5	3	0

2.3.4 Prevalence and abundance and *Asellus* size

In Clogher Lough, there were a total of 57 infected *Asellus*, while in Lough Feeagh 430 infected individuals were recovered. *Asellus* were divided into 1mm length categories. In Clogher Lough, prevalence of *A. clavula* increased with isopod size to a peak in 4 - 5mm isopods and declined thereafter (Figure 2.11). In Clogher Lough, larvae were not observed in isopods of <3mm or >7mm. In Lough Feeagh just 0.27% of infected isopods were in the <3mm category, while 4.9% of infected isopods were in the >7mm category (Figure 2.12).

Table 2.6 Abundance of *A. clavula* cystacanths in *A. meridianus* in Lough Feeagh from February 2007 to September 2008

Month	0	1	2	3	4
February	53	0	0	0	0
May	299	38	7	0	0
July	513	57	18	3	0
November	459	60	10	5	1
February	609	32	3	0	0
May	211	58	13	0	0
July	516	70	12	3	0
September	710	33	5	2	0

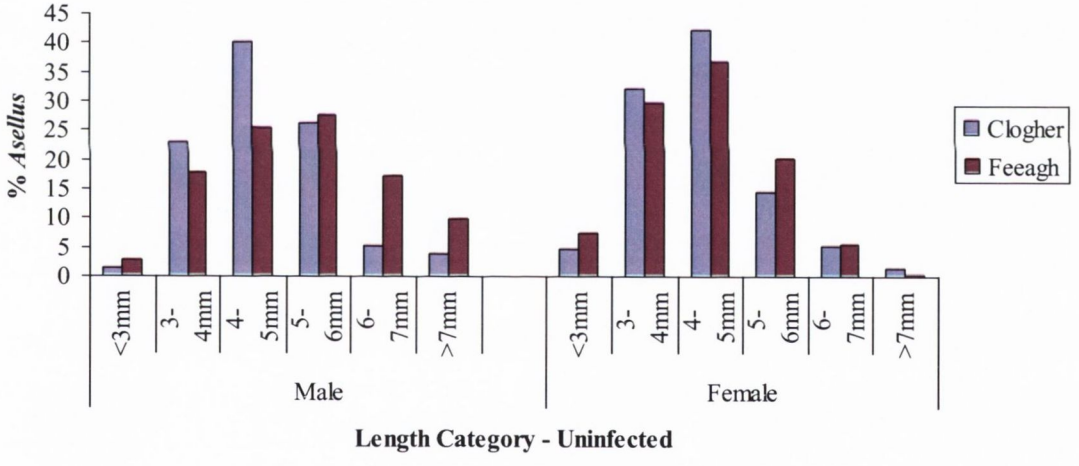


Figure 2.11 Size distribution of uninfected *A. meridianus* in Clogher Lough and Lough Feeagh

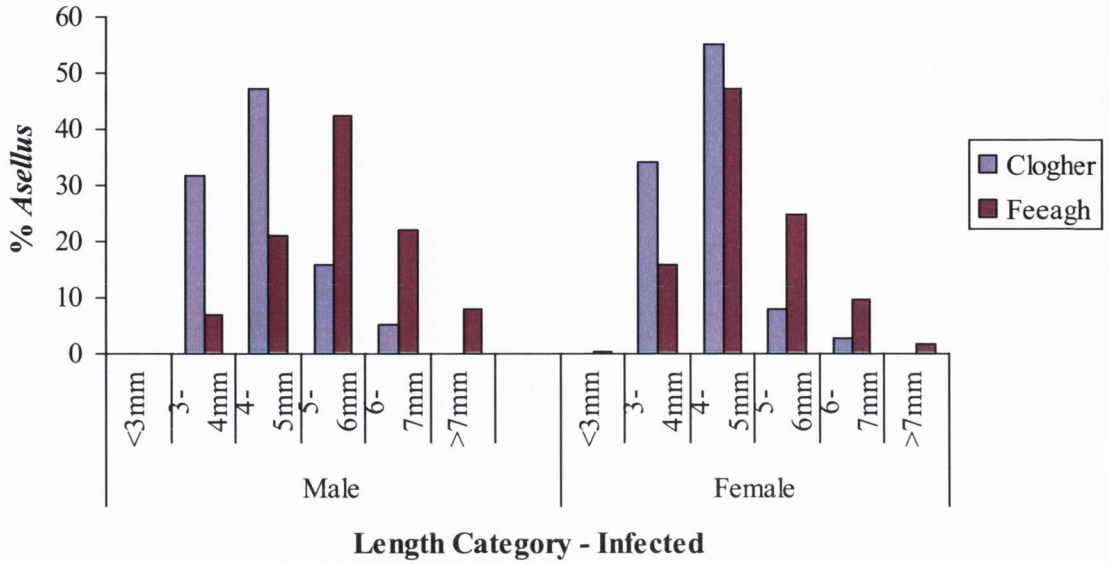


Figure 2.12 Size distribution of infected *A. meridianus* in Clogher Lough and Lough Feeagh

The distribution of multiple infections among the length categories was also explored. In Lough Feeagh, the distribution of multiple infections among length categories follows a similar pattern to that of prevalence, with the occurrence of multiple infections reaching a peak in individuals measuring between 5mm and 6mm and declining thereafter (Table 2.7 & Figure 2.13).

Table 2.7 Distribution of multiple infections of *A. clavula* among *A. meridianus* in Lough Feeagh

Abundance (# cysts)	**<3mm (n)**	**3-4mm (n)**	**4-5mm (n)**	**5-6mm (n)**	**6-7mm (n)**	**>7mm (n)**
2	0	7	25	21	11	2
3	0	0	4	5	3	1
4	0	0	0	1	0	0

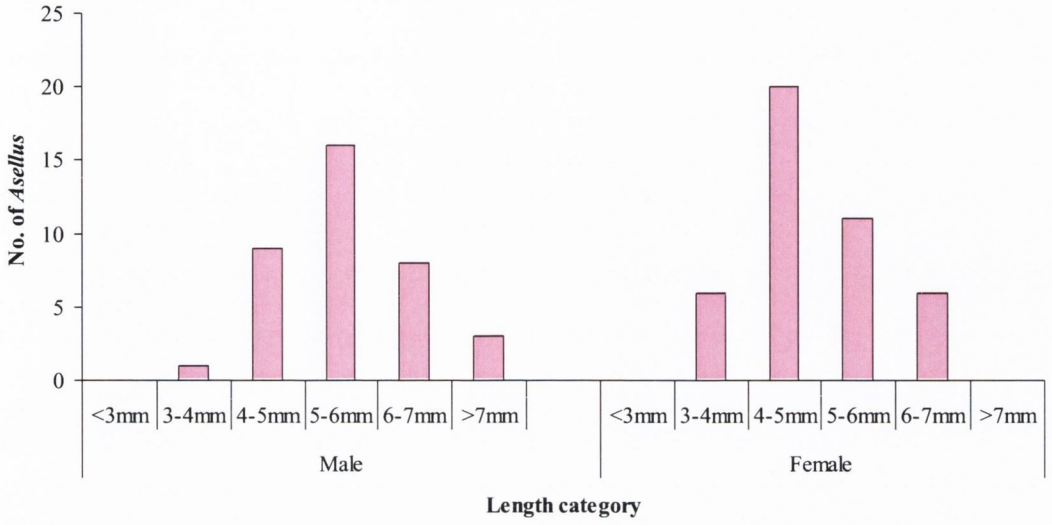


Figure 2.13 Distribution of multiple infections of *A. meridianus* by *A. clavula* in Lough Feeagh among size categories of *A. meridianus*

2.3.5 Sex breakdown of the population of *A. meridianus* in Clogher Lough and Lough Feeagh

The sample of *A. meridianus* from Clogher Lough consisted of 2961 individuals, of which 1791 (60.5%) were female and 1170 (39.5%) were male. The sample of *A. meridianus* from Lough Feeagh consisted of 3799 individuals, of which 2167 (57%) were female and 1632 (43%) were male.

The sex breakdown of the population within the lakes differed significantly between seasons, with the exception of Lough Feeagh in 2008, although females comprised the majority of the population in every season in both lakes (Figures 2.14 & 2.15).

Table 2.8 Results of χ^2 analysis on sex of *Asellus* and season in Clogher Lough and Lough Feeagh in 2007 and 2008

Lake	N	χ^2	Df	P
Clogher				
2007	1342	14.86	3	0.0019
2008	1619	46.64	3	≤0.0001
Feeagh				
2007	1523	21.7	3	≤0.0001
2008	2277	7.01	3	0.0717

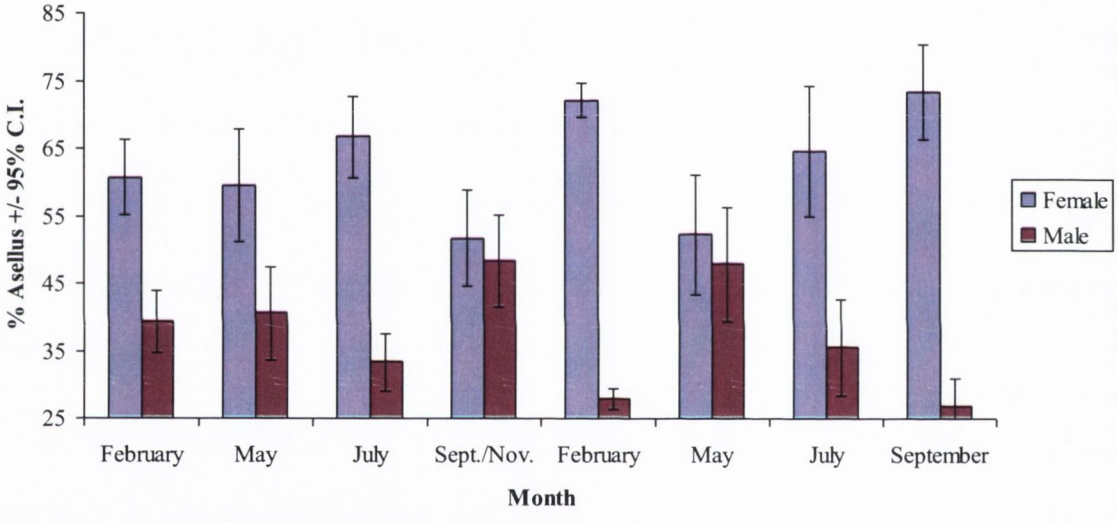


Figure 2.14 Sex breakdown of the population of *A. meridianus* from Clogher Lough from February 2007 to September 2008

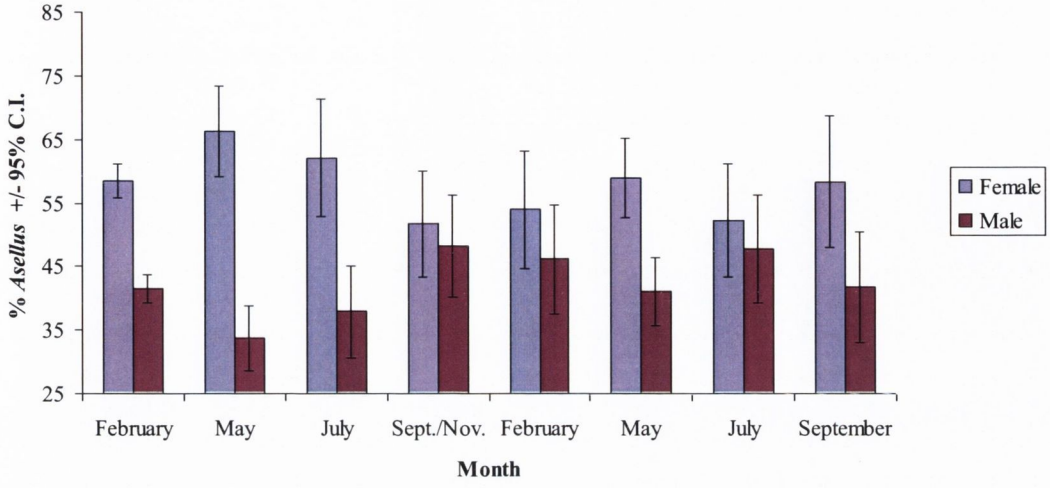


Figure 2.15 Sex breakdown of the population of *A. meridianus* from Lough Feeagh from February 2007 to September 2008

2.3.6 Factors affecting size of *A. meridianus* in Clogher Lough and Lough Feeagh

The effect of various factors (year, sex, infection status and sample month) on *Asellus* length was explored using Kolmogorov-Smirnov tests. Clogher Lough and Lough Feeagh were analysed separately due to the differences in parasite prevalence and abundance levels between them (Table 2.2).

Asellus length was found to differ significantly between sample years in both Clogher Lough and Lough Feeagh (Table 2.8). As a result, data were split by year for all further analyses of factors affecting *Asellus* length.

In Clogher Lough, more large (≥6mm) *A. meridianus* individuals were found in 2007 than in 2008 (Table 2.9, Figure 2.16), whereas in Lough Feeagh, more large *A. meridianus* individuals were recovered in 2008 (Table 2.9, Figure 2.17).

Table 2.9 Results of Kolmogorov-Smirnov tests investigating factors affecting *A. meridianus* length.

Factor	**Clogher**	**Feeagh**
	p	p
Year	0.000	0.000
Sex		
2007	0.001	0.000
2008	0.000	0.000
Infection status		
2007	0.210	0.000
2008	0.032	0.021

Table 2.10 Number of *A. meridianus* individuals in various length categories in Clogher Lough and Lough Feeagh.

	6- 6.99 mm	**7-7.99m**	**8-8.99mm**	**9-9.99mm**
Clogher				
Lough				
2007	93	36	7	1
2008	62	14	5	0
Lough Feeagh				
2007	163	42	10	2
2008	252	83	25	3

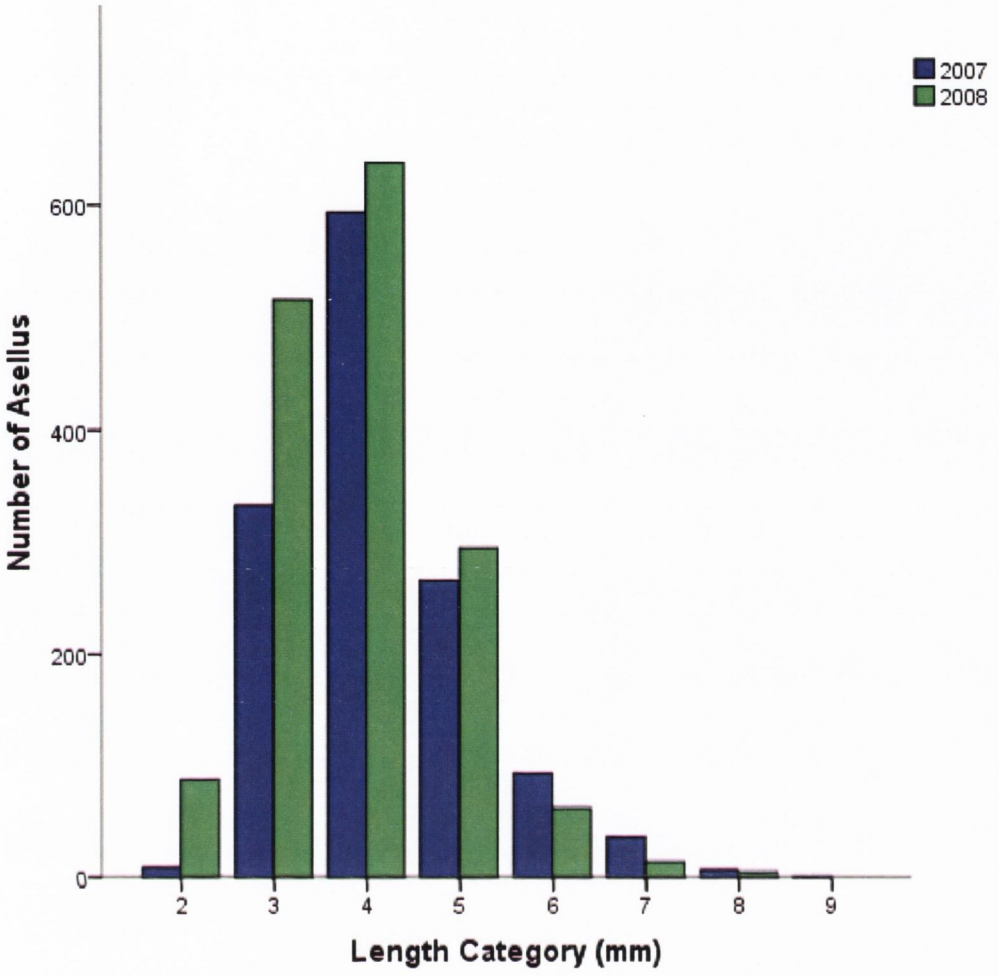


Figure 2.16 Length distributions of *A. meridianus* in Clogher Lough by sample year.

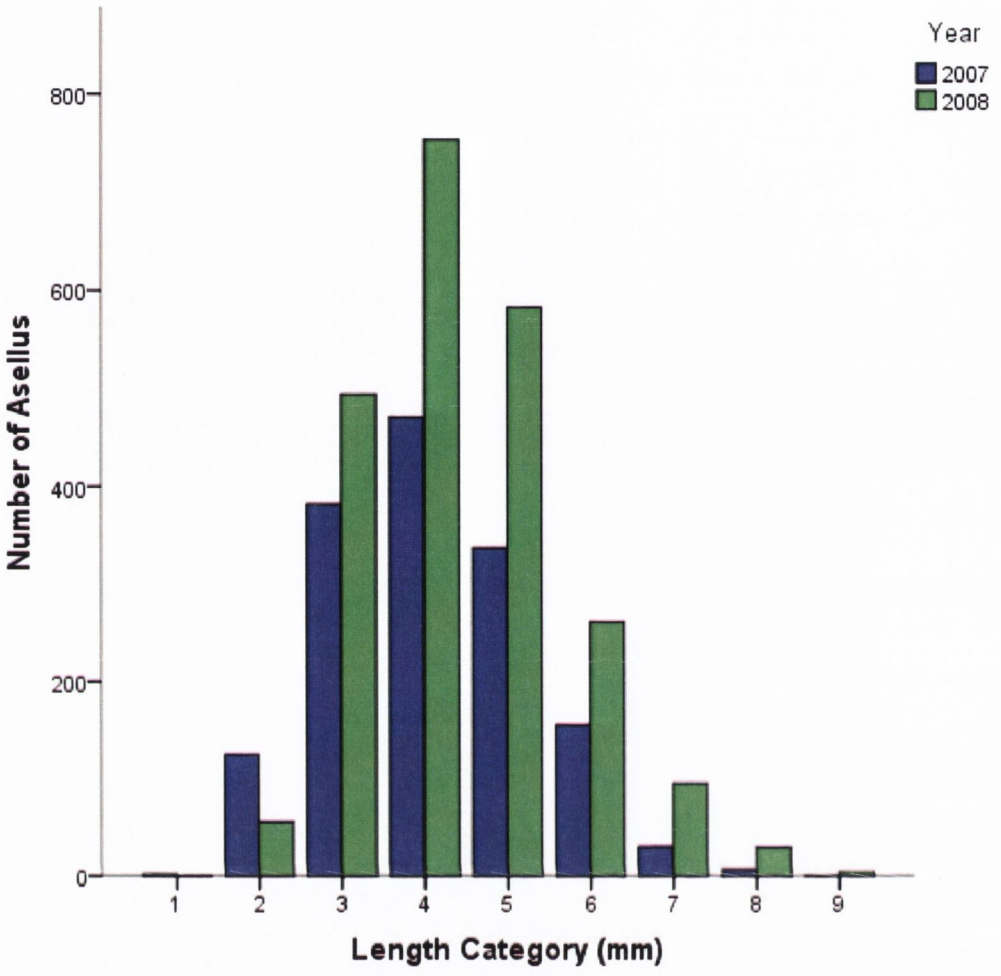


Figure 2.17 Length distributions of *A. meridianus* in Lough Feeagh by sample year.

Sex

In Clogher Lough and Lough Feeagh in both 2007 and 2008 significant differences in *Asellus* length were observed between the sexes (Table 2.11). This difference was due to the fact that males were significantly larger than females in both lakes in both sample years (Figures 2.18, 2.19, 2.20 & 2.21; Table 2.11).

Table 2.11 Mean lengths of male and female *A. meridianus* in Clogher Lough and Lough Feeagh in 2007 and 2008.

	Mean male length ± SD (mm)	**Mean female length ± SD (mm)**
Clogher Lough		
2007	4.81 ± 1.09	4.59 ± 0.93
(n)	(554)	(785)
2008	4.67 ± 0.97	4.19 ± 0.88
(n)	(616)	(1002)
Lough Feeagh		
2007	5.01 ± 1.38	4.32 ± 1.05
(n)	(612)	(897)
2008	5.40 ± 1.21	4.48 ± 0.92
(n)	(1012)	(1263)

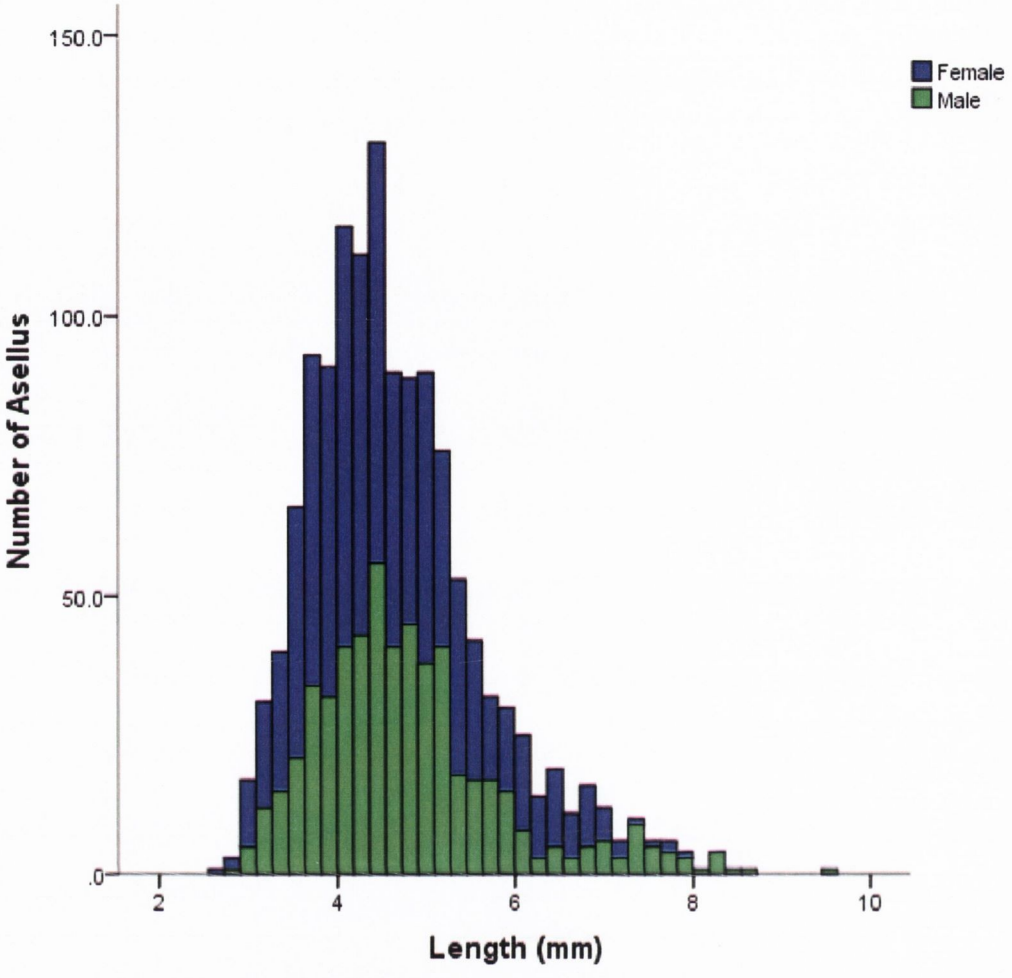


Figure 2.18 Length distributions of male and female *A. meridianus* in Clogher Lough in 2007

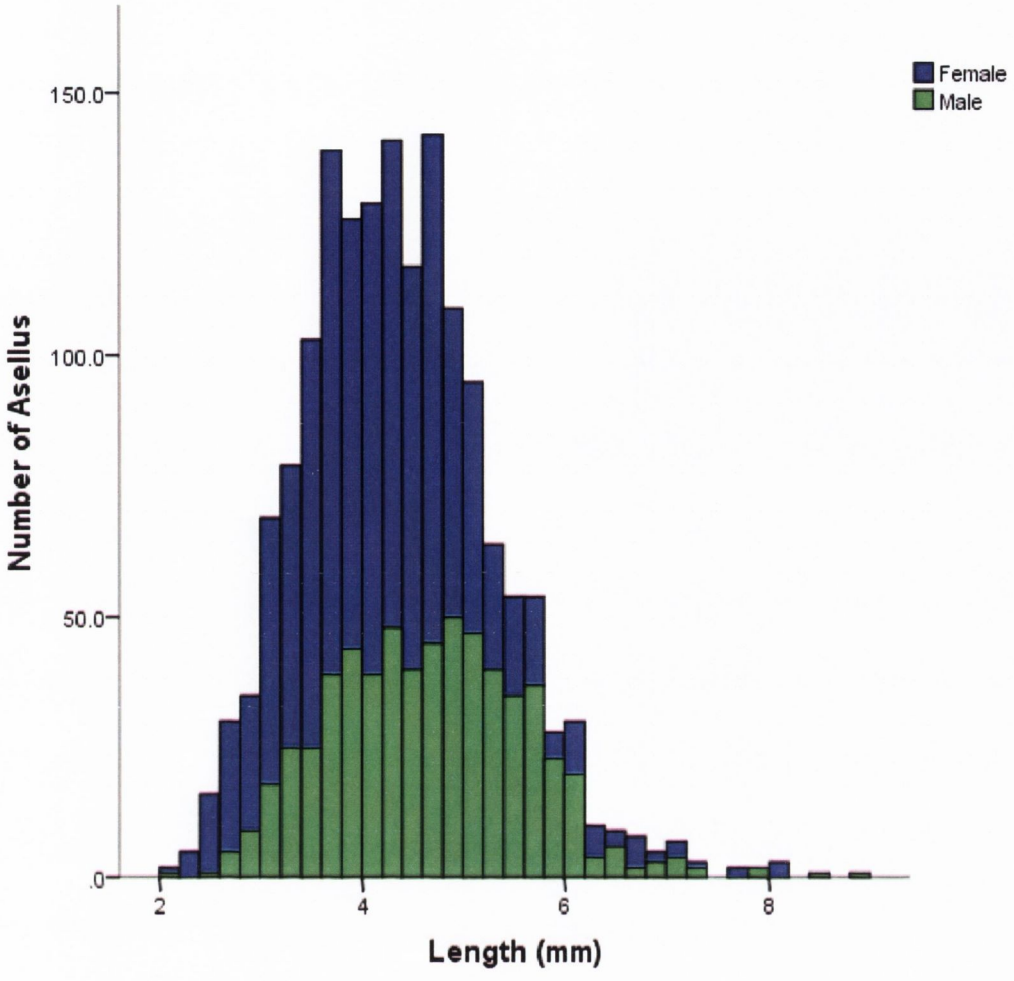


Figure 2.19 Length distributions of male and female *A. meridianus* in Clogher Lough in 2008

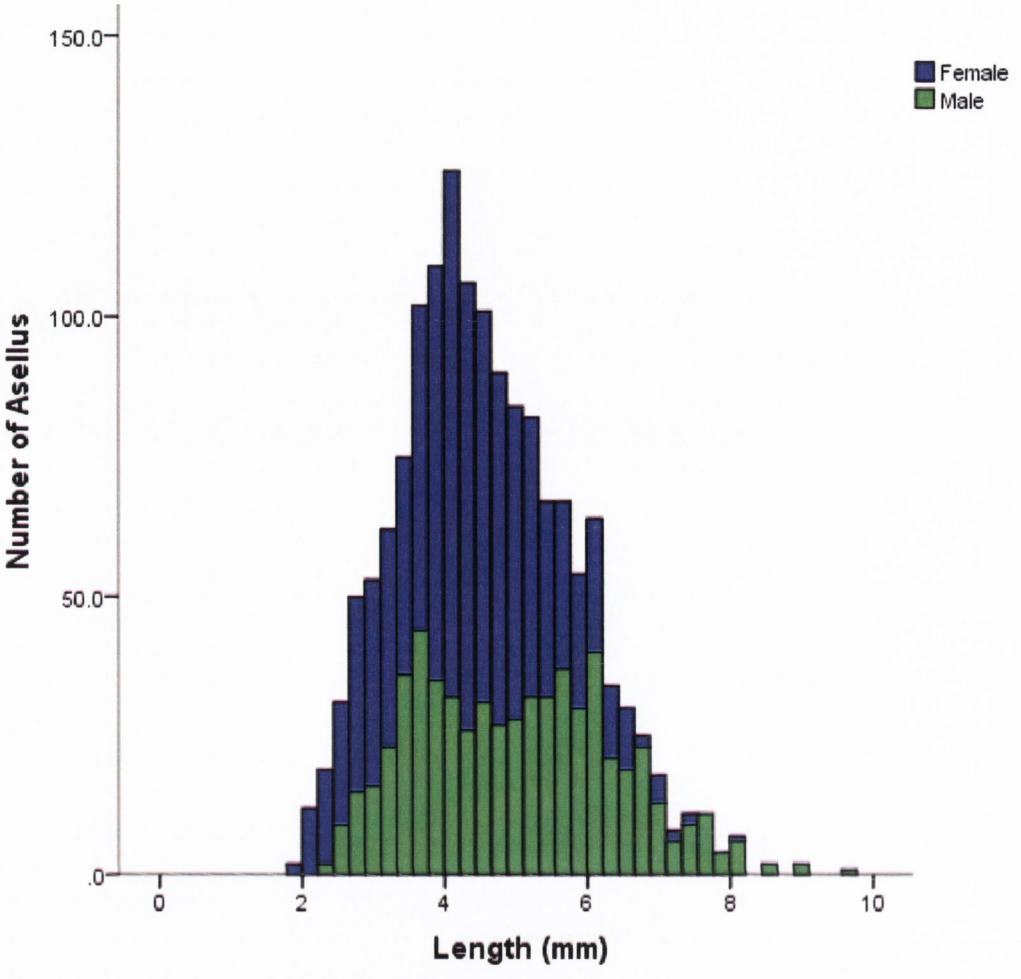


Figure 2.20 Length distributions of male and female *A. meridianus* in Lough Feeagh in 2007

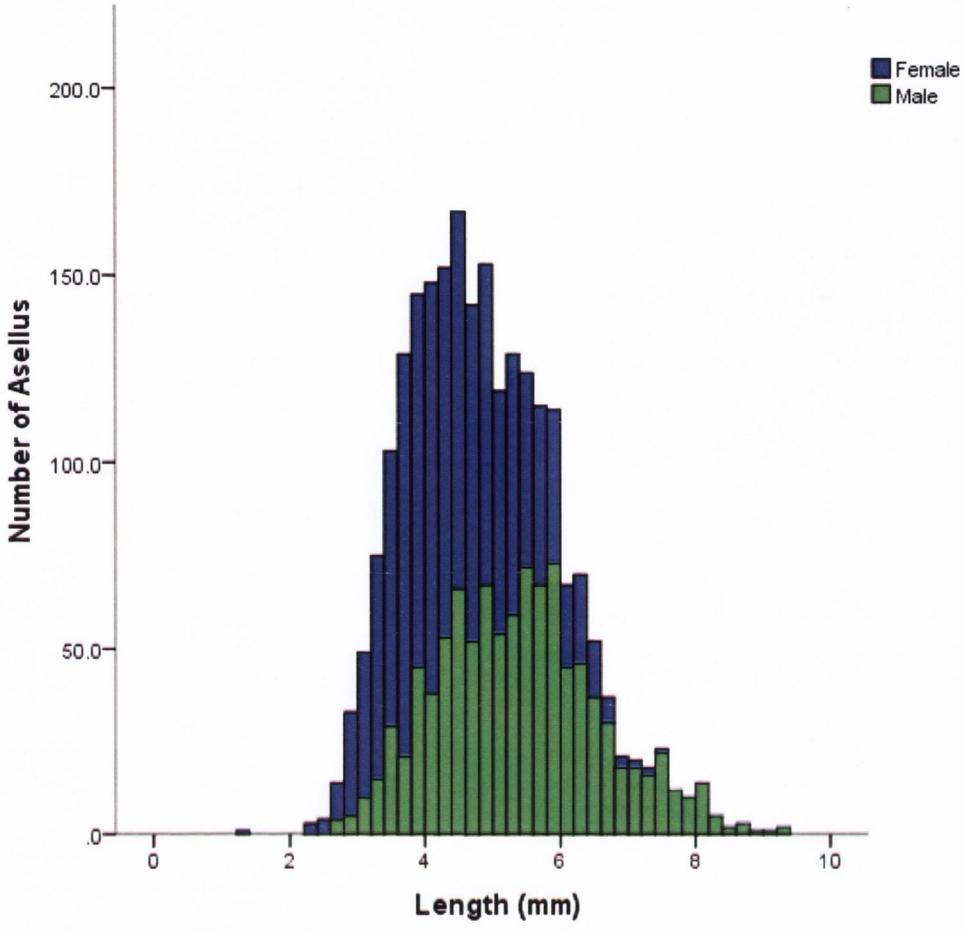


Figure 2.21 Length distributions of male and female *A. meridianus* in Lough Feeagh in 2008

Infection status

In Clogher Lough, contrasting results were found between sample years (Table 2.12). In 2007, no significant difference in *Asellus* length was observed between infected and uninfected individuals, while in 2008 a significant difference was noted (Table 2.12). Infected isopods of both sexes were found to be significantly shorter in length than their uninfected counterparts. Due to the small sample sizes of infected isopods available however, this result should be analysed with caution (Figures 2.22, 2.23, 2.24 & 2.25; Table 2.12).

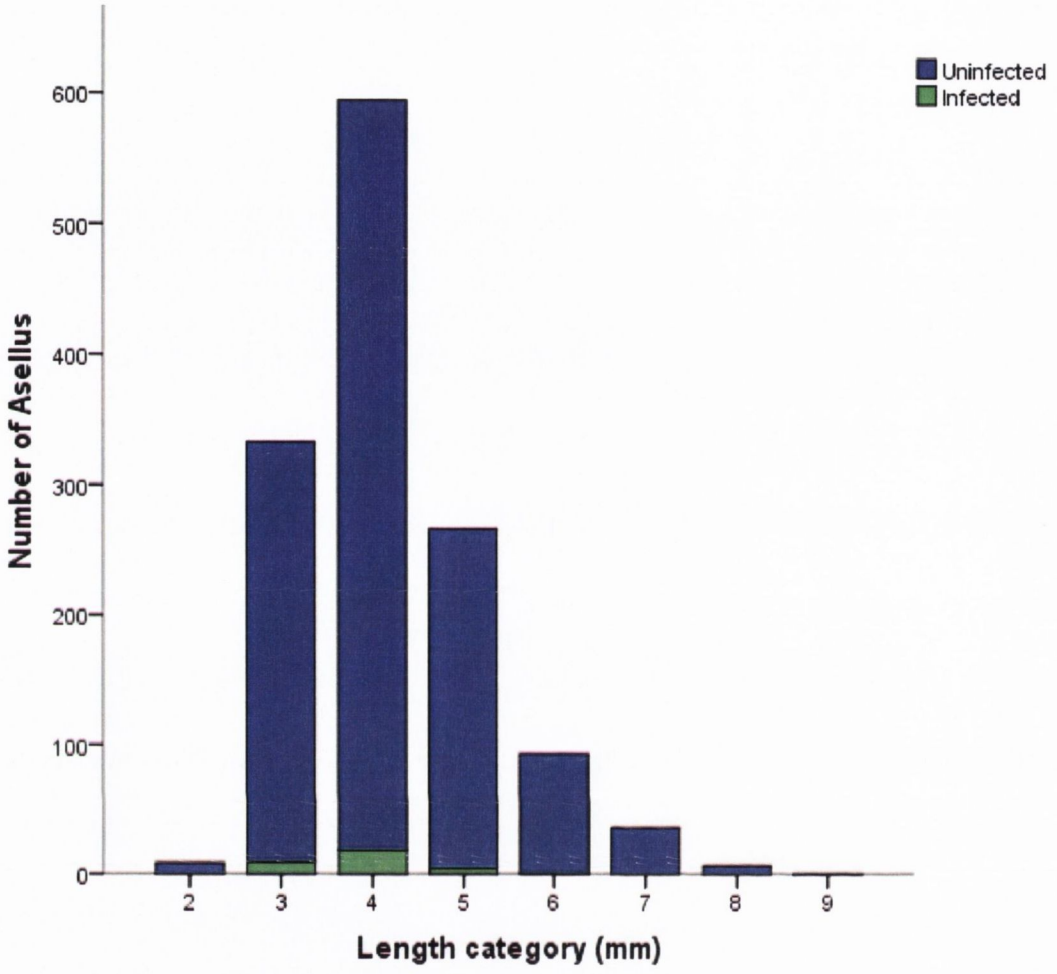


Figure 2.22 Length distributions of infected and uninfected *A. meridianus* in Clogher Lough in 2007.

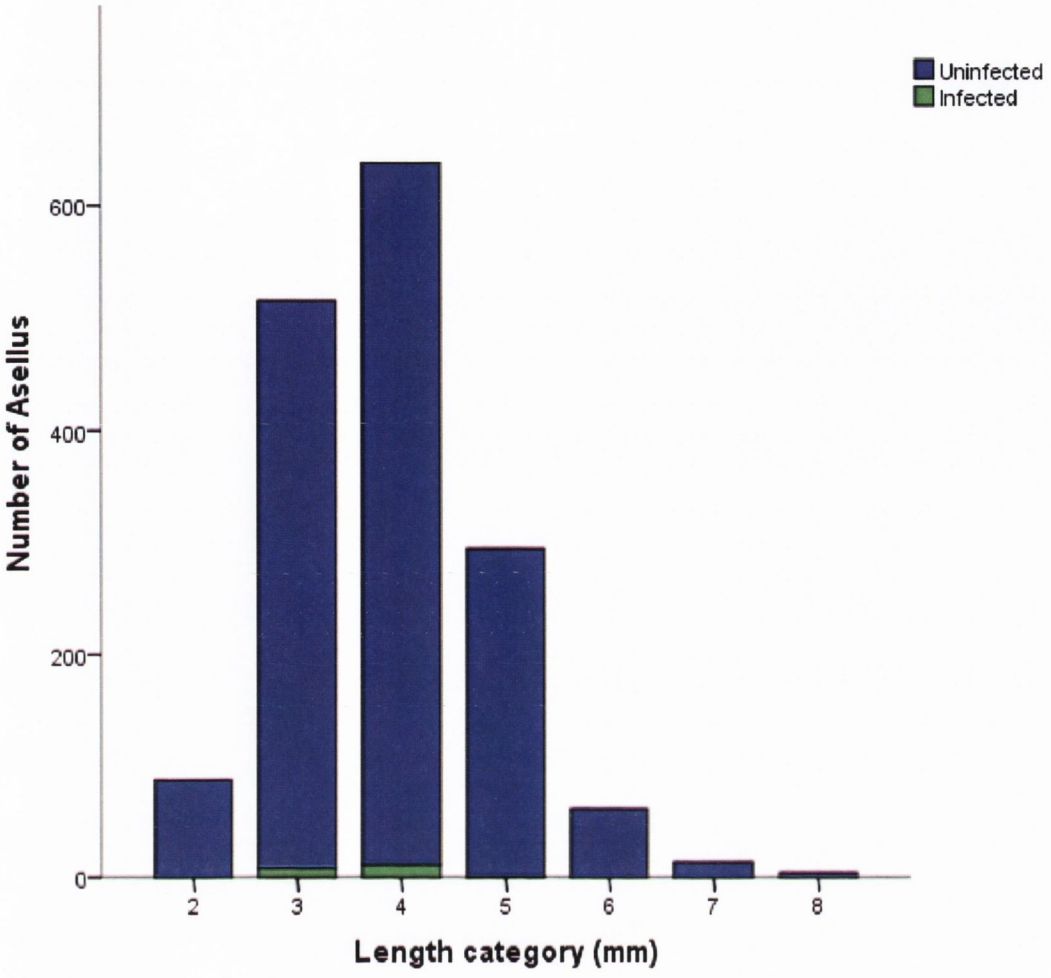


Figure 2.23 Length distributions of infected and uninfected *A. meridianus* in Clogher Lough in 2008.

Table 2.12 Mean lengths of infected and uninfected *A. meridianus* in Clogher Lough in 2007 and 2008.

	Mean infected length ± SD (mm)	**Mean uninfected length ± SD (mm)**
2007		
Male	4.62 ± 0.77	4.81 ± 1.09
(n)	(12)	(542)
Female	4.30 ± 0.47	4.60 ± 0.94
(n)	(23)	(762)
2008		
Male	4.26 ± 0.64	4.67 ± 0.97
(n)	(7)	(609)
Female	4.00 ± 0.50	4.19 ± 0.89
(n)	(15)	(987)

In Lough Feeagh, a significant difference in *Asellus* length was observed between infected and uninfected individuals in both sample years. In 2007, infected individuals of both sexes were significantly larger than uninfected individuals (Table 2.13; Figure 2.24). In 2008 however, while infected female isopods were again larger in size than their uninfected conspecifics, infected male isopods were in fact, on average, smaller than uninfected male isopods (Table 2.13; Figure 2.25).

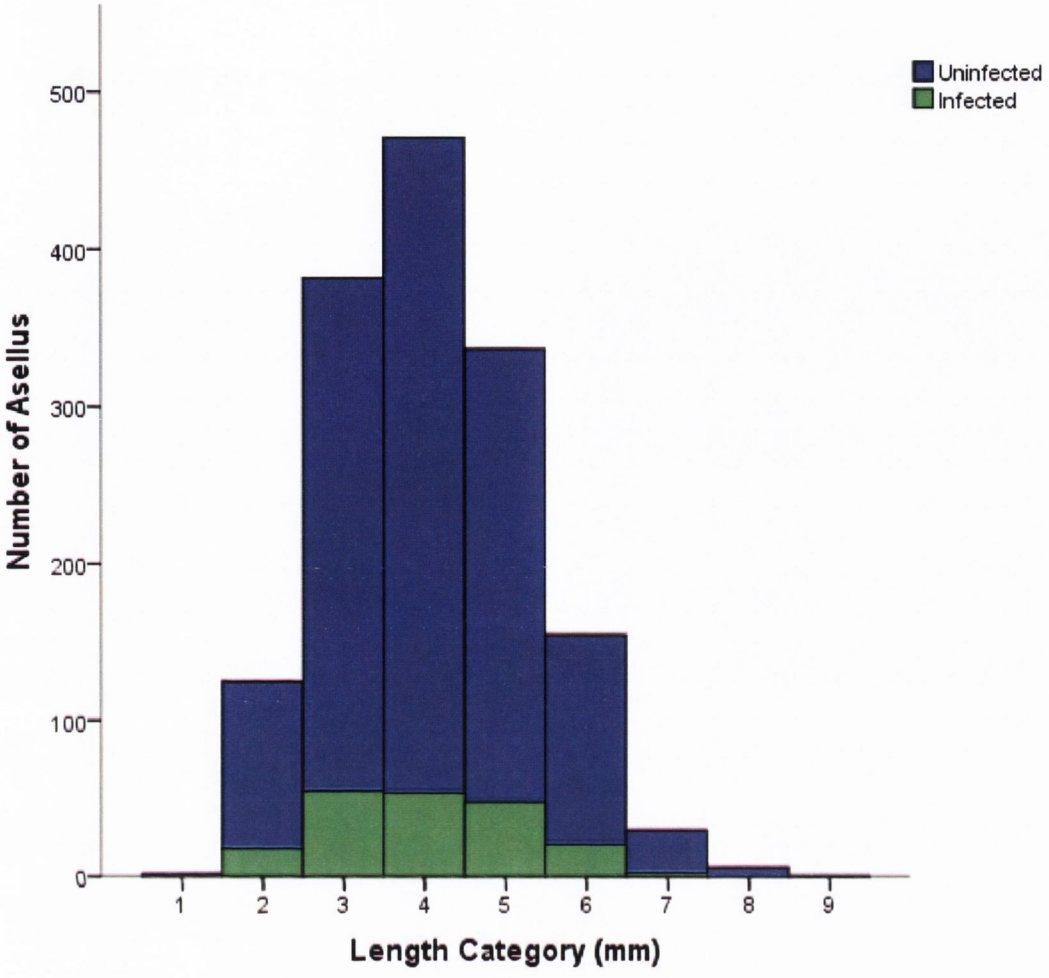


Figure 2.24 Length distribution of infected and uninfected *A. meridianus* in Lough Feeagh in 2007.

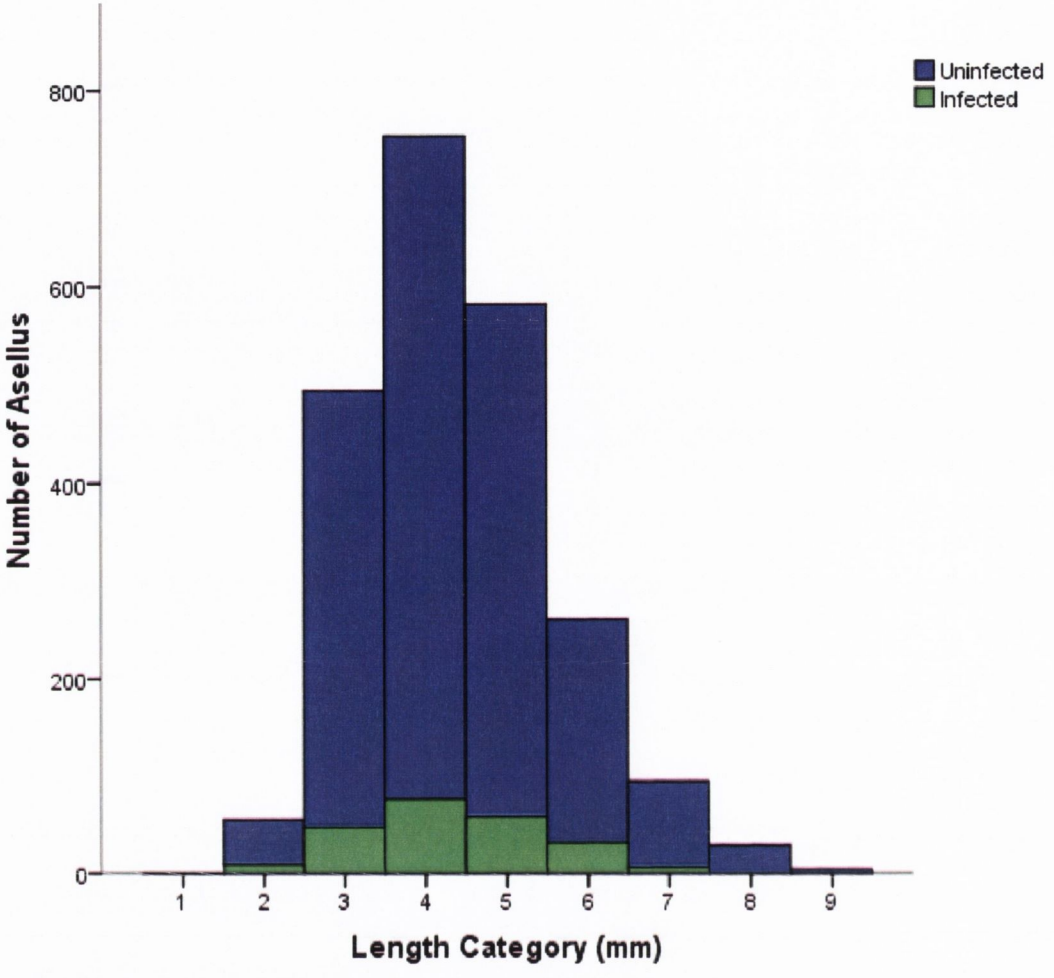


Figure 2.25 Length distributions of infected and uninfected *A. meridianus* in Lough Feeagh in 2008.

Table 2.13 Mean lengths of infected and uninfected *A. meridianus* in Lough Feeagh in 2007 and 2008.

	Mean infected length (mm) ± SD	**Mean uninfected length (mm) ± SD**
2007		
Male	5.93 ± 1.22	4.86 ± 1.34
(n)	(83)	(529)
Female	4.99 ± 1.01	4.23 ± 1.02
(n)	(104)	(104)
2008		
Male	5.23 ± 0.96	5.41 ± 1.23
(n)	(108)	(904)
Female	4.69 ± 0.76	4.45 ± 0.93
(n)	(123)	(1140)

Season

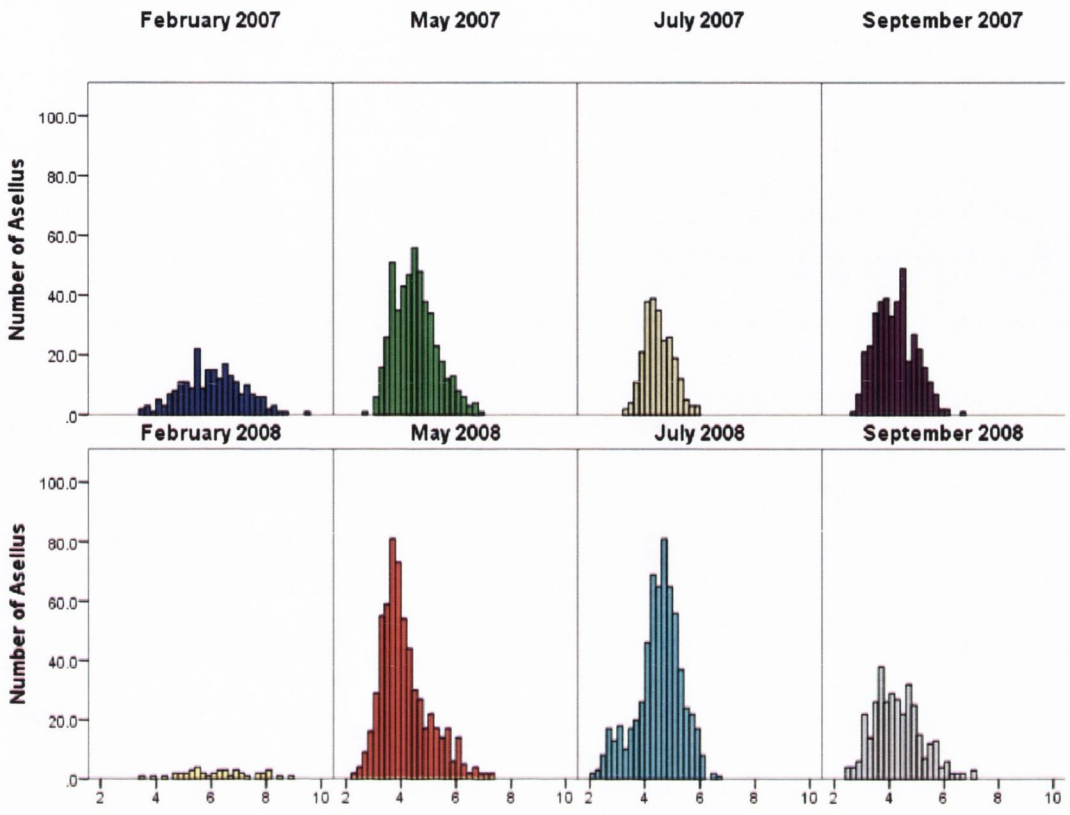
As two samples are the maximum which can be utilised in a Kolmogorov-Smirnov test, pairwise comparisons of sample months were undertaken (Table 2.14).

