A STUDY OF THE MEDICINAL LEECH, *HIRUDO MEDICINALIS* (L), WITH A STRATEGY FOR ITS CONSERVATION

PHILIP JOHN WILKIN

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WYE COLLEGE, UNIVERSITY OF LONDON

ABSTRACT

The study of a natural population of the Medicinal Leech, Hirudo medicinalis (L) is combined with a series of laboratory experiments with feeding and reproduction as two main themes for survival.

An introduction establishes a broad, conservation rationale and a literature review describes the past and present medical importance of the leech, the leech as a highly adapted blood-feeder and the animal's ecology.

A detailed management plan describes the primary leech site at Dungeness, Kent and provides recommendations for its conservation.

The development of a simple technique for marking animals is described and a marking system allows the estimation of population size (using Fisher-Ford and Jolly's models), dispersal and growth of individual leeches in the wild. Weight classes are constructed to described population structure.

Aspects of the leech's general ecology, and behaviour in the wild are observed and the leech's roles as predator, ectoparasite and scavenger in the community are discussed.

An experimental study of the diet of the leech population is based on serological and observational analyses of gut bloodmeals.

An examination of the growth dynamics of the leech under laboratory conditions provides ideas about the role of feeding in survival and is linked with experiments to develop optimum conditions for captive breeding. These breeding experiments are supported by cocoons obtained from a survey in the wild.

A survey of the occurrence of the leech on Dungeness and at three previously known sites in England and France is described. A proposal for re-stocking at two sites on Dungeness is presented.

A Conservation Strategy for the Medicinal Leech describes individual and population traits for survival and discusses the future role of conservation organization, legislation and the potential of the world's first commercial leech farm.

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PREFACE

In July 1978 the Veterinary Investigation Centre at Wye, Kent received a specimen which had attached itself to the side of an Alsatian dog. The dog had been swimming in the shallows of a disused, flooded gravel pit at Dungeness, Kent. The specimen was identified, using external characteristics, by Dr. A. M. Scofield, as the European Medicinal Leech, Hirudo medicinalis (L).

During the summer of 1981 several visits to the site revealed further specimens which were dissected and positively identified by Dr. R. T. Sawyer, as the Medicinal Leech. In August of the same year 26 specimens were collected in a short sampling period and large cocoons were found in the roots of vegetation just above the waterline. Four leeches were preserved and sent to the British Museum (Natural History) along with a grid reference to the site, (BM(NH) Reg. No. 19815-1-4).

At the same time information on the distribution and abundance of the Medicinal Leech, throughout its range, was being co-compiled by Sue Wells at the Conservation Monitoring Centre of the IUCN (International Union for the Conservation of Nature and Natural Resources) in Cambridge. The increased concern over the status of Hirudo medicinalis was due to such publications as 'Why we need to save the Medicinal Leech' (Oryx 16(2) 165-168) by Dr. R. T. Sawyer. This concern was formalised by the inclusion of the Medicinal Leech in the IUCN Invertebrate Red Data Book in 1982, together with a proposal for its listing in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora in Commerce), in order to limit the large trade in wild leeches for scientific, medical and pharmaceutical research.

While information was gathering to highlight the decline of leech populations, the lack of information concerning the animal's life-history and ecology was felt by Sue Wells and Dr. J. M. Elliott (co-author of the Freshwater Biological Association's Key to the British Freshwater Leeches), to be an obstacle to the formulation of a plan for its conservation.

In November 1981 the owners of the gravel pit were informed of the significance of the discovery, and with their enthusiastic support, permission was granted for access to be made to the site for research purposes.

In March 1982 the Natural Environment Research Council awarded a 3 year Research Studentship for the study of the Dungeness population of the Medicinal Leech, H. medicinalis.

"Having acquired from my father, in early life, a taste for Natural History, I have felt the greater interest since I took this subject in hand; but am constrained to observe that an expression of regret was unwillingly drawn from me on finding, notwithstanding the important services the leech renders in Medicine and its extensive employment among the Greek and Roman Physicians, that we should still be ignorant of its Natural History. And such regret will, it is probable, long continue; for what is greatly to our disadvantage, all the operations of this useful animal are carried on beneath the waters and thus evade our research; and the habits of the leech, influenced as they must necessarily be in a state of confinement, will scarcely advance us a single step in obtaining anything like satisfactory information."

From "A treatise on the Medicinal Leech"
by J. R. Johnson, 1816

I N T R O D U C T I O N

BACKGROUND

(i) The environmental crisis and the disappearance of species

"He with a smile did then his words repeat
And said that gathering leeches far and wide,
He travelled, stirring thus about his feet
The waters of the pools where they abide.
Once I could meet with them on everyside
But they have dwindled long by slow decay
Yet still I persevere and find them where I may."

From Resolution and Independence
by William Wordsworth, 1802

It is curious that in Wordsworth's description of an encounter with a Cumbrian leech gatherer he should refer to the scarcity of the Medicinal Leech. It would appear that, since this reference, their numbers have continued to decline drastically, reflecting what has become one of today's most pressing of global problems - the decline and disappearance of species diversity.

Recent global surveys, encompassing population growth and ecosystem destruction, have predicted rapidly increasing rates of species extinctions (Allen, 1980; Cousteau, 1981; Barney, 1982). Some projections, based on the assumption that man-induced extinction rates will continue to rise as they have in recent decades, indicate that perhaps as much as one-fifth of all the species on Earth today will have vanished by the end of the century (Myers, 1979). In fact so incomplete is our knowledge of the genetic diversity of the planet, particularly amongst tropical plants and insects (Elton, 1975), it is already likely that new species will become extinct before their discovery. Quite apart from the moral imperative of maintaining a rich and unique combination of genetic material which has developed over a long period of time, we still do not know the potential long-term damage to a complex and sensitive ecosystem which may result from a loss of genetic resource. Indeed, if we were to value species only on the criterion of their utility to our well being, we are still in danger of not fully realising the tremendous wealth of the genetic resource as alternative food supplies, medicines and energy requirements (Evans, 1976; Myers, 1983).

The disappearance of species has always been a natural process resulting from the selective loss of ill-adapted genomes (Ziswiler, 1967; Frankel and Soule, 1981). By far the most dramatic processes causing extinction are the actions of man on the environment. The most serious effect is the direct destruction of natural habitats resulting from, for example, urbanisation, de-forestation, pollution and increasing conversion to agriculture (Detwyler, 1971; Ehrenfeld, 1972; Goudie, 1981). Much of this destruction is occurring in the Third World where economic inequalities with the developed world have resulted in damaging, inappropriate technology, such as unnecessary dam projects and a dependence on fertiliser and pesticide-based agriculture (Schumacher, 1974; ICIDI, 1980; Eckholm, 1982). In the developed world rapidly changing patterns of land use, particularly in farming practice, are causing drastic habitat loss (Mellanby, 1967; Mabey, 1980; Shoard, 1980; Green, 1981; Pye-Smith and Rose, 1985). Another increasing threat to the survival of species through the actions of man comes from organised and often indescriminate trade in wildlife (Domalain, 1971; Inskipp and Wells, 1979).

(ii) <u>The historical legacy of man's relationship with nature and strategies for combatting species extinction</u>

The historical and philosophical relationship between man and his environment, as providing an explanation for the present crisis, has been discussed by White (1967) and notably by Passmore (1974). Most writers seem to agree that many of the present day environmental problems, including the disappearance of species, have resulted from man's historically despotic role towards the environment. This has been linked by some authors to the historical legacy of Christian dogma, which has promoted Man's dominance over nature (White, 1967; Pollard, 1984). This dominance ethic is sharply contrasted in other religions such as Hindu teaching (Chaitanya, 1983), which regards man's position in nature as equal to all its other components. This holistic view of man's place in nature has been recently encapsulated in a radically new 'Gaian' perspective which regards the total Biosphere as a huge, single, self-regulating system (Lovelock, 1979; Hughes, 1983; Sagan and Margulis, 1983).

Our knowledge of the scientific basis for natural species extinction, including problems of rarity, population genetics and island biogeography, has greatly increased (Drury, 1974; Soule and Wilcox, 1980; Ehrlich and Ehrlich, 1981). As a complement to these developments, the prevention of habitat destruction and new ideas about active management to maintain biotic diversity have increased our ability to prevent species loss (Duffey and Watt, 1971; Usher, 1973; Holdgate and Woodman, 1978). For example, the deliberate movement or introduction of an animal or plant from a threatened site to a more secure one has proved to be a valuable management tool to prevent local species extinction (Green, 1979; Rose, 1979; Campbell, 1980; Pinder, 1981). Another increasingly important tool for combatting species extinction is the work of zoos and nature reserves (Diamond, 1975; Cherfas, 1984; Mallinson, 1984). In addition to their educative role in allowing access to the general public, their importance as centres for captive breeding (Pinder and Barkham, 1978) and the maintenance of frozen genetic material for artificial breeding (Moore, 1984) is becoming paramount. A recent development in ideas about the management of our biotic diversity has been the view that wild species can act as sustainable, economic resources (Myers, 1983). These ideas have generated examples of projects which, despite involving captive breeding for commercial reasons, provide direct benefits to the prevention of species loss. These have ranged from butterfly and crocodile farming to the use of little known Asian mammals as alternatives to accepted animal husbandry practice (see BOSTID reports No. 44, 45 and 46, 1983).

(iii) The growth of the Conservation Movement

The response by man to the ecological crisis has been slow, despite a series of polemic works on man's effect on the environment in the last 40 years. The pioneering ideas of Leopold (1949) who, in his concept of the 'Land Ethic', expounded a radically new and caring approach to the environment and Carson (1962), who served warning on the catastrophic effects of pesticide misuse, stimulated the development of conservation ideas. The work of McHarg 1969) was the first attempt at putting forward the need for more careful town planning within a conservati n framework. An awareness of the need for conservation was further promoted by a series of works during the 1970's, more radical

in approach but still with the same 'Doomsday' message, against continued destruction of the natural resource (Allen and Goldsmith, 1972; Meadows and Meadows, 1972; Hardin and Baden, 1977). A recent book by O'Riordan (1981) provides a useful review of these works. One of the most significant developments for tackling the environmental crisis has been the organisation of the conservation movement. The work of Stamp (1974); Sheail (1976) and Lowe and Goyder (1983) has charted this development in the U.K. while Boardman (1981) has viewed the international scene.

The main achievement of this organization has been the provision of a framework for effecting conservation action. This is occurring, not only through a range of non-governmental groups such as the Royal Society for Nature Conservation (RSNC) but also the Nature Conservancy Council with its aim to conserve endangered species (Stubbs, 1981) and sites of special scientific interest in the U.K. (Ratcliffe, 1977). On the international scene, the formation of the International Union for the Conservation of Nature and Natural Resources (IUCN) has been instrumental in the production of the Red Data Books, important as a monitor of the status of scarce species (Fisher, Simone and Vincent, 1969), the Species Survival Commission (SSC), with its worldwide network of species specialist groups, and the Trade Records Analysis of Flora and Fauna in Commerce group (TRAFFIC) responsible for monitoring the trade in endangered species. The IUCN has also co-ordinated a series of major international conventions for the protection of Wetlands, Wildfowl and the designation of Global Heritage areas. It has also, through its World Conservation Strategy in 1980 (Allen, 1980) prompted many governments to respond with detailed future development plans with conservation as a central theme (The Conservation and Development Programme for the U.K., 1983).

The great challenge for the environmental movement in the future is the creation of greater public awareness of the scale of our environmental problems. These problems continue to occupy academic debate (Helliwell, 1973; Anon., 1974; Ratcliffe, 1976), but it is only recently that more outspoken challeng s have been made to arouse public attention (Ehrlich, 1980; Shoard, 1980; Pye-Smith and Rose, 1985).

The popularisation of conservation ideas, making them more accessible to the public, is an area for further development. Certainly the work of Attenborough (1979, 1984) and Myers (1985) are outstanding examples and two recent publications by Sitwell (1984) and Hywel-Davies and Thom (1984) bring the public into direct contact with the problem and its possible cures.

It certainly remains for the multiplicity of conservation organisations to enhance public understanding by increasing programmes for interpretation and education. The style and presentation at national museums and zoological and botanical gardens are changing to meet these needs (Cherfas, 1984; Whitehead, 1984) and media coverage is now extensive. It is perhaps in the area of international environmental education that the beginnings of a solution to our global problems lies (Carson, 1978; Connect, 1984).

THESIS RATIONALE AND SYNOPSIS

The references reviewed in the last section have provided a valuable background of ideas for formulating the pattern of work presented in this study. The specific areas of work arose from an appreciation of two important factors concerning the Medicinal Leech, H. medicinalis. One is the recognition of its dramatic decline in numbers throughout its geographical range (Collins and Wells, 1983; Wells, Pyle and Collins, 1983) and the need to understand some of the reasons behind this decline (Sawyer, 1976, 1981). The other is the urgent need for more information about the leech's biology and ecology in order to make recommendations for its conservation (Elliot and Tullett, 1984).

Species are not equally likely to disappear under the pressures of environmental change. The vulnerability of a species depends on a wide range of factors such as population size, reproductive ability and its ecological relations with other species. A more subtle factor affecting vulnerability is the way individuals of a p pulation arrange themselves in space - that is their population structure. This characteristic may have a profound influence on the susceptibility of a population to extinction. Understanding this range of characteristics of a threatened species is essential for designing strategies for its

conservation. The aim of this work has been to suggest answers to some of these unknowns concerning the Medicinal Leech, H. medicinalis.

It is the intention of the literature review to provide a broad introduction to the Hirudinea together with a description of the genus Hirudo and close relatives in the family Hirudinidae. A small section describes the part played by the Medicinal Leech, H. medicinalis in ancient and modern medicine, literature and anecdote. The main part of the review is devoted to selected references on the animal's anatomy and physiology and, where relevant, a comparison with other leech species. The aim has been to view the leech as a highly adapted blood feeder and to provide a physiological basis for field observations, such as behaviour. The review is concluded by an examination of the status of the leech in the wild and British and European records containing ecological information.

The Management Plan provides a detailed description of Lade gravel pit, the site supporting the Dungeness population of the Medicinal Leech. Using a format devised by the Ecology and Conservation Unit of University College, London (Wood and Warren, 1976; Wood, 1983), it examines the wider issues of future land use policy at Dungeness and in a prescriptive section suggests a series of management proposals for improving and conserving the site.

Chapter 1 estimates the size, structure and dispersal of the main Dungeness population of the Medicinal Leech over a period of two years. The size of a population discovered at another site in the area, is also estimated during a single field season. A time sampling method is used and a criterion of wet weight is employed to describe population structure. A novel method of marking is developed and a system of unique and date/area specific marks allow the estimation of population size and dispersal using two Mark/Release/Recapture (MRR) models. The assumptions underlying the use of these models are discussed using results from preliminary experiments on the effects of feeding and marking, as well as the implications of population size, structure and dispersal on potential survival.

The following five chapters explore two themes for survival; feeding and reproduction. Ch pter 2 describes observatıons made on the behaviour of the leech in the wild and its interaction, as prey, predator, parasite and possible scavenger, within the community. Ideas about these roles are tested in simple laboratory experiments.

Chapter 3 investigates the diet of wild leeches using observational and serological techniques. The results provide important information on host selection and the implications of host availability is discussed.

Chapter 4 describes a laboratory study of feeding with particular reference to the effects of bloodmeal frequency and quality on growth. Different methods of feeding the leech through a range of membranes are described as well as preliminary experiments on jaw development. A discussion is made on the implications of feeding on survival together with ideas about development and reproduction as a function of feeding rather than size or age.

Chapter 5 describes the distribution of leech cocoons in a restricted survey of littoral areas at the main field site. The problems of sampling and a description of the microhabitat where cocoons were found are discussed.

Chapter 6 provides a short review of the biology of reproduction of the Medicinal Leech, H. medicinalis and compares fecundity with other close relatives. Methods are described for the maintenance of a breeding laboratory population and the conditions required for mating, cocoon laying and successful hatching of cocoons. Ideas concerning the fecundity of the leech and its possible life-cycle are discussed in the context of survival.

The final chapter presents the results of a survey of the Dungeness area with records from several sites. A proposal for re-introducing the leech to two favourable sites on Dungeness is discussed together with sampling for the Medicinal Leech at three sites where it has previously been recorded; The New Forest, Hants; Bookham Common, Surrey and La Sangsurière, Normandy, France. In each case full photographic coverage is provided.

The discussion takes the form of a multi-level conservation strategy for the Medicinal Leech based on present ideas about its physiology and ecology together with the findings of this study. The strategy is viewed at a series of levels including the individual animal, the population, the protection of the primary leech site on Dungeness, the future land use policy for Dungeness, the wider problem of wetland habitat loss, and the potential role of relevant legislation. The development of the company Biopharm in Swansea, which is attempting to create a commercially viable leech farm based on captive breeding, is discussed in its role as a possible source of the animal for research, thus replacing the continued drain on wild populations. In economic terms the success of this enterprise depends very much on the establishment of a market for leech biochemicals and this potential is discussed.

L I T E R A T U R E R E V I E W

1. A Gener l Account of the Hirudınea Group

(i) Structure, function and evolution

The leeches, or Hirudinea, belong to the phylum Annelida, a group of invertebrate animals characterised by a thin, flexible cuticle and a metameric body plan of segments arranged in a linear series along the anteroposterior axis (Barnes, 1974). As a group the leeches are certainly the most specialised Annelids and most of the distinguishing characteristics have no counterpart in the other two Annelid groups.

The number of body segments is, unlike other Annelids, fixed at 34, but secondary, external annulation has obscured the original segmentation and often makes precise estimations of the number of segments difficult (Mann, 1962). The head region, with paired eyes on the dorsal surface, includes a circum-oral sucker with the mouth aperture on the ventral surface while the pharynx is muscular with chitinous jaws sometimes present (Fernandez and Olea, 1982). The posterior segments have been modified to form a sucker which is disc-shaped and turned ventrally (Barnes, 1974). Intersegmental septa and chaetae are absent, except in the primitive Acanthobdellae, while a striking difference between leeches and other Annelids is the loss of a distinct coelom (Elliott and Mann, 1979).

These major differences can all be related to two evolutionary developments; the abandonment of burrowing and the development of a carnivorous, and later, parasitic habit (Clark, 1978). Indeed this change from peristaltic burrowing to creeping over a solid substrate, with the aid of suckers, removes the functional necessity for a spacious coelom, septa, chaetae and many segments (Clark, 1964).

The debate concerning phylogenetic links between the Hirudınea and other groups has recently been re-assessed by Sawyer (1984). Certainly within the Annelıds leeches are closely related to the Oligochaete worms. Both groups lack parapodia and head appendages, both are hermaphroditıc with gonads and gonoducts restricted to a few segments, while both undergo direct development within cocoons secreted by a clitellum (Barnes, 1974). Indeed because of this last similarity

the Oligochaeta and Hirudinea are often regarded as sub-classes of the class Clitellata.

In a discussion of the evolutionary relationships of the major families in the Hirudinea, Mann (1962) and Sawyer (1984) identified an Oligochaete/Lumbriculid-like ancestor. Sawyer (1984) took the discussion further by listing a series of characters which would appear to link the Hirudinea with the Uniramia (Hexapoda, Myriapoda and Onychophora). Certainly this 'arthropodization' of the Hirudinea is supported by evidence from the work of Sauber, Reuland, Berchtold, Hetru, Tsoupras, Luu, Moritz and Hoffman (1983), who found that the ecdysteroids in the moulting cycle of the Medicinal Leech, H. medicinalis are similar to those in insects.

For general information about the Hirudinea, reference can be made to most textbooks of Invertebrate Zoology, whether using a taxonomic approach (Buchsbaum, 1947; Borradaile, Eastham, Potts and Saunders, 1958) or a functional approach (Gardiner, 1972; Barrington, 1979). For the last twenty years Mann (1962) has provided the only specialist English language account of the structure, physiology and ecology of leeches, but a timely and up-to-date revision of the biology of the Hirudinea has recently been provided by Sawyer (1985).

(ii) Distribution and classification

Leeches have a worldwide distribution, inhabiting freshwater, marine and terrestrial habitats, particularly in the tropics. They have been classified to approximately 500 species, 140 genera and 10 families (Elliott and Mann (1979). The work of Soos (1965, 1966, 1969a, 1969b) and Richardson (1969, 1975) provides descriptions and keys for the leech genera of the world and a catalogue of species. Harding and Moore (1927), provide a taxonomic study of leeches from the Indian sub-continent, while Moore (1939) provides additional taxonomic knowledge of African leeches. Sawyer (1972) has provided a key to North American species, while in Europe, Elliott and Mann (1979) remains a standard reference and key for British species. Recently Sladecek and Kosel (1984) have made a contribution to the identification of European species.

The Hirudinea can be divided into four distinct orders: Acanthobdellae, Rhynchobdellae, Pharyngobdellae and the Gnathobdellae (Barnes, 1974), although the last two orders are sometimes combined to form the Arynchobdellae (Elliott and Mann, 1979).

The Acanthobdellae contains a single species from Northern Europe which is an ectoparasite of salmonid fish. It represents a 'living relic' connecting the leeches with the Oligochaetes (Sawyer, 1984).

The Rhynchobdellae are strictly aquatic comprising marine and freshwater species. They are characterised by an eversible proboscis which is capable of penetrating host tissues (Hatto, 1968; Wilkialis, 1973; Sawyer, Damas and Tomic, 1982). The order is divided into the families Piscicolidae and Glossiphoniidae.

The Piscicolids are cylindrical leeches (Sawyer, 1972) that are predominantly ectoparasites of marine fish (Mace and Davies, 1972; Sawyer and Hammond, 1973; Daniels and Sawyer, 1975). They also occur as parasites of freshwater fish and can be a serious pest in commercial fisheries (Mann, 1955). Piscicolids are almost entirely resident on their hosts except during their breeding season, when they attach their cocoons to stones and plants (Malecha and Vinckier, 1983).

Glossiphoniid leeches are dorso-ventrally flattened (Dall, 1982) and are either free-living carnivores that prey on invertebrates (Wilkialis, 1964; Young, 1980, 1981b; Wrona, Davies, Linton and Wilkialis, 1981), or temporary ectoparasites of fish and amphibians (Elliott, Mugridge and Stallybrass, 1979) and birds (Davies and Wilkialis, 1981; Wilkialis and Davies, 1981b; Elliott and Tullett, 1982b; Van der Lande, 1983). They are restricted to freshwater and are the only leeches that brood their fertilised eggs and young (Sawyer, 1971).

The Pharyngobdellae leeches have a weakly muscularized pharynx and lack an eversible proboscis, jaws and teeth (Elliott, 1973a). All species are carnivorous/scavengers and their invertebrate prey is usually swallowed whole (Sawyer, 1970; Elliott, 1973b; Greene, 1974). Most species occur in freshwater but amphibious species may spend

considerable periods in damp conditions on land (Hartley, 1962). They all return to freshwater for cocoon deposition (Brumpt, 1900).

The Gnathobdellae leeches lack an eversible proboscis but possess a short, muscular pharynx usually with toothed, chitinous jaws (Worth, 1951; Dev and Mishra, 1971). This order includes the sanguivorous land leeches from the family Haemadipsidae (Stammers, 1950; Chandra, 1977; Bhatt, 1960; Bhatia and Singh, 1973). The family Hirudinidae comprises freshwater and amphibious species which are predominantly blood-sucking although some species have become macrophagous (Mann, 1955). The most authoritative contribution to the systematics of the family Hirudinidae is given by Richardson (1969) and Soos (1969a). The latter identified 15 species within the genus Hirudo (using Hirudo medicinalis, Linnaeus 1758 as a type species) and their geographical distribution is shown in Table 1.

Table 1. Species list and zoogeographical distribution of the genus Hirudo (from Soos, 1969a).

Species	Distribution
H. capensis	South Africa
H. chaversei	The Azores
H. granviki	Kenya
H. grisea	Angola
H. hildebrandti	Namibia
H. intermedia	South Africa
H. lefevrei	Ruanda
H. medicinalis	Europe
H. michaelseni	South Africa, Kenya
H. morrisii	South Africa
H. notabilis	South Africa
H. septemstriata	South Africa
H. sjoestedfi	Zimbabwe, Urundi
H. troctina	West Mediterranean

Three previously regarded Hirudo species, asiatica and birmanica from Iran, India, Sri Lanka and Burma (Moore, 1923; Jalka and Ghosh, 1976; Mahajan and Chandra, 1976) and nipponia from Japan (Whitman, 1886; Toshioka, 1977) are now regarded by Richardson (1969) as species in a new genus Asiaticobdella. The Australian Medicinal Leech, Hirudo elegens (Richardson, 1968) is now regarded as a species in the genus Goddardobdella. Two other important Hirudinid genera are Macrobdella (type species decora) and Poecilobdella (Synonym: Hirudinaria), the latter comprising three important species, granulosa, javanica and mallinensis from the Oriental region (Soos, 1969a).

The importance of all these close relatives to the European Medicinal Leech, H. medicinalis, will be clear when information concerning their fecundity and general ecology is used in comparison with H. medicinalis. They also represent, in addition to H. medicinalis, the group of species which have been predominantly used by many cultures, in the ancient therapeutic method of phlebotomy or bloodletting (Shipley, 1927; Richardson, 1970). It appears, however, that H. medicinalis has been the single most used species for the purpose of bloodletting in history (Fermond, 1854). Consequently much has been written about the medicinal use of the animal and because it is so much a part of the animal's reputation, and has provided the subject for anecdotal, literary and artisitc interest, a short review is included here.

2. The Relationship between the Leech and Man

(i) Historical medical use

Modern accounts of the development of bloodletting, employing venesection, cupping and leeching, are given by Brockbank (1954) and Ackernecht (1973). Among the older references, Bayfield (1823) described the apparatus and techniques in use at the time. The rationale for the historical use of leeching was based both on the humoral theory of disease and the theory of counter irritation (Brockbank, 1954; Hartnett, 1972). Early medical references to the use of leeches are reviewed by Payton (1981) who concluded that despite occasional references to leeches in ancient texts (e.g. Pliny's

Natural History A.D. 79), the importance of their use did not increase until the late 18th Century. One of the reasons for the widely held belief that H. medicinalis was used in medieval times is that the term 'leech' was used then to describe a medical man or physician (Shipley, 1927; Strauss, 1968; Smolnikoff, 1972). Indeed the word leech meaning doctor is very common and occurs in all early Teutonic languages as well as Celtic from which it is believed to have been borrowed from some centuries B.C. (Shipley, 1927). This quote from Homer's Iliad XI 514 (translated by A. T. Murrey, in Strauss (1968)) illustrates this point.

> "A leech is of a worth of many men for the cutting out of arrows and the spreading of soothing simples".

The great increase in the practice of leeching occurred in the 18th and 19th centuries. During this period the practice spread throughout Europe and major treatises on H. medicinalis were written around this time, for example, Moquin-Tandon (1846) and Fermond (1854) in France and in England by Horn (1798) and Johnson (1816).

The leech became popular as it represented a more controlled method of exsanguination, in comparison to the unpredictable techniques of venesection and cupping, and could be applied to areas of the body such as the temple which proved unsuitable for the application of other methods. The employment of leeches was often regarded as more desirable for women and children (J. Ford, pers. comm.). It appears that leeches could be applied for almost any illness, from the treatment of intractible pain during infant teething, pleurisy, whooping cough, smallpox and gout, to perhaps the most bizarre suggestion by Payton (1982) that H. medicinalis may have been applied to treat the haemorrhoids of Napoleon! In the 19th Century, France was the biggest user of H. medicinalis (Sawyer, 1981b) due to two influential physicians, Broussais (1772-1838) and Bouillard (1798-1881) known at the time as 'the most sanguineous physicians in history' (Castiglioni, 1975). The sheer size of numbers used thr ughout Europe is described in Sawyer (1976) and compares figures fr m several countries. Hare (1883) quotes figures of the number of leeches used in some of the London Teaching Hospitals between 1832-1882 and since they have not appeared in the recent literature they are included here in Table 2.

Table 2. Numbers of H. medicinalis used in four London teaching hospitals in certain years during the period 1832-1882 (from Hare, 1883)

Year	St. George's	University College	St. Thomas'	St. Barts
1832	21800	-	-	97300
1842	19600	-	21000	48100
1852	4050	8000	12000	27300
1862	1360	2000	2550	6200
1872	500	300	450	1000
1882	754	200	800	1700

Although the application of leeches was usually made by physicians, regarded as the top of the medical hierarchy, it was very much the role of the apothecary to maintain a fresh supply from collections held in leech jars in the apothecary's shop (Trease, 1964; Copeman, 1967). In turn the apothecary would be supplied from small, local leech farms (Shipley, 1927; Fermond, 1854) or more often from 'common' folk engaged in leech gathering from wild populations (Wordsworth, 1807; Huws, 1959; Einstein, 1978). Much of this 'sociological' information surrounding H. medicinalis remains to be extracted from the great treatises mentioned as well as historical medical casebooks (J. Ford, pers. comm.).

(ii) Anecdote, literature and art

Arising from the historical medical relationship between the leech and man have been various anecdotal references to H. medicinalis (Shipley, 1915; Drabble, 1979), and the potential information in the area of folklore must be considerable, (Simpson, pers comm).

H. medicinalis has been the subject of poetic reference in William Wordsworth's account of a meeting with a Cumbrian leech gatherer while the same encounter is also described in his sister Dorothy's diaries.

Recently the leech was the subject of the 1983 annual meeting of the Institute of Medical and Biological Illustration where an exhibition entitled "The Medicinal Leech - an Historical Survey of its Illustration" was held at the Wellcome Institute for the History of Medicine (Shipbach, pers. comm.). H. medicinalis is also featured in a permanent exhibition on the History of Medicine at the Science Museum in London.

The problem of storing leeches in the apothecary's shop was often solved by the production of special ceramic 'leech jars' many of which were highly ornate both in design and colour (Lothian, 1959). Special glass and wire paraphernalia were also designed specially to ease the application of leeches. These articles are now valuable medical antiques. It seems that H. medicinalis has even influenced the design of fashion as Shipley (1927) relates:

> "So beautiful are they (Hirudo medicinalis L) that the fastidious ladies, who adorned the salons at the height of the leech-mania during the beginning of the 19th Century, used to deck their dresses with embroidered leeches".

Today H. medicinalis is often the subject of topical conversation and has featured regularly in the press mainly in connection with its occasional present day use in medicine (The Times, March 17th 1982) and recently as the subject for a commercial farming and small-scale pharmaceutical project in Swansea known as Biopharm.

(iii) Modern medical use

The m dern medicinal applicatıon of H. medicınalis is now based on a thorough knowledge of the biochemistry of the salivary gland extracts as well as an increased knowledge of pathology, for example in the relationship between blood viscosity and thrombosis (Pitcher and Benson, 1981). The leech has been employed for the reduction of severe bruising or haematoma (Bunker, 1981; Barnett, 1983) and for inducing blood flow in transplanted skin grafts or replaced digits (J. Gilby, pers. comm.). Despite these successful applications recent doubts have been raised concerning the potential risk of infection by direct transmission of the leech gut symbiont, Aeromonas hydrophila.

Work by Whitlock (1983) and Dickson, Boothman and Hare (1984) reported the presence of this bacterium in the haematoma fluid from a post-operative mastectomy after the application of H. medicinalis. Ross (1983) raised the question of the possible risk of secondary infection, due to ulceration in the area of the leech bite, and anaphylactic reactions. There certainly appears to be a difference in opinion over the relative costs and benefits from the modern-day use of H. medicinalis.

(iv) Other uses

Although the extensive medicinal use of H. medicinalis diminished rapidly towards the end of the 19th century due to the loss in popularity of the humoral theory of disease and its replacement by drug-based therapeutics, the animal itself became a subject for more serious scientific research.

H. medicinalis is now regarded as a 'model' invertebrate for many undergraduate studies (Freeman and Bracegirdle, 1971; Bullough, 1958), and continues to provide material for specialised research into neuroanatomy and physiology (Muller, Nicholls and Stent, 1981). The leech has been used for the identification of pesticides in water (Lapkina and Flerov, 1980) and in the culture of Trypanosoma cruzi for the study of Chagas diseas (Marsden, 1969).

H. medicinalis provides the raw material for the production of the anticoagulant Hirudin. The German pharmaceutical industry uses huge numbers of animals for a comparatively small return on this product (Markwardt, 1970; Wells, pers. comm.).

The increased use of the Medicinal Leech in recent years continues to rely largely on supplies from wild populations with the consequence, as discussed later, that numbers have been drastically reduced.

3. The Medicinal Leech as a Bloodfeeding Animal

It is beyond the scope of this study to provide a comprehensive review of the physiological and anatomical literature relating to

H. medicinalis. Instead references have been selected which illustrate direct and indirect adaptations to an haematophagous habit and which have provided a general background to the areas of research and observations later described.

(i) The function of the salivary glands and biting apparatus in H. medicinalis and its relatives

It must have been noticed from early times that even after the removal of H. medicinalis from the skin surface, protracted bleeding occurs at the wound site. In the author's own experience a bite in the vicinity of a vein passing over the carpal bones of the wrist continued to bleed for several hours after the leech was removed. This powerful ability to cause anticoagulation of vertebrate blood, a characteristic of other haematophagous animals (Gooding, 1972; McNab, 1972), was first investigated in H. medicinalis by Haycraft (1884) who examined the effects of an extract, from the head region, on the clotting of blood. This potentially valuable anticoagulant, known as Hirudin, had been the subject of much research in recent years (Bagdy, Barabus, Graf, Petersen and Magnusson, 1976), especially in Germany.

In the enzyme generated cascade and subsequent clotting reaction in the plasma of vertebrates (See Lorand, 1976), in vitro studies of the Hirudin polypeptide molecule have shown it to be an inhibitor of the thrombin-catalyzed conversion of fibrinogen to fibrin (Markwardt, 1970). Recent work by Markwardt, Nowak, Stürzebecher, Griesbach, Walsmann and Vogel (1984), has shown that the antithrombin action of Hirudin in man can have definite advantages over the much more widely used anticoagulant Heparin (also an antithrombin). To have an effect Hirudin does not require the presence of a cofactor and is not bound or inactivated by platelet factors or other proteins as is the case with Heparin.

The antithrombin action of Hirudin is not unique among natural anticoagulants as several other bloodfeeding animals show this activity (Landmann, 1972). Hirudin is, however, diff rent in its action from those anticoagulants which have been studied in other species of leech.

Stammers (1950) describes protracted bleeding from a bite from the land leech Haemadipsa sp. while Lal and Choudhury (1950) describe prolonged coagulation times in vitro reactions between mammalian blood and head extracts from Poecilobdella (Hirudinaria) granulosa. In both cases the precise action of the anticoagulants were not elucidated. Recently the composition of the salivary extracts from the giant Glossiphoniid leech, Haementeria ghilianii has been extensively studied by Budzynski, Olexa and Sawyer (1981). The anticoagulant, hementin, does not inactivate thrombin but instead attacks the clot forming substrate, fibrinogen rendering it uncoagulable (Budzynski, Olexa, Brizuela, Sawyer and Stent, 1981; Malinconico, Katz and Budzynski, 1984). Work by Kelen and Rosenfeld (1975) on the anticoagulant hementerin, from Haementeria lutzi, found it to have yet another subtle difference in its mode of action as compared to hirudin and hementin. Hementerin does not have any proteolytic effect on fibrinogen or fibrin and does not act as an antithrombin. Instead it promotes fibrinolysis by activating host plasminogen. The whole area of leech anticoagulants has potential importance to the clinical treatment of thromboembolic disease (Budzynski et al., 1981).

The salivary secretions of H. medicinalis have been found to contain a complex of chemicals in addition to hirudin. All are specialised adaptations to the haematophagous habit. Lenggenhager (1936) applied tincture of iodine to a leech bite without feeling pain and concluded that the saliva has a local anaesthetic effect. Lindemann (1939) found that extracts from H. medicinalis contained a histamine-like substance capable of causing the dilatation of capillaries and postulated that the free flow of blood was due as much to the enlargement of the blood vessels as to the inhibition of clotting. In a study on tissue permeability, Claude (1937) found that a fraction from H. medicinalis head extracts caused the spreading of an intradermal injection of test solution, marked with Indian ink, in the rabbit. This spreading factor, or hyaluronidase (Linker, Meyer and Hoffman, 1957) was found to be an enzyme capable of hydrolyzing hyaluronic acid, an important constituent for binding animal cells (Linker, Hoffman and Meyer, 1960).

Recently, specific protease inhibitors have been isolated from the salivary glands of H. medicinalis. Fritz and Krejci (1976) described

trypsin, chymotrypsin and plasmin inhibitors (Bdellins) which have been isolated as impurities in commercially produced hirudin. Baici and Seemüller (1984) described the kinetics of a potent inhibitor of neutral elastase and cathepsin G, from polymorphonuclear leucocytes, known as eglin C. Those protease inhibitors may serve as devices to prevent the attack on the crop wall of the leech by the leucocyte fraction in the bloodmeal (Sawyer, pers comm.). It appears that eglin C may have a therapeutic value in such conditions as lung emphysema and other inflammatory processes (Baici and Seemüller, 1984). A regard for its potential is illustrated by Rink, Liersch, Sieber and Meyer (1984) who have constructed a synthetic gene which codes for eglin C. Protease inhibitors, studied in other species of leech by Murer, James, Budzynski, Malinconico and Gasic (1984), may also have potential therapeutic value and more specifically extracts from H. ghilianii have been shown to inhibit lung tumour colonization (Gasic, Viner, Budzynski and Gasic, 1983).

Recently Baskova et al. (1984) have described lipase and cholesterol esterase activity in the salivary secretions of H. medicinalis , adding another function to its versatility.

All these studies, describing the action of leech salivary fractions, have been based on extracts obtained from macerated heads. Comparatively little work has been done on the anatomy of the salivary glands, their relationship to other structures in the buccal cavity or to an histological and histochemical correlation of sailvary function with anatomical structure. Damas (1974) found, by dissection and light microscopy, that the salivary glands in H. medicinalis are distributed throughout the head musculature, with many individual linking canals draining to a single medio-lateral jaw and two latero-ventral jaws. (cf in H. ghilianii, where salivary glands are composed of discrete bundles draining through a common duct into the base of the proboscis (Sawyer, Damas and Tomic, 1982). Damas (1974) also distinguished two main types of gland. A mucous type to provide lubrication for the jaws and possible to maintain the bloodmeal in a 'hydrated' state and a 'proteic' type secreting the complex hyaluronidase, histamine and hirudin together with inhibitory and digestive proteases. Zapkuvene and Sinyavichene (1975) looked at the development of the salivary glands

with reference to the effect of starvation. They identified three main developmental phases and suggested that the salivary glands in leeches 150 days after last feeding contained the maximum level of hirudin although further starvation to 460 days caused gland atrophy.

A detailed study of the anatomy of the jaw system and associated musculature in H. medicinalis by Damas (1972) found that each of the jaws supported a row of approximately 60 teeth. She identified a central canal running through each tooth, each representing a continuation of a canal draining directly from the salivary glands. A detailed study of the mechanism of biting, synchronous secretion of saliva and the neuro-chemical integration and control of feeding has hardly been discussed in H. medicinalis. Lindemann (1939) suggested that the act of biting and the secretion of the salivary glands is divisible into two phases; the biting phase when incision is being made and the histamine-like compound is being injected, and the sucking phase when the blood, flowing past the jaws is mixed with a secretion containing hirudin. Recently, Lent and Dickinson (1984) suggested that serotonin is responsible for pharyngeal peristalsis, salivation and jaw movement in H. medicinalis and that the neurochemical integrity of certain serotonin containing neurons is both necessary and sufficient for the expression of integrated feeding behaviour.

Some detailed work on the biting sequence and neurochemical control of feeding behaviour has been done for Poecilobdella granulosa and, because of its systematic links with H. medicinalis, may represent a useful model. Dev and Mishra (1971) distinguished four successive phases in the biting sequence; (1) making one to a few microincisions, (2) tri-radiate cut, (3) wound embedding stroke, and (4) wound widening phase. They also observed that the pattern of salivary ductules was distributed in such a way as to pour saliva at the four sites intimately associated with the four successive phases of injury. In a later study, Dev and Mishra (1972) provided a detailed account of the attachment-biting-sucking sequence and suggested a division of labour within the salivary glands as a consequence of the three-fold function of the saliva, i.e. anaesthesia, vasodilation and anticoagulation. They also suggested that these salivary components can be functionally correlated with the biting phases, i.e. the anaesthetic component has a triphasic release

correlated with the phases of injury; microincision, tri-radiate cut, and wound embedding. Mishra and Dev (1976) found that the secretion of these salivary components have different controlling neurosecretory esterases. There do appear to be parallels with H. medicinalis since observations made by this author, of the effect of biting on the skin surface after the removal of animals at different times after biting phase initiation, showed similar stages to those described for Poecilobdella.

Two important characteristics of the haematophagous habit have been discussed so far; the ability to pierce the integument of a host and the production of biologically active salivary secretions which enhance biting efficiency and encourage the flow of bloodmeal from the host. Although these characteristics are central to an haematophagous habit, they represent only part of a broader behavioural and physiological repertoire designed, although not exclusively, to obtain and utilise a bloodmeal. The location of a host, implying active search in response to stimuli must occur; decisions about the surface to be pierced and the quality of the fluid extracted must be made while, as a result of characteristic gorging, specialised physiological and anatomical adaptations to accommodate a large bloodmeal must be present. Each of these adaptations will now be considered in turn.

(ii) The response of the Medicinal Leech, H. medicinalis, to external stimuli

Early work on the reaction of H. medicinalis to such basic stimuli as light, heat, gravity, mechanical stimuli and chemical substances, has been reviewed by Mann (1962), who also included results from his own simple experiments. Many of the experimental methods, however, appear crude and often results appeared inconclusive. For example, he makes conclusions about the preference of H. medicinalis for lighted or shaded parts of an aquarium, based on experiments with individuals of unknown satiation level or size/age, two possible factors which could affect results. He did show significant differences between the response of sanguiverous and macrophagous British leech species to light. In the former, leeches settled in lighted areas of an aquarium

more often, and he suggested that this response was an adaptation for seeking hosts. He also observed that when a shadow passes over H. medicinalis in its natural habitat, the response is often a searching movement followed by swimming.

In a further set of experiments, Mann (1962) also found that H. medicinalis was stimulated to attach to an object warmed to 33-35°C and retained a hold until the temperature was raised to an average of 41.5°C. He exposed H. medicinalis to a series of extreme temperatures which elicited shock reactions at 39°C and paralysis at 43.5°C. He found that in comparison with other British species H. medicinalis showed the greatest tolerance to high temperatures and suggested that this could be an adaptation to its usual habitat of small enclosed ponds prone to high temperatures in summer and to enable it to survive contact with warm blooded hosts. These do appear to be very simplistic conclusions since H. medicinalis has been found in a cold Scottish loch (Warwick, 1961) and to feed on such poikilotherms as toads (Hoffman, 1960). Other simple work on the response of H. medicinalis was done by Kaiser (1954). He found that when leeches were placed in a water temperature gradient they congregated in water at 21°C and he concluded that they were capable of distinguishing between temperature differences of 1.5°C.

The tendency for H. medicinalis to actively take up a position in a crevice, i.e. positive thigmotaxis, was noted by Mann (1962). This behaviour has also been noted by Dickinson and Lent (1984) who found a thigmotactic component in the biting response of H. medicinalis. They suggested that this response probably causes the selection of folded surfaces for biting and that these folded, protected positions would assist the leech in remaining attached to its mammalian host during its prolonged feeding bouts.

More recently behavioural studies on the response of H. medicinalis to light and water disturbances have been more quantitative in approach and supported by detailed knowledge of the anatomy (Nicholls and Van Essen, 1974; Payton, 1981) and physiology (Friesen, 1981) of neuronal control. Indeed, because of the relative simplicity of the leech's nervous system, even among other invertebrates, any behaviour can be completely characterized, i.e. the contribution of every neuron in a

behavioural response can be evaluated (Müller, Nicholls and Stent, 1981; Kristan, McGirr and Simpson, 1982; Kristan, 1983).

A series of behavioural experiments on H. medicinalis in natural and unnatural environments as part of a space programme was performed by Lotz (1969, 1970a, 1970b). He examined the effects of long-term weightlessness on metabolism and biological rhythm. In a series of experiments concerned with diurnal rhythm, 8-9 leeches were placed in four different dark-light-shade regimes for 15 days. All the experiments were performed at a constant temperature of 19-21^{o}C, although the satiation level was unknown. The degree of activity during the experimental period was measured by the frequency of interruptions of a continuous, weak electrical current passing through the chamber, resulting from the movements of the leech. These interruptions were regarded as an estimate of the amount of activity. He found that this was greater in light-dark conditions than for either constant light or constant dark indicating a possible diurnal rhythm. Over a 24 hr period there was greater activity during periods of diffuse lighting rather than direct artificial light and he concluded that this may indicate an adaptation to twilight. Unfortunately the results were again based on a relatively small sample with no statistical significance attached.

Dickinson and Lent (1984) exposed hungry leeches to moving bars of light and flashing stroboscopic light. They found that parallel moving bars of light initiated an orientation behaviour in resting leeches, e.g. release of anterior sucker and head waving, followed by swimming into the oncoming waves. They found that stroboscopic flashing light did not provide an adequate stimulus for significant orientation and concluded that H. medicinalis responds to light waves by orientating into them and swimming towards their apparent source.

An important stimulus which initiates orientation and swimming appears to be water disturbance and this has been quantitatively studied by Young, Dedwylder and Friesen (1981). They found that unfed H. medicinalis responded to low amplitude surface waves by initiating swimming in 95% of tests. This response was not affected by the amount of illumination but was depressed in tests carried out on satiated leeches, and at greater water depths. They ruled out visual cues as the

stimulus initiating locomotory movements and concluded that the effective stimulus was surface water waves, of low-velocity, rather than high-velocity compression waves in the water column. The difference in sensitivity between these two wave types has been shown in dissected preparations by Friesen (1981). Young et al. (1981) concluded that the reduced response to water disturbance in satiated leeches could be explained by their exceeding a minimum satiated level below which water disturbance triggered a response and their general movement to greater depth, a behaviour which has also been noted by Dickinson and Lent (1984).

As a result of increased knowledge of the structure and function of the leech's nervous system many of the behavioural traits observed have been linked to definite anatomical structures and physiological processes. Indeed the distribution, structure and physiology of a series of epidermal and subepidermal sensory structures has been well documented (Mill, 1978).

Work by Kretz, Stent and Kristan (1976) showed two types of photosensory organ; five bilateral pairs of eyes embedded in two longitudinal rows in the dorsal surface of the head; and seven bilateral pairs of sensillae situated in both the dorsal and ventral surface of each body segment. They described the hirudinid eye as consisting of a pigmented, cylindrical cup whose axis is perpendicular to the body wall. It appears to make a selective response to light coming from various directions rather than a crude differentiation between light and dark. It has been suggested by Peterson (1983) that it may provide a very simple form of vision. Kretz et al. (1976) found that the leech sensilla structures had low directional selectivity towards light stimuli and that their function was to provide information about the intensity of light falling on the body wall. They suggested that the amount of information which the leech nervous system abstracts is simply a knowledge of which parts of the body are in light or dark.

In addition to these photosensory structures several types of sensory receptors occur in the leech body wall. One type, free nerve endings in the epidermal layer, has recently been shown to be the sensory terminals of touch-sensitive neurons (T-cells), (Blackshaw, 1981). Despite a suggestion by Mistick (1978) that the T-cells are activated by water

waves, Friesen (1981) found them to be relatively insensitive to this stimulation and concluded that, due to their morphology and position in the body wall they were not directly activated by water waves. Other cutaneous, receptive endings mediate sensitivity to local pressure (P-cells) and noxious stimuli (N-cells), (Kristan, 1982; Kristan et al., 1982.

Recent physiological experiments have shown that the body wall sensillae are the loci for the sensory receptors mediating the sensitivity to water movements (Friesen, 1981). The sensilla, therefore performs a dual role in responding to both light and mechanical stimuli. Their gross structure is discussed in Mann (1962) who described them as consisting of elongated sensory cells, some of which appear to have filiform processes beyond the overlying cuticle. In a more recent examination, using a scanning electron microscope, Derosa and Friesen (1981) found that the midbody sensillae are composed of two morphologically distinct regions, one of which is a central disc bearing two types of filiform processors; a single, relatively long 'hair' (S-hair) and shorter hairs (G-hairs) found in groups of two or more.

They suggested that the length of the S-hairs extends beyond a stationary boundary layer of water against the leech's body, thus suiting them to the role of water movement receptors. These observations have been supported by Phillips and Friesen (1982) who have studied the ultrastructure of the sensillae using transmission electron microscopy. They have also identified a third type of multiciliate sensory cell parallel to the body wall surface but lying within the cuticle.

Recently, Brodfuehrer and Friesen (1983) examined the sensitivity of the S-hairs or sensilla movement receptors (SMRs) and their role in the initiation of swimming. They found that swimming, in response to water vibrations, occurs only in response to wave stimulation and not to near-field stimulation of one or two sensillae. The most likely explanation for this is not that wave stimulation is more intense but rather that it is much more widespread. They suggest that, in their experiments, up to 130 sensillae may be stimulated by each wave packet. They also concluded that the frequency domain of SMR sensitivity extends

as low as 1Hz and that leeches might receive self-stimulation from the water vibrations created by their own swimming movements. Dickinson and Lent (1984) concluded that the segmental sensillae, comprising mechanosensitive hair cells and a photoreceptor cell, combine to enable the leech to process parallel mechanical and photic stimuli in order to orientate itself towards the source of surface ripples.

Many of the behavioural and neurophysiological observations described so far have been obtained from artificial arena experiments and the examination of sections of body wall. Often it appears, from observations in the wild, that conclusions and explanations for certain behaviours are too simplistic. For example, the movements involved in host location are complex and are best described as a repertoire of distinct stages. The early work of Gray, Lissman and Pumphrey (1938) described two distinct types of locomotory rhythm; one of which emerges whenever the body is deprived of all extrinsic, tactile stimulation to its ventral surface or suckers and the other when such tactile stimulation is present. In the first case the animal swims while in the second crawling along a surface occurs. Recently Sawyer (1981a) examined the elementary behaviour used in the foraging repertoire of H. ghilianii in a semi-natural environment with a turtle host present. He identified 7 distinct elementary behaviours used in differing frequencies according to feeding stage. In a parallel experiment, also by Sawyer (1981a) on the behaviour of hungry, adult H. medicinalis, three distinct locomotory behaviours were used; inchworm and vermiform crawling and swimming (with an 'alert' posture as a precursor to swimming). He found that in comparison with H. ghilianii, there was a much greater tendency to swim by H. medicinalis. This author has observed the tendency of all age/size classes of H. medicinalis to use the swimming mode most frequently in its repertoire of movements. Simple experiments have indicated that, in the presence of a continuous stimulus causing water disturbance, the response by H. medicinalis is not to swim directly to the source but to perform a series of circular swimming episodes punctuated with a series of 'rests' followed by 'alert' posture to relocate the stimulus from the new position. Observations in the field support these semi-natural and laboratory observations and it seems likely that the neurophysiological integration and control of host-locating behaviour is considerably more complex than is believed at present.

(iii) The post-orientation feeding pattern of H. medicinalis

So far the ability of Hirudo to orientate and actively locate a host has been described. Galun (1975) recognised this as being the first phase in a generalised feeding pattern characteristic of bloodsucking invertebrates. The next phase is actual contact with the host and in H. medicinalis this may occur in two ways; attachment by anterior suction and jaws from a crawling position or directly during swimming. In the latter it is usual for the jaws to be slightly everted in preparation for host contact. Once orientation and contact has been achieved, Galun (1975) described three successive phases in her generalised haematophagous pattern. Firstly, feeding is initiated by probing and 'tasting', followed by continuation (ingestion) and finally termination. Each of these stages will now be examined in H. medicinalis before describing the excretory, digestive and metabolic adaptions for accommodating a large bloodmeal.

The initiation of a biting and sucking response, in the absence of a living host, was shown by Loëhner (1916), who fed H. medicinalis on whole bovine blood through cattle skin stretched across a glass tube. More recently Galun and Kindler (1966) fed H. medicinalis through processed ox caecum membrane while Dickinson and Lent (1984) successfully used parafilm in feeding experiments. In fact it has been found that other haematophagous invertebrates such as Culex tarsalis will feed readily through artificial membranes (Tarshis, 1959). At present it is not known whether the membrane/skin structure or 'texture' plays an important role in initiating the biting and sucking response in H. medicinalis. Dickinson and Lent (1984) observed that should an exploratory leech bite upon a surface which fails to produce blood, such as calloused thick skin, it will re-explore and bite again.

Most attention has been given to the thermal and chemical stimulation of the biting response. Dickinson and Lent (1984) examined the pattern of thermal preference to biting frequency using parafilm at different temperatures. They found that when kept in pond water at 25°C groups of H. medicinalis rarely bit on parafilm at the same temperature. Most bites occurred when the parafilm was at a higher temperature of 37-40°C. In a later study, Dickinson and Lent (1984) found that thermal stimulation of the prostomium evokes pharyngeal peristalsis, jaw

movements and an obvious salivation in semi-dissected preparations. This response to a typical mammalian core body temperature led these authors to make exaggerated conclusions about the degree of adaptation by H. medicinalis to mammalian hosts. Certainly it has been shown, in the wild, that the leech attacks toads (Hoffman, 1960) and newts (Litton, 1962), both of which are poikilotherms.

Once the membrane/skin has been pierced, the region of the anterior sucker and buccal cavity comes into contact with the potential meal. The decision to reject or continue ingestion and the range of chemical stimulants which cause these two alternative responses, has been studied by Galun and Kindler (1968) and Galun (1975). Early work by Loëhner (1916) showed that H. medicinalis will stop sucking if a mixture of sodium chloride, sucrose and quinine was added to blood. On the basis of this work, Mann (1962) concluded that H. medicinalis possesses chemoreceptors in the buccal cavity. Galun and Kindler (1966) attempted to determine which of the blood components would elicit the leech's feeding response. They found that the leech would suck four different blood fractions, but with significantly different gains in weight after feeding. Results from experiments on the effects of various combinations of chemicals on feeding response showed that H. medicinalis would imbibe considerable amounts of glucose solution (1 mg ml^{-1}) in 0.15 M sodium chloride but would not feed on glucose in water. Further, the leech fed to a limited extent on sodium chloride solution alone. They found that the sodium ion in other salt/sugar mixtures appeared to be an important stimulus for feeding. Overall the results were inconclusive although Galun and Kindler (1966) suggested that the sodium ions were responsible for activating chemoreceptors which stimulate the sucking action. They assumed that the entry of sodium ions into the chemoreceptors was facilitated by membrane-bound glucose. In the event they found that the volume of solutions imbibed was significantly smaller than with whole blood and concluded that additional factors in the bloodmeal are required to elicit an optimum feeding response.

A study by Jennings and Van der Lande (1967) showed that H. medicinalis will feed on clotted frogs' blood indicating a flexibility in bloodmeal requirements. Recently Dickinson and Lent (1984) fed hungry, adult leeches to repletion on a 1:1 mixture of washed erythrocytes and Liebovitz culture medium supporting the idea of dietary flexibility.

Galun (1975) examined the maintenance of gorging in a Rhodnius bug and H. medicinalis. She found that once gorging had started Rhodnius sp proceeded to repletion in the absence of further phagostimulation with adenosine triphosphate (ATP). In contrast, she found that when an application of arginine solution to H. medicinalis was replaced by saline the leech stopped sucking.

In a previous study by Galun and Kindler (1968), the regulation of feeding in H. medicinalis and the mechanisms involved in the termination of ingestion were investigated. They compared it with Rhodnius prolixus where ingestion is normally terminated by the actinn of proprioreceptors in the gut wall which, as the abdomen increases in size, provides negative feedback to the feeding response. They allowed leeches to feed on arginine solution, through an artificial membrane, and after sucking was established cut the posterior end of the leech just above the anal sphincter. They found that leeches thus treated fed for longer than controls and passed 13 fold their own body weight of solution through their gut. They concluded that H. medicinalis probably possesses a gut proprioreceptor system similar to that in Rhodnius. Recent work by Lent and Dickinson (1984) has shown that serotonin significantly alters the feeding response. When compared with control animals, serotonin treated individuals bit a warm surface more frequently and ingested more blood. This may suggest that serotonin can override feedback to terminate the feeding response.

(iv) The accommodation and metabolism of a large bloodmeal by H. medicinalis

It is a characteristic for H. medicinalis to increase its body weight several times after the intake of a single bloodmeal (Putter, 1907, 1908; Bialaszewicz, 1924; Dickinson and Lent, 1984). This gorging behaviour requires an anatomical capacity sufficient to accommodate a large volume and this can be clearly seen in a comparison of the structure of the crop in H. medicinalis and in the carnivorous Haem pis sanguisuga. H. medicinalis possesses an additional 10 pairs of gut diverticula, in addition to the main crop, the last pair of which reach the posterior end of the body (Hammersen and Pokahr, 1972). In contrast, the crop of H. sanguisuga possesses only a single, short posterior diverticula (Herter, 1968).

As well as anatomical adaptations to a large bloodmeal, specialised physiological mechanisms exist to balance the great increase in body volume as a result of gorging. Much work on the physiology of leech excretion and osmoregulation has been carried out by Zerbst-Boroffka and her co-workers. They have made detailed studies of the structure and function of the nephridia of H. medicinalis (Zerbst-Boroffka, 1975).

Early work by Braconnier-Fayenendy (1933) and later by Zerbst-Boroffka (1973) found that after the ingestion of a bloodmeal, urine volume and body osmolarity increased by up to 9 times, but within 12-24 hrs the extra fluid volume and salt load, taken in with the blood, was eliminated. This rapid diuresis was noticed by Büsing, Dölle Freytag (1953), who found a 40% decrease in the weight of blood in the crop after 10 days. Worth (1951) also noticed that land leeches became surrounded by a pool of clear fluid during feeding, possibly indicating a contemporaneous diuretic compensation. After the removal of the bulk fluid only the solid, corpuscular fraction of the blood remains in the crop (Zerbst-Boroffka, 1973). This can be seen by a change in the appearance of the bloodmeal if disgorgement occurs. The blood appears dark and viscous. Zerbst-Boroffka (1973) showed that diuresis, after food intake, cannot be attributed to either an alteration of the osmotic or ionic concentration of the leech blood system or to the stretching of the gastro-intestinal tract. In later work, Zerbst-Boroffka (1978) postulated that the change in blood volume of the leech controls diuresis. She raised the blood volume in one set of leeches by transfusing blood from another donor set. This resulted in a temporary increase in urinary flow. In another experiment blood was displaced within the body of an individual leech by massage. This produced a temporary increase in urinary flow in those segments where blood volume had been elevated. Wenning, Zerbst-Boroffka and Bazin (1980) showed that volume regulation is accomplished within several hours, but salt excretion takes longer. They found that mechanisms controlling urine volume are independent of those controlling urine concentration. They infused the cr p with hyper- and hypo-osmotic salt solutions and found that they affected salt and water balance in different ways. The leech pparently has no problem with large volumes, but does have a problem in dealing with large amounts of salt. More recent work by Zerbst-Boroffka, Wenning and Bazin (1982) on the mechanisms underlying

diuresis in H. medicinalis showed that diuresis after feeding is probably due to accelerating primary urine formation rather than to a decrease in reabsorption of primary urine volume. They suggested that blood volume and body water homeostasis are controlled by a feedback mechanism.

Early analysis, by Heidermanns (1937), of the urine produced by H. medicinalis showed that under normal conditions about 72% of the nitrogen excreted is in the form of ammonia while amino acids, purines, urea and creatine account for 5-10%. This is a much higher proportion of ammonia than is produced by earthworms and he explained this by the presence of bacterial action in the leech nephridial capsule which convert complex nitrogenous compounds into ammonia. Later work by Büsing et al. (1953) confirmed the presence of two symbiotic Corynebacteria species, one located in the nephridial bladder wall, the other floating freely in the nephridial fluid.

After the removal of the fluid bulk from a bloodmeal, the solid 'corpuscular' fraction remains and it is this which provides the substrate on which the digestive process can operate. Mann (1962) noted that the haemoglobin soon becomes deoxygenated but the erythrocytes remain intact for a long time. He was able to identify intact erythrocytes and white corpuscles 18 months after the ingestion of a bloodmeal. He attributed this slow, controlled haemolysis to freedom from putrefaction and indeed the longevity of the bloodmeal in the gut of H. medicinalis contrasts sharply with other haematophagous invertebrates (Gooding, 1972).

Studies on the actual process of digestion in H. medicinalis were made by Diwany (1925). He found that starved leeches could not digest milk, egg protein or peptones injected aseptically into the gut. Later work by Graetz and Autrum (1935) failed to demonstrate proteases in the leech gut wall and they suggested that bacteria may be involved in the digestive process.

Subsequently Büsing (1951) described a single species of bacterium, Pseudomonas hirudinis associated with the gut of H. medicinalis. This species was later shown by Bullock (1961) to be one from the genus Aeromona .

The properties of this gut symbiont were first investigated by Büsing et al. (1953) and some of the experimental results are described in Mann (1962). Samples of defribinated sheep's blood were inoculated with isolated P. hirudinis. In comparison with controls, the nitrogen content in treated samples rose gradually indicating that the bacterium was capable of digesting the bloodmeal. They also found that P. hirudinis was capable of breaking down fats. Büsing et al. (1953) also studied the apparent antibiotic action of P. hirudinis by inoculating isolated cultures into sheep's blood which had previously been infected with Corynebacterium diptheriæ and Staphylococcus aureus. They found that the pathogens were eliminated. These results were supported by earlier work by Weiler (1949) who also found this effect in experiments with bacterium-free filtrates from P. hirudinis cultures.

Jennings and Van der Lande (1967) made a histochemical and bacteriological study on digestion in nine species of leech, including H. medicinalis. They found that digestion in the gut lumen resulted in the visible breakdown of the erythrocytes involving the degradation of haemoglobin into brown, insoluble granules of haematin. Mann (1962) suggested that it was the globin which formed a major source of nutriment for the leech, while the haematin was further degraded into proto-porphyrin and inorganic iron. Windsor (1970) examined faeces from H. medicinalis fed on rat blood and found it to consist almost entirely of haem (a porphyrin containing bound iron). Jennings and Van der Lande (1967) found that significant amounts of digestion in H. medicinalis occurred in the intestine, food being passed into this region a little at a time from the crop. Their chemical analyses showed that endopeptidases, lipases and carbohydrates were absent in the crop and intestine. They also examined the hydrolytic capacity of the gut bacterium and found it to have an extensive role in the digestive process. The hydrolytic activity was divided into the production of lecithinases, concerned with the breakdown of erythrocyte walls and haemolysins, important in the degradation of haemoglobin. Little work has been done on the absorption of digestive products although Jennings and Van der Lande (1967) suggested that soluble metabolites, such as glucose, may be absorbed from the bloodmeal as it lies in the crop.

The maintenance of a digestive physiology based largely on the enzymic properties of a single bacterium has been discussed by Mann (1962). He noted that young leeches have P. hirudinis in their gut before leaving the cocoon. He suggested that the adult leech, during the act of plugging the cocoon after its formation, passes some of the pseudomonads into the nutritive fluid surrounding the eggs.

The extremely slow process of digestion allowing gorged, adult H. medicinalis to survive for over a year after feeding, has led several workers to examine those factors which influence the animal's respiratory metabolism. Mann (1962), quoting from studies by Pütter (1907-1908) on the metabolism of the leech, quoted an estimate of the energy consumption of a gorged leech to be 15 calories per day at 18°C. During starvation energy consumption dropped to about 7 calories per day. This is an interesting metabolic adaptation and could, in part, explain the ability of H. medicinalis to withstand long periods of starvation. Early work by Bialaszewicz (1924) examined the influence of feeding on the chemical metabolism and energetics of H. medicinalis. In a preliminary experiment he measured oxygen uptake per leech per 24 hrs over a period of 11 days after feeding individuals an average weight of blood of 7.23 g (an average of 37% increase of body weight). He found that oxygen consumption doubled during the first 24 hours rising to a peak on the 8th day after feeding. This peak represented an oxygen consumption over 3 times greater than in starving leeches before the experiment. The oxygen consumption fell after the 8th day and due to the short duration of the experiment no further trends were apparent. In a further experiment to measure the energy output of the leech, energy values were measured at 3-4 day intervals for 20 days, after a known blood volume intake. Energy production increased over 5 times in the first 24 hrs as compared to the pre-fed, starved state. Peak energy production occurred on the 12th day after feeding (Bialaszewick, 1924). Büsing et al. (1953) found this to be the average period during which most body weight loss due to diuresis occurred. This intense diuretic activity could possibly explain the increase in energy production. Bialaszewicz (1924) studied the production and consumption of carbon dioxide and oxygen in an experiment lasting 30 days at an average temperature of 25°C. The

average blood volume intake per leech was 8.2 g (approx. 300% body weight increase). He found that the production of carbon dioxide and the consumption of oxygen increased 3.4 times and 2.7 times respectively as compared to the pre-fed, starved state. Both values peaked 2 days after feeding and subsequently fell and stabilised to similar values 5-6 days after feeding. These results seemed to indicate that metabolism was very intense during the first few days after feeding but equilibriates rapidly thereafter. In a separate experiment Bialaszewicz (1924) measured the production of excretory nitrogen and carbon dioxide after feed ing leeches on different, known amounts of blood. Metabolism was shown to be much higher in those leeches which increased their body weight by over 300% as compared to leeches which increased their body weight by 60%.