In Clogher Lough, *Asellus* length differed significantly between all compared months. In Lough Feeagh, *Asellus* length differed significantly between all compared months, with the exception of February v November in 2007 and July v September in 2008 (Table 2.14)

Table 2.14 Results of Kolmogorov-Smirnov tests investigating the effect of sample month on *A. meridianus* length.

	Clogher Lough	**Lough Feeagh**
	p	**P**
2007		
February v May	0.000	0.000
February v July	0.000	0.000
February v Sept./Nov.	0.000	0.104
May v July	0.001	0.000
May v Sept./Nov.	0.000	0.000
July v Sept./Nov.	0.000	0.000
2008		
February v May	0.000	0.000
February v July	0.000	0.000
February v September	0.000	0.000
May v July	0.000	0.000
May v September	0.000	0.000
July v September	0.000	0.132

In Clogher Lough, in February and May of both sample years there were more worms in the larger size classes than in July and September (Table 2.26).


February 2007
May 2007
July 2007
September 2007
Number of Asellus
100.0
80.0
60.0
40.0
20.0
.0
February 2008
May 2008
July 2008
September 2008
Number of Asellus
100.0
80.0
60.0
40.0
20.0
.0
2
4
6
8
10

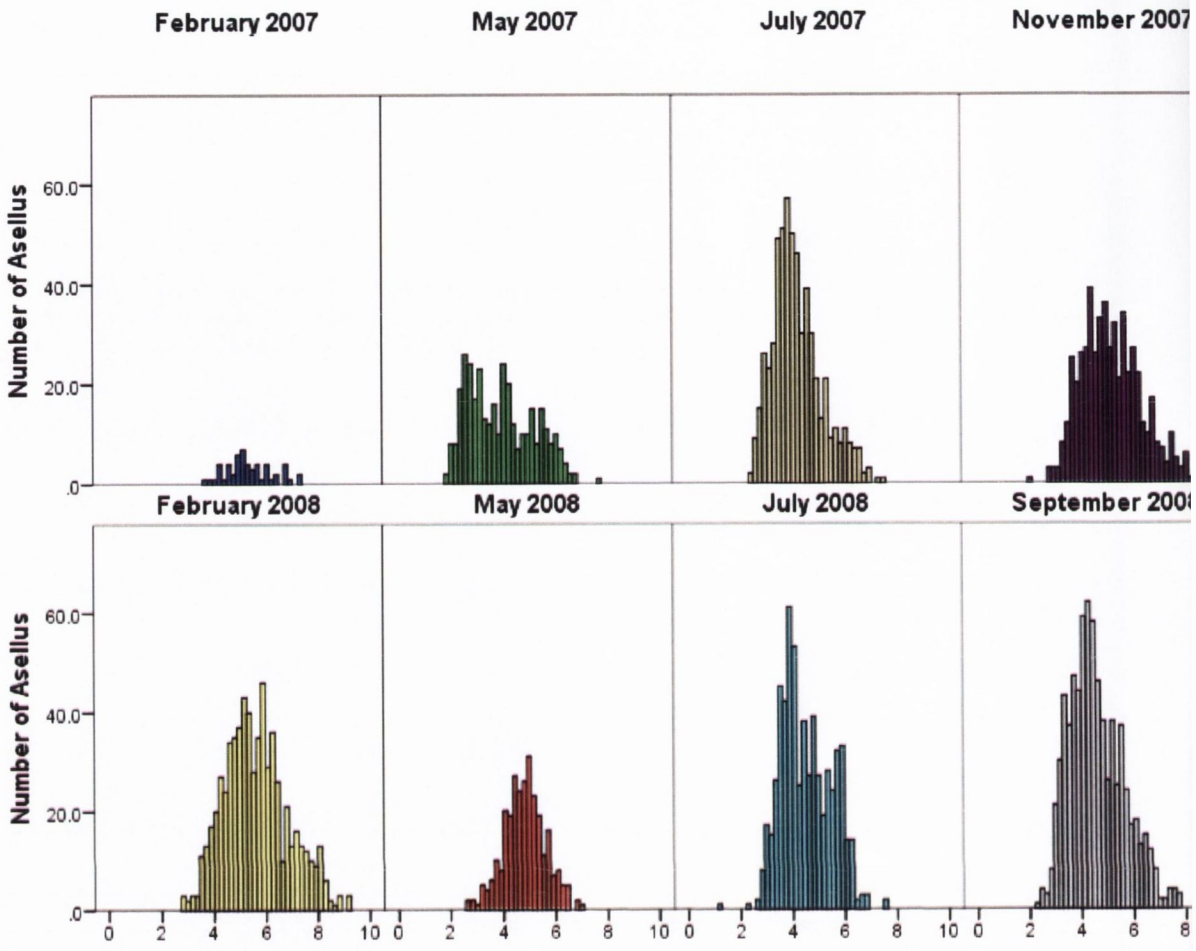


Figure 2.27 Length distributions of *A. meridianus* in Lough Feeagh by sample month.

In Lough Feeagh, individuals in the largest size classes were recovered most frequently in November 2007 and February 2008. In the summer months (May and July) of both 2007 and 2008 the majority of individuals recovered were in the smaller size classes (Table 2.15, Figure 2.27).

Table 2.15 Mean length of male and female *A. meridianus* in Clogher Lough and Lough Feeagh in all sample months.

	Clogher Lough		**Lough Feeagh**	
	Mean male length ± SD (mm)	**Mean female length ± SD (mm)**	**Mean male length ± SD (mm)**	**Mean female length ± SD (mm)**
2007				
February	6.51 ± 1.25	5.76 ± 0.93	5.91 ± 0.95	5.07 ± 0.68
May	4.50 ± 0.67	4.55 ± 0.84	4.40 ± 1.30	3.80 ± 1.17
July	4.82 ± 0.51	4.33 ± 0.43	4.44 ± 1.14	4.17 ± 0.80
Sept./Nov.	4.35 ± 0.74	4.10 ± 0.68	5.68 ± 1.27	4.87 ± 0.96
2008				
February	6.81 ± 1.4	6.03 ± 1.21	6.21 ± 1.31	5.12 ± 0.88
May	4.34 ± 0.91	3.95 ± 0.85	5.01 ± 0.84	4.66 ± 0.72
July	4.96 ± 0.77	4.25 ± 0.74	5.01 ± 0.94	4.07 ± 0.74
September	4.73 ± 0.95	4.15 ± 0.83	5.12 ± 1.10	4.18 ± 0.84

2.3.7 Reproductive cycle of *A. meridianus* in Clogher Lough and Lough Feeagh

Seasonal differences in the reproductive cycle of *A. meridianus* were investigated using the proportion of females with a brood pouch, which gives an approximation of the intensity of breeding at different times of the year (Figures 2.29 & 2.30). Brooding females were present in all months, with the exception of November 2007 in Lough Feeagh. The pattern of occurrence of brooding females was different in each year and in each lake, although the lowest levels of brooding females were always noted in September and November, suggesting that brooding slows or even stops with decreasing water temperatures in winter. In Clogher Lough in 2007, the level of brooding females peaked in February. A small decline was observed in May and a similar level was

maintained in July, before a sharp dip in the level of brooding females was observed in September. In 2008, the peak in brooding females was again seen in February, however there was then a sharp decline in May followed by an increase in July and another sharp decline in September (Figure 2.28).

In Lough Feeagh in 2007, similarly high levels of brooding were observed in February and July, with a dip in May and no brooding females observed in November. In 2008, however, similar levels of brooding were observed in February, May and July, followed by a sharp dip in September (Figure 2.29). In both lakes, the levels of brooding females differed significantly between sample months (Table 2.16).

Table 2.16 Results of χ^2 analysis on *Asellus* brooding and sample month in Clogher Lough and Lough Feeagh.

Lake	**N**	χ^2	**Df**	**P**
Clogher	1790	190.6	7	≤ 0.0001
Feeagh	2166	241.4	7	≤ 0.0001

The percentage of brooding females was higher in Clogher Lough than in Lough Feeagh in all months, with the exception of May 2008.

With the exception of three individuals (two of which carried empty brood pouches, while one carried eggs), no infected female *A. meridianus* were brooding. There was a significant difference in brooding between infected and uninfected isopods in both lakes (Table 2.17).

Table 2.17 Results of χ^2 analysis on *Asellus* brooding and infection status in Clogher Lough and Lough Feeagh.

Lake	**N**	χ^2	**df**	**p**
Clogher	1790	18.98	1	≤ 0.0001
Feeagh	2167	71.08	1	≤ 0.0001

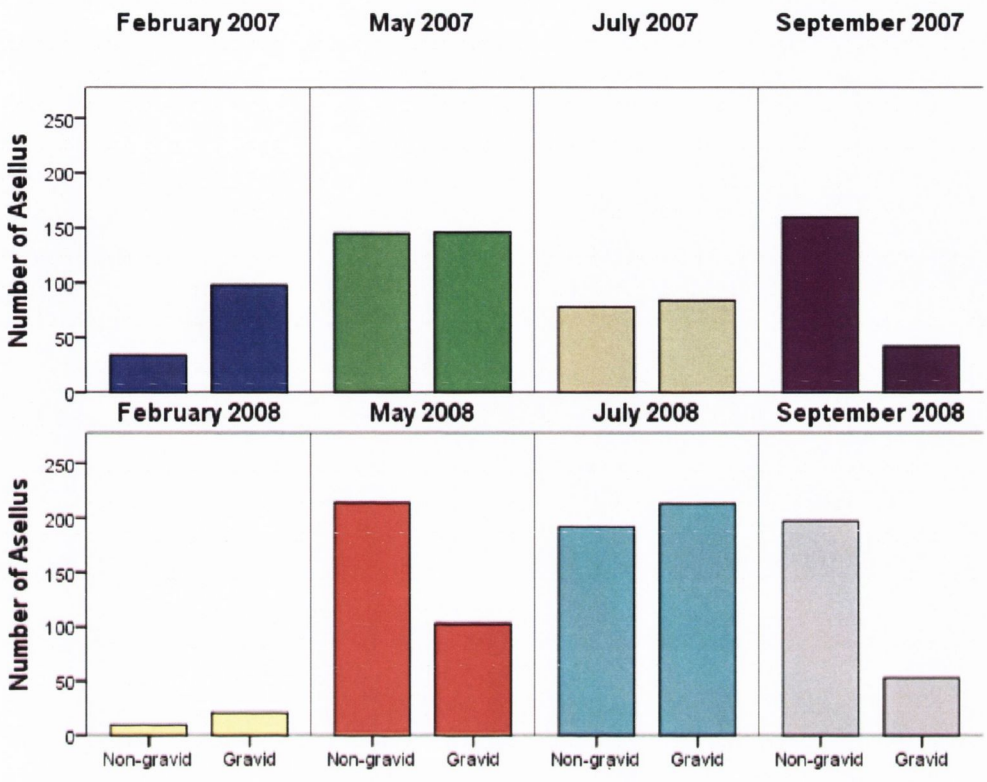


Figure 2.28 Seasonal changes in the reproductive cycle of *A. meridianus* in Clogher Lough from February 2007 to September 2008

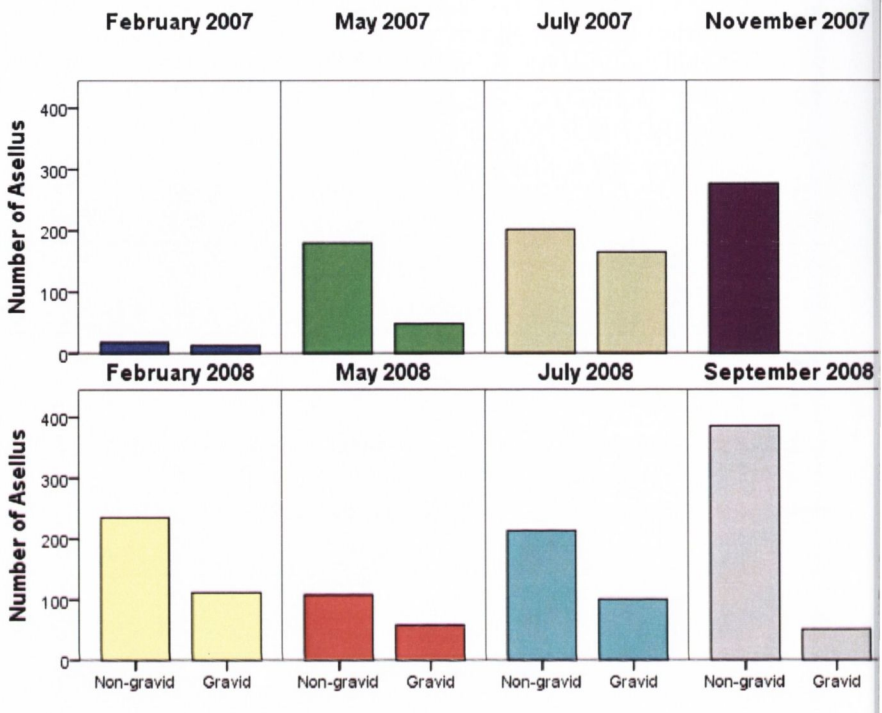


Figure 2.29 Seasonal changes in the reproductive cycle of *A. meridianus* in Lough Feeagh from February 2007 to September 2008

2.3.8 Behavioural changes of *A. clavula*-infected *A. meridianus*

2.3.8.1 Light/dark preference experiment

The effect of *Asellus* sex and infection status on the amount of time individuals spent in the light half of the tank was investigated using a two-way GLM. Infected individuals spent significantly more time in the light half of the tank than uninfected individuals (Table 2.18). The amount of time spent in the light half of the tank did not differ significantly between male and female *A. meridianus*. There was no significant effect of the interaction of sex and infection status on the amount of time spent by *A. meridianus* individuals in the light half of the tank (Table 2.19).

Table 2.18 Mean amount of time spent in the light half of the tank by infected and uninfected *A. meridianus*

Group	N	Mean ± S.E. (secs)	Range
Uninfected	27	1.08 ± 0.22	2.73
Infected	30	1.84 ± 0.20	2.78

Table 2.19 GLM table of the effect of various factors on the time spent by test individuals in the light half of the tank

	F-ratio	p	df
Factors			
Infection status	**11.165**	**0.0015**	**1**
Sex	1.725	0.1948	1
Interaction			
Sex * Inf. status	0.676	0.4147	1

Boundary crossings

The effect of sex and infection status on the amount of times *Asellus* crossed from one half of the tank to the other was investigated using a two-way GLM. Neither sex, infection status, nor the interaction between sex and infection status had a significant effect on the number of boundary crossings made by test individuals (Table 2.20).

Table 2.20 GLM table of the effect of various factors on the number of boundary crossing made by test individuals

	F-ratio	p	df
Factors			
Sex	3.32	0.074	1
Infection status	1.15	0.289	1
Interaction			
Sex*Inf. Status	0.52	0.473	1

2.3.8.2 Activity Experiment

The effect of various factors on *Asellus* activity was investigated by counting the number of 2x2cm squares each individual crossed during the test period. The number of squares crossed was used as a measure of *Asellus* activity. The mean activity of uninfected individuals was higher than that of infected individuals (Table 2.21). The effect of sex and infection status on *Asellus* activity was investigated using a two-way GLM. Infection status had a significant effect on *Asellus* activity, with infected individuals being less active than uninfected individuals (Table 2.22).

Table 2.21 Mean number of squares crossed by infected and uninfected *Asellus*

Group	**N**	**Mean ± S.E.**	**Range**
Uninfected	31	98.5 ± 6.7	149
Infected	24	62.8 ± 7.0	112

Table 2.22 GLM table of the effect of sex and infection status on *Asellus* activity

	F-ratio	**p**	**Df**
Factors			
Infection status	**15.64**	**0.0002**	**1**
Sex	0.61	0.4367	1
Interactions			
Sex*Infection Status	1.96	0.1672	1

2.4 Discussion

The levels of prevalence and abundance of *A. clavula* infection are very different in Clogher Lough and Lough Feeagh, with those of Clogher Lough being much lower. The reasons for this difference are not yet known, but may be related to one or more of the following: lake size, *Asellus* abundance, size of the fish populations, composition of fish populations and varying water temperatures. Clogher Lough shows the lowest overall prevalence of infection of any isopod-acanthocephalan system in the published literature (Table 2.23), while Lough Feeagh shows a value intermediate between those found in other parts of Europe and those from the USA and Japan. Abundance values are not comparable across studies, as different definitions of 'abundance' were used in different studies.

Table 2.23 Prevalence values of *Acanthocephalus* sp. in isopod intermediate hosts

Host-Parasite System	Location	Prevalence (%)	Reference
A. clavula /A. meridianus	Ireland	2.7	Present study
A. clavula /A. meridianus	Ireland	17.9	Present study
A. lucii/ A. aquaticus	Scotland	5.9	Brattey, 1986
A. clavula/A. coxalis	Italy	17	Fresi, 1967
A. anguillae/ A. aquaticus	Italy	3.1	Dezfuli *et al.*, 1994
Acanthocephalus sp./ A. hilgendorfi	Japan	24.4	Kakizaki *et al.*, 2003
A. dirus/ A. intermedius	USA	26.5	Seidenberg, 1973
A. dirus/ A. intermedius	USA	32	Camp & Huizinga, 1980
A. jacksoni/ L. lineatus	USA	16.7	Muzzall & Rabalais, 1975a
A. dirus/ Caecidotea intermedius	USA	55	Oetinger, 1987

The lower prevalence of infection in the smallest isopods is most likely due to feeding behaviour: smaller isopods have a lower intake of food than larger individuals and

therefore have a lower probability of ingesting shelled acanthors (Moore, 1975). Alternatively, juvenile isopods may have a lower susceptibility to infection than older isopods, although there are no data available as yet to support this theory.

The declines in prevalence and abundance in larger (therefore older) isopods are unexplained. Similar declines have, however, been observed in other acanthocephalan-intermediate host systems (Brattey, 1986, Hasu *et al.*, 2007). Brattey (1986) did not observe *A. lucii* infection in the largest field-caught *A. aquaticus*, while the largest experimentally-infected individuals died before cystacanth formation. Hasu *et al.* (2007) observed increased mortality of the largest *A. aquaticus* individuals infected with *A. lucii* in the laboratory.

Declines in prevalence and abundance in larger individuals may be an indication of parasite-induced host mortality (Anderson & Gordon, 1982). Alternatively, experimental studies have shown that declines in prevalence and abundance in larger individuals may be owing to age-related increases in resistance to infection (Moore, 1962; Nickol & Dappen, 1982; Oetinger & Nickol, 1981). Differences in parasite abundance across size classes may also be owing to changes in the numbers of parasites available for uptake at different stages of the *A. meridianus* life cycle, although, to my knowledge, no study has yet tested this possibility.

In Clogher Lough, multiple infections occurred in just five individuals, to a maximum of three larval acanthocephalans per isopod. In Lough Feeagh, however, multiple infections occurred in all months, with the exception of February 2007, up to a maximum of four larval acanthocephalans per isopod. Multiple infections of isopods by larval acanthocephalans are common and occur in almost every host-parasite system studied (Seidenberg, 1973; Muzzall & Rabalais, 1975a; Camp & Huizinga, 1980; Nickol & Dappen, 1982; Brattey, 1986; Oetinger, 1987 & Dezfuli *et al.*, 1994), ranging from a maximum of two acanthocephalans per isopod (Nickol & Dappen, 1982; Dezfuli *et al.*, 1994) to a maximum of 31 acanthocephalans per isopod (Seidenberg, 1973). It has been observed that growth and development of one or all cysts in a multiple infection may be

retarded, possibly due to lack of space and resources (Seidenberg, 1973, Pilecka-Rapacz, 1986), however, when the size ranges of a number of isopod species are compared (Table 2.24), it may be seen that the size range of the isopod species has no discernible bearing on the maximum number of cystacanths harboured.

Table 2.24 Summary of data on size of isopods and maximum number of acanthocephalan larvae harboured

Location	Isopod species	Acanthocephalan species	Size range (mm)	Maximum no. of larvae	Reference
Japan	*A. hilgendorfi*	*Acanthocephalus sp.*	NA	7	Kakizaki *et al.*, 2003
Scotland	*A. aquaticus*	*A. lucii*	3.0 – 15.0	5	Brattey, 1986
USA	*A. intermedius*	*A. dirus*	6.5 – 14.5	> 1	Oetinger, 1987
USA	*A. intermedius*	*A. dirus*	2.0 – 17.9	> 4	Camp & Huizinga, 1980
USA	*A. militaris*	*A. parksidei*	3.0 – 23.0	10	Amin *et al.*, 1980
USA	*A. intermedius*	*A. dirus*	5.0 – 14.0	31	Seidenberg, 1973
Poland	*A. aquaticus*	*A. anguillae*	3.0 – 15.0	16	Pilecka-Rapacz, 1986
Poland	*A. aquaticus*	*A. lucii*	3.0 – 15.0	7	Pilecka-Rapacz, 1986
Poland	*A. aquaticus*	*A. ranae*	3.0 – 15.0	9	Pilecka-Rapacz, 1986
Ireland	*A. meridianus*	*A. clavula*			Present study

A significant difference in brooding was observed between infected and uninfected isopods (Table 2.17).With the exception of three individuals (two of which carried empty brood pouches, while one carried eggs), no infected female *A. meridianus* were brooding. Lyndon (1996) also found small numbers of brooding *A. aquaticus* infected with *A. lucii* and *A. anguillae.* Sterilisation of female hosts by larval acanthocephalans has been observed in a number of other isopod- acanthocephalan systems (Hynes, 1955; Schmidt & Olsen, 1964; Muzzall & Rabalais, 1975a; Brattey, 1983; Oetinger, 1987; Dezfuli *et al.*, 1994 & Kakizaki *et al.*, 2003).

A small number of infected male *A. meridianus* were observed *in amplexus* in this study, from both Clogher Lough and Lough Feeagh, always with uninfected females. These observations were made in the laboratory, following *A. meridianus* collection and prior to dissections. Brattey (1983) found that *A. lucii*-infected male *A. aquaticus* could successfully fertilise uninfected females in the laboratory. Kakizaki *et al.* (2003) also found no visible effects of *Acanthocephalus* sp. infection on male isopods *Asellus hilgendorfi*. As infected males are capable of mating and fertilisation, it would appear that changes in morphology or reproduction of isopods caused by acanthocephalans appear to be lesser in male than female hosts.

In Clogher Lough in 2007, no significant difference in length between infected and uninfected isopods was found. In contrast, in 2008 in Clogher Lough, infected isopods were found to be significantly shorter than uninfected individuals. These results should be analysed with caution, as the sample size of infected isopods in both years was extremely small.

In Lough Feeagh in 2007, infected isopods of both sexes were found to be significantly larger (in terms of total length) than uninfected isopods. In 2008, however, only infected female *A. meridianus* were found to be larger than uninfected females. No significant difference in the lengths of infected and uninfected male isopods was noted in 2008.

Kakizaki *et al.* (2003) found that female *A. hilgendorfi* infected with *Acanthocephalus* sp. were significantly larger than uninfected females, and suggested that infection with acanthocephalan larvae induced the increase in body size by suppressing reproductive development. Oetinger & Nickol (1981) also observed that female *Asellus intermedius* isopods infected with *Acanthocephalus dirus* were significantly larger than their male counterparts, while Hasu *et al.* (2007) found that both male and female *A. aquaticus* infected with *A. lucii* grew larger than uninfected individuals in the laboratory.

The results of Hasu *et al.* (2007) from experimentally infected isopods, coupled with data from the field in this study, suggest that the growth of isopods may be enhanced by

infection with an acanthocephalan parasite. The method by which the parasite achieves this growth enhancement is as yet unknown, but may involve interference with resource allocation or manipulation of foraging behaviour (Hasu *et al.*, 2007).

The absence of dead or encysted larvae in *A. meridianus* from Clogher Lough and Lough Feeagh supports Anderson and Gordon's (1982) hypothesis that, after becoming established in the isopod host, mortality of larval *A. clavula* tends to result from host death rather than the activation of a host response.

Analyses of variance-to-mean ratios indicated an aggregated distribution of larval *A. clavula* among isopods in Lough Feeagh, which is in accordance with previous findings from other larval acanthocephalan species (Hine & Kennedy, 1974; Amin *et al.*, 1980; Hirsch, 1980; Brattey, 1986). In Clogher Lough, where variance to mean ratios did not differ from unity, the abundance of the parasite was below the level at which aggregation can be detected (i.e. a maximum of one parasite per host), and therefore randomness occurs as a statistical consequence (Taylor *et al.*, 1978).

Seasonal cycles have been demonstrated in acanthocephalans from all continents and in all biomes. The cycles are thought to closely correlate with the life history of the host, seasonal changes in biotic factors, for example host diet, and abiotic factors, for example temperature (Chubb, 1982). The occurrence of *A. clavula* in its isopod intermediate host in Clogher Lough and Lough Feeagh showed clear seasonal periodicity, however this pattern differed both between lakes and between years within the lakes. In Clogher Lough, prevalence was generally so low that elucidating a seasonal pattern was unrealistic; however a notable peak in prevalence did occur in July 2007. For this reason, all further discussion of seasonal cycles in prevalence and abundance will centre on data from Lough Feeagh.

In Lough Feeagh, a seasonal cycle of infection was evident in both 2007 and 2008, due to the much higher prevalence of infection; however this cycle differed between years. In 2007, prevalence remained at a similar level (13.1 – 14.2%) for all months in which a

satisfactory sample size was achieved. In 2008 however, a clear cycle was observed, with lows in February and September and peaks in prevalence of infection in the summer months.

The disparity in prevalence cycles between 2007 and 2008 is most likely due to changes in the life history of *A. meridianus* in Lough Feeagh between the two sampling years. Potential life history changes may be investigated through an exploration of seasonal changes in *Asellus* reproduction and size. The proportion of brooding females may be used as a relatively accurate estimate of the level of reproduction in the *A. meridianus* population in each sample month. While brooding females may occur in all months sampled, any peaks in the proportion of brooding females may be thought of as large-scale breeding events, and thus indicate the recruitment of a new *A. meridianus* generation into the population. Recording seasonal changes in length distribution of *A. meridianus* is a simple method of detecting both the recruitment of newborn individuals into the population and the occurrence of long-lived individuals. Recruitment of newborn individuals into the population results in an increase in the numbers of *A. meridianus* in the larger length categories, i.e. the newborn individuals 'dilute' the size distribution. As *A. meridianus* increase in age, so they increase in size, and so large groups of long-lived individuals, e.g. an overwintering generation, result in an increase in the numbers of *A. meridianus* in the larger size categories size.

In 2007, brooding females were observed in all months except November; however, peaks in brooding occurred in February and July, suggesting that there were two new generations of *A. meridianus* in Lough Feeagh in 2007. The large number of isopods the larger size categories in February 2007 suggests that these individuals overwintered before breeding in spring. These overwintering individuals then died off after breeding, as evidenced by the near absence of isopods greater than 6mm in length in May 2007. The population was then made up of the offspring of the overwintering isopods. There was then a second breeding event in July 2007, although on a smaller scale than in February 2007. The evidence for this second breeding event is the second peak in brooding isopods. Spring-born isopods bred at this point, releasing a second cohort of

isopods, and then remained in the population, as shown by the gradual increase in larger isopods throughout July and September. We can observe the spring born isopods growing throughout the summer months. No brooding females were recovered in November 2007, suggesting that water temperatures and *Asellus* feeding activity were too low to support breeding. The remaining isopods in the population overwintered before breeding again in spring, suggesting that perhaps spring-born isopods may produce two broods in their lifetime. We may observe these overwintering isopods in the larger size catergories in February 2008. These isopods again died off after breeding, leaving very few large isopods in the population by May 2008.

Similar proportions of brooding females were observed in all months in Lough Feeagh in 2008, with the exception of September, when a lower level was observed. This would suggest that breeding was taking place throughout the year, without any significant peaks, which explains the relatively consistent high numbers of isopods in the smaller size categories in May, July and September 2008. We may again observe the growth of these isopods by means of the increase in the numbers of isopods in the larger size categories as the year progresses.

Once the life history pattern of *A. meridianus* is elucidated, it is then possible to explain the life history pattern of *A. clavula*. In 2007, similar prevalence values were observed in all months for which a satisfactory sample size was obtained. Prevalence values remained at a relatively constant level, as there were a high number of isopods of intermediate size (the most frequently infected size categories) in every collection. The relative constancy of prevalence levels would also suggest that shelled acanthors were being deposited in the environment at a similarly constant rate, meaning that fish may have been ingesting *Asellus* at a similar rate in all sample months. In May and July 2007 these intermediately-sized isopods were those born in spring, while in September, isopods from the second cohort were most likely to be infected with *A. clavula*. This would suggest that isopods pick up infection at a very young age while feeding, which is quite possible, as isopods have been shown to ingest similar size particles of food, regardless of their size (Moore, 1975).

The relatively constant level of *A. meridianus* reproduction in Lough Feeagh in 2008 would suggest that prevalence values of *A. clavula* would remain at a similar level throughout the year, due to the consistent appearance of newborn isopods available for infection; however, this is not the case. In 2008, prevalence values reach a peak in May before declining in July and September, a very different situation to that observed in 2007. The life history pattern of *A. clavula* in Lough Feeagh in 2008 thus appears to be independent of the life history of *A. meridianus*. The difference in infection patterns between the two years must therefore arise from disparity in the deposition rate of shelled acanthors, and thus a difference in the rate of ingestion of *A. meridianus* by fish between studied months.

The results from the current study differ quite markedly from those found in previous work on acanthocephalan – intermediate host systems, where seasonal periodicity of the parasite has been found to be closely related to the life history of the host. Where the host is able to breed throughout the year and shows no evidence of discrete generations (as found in Lough Feeagh in 2008), there is no seasonal pattern of infection of the host (Hine & Kennedy, 1974). In Lough Feeagh in 2008, however, a clear seasonal pattern of infection was observed. When the host life history involves either one or two discrete generations per year (as in Lough Feeagh in 2007), clear seasonal periodicity of the parasite can be observed (Awachie, 1965; Camp & Huizinga, 1980; Brattey, 1986). In Lough Feeagh in 2007, however, a relatively constant prevalence of infection was maintained, despite clear evidence of discrete *A. meridianus* generations.

In addition, in the majority of previous studies of seasonal cycles in acanthocephalan-isopod systems, very different cycles were observed. In both Britain (Brattey, 1986) and the USA (Seidenberg, 1973; Camp & Huizinga, 1980; Muzzall & Rabalais, 1975c; Amin *et al.*, 1980), a trough in prevalence was observed in early summer, with peaks generally found in late summer or early autumn, while in Lough Feeagh, prevalence either remained constant or peaked in summer, with troughs in the winter months.

It is likely that the difference in seasonal cycles between studies results from variations in biotic and abiotic factors in the study sites. Potential varying biotic factors include (1) the type of water body, (2) the trophic status of the water body, (3) the size and composition of the fish population in the water body, (4) vegetation, both within and surrounding the water body, (5) the invertebrate community in the water body and (6) the pollution levels in the water body. Potential abiotic factors include (1) the local climate and, connected to this, (2) the changes in water temperature throughout the seasons.

Behavioural changes of isopod intermediate hosts infected with acanthocephalans have been observed in almost every host-parasite system studied (Table 2.19), and the *A. meridianus -A. clavula* system is no different. *A. clavula*-infected isopods were found to exhibit decreased activity levels and an increased phototactic response, when compared to their uninfected conspecifics. Photographs were taken of a sample (100) of infected and uninfected isopods and pigmentation alterations with infection were not observed in the *A. meridianus -A. clavula* system.

Increased phototactism of acanthocephalan-infected isopods has been previously observed in the *A. aquaticus-A. anguillae* system (Lyndon, 1996). The phototactic response of acanthocephalan-infected isopods has not been investigated in any other host-parasite systems. It is customary for freshwater isopod species to spend large portions of time on the bottom, hidden under leaves or debris, and uninfected isopods are in fact photophobic when tested (Lyndon, 1996). Increased phototactism may therefore result in increased visibility of isopods to fish predators, and thus increased transmission of the parasite. However, in this study, isopods were found only in shallow water areas with sandy/muddy bottoms. In addition, isopods were observed to crawl along the bottom (in the laboratory), never swimming in the water column. In this case, increased phototactism of infected individuals may have no benefit to the parasite, as isopods remain on the bottom, which is, by nature, dark in colour, at all times.

In the majority of isopod-acanthocephalan systems studied, infected isopods were hyperactive (Table 2.25), which is in contrast to the result found in the present study,

where infected *A. meridianus* were less active than uninfected individuals. Increased activity makes isopods more conspicuous to fish predators, thus facilitating transmission of the parasite. This phenomenon has been demonstrated through predation experiments (Camp & Huizinga, 1980; Brattey, 1983; Hechtel *et al.*, 1993). For example, significantly more *A. lucii*-infected *A. aquaticus* were consumed by perch in feeding trials carried out by Brattey (1983). A decrease in activity of infected *A. meridianus* may also facilitate transmission of *A. clavula*, as slow-moving isopods may be more easily caught by fish predators. An alternative explanation for the decreased activity of infected isopods is simply that they are sick, and are unable to maintain the levels of movement of uninfected individuals. Predation experiments are necessary to further explore whether or not *A. clavula*-infected *A. meridianus* are more frequently eaten than uninfected individuals, however the process for investigating the adaptive nature of behaviour changes of parasitised animals is complex and not always easily achieved (Poulin, 1995).

In conclusion, this chapter has supported the findings of previous studies, for example by showing declines in the prevalence of *A. clavula* in larger, and therefore, older isopods, as well as showing again that the reproductive capabilities of female isopods are greatly hampered by infection with an acanthocephalan parasite, possibly in order to divert resource allocation to an isopod growth increase which would benefit the parasite. *A. clavula* has been shown to modify the behaviour of *A. meridianus*, although further work is necessary in order to establish whether these behavioural modifications are adaptive or simply by-products of infection.

Table 2.25 Behavioural and pigmentation changes of acanthocephalan-infected isopod species

	Isopod species	Acanth. species	Pigment-ation change	Behavioural changes	Reference
England	*A. aquaticus*	*A. anguillae*	Dark	Phototactic, attracted to disturbance	Lyndon, 1996
England	*A. aquaticus*	*A. lucii*	Dark	No changes	Lyndon, 1996
Poland	*A. aquaticus*	*A. anguillae*	Dark	Hyperactive, increased time at water surface	Pilecka-Rapacz, 1986
Poland	*A. aquaticus*	*A. lucii*	Dark	Hyperactive, increased time at water surface	Pilecka-Rapacz, 1986
Poland	*A. aquaticus*	*A. ranae*	-	Hyperactive, increased time at water surface	Pilecka-Rapacz, 1986
USA	*L. lineatus*	*A. jacksoni*	Light	Hyperactive	Muzzall & Rabalais, 1975c
USA	*A. intermedius*	*A. dirus*	Light	Hyperactive	Camp & Huizinga, 1979
USA	*A. intermedius*	*A. dirus*	Light	Hyperactive	Hechtel *et al.*, 1993

In addition, some new aspects of acanthocephalan-isopod infection dynamics have been brought to light. This chapter has shown that a variety of results may be found when investigating a host-parasite system in multiple locations and at multiple time points. It has shown that seasonal cycles of infection may differ by location and may not occur in a repeatable pattern. This study has also shown that the seasonal periodicity of a parasite may be independent of host life history, despite the occurrence of discrete host generations.

Overall, this chapter has shown that acanthocephalan-isopod population biology is dependent on many factors, not all of which are measurable by any one study, and underlined the need for sampling the same host-parasite system in multiple locations with temporal variation in order to obtain a clear and reliable picture of infection dynamics.

Chapter 3

A. clavula in eels, *Anguilla anguilla*

3.1 Introduction

While *A. clavula* is able to grow and reproduce in many fish species, its preferred definitive host is the eel, *Anguilla anguilla*. This designation is based on a number of studies (Chubb, 1964; Rojanapaibul, 1977; Lyndon & Kennedy, 2001; Byrne *et al.*, 2004) which found that *A. clavula* shows an increased ability to mature and reproduce in eel compared with other fish hosts. Chubb (1964) explored *A. clavula* infection in eel, grayling (*Thymallus thymallus*), pike (*Esox lucius*) and roach (*Rutilis rutilis*) in Bala Lake (Wales), and found that, while grayling showed the highest prevalence of infection, the highest intensity of infection was observed in eels. In addition, 82% of female *A. clavula* from eel were found to be reproductively mature, compared to just 23% from grayling (Chubb, 1964). Rojanapaibul (1977) found similar results, also in Bala Lake. 47% of female *A. clavula* from eels were reproductively mature, compared with just 3.5% from gwyniad (*Coregonus lavaretus*), 11.5% from bullhead (*Cottus gobio*) and 4% from roach. Byrne *et al.* (2004), in a study of *A. clavula* in eel and trout in Clogher Lough found that, while 61% of female *A. clavula* from eels were reproductively mature, just 2% of female worms from trout (*Salmo trutta*) reached this stage.

A. clavula has been reported from eels in various locations around Europe on numerous occasions (Conneely & McCarthy, 1986; Kennedy, 1993; Callaghan & McCarthy, 1996; Kennedy *et al.*, 1998; Kennedy, 2001; Outeiral *et al.*, 2002; Aguilar *et al.*, 2005), generally as part of a larger exploration of helminth community structure, although a small number (Chubb, 1964; Rojanapaibul, 1977; Kennedy & Lord, 1982; Kennedy, 1984. Byrne *et al.*, 2004) focus specifically on various aspects of *A. clavula* ecology. Prevalence, abundance and intensity values for *A. clavula* vary widely in these studies (Table 3.1), ranging from a prevalence of 0.85%, with just one eel infected (Callaghan & McCarthy, 1996), to a prevalence of 97% (Byrne *et al.*, 2004). *A. clavula* also has the

capacity to completely dominate helminth communities in which it is found (Kennedy *et al.*, 1998).

Table 3.1 Population parameters of *A. clavula* in eels from various European locations. NA = not available

Location	Year	N	Prevalence (%)	Mean abundance ± S.E.	Mean intensity ± S.E.	Reference
Ireland						
Lough Derg	1987	146	1.4	NA	2.5	Kennedy & Moriarty, 2002
Lough Derg	1988	75	2.7	NA	1.0	Kennedy & Moriarty, 2002
River Dunkelin	1992	117	0.85	0.04 ± 0.67	5 ± 0.04	Callaghan & McCarthy, 1996
Lough Rea	1992	32	12.5	0.13 ± 0.58	1 ± 0.06	Callaghan & McCarthy, 1996
River Drimneen	1984	49	12	17.5	NA	Conneely & McCarthy, 1986
Lough Corrib	1984	39	59	3.4	NA	Conneely & McCarthy, 1986
Clogher Lough	2002	30	97	7.7 ± 2.49	12.5 ± 2.41	Byrne *et al.*, 2004
Great Britain						
Bala Lake	1964	54	27.7	NA	18.4	Chubb, 1964
Bala Lake	1977	58	53.5	2.79	5.23	Rojanapaibul, 1977
River Culm	1997	74	16.2	0.2 ± 0.83	NA	Kennedy, 2001
River Clyst	1997	19	63.1	1 ± 1.41	NA	Kennedy, 2001
Spain						
River Ulla	1999/2000	323	3.7	0.09 ± 0.55	2.5 ± 1.51	Aguilar *et al.*, 2005
River Tea	1999/2000	200	2	0.03 ± 0.22	1.5 ± 0.58	Aguilar *et al.*, 2005
Arousa (estuary)	1995/1996	956*	0.4	0.01 ± 0.28	3.5	Outeiral *et al.*, 2002

Table 3.1 contd Population parameters of *A. clavula* in eels from various European locations. NA = not available.

Location	Year	N	Prevalence (%)	Mean abundance ± S.E.	Mean intensity ± S.E.	Reference
Italy						
River Tiber	1980 (Summer)	58	58.6	5.6 ± 3.85	NA	Kennedy *et al.*, 1998
River Tiber	1980 (Autumn)	57	61.4	9.2 ± 3.95	NA	Kennedy *et al.*, 1998
River Tiber	1996 (Summer)	41	63.4	2.5 ± 1.73	NA	Kennedy *et al.*, 1998
River Tiber	1996 (Autumn)	60	65	4.6 ± 3.08	NA	Kennedy *et al.*, 1998
Netherlands						
North Ijsselmeer	1993	92	57.2	NA	8.2	Borgsteede *et al.*, 1999
South Ijsselmeer	1993	72	57.5	NA	13.4	Borgsteede *et al.*, 1999
Markermeer	1993	99	44.2	NA	12.8	Borgsteede *et al.*, 1999
Volkerak (brackish)	1993	98	31.9	NA	16.6	Borgsteede *et al.*, 1999

* = sample size for two estuaries

In several other studies examining the helminth communities of eels in Great Britain (Norton *et al.*, 2003), Germany (Sures *et al.*, 1999a; Sures & Streit, 2001; Thielen *et al.*, 2007), Austria (Schabuss *et al.*, 2004) and Italy (DiCave *et al.*, 2001), *A. clavula* was absent, more than likely owing to the absence of the appropriate intermediate host.

In addition to the studies detailed above, a review of eel helminth communities by Kennedy (1986), using data from 50 localities (rivers and lakes) in Great Britain and Ireland found *A. clavula* in 13 (26%) of them. *A. clavula* occurred in 101 (12%) of 842 eels examined and was dominant in 38.5% of the localities in which it occurred. All localities in which *A. clavula* was found to be dominant were in Great Britain. No prevalence, abundance or intensity data were provided for individual localities in this review.

As is clear from Table 3.1 and the above data, there is no clear and consistent pattern of *A. clavula* infection, rather infection levels appear to be determined by biotic and abiotic factors in individual sample locations.

Of the studies focussing specifically on *A. clavula* ecology, one (Kennedy & Lord, 1982) explores the habitat specificity of *A. clavula*, a second (Kennedy, 1984) looks at the dynamics of a declining population of *A. clavula* in an English stream, while the remainder (Chubb, 1964; Rojanapaibul, 1977) explore *A. clavula* population biology in several fish hosts.

The aims of this chapter are (a) to explore various aspects of the population biology of *A. clavula* in eels from Clogher Lough and Lough Feeagh, including prevalence, abundance, intensity, seasonal patterns of infection as well as the niche of the parasite and (b) to measure the relative success of the parasite in its preferred definitive host. Relative success of *A. clavula* will be measured in terms of parasite size and the proportion of reproductively mature females.

3.2 Materials and Methods

Primary sample site descriptions and information regarding descriptive terms and statistics may be found in Chapter 2.

3.2.1 Fish capture

Eels and trout were collected from the primary sites in the latter half of the months of February, May, July and September 2007. Samples were taken to reflect seasonal differences and also as a function of fish availability. It has previously been shown that sampling in late winter (December and January) is unproductive and yields very few fish (Byrne, 2000). Where possible, a minimum of 30 eels and trout were taken at each sampling occasion (Kennedy, 1993).

Eels (and trout) were caught by fyke netting. A fyke net is a tube of netting, supported by hoops with three or more internal funnels, each with a smaller opening than the last, which makes it impossible for the eel to turn and swim out again (Moriarty, 1974). Eels were caught using two trains of ten fyke nets, and, as they are nocturnal animals, with peaks of activity after dusk and before dawn, the fyke nets were left overnight and removed the following morning.

3.2.2 Post-catching procedures

As soon as fish were caught (or collected from nets) they were transported back to the laboratory where they were killed using an excess of anaesthetic (1,1,1-trichlor-2-methy-2-propanol hemihydrate). The fish were weighed (± 0.1g) and measured (fork length ± 0.1cm) and then individually bagged, labelled and frozen until ready for dissection.

3.2.3 Fish dissection protocol

Fish were defrosted at room temperature. Following a ventral incision from the lower jaw to the anus the entire gut, was removed, laid in a dissecting tray and measured from the anterior end of the oesophagus to the anus. This facilitated accurate positioning of individual parasites. Each parasite was assigned a percentage location, with 0% corresponding to the anterior oesophagus and 100% corresponding to the anus.

The gut was initially rinsed with 0.9% saline, and then visually examined for the presence of parasites. Gills were individually removed from each fish, washed in saline and examined for parasites. The heart, swimbladder, liver and spleen were removed, weighed and examined for parasites. The body cavity was also examined for encysted parasites.

3.2.4 Treatment of parasites

All parasites recovered were fixed in 6% formalin solution for 24 to 48 hours. Acanthocephalans were first placed in tap water for 24 to 48 hours in order to evert the proboscis and then transferred to formalin. After fixation, parasites were transferred to 70% ethanol with 4% glycerol for long-term storage. Parasites were individually stored in sealed and labelled tubes. A separate written record was kept of the number of parasites found as well as the percentage locations of parasites occurring along the intestine.

3.2.5 Identification of parasites

Temporary mounts of all acanthocephalans were prepared. Each worm was placed on a glass slide and flooded with tap water. Using a binocular microscope, the numbers of rows of hooks on the proboscis, as well as the number of hooks per row, were counted (Figure 3.1). Using these data, as well Brown's (1986) identification key, it was possible to identify all acanthocephalans to species level. In cases where the proboscis was not fully everted, other characteristics were used, including colouration, hook shape, neck shape, and length.

Following identification, each parasite was sexed. Males were identified by the presence of cement glands and testes, which appear as small black/dark ovoid structures when examined under the binocular microscope (Figures 3.1b and 3.2). In a small proportion of males a copulatory bursa was observed attached to the end of the body. Females were identified by the presence of reproductive structures, such as eggs and ovarian balls, as well as the absence of testes and cement glands (Figures 3.1a and 3.3).

a) female b) male c) proboscis

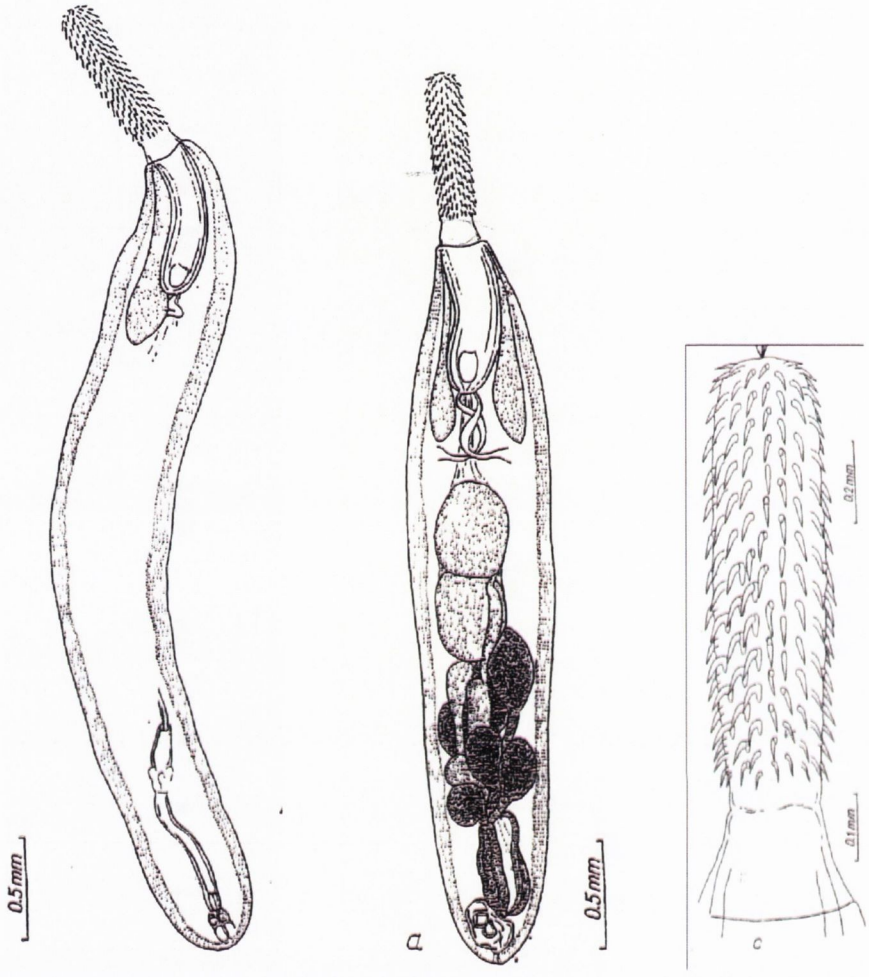


Figure 3.1 *Acanthocephalus clavula* [images modified from Grabda-Kazubska and Chubb, 1968)]



Figure 3.2 Male *Acanthocephalus clavula*. Note the presence of two large, oval-shaped testes, leading to three pairs of cement glands (Image: Naiara O' Mahony)

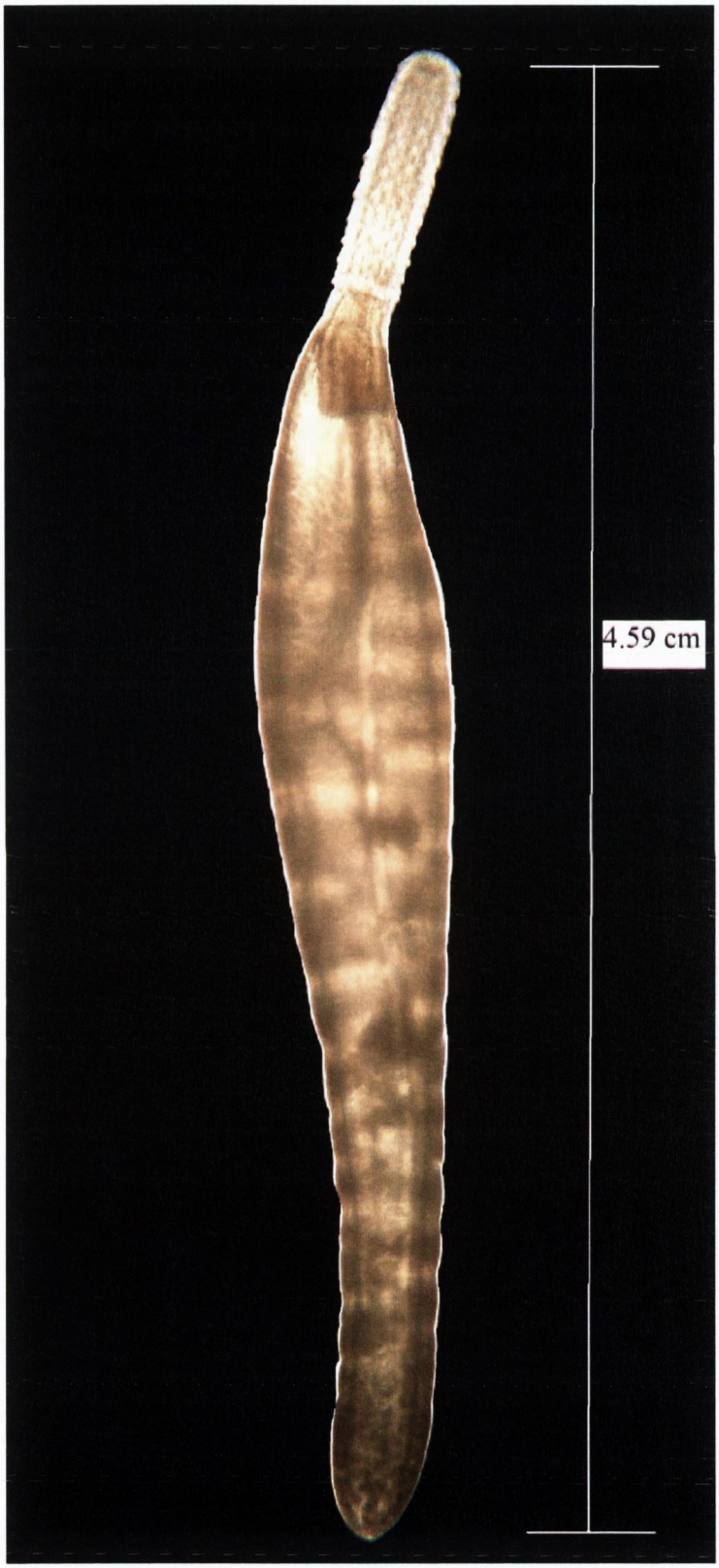


Figure 3.3 Female *Acanthocephalus clavula*. (Image: Naiara O' Mahony)

3.2.6 Weighing, measuring and reproductive status of acanthocephalans (modified from Byrne, 2000)

Parasites were removed from the storage medium, blotted on adsorbent tissue paper to remove excess moisture and weighed on a Mettler-Toledo AG245 balance (±0.001mg). Parasites were measured to the nearest millimetre using a map reader, via a microscope with attached *camera lucida*.

Each worm was placed on a glass slide, flooded with tap water and cut in half, in order to release the contents of the body cavity. In females, the single ligament sac containing the reproductive structures disintegrates when the ovary fragments, allowing eggs and ovarian balls to float free of the body cavity. Where necessary, the contents of the body cavity were squeezed out using a fine forceps.

The sex ratio of parasites from each fish was recorded. The maturity status of female parasites was assessed, using the three-stage classification system of Bates & Kennedy (1990). Under this system, at Stage 1 females contain ovarian balls only (Figure 3.4), at Stage 2 ovarian balls and immature acanthors (Figure 3.5) and at Stage 3 ovarian balls, immature acanthors and mature, shelled acanthors (Figure 3.6). The proportions of females at each stage in a particular host may then be used to determine whether the species is a preferred or suitable host for the parasite (Lyndon & Kennedy, 2001).

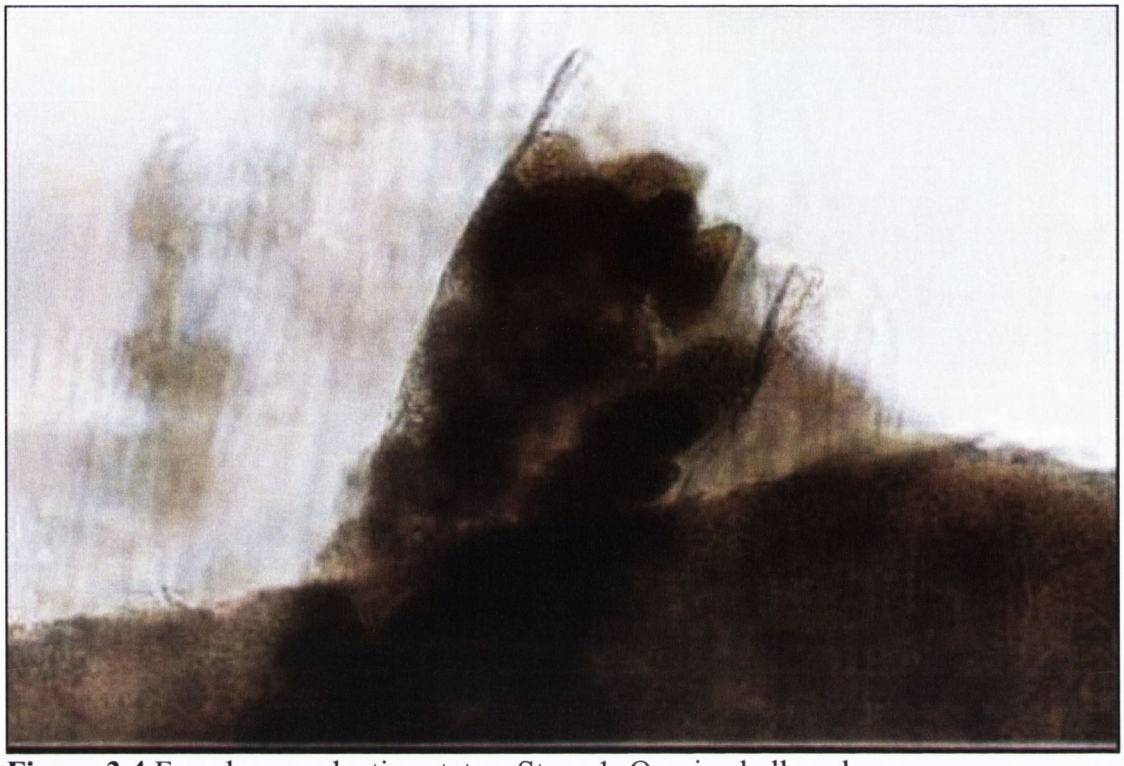


Figure 3.4 Female reproductive status, Stage 1: Ovarian balls only



Figure 3.5 Female reproductive status, Stage 2: Ovarian balls and immature acanthors. Note that, in this image, only immature acanthors are present



Figure 3.6 Female reproductive status, Stage 3: Ovarian balls, immature acanthors, and mature shelled acanthors. Note the mature shelled acanthors are pinched at the ends. Ovarian balls present (top right) and immature acanthors present (top right of ovarian ball)

3.2.7 Infection Intensity

Infections were split into three intensity levels: low (0-10 worms), medium (11-29 worms) and high (> 30 worms). These levels were chosen following an examination of the number of parasites per fish host where it was observed that the majority of infections fell naturally into one of the three intensity brackets. In addition, these intensity levels correspond to those used by Byrne (2000) and allow for comparison.

3.2.8 Data Analysis

For descriptions of GLMs, Kolmogorov-Smirnov tests and all other statistical tests, please refer to Section 2.2.6.

Parasite niche

The ecological niche of a parasite species is the multidimensional habitat volume occupied by parasites and is defined by numerous biotic and abiotic variables (Hutchinson, 1957). Most of these variables are extremely difficult to quantify and, as a result, most research on parasite niches has focussed on the spatial dimension of the niche (Poulin, 2007a). Most parasites tend to be restricted to particular sites on or in the host. Measurements of parasite attachment sites can therefore be used to describe the spatial niche of a parasite species in both single and mixed species infections. In this thesis, the distribution of *A. clavula* along the length of the intestine of eel and trout was examined. In most cases, the percentage location of each parasite in an intestine was reported, however, in some cases, due to the sheer number of parasites present, it was not always possible to give an exact location for each parasite. In such cases the number of individuals occurring along a specific length of the intestine was recorded. The midpoint of this range was then used for all further analyses.

Summary statistics provided the mean position values, lower, upper and interquartile ranges and the minimum and maximum location values of *A. clavula* along the intestine. Frequency histograms summarising the distribution of each species were presented. Frequency histograms were calculated by dividing the intestine into 50 discrete sections, with each section comprising 2% of the total intestine length.

3.3 Results

3.3.1 Eel sample sizes

A. clavula was never found in eels from Bunaveela Lake, so all results presented are from Clogher Lough and Lough Feeagh. Adequate numbers of eels (approximately 30 individuals) were obtained for all months in Clogher Lough and Lough Feeagh except February 2007 and February 2008 (Table 3.2). The low sample sizes obtained in these months were expected, and were due to unsuitable weather conditions and low fish availability.

Table 3.2 Eel sample sizes from Clogher Lough and Lough Feeagh in 2007 and 2008

	Clogher Lough	**Lough Feeagh**
	(n)	**(n)**
2007		
February	6	13
May	30	29
July	25	30
September	31	33
2008		
February	7	6
Total	**99**	**111**

As expected, the sex ratio was heavily biased towards female eels in both Clogher Lough (3.29:1) and Lough Feeagh (17.33:1) (Table 3.3).

Table 3.3 The sex of eel samples from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
	(n)	**(n)**
Male	21	6
Female	69	104

3.3.2 Eel length and mass

As eels are sexually dimorphic, with females being, on average, longer and heavier than males, mean length and weight data for eels sampled from Clogher Lough and Lough Feeagh were separated by sex (Tables 3.4 & 3.5).

In both lakes the mean length of female eels was significantly greater than the mean length of male eels (t-tests: Clogher Lough: $t = 7.92$, $df = 71$, $p \leq 0.0001$; Lough Feeagh: $t = 6.981$, $df = 9$, $p \leq 0.0001$) (Table 3.4).

Table 3.4 Mean length of eels sampled from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
Mean male length (cm) ± S.E.	35.61 ± 0.958	34.17 ± 1.534
Mean female length (cm) ± S.E.	47.18 ± 1.104	46.77 ± 0.973

In both lakes the mean mass of female eels was also significantly greater than the mean mass of male eels (t-tests: Clogher Lough: $t = 8.217$, $df = 52$, $p \leq 0.0001$; Lough Feeagh: $t = 9.039$, $df = 9$, $p \leq 0.0001$) (Table 3.5).

Table 3.5 Mean mass of eels sampled from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
Mean male mass (g) ± S.E.	81.05 ± 6.571	69.50 ± 8.690
Mean female mass (g) ± S.E.	205.42 ± 17.838	205.22 ± 16.497

3.3.3 *A. clavula* from eels from Clogher Lough and Lough Feeagh

Numbers of *A. clavula* recovered

A total of 458 *A. clavula* individuals were recovered from eels sampled from Clogher Lough, ranging from 25 individuals in February 2007 to 203 in May 2007. A total of 2306 *A. clavula* individuals were recovered from eels sampled from Lough Feeagh, ranging from 332 in February 2008 to 807 in May 2007 (Figure 3.7).

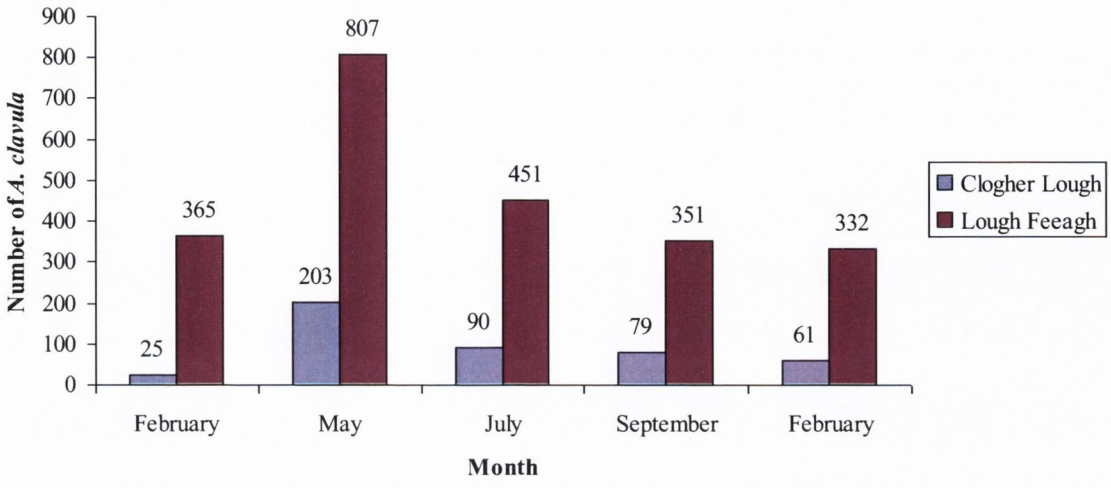


Figure 3.7 Numbers of *A. clavula* recovered from eels from Clogher Lough and Lough Feeagh

3.3.4 Overall prevalence, abundance and intensity of *A. clavula*

Overall prevalence, abundance and intensity of *A. clavula* in eels varied greatly between the two lakes, with Lough Feeagh showing much higher values in all measures than those from Clogher Lough (Table 3.6).

The overall prevalence of *A. clavula* differed significantly between Clogher Lough and Lough Feeagh ($\chi^{2:}\chi = 24.75$, df = 1, $p \leq 0.0001$).

The abundance of *A. clavula* also differed significantly between the two lakes (t-test: t = 4.792, df = 139, $p \leq 0.0001$), as did the intensity (t-test: t = 3.824, df = 140, p = 0.0002) with Lough Feeagh eels carrying heavier burdens than eels from Clogher Lough (Table 3.6).

Table 3.6 Overall prevalence, mean abundance and mean intensity of *A. clavula* from eels from Clogher Lough and Lough Feeagh

Lake	N	Prevalence (%)	Mean abundance ± S.E.	Mean intensity ± S.E.
Clogher	90*	58.9	5.69 ± 1.19	9.66 ± 1.83
Feeagh	111	89.2	22.04 ± 3.20	24.47 ±3.45

*Sample size for which parasite data were available (As the intestines of 9 eels were so degraded when dissected that removal and identification of parasites was impossible).

3.3.5 Seasonal patterns in prevalence, abundance and intensity of *A. clavula* in eels in Clogher Lough and Lough Feeagh

Prevalence

While infection levels are very different between Clogher Lough and Lough Feeagh, within each lake, similar levels are maintained throughout the year. Indeed, there are no significant differences in prevalence between seasons in either Clogher Lough or in Lough Feeagh (χ^2 analysis: Clogher Lough: $\chi^2 = 3.751$, df = 4, p = 0.4407; Lough Feeagh: $\chi^2 = 3.483$, df = 4, p = 0.4805) (Table 3.7).

Table 3.7 Prevalence (± 95% C.I.), abundance (± S.E.) and intensity (± S.E.) of *A. clavula* in eels from Clogher Lough and Lough Feeagh by month

Month	Lake	No. eels infected (n)	Prevalence (%)	Abundance ± S.E.	Intensity ± S.E.
2007					
February	Clogher	3 (6)	50	4.8 ± 3.7	9.7 ±6.7
	Feeagh	11 (13)	84.6	29.7 ± 11. 1	33.2 ± 11.8
May	Clogher	13 (27)	48.1	7.5 ± 2.9	15.6 ± 5.2
	Feeagh	28 (29)	96.6	30.8 ± 9.1	31.2 ± 9.1
July	Clogher	17 (25)	68	3.7 ±1.2	5.5 ± 1.6
	Feeagh	26 (30)	86.7	15.8 ± 2.7	18.3 ± 2.8
September	Clogher	18 (30)	60	4.2 ± 1.1	7.0 ± 1.5
	Feeagh	28 (33)	84.8	10.9 ± 2.1	12.8 ± 2.3
2008					
February	Clogher	2 (2)	100	30.5 ±28.5	30.5 ± 28.5
	Feeagh	6 (6)	100	55.3 ± 20.7	55.7 ±20.9
2007 & 2008					
February	Clogher	5 (8)	62.5	11.3 ± 7.3	18.0 ± 11.0
	Feeagh	17 (19)	89.5	37.8 ± 10.1	40.6 ± 10.5

Abundance and Intensity

Abundance and intensity, however, show a more pronounced seasonal pattern. As the sample sizes in February 2007 and 2008 are low in both lakes, data for these months were combined for further analysis, after it was noted that there was no significant difference in infection levels in either lake (t-test: Clogher Lough: $t = 0.8931$, $df = 1$, $p = 0.5319$; Lough Feeagh: $t = 1.09$, $df = 8$, $p = 0.3073$). From this point on, the February 2007 and 2008 samples will be referred to collectively as 'February'.

The seasonal patterns of abundance in Clogher Lough and Lough Feeagh were compared using a two-way GLM (Table 3.8). Abundance differed significantly between lakes and also between months.

The seasonal pattern is the same for both lakes, with the highest mean abundance and mean intensity values recorded in February, followed by a gradual decline in both measures for the remainder of the year (Figures 3.8 & 3.9).

Table 3.8 GLM table of the effect of lake and month on the abundance of *A. clavula* in eels in Clogher Lough and Lough Feeagh

	***F*-ratio**	***p*-value**	***df***
Factors			
Lake	**19.074**	**≤ 0.0001**	1
Month	**3.9199**	**0.0095**	3
Interactions			
Lake * Month	1.5184	0.2110	3

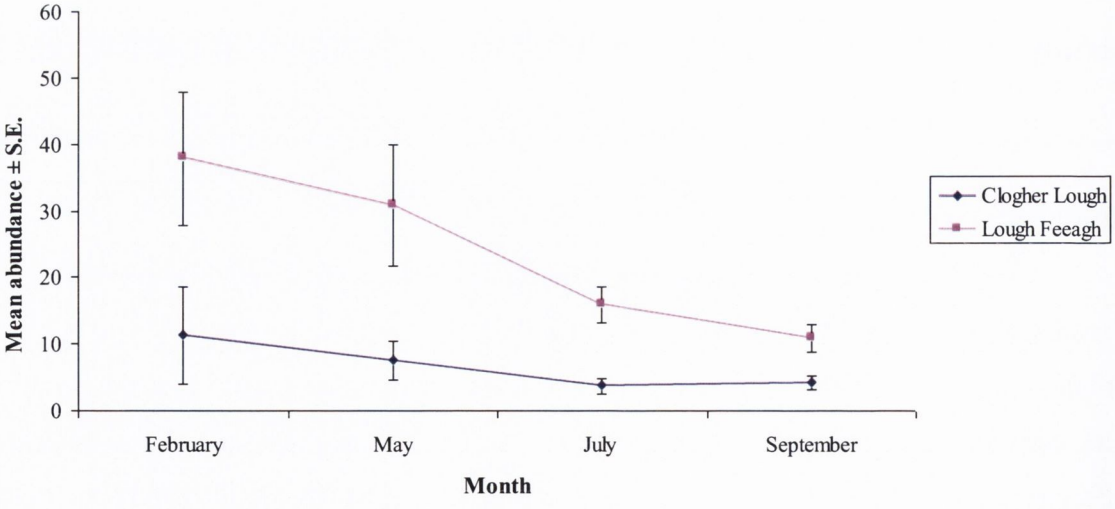


Figure 3.8 Mean abundance (± S.E.) of *A. clavula* in Clogher Lough and Lough Feeagh

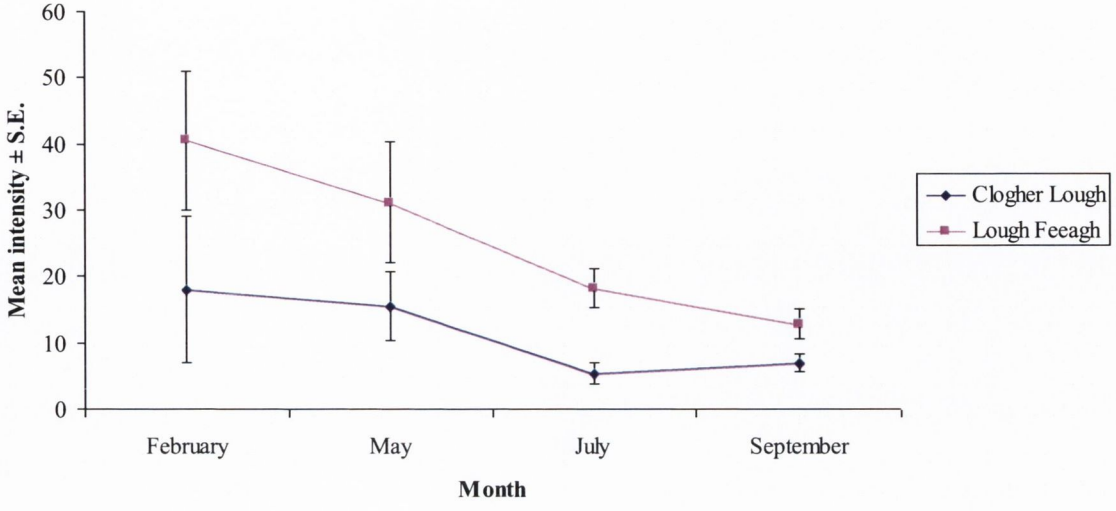


Figure 3.9 Mean intensity (± S.E.) of *A. clavula* in Clogher Lough and Lough Feeagh

3.3.6 Aggregation

In Table 3.9, the mean abundance by month was calculated for Clogher Lough and Lough Feeagh, along with the variance and variance to mean ratio. The *A. clavula* population in eels in both lakes was highly aggregated in all months.

Table 3.9 Seasonal changes in infection of eels by *A. clavula* in Clogher Lough and Lough Feeagh. C denotes Clogher Lough; F denotes Lough Feeagh

Month	No. examined		No. infected		Total no. of *A. clavula*		Mean abundance		Variance		Variance: mean ratio	
	C	F	C	F	C	F	C	F	C	F	C	F
February	8	19	5	18	86	697	11.25	37.79	431.64	1933.73	11.42	51.17
May	27	29	13	29	203	807	7.52	30.83	226.95	2391.86	30.18	77.58
July	25	30	17	26	90	451	3.72	15.83	34.88	218.63	9.38	13.81
September	30	33	18	28	79	351	4.2	10.88	34.58	143.22	8.23	13.16

3.3.7 *A. clavula* length and mass in Clogher Lough and Lough Feeagh

In both Clogher Lough and Lough Feeagh, female *A. clavula* were found to be longer (t-test: Clogher: $t = 9.218$, $df = 149$, $p \leq 0.0001$; Feeagh: $= 12.79$, $df = 210$, $p \leq 0.0001$) and heavier (t-test: Clogher: $t = 11.24$, $df = 247$, $p \leq 0.0001$; Feeagh: $t = 7.987$, $df = 163$, $p \leq 0.0001$) than male *A. clavula* (Table 3.10).

Table 3.10 Mean body length (± S.E) and mass (± S.E.) of male and female *A. clavula* from Clogher Lough and Lough Feeagh

	N	Body length ± S.E. (mm)	N	Mass ± S.E (mg)
Clogher Lough				
Male	57	2.862 ± 0.092	78	0.280 ± 0.016
Female	97	4.157 ± 0.106	179	0.661 ± 0.029
Lough Feeagh				
Male	150	3.24 ± 0.05	152	0.114 ± 0.006
Female	132	4.59 ± 0.09	124	0.234 ± 0.01

The effect of extrinsic and intrinsic factors on *A. clavula* length in Clogher Lough and Lough Feeagh

A four-way GLM was carried out to explore the effect of lake, parasite sex, intensity status and month on parasite body length (Table 3.11).

Table 3.11 GLM table of the effect of various factors on length of *A. clavula* in the intestine of eels in Clogher Lough and Lough Feeagh

	F-ratio	*p*-value	*Df*
Factors			
Lake	**17.478**	**≤ 0.0001**	**1**
Season	**2.9748**	**0.0316**	**3**
Parasite sex	**38.817**	**≤ 0.0001**	**1**
Intensity status	**5.2389**	**0.0057**	**2**
Interactions			
Lake * Season	0.4401	0.7244	3
Lake * Sex	**5.5181**	**0.0193**	**1**
Lake * Int. Status	1.7757	0.1707	2
Season * Sex	0.34885	0.7900	3
Season * Int. status	0.62445	0.7107	6
Sex * Int. Status	**3.1137**	**0.0455**	**2**
Lake * Season * Sex	0.81862	0.4851	3
Lake * Season * Int. status	1.0772	0.3674	4
Lake * Sex * Int. status	0.55332	0.5755	2
Season * Sex * Int. status	0.51083	0.8002	6
Lake * Season * Sex * Int. Status	0.36067	0.7814	3

Lake was a highly significant factor, as *A. clavula* were significantly longer in length in Lough Feeagh than in Clogher Lough.

Parasite sex was a highly significant factor as female worms were significantly longer than male worms in both lakes (Table 3.10).

A significant interaction between lake and parasite sex was observed, which is as result of the fact that, while male *A. clavula* are shorter than females in both lakes, in Clogher Lough, males are, on average, significantly shorter than those from Lough Feeagh(Table 3.10, Figures 3.10 & 3.11).

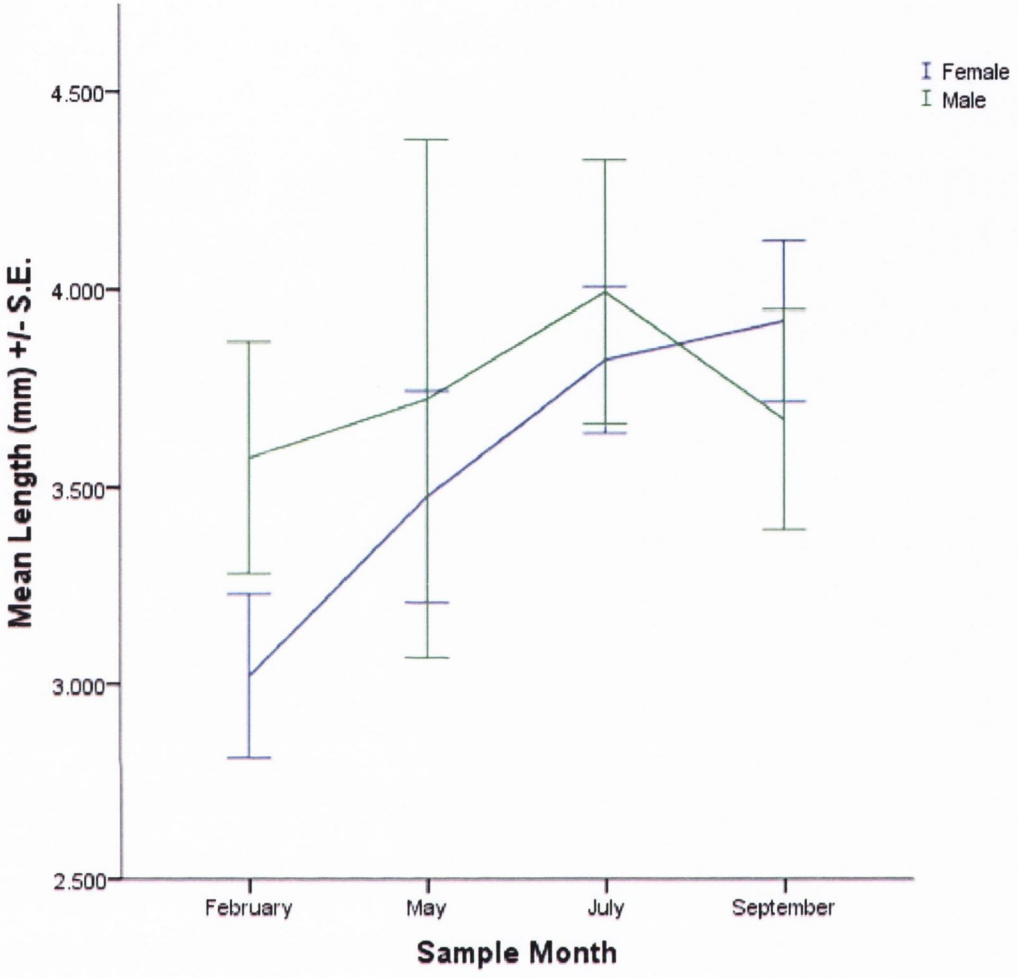


Figure 3.10 Mean length of male and female *A. clavula* in Clogher Lough

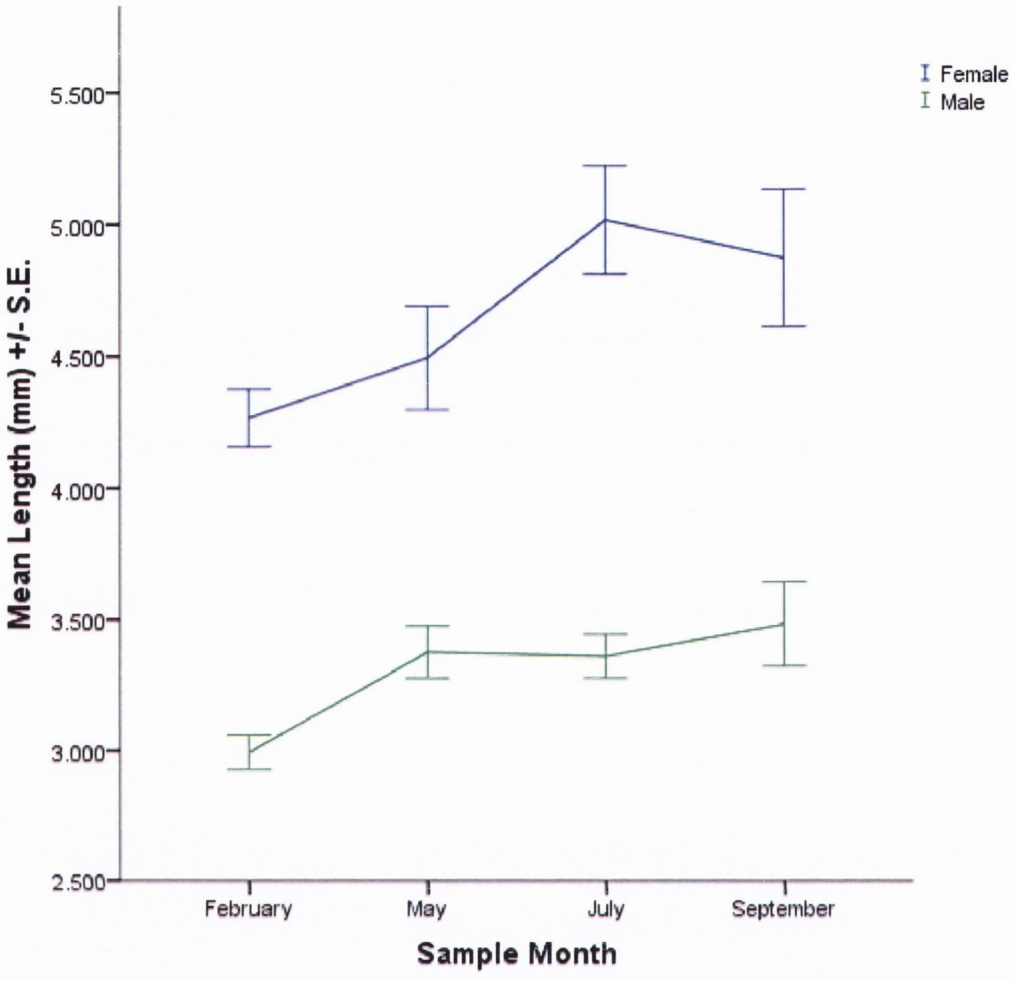


Figure 3.11 Mean length of male and female *A. clavula* in Lough Feeagh

Intensity status was a significant factor as worms from low intensity infections (1- 10 worms) were significantly longer in length than worms from medium (11 – 29 worms) and high (> 30 worms) intensity infections (Table 3.12).

The interaction of sex and intensity status had a significant effect on parasite length, as more large female *A. clavula* were recovered in low and medium intensity infections, while the largest male worms were recovered from high intensity infections (Table 3.12, Figures 3.12, 3.13, 3.14 & 3.15).

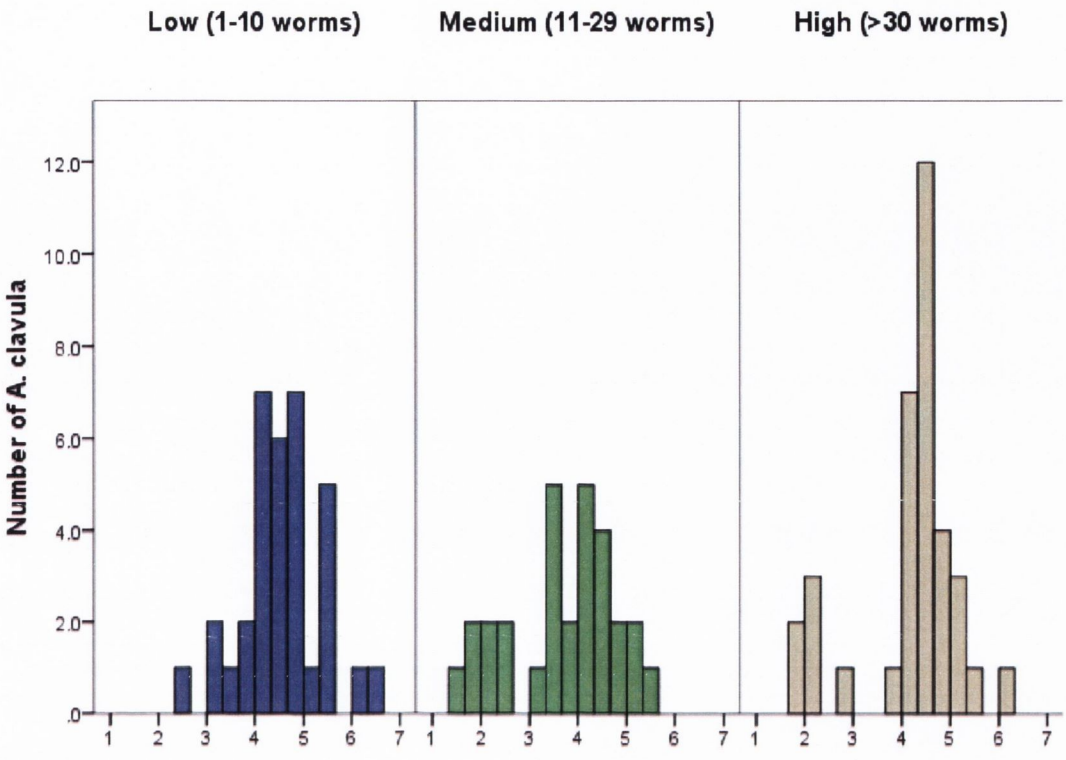


Figure 3.12 Length of female *A. clavula* from low, medium and high intensity infections in Clogher Lough

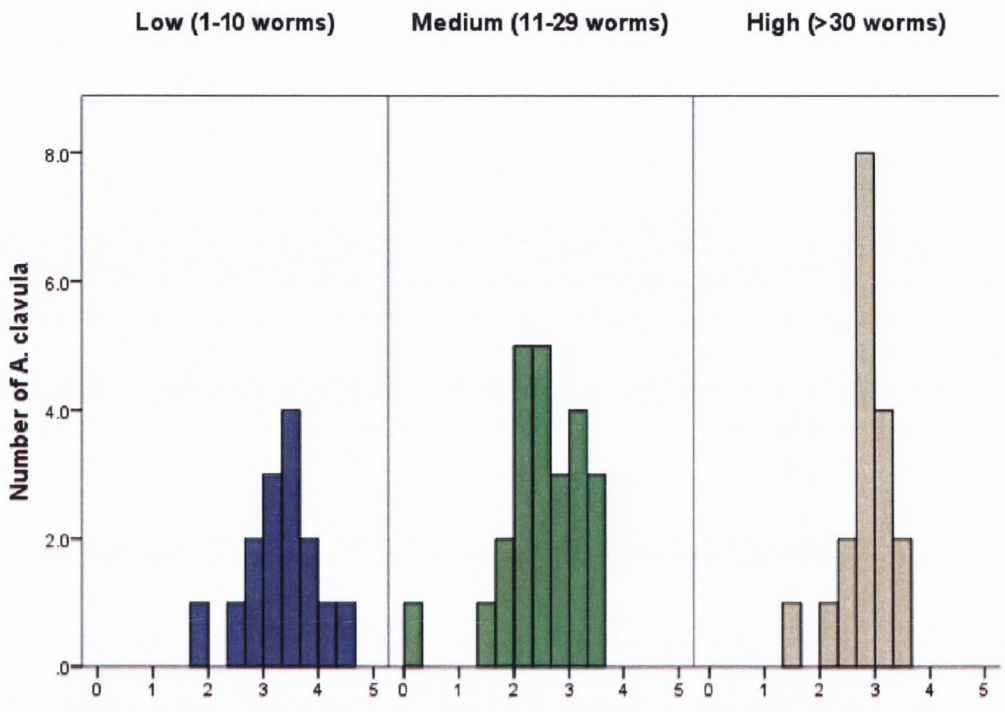


Figure 3.13 Length of male *A. clavula* from low, medium and high intensity infections in Clogher Lough

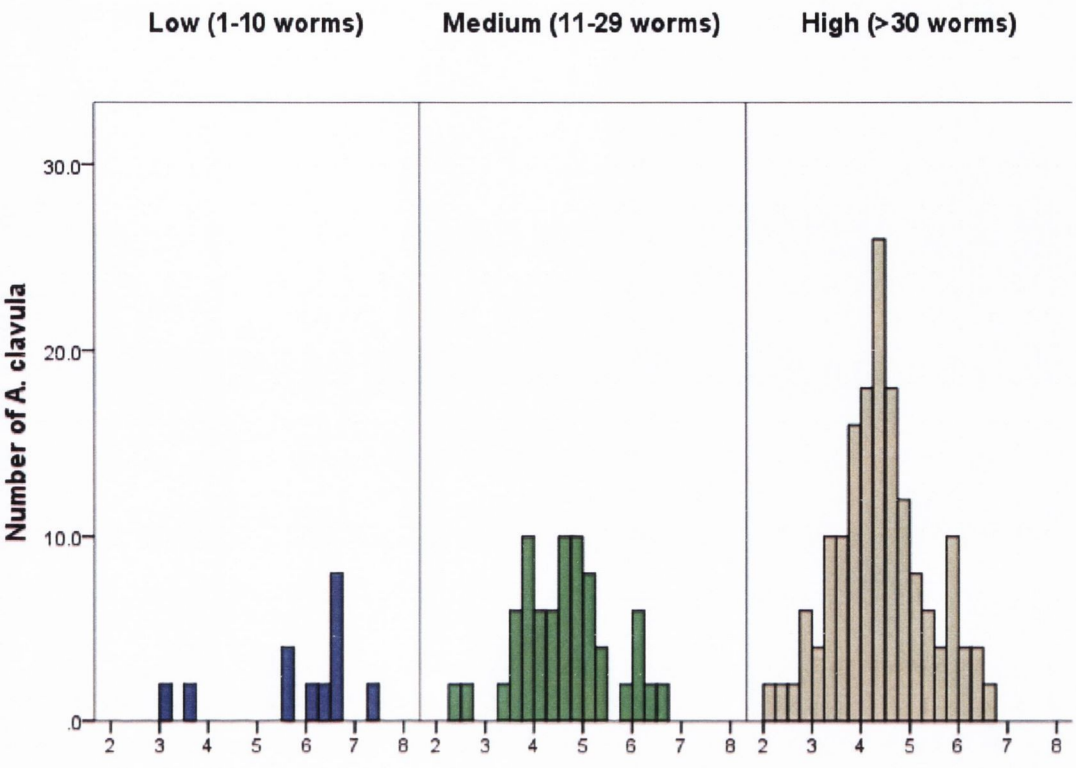


Figure 3.14 Length of female *A. clavula* from low, medium and high intensity infections in Lough Feeagh

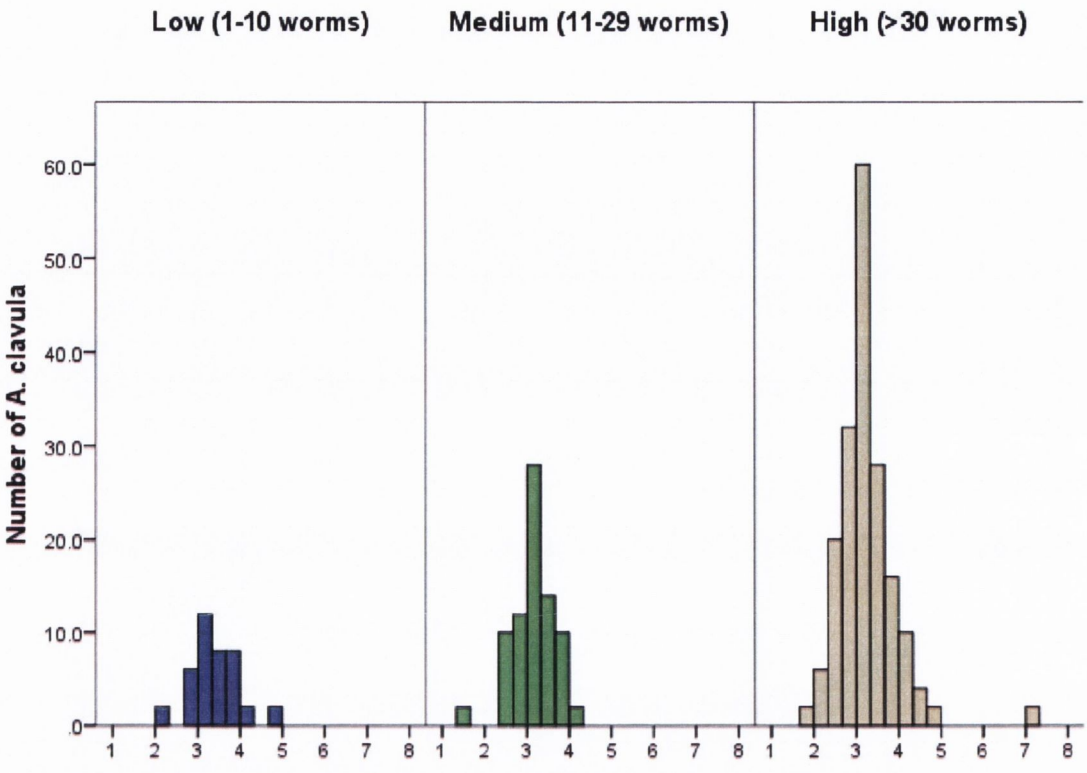


Figure 3.15 Length of male *A. clavula* from low, medium and high intensity infections in Lough Feeagh

Table 3.12 Mean length (mm) ± S.E. of male and female *A. clavula* in infections of varying intensity from eels in Clogher Lough and Lough Feeagh

	Low intensity (1-10 worms)	**Medium intensity (11-29 worms)**	**High intensity (> 30 worms)**
Clogher Lough			
Male length (mm) ± S.E.	3.32 ± 0.16	2.56 ± 0.15	2.89 ± 0.11
Female length ± S.E.	4.56 ± 0.14	3.69 ± 0.20	4.17 ± 0.18
Lough Feeagh			
Male length (mm) ± S.E.	3.42 ± 0.13	3.17 ± 0.08	3.24 ± 0.07
Female length ± S.E.	5.86 ± 0.40	4.63 ± 0.15	4.40 ± 0.10

Season was also a significant factor, as a seasonal pattern in *A. clavula* length was observed in both lakes. The shortest individuals were recovered in February; length then increased in May before levelling off in July and September (Figure 3.16).

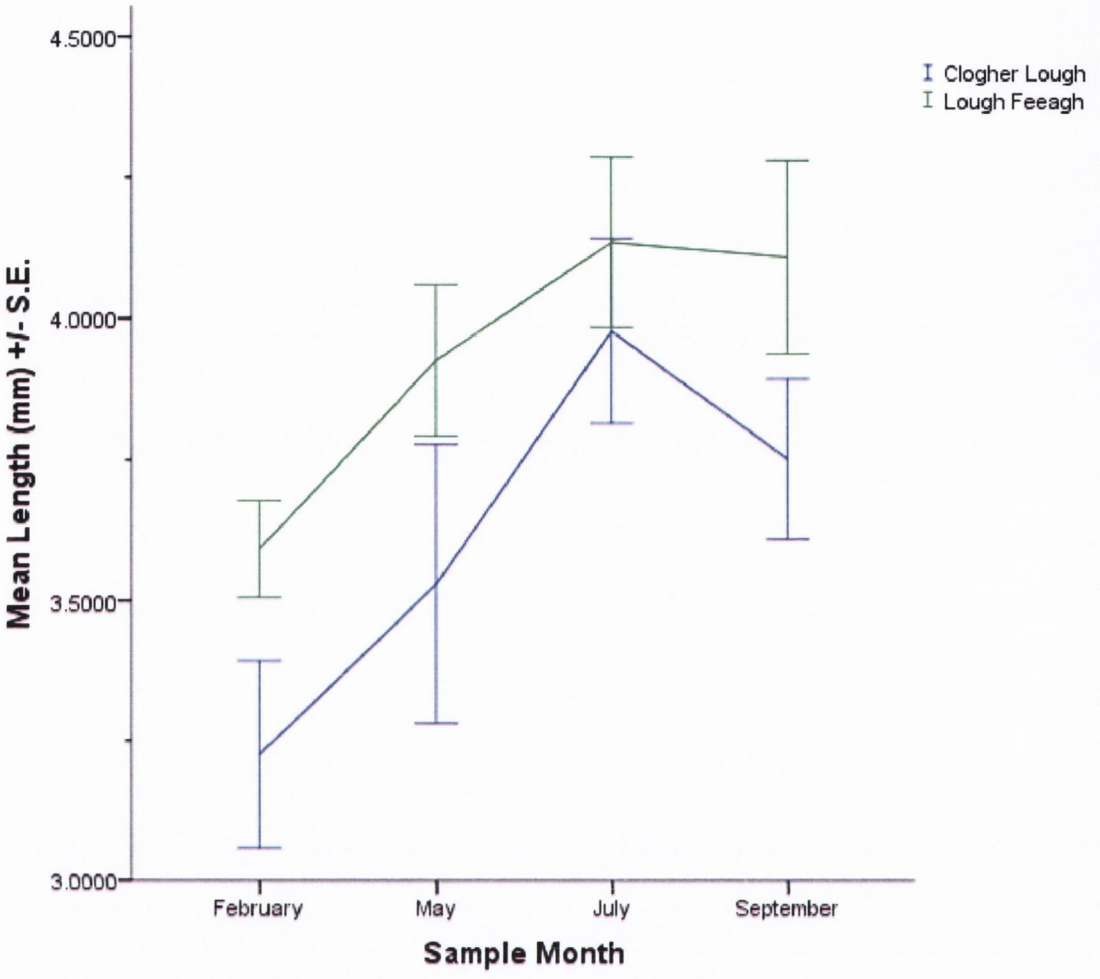


Figure 3.16 Seasonal variations in *A. clavula* body length in Clogher Lough and Lough Feeagh

Seasonal pattern in *A. clavula* mass in Clogher Lough and Lough Feeagh

A four-way GLM was carried out to explore the effect of lake, parasite sex, intensity status and month on parasite body mass (Table 3.13).

Table 3.13 GLM table of the effect of various factors on mass of *A. clavula* in the intestine of eels in Clogher Lough and Lough Feeagh

	***F*-ratio**	***p*-value**	***df***
Factors			
Lake	**69.347**	**≤ 0.0001**	**1**
Season	0.11348	0.9401	3
Parasite sex	**17.821**	**≤ 0.0001**	**1**
Intensity status	2.3462	0.0968	2
Interactions			
Lake * Season	0.068505	0.9767	3
Lake * Sex	**15.823**	**≤ 0.0001**	**1**
Lake * Int. status	1.9872	0.1382	2
Season * Sex	0.24558	0.8643	3
Season * Int. status	0.47931	0.8239	6
Sex * Int. status	0.27497	0.7597	2
Lake * Season * Sex	0.84157	0.4715	3
Lake * Season * Int. status	0.56246	0.6900	4
Lake * Sex * Int. status	1.5123	0.2214	2
Season * Sex * Int. status	0.46181	0.8366	6
Lake * Season * Sex * Int. Status	0.72441	0.5378	3

Lake was a highly significant factor, as *A. clavula* individuals from Clogher Lough were significantly heavier than those from Lough Feeagh (Figure 3.17).

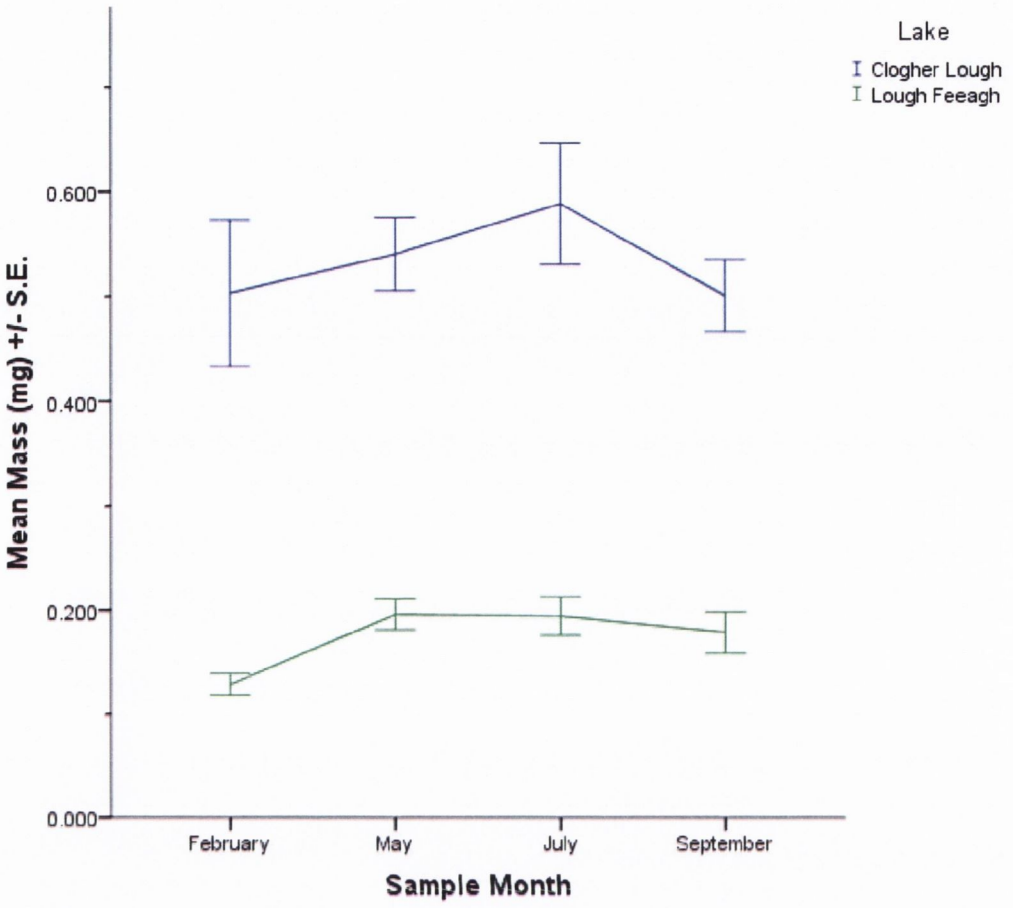


Figure 3.17 Seasonal variations in *A. clavula* mass in Clogher Lough and Lough Feeagh

Parasite sex was also a highly significant factor, due to the fact that females from both lakes were significantly heavier than males.