It has been known for some time that H. medicinalis is able to withstand low dissolved oxygen concentrations and even periods of anoxia (Lindemann, 1932; 1935). In comparison with other British leeches this tolerance is high and the adaptation has been attributed to its usually recorded habitat of small ponds which tend to anoxia, especially during the summer months (Mann, 1962). Heistand and Singer (1934) examined the effect of altering the balance of dissolved oxygen and carbon dioxide above and below the normal air/water equilibrium on the rate of respiration in H. medicinalis. They found a constancy in oxygen consumption in water of high and low oxygen concentration indicating an adaptive tolerance to fluctuating environmental conditions. Recently Zebe, Salge, Wiemann and Wilps (1981) examined the tolerance of H. medicinalis to anoxia and the mode of energy production under anoxic conditions by measuring changes in the concentration of the key respiratory metabolites, succinate and propionate. They found that the leech was very tolerant of anoxia and that during these periods there was a quantitative difference in the utilization of reserve substances. This metabolic adaptation was considered unusual and similar to the extremely anoxia tolerant midge, Chaoborus.

4. The Zoogeography and Ecology of the Medicinal Leech, H. medicinalis

Despite the huge historical medicinal use of H. medicinalis and the recent rapid accumulation of detailed knowledge about its anatomy and physiology, comparatively little is known about the leech's distribution, ecology and life-history. In fact any review of the ecology of the leech depends much on information from anecdotal or 'natural history' sources since little exists, in comparison with other European leech species, in the major reviews, for example, Herter (1937), Mann (1955, 1962) and Elliott and Mann (1979). Little published information relating to the life-history of H. medicinalis in the wild exists and previous data on fecundity has been largely obtained from captive breeding experiments. This information is included in a review of the reproductive biology of the leech in a later chapter on captive breeding and cocoon culture. Indeed, to date, no published work has been found based on a quantitative study of a natural population of H. medicinalis and so findings relating to population parameters such as size, structure and dispersal have awaited this study.

(i) The distribution of the Medicinal Leech, H. medicinalis

The uncertainty surrounding the status of the leech in the wild and the need for some measure of protection has been highlighted by Sawyer (1976, 1981b) and Wells, Pyle and Collins (1983). Although there is some dispute about the possible explanations for the decline in abundance of leech populations in the last two hundred years (see Elliott and Tullett, 1984) all the authorities agree that this decline has been enormous. In recognition of this fact the status of H. medicinalis in the wild has been listed in the Invertebrate Red Data Book, published by the International Union for the Conservation of Nature and Natural Resources (IUCN), as 'Indeterminate', i.e. taxa known to be endangered, vulnerable or rare but where there is not enough information to say which of these categories is appropriate (Wells et al., 1983). In fact in an earlier draft of the leech section in the Red Data Book, its status was more urgently assigned 'Endangered', i.e. taxa in danger of extinction and whose survival is unlikely if the causal factors continue operating, Wells (in litt).

The known distribution of H. medicinalis in Britain and throughout the rest of its European range has been reviewed by Wells et al. (1983) and Elliott and Tullet (1982a, 1984). In the British Isles published records number only 18 (Elliott and Tullet, 1984; Harding, in litt), dating from 1853. Elliott and Tullet (1982a) have mapped most of these records on a 10 km tetrad system. The distribution of these records, although widespread, are each very isolated and extend from Northern Scotland to Dorset in Southern England. Because of the relatively small number of records (covering only 1% of the total number of 10 km squares) no geographical trend can be discerned. A general problem with this approach to mapping the distribution of an organism, based on submitted records, is that it often represents the location of recorders rather than a true picture of the recorded. In the case of H. medicinalis, whose presence is possibly more obvious than other British leech species (i.e. activity in response to water disturbance, relatively large size, and history of attack on domestic animals), one would expect more records if indeed the animal is more widespread. The scattered and infrequent records of H. medicinalis in the British Isles may therefore be an accurate representation of the distribution rather than a lack of map coverage and would support the view of its decline and scarcity. In fact it was regarded by Harding (1910) as being extinct in Britain, while McCarthy (1975) has suggested that it has been absent in Ireland for at least 100 years. Certainly an up to date assessment of the distribution of H. medicinalis in the British Isles is overdue and could possibly be co-ordinated by such organisations as County Field Clubs and Trusts for Nature Conservation.

A further question mark against the suggested distribution and status of H. medicinalis in Europe exists as a result of a recent review by Elliott and Tullet (1984). Their main conclusion was that the leech is possibly more common than other authors, such as Sawyer (1981b), believe. They have used the records simply as indicators of presence or absence and a re-examination of several records seems to indicate the danger of equating a record with improved abundance/status in the wild. For example Russev and Janeva (1976) quoted only older records of H. medicinalis in Bulgaria. They did not find the leech in more recent surveys. Several studies in Poland by Serafinska (1958), Kufel

(1966), Wilkialis (1970) and Radkiewicz (1972) showed specimens of H. medicinalis representing very small proportions of total numbers of leeches sampled (for example in Serafinska (1958), 0.8%, Kufel (1966), 0.2% and Radkiewicz (1972), 0.8%). Manoleli (1974) made an extensive survey in swampy areas of the Rumanian plain and noted its rarity on finding only a single specimen. A survey by Higler (1977) of the macrofauna cenoses on the Water Soldier, Stratiotes aloides in the Dutch broads, found nearly 2000 leeches of 10 species by hand sampling. This sample did not include H. medicinalis although a single specimen was seen swimming at one sampling station out of 72. Jimenez and Garcia-Mas (1980-81) reviewed Spanish records for H. medicinalis and noted only 9 sites throughout the provinces. While on a recent serological study of bloodmeals from wild and trade H. medicinalis, Keim (1979) was unable to locate the leech in Germany and depended on supplies from Istria in Yugoslavia, Lakes Neusiedlersee and Seewinkel in Austria and the National Park Kiskunsag in Hungary.

Certainly this author believes that the sampling activity involved in these surveys mentioned would be sufficient to attract hungry H. medicinalis if present in large numbers. It seems likely that the very low numbers encountered supports the idea that the leech continues to decline in numbers across its range.

(iii) The habitat and host range of the Medicinal Leech, H. medicinalis

In his reviews of the ecology of British freshwater leeches, Mann (1955, 1962) concluded that the availability of a suitable host is the most important factor affecting the distribution and abundance of sanguiverous species, whilst physico-chemical factors tended to be more important in the non-sanguiverous species. Sawyer (1974) listed 11 environmental factors which affect the ecology of leeches and again considered food availability as being the most important. It is likely that H. medicinalis is no exception and its occurrence in a wide range of habitats, throughout its geographical range, points to the availability of hosts as being of prime importance.

Most of the published records of the occurrence of H. medicinalis contain short references to the type of habitat and occasionally mention

the possible range of hosts utilised. Many of the habitat records are not consistent in the accuracy of description ranging from vague terms such as 'pond' or 'ditch' to some which provide supporting lists of flora and fauna and chemical parameters such as pH. It has not been possible, therefore, to summarise and group these records into specific habitat types and so they are reviewed in full from Britain and Europe.

A description of a breeding population of H. medicinalis is given by Blair (1927). He found the leech inhabiting 'most streams and ponds' in the New Forest area in Southern England. He noted that young leeches fed on frogs, tadpoles and small fish, many of which were found dead in the ripple of ponds and streams, each with a characteristic Y-shaped bite mark. When adult he suggested that the leech will feed on the wild ponies and cattle which enter these wet areas.

Quick (1938) found 4 specimens of H. medicinalis in the shallows of Llangorse Lake in Brecon, South Wales. This lake is internationally significant as a nutrient rich lake and is designated as a Site of Special Scientific Interest (SSSI). Recently Parker and Penning-Rowsell (1980) discussed the risk of habitat deterioration at Llangorse due to gradual eutrophication of its waters. Although mentioning its continued importance to wintering and summer breeding wildfowl and a rich, 'classical', zoned lake edge flora they do not record the presence of the leech. Recent visits by Dr. Roy Sawyer, (pers. comm.) have also failed to find H. medicinalis at a number of stations around the lake.

An old record (1885) in North Yorkshire is described by Whitehead (1948). He described the site at Randymere, near Whitby, as 'marshy' and despite disturbance, due to excavations for a reservoir, the population of H. medicinalis continued until recent times in the remaining marshy areas. He was unable, however, to find any sign of the leech in 1948. A further record in Yorkshire was described by Whitehead (1952) in a pond on Strensall Common near York. Only a single specimen was found in April feeding on a toad, Bufo bufo. This author visited the general locality in April 1983 but was unable to gain access to the site. The pH value in a nearby pond on the common was slightly acidic at 6.0.

During a survey of the lochs of Islay, one of the Western Isles of Scotland, Reynoldson (1952) found a single specimen of H. medicinalis in loch nan Diol, a small body of water lying 300 yds inland from the coast. He classified the loch as a poor moorland type, poor in dissolved calcium and low in dissolved solutes. Two thirds of the loch was covered by water lily while there were emergent beds of Phragmites communis and Equisetum sp. An examination of a further twenty-two lochs on Islay did not produce any further specimens. A further record from Reynoldson (1956) described a single gorged specimen obtained from Beaumaris reservoir, Anglesey. A stained blood smear showed large, nucleated erythrocytes and he concluded that the leech had fed on a frog. He noted that periodic flooding from the reservoir onto adjacent stock grazing areas possibly afforded contact with a mammalian blood source. He did not, however, comment on the potential long term survival of the leech in this new displaced habitat.

In a survey of the vice-county distribution of Scottish fresh-water leeches, Warwick (1961) noted, in addition to the Islay record, another site where a single large leech and cocoons were found. The site, in the Kinlochbervie area of Sutherland, was a roadside marsh traversed by a small ditch. In the following year H. medicinalis was found in a small loch about 1 mile from the marsh. It was near the sea and surrounded by moorland. The water was 'brown with peat' and supported a rich fauna. The characteristic flora were Potamogeton crisous, Utricularia, Lobelia and Littorella. Another leech survey in several Scottish vice counties, by Maitland (1966), failed to record any H. medicinalis.

Relatively recent references by Ellis (1973), Le Gros (1974) and Groves (1975) in fact describe past records of H. medicinalis inhabiting several ponds on Bookham Common in Surrey. All were acidic woodland ponds and one was heavily choked with leaves. A series of non-mammalian hosts were suggested as the practice of grazing cattle on this commonland ceased in the 1920's. They included frogs, toads, newts and possibly fish and waterfowl. It is interesting that these references suggested fish and birds as possible hosts, since this extension of the host range has been a definite finding in this study. Indeed fish have been observed as important hosts for the American

Medicinal Leech, Macrobdella decora (Mathers, 1948, Rupp and Meyer, 1954).

Several authors have extended ideas about the host range utilised by H. medicinalis. For example Litton (1962) described an attack on a smooth newt, Triturus vulgaris, while Jennings and Van der Lande (1967) included 'reptile' in a list of possible hosts. Unfortunately they did not provide any details to support this idea although this author found that large, hungry leeches are able to penetrate the scales of the Grass Snake, Natrix natrix.

It has been possible to trace several recent unpublished British records of H. medicinalis. Sawyer (pers. comm.) found specimens in a pond near Swansea, South Wales in 1984, while Tullet (in litt.) describes a record from a pond at Stalham in Norfolk. Records supplied by Harding (in litt.) include two from the River Frome in Dorset (1964/65 and 1970) and a 1981 record from a Lake District brook. H. medicinalis is still thought to inhabit several ponds in the New Forest, Hants (Tubbs, pers. comm), although the last official record dates from 1919.

It would seem likely that small, local populations of the Medicinal Leech, H. medicinalis, are still to be found, probably widely scattered and isolated from each other. If this is the case, it appears very necessary for there to be a nationwide survey, possibly co-ordinated through the County Trusts for Nature Conservation, to improve radically the known distribution of the leech in Britain.

The few published records of H. medicinalis in Europe, describe an equally wide range of habitats and a similar host range. The majority of records refer to a range of standing water and wetlands, although Scharff (1898) and Lukin (1957) describe its occurrence in slow flowing rivers and major tributaries in the basin of the River Donetz in Russia.

In Poland, Matysiak (1975) found the leech in standing water with emergent Phragmites reed. While Kijek (1978) recorded a flooded clay-pit habitat in Silesia. Here the pH was 7.9 with an annual temperature range of 4-20°C. Typical vegetation included Phragmites c mmunis and P tamogeton

natans, while significantly amongst the fauna were Rana temporaria (Common Frog), Rana esculenta (Edible Frog) and Triturus vulgaris (smooth newt). On the Baltic coast of Poland, Hadjuk (1980) found H. medicinalis (and Haementeria costata) in Lake Kopan which he described as 'saltish'. He concluded that the low numbers from few species of leeches sampled at this site resulted from the increased chloride concentration of the water (1.80 - 3.53o/oo).

Another coastal record of H. medicinalis was described by Champeau (1966) in the Carmargue region of Southern France. At three locations the salinity values were high and at one site the pH averaged 7.6. Although there is no published reference to possible hosts at this site it is generally believed that the traditional practice of grazing cattle and horses in the flooded pastures sustains the Medicinal Leech, although the enormous numbers of birds associated with the area almost certainly provide a food supply. An important environmental factor is the high annual range of water level with considerable drying out of much of the wetland in the summer. Champeau (1966) suggested that resistance to drying out by H. medicinalis may be achieved by burying themselves in the bottom muds. Keve (1968) and Feliciano (in litt.) have also suggested that this behaviour enables survival of dry periods and indeed the latter author found the leech buried to a depth of 1 metre in dried out, previously marshy, areas at an undisclosed site in Italy.

A further record of the Medicinal Leech, H. medicinalis, in France, was described by Debout and Provost (1981), from a region in Normandy. It is unfortunately an historical record of a river valley, drained by numerous, slow flowing dykes interspersed with rough grazing pasture, which once supplied the animal in great numbers for medicinal use. The area, known as La Sangsuriere, is now threatened with drainage schemes for arable improvement. These authors did not mention a recent record of the leech in this area and this author failed to find H. medicinalis during samples made in September, 1984.

Another important mid-European record of H. medicinalis was from Lake Neusiedlersee in Austria (Kürkrelf, in litt.). He described the leech as being associated with extensive Phragmites beds and suggested that frogs were an important food source. In a serological analysis of

the gut contents of H. medicinalis sampled from this lake, Keim (1979) confirmed a frog host and in addition the Mallard Duck, Anas platyrhynchus. He also observed the leech attached to an unidentified species of snake and in the nostrils of cattle.

H. medicinalis has been the subject of much description in Russian scientific literature. Unfortunately it has only been possible to abstract a small amount of information concerning the leech's distribution and ecology from this eastern European part of its range. Lukin (1957) mentions its occurrence in larger lakes while in Rumania, Cristea and Manoleli(1977) found the leech restricted to ponds and marshy areas.

At the northern edge of its distribution in Scandinavia several authors have extended our ideas about the range of habitats where the leech has occurred. In an extensive study of the leech fauna of Denmark, Bennike (1943) only found H. medicinalis in 4 out of 34 ponds of differing type. He described one of the sites as an intensely eutrophic village pond type with high biological oxygen demand and a high pH of 9.0. Another pond was described as a temporary pool which dried up completely by mid-summer. It is interesting to note that despite a wide coverage of habitat types, Bennike (1943) did not find H. medicinalis in alkaline or acidic bogs, lakes or running water. He did note that the leech fed on Triturus vulgaris and 'various' species of Rana in the laboratory. More recent references to the leech in Scandinavia have been described by Forselius (1952) in the Baltic area of Sweden and Tvermyr (1965) in Norway. They both noted its occurrence in ponds in coniferous woodland openings.

The description of H. medicinalis in the southern part of its European range has often been unclear since several specimens have been classified as Hirudo troctina. These individuals had a bright olive-green ground colour with a series of orange spots along the dorsal surface instead of more characteristic longitudinal stripes. Indeed a specimen identified as H. medic'nalis was recorded by Ali (1948) in the larynx of an infant in Iraq. This is certainly a questionable identification and it was more likely to have been a common leech in the area, Limnatis nilotica (Almallah, 1968). There have, however, been more definite descriptions in the southern European countries of Spain, Italy and Greece.

In a thorough survey of freshwater communities in N. W. Spain, Marçalef (1955) found very few specimens of H. medicinalis in the shallows of Lake Antela, at an altitude of 615 metres. Typical vegetation included Utricularia sp, Myriophyllum sp and Scirpus sp. The pH at one station was 6.5. In another lake, at an altitude of 483 metres both H. medicinalis and Rana ridibunda (Marsh Frog) were noted although no evidence of the latter acting as a host was mentioned.

During an invertebrate survey in an area around Madrid, Alvarez and Selga (1967), located 'great numbers' of H. medicinalis in Lake Ortigola at an altitude of 540 metres. They described rich emergent stands of Juncus sp, Phragmites communis and Scirpus sp and suggested that the leech was utilising the enormous numbers of birds as hosts.

In Italy, Taticchi (1968) recorded H. medicinalis from the littoral zone of Lake Trasimeno. He described typical emergent vegetation and the presence of Rana esculenta.

Several records have been described of the leech in Greece. Stephanides (1948) found H. medicinalis in 'swamps' on the island of Corfu, while a survey of the Aegean lakes by Sapkarev (1970) recorded it, together with Haemopis sanguisuga, in the littoral zone of the Karstic Lake Ostrova (74 km^2 in area, with a maximum depth of 65 metres). This contrasted with two other species, Erpobdella octoculata and Glossiphonia complanata which were found at depths of 30 metres.

To summarise, it appears that H. medicinalis cannot be assigned to a specific habitat type and it would be incorrect to describe as typical a 'eutrophic pond with dense stands of macrophytes' (Elliott and Tullet, 1984). The host range appears to be wider than the traditionally believed mammalian host. Certainly many of the sites where H. medicinalis has been recorded have also supported noticeable populations of amphibia and this has an interesting parallel with records of the American Medicinal Leech, Macr bdella decora (Brocklemann, 1969).

It should be mentioned as a concluding remark to this section, that man himself has been recorded as a host. Both Katsulov (1968) and Guseinov et al. (1973) have recorded serious attacks in ponds which

have been utilised for bathing. There is a whole catalogue of attacks on man by the North American and Asian relatives of H. medicinalis (Sawyer, 1973; Mukherjee, 1974; Chaudhuri, 1971; and Lepage et al. 1981).

(iv) Other aspects of the ecology of H. medicinalis

So far the ecology of H. medicinalis has been largely described in terms of habitat and host range. Very little documented information exists on other aspects of the ecology of the leech, such as predation and behaviour in the wild. It is likely that many descriptions of its natural history are in an unpublished form as well as contained in some of the older treatises.

For example, Johnson (1816) made the interesting observation that 'the leech should be taken from waters where there is an abundance of frogs'. He also performed simple feeding studies and found that some leeches, after attacking and killing a frog, fed on a lizard, Lacerta sp. In simple experiments on the predation of hatchling leeches he 'often witnessed the larvae of the Great Diving Beetle, Dytiscus marginalis in the act of seizing the leeches by means of the mandibles' and observed hungry H. sanguisuga devouring a small, gorged Medicinal Leech. To counter this, his observations on the life-cycle were certainly erroneous when he suggested that H. medicinalis was viviparous. It is curious, however, that a later treatise by Fermond (1854) also referred to this phenomenon of live birth 'in a situation where suitable cocoon laying sites are not available'. Certainly this author has maintained gravid H. medicinalis in a completely aquatic environment for several weeks in the laboratory with the result that the gravid condition receded without live birth.

Johnson (1816) also suggested that the leech will bite on the skin of a dead animal in the water, 'for it is certain that when a leech comes to bite the skin of a corpse it is slow to let go and to distance itself from the place'. Certainly this author has found H. medicinalis (together with Haemopis sanguisuga) attached to corpses of coot (Fulica atra) and discarded domestic chickens.

Fermond (1854) discussed the incidence of cannabalism by hungry leeches on gorged individuals. Even after deliberate cutting of the cuticle of the latter attacks did not occur, although this author has observed attacks in crowded laboratory aquaria, as has Dr. Roy Sawyer (pers. comm.). It is unlikely to be a significantphenomenon in the wild, although Keegan et al. (1968) found that it occurred in two relatives. In this instance hungry Hirudo nipponica attacked a gorged Hirudinaria sp.

More recent references to aspects of the ecology of H. medicinalis exist mostly as brief notes. For example, several authors have described a seasonal affect on the activity of the leech in the wild. In the southern part of its range, Alvarez and Selga (1967) obtained large numbers of H. medicinalis in early March and at a similar latitude, Sapkarev (1970), found the leech during October. Indeed Stephanides, (1948) found that the activity of H. medicinalis on Corfu was year-round. This extended active season, in the southern part of its range, is significant since it contrasts with this author's findings of a shorter 'active' season between April and September with winter inactivity. A similar pattern was also found by Hoffman (1960) at a site in Luxembourg. It seems likely that water temperature may play an important part in determining activity in the wild.

A long-running question mark has arisen from remarks first made by Scriban and Autrum (1932-34) and described in Bennike (1943). This concerns the suggestion that H. medicinalis does not attain sexual maturity unless it has fed at least once, on mammalian blood. In fact later work by Sineva (1944, 1949) showed that the leech could be raised to sexual maturity on frog's blood alone, while fieldwork by this author has been at a site where access by mammals (i.e. grazing animals) is severely restricted, yet sexually mature adults have been obtained.

Scriban and Autrum (1932-34) also suggested that hatchling leeches fed on whole enchytraeid worms and small insect larvae, although laboratory studies by this author do not support this suggestion. It is interesting, however, to draw a parallel with Macrobdella decora where Cargo (1960) found predation on salamander eggs by this leech, suggesting an element of macrophagy in a largely haemotophagous diet.

A major work on the ecology of leeches by Herter (1937) contains several important observations relating to parasitism and predation of H. medicinalis. He found individuals with trypanosome bacilli, a trematode and nematode (Filaria rubella) parasites, the latter obtained as a result of feeding on an infected frog. Despite this he did not regard the leech as being an important vector in the life-cycle of disease organisms.

Herter (1937) made many observations on the predators of H. medicinalis and noted that Coleoptera:Hydrophiilidae larvae (water beetles) and Odonata:Libellulidae (dragon flies) larvae were active predators of young H. medicinalis. However, he suggested that the most active predators of all size groups of leech were fish, including the European Perch (Perca fluviatilis) and the Common Eel (Anguilla anguilla). The latter species, in addition to the River Lamprey, Lampetra fluviatilis, were also suggested as predators by Blair (1927). Herter (1937) also attributed the rare occurrence of H. medicinalis in an unspecified Hungarian lake to heavy predation by Catfish which, incidentally, are probing, bottom feeders.

The other significant predator of H. medicinalis suggested by Herter (1937) were birds. He described an occasion at a 19th century leech farm in France, where ducks were purported to have predated at least 20,000 leeches. An interesting parallel of ducks predating a relative of H. medicinalis was described by De Jesus (1934). Here the duck, Anas boschas, was actively used in the control of Hirudinaria malinensis. The leech was crushed by the stones of the gizzard and no internal bite marks were found. An interesting predator of H. medicinalis, noted by Herter (1937) was the Bittern, Botaurus stellaris which was observed attacking and eating the leech whole. Other, unusual, bird predators of the leech, mentioned by the same author, included chickens and crows.

In a reference by Keve (1968), quoting Dr. A. Soos, it was suggested that H. medicinalis may play a significant role in the diet of the Glossy Ibis, Plegadis falcinellus, in the marshlands of Hungary, while more recently Schmidt (1983) observed Cattle Egrets, Ardeola ibis, pecking unidentified leeches fr m the und rside

of cattle wading in the flooded pastures of the Marismas wetlands in Andulusia, Southern Spain. It is interesting to speculate that the species might have been H. medicinalis.

It has been known for some time that H. medicinalis leaves the water to deposit cocoons above the water line. Herter (1937) suggested that hedgehogs, Erinaceus europaeus and voles (Microtus sp) may be important predators of cocoons. Several authors, including Bennike (1948), have found Dipteran larvae within the cocoons of H. medicinalis. This author recorded this on one occasion where the cocoon failed to develop. It is difficult to assess whether this represents a case of parasitism or the Dipteran utilising a moribund cocoon.

An interesting series of records by Hoffman (1955a, 1955b and 1960) described a population of H. medicinalis in a toad spawning pond in Luxembourg. He described the pond as 'spring fed' from limestone rock with some 'humic' enrichment, and lists a wide range of lentic vegetation species. In addition to the leech a wide range of aquatic invertebrates were found with an abundance of fish, including carp species. Also present were two species of frog and toad. Of particular interest at this site was the enormous mortality of the toad population caused by the leech at the beginning of April. Hoffman (1960) observed H. medicinalis attacking mating toads in great numbers. The female toads, which were immobilised by the embrace of the male, were particularly affected. He observed that, despite the physical efforts of the toads to remove the leeches, including actually leaving the water, the leeches remained attached until gorging had occurred. With such an infestation of H. medicinalis mortality always resulted. After the termination of feeding the leeches were observed to return to the pond. The toad corpses were then scavenged in great numbers by the Horse Leech, H. sanguisuga. He concluded that practically no pairs of toads achieved spawning since attacks on females usually resulted in the premature release of egg strands. Consequently very few toad tadpoles were observed later in the season. He did not mention any attacks on other hosts and so this annual event could well have been highly significant to the maintenance of the population of H. medicinalis.

It seems appr priate to conclude this literature review with a description of the work of Hoffman, who r presents a surprisingly small number of documented workers who have observed, first-hand, the behaviour and some aspects of the ecology of the leech in the wild. It is hoped that some of the findings by this author will build on previous observation and, more importantly, add to our knowledge of the biology and ecology of H. medicinalis.

A MANAGEMENT PLAN FOR
LADE GRAVEL PIT, DUNGENESS, KENT

BY

PHILIP JOHN WILKIN

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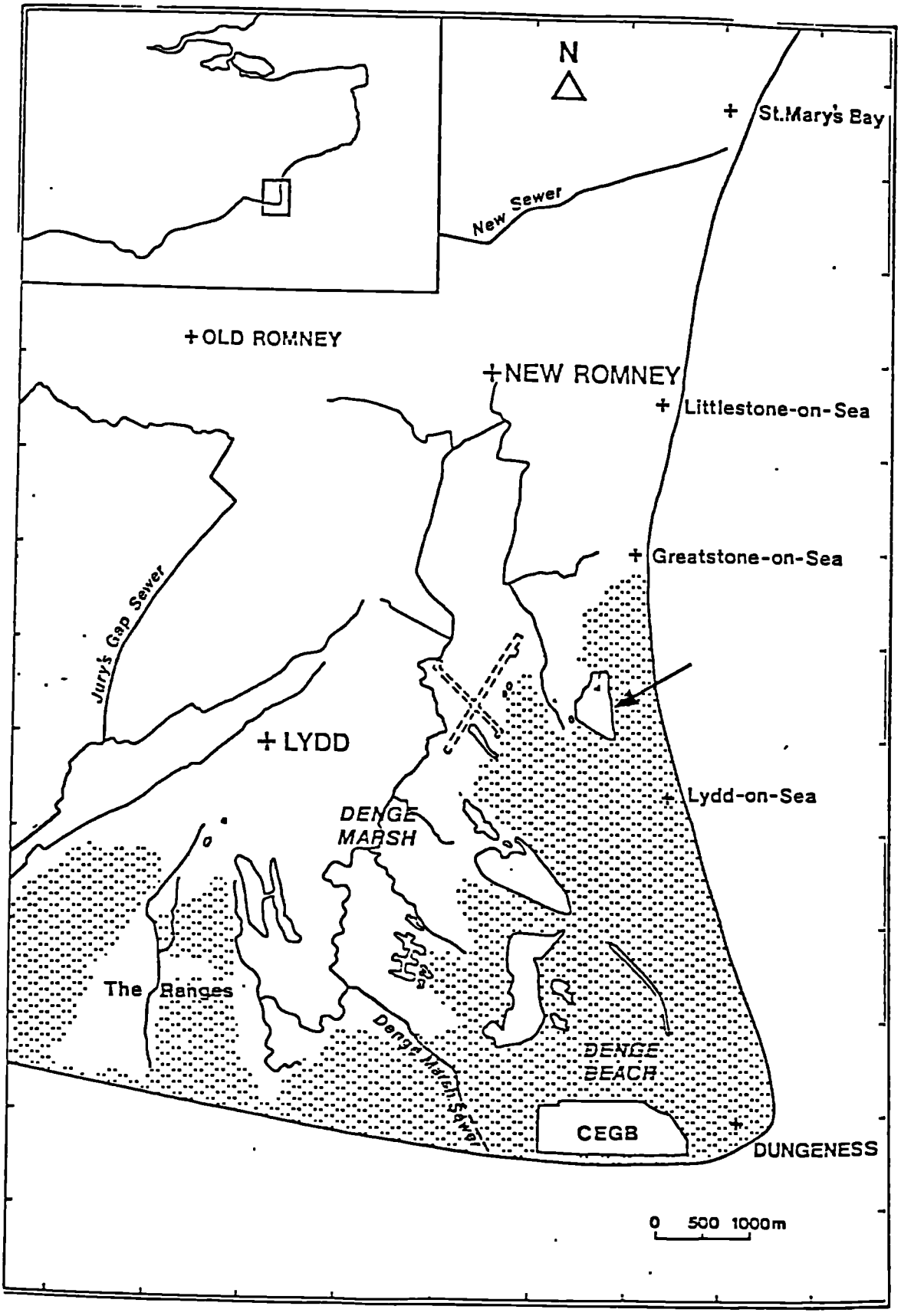


Figure 1.1.1. The location of Lade gravel pit in S.E. England and on the Dungeness foreland (arrowed)
(adapted from The Denge Hydrogeological Survey, Southern Water Authority et al (1984))

PART I: DESCRIPTION

CHAPTER 1.1 GENERAL INFORMATION

1.1.1 Location

Lade, or Hall's, gravel pit (Longitude 0°58'E, Latitude 50°57'N, Ordnance Survey map reference TR077217) is situated at the northern end of the Dungeness shingle foreland in Kent (Fig. 1.1.1). It lies approximately 600 metres from the coast to the east while the western margin is adjacent to rough grazing pasture land. Lade gravel pit forms part of an age series of gravel pits which have developed largely from post-war excavations of the Dungeness shingle.

1.1.2 Status

Lade gravel pit is included within the boundary of the Dungeness Special Site of Scientific Interest (SSSI), designated by the Nature Conservancy Council in 1954, and within an Area of High Nature Conservation value recognised by Kent County Council (1978). It is not recognised as Nature Reserve status by either the Kent Trust for Nature Conservation or the local district authority.

1.1.3 Tenure

The original shingle area, now replaced by the open water gravel pit, was owned by Romney Marsh Ballast Company Ltd. who started excavations at the northern end in the late 1940's. Lade gravel pit now forms part of an area of 83 hectares owned by Hall Aggregates (South East) Ltd., part of the Ready Mix Concrete group (RMC).

1.1.4 Maps and Photographic Coverage

Ordnance survey:

Scale	Sheet
1 : 50,000 (2 cm to 1 km)	No. 189 'Ashford and R mney Marsh'
1 : 25,000	TR01 New Romney and Dungeness
1 : 2,500	Plan TR 621-0721

Original beach formation and local soil conditions shown on colour-coded map in Green (1968).

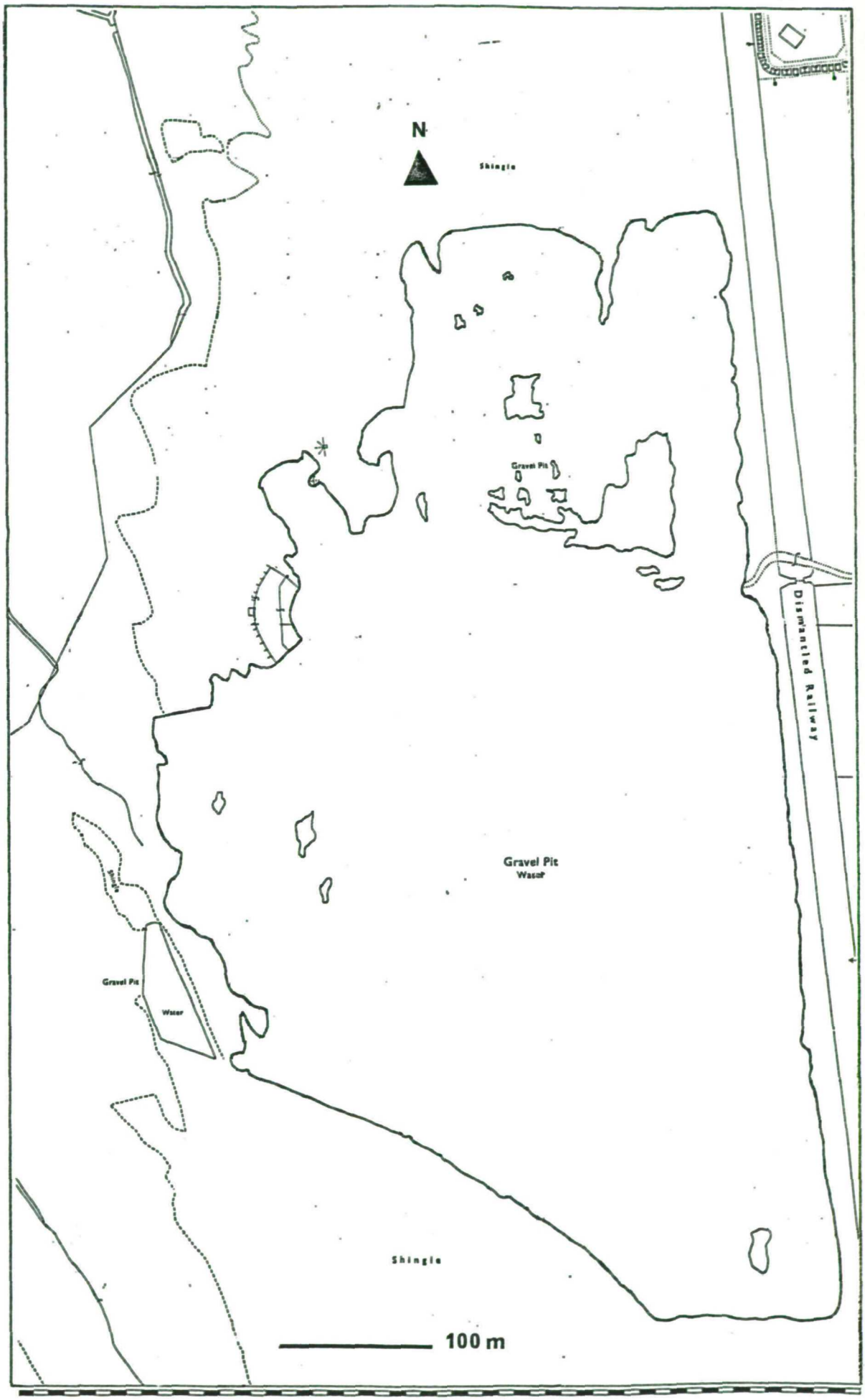


Figure 1.2.1. The Lade gravel pit site and associated small pond on its Western margin (adapted from Ord nance Survey)



Plate 1.2.1.(a) A view, from the Western margin, of the Northern, vegetated end of Lade gravel pit and the main open water area.



Plate 1.2.1.(b) The 'Small pond' at Lade gravel pit

A photogrammetric survey by the Central Electricity Generating Board (1959) represents the first full coverage of Dungeness. A series of photographs showing Lade gravel pit in 1962 and 1967 are held in the Planning Department of Kent County Council, while a series of closeup photographs of the site (contact scale 1:5000) was commissioned in 1981 by Southern Water Authority (SWA) as part of a larger Denge Hydrogeological Survey (SWA, Folkestone and District Water Authority (FDWC), Amey Roadstone Corporation (South East) Ltd., and Kent County Council, 1984).

CHAPTER 1.2 DESCRIPTION

1.2.1 General Description

The total surface area of Lade gravel pit is 35.6 hectares, of which 8.9 hectares, at its northern end, is predominantly shallow (<1.5 metres) and well vegetated with emergent macrophytes and typical marsh species. It is this small area which forms the habitat for the main population of the Medicinal Leech, H. medicinalis on Dungeness. The remaining 26.7 hectares comprise open water at a depth of 2-2.5 metres. Temporary, bare shingle islands are present in the summer months when water level falls. Little emergent vegetation has developed in this area, probably due to the recent age of excavation, strong wave action and a shifting, shingle shoreline. Only occasional, individual leeches have been found in this area. Adjacent to the western edge of the main open water area is a smaller shallow excavation of 0.19 hectares, referred to in this study as the 'small pond'. It was regarded as being continuous with the main site for the purposes of this study. It supported good numbers of the Medicinal Leech and, together with the northern end of Lade gravel pit, formed the main area for the whole study (Fig. 1.2.1 , Plates 1.2.1(a) and (b)).

1.2.2 Site Development

It is likely that some excavation at the northern end of Lade gravel pit occurred prior to the first official application to excavate gravel in 1948 by the Romney Marsh Ballast Company. By matching the dates of granted planning applications with the aerial photographs available, it has been possible to build up a picture of the development

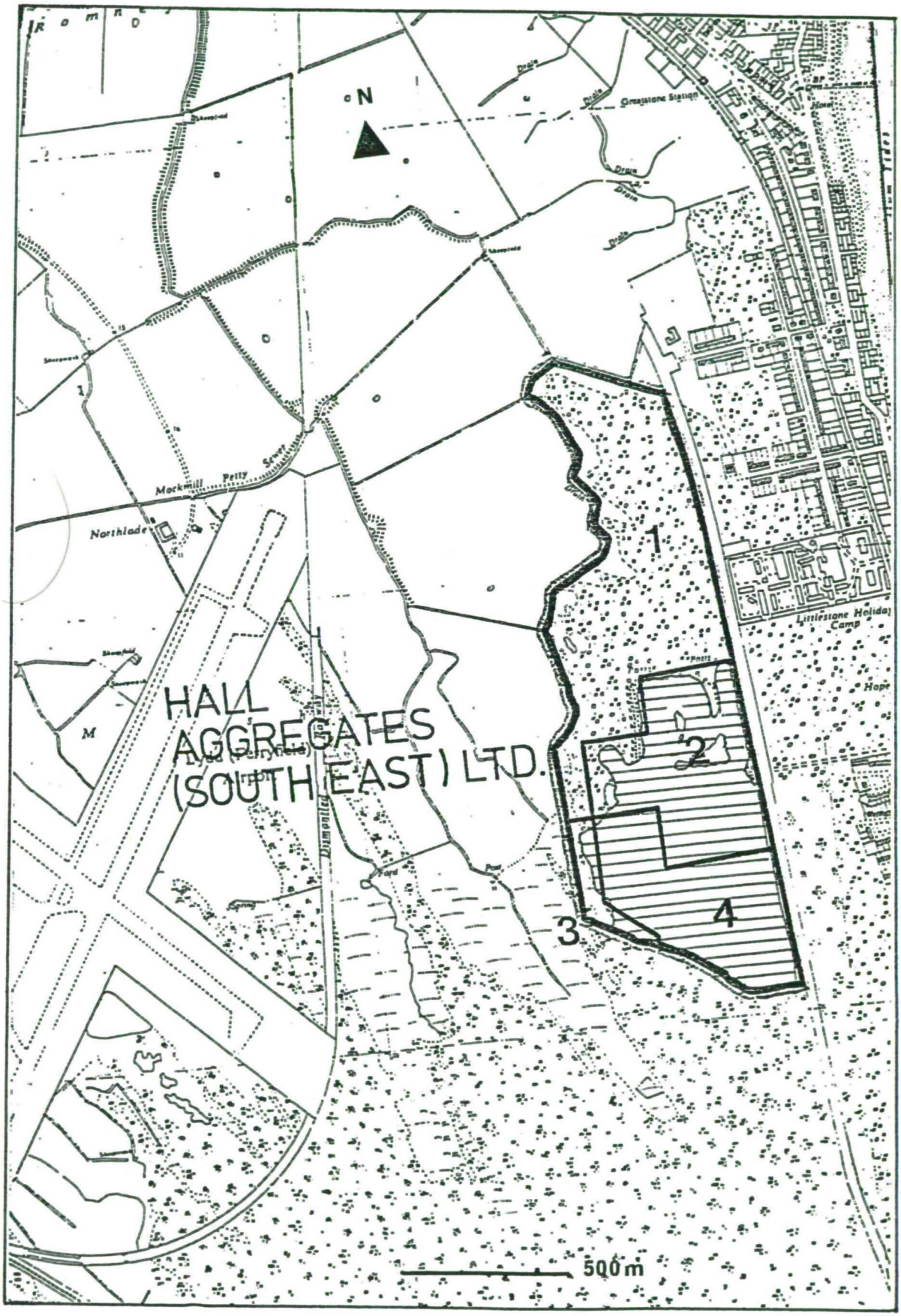
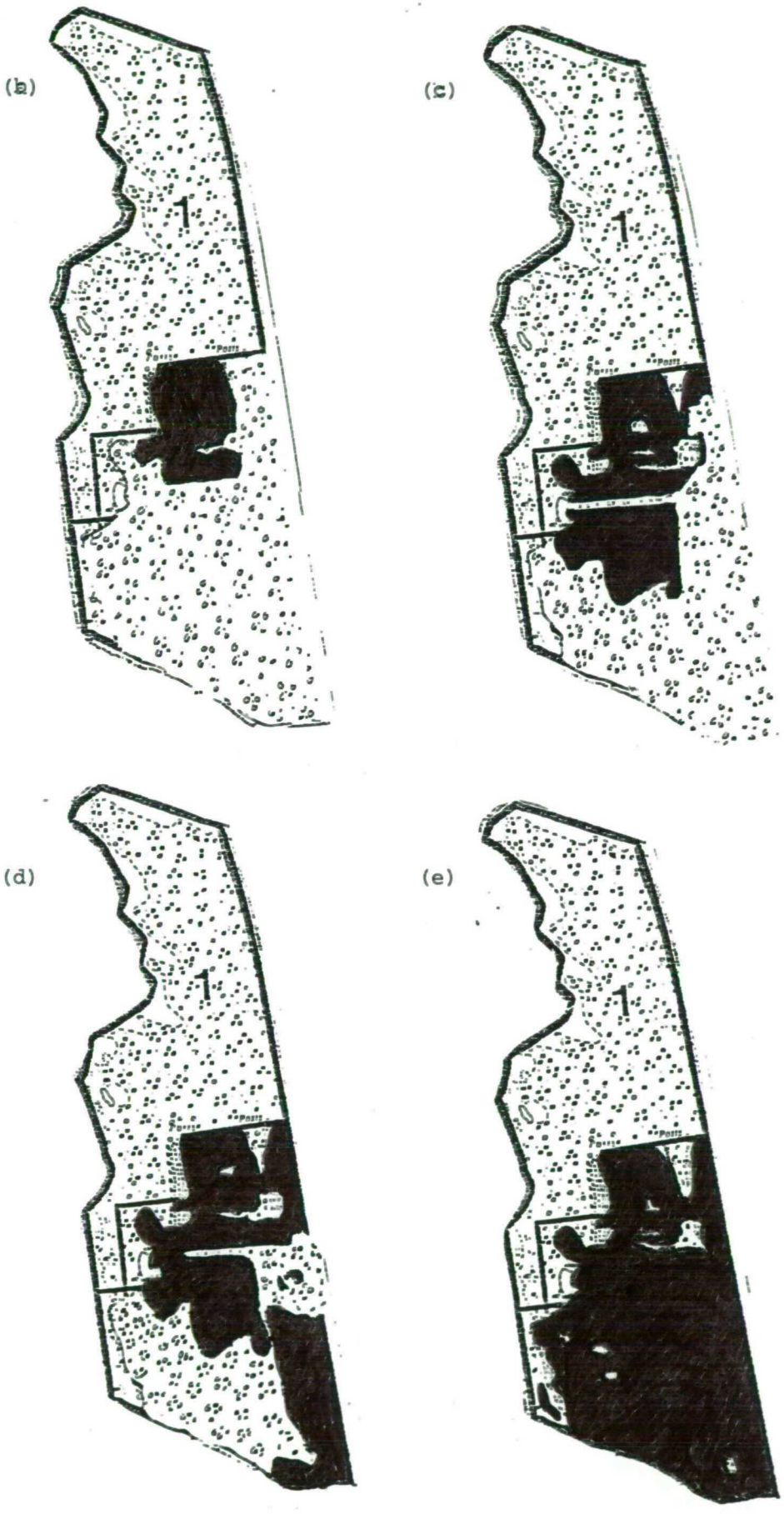


Figure 1.2.2.(a) The four area planning permissions for gravel extraction at Lade gravel pit, Dungeness (courtesy of Hall Aggregates(South East) Ltd)



Dark areas indicate open water

of the site over the last 30 years. It should be noted that there is often a gap of several years between the granting of planning permissions and actual gravel excavation. Fig. 1.2.2(a) shows the 4 planning permissions relevant to Lade gravel pit. Notice that area 1 is shingle awaiting excavation as a result of permission won on appeal (see Table 1.2.2 below). This area will be discussed at length in Section 3.1.1.

Table 1.2.2 Planning permissions for gravel extraction at Lade gravel pit

Area of Planning Permission	Date of Planning Permission
1	1976 (won on appeal)
2	1948
3	1971
4	1953

Fig. 1.2.2 (b-e) shows the gradual process of shingle excavation from about 1948 to 1978 estimated from aerial photographs. It shows that the main nucleus of the leech population is inhabiting the oldest part of Lade gravel pit (although this does not indicate the exact point when colonization/introduction occurred). It should also be noted that the 'small pond', included in area 3 (Fig. 1.2.2(a)), was not shown or excavated on a 1972 aerial photograph and so the colonization of this site is relatively recent.

1.2.3 Climate

The climate of Dungeness is characterised by its uniformity; low annual rainfall, small temperature range and above average hours of sunshine. The fact that Dungeness extends well into the English Channel means that there is considerable oceanic influence. Although sea fogs do occur at times, strong winds from the South-West and North-East predominate for much of the year. These winds carry salt spray and cause almost constant wave action at Lade gravel pit. For detailed climatic data on Dungeness see Frith (1984).

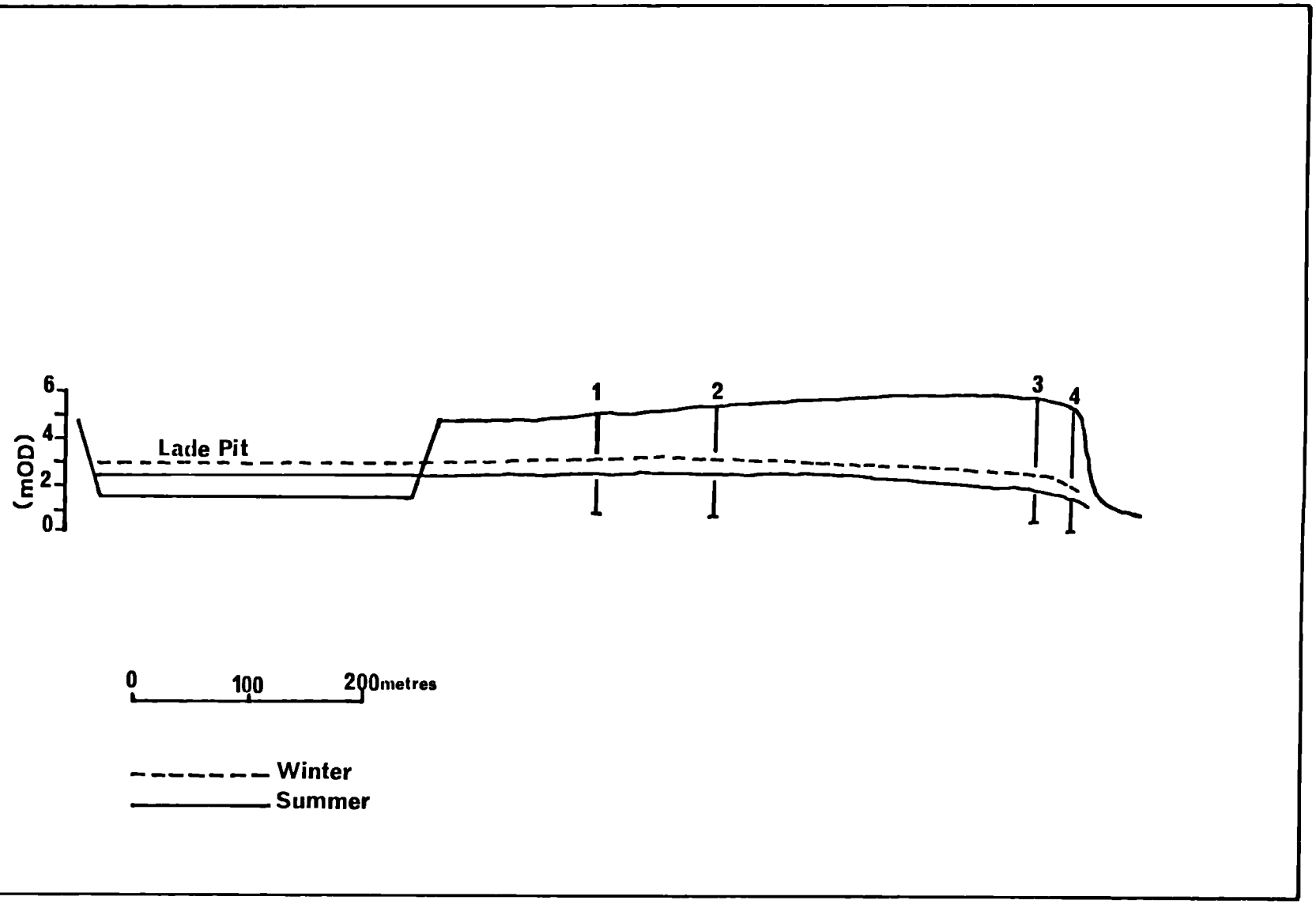


Figure 1.2.4.(a) The position of four boreholes, prepared as part of the Denge Hydrgeological Survey, to monitor saline intrusion at Lade grave pit. Summer minimum and Winter maximum 'fresh' groundwater levels

1.2.4 Hydrogeology

All the information on the Hydrogeology at Lade gravel pit was obtained from the Denge study (SWA et al., 1984). A succession of 4 strata are encountered at Dungeness but it is only the top two, younger deposits which are relevant to the Lade pit site. A recent alluvium of soft clays and silts up to 2 metres deep has developed in a series of natural freshwater pits in the area known as the Oppen pits (Harden, 1963). Although this layer at Lade pit is negligible, mainly due to its recent excavation, some siltation has occurred at the northern, sheltered end. Dungeness is characterised by its area of beach shingle deposit varying in depth from 4-19 metres thick. The Lade pit site lies on an area of the beach where the shingle deposit is relatively thin and explains the northern shallow areas of the site and the increased sand fraction which has allowed consolidation and the development of vegetation.

The Dungeness shingle acts as an aquifer and is an important source of water supply. Around the whole of the southern and eastern seaboard there is a coastal, saline zone which decreases uniformly inland from the beach margin, where groundwater is entirely saline to a point inland where it merges with normal groundwater of low conductivity. The eastern margin of Lade gravel pit is close to this boundary between fresh and saline groundwater. It is recognised that the thin shingle aquifer at Dungeness is vulnerable to saline intrusion. This has been demonstrated by periodic and sharp rises in chloride concentrations at Lade gravel pit - due to tidal surges. For example, the chloride value rose from 52 $mg.l^{-1}$ to 440 $mg.l^{-1}$ during the winter of 1977/78 and only recovered to a value of 196 $mg.l^{-1}$ in November 1984.

As part of the Denge survey a series of coastal boreholes were dug between Lade gravel pit and the coast to monitor changes in groundwater chloride gradients (Fig.1.2.4(a) shows the position of the boreholes). Average chloride values show a decrease inland from 10,000 $mg.l^{-1}$ (borehole 4) to < 1000 $mg.l^{-1}$ (borehole 1). The results indicate that steep groundwater gradients exist towards the sea throughout the year at Lade gravel pit with the saline/freshwater divide lying between 280-350 metres from high water mark in the Autumn and Spring respectively. The steep gradient suggests the presence of a zone of relatively low

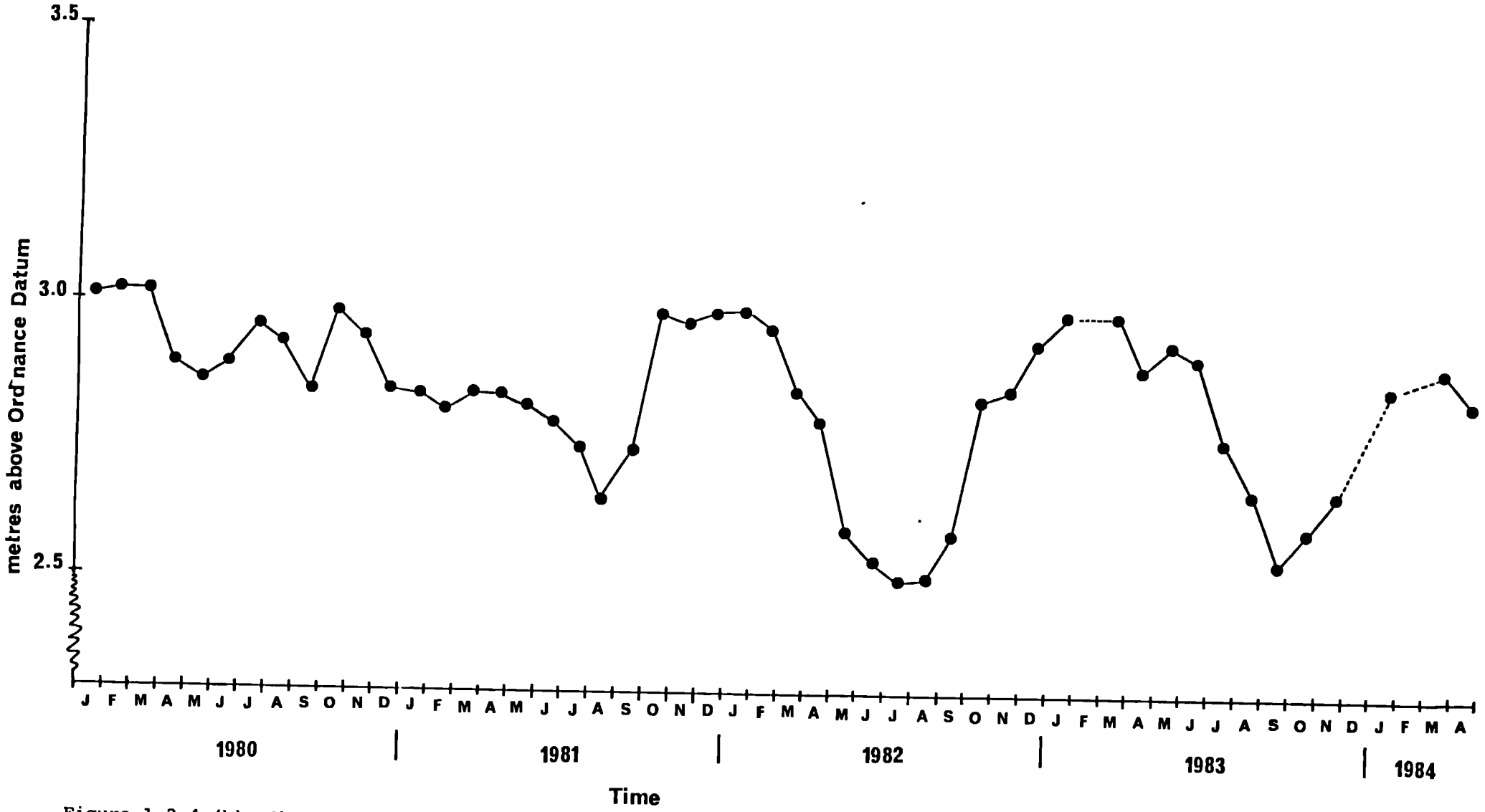


Figure 1.2.4.(b) Changes in groundwater levels(lake surface) at Lade gravel pit between Jan.1980-April 1984
(based on data provided by Folkestone and District Water Company)

permeability very close to the eastern margin of Lade gravel pit which retains high groundwater levels. This zone could have been formed by silt being driven into the face of the storm beach during rough seas. These results also suggest that in the area of Lade gravel pit the effects of tidal surge is reduced.

Groundwater levels have been recorded, periodically, at Lade gravel pit since 1973. Figure 1.2.4(b) shows the variation in groundwater level (lake surface) at Lade gravel pit between January 1980-April 1984. Measurement was recorded from a permanent scaleboard at the sou thern end of the gravel pit and transformed to standard metres above ordnance datum (mOD). The mean groundwater level of 2.51 mOD and small variation (SD = 0.16 mOD), gives a comparatively stable lake level. The effects, however, of a fall of 0.5 metre between winter and summer levels are that small patches of typical wetland plants have developed at the northern end of Lade gravel pit. The fall in groundwater level during the summer increases the extent of shallow areas suitable for roosting and dabbling wildfowl and gulls. This could be of indirect benefit to the Medicinal Leech as numbers of birds (acting as hosts) and their activity increases. Summer water level falls also have the consequence of increasing the distance between optimum leech cocoon laying sites and the water margin. Both these factors are discussed at length in the body of the thesis.

1.2.5 Water Chemistry and Temperature

Full chemical analyses of 25 parameters have been performed on water samples obtained periodically from the southern end of Lade gravel pit since 1975. Samples, taken by Folkestone and District Water Company, are analysed at the Snodland laboratories of the Mid-Kent Water Company and the methods used are summarised in Appendix 1.4.2. The results of analyses made on the main chemical parameters are summarised between 1980-84 in Appendix 1.4.1. It should be stressed that the precision, accuracy and repeatability of the results are with reference to the requirements of potable water quality.

It was difficult to assess precisely the significance of the range and variation of the main chemical parameters in terms of any

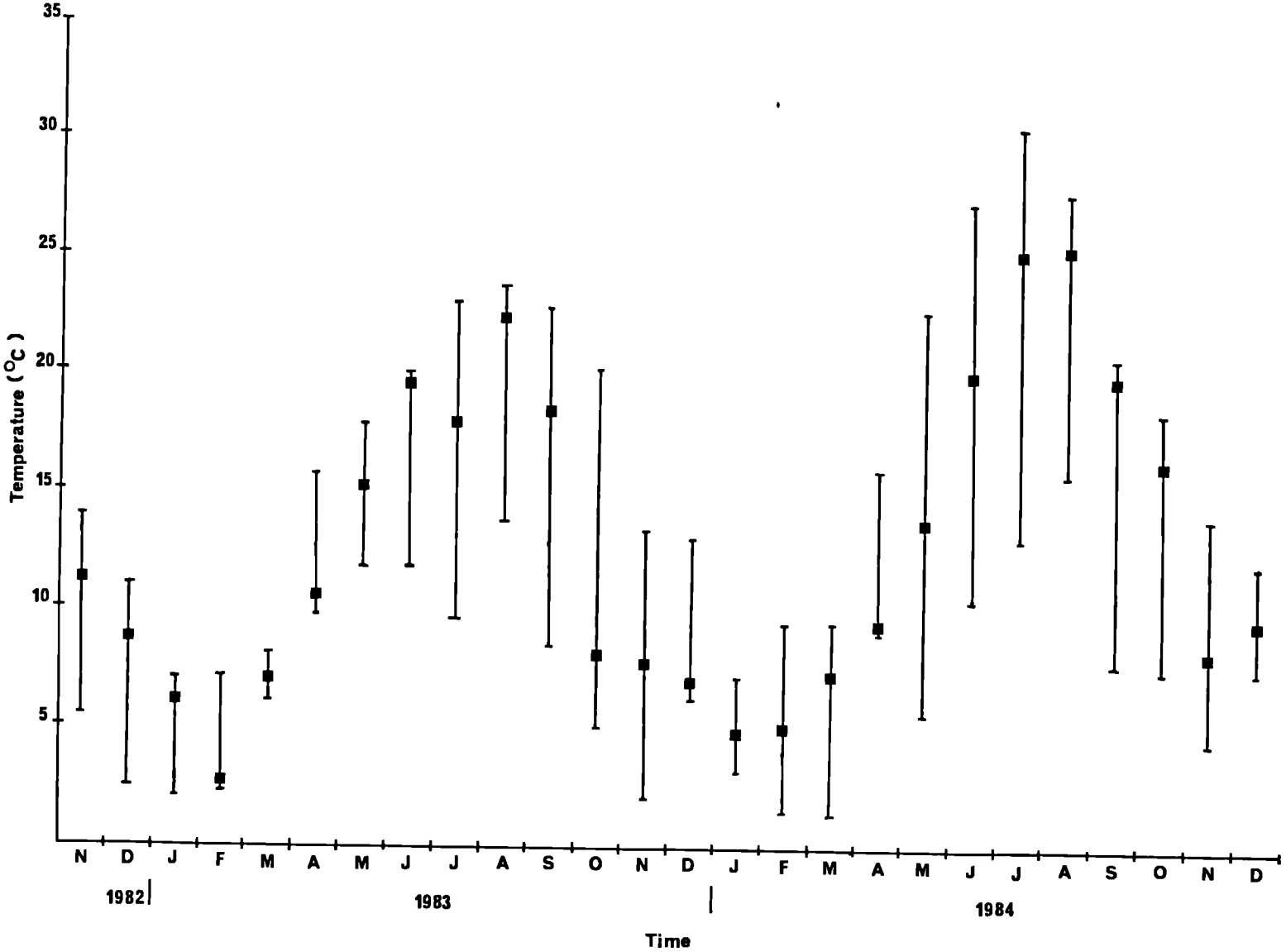


Figure 1.2.5. Maximum, Minimum and Mean mid-day surface water temperatures recorded in a stand of Lesser Reedmace, Typha angustifolia, at Lade gravel pit, Nov. 1982- Dec. 1984

effect they might have on the biota at Lade gravel pit. The mean chloride level during 1980-84 certainly exceeded values normally associated with freshwater (Bowen, 1979) but at a value of 243.5 $mgl.l^{-1}$ falls well below a level which would be described as saline. Perhaps of more significance was the periodic sharp change in chloride level, possibly associated with tidal surges. For example, during a period of a week between January and February 1980 the chloride level rose from 180 $mg.l^{-1}$ to 392 $mg.l^{-1}$ while during a period of 8 days in December 1981 the level fell from 214 $mg.l^{-1}$ to 108 $mg.l^{-1}$.

During the period 1980-84 the mean pH value at Lade gravel pit was 8.31. Interestingly, summer values rose to 9.2-9.6 suggesting the removal of carbon dioxide, possibly by planktonic algae. It also indicates that the water may have a reduced buffering capacity, possibly due to relatively low levels of calcium.

The plant nutrients nitrate (NO_3^{2-}) and phosphate (PO_4^{2-}) are low in value and are likely to limit plant growth. Lade gravel pit, as a lowland water, can be regarded as being poor in nutrient status, probably with low primary productivity. This is almost certainly due to its position on the beach shingle. It is a characteristic of the shingle that soil formation is severely reduced, therefore providing less nutrient loading to the gravel pit waters from runoff, despite its proximity to grassland pasture on the western margin. Indeed the low nitrate value indicates no evidence of recent agricultural input which is becoming such a feature of lowland waterways. The consequence of this is that extracted potable water from Dungeness is of high quality requiring very little chlorination.

Maximum, minimum and mean mid-day temperatures were recorded between the period November 1982-December 1984 (see Fig. 1.2.5). All readings were taken from the surface water layer in a small stand of Typha augustifolia L.Reedmace). Maximum and minimum values were recorded by a resident mercury thermometer while mid-day temperatures were recorded, on each visit to the site, using an electronic thermometer (accuracy $\pm$ 0.2°C). The most significant feature was the increased range between summer and winter temperatures. For example, in July 1984 the mean (n = 4) mid-day surface water temperature was 24.4°C with a

range of 17.5 C. It was a feature, during the summer months, for the shallow areas of Lade gravel pit to reach high temperatures exceeding 30°C.

1.2.6 Flora and Fauna

Flora

The flora of Lade gravel pit is represented by common aquatic and wetland species (see appendix 1.4.3 for a full list). The botanical interest is in the rate of development and the composition/structure of the vegetation restricted mainly to the older, northern end of the pit. In this largely shallow area a secondary hydrosere is developing despite its relatively recent excavation. In quality it is second to a botanically rich, secondary site at Lydd Airport (TR065210) and third to a series of naturally occurring freshwater ponds on Dungeness known as the Oppen pits (Ferry and Henderson, 1984; Ferry and Waters, 1984). Here, the primary hydrosere, in the larger ponds, has progressed to a more mature stage with extensive reed beds (Phragmites communis and Sallow Carr).

For the purpose of the main sampling study the northern end of Lade gravel pit was sub-divided into 9 discrete zones corresponding to discontinuities in the distribution of emergent vegetation (see Fig. 1.2.6). The most common, larger emergent macrophyte is the lesser Reedmace, Typha augustifolia. Although it has not formed an extensive area of reedbed it is extending into the shallow areas by rhizomatous growth. Because of the moderately deep root system and diffuse network of rhizomes the stands of T. augustifolia permit the presence of more species, e.g. Alisma plantago-aquatica. This open characteristic of T. augustifolia stands appears to prevent excessive 'choking' resulting from the development of a thick, impenetrable sub-surface root system and trapped debris, (cf. P. communis at the Oppen pits), and is probably an important factor in the ecology of the Medicinal Leech, by allowing free swimming for the location of their food.