The interaction between lake and parasite sex had a significant effect on parasite length, due to the fact that more large females were found in Clogher Lough than in Lough Feeagh, while more large male *A. clavula* were found in Lough Feeagh than in Clogher Lough (Figures 3.18 & 3.19).

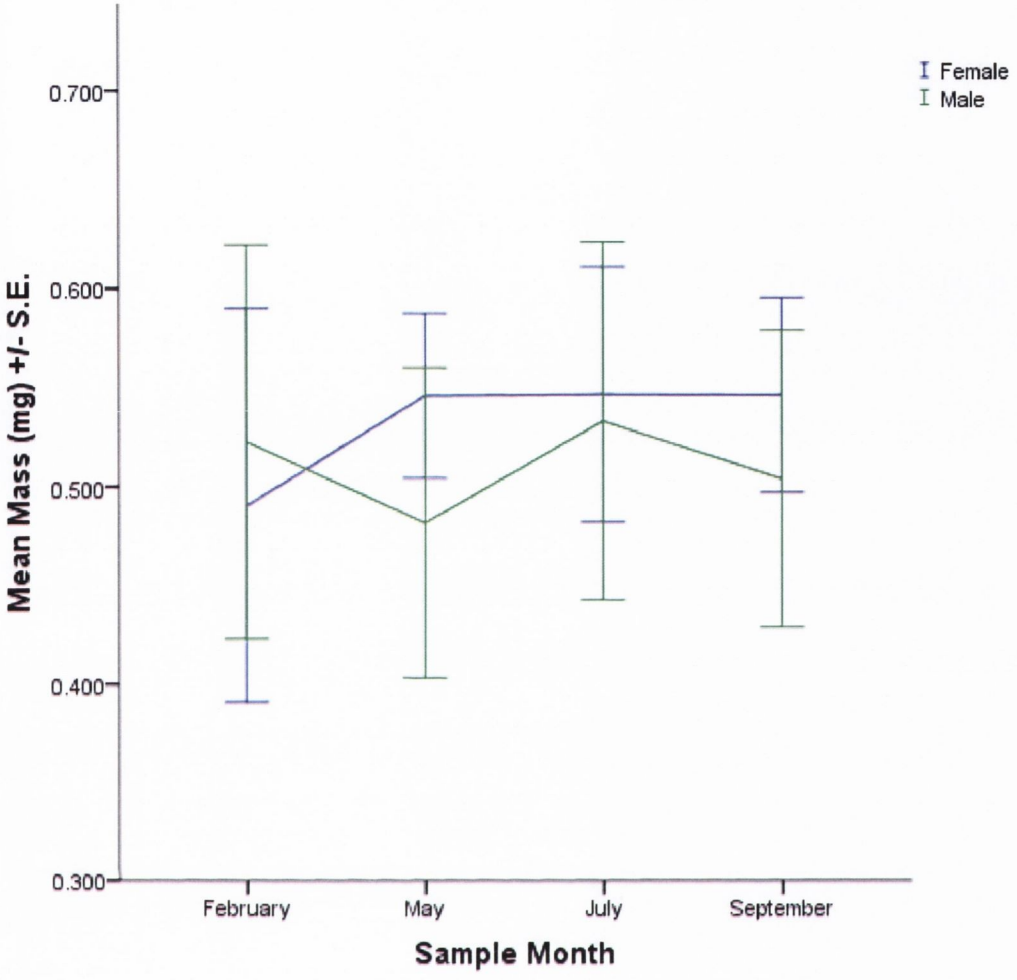


Figure 3.18 Mass of male and female *A. clavula* in Clogher Lough

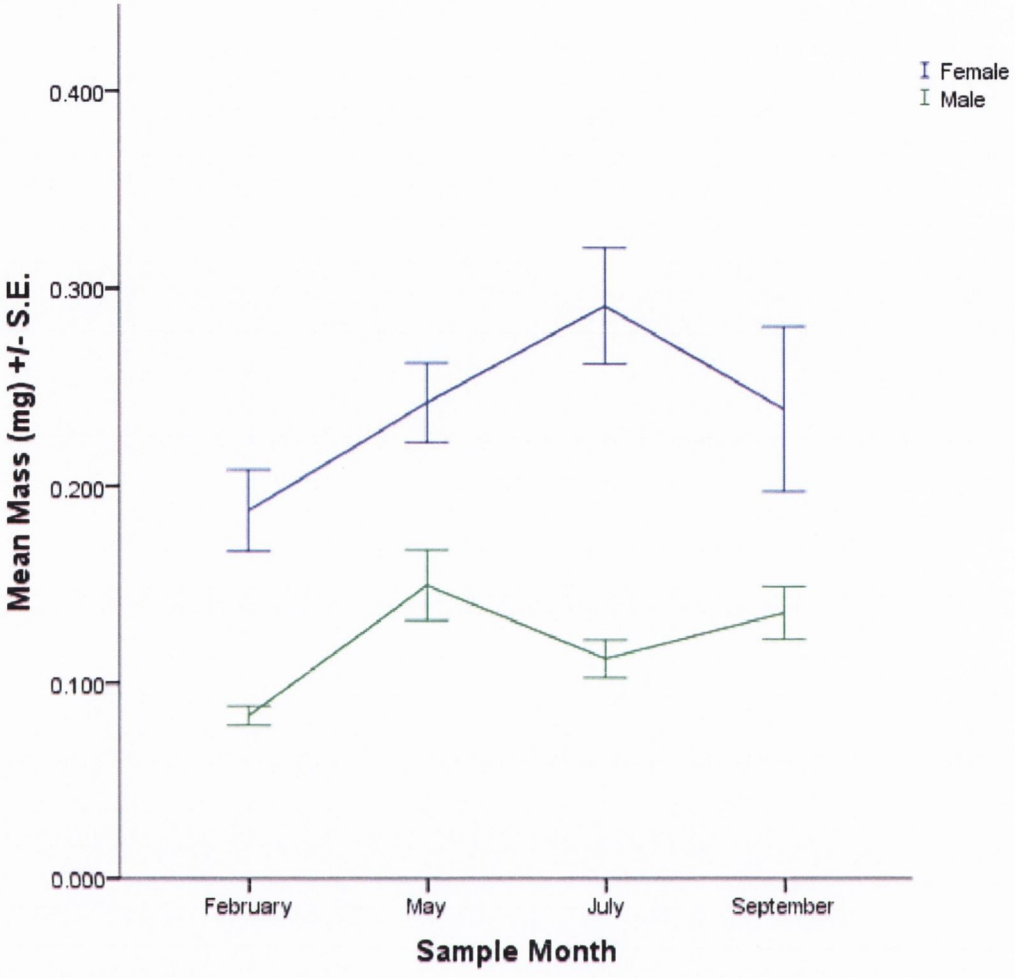


Figure 3.19 Mass of male and female *A. clavula* in Lough Feeagh

3.3.8 *A. clavula* reproductive status

An examination of the maturity status of a sample of female *A. clavula* found that the majority of worms in both lakes were reproductively mature, containing ovarian balls, immature acanthors and mature, shelled acanthors (i.e. they were Stage 3 females) (Table 3.14).

Table 3.14 Reproductive status of *A. clavula* from eels sampled from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
Stage 1 (%)	22.98	23.02
(n)	(20)	(29)
Stage 2 (%)	12.09	19.05
(n)	(11)	(24)
Stage 3 (%)	65.93	57.94
(n)	(60)	(73)

No significant difference in levels of the various reproductive stages was observed between the lakes (χ^2: $\chi^2 = 2.163$, df = 2, p = 0.339).

A. clavula reproductive status: by season

The pattern of occurrence of *A. clavula* reproductive stages in eels was explored (Figures 3.20 & 3.21). All reproductive stages were found in all months in both lakes, with the exception of July in Lough Feeagh, when no Stage 1 females were recovered. Stage 3 (reproductively mature) females predominated in all months in both lakes, with the exception of February in Lough Feeagh, when equal numbers of Stage 1 and Stage 3 females were recovered.

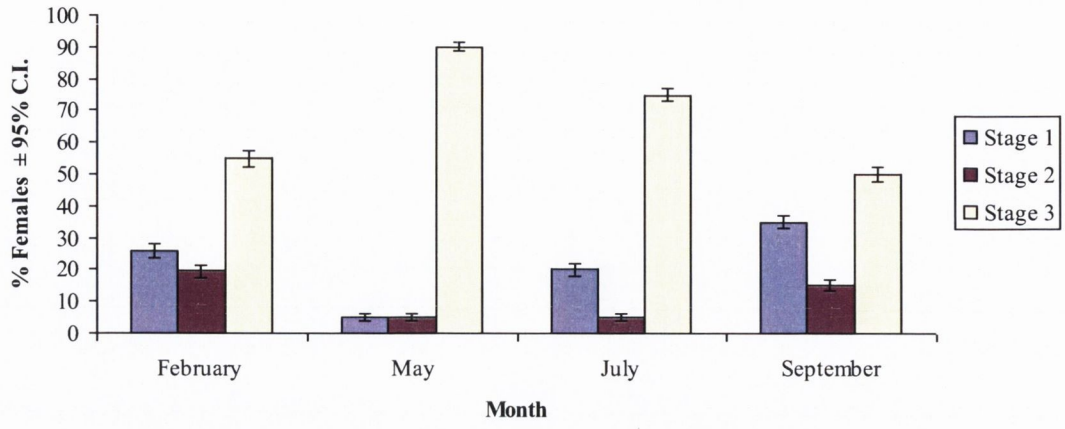


Figure 3.20 Reproductive status of female *A. clavula* (± 95% C.I.) from eels sampled from Clogher Lough

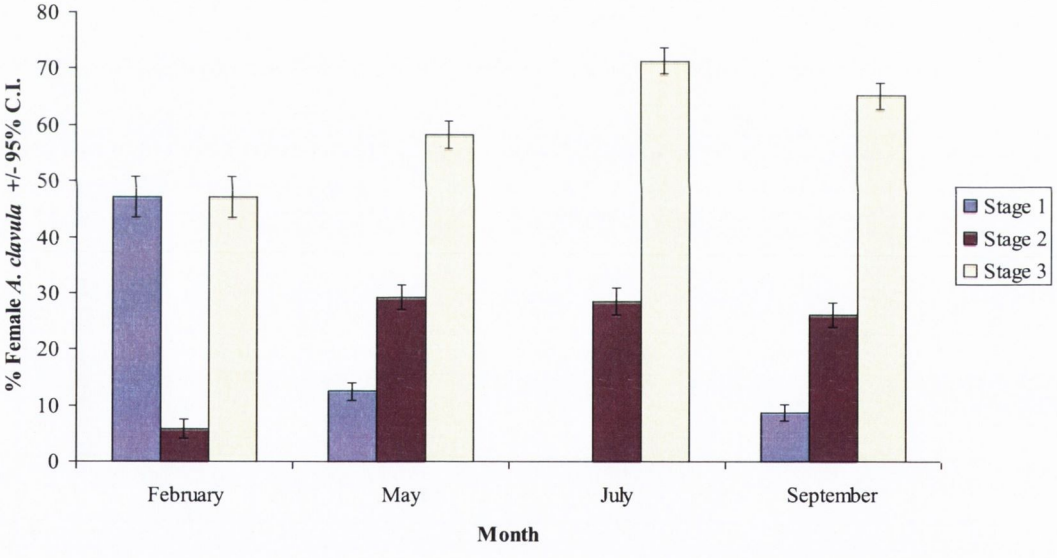


Figure 3.21 Reproductive status of female *A. clavula* (± 95% C.I.) from eels sampled from Lough Feeagh

When differences in the pattern of occurrence of reproductive stages of *A. clavula* were analysed by season (using a G-test), a highly significant difference in the pattern of occurrence was observed in Lough Feeagh, but not in Clogher Lough (Table 3.15).

In Lough Feeagh, the level of Stage 3 females was relatively high in all months, but the level of Stage 1 females varied throughout the year, with a high level (~50%) recovered in February, followed by a decline to low levels (0-10%) in subsequent months (Figure 3.21).

Table 3.15 Results of G-test on the pattern of occurrence of *A. clavula* reproductive stages in Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
G	12.05	38.086
df	6	6
p	0.061	<0.0001

3.3.9 *A. clavula* niche

The niche occupied by *A. clavula* in eels from Clogher Lough and Lough Feeagh was examined in detail. The distribution of *A. clavula* along the eel intestine was calculated for the total sample from each lake, as well as for each sample month. Distribution data were also examined with respect to low (1- 10 worms), medium (11-29 worms) and high intensity (≥30 worms) infections. Finally, the distribution of *A. clavula* in eels was compared between eels from the two lakes.

The *A. clavula* niche: the fundamental niche

The fundamental niche (the niche of all worms present in single species infections) of *A. clavula* along the intestine was first examined (Figures 3.22 & 3.23). The mean percentage position of *A. clavula* in the intestine of eels in Clogher Lough was 52.68% ± 0.78 S.E. The minimum position in which *A. clavula* was found was 9.91%, while the maximum was 100%, indicating that *A. clavula* occupies an extremely broad fundamental niche in the eel intestine in Clogher Lough. Interestingly, three peaks in *A. clavula* percentage position are present in Clogher Lough, at points 30%, 50% and 70% of the way along the eel intestine (Figure 3.22).

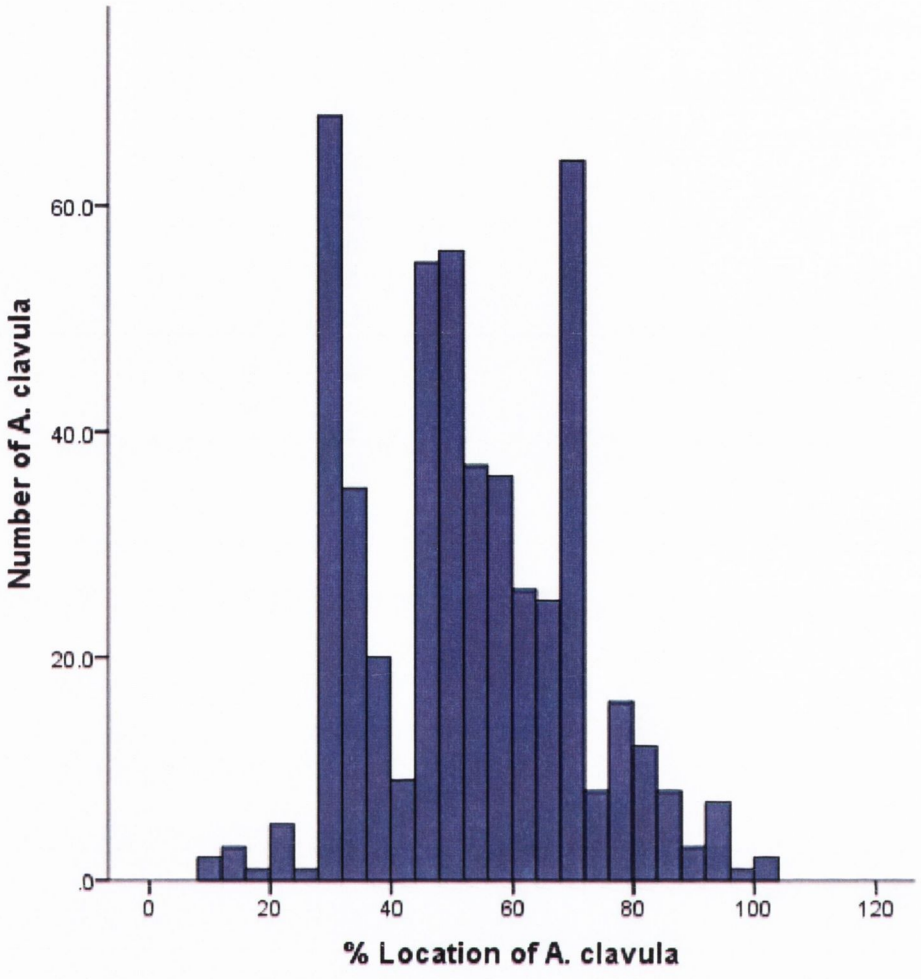


Figure 3.22 The fundamental niche of *A. clavula* along the intestine of eels in Clogher Lough

In Lough Feeagh, the mean percentage position of *A. clavula* was 46.97% ± 0.37 S.E. The minimum position in which *A. clavula* was found was 7.79%, while the maximum was 99.34%, indicating that, again, *A. clavula* occupies an extremely broad fundamental niche in the eel intestine. The occurrence of *A. clavula* along the eel intestine in Lough Feeagh results in a more bell-shaped curve, although multiple smaller peaks are visible, at 35% and 50% of the way along the eel intestine (Figure 3.23).

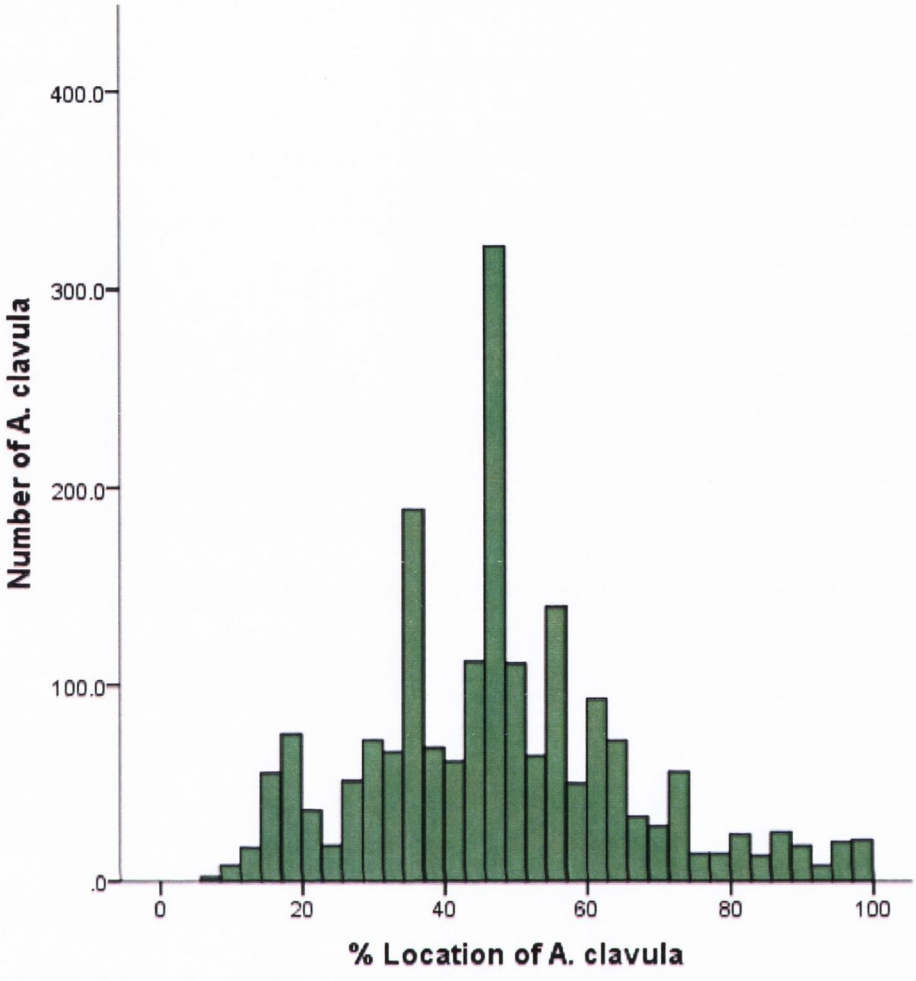


Figure 3.23 The fundamental niche of *A. clavula* along the intestine of eels in Lough Feeagh

The effect of various factors on the niche of *A. clavula* was then explored using Kolmogorov-Smirnov tests. The factors examined were lake, season, parasite sex and intensity status (Tables 3.16a & 3.16b). The dependent variable was percentage location of *A. clavula.*

Table 3.16a Details of factors involved in Kolmogorov-Smirnov tests of *A. clavula* niche

Factor	**Details**
Lake	Clogher Lough, Lough Feeagh
Season	February (2007 & 2008 combined), May, July, September
Parasite sex	Male, Female
Intensity status	Low (1-10 worms)
	Medium (11-29 worms)
	High (> 30 worms)

A significant difference in *A. clavula* percentage position was observed between the lakes ($p < 0.001$, with *A. clavula* from Lough Feeagh occupying a more anterior position in the eel intestine than those from Clogher Lough (Table 3.17).

Table 3.16b Results of Kolmogorov-Smirnov tests investigating factors affecting *A. clavula* percentage position in the intestine of eels from Clogher Lough and Lough Feeagh.

Factor	**Clogher Lough**	**Lough Feeagh**
	p	p
Sex	0.018	<0.001
Intensity level		
Low v Medium	<0.001	0.018
Low v High	<0.001	<0.001
Medium v High	<0.001	0.012

Table 3.17 Mean *A. clavula* percentage position in the intestine of eels from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
Mean male % position ± S.D. (n)	49.01± 16.93 (134)	44.38 ± 17.13 (611)
Mean Female % position ± S.D. (n)	54. 58 ± 17.49 (361)	49.66 ± 18.75 (1265)

Parasite sex was a highly significant factor, with male *A. clavula* in both lakes occupying a more anterior mean position in the eel intestine than females (Table 3.18, Figures 3.24. & 3.25). Indeed, when *A. clavula* distribution is examined by sex, it may be seen that more female worms than male worms are found in posterior positions (>70%) in the intestine (Table 3.19, Figures 3.24 & 3.25).

Table 3.18 Mean percentage position of male and female *A. clavula* in eels from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
Mean male position ± S.E. (%)	49.01 ± 0.91	44.38 ± 0.66
Mean female position ± S.E. (%)	54.51 ± 1.46	48.41 ± 0.47

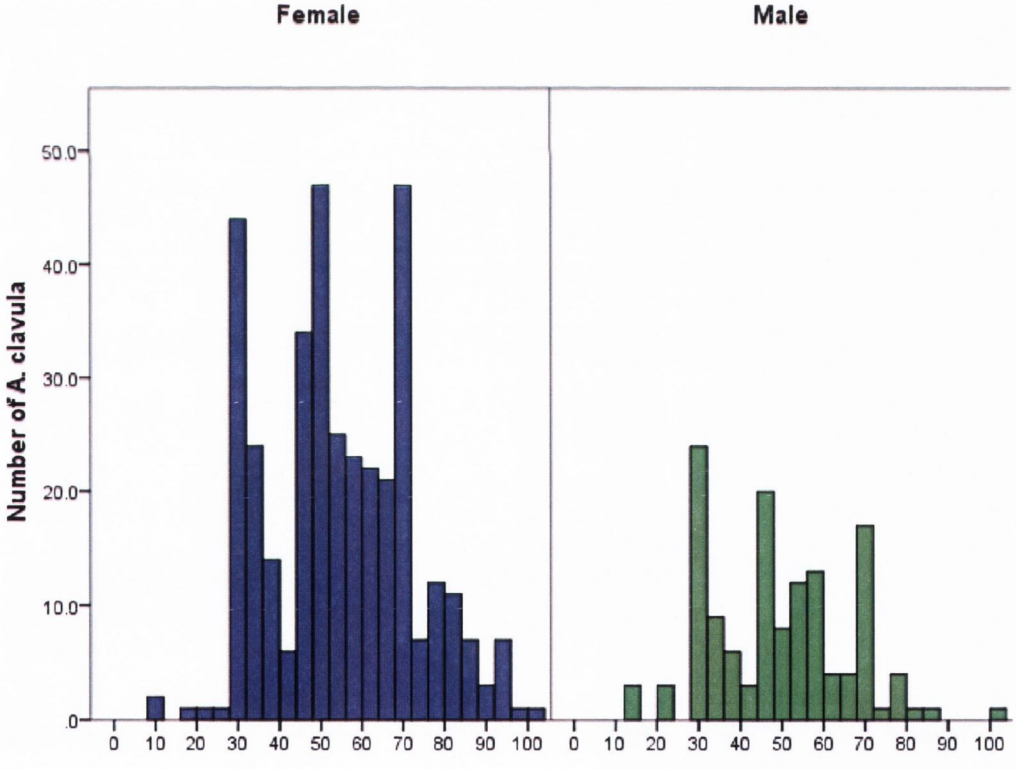


Figure 3.24 Distribution of male and female *A. clavula* in the intestine of eels from Clogher Lough

Table 3.19 Percentage of male and female *A. clavula* in the posterior region of the eel intestine in Clogher Lough and Lough Feeagh

	% Male *A. clavula*	% Female *A. clavula*
Clogher Lough		
71-80%	8.96	6.93
81-90%	1.49	5.00
91-100%	0.75	3.05
Total	**11.2**	**14.98**
Lough Feeagh		
71-80%	2.95	7.00
81-90%	2.13	3.95
91-100%	1.15	3.32
Total	**6.32**	**14.27**

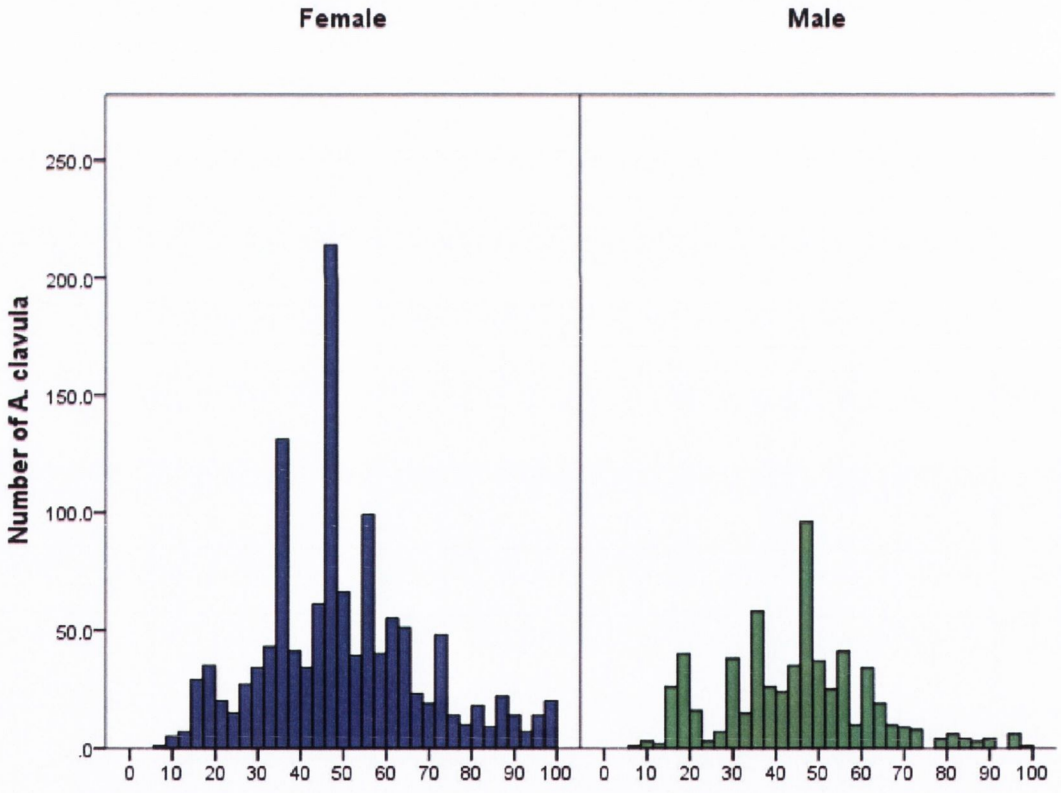


Figure 3.25 Distribution of male and female *A. clavula* in the intestine of eels from Lough Feeagh

As Kolmogorov-Smirnov tests permit the use of two samples only, pairwise comparisons of intensity levels were undertaken. Significant differences in *A. clavula* percentage position were observed in all intensity level comparisons (Table 3.16b).

In Clogher Lough, of the 519 worms recovered, 138 were in low intensity infections, 188 were in medium intensity infections and 193 were in high intensity infections.

The mean percentage position of *A. clavula* declined with increasing infection intensity (Table 3.20). In high intensity infections, three peaks in *A. clavula* occurrence may be observed, at 30%, 55% and 70%. These peaks are likely owing to the occurrence of large numbers of closely packed individuals in a small area of intestine (Figure 3.26).

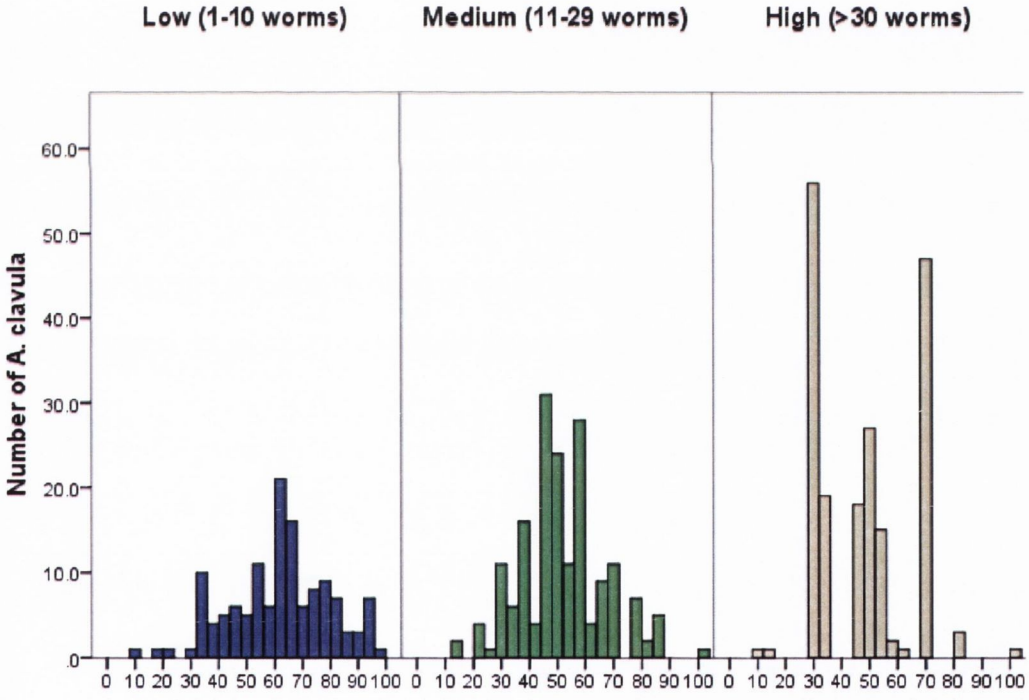


Figure 3.26 Distribution of *A. clavula* according to intensity level along the intestine of eels from Clogher Lough

The lowest minimum position of *A. clavula* in the intestine (9.71%) occurred in a low intensity infection, while the highest (100%) occurred in a both a medium and a high intensity infection.

Table 3.20 Summary statistics for the distribution of *A. clavula* in the intestine of eels in Clogher Lough by intensity level

	Low	**Medium**	**High**
Count	120	151	191
Mean (%) ± S.E.	60.70 ± 1.55	51.50 ± 1.12	48.08 ± 1.24
Inter-quartile range	24.35	13.91	39.75
Minimum (%)	9.7	13.6	9.91
Maximum (%)	96.6	100	100
Range	86.89	86.4	90.09

In Lough Feeagh, of the 2345 worms for which intensity level data were available, 213 were in low intensity infections, 605 were in medium intensity infections and 1527 were in high intensity infections.

The mean position of *A. clavula* was almost identical for low (45.28%) and high (45.99%) intensity infections, while it was slightly higher (50.03%) for medium intensity infections (Table 3.21). In low intensity infections, less worms were found in more posterior locations (>70%) than in medium or high intensity infections (Figure 3.27).

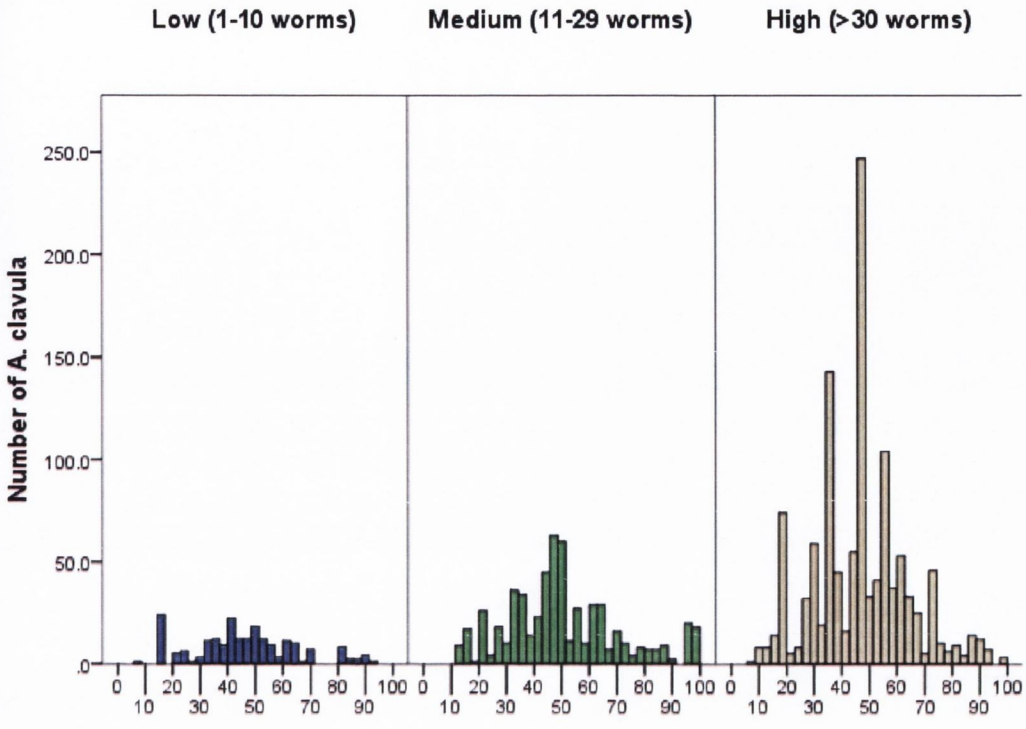


Figure 3.27 Distribution of *A. clavula* according to intensity level along the intestine of eels from Lough Feeagh

The lowest minimum position of *A. clavula* in the intestine (7.8%) occurred in a low intensity infection, while the highest (99.3%) occurred in a medium intensity infection.

Table 3.21 Summary statistics for the distribution of *A. clavula* in the intestine of eels in Lough Feeagh by intensity level

	Low	**Medium**	**High**
Count	213	605	1527
Mean (%) ± S.E.	45.3 ± 1.3	50.0 ± 0.8	46.0 ± 0.4
Inter-quartile range	19.7	26.5	20.5
Minimum (%)	7.8	12.0	7.9
Maximum (%)	93.0	99.3	98.2
Range	85.2	87.3	90.3

Differences in *A. clavula* percentage location between sample months were also investigated. Again, pairwise comparisons were undertaken. Significant differences were observed between all pairwise comparisons in both lakes (Table 3.22).

Table 3.22 Results of Kolmogorov-Smirnov tests investigating the effect of sample month on *A. clavula* percentage location in eels from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
	P	**p**
2007		
February v May	<0.001	<0.001
February v July	<0.001	<0.001
February v September	<0.001	<0.001
May v July	<0.001	<0.001
May v September	0.001	<0.001
July v Septmber	<0.001	0.014

In Clogher Lough, more *A. clavula* individuals are found in more anterior locations in February and May, while in July and September, large numbers of worms are found in more posterior locations (Figure 3.28). Indeed, the lowest mean *A. clavula* position observed was in February and was significantly higher in all other months (Table 3.23).

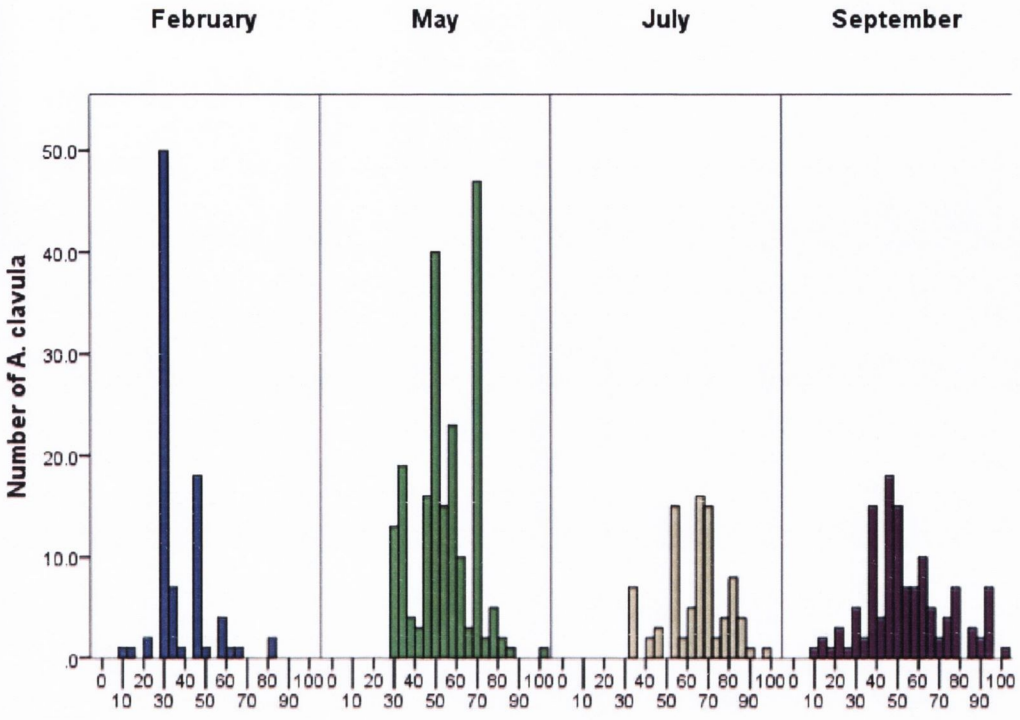


Figure 3.28 Distribution of *A. clavula* in the eel intestine by sample month in Clogher Lough

Table 3.23 Summary statistics for the distribution of *A. clavula* in the intestine of eels in Clogher Lough for each sample month

	February	**May**	**July**	**September**
Count	89	204	85	84
Mean (%) ± S.E.	36.13 ± 1.33	55.02 ± 0.97	64.08 ± 1.61	52.99 ± 1.78
Inter-quartile range	16.85	22	17.8	21.1
Minimum (%)	9.9	28.0	32.3	9.7
Maximum (%)	83.4	100	96.6	100
Range	73.5	72.0	64.3	90.3

In Lough Feeagh, a similar pattern may be observed, with increasing numbers of *A. clavula* individuals occupying more posterior positions throughout the year (Figure 3.29).

In addition, the mean position occupied by *A. clavula* individuals also increased gradually from February to September (Table 3.24).

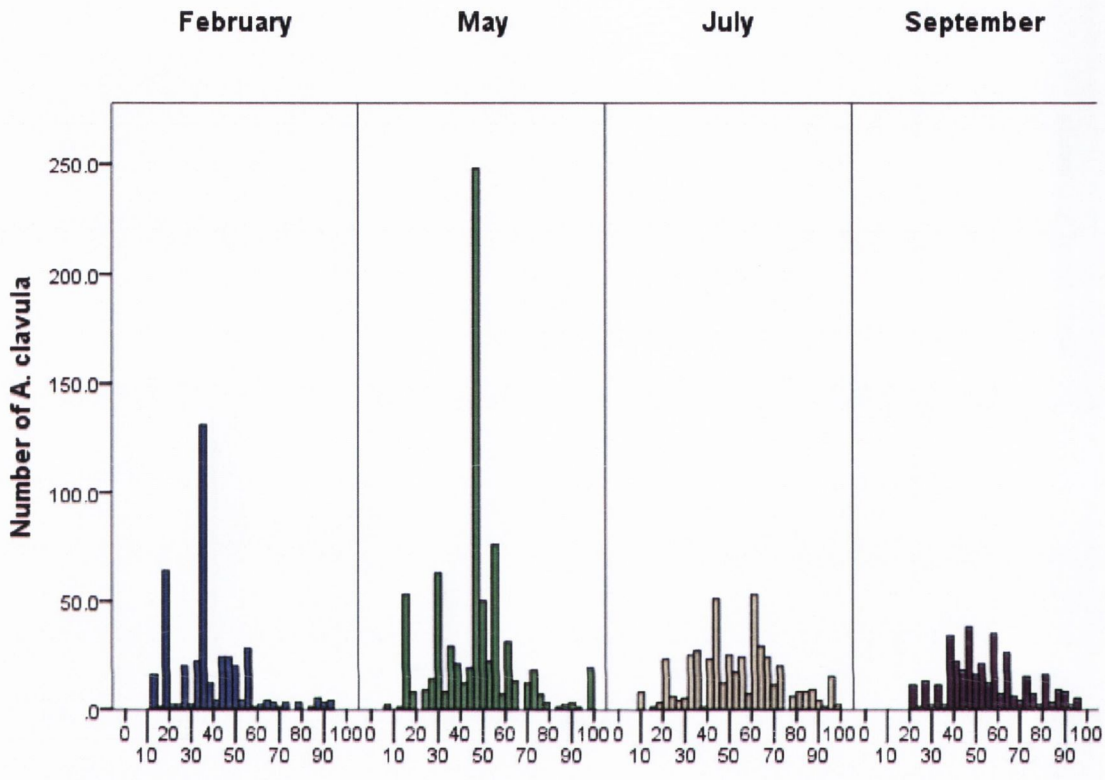


Figure 3.29 Distribution of *A. clavula* in the eel intestine by sample month in Lough Feeagh

Table 3.24 Summary statistics for the distribution of *A. clavula* in the intestine of eels in Lough Feeagh for each sample month

	February	**May**	**July**	**September**
Count	705	826	466	348
Mean (%) ± S.E.	40.3 ± 0.6	46.0 ± 0.6	53.3 ± 0.9	54.3 ± 0.9
Inter-quartile range	10.9	16.2	23.7	22.3
Minimum (%)	13.1	7.8	10.5	20.6
Maximum (%)	98.0	98.8	99.3	96.9
Range	84.9	91.0	88.8	76.3

3.3.10 *Pomphorhynchus laevis* and other acanthocephalan species in eels in Clogher Lough and Lough Feeagh.

Clogher Lough

Neither *P. laevis* nor any other acanthocephalan species with the exception of *A. clavula* was found in the intestine of eels sampled from Clogher Lough.

Lough Feeagh

P. laevis occurred in Lough Feeagh eels at low levels of prevalence and abundance (Table 3.25).

P. laevis was observed only in February 2007 & 2008, when five eels were infected and May 2007, when three eels were infected.

Table 3.25 Population parameters of *P. laevis* from eels from Lough Feeagh

Lake	**N**	**Prevalence (%)**	**Mean abundance ± S.E.**	**Mean intensity ± S.E.**	**Range**
Feeagh	111	7.2	0.23 ± 0.09	3.1 ± 0.69	0 - 6

No other acanthocephalan species were found in the intestine of eels from Lough Feeagh.

3.4 Discussion

Distribution and abundance of *A. clavula*

It has been proposed that various physical and chemical features of lakes, including lake size, depth and altitude, as well as trophic status, may have an influence on parasite assemblages and abundance. Biotic factors, such as the abundance of intermediate hosts (Sures & Streit, 2001) and post-cyclic transmission (Kennedy, 1999), have also been explored as potential determinants of distribution and abundance patterns (Carney & Dick, 2000).

Dogiel (1961) first observed the relationship between the parasite fauna of a fish species and the size of a water body. Larger water bodies by nature have greater habitat diversity within them and thus have greater faunal diversity, which leads to a greater abundance of potential host species for parasites.

The majority of studies exploring the relationship between lake size and parasite assemblages deal only with the number of parasite species present, and not with the prevalence and abundance of individual species (Kennedy, 1978; Marcogliese & Cone, 1991; Hartvigsen & Kennedy, 1993), however we can logical assume that higher numbers of individual parasite species would be present in large lakes when compared with lakes of a smaller size, due to increased micro-habitat diversity as well as increased numbers of intermediate and definitive host species.

With an area of 410 hectares, Lough Feeagh is almost seven times larger than Clogher Lough, and so it is to be expected that eels from Lough Feeagh would harbour much heavier burdens of *A. clavula* than eels from Clogher Lough, due to the more diverse host populations of Lough Feeagh (R. Poole, pers. comm.) Indeed, the number of *A. clavula* individuals recovered from eels from Lough Feeagh (2306) was approximately five times greater than that recovered from eels from Clogher Lough (458). The prevalence, mean

abundance and mean intensity of *A. clavula* infection in eels were also significantly higher in Lough Feeagh.

The relationship between lake trophic status and the parasite fauna of freshwater fish has been extensively explored. Many studies have found a positive relationship between eutrophication and fish parasitism (Dogiel *et al.*, 1961; Dechtiar, 1962; Snieszko, 1972; Hartmann & Numann, 1977; Schupp & Macins, 1977; Hanek & Fernando, 1978) and a number of authors (Wiesniewski, 1958; Esch, 1971) have succeeding in predicting the parasite species present in a lake based on its trophic status. Halvorson (1971) however, found fish species which harbour the same parasite species in lakes of differing trophic status. Moser & Cowen (1991) reported a higher prevalence of *Echinorhynchus* species on the eutrophic east side of McMurdo Sound in Antarctica than on the oligotrophic west side. The higher level of prevalence was attributed to the much higher abundance of species which function as intermediate hosts in the eutrophic location (Moser & Cowen, 1991).

Here, a conflicting result was found: Lough Feeagh, which is classed as oligotrophic, was found to harbour much greater numbers of *A. clavula* than Clogher Lough, which is mesotrophic. In this case, the size of the lake may be of more importance in determining *A. clavula* abundance than its trophic status.

However, if the situation is examined more closely, a different picture emerges. In Moser & Cowen's (1991) study, the acanthocephalan species was most prevalent in the location where there was a greater abundance of potential intermediate hosts. In Clogher Lough and Lough Feeagh the intermediate host of *A. clavula* is the isopod *A. meridianus*, a species known to flourish in polluted environments (Chambers, 1977). *A. meridianus* in both lakes had an extremely patchy distribution, occurring only in areas containing dense vegetation and a large amount of detritus and debris, i.e. the more 'eutrophic' areas of the lake (see Chapter 2). Lough Feeagh contains more of these 'eutrophic' areas and thus harbours a greater abundance of *A. meridianus*.

Lough Feeagh, due to its size, also contains a greater number of eels than Clogher Lough. The higher number of eel individuals present means that more shelled acanthors are shed into the environment in Lough Feeagh, which results in a higher prevalence of *A. clavula* in both its intermediate and definitive hosts in Lough Feeagh.

The higher level of *A. clavula* infection in eels from Lough Feeagh also suggests that eels in Lough Feeagh may be feeding intensively on *A. meridianus*. However, when stomach contents of eels from Lough Feeagh were examined (for the months May, July and September), it was found that just 11% of stomachs contained *A. meridianus*, with an average occurrence of just 0.34 *A. meridianus* per eel (Maher, 2009). This is in stark contrast to the high levels of *A. clavula* prevalence and abundance in Lough Feeagh eels, which range from 85% to 97% and 11 to 38 individuals, respectively.

Chubb (1964) reported a similar discrepancy between *A. meridianus* ingestion by eels and *A. clavula* infection, with just 4% of stomachs examined containing *A. meridianus*, while *A. clavula* prevalence in eels was 28%. In grayling, a non-preferred definitive host of *A. clavula*, a different situation was observed, with 50% of examined stomachs containing *A. meridianus* and 46% of grayling infected with *A. clavula*. However, the mean intensity of infection in eels was almost five times greater in eel than grayling, which suggests that it is host suitability, and not volume of intake of intermediate host, which is important for *A. clavula* infection.

Seasonal patterns in prevalence, mean intensity and mean abundance

Kennedy (1984) examined a population of *A. clavula* in eels in a British river. During the 4.5 year study, both prevalence and mean abundance showed a progressive decline and by the end of the study, *A. clavula* had disappeared from the river. No seasonal periodicity in either measure was evident during the decline.

Rojanapaibul (1977) observed a seasonal cycle of infection of *A. clavula* in gwyniad and roach in Llyn Tegid, with the highest levels of prevalence and abundance occurring in

the summer months; however neither species is a preferred definitive host for the parasite. Indeed, just 15% of females in gwyniad reached full reproductive maturity and no mature females were reported from roach. The majority of *A. clavula* infecting these species are therefore unable to complete their life-cycle. Infection of gwyniad and roach by *A. clavula* is thus accidental. As infection of these accidental host species is of little or no benefit to the parasite, utilising data from these hosts for analysis of patterns of infection of *A. clavula* is therefore of little use to the exploration of the population dynamics of *A. clavula*.

In his exploration of *A. clavula* population biology in eels, however, Rojanapaibul (1977) found a similar pattern of infection to that found in Clogher Lough and Lough Feeagh. Prevalence was maintained within relatively narrow limits (35 – 53%) for the majority of the year (with the exception of the period January – March, when a peak in prevalence of 77% was observed). Mean abundance reached its highest level in the period January – March (7.69 individuals), then declined for the rest of the year.

Chubb (1964), also working in Llyn Tegid, found that the prevalence pattern of *A. clavula* in eels was more variable than those found by Rojanapaibul (1977) as well as in the current study (ranging from 14% in the period January to March to 84% in the period October – December). Mean intensity was highest in the periods January – March and April – June (20.2 and 26.3 individuals, respectively) and declined thereafter. The results of Chubb (1964) should be analysed cautiously however, as the sample sizes used were very small (< 10 individuals). It is interesting to note that *A. clavula* appears to have declined in Llyn Tegid in the period between the two studies, which may be owing to a decline in *A. meridianus*, possibly due to replacement by its congener and superior competitor *A. aquaticus* (Williams, 1962; Wolff, 1973).

Byrne *et al.* (2004) reported *A. clavula* from eels in Clogher Lough at a prevalence of 97% and mean abundance of 7.7 individuals; however these values are from June and September samples combined and no other samples were taken.

Prevalence of *A. clavula* from eels from Clogher Lough and Lough Feeagh in the current study showed no evidence of seasonal periodicity and prevalence was maintained within very narrow limits for all months in both lakes (Clogher Lough: 60-68%; Lough Feeagh: 84-97%, with the exception of May 2007 in Clogher Lough, when prevalence dipped to 48.1%). Despite the lack of seasonal periodicity in prevalence, a clear seasonal cycle of mean abundance and mean intensity was observed. The cycle was the same in both lakes. The highest mean abundance (11.3 and 37.8 individuals, respectively) and mean intensity values were recorded in February, followed by a gradual decline in both measures for the remainder of the year.

This is an unexpected result and one which is in contrast to previously found cycles of infection in acanthocephalans of fish. Many acanthocephalan species have been shown to have clear seasonal cycles in prevalence and abundance in intermediate and/or definitive hosts (see review by Chubb, 1982). The cycles are generally closely correlated with seasonal changes in biotic factors, such as host diet and the life-cycle of the intermediate host, and abiotic factors, such as water temperature (Kennedy, 2006). Most often, infection levels peak in the summer months, when fish feeding activity is at its highest levels (Awachie, 1965; Brattey, 1986, 1988; Camp & Huizinga, 1980). A small number of studies, however, report no evidence of such cycles (Kennedy, 1985a; Brown, 1989). In these cases hosts were able to acquire parasites throughout the year and a dynamic equilibrium between gain and loss of parasites existed. This equilibrium is related to fish feeding intensity and parasite establishment, both of which, in turn, are related to water temperature. In the summer months, parasite establishment is lowered due to higher water temperatures, but fish feed more actively so parasite intake is high, while in the winter months fish feeding rates decline, so fewer parasites are ingested, but those that are ingested establish better. Infection levels thus remain at relatively similar levels throughout the year (Kennedy, 2006).

In Clogher Lough and Lough Feeagh, however, while a seasonal cycle in abundance was observed, it was highly unusual. As adult *A. clavula* are not long-lived, living for a maximum of 48 days in the definitive host (Rojanapaibul, 1977), it appears that the

majority of *A. clavula* are recruited into infrapopulations during the winter months, when both water temperature and fish feeding intensity are at their lowest levels.

It is possible that *A. clavula* is sensitive to temperature and shows decreased survival at higher temperatures. This would explain the decrease in mean abundance throughout the year, despite the fact that prevalence remained at similar levels. Indeed, in Lough Feeagh, water temperature rose steadily from February to September (mean temperatures: February: 5.5°C; May 9.0°C; July 12.0°C; September 13.0°C) (Russell Poole, pers. comm.). While eels feed more intensively as water temperature rises, *A. clavula* would be less able to establish, so in spite of potentially ingesting large numbers of *A. meridianus*, *A. clavula* abundance decreases as water temperature increases. While no data on temperature-dependent establishment of *A. clavula* exist, Kennedy (1972) investigated establishment of *P. laevis* in goldfish *Carassius auratus* at varying temperatures and found that establishment did in fact decrease as water temperature increased.

While *A. clavula* abundance in *A. meridianus* is lowest in the month of February, it is possible that *A. meridianus* abundance is at its highest level in the lakes. The *A. meridianus* population in February contains many large, over-wintered individuals. As adult eels use visual location of prey, these larger individuals would be more easily seen, and thus it is possible that eels are ingesting higher numbers of *A. meridianus* in February than in other months. This higher rate of ingestion would counteract the fact that prevalence of *A. clavula* is low in *A. meridianus*. The ingestion of high numbers of isopod individuals therefore ensures high abundances of *A. clavula* in eels.

Unfortunately, no data are available on either *A. meridianus* abundance or eel diet in Clogher Lough and Lough Feeagh for the month of February, so it is not possible to determine if *A. meridianus* were more abundant or indeed if eels were ingesting high numbers of *A. meridianus* in February.

Rojanapaibul (1977) suggests that intensity may decrease as female worms are discharged after acanthor release. As the sex ratio remained relatively constant in Clogher Lough and Feeagh throughout the current study, the loss of female *A. clavula* is unlikely to have contributed to the decline in abundance.

A. clavula length, weight and reproductive status

The majority of acanthocephalan species exhibit sexual dimorphism, with females being larger than males (Crompton, 1985). Sexual dimorphism has been previously observed in *A. clavula* (Rojanapaibul, 1977) and was also observed in *A. clavula* from eels in Clogher Lough and Lough Feeagh; females were both significantly longer and significantly heavier than males in both lakes.

Females achieve a larger size than males due to their increased longevity in the definitive host. Males are lost soon after mating takes place, while females remain until acanthors have matured and been released (Awachie, 1965; Chubb, 1964). In Clogher Lough and Lough Feeagh, the sex ratio was in favour of females in all sample months in both lakes. The sex ratio in both lakes was maintained at similar levels in all sample months, which suggests that *A. clavula* were mating throughout the year.

As the European eel is the preferred definitive host of *A. clavula*, it is to be expected that worms should therefore reach their maximum size and reproductive potential in this host. This phenomenon has been previously observed in *A. clavula* from England (*A. clavula* mass and reproductive potential were found to be greater in eels than in perch), as well as in other acanthocephalan species (Lyndon & Kennedy, 2001).

A. clavula recovered in this study were much smaller in size than their British counterparts. A growth differential between Britain and Ireland has also been reported for the acanthocephalan *Pomphorhynchus laevis* in brown trout. The mean mass of female *P. laevis* from brown trout in Britain (38.3 ± 3.1 mg) (Lyndon & Kennedy, 2001) was much greater than that recorded from brown trout in Ireland (7mg, but males and females combined) (Molloy *et al.*, 1995b). Lyndon & Kennedy (2001) reported mean

mass of female *A. clavula* of 3.07 ± 0.38 mg from the River Clyst, while in Clogher Lough and Lough Feeagh, much lower mean mass values (0.661 ±0.03 and 0.234 ± 0.01, respectively) were observed. While these mean mass values are in accordance with those reported by Byrne *et al.* (2004) from Clogher Lough (0.34 ± 0.27), in terms of British *A. clavula*, the values are more comparable to those recorded from perch (0.63 ± 0.02) (Lyndon & Kennedy, 2001).

Despite their small size, female *A. clavula* in Irish eels are capable of reaching full reproductive maturity and Stage 3 females predominated in all sample months in both Clogher Lough and Lough Feeagh (except February in Lough Feeagh, when equal numbers of Stage 1 and Stage 3 females were recovered). In the one other exploration of *A. clavula* maturity status in Irish eels, Stage 3 females were also found to predominate (Byrne *et al.*, 2004).

Interestingly, *A. clavula* from eels in Lough Feeagh were significantly smaller in terms of mass than their conspecifics from Clogher Lough, however their small size did not appear to hamper their reproductive capabilities. Indeed, 57.9% of female *A. clavula* from Lough Feeagh eels reached full reproductive maturity, despite an average size just one-third that of worms from Clogher Lough. The proportion of eels reaching reproductive maturity in eels was, however, lower in Lough Feeagh than in Clogher Lough (65.9%). In addition, the abundance of *A. clavula* in Lough Feeagh eels was significantly higher than in eels from Clogher Lough. The high levels of abundance of *A. clavula* in Lough Feeagh, in combination with the smaller size and lower proportion of Stage 3 females, suggests that the parasite population may be undergoing regulation at the infrapopulation level. It appears that fecundity of *A. clavula* is being regulated through a reduction in growth and biomass of the parasite, i.e. a 'crowding effect' is in operation.

Seasonal pattern in *A. clavula* size and reproductive status

While length and mass of *A. clavula* remained at similar levels throughout the year in Clogher Lough, a significant size difference between seasons was observed in Lough Feeagh. In Lough Feeagh, the shortest and lightest *A. clavula* were observed in February. This was followed by an increase in size in May; size was then maintained at similar levels for the remainder of the year.

Reproductively mature female *A. clavula* occurred throughout the year in both lakes, but were more common in the summer months, despite the decline in mean abundance after February. The maintenance of similar size and the occurrence of mature females in all sample months suggest that *A. clavula* were most likely being acquired by eels in both lakes throughout the year.

***A. clavula* niche**

There is just one prior study of the niche of *A. clavula* in eels with which the results of the current study may be compared. Kennedy & Lord (1982) investigated the habitat preferences of *A. clavula* in eels from the River Clyst in Britain and concluded that *A. clavula* is as adaptable and versatile as its host, able not only to withstand changes in temperature and salinity, as well as fish starvation, but to persist in its preferred habitat during the course of these changes.

The habitat selection of *A. clavula* from the River Clyst parallels that of *A. clavula* from the present study in several ways: *A. clavula* from both locations in the present study and from the River Clyst exhibited a preference for the midsection of the alimentary canal, but were able to inhabit a very wide range of the intestine, although very few, or no, individuals were observed in the first 10% of the intestine (Kennedy & Lord, 1982). Preference for a particular region of the intestine is widespread among fish acanthocephalans (Crompton, 1973), although many species exhibit a more precise location preference than *A. clavula* (Awachie, 1966; Chappell, 1969; Amin, 1975; Muzzall, 1980; Valtonen, 1980).

In Clogher Lough and the River Clyst, *A. clavula* were found to occupy a more anterior location in the intestine with increasing intensity of infection, while no difference in mean percentage location was observed between low and high intensity infections in Lough Feeagh. Extension of the parasite range in high intensity infections has been observed frequently (Amin, 1975; Muzzall, 1980), as has the change to a more anterior position (Kennedy *et al.*, 1976).

In Clogher Lough, Lough Feeagh and the River Clyst, males were found to occupy a more anterior position to females (Kennedy & Lord, 1982). This situation has also been observed in other acanthocephalan-host systems (Amin, 1975; Valtonen, 1980).

Unlike *A. clavula* from the River Clyst however, the niche of *A. clavula* from Clogher Lough and Lough Feeagh differed significantly between sample months. In Clogher Lough the mean position of *A. clavula* became more posterior between February and July, before declining again in September. In Lough Feeagh, the mean position of *A. clavula* showed a slight increasing trend throughout the year, nevertheless it was maintained within very narrow limits (40 – 54%). Despite these changes in mean position throughout the year, the range of location of *A. clavula* remains similar in both lakes in all sample months. *A. clavula* have previously been shown to be extremely tolerant of environmental changes (Kennedy & Lord, 1982), and so the possible reason for the significant difference in mean percentage position between sample months is currently unclear.

A. clavula was the only acanthocephalan found in eels from Clogher Lough, so the niche occupied in this location may be close to the fundamental niche of *A. clavula*. In Lough Feeagh, *P. laevis* was also present, albeit in low numbers and only in the months of February and May, however, all niche analysis was carried out only on eels which were infected only with *A. clavula*, thus allowing comparison of the *A. clavula* niche from Clogher Lough and Lough Feeagh.

In conclusion, this chapter has again underlined the need for sampling in multiple locations when exploring acanthocephalan population biology, due to the significant differences in prevalence, abundance and parasite size observed between Clogher Lough and Lough Feeagh. The scope and magnitude of the differences between the two lakes suggest that environmental factors and intermediate host biology have a significant influence on acanthocephalan population biology. It is thus of the utmost importance to incorporate investigations of these factors into future studies in this area.

This chapter has also confirmed that the eel is the preferred definitive host of A. clavula in Ireland, despite the smaller size of Irish worms relative to their counterparts from Great Britain.

A. clavula has been shown to occupy a broad niche in the eel intestine, with the majority of individuals occupying the midsection of the gut. Males were found to occupy a more anterior position than females and the niche range was observed to extend in high intensity infections in Clogher Lough. These findings support previous work on A. clavula niche by Kennedy & Lord (1982). The significant differences in A. clavula niche between sample months are a new finding, however, and further work is necessary in order to establish the purpose (or lack thereof) of the observed shifts in mean position by sample month.

The seasonal changes in A. clavula abundance observed in this study were highly unusual, with decreases in parasite abundance in the summer months. These decreases are possibly owing to a combination of increases in water temperature and changes in the size of the A. meridianus population; however it was not possible to investigate these variables in the current study. This again points to the need for a multi-disciplinary approach to the study of parasite population dynamics.

Chapter 4

A. clavula in Trout, *Salmo trutta*

4.1 Introduction

The brown trout (*Salmo trutta* L.) has been described as a suitable host for *A. clavula* (Lyndon & Kennedy, 2001).

Outside Ireland, this host-parasite combination is rarely observed (Table 4.1). Indeed, Kennedy & Hartvigsen (2000) reported it in only three of 72 localities sampled for trout in Ireland, Great Britain and Norway. In northern Italy, *A. clavula* was recorded in trout in only one of three streams, and then only at a very low prevalence (Dezfuli *et al.*, 2001). In Ireland however, *A. clavula* has been reported from trout on several occasions (Conneely & McCarthy, 1984, 1988; Molloy *et al.*, 1993; Byrne *et al.*, 2002b; Byrne *et al.*, 2004).

In several other studies exploring the helminth community of brown trout in Great Britain, Ireland and mainland Europe, *A. clavula* was absent (for example Dorucu *et al.*, 1995; Conneely & McCarthy, 1988; Kennedy & Hartvigsen, 2000).

The infection of brown trout by *A. clavula* is a rare occurrence (Kennedy & Hartvigsen, 2000). Undoubtedly, this host-parasite combination occurs more frequently in the west of Ireland (specifically the Burrishoole catchment) than in other European locations (Molloy *et al.*, 1993; Byrne *et al.*, 2002b; Conneely & McCarthy, 1988, Kennedy & Hartvigsen, 2000), and while the reason for this is, as yet, unknown, the depauperate nature of the Irish fish fauna is a potential explanation (Griffiths, 1997). This paucity of fish species (in comparison with Britain and mainland Europe) results in unusual host-parasite combinations, as well as forced host-sharing by parasite species, in the absence of their preferred definitive hosts from Ireland (Lyndon & Kennedy, 2001).

Table 4.1 Population parameters of *A. clavula* in trout from various European locations. NA = not available

Location	Year	N	Prevalence (%)	Mean abundance ± S.E.	Mean intensity ± S.E.	Reference
Ireland						
Burrishoole river system (smolts)	1989/1990	60	8.3	0.1 ± 0.6	NA	Molloy *et al.*, 1993
Burrishoole river system (kelts)	1989/1990	17	23.5	2.0 ± 2.3	NA	Molloy *et al.*, 1993
Lough Feeagh (wild trout)	1997/1998	217	25.8	3.0 ± 3.3	11.7 ± 4.4	Byrne *et al.*, 2002b
Lough Feeagh (stocked trout)	1997/1998	122	39.3	3.7 ± 3.3	9.3 ± 4.0	Byrne *et al.*, 2002b
Clogher Lough (wild trout)	1997/1998	161	86.3	53.2 ± 9.0	90.1 ± 9.7	Byrne *et al.*, 2003
Italy						
San Giorgio (stream)		68	4.4	NA	NA	Dezfuli *et al.*, 2001

* Powell (1966): unavailable at time of writing

An alternative explanation is that the intermediate host of *A. clavula*, the isopod *A. meridianus*, is more common in the west of Ireland than in other locations, and thus *A. clavula* is more common in fish in this region, possibly as a result of parasite manipulation of the intermediate host.

In Irish brown trout, the prevalence of *A. clavula* varied considerably, from just 8.3% (Molloy *et al.*, 1993) to 86.3% (Byrne *et al.*, 2003), in spite of the fact that the majority of reports occur in the Burrishoole catchment in the west of Ireland (Molloy *et al.* 1993, Byrne *et al.*, 2002b). A low level of mean abundance and mean intensity were maintained in the Burrishoole catchment, while an extremely high mean abundance (53.2 ± 9.0) of *A. clavula* was observed in Clogher Lough, which is located outside the catchment.

This high level of infection was further investigated by Byrne *et al.* (2004), who found that, while prevalence and intensity were particularly high in Clogher Lough, the proportion of females reaching reproductive maturity was low (2%). It was postulated that either (a) *A. clavula* may have been undergoing a host shift from eel to trout, or (b) that infection levels were due to intensive feeding by trout on the intermediate host of *A. clavula*, the isopod *A. meridianus*.

The aims of this chapter are (1) to explore various aspects of the population biology of *A. clavula* in trout from Clogher Lough and Lough Feeagh, including (a) the prevalence, abundance and intensity of *A. clavula*, (b) the seasonal patterns in infection, (c) aggregation and (d) the parasite niche, and (2) to investigate the relative success of *A. clavula* in trout. Relative success of the parasite will be determined by investigating the size of *A. clavula* individuals in trout as well as the proportion of reproductively mature females, according to the guidelines set out by Lyndon and Kennedy (2001). The possibility of a host shift will then be further examined through a comparison of the results of this thesis with those of Byrne *et al.* (2004).

4.2 Materials & Methods

Materials and methods are as for Chapter 3, with the exception of the fish dissection protocol.

4.2.1 Fish dissection protocol

Trout were defrosted at room temperature. Following a ventral incision from the lower jaw to the anus the entire gut, including the pyloric caecae, was removed, laid in a dissecting tray and measured from the anterior end of the oesophagus to the anus. This facilitated accurate positioning of individual parasites. Each parasite was assigned a percentage location, with 0% corresponding to the anterior oesophagus and 100% corresponding to the anus.

The gut was initially rinsed with 0.9% saline, and then visually examined for the presence of parasites. Gills were individually removed from each fish, washed in saline and examined for parasites. The heart, swimbladder, liver and spleen were removed, weighed and examined for parasites. The body cavity was also examined for encysted parasites.

4.3 Results

4.3.1 Trout sample sizes

A. clavula was never found in trout from Bunaveela Lake, so all results presented are from Clogher Lough and Lough Feeagh. Satisfactory samples (approximately 30 trout) were obtained for all months in Clogher Lough and Lough Feeagh except February 2008 in Clogher Lough (Table 4.2).

Table 4.2 Trout sample sizes from Clogher Lough and Lough Feeagh in 2007 and 2008

	Clogher Lough	**Lough Feeagh**
	(n)	**(n)**
2007		
February	30	33
May	30	26
July	29	31
September	28	40
2008		
February	8	21

The sex of trout sampled from Clogher Lough and Lough Feeagh

Similar numbers of male and female trout were recovered from both Clogher Lough and Lough Feeagh, with slightly more females sampled from each lake (Table 4.3).

Table 4.3 The sex of trout samples from Clogher Lough and Lough Feeagh

	Clogher Lough	Lough Feeagh
	(n)	(n)
Male	57	66
Female	64	77

4.3.2 Trout length and mass

There was a marginally significant difference in length between male and female trout in Clogher Lough (t-test: t = 1.991, df = 81, p = 0.0498) (Table 4.4), with males being longer. There was no significant difference in length between male and female trout in Lough Feeagh (t-test: t = 0.2243, df = 136, p = 0.8228).

Table 4.4 Mean length of trout sampled from Clogher Lough and Lough Feeagh

	Clogher Lough	Lough Feeagh
Mean male length (cm) ± S.E.	17.8 ± 0.5	17.1 ± 0.4
Mean female length (cm) ± S.E.	16.6 ± 0.3	17.3 ± 0.3

There was a marginally significant difference in weight of male and female trout in Clogher Lough (Table 4.5), with males being heavier (t-test: t = 2.054, df = 84, p = 0.0431). There was no significant difference in weight of male and female trout in Lough Feeagh (t-test: t = 0.7313, df = 138, p = 0.4658).

Table 4.5 Mean mass of trout sampled from Clogher Lough and Lough Feeagh

	Clogher Lough	Lough Feeagh
Mean male mass (g) ± S.E.	69.7 ± 7.8	59.5 ± 6.8
Mean female mass (g) ± S.E.	51.0 ± 2.6	61.5 ± 4.2

The significant differences in length and weight of male and female trout in Clogher Lough may be owing to the occurrence in the samples of one exceptionally large individual recovered in September 2007, and four exceptionally large individuals recovered in May 2007.

4.3.3 *Acanthocephalus clavula* from trout from Clogher Lough and Lough Feeagh

A total of 428 specimens of *A. clavula* were recovered from trout in Clogher Lough, ranging from 13 in February 2008 to 243 in July 2007. A total of 3227 specimens were recovered from trout in Lough Feeagh, ranging from 162 in February 2008 to 1838 in May 2007 (Figure 4.1). Almost half (815) of the *A. clavula* specimens recovered in Lough Feeagh in May 2007 originated from one heavily infected individual.

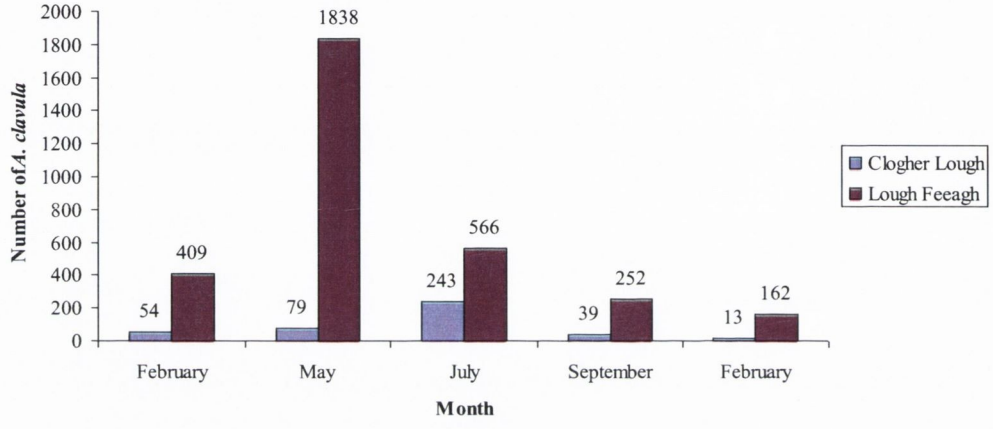


Figure 4.1 Numbers of *A. clavula* recovered from trout in Clogher Lough and Lough Feeagh

4.3.4 Overall prevalence, abundance and intensity of *A. clavula*

Prevalence, mean abundance and mean intensity differ greatly between Clogher Lough and Lough Feeagh, with higher values of all measures reported from Lough Feeagh (Table 4.6).

The overall prevalence of *A. clavula* differed significantly between Clogher Lough and Lough Feeagh (χ^2: $\chi^2 = 18.15$, df = 1, $p \leq 0.0001$).

The abundance of *A. clavula* also differed significantly between the two lakes (t-test: t = 2.72, df = 156, p = 0.0073), as did the intensity (t-test: t = 2.359, df = 107, p = 0.0202)

with Lough Feeagh trout carrying heavier burdens than trout from Clogher Lough (Table 4.6).

When the data were re-analysed with the individual trout recovered in May harbouring 815 worms omitted, the results of the above tests of differences in infection parameters remained unchanged, i.e. all significant results retained significance.

Table 4.6 Overall prevalence, mean abundance and mean intensity of *A. clavula* from trout from Clogher Lough and Lough Feeagh

Lake	**n**	**Prevalence (%)**	**Mean abundance ± S.E.**	**Mean intensity ± S.E.**
Clogher	125	39.2	3.4 ± 0.9	8.8 ± 2.1
Feeagh	151	65.3	14.8 ± 2.9	30.9 ± 9.1
Feeagh*	150	64.7	14.7 ± 2.9	22.8 ± 4.3

*= with the trout containing 815 *A. clavula* individuals from the May sample omitted

4.3.5 Seasonal patterns in prevalence, abundance and intensity of *A. clavula* in trout in Clogher Lough and Lough Feeagh

Prevalence

While there were no significant differences in the prevalence of *A. clavula* between seasons in Clogher Lough (χ^2 -test: $\chi^2 = 6.513$, df = 3, p = 0.0891) or Lough Feeagh (χ^2-test: $\chi^2 = 2.985$, df = 3, p = 0.3939; again, re-analysis of these data with the omission of the heavily infected individual from the May sample did not alter the test result), differing seasonal patterns in prevalence were observed between the lakes (Table 4.7, Figure 4.2). In Clogher Lough, prevalence was maintained at similar levels for February and May, followed by a gradual decline in July and September. In Lough Feeagh, however, prevalence remained at similar levels throughout the year, with the exception of a sharp decline in July.

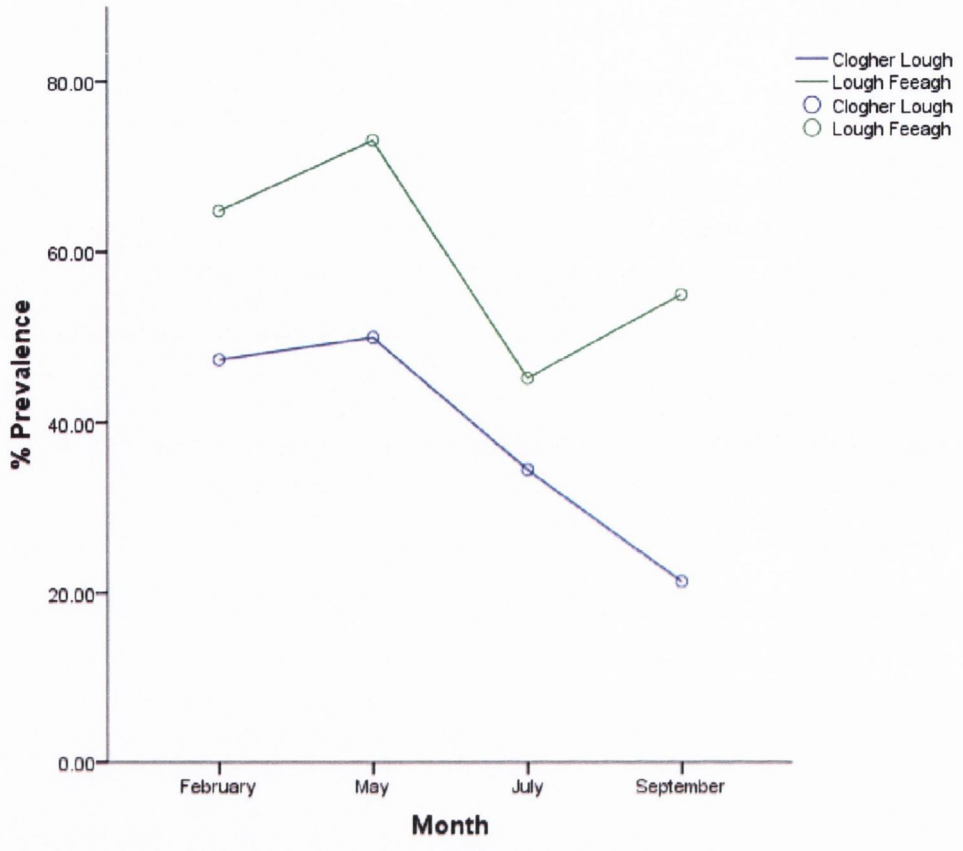


Figure 4.2 Prevalence of *A. clavula* (± 95% C.I.) in Clogher Lough and Lough Feeagh

Mean abundance and mean intensity: Seasonal patterns

Seasonal patterns in mean abundance and mean intensity also differed between Clogher Lough and Lough Feeagh. In Clogher Lough, both mean abundance and mean intensity remained at relatively low levels throughout the year, with the exception of a peak in both measures in July. In Lough Feeagh, mean abundance was maintained at a similar level (always higher than values from Clogher Lough) throughout the year, with the exception of a large peak in May (Figure 4.3). In both lakes, mean intensity remained at relatively similar levels throughout the year, with the exception of a large peak in May in Lough Feeagh (Figure 4.4).

Table 4.7 Prevalence, abundance and intensity of *A. clavula* in trout from Clogher Lough and Lough Feeagh by month

Month	Lake	No. trout infected (n)	Prevalence (%)	Mean Abundance ± S.E.	Mean Intensity ± S.E.
2007					
February	Clogher	15 (30)	50.0	1.7 ± 0.5	3.4 ± 0.7
	Feeagh	20 (33)	60.6	10.9 ± 3.0	18.0 ± 4.2
May	Clogher	15 (30)	50.0	2.6 ± 0.9	5.3 ± 1.4
	Feeagh	19 (26)	73.1	67.1 ± 33.2	91.8± 44.4
	Feeagh*	18 (26)	72.0	37.2 ± 15.2	85.6 ± 20.2
July	Clogher	10 (29)	34.5	8.5 ± 3.6	24.8 ± 8.6
	Feeagh	22 (31)	45.2	16.8 ± 4.2	23.6 ± 5.3
September	Clogher	6 (28)	21.4	1.4 ± 0.7	6.5 ± 2.4
	Feeagh	22 (40)	55.0	6 ± 1.9	10.9 ± 3.1
2008					
February	Clogher	3 (8)	37.5	1.6 ± 1.0	4.3 ± 2.0
	Feeagh	15 (21)	71.4	7.7 ± 2.7	10.7 ± 3.5
2007 & 2008 February					
	Clogher	18 (38)	47.4	1.7 ± 0.4	3.6 ± 0.6
	Feeagh	35 (54)	64.8	9.7 ± 2.1	14.9 ± 2.9

*= with the trout containing 815 *A. clavula* individuals from the May sample omitted

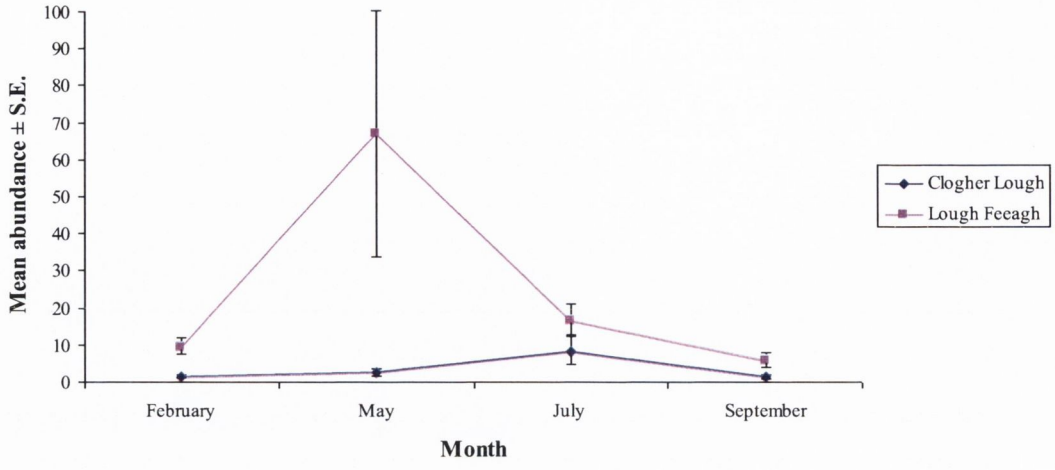


Figure 4.3 Mean abundance (± S.E.) of *A. clavula* in trout in Clogher Lough and Lough Feeagh

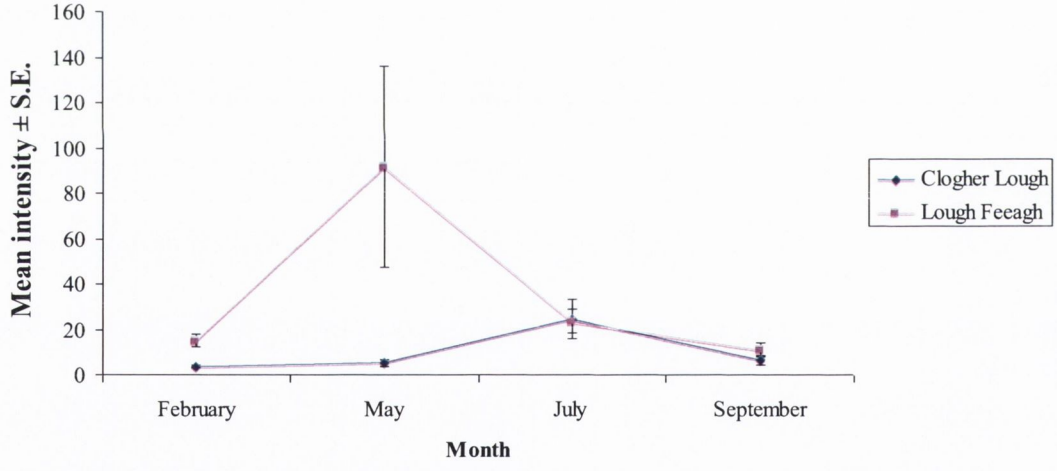


Figure 4.4 Mean intensity (± S.E.) of *A. clavula* in trout in Clogher Lough and Lough Feeagh

A two-way GLM was carried out to explore the effect of lake and sample month on the abundance of *A. clavula* infecting trout in Clogher Lough and Lough Feeagh. A significant difference in mean abundance of *A. clavula* was observed between the lakes, due to the higher numbers of *A. clavula* infecting trout in Lough Feeagh in all sample months. The abundance of *A. clavula* in trout differed significantly between sample months, due to the large peak in mean abundance in Lough Feeagh observed in May

2007. The abundance of *A. clavula* in trout also differed significantly between lakes, with trout from Lough Feeagh carrying higher numbers of *A. clavula* than those from Clogher Lough. These differences explain the significant interaction observed between lake and month (Table 4.8).

Table 4.8 GLM table of the effect of various factors on the abundance of *A. clavula* infecting trout from Clogher Lough and Lough Feeagh

	***F*-ratio**	***p*-value**	***df***
Factors			
Lake	10.441	**0.0014**	1
Month	4.3396	**0.0052**	3
Interaction			
Lake * Month	4.2848	**0.0056**	3

Aggregation

In Table 4.9, the mean abundance by month was calculated for Clogher Lough and Lough Feeagh, along with the variance and variance-to-mean ratio. The *A. clavula* population in trout in both lakes was highly aggregated in all months, with a particularly high value in May 2007 in Lough Feeagh.

Table 4.9 Seasonal changes in infection of trout by *A. clavula* in Clogher Lough and Lough Feeagh. C denotes Clogher Lough; F denotes Lough Feeagh

Month	No. examined		No. infected		Total no. of *A. clavula*		Mean abundance		Variance		Variance: mean ratio	
	C	F	C	F	C	F	C	F	C	F	C	F
February	30	33	15	20	54	366	1.7	10.9	6.4	291.6	3.7	26.7
May	30	26	15	19	79	1696	2.6	67.1	21.7	28723.5	8.3	427.9
July	29	31	10	22	243	509	8.6	16.8	381.4	552.5	44.6	32.9
September	28	40	6	22	39	235	1.4	6.0	13.9	144.8	10.0	24.2
February	8	21	3	15	13	161	1.6	7.7	8.6	151.4	5.3	19.7

4.3.6 *A. clavula* length and mass in Clogher Lough and Lough Feeagh

Ideally, length and mass of *A. clavula* from both lakes would have been analysed using samples from fish which were infected with *A. clavula* only, in order to ensure that the presence of another fish species in the trout intestine did not affect the size of the worms. This was possible for Clogher Lough as *A. clavula* was the only acanthocephalan species found. In Lough Feeagh however, *P. laevis* was also recovered, and the vast majority of the *A. clavula* individuals from which length and mass measurements were taken were from fish infected with both *P. laevis* and *A. clavula*, resulting in extremely small sample sizes of *A. clavula* from fish infected with this species only. The mean values for length and mass of worms from fish with and without *P. laevis* infections were compared and found to be almost identical with respect to sex and month (t-test: all p-values were non-significant), and so length and mass data from fish infected with both *A. clavula* and *P. laevis* (i.e. the larger sample size) were used for further analysis

In both Clogher Lough and Lough Feeagh, female *A. clavula* were found to be longer (t-test: Clogher: $t = 11.72$, $df = 177$, $p \leq 0.0001$; Feeagh: $= 14.74$, $df = 402$, $p \leq 0.0001$) and heavier (t-test: Clogher: $t = 10.2$, $df = 251$, $p \leq 0.0001$; Feeagh: $t = 5.616$, $df = 164$, $p \leq 0.0001$) than male *A. clavula* (Table 4.10).

Table 4.10 Mean body length (± S.E) and mass (± S.E.) of male and female *A. clavula* from trout from Clogher Lough and Lough Feeagh

	n	Body length ± S.E. (mm)	n	Mass ± S.E (mg)
Clogher Lough				
Male	68	2.58 ± 0.06	104	0.18 ± 0.007
Female	112	3.70 ± 0.07	195	0.34 ± 0.01
Lough Feeagh				
Male	173	2.74 ± 0.04	67	0.09 ± 0.005
Female	244	3.71 ± 0.05	103	0.15 ± 0.008

The effect of extrinsic and intrinsic factors on *A. clavula* length in Clogher Lough and Lough Feeagh

A four-way GLM was carried out to explore the effect of extrinsic (lake and season) and intrinsic (parasite sex and intensity status) factors on parasite body length (Table 4.11).

Table 4.11 GLM table of the effect of various factors on length of *A. clavula* in the intestine of trout in Clogher Lough and Lough Feeagh

	***F*-ratio**	***p*-value**	***df***
Factors			
Lake	2.3102`	0.1291	1
Season	1.8281	0.1409	3
Parasite sex	**79.56**	**≤ 0.0001**	**1**
Intensity status	0.25117	0.7780	2
Interactions			
Lake * Season	2.404	0.0666	3
Lake * Sex	1.9562	0.1625	1
Lake * Int. status	0.41317	0.6618	2
Season * Sex	1.7271	0.1603	3
Season * Int. status	0.95912	0.4523	6
Sex * Int. status	0.86026	0.4236	2
Lake * Season * Sex	0.89657	0.4427	3
Lake * Season * Int. status	1.0362	0.3555	2
Lake * Sex * Int. status	0.21537	0.8063	2
Season * Sex * Int. status	0.57968	0.7467	6
Lake * Season * Sex * Int. Status	**4.729**	**0.0301**	**3**

Sex was a highly significant factor, as male *A. clavula* were significantly shorter than female *A. clavula* in both lakes (Figures 4.5 & 4.6).

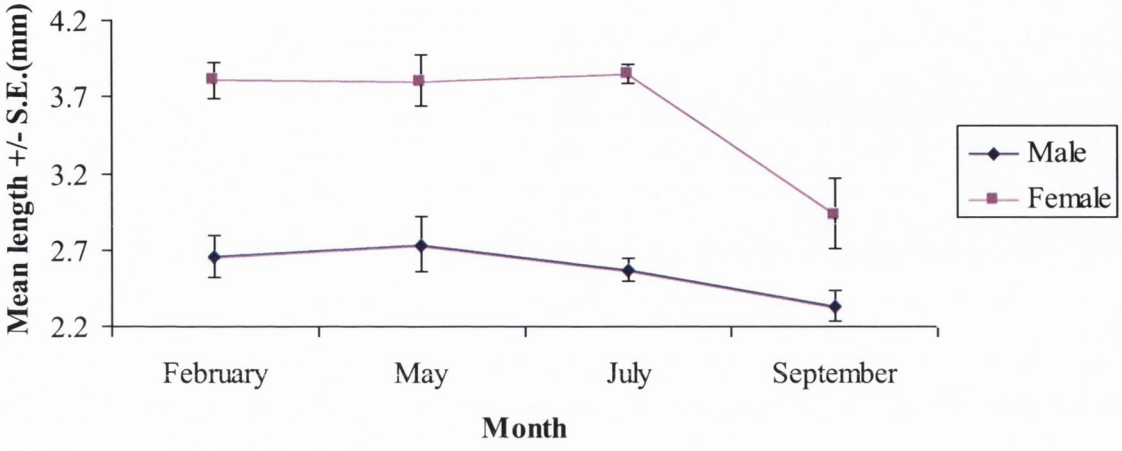


Figure 4.5 Mean length (± S.E.) of male and female *A. clavula* in trout from Clogher Lough

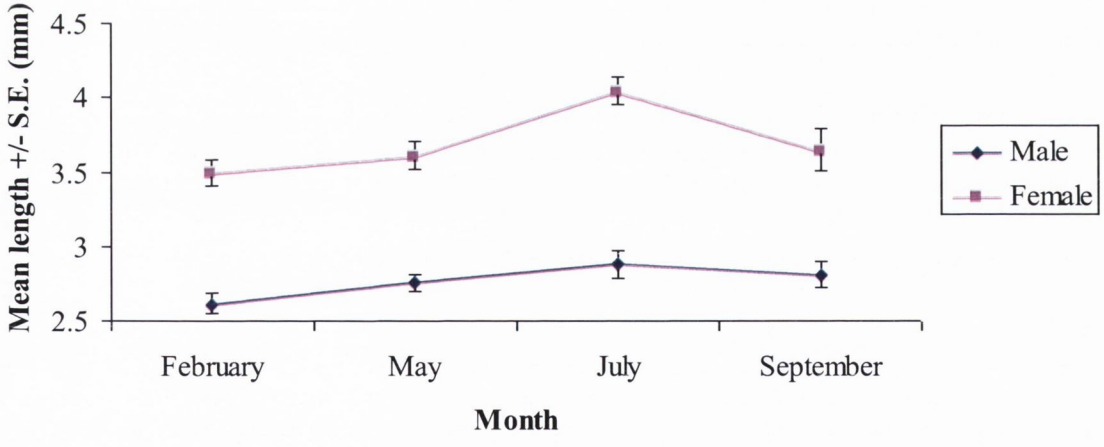


Figure 4.6 Mean length (± S.E.) of male and female *A. clavula* in trout from Lough Feeagh

The effect of extrinsic and intrinsic factors on *A. clavula* mass in Clogher Lough and Lough Feeagh

Initially, a four-way GLM was carried out to explore the effect of lake, parasite sex, intensity status and month on parasite mass; however the model was constrained by

low sample size. Instead, the data were split by lake and two three-way GLMs were carried out to investigate the effect of parasite sex, intensity status and month on parasite mass (Tables 4.12 and 4.13).

Clogher Lough

Table 4.12 GLM table of the effect of various factors on mass of *A. clavula* in the intestine of trout in Clogher Lough

	***F*-ratio**	***p*-value**	***df***
Factors			
Season	**2.9287**	**0.0341**	**3**
Parasite sex	**23.096**	**≤ 0.0001**	**1**
Intensity status	0.28147	0.8388	2
Interactions			
Season * Sex	0.67588	0.5675	3
Season * Int. status	1.5683	0.1973	3
Sex * Int. status	0.36038	0.6977	2
Season * Sex * Int. status	2.8724	0.0582	2

Season was a significant factor, as seasonal variation in *A. clavula* mass was observed in Clogher Lough (Figure 4.7). Both male and female *A. clavula* followed the same pattern of seasonal variation: the heaviest individuals were recovered in February and May, while the lightest individuals were recovered in July and September. Similar mean mass values were recorded for each pair of months.

Parasite sex was a highly significant factor, as male *A. clavula* were significantly lighter than female *A. clavula* in Clogher Lough (Table 4.12).

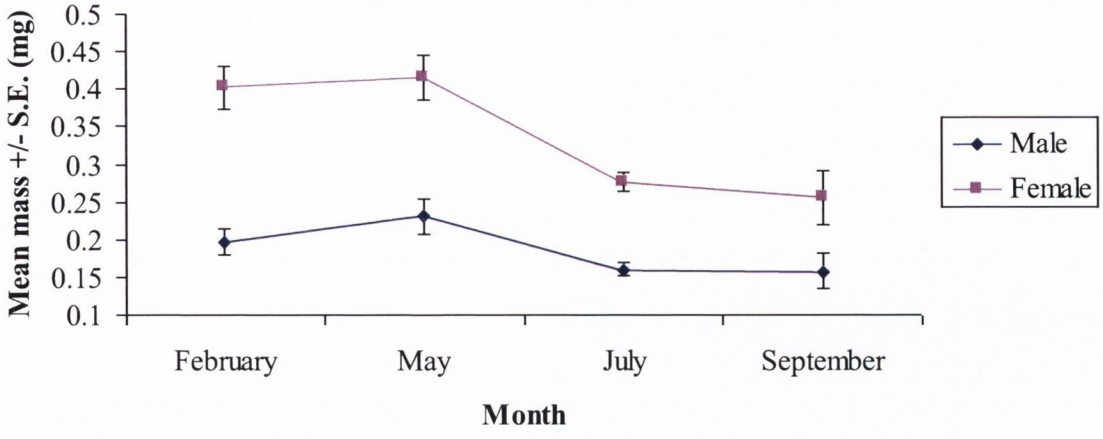


Figure 4.7 Mean mass (± S.E.) of male and female *A. clavula* in trout from Clogher Lough

Lough Feeagh

No factors or interactions were found to affect the mass of *A. clavula* in Lough Feeagh (Table 4.13).

Table 4.13 GLM table of the effect of various factors on mass of *A. clavula* in the intestine of trout in Lough Feeagh

	***F*-ratio**	***p*-value**	***df***
Factors			
Season	1.81	0.1478	3
Parasite sex	0.49522	0.4827	1
Intensity status	0.33544	0.7156	2
Interactions			
Season * Sex	1.1064	0.3485	3
Season * Int. status	0.48796	0.7849	5
Sex * Int. status	0.29953	0.7416	2
Season * Sex * Int. status	0.12636	0.8814	2

4.3.7 *A. clavula* Reproductive Status

An examination of the maturity status of a sub-sample of female *A. clavula* from trout from each sample month (≥20 individuals per month) found that the majority of worms in both lakes were reproductively immature, containing ovarian balls only (i.e. they were Stage 1 females) (Table 4.14). Indeed, just one reproductively mature (Stage 3) female *A. clavula* was found in each lake, in May.

Table 4.14 Reproductive status of *A. clavula* from trout sampled from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
Stage 1 (%)	83.3	95.7
(n)	(80)	(221)
Stage 2 (%)	15.6	3.9
(n)	(15)	(9)
Stage 3 (%)	1.04	0.433
(n)	(1)	(1)

A significant difference was observed in the levels of Stage 1, 2 and 3 females between the lakes (G-test: G= 12.786, df = 2, p = 0.0002). This difference is a result of the greater proportion of immature (Stage 1) females found in Lough Feeagh (95.7%) than in Clogher Lough (83.3%) (Table 4.14). The smaller size of female *A. clavula* in Lough Feeagh may also contribute to the lower proportion of maturing females observed in this lake.

A. clavula Reproductive Status: by season

The seasonal pattern of occurrence of *A. clavula* reproductive stages in trout was explored (Figures 4.8 & 4.9). In both lakes immature (Stage 1) females dominated in all months. In Clogher Lough, maturing (Stages 2 & 3) females were found in all months with the exception of February. In Lough Feeagh, maturing females were found only in May and July and at a lower level than that found in Clogher Lough.

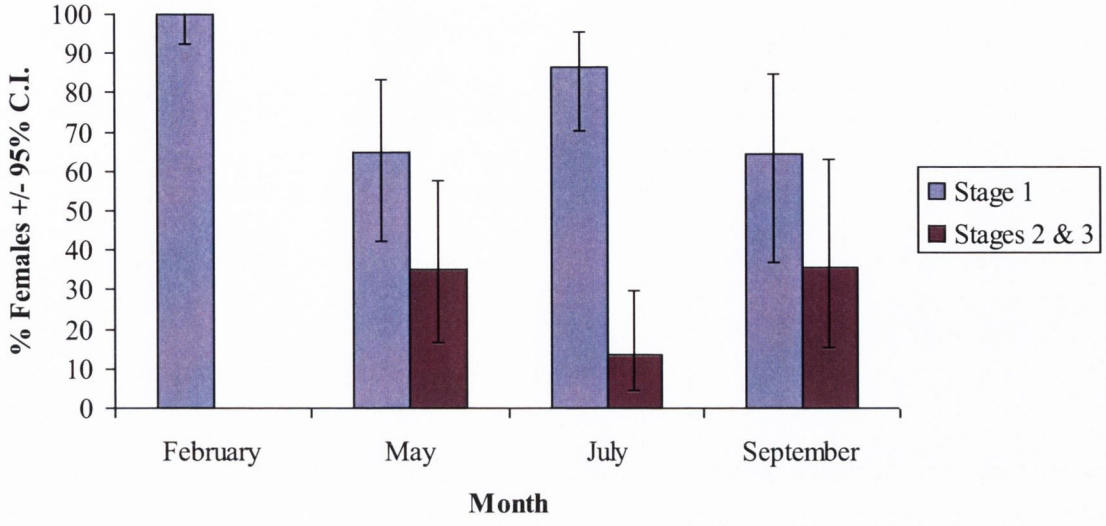


Figure 4.8 Reproductive status of female *A. clavula* (± 95% C.I.) from trout sampled from Clogher Lough

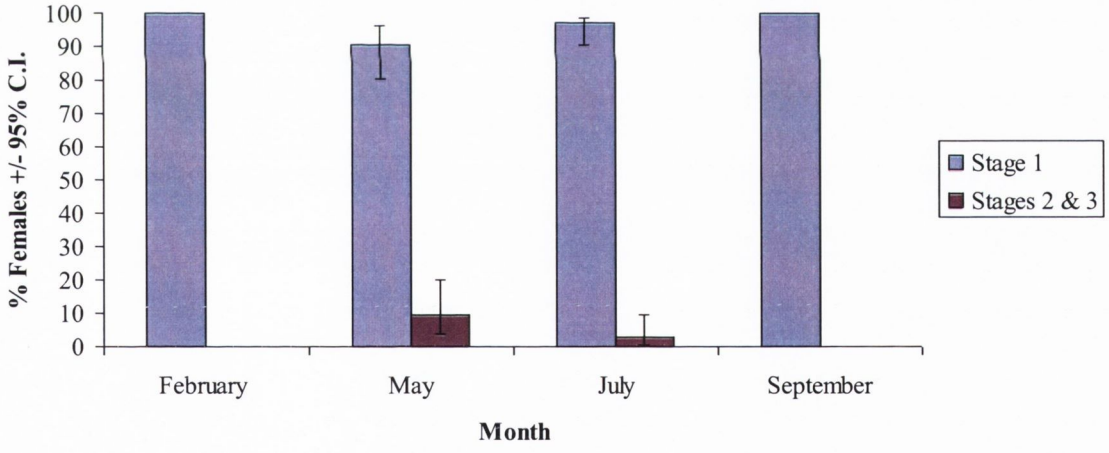


Figure 4.9 Reproductive status of female *A. clavula* (± 95% C.I.) from trout sampled from Lough Feeagh

When differences in the pattern of occurrence of reproductive stages of *A. clavula* were analysed by season (using a G-test), a highly significant difference in the pattern of occurrence was observed in Clogher Lough, but not in Lough Feeagh (Table 4.15).