Another important botanical feature of the site is the abundance of the Great Willowherb, Epilobium hirsutum, which has colonised much of the pit margin. The extensive and finely branching root system consolidates the unstable shingle and it is amongst this fibrous system

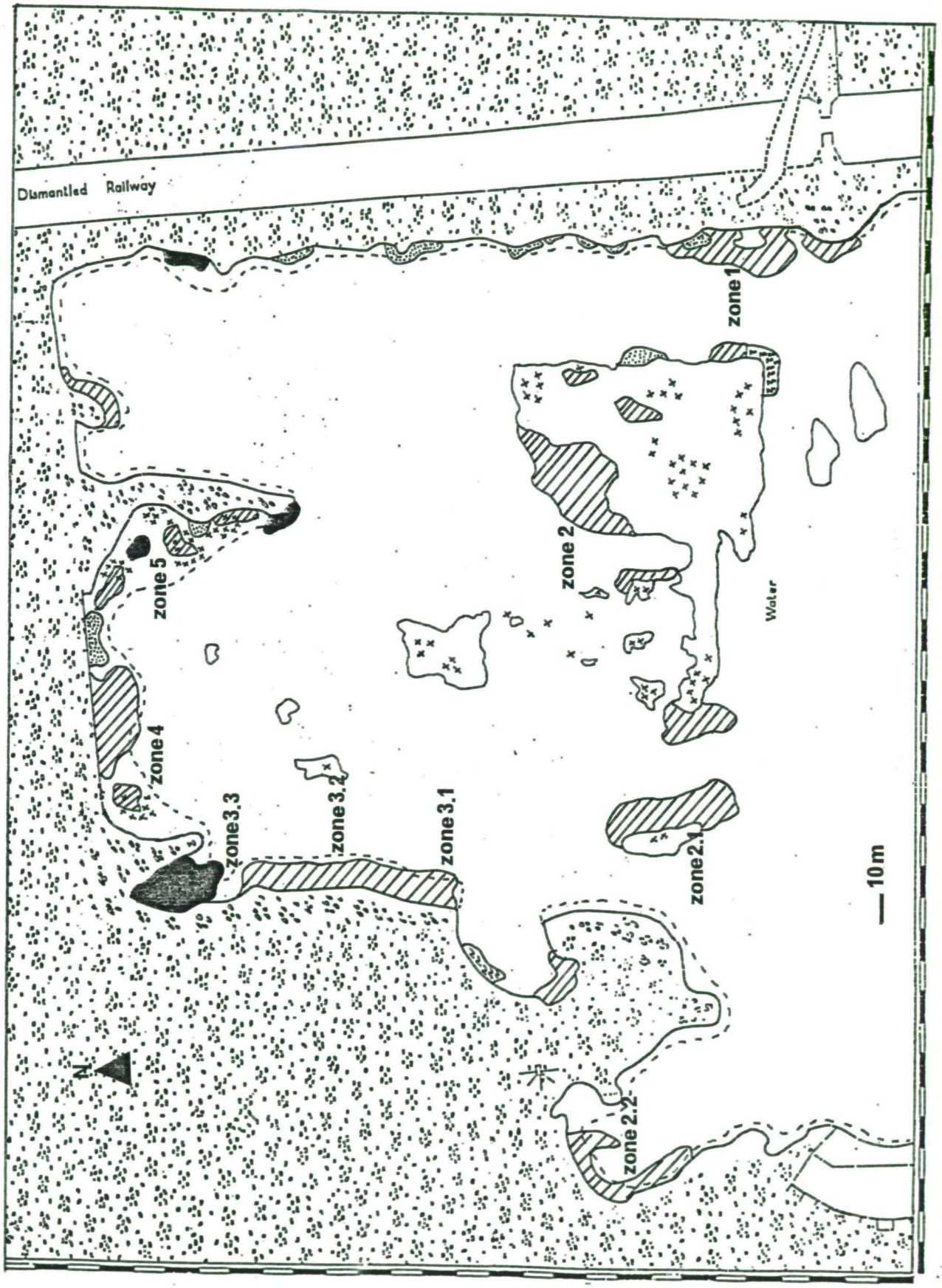

Dismantled Railway
zone 1
zone 2
zone 5
zone 4
zone 3.3
zone 3.2
zone 3.1
zone 2.1
zone 2.2
Water
10 m
N



Plate 1.2.6.(a) A view across the Northern end of Lade gravel pit with Zone 1 in the foreground



Plate 1.2.6.(b) Part of the central island (Zone 2), showing shallow water and individual Salix cinerea



Plate 1.2.6.(c) A view of Zone 5 at the Northern end of Lade gravel pit

that the important cocoon laying sites of the Medicinal Leech are found (see Chapter 5).

Invertebrate Fauna

Due to its extreme rarity in the U.K., the most important species at Lade gravel pit is the Medicinal Leech, H. medicinalis. Among the 6 other leech species represented, the Bird Leech, Theromyzon tessulatum is common. Amongst the freshwater triclads represented the presence of Dugesia tigrina is notable as an American immigrant species mostly recorded from artificial habitats in the London area (Reynoldson, 1978). Amongst the water bugs (Hemiptera-Heteroptera) Ranatra linearis is present although Macan (1976) recognises it as being scarce in the South of England. Also of interest is Notonecta viridis which tends to be associated with brackish water. Sigara sp. and Corixa sp. are the most abundant invertebrate species at Lade gravel pit. For a list of the larger recorded invertebrates see appendix 1.4.3. It does not represent an exhaustive list (no attempt was made to identify the zooplankton or smaller crustaceans). It would be interesting to construct detailed diversity indices to compare the invertebrate fauna at Lade gravel pit with other areas on Dungeness. Periodic sampling suggested that diversity and abundance was higher than the more recent gravel pits and even the older Long pits (TR085785). This could be explained by the development and pattern of vegetation at the northern end of Lade gravel pit and the extensive shallow areas.

Vertebrate Fauna

The two most significant vertebrate species, in terms of numbers, are the Marsh Frog, Rana ridibunda and the Smooth Newt, Triturus vulgaris. Although precise population size estimates were not calculated, estimates of spring mating pairs of smooth newt was in the order of several thousand while an assessment of individual vocalisations from rand m walk exercises estimated the Marsh Frog population several hundred. These could represent important populations given the general decline in the numbers of British amphibia (Beebee, 1973; Frazer, 1983) and the possible threat to the Marsh Frog by changing agricultural practice (Phillips, 1975; Beebee, 1980). The Marsh Frog is an introduced species,

the first pairs having been released at Stone-in-Oxney in Kent in 1939. Since that time they have spread rapidly throughout the dyke system of Romney and Walland Marsh, (Menzies, 1962), and to lowland marshes elsewhere (Beebee, 1977). Of the excavated gravel pits on Dungeness, Lade gravel pit appears to support the most significant populations of Marsh Frog and possibly Smooth Newt.

Lade gravel pit supports large numbers of resident and migrant birds throughout the year. Although the network of open water sites on Dungeness all represent important areas for birds, the proximity of Lade gravel pit to the sea provides a valuable sheltering area for sea-going birds during rough weather, particularly the Oyster Catcher, Haematopus ostralegus. The shallow, northern end of Lade gravel pit attracts large numbers of dabbling duck and several species of gull. The resident, nesting species in this area include Coot, Fulica atra, Moorhen, Gallinula chloropus, Mallard, Anas platyrhynchos, Mute Swan, Cygnus olor, Canada Goose, Branta canadensis, Reed Warbler, Acrocephalus scirpaceus and Reed Bunting, Emberiza schoeniclus. A list of the more common birds at Lade gravel pit is given in appendix 1.4.3.

Information from local anglers indicated that Rudd, (Scardinius erythropthalamus), and Perch (Perca fluviatilis) are the two most common fish species. It is significant that, during the period of study, no aquatic mammals or grazing domestic animals were observed at the Lade pit site.

1.2.7 Public Pressure

Public pressure is intense at Lade gravel pit and arises from its proximity to residential housing, a seasonal holiday camp and relatively easy access from nearby roads. The two most serious consequences are disturbance and damage to the habitat and wildlife. The leisure activities of walking and birdwatching probably do not cause significant disturbance to the area. There is continual evidence of damage to the habitat by the removal and trampling of vegetation and the dumping of oil and chemical containers. There is evidence of damage to wildlife as a result of angling and Marsh Frog mortality due to stone throwing. Despite this there is, as yet, no direct evidence to suggest that disturbance to the Medicinal Leech

population has occurred. The threat remains however, and could increase as the public becomes aware of the significance of the population.

1.2.8 Past Management

Under a directive from Kent County Council Planning Department in 1981, the owners of Lade gravel pit were requested to take a series of measures to enhance the appearance of the site once excavation was complete. As a result pit margins, at the north end, were smoothed to some extent and direct vehicle access prevented.

1.2.9 Recent Issues

Four major issues have arisen recently with important implications for the Lade gravel pit site. The Dungeness Countryside Plan, prepared by Kent County Council, has earmarked an extended Lade gravel pit exclusively as a water recreation area. This would involve the flooding of the existing northern end of the pit with the probable destruction of the Medicinal Leech population. The Denge hydrogeological survey concluded that there is a risk of an extensive saline intrusion into the aquifer with unpredictable biological consequences. A recent reappraisal, by the Nature Conservancy Council, of the Dungeness SSSI included an evaluation of the biological interest of the area with implications for the Medicinal Leech (see Section 2.1.1). Their major concern is the likelihood of further, extensive gravel extractions and this is of direct concern to Lade gravel pit, where permission has been granted to Hall's Aggregates Ltd. to excavate shingle north of the present site. Recently the rebirth of the long running Channel Tunnel issue (see KCC, 1968) and a recent decision by the British and French governments to proceed with the project, could have an irreversible impact on Dungeness. It could mean an acceleration in gravel extraction to supply building materials or infilling of present open water sites with materials removed from the tunnel.

CHAPTER 1.3 BIBLIOGRAPHY AND RESEARCH REGISTER

1.3.1 Bibliography

The bibliography includes those references quoted in this section of the Management Plan together with other general references to

Dungeness. Those references marked * specifically refer to Lade gravel pit.

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Appendix 1.4.1.

Chemical analyses on samples from Lade gravel pit 1980-84 (data kindly supplied by Folkestone and District Water Company)

Parameter (units)	Maximum	Minimum	Mean (sample size)	Standard Deviation
Ammoniacal N (mg/lN)	0.109	0.000	0.028 (19)	0.035
Albuminoid N (mg/l N)	0.816	0.016	0.273 (19)	0.028
Nitrate (mg/l NO_3)	4.3	0.4	1.19 (19)	0.99
Phosphate (mg/l PO_4)	1.04	0.00	0.07 (19)	0.23
Sulphate (mg/l SO_4)	112	15	41.52 (19)	38.21
Calcium (mg/l Ca)	366	19	88.18 (18)	84.81
Magnesium (mg/l MG)	99	9	76.3 (19)	53.78
Sodium (mg/l Na)	179	39	119.89 (19)	37.89
Potassium (mg/l K)	6.0	0.00	4.48 (19)	1.37
Iron 2,3 (mg/l Fe)	2.03	0.00	0.303 (19)	0.56
Silica (mg/l SiO_2)	16	108	243.53 (84)	72.78
Fluoride (mg/l F)	0.21	0.05	0.17 (19)	0.033
Hardness (total) (mg/l $CaCO_3$)	401	39	180.78 (18)	18.06
C nductivity (us/cm)	1300	650	874.73 (19)	271.02
pH (pH units)	9.6	7.1	8.31 (19)	0.650
Turbidity (FTU)	25.0	3.20	12.8 (10)	10.35
T tal Permanganate value (PV) 4 hrs (mg/l O)	3.92	0.01	1.94 (19)	0.947
Total Solids (180°C) (mg/l)	865	411	601.27 (18)	134.75

Zinc and Copper detected in trace amounts 0.02 mg/l

N.B. Zero values may reflect quantities less than the limit of detection (see Appendix 1.4.2)

Appendix

1.4.2 Methods for Ch mical Analyses - kindly supplied by the Snodland laboratory of the Mid Kent Water Company

Determinand	Ref.		Method Used	Limit of Detection
Ammoniacal N	(1)	(3)	Distillation of ammonia under mildly alkaline conditions followed by addition of Nessler reagent and colourimetry (Nessleriser).	0.002 mg/l N
Albuminoid N	(1)	(3)	Alkaline permanganate is added to the residues after the determination of ammoniacal N. Liberated ammonia is distilled and measured as above.	0.002 mg/l N
Permanganate Value; 4 hours at 27°C	(1)		Standard potassium permanganate solution is added to the acidified sample, this is incubated at 27°C for 4 hours. The remaining permanganate concentration is determined by iodiometry using standard sodium thiosulphate with 'Iotect' as indicator.	0.04 mg/l O
Alkalinity	(1)	(2)	Titration against 0.01 M sulphuric acid using BDH 4.5 indicator	1 mg/l $CaCO_3$
Free Carbon Dioxide	(1)	(2)	Titration against standard sodium carbonate solution with phenolphthalein as indicator.	1 mg/l CO_2
Total Hardness			Addition of calcium and magnesium determinations expressed as $CaCO_3$	
Chloride	(1)	(2)	Titration against standard mercuric nitrate solution with diphenylcarbazone as indicator	1 mg/l Cl
Fluoride	(4)		Specific ion meter	0.1 mg/l F
Nitrate	(2)		Measurement of u.v. absorbance at 230 nm. Correction for organic content.	0.2 mg/l N

Determinand	Ref.	Method Used	Limit of Detection
Nitrite	(1) (3)	Diazotisation method using modified Greise Ilosveys reagent. Absorbance measured spectrophotometrically at 525 nm.	0.001 mg/l N
Phosphate ortho	(1) (2) (3)	Molybdenum blue method using stannous chloride reducing agent. Absorbance measured spectrophotometrically at 680 nm.	0.02 mg/l P
Silica	(2) (3)	Molybdenum yellow method. Absorbance measured spectrophotometrically.	1 mg/l SiO_2
Sulphate	(2)	Turbidity of acidified sample measured spectrophotometrically after the addition of barium chloride reagent.	2 mg/l SO_4
Calcium	(1)	Titration against standard EDTA solution using B.D.H. Calcium Hardness indicator tablets after the addition of 1 ml NaOH.	1 mg/l Ca
Magnesium	(5)	Atomic Absorption. A 50/50 dilution of sample with solution containing HCl, NaCl and La_2O_3. Burner turned at 90° to lamp.	1 mg/l Mg
Iron Total	(1)	Acidification of sample, followed by addition of Thioglycollic acid. The purple colour produced in the presence of ammonia is measured by comparison in a Nessleriser.	0.02 mg/l Fe
Manganese	(5)	Atomic Absorption. Sample acidified with HCl to 2%.	0.02 mg/l Cu
Zinc	(5)	Atomic Absorption. Background correction. Sample acidified with HCl to 2%.	0.02 mg/l Zn
Sodium	(5)	Atomic Absorption. Sample acidified with HCl to 2% = 330.0 nm.	1 mg/l Na

Determinand	Ref.	Method Used	Limit of Detection
Potassium	(5)	Atomic Absorption. Sample acidified with HCl to 2%. Burner turned at 90° to lamp.	0.1 mg/l K
pH	(1)	Electrometric determination using Orion 611 pH meter	0.1 pH unit
Conductivity	(1)	Measurement using a Philips PW 9505 conductivity meter.	1 us/cm
Total Solids	(1)	Weight of residues following evaporation and drying at 180°C determined.	1 mg/l
Turbidity	(1)	Measured by Hach analytical Nephelometer	0.05 NTU

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(5) Instrumentation Laboratory Atomic Absorption Methods manual.
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Appendix
1.4.3 Species Lists

Flora:

The following list includes species identified from open water and wetland areas of Lade gravel pit.

Typha angustifolia	Lesser Reedmace
T. latifolia	Common Reedmace
Phragmites communis	Common Reed
Sparganium erectum	Branched Bur-reed
Scirpus lacustris	Common Bulrush
S. maritimus	Seaclub-rush
Eleocharis palustris	Common Spike Rush
Juncus effusus	Soft Rush
J. inflexus	Hard Rush
Schaenoplectus tabernaemontani	Glaucous Club Rush
Carex divisa	Salt Meadow Sedge
Carex sp.	
Agrostis stolonifera	Creeping Bent
Alisma plantago-aquatica	Water Plantain
Polygonum amphibian	Amphibious Bistort
Hydrocotyle vulgaris	Marsh Pennywort
Mysotis arvensis	Common Forget-me-not
M. caespitosa	Lesser Water Forget-me-not
Mentha aquatica	Water Mint
Galium Palustre	Marsh Bedstraw
Solanum dulcamara	Woody Nightshade
Lemna minor	Common Duckweed
Elodea canadensis	Canadian Pondweed
Ranunculus spp.	
Potamogeton lucens	Shining Pondweed
Ceratophyllum submersum	Soft Hornwort
Potentilla palustris	Marsh Cinquefoil
Rumex crispus	Curled Dock
Epilobium hirsutum	Great Willowherb
Lythrum salicaria	Purple Loosestrife
Lotus uliginosus	Greater Birds-foot-trefoil
Salix cinerea ssp.	Fen Sallow
Salix alba	White Willow
Urtica dioica	Stinging Nettle
Cirsium palustre	Marsh Thistle
C. arvensis	Creeping Thistle
Sonchus arvensis	Corn Sowthistle
S. asper	Prickly Sowthistle
Senecio jacobaea	Ragwort
Senecio sp.	
Orchis mascula	Early Purple Orchid
Dactylorhiza spp.	Marsh/Spotted Orchid hybrids
Rubus fruticosus	Bramble

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Fauna:

Hirudinea

Hirudo medicinalis	Medicinal Leech
Theromyzon tessulatum	Bird Leech
Erpobdella testacea	
E. octoculata	
Helobdella stagnalis	
Glossiphonia complanata	
Haemopis sanguisuga	Horse Leech

Turbellaria

Polycelis nigra
Dendrocoelum lacteum
Dugesia tigrina
Dugesia sp.

Bivalvia

Pisidium sp.	Pea-shell Cockle

Gastropoda

Planorbarius corneus	Giant Ram's Horn Snail
Lymnaea stagnalis	Great Pond Snail
Planorbis leucostoma	Button Ram's Horn
Lymnaea peregra	Wand ring Snail

Crustacea

Cladocera

Daphnia sp.

Isopoda

Asellus aquaticus

Amphipoda

Gammarus pulex

Hemiptera-Heteroptera

Sigara sp.	
Corixa sp.	Water Boatmen
Notonecta viridis	
Ranatra linearis	Water Stick Insect
Nepa cincera	Water Scorpion
Gerris lacustris	Common Pond Skater
Ilyocoris cimicoides	Saucer Bug

Chironomidae and Ceratopogonid midge larvae

Coleoptera

Gyrinus natator	Whirligig Beetle
Hyrobia hermanni	Screech Beetle
Dytiscus marginalis	Great Diving Beetle
Hippodamia tredecinpunctata	13 Spot-ladybird

Odonata

Libellula depressa	Broad-bellied Libellulid

Zygoptera

Agrion virgo	Demoiselle Damselfly

Arachnida

Argyroneta aquatica	Water Spider

Pisces

Rutilus rutilus	Roach
Scardinius erythropthalamus	Rudd
Gasterosteus aculeatus	Stickleback
Perca fluviatilis	Perch

Amphibia

Bufo bufo	Toad
Rana ridibunda	Marsh Frog
Triturus vulgaris	Smooth Newt

Reptilia

Natrix natrix	Grass Snake

Aves

Anas platyrhynchas	Mallard
Aythya ferina	Pochard
Aythya fuligula	Tufted Duck
Cygnus olor	Mute Swan
Branta canadensis	Canada Goose
Podiceps cristatus	Great Crested Grebe
Tachybaptus ruficollis	Little Grebe
Larus ridibundus	Black-headed Gull
Gallinula chloropus	Moorhen
Fulica atra	Coot
Sterna paradisaea	Arctic Tern
Tadorna tadorna	Shelduck
Ardea cinerea	Heron
Acrocephalus scirpaceus	Reed Warbler
Emberiza schoeniclus	Reed Bunting
Haematopus ostralegus	Oyster Catcher

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Freshwater Biological Association

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Rivers, Lakes and Marshes
Hodder and Stoughton, London.

1.4.4 Interested persons and acknowledgements

The following persons have a specific interest in the Lade gravel pit site:

Dr. A. M. Scofield,
Department of Physiology,
Wye College,
Ashford,
Kent TN25 5AH

Mr. R. Findon,
Assitant Regional Officer,
Nature Conservancy Council,
'Zealds',
Church Street,
Wye, Kent TN25 5BN.

Mr. D. Bush,
Production Manager,
Hall Aggregates (South East) Ltd.,
RMC House,
Canning Street,
Maidstone,
Kent ME14 2RX

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Dr. D. Frazer
Mr. P. Makepiece - RSPB Dungeness Reserve Warden.

CHAPTER 2.1 EVALUATION

The main aim of an ecological evaluation is to apply a system of worth to an area in order to base decisions on its conservation value. Many schemes of evaluation have been suggested for use at local and national level for the designation of protected areas or to predict the effect or impact on wildlife resulting from a change in land use. The works of Spellerberg (1981) and Margules and Usher (1981) provide a good general introduction to the field while a recent revision of Policy Procedure Guidelines on the Selection of Biological SSSI's by the Nature Conservancy Council has been applied to the Dungeness area.

At present Lade gravel pit is not a nature reserve but it is hoped that the evaluation which follows will assist in formulating a policy for its future.

2.1.1 Evaluation of Species

The most important species at Lade gravel pit is the Medicinal Leech, H. medicinalis, and from the work of Elliott and Tullet (1982) and Wells, Pyle and Collins (1983) there is no doubt a continued need for some measure of protection. It is useful to consider a series of general species characteristics, devised by Adamus and Clough (1978), as applied to the leech. They are:

- Site tenacity
- Seasonal mobility
- Area size needs
- Spatial distribution
- Relative scarcity
- Status changes
- Endemicity
- Peripherality
- Habitat specialisation
- Habitat scarcity
- Unusual or unique scientific value
- Aesthetic value

Site tenacity can be regarded as the probability that a species will occur at the same general site, or natural area for a significant period of time. The site tenacity of the leech should be regarded as low and further that it is prone to rapid disappearance as suggested by the general decline in populations throughout its range.

The degree to which a species remains at the same general site throughout the year is a measure of its seasonal mobility. Under the scheme proposed by Adamus and Clough (1978) the leech can be regarded as having 'confined mobility' i.e. most members of a population spend their entire lifespan within a small area. Species with such a confined mobility should be considered to be more suitable for nature reserve protection because a greater proportion of their seasonal habitat needs can be protected.

Area size needs is a measure of the area required by a typical breeding unit, e.g. breeding pair or population, to accomplish all the life functions, e.g. breeding areas, refuges etc. The Medicinal Leech can be regarded as having 'low area needs', i.e. less than 1 km^2 of relatively continuous, suitable habitat required for a population. A complicating factor could be the area size needs of hosts although in the case of Lade gravel pit the area involved appears to support a high concentration of Marsh Frog, Smooth Newt and breeding birds, despite territoriality and display areas important in their area size needs.

Spatial distribution can be considered as a measure of the pattern of localisation or dispersion of a species over its geographic range. Drury (1974) described three major kinds of spatial distribution which characterise rare species. The Medicinal Leech in Britain may be included under the following category:

> "a species occurring as very few individuals or small groups at widely scattered localities over a large geographic area of what appears to be suitable habitat".

The relative scarcity of the Medicinal Leech has been mentioned and obviously a rarer species should be given higher priority than common ones for the protection of a natural area.

The status change of the leech, in terms of distribution and abundance, has declined dramatically. Obviously species declining at sites and in numbers should receive higher consideration for natural area protection, particularly when human activities are responsible for the decline.

Endemicity is a measure of the significance of a population in relation to a larger area of reference. Certainly the Lade gravel pit population is the first record of the Medicinal Leech in Kent (Philp, pers. comm.) and is probably the most significant in Britain in terms of size.

Peripherality is the condition of a population being at the edge of a normal geographic breeding range. Peripheral populations are of concern to nature reserve programmes because they may represent one which is a forerunner of species range expansion or a remnant group of species range contraction. In the case of the Medicinal Leech, Britain represents the western edge of a distribution stretching eastwards to the Ural mountains in Russia. It has been extinct in Ireland for over 100 years (McCarthy, 1975). Certainly the leech was more common in Britain at one time and so the Lade pit population, despite its comparatively recent origin (post 1947) represents a remnant of range contraction.

Ideas about habitat specialisation are determined from the requirements of a species and these may vary seasonally, in different parts of their range or during the population lifecycle. The Medicinal Leech is usually associated with shallow areas of lakes and ponds although it appears to tolerate a wide range of physico-chemical factors.

Habitat scarcity has probably been an important factor in the decline of the Medicinal Leech. The general decline in area and continuity of unimproved, wet, grazing pasture, resulting from drainage schemes, has probably been the single most significant habitat change for the Medicinal Leech.

A species may be desirable for a natural area because it fulfils a unique scientific function. The Medicinal Leech is still applied in certain areas of modern medicine and it is expected that the array of salivary biochemicals will have important application in tissue culture research and the clinical treatment of circulatory problems.

Recently Wood (1983) proposed a scheme of relative worth (aesthetic

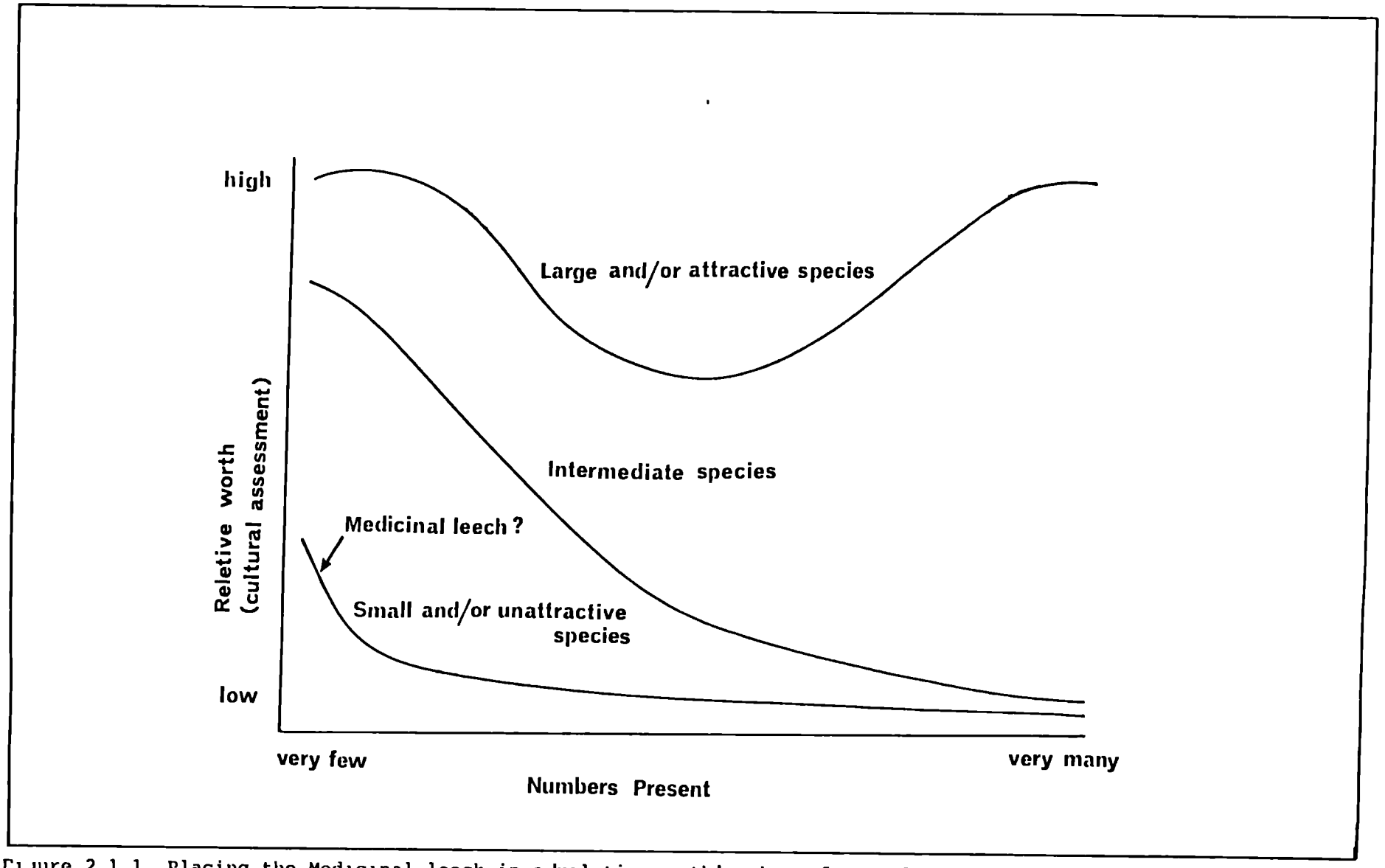


Figure 2.1.1. Placing the Medicinal leech in a 'relative worth' scheme for evaluating the conservation value of a species, (adapted from Wood, (1983))

value) for evaluating the conservation value of a species.(Fig. 2.1.1 is adapted from Wood's Scheme and shows a possible position for the leech.

2.1.2 Evaluation of Biotic Communities

It has been beyond the scope of this work to make a detailed evaluation of the vegetation cover and diversity at Lade gravel pit although as a secondary succession it is not of high value, especially in comparison with the internationally acclaimed Oppen pits. It would be useful to apply a scheme suggested by De Lange and Van Zon (1983) or Harris, Milligan and Fewless (1983) to evaluate botanically Lade gravel pit in comparison to other sites on Dungeness.

Dungeness is internationally famous for its bird communities. The Nature Conservancy Council recognises it as outstanding for its assemblages of winter migrants, passage and summer breeding birds. Lade gravel pit accommodates birds in these three categories and is regularly mentioned in the Bulletin of the Kent Ornithological Society and the Dungeness Bird Observatory. There is no doubt that human disturbance is a problem and if this were reduced Lade gravel pit could possibly rank with the other important sites on Dungeness (Makepiece, pers. comm). Fuller (1980) employed an objective scheme to assess the ornithological interest of a series of mature, flooded gravel pits and it would be valuable to apply a similar scheme to evaluate Lade gravel pit.

The quality of the aquatic, invertebrate assemblage at Lade gravel pit is difficult to assess due to insufficient data from other areas, although the incidence of larger, carnivorous invertebrates such as Tricladida, Hirudinea and Hemiptera indicates a mature assemblage.

2.1.3 Evaluation as a Potential Nature Reserve

It is important that the status of nature reserve is assigned to, at least, the northern section of the present Lade pit site. This would further the protection of the population of Medicinal Leech, already afforded under the Dungeness SSSI designation, and add an important component to the present areas managed by conservation organisations. If

it were possible to extend consideration beyond the gravel pit itself a valuable addition would be a nearby area of undisturbed shingle vegetation and a line of Blackthorn, Prunus spinosa, supporting an important lichen assemblage.

CHAPTER 2.2 MANAGEMENT OBJECTIVES

2.2.1 Broad Basis

To achieve a set of management objectives requires those involved to see clearly how their individual actions can best be employed within a framework of co-operation. The commercial interests of the owners of Lade gravel pit need not conflict with the very important conservation value of the area. Indeed Hall Aggregates have co-operated fully with the research work at the site and it is to be expected that this will be continued in the future. The main objective must be to retain the value of the site by preventing any major disturbance due to future infilling or gravel extraction.

2.2.2 Long Term

It is difficult to project long term management objectives since within the next 10 years Hall Aggregates will have begun excavating shingle to the north of the present Lade gravel pit site. Indeed the future conservation value of the whole area of Dungeness is in question as a result of the recent Channel Tunnel agreement.

Assuming no disturbance occurs an important long term management objective would be to retain the important open water shallow areas, at the northern end of the pit, by preventing extensive development of Typha augustifolia. The area would then continue to attract birds and, it is believed, enhance the survival of the Medicinal Leech. Another major long term objective would be to encourage the development throughout the site of emergent macrophytes along an altered gravel pit margin. (Some suggestions for site management projects are discussed in Section 3.1.4).

2.2.3 Short Term

The prevention of human disturbance and damage to both wildlife and and habitat at Lade gravel pit is an important short term manag ment objective. This could be achieved by a regular wardening presence either on a voluntary basis by volunteers from the K nt Trust for Nature Conservation or as part of the job of a full-time warden employed by the Royal Society for the Protection of Birds at its Dungeness reserve. The role of a site warden could be extended to c ntinue the site evaluation process and to organize site management projects.

An important, short-term habitat management objective is to ensure that Zone 2, at the northern end of Lade gravel pit (see Fig. 12.6) is only accessible by authorised boats. A refuge from man and fox could be created including the provision of duck nesting boxes.

CHAPTER 2.3 TRENDS

2.3.1 Ecological Trends

The 'open' system of vegetation at Lade gravel pit is an important factor in the ecology of the range of birds, Marsh Frog and Smooth Newt which are resident to the area. The trend towards increased dominance by a single macrophyte (T. augustifolia) should be prevented in order to retain the present niche structure favourable to the likely hosts of the Medicinal leech.

The Marsh Frog population in the dykes of Romney Marsh are believed to be declining due to the increasing pace of conversion from grazing pasture to arable, resulting in increased runoff from pesticide and fertilizer applications and a less open dyke system (dykes, in the traditionally sheep grazed areas, are prevented from 'clogging' by Phragmites communis by sheep feeding on the young macrophyte shoots). It may be that the trend in population size of the Marsh Frog (and Smooth Newt) will be more stable at Lade gravel pit where agricultural enrichment is unlikely and water quality is high.

It is difficult to predict the trend in population size of the Medicinal Leech. It is believed that by enhancing the conditions for an upward trend in host populations, the chances of survival of the leech will be increased.

2.3.2 Man-induced Trends

An important development in the after-use of wet gravel pits, throughout the country has been their commercial use for water recreation (e.g. Thorpe Park on the Thames Valley gravel pit complex). Despite the existence of Lydd Watersports Centre in the South-West of Dungeness, Kent County Council, in the Dungeness Countryside Plan, have proposed that the after-use of Lade gravel pit should be developed for water recreation (N.B. they do not regard the site under a category of wildlife conservation).

In 1974 permission was granted by Hall aggregates for the use of Lade gravel pit by Lydd Sailing Club, but was later revoked for commercial reasons. In 1982 the company was approached by the Cinque Ports Angling

Society for fishing rights to the northern end of Lade gravel pit. This was rejected on safety grounds due to the future commercial operations north of the present site. It seems that in the near future the pressure f r recreational use will be lessened by commercial priorities. The trend, however, of formulating multipurpose amenity plans for such man-made water spaces will remain an attractive commercial option for the owners. The entire Lade gravel pit site, with suitable management, could be an important conservation area, but it seems likely that a mix between recreation and conservation use may have to be agreed long term. It is, therefore, worth noting some of the possible ecological implications of such recreational use.

Liddle and Scorgie (1980) concluded that the physical impacts of erosion due to wash, an increase in turbidity due to propeller turbulence and damage to emergent macrophytes by cutting and direct contact, could be serious consequences of water based recreation. They found that Great Willowherb, Epilobium hirsutum was particularly susceptible to erosion by wash. This marginal species has already been described as occurring at Lade gravel pit and further that it provides the site for leech cocoon deposition. The major impact of shore based activities such as angling would be trampling and bank erosion. These obvious effects on vegetation compare with less understood effects of recreation on aquatic animals, including disturbance by sight and sound. Animals are strongly dependent on plants f r food, shelter and breeding sites etc. and would probably suffer indirectly if plants were affected.

Batten (1977) examined the effects of disturbance by sailing on water birds. He emphasised the importance on the response of birds to the proximity of a disturbance factor in order to assess the area needs of a wildfowl refuge. He found that the Great Crested Grebe, Podiceps cristatus and general wildfowl were highly sensitive to the presence of sailing boats, while moorhen, Gallinula chlor pus and Coot, Fulica atra, tended to be unaffected by remaining close to the banks. Any reduction in the presence and activity of birds at Lade gravel pit would reduce the availability of birds as hosts for the Medicinal Leech and could have serious implications for the population.

Mudge (1983) examined the incidence of ingested lead pellet poisoning in British Wildfowl and found that it was greatest in diving ducks such as Pochard, Aythya ferina and Tufted Duck, Aythya fuligula, and high in Mallard, Anas platyrhynchus and the Mute Swan, Cygnis olor. The development of angling at Lade gravel pit would certainly result in lead input from discarded lines and could threaten the resident birds.

2.3.3 External Factors

The three most important external factors with implications for the Lade gravel pit site are recent proposals for the Romney Marsh and Rye Rural Development area (RDA), the recent agreement on a Channel link and rapidly changing land use patterns in the Romney Marsh area.

The aim of the RDA is to encourage local business enterprise, housing and tourism in an area regarded as needing priority attention. The hope is to increase local services and employment, but this development will, of necessity, involve greater human demands on the landscape. A balance must be found between these demands and conserving the considerable ecological interest of the area.

Kent County Council's Minneral Planning Department regard Dungeness as a long-term, strategic supply of aggregate for the construction industry. The demands on this local supply resulting from the construction of a Channel tunnel would almost certainly mean a dramatic increase in shingle removal. Some authorities believe that a form of compulsory purchase could be enforced on those areas presently managed by conservation organizations to fulfil the supply. A byproduct from the construction of a Channel tunnel will be enormous quantities of excavated spoil. There have been suggestions that the present open water sites on Dungeness represent attractive sites for infilling followed by agricultural reclamation. The implication of this to the Medicinal Leech is obvious.

The historical development of the drainage and establishment of grazing pasture on Romney Marsh is well d cumented. In recent years an increasing change from sheep grazing to arable has been occurring throughout Walland and Romney Marsh. The dyke syst m, which was once kept clear of invasive, emergent vegetation by sheep grazing, is becoming

increasingly choked, while arable runoff (nitrate enrichment) and pesticide spraying is increasing. The Lade gravel pit site is adjacent to an area of traditional grazing pasture (on thin soils overlying the shingle beach) which may be an important buffer against arable impacts. In addition, the Medicinal Leech has been found in the more open dykes in this area. It is very important that this traditional land use is maintained adjacent to Lade gravel pit in order to maintain its high water quality.

CHAPTER 2.4 CONSTRAINTS

2.4.1 Obligations

In the event of a nature reserve designation (or management agreement for nature conservation) at Lade gravel pit, a requirement by Hall Aggregates would be for continued access to the area north of the present site. This would involve the movement of heavy plant plus the construction of a conveyer belt along the track of the old railway to carry excavated shingle to their processing plant at Lydd-on-Sea. This would involve secure fencing along its length and this could be beneficial to the site by reducing public access despite initial disturbance from construction.

2.4.2 Public

A long term constraint on any nature conservation plan at Lade gravel pit would be the ease of access by the public from nearby housing and holiday camp. For example, markers for delimiting areas or locating specimens for research or management purposes would almost certainly be disturbed. Experimental monitoring equipment left in the field during this study was repeatedly broken or removed.

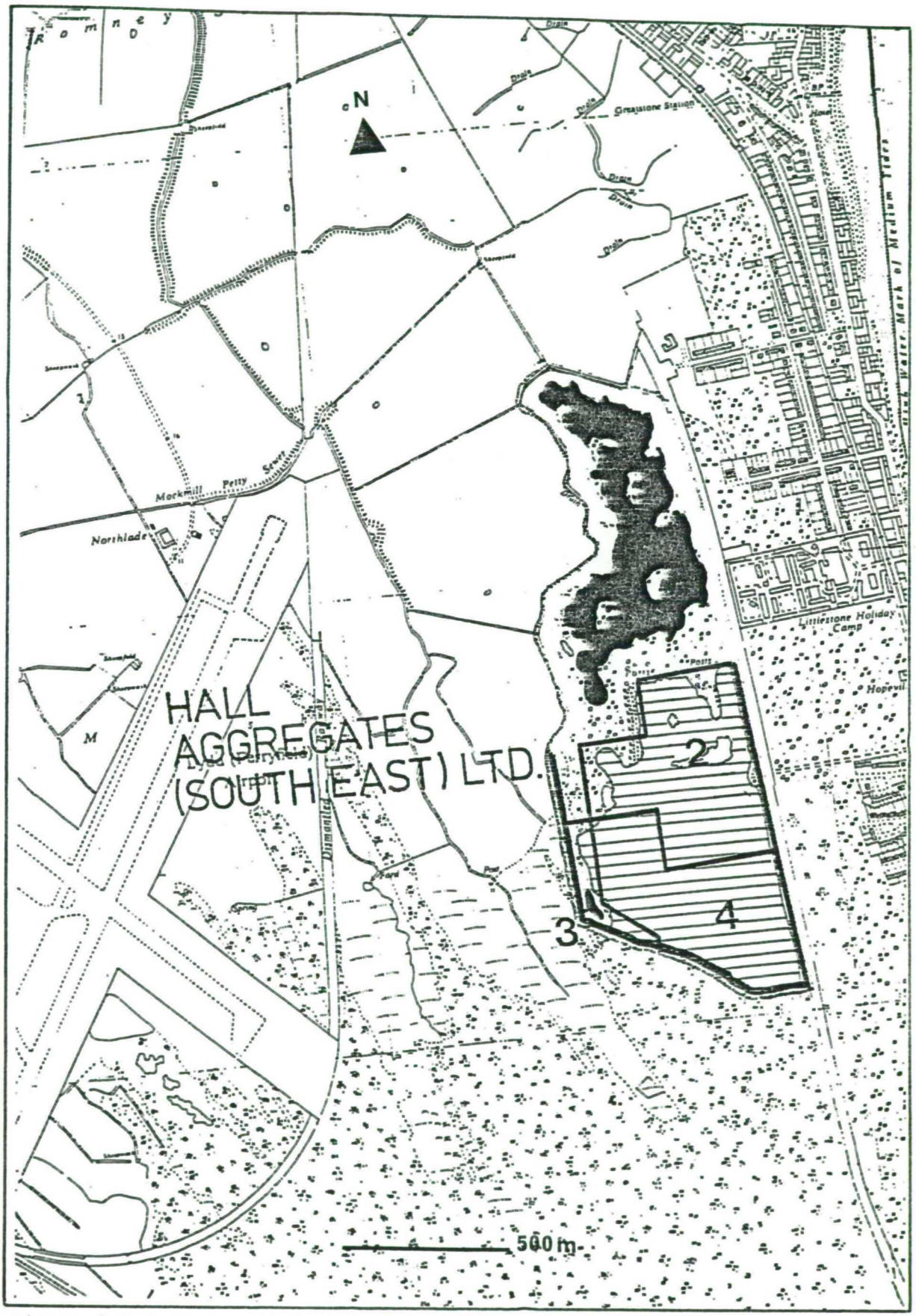


Figure 3.1.1. A proposed outline for the potentially new gravel pit North of the present Lade gravel pit site

PART III: PRESCRIPTION

CHAPTER 3.1 FUTURE SITE DEVELOPMENT

3.1.1 Site Owners and Development Plans

It is vital that an agreement is reached with Hall Aggregates to retain the conservation value of Lade gravel pit. This must involve a balance between conservation interests and the commercial extraction of shingle north of the present site. It is proposed that a condition be added to the 1976 planning permission which would require Hall Aggregates to retain an area of shingle between the present Lade pit site and the newly excavated area in order to retain the present habitat of the Medicinal Leech. A possible plan is shown in Figure3.1.1 . It assumes that shingle extraction occurs down to groundwater level. Notice that the proposal is for two adjacent gravel pits rather than the large single gravel pit currently projected by Kent County Council in the Dungeness Countryside Plan.

The following proposals for the newly excavated site should also be incorporated as additional conditions to the 1976 planning permission.

(i) pit margins should be shallow to enable colonization by emergent vegetation (cf very steep gradients on eastern and southern margins of Lade gravel pit.

(ii) creative pit 'edge sculpturing', (Kelcey, 1978), forming more sheltered inlets and a variety of pit margin profiles, thus encouraging the development of marginal and emergent vegetation.

(iii) the provision of more islands for roosting and nesting birds. Relatively bare shingle with management to reduce scrub formation could attract nesting terns.

These conditions would not be incompatible with the commercial requirements and could allow dispersal of the Medicinal Leech by the creation of a suitable habitat for birds and amphibia.

3.1.2 Site Tenure and Management Agreements

The ownership of Lade gravel pit will remain with Hall Aggregates until all commercial operati ns in the area have been completed (at least 10 years). It is pr p sed that a manag ment agreement is reached as soon

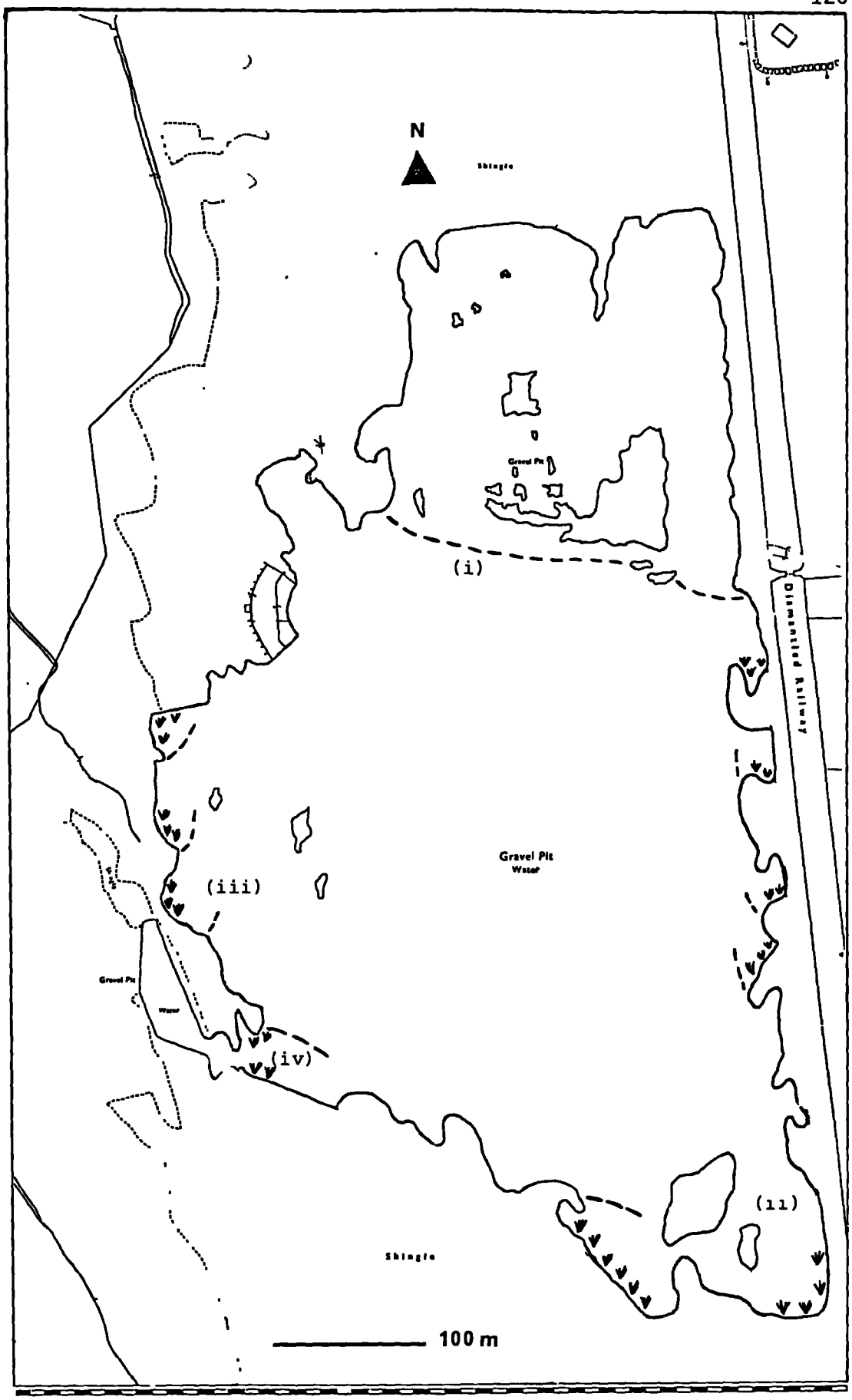


Figure 3.1.3 An improved Lade gravel pit after the implementation of the management proposals (i)-(iv) refer to specific proposals in text)

----anchored log for shelter

planting programme for emergent macr phyte

as possible with a c nservation organization. At present the RSPB is a major land owner on Dungeness and is seeking a leasehold arrangement for access and management of a high quality shingle area to the south of Lade gravel pit. This area is presently owned by Folkestone and District Water Company. It is proposed that the RSPB enters into a similar agreement with Hall Aggregates eventually leading to purchase. The ornithological interest of Lade gravel pit has been described and it could provide a supporting role, and relieve public pressure on Burrowes pit.

3.1.3 Site Management Projects

Much of the management of flooded gravel pits has been involved with the creation of habitats favourable to wildfowl (Harrison, 1972; Milne, 1974; Game Conservance, 1982; Scott, 1982). The management proposals described below are designed to indirectly enhance the survival potential of the Medicinal Leech at Lade gravel pit. By creating conditions which are attractive for more birds and extending favourable habitats for the resident amphibian populations, it could increase the food supply of the leech which has been found to feed on these hosts.

Figure 3.1.3 shows an improved Lade gravel pit after the implementation of the following management proposals.

(i) The northern end of Lade gravel pit requires protection from the erosive action of waves created by S.W. prevailing winds. In bad weather emergent vegetation is often dislodged. Increasing the shelter, possibly by anchoring heavy logs (see Game Conservancy, 1981), could create additional areas for nesting sites and amphibian display areas.

(ii) The unstable shingle along the steep eastern and southern margins of Lade gravel pit is not conclusive to the establishment of emergent vegetation. These gradients could be sm othed by a bulldozer to provide a range of profile gradi nts and create a more indented margin. In the sheltered inlets formed a planting programme f emerg nts would increase the rate of natural colonization. Increasing the extent of vegetated pit margin could result in further colonization by the Medicinal Leech.

(iii) Although the gradient of the western margin of Lade gravel pit is shallow, considerable erosion, by wave action, has created an abrupt shoreline, severely reducing colonization by the larger emergent macrophytes. The creation of sheltered conditions using anchored, floating logs and the subsequent planting of, for example, Phragmites communis, would provide an extension of those favourable conditions already present at the northern end of the pit.

(iv) The 'small pond' site, adjacent to Lade gravel pit contains important numbers of Medicinal Leech. At present it is separated from an inlet of the main area by a narrow width of shingle (2-3 metres wide). Removing this shingle would convert the small pond into a sheltered inlet of the main site and could allow the leech to disperse into those favourably managed areas along the western and southern margins of the pit.

The following three management proposals would also be designed to enhance indirectly the survival of the leech:

(v) Although zone (2) at the northern end of Lade gravel pit provides cover for birds and nesting sites for Coots, Moorhens and a pair of Mute Swans, nesting Mallard duck could be encouraged by the provision of suitable artificial nesting boxes, either mounted on stakes (woven, basket types) or on nesting rafts (timber type).

(vi) Chemical analyses of Lade gravel pit waters indicate low levels of dissolved nutrients. It is in 'closed system' with no inflowing streams to provide nutrient replenishment. There are few mature trees to supply significant amounts of leaf litter and bottom samples from several areas throughout the site indicate that little detritus has accumulated. Experiments at a complex of gravel pits at Great Linford, Bucks, indicated that the addition of waste barley straw stabilized and added to bottom silts. Andersson and Danell (1982) and Street (1982) found that decaying straw was rapidly invaded by large numbers of macro-invertebrates, especially Asellus

aquaticus and Chironomus sp larvae. This increase in invertebrate productivity, if encouraged by similar methods at Lade gravel pit, could provide additional food for birds, fish and amphibian populations, all important sources of food for the Medicinal Leech.

(vii) Fish populations may have severe consequences on vegetation and invertebrate populations and compete directly with waterfowl (Anderson, 1982). Therefore fish populations should be carefully managed at Lade gravel pit. At present there is no evidence to suggest the presence of such cyprinid species as Carp which can destroy submerged vegetation by grazing (Britton, 1982). The consequences of such an introduction could be a reduction in the invertebrate assemblage associated with the submerged vegetation which also offers cover for the Medicinal Leech, which otherwise appears conspicuous against the light background of a bare shingle bottom.

3.1.4 Public Education and Interpretation

A careful programme of public education and interpretation of the general wildlife value of Lade gravel pit could reduce the degree of damaging public pressure in the area. As Catchpole and Tydeman (1975) emphasised, a gravel pit can offer more than just an intrinsic scientific value by acting as a site for public education, interpretation and constructive recreation.

In the long term, after commercial operations have ceased and a programme of management projects has been established, Lade gravel pit could provide an important educational resource for local field studies. A warden could co-ordinate visits and allow access to designated areas. The design of access, involving such techniques as nature trails, pathing, hides, refuges and screening, is discussed by Haslam (1973) and a detailed review of interpretive planning is provided by Beatty (1978).

Kent County Council's Dungeness Countryside plan has identified a need for an Interpretation Centre for Romney Marsh and Dungeness to increase public understanding of the scenic and conservation value of the area. Land between Lade gravel pit and the coast road could be a valuable site for such a centre.

CHAPTER 3.2 MANAGEMENT RESOURCES

3.2.1 Finance

The model for gravel pit management with the creation of a Wildfowl Centre for research and education is based at Great Linford, near Milton Keynes in Buckinghamshire. This series of gravel pits is managed by the Game Conservancy and financed entirely by the Amey Roadstone Company. It represents an excellent example of Industry sponsoring landscape restoration and research for conservation.

The finance to support the habitat management proposals at Lade gravel pit could be provided by the present owners, Hall Aggregates (Ready Mix Concrete). Indeed it is becoming increasingly important for industries which make an impact on the landscape to be seen as caring for the environment. If a plan for an interpretation centre at Lade gravel pit materialised the RMC group should consider a major financial sponsorship.

3.2.2 Equipment

The major relandscaping proposals involving 'edge sculpturing' and island creation would require heavy land moving equipment. This could be provided by Hall Aggregates while their commercial operations continue in the area. Equipment for small scale work, such as vegetation planting, construction of nesting sites, hides and paths, etc. could be provided by the RSPB and other interested conservation organisations.

3.2.3 Manpower

It is hoped that, as possible future leaseholders of Lade gravel pit, the RSPB will provide regular wardening in the area. Volunteers from such organisations as the Kent Trust for Nature Conservation or the British Trust for Conservation Volunteers (BTCV) could be employed on management projects.

CHAPTER 3.3 THE ROLE OF ORGANISATIONS

3.3.1 The Nature Conservancy Council (NCC)

At present the NCC is involved with the renotification of the Dungeness SSSI, using wildlife evaluation techniques and environmental impact assessment of potentially damaging operations. The NCC recognises Dungeness as having an outstanding assemblage of invertebrates but at present the Medicinal Leech, H. medicinalis is not included in the official species lists. This should be amended. The Medicinal Leech should also be regarded as having the status of 'a species in need of special protection', which has so far only applied to two rare species of Lepidoptera.

As the government department with responsibility for nature conservation, the NCC has a statutory obligation to enforce the 1981 Wildlife and Countryside Act. It should ensure that the Medicinal Leech is included in schedule 5 of the Act, 'animals which are protected', which is presently undergoing revisions.

In the NCC's contribution to the U.K. response to the World Conservation Strategy (Wilkinson, 1984), an increase in public awareness of conservation was regarded as a major objective. The NCC should, therefore, have an important role to play in the establishment of an education and interpretation programme for Dungeness.

3.3.2 Kent County Council

At the present time the Planning department of KCC is revising the Dungeness Countryside Plan. It should be more fully aware of the ecological significance of Lade gravel pit and consequent to this replace the water recreation status with that of wildlife conservation.

There should be a wider reassessment of the county and local council's policies towards the use of ecological evaluation in planning (Selman, 1982), and more resourc s should be made available to employ a team of full-time ecologists within the planning department (Gordon, 1983).

3.3.3 Wye College

The problems of Dungeness should receive greater attention from undergraduate and postgraduate studies within the Department of Environmental Studies and Countryside Planning. An examination of conflicting land uses in the area would provide the subject of a special or research study. The responsibility for monitoring the Medicinal Leech population at Lade gravel pit should be continued by the College with the assistance of the Kent Trust for Nature Conservation and the Kent Field Club.

3.3.4 Non-governmental Organisations

A much more detailed, comparative examination of the aquatic, invertebrate fauna of the wetland areas on Dungeness should be organized by the Kent Field Club.

A series of special reports should be compiled by the Kent Trust for Nature Conservation, on the threats to wildlife and conservation value at Dungeness to increase public awareness of the problem. Finally, an all party conservation committee should be established to include academics, amateur naturalists and representatives from the Nature Conservancy Council, Kent County Council, the extractive and water Industry and the Ministry of Defence, to formulate a future conservation plan for the area.

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CHAPTER ONE

AN EXPERIMENT USING MARK, RELEASE AND RECAPTURE TECHNIQUES TO ESTIMATE THE SIZE, STRUCTURE, DISPERSAL AND GROWTH, IN THE WILD OF A POPULATION OF HIRUDO MEDICINALIS AT LADE GRAVEL PIT, DUNGENESS, KENT.

1. Sampling methods and estimating population size

A sampling programme, designed to be an absolute estimate of population size (Southwood, 1978), was developed against a background of incomparable quantitative methods used in previous studies of other leech species in Britain (Mann, 1953, 1957; Elliott, 1973a; Aston and Brown, 1975 and Murphy and Learner, 1982). The absence, in the published literature, of a quantitative study of another population of H. medicinalis also added to the problem of devising a sampling programme.

The problem of designing a sampling programme lay in defining precisely the population and the application of an appropriate sampling method. This required a consideration of the behaviour (i.e. the relative mobility as defined by Blower, Cook and Bishop, (1981)) and the possible effects of feeding on the activity of H. medicinalis in the wild.

Laboratory studies by Young, Dedwylder and Friesen (1981) showed the variable response of the leech to surface water waves and the effects of feeding on this response. Dickinson and Lent (1984) found that satiated leeches cease their repertoire of locomotory movements in response to surface water waves and seek a resting place. These laboratory findings, together with preliminary observations by this author, of small leeches (<0.5 g) feeding on the smooth newt (Triturus vulgaris) in the wild, suggested that in a wild population a large fraction will be below an 'optimum satiated level' and could be expected to respond to surface wave stimulation. A smaller fracti n of the population, having fed recently, may have reached an optimum satiated level and might be expected to remain sessile. The problem was further compounded by the

difficulty of defining exactly 'optimum satiated level' since this would certainly vary with each individual and depend on an unknown dietary history.

The central problem for constructing a valid sampling method was that the population fraction below an optimum satiated level could be regarded as being relatively mobile (i.e. able and likely to enter or leave a sampling quadrat during the sampling interval) and whose absolute size could best be estimated by Marking, Release and Recapture methods (MRR). Here animals are captured within an entire sampling area (not a known sampling fraction), marked and released and a further sample taken at a later time. From this an estimate of the sample fraction (i.e. the fraction of the entire population within the sampling area) can be derived from the proportion of marked animals recaptured in the subsequent sample. A clear and full discussion of these methods is given by Begon (1979) and Blower, Cook and Bishop (1981).

The population fraction which has reached an optimum satiated level could be regarded as relatively immobile (i.e. unlikely to leave or enter the sampling quadrat during the sampling interval) and whose absolute size could be estimated by sampling a known fraction (area/quadrat) of the habitat (Elliott, 1971; Elliott and Tullet, 1978; Randall, Spelling and Young, 1985).

A series of preliminary samples, using a 1 metre quadrat in areas of stoney bottom and macrophyte ($<$0.5 m depth), did not recover any satiated, sessile leeches, but the water disturbance created during the sampling period attracted swimming individuals from outside the quadrat area. It proved impossible to count accurately those individuals which may have originally been within the quadrat area from those which had entered from outside. This area sampling method, to estimate the size and dispersion of the population, therefore, proved unsuitable. Against this background, a relative sampling meth d, combining elements of catch-per-unit effort and trapping based on attraction by water disturbance was developed to collect leeches for an MRR programme.

Although MRR methods represent an absolute estimate of population

size, limitations apply to relative methods of collection; the most important, according to Southwood (1978), is that collection is based on the proportion of the population that is in a 'phase' to respond to the sampling method and that did so under prevailing climatic conditions and the current level of efficiency of the method. The following factors were considered to be important in this study.

(i) Changes in the numbers of animals in a particular 'phase';

(ii) Changes in activity;

(iii) Changes in efficiency of the sampling method.

(i) The 'phase' of an animal refers to the susceptibility of it being caught as behavioural attributes and responses vary over time. Southwood (1978) reviewed a series of studies which showed phase changes in different species of insect. Many found that the phase of the animal was dependent on age and reproductive cycle. For example, a population study of the mosquito, Aedes aegypti (L) by Conway, Trpis and McClelland (1974) showed that the behaviour of adult females was closely linked to the gonotrophic cycle and level of satiation, i.e. there was an obligatory resting stage after gorging.

The possible effects of satiation level on the response of the leech to a sampling method of water disturbance has already been discussed. It was felt that the concept of age as applied to the leech was not an important factor likely to affect the sampling method. Laboratory studies, described in Chapters 4 and 6, indicated that size and possibly sexual maturity were determined more by dietary history than time. The effects of the leech reproductive cycle on the sampling method were uncertain although it was accepted that the susceptibility of gravid leeches being caught would be reduced to zero during the period of cocoon laying.

(ii) Southwood (1978) suggested that the level of activity of an insect will be governed by its diurnal cycle conditi ned by prevailing climatic c nditions. From preliminary survey work on leech behaviour in the wild (see Chapter 2), it was possible to include in addition to diurnal effects, seasonal factors affecting activity (e.g. water temperature).

(iii) The efficiency of a sampling method is measured in terms of the percentage of those animals present that are recorded (Southwood, 1978). In laboratory trials, to test certain assumptions about the use of MRR methods, a 90-95% response rate to water disturbance was recorded in experimental groups of hungry leeches (see 'testing MRR assumptions' in the method section of this chapter). It was decided that water disturbance was an acceptably efficient, relative sampling method compared to attraction by a baited trap design.

2. Mark, Release and Recapture programme

(i) Marking techniques

A basic prerequisite for the use of this method of population estimation is a technique for marking the animals so that they can be released, unharmed into the wild and recognised again on recapture. Such marking techniques can also be employed to study dispersal, longevity and growth, particularly if animals carry a unique mark (Cook, Thomason and Young, 1976; Brakefield, 1982 (a), 1982 (b)).

Many techniques have been developed for marking animals. Southwood (1978) provides a comprehensive review of methods applied to insects while a series of papers in Stonehouse (1978) describe techniques applicable to vertebrates. Very careful consideration was given before initiating a marking programme on the leech population as many of the techniques for marking invertebrates were developed for the hard, arthropod skeleton. Techniques, for example, involving the use of non-toxic paints, suggested by Corbet (1952) and Holt (1955), or mutilation (Edwards ,1958) were not appropriate for the Medicinal leech.

The possibility of recognising individual leeches using the animal's natural markings was considered first. This type of recognition has been used successfully in the study of larger animals, most notably the Bewick's swan (Scott, 1978), and the larger African game animals (Pennycuick, 1978). Within the leech population the ground colour on the dorsal surface varied from alm st black, to shades of brown and green. The degree of variability in this one feature alone was

insufficient to provide unique recognition. There was, however, great variation of patterning on the ventral body surface, comprising a black and yellow mottled appearance. (cf. a uniform, olive-green ventral surface was noted in individuals purchased from suppliers in France and Germany). The two pairs of orange, longitudinal stripes on the dorsal surface had a variable degree of black marking along their length. In a resting leech this highly distinctive combination of natural marks, if carefully mapped against the body plan of segments, provided a potentially unique recognition system. The major disadvantage was the continual change in body shape resulting in distortion of the marks. It was also impossible to use this method as a quick means of identification, important to reduce handling and captivity time. It was therefore decided that the application of a mark was necessary.

It was important to apply a mark which did not affect the leech's behaviour and which overcame the problems of a completely flexible body surface. Rigid marks, such as tagging, or the impregnation of latex, were rejected as being likely to disrupt movement. A system of radiotracking the giant leech, Haementeria ghilianii, using a transmitter attached to the body surface, was used with some success by Sawyer (pers. comm.). The possibility of using radiotracking with H. medicinalis was not possible due to limits on the minimum size of transmitters and batteries (Macdonald, 1978 and Linn, pers. comm.). The use of coloured paints and dyes was precluded by the moist body surface and, in certain individuals, a very dark body colour.

It was decided, after a suggestion by Dr. R. T. Sawyer (pers. comm.) to develop a system of careful branding of the body surface after preliminary experiments had shown that recovery was good and the resulting scar tissue provided an easily recognizable and long lasting mark. Freeze-branding was used successfully on Amphibia and Reptiles (Woodbury, 1956; Daugherty, 1976) and with Nudibranch molluscs (Anderson, 1973). Work by Legore and Sparks (1971) and Sparks (1972) on the ability of the Rhynchobdellid leech Pisciola salmostica to repair its body wall after incision and hot branding, sh wed an inflammatory response which sealed off the damaged area. The survival period after this treatment was however, short.

Due to the necessity of developing a marking technique to estimate population size and other parameters, together with the absence of viable alternatives, a system of marking, using hot branding, was developed. A series of preliminary experiments to test the effect of this marking method and its use in the MRR study are described in the methods section, together with the marking design and system.

(ii) MRR models and assumptions

A range of models has been developed differing in assumptions about the pattern of survival within the population, the statistical approach underlying them and their relative efficiencies at different sampling intensities. These models have been described in detail by Cormack (1968, 1973), Seber (1973), Begon (1979) and Blower et al. (1981). There are four basic assumptions which apply to all the MRR models.

(1) That all marks are permanent and are noted correctly on recapture.

(2) That being caught, handled and marked has no effect on an individual's subsequent chance of capture.

(3) That the population is sampled randomly with respect to its mark status.

(4) That sampling is at discrete time intervals and the actual time involved in taking the samples is small in relation to the total time.

The selection of an MRR model depends on the design of the marking system and the data obtained. A series of single, date-specific marks were used on the main fraction of the population at Lade gravel pit. This enabled the use of more complex models based on multiple mark and recaptures. They have an advantage over the simple Petersen estimate of population size, which depends on a single mark and gives only one estimate; a relatively small amount of information per unit effort. It was felt that there was a limit to the number of date specific marks which could be applied to the dorsal side of the leech body surface which did limit the use of some of the multiple MRR models.

The selection of the MRR models depends on certain assumptions about the population and particularly the quality of data obtained. The selection of two models for this study is discussed in the results section.