In Clogher Lough, the level of Stage 1 females was relatively high in all month. Females at Stages 2 were found in all months with the exception of February, while

only one Stage 3 female was recovered, in May. Levels of Stage 2 females were at their highest in May and September, and dipped in July.

Table 4.15 Results of G-test on the pattern of occurrence of *A. clavula* reproductive stages in Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
G	20.54	11.618
df	6	6
p	0.002	0.071

4.3.8 The *A. clavula* niche

Despite the fact that trout are not a preferred definitive host for *A. clavula*, the niche occupied by *A. clavula* in trout from Clogher Lough and Lough Feeagh was examined in detail, in order to compare it with that of *A. clavula* from eels from Clogher Lough and Lough Feeagh. The distribution of *A. clavula* along the trout intestine was calculated for the total sample from each lake, as well as for each sample month. Distribution data were also examined with respect to low (1- 10 worms), medium (11-29 worms) and high intensity (≥30 worms) infections. Finally, the distribution of *A. clavula* in trout was compared between trout from the two lakes.

The *A. clavula* niche: the fundamental niche

The fundamental niche (the niche of all worms present in single species infections) of *A. clavula* along the intestine was first examined (Figures 4.10 & 4.11). The mean percentage position of *A. clavula* in the intestine of trout in Clogher Lough was 53.1% ± 1.3 S.E. The minimum position in which *A. clavula* was found was 5.5%, while the maximum was 99%, indicating that *A. clavula* occupies an extremely broad fundamental niche in the trout intestine in Clogher Lough. It is interesting to note that the distribution of *A. clavula* in the intestine of trout from Clogher Lough is bimodal in nature, with peaks in occurrence at ~25% and ~75% of the way along the gut. This bimodal distribution is possibly due to the concentrated occurrence of worms from high intensity infections in small segments of the trout intestine, although further investigation of the data shows that this is not the case.

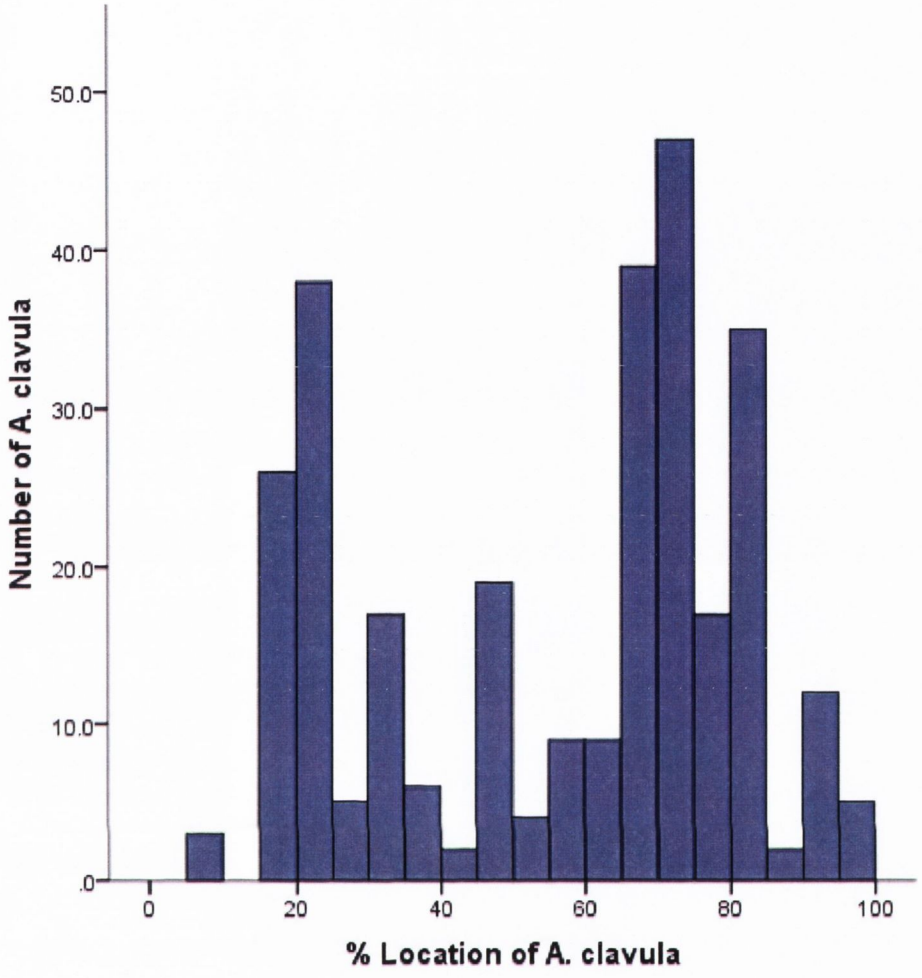


Figure 4.10 The fundamental niche of *A. clavula* along the intestine of eels in Clogher Lough

In Lough Feeagh, the mean percentage position of *A. clavula* was much more anterior than in Clogher Lough, at 34.3% ± 0.4 S.E. The minimum position in which *A. clavula* was found was 6%, while the maximum was 100%, indicating that, again, *A. clavula* occupies an extremely broad fundamental niche in the trout intestine in Lough Feeagh.

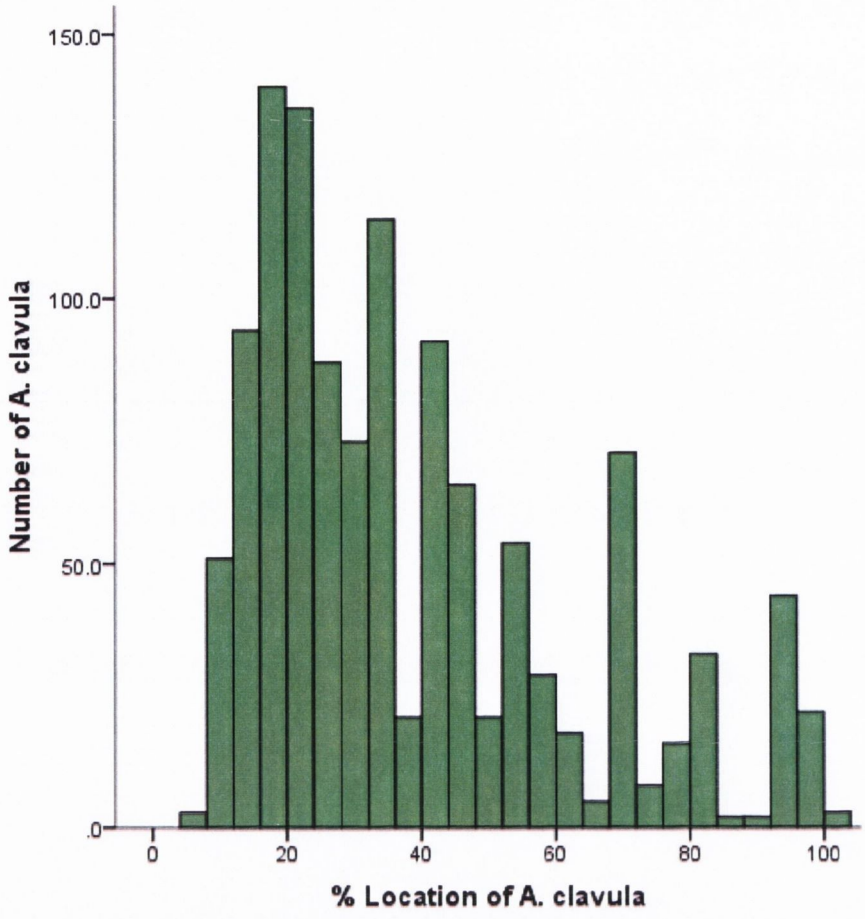


Figure 4.11 The fundamental niche of *A. clavula* along the intestine of trout in Lough Feeagh

The effect of various factors on the niche of *A. clavula* was then explored using Kolmogorov-Smirnov tests. The factors examined were lake, season, parasite sex and intensity status (Tables 4.16a & 4.16b). The dependent variable was percentage location of *A. clavula*.

Table 4.16a Details of factors involved in Kolmogorov-Smirnov tests of *A. clavula* niche

Factor	**Details**
Lake	Clogher Lough, Lough Feeagh
Season	February (2007 & 2008 combined), May, July, September
Parasite sex	Male, Female
Intensity status	Low (1-10 worms)
	Medium (11-29 worms)
	High (> 30 worms)

A significant difference in *A. clavula* percentage position was observed between the lakes ($p < 0.001$), with *A. clavula* from Lough Feeagh occupying a more anterior position in the eel intestine than those from Clogher Lough (Table 4.17).

Table 4.16b Results of Kolmogorov-Smirnov tests investigating factors affecting *A. clavula* percentage position in the intestine of eels from Clogher Lough and Lough Feeagh

Factor	**Clogher Lough**	**Lough Feeagh**
	p	P
Sex	0.324	0.205
Intensity level		
Low v Medium	0.068	<0.001
Low v High	<0.001	<0.001
Medium v High	<0.001	<0.001

Table 4.17 Mean *A. clavula* percentage position in the intestine of eels from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
Mean male % position ± S.D. (n)	58.06± 24.89 (108)	39.17 ± 24.14 (487)
Mean Female % position ± S.D. (n)	54. 43 ± 25.79 (185)	37.58 ± 22.88 (709)

Parasite sex had no significant effect on the percentage location of *A. clavula* in either lake, (Table 4.17). Indeed, it may be seen that male and female *A. clavula* occupy a similar distribution in the trout intestine in Clogher Lough and Lough Feeagh (Figures 4.12 & 4.13).

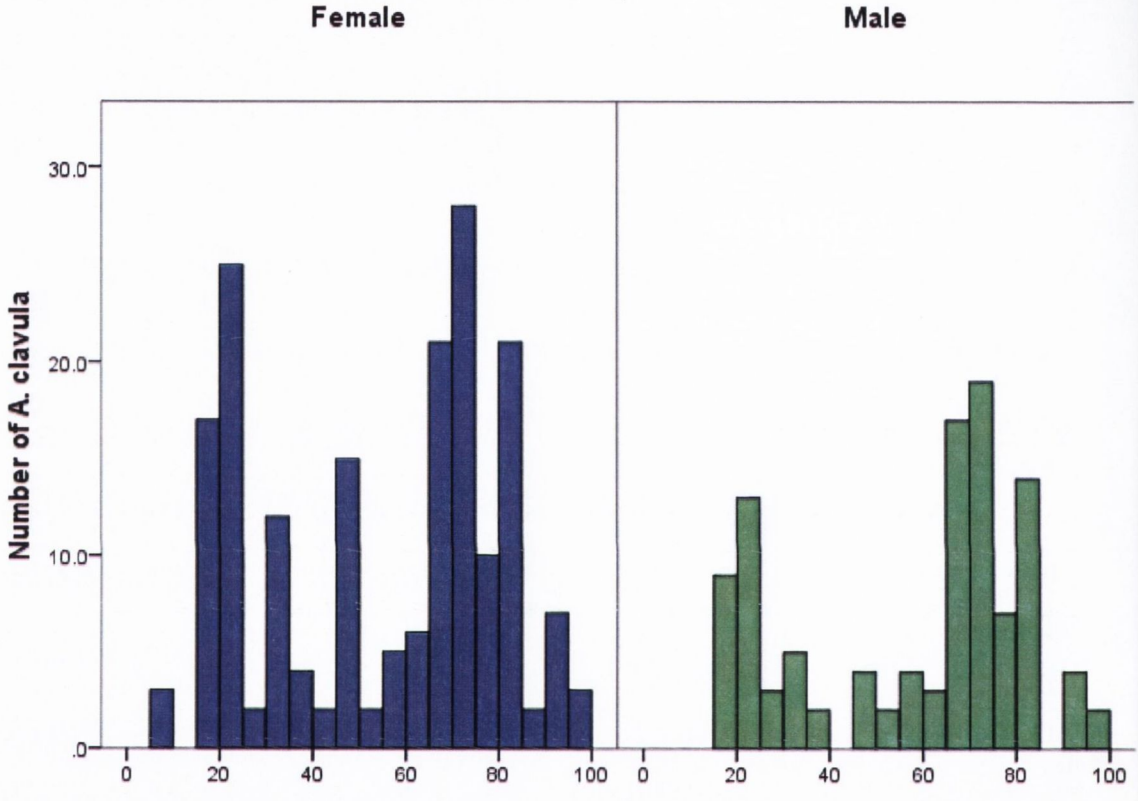


Figure 4.12 Distribution of male and female *A. clavula* in the intestine of trout from Clogher Lough

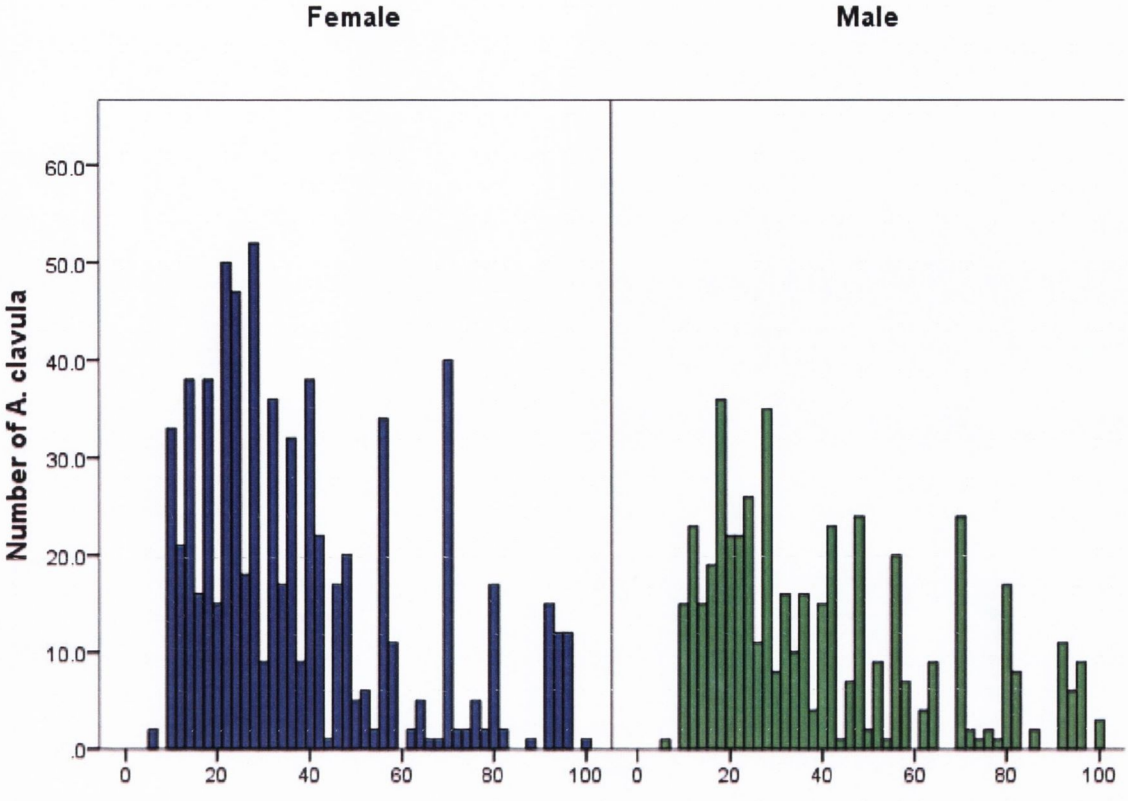


Figure 4.13 Distribution of male and female *A. clavula* in the intestine of trout from Lough Feeagh

As Kolmogorov-Smirnov tests permit the use of two samples only, pairwise comparisons of intensity levels were undertaken. Significant differences in *A. clavula* percentage position were observed in all intensity level comparisons in Lough Feeagh, while in Clogher Lough no significant difference was observed in *A. clavula* percentage position between worms in low and medium intensity infections (Table 4.16b).

In Clogher Lough, of the 374 worms for which intensity level data were available, 107 were in low intensity infections, 94 were in medium intensity infections and 173 were in high intensity infections. It should be noted that high intensity infections were found only in July in Clogher Lough. The majority of worms from low intensity

infections were found in the anterior of the intestine, while two peaks of occurrence were noted in medium intensity infections, at 20% and 65%. In high intensity infections, the majority of worms were situated in the posterior of the intestine (>70%) (Figure 4.14).

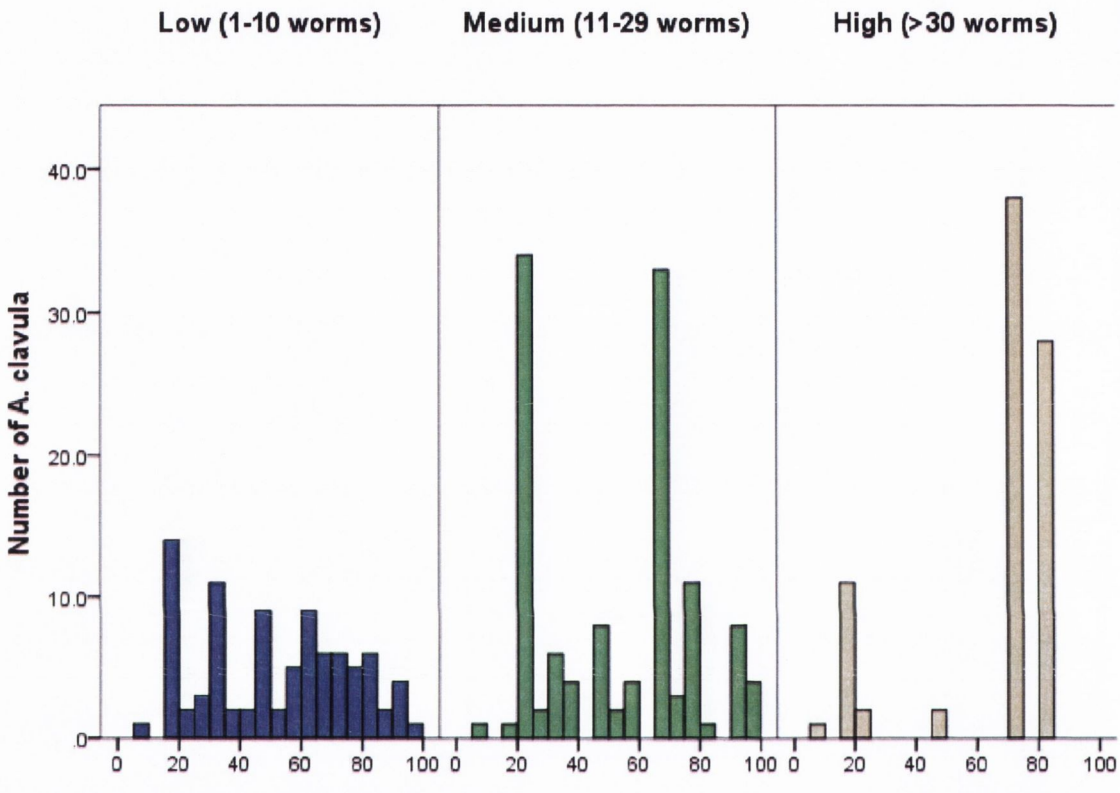


Figure 4.14 Distribution of *A. clavula* according to intensity level along the intestine of trout from Clogher Lough

The mean position of *A. clavula* was similar for low, medium and high intensity infections (Table 4.18a). The lowest percentage position of *A. clavula* in the intestine (5.5%) occurred in a medium intensity infection, while the highest (99%) occurred in both a medium and a high intensity infection.

Table 4.18a Summary statistics for the distribution of *A. clavula* in the intestine of trout in Clogher Lough by intensity level

	Low	**Medium**	**High**
Count	107	94	173
Mean ± S.E.	52.5 ± 2.3	53.2 ± 2.6	53.4 ± 2.1
Inter-quartile range	39.2	38.9	52.1
Range	88.2	93.5	92.2

In Lough Feeagh, of the 2926 worms for which intensity level data were available, 242 were in low intensity infections, 314 were in medium intensity infections and 2370 were in high intensity infections. The mean percentage position of *A. clavula* was similar for low (50.7%) and high (53.4%) intensity infections in Lough Feeagh, however a significantly more anterior position (39.3%) was occupied by worms in medium intensity infections (Table 4.18b). The lowest percentage position of *A. clavula* in the intestine (6%) occurred in a low intensity infection, while the highest (100%) also occurred in a low intensity infection.

Table 4.18b Summary statistics for the distribution of *A. clavula* in the intestine of trout in Lough Feeagh by intensity level

	Low	**Medium**	**High**
Count	242	314	2370
Mean ± S.E.	50.7 ± 1.7	39.3 ± 1.2	53.4 ± 2.1
Inter-quartile range	39.2	38.9	52.1
Minimum	6	7	10
Maximum	98	88	99
Range	94	81	89.2

In low intensity infections, worms were recovered throughout the intestine, while in medium intensity infections, the majority of worms were found in the anterior of the intestine (<50%).

In high intensity infections in Lough Feeagh, there are two notable peaks in *A. clavula* occurrence in the trout intestine, at 25% and 70% (Figure 4.15).

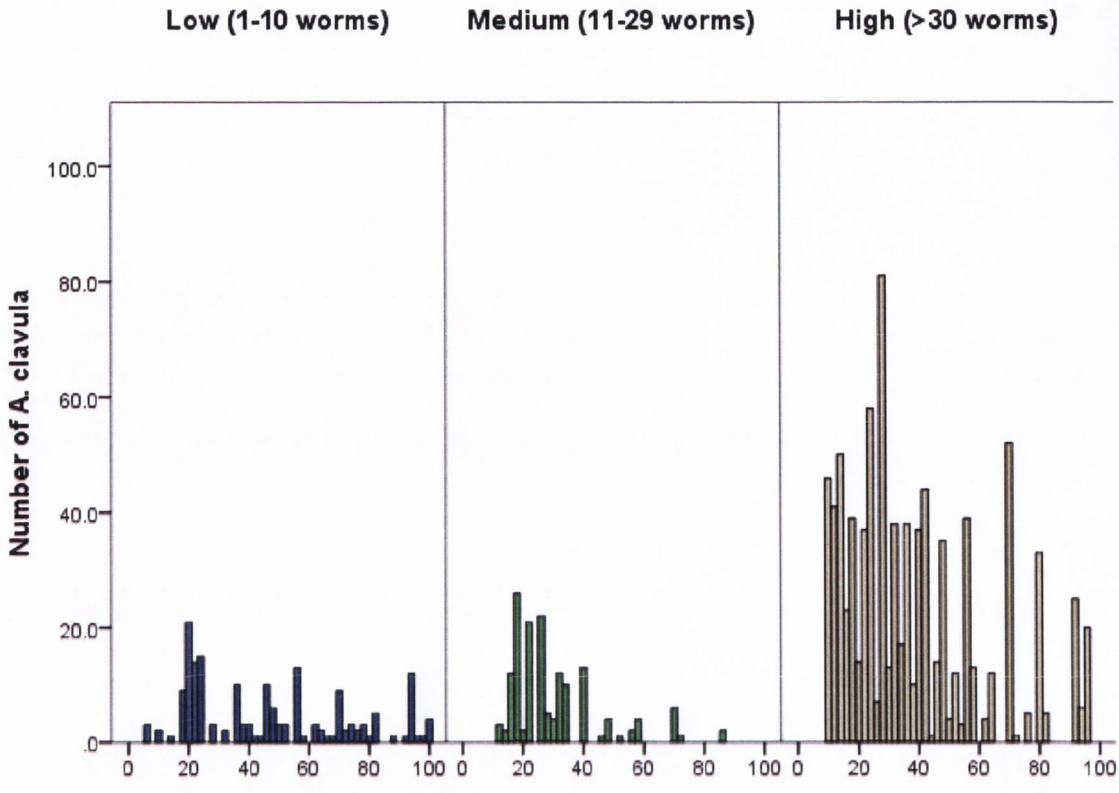


Figure 4.15 Distribution of *A. clavula* according to intensity level along the intestine of trout from Lough Feeagh

Differences in *A. clavula* percentage location between sample months were also investigated. Again, pairwise comparisons were undertaken. Significant differences were observed between all pairwise comparisons in Lough Feeagh, and between all comparisons with the exception of February v September in Clogher Lough (Table 4.19).

Table 4.19 Results of Kolmogorov-Smirnov tests investigating the effect of sample month on *A. clavula* percentage location in trout from Clogher Lough and Lough Feeagh

	Clogher Lough	**Lough Feeagh**
	p	**P**
2007		
February v May	<0.001	<0.001
February v July	0.002	<0.001
February v September	0.051	<0.001
May v July	<0.001	<0.001
May v September	<0.001	<0.001
July v Septmber	<0.001	<0.001

In Clogher Lough in February and September, the majority of worms are found in the anterior region of the intestine (Figure 4.16), while in May the vast majority of *A. clavula* individuals occur in more posterior locations (>40%). In July, an unusual situation was encountered, whereby there are two peaks in *A. clavula* occurrence in the trout intestine, one at 25% and one at 70%, with very few worms present in the midsection of the intestine (40-60%) (Figure 4.16). The mean percentage position of *A. clavula* was similar in February and July, while in May it was significantly more posterior than other sample months (Table 4.20a).

Table 4.20a Summary statistics for the distribution of *A. clavula* in the intestine of trout in Clogher Lough for each sample month

	February	**May**	**July**	**September**
Count	54	69	212	39
Mean (%) ± S.E.	50.2 ± 3.5	68.7 ± 1.9	51.4 ± 1.8	38.4 ± 3.5
Inter-quartile range	44.2	18.3	52.1	23.5
Minimum (%)	15.3	18.2	6.8	5.5
Maximum (%)	92.7	99	99	96.6
Range	77.4	80.8	92.2	91.1

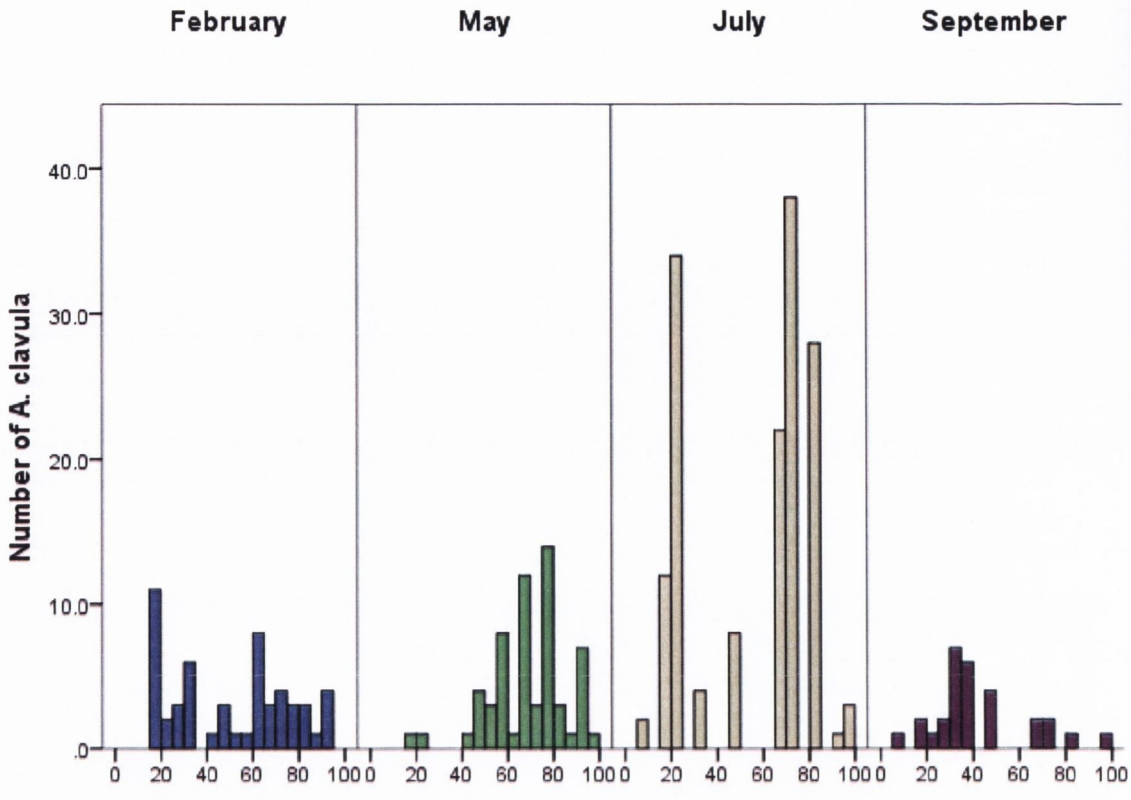


Figure 4.16 Distribution of *A. clavula* in the trout intestine by sample month in Clogher Lough

In Lough Feeagh in February, July and September, the majority of worms were recovered in the anterior region of the intestine (<40%), while in May, *A. clavula* were more evenly distributed along the trout intestine (Figure 4.17). Similar *A. clavula* mean percentage positions were noted in all sample months (Table 4.20b).

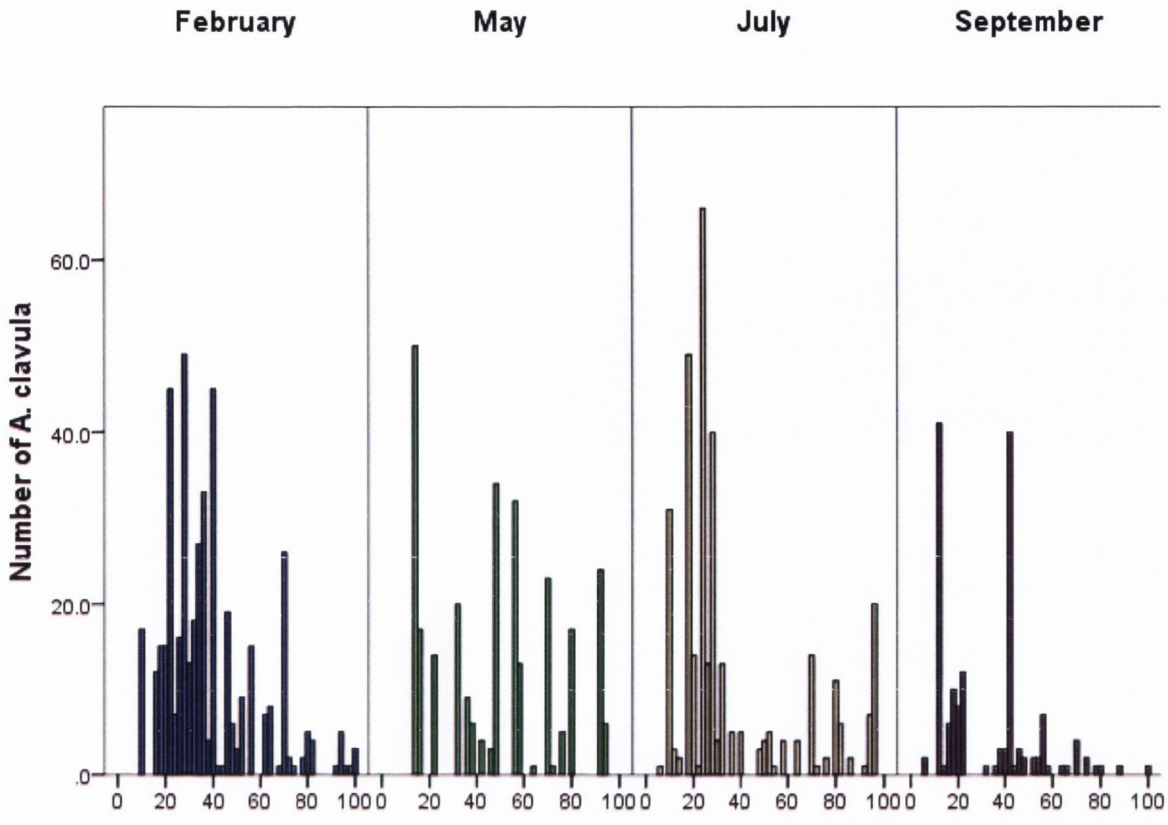


Figure 4.17 Distribution of *A. clavula* in the trout intestine by sample month in Lough Feeagh

Table 4.20b Summary statistics for the distribution of *A. clavula* in the intestine of trout in Lough Feeagh for each sample month

	February	**May**	**July**	**September**
Count	485	1646	547	248
Mean (%) ± S.E.	39.3 ±0.9	32.6 ± 0.4	36.2 ± 1.1	31.5 ± 1.3
Inter-quartile range	24	3.5	41	28
Minimum (%)	9.0	10.0	6.0	6.0
Maximum (%)	100	99.2	96.0	100
Range	91	89.2	90	94

4.3.9 *Pomphorhynchus laevis* and other acanthocephalan species in trout from Clogher Lough and Lough Feeagh

Clogher Lough

Neither *P. laevis* nor any other acanthocephalan species was found in the intestine of trout sampled from Clogher Lough.

Lough Feeagh

P. laevis was found in the intestine of trout sampled from Lough Feeagh in all sample months. A total of 210 *P. laevis* individuals were recovered from trout sampled from Lough Feeagh, ranging from 12 in September 2007 to 121 in May 2007 (Figure 4.18).

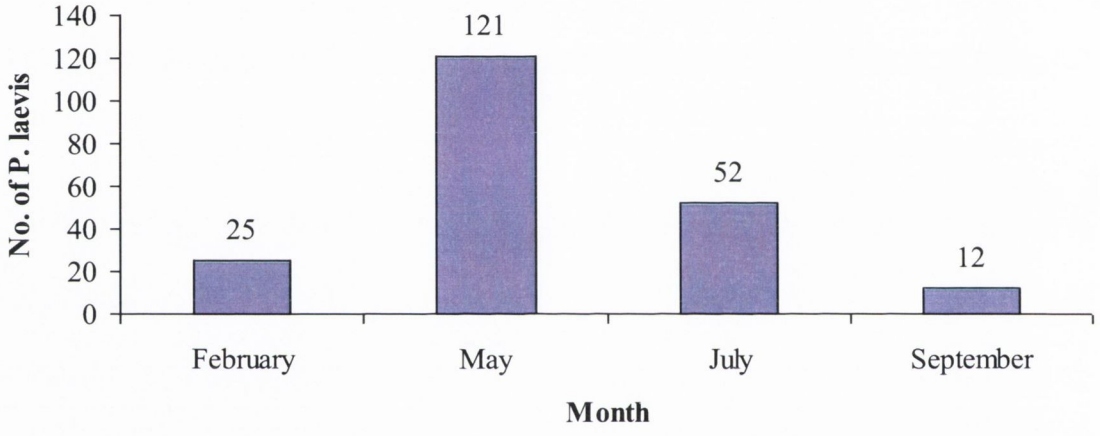


Figure 4.18 Numbers of *P. laevis* recovered from trout from Lough Feeagh

Overall prevalence, abundance and intensity of *P. laevis*

P. laevis was found at intermediate levels of prevalence, mean abundance and mean intensity in Lough Feeagh (Table 4.21).

Table 4.21 Population parameters of *P. laevis* from trout from Lough Feeagh

Lake	n	Prevalence (%)	Mean abundance ± S.E.	Mean intensity ± S.E.	Range
Feeagh	151	27.2	1.38 ± 0.35	5.1 ± 1.1	0 – 41

Seasonal patterns in prevalence, abundance and intensity of *P. laevis* in trout in Lough Feeagh

Prevalence of *P. laevis* differed significantly between seasons (χ^2-test: $\chi^2 = 34.2$, df = 3, $p \leq 0.0001$). Prevalence reached a peak in May and remained at lower levels in other sample months (Table 4.22, Figure 4.19). Mean abundance and mean intensity followed the same seasonal pattern (Table 4.22, Figures 4.20 & 4.21), however while mean abundance was found to differ significantly between sample months (One-way GLM: F = 7.3311, df = 3, p = 0.0001), there was no significant difference in mean intensity of *P. laevis* between sample months (One-way GLM: F = 0.79171, df = 3, p = 0.5063).

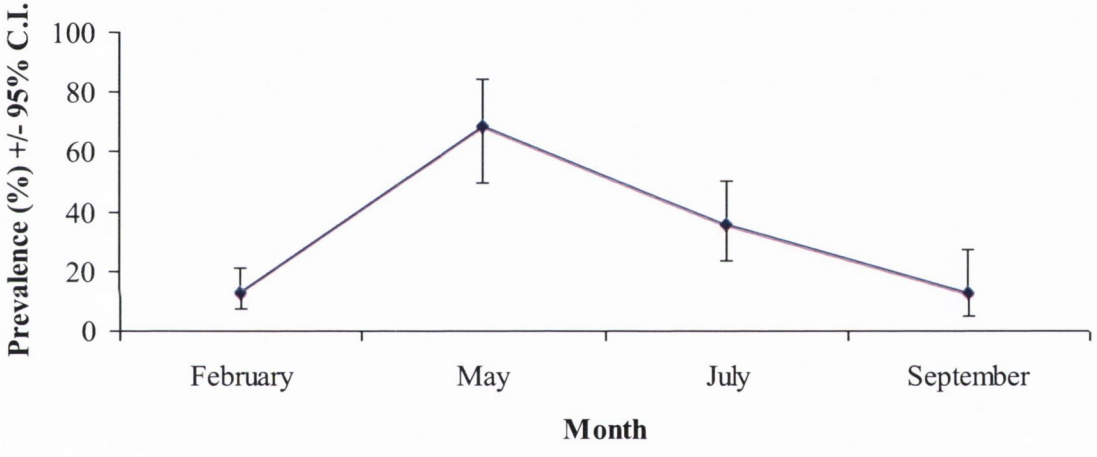


Figure 4.19 Prevalence (± 95% C.I.) of *P. laevis* from trout in Lough Feeagh

Table 4.22 Prevalence (± 95% C.I.), abundance (± S.E.) and intensity (± S.E.) of *P. laevis* in trout from Lough Feeagh by month

Month	No. trout infected (n)	Prevalence (%)	Abundance ± S.E.	Intensity ± S.E.
2007				
February	6 (27)	18.2	0.73 ± 0.4	5.6 ± 2.1
May	18 (26)	69.2	4.6 ± 1.7	2.3 ± 0.9
July	11 (31)	35.5	1.7 ± 0.7	2.6 ± 0.9
September	5 (40)	12.5	0.3 ± 0.2	6.8 ±2.2
2008				
February	1 (21)	4.8	0.04 ± 0.05	2.6 ± 0.9
2007 & 2008 February	7 (48)	13.0	0.46 ± 0.3	4.5 ± 1.4

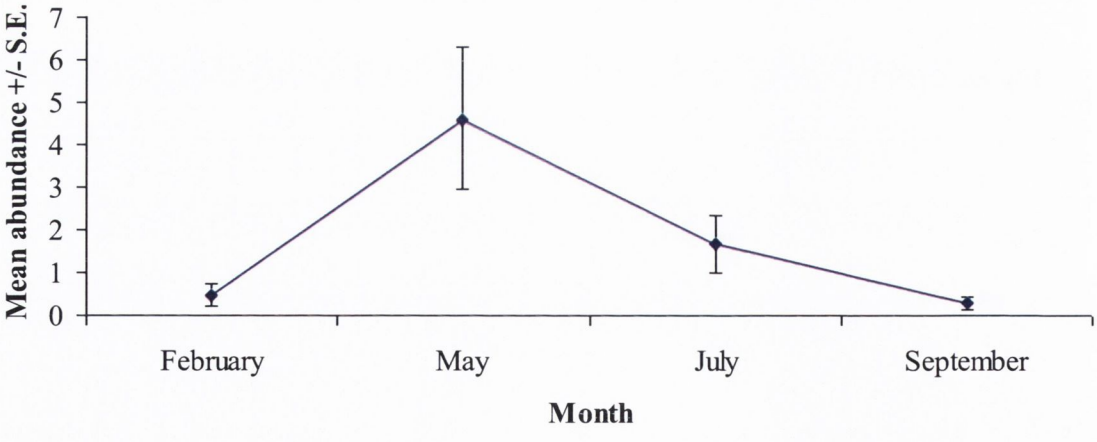


Figure 4.20 Mean abundance (± S.E.) of *P. laevis* in trout from Lough Feeagh

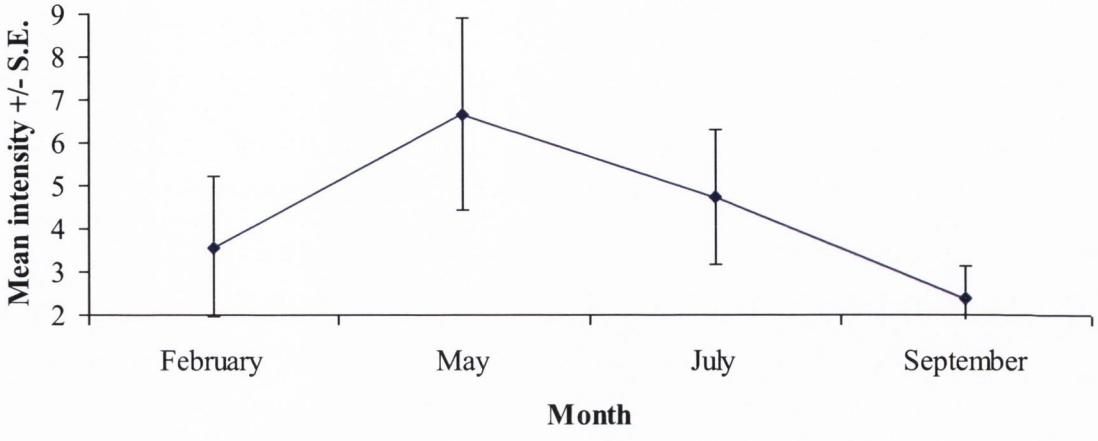


Figure 4.21 Mean intensity (± S.E.) of *P. laevis* in trout from Lough Feeagh

4.4 Comparative analysis of *A. clavula* in eels and trout

The abundance, female mass and reproductive status of *A. clavula* from Clogher Lough and Lough Feeagh were analysed comparatively. The results of this analysis are presented below.

4.4.1 Abundance of *A. clavula* in eels and trout

The seasonal patterns of *A. clavula* abundance in eel and trout in Clogher Lough and Lough Feeagh were compared using a three-way GLM (Table 4.23).

Abundance differed significantly between lakes, as discussed in Chapters 3 and 4, with much higher values reported from Lough Feeagh from both eel and trout.

There was a significant interaction between fish species and month, which was due to the differing seasonal patterns of *A. clavula* abundance in eel and trout (Table 4.24). In eel, the abundance of *A. clavula* reached a peak in February and declined throughout the remainder of the year, although infection levels were much higher in Lough Feeagh, whereas in trout the pattern of *A. clavula* infection was irregular and differed between the two lakes.

Table 4.23 GLM table of the effect of lake, season and fish species on abundance of *A. clavula*

	***F*-ratio**	***p*-value**	***df***
Factors			
Lake	**34.432**	**≤ 0.0001**	**1**
Season	2.2417	0.0836	3
Fish species	1.1053	0.2940	1
Interactions			
Lake * Season	2.604	0.0522	3
Lake * Fish species	0.24601	0.6203	1
Fish species * Season	**4.0683**	**0.0075**	**3**
Lake * Species * Season	2.2548	0.0822	3

Table 4.24 Mean abundance (± S.E.) of *A. clavula* in eel and trout in Clogher Lough and Lough Feeagh

	n	**Clogher Lough**	**n**	**Lough Feeagh**
Eel				
February	8	11.25 ± 7.3	19	37.8 ± 10.1
May	27	7.5 ± 2.9	29	30.8 ± 9.1
July	25	3.7 ± 1.2	30	15.8 ± 2.7
September	30	4.2 ± 1.1	33	10.9 ± 2.1
Trout				
February	38	1.7 ± 0.4	54	9.6 ± 2.1
May	30	2.6 ± 0.9	26	67.1 ± 33.2
July	29	8.6 ± 3.6	31	16.8 ± 4.2
September	28	1.4 ± 0.7	40	6.0 ± 1.9

4.4.2 *A. clavula* Mass

The effect of various factors on the mass of female *A. clavula* in eel and trout was explored using a four-way GLM (Table 4.25).

Table 4.25 GLM table of the effect of various factors on mass of female *A. clavula* in the intestine of eels and trout in Clogher Lough and Lough Feeagh

	***F*-ratio**	***p*-value**	***df***
Factors			
Lake	**99.886**	**≤ 0.0001**	**1**
Season	**3.1158**	**0.0257**	**3**
Fish species	**32.917**	**≤ 0.0001**	**1**
Intensity status	1.7009	0.1834	2
Interactions			
Lake * Season	0.94496	0.4185	3
Lake * Fish species	0.11052	0.7397	1
Lake * Int. Status	1.0781	0.3409	2
Season * Fish species	2.1747	0.0899	3
Season * Int. Status	0.56129	0.7612	6
Fish species * Int. status	1.3228	0.2672	2
Lake * Season * Fish species	1.1527	0.3271	3
Lake * Fish species * Int. status	1.8016	0.1659	1
Lake * Season * Int. status	0.30387	0.8754	5
Season * Fish species * Int. status	0.76755	0.5941	6
Lake * Season * Fish species * Int. Status	1.4349	0.2314	1

The mass of female *A. clavula* in eels and trout from Clogher Lough and Lough Feeagh differed significantly both between lakes and between seasons. These factors have been previously discussed in relation to *A. clavula* mass (Chapters 3 and 4) and so they will not be discussed further here.

The mass of female *A. clavula* was found to differ significantly between fish species. Female *A. clavula* reached a larger size in eels, indeed female *A. clavula* from eels were, on average, double the mass of *A. clavula* from trout (Table 4.26).

Table 4.26 Mass of female *A. clavula* from eel and trout (data from Clogher Lough and Lough Feeagh combined)

	n	Eel	n	Trout
Mean female mass ± S.E. (mg)	303	0.49 ± 0.02	348	0.25 ± 0.009

4.4.3 *A. clavula* reproductive status

An examination of the maturity status of a sample of female *A. clavula* found that the majority of worms from trout from Clogher Lough and Lough Feeagh were reproductively immature, containing ovarian balls only (i.e. they were Stage 1 females), while the majority of worms from eels from the two lakes were reproductively mature, containing ovarian balls, immature acanthors and mature, shelled acanthors (i.e. they were Stage 3 females) (Table 4.27). A significant difference was observed between eel and trout with respect to the levels of the various reproductive stages of *A. clavula* (χ^2: $\chi^2 = 309.1$, df = 2, $p \leq 0.0001$).

Table 4.27 Reproductive status of *A. clavula* from eel and trout (data for Clogher Lough and Lough Feeagh combined)

	Eel	Trout
Stage 1 (%)	22.6	92.3
(n)	(49)	(312)
Stage 2 (%)	16.1	7.1
(n)	(35)	(47)
Stage 3 (%)	61.3	0.6
(n)	(133)	(2)

4.4 Discussion

The brown trout has been termed a 'suitable host' for *A. clavula* (Lyndon & Kennedy, 2001); however the occurrence of this host-parasite combination is extremely rare in Britain and mainland Europe. In western Ireland, however, the trout - *A. clavula* combination has been reported on several occasions (Conneely & McCarthy, 1986, 1988; Byrne, 2000; Molloy *et al.*, 1993), most often from the Burrishoole catchment in Co. Mayo. Previous studies investigating trout-helminth communities in this catchment (Molloy *et al.*, 1995a; Byrne, 2000) have found a variety of results with respect to the presence and population biology of *A. clavula*.

Molloy *et al.* (1995a) found no evidence of *A. clavula* in 549 trout from two lakes in the Burrishoole catchment (Bunaveela Lake and Lough Feeagh), however Byrne (2000), in an examination of trout-helminth community structure in Lough Feeagh (sampling for which took place during 1997 – 1998) found that *A. clavula* were infecting trout, at a prevalence of 25-40% and a mean abundance of approximately 3.5 worms per fish. The present study has found that *A. clavula* is currently infecting the trout of Lough Feeagh at high levels of prevalence and abundance.

A. clavula was not found in trout from Bunaveela Lake during this study, however since the work of Molloy (1995a), the parasite has been found in ever-increasing numbers in Lough Feeagh. Kennedy (1984), in an investigation of the population dynamics of *A. clavula* in eels in Britain, found that *A. clavula* declined in the eel population in tandem with a decline in *A. meridianus*, its intermediate host. The decline in *A. meridianus* found by Kennedy (1984) was attributed to competition with its congener, *Asellus aquaticus*. In two recent macro-invertebrate surveys of Lough Feeagh (May, 1994; Boisson, 1997) however, *A. meridianus* was the only isopod species recorded, thus competition of *A. meridianus* with *A. aquaticus* cannot explain the absence of *A. clavula* from the trout examined by Molloy *et al.* (1995a).

An alternative explanation is that *A. meridianus* was absent, or present only at very low density, in Lough Feeagh when Molloy *et al.* (1995a) recovered their trout samples and that the population had increased by 1997, when Byrne (2000) sampled.

When the data of May (1994) and Boisson (1997) are further explored, it can be seen that in 1994, *A. meridianus* made up just 0.2% of the total number of invertebrates (excluding *Gammarus duebeni*) recovered from 13 sites around Lough Feeagh, while in 1997 (Boisson, 1997) this number had risen to 5.6%. The near-absence of *A. meridianus* from the lake in 1994 may explain the results found by Molloy *et al.* (1995a). By 1997, the *A. meridianus* population had most likely increased to the extent that trout in Lough Feeagh became infected with *A. clavula*, albeit at relatively low levels of prevalence (15 – 35%, sampled in April, May, August and September) and mean abundance (0.7 – 4.9 *A. clavula* individuals) (Byrne, 2000). The much higher levels of prevalence (45 – 74%, sampled in February, May, July and September) and mean abundance (6 – 68 *A. clavula* individuals) of *A. clavula* in trout found in this study may indicate that the *A. meridianus* population continued to expand from 1997 to 2007, and that this larger population is responsible for the increase in population parameters of *A. clavula* in trout in the period between the work of Byrne (2000) and the current study. The reasons for a possible population expansion of *A. meridianus* are as yet unknown, but the increasing trophic status of Lough Feeagh (R. Poole, pers. comm.) may be partially responsible.