A unique marking system was employed on a small fraction of the population in the small pond adjacent to Lade gravel pit. The aim was to obtain recaptures of recognizable individuals over time and, from successive weighings, provide ideas about feeding and growth in the wild.

3. Population structure and dispersal

In the many ecological studies on other British freshwater leeches (see Elliott and Mann, 1979, for a short review), it has been standard practice to show seasonal changes within the population, in terms of changes in the frequency of body weight classes. This description of population structure, in terms of the proportion of different weight classes, has been important to our understanding of factors such as survival, particularly during overwintering periods, and the proportion and stage at which certain weight classes attain sexual maturity. The estimation of population size is important for assessing the viability and potential threat of extinction. The distribution of these numbers gives an idea of structure and represents a more dynamic picture of the population. An assessment of the population structure of the Medicinal Leech at Lade gravel pit was based on samples obtained over field seasons 1983, 1984 and 1985.

While population structure gives an idea of changes over time, dispersal is an important description of a population in space. No population is static and all individuals move to a greater or lesser extent. While the application of marks to individuals in a population is a useful tool for using models to estimate size, the mov ment of these marks in space provides useful information on dispersal. The boundaries of the Medicinal Leech population were clearly defined by Lade gravel pit and it was evidence of movement within this area that was of particular interest.

Methods

1. Sampling methods

Leeches were obtained in the hand by attraction to water disturbance at the pit margins in a water depth of less than 1 metre. This involved rhythmic splashing by hand, at the water surface, and displacement of the water column using leg movements. After a variable time period leeches either appeared as free-swimming individuals or affixed to waders. Care was required to recover individuals attached to the waders, since lifting them from the water often induced the animals to drop off. After collection leeches were placed in securely fastened plastic bags. The ability of the leech to push itself through the smallest hole was not underestimated and so the collection bags were further secured in screw top plastic containers.

The sampling period varied according to the objective. During the first field season in 1983, when preliminary sampling trials were made, no fixed time period was established while collecting for serological samples as well as the unique marking programme of leeches in the small pond. In the following field season specimens for unique marking and serological samples were obtained from the small pond during two hour periods.

For the main Mark, Release and Recapture experiment, during the summer months of 1984 and 1985, the sampling period was determined by the area covered. Figure 1.1 shows the northern end of Lade gravel pit divided into nine areas. The whole area was regarded as continuous and a full description of the site is given in the Management plan. The following sampling times were spent in each zone according to approximate relative size:

Zones 1, 2/1, 2/2, 3/1, 3/2, 3/3	=	½ hour
Zones 4, 5	=	1 hour
Zone 2	=	2 hours

Each area was covered at random with 10-15 minute sampling activities at a series of points in the area. The total 7 hour sampling period was carried out on a series of single days per month between April and July 1984, with the final sample taken on a single day in June 1985.

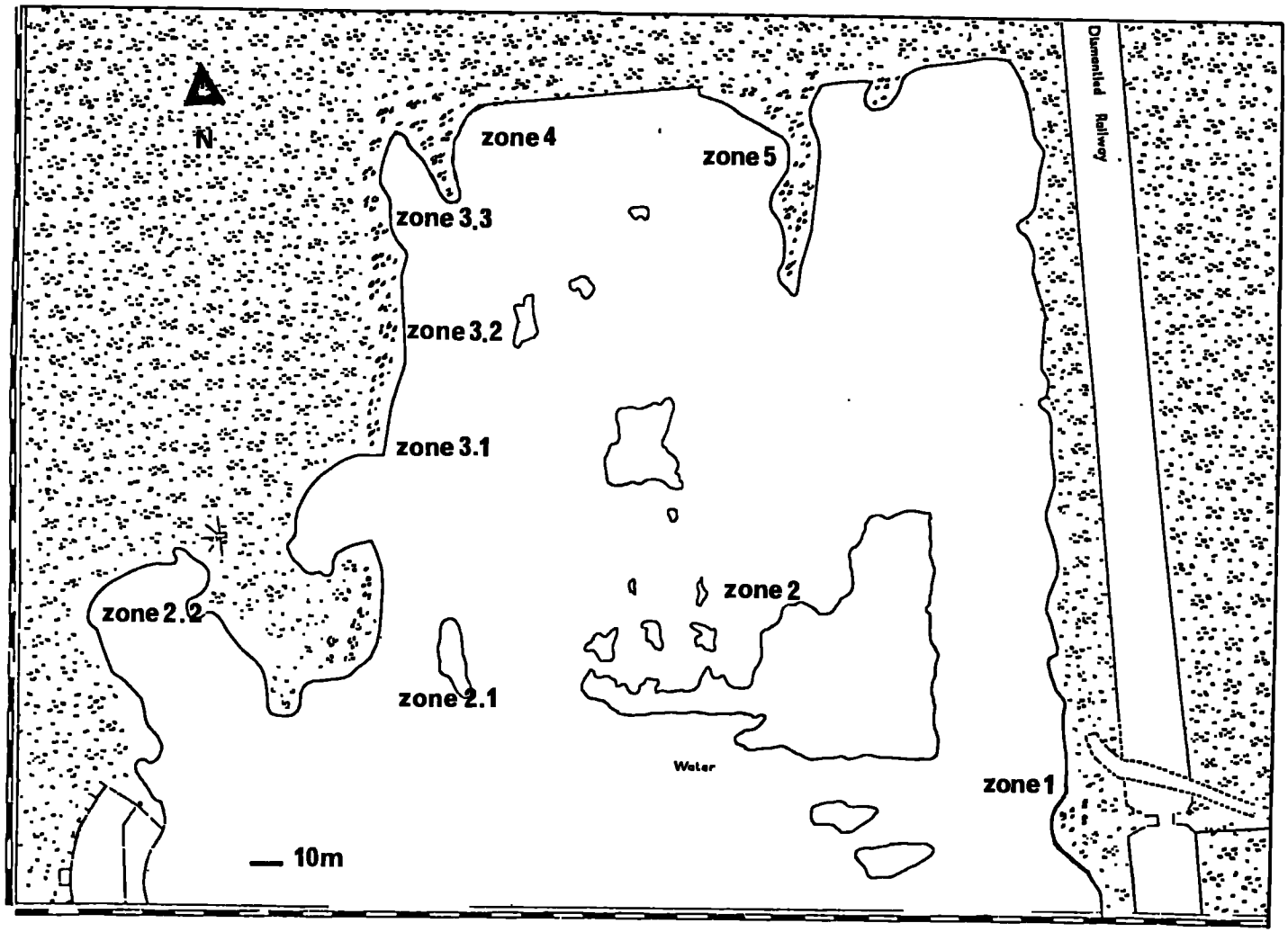


Figure 1.1 The Northern end of Lade gravel pit showing the nine sampling zones for the Mark, Release, Recapture experiment

2. Marking methods and patterns

It was essential that the technique of hot branding was fast, effective and did not affect the survival of the leech. All marks were applied on or around the mid-line of the dorsal surface, well clear of the head and posterior sucker regions and avoiding ventro-lateral nephridiopores and ventral reproductive apertures.

A satisfactory method for applying a hot brand was developed from the internal tungsten filaments of an ordinary domestic light bulb. The filament proved sufficiently malleable to fashion different shaped branding marks and, most importantly, retained sufficient heat, after removing from a flame, to apply a rapid and clear mark. The base of the light bulb also provided a useful handle. A large individual, for marking, was removed from its container, blotted dry and held firmly over the index finger with the required section of dorsal body surface upwardly exposed. The tungsten filament was held in a flame until red hot, removed and allowed to cool momentarily. The brand was quickly applied with a light touch to the body surface and the leech was immediately replaced into its water filled container. Leeches obtained from the wild and marked in this way were retained in the laboratory for at least 24 hours before returning to the wild. There was a mortality rate during captivity of 0.5% which was felt to be at a reasonable level.

Two systems of mark pattern were devised, one acting as a unique mark for individuals in the small pond and the other specifying the date captured and, for leeches in excess of 1 g body weight, the designated zone at the north end of Lade gravel pit. For leeches weighing less than 1 g in the second marking system, a series of date specific spot marks only were used instead of brand shapes. It was felt that the size of the brand shapes would cause a serious impediment to recovery in this weight class of leech. All marks, with the exception of Day 3 and 4 spot marks to leeches weighing less than 1 g in the MRR experiment, were applied t the dorsal surface. Figure 1.2(a) shows the pattern of spot marks used for marking individuals uniquely in the small pond. The maximum number of individuals which could be marked with this system was 999. At all times the least number of spot marks were used to compose the unique numbered mark. Figure 1.2(a)

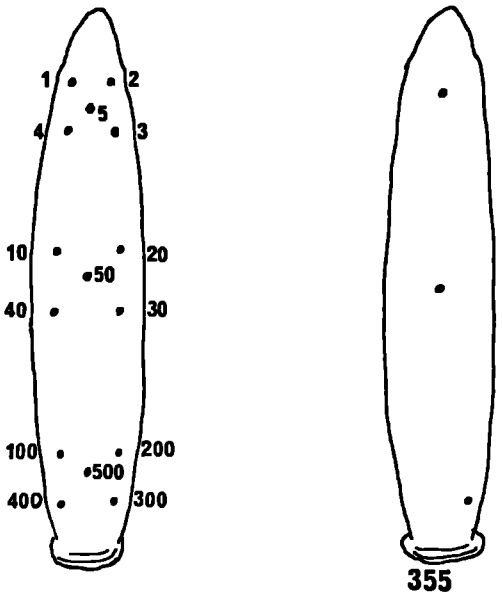


Figure 1.2(a) The system of spot marks used to uniquely mark Medicinal leeches in the 'Small Pond'

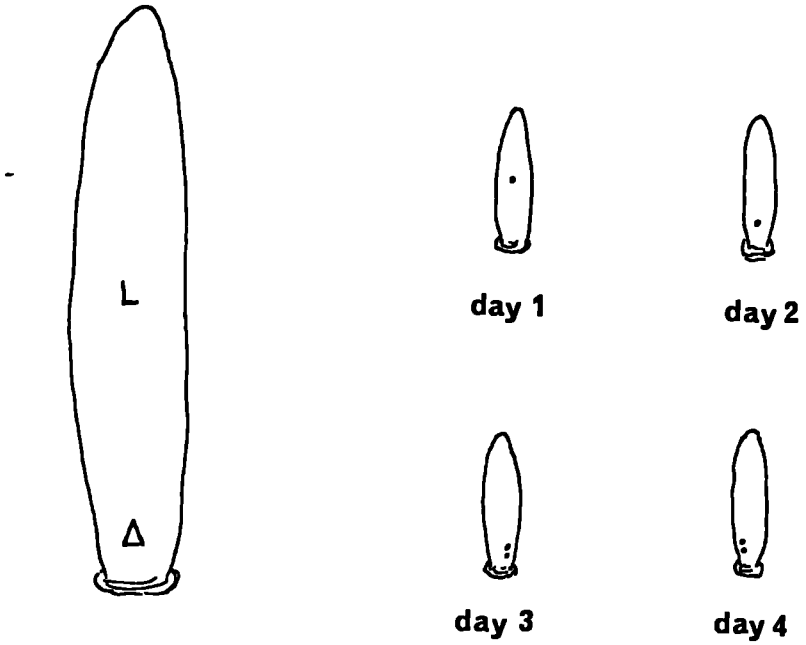


Figure 1.2(b) The system of Zone and date specific marks used in the Mark, Release, Recapture experiment

Table 1.1 The system of Day Zone specific brand marks used for the Mark, Release, Recapture experiment

	Zones									
Day	1	2	2/1	2/2	3/1	3 2	3 3	4	5	
1	L	∩	C	⊃	⅂	≺	^	U	Δ	
2	|	—	|·	÷	⁄	∖	¯	/	\	
3	L	∩	C	⊃	⅂	≺	^	U	Δ	Po terior
4	|	—	|·	÷	⁄	∖	¯	/	\	

illustrates this with an example for leech number 355. Figure 1.2 (b) shows an example of a combination of two brand shapes on an individual exceeding 1 g in weight and the pattern of date specific spot marks used for leeches weighing less than 1 g. In this example the large individual was marked initially on day 1 in zone 1 and later recaptured on day 3 in zone 5. Day 3 and 4 spot marks were applied ventrally. Below the figures Table 1.1 shows the complete set of brand shapes used for date/day and zone specific marking. Day 1 and 2 marks were applied in the mid-dorsal region of the body surface while day 3 and 4 marks were applied in the posterior region of the mid-line.

3. Experiments to test the MRR assumptions

The main concern in this set of experiments was to examine the possible effects of the marking technique on behaviour and survival before using it to study the wild population.

Single and multiple shaped brand marks were applied to 25 large hungry leeches obtained from a commercial supplier. After marking and replacing leeches into a separate water filled container, each individual's behaviour was observed for 1 hour after marking and on subsequent days. For a short period immediately after branding, behaviour was subdued but soon returned to active searching behaviour. A simple test to examine whether individuals were able to continue searching involved placing them into an aquarium full of water. Rapid water disturbance continued to initiate a typical swimming/searching behaviour in 95% of trials 2-3 hrs after markings.

Two further factors were investigated which were important for the successful use of the MRR models. One was the ability of the leech to repair the wound site and the other was the effect of branding on long term survival. Preliminary experiments showed a good recovery of the brand site resulting in a discrete area of scar tissue. The arrow on plate 1.1 shows an L-shaped area of such tissue on the mid-dorsal surface (corresponding to Day 1, Zone 1 in the MRR programme). Of the trial group of 25 leeches, 80% survived after branding for the period of 12 months up to the time when the main MRR experiment was undertaken. At present six individuals survive over 3 years after

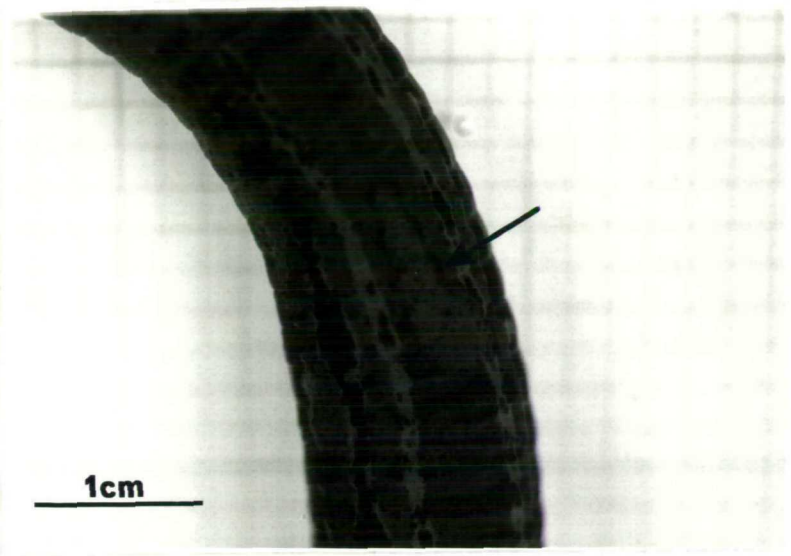


Plate 1.1 L-shaped scar tissue on the mid-dorsal body surface of a Medicinal leech, 24 hours after hot-branding.

initial branding. Mortality in the rest of this group was linked to laboratory feeding experiments and apparently natural ageing effects rather than any effect from the brand. A similar set of experiments, examining the effect of single and multiple spot brands on the behaviour and survival of hatchling H. medicinalis, gave similarly favourable results indicating that branding was an acceptable method for all sections of the wild population.

Results

1. Mark, Release, Recapture models

The determining factors for using an MRR model was the quality of the data obtained, defined as the incidence of recaptured marks, and certain preconceived assumptions about the dynamics of the population of H. medicinalis.

The most widely used models, based on data obtained from more than three mark and recapture events, are those of Fisher-Ford, Jolly and Manly and Parr. Each differs in its statistical approach and assumptions about the population.

The Fisher-Ford method assumes that survival rate in the population is constant and independent of the age of the animal. It is particularly robust in situations where relatively little data is accumulated, particularly in terms of recaptures. Its estimates of population size, gain and loss, are derived by grouping data together to produce a single survival rate. The effect of grouping data also reduces sampling error. If the central assumption of constancy of survival-rate is subject to variation in the real population, the strength of this model is weakened and precautionary tests of significance for variation in survival rate should be undertaken.

If it is believed that, over the period of MRR study, survival rate varies from day to day, then Jolly's method becomes more appropriate than Fisher-Ford. Related to this, survival is probablistic

or stochastic, rather than deterministic - an important gain in realism. Although Jolly's method assumes age-independent survival, it is largely unaffected by age-dependent survival, as long as survival is independent of mark status and capture probability is independent of age. Thus Jolly's method is appropriate whenever there is sufficient data, except in cases of strongly age-dependent survival.

The Manly-Parr method assumes less than the two previous models discussed. It allows a survival rate which is both variable and age-dependent. it is therefore preferable to Jolly only if survival is strongly age-dependent. This is an important advantage since survival is probably never strictly independent of age and is sometimes very far from independent as in cases of dramatic infant mortality. Its main disadvantage is that large numbers of multiple-recaptures are necessary before population estimates can be relied upon. For the benefit of the study, therefore, the Manly-Parr model was considered unsuitable for an experiment involving only four marking occasions and five captures.

It is useful to note Begon (1979) who stated that it is often impossible to know if one chosen MRR model is unequivocally the best, particularly if ecological information on survival is uncertain. At such times the reconciliation of answers from different models becomes important although he suggests that there should never be more than two models used for the same multiple capture-recapture data. On this basis the models of Fisher-Ford and Jolly were applied to the data obtained from the MRR experiment on the population of H. medicinalis.

For the purpose of data analysis the totals of zone/date and date specific marks were used. (N.B. the zone specific element of the mark provided important evidence of dispersal rather than being of particular relevance to the MRR experiment.) Population estimates were calculated for the whole sampling area rather than for each zone. The notation used is the same as that used by Beg n (1979) and Table 1.2 shows an extracted summary. Although the time between the first four successive captures was on average 30 days, for the purp se of analysis sampling was descrıbed as occurring on day 1, day 2, etc.

Table 1.2 Summary of notation used in the mark, release, recapture models - adapted from Begon (1979) - "Investigating animal abundance - capture - recapture for biologists".

m_i	the number of marked individuals caught on day i
m_{ij}	the number of individuals caught on day i with day j mark
n_i	the number of individuals caught on day i
r_i	the number of (marked) individuals released on day i
y_i	the number of individuals marked (and released) on day i and caught again subsequently
z_i	the number of individuals marked before day i, not caught on day i, but caught again subsequently
A_i	the mean age of marks on day i
B_i	the number of additions to the population between days i and i + 1
L_i	the number of losses from the population between days i and days i + 1
M_i	the number of marks at risk on day i
M_{ij}	the number of day j marks at risk on day i
N_i	the population size on day i
ϕ_i	the proportion of the day i population surviving until day i + 1; or the chances of an individual in the day i population surviving until day i + 1

The Fisher-Ford model estimates population size from the modified Petersen estimate by assuming that the ratio of marks to total individuals in the day i sample sample is the same as in the total population:

$$N_i = \frac{(n_i + 1)}{(m_i + 1)} M_i$$

However M_i - the marks at risk on day i - is unknown and must be estimated before N_i can be calculated. This involves comparing the days survived by marks in the population. These should, on average, be the same, but because of sampling error, this equality is unlikely to be exact on any particular day. It is better to add the days survived from different sampling occasions together, and then compare the sum from the samples with the sum from the populations. By doing this, discrepancies should cancel each other out to some extent.

Consider the samples first. The total number of marks caught on day i is given by

$$m_i = \sum_j m_{ij}$$

where j = the day on which the mark was given - varies from i - 1. Each mark is (i - j) old and the total age of all marks is:

$$\sum_j m_{ij} (i - j)$$

If this quantity is summed over all days, we obtain the'total days survived'by marks throughout the experiment.

$$\sum_i \sum_j m_{ij} (i - j)$$

This is not an estimate but the calculation of a quantity, that is actually observed; 'observed total days survived' or ' bserved TDS'.

Consider the whole population, where A_i is the mean age of marks on day i. Immediately before the day i sample there are M_i marks at

risk. During the sample some are removed and examined but at the end they are replaced - along with r_i new marks. Immediately after the day i sample there are therefore $(M_i + r_i)$ marks at risk in the population. At this time the M_i marks have a total age of A_iM_i and the r_i marks - released immediately before - a total age of zero. The mean age of all the marks is therefore given by:

$$\frac{A_iM_i + 0}{M_i + r_i}$$

One day later, immediately before the day i + 1 sample, all these marks are one day older and consequently:

$$A_i + 1 = \frac{A_iM_i}{M_i + r_i} + 1$$

All the r_i's are known. Assume initially that all M_i's are also known.

A_2 - the mean age of marks one day after the first (and only previous) sample - is obviously 1. A_3 can therefore be calculated. This in turn means that A_4 can be calculated and so on throughout the sequence of A_i's. We now have a term, A_im_i which is an estimate of the days survived by the marks caught in the day i sample. Similarly, we can compute $\sum A_im_i$, the estimated total days survived (estimated TDS), which should equal the observed TDS previously calculated from the samples.

In other words, a sequence of M_i's will lead to a sequence of A_i's and finally to a value of $\sum A_im_i$. We are attempting, however to estimate the M_i's themselves. The estimates needed are those that give a $\sum A_im_i$ which is equal to the observed TDS.

Immediately after the day i sample there are $(M_i + r_i)$ marks at risk. Yet between day i and day i + 1 some of these are lost; only $\phi(M_i + r_i)$ survive. (ϕ is the daily survival rate, assumed to remain constant). In other words immediately before the day i + 1 sample there are $(M_i + r_i)$ marks at risk, or

$$M_i + 1 = \phi(M_i + r_i)$$

All r_i's are known. Assume initially that ϕ is also known. M_1 - the marks at risk before the first sample is obviously zero. M_2 can be calcualted ($= \phi r_i$). Now r_2, ϕ and M_2 are all known and M_3 can also be calculated, followed by all the other M_i's in sequence.

In other words, a particular value of ϕ has led, via a sequence of M_i's to a particular value of $\Sigma A_i m_i$. Therefore the final step is to plot a graph of ϕ against $\Sigma A_i m_i$. The ϕ-value that would lead to a $\Sigma A_i m_i$ - value equal to the observed TDS can be read from this graph. This is our best estimate of ϕ and it can be used to obtain our best sequence of M_i's The N_i's can then be calculated.

The number of losses between days i and i + 1 (L_i), and the number of gains between days i and i + 1 (B_i) can be estimated from the following equations:

$$L_i = (1 - \phi)N_i$$

$$B_i = N_i + 1 - \phi N_i$$

Table 1.3 (below) shows the data obtained from the MRR experiment on H. medicinalis at Lade gravel pit.

Table 1.3 Capture-recapture data for H. medicinalis at Lade gravel pit

Day (i)	Captured (n_i)	Released (r_i)	Time of release of marks (j)			
			1	2	3	4
			Recaptured marks m_{ij}			
1	683	683				
2	797	796	89			
3	878	877	63	63		
4	833	830	53	62	55	
5	613	-	30	30	22	26

There were, for example, 62 day 2 marks caught on day 4.

Table 1.4 Observed days survived computed from data in Table 1.3

Day (i)	(m_i)	$\sum_j m_{ij}(i - j)$	
1	0	0	
2	89	89	
3	126	189	
4	170	338	
5	108	280	
		896 =	$\sum_i \sum_j m_{ij}(i - j)$ (observed TDS)

The first step is to calculate the days survived by marks in the samples. For example

$$\sum_j m_{4j}(4 - j) = (53 \times 3) + (62 \times 2) + (55 \times 1) = 338$$

The next step is to find a survival-rate, ϕ, that will give an estimated TDS approximating to 896.

Selecting an arbitrary survival-rate of 0.8 gives:

$$M_1 = 0$$
$$M_2 = 0.8(0 + 796) = 636.8$$
$$M_3 = 0.8(636.8 + 877) = 1211$$

Table 1.5 Estimated days survived computed from data in Table 1.3 with a survival-rate (ϕ) of 0.8

Day (i)	M_i	A_i	$A_i m_i$	
1	0	-	-	
2	636.8	1	89	
3	1211	1.44	181.4	
4	1632.8	1.83	311.1	
5	1306.2	2.21	238.7	
			820.2	(estimated TDS)

A_i was calculated, for example:

$$A_2 = 1$$

$$A_3 = \frac{1 \times 636.8}{636.8 + 796} + 1 = 1.44$$

$$A_4 = \frac{1.44 \times 1211}{1211 + 877} + 1 = 1.83$$

The ϕ value of 0.8 gives an estimated TDS which is considerably lower than the observed TDS.

A value of 1.2 for ϕ gave an estimated TDS of 900.4, slightly too high.

Further ϕ values gave

ϕ	A_iM_i
0.9	843.9
1.0	866.5
1.1	883.4
1.18	899.1

Figure 1.3 shows the resulting graph from plotting the four points of ϕ against $\sum A_i m_i$ and suggests that a ϕ value of 1.176 would lead to a $\sum A_i m_i$ value of approximately 896.

Table 1.6 checks this suggestion below.

Table 1.6 Estimated days survived computed from the data in Table 1.3 with a survival-rate (ϕ) of 1.176.

Day i	M_i	A_i	A_im_i
1	0	-	-
2	936.09	1	89
3	1783.75	1.52	191.52
4	2402.56	2.02	343.40
5	1922.40	2.50	270.00
			893.92

This required sequence of M_i's can now be used to estimate the N_i's, L_i's and B_i's and is shown in Table 1.7 below.

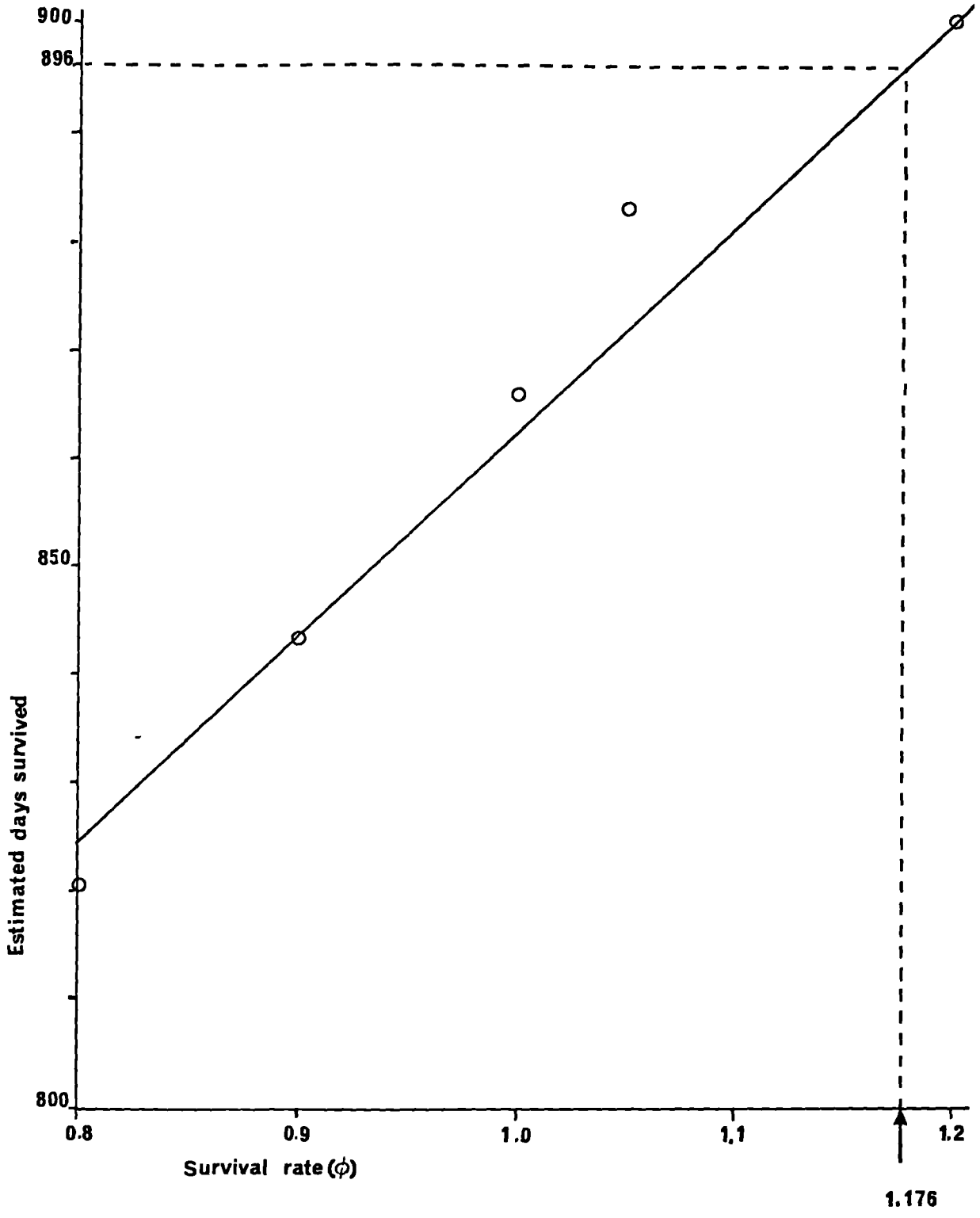


Figure 1.3 Estimating the survival-rate Ø),(Fisher-Ford) for data computed from Table 1.3

Table 1.7 Population parameters estimated from the MRR data obtained from Table 1.3

Day i	N_i	L_i	B_i
1			
2	8293	-1497	2591
3	12343	-2172	-2743
4	11772	2071	-2914
5	10929	-1923	-

From the application of the Fisher-Ford model to the data in Table 1.3, the estimated size of the Medicinal Leech population at Lade gravel pit rose during May and June 1984 to 12,343, dropping slightly during July to 11,772, but showing good survival over the winter period through to June 1985 (Day 5) when it stood at 10,929; the estimated daily survival rate was 1.176.

It is obvious that the assumption of a constant survival rate has been a central feature of the above computations. We can test this assumption by applying a χ^2 test of significance to observed and expected values.

Day-to-day differences in the survival rate of the whole population can be examined by comparing individual $A_i m_i$'s with their corresponding $\Sigma m_{ij}(i-j)$'s. Although the sums of these two quantities have been equated, large inequalities on particular days will indicate differences in survival rate.

The Σm_{ij} $(i-j)$'s were actually observed; the $A_i m_i$'s are what would be expected if ϕ had remained constant. The two sequences shown in Table 1.8 below can be compared using χ^2 with 3 degrees of freedom (4 days minus 1).

Table 1.8 Observed and expected days survived (H.medicinalis data, Fisher-Ford model).

Day i	$A_i m_i$	m_{ij} $(i-j)$
2	89	89
3	181.4	189
4	311.1	338
5	238.7	280

χ^2 = 9.78 with 3 d.f.

P = 0.02

The assumption that ϕ remained constant is disputed by this χ^2 test and Jolly's model may be more applicable

In Jolly's method only the most recent mark is noted, all previous marks are ignored. The estimation of M_i is the central feature of this method,

$$M_i = m_i + (M_i - m_i)$$

m_i is known and so M_i can be estimated if we can estimate $(M_i - m_i)$; the number of marked individuals at risk on day i that were not caught.

Of these $(M_i - m_i)$ individuals, some will be caught subsequently (on day i + 1 or day i + 2, or day i + 3 etc.). They are recognizable because their most recent mark will be one given to them on a day prior to day i. We can define z_i as the number of individuals marked before day i, not caught on day i itself, but caught subsequently. In other words, of the $(M_i - m_i)$ individuals at risk on day i, z_i are caught subsequently.

Immediately after the day i sample, these $(M_i - m_i)$ individuals are joined by the r_i individuals released on day i with a day i mark. We can define y_i as the number of these r_i individuals that are caught again subsequently.

Assume that the fate of these two groups - r_i and $(M_i - m_i)$ - runs parallel after day i, then

$$\frac{z_i}{M_i - m_i} = \frac{y_i}{r_i}$$

m_i, r_i, y_i and z_i can all be obtained directly from the data and the M_i's can, therefore, be estimated

$$M_i = m_i + \frac{z_i r_i}{y_i}$$

Population size is then given by a modified Petersen estimate

$$N_i = \frac{M_i(n_i + 1)}{(m_i + 1)}$$

Immediately after the day i sample there are $(M_i - m_i + r_i)$ marked individuals in the population: the number at risk before the sample, minus those removed during it, plus the new marks added at the sample's end. Of these marked individuals there will be M_{i+1} individuals that survive until the day i + 1. The survival rate from day i until i + 1 (ϕ_i) can be estimated by

$$\phi_i = \frac{M_{i+1}}{M_i - m_i + r_i}$$

Finally, the number of additions to the population between days i and i + 1 is given by

$$B_i = N_{i+1} - \phi_i N_i$$

Table 1.9, shown below, is the same data obtained from the MRR experiment on H. medicinalis at Lade gravel pit with the modification that only the most recent marks are considered.

Table 1.9 Capture-recapture data for Jolly's annalysis

Day i	Captured n_i	Released r_i	Time of release of mark j			
			1	2	3	4
			Recaptured marks			
1	683	683				
2	797	796	89			
3	878	877	57	63		
4	833	830	41	58	55	
5	613	-	26	29	21	26

Remember that only the most recent marks were considered in cases where recaptured individuals carried multiple marks; for example, the actual breakdown of recaptured marks on Day 5 was composed of: 26 day 1 marks only; 1 day 1 and 2 marks; 1 day 1 and 3 marks; 2 day 1 and 4 marks; 28 day 2 marks only; 1 day 2 and 4 marks; 23 day 4 marks only. Note that the totals for each day marks are equivalent to the totals in Table 1.3

Treating the recapture results in this way illustrates the very small proportion of individuals which were recaptured and marked on more than one occasion.

The m_i's, y_i's and z_i's must now be calculated

m_i - the total number of marked individuals captured on day i is the sum of the ith row of the trellis, for example

$m_3 = 57 + 63 = 120$

y_i - the total number of the r_i individuals that are caught subsequently is the sum of the ith column of the trellis, for example

$y_1 = 89 + 57 + 41 + 26 = 213$

z_i - the total number of individuals marked before day i, not caught on day i, but caught subsequently. It is the sum of these m_{ij}'s which are both in columns to the left of column i, and in rows below row i, for example

$z_3 = 41 + 58 + 26 + 29 = 154$ (see boxed area in Table 1.9)

From these series of computations, Table 1.10 can thus be drawn up (see below).

Table 1.10 Preliminary computations for Jolly's method

Day i	r_i	m_i	y_i	z_i
1	683	-	213	-
2	796	89	150	124
3	877	120	76	154
4	830	155	26	76
5	-	102	-	-

Calculation of the M_i's and thus N_i's, ϕ_i's and B_i's is possible by substitution into the appropriate equations.

$$M_i = m_i + \frac{z_i r_i}{y_i}$$

$$N_i = \frac{M_i (n_i + 1)}{(m_i + 1)}$$

$$\phi_i = \frac{M_i + 1}{M_i - m_i + r_i}$$

$$B_i = N_i + 1 - \phi_i N_i$$

For example

$$M_2 = 89 + \frac{124\ (796)}{150} = 747$$

$$M_3 = 120 + \frac{154\ (877)}{76} = 1897$$

$$N_2 = \frac{747\ (797 + 1)}{(89 + 1)} = 6623$$

$$N_3 = \frac{1897\ (878 + 1)}{(120 + 1)} = 13780$$

$$\phi_2 = \frac{747 + 1}{747 - 89 + 796} = 0.514$$

$$\phi_3 = \frac{1897 + 1}{1897 - 120 + 877} = 0.715$$

Furthermore Begon (1979) gives the following formulae for calculating standard errors on the population size and survival rate estimates (based on Jolly):

$$SE_{N_i} = \sqrt{N_i (N_i - n_i) \left\{ \frac{M_i - m_i + r_i}{M_i} \left(\frac{1}{y_i} - \frac{1}{r_i} \right) + \frac{1}{m_i} - \frac{1}{n_i} \right\}}$$

$$SE_{\phi_i} = \phi_i \left\{ \frac{(M_{i+1} - m_{i+1})\ (M_{i+1} - m_{i+1} + r_{i+1})}{M_{i+1}^2} \left(\frac{1}{y_{i+1}} - \frac{1}{r_{i+1}} \right) + \frac{M_i - m_i}{M_i - m_i + r_i} \left(\frac{1}{y_i} - \frac{1}{r_i} \right) \right\}^{\frac{1}{2}}$$

Thus, the complete computations using Jolly's model can be prepared and are shown in Table 1.11 below.

Table 1.11 Final computations using Jolly's method, based on data from Table 1.9

Day i	M_i	N_i	SE_{N_i}	ϕ_i	SE_{ϕ_i}
1	0				
2	747	6623	1020.7	0.514	0.067
3	1897	13780	2166.8	0.715	0.162
4	2581	13781	2764.1	0.792	-

The application of the Jolly model does produce comparable population size estimates to the Fisher-Ford model and this does indicate a consistency in the original data. There is, however, no estimate of population size after the period of Winter 1984/85. The Jolly estimates show a population rise between May and June 1984 but, in contrast to the Fisher-Ford, this remains stable during July.

The change in the number of individuals captured on each of the five sampling occasions and the rise in population size estimates requires some interpretation. The steady rise from early April to July cannot be explained by recruitment due to births into the population. As Chapter 5 describes, cocoons were only found in the wild from late July/ early August with a rise in the proportion of empty cocoons into mid-September. This late summer period is probably the main peri d during which

recruitment, by new hatchlings, occurs in the population. It is therefore likely that these results were affected by changes in the behaviour of individuals actively searching for food (note that the sampling method was applicable primarily to the 'hungry' fraction of the population). As described qualitatively in Chapter 2, general swimming activity increased from April to June with a decline during July and August. Therefore the rise in the estimated population size could be a measure of the increase in the numbers of hungry leeches responding to water stimulation rather than a 'real' increase in population size. Nevertheless, as discussed below, the most important objective of calculating population size was to gain some idea of the order of magnitude of the population.

The other common parameter to both models is an estimate of survival rate, i.e. the proportion of the day i population surviving until day i + 1, or the chances of an individual in the day i population surviving until i + 1.

The accuracy of the estimate of constant survival rate (1.176) from the Fisher-Ford model is questionable and indeed the χ^2 test rejects the assumption of constancy. A survival rate of 1.00 indicates a 100% survival between i and i + 1. The more realistic estimates of variable survival rates in Jolly's model show an increase from 0.514 to 0.792. This may indicate that the survival rate of individuals may improve during the active season.

2. Population Structure

The main source of data, which could be constructed into population structure diagrams, was the main MRR programme. Data were also obtained from sampling for serological samples (see Chapter 3) and the unique marking experiment. All samples were obtained using the sampling method described. It was not possible to construct population structure diagrams during winter months due to the inactivity of the leech (see Chapter 2 for details of seasonal behaviour).

The construction of body weight classes in increments of 0.5 g was arbitrary. The body weight was obtained after blotting individuals dry before weighing. The number of gravid leeches at different times and in different weight classes are also shown.

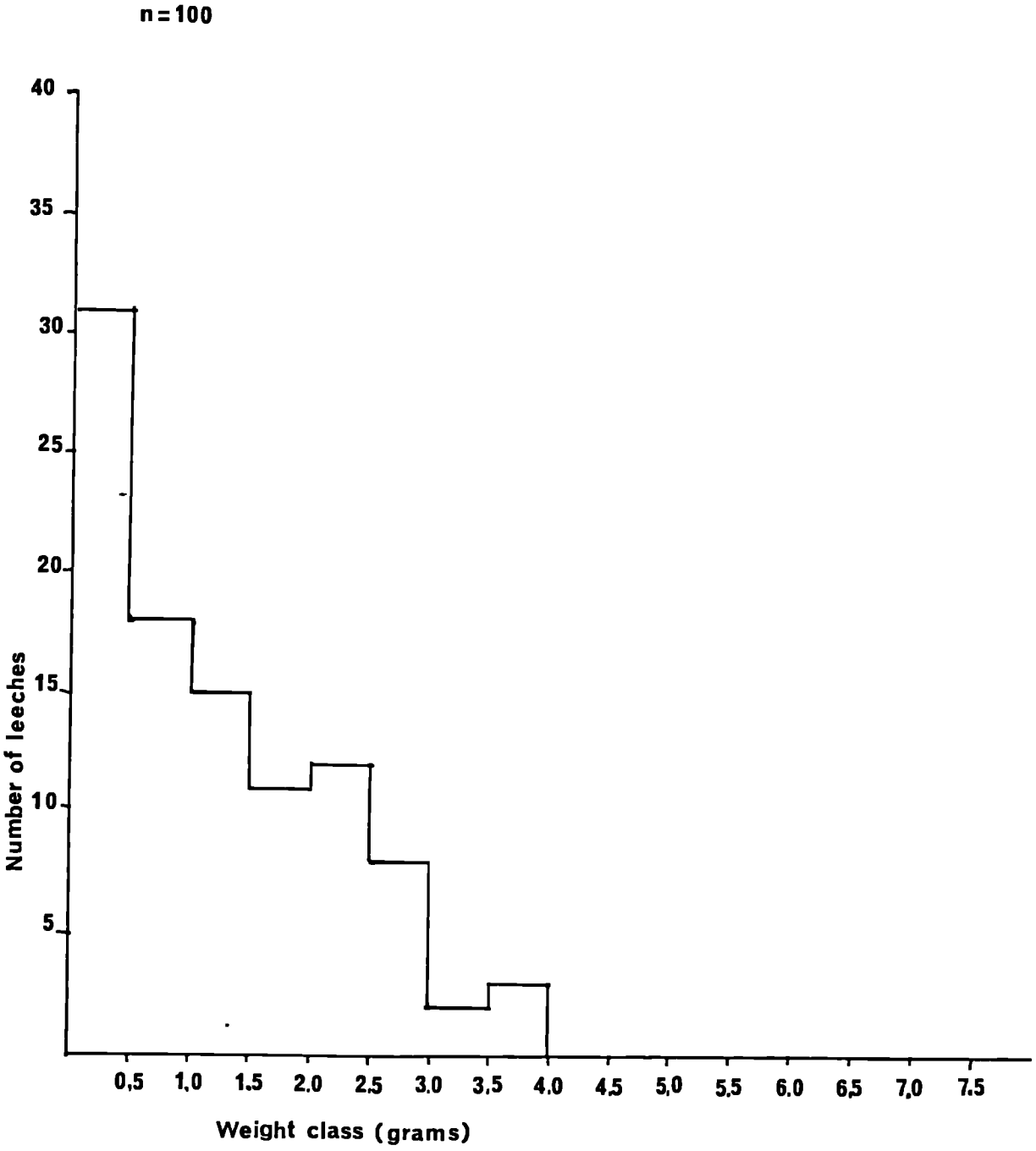


Figure 1.4 a The population structure of the Medicinal leech at Lade gravel pit; estimated from a 2hr rand m sample May 1983

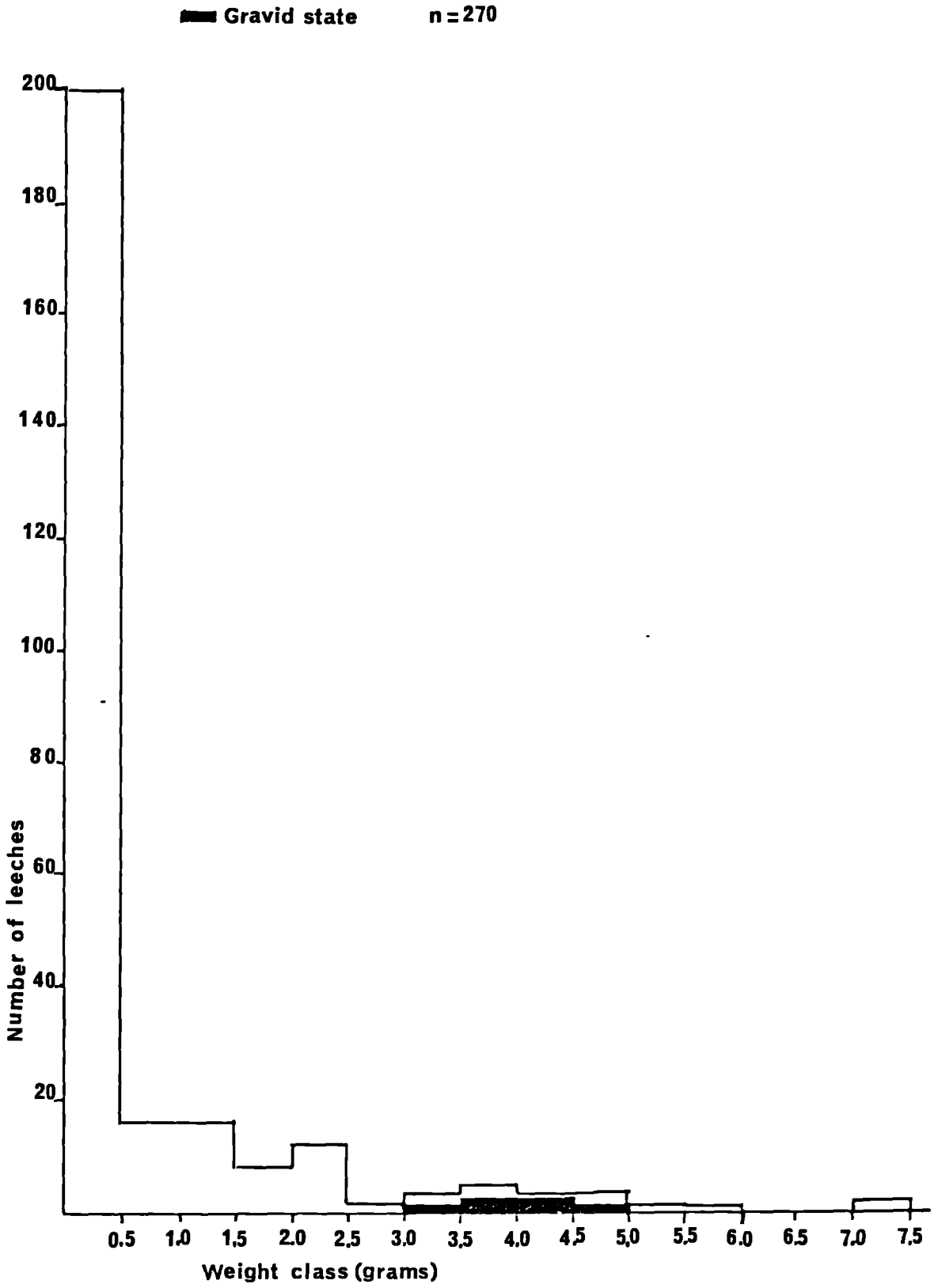


Figure 1.4(b The population structure of the Medicinal leech at Lade gravel pit; estimated from a 2½hr random sample June 1983

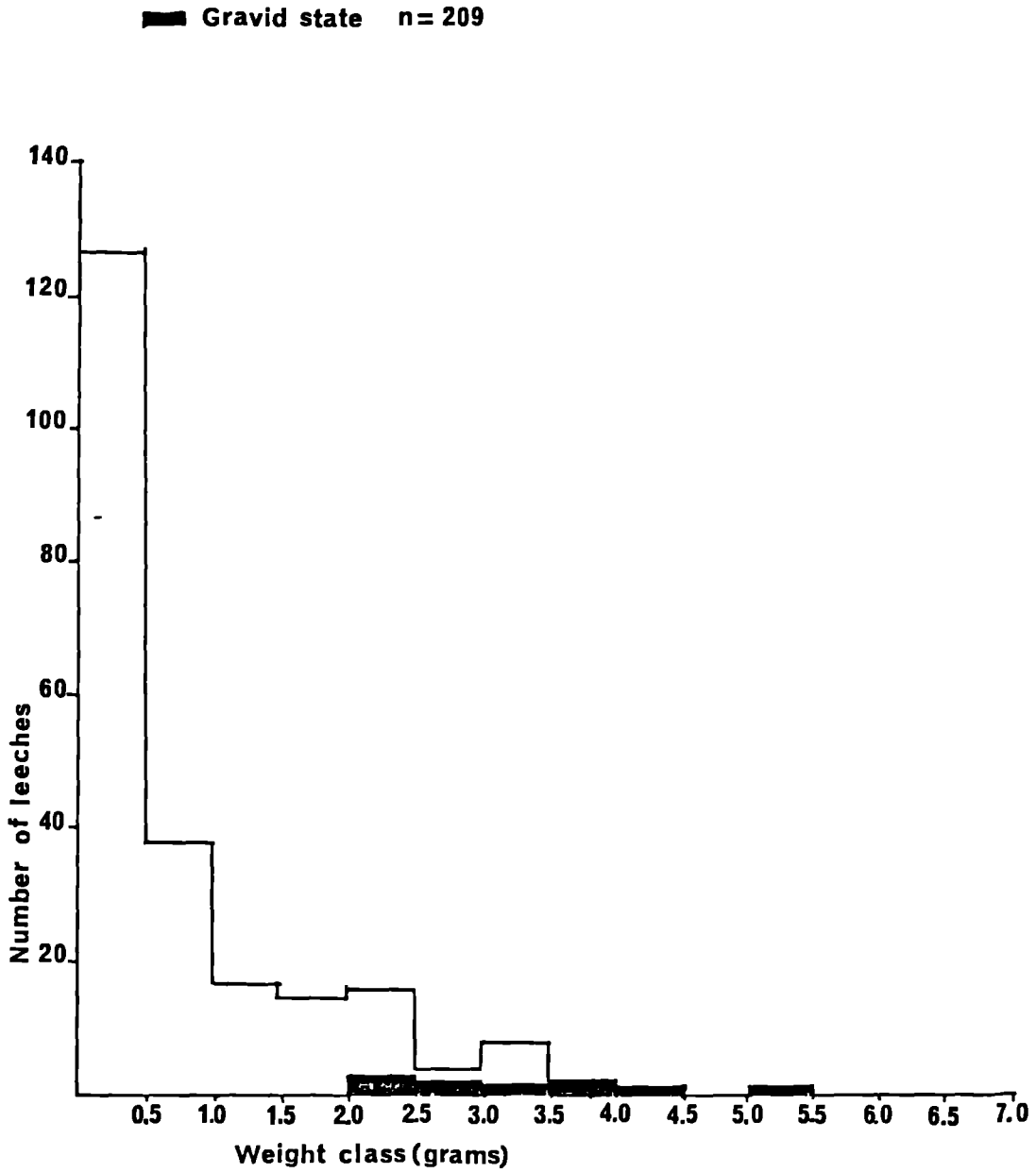


Figure 1.4 c The population structure of the Medicinal leech at Lade gravel pit; estimated fr m a 3hr random sample July 1983

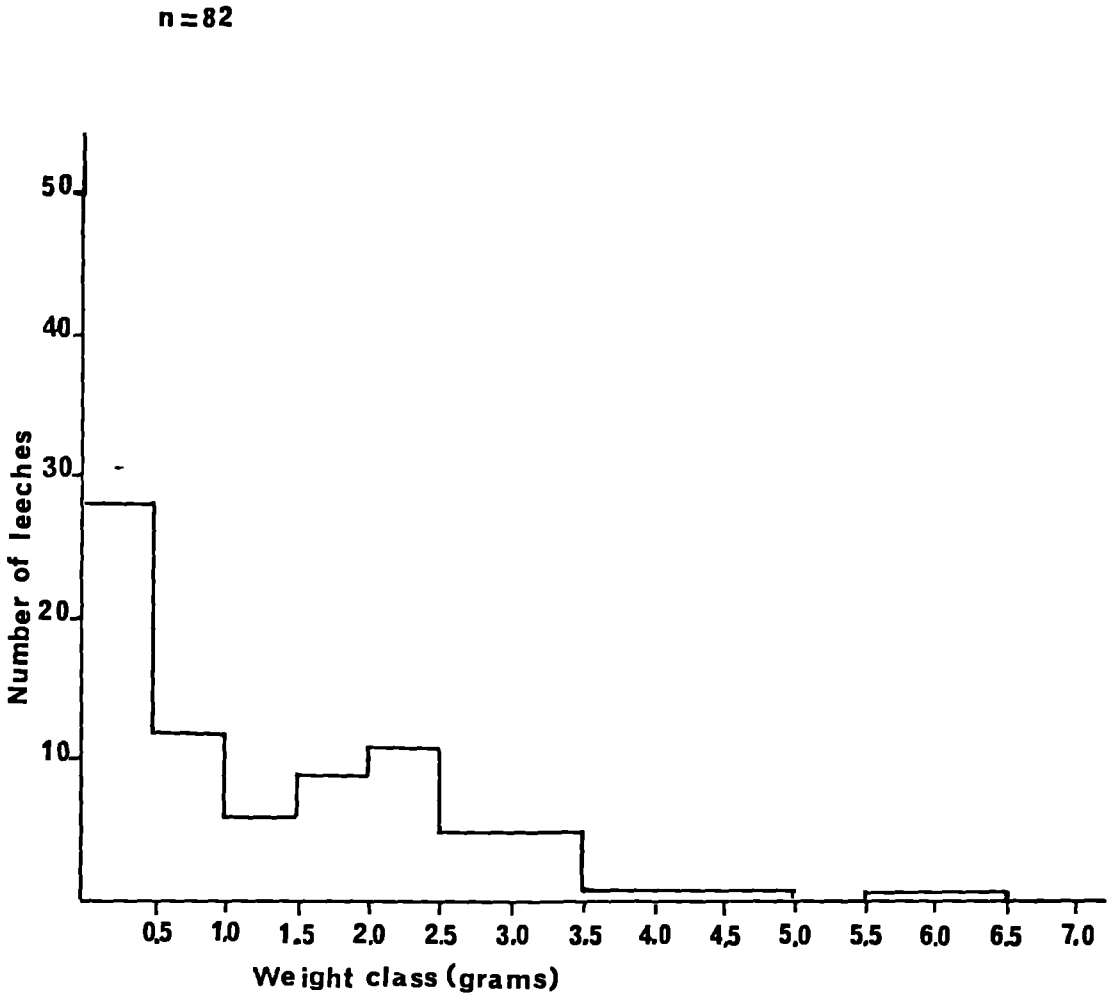


Figure 1.4(d The population structure of the Medicinal leech at Lade gravel pit; estimated from a 2hr rand m sample August 1983

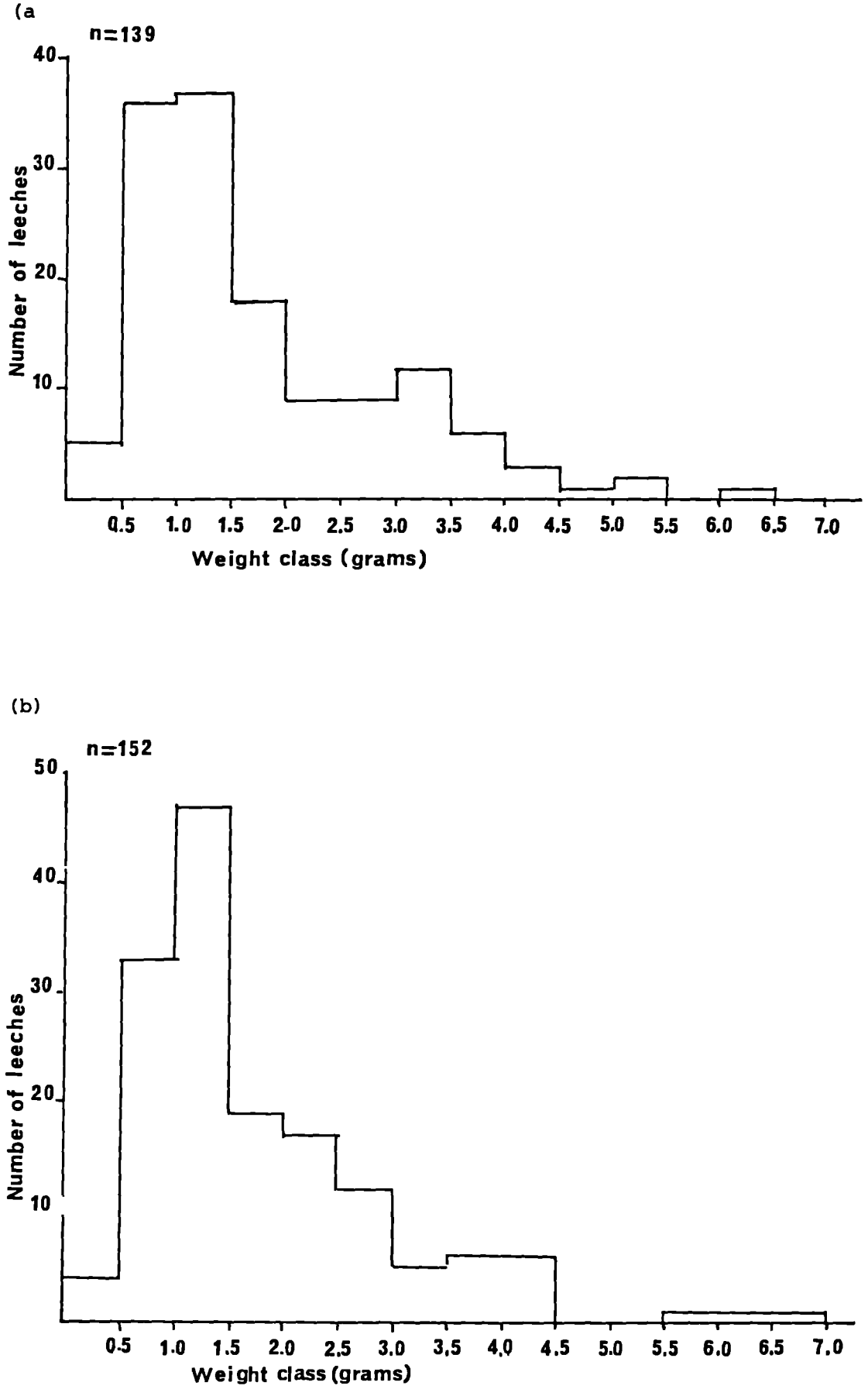


Figure 1.5 a , b The population structure of the Medi inal leech in the 'Small Pond', Lade gravel pit; estimated from a random sample, a) 3rd May 1984, b) 3 th May 1984

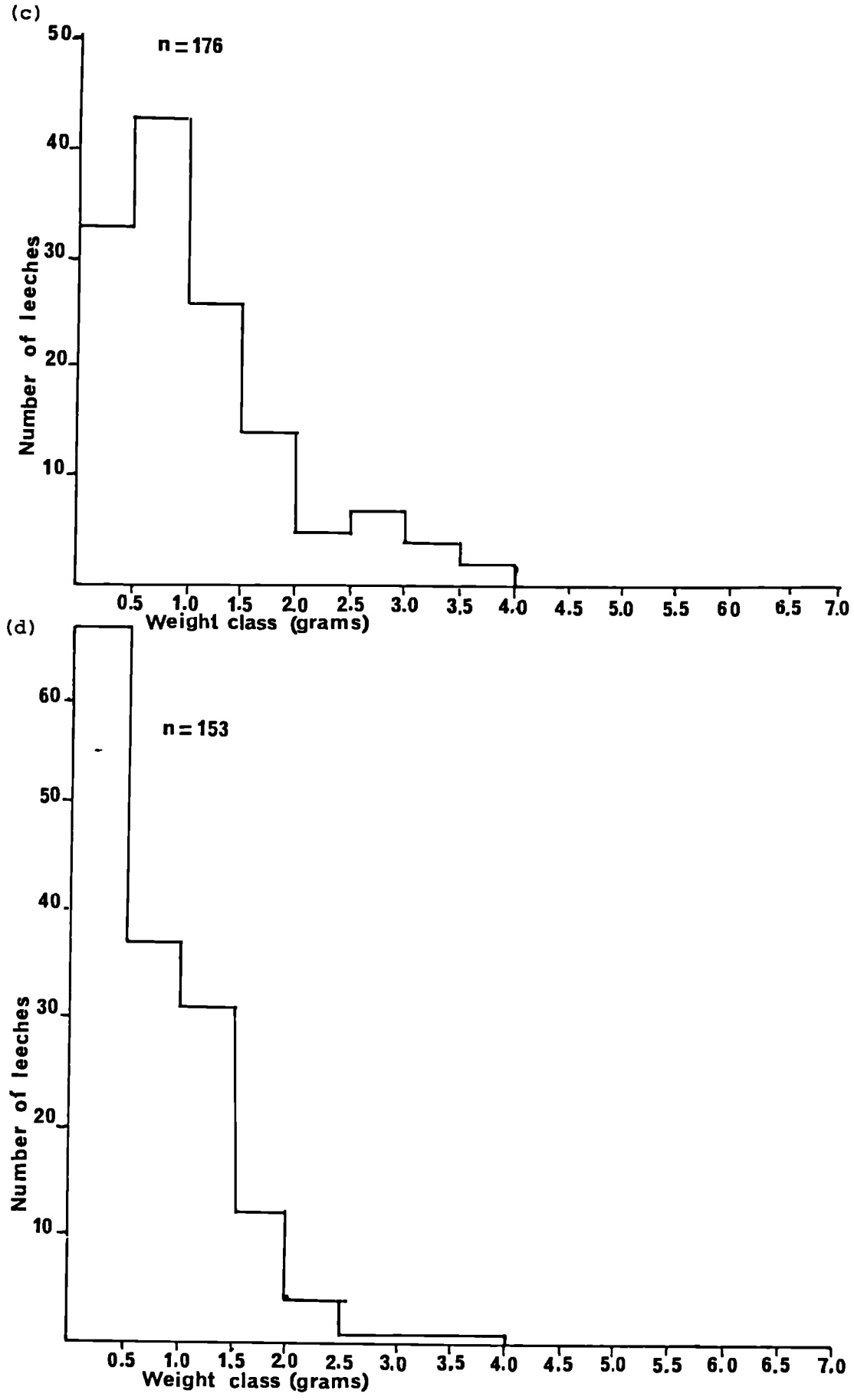


Figure 1.5 c , d The p pulation structure of the Medicınal leech in the 'Small Pond',Lade gravel pit; estımated from a random sample,(b 3rd July 1984,(d) 18th July 1984

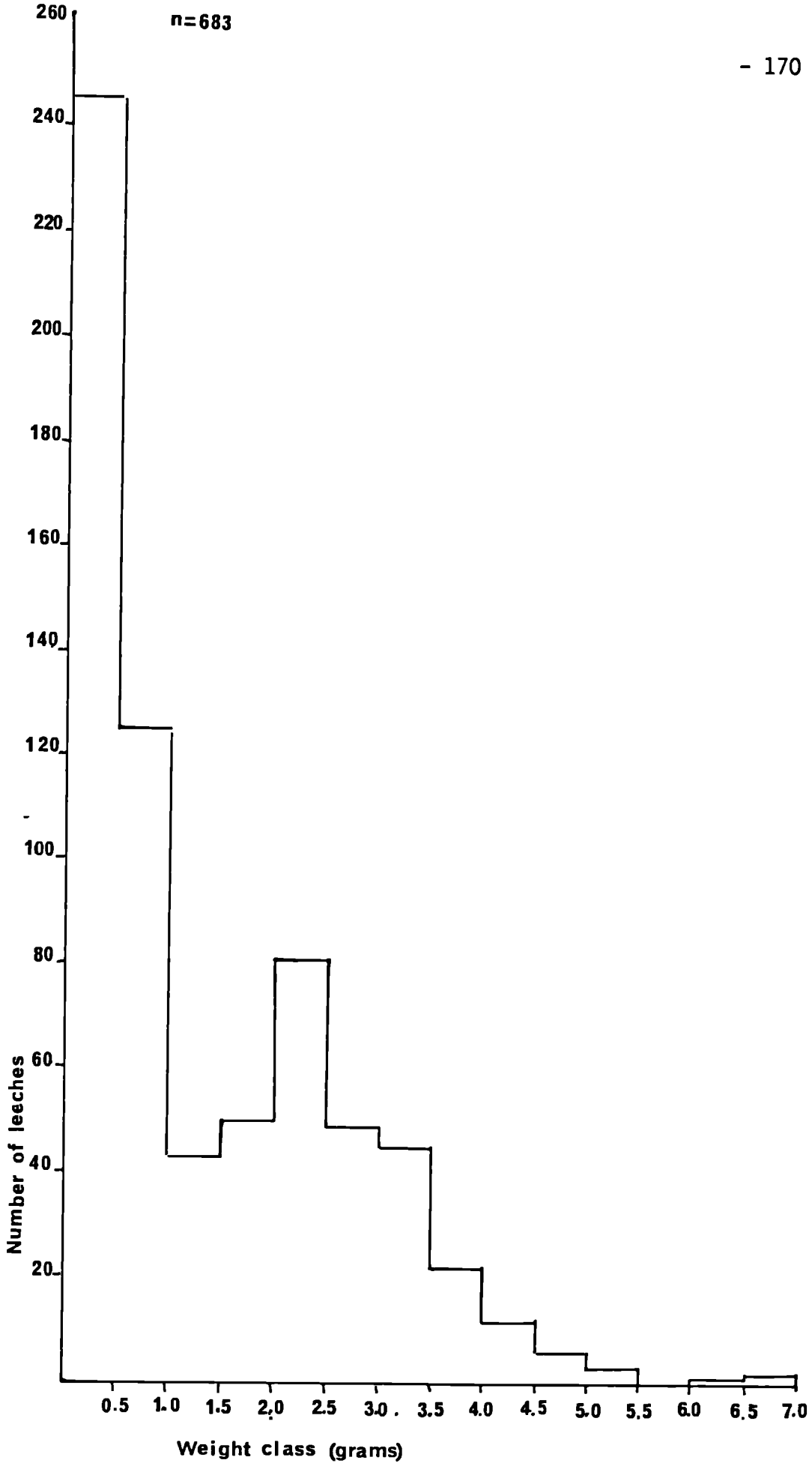


Figure 1.6 a) The population structure of the Medicinal leech at Lade gravel pit; estimated from a 7hr random sample, as part of the Mark,Release,Recapture experiment, April 1984