Byrne (2000) also investigated the helminth community of brown trout in Clogher Lough, and extremely high values of prevalence (93 – 100%) and mean abundance (45 – 95 individuals) of *A. clavula* were found during the majority of the sampling period (1997-1998). The exception occurred in August 1997, when prevalence (43%) and mean abundance (6.6) decreased considerably. That the values of these population parameters were so elevated led the author to suggest that the parasite was undergoing a host shift from eel, its preferred definitive host, to trout, a suitable host (Byrne *et al.*, 2004). However, while high numbers of *A. clavula* were found in trout by Byrne (2000) and Byrne *et al.* (2004), the parasite was not reaching maturity, with just 2% of females achieving full reproductive maturity.

When compared with the results of the present study, it is clear that the situation found by Byrne (2000) and Byrne *et al.* (2004) in Clogher Lough was an anomaly. If a host shift of *A. clavula* had occurred in Clogher Lough, an increase in the proportion of reproductively mature female *A. clavula* in trout would have to be

observed when the system was examined at a later date. Just one reproductively mature female was found among the *A. clavula* recovered from Clogher Lough in the current study.

The situation found by Byrne (2000) and Byrne *et al.* (2004) was therefore most likely an irregularity, caused by a population explosion of *A. meridianus* in the lake, followed by intensive, opportunistic, feeding of trout on isopods. Unfortunately, no macro-invertebrate surveys of Clogher Lough have been carried out, and thus it is not possible to compare the abundance of *A. meridianus* in Clogher Lough through time. It will therefore never be known definitively whether a population explosion of *A. meridianus* did, in fact, occur, but this does appear to be the most likely explanation.

A number of parallels may be drawn between the population parameters of *A. clavula* in eel and those in trout. Similar to the situation for *A. clavula* in eels (Section 3.3), a greater number of parasites, as well as higher levels of prevalence, mean abundance and mean intensity were recorded from Lough Feeagh than from Clogher Lough. More than seven times the number of parasites was recovered from Lough Feeagh (3227) than from Clogher Lough (428). Indeed, the numbers of parasites recovered from the lakes are directly proportional to their size, with Lough Feeagh (410 ha) being seven times larger than Clogher Lough (60 ha). Due to its larger size, we would expect Lough Feeagh to contain more potential host species for *A. clavula* than Clogher Lough, and thus that the parasite population in Lough Feeagh would be larger than that in Clogher Lough. Indeed, Lough Feeagh does contain a greater diversity of fish species than Clogher Lough (Joseph Cooney, pers. comm.).

The relationship between lake size and fish parasite communities was first observed by Dogiel (1961). The larger the water body, the greater the habitat and faunal diversity, and, following from this, the greater the diversity of potential hosts for parasites. Most subsequent work, however, has focussed on the relationship between lake size and the number of parasite species present (e.g. Kennedy, 1978), not the prevalence and abundance of individual parasite species. Kennedy (1978) found a correlation between the number of parasite species in trout and lake size, with larger lakes harbouring more parasite species, however Carney & Dick (1999) found that

the richest yellow perch (*Perca flavescens*) helminth communities occurred in the smallest lakes, and while Marcogliese & Cone (1991) discovered that salmonids in small lakes in Newfoundland harboured more depauperate helminth communities than medium and large lakes, they also proposed that lake depth was the more crucial structuring factor in salmonid helminth communities.

Halmetoja *et al.* (2000) reported that the chemical condition of a water body is a critical factor in determining helminth community richness and abundance of perch in Finland, and found that both richness and abundance decreased markedly with increasing acidity of the water body, however in Clogher Lough (the pH of Clogher Lough is currently unknown, however the adjacent Ballin Lough has comparable geology and would therefore be expected to be very similar chemically to Clogher Lough; it has a pH of 8.1) and Lough Feeagh the opposite is true: Clogher Lough is more basic, with a pH of approximately 8.1 (extrapolated from the pH of Ballin Lough on the basis of comparable geology), while Lough Feeagh has a mean pH of 6.7 (E. DeEyto, pers. comm.). Thus in the present study the more acidic lake, Lough Feeagh, harbours a larger population of *A. clavula* than Clogher Lough.

Most likely more important than all of the previously discussed factors in determining the prevalence and abundance of *A. clavula* in trout is intermediate host abundance. Carney & Dick (1999) have shown that patterns of helminth prevalence and abundance in yellow perch are best explained by the composition of the invertebrate community on which the host feeds. If the invertebrate community is rich and diverse, then the helminth community of the host will be correspondingly rich and vice versa. The richness (or lack thereof) of the invertebrate community is in turn explained by the ability of its species to withstand their environmental conditions. *A. meridianus*, the isopod intermediate host of *A. clavula*, has been shown to thrive in eutrophic, and even polluted, conditions (Chambers, 1977). For this reason, a higher density of *A. meridianus* would be expected in mesotrophic Clogher Lough than in oligotrophic Lough Feeagh; however it is not certain whether this is the case as accurate data on *A. meridianus* density are not available.

A. clavula has been shown to occupy a broad distribution in the trout intestine, although worms from Lough Feeagh were found to occupy a more anterior mean position than those from Clogher Lough. The sex of *A. clavula* had no significant effect on its percentage location in the gut, a finding which is in contrast to that found in eels in the present study as well as that found by Kennedy & Lord (1982). This finding may be owing to the fact that trout are not a suitable host for *A. clavula* and thus they do not occupy the intestine in the same manner as they would in a preferred definitive host, like the eel.

The bimodality in *A. clavula* distribution in the trout intestine in Clogher Lough is extremely interesting. 65.2% of worms in the trout intestine were recovered from the area where the peaks in *A. clavula* distribution occur (20-29% contains 29.5% of worms, while 61-80% contains 35.7% of worms). Upon further exploration of this situation, it may be seen that the bimodality is present in medium intensity infections, and is particularly evident in the month of July. The bimodality recorded in Clogher Lough is unusual, and to my knowledge, has not been recorded from other acanthocephalan systems (see Kennedy, 2006 for a review). Possible reasons for the occurrence of bimodality in *A. clavula* distribution include clumped distribution of resources in the trout intestine or an inhospitable environment in the midsection of the gut. Alternatively, this result may be an artefact, although much further work is necessary to adequately explore this phenomenon.

This chapter has highlighted the significant differences in prevalence, abundance and parasite size observed between *A. clavula* in trout from Clogher Lough and Lough Feeagh, which again underlines the need for sampling in multiple locations.

This chapter has also shown definitively that the brown trout is not a suitable host for *A. clavula*, with just one Stage 3 female recovered from each lake. This is despite the high levels of prevalence and abundance recorded from both lakes.

The seasonal changes in mean abundance and intensity of *A. clavula* in trout were again unusual, with similar levels of both maintained in both lakes, with a peak in May in Lough Feeagh and July in Clogher Lough. There were no significant

differences in prevalence between seasons in Clogher Lough, while in Lough Feeagh a significant decline in prevalence was noted in July, in conjunction with a decline in mean abundance.

Again, changes in the abundance of *A. meridianus*, the intermediate host of *A. clavula*, are thought to be the primary cause of variation in infection parameters in fish hosts.

Chapter 5

A. clavula in Eels from Regional Sites

5.1 Introduction

Previous studies have shown that, outside the Burrishoole catchment and Clogher Lough, the occurrence of *A. clavula* in its preferred definitive host, the eel, in Ireland is rare and uncommon (Conneely & McCarthy, 1986; Kennedy & Moriarty, 2002; C.R. Kennedy, pers. comm.). When *A. clavula* has been reported from eels in Ireland, it has occurred most often at relatively low levels of prevalence and abundance (Table 5.1).

Table 5.1 Population parameters of *A. clavula* in eels from various Irish locations. NA = not available

Location	Year	N	Prevalence (%)	Mean abundance ± S.E.	Mean intensity ± S.E.	Reference
Ireland						
Clogher Lough	2002	30	97	7.7 ± 2.49	12.5 ± 2.41	Byrne *et al.*, 2004
River Dunkelin	1992	117	0.85	0.04 ± 0.67	5 ± 0.04	Callaghan & McCarthy, 1996
Lough Rea	1992	32	12.5	0.13 ± 0.58	1 ± 0.06	Callaghan & McCarthy, 1996
Lough Derg	1988	75	2.7	0.03 ± 0.02	1.0*	Kennedy & Moriarty, 2002
Lough Derg	1987	146	1.4	0.03 ± 0.02	2.5 ±0.5	Kennedy & Moriarty, 2002
Lough Corrib	1985	39	59	3.4*	NA	Conneely & McCarthy, 1986
River Drimneen	1984	49	12	17.5*	NA	Conneely & McCarthy, 1986

* S.E. not available

In a number of other studies exploring eel helminth communities in Ireland, while other acanthocephalan species were present in most locations, *A. clavula* was absent

(C.R. Kennedy, pers. comm.) (Table 5.2). In the majority of these surveys, helminth communities were heavily dominated by the generalist acanthocephalan, *A. lucii* (Kennedy & Hartvigsen, 2000).

Table 5.2 Summary of data on *Acanthocephalus* sp. in Ireland (Data from C.R. Kennedy, pers. comm.)

Lake/River	Year	N	Acanthocephalan species present
River Bann	1992	9	*A. lucii*
River Nore	1988	28	*A. lucii*
Lough Corrib	1991	62	*A. lucii*
Lough Garton	1990	6	None
Lough Derg	1990	3	None
Lough Allua	1989	18	None
Lough Bofin	1986	2	*A. anguillae*
Lough Boderg	1986	9	*A. anguillae, A. lucii*
Lough Coosan	1986	6	*A. anguillae, A. lucii*
Lough Ree	1986	31	*A. anguillae, A. lucii*
Lough Conn	1988	20	*A. lucii*
Lough Bunny	1987	11	*A. lucii*
Lough Dromore	1987	17	*A. lucii*
Lough Neagh	1986	30	*A. lucii*
Lough Neagh	1987	12	*A. lucii*
Lough Neagh	1988	18	*A. lucii*

In addition, in a number of locations where repeated sampling took place, *A. clavula* was found only sporadically (Table 5.3), and, when found, was observed at low levels of prevalence and abundance (C.R. Kennedy, pers. comm.).

Table 5.3 Summary of data on the sporadic occurrence of *A. clavula* in Irish locations

Lake	Years sampled	*A. clavula* present	Reference
Lough Corrib	1984	Y	Conneely & McCarthy, 1984
	1985	Y	Conneely & McCarthy, 1986
	1991	N	C.R. Kennedy, pers. comm.
	2008	N	Present study
Lough Derg	1983-1986	N	Kennedy & Moriarty, 2002
	1987	Y	Kennedy & Moriarty, 2002
	1988	Y	Kennedy & Moriarty, 2002
	1989-2001	N	Kennedy & Moriarty, 2002
Lough Conn	1988	N	C.R. Kennedy, pers. comm.
	2008	Y	Present study

Aims

The principal aim of this chapter is to explore the occurrence of *A. clavula* throughout Ireland and to compare patterns of occurrence with those found in the primary sampling sites in Co. Mayo. The comparison will be undertaken in order to investigate whether the situation with regard to *A. clavula* infection found in Lough Feeagh and Clogher Lough in unique in Ireland. Various aspects of the population biology of *A. clavula* in eels from seven locations around Ireland will be examined, including (a) the prevalence, abundance and intensity of *A. clavula*, (b) aggregation and (c) the parasite niche, as well as the relative success of the parasite in its preferred definitive host. Relative success of the parasite will be determined by investigating the size of *A. clavula* individuals in eels as well as the proportion of reproductively mature females, according to Lyndon and Kennedy (2001).

5.2 Materials and Methods

5.2.1 Regional sampling

Eels were collected from various locations around Ireland in August 2008 (Table 5.4) by commercial fishermen as part of a larger study carried out by the Marine Institute. These lakes were selected based on the requirements of other research programmes. Eels were recovered by fyke netting over the course of one or two nights and maintained alive at the sample site. Two hundred eels were recovered from each site. Of this sample, one hundred were sedated on-site, then weighed, measured and released. The remaining one hundred individuals were killed by an excess of anaesthetic. Each individual was then weighed, measured and placed in a sealed and labelled plastic bag. The sample was transported back to the laboratory and frozen until ready for dissection.

A random sample of 30 eels from each location was dissected and examined for the presence of *A. clavula*. Fish dissections and parasite processing and identification were carried out as per Section 3.2.

5.2.2 Sample site descriptions

Various descriptive parameters for each sample site are given in Table 5.4. The site locations are shown in Figure 5.1.

Table 5.4 Descriptive parameters for regional sample sites (NA = not available).

Lake/Estuary	County	Area (ha)	Mean depth (m)	Altitude (m ASL)	Trophic status	Location on map (Figure 5.1)
Lough Conn	Mayo	4684	7.7	7.9	Mesotrophic*	A
Lough Corrib	Galway	5042	3.9	1.8	Mesotrophic*	B
Lough Erne	Fermanagh	10384	11.9	45.0	Eutrophic*	C
Lough Oughter	Cavan	658	2.6	47.7	Eutrophic-hypereutrophic*	D
Lough Mask	Mayo	8183	5.0	11.8	Mesotrophic-eutrophic*	E
Waterford Harbour	Waterford	NA	NA	0	-	F
Slaney estuary	Wexford	NA	NA	0	-	G

* Ian Donohue, pers. comm.

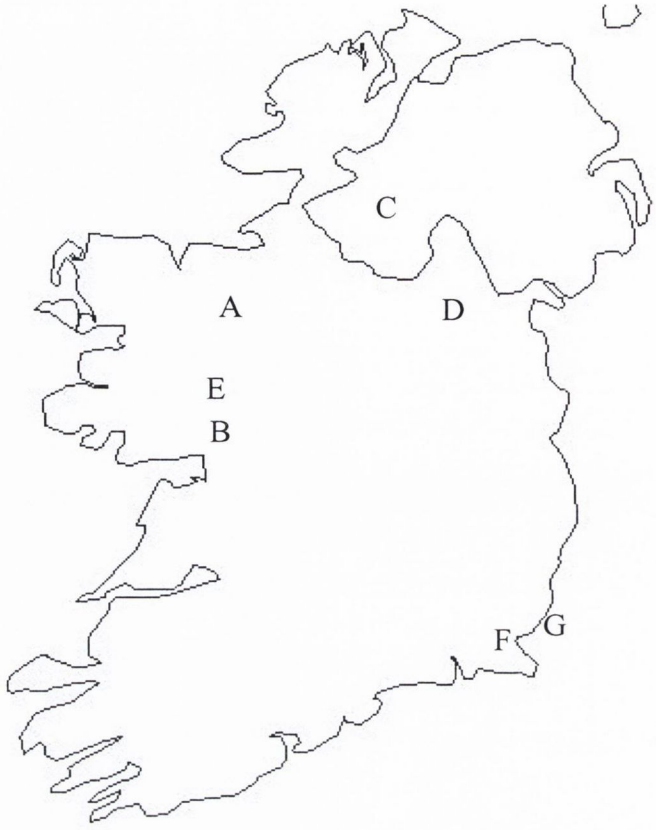


Figure 5.1 Map of Ireland showing regional eel sampling sites. For key see Table 5.4.

5.3 Results

A. clavula was recovered from eels in just one regional sample site, Lough Conn, and was not found in eels sampled from Lough Corrib, Lough Erne, Lough Oughter, Lough Mask, Waterford Harbour or the Slaney Estuary.

Lough Conn

A total of 34 *A. clavula* individuals were recovered from eels sampled from Lough Conn in August 2008, with infections ranging from 1 to 11 individuals. Of the 34 individuals recovered, 15 were female and 19 were male.

5.3.1 Overall prevalence, abundance and intensity of *A. clavula*

A. clavula was recovered from eels sampled from Lough Conn at an intermediate level of prevalence and relatively low levels of mean abundance and mean intensity (Table 5.5).

Table 5.5 Population parameters of *A. clavula* from Lough Conn

Lake	N	Prevalence (%)	Mean abundance ± S.E.	Mean intensity ± S.E.
Lough Conn	30	37.9	1.2 ± 0.5	3.2 ± 1.1

The population of *A. clavula* in eels from Lough Conn was aggregated, with a variance to mean ratio of 5.98.

5.3.2 *A. clavula* reproductive status

An examination of the maturity status of the female worms found that 13% (n = 2) of all worms were stage 1 females, 20% were stage 2 females (n = 3), and 67% were stage 3 (n = 15) females.

5.3.3 *A. clavula* niche

The niche occupied by *A. clavula* in eels from Lough Conn was examined in detail. The distribution of *A. clavula* along the eel intestine was calculated for the total sample. Due to the low number of individuals recovered, it was not possible to analyse the niche of *A. clavula* in low, medium and high intensity infections, indeed no eel recovered was infected at high intensity.

The mean percentage position of *A. clavula* in the intestine of eels in Lough Conn was 50.1% ± 2.4 S.E. The minimum position in which *A. clavula* was found was 4.4%, while the maximum was 73.2%, indicating that *A. clavula* occupies a broad fundamental niche in the eel intestine in Lough Conn.

The mean percentage position did not differ significantly between male and female parasites ($F = 0.682$, $df = 1$, $p = 0.4152$).

5.4 Discussion

A. clavula has a wide distribution in its preferred definitive host, the eel, extending across Ireland, Britain and continental Europe (Table 3.1). In several studies examining the helminth communities of eels in Great Britain (Norton *et al.*, 2003), Germany (Sures *et al.*, 1999a; Sures & Streit, 2001; Thielen *et al.*, 2007), Austria (Schabuss *et al.*, 2004), the Netherlands (Outeiral, 2002) and Italy (DiCave *et al.*, 2001), *A. clavula* was absent. In the present study, of the seven regional sites investigated, *A. clavula* was recovered only in eels from Lough Conn.

The presence or absence, as well as the rarity or dominance of *A. clavula*, is most likely correlated with the presence and abundance of its intermediate host, *A. meridianus* (Kennedy, 1984).

A. meridianus has a patchy distribution in Ireland and Great Britain. An apparent decline in the occurrence of *A. meridianus* corresponding with an increase in the occurrence of *A. aquaticus* in Great Britain is postulated to result from a competitive interaction between the two species (Moon, 1957a, b; Williams, 1962a, b, 1979) although the nature of this interaction has yet to be elucidated and populations of the two species have been shown to co-exist successfully in the laboratory (Dupey, 1967). It is thought that *A. meridianus* was the first of the two species to re-colonise Great Britain and Ireland after the end of the last glaciation (Williams, 1962b). *A. aquaticus* is thought to have arrived subsequently, and then competed with, and replaced *A. meridianus* in some locations (Williams, 1962b).

The gradual replacement of *A. meridianus* by *A. aquaticus* may also be responsible for the overwhelming dominance of *A. lucii* in the vast majority of eel helminth communities in Ireland (Table 5.2). Co-occurrences of *A. lucii* and *A. clavula* are observed infrequently, despite the fact that concurrent infections of acanthocephalans are more frequent in Ireland due to the absence of certain definitive hosts (Lyndon & Kennedy, 2001) as well as the fact that concurrent infections by two or more species of acanthocephalans are more frequently observed in aquatic hosts, including fish

(Kane, 1966; Conneely & McCarthy, 1984, 1986; Holland & Kennedy, 1997). This suggestion is backed up by observations of stable populations of *A. lucii* and *A. anguillae*, two species which share *A. aquaticus* as an intermediate host, in eels over a 3-year period in Lough Derg (Kennedy & Moriarty, 1987).

The paucity of observations of concurrent infections of *A. lucii* and *A. clavula* is likely due to the rarity of co-occurrence of the intermediate hosts of the two acanthocephalan species (*A. aquaticus* and *A. meridianus*, respectively) in the same location. While no concurrent infections of *A. lucii* and *A. clavula* have yet been recorded in Great Britain, two instances have been noted in Ireland. Kennedy & Moriarty (2002) recorded *A. clavula* at low levels of prevalence in eels from Lough Derg on two occasions in an 18-year study, where the helminth community was overwhelmingly dominated by *A. lucii* in all sample years. In contrast, Byrne *et al.* (2004) investigated eel helminth communities in Clogher Lough and found that, despite relatively high prevalence levels of *A. lucii* (57%), helminth communities were heavily dominated by *A. clavula* in terms of both prevalence (97%) and intensity of infection (12.5 ± 5.8 individuals). The disparity in results of these studies underlines the need for an investigation of potential interactions between the two species.

In locations where *A. meridianus* is present, the presence of *A. clavula* may be further restricted through competitive interactions with *P. laevis* (Byrne *et al.*, 2003), although this interaction has thus far only been observed in brown trout (Byrne *et al.*, 2003).

For the majority of the regional sites, the absence of *A. clavula* may be explained as a function of *A. meridianus* presence or absence. The reasons for the patterns of occurrence of *A. clavula* observed in the present study will now be explored on a site-by-site basis.

A. clavula was present in Lough Corrib in 1983 and 1985 (Conneely & McCarthy, 1984, 1986), however, in the present study (2008), *A. clavula* was absent from the lake. A similar situation was observed by Kennedy (1984) in the River Clyst in

England, where the prevalence and abundance of *A. clavula* declined in tandem with the abundance of its intermediate host, *A. meridianus*. This decline was observed during a 4.5 year study during which the *A. clavula* population in eels and flounder *Platyichthus flesus* underwent a steady and continuous decline. This decline was linked to a corresponding decline in *A. meridianus*, which were also sampled throughout the study, due to competition with its congener, *A. aquaticus*. Indeed, by the end of the study, *A. meridianus* was completely absent from some parts of the river, resulting in the inability of *A. clavula* to complete its lifecycle in these locations. It is quite possible that a similar decline of *A. meridianus* has occurred in Lough Corrib, and that this is the reason for the absence of *A. clavula* in the eels sampled in this study.

While there are no specific data on *A. clavula* from eels from Lough Erne, one study explored the ecology of the eel in Lough Erne (Moriarty, 1973) and found that, while *A. aquaticus* comprised an important part of the eel diet, no *A. meridianus* individuals were found. *A. clavula* may therefore be absent from Lough Erne due to the absence of its intermediate host.

Unfortunately, no data detailing *A. clavula* and *A. meridianus* abundance or eel diet are available for Lough Oughter; however, its trophic status may explain the absence of *A. clavula* from the lake. Lough Oughter is classified as eutrophic-hypereutrophic (I. Donohue, pers. comm.) and so if any *Asellus* species is present in the lake, it is most likely to be *A. aquaticus*, owing to its greater tolerance of pollution, relative to *A. meridianus* (Moon, 1968). *A. clavula* would then be absent from the lake due to the absence of its intermediate host.

In a survey of eel ecology in Lough Mask (Moriarty, 1972), the diet of eels was examined in detail. Large numbers of *A. aquaticus* were consumed by Lough Mask eels, but *A. meridianus* was not recovered. If the situation remained the same up to 2008, then *A. clavula* is absent from Lough Mask eels due to the absence of its intermediate host from the lake.

A. clavula has previously been recorded from both brackish water (Borgsteede *et al.*, 1999) and an estuary (Outeiral *et al.*, 2002), although it was not found in either of the saltwater sites sampled in the present study. *A. meridianus* has also been found in brackish and saltwater habitats (Wolff, 1973), and so in the absence of current data on *A. meridianus* distribution from Waterford Harbour and the Slaney estuary, it is not possible to speculate about the reason for the absence of *A. clavula* from eels from these locations.

In locations where *A. clavula* does occur in eels, it tends to occur as the sole and/or dominant acanthocephalan species (Kennedy *et al.*, 1998, Byrne *et al.*, 2004), or is present at low levels of prevalence and abundance (Callaghan & McCarthy, 1996; Kennedy & Moriarty, 2002; Outeiral *et al.*, 2002). *A. clavula* was the sole acanthocephalan species recorded from Lough Conn and was present at an intermediate level of prevalence but low levels of abundance and intensity. This would suggest that *A. meridianus* is a minor, but constant, part of the eel diet in this location; however in a study of eel ecology (Moriarty, 1973) in Lough Conn, *A. meridianus* was a frequent occurrence in eel stomachs and was eaten in substantial numbers. Unfortunately, acanthocephalan infection of eels was not investigated at this time, although in a parasitological survey of Lough Conn eels carried out in 1988, *A. clavula* was not recorded (C.R. Kennedy, pers. comm.).

Chapter 6

General Discussion

The overall aims of this study were to investigate whether brown trout are a suitable definitive host (as defined by Lyndon & Kennedy, 2001) for *A. clavula* and to explore the seasonal population dynamics of the parasite in three host species: eel, trout and *A. meridianus*. While acanthocephalan parasites may infect many species of definitive host, they are generally successful with regard to growth and reproduction in just one or two species (Lyndon & Kennedy, 2001). For example, Holmes *et al.* (1977) recovered *Metechinorhynchus salmonis* from all ten fish species in Cold Lake, Canada, however the parasite reached maturity in just four of the species present. *A. clavula* has been recorded from more than 25 freshwater fish species (Kennedy, 2006) but thus far large proportions of worms have been found to mature only in eel and perch *Perca fluviatilis* (Lyndon & Kennedy, 2001), with other species acting as accidental or additional hosts.

Bates & Kennedy (1990) developed a method of assessing the maturity status of female acanthocephalans, which was later incorporated, in conjunction with parasite size, into a system for the determination of the suitability of hosts for acanthocephalan species by Lyndon & Kennedy (2001). Even prior to the introduction of this system, a number of authors investigated host suitability for acanthocephalan parasites. Much of this work focussed on *P. laevis* (Kennedy *et al.*, 1978; Kennedy, 1984; Munro *et al.*, 1989); however a small number of studies explored the maturity status of *A. clavula* in various fish species, as part of larger studies of the population biology of the parasite (Chubb, 1964; Rojanapaibul, 1977) and other acanthocephalan species (Holmes *et al.*, 1977).

For example, Kennedy *et al.* (1978) examined the suitability of brown trout and rainbow trout *Salmo gairdneri* as hosts of *P. laevis* in Britain and found in both field studies and experimental infections that the establishment, growth and proportion of mature female worms was lower in brown trout than in rainbow trout. It was proposed that rainbow trout were preferred hosts for *P. laevis*. *P. laevis* alters both the

behaviour and the appearance of its intermediate host, *Gammarus pulex*, making them more vulnerable to surface or mid-water feeding predators, such as trout. This means that *G. pulex* are more susceptible to predation by these species, which explains the infection of brown trout by *P. laevis*, despite the fact that this species is unsuitable and a reproductive dead-end as a definitive host for the parasite (Kennedy *et al.*, 1978).

According to Lyndon & Kennedy (2001), the preferred definitive host of an acanthocephalan species is one in which the parasite reaches maximum size and females reach reproductive maturity. In the case of *A. clavula*, the eel is widely acknowledged to be its preferred definitive host in Ireland and Great Britain (Chubb, 1964; Rojanapaibul, 1977; Lyndon & Kennedy, 2001; Byrne *et al.*, 2004).

Chubb (1964) investigated the ecology of *A. clavula* in four species of fish in Llyn Tegid, Wales: grayling *Thymallus thymallus*, pike *Esox lucius*, roach *Rutilus rutilus* and eel. *A. clavula* was present only at very low levels of infection in pike and roach in all samples taken. The infection of these species was thus most likely accidental. While grayling had the highest prevalence of infection, eels harboured the highest mean intensity and the greatest proportion of reproductively mature females. When stomach contents of each fish species were analysed, it was found that the grayling stomachs harboured the greatest numbers of *A. meridianus*, and eels the lowest, which confirms that host suitability, rather than diet is the more important factor for *A. clavula* infection.

Hosts in which parasites reach a smaller size and a smaller proportion of females become gravid are termed suitable hosts (Lyndon & Kennedy, 2001). Lyndon & Kennedy (2001) proposed that brown trout are a suitable host for *A. clavula*; however they did not present any evidence for this proposal with respect to the size and reproductive status of *A. clavula* from brown trout.

Indeed, the *S .trutta-A. clavula* host-parasite combination is a rare one: in a study of trout helminths from 72 localities in Ireland, Great Britain and Norway, Kennedy &

Hartvigsen (2000) reported *A. clavula* from trout in just three of them, and all three reports were from the west of Ireland.

When the *S .trutta-A. clavula* combination was examined further by Byrne *et al.* (2004) in Clogher Lough, Co. Mayo, an unusual situation was encountered: while the parasite was reported at high levels of prevalence and abundance, just 2% of females reached reproductive maturity. In eels from Clogher Lough, however, the majority of *A. clavula* (61%) reached reproductive maturity, but the worms reached a smaller size than those infecting trout.

Byrne *et al.* (2004) postulated that a host shift of *A. clavula* from eel to brown trout was in operation in Clogher Lough. While no host shift of a helminth parasite has been reported in the literature to date, it should be noted that the observation of such an event is highly unlikely, as long-term monitoring of population biology, as well as reproductive status and size of acanthocephalan species is unfortunately an extremely rare occurrence.

A primary aim of the present study was to explore whether the postulated host shift had in fact occurred and also to revisit the question of the status of eels and brown trout as hosts for *A. clavula* in Ireland. To this end, eels and trout were sampled again from Clogher Lough, as well as from Lough Feeagh and Bunaveela Lake (*A. clavula* was not found in either species from this location), two lakes from a nearby catchment.

If a host shift had taken place, it was hypothesised that the proportion of females reaching full reproductive maturity in trout would increase. The results obtained from the present study suggest that no host shift of *A. clavula* has taken place in the period between Byrne *et al.* (2004) and 2008, when sampling for the present study ceased. While the prevalence of infection in trout remained relatively high, the proportion of females reaching full reproductive maturity was again extremely low, with just 0.6% of *A. clavula* females reaching Stage 3 in the present study. The eel thus remains the preferred definitive host of this species. This result is similar to that found by Chubb (1964) when grayling were found to have a high prevalence of *A. clavula* infection,

despite extremely low levels of mature females. The results of the present study, in combination with those of Chubb (1964), underline the unusual nature of *A. clavula*, in that this species appears to show a narrower specificity to its definitive hosts than many other fish acanthocephalans.

With respect to *A. clavula* mass, in the present study, female worms in eels from Clogher Lough grew to almost double the size of worms from trout, however in Lough Feeagh female *A. clavula* in eels and trout were of similar size. Interestingly, *A. clavula* from Lough Feeagh were statistically significantly smaller than those from Clogher Lough, for both fish species (Table 4.26). The small size of worms from Lough Feeagh did not appear to hamper their reproductive capability in eels. Indeed, 57.9% of female *A. clavula* from Lough Feeagh eels reached full reproductive maturity, despite an average size just one-third that of females from Clogher Lough. The disparity in *A. clavula* size between the lakes may be owing to the operation of a crowding effect, as the intensity of infection was much higher in Lough Feeagh than in Clogher Lough in all sample months.

In contrast to the present study, Byrne *et al.* (2004) reported that *A. clavula* from Clogher Lough attained a similar size in both eels and trout. While the mean size of *A. clavula* from trout in the present study remained similar to that found by Byrne *et al.* (2004), the mean size of *A. clavula* from eels has almost doubled in the period between the two studies.

Despite the size increase noted in the present study, *A. clavula* from eels in Ireland are a great deal smaller than their conspecifics from Great Britain (Lyndon & Kennedy, 2001) (Table 6.1), and are in fact closer in size to worms from perch (Table 6.1). This phenomenon has previously been encountered: the mean mass of *P. laevis* from Irish brown trout (7.1mg, n= 731, males & females combined) was much lower than that reported from Great Britain (38.3mg, n= 10, gravid females only). Therefore there is a precedent for differential growth of acanthocephalans between the two locations. While the reasons for the growth disparity are as yet unknown, O'Mahony *et al.* (2004) found that strain differences of *P. laevis* were based on fish species

rather than geographical distribution, so perhaps multiple strains of *A. clavula* are also in existence.

As the small size of *A. clavula* from eels does not appear to affect their reproductive capabilities, it may be said that eels from Clogher Lough and Lough Feeagh examined in the present study fulfil the criteria for preferred definitive host status of *A. clavula*, as laid out by Lyndon & Kennedy (2001).

In contrast to this, brown trout do not fulfil the criteria for suitable host status. In order to be designated as a suitable host for *A. clavula*, as per Lyndon & Kennedy (2001), worms should grow to a smaller adult size than in their preferred definitive host and many females should reach full reproductive maturity. While the size criterion has been fulfilled in the present study, very few females reached reproductive maturity and thus it is now clear that brown trout are not in fact a suitable host for *A. clavula*.

Table 6.1 Prevalence, abundance and size and reproductive status of *A. clavula* from various locations

Location	Fish species	Prevalence (%)	Mean Abundance ± S.E.	Mean Mass ± S.E. (mg)	% Stage 3	Ref.*
Clogher Lough	Eel	58.9	5.69 ± 1.19	0.66 ±0.03	65.9	1
Clogher Lough	Eel	97.0	7.7	0.34	61.0	2
Lough Feeagh	Eel	89.2	22.04 ± 3.20	0.23 ±0.01	57.9	1
Keadby Drain	Eel	NA	NA	2.69	NA	3
River Clyst	Eel	NA	NA	3.07	NA	3
Clogher Lough	Trout	39.2	3.4 ± 0.9	0.34 ± 0.01	1.0	1
Clogher Lough	Trout	86.3	53.2	0.39	2.0	2
Lough Feeagh	Trout	74.8	14.8 ± 2.9	0.15 ± 0.008	0.43	1
Slapton Ley	Perch	NA	NA	0.63	NA	3

* = 1: Present study; 2: Byrne *et al.* (2004); 3: Lyndon & Kennedy (2001)

While the brown trout-*A. clavula* combination has been reported on numerous occasions from the west of Ireland (Conneely & McCarthy, 1984, 1988; Molloy *et al.*, 1993; Byrne *et al.*, 2002b; Byrne *et al.*, 2004; present study) *A. clavula* has only been recovered in relatively high numbers from trout in the Burrishoole catchment and Clogher Lough. The *A. clavula*-brown trout combination is thus not, in fact, more common in Ireland than in other locations, it is simply more common in the Burrishoole catchment and its environs. This is likely a result of the occurrence of the intermediate host of *A. clavula*, *A. meridianus*, in high numbers in this area, which is in turn a result of the local conditions.

Despite the occurrence of *A. clavula* in high numbers in trout from the Burrishoole catchment and Clogher Lough, the worms do not reproduce. The infection of trout by *A. clavula* thus results in an end to the parasite life cycle.

Brown trout should therefore be designated as an additional host for *A. clavula*, defined as one in which the parasite makes some growth and some females reach Stage 3 (Lyndon & Kennedy, 2001). Additional host status is equivalent to secondary host status as defined by Holmes *et al.* (1977). As *A. clavula* occur at relatively high levels of prevalence and abundance in trout, this species may be of more importance than simply being accidental hosts. The high infection levels may be of importance for the flow of the parasite through the fish populations of the lakes, despite the lack of mature worms, especially if post-cyclic transmission is in operation.

While convincing field data on population parameters and reproductive maturity from multiple studies (present study, Byrne *et al.*, 2004) have been presented, experimental work on parasite growth in this species is necessary in order to definitively designate the status of brown trout as an additional host for *A. clavula*. Further work is also required on the importance of trout in the diet of eels as well as laboratory studies on the ability of *A. clavula* from brown trout to mature in eels following ingestion by the latter, in order to establish whether *A. clavula* is capable of undergoing post-cyclic transmission.

Alternatively, the high levels of prevalence and abundance of *A. clavula* in trout observed in Clogher Lough and Lough Feeagh may be due to intensive, opportunistic feeding by trout on an invertebrate species which happens to be present in large numbers in Clogher Lough and Lough Feeagh, and the resultant acanthocephalan infection is potentially a by-product of this dietary choice. It has been shown by Chubb (1964) and Rojanapaibul (1977) however, that the volume of intake of *A. meridianus* by fish is less important than host suitability for *A. clavula* infection. In addition, the dietary choice proposal is not backed up by analysis of the diet of eels and trout from Clogher Lough and Lough Feeagh (see Appendix). Both the percentage of eel and trout stomachs containing *A. meridianus* and the mean number of *A. meridianus* per stomach were very low, therefore if *A. meridianus* is present at

high density in the lakes, this is not reflected in the diets of eel and trout. In contrast to the results found in the present study, *Asellus* sp. have previously been found to be one of the dominant food items in the diet of brown trout in Irish lakes (O' Grady, 1983).

The ecology of freshwater fish parasites has been studied intensively for five decades and the sheer volume of work in this area (the overwhelming majority carried out in Europe and North America) is a testament to the ease of utilising fish hosts to study the ecology of parasites, however the bulk of research takes the form of short-term or seasonal field studies, and true long-term studies are rare (Kennedy, 2006). As it is often not possible to test the conclusions derived from such field studies in the laboratory, the custom has generally been to search for patterns that are replicable in time and/or space (Kennedy, 2009).

A number of studies have reported patterns in helminth community structure and population biology (Awachie, 1965; Camp & Huizinga, 1980; Kennedy & Guegan, 1994; Carney & Dick, 2000). Carney & Dick (2000) found that the complexity of the invertebrate community in lakes was a predictable determinant of the parasite community structure of perch in the USA.

In contrast, other studies have found no evidence of seasonal patterns of occurrence. Both Chubb (1964) and Rojanapaibul (1977) found that there was no discernible seasonal pattern of infection of the fish of Llyn Tegid, Wales, with *A. clavula*. Still other studies have reported patterns from fish species which are inconsistent between locations (Shulman & Shulman-Albova, 1953; Polyanski, 1955; Vidal-Martinez & Poulin, 2003). For example, while Shulman & Shulman-Albova (1953) reported a seasonal cycle in development of *Echinorhynchus gadi* in the fish of the White Sea, Polyanski (1955) found no evidence of a seasonal cycle of *E. gadi* in the fish of the Barents Sea.

In the few long-term studies of fish parasite community structure and population biology in existence the patterns observed are inconsistent. Both Kennedy & Rumpus (1977) and Kennedy & Moriarty (2002) found extremely stable acanthocephalan communities which were maintained over long periods. No regulatory mechanisms

were identified, and the observed stability may be owing to constancy of environmental conditions in the rivers sampled (River Avon and River Shannon, respectively), both of which are closely regulated by man (Kennedy, 2009).

In other studies, different patterns in parasite population dynamics were observed. Kennedy (1984) observed a continuous decline in the prevalence and abundance of *A. clavula* in the River Clyst, which was postulated to have resulted from a decline in the abundance of the intermediate host *A. meridianus* in the river, while Kennedy *et al.* (2001) observed three cycles of the cestode *Ligula intestinalis* in roach *R. rutilus* over a period of 32 years. It was found, however, that the cycles were independent and unregulated, as at the end of each cycle the host and parasite populations collapsed and the parasite was later reintroduced into the habitat by aquatic birds.

It has been suggested that the lack of consistent patterns reported from parasite populations in freshwater fish is due to local the influence exerted by local factors on population biology and community structure of freshwater fish parasites (Hartvigsen & Kennedy, 1993), however Kennedy (2009) has warned that caution must be exercised when drawing conclusions from studies of parasite ecology in freshwater fish, as most have been carried out in temperate regions of Europe and North America.

In the present study, the seasonal population dynamics of *A. clavula* in three hosts was investigated. Previous studies have reported linked cycles in population dynamics between intermediate and definitive hosts (Awachie, 1965; Camp & Huizinga, 1980), although this was not the case for *A. clavula* in eels, trout and *A. meridianus* in Clogher Lough and Lough Feeagh.

Awachie (1965) explored the seasonal dynamics of infection of *G. pulex* and brown trout by *E. truttae* in Wales. The intensities of infection were seasonal and bore an inverse relationship to each other. In brown trout, intensities were highest in the summer months (June to September), while the intensity of *E. truttae* in *G. pulex* was highest from October to April. These cycles were thought to correspond to the feeding activity of trout and the life history of *G. pulex*. The intensity of infection

in the intermediate host was lowest in summer due to the influx of a new generation of gammarids, but feeding intensity of brown trout increased with increasing water temperature and counteracted the low infection levels in *G. pulex* at this time, which explains the peak in *E. truttae* intensity in trout in the summer months.

Camp & Huizinga (1980) reported seasonal cycles in prevalence of *A. dirus* in the isopod *A. intermedius* and creek chub *S. atromaculatus*. Prevalence in fish reached its highest level in the period March to May and declined gradually thereafter, whereas in isopods, cystacanth prevalence reached a peak in the winter months (December to February) and declined rapidly throughout the remainder of the year. These seasonal cycles were interdependent, with adult worm prevalence in fish lagging approximately two months behind cystacanth prevalence in isopods.
In the present study, the population dynamics of *A. clavula* in its intermediate and definitive hosts appeared to show no signs of linkage. While seasonal cycles in infection parameters were observed in both isopod and fish hosts, they were not correlated and in isopods even differed between sample years. In 2007, the prevalence of *A. clavula* in *A. meridianus* remained at similar levels in all sample months, while in 2008 prevalence reached its highest levels in the summer months and declined in February and September.

If infection levels of *A. clavula* in isopod and fish hosts were interdependent, it would be expected that the prevalence of *A. clavula* in fish hosts would follow a similar pattern to that in *A. meridianus* in 2007, with similar levels of infection throughout the year; however this was not the case. Prevalence of *A. clavula* in fish was maintained within very narrow limits in all sample months. There was a clear seasonal cycle in mean abundance and mean intensity, with the highest levels observed in February, followed by a gradual decline for the remainder of the year. The disparity between the cycles of infection would suggest either (a) a differential uptake of *A. meridianus* by fish throughout the year, or (b) a difference in the ability of *A. clavula* to establish in fish hosts with increasing water temperature.

Differential uptake of *A. meridianus* by eels and trout throughout the year could occur in one of two ways: variation in feeding intensity of fish or alternatively, changes in

the population density of *A. meridianus* throughout the year. As population density of *A. meridianus* was not measured in this study, any changes in abundance of *A. meridianus* which may have occurred during the sampling period are unknown.

Temperature-dependent establishment of acanthocephalans has previously been reported from *P. laevis* in goldfish *C. auratus* (Kennedy, 1972). Goldfish were experimentally infected with different population densities of *P. laevis* and maintained at different temperatures. The density of infection had no effect upon the establishment of the parasite, but a 12°C rise in water temperature reduced parasite recovery after one week by 30%. It was postulated that temperature, in conjunction with changes in fish diet, had the potential to regulate the flow of parasites through acanthocephalan-fish parasite systems. Temperature-dependent establishment is the more likely cause of abundance changes of *A. clavula* in fish, as feeding activity of both brown trout and eel is widely acknowledged to increase with increasing temperature (Bagliniere and Maisse, 1991; Tesch, 2003)

In Lough Feeagh in 2008, *A. meridianus* reproduction occurred throughout the year, with a constant influx of juveniles available to be infected with *A. clavula*. This would suggest that relatively constant levels of infection would be observed. Instead, there was a clear seasonal cycle of infection, with a peak in prevalence in May, followed by a decline in July and September. The changes in prevalence of *A. clavula* may be owing to changes in acanthor deposition by fish or to the death of heavily infected *A. meridianus* individuals, i.e. parasite-induced host mortality. As fish were not sampled in 2008, it is not possible to look at potential changes in the proportion of gravid females as a measure of acanthor deposition.

Overall, the results of this study agree with the conclusions reached by Kennedy (2009) in his review of the ecology of freshwater fish parasites: first, parasite populations in most freshwater fish species exist in non-equilibrial conditions and second, that host and parasite populations have not yet been unequivocally shown to undergo linked and stable cycles. He states that local habitat conditions are of the utmost importance and that observed population stability may be due simply to environmental stability. To-date, aggregation remains the sole measure of parasite populations which is repeatable in time and space (Poulin, 2007b). Indeed, there was

no evidence of a linked cycle in the population dynamics of *A. clavula* in intermediate and definitive hosts in Clogher Lough and Lough Feeagh.

It is clear that *A. clavula* is unusual for an acanthocephalan species for several reasons. First, it shows narrower specificity to its definitive hosts than many other fish acanthocephalan species, to date being shown to mature only in eel and perch.

Second, *A. clavula* does not appear to use paratenic hosts, as several other acanthocephalan species tend to do (Amin, 1987; Valtonen *et al.*, 2004). For example, *Pomphorhynchus bulbocolli* has been found to utilise as many as 13 paratenic hosts in lakes in Wisconsin, USA (Amin, 1987). Thus far, larval *A. clavula* have been found only in the invertebrate species it uses as intermediate hosts.

Third, *A. clavula* does not appear to undergo post-cyclic transmission. Post-cyclic transmission occurs when a parasite survives, grows and matures in a predator of their definitive host (Rauque *et al.*, 2002). This phenomenon that been observed in several other fish acanthocephalan species (Nickol, 1985; Lassiere & Crompton, 1988; Kennedy, 1999; Rauque *et al.*, 2002; McCormick, 2004). If post-cyclic transmission was in operation, mature adult *A. clavula* would be observed to infect a wider variety of definitive host species. In Clogher Lough and Lough Feeagh for example, trout are prey for several fish species, including eel and salmon. In addition, if post-cyclic transmission was in operation, there would not be such a clear size disparity between *A. clavula* from eel and their counterparts from trout.

Finally, *A. clavula* may not in fact be a single species at all, but a complex of species. Species complexes have been previously discovered in acanthocephalans. For example, different strains of *P. laevis* are now known to exist in Ireland and Great Britain (O'Mahony *et al.*, 2004). Strain differences were noted in *P. laevis* based on host species, not geographical location, so it is quite possible that there are two *A. clavula* strains present in the Burrishoole system, one in eels and one in trout.

In addition, it is now clear that *A. meridianus* is the driving force behind both the *A. clavula*-eel and *A. clavula*-trout systems. The evidence for this is strong and is as

follows: (1) there is no correlation in the population dynamics of the host and parasite, (2) there is no repeatable, consistent seasonal cycle of infection parameters and (3) changes in infection levels in eels were observed during a period when eels were declining in the area (Poole, 2008). Thus, the patchy distribution of *A. clavula* in Ireland (and probably Britain also) is most likely owing to the patchy distribution of A. meridianus and not any parasite-specific factors.

In conclusion, the lack of consistency in seasonal cycles of *A. clavula* in *A. meridianus* between years, as well as the absence of a linked cycle between the parasite and its intermediate and definitive hosts suggests that the occurrence of *A. clavula* in its hosts is stochastic, unregulated, and solely dependent on the abundance and life cycle of the intermediate host, *A. meridianus*, which in turn is dependent on environmental factors, particularly water temperature and abundance of vegetation and detritus.

References

Aguilar, A., Alvarez, M.F., Leiro, J.M. and Sanmartin, M.L. (2005) Parasite populations of the European eel (*Anguilla anguilla* L.) in the Rivers Ulla and Tea (Galicia, northwest Spain). *Aquaculture* **249**: 85-94.

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

Amin, O. M., Burns, L. A. and Redlin, M. J. (1980) The ecology of *Acanthocephalus parksidei* (Acanthocephala: Echinorhychidae) in its isopod intermediate host. *Proceedings of the Helminthological Society of Washington* **47**: 124-131

Anderson, R. M. and Gordon, D. M. (1982) Processes influencing the distribution of parasites numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**: 373-398.

Awachie, J. B. E. (1965) The ecology of *Echinorhyncus truttae* Schrank, 1788 (Acanthocephala) in a trout stream in North Wales. *Parasitology* **55**: 747-762.

Awachie, J. B. E. (1965) The development and life history of *Echinorhynchus truttae* Schrank, 1788 (Acanthocephala). *Journal of Helminthology* **40**:11-32.

Bagliniere, J.L. and Maisse, G. (1991) *Biology and Ecology of the Brown and Sea Trout*. Springer, New York.

Balesdent, M.L. (1965) Recherches sur la sexualite et le determinisme des caracteres sexuals d'*Asellus aquaticus* Linne (Crustace: Isopode). *Bulletin de l'Academie et de la Societe Lorraines des Sciences* **5**: 1-273.

Barker, D.E., Marcogliese, D.J. and Cone, D.K. (1996) On the distribution and abundance of eel parasites in Nova Scotia; local versus regional patterns. *Journal of Parasitology* **82**: 697-701.

Bates, R.M. and Kennedy, C.R. (1990) Interactions between the acanthocephalans *Pomphorhyncus laevis* and *Acanthocephalus anguillae* in rainbow trout: testing an exclusion hypothesis. *Parasitology* **100**: 435-444.

Bates, R.M. and Kennedy, C.R. (1991) Potential interactions between *Acanthocephalus anguillae* and *Pomphorhynchus laevis* in their natural hosts chub, *Leuciscus cephalus* and the European eel, *Anguilla anguilla*. *Parasitology* **102**: 289-297.

Boisson, A. (1997) *Macroinvertebrate study of Lough Feeagh and Clogher Lake in 1997*. Salmon Research Agency of Ireland, Newport, Co. Mayo.

Borgsteede, F.H.M., Haenen, O.L.M., DeBree, J and Lisitsina, O.I. (1999) Parasitic infections of European eel (*Anguilla anguilla* L.) in the Netherlands. *Helminthologia* **36**: 251-260.

Brattey, J. (1983) The effects of larval *Acanthocephalus lucii* on the pigmentation, reproduction and susceptibility to predation of the isopod *Asellus aquaticus*. *Journal of Parasitology* **69**: 1172-3.

Brattey, J. (1986) Life history and population biology of larval *Acanthocephalus lucii* (Acanthocephala: Echinorhyncidae) in the isopod *Asellus aquaticus*. *Journal of Parasitology* **72**: 633-645.

Brattey, J. (1988) Life history and population biology of *Acanthocephalus lucii* (Acanthocephala: Echinorhyncidae). *Journal of Parasitology* **74**: 72-80.

Briand, C., Fatin, D., Fontenelle, G. and Feunteun, E. (2003) Estuarine and fluvial recruitment of the European glass eel, *Anguilla anguilla*, in an exploited Atlantic estuary. *Fisheries Management and Ecology* **10**: 377-384.

Brown, A.F. (1986) Seasonal dynamics of the acanthocephalan *Pomphorhynchus laevis* (Muller, 1776) in its intermediate and preferred definitive hosts. *Journal of Fish Biology* **34**: 183-194.

Brown, A.F., Chubb, J.C. and Veltcamp, C.J. (1986) A key to the species of Acanthocephala parasitic in British freshwater fishes. *Journal of Fish Biology* **28**: 327-334.

Brown, A.F. (1989) Seasonal dynamics of the acanthocephalan *Pomphorhynchus laevis* (Muller, 1776) in its intermediate and preferred definitive hosts. *Journal of Fish Biology* **34**: 183-194.

Brusca, R.C. and Brusca, G.J (2002) *Invertebrates*. Sinauer Associates, Inc. U.S.A. pp.936.