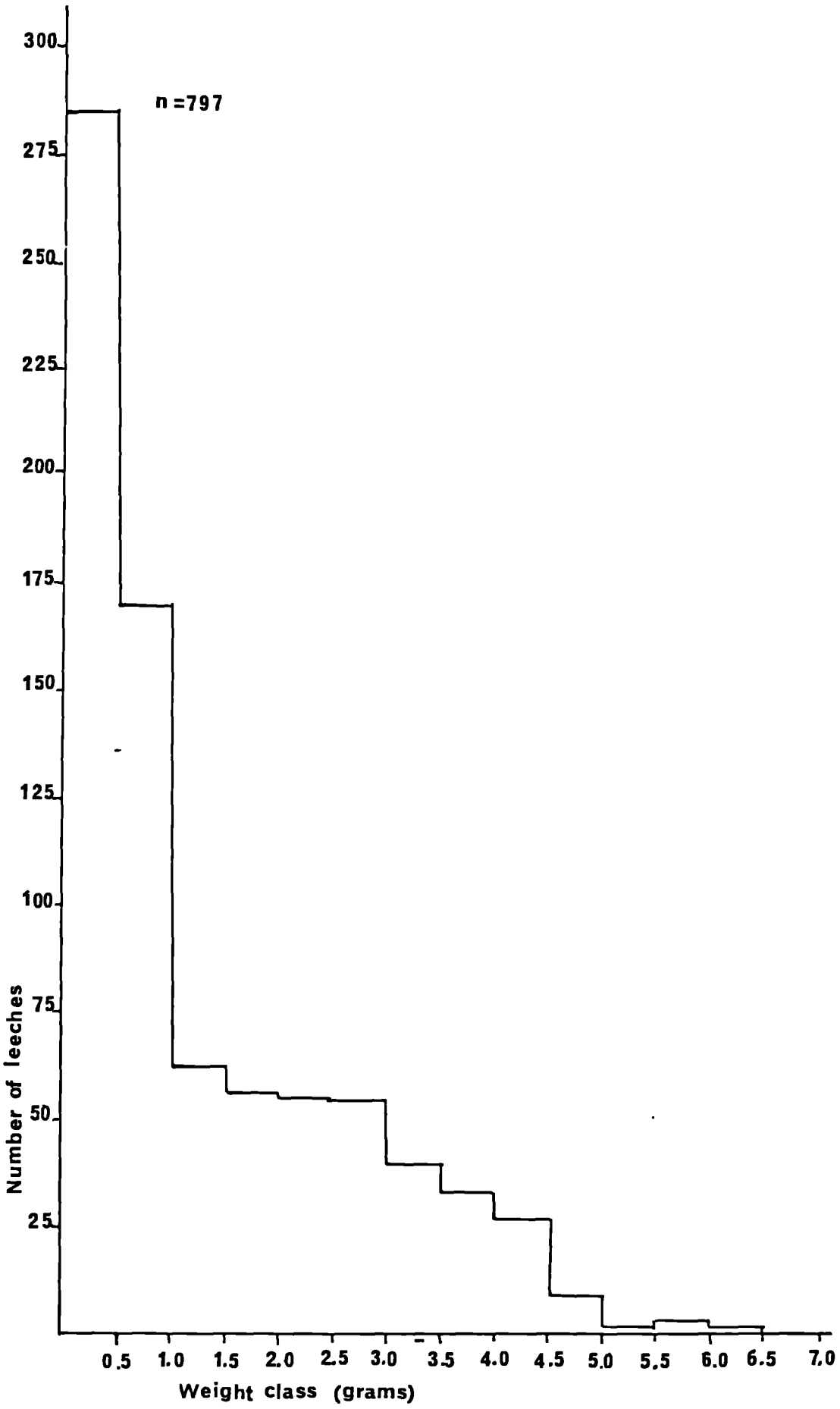


Figure 1.6 b The population structure of the Medicinal leech at Lade gravel pit; estimated from a 7hr random sample, as part of the Mark,Release,Recapture experiment, May 1984

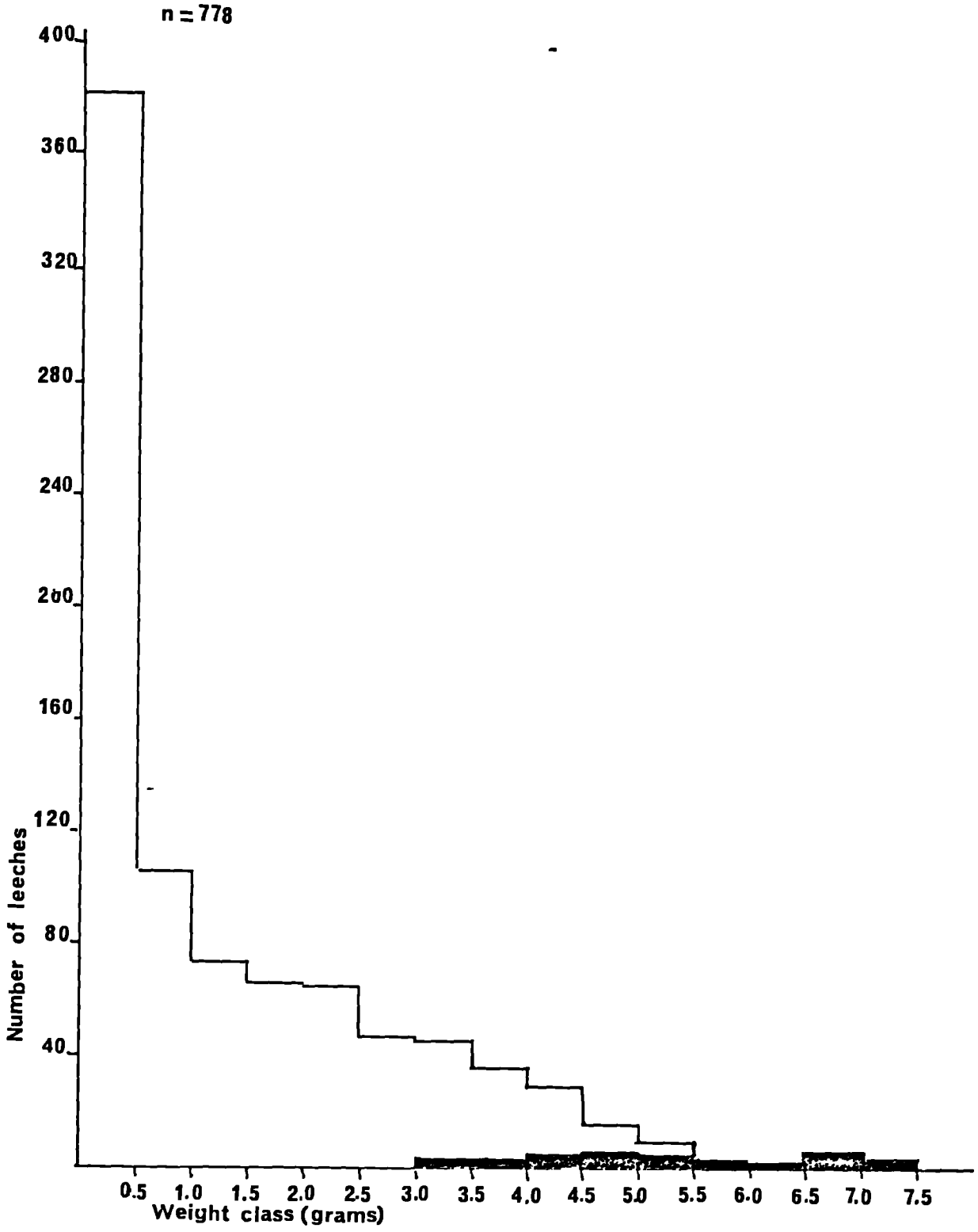


Figure 1.6(c The population structure of the Medicinal leech at Lade gravel pit; estimated from a 7hr random sample as part of the Mark,Relea e,Recapture experiment, June 1984

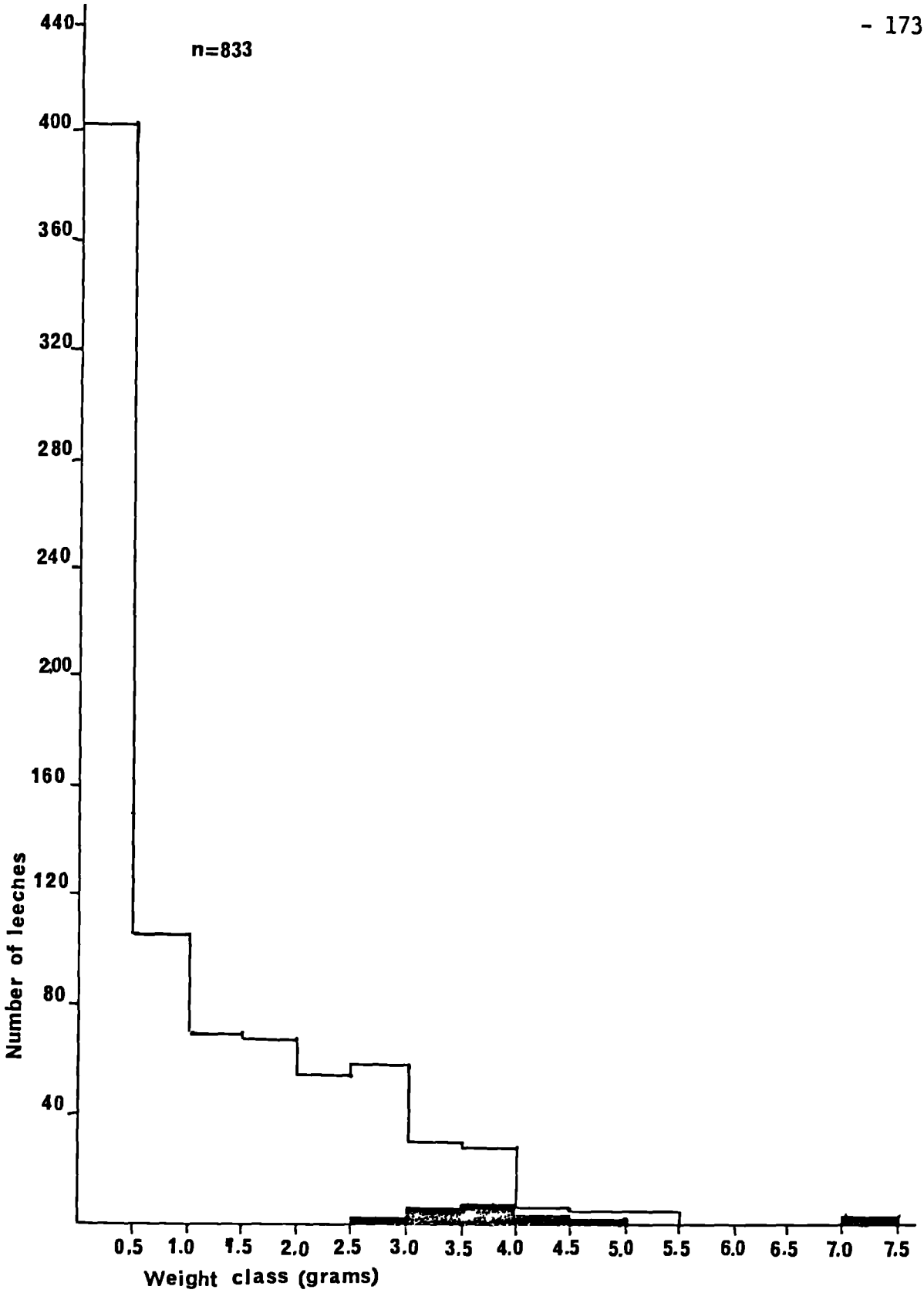


Figure 1.6 d The population structure of the Medicinal leech at Lade gravel pit; estimated from a 7hr random sample for the Mark-Release-Recapture experiment, July 1984

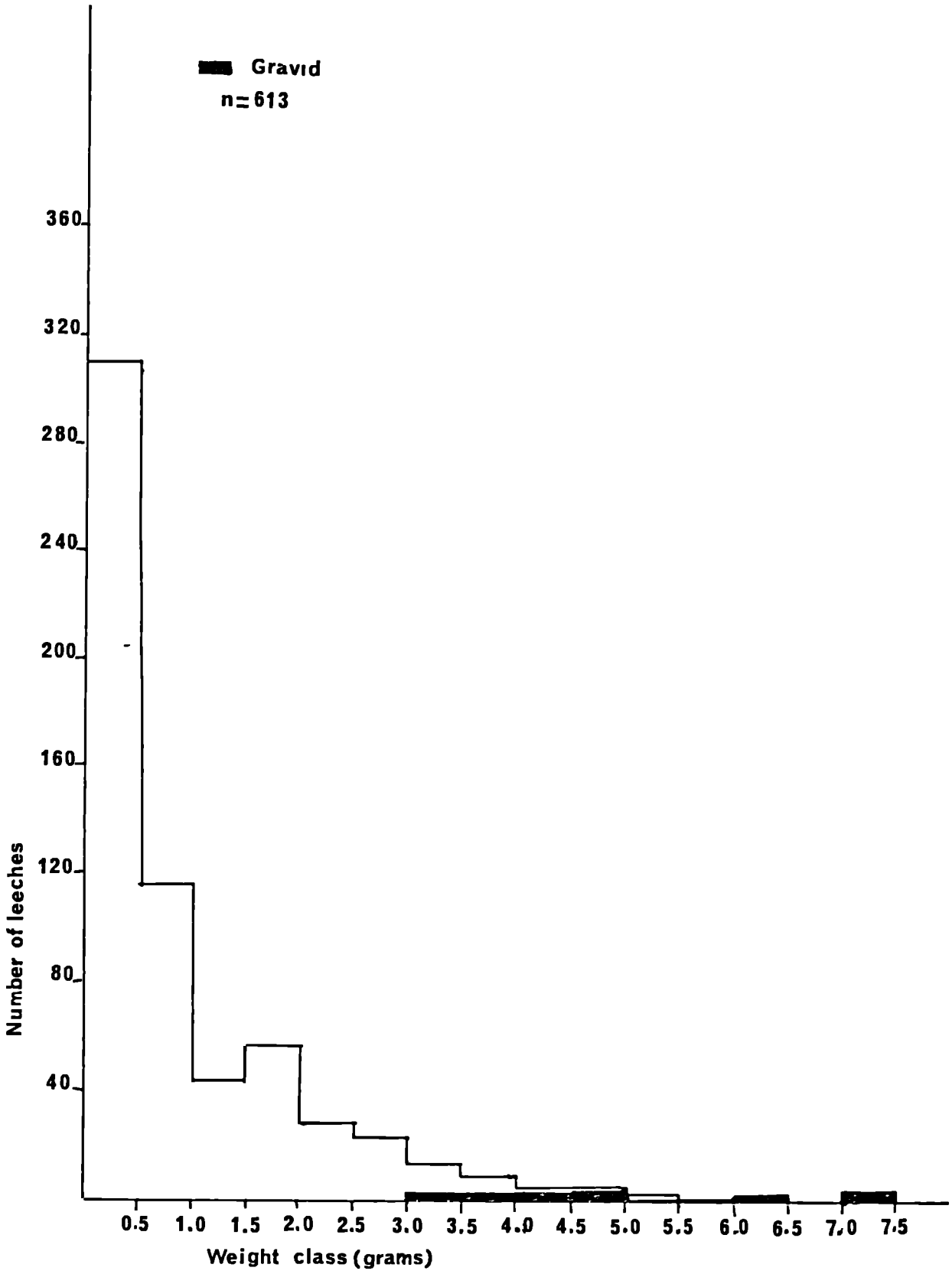


Figure 1.7 The population structure of the Medicinal leech at Lade gravel pit; estimated from a 7hr random sample for the Mark, Release, Recapture experiment, June 1985

The series of Figures 1.4 (a-d) show changes in the population structure of the Medicinal Leech at the main sampling sites during the Summer of 1983. The series of Figures 1.5 (a-d) show the population structure of the leech in the small pond isolated from the main site (see Management Plan for detailed description of this site), during Summer 1984. Figures 1.6 (a-d) show the population structure from the samples obtained for the MRR programme at the main site during April-July 1984. Figure 1.7 shows the population structure in June 1985.

The two most important observations from the series of figures showing population structure are the very high proportion of captured individuals weighing less than 1 g and the very small numbers of gravid leeches obtained in all samples. These points will be discussed in more detail in the Discussion section.

3. Dispersal

The main aim of applying different zone specific marks to large individuals was to examine whether any significant 'within site' movements or dispersal occurred between the five days on which the population was sampled. Table 1.12 below shows the recapture of Day 1, 2 and 3 zone marks on Day 4. It indicates dispersal throughout the area between zones and although this is to be expected, by the very nature of a mobile animal, the distance between (for example) zones 1 and 5 is significant. Similar 'between zone' movements were observed on each sampling occasion and Fig.1.8 shows a diagrammatic representation of mark movements from recaptures obtained on Day 2. (Note that dispersal was not exactly from one focal point to another but from the general area of one zone to another). The dispersal observed at the site is discussed further in the Discussion section.

4. Growth Rate in the Wild

The main aim of uniquely marking individual leeches from the 'small pond' with each mark designating a number from 1-999 was to enable recognition of individuals after successive recaptures (see Figure 1.2(a)).

A total of 494 leeches were marked uniquely from seven samples taken over a period of a year between September 1983 and August 1984.

Table 1.12 The dispersal of zonal marks shown by the number of recaptures of previous marks Samples on Day 4.

Zone in which mark was re-captured on Day 4	Previous zone mark/Day (1, 2, 3) marked																										
	1			2			2/1			2/2			3/1			3/2			3/3			4			5		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1		2		1													1						1				
2		2	1		2									1	1								2			1	1
2/1				2	1									1	1								1	1	2		
3/1																										1	1
3/3					1					1													1	1		1	1	1
4				1	3	1									2								1				1
5			1	1		1							1	1			1						1	1		3	1	2

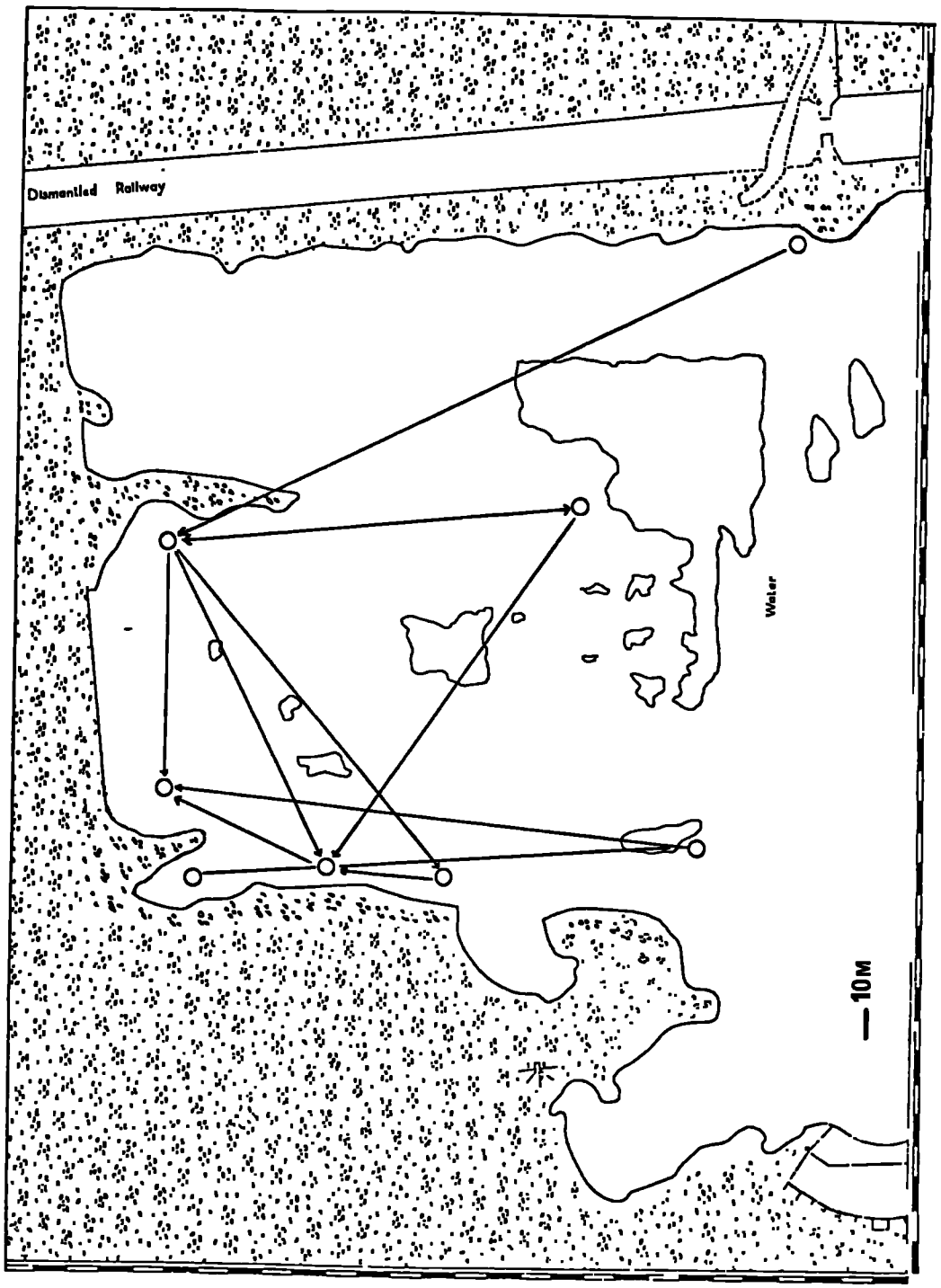


Figure 1.8 A diagrammatic representation of mark movements between Day 1 and Day 2 based on recaptures obtained on Day 2 of the Mark, Release, Recapture experiment

Of this number, 114 leeches were marked in Sept mber 1983. In subsequent samples, taken in the following year after an overwintering period, a total of 72 leeches from the September group were recaptured. This indicated at least a 63% survival rate in this group between two successive seasons.

Of the total number of leeches uniquely marked (494), 162 were recaptured on at least one subsequent sampling occasion. From this number 39% showed a net decrease in body weight from that recorded on the initial day of capture. The predominant pattern of growth in the other 61% of recaptures showed relatively small increases in body weight, not indicative of saltatory feeding with large, single increases in body weight, as was found with specimens fed optimally under laboratory conditions (for details see Chapter 4). The average net body weight increase (measured as a percentage of the initial body weight) in the sample of wild H. medicinalis was only 79%, significantly lower than those acheived by equivalent weight specimens over a similar time period, under optimum feeding conditions in the laboratory (see Chapter 4). Figure 1.9 shows the changes in body weight of five leeches, all initially captured in September 1983, and on four subsequent occasions in the following year. The broken lines, linking the first and second captures, only represent the actual change in body weight between the two sampling occasions. The 'pattern' of growth between the two points being unknown. A single sample taken from the small pond in July 1985 included 10 leeches originally captured in September 1983. Details of body weights recorded for this group during this period of time are given in Table 1.13 below.

These results suggest that body weight increase over a period of nearly two years was, for most of the group, small. The full significance of these results is discussed in the next section.

A single recapture of one particular individual leech is worthy of special mention. This individual was initially captured in May 1984 at a body weight of 0.79 g. The same individual was later captured in early August 1984 at a body weight of 3.24 g and in a gravid state. Only four other individuals showed a similar net body weight increase

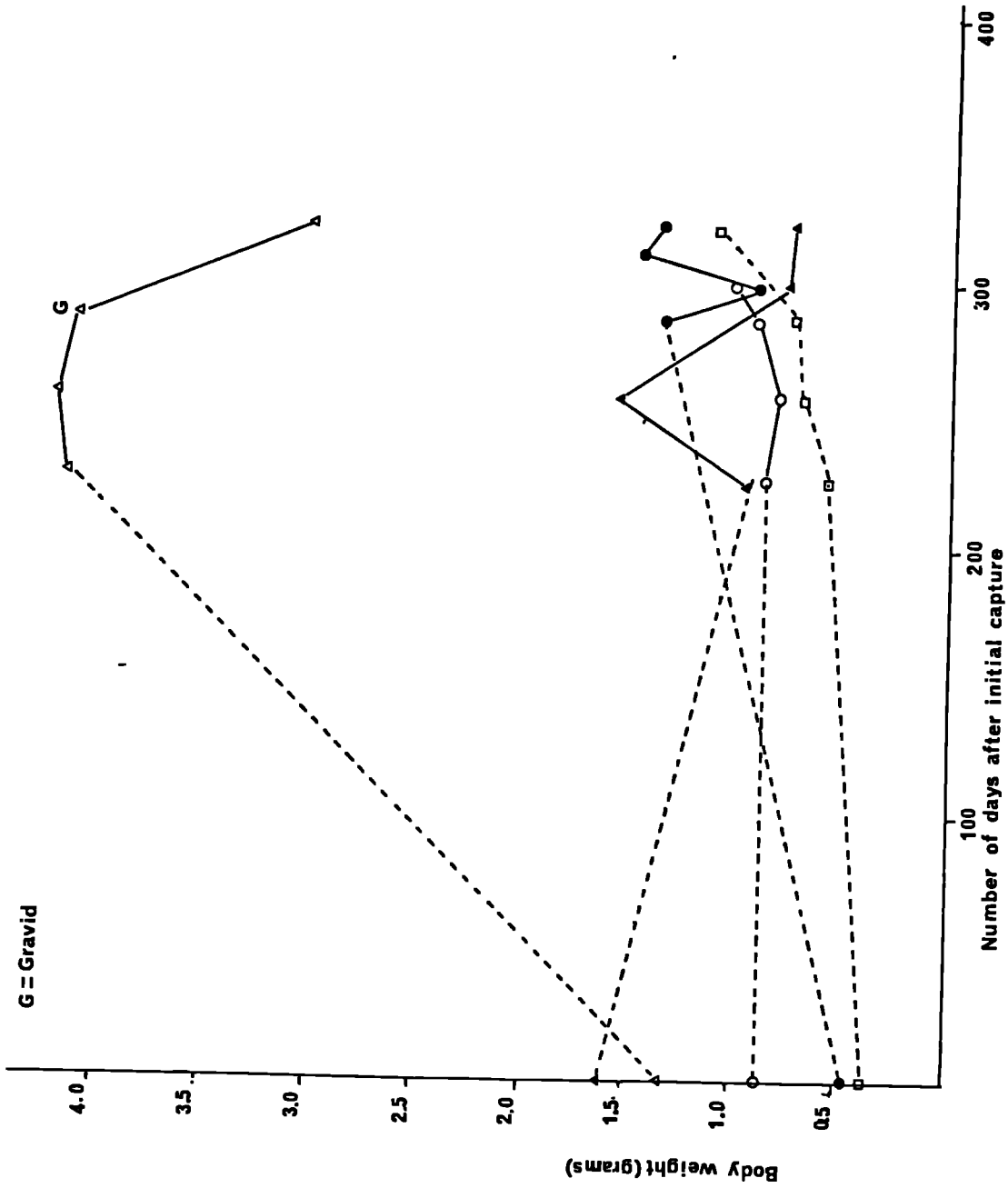


Figure 1.9 Changes in the bodyweight of five uniquely marked Medicinal leeches captured on five occasions in the 'Small Pond' between Sept.1983-August 1984

in excess of 250% possibly indicating that 'gorging' had occurred on one or more feeding occasions in the wild. The significance of these findings is discussed in the next section.

Table 1.13 Change in body weight (g) of 10 uniquely marked leeches in the small pond between September 1983-July 1985

	Date of capture/weight (g)							
Leech	18.9 '83	3.5 '84	30.5 '84	3.7 '84	18.7 '84	30.7 '84	19.8 '84	19.7 '85
5	0.51		0.87					1.50
8	1.16	0.70		1.10				1.47
9	1.76							2.84
19	0.60	0.79					2.03	1.11
32	0.58		1.80					1.10
42	1.30	1.23		1.58			1.39	1.27
50	0.65	1.03			0.36			0.71
54	1.43	1.41						1.59
64	0.68		1.48					1.68
74	0.83				3.14			1.14

Discussion

The main aim of estimating the size of the leech population at Lade gravel pit was to make an assessment of the order of magnitude rather than absolute numbers. In the context of formulating a conservation strategy it was necessary to find out whether this population could be regarded in terms of hundreds, thousands or tens of thousands of individuals and, more importantly, whether it is self-sustaining and viable given the continuance of the present ecology of the area.

The results obtained, indicating a population size in the order of 6-12,000 individuals, must be a conservative estimate given that a certain, unknown, proportion of the population in a satiated and sessile state would have been unresponsive to the sampling method employed. As discussed later, however, this proportion is likely to

be small and the results for population size are probably a fair estimate of the total numbers present at the site.

In terms of its size, it is difficult to place the Lade gravel pit population of the Medicinal Leech into the context of its presently known geographical distribution, since no comparable work exists in the published literature. All of the older British records describe individuals, although a recent reference to the leech in the Lake District by Elliott & Tullet (1986) counted a total of 842 individuals over two summer field seasons.

Due to the behaviour of the leech to water disturbance and the animal's relatively large size, it might be expected to be noticed if present at a site in large numbers. The Lade gravel pit population was in fact 'discovered' by a member of the public who noticed that a pet dog had been attacked after entering the water. In addition, the survey and conservation work of the County Nature Conservation Trusts on ponds and wetlands have not discovered the leech in any numbers at other sites.

It therefore seems likely that the Lade gravel pit site supports the largest extant population of the Medicinal Leech in the British Isles and possibly in Western Europe.

The way the population size is distributed in time is illustrated by population structure and in this study the results indicate that the Lade gravel pit population is viable and self-sustaining. The high numbers of individuals in the weight classes of less than 1 g clearly represent mainly recent hatchlings, good evidence, in addition to the occurrence of cocoons at the site, of recruitment into the population. The occurrence of this weight class in samples early in the active season (early April) indicates a good capacity for the previous season's hatchlings to survive over winter. The results obtained from the feeding experiments with the Medicinal Leech in the laboratory (see Chapter 4 for detail) suggested that some individuals could survive on a sub-optimal feeding regime. It seems certain, therefore, that an unknown fraction of leeches in the weight class between 0-1 g may have been older than the previous season's hatchlings.

The presence of gravid leeches during the summer months of June, July and August and the presence of viable cocoons until end-August is an obvious sign that a proportion (unknown in size) of the population is reaching sexual maturity. As discussed elsewhere, this gravid proportion of the population is likely to be relatively small. This is illustrated by the population structure histograms although it may be that gravid individuals are under-represented using the sampling method described. It does appear that a certain proportion of the population does become sexually mature and gravid each season. What this study does not show is whether some individual leeches become gravid in more than one season and for how long reproductive potential lasts in the Medicinal Leech. The point at which any one individual attains sexual maturity and becomes gravid for the first time is almost certainly linked to its past feeding history (see Chapter 4 for a discussion) and this is likely to take several seasons if the feeding regime is sub-optimum.

An interpretation of the consistent skewing of the population structure histograms towards the lower body weight classes can be made in two ways. It may be a true reflection of the proportion of this weight class in the population with a relatively small number of individuals in weight classes above 2 g (all gravid leeches were found in this group). An alternative interpretation could be that the heavier weight classes of leech were under-represented in the samples, possibly due to longer periods of sessile behaviour between feeds or reproductive behaviour (it should be noted that laboratory experiments indicated that searching behaviour in response to water disturbance was not initiated during periods of reproductive behaviour and copulation).

Looking in detail at the three sets of population structure histograms obtained from the main site during the 1983/84 field seasons and the small pond in 1984, there are slight differences in the proportion of weight classes represented which could be significant.

During 1983 at the main site, the proportion of individuals in the hatchling weight class of 0-0.5 g steadily declined from 74% of the sample in June to 7% in September. The main incidence of gravid leeches in the population occurred during early June to early August and, as Chapter 5 describes, viable cocoons were collected from the wild during

late July and August. The incidence of empty cocoons found in the wild increased during August until no viable cocoons were found in September. From this it might be concluded that the major recruitment of hatchlings into the population occurred during August and early September The reduced proportion of the hatchling weight class during these months might be explained by the vast majority of hatchlings being satiated on yolk from the cocoon and hence unresponsive to water disturbance. Certainly leeches hatched in the laboratory, for growth experiments described in Chapter 4, required a period of 'starvation' ranging from 4-6 weeks before an initial bloodmeal was accepted. For new recruits hatched into the wild population during late August or early September the end of this period of 4-6 weeks before feeding would coincide with a time when little activity was found in the leech population (see Chapter 2), a drop in daily water temperature and the absence of an adult and larval smooth newt food supply and the onset of the inactive hibernation period of the Marsh Frog. These factors, coupled with laboratory findings of a good overwintering survival rate of unfed hatchlings, suggest that a proportion of the hatchling recruits to the population probably take their first bloodmeal at the beginning of the following active season (early April)

An interesting finding in the small pond adjacent to the main Lade gravel pit site, during sampling in 1984, was a reduction in the proportion of leeches in the body weight class 0-1 g, especially during May, compared with the main site. During this period of the active season this group was the predominant weight class in all samples. A possible explanation for this difference, observed in the small pond, may have been in the much higher concentration of Marsh Frogs inhabiting this small area. The likely increased probability of a successful feeding encounter by the leech with this important host might mean that hatchlings were obtaining a series of bloodmeals earlier in the season and consequently moving into heavier body weight classes. The results could also be explained by the absence, in detailed searches of the littoral areas, of cocoons indicating that the small pond is not an important recruitment site to the population.

The re-emergence of the 0-0.5 g hatchling weight class in the small pond in mid-July 1984 is a puzzle since this certainly precedes

the main cocoon laying period at the main site by 2-3 weeks. It probably represents a proportion of the population which failed to obtain a bloodmeal during the season, thus losing body weight rather than genuine recruitment by new 1984 hatchlings. This series of population structure histograms illustrates the difficulties of interpreting changes in the proportion of weight classes during the active season.

An important finding from the series of population histograms obtained from the MRR samples and the previous 1983 samples at the main site was the very small proportion of gravid leeches obtained. It was difficult to interpret this finding in the context of the relatively small numbers of leeches captured in the same heavier weight classes ($>$2.5 g). It was an interesting behavioural finding that leeches in a progressive gravid state (i.e. heavy swelling of anterior segments preceding cocoon laying rather than the early gravid appearance of yellow, discoloured segments) were obtained in samples by responding to water disturbance with searching movements. It is likely that the proportion of the population at Lade gravel pit which reach the required minimum weight class develop to sexual maturity and successfully mate with each other to reach the gravid state, is probably very small. The disappearance of gravid leeches in August and September samples in 1983 at the main site might indicate that individuals had laid their complement of cocoons or were unavailable being out of the water in the act of laying cocoons.

The movement of zonal marks between successive samples taken at the main site during the active season in 1984, was an interesting finding. It indicated that the leech was not confined to a particular niche and indeed individuals, although predominant in stands of emergent macrophytes, were also found under bottom shingle pebbles in shallow ($<$1 m) open water areas. It is likely that the activity of potential hosts is an important factor in determining the movement and distribution of the leech at Lade gravel pit rather than any strict physico-chemical or niche preference. The status of the leech as a sit-and-wait or searching predator, and a temporary ect parasite is discussed at length in Chapter 2. The extent of movement of individual leeches will be closely linked with the type and activity of host in the immediate vicinity. The results of Chapter 2 & 3 show that the Medicinal Leech at Lade gravel

pit utilises a range of non-mammalian hosts including specifically the Smooth Newt, Triturus vulgaris, the Marsh Frog, Rana ridibunda and fish and birds.

Each host type will probably initiate searching behaviour in the leech to a varying degree depending on the extent of water disturbance. For example, the behaviour of male and female smooth newts during courtship displays from early April create very local water disturbances (the male displays with characteristic waving movements of his tail). Attacks on smooth newts in the wild by the leech was restricted to individuals which came into almost direct contact with a sessile leech. The extent of movement of the leech in this situation was minimal. In contrast, the behaviour of resident birds at Lade gravel pit involved wide ranging movements creating centres of water disturbance. It was observed, for example, that a pair of squabbling coots created surface water disturbance which, in shallow conditions was propagated up to 30 metres. Simulated trials of water disturbance in the same region were observed to attract individual leeches from distances of 5-10 metres. In a situation where an individual leech gets into a position close to a potential host after swimming such a distance,but is subsequently unsuccessful in making actual contact, the searching behaviour would again be initiated, possibly after a period of rest. A succession of movements such as these could explain the considerable displacement of marks within the site area.

Preliminary laboratory feeding experiments using live fish, newts and frogs indicated that a leech which had successfully made contact with the host remained attached for periods of 20-60 minutes. The initial response of the host to the leech's presence was rapid extensive movements, especially by fish and frogs. (The behaviour patterns of the range of hosts available to the Medicinal leech at Lade gravel pit are discussed in more detail in Chapter 2.) The successful contact by a leech with a bird host could involve a residence time of up to 90 minutes, (i.e. feeding times attained by large leeches fed in the laboratory; see Chapter 4). In both these situations the transit of the leech on the host could be extensive. Indeed several individuals were found at the Burrowes gravel pit

2 miles away, almost certainly as a result of bird dispersal (see Chapter 7 for details).

The results obtained from the recapture of uniquely marked individuals in the small pond over a period of nearly two years provide some evidence of growth patterns of the Medicinal leech in the wild. A remarkable finding was the very small increases in body weight (regarded in this study as growth) after two field seasons. Only one individual had reached a weight class in which gravid leeches had been found elsewhere. It certainly represents very restricted food availability over the period and this is emphasised in comparison with optimum feeding in the laboratory described in Chapter 4 where several hatchling leeches reached body weights of over 20 g. The combination of very little body weight increase in wild Hirudo medicinalis and the high proportion of leeches in the population samples in weight classes of less than 2.0 g suggests that the majority of the population survives on a restricted food supply. With the high numbers of potential hosts in the area, this may indicate that many incidences of searching behaviour for a host result in unsuccessful contact and feeding.

CHAPTER TWO

OBSERVATIONS ON THE NATURAL HISTORY OF THE MEDICINAL LEECH, HIRUDO MEDICINALIS (L) AT LADE GRAVEL PIT; AN ACCOUNT OF DIURNAL AND SEASONAL BEHAVIOUR, INTERACTION WITH THE COMMUNITY AND PRELIMINARY LABORATORY EXPERIMENTS IN SUPPORT OF FIELD OBSERVATIONS

Introduction

One of the most important objectives in this study was to gain information about the leech at Lade gravel pit. This involved a considerable amount of time in the field accumulating largely qualitative observations on the leech in the wild and its interaction with the community. Although there was evidence of diurnal and seasonal differences in behaviour it was the role of the leech in the community, especially as predator, parasite and prey, which was of particular interest.

One set of laboratory experiments were designed to further investigate field observations and ideas about predator/prey relationships. A further laboratory experiment was designed to try to determine if hatchling H. medicinalis are obligate feeders on amphibia because of a limitation in the function of the jaws. The format of this chapter is made up of separate sections brought together in a concluding discussion.

Seasonal Behaviour of the Leech

The Medicinal Leech at Lade gravel pit shows a definite seasonality in its behaviour. This was noticed from the number of active leeches responding to water disturbance and evidence of leech predation on the smooth newt, Triturus vulgaris.

During the period between mid-November and mid-March in 1982/83/84, there were no captures of leeches in twice monthly samples in zones 4 and 5 (see Management Plan for details of sampling zones) by the meth d of water disturbance described inChapter 1. During these periods, the water temperature remained below 8-10^{o}C and it is interesting that this "threshold" temperature range for activity was similar to that found in a leech population in a Lake District tarn by Elliott & Tullet (1986). In each of the years 1983/84/85, significant activity by hungry leeches in

zones 4 and 5 occurred between the period mid-April and mid-October and corresponded to average water temperatures above 10°C.

It still remains uncertain why temperature should limit swimming activity and hence the search for food by the Medicinal leech. This seasonal tendency is in contrast to other leech species at Lade gravel pit such as Theromyzon tessulatum, a sanguiverous ectoparasite on birds, which was observed throughout the year in typical anchored body waving behaviour designed to make contact with the beaks of dabbling duck and wildfowl. Observations on the availability of possible hosts and their seasonal behaviour also added to the puzzle of why the leech does not appear to take advantage of hosts throughout the year. The availability of birds at Lade gravel pit is year round and as the results in Chapter 3 show, birds are utilised by the leech at Lade gravel pit as hosts. The return of the smooth newt, T. vulgaris, from winter hibernation to the water occurs from late February when water temperature is still below 10°C yet evidence of significant predation by the leech (see section below), was not evident until early April. These examples of host availability outside of the apparent active season of the leech population again indicate the likelihood of water temperature being a primary factor controlling activity.

Diurnal Behaviour of the Leech

It was necessary to investigate whether the leech showed any behavioural differences to the stimulus of water disturbance during a period of 24 hours in order that planned daytime sampling provided adequate samples for the study.

During June 1983 two separate visits were made to the site for a period of 24 hours. Sampling was restricted to zone 5 (see Management Plan for description of this zone) for a period of ½ hour every six hours. Additional sampling was made at dusk and dawn to examine the laboratory finding by Lotz (1970) that activity increases during the transition between light and dark. On each sampling occasion surface water temperature was measured and, with the exception of 0430 and 0600 hour samples, this remained above 10°C. It was assumed that, for most of the 24 hour period, temperature was unlikely to affect activity.

The method of sampling was identical to that described in Chapter 1 and specimens were collected in plastic bags for counting. All individuals were replaced after sampling. Table 2.1 below shows the numbers of leeches captured during a 24 hour period on two separate occasions at the site.

Table 2.1 The number of H. medicinalis captured during ½hr sampling periods during 24 hours on two separate occasions in June 1983 at Lade gravel pit

Time (24 hour clock)	Number of leeches captured	
18.00	121	117
21.45 (dusk)	138	86
24.00	95	104
04.30 (dawn)	87	93
06.00	89	71
12.00	133	148
18.00	118	126

During the hours of darkness torchlight was used to pick out swimming leeches and individuals attached to waders. The slightly lower numbers of captures at these times may be partly due to sampling error. The results however do not indicate any major differences in the number of leeches captured over a period of 24 hours. The reduction during the early hours of the morning may also have been slightly influenced by water temperature (7-8^{o}C). It was an interesting finding that the activity of the leech was maintained throughout the 24 hour period in response to water disturbance. It would indicate that this stimulus overrides any diurnal effects and this agrees with similar, general conclusions made by Sawyer (1986) about other Hirudinid, sanguiverous species. It certainly makes sense for the Medicinal Leech to be in continued readiness for host activity as successfully obtaining a bloodmeal in the wild is likely to be infrequent in proportion to the number of attempts at host contact (see below for a full discussion of this idea).

It is also worth noting that the activity of the smooth newt, T. vulgaris is largely nocturnal (Halliday, 1974, Dolmen, 1983). The other important amphibian at the site, the Marsh Frog, R. ridibunda, has been found by other authors to be highly vocal and active in defence of territory (Tunner, 1976; Brzoska, 1982). At Lade gravel pit vocalisation continued after dark although it did not continue throughout the night. There appeared to be considerable movement after dark, possibly involving territorial behaviour. All this activity is likely to provide H. medicinalis with nocturnal local water disturbances. In contrast, the activity of the resident, avian populations at Lade gravel pit was restricted to the period between dawn and dusk.

On the basis of this preliminary study, it was decided that daytime sampling for the leech was likely to provide a satisfactory number of specimens.

The Medicinal Leech as a Predator in the Community

The widely accepted definition of a predator is a species which kills and consumes another and hence one benefits while the other loses (Krebs, 1978). The central assumption in the literature is that Hirudo medicinalis is primarily an ectoparasite of large mammals and amphibia and has not been regarded as a predator.

Early field observations in April, 1983, indicated that the leech at Lade gravel pit acts as a predator on the adult smooth newt, T. vulgaris. This was evidenced by dead specimens found in the shallows with single and multiple triradiate marks characteristic of the leech bite (λ). In addition to this indirect evidence, small leeches (<0.5 g) were observed attached to adult newts. These observations were monitored and, in each case, the newt was bitten in several places on the body surface and subsequently died from these injuries. On each occasion the leech departed from the newt before death occurred. Dead specimens of newt were collected throughout the site when found rather than during a defined sampling period.

A total of 166 dead adult smooth newts were found between April and July 1983/84 in a 1:1 ratio of male to female. Within this total, 58% were collected during April, 17% in May, 17% in June and 8% in July. It

was interesting that mortality may have been greatest in April, a period of intense activity by pairs of adult newts engaged in mating behaviour and a general increase in the activity of the leech population (indicated by a greater response to water disturbance). Previous work by Halliday (1974) and Houston, Halliday and McFarland (1977) elucidated a complex repertoire of displays, particularly by the male, during mating behaviour in the smooth newt. One behaviour by the male involves a vigorous tail flexing action in front of the female. This repertoire was observed by this author in the laboratory and it was felt that very local small water disturbances by male and female newts involved in mating behaviour could act as sufficient stimulus for a leech in close proximity. It was decided to investigate this interaction further in an artificial environment in the laboratory (see later).

Further analysis from the 166 dead newts collected showed an average of 4.6 bites on males (range 1-15 bites) and 2.8 bites on females (range 1-14 bites). Plate 2.1 shows multiple bites on a male and female smooth newt (arrows). Note that the female (top) had been bitten on the eye and this was a particularly common site of attack. On several individuals with multiple bites, there was a clear indication that more than one leech was involved in the attack. An examination of bite sizes indicated that the majority of attacks were by small leeches (<1.0 g). Plate 2.2 shows multiple bites on a male smooth newt. The two distinct size differences of bites (see arrows) may indicate that this individual had been attacked by at least two leeches. As described below, the Medicinal Leech at Lade gravel pit does appear to have developed an ability to scavenge and in the case of the individual in Plate 2.2, initial attack and subsequent mortality of the newt may have been caused by one leech and afterwards scavenged upon by a second. It could also represent a multiple attack on the live newt, although this was not observed in trials in the laboratory or in the wild.

These predatory attacks by the leech on the smooth newt were not restricted to the adult stage of the life-cycle but were also observed later in the season, during July and August on the larval stages. Plate 2.3 shows larval stages of the smooth newt, found in early July at Lade gravel pit (note the presence of external gills). Each individual has been attacked at a single site on the body which is likely to have resulted in mortality.

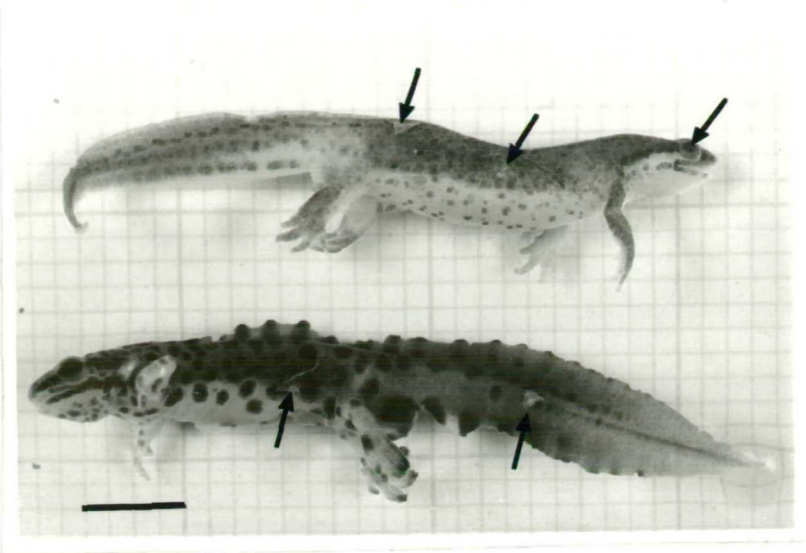
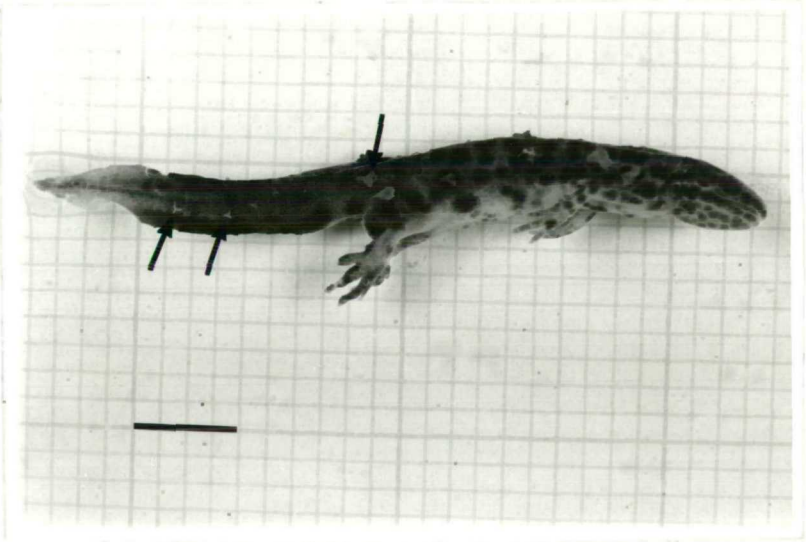


Plate 2.1 Multiple Medicinal leech bites, (arrowed) on a male, (belo and female Smooth newt, Triturus vulgaris

Scale bar - 1cm

Plate 2.2 Multiple Medicinal leech bites, (arrowed) on a male Smooth newt, T.vulgaris, showing differennt bite sizes.

Scale bar - 1cm



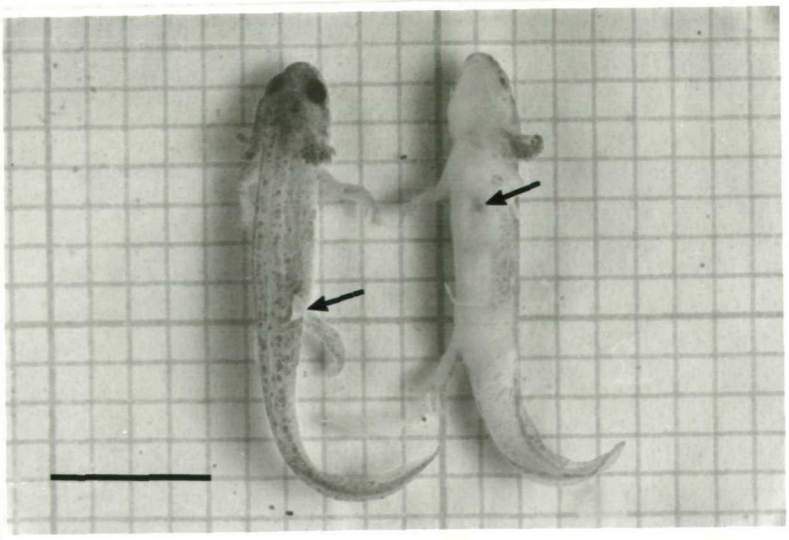


Plate 2.3 Medicinal leech attack on the larva stages of the Smooth newt, T.vulgaris, (arrowed)

Scale bar - 1cm

The first observation of the Medicinal Leech utilising fish as hosts was made in June 1983. A small Roach, Rutilus rutilus, was observed with a leech attached by its posterior sucker in the area of the pectoral fin and biting the eye. The fish, although still alive, had been effectively immobilised but continued to swim weakly on its side. The attack was monitored for approximately 30 minutes during which time the fish was killed. The leech departed soon after mortality occurred.

Other examples of predation by the leech on fish were observed periodically throughout the study, mainly indicated by dead specimens found with leech bites. Plate 2.4 shows a clear leech bite on this unidentified fish fry (possibly a Perch, Perca fluviatilis). The most interesting specimen collected is shown in Plate 2.5. This large specimen of Rudd, Scardinius erythropthalmus, was collectected by net at the water surface in July 1984. On inspection there appeared to be no major damage to the body surface indicating attack by birds or death due to fungal infection. A lack of damage to the mouth region eliminated death due to an angler's returned catch. The only mark on the body surface was a large, tri-radiate leech bite at the base of the pectoral fin (see arrow). This bite size indicated an attack by a large leech and it appeared likely that this single-site attack was sufficient to cause mortality.

Observations on the behaviour of the fish population at Lade gravel pit showed that considerable time was spent, by adults and shoaling fry, grazing in amongst the emergent macrophytes. This obviously represents niche overlap with the leech. A common fish species at the site was the Perch, P. fluviatilis, and large adults were frequently observed feeding in the shallows. A previous author noted that this species is a predator on the leech (Herter, 1937), although no evidence of this was found, despite a series of 14 gut analyses, in specimens provided by local angler for this study.

Another important host species at Lade gravel pit is the Marsh Frog, Rana ridibunda. This conclusion was based almost entirely on the results of serological analyses described in Chapter 3. Throughout the field study there was no evidence of the leech causing mortality to the adult stage of the frog life-cycle.

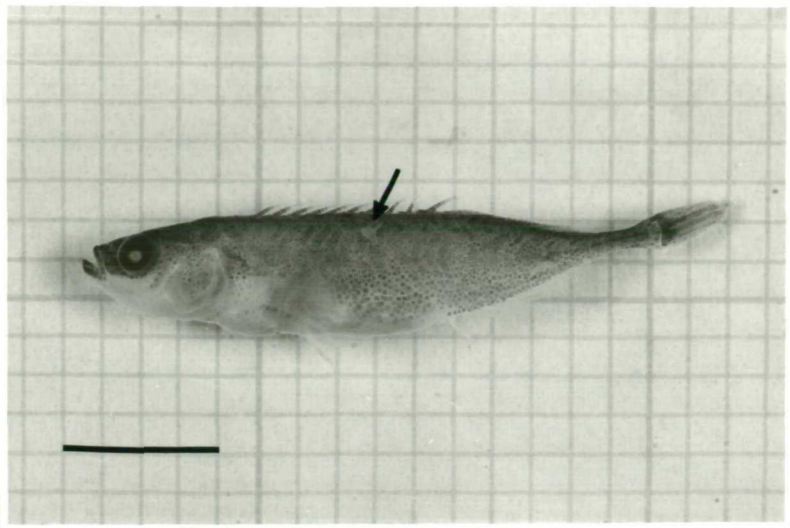


Plate 2.4 Medicinal leech bite on an unidentified fish fry,(arrowed)

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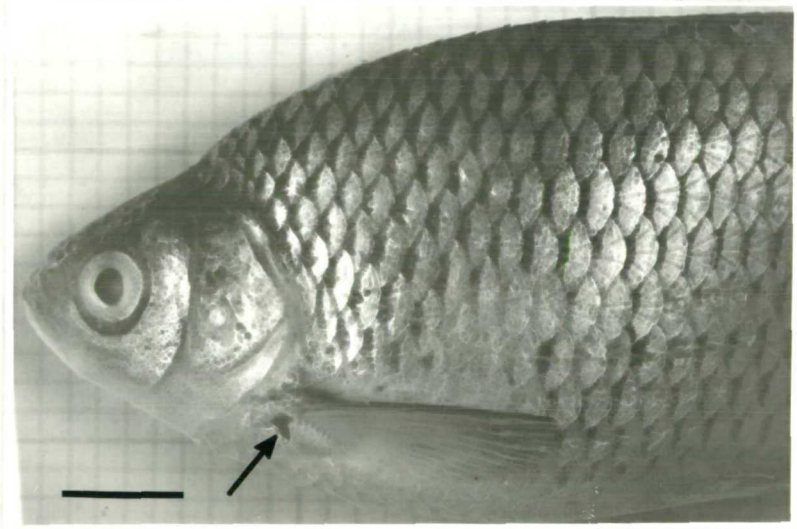


Plate 2.5 A single,large Medicinal leech bite at the base of the Pectoral fin of a Rudd, Scardinius erythropthalmus

Scale bar - 1cm

In early June 1983, a pair of adult Marsh frogs were observed in a mating posture in the shallows at Lade gravel pit (Plate 2.6). This pair was gently removed by hand and placed in a small cage which was half submerged in a small stand of emergent Phragmites communis (Common Reed). The cage was inspected on the following day. Both adults appeared to have died from multiple leech bites. The different sized bites shown in Plate 2.7 suggested that attacks had been made by several leeches. It is possible that prevention of escape caused the fatal effects as observations under natural conditions suggested that the frog was very successful in evading the leech. Even slight contact by the leech caused rapid movements by the frog. It is interesting to contrast this with the smooth newt whose escape movements were sluggish and resulted in almost a 100% successful attack rate by the leech.

Like the Smooth newt, T. vulgaris, the larval stages of the Marsh frog were also predated on by the leech The specimens shown in Plate 2.8 show two larval stages in the life-cycle of the frog. One showed signs of leech attack; the specimen on the left has a large hole where the gut has extruded.

This section has described the leech as a true predator in the community at Lade gravel pit. In situations where an attack on the host does not result in its mortality, the Medicinal Leech can be regarded as acting as an ectoparasite.

The Medicinal Leech as an Ectoparasite in the Community

The leech leaves a very characteristic tri-radiate bite mark after its attack and during the field study any evidence of this mark on living animals would have been a good indication of survival by the host and hence an act of ectoparasitism on the part of the leech. During June 1984 a total of 200 adult Smooth newts T. vulgaris were captured and the body surface of each examined for signs of bite scars. There was no evidence of any marks resembling the typical tri-radiate pattern. It seems reasonable to conclude that a leech attack on the Smooth newt nearly always results in mortality. The general difficulty in obtaining live fish specimens meant that no conclusion could be made on whether fish, as a group, can survive a leech attack.



Plate 2.6 A pair of mating Marsh frogs, Rana ridibunda, observed at Lade gravel pit in early June 1983, (female is below)

Scale bar - 5cm

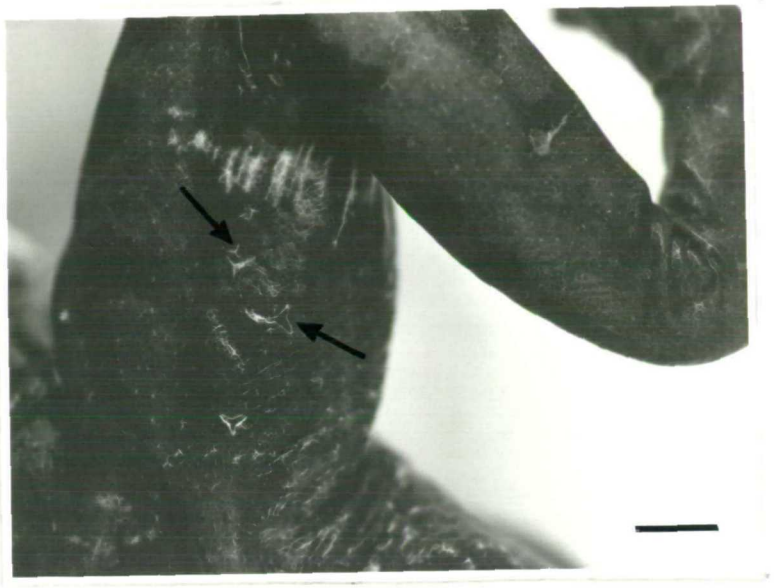


Plate 2.7 Multiple Medicinal leech bites on the hind limb of the male Marsh frog, R.ridibunda, observed in plate 2.6

Scale bar - 1cm

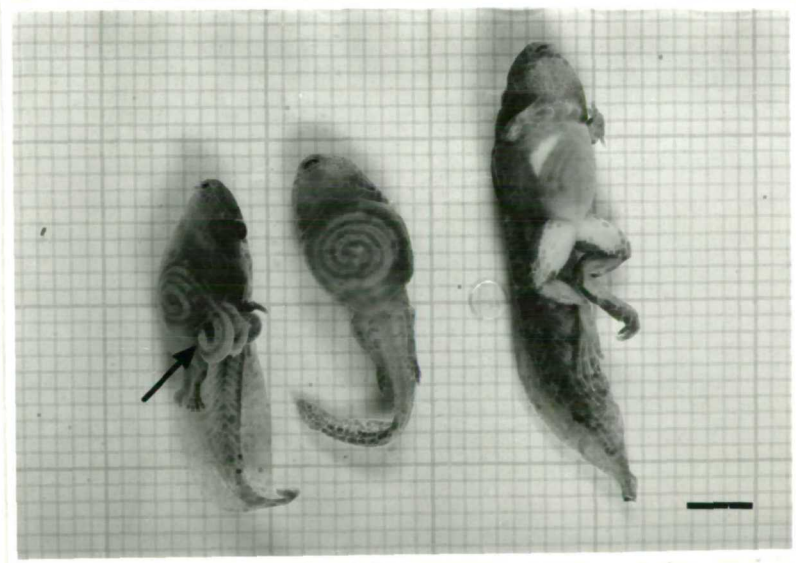


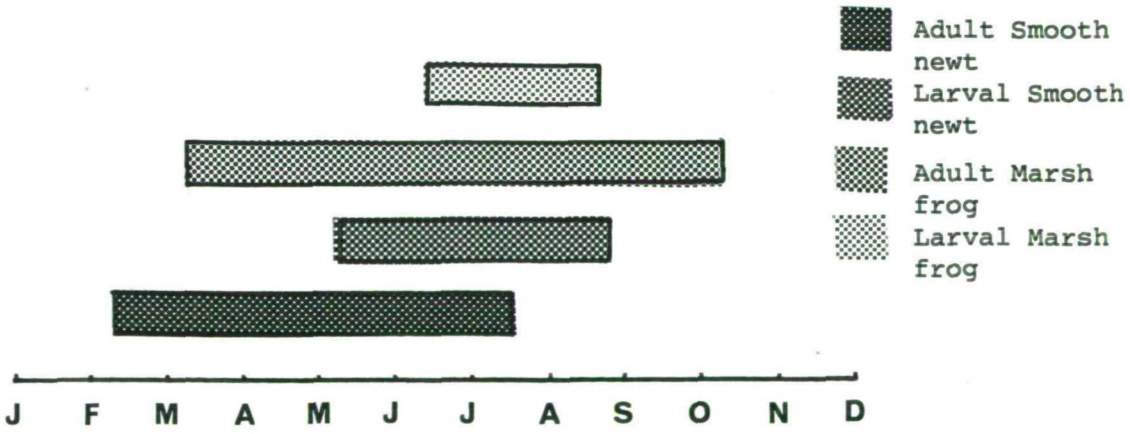
Plate 2.8 Larval stages of the Marsh frog, R. ridibunda showing signs of Medicinal leech attack

Scale bar - 1cm

As mentioned in the last section, no dead Marsh frogs were found in the wild under natural conditions. From a sample of 50 live frogs captured during June 1984 only a single specimen was found with a leech bite scar. The position of the bite scar is shown in Plate 2.9 (see arrow). The anomaly in these field observations is that the serological analyses, described in Chapter 3, suggest that the frog does play an important role as a host for the leech. It seems likely that at Lade gravel pit the Marsh frog population is able to tolerate the parasite load of the leech without significant mortality. This contrasts with observations by Hoffman (1960) on a population of common toads, Bufo bufo, where dramatic mortality of spawning adults occurred in the spring as a result of leech attack.

Having found that the Medicinal Leech at Lade gravel pit utilises the Smooth newt and Marsh frog populations, it is interesting to note that the availability of these hosts, in seasonal terms, extends from early February right to mid-October. Figure 2.1 below shows the approximate seasonal availability to the leech of the Smooth newt and Marsh frog.

Figure 2.1 The seasonal overlap of life-cycle stages of the Smooth newt and Marsh frog at Lade gravel pit.



It is interesting that mating/spawning in the Marsh frog occurs much later in the season than the Common frog, Rana temporaria (note: R. temporaria is absent from Lade gravel pit). The presence of larval



Plate 2.9 A Medicinal leech bite scar on the ventral surface of a live Marsh frog R. ridibunda

Scale - 1cm

amphibia well into July, August and early September, probably provides an important food supply to hatchling H. medicinalis which appear during the same period.

The other important host for the leech at Lade gravel pit appears to be birds. This conclusion is based almost entirely on serological results, described in Chapter 3, as no direct field observations were made of leeches acting as temporary ectoparasites on birds. It is worth mentioning here the leech specimens obtained at another site on Dungeness (Burrowes pit) which had recently gorged on bird blood. On this occasion, t mporary, ectoparasitic feeding by the leech may have resulted in the animals' dispersal by birds (Chapter 7 describes, in detail, the occurrence of the Medicinal Leech on Dungeness).

The Medicinal Leech as a Scavenger in the Community

Scavenging is a feeding strategy that has not previously been attributed to the Medicinal Leech but strong evidence was found at Lade gravel pit to indicate that the leech had been attracted to previously dead, decaying animal matter. Indeed the leech was observed biting and apparently feeding, as shown by rhythmic contraction of the buccal cavity, on dead animal material.

In April 1984 several chicken carcasses were found dumped at the site. On closer inspection 20 Medicinal Leeches were found attached to areas of skin between the feather tracts. In addition three specimens of the Horse Leech, Haemopis sanguisuga were also found associated with the carcasses, although they were not observed feeding. Plate 2.10 shows both the Medicinal and Horse Leech on one of the chicken carcasses, while Plate 2.11 shows a large Medicinal Leech in typical feeding posture between the feather tracts on the carcass. Plate 2.12 shows the exposed neck region of one of the carcasses with multiple Medicinal Leech bites.

Two other instances of possible scavenging behaviour by the Medicinal Leech were observed in the previous field season of 1983.