Bullock, W.L. (1963) Intestinal histology of some salmonid fishes with particular reference to the histopathology of acanthocephalan infections. *Journal of Morphology* **112**: 23-44.

Burlingame, P.L. and Chandler, A.C. (1941) Host-parasite relations of *Moniliformis dubius* (Acanthocephala) in albino rats, and the environmental nature of resistance to single and superimposed infections with this parasite. *American Journal of Hygiene* **33**: 1-21.

Bush, A. O., Heard, R.W. and Overstreet, R.M. (1993) Intermediate hosts as source communities. *Canadian Journal of Zoology* **71**: 1358-1363.

Bush, A.O. and Holmes, J.C. (1986) Intestinal helminths of lesser scaup ducks: an interactive community. *Canadian Journal of Zoology* **64**: 142-152.

Bush, A.O., Aho, J.M. and Kennedy, C.R. (1990) Ecological versus phylogenetic determinants of helminth parasite community richness. *Evolutionary Ecology* **4**: 1-20.

Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**: 575-583.

Byrne, C.J. (2000) The parasite community structure of stocked and wild brown trout (*Salmo trutta* L.) *Unpublished Ph.D thesis*. University of Dublin, Trinity College.

Byrne, C.J., Grey, C., Holland, C. and Poole, R. (2002) Parasite community similarity between four Irish lakes. *Journal of Helminthology* **74**: 301-305.

Byrne, C.J., Holland, C.V., Poole, W.R. and Kennedy, C.R. (2002b) Comparison of the macroparasite communities of wild and stocked brown trout *Salmo trutta* L. in the west of Ireland. *Parasitology* **124**: 435-445.

Byrne, C.J., Holland, C.V., Kennedy, C.R. and Poole, W.R. (2003) Interspecific interactions between Acanthocephala in the intestine of brown trout: are they more frequent in Ireland? *Parasitology* **127**: 399-409.

Byrne, C.J., Holland, C.V., Walsh, E., Mulligan, C., Kennedy, C.R. and Poole, W.R. (2004) Utilization of brown trout *Salmo trutta* in an Irish lake: is this evidence of a host shift? *Journal of Helminthology* **78**: 201-206.

Callaghan, R. and McCarthy, T.K. (1996) Metazoan parasite assemblages of eels in the Dunkelin catchment, western Ireland. *Archives of Polish Fisheries* **4**: 147-174.

Camp, J. W. and Huizinga, H. W. (1979) Altered color, behaviour and predation susceptibility of the isopod *Asellus intermedius* infected with *Acanthocephalus dirus*. *Journal of Parasitology* **65**: 667-669.

Camp, J. W. and Huizinga, H. W. (1980) Seasonal population interactions of *Acanthocephalus dirus* (Van Cleave 1931) in the creek chub, *Semiotilus atromaculatus*, and isopod, *Asellus intermedius*. *Journal of Parasitology* **66**: 299-304.

Carney, J.P. and Dick, T.A. (1999) Enteric helminths of perch (*Perca fluviatilis* L.) and yellow perch (*Perca flavescens* Mitchill): stochastic or predictable assemblages? *Journal of Parasitology* **85**: 785-795.

Carney, J.P. and Dick, T.A. (2000) Helminth communities of yellow perch (*Perca flavescens* (Mitchill)): determinants of pattern. *Canadian Journal of Zoology* **78**: 538-555.

Caswell, H. (1978) Predator-mediated co-existence: a nonequilibrium model. *American Naturalist* **112**: 127-154.

Chambers, M.R. (1977) A comparison of the population ecology of the *Asellus aquaticus* (L.) and *Asellus meridianus* Rac. in the reed beds of the Tjeukemeer. *Hydrobiologia* **53**: 147-154.

Chappell, L.H. (1969) Competitive exclusion between two intestinal parasites of the three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Parasitology* **55: 775-778.**

Choudhury, A. and Dick, T.A. (2000) Richness and diversity of helminth communities in tropical freshwater fishes: empirical evidence. *Journal of Biogeography* **27**: 936-956.

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

Chubb, J. C. (1982) Seasonal occurrence of helminths in freshwater fishes: IV. Adult Cestoda, Nematoda and Acanthocephala. *Advances in Parasitology* **20**: 1-293.

Combes, C. (2001) *Parasitism: The Ecology and Evolution of Intimate Interactions*. University of Chicago Press, Chicago.

Conneely, J.J. and McCarthy, T.K. (1984) The metazoan parasites of freshwater fishes in the Corrib catchment area, Ireland. *Journal of Fish Biology* **24**: 363-375.

Conneely, J.J. and McCarthy, T.K. (1986) Ecological factors influencing the composition of the parasitic fauna of the European eel, *Anguilla anguilla* (L.), in Ireland. *Journal of Fish Biology* **28**: 207–219.

Conneely, J.J. and McCarthy, T.K. (1988) The metazoan parasites of trout (*Salmo trutta* L.) in western Ireland. *Journal of Fish Biology* **28**: 207-219.

Connors, V.A. & Nickol, B.B. (1991) Effects of *Plagiorhynchus cylindraceus* (Acanthocephala) on the energy metabolism of adult starlings, *Sturnus vulgaris*. *Parasitology* **103**: 395-402.

Conway-Morris, S. and Crompton, D.W.T. (1982) The origin and evolution of the Acanthocephala. *Biological Reviews* **57**: 85-115.

Crompton, D.W.T and Lockwood, A.P.M. (1968) Studies on the absorption and metabolism of D-(U-^{14}C) glucose by *Polymorphus minutus* (Acanthocephala) in vitro. *Journal of Experimental Biology* **48**: 411-425.

Crompton, D.W.T and Walters, D.E. (1972) An analysis of the course of infection of *Moniliformis dubius* (Acanthocephala) in rats. *Parasitology* **64**: 517-523.

Crompton, D.W.T (1973) The sites occupied by some parasitic helminths in the alimentary tract of vertebrates. *Biological Reviews* **48**: 27-83.

Crompton, D.W.T, Singhvi, A. and Keymer, A. (1982) Effects of host dietary fructose on experimentally stunted *Moniliformis* (Acanthocephala). *International Journal for Parasitology* **12**: 117-121.

Crompton, D.W.T, Keymer, A., Singhvi, A. and Nesheim, M.C. (1983) Rat dietary fructose and the intestinal distribution and growth of *Moniliformis* (Acanthocephala). *Parasitology* **86**: 57-81.

Crompton, D.W.T. (1985) Reproduction. In *Biology of the Acanthocephala* (edited by Crompton, D.W.T. and Nickol, B.B.), Cambridge University Press, Cambridge. pp 213-263.

Crompton, D.W.T. and Nickol, B.B. (1985) *Biology of the Acanthocephala.* Cambridge University Press, Cambridge.

Culver, D.C. (1972) A niche analysis of Colorado ants. *Ecology* **53**: 126-131.

Dauod, H.A., Bolger, T. and Bracken, J.J. (1986) Age, growth and diet of the brown trout, *Salmo trutta* L. in the Roundwood Reservoir System. Irish Fisheries investigations No. 28.

Dechtiar, A.O. (1972) New parasite records for Lake Erie fish. *Great Lakes Fisheries Commission Technical Report* **17**: 1-20.

Dekker, W. (2003) Did lack of spawners cause the collapse of the European eel, *Anguilla anguilla*? *Fisheries Management and Ecology* **10**: 365-376.

Dekker, W. (2004) What caused the decline of the Lake Ijsselmeer eel stock after 1960? *ICES Journal of Marine Science* **61**: 394-404.

Dezfuli, B. S., Rossetti, E., Zanini, N. and Rossi, R. (1991) Occurrence of *Acanthocephalus clavula* (Acanthocephala) in *Echinogammarus pungens* (Amphipoda) from the River Adige. *Parasitologia* **33**: 121-126.

Dezfuli, B. S., Rossetti, E. and Fano, E. A (1994) Occurrence of larval *Acanthocephalus anguillae* (Acanthocephala) in the *Asellus aquaticus* (Crustacea, Isopoda) from the River Brenta. *Bolletin Zoologia* **61**: 77-81.

Dezfuli, B.S., Rossetti, E., Bellettato, C.M. and Maynard, B.J. (1999) *Pomphohynchus laevis* in its intermediate host *Echinogammarus stammeri* in the River Brenta, Italy. *Journal of Helminthology* **73**: 95-102.

Dezfuli, B.S., Giari, L., De Biaggi, S. and Poulin, R. (2001) Associations and interactions among intestinal helminths of the brown trout, *Salmo trutta*, in northern Italy. *Journal of Helminthology* **75**: 331-336.

Di Cave, D., Berrilli, F., De Liberato, C., Orecchio, P. and Kennedy, C.R. (2001) Helminth communities in eels from Adriatic coastal lagoons in Italy. *Journal of Helminthology* **75**: 7-13

Dobson A. P. (1985) The population dynamics of competition between parasites. *Parasitology* **66**: 317-347.

Dogiel, V.A. (1961) Ecology of the parasites of freshwater fishes. In *Parasitology of Fishes* (edited by Dogiel, V.A., Petrushevski, G.K. and Polyanski, Yu.I) Oliver and Boyd, Edinburgh and London. pp 1-47.

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

Dogiel, V.A. (1964) *General Parasitology*. Oliver & Boyd. pp. 516.

Dorucu, M., Crompton, D.W.T., Huntingford, F.A. and Walters, D.E. (1995) The ecology of endoparasitic helminth infections of brown trout (*Salmo trutta*) and rainbow trout (*Onchorhynchus mykiss*) in Scotland. *Folio Parasitologica* **42**: 29-35.

Dupey, J.R. (1967) An experimental investigation into the ecology of two freshwater isopods *Asellus aquaticus* L. and *Asellus meridianus* Racovitza. *Unpublished Ph.D. Thesis*. University of Leicester.

Edmonds, S.J. (1965) Some experiments on the nutrition of *Moniliformis dubius* Meyer (Acanthocephala). *Parasitology* **55**: 337-344.

Esch, G.W. (1971) Impact of ecological succession on the parasite fauna in centrarchids form oligotrophic and eutrophic ecosystems. *American Midland Naturalist* **86**: 160-168.

Esch, G.W., Kennedy, C.R., Bush, A.O. and Aho, J.M. (1988) Patterns in helminth communities in freshwater fish in Great Britain: Alternative strategies for colonization. *Parasitology* **96**: 519-532.

Esch, G.W., Bush, A.O. and Aho, J.M. (editors) (1990) *Parasite Communities: Patterns and Processes*. Chapman and Hall, London.

Feunteun, E. (2002) Management and restoration of European eel (*Anguilla anguilla*): An impossible bargain. *Ecological Engineering* **18**: 575-591.

Fitzgerald, R.D. and Mulcahy, M.F. (1983) Parasites of salmon *Salmo salar* L. and trout *Salmo trutta* L. in the River Shournagh. *Irish Fisheries Investigations* **23**: 24-31.

Franceschi, N., Bauer, A., Bollache, L. & Rigaud, T. (2008) The effects of parasite age and intensity on variability in acanthocephalan-induced behavioural manipulation.*International Journal for Parasitology* **38**: 1161-1170.

Freeland W.J. (1983) Parasites and the coexistence of animal species. *American Naturalist* **121**: 223-236.

Fresi, E. (1967) Richerche sulla sessualita di *Asellus (ProAsellus) coxalis* Dollfus (Crust. Isop.) I. Effeti del parassitismo. *Archives Zoologica Italia* **52**: 287-303.

Freyhof, J. & Kottelat, M. (2008) *Anguilla anguilla*. In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2. <www.iucnredlist.org>.

Garey, R.J., Nonnemacher, M.R., Near, T.J. and Nadler, S.A. (1996) Molecular evidence for acanthocephalans as a sub-taxon of rotifers. *Journal of Molecular Evolution* **43**: 45-54.

Gledhill, T., Sutcliffe, D.W. and Williams, W.D. (1976) Key to British Freshwater Crustacea: Malacostraca. Scientific Publications of the Freshwater Biological Association **32**:1-72.

Goater, T.M., Esch, G.W. and Bush, A.O. (1987) Helminth parasites of sympatric salamanders: ecological concepts at infracommunity, component and compound community levels. *American Midland Naturalist* **118**: 289-300.

Grabda-Kazubska, B. and Chubb, J.C. (1968) *Acanthocephalus*, the correct generic nme for *Echinorhynchus clavula* Dujardin 1845 (Acanthocephala). *Acta Parasitologica Polonica* **15**: 305-311.

Graca, M.A.S., Maltby, L. and Calow, P. (1993) Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus* I: feeding strategies. *Oecologia* **93**: 139-144.

Griffiths, D. (1997) The status of Irish freshwater fish fauna: a review. *Journal of Applied Ichthyology* **13**: 9-13.

Halmetoja, A., Valtonen, E.T. and Koskenniemi, E. (2000) Perch (*Perca fluviatilis* L.) parasites reflect ecosystem conditions: a comparison of a natural lake and two acidic reservoirs in Finland. *International Journal for Parasitology* **30**: 1437-1444.

Halvorson, O. (1971) Studies on the helminth fauna of Norway. XVIII: On the composition of the parasitic fauna of coarse fish in the river Gloma, southeastern Norway. *Norwegian Journal of Zoology* **19**: 181-192.

Hanek, G. and Fernando, C.H. (1978) The role of season, habitat, host age and sex on gill parasites of Lepomis gibbosus. *Canadian Journal of Zoology* **56**: 1247-1250.

Hanski, I. (1982) Dynamics of regional distribution: the core and satellite species hypothesis. *Oikos* **38**: 210-221.

Hartmann, J. and Numann, W. (1977) Percids of Lake Constance, a lake undergoing eutrophication. *Journal of the Fisheries Research Board of Canada* **34**: 1676-1677.

Hartvigsen, R. and Kennedy, C.R. (1993) Patterns in the composition and richness of helminth communities in brown trout *Salmo trutta* in a group of reservoirs. *Journal of Fish Biology* **43**: 603-615

Hasu, T., Holmes, J. C. and Valtonen, V. E. (2007) Isopod (*Asellus aquaticus*) size and acanthocephalan (*Acanthocephalus lucii*) infections. *Journal of Parasitology* **93**: 450-457.

Hechtel, L.J., Johnson, C. and Juliano, A. (1993) Modification of antipredator behavior of *Caecidotea intermedius* by its parasite *Acanthocephalus dirus*. *Ecology* **74**: 710-713.

Hine, P. M. and Kennedy, C. R. (1974) The population biology of the acanthocephalan *Pomphorhynchus laevis* (Muller) in the River Avon. *Journal of Fish Biology* **6**:665-679.

Hirsch , R. P.(1980) Distribution of *Polymorphus minutus* among its intermediate hosts. *International Journal for Parasitology* **10**: 243-248.

Holland, C.V. and Kennedy, C.R. (1997) A checklist of parasitic helminth and crustacean species recorded in freshwater fish from Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy* **97B**: 225-243.

Holloway, H.L. (1966) *Prosthorhynchus formosum* (Van Cleave, 1918) in songbirds, with notes on acanthocephalans as potential parasites of poultry in Virginia. *Virginia Journal of Science* **17** (new series) 149-154.

Holmes, J.C. (1961) Effects of concurrent infections on *Hymenolepis diminuta* (Cestoda) and *Moniliformis dubius* (Acanthocephala). I. General effects and comparison with crowding. *Journal of Parasitology* **47**: 209-216.

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

Holmes, J.C. (1973) Site selection by parasitic helminths: interspecific interactions, site segregation, and their importance to the development of helminth communities. *Canadian Journal of Zoology* **51**: 333-347.

Holmes, J.C., Hobbs, R.P. and Leong, T.S. (1977) Populations in perspective: community organization and regulation of parasite populations. In *Regulation of Parasite Populations* (edited by Esch, G.W.) Academic Press Inc, New York. pp 209-246.

Holmes, J.C. and Price, P.W. (1986) Communities of Parasites. In *Community Ecology: Pattern and Process* (edited by Kikkawa, J. and Anderson, D.J. Blackwell Scientific Publications, Oxford. pp 187-213.

Hurlbert, S.H. (1997) Functional importance versus keystoneness: reformulating some questions in theoretical biocenology. *Australian Journal of Ecology* **22**: 369-382.

Hutchinson, G.E. (1957) Concluding remarks. Cold Spring Harbor Symposium on Quantitative Biology **22**: 415-427.

Hynes, H. B. N. (1955) The reproductive cycle of some British freshwater *Gammaridae. Journal of Animal Ecology* **24**: 352-387.

Hynes, H.B.N. (1965) Experiments on competition between two *Asellus* species (Isopoda Crustacea). *Hydrobiologia* **26**: 203-210.

Hyvarinen, P. and Huusko, A. (2006) Diet of brown trout in relation to variation in abundance and size of pelagic fish prey. *Journal of Fish Biology* **68**: 87-98.

Itamies, J., Valtonen, E.T. and Fagerholm, H.P. (1980) *Polymorphus minutus* infection in eiders and its role as a possible cause of death. *Annales Zoologici Fennici* **17**: 285-289.

Ivashkin, V.M. (1972) Infections of Amphipoda from streams in the Carpathian mountains by acanthocephalan larvae. In *Parazity vodnykh bespozvonochnykh zhivotnkyh. I Vsesoyuznyi Simpozium*. L'vov, USSR: Izdatel'stvo L'vovskogo Universiteta. pp 31-33.

Kakizaki, T., Saito, T., Ohtaka, A. and Nagasawa, K. (2003) Effects of *Acanthocephalus* sp. (Acanthocephala: Echinorhyncidae) on the body size and reproduction of isopods (*Asellus hilgendorfi*). *Limnology* **4**: 43-46.

Kane, M.B. (1966) Parasites of Irish fishes. *Scientific Proceedings of the Royal Dublin Society* **1B**: 205-220.

Kates, K.C. (1942) Viability of the eggs of the swine thorn-headed worm (*Macroacanthorhynchus hirudinaceus*). *Journal of Agricultural Research* **64**: 93-100.

Kates, K.C. (1944) Some observations on experimental infections of pigs with the thorn-headed worm *Macroacanthorhynchus hirudinaceus*. *American Journal of Veterinary Research* **5**: 166-172.

Kelly-Quinn, M. and Bracken, J.J. (1989) A comparison of the diet of wild and stocked hatchery-reared brown trout, *Salmo trutta* L., fry. *Aquaculture and Fisheries Management* **20**: 325-328.

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

Kennedy, C.R. (1974) The importance of parasite mortality in regulating the population size of the acanthocephalan *Pomphorhynchus laevis* in goldfish. *Parasitology* **68**: 271-284.

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

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

Kennedy, C. R. (1978) An analysis of the metazoan parasitocenoses of brown trout *Salmo trutta* from British Lakes. *Journal of Fish Biology* **13**: 255-263.

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

Kennedy, C.R. and Lord, D. (1982) Habitat specificity of the acanthocephalan *Acanthocephalus clavula* (Dujardin, 1845) in eels *Anguilla anguilla* (L). *Journal of Helminthology* **56**: 121-129.

Kennedy, C. R. (1984) Dynamics of a declining population of the acanthocephalan *Acanthocephalus clavula* in eels *Anguilla anguilla* in a small river. *Journal of Fish Biology* **25**: 665-677.

Kennedy, C.R. (1985a) Regulation and dynamics of acanthocephalan populations. In *Biology of the Acanthocephala* (edited by Crompton, D.W.T. and Nickol, B.B.), Cambridge University Press, Cambridge. pp 385-414.

Kennedy, C.R. (1985b) Site segregation by species of Acanthocephala in fish, with special reference to eels, *Anguilla anguilla*. *Parasitology* **90**: 375-390.

Kennedy, C.R. and Moriarty, C. (1987) Co-existence of congeneric species of Acanthocephala: *Acanthocephalus lucii* and *A. anguillae* in eels *Anguilla anguilla* in Ireland. *Parasitology* **95**: 301-310.

Kennedy, C.R. (1993) The dynamics of intestinal helminth communities in eels *Anguilla anguilla* in a small stream: long term changes in richness and structure. *Parasitology* **107**: 71-78.

Kennedy, C.R. and Guegan, J.-F. (1994) Regional versus local helminth parasite species richness in British freshwater fish: saturated or unsaturated parasite communities? *Parasitology* **109**: 175-185.

Kennedy, C.R. (1995) Richness and diversity of macroparasite communities in tropical eels *Anguilla reinhardt*ii in Queensland, Australia. *Parasitology* **111**: 233-245.

Kennedy, C.R. and Guegan, J.-F. (1996) The number of niches in intestinal communities of *Anguilla anguilla*: are there enough spaces for parasites? *Parasitology* **113**: 293-302.

Kennedy, C.R. (1997) Long-term and seasonal changes in composition and richness of intestinal helminth communities in eels *Anguilla anguilla* of an isolated English river. *Folia Parasitologica* **44**: 267-273.

Kennedy, C.R., Berrilli, F., DiCave, D., DeLiberato, C. and Orecchia, P. (1998) Composition and diversity of helminth communities in eels *Anguilla anguilla* in the

River Tiber: long-term changes and comparison with insular Europe. *Journal of Helminthology* **72**: 301-306.

Kennedy, C.R. (1999) Post-cyclic transmission in *Pomphorhynchus laevis* (Acanthocephala). *Folia Parasitologica* **46**: 111-116.

Kennedy, C.R. and Hartvigsen, R.A. (2000) Richness and diversity of intestinal metazoan communities in brown trout *Salmo trutta* compared to those of eels *Anguilla anguilla* in their European heartlands. *Parasitology* **121**: 55-64.

Kennedy, C.R. (2001) Metapopulation and community dynamics of helminth parasites of eels *Anguilla anguilla* in the River Exe system. *Parasitology* **122**: 689-698.

Kennedy, C.R., Shears, P.C. and Shears, J.A. (2001) Long-term dynamics of *Ligula intestinalis* and roach *Rutilus rutilus*: a study of three epizootic cycles over thirty-one years. *Parasitology* **123**: 257-269.

Kennedy, C.R. and Moriarty, C. (2002) Long-term stability in the richness and structure of helminth communities in eels, *Anguilla anguilla*, in Lough Derg, River Shannon, Ireland. *Journal of Helminthology* **76**: 315-322.

Kennedy, C.R. (2006) *Ecology of the Acanthocephala.* Cambridge University Press. Cambridge. pp.249.

Kennedy, C.R. (2009) The ecology of parasites of freshwater fishes: the search for patterns. *Parasitology* **136**: 1653-1662.

Knights, B. (2003) A review of the possible impacts of long-term oceanic and climate changes and fishing mortality on recruitment of anguillid eels of the Northern Hemisphere. *The Science of the Total Environment* **310**: 237-244.

Komarova, T.I. (1969) Acanthocephalan larvae, parasites of benthic Crustacea in water-reservoirs in the Danube delta. *Problemy Parazitologiya* **1969**: 122-123.

Kurandina, D.P. (1975) The parasite fauna of Amphipoda in the Kremenchug Reservoir. In *Problemy Parazitologii. Materialy VIII Nauchnoi Konferentsii Parazitologov UkSSR. Chast' 1.* Kiev: Izdatel'stvo 'Nauka Dumka' pp 296-297.

Loreau, M., Roy, J. and Tilman, D. (2005) Linking ecosystem and parasite ecology. In *Parasitism and Ecosystems* (edited by Thomas, F., Renaud, F. and Guegan, J.-F.). Oxford University Press, New York. pp 13-21.

Luhe, M. (1911) Acanthocephalen. Register der Acanthocephalen und parasitischen Plattwurmer geordnet nach ihren Wirten. *Susswasserfauna Deutschlands Exkursionfauna* **16**: 114.

Lyndon, A.R. (1996) The role of acanthocephalan parasites in the predation of freshwater isopods by fish. In *The Aquatic Predators and their Prey* (edited by Greenstreet, S.P.R. and Tasker, M.L.). Blackwell Scientific, Oxford. pp 26-32.

Lyndon, A.R. and Kennedy C.R. (2001) Colonisation and extinction in relation to competition and resource partitioning in acanthocephalans of freshwater fishes of the British Isles. *Folia Parasitologica* **48**: 37-46.

Maher, K. (2009) *Unpublished moderatorship thesis.* University of Dublin, Trinity College.

Marcogliese, D.J. and Cone, D.K. (1991) Importance of lake characteristics in structuring parasite communities of salmonids from insular Newfoundland. *Canadian Journal of Zoology* **69**: 2962-2967.

Marcogliese, D.J. and Cone, D.K. (1997) Food webs: a plea for parasites. *Trends in Ecology and Evolution* **12**: 320-325.

Marcogliese, D.J. and Cone, D.K. (1998) Comparison of richness and diversity of macroparasite communities among eels from Nova Scotia, the United Kingdom and Australia. *Parasitology* **116**: 73-83.

Marcus, J.H. and Willoughby, L.G. (1978) Fungi as food for the aquatic invertebrate *Asellus aquaticus*. *Transactions of the British Mycological Society* **70**: 143-146.

May, H. (1994) Investigation into the effects of peat erosion on the aquatic ecosystem of Lough Feeagh in the Burrishoole Catchment. *Unpublished M.Sc Thesis*. Queen's University Belfast.

McCormick, A.L. and Nickol, B.B. (2004) Postcyclic transmission and its effect on the distribution of *Paulisentis missouriensis* (Acanthocephala) in the definitive host *Semotilus atromaculatus*. *Journal of Parasitology* **90**: 103-107.

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

Miller, M.A. (1943) Studies on the developmental stages and glycogen metabolism of *Macroacanthorhynchus hirudinaceus* in the Japanese beetle larva. *Journal of Morphology* **73**: 19-41.

Minchella, D.J. and Scott, M.E. (1991) Parasitism: a cryptic determinant of animal community structure. *Trends in Ecology and Evolution* **6**: 250-253.

Molloy, S., Holland, C. and Poole, R. (1993) Helminth parasites of brown and sea trout *Salmo trutta* L. from the west coast of Ireland. *Biology and Environment: Proceedings of the Royal Irish Academy B* **93B**: 137-142.

Molloy, S., Holland, C. and Poole, R. (1995) Metazoan parasite community structure in brown trout from two lakes in western Ireland. *Journal of Helminthology* **69**: 237-242.

Molloy, S., Holland, C. and O'Regan, M. (1995) Population biology of *Pomphorhynchus laevis* in brown trout from two lakes in the west of Ireland. *Journal of Helminthology* **69**: 229-235.

Moon, H.P. (1957a) The distribution of *Asellus* in Windermere. *Journal of Animal Ecology* **26**: 113-123.

Moon, H.P. (1957b) The distribution of *Asellus* in the English Lake District and adjoining areas. *Journal of Animal Ecology* **26**: 403-409.

Moon, H.P. (1968) The colonisation of Esthwaite Water and Ullswater, English Lake Dsitrict, by *Asellus* (Crustacea, Isopoda). *Journal of Animal Ecology* **37**: 405-415.

Moore, D. V. (1962) Morphology, life history, and development of the acanthocephalan *Mediorhynchus grandis* Van Cleave, 1916. *Journal of Parasitology* **48**: 76-86.

Moore, J.W. (1975) The role of algae in the diet of *A. aquaticus* and *G. pulex*. *Journal of Animal Ecology* **44**: 719-730.

Moore, J.K. (1984) Altered behavioural responses in intermediate hosts: an acanthocephalan parasite strategy. *American Naturalist* **123**: 572-577.

Moravec, F. (1985) Occurrence of endoparasitic helminths in eels (*Anguilla anguilla* (L.)) from the Macha Lake fishpond system, Czechoslovakia. *Folia Parasitologica* **32**: 113-125.

Moser, M. and Cowen, R.K. (1991) The effects of periodic eutrophication on parasitism and stock identification of *Trematomus bernacchii* (Pisces: Nototheniidae) in McMurdo Sound, Antarctica. *Journal of Parasitology* 77**:** 551-556.

Moriarty, C. (1972) Studies of the eel *Anguilla anguilla* in Ireland: 2. In the lakes of the Corrib System. *Irish Fisheries Investigations* No. 10.

Moriarty, C. (1973) Studies of the eel *Anguilla anguilla* in Ireland: 2. In Lough Conn, Lough Gill and North Cavan Lakes. *Irish Fisheries Investigations* No. 13.

Moriarty, C. (1974) Studies of the eel *Anguilla anguilla* in Ireland: 2. In the Shannon catchment area. *Irish Fisheries Investigations* No. 14.

Mouritsen, K.N. & Poulin, R. (2002) Parasitism, community structure and biodiversity in intertidal ecosystems. *Parasitology* **124**: 101-117.

Munro, W.R. (1953) Intersexuality in *Asellus aquaticus* L. parasitized by a larval Acanthocephalan. *Nature* **238**: 333.

Munro, M.A., Whitfield, P.J., and Diffley, R. (1989) *Pomphorhynchus laevis* (Muller) in the flounder, *Platyichtys flesus* L., in the tidal River Thames: population structure, microhabitat utilization and reproductive status in the field and under conditions of controlled salinity. *Journal of Fish Biology* **35**: 719-735.

Muzzall, P. M. and Rabalais, F. C. (1975a) Studies on *Acanthocephalus jacksoni* Bullock, 1962 (Acanthocephala: Echinorhyncidae). I. Seasonal periodicity and new host records. *Proceedings of the Helminthological Society of Washington* **42**: 31-34.

Muzzall, P. M. and Rabalais, F. C. (1975b) Studies on *Acanthocephalus jacksoni* Bullock, 1962 (Acanthocephala: Echinorhyncidae). II. An analysis of the host-parasite relationship of larval *Acanthocephalus jacksoni* in *Lirceus lineatus* (Say). *Proceedings of the Helminthological Society of Washington* **42**: 35-38.

Muzzall, P. M. and Rabalais, F. C. (1975c) Studies on *Acanthocephalus jacksoni* Bullock, 1962 (Acanthocephala: Echinorhyncidae). III. The altered behavior of *Lirceus lineatus* (Say) infected with cystacanths of *Acanthocephalus jacksoni. Proceedings of the Helminthological Society of Washington* **42**: 116-118.

Muzzall, P. M. (1980) Ecology and seasonal abundance of three acanthocephalan species infecting white suckers in SE New Hampshire. *Journal of Parasitology* **66:** 127-133.

Needham, A.E. (1974) *The Significance of Zoochromes*. Springer-Verlag, New York.

Nickol, B.B. (1985) Epizootiology. In *Biology of the Acanthocephala* (edited by Crompton, D.W.T. and Nickol, B.B.), Cambridge University Press, Cambridge. pp 307-346.

Nickol, B. (2003) Is postcyclic transmission under estimated as an epizootiological factor for acanthocephalans? *Helminthologia* **40**: 93-95.

Nickol, B.B. and Heard, R.W. (1973) Host parasite relationships of *Fessisentis necturorum* (Acanthocephala: Fessisentidae). *Proceedings of the Helminthological Society of Washington* **40**: 204-208.

Nickol, B. B. and Dappen, G. E. (1982) *Armadillium vulgare* (Isopoda) as an intermediate host of *Plagiorhynchus cylindraceus* (Acanthocephala) and isopod response to infection. *Journal of Parasitology* **68**: 570-575.

Norton, J., Lewis, J.W. and Rollinson, D. (2004a) Temporal and spatial patterns of nestedness in eel macroparasite communities. *Parasitology* **129**: 203-211.

Norton, J., Rollinson, D. and Lewis, J.W. (2004b) Patterns of infracommunity richness in eels *Anguilla anguilla*. *Journal of Helminthology* **78**: 141-146.

Oetinger, D. F. (1987) Effects of *Acanthocephalus dirus* (Acanthocephala) on morphometrics and reproduction of *Caecidotea intermedius* (Crustacea: Isopoda). *Transactions of the American Microscopy Society* **106**: 240-248.

Oetinger, D. F. and Nickol, B. B. (1981) Effects of acanthocephalans on pigmentation of freshwater isopods. *Journal of Parasitology* **67**: 672-684.

Oetinger, D. F. and Nickol, B. B. (1982) Developmental relationships between acanthocephalans and altered pigmentation in freshwater isopods. *Journal of Parasitology* **68**: 463-469.

O' Grady, M.F. (1983) Observations on the dietary habits of wild and stocked brown trout, *Salmo trutta* L. in Irish lakes. *Journal of Fish Biology* **22**: 593-601.

Ohtaka, A., Saito, T., Kakizaki, T, Ogasawara, S., Ohtomo, C. and Nagasawa, K. (2002) Seasonal and regional occurrence of *Acanthocephalus* sp. (Acanthocephala: Echinorhynchidae) in fishes and isopods (*Asellus hilgendorfi*) in a lake system in northern Japan. *Limnology* **3**: 143-150.

O'Mahony, E.M., Bradley, D.G., Kennedy, C.R. and Holland, C.V. (2004) Evidence for the hypothesis of strain formation in *Pomphorhynchus laevis* (Acanthocephala): an investigation using DNA sequences. *Parasitology* **129**: 341-347.

Outeiral, S., Alvarez, M.F., Iglesias, R., Paniagua, E. and Sanmartin, M.L. (2002) Non-digenean parasites of eels from estuaries in North-west Spain. *Helminthologia* **39**: 91-97.

Petridis, D. (1990) Utilization of grass carp faeces by the isopod *Asellus aquaticus* (L.) in the laboratory. *Hydrobiologia* **194**: 47-56.

Pilecka-Rapacz, M. (1986) On the development of acanthocephalans of the genus Acanthocephalus Koelreuther, 1771, with special attention to their intermediate host *Asellus aquaticus* L. *Acta Parasitologica Polonica* **30**: 233-250.

Polyanski, Yu. I. (1955) Contribution to parasitology of fishes of the northern seas of the USSR. Fish Parasites of the Barents Sea. Trudy zoology Institute Leningrad **19**.

(Quoted from *Parasitology of Fishes*. Translation by Z. Kabata. Oliver and Boyd, Edinburgh).

Poole, W.R. (1994) A population study of the European eel (Anguilla anguilla L.) in the Burrishoole system, with special reference to growth and movement. *Unpublished Ph.D. Thesis*. University of Dublin, Trinity College.

Poulin, R. (1995) "Adaptive" changes in the behaviour of parasitised animals: a critical review. *International Journal for Parasitology* **25**: 1371-1383.

Poulin, R. (1999) The functional importance of parasites in animal communities: many roles at many levels? *International Journal for Parasitology* **29**: 903-914.

Poulin, R. (2001) Interactions between species and the structure of helminth communities. *Parasitology* **122**: S3-S11.

Poulin, R. (2007a) *Evolutionary Ecology of Parasites*. Princeton University Press, Princeton, USA.

Poulin, R. (2007b) Are there general laws in parasite ecology? *Parasitology* **134**: 763-776.

Poulin, R. (2010) Parasite manipulation of host behavior: an update and frequently asked questions. In *Advances in the Study of Behavior* (ed. H. Jane Brockmann) Vol. 41, Burlington: Academic Press pp. 151-186.

Poulin, R. and Mouillot, D. (2005) Host specificity and the probability of discovering species of helminth parasites. *Parasitology* **130**: 709-715.

Rauque, C.A., Semanas, L.G. and Viozzi, G.P. (2002) Post-cyclic transmission in *Acanthocephalus tumescens* (Acanthocephala: Echinorhynchidae). *Folia Parasitologica* **50**: 72-78.

Rohde, K. (1994) Niche restriction in parasites: proximate and ultimate causes. *Parasitology* **109**: S69-S84.

Rojanapaibul, A. (1977) The biology and the life history of *Acanthocephalus clavula* Dujardin, 1845 in Llyn Tegid (Bala Lake), North Wales. *Unpublished Ph.D Thesis.* University of Liverpool.

Rosell, R., Evans, D. and Allen, M. (2005) The eel fishery in Lough Neagh, Northern Ireland – an example of sustainable management? *Fisheries Management and Ecology* **12**: 377-385.

Rossi, L. and Vitagliano-Tadini, G. (1978) Role of adult faeces in the nutrition of larvae of *Asellus aquaticus* (Isopoda). *Oikos* **30**: 109-113.

Rumpus A. E and Kennedy C.R. (1974) The effect of the acanthocephalan *Pomphorhynchus laevis* upon the respiration of its intermediate host *Gammarus pulex*. *Parasitology* **68**: 271-284.

Salgado-Maldonado, G. and Kennedy, C.R. (1997) Richness and diversity of helminth communities in a tropical cichlid fish in Mexico. *Parasitology* **114**: 581-590.

Schabuss, M., Konecny, R., Belpaire, C. and Schiemer, F. (1997) Endoparasitic helminths of the European eel, *Anguilla anguilla*, from four disconnected meanders from the rivers Leie and Scheldt in western Flanders, Belgium. *Folia Parasitologica* **44**: 12-18.

Schabuss, M, Kennedy, C.R., Konecny, R., Grillitsch, B., Schiener, F. and Herzig, A. (2005) Long-term investigation of the composition and richness of intestinal helminth communities in the stocked population of eel, *Anguilla anguilla*, in Neusiedler See, Austria. *Parasitology* **130**: 185-194.

Schmidt, G. D. and Olsen, O.W. (1964) Life cycle and development of *Prosthorhynchus formosus* (Van Cleave, 1918) Travassos, 1926, an acanthocephalan parasite of birds. *Journal of Parasitology* **50**: 721-730.

Schmidt, G. D. (1985) Development and life cycles. In *Biology of the Acanthocephala* (edited by Crompton, D.W.T. and Nickol, B.B.) Cambridge University Press. Cambridge. pp. 273-305.

Schmidt, G. D., Walley, H.D. and Wijek, D.S. (1974) Unusual pathology in a fish due to the acanthocephalan *Acanthocephalus jacksoni* Bullock, 1962. *Journal of Parasitology* **60**: 730-731.

Schmidt, J. (1922) The breeding places of the eel. *Philosophical Transactions of the Royal Society of London B* **211**: 179-211.

Schmidt, J. (1923) Breeding places and migration of the eel. *Nature* **111**: 51-54.

Schmidt, J. (1925) The breeding places of the eel. Annual Report of the Sminthsonian Institute for 1924.

Schmidt, J. (1935) Danish eel investigations during 25 years (1905-1930). The Carlsberg Foundations Oceanographical Expeditions under the leadership of the late Professor Johannes Schmidt. The Carlsberg Foundation, Copenhagen.

Schupp, D.H. and Macins, V. (1977) Trends in percid yields from the Lake of the Woods, 1888-1973. *Journal of the Fisheries Research Board of Canada* **34**: 1784-1791.

Seidenberg, A. J. (1973) Ecology of the Acanthocephalan, *Acanthocephalus dirus* (Van Cleave, 1931), in its intermediate host, *Asellus intermedius* Forbes (Crustacea: Isopoda). *Journal of Parasitology* **59**: 957-962.

Shtein, G.A. (1959) The parasite fauna of aquatic arthropods from some lakes in Karelia. III. Larval acanthocephala from crustaceans. In *Ekologisheskaya Parazitologiya.* Leningrad.

Shulman, S.S. and Shulman-Albova, R.E. (1953) Parazity ryb Belogo moray. Izdatel'stvo AN SSSR. (Quoted from *Parasites of Freshwater Fish and the Biological Basis for their Control.* Translation by Kochva. Sivan Pres, Jerusalem).

Slack, H.D. (1934) The winter food of brown trout (*Salmo trutta* L.). *Journal of Animal Ecology* **3**: 105-108.

Sniesko, S.F. (1974) The effects of environmental stress on outbreaks of infectious diseases in fishes. *Journal of the Fisheries Research Board of Canada* **34**: 1784-1791.

Sorensen, M.V. & Giribet, G. (2006) A modern approach to rotiferan phylogeny: combining morphological and molecular data. *Molecular Phylogenetics and Evolution* **40**: 585-608.

Sousa, W.P. (1994) Patterns and processes in communities of helminth parasites. *Trends in Ecology and Evolution* **9**: 52-57.

Starkie, A. (2003) Management issues relating to the European eel, *Anguilla anguilla. Fisheries Management and Ecology* **10**: 361-364.

Starling, J.A. (1985) Feeding, nutrition and metabolism. In *Biology of the Acanthocephala* (edited by Crompton, D.W.T. and Nickol, B.B.), Cambridge University Press, Cambridge. pp 125-212.

Stock, T.M. and Holmes, J.C. (1987) *Diococestus asper* (Cestoda: Diococestidae): an interference competitor in an enteric helminth community. *Journal of Parasitology* **73**: 1116-1123.

Styczynska, E. (1958) Acanthocephala of the biocenosis of Druzno Lake (Parasitofauna of the biocenosis of Druzno Lake part 6). *Acta Parasitologica Polonica* **6**: 195-211.

Sures, B., Taaschewski, H. and Siddal, R. (1997a) Heavy metal concentrations in adult acanthocephalans and cestodes compared to their fish hosts and to established free-living bioindicators. Parassitologia **39**: 213-218.

Sures, B., Knopf, K., Wurtz, J. and Hirt, J. (1999a) Richness and diversity of parasite communities in European eel *Anguilla anguilla* of the River Rhine Germany, with special reference to helminth parasites. *Parasitology* **119**: 323-330.

Sures, B., Siddall, R. and Taraschewski, H. (1999b) Parasites as accumulation indicators of heavy metal pollution. *Parasitology Today* **15**: 16-21.

Sures, B. and Streit, B. (2001) Eel parasite diversity and intermediate host abundance in the River Rhine, Germany. *Parasitology* **123**: 185-191.

Taylor, L. R., Woiwood, I. P. and Perry, J. N. (1978) The density-dependence of spatial behaviour and the rarity of randomness. *Journal of Animal Ecology* **47**: 383-406.

Taraschewski, H. (2000) Host-parasite interactions in Acanthocephala: a morphological approach. *Advances in Parasitology* **46**: 1-179.

Tesch, F.-W. (2003) *The Eel.* Blackwell Publishing, Oxford.

Thielen, F., Munderle, M., Taraschewski, H. and Sures, B. (2007) Do eel parasites reflect the local crustacean community? A case study from the Rhine river system. *Journal of Helminthology* **81**: 179-189.

Thompson-Cowley, L.L., Helfer, D.H., Schmidt, G.D. and Eltzroth, E.K. (1979) Acanthocephalan parasitism in the wester bluebird (*Sialia mexicana*). *Avian Diseases* **23**: 768-771.

Valtonen, E.T. (1980) *Metechinorhynchus salmonis* (Muller, 1780) (Acanthocephala) as a parasite of the whitefish in the Bothnian Bay. I. Seasonal relationships between infection and fish size. *Acta Parasitologica Polonica* **27**: 293-300.

Valtonen, E.T. and Crompton, D.W.T. (1990) Acanthocephala in fish from the Bothnian Bay, Finland. *Journal of Zoology* **220**: 619-639.

Van Ginneken, V.J.T. and Maes, G.E. (2005) The European eel (*Anguilla anguilla*, Linnaeus), its lifecycle, evolution and reproduction: a literature review. *Reviews in Fish Biology and Fisheries* **15**: 367-398.

Van Maren, M.J. (1979) The amphipod *Gammarus fossarum* Koch (Crustacea) as an intermediate host for some helminth parasites, with notes on their occurrence in the final host. *Bijdragen tot de Diekunde* **48**: 97-110.

Vidal-Martinez, V.M. and Poulin, R. (2003) Spatial and temporal repeatability in parasite community structure of tropical fish hosts. *Parasitology* **127**: 387-398.

Ward, H.L. (1940) Studies on the life history of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) (Acanthocephala). *Transactions of the American Microscopical Society* **59**: 327-347.

Whelan, K.F. (1995) The role of peatlands in the management of freshwater fisheries. In *Seeking a Partnership Towards Managing Ireland's Uplands*. University College Galway.

Whitfield, P.J. (1970) The egg-sorting function of the uterine bell of *Polymorphus minutus* (Acanthocephala). *Parasitology* **61**: 671-682.

Whitfield, P.J. (1979) *The Biology of Parasitism*. Edward Arnold, London.

Whittow, J.B. (1974) *Geology and scenery in Ireland*. Pelican Books, London.

Williams, W.D. (1960) The ecology of *Asellus aquaticus* (Linnaeus) 1758 and *A. meridianus* Racovitza 1919. *Unpublished Ph.D. Thesis*, University of Liverpool.

Williams, W.D. (1962a) The geographical distribution of the isopods *Asellus aquaticus* (L.) and *A. meridianus* Rac. *Proceedings of the Zoological Society of London* **139**: 75-96.

Williams, W.D. (1962b) Notes on the ecological similarities of *Asellus aquaticus* (L.) and *A. meridianus* Rac. (Crust., Isopoda). *Hydrobiologia* **20**: 1-30.

Williams, W.D. (1979) Distribution of *Asellus aquaticus* and *Asellus meridianus* (Crustacea: Isopoda) in Britain. *Freshwater Biology* **9**: 491-501

Wirth, T. and Bernatchez, L. (2003) Decline of North Atlantic eels: a fatal synergy? *Proceedings of the Royal Society of London B*: **270**: 681-688.

Wisniewski, W. (1958) Characterisation of the parasitofauna of an eutrophic lake. *Acta Parasitologica Polonica* **6**: 1-64.

Wolff, W.J. (1973) The distribution of *Asellus aquaticus* (L.) and *ProAsellus meridianus* (Rac.) in the southwestern part of the Netherlands. *Hydrobiologia* **42**: 381-392.

Yalynskaya, N.S. (1980) Protein changes in the body cavity of Gammarus (Crustacea: Amphipoda) infected with helminths. In *Voprosy parasitologii vodnykhnbespozvonochnykh zhivotnykh. (Tematicheskii Sbornik*). Vilnyu, USSR: Akademiya Nauk Litovskoi SSR, Institut Zoologii I Parazitologii. pp108-110.

Appendix

Methods, Results and Discussion of Stomach Contents Analysis of Eels and Trout

Materials & Methods

Analysis of fish stomach contents

Stomachs were removed from fish when dissected and stored in a solution of 70% ethanol with 4% glycerol. A sample of the stomachs from Lough Feeagh eels and trout were randomly selected and their contents were later analysed, with a particular focus on the presence of *A. meridianus*. 30 stomachs (from each of eel and trout) were randomly chosen for the months May, July and September 2007. The small fish sample sizes in February 2007 and February 2008 precluded their inclusion in the dietary study.

Stomach processing

Stomachs were removed from storage, drained and weighed to the nearest gram. After weighing, each stomach was placed in a Petri dish filled with tap water and placed under a dissection microscope. The stomach was opened by means of a vertical incision from the posterior end of the oesophagus to the intestinal opening of the stomach and the contents removed to the water by scraping. All detritus and plant material were removed. Stomach contents were sorted and identified to the lowest possible taxonomic level. In some cases, identification was not possible due to digestive processes. The number of individuals of each species/taxon were counted and weighed.

Results

A. meridianus in the diet of eels and trout from Lough Feeagh

Stomach contents of a selection of eels and trout from Lough Feeagh were analysed, with particular focus on the presence of *A. meridianus*. While similar proportions of the stomach contents of both species contained *A. meridianus*, the mean number of individuals per fish was much higher in trout (Table 2.16), owing to the ingestion by one fish of 61 *A. meridianus*. *A. meridianus* had a similar frequency of occurrence in the stomach contents of both species (Table 2.16).

Table A1 Details of *Asellus* presence in eel and trout stomachs from Lough Feeagh

Species	**N**	**No. stomachs containing *Asellus* (%)**	**Frequency of occurrence**	**Mean no. *Asellus*/stomach ± S.E.**	**Range**
Eel	80	9 (11.3)	0.04	0.34 ± 0.17	11
Trout	82	10 (12.2)	0.06	1.5 ± 0.82	61

A two-way GLM was carried out in order to explore the effect of month and fish species on the number of *A. meridianus* in eel and trout stomachs (Table 2.17). No significant difference in the number of *A. meridianus* in eel and trout stomachs was observed between months. The number of *A. meridianus* present was not significantly affected by either fish species or the interaction of fish species and month.

Table A2 GLM table of the effect of various factors on the number of *A. meridianus* present in eel and trout stomachs from Lough Feeagh

	F-ratio	**P**	**Df**
Factors			
Month	**4.8218**	**0.0093**	**2**
Species	2.3523	0.1271	1
Interactions			
Species*Month	2.464	0.0884	2

In both species, *A. meridianus* occurs in stomachs most frequently, and in higher numbers, in May than in July or September (Table 2.18).

Table A3 Distribution of *A. meridianus* in eel and trout stomachs from Lough Feeagh throughout 2007

	May	**July**	**September**
EEL			
No. stomachs containing *Asellus* (%*)	6 (20.7)	1 (3.9)	2 (8.0)
Total no. of *Asellus*	24	1	2
Mean no. of *Asellus* ± S.E.	0.83 ± 0.46	0.04 ± 0.04	0.08 ± 0.06
Range	11	1	1
TROUT			
No. stomachs containing *Asellus* (%*)	8 (31)	2 (7.1)	0 (0)
Total no. of *Asellus*	121	2	0
Mean no. of *Asellus* ± S.E.	4.65 ± 2.5	0.07 ± 0.05	0
Range	61	1	0

* = percentage of stomachs for the month in question

Discussion

In the present study, *A. meridianus* was found infrequently in the diet of eel and trout from Lough Feeagh. A seasonal pattern in the occurrence of *A. meridianus* in the diet of eel and trout was observed, with significantly higher numbers of *Asellus* consumed in May than in July or September. The numbers of *A. meridianus* consumed did not differ significantly between eel and trout.

A. meridianus has frequently been recorded as an important component of the diet of both eels and trout in Ireland (Moriarty, 1972, 1973, 1974) and Great Britain (Slack, 1934) (Table 2.20). *A. meridianus* occurs at lower levels of abundance in Lough Feeagh than in the majority of other sites investigated (Table 2.20). It was not possible to compare seasonal patterns in *A. meridianus* occurrence in eel and trout diet in Lough Feeagh with other locations as no seasonal study of brown trout diet which contained *A. meridianus* could be found.

Table A4 Summary of data on *A. meridianus* occurrence in eel and trout stomachs in Ireland

Location	**Fish species**	**Mean no. *A. meridianus*/ fish**	**Reference**
Lough Corrib North	Eel	10.7	Moriarty, 1972
Lough Corrib South	Eel	26.4	Moriarty, 1972
Lough Conn	Eel	18.7	Moriarty, 1973
Fergus system lakes	Eel	54.8	Moriarty, 1974
Upstream of Lough Derg	Eel	1.0	Moriarty, 1974
Lough Feeagh	Eel	0.34	Present study
Lough Feeagh	Trout	1.5	Present study
Roundwood Reservoir System	Trout	+	Dauod *et al.*, 1986
River Test	Trout	+	Slack, 1934

+ = presence/absence data only