During each year of the field study 1983/84/85 a resident pair of Mute swans, Cygnus olor, nested successfully at Lade gravel pit. The male swan was vigorous in the defence of territory and nest to the point that due care in certain sampling areas was required by this author. In June 1983 the male swan was observed fatally attacking an adult coot,

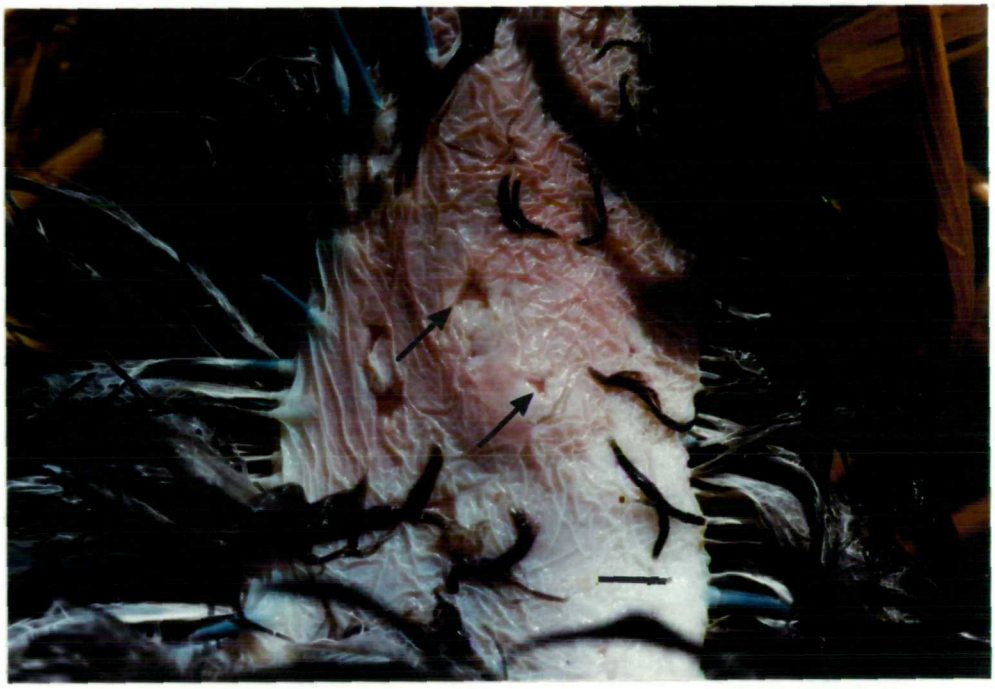


Plate 2.12 The exposed neck region of one of the chicken carcasses showing multiple Medicinal leech bites,(arrowed)

Scale bar - 1cm

Fulica atra. The position of the attack was noted and the following day the coot carcass was found lodged in a stand of emergent macrophytes. On inspection three large Medicinal Leeches were found on the carcass although they were not observed biting. Previously, in the same season, several coot chicks were found dead near the nest. Their injuries suggested that death had occurred by attacks to the head, possibly by raiding gulls or the male mute swan. On closer examination one hatchling had several Medicinal Leech bite scars on the webbed tissue of the feet. Again this could have represented an instance of leech scavenging. The possible significance of this adaptation is discussed later.

Predation on the Medicinal Leech in the Community

Throughout the field study no direct evidence was found of predation on the Medicinal Leech. This aspect of the animal's ecology still remains relatively unknown, although significant predators are thought to be birds and carniverous fish (e.g. Perch, P. fluviatilis).

A single piece of evidence, possibly indicating attack by birds on the leech, is shown in Plate 2.13. This large leech was found on the shoreline with apparent peck marks (arrowed) on the body surface.

An important predator on small frogs and newts at Lade gravel pit is the Grass snake, Natrix natrix. There is no evidence of the snake acting as a predator, or host to the Medicinal Leech, despite considerable niche overlap, i.e. the Grass snake is highly aquatic and stalks its prey in shallow water areas. The capture of an individual Grass snake in June 1984 formed the subject of an interesting series of laboratory experiments on the interaction of the leech with the snake.

There was no available evidence in the field of predation on leech hatchlings and cocoons and a series of laboratory trials were set up to investigate whether other aquatic invertebrate predators will attack the leech after hatching and whether selected predators from the litter, below the shoreline stands of Great Willow Herb, Epilobium hirsutum, will utilise the leech cocoon as found (see later).



Plate 2.13 A dead Medicinal leech found on the shoreline at Lade gravel pit with possible peck marks (arrowed) indicating attack by birds.

Natural size

The Interaction of the Leech Helobdella stagnalis and the Medicinal Leech, H. medicinalis

The interaction between these two leech species was highly seasonal and was only observed between late May and late June. During this period in both field seasons 1984/85, 63 large specimens of H. medicinalis (2.34 g - 7.01 g) were captured with infestations of Helobdella stagnalis. These small, transparent leeches were all attached to the Medicinal Leech by their posterior suckers and, in some cases, apparently by anterior mouthparts.

After capturing each individual Medicinal Leech infested with H. stagnalis it was placed carefully in a separate plastic container so as not to dislodge any of the attached leeches. On return to the laboratory the specimens of H. stagnalis were carefully removed from the body of the Medicinal Leech and maintained in a small plastic pot with a small amount of water. From the sample of 63 Medicinal Leeches, a total of 1449 attached H. stagnalis were obtained, ($\bar{x} = 23 \pm 5.25$ per leech, range 7-64). An interesting finding was that from this sample of H. stagnalis 1260 individuals (87%) were brooding either eggs or young. Further, none of these young were observed detached from the parent and all the H. stagnalis attached to the Medicinal Leeches appeared to be of adult size.

It remained unclear whether this relationship between the two species of leech was commensal or whether H. stagnalis was utilising the Medicinal Leech as a food supply. The feeding ecology of the former species has been studied by Young (1980) who found a wide prey selection mainly consisting of aquatic oligochaetes, molluscs and amphipods. There was no evidence suggesting predation on other small species of leech, e.g. Glossiphonia sp, and certainly no reference to ectoparasitism on the Medicinal Leech.

A small scale laboratory trial was set up to investigate whether 20 starved specimens (on the criterion of an apparently empty gut) of H. stagnalis would re-attach and feed from a Medicinal Leech introduced into a water filled container. Initial observations indicated that attachment by H. stagnalis was a result of direct contact with the body

of the Medicinal Leech as it moved around the container rather than positive movement by H. stagnalis to the Leech. After a period of approximately 2 hours, all the H. stagnalis were attached by their posterior sucker to the Medicinal Leech. The container was observed on the following day when three specimens of H. stagnalis had become detached. It was not possible to determine clearly under the microscope whether darkening in the guts of these individuals was due to feeding from the Medicinal Leech.

The remaining H. stagnalis were only observed attached by their posterior suckers. The only sure observation was that this association did not appear to be detrimental to the Medicinal Leech and until further study is made, possibly by a serological analysis of H. stagnalis gut contents against an anti-Medicinal Leech serum, a commensal relationship must be assumed. It is possible that the Medicinal Leech acts as a dispersal mechanism for H. stagnalis. Recent work by Tullet & Elliott (1986) also described similar behaviour by H. stagnalis and they concluded that it represented hyperparasitism of H. medicinalis.

A laboratory experiment to investigate predation by the Medicinal Leech on a pair of smooth newts, Triturus vulgaris, a small Marsh frog, Rana ridibunda and fish fry

An experimental arena was constructed consisting of a 1 metre square perspex container with shallow walls of 15 cms. A thin layer of fine gravel was used to cover the smooth base and this was covered with water to a depth of 10 cm. It was found by trial and error that this area accommodated up to three pairs of courting smooth newts (more than three males in this area resulted in fighting which disrupted the full expression of mating behaviour). The newts were allowed to acclimatise to the arena for several hours during which they were provided with a meal of chopped earthworm. All experiments were carried out in dim light which favoured the natural crepuscular behaviour of the newt. The pairs of newts readily engaged in the mating repertoire leading up to the male depositing a spermatophore followed by pickup by the female. During this series of behaviours the male was very active, constantly swimming in front of the creeping female and performing the characteristic tail fanning and whipping behaviour. The previous work of Houston, Halliday and McFarland (1977), which modelled the courtship of the smooth newt, provided a very useful diagrammatic representation of the behaviour sequences which were

all observed in this author's study. This diagram has been modified to include the involvement of the leech and is shown in Figure 2.2.

It has already been noted that evidence of leech attacks on the smooth newt were most often found during April and May, a period of intense mating activity by the Smooth newt. It was thought that the Medicinal Leech may home in on this activity and so this was tested.

In a series of 10 separate trials using a different pair of newts the behaviour of a single small Medicinal Leech was observed (a different leech was used for each trial). A trial commenced with the establishment of courtship display by the newts at which point the leech was introduced at the furthest point from the newts. In each trial, the leech adopted an alert posture and swimming was initiated, when newt courtship activity was within approximately 50 cms of the resting leech. The activity of the male newt was at times very vigorous (i.e. tail whipping and fanning), and it was clear that these movements caused sufficient local water disturbances to attract the leech. It was noticed, however, that the slower creeping stages in the newt courtship did not provide a sufficient stimulus to initiate swimming by the leech. There appeared to be a sequence of four main events which described the interaction between the newts and the leech.

1) Vigorous body and tail movements by the male Smooth newt

2) Leech initiates swimming and moves towards source of local water disturbance

3) Male and female newts engage in slow creeping

4) Leech rests and adopts an alert positon.

The sequence 1-4 was repeated several times in each trial until the leech was in close proximity to the pair of newts. Actual contact by the leech, with either the male or female newt was never achieved while the leech was actually swimming. Instead, in all instances of a successful leech attack, the newt actually touched the leech during slow creeping. The whole newt courtship repertoire was immediately disrupted if either partner was attacked by the leech. On no occasion was the leech dislodged after initial contact, despite vigorous body

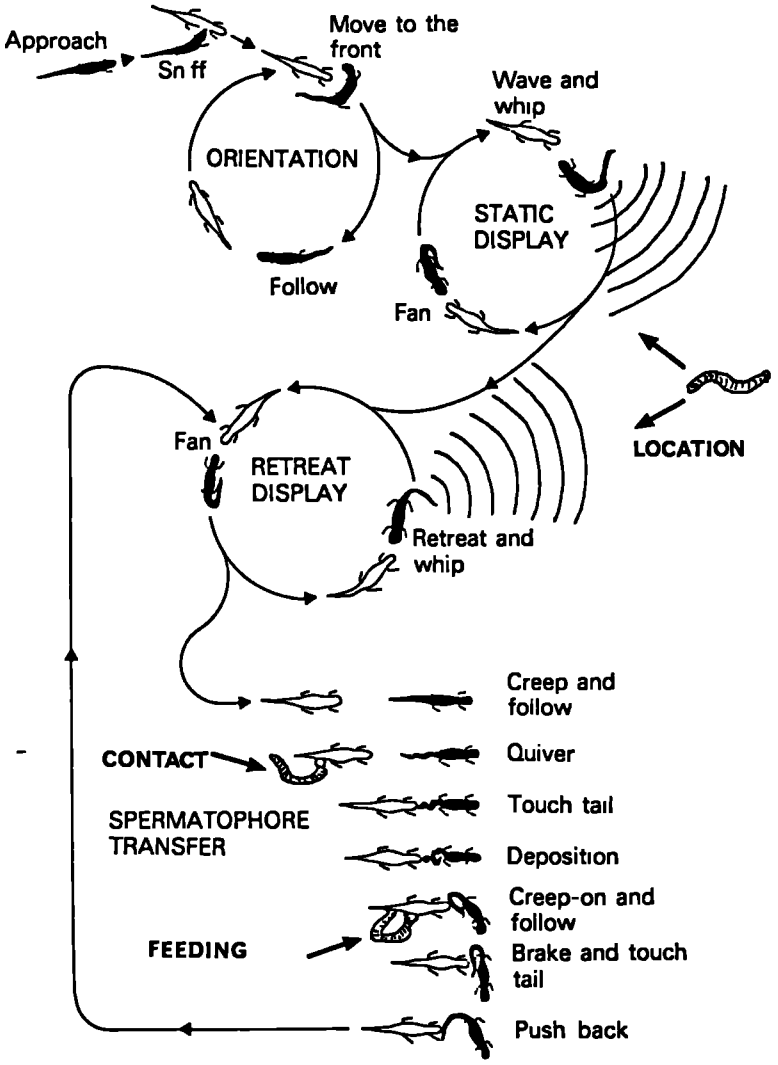


Figure 2.2 The involvement of the Medicinal leech in the sexual behaviour of the Smooth newt, Triturus vulgaris, (adapted from Houston, Halliday and McFarland, (1977)

contortions by the newt.

Each attack by the leech resulted in the death of the newt and this seemed to coincide with the departure of the leech from the body 35-75 minutes after the initial bite. It does appear that the leech maximises its residence time on the living host as a return on the energy invested in host location. It was interesting that during this residence period all the leeches involved inflicted multiple bites on the newt body although it was unclear why the leech ceased biting at one site only to re-attach at another site on the body surface after up to 1-2 minutes inspection by the anterior (mouth) end. In each of 10 trials, contact was eventually made by each leech and the arena size did not appear to restrict the courting newts nor prevent their escape.

The same arena was used to investigate the interaction of 10 different Medicinal Leeches with 10 small Marsh frogs, R. ridibunda and a small group of fish fry (Roach R. rutilus).

The interaction between the Marsh frog and leech was more difficult to interpret since both remained separate and sedentary for long periods. An encounter between the two only occurred after random movements by the foraging leech rather than positive location. The most significant difference in the behaviour of the Marsh frog compared to the Smooth newt was the immediate and rapid escape reflex behaviour on the part of the frog when the leech came into contact with the body surface. There was a period of inspection by the leech, which lasted several seconds, before firm attachment was attempted and this period was always sufficient, in a series of 10 trials, to enable the frog to successfully evade the leech. Indeed sampling for the Marsh frog in the wild was very difficult due to their elusiveness and rapid movement into deeper water when disturbed. It does seem to be an anomaly that serological analyses indicated that the Marsh frog is a significant host to the Medicinal Leech and yet it appears to be a highly evasive quarry. It could be that the Marsh frog becomes particularly vulnerable to attack during the mating period in late May-June although no field evidence was found of this possible seasonal utilisation of a host.

The movements of a small group of fish fry in the arena did not stimulate the Medicinal Leech in searching behaviour. In a series of

10 trials, where a different leech was introduced to the arena containing 10 different fish fry for each trial, only three successful attacks occurred as a result of foraging movements by the leech.

These preliminary experiments could be extended to examine the interaction of the leech with its hosts in quantitative behavioural terms.

Laboratory experiments to investigate invertebrate predation in the hatchlings and cocoons of the Medicinal Leech

A series of potential invertebrate predators were obtained from net and hand sampling in the shallows of Lade gravel pit and from the litter below stands of the Great Willowherb, Epilobium hirsutum (Chapter 5 describes in detail the microhabitat selected by the leech for laying cocoons). The following potential aquatic and terrestrial invertebrate predators were obtained.

Aquatic predators (hatchlings)

Corixa sp (Water Boatmen)
Notonecta viridis
Dystiscus marginalis (Great Diving Beetle larva)
Libellula depressa (Dragonfly Nymph)
Argyroneta aquatica (Water Spider)
Helobdella stagnalis

Litter predators (on cocoons)

Haemopis sanguisuga (Horse Leech)
Carabus nemoralis (Ground Beetle)
Staphylinus olens (Rock-tail Beetle)
Lithobius sp (Centipede)
Cylisticus convexus (Woodlouse)

All Medicinal Leech hatchlings and cocoons were obtained by laboratory culture. Trials were set up in small plastic sealed containers and comprised a single hatchling, or cocoon, for each potential predator. All trials were initially observed for an hour and left for a total of 24 hours (14 hr light/24^{o}C laboratory regime).

There was clear evidence that Notonecta viridis and the dragonfly nymph Libellula sp would attack leech hatchlings. On each occasion where the hatchling was actively grabbed and held in the mouthparts of these insects it was able to escape. After the 24 hour period there was no mortality recorded. There did not appear to be any evidence of attack by the other aquatic predators.

In the series of trials with litter invertebrates there was no sign of any attack on the leech cocoon. Only a single piece of evidence was obtained from the wild of invertebrate utilisation of the leech cocoon. This involved a failed cocoon, obtained from the Lade gravel pit site, which contained the larvae of an unidentified Dipteran fly. It was uncertain whether this represented parasitism on the cocoon or the use of an already moribund cocoon.

The Interaction of the Grass Snake, Natrix natrix and the Medicinal Leech

The Grass snake was active in the shallow, vegetated areas of Lade gravel pit between May and August and the capture of an individual provided the opportunity of observing any interaction between the snake and the leech in the laboratory.

The Grass snake was housed in an aquarium (100 x 45 x 45 cm) which was partly filled with water and a sloping bank of dry shingle with leaf litter and driftwood as shelter. A tight fitting lid was applied with necessary air perforations. The snake was maintained on its natural diet of Smooth newts and small Marsh frogs which were both readily accepted.

To examine whether the Grass snake would utilise the Medicinal Leech as food the snake was starved for several days prior to the experiment. A large leech was then introduced into the aquarium and the behaviour of the snake and leech observed. After a period of 48 hours the snake had not fed on the leech although two introduced Smooth newts were immediately fed upon by the snake. This trial was repeated twice and on both occasions the snake failed to feed on the leech despite appearing to be aware of the leech's presence.

To examine whether the Medicinal Leech utilised the Grass snake as a host, three large hungry H. medicinalis were added to the aquarium. The leeches remained in the water-filled section of the aquarium and movements by the snake did arouse searching behaviour. After a short time one of the leeches became attached by the ventral sucker to the body of the snake. Vigorous movements by the snake did, on this occasion, successfully dislodge the leech. A further contact was made by another



Plate 2.14 A view of part of the body of a Grass snake, Natrix natrix, (held in a gloved hand), showing the site of a recent Medicinal leech bite, (arrowed)

Scale bar - 1cm

leech and on this occasion biting was initiated. There appeared to be some buccal pumping indicating that penetration of the snake scales had been achieved and feeding was taking place. Again this leech was dislodged while feeding. Plate 2.14 shows a section of the Grass snake body with the bite mark arrowed (it was necessary to handle the snake with gloves to protect against the defensive emission of noxious slime).

This laboratory experiment, showing that the Medicinal Leech is able to penetrate the scales of the Grass snake is an interesting finding and does add to our ideas about the effectiveness of the jaw apparatus but the interactions observed may not be a realistic representation of what occurs in the wild.

A few observations of the Grass snake in the wild showed it to move very rapidly in the shallow water areas and it would be unlikely for a leech to make successful contact.

Although the Grass snake is highly aquatic, it is only a temporary visitor to the water spending most of its time on land. Therefore, even if the leech were able to utilise the snake as a host on rare occasions, the general availability of the Grass snake to the leech population as a whole would remain very restricted.

An Experimental Study of the Jaw Apparatus of the Medicinal Leech

This preliminary study involved an examination, using scanning electron microscopy, of the jaws of a pre-fed leech hatchling, which was unable to penetrate the author's skin, and two other small leeches which were able to bite the author's skin and feed. The aim was to investigate the idea that limitations of the jaw apparatus of the hatchling leech may restrict them to feeding on the relatively thin integument of amphibia during their early period of growth while being able to penetrate the thicker bird or mammalian integument only after a period of jaw development. Although a recent detailed description of the structure of the teeth of the Medicinal Leech was given by Damas (1985), no comment was made on the efficiency of the jaws or teeth for penetrating different surfaces or whether there were any differences in the development of the teeth in leeches of different ages.

For this experiment three preparations were made from the following specimens:

Specimen 1 a pre-fed hatchling leech weighing 36 mg, which was unable to bite the author's skin

Specimen 2 a wild leech weighing 0.27 g of unknown dietary history but which was able to bite the author's skin

Specimen 3 a laboratory hatched leech fed infrequently over a period of two years weighing only 80 mg but able to bite the author's skin.

The jaws of each leech were obtained by anaesthetising the animal in carbonated water followed by 8% ethanol. The head region was dorsally dissected under saline and the three jaws were carefully removed. The jaws were observed using a scanning electron microscope and the methods involved for the preparation of these specimens were identical to those described fully for the preparation of leech gut blood samples in Chapter 3.

The results of this preliminary investigation were interesting as the following series of plates show.

The general position of the three jaws in Specimen 1 is shown in Plate 2.15. A closer view of one of these jaws is shown in Plate 2.16 and it is clear that the row of teeth (arrowed) does not extend along the full length of the jaw. This contrasts with the appearance of a jaw from specimens 2 and 3 shown in Plates 2.17 and 2.18 respectively. They both show a long dental arcade with well formed sharp teeth. Scanning in close-up along the contrasting jaws also showed differences in the appearance of teeth on the jaw. Plate 2.19 shows an irregular arrangement of teeth on the jaw of specimen 1 contrasting with those of specimen 2 which are evenly arranged and appear sharp (Plate 2.20).

Many more samples need to be quantitatively observed for any significant differences in morphology and in total number of teeth between leeches of different ages and biting ability. This study obviously did not compare any differences in the muscular action of the jaws in situ and this is obviously an important factor in overall efficiency.

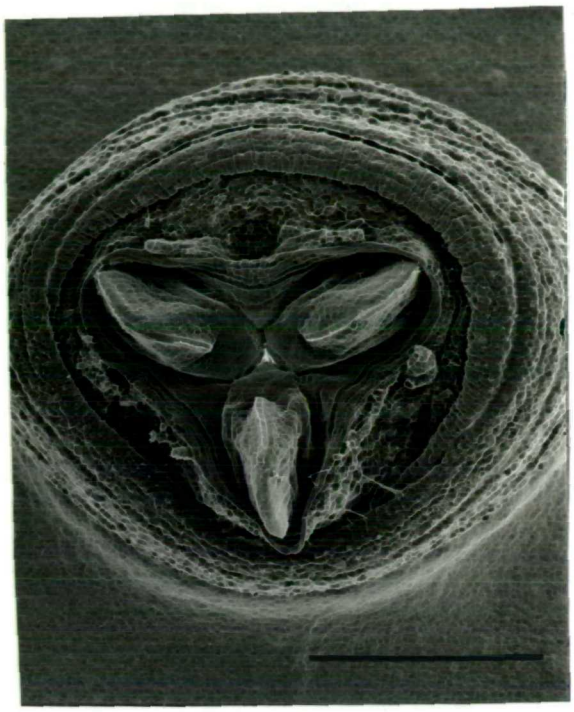
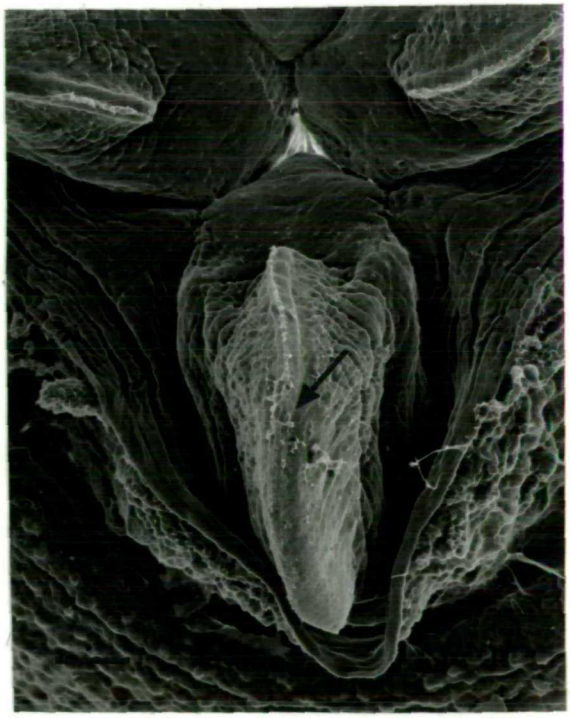


Plate 2.15 A Scanning Electron Micrograph (SEM) showing the positio of the three jaws in the pre-fed hatchling Medicinal leech.

Scale bar- 500µ

Plate 2.16 A closer SEM of one of the jaws shown in plate 2.15. It shows a short dental arcade which does not extend right along the jaw, (arrowed)

Scale bar - 50µ



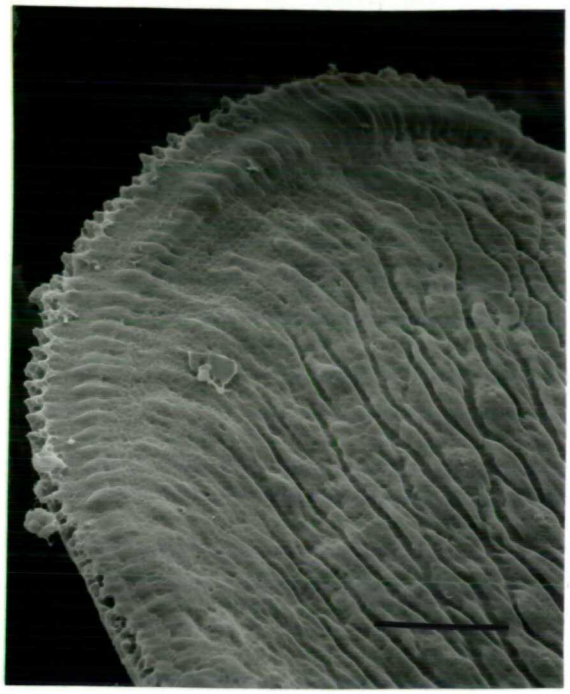


Plate 2.17 A SEM showing a long dental arcade in the Medicinal leech obtained from Lade gravel pit, (Specimen 2)

Scale bar - 50µ

Plate 2.18 A SEM showing a long dental arcade in the Medicinal leech hatched in the laboratory and fed over two years, (Specimen 3)

Scale bar - 50µ



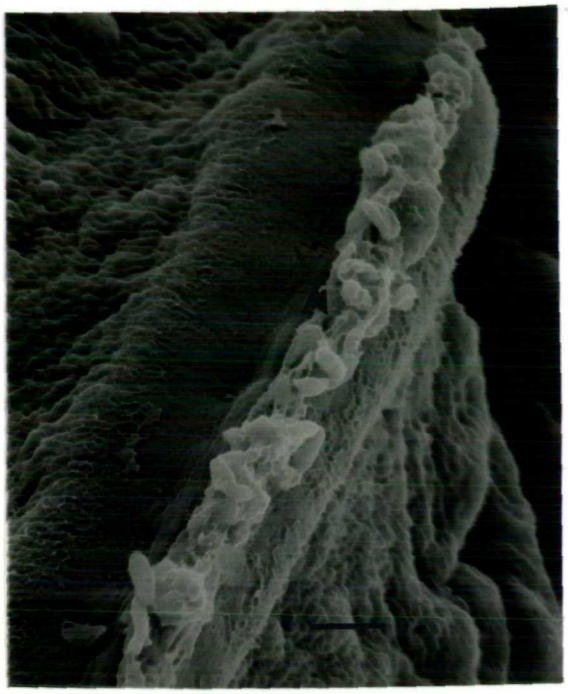
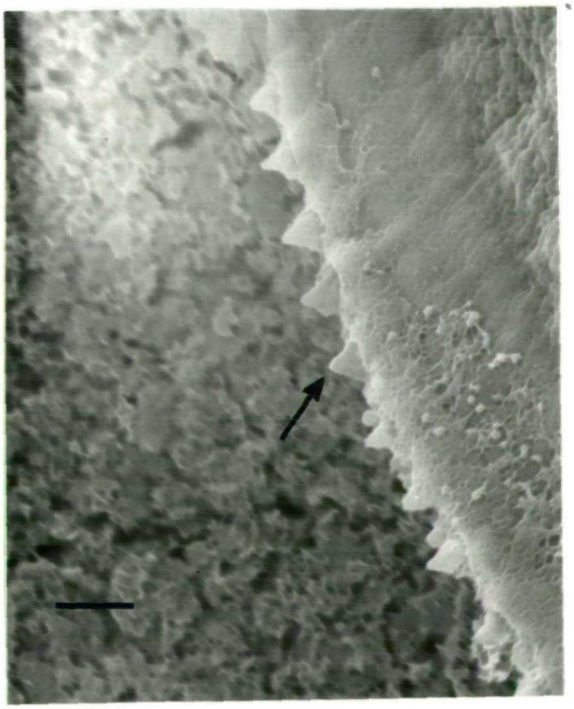


Plate 2.19 A SEM showing an irregular arrangement of teeth on the jaw of the pre-fed hatchling Medicinal leech, (Specimen 1), (arrowed)

Scale bar - 5μ

Plate 2.20 A SEM showing the regular arrangement of sharp teeth on the jaw of the wild Medicinal leech, (Specimen 2), (arrowed)



Nevertheless, this is the first attempt at a comparative look at differences in the dental arcade of leeches of different ages and development and deserves further investigation.

Discussion

It has already been noted in the literature that the Medicinal Leech is seasonal in its activity, or, more particularly, its response to water disturbance and that this stimulus seems to override any diurnal effects. It still remains a puzzle why water temperature appears to be the important controlling factor of activity, particularly at Lade gravel pit where the activity of at least one of its hosts, birds, is year round. It is likely, however, that the main hosts at the site are amphibia and these are absent from the water during the period late September - early March. This seasonal activity by the Medicinal Leech is in contrast to other leech species at the site particularly Theromyzon tessulatum, the sanguiverous ectoparasite of birds.

There does appear to be an energy investment by the leech in its body activity during the most favourable part of the year when the probability of obtaining a bloodmeal is greatest. Indeed leeches kept in an aquarium at an ambient temperature of 2-6^{o}C during the winter months remained virtually immobile for the whole of this period. This contrasted with leeches maintained in the laboratory at 15^{o} $\pm$ 2^{o}C during the winter months (November - February) at an average winter daylength of 8 hours. These leeches remained active during this period and even received a bloodmeal as part of breeding experiments described in Chapter 6. This tended to support the idea that seasonal photoperiodic cues were not important factors determining leech activity in the wild.

The most important results from the field work were the observations that in the wild the Medicinal Leech acts as predator, ectoparasite and apparent scavenger. This is an important extension on previous ideas about the leech feeding in the wild. It is believed by this author that the leech in the wild invests much energy in its search for hosts with only occasional successful contacts and, even then, probably only resulting in a sub-optimal bloodmeal (i.e. the leech is not gorged after the meal). Obviously an adaptation to a strategy of scavenging

could supplement this infrequency in the supply of its normal bloodmeal.

It certainly seems likely that, in the wild, the leech, despite being a specialist feeder on vertebrate blood, is opportunist in its approach and is not host selective. Once the leech has made contact with a host its handling-and-feeding time is extended and, in the sense that it is an energy maximiser (Schoener, 1971), the more time spent feeding the better it is for the animal's survival (fitness).

The very slow utilisation of a bloodmeal by the leech has been well documented but an explanation as to why the animal has adapted this strategy of slow digestion remains uncertain. It would appear to make sense to digest a bloodmeal slowly if the probability of successful, subsequent contacts with hosts was small. These aspects of adaptation by the leech are discussed in more detail in the main discussion at the end of the thesis in connection with survival and conservation potential.

During the arena experiments on the predator-prey model of the Medicinal Leech and Smooth newt, T. vulgaris, it was clear that the leech was investing energy in foraging only during active stages of the Smooth newt courtship repertoire. The pattern of location was: swimming - rest - alert posture - swimming - rest etc. Therefore, during foraging, the leech apparently only makes significant energy investments in discrete bursts of swimming rather than a possibly more inefficient investment in continual swimming until contact is made with the host. In the wild a relatively small patch of shallow bottom, (approx $2\,m^2$) will accommodate several pairs of mating Smooth newts. This represents a changing distribution of prey for the leech and it is interesting to speculate whether a leech would optimise its foraging activity within the patch.

These ideas concerning feeding strategy and optimal foraging, as applied to the Medicinal Leech, could be an extremely interesting area for future research. The reviews provided by Schoener(1971), Pyke, Pulliam and Charnov (1977) and Townsend and Hughes (1981) together with model work, on a predatory net-spinning Caddis larva, by Townsend and Hildrew (1980) could provide a good starting point.

CHAPTER 3

AN INVESTIGATION OF THE DIET OF H. MEDICINALIS USING OBSERVATIONAL AND SEROLOGICAL TECHNIQUES

Introduction

The analysis of diets, involving the examination of the gut contents of a predator or parasite, has found great application in the study of prey-predator relationships (Boreham and Ohiagu, 1978) and for the identification of arthropod bloodmeals in relation to the epidemiology of vector-borne disease (T mpelis, Reeves, Bellamy and Lofy, 1965; Edman and Kale, 1971; Boreham, 1972, 1975). The direct examination of gut contents, for the detectable remains of prey, is only of value where sufficient hard parts remain for positive identification (Sunderland, 1975). Although the blood cellular characteristics in the vertebrate classes have been described (Andrews, 1965; Archer and Jeffcott, 1977), direct examination of erythrocyte size and nucleation, in the meal of blood feeders, has only been used as a general guide for the identification of host range (Lloyd, Johnson and Young, Morrison H,1924). Often results have proved misleading (Weitz, 1956), probably due to erythrocyte change in the gut environment (Rahman, Wright and Cerny, 1973).

Amongst the range of experimental and analytical approaches to the study of prey-predator interactions suggested by Kiritani and Dempster (1973) and Southwood (1978), serological techniques have been used extensively as highly sensitive methods for the detection of such interactions (Dempster, 1960; Ashby, 1974; Healy and Cross, 1975).

These methods have been largely adapted fr m the use of serology for the identification of the host-range of haematophagous arthropods (Weitz, 1960; Gooding, 1972; Tempelis, 1975).

Early work by Nuttal (1904), which was later added to by Hubalek (1982), on the taxonomic relationships between animal groups, showed that specific proteins or glycoproteins (antigens) of one organism react more strongly with an antiserum (antibodies) prepared against the antigens of a similar organism than with an antiserum against the

antigens of a dissimilar organism. This antigenic relationship between sera was demonstrated by the formation of an insoluble precipitate at the boundary of a mixture of antigen and corresponding antibody. This serological test has been developed to study the interaction between a saline extract from an arthropod bloodmeal and a suitable antiserum (i.e. antisera corresponding to likely hosts) prepared in rabbits or chickens (Bull and King, 1925; Proom, 1943; Eligh, 1952; Tempelis and Lofy, 1963; Cupp and Stokes, 1973).

Today, a wide range of serological techniques exist for not only the formation of a precipitation reaction (Kabat, 1968; Ouchterlony and Nilsson, 1973), but also agglutination reactions (Greenstone, 1977; Boreham and Ohiagu, 1978). The latter reaction uses the principle that particulate antigens such as erythrocytes, or soluble antigens, will attach to inert particles such as latex and agglutinate in the presence of specific antibody. The development of serological techniques in the study of prey-predator relationships have simply replaced the bloodmeal antigen with a saline extract of antigen prepared from a part of, or whole prey organism. The precipitation principle remains the same.

In the freshwater environment, serological techniques have been used extensively to identify the 'food niches' of lake dwelling triclads by Young, Morris and Reynoldson (1964), Reynoldson and Davies (1970), Reynoldson and Sefton (1976) and Reynoldson and Pierce (1979). Recently, the diets of some Rhynchobdellid leeches have been studied by Davies, Wrona and Everett (1978), Wrona, Davies and Linton (1979), Davies, Wrona and Linton (1979) and Young (1980, 1981a, 1981b). Little work has been done on the diets of blood-feeding Gnathobdellid leeches. Jung (1955) attempted a microscopical and serological approach to the study of wild H. medicinalis gut contents and was able, on the basis of erythrocyte size and limited serological results, to distinguish the class of vertebrate host. An unpublished, serological and microscopical study of the bloodmeals of H. medicinalis was made by Keim (1979). He analysed the gut c ntents from wild and trade leeches using Ouchterlony diffusion and disc-electrophoretic techniques. He identified a range of hosts and, interestingly, found the electrophoretic technique less sensitive than Ouchterlony diffusion

(cf. Healy and Cross, 1975). He also used microscopical observation to examine the change in appearance of human erythrocytes, fixed at various times after a known feed. He observed them becoming 'paler' until the 13th day after feeding, when the majority appeared to have undergone haemolysis. It is interesting that he observed a granulation and emptying and dissolving of lymphocytes on the sixth day after feeding, possibly indicating the rapid action of the protease inhibitor eglin C (Baici and Seemüller, 1984).

Materials and Methods

In this study pilot experiments were performed to examine the validity of observational methods for identifying bloodmeals from wild H. medicinalis using light microscopy and scanning electron microscopy (SEM), (Hayat, 1978). The work of Weitz (1956), Pickavance (1970), Ouchterlony and Nilsson (1973) formed the basis of the serological methods used. Some modifications were adopted at certain stages of the preparations.

(i) Method for obtaining bloodmeal samples

Due to the significance, and likely fragility of the Medicinal Leech population , a non-destructive method for obtaining a small sample of bloodmeal from the gut was developed. This contrasts with Keim (1979) who obtained larger samples from dorsal dissections of all tested specimens.

Leeches were obtained from two populations on Dungeness (Lade gravel pit, TR077217, and Lydd Airport Marsh, TR065210) by a sampling method described in Chapter 1.

Narcotisation with 8% ethanol, although resulting in occasional disgorgement, proved an unreliable method of obtaining a bloodmeal due to some mortality and the problem of bloodmeal recovery and possible dilution of the antigen in the ethan 1. The use of micro-capillary tubing to obtain blood from the crop caecae, was rejected as being likely to cause internal damage. Instead, the leeches were blotted dry and gently rolled across the palm of the hand.

Standard, plastic, laboratory gloves were worn to ease the recovery of the bloodmeal and to prevent possible contamination. Occasional, light finger pressure was applied ventrally in a posterior to anterior direction. This rolling process was continued for no longer than 1-2 minutes regardless of whether disgorgement had taken place. This was to avoid overstressing the animal. Occasionally mortality did result from this technique but usually the leech, although producing much mucous, did return to normal behaviour after several hours. If disgorgement occurred a sample of bloodmeal was applied to a microscope slide and to a fragment of cover slip for the light and SEM work respectively. Another sample was recovered in 1-2 drops of 0.9% saline, in a small plastic specimen pot with a clip-top and refrigerated at -20°C to await serological analysis. The amount of bloodmeal obtained from the leeches was very variable and it was not possible to standardise volumes used for serological analysis.

(ii) Observational techniques

(a) Light microscopy:

A small sample of bloodmeal ranging from a smear to 1-2 drops was obtained as described above and applied to a clean, dry microscope slide. A drop of 0.9% saline was applied to one side of the bloodmeal sample and the two gently mixed with the edge of a cover slip. This achieved some spreading of the dark red, viscous bloodmeal. A smear was made and allowed to dry. One series of smears was left unstained, while another series was stained with Leishman's stain (Drury and Wallington, 1967). These smears were covered for 20 seconds with stain followed by a mixture of two parts distilled water (phosphate-buffered at pH 6.8) and one part stain. Mixing was achieved by gentle rocking or blowing on the slide. After about 8 minutes the smear was washed with distilled water. Overstained smears were differentiated in the buffered distilled water for 2 minutes before being washed in distilled water. Stained smears were dried vertically in air. Both series of smears were observed using a Nik n Optiphot Microscope with Differential Interference Contrast lighting (after Nomarski).

(b) Scanning Electron Microscopy:

A bloodmeal sample, ranging from a smear to 1-2 drops was applied to a fragment of coverslip, and immediately placed in 10 mls of 2.5% phosphate buffered glutaraldhyde fixative for 12 hours (Appendix 3,1) The sample was then washed in two successive 10 minute baths of 0.1M Sorensen's phosphate buffer at pH 7.2
The sample was then immersed in 50 ml of 50% acetone for 15 minutes followed by successive dehydration in 70%, 80% 90% acetone and twice in 100% acetone, each stage lasting 15 minutes. The dehydration process was completed by placing the sample in a Polaron Critical Point Dryer for 1½ hours. The sample was then mounted on a stubb using silver dag and gold coated for 5 minutes in a Nanotech Sputtercoater. These bloodmeal samples were then stored in a dry cabinet until viewed with an Hitachi S430 Scanning Electron Microscope.

(iii) Serological techniques

(a) Preparation of the antigen:

It was hoped to prepare antisera specific to the more common, likely host species at the two sites such as mallard (Anas platyrynchos), coot (Fulica atra), moorhen (Gallinula chloropus), roach (Rutilus rutilus), rudd (Scardinius erythrophthalamus) and perch (Perca fluviatilis). Unfortunately due to the lack of availability and the law relating to the capture and handling of wild birds, these specific blood antigens were not obtained. An anti-newt (Triturus vulgaris) serum was not produced because it would have involved the sacrifice of excessive numbers of animals to produce the required volume of newt antigen.

In order to produce general antisera to bird, fish, amphibia, and mammal, blood samples were obtained from a domestic duck, a farmed brown trout, several marsh frogs and from cattle, sheep, human, dog and rat. These mammal antigens were combined to produce an anti-mammal serum. These whole blood samples were allowed to clot for ½ hour in a 37°C waterbath and then centrifuged at 3,500 g for 10 minutes. The blood serum fraction was pipetted off and stored as 2 ml aliquots in small plastic containers with sealed plastic tops, at -20°C.

(b) Determination of the protein content of the serum antigens:

An estimation of the amount of protein present in the sera to act as antigens was required to interpret any possible variation in the behaviour of the immune system of the rabbit, the animal in which the antibodies were raised, which might have affected the titre of the antisera. If too little antigen is used the rabbit's immune system will not be stimulated sufficiently while conversely if an excess of antigen is used a state of immunological tolerance may be induced (Weir, 1977).

For the determination of serum protein concentration an improved Biuret reagent was prepared as suggested by Yatzidis (1977). The advantage over the original preparation by Layne (1957) was that a maximum colour intensity was produced within 5 minutes at room temperature which remains stable for hours. A calibration curve was produced by mixing varying proportions of a standard protein solution (8g/100 ml) and 0.9% saline. The scheme used is shown in Table 3.1.

Table 3.1 The combination of protein solutions used to calculate the calibration curve

	Test	Calibration Curve	Control	Blank
Serum (antigen)	50 µl			
0.9% Saline	50 µl	50 µl or less	50 µl	100 µl
Standard Protein	-	50 µl or more	50 µl	
		100 µl		
Biuret Reagent	2.5 µl	2.5 µl	2.5 µl	2.5 µl

The optical d nsities of the solutions were measured with a Pye Unicam SP6-450 uv/vis spectrophotometer. The protein concentration of the antigen was measured from the calibration curve.

(c) Preparation of the antisera:

For this preparation 4 adult, male, non-pedigree white rabbits were used. They were housed and handled in accordance with the recommendations of the Universities Federation for Animal Welfare (UFAW, 1972) and maintained on a diet of G.421.HE. Rabbit pellets ACS (Appendix 3.2).

As a preliminary it was important to test the serum of the rabbits to be used against each of the serum antigens in order to detect any pre-existing antibodies in the rabbits which might interfere with the properties of the antisera to be raised. A 5 ml blood sample was obtained from a nick in the marginal ear vein of each rabbit and the serum prepared as described above. A 50 µl aliquot of rabbit serum was pipetted into a small length of corked capillary tubing followed by a 50 µl aliquot of the serum antigen. This capillary ring test was allowed to stand at room temperature for 4 hours. In each of the 32 tests no precipitate was formed at the interface of the rabbit serum and serum antigens indicating that no pre-existing antibodies were present in the experimental rabbits to the range of serum antigens to be used.

The literature contains many recommendations on the amount, site, number and period for the inoculation of antigen. The method used in this study was based on that suggested by Boreham and Ohiagu (1978) for the production of an antiserum of high titre and broad specificity.

A series of 5 injections of an emulsion of 1 ml Freund's complete adjuvant (FCA) (Appendix 3.3) and 1 ml of serum antigen, were given to each rabbit over a period of 17 days, i.e. 3 clear days between successive injections. In the case of mammal serum antigen, the 1 ml aliquot was composed of a mixture of 0.2 ml fractions of cattle, sheep, dog, human and rat sera. It was considered that the degree of shared protein antigen would give an overall anti-mammal response while also including a reaction to more specific protein antigens. Injections were made in the region of the inguinal and axillary lymph nodes. They were made in rotation so that each node received at least one dose of

antigen. The theory behind this technique is that most of the antigen is presented to the primary site of antibody production instead of having to drain to the nodes, as in the case of intramuscular and intradermal injections.

Preliminary titre and specificity tests (described in Section (d) below), were made on small samples (5 ml) of blood obtained from the marginal ear vein of each rabbit 20 days after the 5th injection of antigen. Positive results were obtained for reactions to mammal and duck antigen and so these two rabbits were bled for maximum yield of antibodies. However, the response of the other two rabbits to fish and frog antigens gave an initially low titre (1:1000) after the same period so a further 'booster' injection was given 22 days after the 5th injection. A satisfactory antiserum titre for frog and bird was found from a small blood sample taken seven days after the booster injection and so these rabbits were bled for maximum antibody yield at this stage.

Two methods for bleeding the rabbits were used. One involved bleeding from the marginal ear vein, followed by heart puncture after a fatal, intravenous injection of anaesthetic. Both these methods were recommended by the UFAW (1972).

The rabbit was restrained and an area around the marginal ear vein was shaved and bathed with 70% ethanol. A transverse slice was made with a razor blade along a 2 cm length of the vein. The blood was collected in a clean, 50 ml flat-bottomed tube placed next to the ear. The blood was allowed to flow for several minutes until clotting around the cut made it difficult to collect. At this point a fatal injection of the anaesthetic Nembutal was given intravenously into the intact marginal ear vein. As soon as death had occurred the heart was revealed by dissection, punctured with a large needle (1.85-2.00 mm x 5 cm) and the blood collected in a syringe and transferred to a 50 ml flat-bottomed tube. Once all the blood had been collected, it was allowed to clot for ½ hour in a 37^{o}C waterbath, transf rred to centrifuge tubes and spun for 10 minutes at 3,500 g). The serum was pipetted off and stored at

$-20^{o}C$ as small 1 ml aliquots in labelled, plastic, clip-top tubes. At this temperature the antisera was found to remain viable for at least 18 months.

Two series of tests were performed on the antisera. Firstly a titre test for antiserum sensitivity and secondly a test for specificity. These are described in the following section.

(d) Testing the titre and specificity of the antisera

Testing the antisera titre and specificity was an important preliminary before the decision to bleed each rabbit for maximum yield of optimum antisera.

A small blood sample was obtained from a nick in the marginal ear vein of the rabbit and a sample of antiserum prepared as described above. A 0.1 ml aliquot of corresponding serum antigen was added to 10 ml of 0.9% saline to produce a 1:100 dilution of antigen. A serial dilution of antigen was prepared from this in the ratios 1:100, 1:2000, 1:4000, 1:8000, 1:16,000 and 1:32,000. A series of capillary ring tests were set up as follows. 50 µl aliquots of pure antigen and the 7 dilutions were placed in 8 separate small glass capillary tubes, with an additional tube containing 50 µl of 0.9% saline as a control. A 50 µl aliquot of corresponding antiserum was added to each tube and the mixtures allowed to stand at room temperature. The greatest dilution of antigen giving a positive result (i.e. formation of precipitate) was the titre of the antiserum.

Antisera may cross-react with closely related species and although the range of antisera produced were to animals from different vertebrate classes, their specificities were checked as follows.

A 50 µl aliquot of each antiserum raised (4) was tested against the whole range of serum antigens (8). A capillary ring test was used to sh w any reaction, followed by a more sensitive test; the modified miniature Ouchterlony agar diffusion method (described in the next section).

(e) Serological tests

A modified, miniature Ouchterlony double diffusion test, based on the method used by Pickavance (1970), was employed using equipment from an LKB 2117-401 Multiphor Immunoelectrophoresis kit (Appendix 3.4).

Very clean microscope slides were prepared by boiling them in weak detergent followed by a 15 minute rinse in tap water and several changes of distilled water. They were allowed to drain vertically in slide racks and then sluiced in hot 0.2% agar solution (in distilled water). They were allowed to dry in a dust free place at room temperature for about 20 minutes. This preliminary agar coat acted as a cement preventing the experimental top agar layer from slipping on the glass. For the serological tests a high quality, purified agar was used to produce a very clear medium which showed even a slight precipitation reaction.

Two types of agar solution were prepared. A 1.5% (w/v) agar solution was prepared, as recommended in the LKB kit, in an Erlenmeyer flask by adding 1.8 g of purified agar powder to 120 ml of 0.3 M potassium phosphate buffer pH 8.0 (Appendix 3.5). The flask was placed in a large beaker of water on a hotplate/stirrer and maintained at a temperature of about 80^{o}C. After 5-10 minutes the agar dissolved and clarified. A 1% agar solution (in 0.9% saline) was also prepared and clarified in the same way. It was found that this second preparation produced a clearer medium than that suggested by the LKB kit and was used for the rest of the experiment.

The pre-coated microscope slides were placed on a level table and 5 ml of molten 1% agar solution was carefully pipetted onto each. It was necessary to use a pre-heated pipette retained in hot water, to avoid clogging by solidifying agar. The agar layer was allowed to dry and the slide placed on the LKB plate holder. The LKB template for double diffusion was aligned over the micr sc pe slide in order that a central well with six surrounding wells, forming a hexagonal pattern, could be cut in the agar (Figure 3.1(a) and (b)).

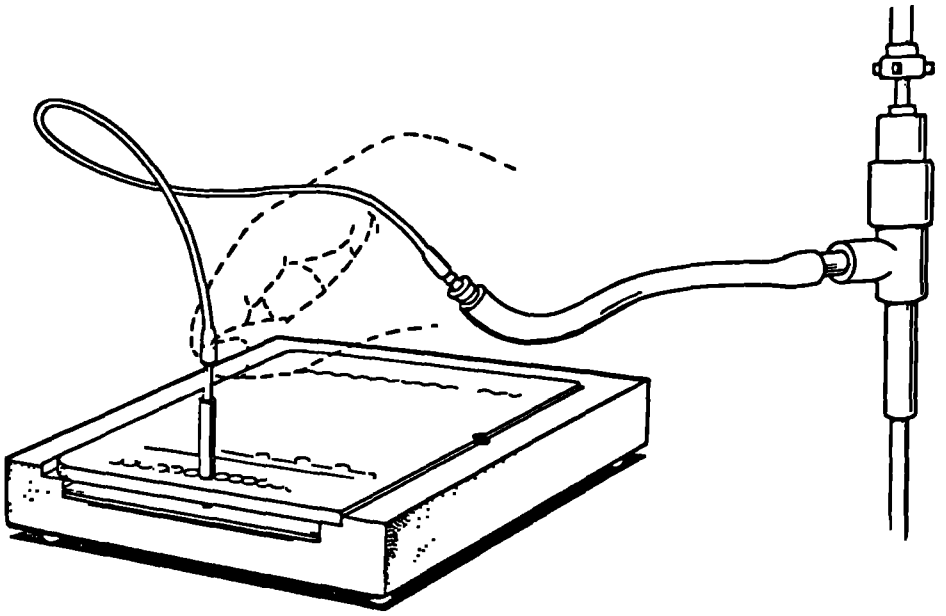


Figure 3.1(a) Experimental technique for cutting serological wells in agar gel; note the agar gel is removed by suction, (adapted from the Introduction manual for the LKB kit)

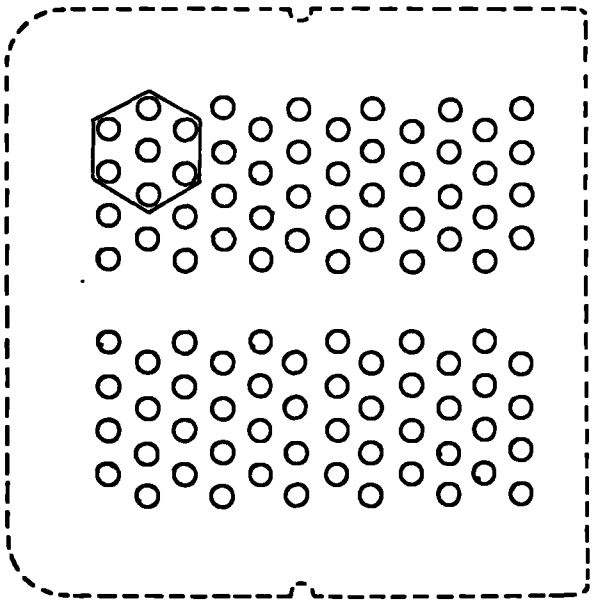


Figure 3.1 b LKB template for Double Diffusion, showing the hexagonal pattern of wells, (adapted from the Introduction manual for the LKB kit

These wells were cut at a diameter of 2.5 mm using the LKB puncher. It was possible to fit two hexagons on each microscope slide and hence two serological tests. Each slide was then placed on damp filter paper in a sterilin petri dish and each hexagon labelled. The petri dishes were stored for a few days at 4°C in the fridge. The cool, humid atmosphere prevented the agar from drying out.

The microscope slides were removed from the fridge, as required, and 25 µl aliquots of each antiserum were placed in four separate peripheral wells in the agar. Another peripheral well was filled with 25 µl of 0.9% saline while the other was left empty. A 25 µl sample of the leech bloodmeal under test was placed in the central well. Figure 3.2 shows the position of the antigen and antisera. The microscope slides were stored in closed petri dishes and kept at room temperature for 24-36 hours, after which time it was usually possible to see the formation of any precipitate as a band between the central well and one or more of the peripheral antisera wells. At this stage it was usual to wash and stain the gels to enhance the appearance of the bands for photography. It was found on a number of occasions, however, that soaking overnight in a balanced salt solution removed not only the non-precipitated proteins but also the precipitate bands. Therefore, this washing stage was discontinued and slides were stained directly.

Each petri-dish was filled with enough Dulbecco's phosphate buffered saline (Appendix 3.6) to completely cover the agar gel. This was allowed to stand overnight. The gels were then soaked in two successive 10 minute baths of distilled water. Since this stage served only to remove excess buffer from the gels, the time that they remained in distilled water was not critical.

Two methods of staining the gels were used but only one proved satisfactory for photographic purposes. Firstly, several gels were stained for 0.5 h in 0.02% Amide black solution followed by de-staining for 0.5 h in 7% acetic acid. Although precipitate bands were visible the process was time-consuming and did not give satisfactory photographic results. The bulk of the agar gels, therefore, were either soaked for 10 minutes in a 0.1% thiazine red s lution (in 1% acetic acid) with no acetic acid wash or soaked for 2-5 minutes in a

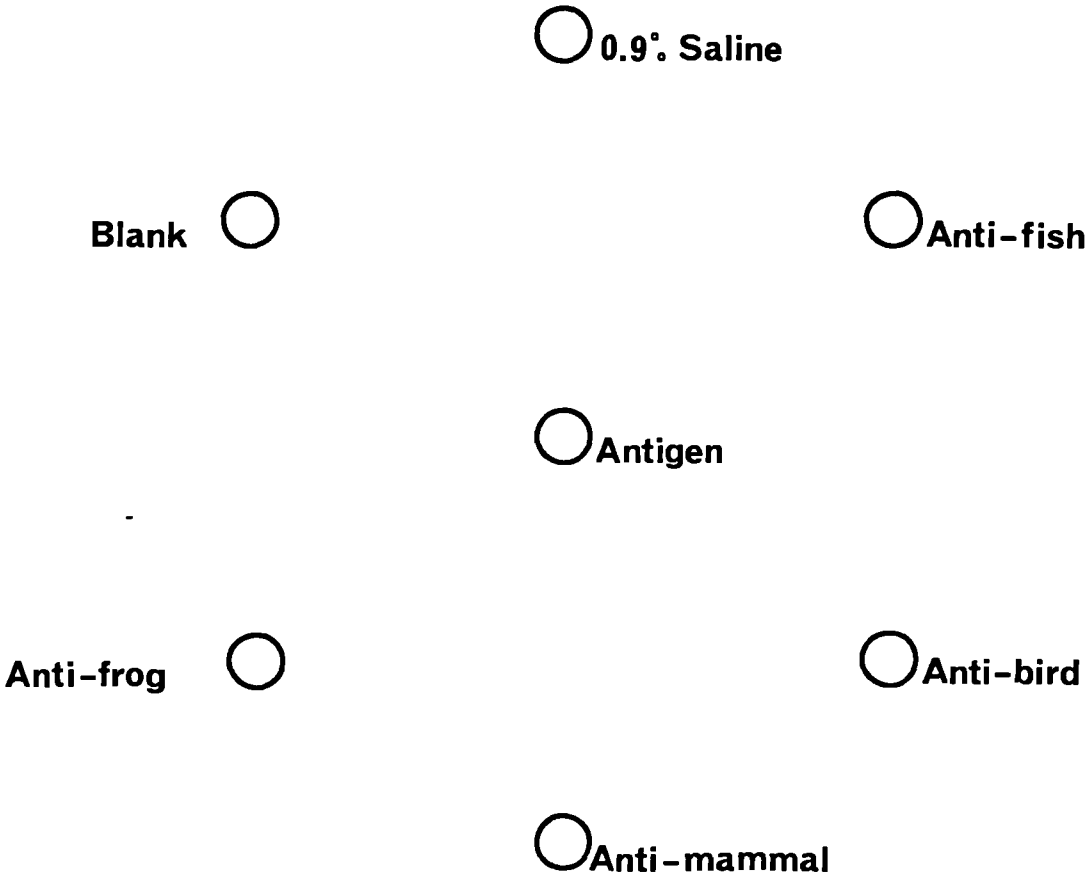


Figure 3.2 The Hexagonal pattern of wells in the agar gel, showing the position of the leech bloodmeal antigen and the prepared antisera.

1% thiazine red solution followed by a series of 3 minute washes in baths of 1% acetic acid. In both cases satisfactory results were obtained. The background colour in the gel was a pale pink while the precipitate bands stood out as dark red arcs. The microscope slides were allowed to dry and stored at room temperature for a short period.

The microscope slides were placed in the negative carrier of an enlarger and projected onto Ilfospeed Multigrade II paper at a magnification of approximately X5.

Results

(i) Observational techniques

(a) Light microscopy

A total of 100 stained and unstained bloodmeal smears were observed and the results indicated that only limited use could be made of this technique for identifying hosts. The appearance of the smears fell into two main categories:

(i) a high level of blood cellular fragmentation possibly indicating a well digested/old bloodmeal (n = 95)

and (ii) a proportion of intact blood cellular contents, allowing a more definite identification and possibly representing a more recent meal (n = 5)

A guide for identifying hosts in category (i) was the presence/absence and extent of nuclear material (appearing as dark purple stained bodies). Approximately 98% of these bloodmeals appeared to have large amounts of nuclear material, above that expected from just the 'white' cellular fraction, indicating the possible presence, at one time, of nucleated erythrocytes. It was tentatively concluded that this large fraction (98%) of bloodmeals was from a non-mammalian source.

It was possible to observe in 27 bloodmeals the presence of large nuclear bodies on the scale of that observed in amphibian blood rather than fish or bird. It was not possible on the basis of the appearance of

Plate 3.1

The relative size and degree of nucleation of erythrocytes from four vertebrate classes.

Magnification X 327

(a) Large, nucleated erythrocytes from the smooth newt, (Triturus vulgaris) (Nomarski illumination)

(b) Non-nucleated, biconcave erythrocytes from the rat (Nomarski illumination)

(c) Nucleated erythrocytes from the trout (Salmo trutta) (Nomarski illumination)

(d) Nucleated erythrocytes from a domestic duck showing darkly stained nuclei (bright field illumination)

n = nucleus

Plate 3.2

Bloodsmears of the gut contents of four H. medicinalis from Lade gravel pit and small pond, June 1983 (Nomarski illumination).

All at magnification X 327

(a) Bloodmeal from a leech, weighing 1.14 g, showing large, nuclear bodies - amphibian host? (arrowed)

(b) Bloodmeal from leech, weighing 1.32 g, showing the difficulty of interpretation - large and small nuclear bodies. (arrowed)

(c) Bloodmeal from leech, weighing 4.90 g - showing small nucleated erythrocytes - could represent contracted fish or bird erythrocytes.

(d) Bloodmeal from leech, weighing 3.31 g - showing high proportion of intact, nucleated erythrocytes possibly indicating a recent meal - some cytoplasmic contraction (circular cells) - it could represent either fish or bird origin.

n = nucleus

Plate 3.3 (a-c)

SEM preparations of frog (R. ridibunda), rat and duck erythrocytes

(a) Single large frog erythrocyte - no nucleus visible

(b) characteristic mammalian biconcave erythrocyte

(c) oval duck erythrocytes - no nucleus visible

Scale bar - 5μ

Plate 3.4 (a-c)

SEM preparations of bloodmeals from the guts of three leeches from Lade gravel pit, June, 1983.

Distortion of the surface has occurred and combined with the lack of visibility of nuclear material makes host identification impossible.

Scale bar - 5μ

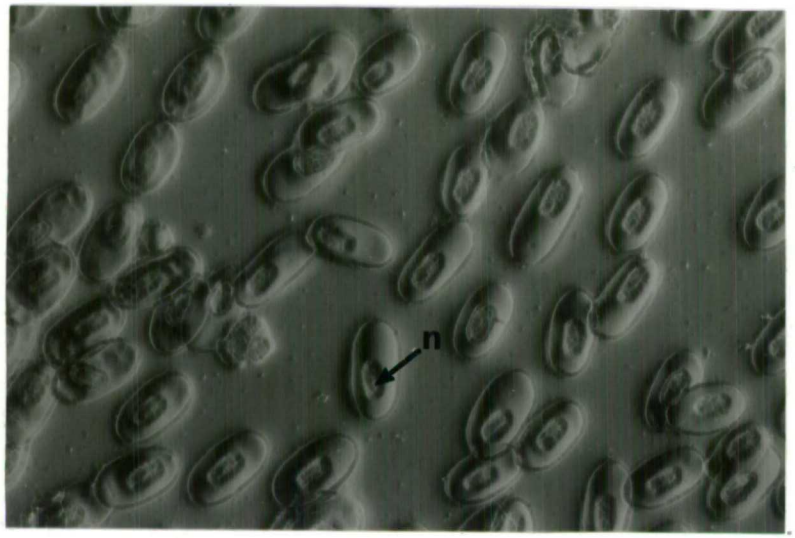


Plate 3.1 (a)

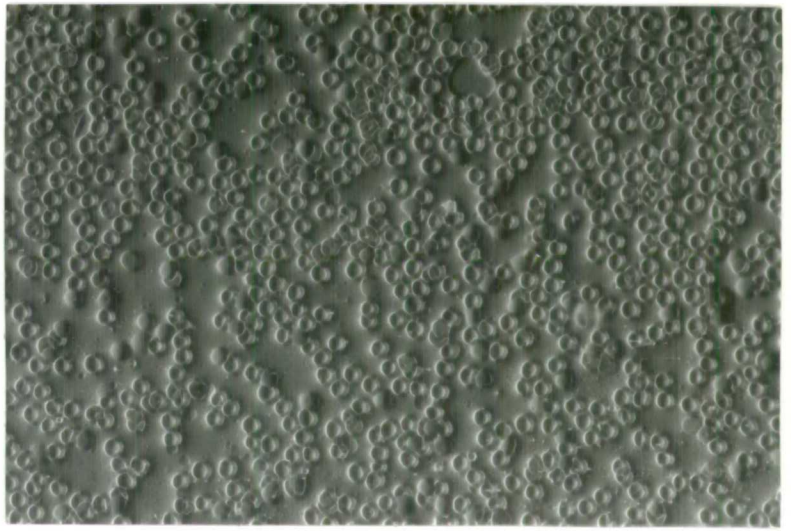


Plate 3.1 (b)

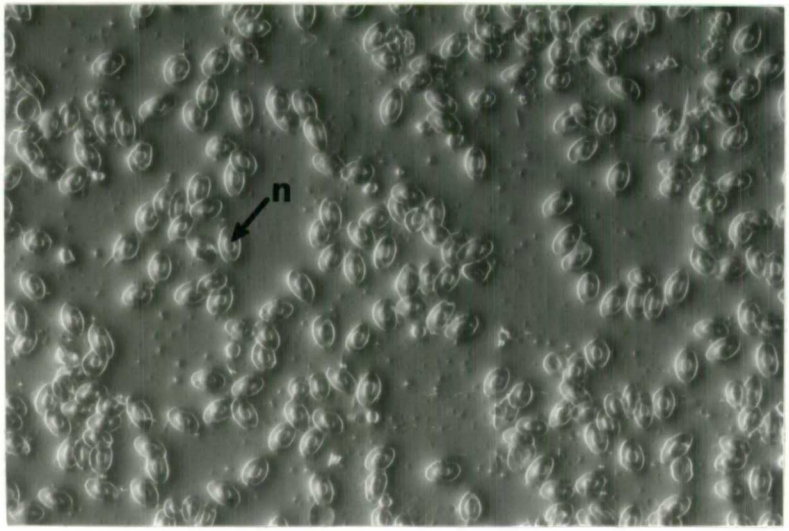


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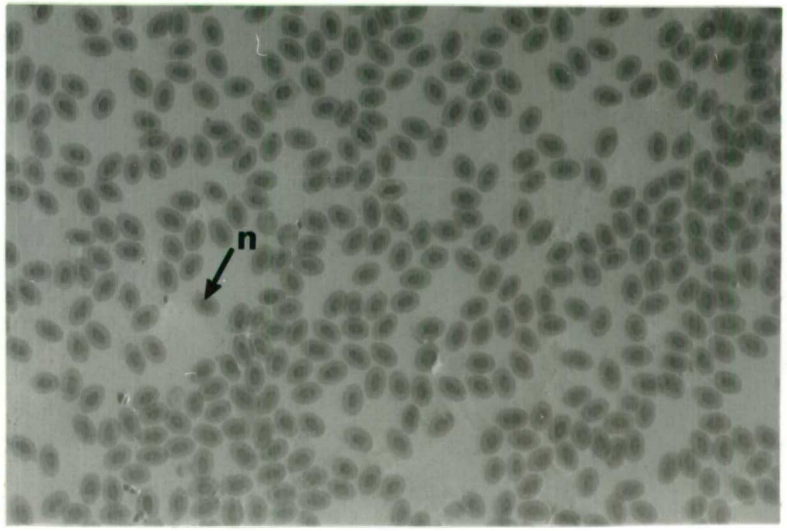


Plate 3.1 (d)

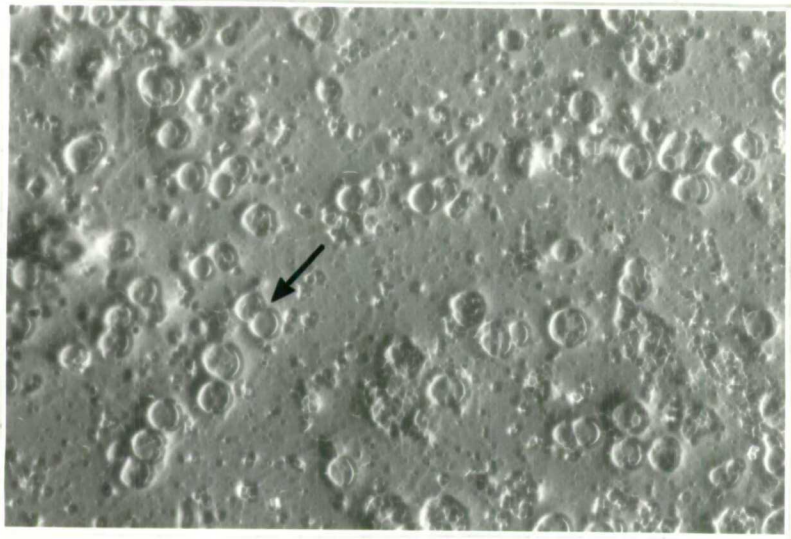


Plate 3.2 (a)

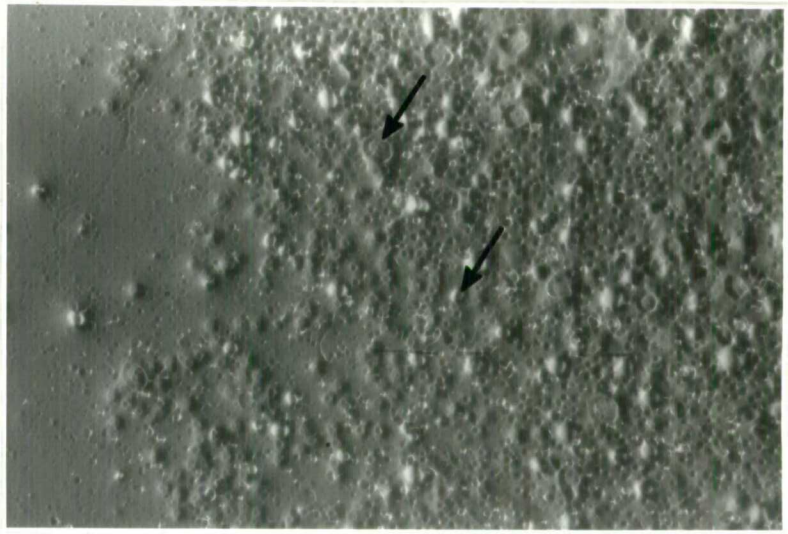


Plate 3.2 (b)

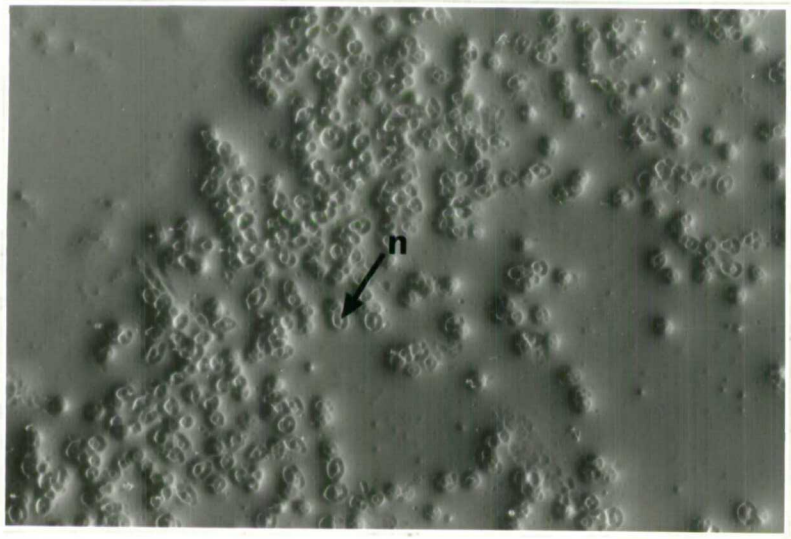


Plate 3.2 (c)

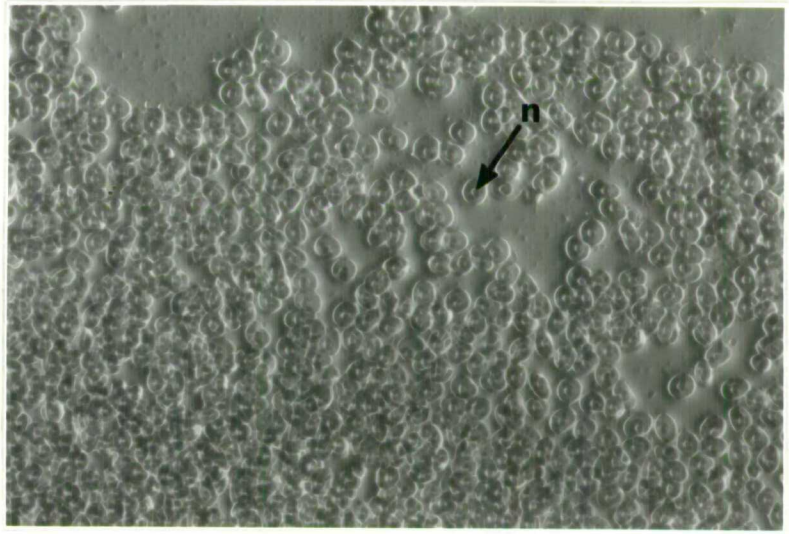


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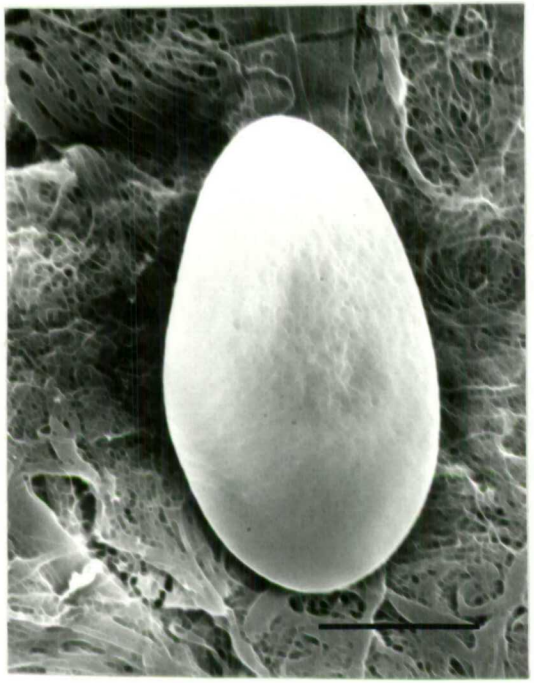


Plate 3.3 (a)

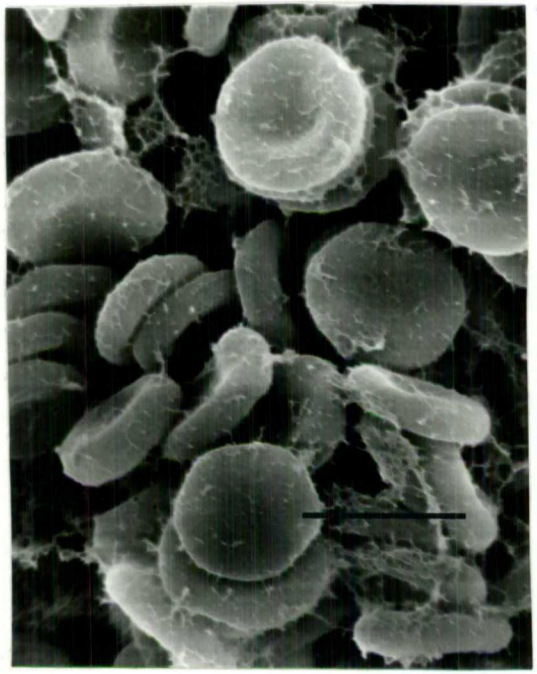


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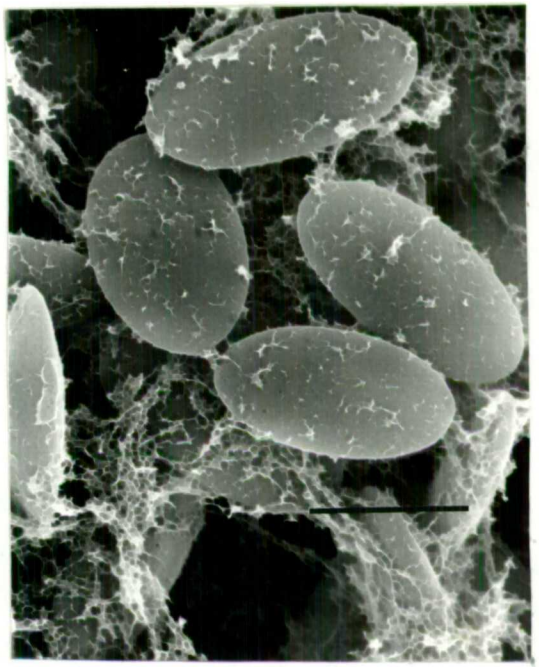


Plate 3.3 (c)

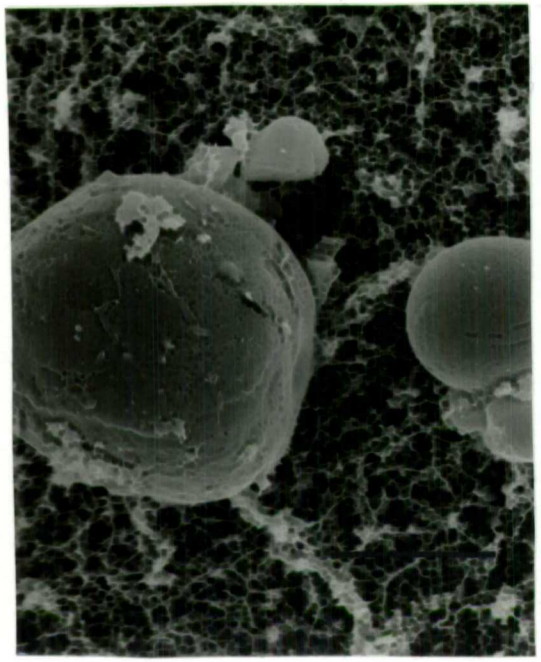


Plate 3.4 (a)

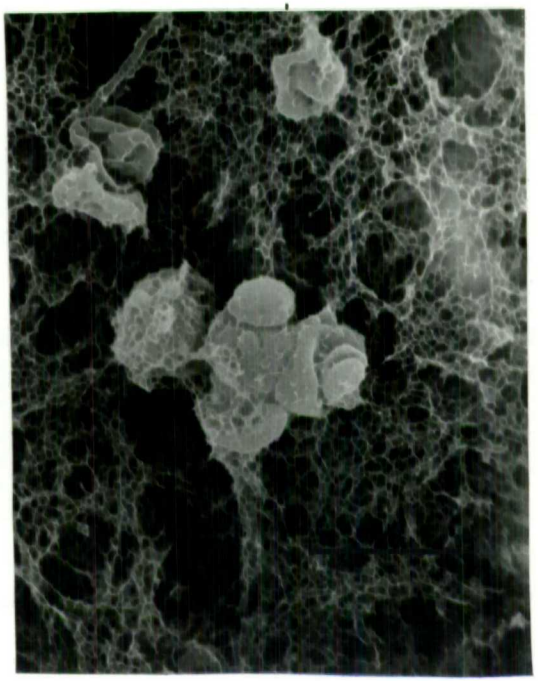


Plate 3.4 (b)

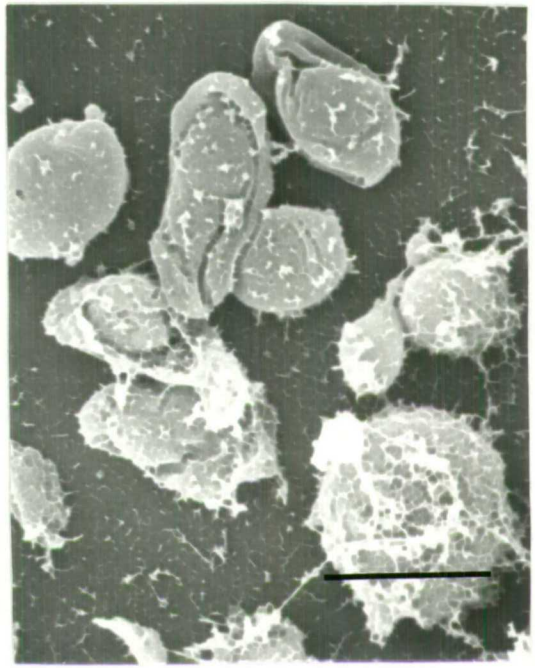


Plate 3.4 (c)

cellular material to distinguish whether a bloodmeal originated from a bird or fish host. Several bloodmeals appeared to have large and small nuclear bodies which could have indicated a mixed meal, composed of more than one host (i.e. the nucleated erythrocytes of frog were significantly larger than those from fish and birds),or it may have represented an artifact resulting from uneven cellular destruction during digestion. Of the five bloodmeal smears in category (ii) all showed nucleated erythrocytes on a scale comparable with fish and bird, although again it was not possible to distinguish between the two.

Plates 3.1 (a-d) show the relative size and nucleation of erythrocytes from amphibia (newt), mammal (rat), fish (trout) and bird (duck). These standard preparations were compared with leech bloodmeal smears from unknown hosts.

Plates 3.2 (a-d) show smears of bloodmeals from four H. medicinalis all viewed using Nomarski enhancement. Each of these smears was taken from a bloodmeal sample which gave a negative serological result. Therefore the observable presence of nucleation does provide a limited idea of host source. Plates 3.2 (a) and (b) are examples from bloodsmear category (i) while Plates 3.2 (c) and (d) are from category (ii). All four indicate the difficulty of accurately determining host origin.

(b) Scanning electron microscopy

Initial results from the preparation of standard frog (R. ridibunda) bird (duck) and mammal (rat) erythrocytes showed that the technique did not reveal nucleation, despite providing a magnified view of the erythrocyte surface (Plates 3.3 (a-c)). With this severe restriction only 10 bloodmeals from wild leeches were prepared. This technique proved to be inadequate for host identification (Plates 3.4 a-c)

(ii) Serological techniques

(a) Determination of the protein content f the serum antigens

Figure 3.3 shows the calibrati n curve obtained fr m standard protein concentrations (Table 3.1). The protein concentration of the serum antigens is shown in Table 3.2 below).

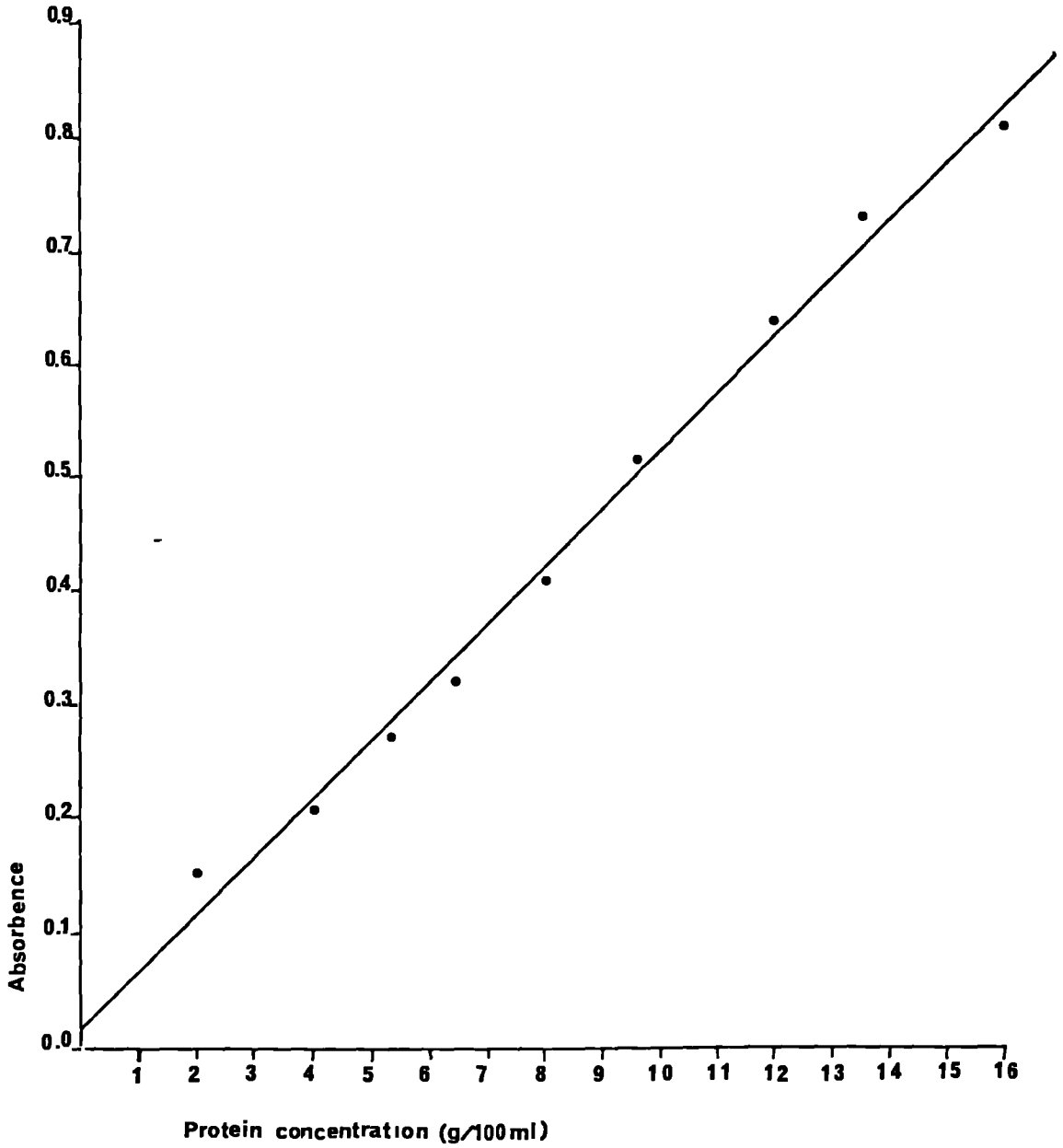


Figure 3.3 A Calibration curve for the determination of protein content of the serum antigens

Table 3.2 Protein concentration of the serum antigens

Serum antigen	Protein concentration (g/100 ml)
frog (R. ridibunda)	1.4
fish (trout)	1.0
bird (duck)	3.9
cattle	6.9
sheep	7.4
dog	7.4
human	8.7
rat	9.1

(b) Testing the titre and specificity of the antisera

After a series of five injections and a subsequent period of 20 days after the last injection, both the anti-mammal and anti-bird sera had satisfactory titres of 1:4000. The initial titres of the anti-frog and anti-fish sera were low, 1:100 and 1:1000 respectively. It is possible that the low protein concentrations, shown in Table 3.2, of these antigens explains the poor initial immune response by the rabbits. The result of the additional 2 ml injection of FCA/frog and fish antigens was a rise of the antiserum titre to 1:2000 and 1:4000 respectively.

The anti-bird and anti-fish sera were specific for their corresponding serum antigens. The behaviour of the anti-mammal serum was erratic. During the titre tests a clearly positive capillary ring reaction was observed between anti-mammal serum and a total mammal antigen. However, specificity results proved negative with both the capillary ring and miniature Ouchterlony methods using anti-mammal serum against a total mammal serum antigen composed of mixtures of three different combinations of mammal antigen fractions. There did not appear to be a clear explanation for the behaviour of the anti-mammal serum especially as a single mammal positive result was obtained with a bloodmeal obtained from a wild leech (Section c). It is possible that the mammalian antigens deteriorated while being held over 48 h at room temperature during the specificity experiments.

The anti-frog serum reacted with both frog and bird serum antigens. Techniques developed by Weitz (1956) for removing cross-reacting antisera by absorption were considered but it was decided that the aberrant behaviour of the anti-frog serum would not affect the general interpretation of results. The anti-frog serum did not react against a preparation of newt (T. vulgaris) serum antigen.

(c) Serological tests

The results of bloodmeals analysed from leeches obtained from Lade gravel pit between June and September 1983 were disappointing. From a total of 647 specimens, 192 bloodmeal samples were obtained. Of these only four gave positive results - three indicating a frog host and one a bird (duck). This may have been due to excessive dilution of the bloodmeals when collected since in these early experiments 2.5 ml of 0.9% saline was used to recover the sample. However the microscopical results discussed refer to this series of bloodmeal samples and the general degree of nucleation indicating non-mammalian hosts, was a significant finding.

From a total of 471 leeches collected between May and August 1984 from Lade gravel pit, 143 bloodmeal samples were obtained. Table 3.3 shows the results of 102 positive serological tests. It shows the numbers of single host bloodmeals and the various combinations of double host bloodmeals. Four results, not included in Table 3.3 indicated a triple host bloodmeal made up of fish, bird and frog.

Table 3.3 The results of 102 positive serological tests showing the composition of single and double host bloodmeals

	Fish	Bird	Mammal	Frog
Fish	[7]	2	0	8
Bird		[4]	0	23
Mammal			[1]	
Frog				[57]

Boxed values indicate single host bloodmeals. Amongst the double host bloodmeals, for example, 23 of the positive serological tests gave precipitate bands between the bloodmeal antigen and both anti-bird and anti-frog sera. This result was difficult to interpret due to the behaviour of the anti-frog serum. It is possible that some of these meals were from a single bird host and that the bird antigens reacted with the anti-frog serum. The result of 57 bloodmeals from a single frog host required some interpretation due to the behaviour of the anti-frog serum described. Since the anti-bird serum was specific to bird antigen any of these 57 bloodmeals which might have contained bird antigen reacting positively with the anti-frog serum, would also have reacted positively with the anti-bird sera. It was therefore possible to be definite about these 57 bloodmeals and say that they originated from a single frog host.

Despite these inconsistencies in the results, Table 3.3 does indicate the significantly high proportion of bloodmeals from a frog host and the important finding that H. medicinalis is obtaining bloodmeals from birds (ducks?).

Figure 3.4 shows the distribution of single and multiple host bloodmeals in five weight classes of leech based on the results in Table 3.3. Certainly the heavier weight classes are under-represented since a much smaller proportion of the total number of these leeches sampled yielded a bloodmeal. Figure 3.4 does indicate that bloodmeals were more easily obtained from leeches less than 2 g in weight (82% of total sample). The availability of a bloodmeal sample may have been linked to how recently the individual leech had fed and the degree to which the meal had been assimilated in the gut. The high proportion of bloodmeals from leeches weighing less than 2 g could indicate that this weight class feeds more frequently than the larger leeches, where samples were only obtained from individuals with a recently gorged appearance. Bloodmeals were never obtained from large leeches (3-4 g) with a flaccid appearance. It is interesting to note that leeches, still containing part of a previous meal, were attracted by the water disturbance sampling method. It might suggest that the leech may adopt a strategy of accumulating bloodmeals when the opportunity arises rather than fully digesting each meal before searching for another.

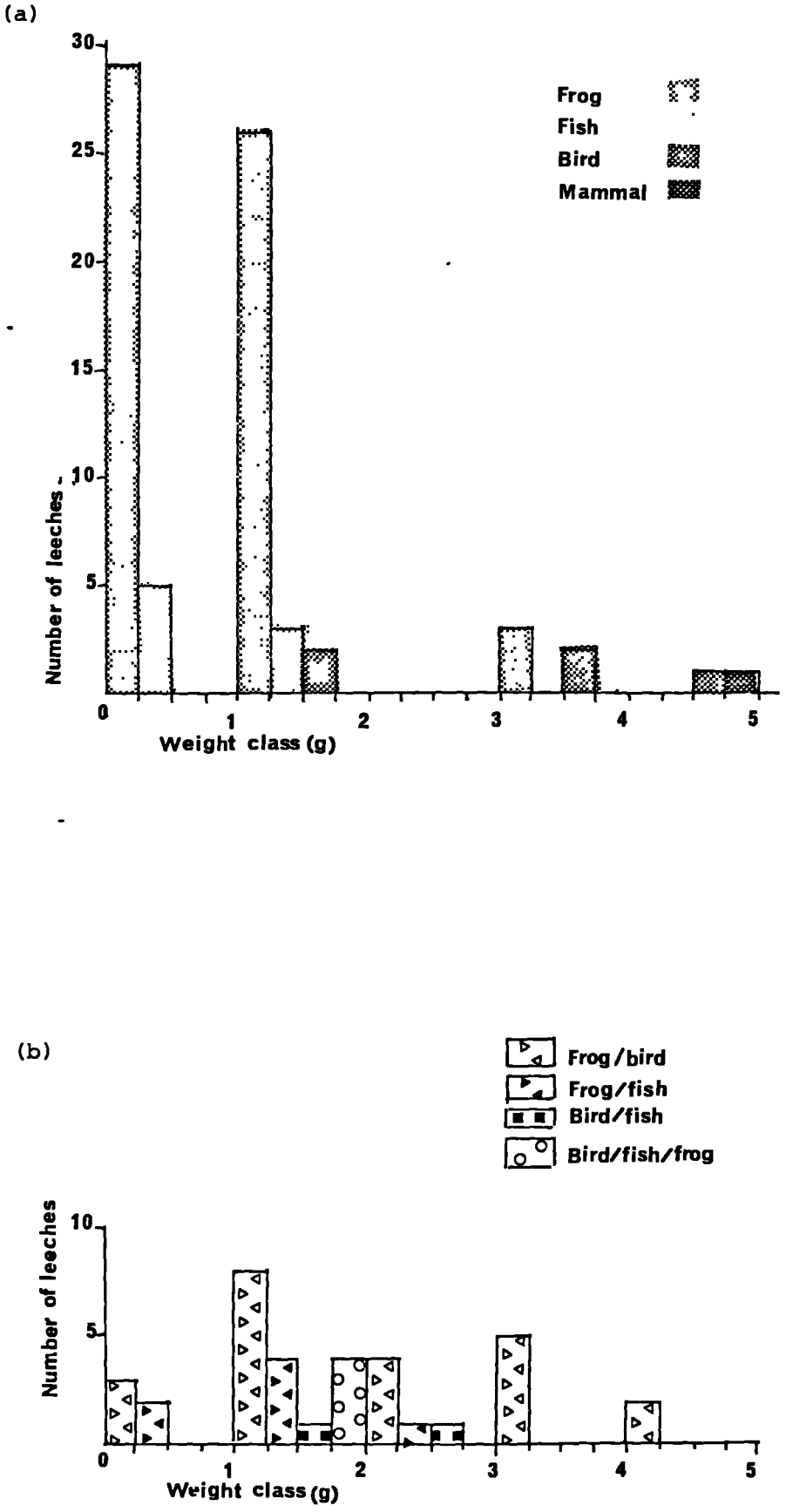


Figure 3.4 The distribution of (a) single, and (b) multiple host bloodmeals in five weight classes of leech based on 106 positive serological tests from samples collected between May-August 1984 from Lade gravel pit.

Plates 3.5 (a-e) show the photographic results of single and multiple serologically positive results. The light arcs indicate the precipitation reaction between antisera and antigen. Below is an example for the interpretation of results:

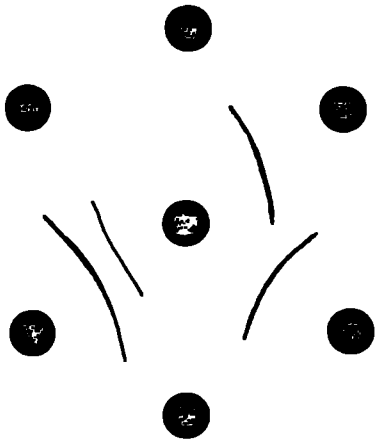


Plate 3.5(e showing serologically positive for fish, bird and frog.

From a total of 276 specimens collected during May-June 1985 from a population at Lydd Airport Marsh (TR065210), 49 bloodmeal samples were obtained, of which 22 gave positive serological results. Table 3.4 shows the composition of these bloodmeals.

Table 3.4 The results of 22 positive serological tests showing the composition of single and double host bloodmeals.

	Fish	Bird	Mammal	Frog
Fish	0	0	0	2
Bird		[3]	0	9
Mammal			0	0
Frog				[8]

Boxed values indicate single host bloodmeals. Although this sample was much smaller than the results from Lade gravel pit, it still indicates the importance of frogs and birds as hosts to the leech.

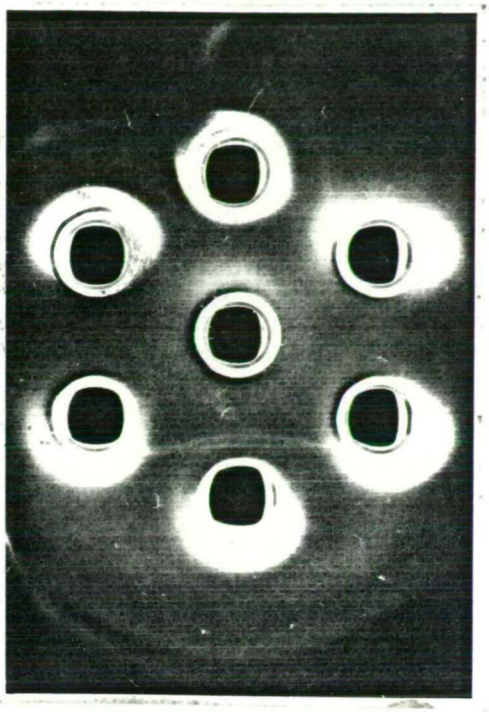


Plate 3.5(c)

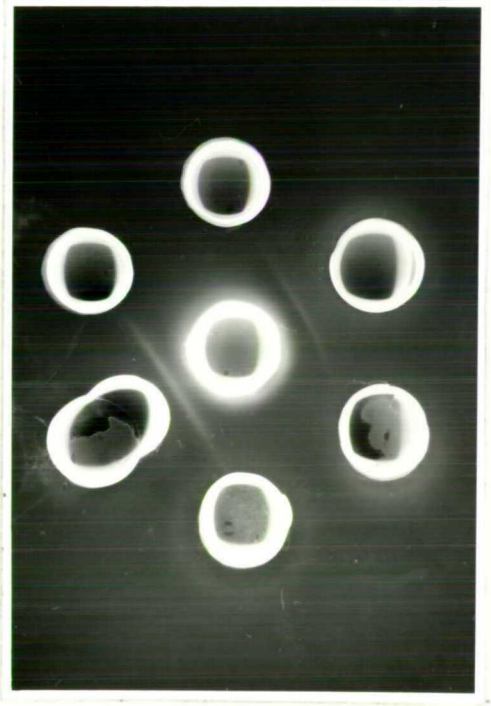


Plate 3.5(d)

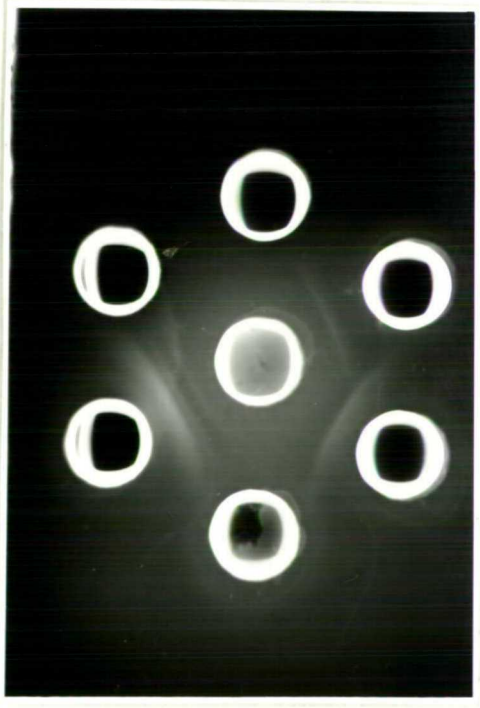


Plate 3.5(e)

DISCUSSION

The results of this analysis of bloodmeals obtained from two populations of H. medicinalis on Dungeness suggests that birds and frogs (R. ridibunda) are the most important source of food for the leech. Although grazing cattle and sheep were allowed access to Lade gravel pit at one time, this was rarely observed during this study. Certainly grazing animals have had no access to the Lydd Airport area for many years. It seems very likely that these leech populations are almost entirely sustained on non-mammalian blood supplies. At present both populations are viable, containing gravid adults during the Summer, and cocoons were found at both sites. It seems that sexual maturity is being attained on non-mammalian diets and the idea that a mammalian bloodmeal is a pre-requisite for attaining such maturity is disputed by these results. Certainly other authors, working with blood-sucking insects, have found that the source of a bloodmeal can affect fecundity (Bennett, 1970; Shelton, 1972; Downe and Archer, 1975). The link between diet and growth and reproduction in H. medicinalis is discussed at length in Chapters 4 and 6.

The finding that H. medicinalis utilises birds as hosts extends the range suggested by Elliott and Mann (1979) and confirms the finding of Keim (1979) whose serological work indicated the mallard, A. platyrhynchus, as an important host. Certainly this, and other species of duck, are frequent visitors to both sites on Dungeness. From a conservation viewpoint this finding is also valuable since many wetland areas are now managed to sustain bird populations and could, potentially, also provide suitable habitats for the Medicinal Leech. Indeed birds have been found to be an agent for the dispersal of the leech in the Dungeness area (see Chapter 7). Therefore the dramatic loss of the traditionally held leech habitat of wet grazing pastures could well be replaced by such sites managed for wildfowl, as well as supporting good amphibian populations.

The prevalence of results showing that individual leeches have fed on more than one type of host is also significant, particularly in the small weight class of less than 1 g. It indicates that it may be possible for even the smallest leeches to obtain a bl odmeal by

penetrating the skin of a bird. Some authors have implied that 'young' H. medicinalis are completely dependent on amphibian hosts which provide a thin integument to pierce.

The evidence of multiple bloodmeals, in all weight classes of leech, may indicate a much broader utilisation of hosts rather than any specific host selection. The idea that H. medicinalis is an opportunistic feeder on a wide range of available vertebrate hosts is discussed further in Chapter 2, where the smooth newt, T. vulgaris is also described as a significant host.

The single positive mammalian result from a total of 128 serological positives was obtained from an individual weighing 5.2 g from the 'small pond' of Lade gravel pit. This single result may reflect the very small part, if any, that mammalian hosts play in sustaining the population. It could represent a rare occasion where a dog or human may have had access to the pond.

Appendix 3.1

Method for preparing 2.5% phosphate buffered glutaraldehyde fixative (from Glauert, 1975).

0.2M Sorensen's phosphate buffer (ml)	50
25% Glutaraldehyde in H_2O (ml)	10
Distilled water	100
Concentration of Glutaraldehyde	2.5%

Appendix 3.2

G421 H.E. Rabbit Pellets ACS

Oil	2.5
Protein	18.0
Fibre	12.5

Appendix 3.3

Freund's Complete Adjuvant.

Each ml contains:

1.0 mg	Mycobacterium tuberculosis (H37Ra, ATCC25177) - heat killed and dried
0.85 ml	Paraffin Oil
0.15 ml	Mannide Monoleate

- Obtained through Sigma Chemical Company Ltd.

Appendix 3.4

LKB2117-401 Multiphor
Immunoelectrophoresis Kit

from: LKB-Produkter AB
S-16125 Bromma 1. Sweden.

Appendix 3.5

0.3 M Potassium phosphate buffer pH 8.0

49.3 g	K_2HPO_4
2.5 g	KH_2PO_4
0.7 g	Sodium azide

Make up to 1000 ml with distilled water

Appendix 3.6

Dulbecco's Phosphate Buffered Saline

Salts	g/litre
$CaCl_2$	0.20
KCl	0.20
KH_2PO_4	0.20
$MgCl_2$	0.047
NaCl	8.00
Na_2HPO_4	1.15

CHAPTER 4

A LABORATORY STUDY OF FEEDING IN THE MEDICINAL LEECH WITH PARTICULAR REFERENCE TO THE EFFECTS OF BLOODMEAL FREQUENCY AND QUALITY ON GROWTH, ATTAINMENT OF SEXUAL MATURITY AND SURVIVAL IN THE WILD

Introduction

The availability of food has been shown to be an important factor in the distribution of leeches, especially amongst the sanguiverous species (Sawyer, 1974). Feeding is obviously an important factor in the growth and development of any living organism but it is apparent that among the Hirudinea the relationship between food intake and growth is very different between sanguiverous and macrophagous species. Calow and Riley (1980) monitored the growth (measured as mean body length) of Erpobdella octoculata at 15°C fed on a diet of Tubifex worms. They found that mean body length increased gradually with time giving a sigmoid growth curve (see Figure 4.1(a)). However, work by Sawyer, Lepont, Stuart and Kramer (1981) and Van der Lande (1983) on two Glossiphoniid, sanguiverous leeches, Haementeria ghilianii and Theromyzon tessulatum showed a completely different saltatory growth curve pattern (measured as wet body weight) in response to optimum feeding regimes (see Figure 4.1(b)). This saltatory pattern appears to have important behavioural consequences as it represents a move away from the macrophagous, continual feeding strategy to a series of large increases in body weight, due to engorgement, followed by diuretic compensation and a period of stable body weight.

It has been known for some time that H. medicinalis increases its body weight several times after a bloodmeal and, due to a slow process of digestion, may survive for over a year on a single bloodmeal (Pütter, 1907, 1908; Bialaszewicz, 1924). However no published work to date has attempted to examine the growth dynamics of H. medicinalis as a function of feeding rather than time, from juvenile hatchling to the sexually mature adult.

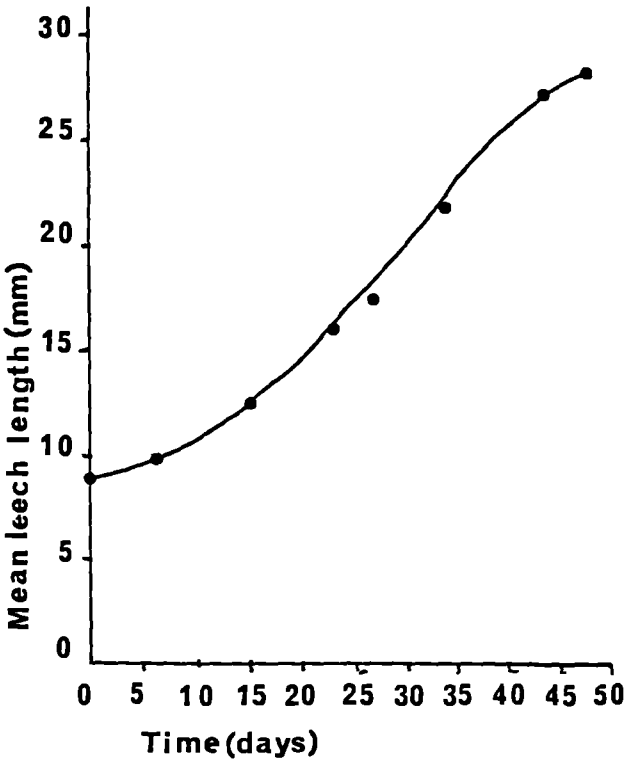


Figure 4.1(a) Growth curve of Erpobdella octoculata at 15°C fed on Tubifex worms, (from Calow and Riley, (1980))

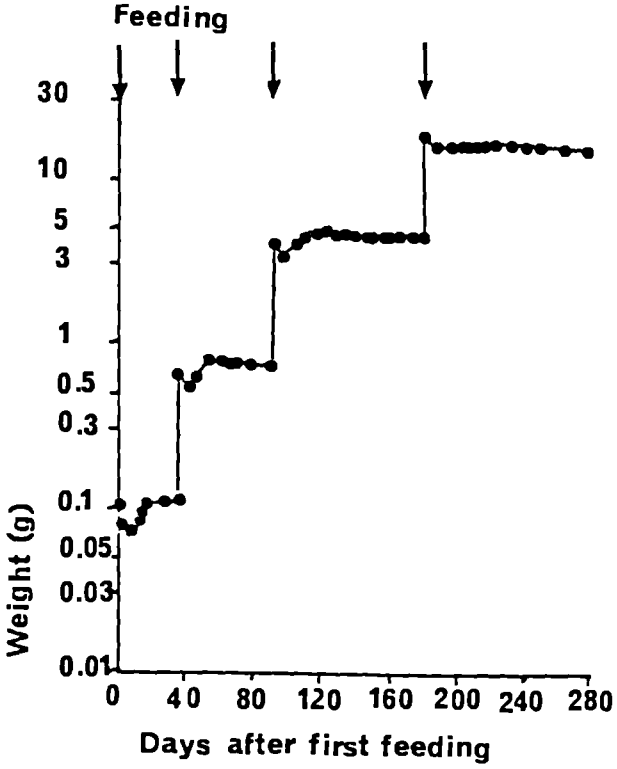


Figure 4.1(b Growth curve of Haementeria ghilianii from juvenile to sexually mature; provid d with four discrete bloodmeals from rabbit hosts, (from Sawyer, Lepont, Stuart and Kramer, (1981

The criteria for assessing sexual maturity, by the presence or absence of a clitellum and spermatophores, in the glossiphoniid leech Nephelopsis obscura was discussed by Collins and Holmstand (1984). There is, however, no discussion of any relationship between feeding and reproduction. In their work on the energetics of several glossiphoniid leeches, Calow and Riley (1980) make the important point that it is difficult to link food intake with reproduction since it is not always possible to assess the period of time during which gametes are formed and hence over what period of food intake an investment is being made in reproductive effort.

There is clear evidence suggesting that the number and frequency of bloodmeals in four species of sanguiverous, glossiphoniid leeches affects not only reproduction but also lifespan in a population study of T. tessulatum. Wilkialis and Davies (1979) concluded that sexual maturity is dependent on the number and frequency of bloodmeals rather than being a function of time or size. For example they found that sexually mature specimens ranged from 150 to 1200 mg, independent of whether they were in their second or third summer, but being dependent on the feeding regime.

The trophic effects of the timing of bloodmeals on the population dynamics and life-cycle of Theromyzon rude were studied by Davies (1984), both in laboratory and wild populations. This parallel study indicated that the feeding regime affected the attainment of sexual maturity, longevity and life-cycle. Similar results were obtained by Wilkialis (1984) from a study of Haementeria costata. He identified at least five distinct life-cycles within a single cohort determined completely by the availability of food, number of feeds and the amount of food consumed. For example leeches which had three or four 'ample' feeds in the first season or had three or four bloodmeals in March or April in the second season, matured and began to reproduce in June or July in the second season. These individuals died at the age of 1.5 years. In contrast, leeches which consumed small quantities of blood in various years of their lives, or did not always consume blood at each feeding opportunity did not become sexually mature but lived for 1-3½ years.

The reason for studying the possible effects of bloodmeal source on growth and the attainment of sexual maturity in H. medicinalis arose from four basic considerations.

The first was the uncertainty surrounding the idea proposed by Scriban and Autrum (1932-34) that the leech does not attain sexual maturity until it has fed at least once from a mammalian host. Later work by Sineva (1944), however, showed that H. medicinalis could be reared to sexual maturity on frogs' blood alone although only after a prolonged period of 17-20 months.

The second consideration arose from observations by other authors of the effects of bloodmeal source on egg production in certain haematophagous insects and it was considered possible a similar response may occur in the leech. For example, Shelton (1972) and Downe and Archer (1975) working on two Culex sp. (Diptera; Culicidae), found that egg production was significantly increased after feeding on chickens as compared to snakes, turtles and guinea pigs. Downe and Archer (1975) suggested that the effects of different bloodmeals may be linked to observed differences in digestion rates in the midgut (digestion was measured by changes in blood colour, viscosity and erythrocyte structure in the midgut). They also suggested that the more rapid digestion of bloodmeals from certain hosts could provide the advantages that a greater amount of nutrient would be made available for egg production within a critical time period.

The third consideration was based on the work of Andrews (1965) who found quantitative differences between blood from different vertebrate classes. For example he found that the concentration of erythrocytes (per ml of blood) in amphibians was 5.10^4-4.10^5 while in mammals it ranged from 5.10^6-10^7. There was also a significant size difference between nucleated amphibian erythrocytes ($\bar{x}$ = 4.99 µm) and non-nucleated mammalian erythrocytes ($\bar{x}$ = 2.45 µm). It was shown by Winds r (1970) that the haemogl bin molecule within the erythrocyte was the important fo d substrate for H. medicinalis. It is interesting to speculate that a mammalian bloodmeal with high erythrocyte c ncentration and small size (large surface area for digestion)

may be of increased nutritive value compared to an amphibian bloodmeal with a lower concentration of erythrocytes of large size.

The fourth consideration arose from attempts by previous workers, including Galun and Kindler (1966) and Dickinson and Lent (1984), who attempted to induce H. medicinalis to feed on non-blood solutions and part whole bloodmeals. They also examined whether similar food substrates would support similar growth.

For this study it was also important to review the different types of feeding methods which have been used in laboratory studies of growth in other sanguivérous leeches.

A common method was the direct application of a leech to the living host. In a study by Wilkialis (1973) on nutrition in H. costata, leeches were allowed to feed on live amphibians while in a recent study by Van der Lande (1984) on nutrition and growth in T. tessulatum, leeches were applied to the nares of ducklings some of which were subsequently killed to recover gorged leeches. In studies on laboratory breeding of H. medicinalis, Sineva (1944) applied leeches to live frogs and rabbits, while recently Fernandez and Stent (1982) allowed leeches to feed from the shaved, ventral skin of unanaesthetized rabbits restrained in an aquarium filled to a depth of 3-4 inches with water.

This author decided that these methods were an unjustified use of living animals in research and so alternative, artificial methods of feeding H. medicinalis were developed.

Some workers have developed artificial methods for feeding leeches. Wilkialis (1973, 1984) found that H. costata would feed on fresh blood from birds and mammals in a petri dish. Sawyer et al. (1981) developed an artificial blood feeding device comprising a chamber sealed at one end with a piece of stretched cow skin. It was based on an original model used by Löehner (1916). The only reference to feeding H. medicinalis through an artificial membrane was Dickinson and Lent (1984) who successfully used warm parafilm through which to feed large leeches. One of the aims of the present study was to investigate a

range of artificial membranes as possible alternatives to natural membranes. The trials using 'artificial' bloodmeals and membranes were designed to show whether they were easily managed techniques applicable to a large scale farming context.

Since the availability of food in the wild is unpredictable and possibly more so in those sanguiverous species of leech reliant on vertebrate hosts (cf. macrophagous species such as Erpobdella octoculata where feeding is probably at an optimum level for much of the time and in those small sanguivores such as Helobdella stagnalis where there is broad prey selection) a series of experiments was set up to examine the survival of hatchling H. medicinalis exposed to complete starvation and very infrequent feeding. Davies (1984) found that populations of T. rude fed once only survived for an average of 12 weeks at 3°C and 6 weeks at 5°C. With populations fed three times and maintained at 20°C survival, after a period of starvation, continued for an average of 28 weeks. In a study of H. costata, Wilkialis (1984) found that leeches hatched during June which were unable to find food, or individuals which fed only once survived for 5-6 months. It was felt that information regarding the survival of hatchling H. medicinalis, particularly over winter, could be an important factor in a wider consideration of the leech's survival and conservation.

Methods

All hatchling leeches used in these experiments were obtained from cocoons collected from the wild or laid under laboratory conditions.

(i) Preparation of bloodmeals

A series of four different bloodmeals was prepared for each feeding occasion. They were developed to replace the use of live hosts and to simulate bloodmeals of different 'quality' as measured by qualitative differences in erythrocyte concentration. All bloodmeals were based on fresh bovine blood collected from a local abattoir (in sterile plastic bottles).

Diet A consisted of heparinised whole blood. Samples of fresh blood were prevented from clotting by adding a 10 ml solution of non-preserved heparin (supplied as anhydrous white crystals, 156.3 USP K-1 units/mg; SIGMA) to the blood. Previous trials with a commercially prepared heparin solution containing the preservative Chlorbutol caused hatchling mortality. The required concentration of heparin, approximately 2 units per ml of blood, was found by trial and error. The blood sample was refrigerated until required but not retained for experiments beyond 24 hr.

Diet B consisted of 'defibrinated' blood prepared at the abattoir. A large volume of whole blood was allowed to clot in a stainless steel bucket and then stirred vigorously for several minutes. The liquid serum fraction was decanted and used as diet B.

Diet C consisted of a preparation similar to that described by Dickinson and Lent (1984). A sample of defibrinated blood was centrifuged at 3500 g. The supernatant serum was poured into a separate sterile glass container while the remaining, cellular fraction of the blood was pooled with several other samples and refrigerated. A 5% solution of L-15 (Leibovitz) culture medium was prepared by dissolving powdered media in distilled water at room temperature (Appendix 4.1 shows the constituents of Leibovitz tissue culture medium). A 1:1 mixture of the blood cellular fraction and L-15 (Leibovitz) culture medium was prepared and used as diet C.

Diet D consisted of the supernatant serum collected during the preparation of diet C. This diet was designed to simulate a bloodmeal containing a low concentration of erythrocytes.

(ii) Feeding methods

A feeding method was developed from trials using a range of membranes and containers and designed for use with both hatchling leeches and adult leeches used in the captive breeding experiments (see Chapter 6). Trial 1 involved stretching a piece of parafilm

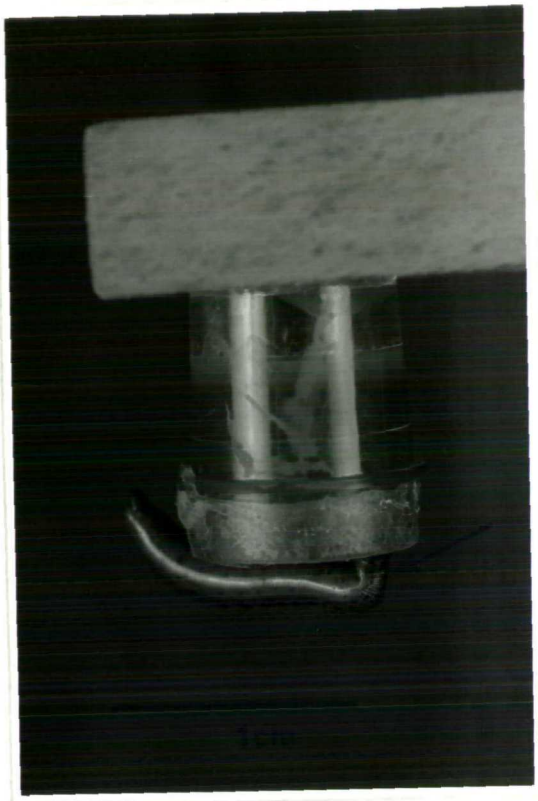
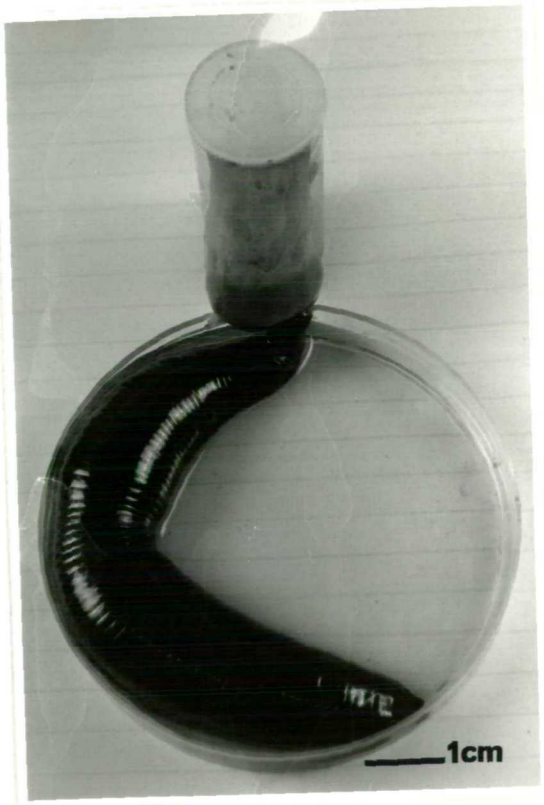


Plate 4.1 Laboratory feeding method for hatchling Medicinal leeches; note the characteristic broadening of the bucca cavity during feeding. (arrowed)

Plate 4.2 Laboratory method for feeding large Medicinal leeches; note the build-up of excretory fluid around the animal which resulted from diuretic compensation during feeding.



across a small, inverted funnel (diam. 5 cm). This was immersed in a waterbath at 37°C for several minutes, removed and blotted dry. The bloodmeal under test, at a temperature of 37°C, was pipetted into the funnel and a leech applied, by hand, to the surface of the parafilm. Although hatchling leeches attempted to bite (indicated by a broadening of the mouth and arching of the buccal cavity, see plate 4.1) all were unable to penetrate the film. Only a few adult leeches were able to bite through the parafilm and so this membrane proved unsuitable for use. Trial 2 involved a similar method except cellophane replaced the parafilm. Leeches from all weight classes were unable to bite this membrane. Trial 3 involved the use of Visking dialysis tubing as a membrane but again all leeches were unable to bite.

The most successful membrane in all feeding trials was natural, cleaned pig ileum. This was obtained as salted sausage skin and soaked overnight in distilled water. It was not possible to use an inverted funnel as a container so this was replaced by a small, plastic vial 7 mm in diameter for hatchling leeches and a larger plastic tube 18 mm in diameter for the adults. Before using the membrane it was soaked in a waterbath at 37°C, cut into short segments and stretched across the mouth of the container. This was allowed to stand for a few minutes to allow the skin to dry against the sides of the container. A leech was applied and allowed to feed to satiation (plates 4.1 and 4.2 show the two experimental feeding methods used).

(iii) Housing and water quality

All hatchling leeches were housed individually in small plastic containers 80 mm in diameter, depth 40 mm with tightly fitting perforated lids. A small stone was provided to give shelter and to allow leeches to rest out of water. When body size exceeded the capacity of this container individuals were transferred to a larger perspex box 160 x 50 mm again with a tightly fitting lid (this standard housing was used during the captive breeding experiments on large adult leeches).

All pre-first fed hatchlings were maintained in aerated tap water prior to the feeding experiments.

Three different water qualities were used in the optimum feeding experiments to examine any effect on post-feeding leech condition. A set of ten leeches in each optimum feeding experiment were maintained in tap water aerated for 24 hr, a further ten in an artificial pond water suggested by Dr. R. T. Sawyer (see Appendix 4.2) and the remainder in unfiltered, natural water from the River Stour. (N.B. in a survey of backwaters of the river six other species of leech were found, including the uncommon Hemiclepsis marginata, and as a result the water quality was considered likely to be suitable for maintaining H. medicinalis).

(iv) Feeding regimes

All hatchling leeches were weighed prior to feeding by removing them carefully with a small paint brush, blotting dry and weighing in a small container. Immediately after feeding leeches were gently blotted dry, avoiding disgorgement, and reweighed.

(a) Optimum feeding frequency experiment

The term 'optimum' was used to describe a schedule of feeding opportunities made available to hatchling leeches over an extended period of time. The idea of an optimum frequency of feeding opportunities was based partly on the results of preliminary feeding experiments which indicated that 30-50 days represented the minimum period of time that the majority of the sample took successive feeds. Also, as the results of this study suggested, very few leeches fed at every feeding opportunity (i.e. 30-50 days) and more often the period between two actual feeds was longer.

For this experiment four groups of 50 hatchling H. medicinalis were offered a series of feeds at regular intervals on one of the four types of bloodmeals described in section (i). After the initial feed, subsequent feeding opportunities on diets A, B and D were offered every 3 -50 days over a period of approximately 300 days. (Table 4 below shows the schedule of feeds offered during the experiment).

Table4.1 The number of feeds on diets A, B and D offered to hatchling *H. medicinalis* over approximately 300 days.

Feeding occasion	Number of days after first feed
1	0
2	30
3	60
4	110
5	150
6	190
7	240
8 (diets A & B only)	290

For example, a fifth feeding opportunity was offered 150 days after the first opportunity to feed. For some individual leeches this corresponded to a fifth feed but for those which failed to feed on previous bloodmeals offered it represented, for example, a second feed 150 days after the initial feed.

The final feed in the diet D experiment was on heparinised blood to prepare the hatchlings for release back into the wild (see Chapter 7). Diet C was less readily accepted by hatchling leeches despite the frequency of feeding opportunities being similar to those on diets A, B and D. A fifth feed, on heparinised whole bovine blood, was offered 195 days after the first feeding opportunity, after which diet C experiment was terminated.

After the first feed, and for the duration of the experiment, the water in each individual container was changed at least twice a week and more frequently if defaecation was noticed (observed as a dark brown haem discharge). It proved essential to maintain the water free from excretory and defaecatory byproducts, especially in conditions of raised temperature where discharges were more frequent. A leech's response to such deterioration of water quality was disgorgement of the bloodmeal.

The effects of raised temperature and increased day length were investigated on a group of leeches from growth experiments using diets A, B and D. All hatchlings fed on diet C were maintained at $15^{o} \pm 2^{o}C$ for the duration of the experiment.

For 125 days after the first feed all hatchlings were maintained at $15^{o} \pm 2^{o}C$ at a controlled daylength of 10 hr under fluorescent lighting. After this period 15 leeches from each of the experiments using diets A, B and D were moved and maintained for the rest of the experiment at a raised temperature of $24^{o} \pm 2^{o}C$ and an increased day-length of 14 hr. These conditions were found by Sineva (1944, 1949) to be favourable to the development and the attainment of sexual maturity. It was found that these conditions tended to cause disgorgement if leeches were returned to these conditions immediately after feeding. Maintaining leeches at the reduced temperature of $15^{o} \pm 2^{o}C$ for 48 hr after feeding before returning to the higher temperature reduced this problem.

An important objective of this series of feeding experiments was to assess the stage at which individual leeches attained sexual maturity. To assess this, 14 hatchlings, fed on diets A and B and previously housed individually, were selected after the sixth feeding opportunity from the sample maintained at the raised water temperature (see above). This corresponded to 200 days after the first feed and at a body weight equivalent to gravid leeches found in the wild, i.e. 2.0-7.5 g. Leeches of comparable body size were paired and maintained in local river water in a sealed, perspex container (160 x 100 x 50 mm) for a period of 30 days. After this period they were parted and housed individually in similar containers for a furth r 30 days during which time a seventh feed was offered. A second series of pairings was made with an additional 53 hatchlings, again taken from growth experiments on diets A and B, approximately 290 days after the first feed. These pairings were maintained in similar housing for a period of 30 days after which all hatchlings were parted, maintained individually and observed for any development of a gravid state (indicated by a yellow discolouration and swelling of anterior segments).

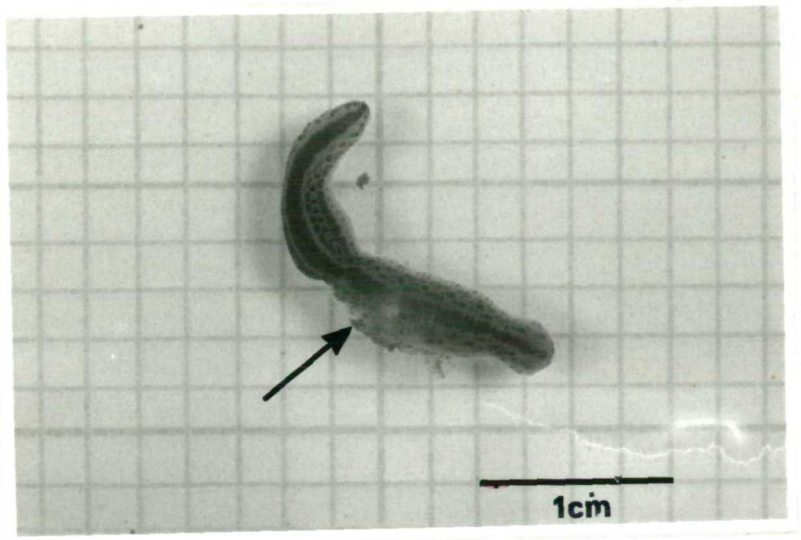


Plate 4.3 Deterioration of a hatchling Medicinal leech maintained in aerated tap water; note the breakdown of tissue may have been due to osmotic stress, (arrowed)

(b) Sub-optimum feeding and starvation experiment

The sub-optimum feeding experiments involved a sample of ten surviving leeches from some 185 hatched in the laboratory from cocoons collected or laid by captured gravid leeches from Lade gravel pit, and from specimens supplied from Germany, during 1983. Very high mortality occurred during feeding trials with these hatchlings while using citrated whole bovine blood and heparin containing the preservative Chlorobutal. During these trials all hatchlings were maintained in aerated tap water and, as later results from optimum feeding experiments showed, this may also have contributed to the high mortality. Plate 4.3 shows the gradual breakdown in body tissue suffered by hatchlings maintained in aerated tap water.

The ten surviving leeches were housed individually in similar conditions as described above and offered infrequent feeding opportunities over a period of 725 days after hatching. During the second year after hatching a series of pairings was made within this group 570 days after hatching and maintained for a period of 50 days in similar conditions already described, after which hatchlings were parted and maintained individually for the rest of the experiment.

The starvation experiments were designed to investigate whether hatchling H. medicinalis were able to survive a long period, including overwintering conditions, without a post-hatching feed and also on a single non-blood meal.

A sample of 50 hatchlings were maintained (individually in small plastic containers already described) in local river water at $15^{o} \pm 2^{o}C$ for 50 days, weighed and offered a single meal of L15 (Leibovitz) culture medium using the method described in Section (ii). These hatchlings were maintained at $15^{o} \pm 2^{o}C$ until death occurred.

The other group of 25 hatchlings was not offered any f d and maintained at $15^{o} \pm 2^{o}C$ for 60 days after hatching after which they were transferred to a room at a temperature and daylength determined by external conditions and maintained in these conditions until death occurred. In both experiments the number of surviving leeches was recorded every 10 days.

Results

(a) Optimum feeding frequency experiments

Hatchling mortality and disgorgement of bloodmeal were significant factors affecting the results from growth experiments using diets A and B. During the 300 day experiment there was 28% and 20% mortality of hatchlings fed on diets A and B respectively. Approximately 90% of these hatchlings were maintained in aerated tap water suggesting that this water quality was unsuitable. In addition a further 40% and 30% of hatchlings fed on diets A and B respectively disgorged during the experiment. Of hatchlings fed on diet C there was a 26% mortality, many of which probably resulted from starvation due to a refusal to feed on the diet. There was only 6% disgorgement. There was a 16% mortality of hatchlings fed on diet D most of which were maintained in aerated tap water. There was no incidence of disgorgement during this 300 day experiment.

The general effects of water quality on the survival of leeches maintained in the laboratory indicated that aerated tap water caused increased disgorgement and mortality, possibly due to chlorination. The survival of hatchling leeches fed on diets A and B and maintained in artificial pond water (Appendix 4.2) and local river water was good (90% survival). Interestingly, survival of hatchlings fed on diet D and maintained in aerated tap water was greater than those fed on diets A and B.

A clear result from feeding hatchling *H. medicinalis* at an optimum frequency of feeding opportunity on diets A, B and D was the development of a saltatory growth pattern. Within each of these experiments there was great variation in the volume of bloodmeal imbibed on each feeding occasion and the frequency that individuals fed over the duration of the experiment. A series of diagrams (Figures 4.2 (a-c) show the growth curves and maximum and minimum final body weights attained by individual hatchling *H. medicinalis* fed on diets A, B and D. They were from the samples maintained at the raised temperature and increased daylength regime during the experiment.

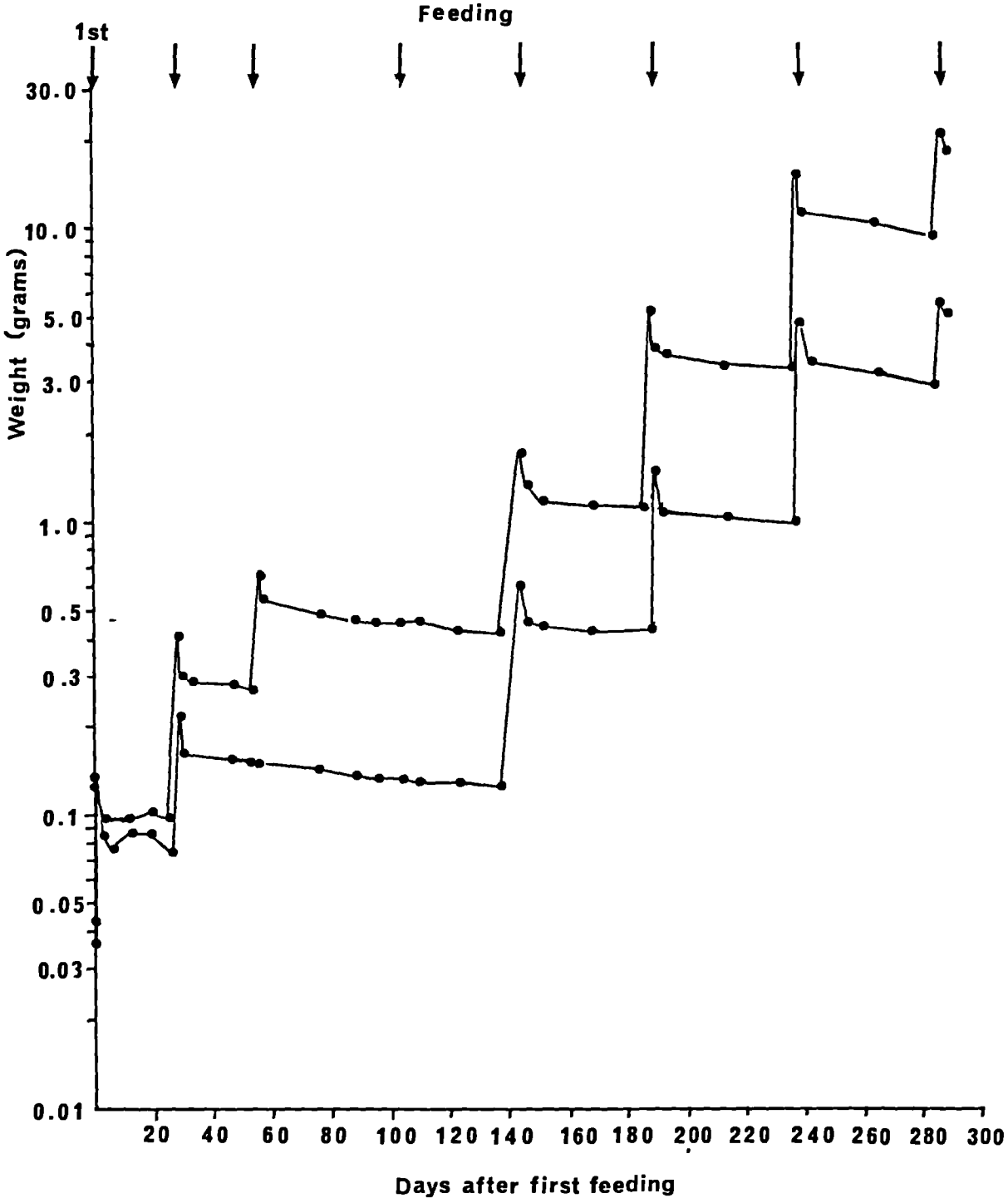


Figure 4.2(a) Growth curves of two Medicinal leeches fed n diet A and maintained at 24± 2°C, 14hrs artificial light(details in text)

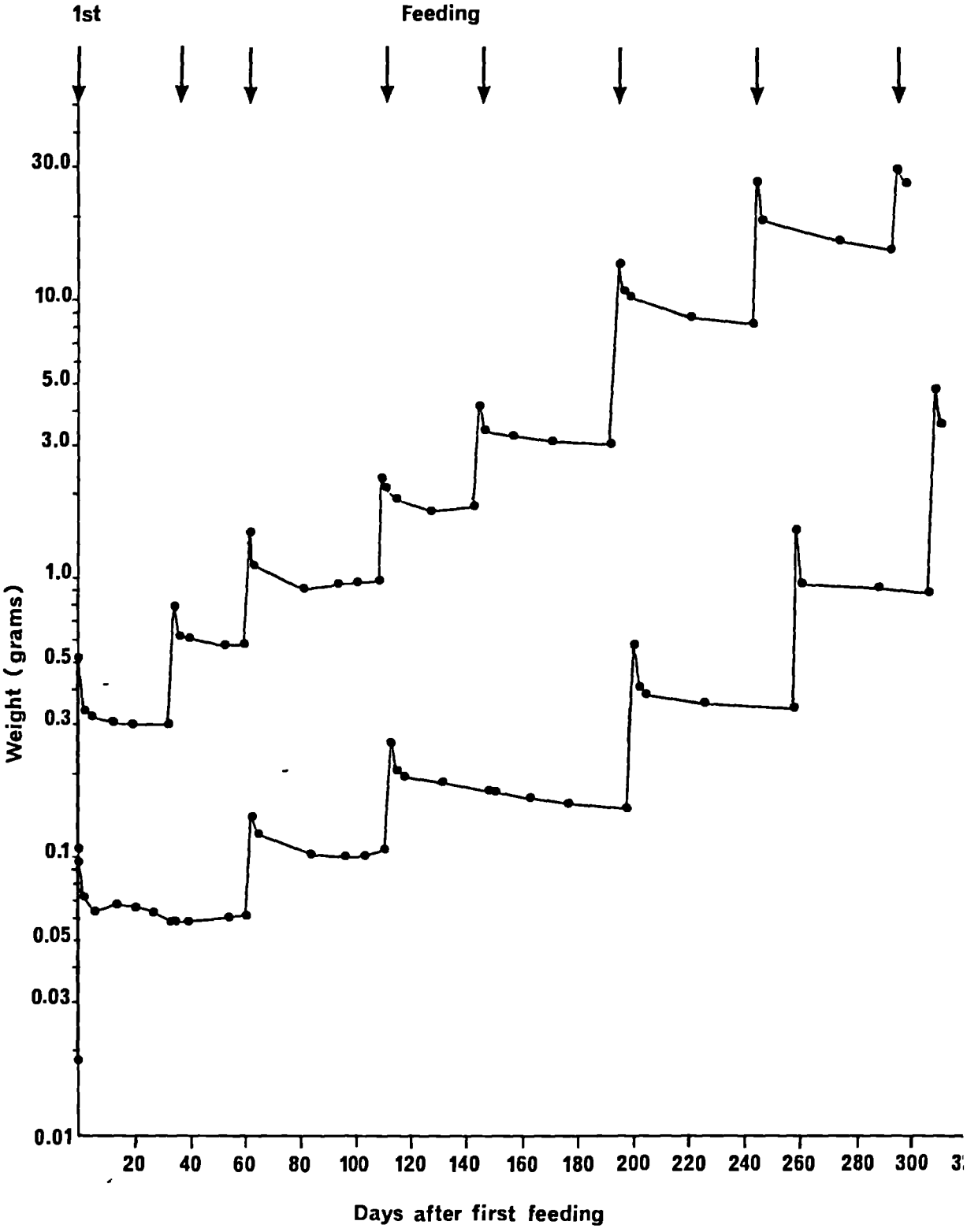


Figure 4.2 b) Growth curves of two Medicinal leeches fed on diet B and maintained at 24±2°C, 14hrs artificial light, (details in text

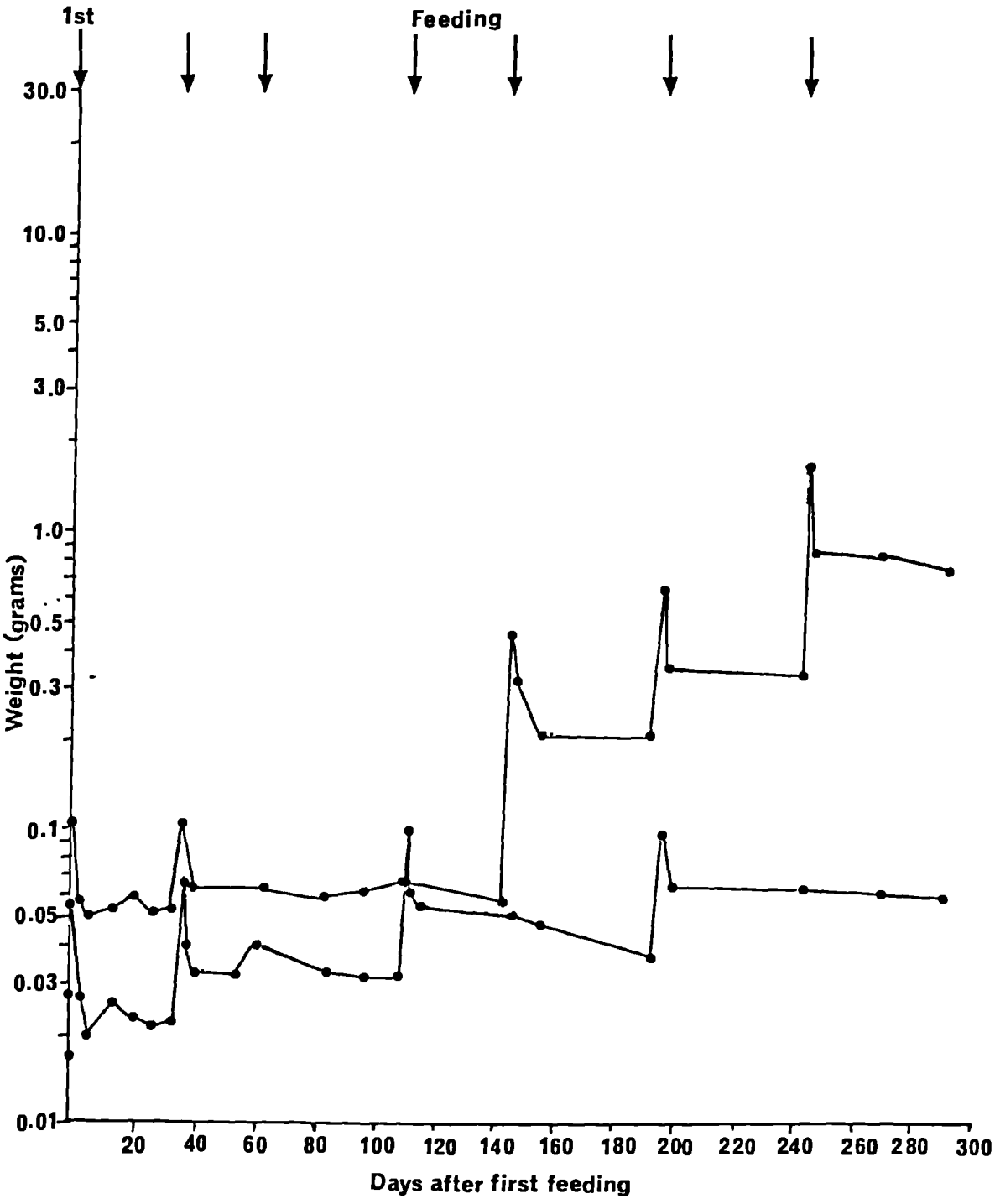


Figure 4.2 (c) Growth curves of two Medicinal leeches fed on diet D and maintained at 24±2°C, 14 hrs artificial light (details in text)

Clearly diets A and B provided a good food substrate for attaining a maximum growth rate and frequency of feeding. The highest body weight attained over the 300 day feeding programme was by a hatchling in the Diet B experiment which reached a weight of 29 g. (A German specimen of H. medicinalis of previously unknown status, i.e. age, number of feedings, etc., attained a maximum body weight of 61 g during experiments described in Chapter 6). These high body weights are comparable with the giant glossiphoniid leech, Haementeria ghilianni (Sawyer et al., 1981), and have not previously been reported for H. medicinalis. The series of plates 4.4 (a-f) show the change in body size of a hatchling leech after three successive feeds on heparinised whole blood and a German specimen which attained a body weight of 61 g after a single feed.

It is interesting to note the lower final body weights obtained by hatchlings fed on diet D and to speculate that, although overall growth was possible, this reduced growth rate and final body weight could have resulted from a much lower concentration of erythrocytes in the diet and hence nutritive value.

An important feature of all the growth curves was the loss of body weight, due to urine elimination, immediately after completing a bloodmeal (in fact droplets of fluid gradually accumulated on the body surface, at the nephridial pores, during the course of feeding). The greatest proportion of body weight loss occurred within a period of several hours after feeding, indicating a rapid compensation for the bulk fluid intake with the bloodmeal. There was no evidence of immediate post-feeding water intake, as shown by Theromyzon tessulatum, (Van de Lande, 1983), or a body weight increase several days after the immediate post-feed diuresis as shown in Haem nteria ghilianii (Sawyer, et al., 1981).

The c nsequence of a general rejection of diet C by hatchling H. medicinalis was that no clear saltatory growth curve was developed. The maximum body weight attained, in a sample of 50 hatchlings fed over 19 days, was 365 mg. Although not an ptimum diet there was 66% survival in this sample after 195 days.

Plate 4.4 (a)

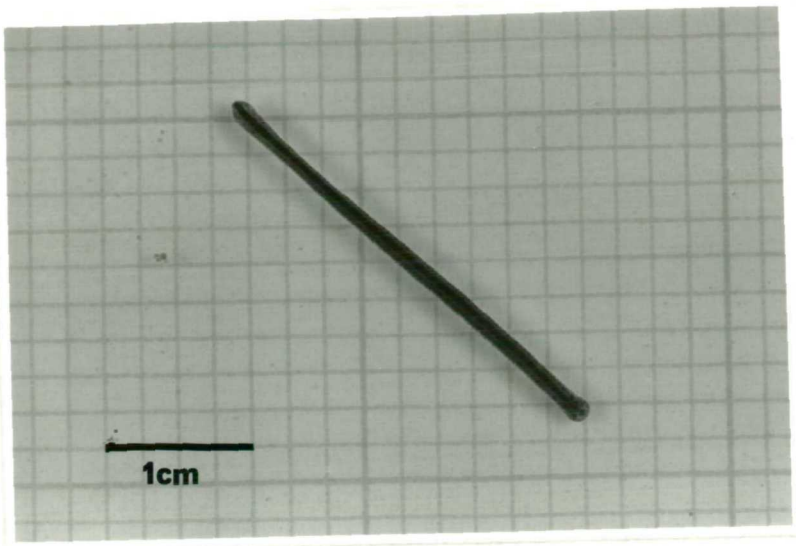


Plate 4.4 (b)

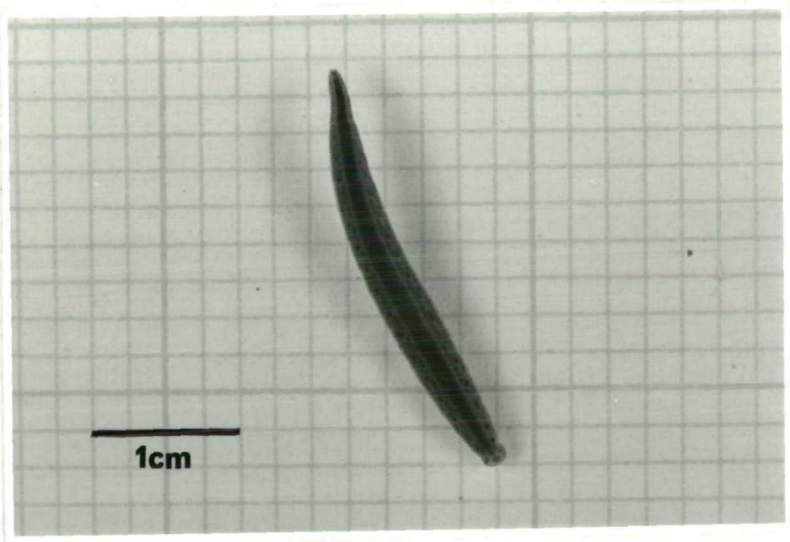


Plate 4.4 (c)

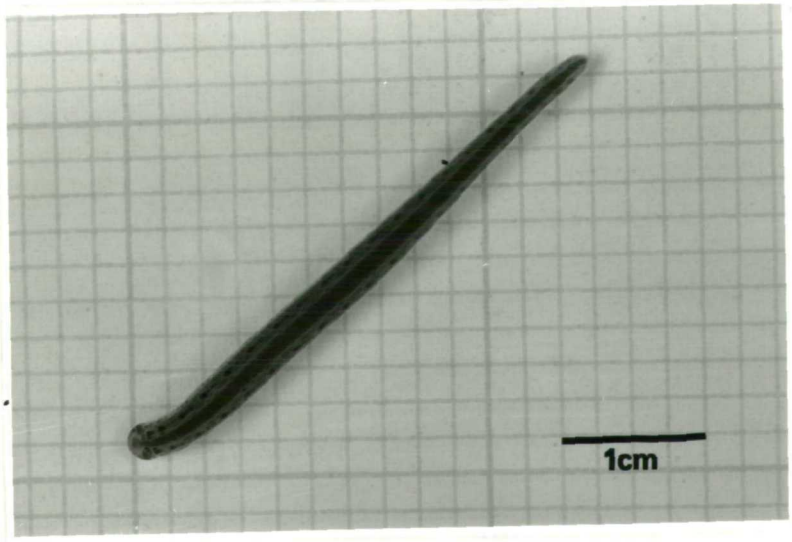


Plate 4.4 (d)

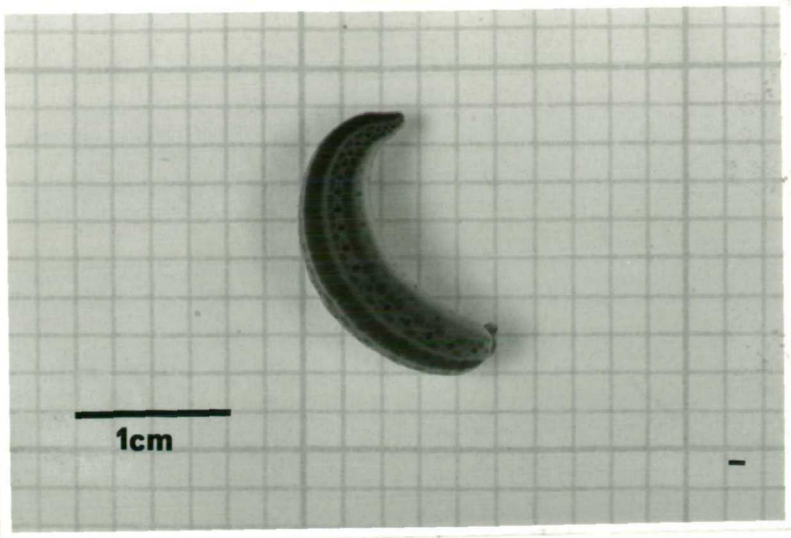


Plate 4.4 (e)

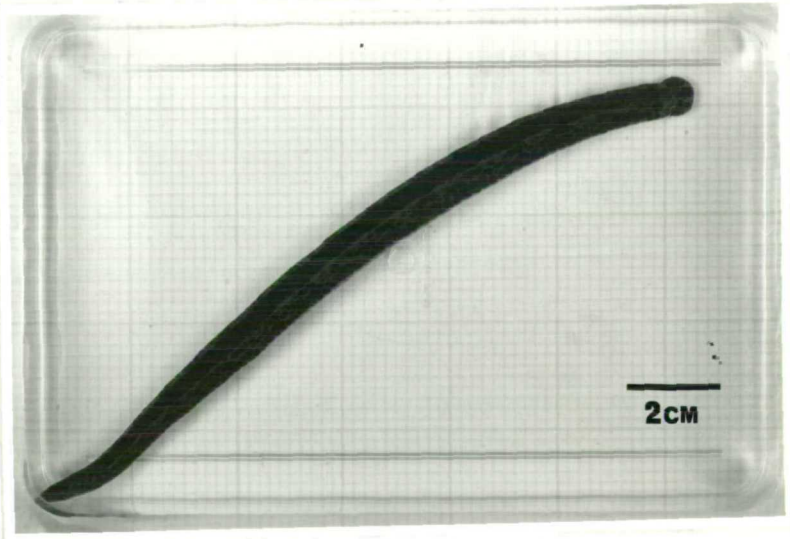
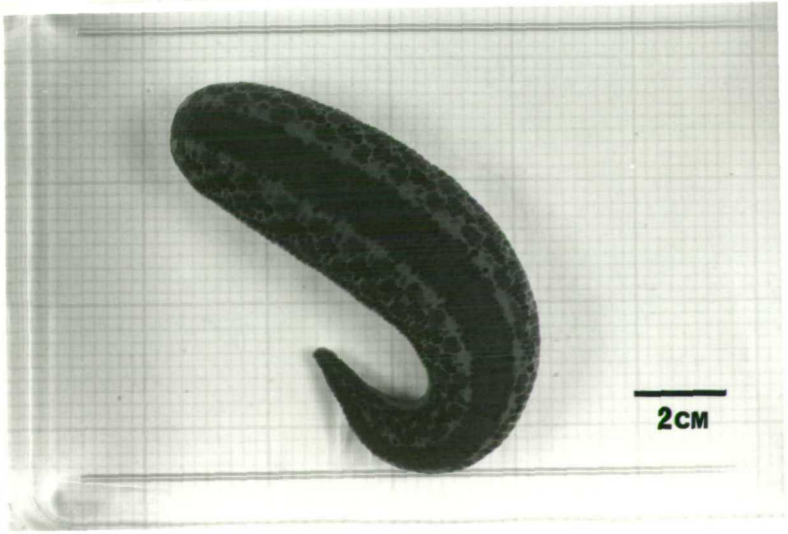


Plate 4.4 (f)



As mentioned, there was considerable variation in the volume of bloodmeal imbibed at each feed and the frequency of feeding during A, B and D diets experiments. The series of tables 4.2-4.6 shows the effects of the different diets and their frequency of intake on the relative increase in body weight after each of five successive feeds. The criteria of relative increase in body weight after feeding and describing the occurrence of feeding as the number of days since the first feed was adopted after the work of Sawyer et al. (1983).

In order to make valid interpretations of these results it was important to ensure that the same individuals were included in successive sample results. The gradual reduction of sample size, after successive feeds, shown in Tables 4.2-4.6 was due to an increase in the variable frequency of feeding. For example, the sample of 20 hatchlings, fed on diet A, which took a third feed after 60 days represented the largest group from the same sample of 34 hatchlings which took a second feed after 30 days. This difference of 14 hatchlings included only a single mortality, the rest feeding after 150 and 190 days. The absence of data on diet B hatchlings after the fifth feed was due to a wide variation in the number of days on which successive feeds occurred.

The tables indicate that the effects of the different diets on the relative increase in body weight (percentages) after successive meals are not significant at the 5% level(student's t). There does seem to be a greater significance between effects of the diets after the first and second feeds.

The effect of an increase in the number of days between successive feeds on relative body weight increase (%) was variable. For example, there was no significant difference (at the 5% levels, student's t) between the relative increase in body weight (%) of hatchlings which took a third feed on diet B after 110 and 150 days. Similarly there was no significant difference (at the 5% level, student's t) between hatchlings taking a fourth feed on diet A, after 110 and 150 days. There was, however, a significant differ nce (at the 5% level, student's t) in the relative body weight (%) of hatchlings fed a diet increase on D after 60 and 110 days.

Table 4.2 Relative increase in body weight (%) of hatchling H. medicinalis after the first feed

	Diet A	Diet B	Diet C	Diet D
Number of hatchlings in sample	50	50	50	50
Mean relative increase in body weight (%) ± standard deviation	414.3 ± 115.8	494.8 ± 164.2	367.8 ± 109.4	344.1 ± 83.5

There was a significant difference (at the 5% level) in the relative increase in body weight (%) between hatchlings fed on diets A and B, A and C, B and C and B and D (student's t).

There was no significant difference (at the 5% level) in the relative increase in body weight (%) between hatchlings fed on diets A and D and D and C

Table 4.3 Relative increase in body weight (%) of hatchling H. medicinalis after the second feed (30 days after the first feed)

	Diet A	Diet B	Diet D	Diet C
Number of hatchlings in sample	34	26	47	15
Mean relative body weight increase (%) ± standard deviation	334.1 ± 95.7	248.6 ± 61.1	326.3 ± 84.7	191.4 ± 39.1

There was a significant difference (at the 5% level) in the relative increase in body weight (%) between hatchlings fed on diets A and B, A and C, B and D, B and C and C and D.

There was no significant difference (at the 5% level) in the relative increase in body weight (%) between hatchlings fed on A and D.

Table 4.4 Relative increase in body weight (%) of hatchling *H. medicinalis* after the third feed

	Diet A	Diet B		Diet D	
	(after 60 days)	(after 110 days)	(after 150 days)	(after 60 days)	(after 110 days)
Number of hatchlings in sample	20	10	8	15	26
Mean relative increase in body weight (%) ± standard deviation	270.7 ± 56.7	283.3 ± 85.1	379.8 ± 117.6	238.7 ± 54.5	318.5 ± 49.3

The number of days given in the table follows the system used by Sawyer *et al.* (1983) and described the number of days after the first feed. The only valid comparisons possible were those between hatchlings which took a third feed the same number of days after the first feed.

There was no significant difference (at the 5% level) in the relative increase in body weight (%) between hatchlings fed on diets A and D and B and D after 60 days and 110 days respectively.

Table 4.5 Relative increase in body weight (%) of hatchling H. medicinalis after the fourth feed

	Diet A		Diet B		Diet D	
	(after 110 days)	(after 150 days)	(after 150 days)	(after 110 days)*	(after 150 days)	(after 190 days)**
Number of hatchlings in sample	11	7	7	11	15	6
Mean relative body weight increase (%) ± standard deviation	285.2 ± 83.5	303.0 ± 59.6	333.0 ± 65.9	324.0 ± 108.2	338.8 ± 74.3	296.8 ± 73.4

The number of days given in the table describes the number of days after the first feed. The only valid comparisons possible were those between hatchlings which took a fourth feed the same number of days after the first feed.

* sample from 15 hatchlings third fed after 60 days
** sample from 26 hatchlings third fed after 110 days

There was no significant difference (at the 5% level) in the relative increase in body weight (%) between hatchlings fed on diets A and D 110 days after the first feed and between hatchlings fed on diets A and B, A and D and B and D 150 days after the first feed.

Table 4.6 Relative increase in body weight (%) of hatchling H. medicinalis after the fifth feed

	Diet A		Diet D		
	(after 150 days)*	(after 190 days)**	(after 150 days)***	(after 190 days)	(after 250 days)****
Number of hatchlings in sample	8	7	6	6	9
Mean relative increase in body weight (%) ± standard deviation	260.5 ± 46.2	459.3 ± 61.1	289.5 ± 102.4	298.5 ± 125.3	407.3 ± 84.8

The number of days given in the table describes the number of days after the first feed. The only valid comparisons possible were those between hatchlings which took a fifth feed the same number of days after the first feed.

* sample from 11 hatchlings fourth fed after 110 days
** sample from 7 hatchlings fourth fed after 150 days
*** sample from 11 hatchlings fourth fed after 110 days
**** sample from 15 hatchlings fourth fed after 150 days

N te The absence of data from hatchlings fifth fed on diet B resulted from the 7 individuals involved (i.e. fourth fed after 150 days) each feeding on a different number of days after the first feed thus reducing the sample size to less than 5.

There was no significant difference (at the 5% level) between the relative increase in body weight (%) between hatchlings fed on diets A and B, 150 days and 190 days after the first feed (student's t).

Tables 4.2-4.6 show the trends in relative body weight increase (%) after five successive feeds on diet A and D. It shows a downward trend with diet A while the trend is more erratic with diet D.

The results of the series of pairings of hatchling leeches from diet A and B experiments, 200 and 290 days after the first feed (described in the methods section iv (a)) yielded some interesting observations. In the first series of pairings actual copulation was not observed, although preliminary stages in the mating repertoire occurred. This included mutual examination of body surfaces with dilated mouth, some gripping of body surfaces with mouth and posterior sucker and body coiling (Chapter 6 illustrates this behaviour in detail).

In the second series of pairings, copulation was observed between three pairs of hatchlings (fed on diet A and body weight 3.50-6.00 g). It involved one individual acting as a recipient of the partners inserted penis. The reciprocal process was not observed, and it was not possible to assess whether any sperm transfer had occurred. Although copulation was observed in only a few individuals in these optimum growth experiments, it was not possible to say definitely whether sexual maturity had been attained at this stage since none of these hatchlings became gravid within the experimental period.

(b) Sub-optimum feeding and starvation experiment

The results of these experiments, involving irregular opportunities of feeding on citrated and heparinised whole bloodmeals over a period of 725 days by a group of ten hatchlings, a group of 50 hatchlings offered a single feed on L-15 (Leibovitz) culture medium and maintained in overwintering condition without subsequent feeding and a further group of 25 hatchlings which were not offered a feed after hatching and also kept in overwintering conditions, indicated the considerable capabilities of the Medicinal Leech to withstand long periods of sub-optimum feeding and starvation. Although several previous workers have recorded this capability in larger adult leeches, it was an important finding in this study that hatchlings also display a high degree of tolerance, especially to overwintering conditions.

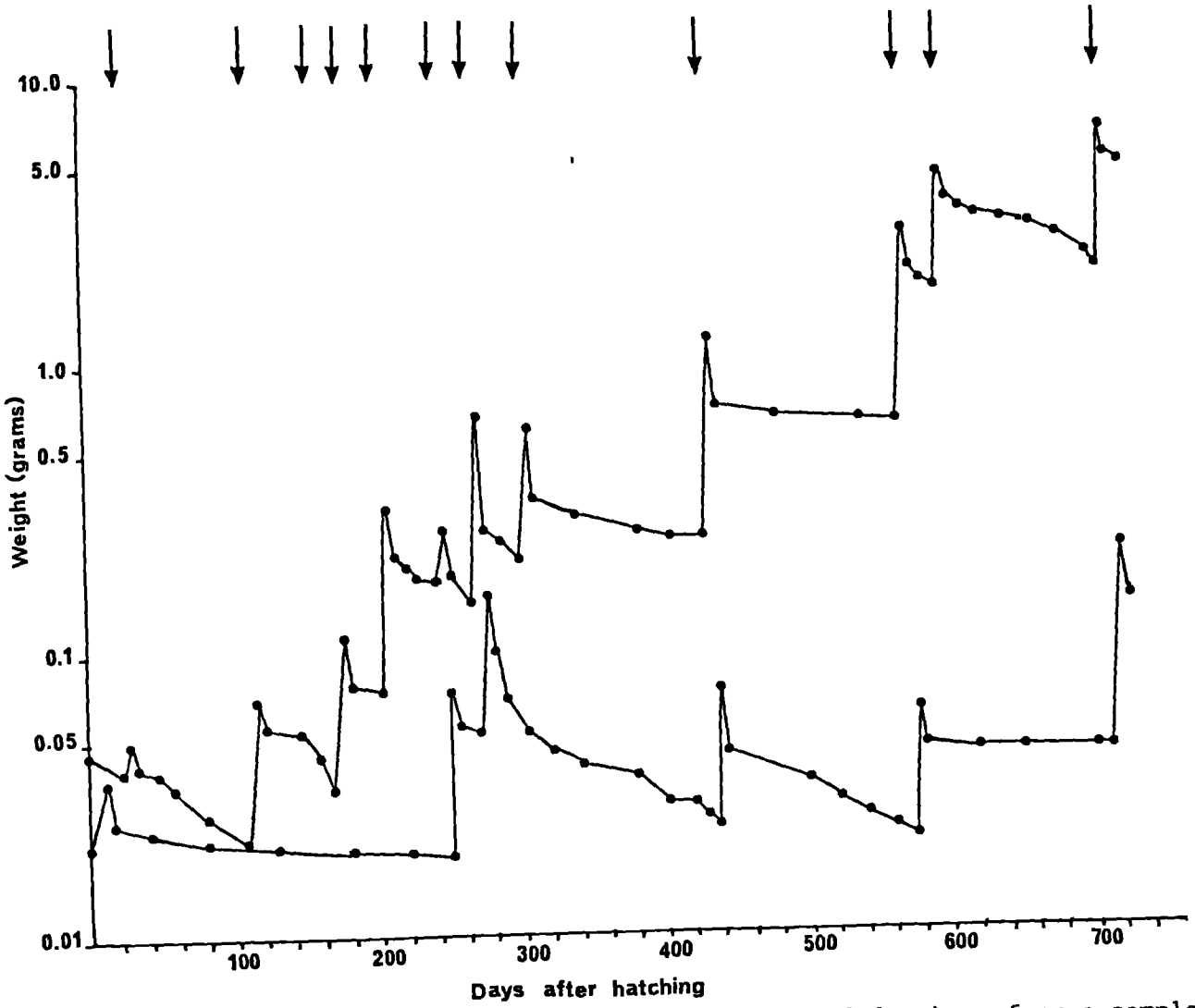


Figure 4.3 Growth curves of two hatchling Medicinal leeches, from a sample of 10, which attained maximum amd minimum bodyweights after feeding on a series of citrated and heparinised whole bovine bloodmeals over a period of 725 days at 15°+2°C and ambient daylength(8-14hrs)

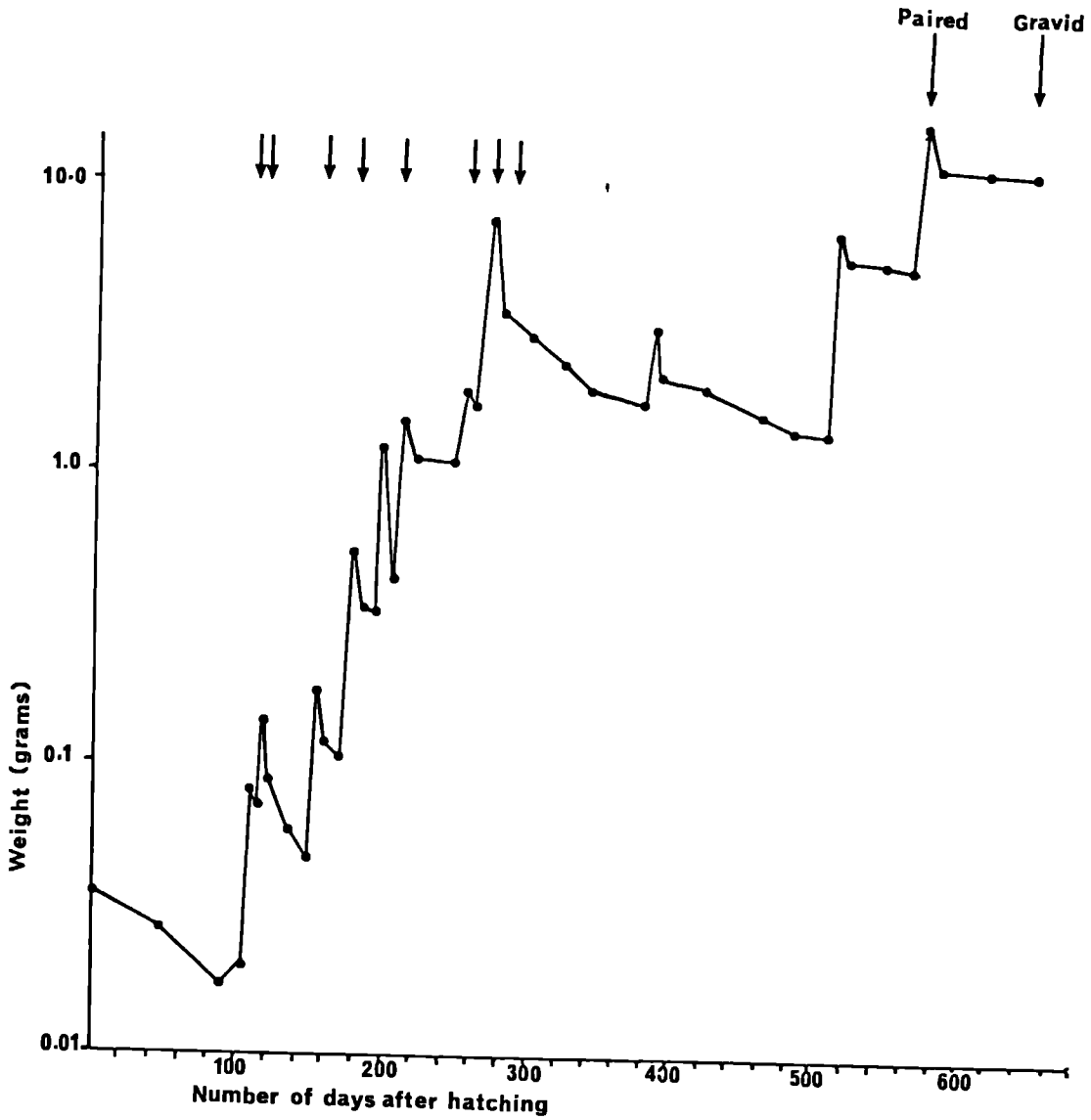


Figure 4.4 Growth curve of a single hatchling Medicinal leech fed on a series of citrated and heparinised whole bovine bloodmeals which became gravid approximately 134 days after initial pairing (15°+2°C, 8-14 hrs dayl...

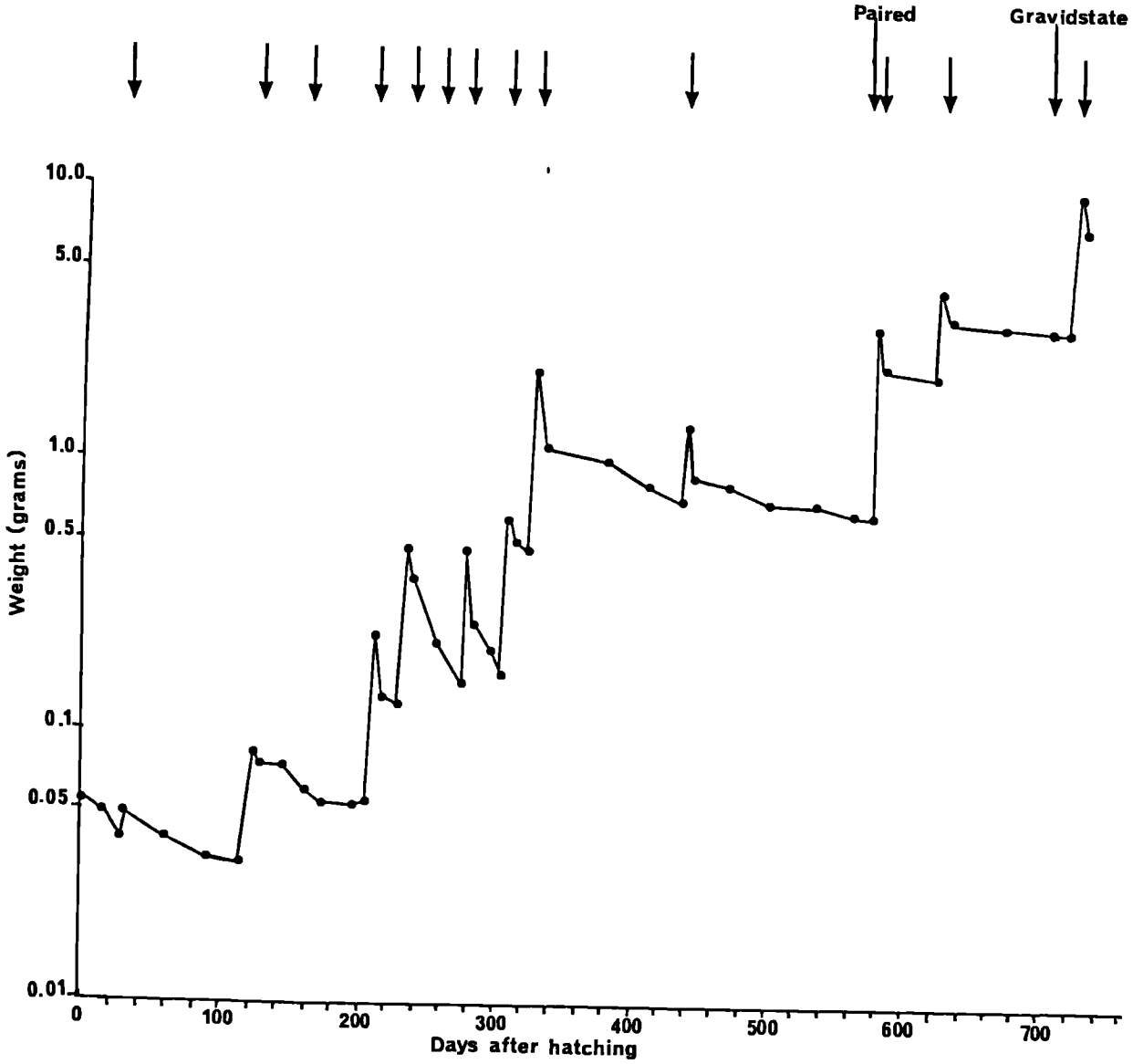


Figure 4.5 Growth curve of a single hatchling Medicinal leech fed on a series of citrated and heparinised whole bovine bloodmeals which became gravid approximately 146 days after initial pairing (15°+2°C.

Figure 4.3 shows the growth curves of two hatchling H. medicinalis which attained a maximum and minimum body weight after feeding on a series of citrated and heparinised whole bloodmeals over a period of 725 days. The arrows indicate the occasions on which a feed was offered, although as the curves show these were not always accepted. The capacity of the hatchling leech, which attained the minimum final body weight, to survive long periods between successive feeds (approximately 240 days, between the first and second feeds, despite four intervening feeding opportunities) has great significance to ideas concerning longevity and survival in the wild. Indeed a weight increase of only 83 mg over a period of 725 days supports the idea that the body weights of leeches captured in the wild (as described in Chapter 1) cannot be used as a guide to the age of the animal. This is well illustrated in Plate 4.5 which shows the size difference between the large individual which fed optimally on defibrinated whole bovine blood over 300 days (final body weight 29 g) and the small individual which fed irregularly over a period of 725 days (final body weight 104 mg).

Of the sample of ten hatchlings fed over a period of 725 days, two individuals developed a clearly gravid state 134 days and 146 days after the initial pairing. The growth curves of these two leeches are shown in Figure 4.4 and 4.5. The stage at which pairing occurred and the attainment of a gravid state is shown. One of these individuals died while gravid (Figure 4.5) while the other, despite providing conditions for cocoon laying (see Chapter 6) failed to produce any cocoons before the end of the experiment.

The results of the starvation experiment indicated the ability of hatchling H. medicinalis to survive very long periods without feeding as well as on a single sub-optimum feed. Figure 4.6(a) shows that over 50% of the sample survived up to 210 days after a single feed after which rapid mortality occurred. Figure 4.6(b) again shows a similar pattern; over 50% of the sample which had no post-hatchling feed, survived up to 230 days. The significance of this result is the high proportion of unfed hatchlings which were able to withstand over-wintering and a period of time equivalent to that between the end of one active season (early October) and the following Spring (early April). The implications of these findings are discussed in the next section.

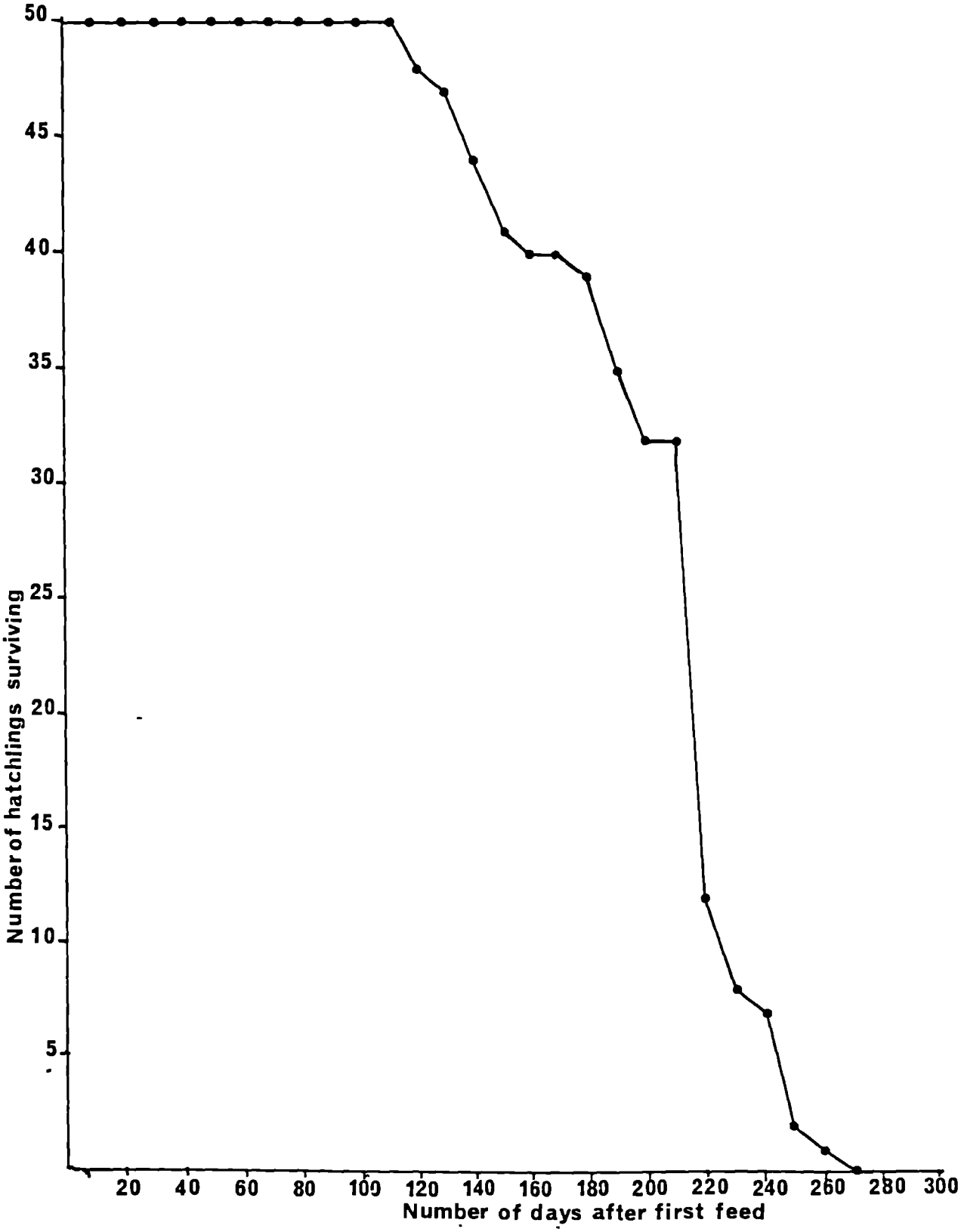


Figure 4.6 a The mortality of 50 hatchling Medicinal leeches provided with a single, post-hatching feed on L-15 Leibovitz culture medium but subsequently tarved maintained at 15°± 2°C)

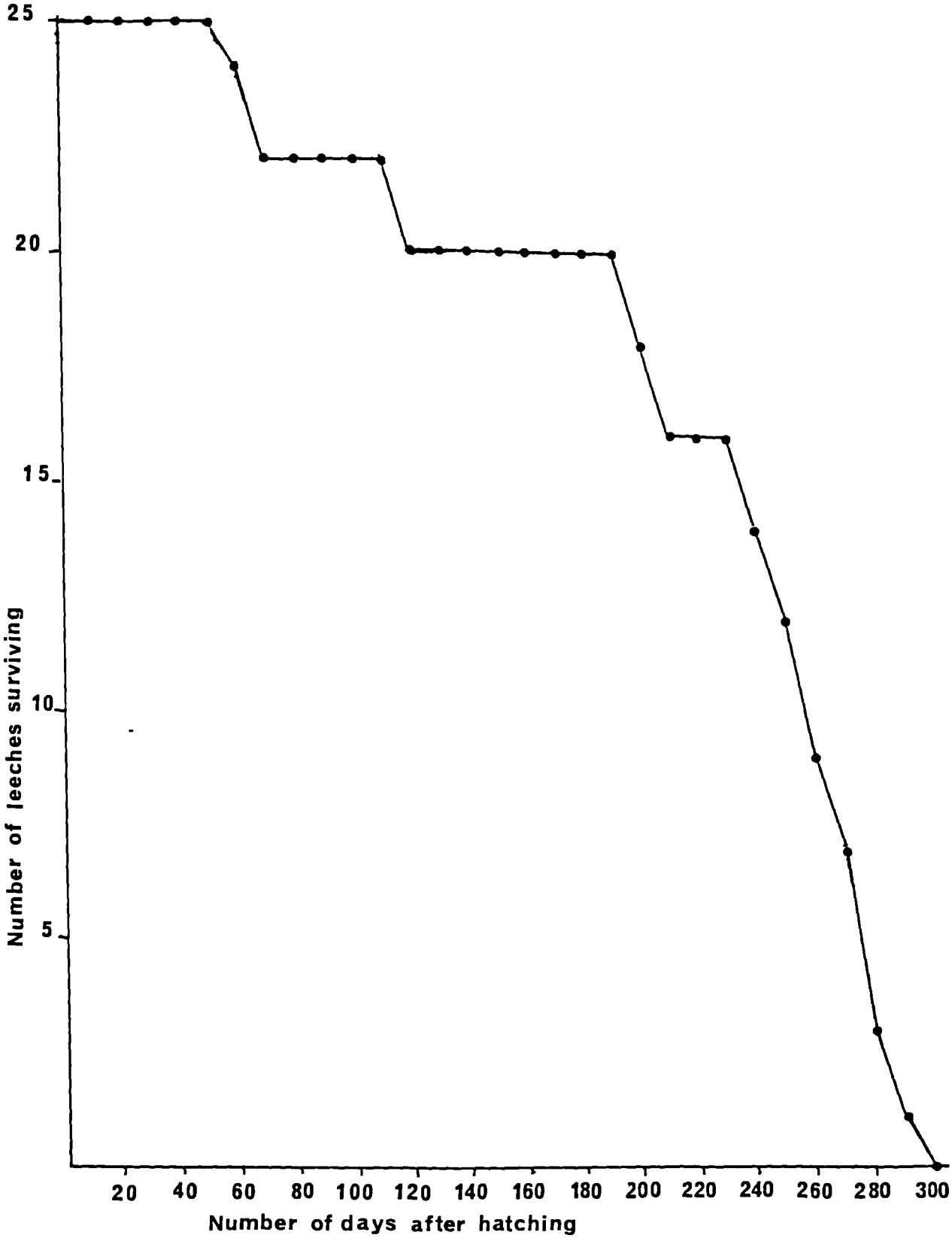


Figure 4.6 b The mortality of 25 hatchling Medicinal leeches which were not fed post-hatching and subs quently starved, (maintained at 15°±2 C for 60 days and thereafter at ambient, overwintering temperatures,2-6 C

Discussion

It was hoped that a method, based on an alternative artificial membrane, could be developed from these feeding experiments. This certainly would have improved the management of mass feeding techniques on a farm scale. Instead the natural, pig gut membrane provided the optimum membrane for feeding hatchling and adult leeches.

The results from the use of different bloodmeals provided some evidence on the possible effects of bloodmeal quality on growth in the wild. The aim of preparing diet D was to simulate a bloodmeal containing a reduced concentration of cellular elements such as amphibian blood. As described the final body weights of hatchlings fed on diet D was significantly less than those fed on diets A and B (simulated bird or mammal blood). Previous work by Sineva (1944, 1949) found in laboratory breeding experiments, that H. medicinalis fed only on frog blood attained a breeding size and condition after 18 months as compared to 9 months for leeches fed on mammalian blood. Although rearing leeches to sexual maturity was largely unsuccessful in these experiments, the results suggested that leeches feeding mainly on amphibia in the wild may take a much longer period of time to attain a weight equivalent to those sexually mature leeches found in the wild than if feeding regularly on a mammalian host.

The final body weights of many of the hatchling leeches fed on diets A and B far exceeded the weights of any individual found from the two wild populations sampled at Dungeness. This result has important implications for interpreting the feeding regimes of the Medicinal Leech in the wild. It should be remembered that a fraction of the wild populations, at the time of sampling, probably remained sessile. It was interesting, however, that individuals of large body weight (i.e. greater than 8 g) were never encountered over three sampling seasons at Lade gravel pit. This time period certainly represented sufficient time for a large, gorged leech to re-enter the active feeding fraction of the populati n.

The results from all the ptimum feeding experiments indicated considerable variation in the frequency of feeding and body weights

over time. Although Wilkialis (1984) and Sawyer (1981) noted similar variations in two Haementeria sp., there has been a t ndency by other authors, when discussing the effects of feeding on growth, to be too rigid in the interpretation of results. For example, Davies (1983) stated, on the basis of laboratory results, that over 80% of T. rude take three bloodmeals in the first six months after hatching, the remainder of the population overwinters after two meals and take their third meal in the following spring. On the basis of very variable results in the feeding frequency and consequent growth curve of hatchling H. medicinalis, it was not possible to be definite about feeding regime in the wild. It is possible to speculate that growth rate is probably very variable in the wild and is determined by an unpredictable food supply.

Sawyer (1981) attempted to link laboratory feeding results in H. ghilianni to weight classes found in wild populations. He suggested that these weight classes corresponded to a certain number of feeds. The results of these experiments with feeding H. medicinalis hatchlings do not permit such a definite interpretation of weight clases found in those wild populations described in Chapter 1. They do, however, indicate the affect of time interval between feeds on body weight. It is possible to speculate that the majority of leeches weighing less than 0.5 g sampled in the early part of the active season (April/May) may represent the previous season's hatchlings, although, as Figure 4.3 and Plate 4.5 illustrate, even individuals weighing 104 mg may represent sub-optimum feeding over an extended time period.

The results from these feeding experiments showed that it was not possible to equate leech b dy size with age and this is illustrated in Plate 4.5. It was n t possible, therefore, to assess the age of leeches obtained in samples from the wild populations studied, particularly individuals weighing more than 0.5 g.

Several authors have speculated on the l ngevity of the Medicinal Leech and have suggested lifespans of over 10 years (Fermond, 1854; Lotz, 1970(b)). It is possible, on the basis of these feeding results, to speculate on longevity by comparing the stage at which a certain body weight was reached in the laboratory and an equivalent weight found in

the wild. For example, Figure 4.2(a) showed maximum and minimum body weights attained by leeches offered a series of eight whole bloodmeals over a period of 300 days. At that frequency of food availability, the minimum body weight was still below 5 g. An equivalent body weight encountered in the wild may well have been attained on a much reduced frequency of feeding and consequently over a much longer time period. The results of population structure studies described in Chapter 1 showed that the majority of individuals sampled were in weight classes less than 3 g. Although the sampling method attracted the hungry fraction of the population, the consistent finding of large numbers of leeches in the lighter weight classes may indicate that the majority of the population survives on a sub-optimum frequency of feeding. The speculation on those large individuals found in the wild (i.e. $>$ 5 g in weight), is that some may be long-lived.

Several authors, who have worked with other leech species which are saltatory feeders, have suggested that the attainment of sexual maturity is more a function of feeding rather than time. Earlier work by Sineva (1944, 1949), on laboratory breeding of H. medicinalis indicated a link between bloodmeal quality and time to reach sexual maturity. More recent work by Fernandez and Stent (1982) on the embryology of H. medicinalis involved a laboratory breeding group. They raised leeches to sexual maturity in nine months by feeding optimally on rabbit blood.

Despite offering hatchling leeches optimum feeding, temperature, light and a cocoon laying environment no individual reached sexual maturity (i.e. gravid state) after 300 days. The result of two individuals becoming gravid 650-700 days after hatching after a series of 12 feeding opportunities and a period in overwintering conditions, may indicate a wide variation in the stage (i.e. time and number of feeds) at which sexual maturity may be attained in the wild. It was, therefore, not possible to be definite about the number of bloodmeals or time period required before sexual maturity is attained. It is possible to combine observations described in Chapter 1, i.e. that all gravid individuals found in the wild were restricted to weight classes over 3.5 g, and speculate that, based on a sub-optimum pattern of feeding, sexual maturity may take several seasons to attain.

The finding that hatchling H. medicinalis can survive for a considerable time without feeding has important implications for survival in the wild. The experimental overwintering conditions described corresponded to the period end October - early April, a time when little or no leech activity was observed in the wild population at Lade gravel pit (i.e. no hungry, searching behaviour was observed at water temperatures below 8-10°C; see Chapter 2 for more details). The laboratory results indicate that a large proportion of hatchlings can survive on the initial food supply from the cocoon, over winter. As Chapter 2 describes, adults of the smooth newt T. vulgaris appear to be a particularly important food supply for the leech during the early part of the active season (April/May). However during August and early September (a period when maximum empty, hatched cocoons were found in the wild), most of the adult newts have left the water to overwinter on land, leaving a sub-adult and larval newt food supply. The general activity of birds and frogs is much reduced during August - October, with the frog Rana ridibunda becoming completely inactive and entering hibernation at the end of this period. It is therefore possible to speculate that towards the end of the leech's active season (mid October) the activity and hence availability of hosts is reduced. It therefore means that the capacity of hatchling H. medicinalis to survive, if necessary, to the following spring without feeding is extremely important for it is during this spring period that maximum host activity recurs.

APPENDIX 4.1

L-15 (LEIBOVITZ MEDIUM)

L-15 (Leibovitz) Medium

Inorganic Salts	mg/L
$CaCl_2$ (anhyd.)	140.00
KCl	400.00
KH_2PO_4	60.00
$MgCl_2:6H_2O$	200.00
$MgSO_4:7H_2O$	200.00
NaCl	8000.00
$Na_2HPO_4:7H_2O$	359.00a
Other Components	
D(+) Galactose	900.00
Phenol red	10.00
Sodium pyruvate	550.00
Amino Acids	
DL-a-Alanine	450.00
L-Arginine (free base)	500.00
L-Asparagine	250.00
L-Cysteine (free base)	120.00
L-Glutamine	300.00
Glycine	200.00
L-Histidine (free base)	250.00
DL-Isoleucine	250.00
L-Leucine	125.00
L-Lysine (free base)	75.00
DL-Methionine	150.00
DL-Phenylalanine	250.00
L-Serine	20 .00
DL-Threonine	600.00
L-Tryptophan	20.00
L-Tyrosine	30 .00
DL-Valine	200.00

APPENDIX 4.1 cont.

	mg/l
Vitamins	
DL-Ca pantothenate	1.00
Choline chloride	1.00
Folic acid	1.00
Inositol	2.00
Nicotinamide	1.00
Pyridoxine HCl	1.00
Riboflavin-5-phosphate, sodium	0.10
Thiamine monophosphate	1.00

References:

Leibovitz, Albert, Am.J.Hyg. 78:173-180 (1963).

Remarks:

a. Original formula lists Na_2HPO_2 at 190.0 mg/litre.

Supplied by K C Biological, Inc.

APPENDIX 4.2

A formula for producing a balanced, artificial pond water suitable for maintaining the Medicinal Leech (suggested by Dr. R. T. Sawyer, pers. comm.).

Basic stock solution per litre: 2.8 g NaCl, 0.05 g KCl, 0.08 g $Ca(NO_3)_2$ 4 H_2O, 0.025 g $MgSO_4 \cdot 7\ H_2O$, 0.56 g Tris buffer (pH to 7.4).

Artificial pond water: Dilute the basic stock solution to 1% with distilled or deionized water.

CHAPTER FIVE

THE DISTRIBUTION OF THE COCOONS OF THE MEDICINAL LEECH, HIRUDO MEDICINALIS AT LADE GRAVEL PIT, DUNGENESS, KENT

Introduction

A preliminary search of restricted areas of the shoreline at Lade gravel pit by Dr. R. T. Sawyer, (pers.comm.), during the Summer of 1981, successfully located several viable cocoons which were identified as those of the Medicinal Leech. On the strength of this evidence it was decided to undertake a more exhaustive search of the site.

Although it has been documented that the Medicinal Leech leaves the water to lay its cocoons (Elliott & Mann, 1979), no detailed description has been made of the characteristics of the microhabitat of a known leech cocooning site. A short review of the literature (for example Putnum & Shklov, 1956, and Stark & Dahlsten, 1961), showed that, in insects at least, there is often careful selection of the microhabitat for egg and cocoon deposition. This is often indicated as a clumped distribution after random sampling or a distribution confined to a limited band or strata related to a physical gradient such as moisture (Ives, 1955).

The aim of this study was twofold. Firstly, to determine the extent of cocooning sites at Lade gravel pit and whether there were any particular areas where cocoon deposition was preferred, and secondly to examine, more closely, cocoon distribution in a small area of shoreline using a quadrat sampling method.

Methods

To assess the extent of cocoon laying areas, the total length of favourable shoreline at the northern end of Lade gravel pit, including zone 2, and the small pond area, at its western edge, was sampled at distance intervals of every 5 metres (note that a detailed description of these areas is given in the Management Plan). All sampling for this study took place during mid-July to mid-August 1983, which was believed to be the main cocoon laying period of the leech.

At each 5 metre interval a transect was marked out perpendicular to the shoreline and extending, on average, for 2 metres from the waterline to a point where the upper layer of shingle, interspersed with a poorly developed soil, was dry and supported more terrestrial vegetation. A 0.5 m^2 area quadrat was laid on the transect line and this area was carefully searched for cocoons down to a depth of 5-10 cms, (it is known that although the Medicinal Leech lays its cocoons above the waterline, they are usually buried slightly below the ground surface). This search involved carefully removing shingle pebbles and examining the root interstices of emergent macrophytes and more terrestrial vegetation. For the purpose of this general survey only the presence of cocoons, their distance from the waterline and depth below ground were noted.

To examine, in detail, the distribution of leech cocoons in a small area of favourable shoreline, two specific areas were selected, one in zone 3/1 and the other in zone 4. It was important that each area was consistent within itself, without sharp changes in shoreline gradient or vegetation cover. Any pattern in the distribution of cocoons would then be more likely due to subtle effects of microhabitat differences.

An area of 10 x 2 metres was marked out using the waterline as one boundary. This area was sub-divided into a series of 2 m^2 blocks and each block regarded as a 10 x 10 grid. A 20 cm^2 wire quadrat was constructed and a series of random numbers were used in pairs as co-ordinate for the 10 x10 grid. Figure 5.1(a) shows the sampling pattern for the whole area, while Figure 5.1(b) shows a series of five pairs of co-ordinates within one block, with corresponding areas sampled. Note that each pair of co-ordinates defined a point on the grid and a convention was adopted that this point represented the bottom left-hand corner of the quadrat. A total of 20 random quadrats were taken in each 2 m^2 block (a total of 100 in the whole area), thus representing approximately a 20% coverage. Plate 5.1 shows part of zone 4 sampled with a dense stand of Great Willowherb, *E. hirsutum* to the left and the emergent Lesser Reedmace, *Typha angustifolia* to the right (note the waterline boundary line). Plate 5.2 is a view down through the *E. hirsutum* stand showing the 20 cm^2 quadrat (note:the smaller 10 cm^2 quadrat inside was a preliminary trial size).

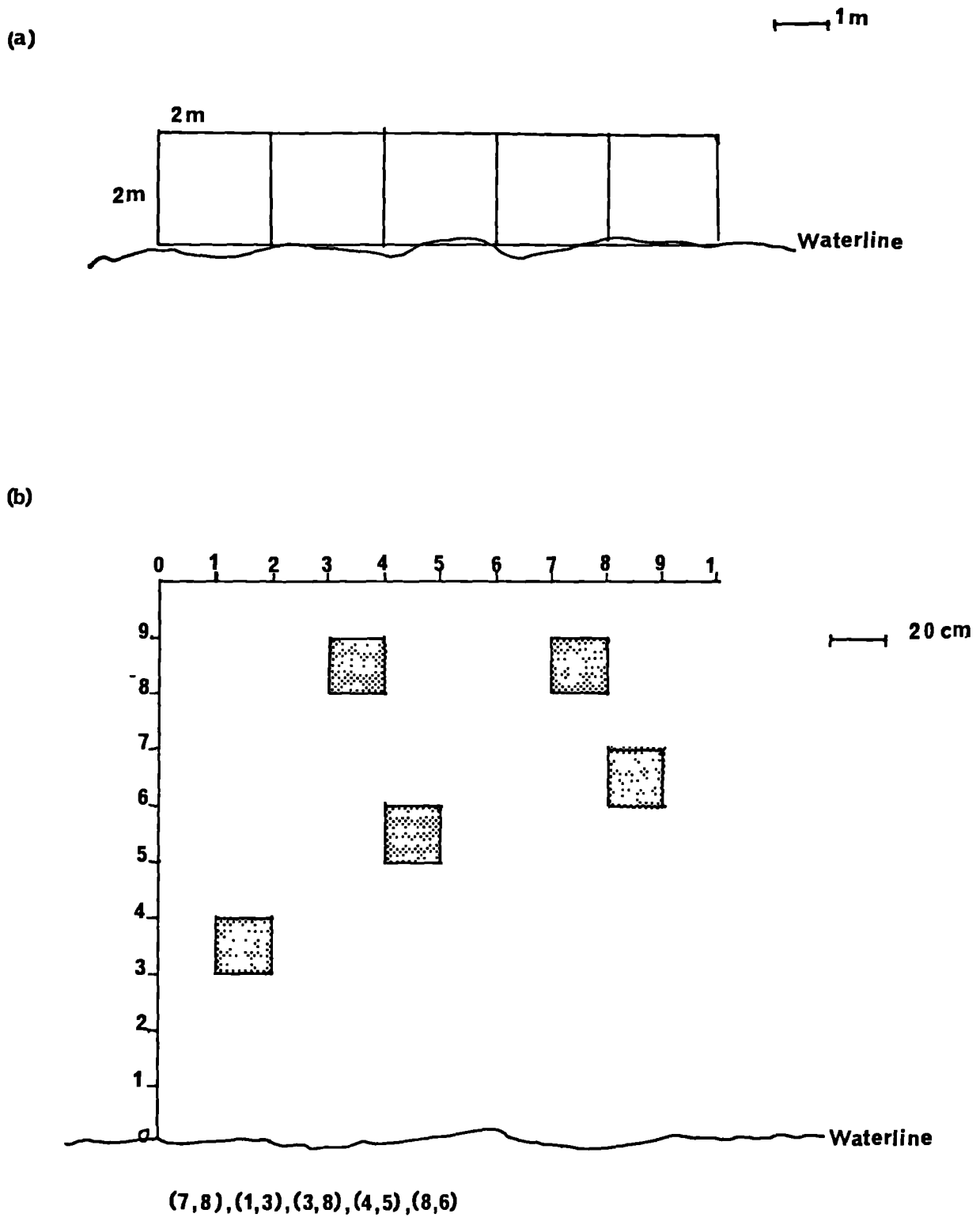


Figure 5.1 The sampling design for locating the cocoons f the Medicinal leech at two specific sit s at Lade gravel pit



Plate 5.1 A 10 metre section of shoreline in Zone 4 marked out for coccon sampling



Plate 5.2 A view down through a stand of Great Willowherb, Epilobium hirsutum showing 10cm² and 20cm² sampling quadrats

Results

There were three main types of shoreline profile at Lade gravel pit:

Type 1 Bare shingle with no aquatic, emergent vegetation or fringing, terrestrial vegetation.

Type 2 Shingle with or without emergent vegetation and, in drier areas, with stands of Great Willowherb, E. hirsutum.

Type 3 Shingle with or without emergent vegetation but with drier areas supporting terrestrial grasses and smaller wetland herbs, e.g. Forget-me-not, Mysotis arvensis.

Plate 5.3 shows Type 2 shoreline profile. Only Types 2 and 3 profiles were regarded as potentially favourable sites for leech cocoons so all areas of bare shingle in the general survey were not sampled.

In the general survey, a total of 108 transects were sampled. The results were disappointing but significant. In the total of 108 transects only 16 cocoons were found at the northern end of Lade gravel pit. Of the 16 cocoons found, 11 were found singly while the other five were found in two separate clusters in different transects. One cluster consisted of a group of three cocoons, all within a radius of 5-10 cms of each other, while the other pair were 3-4 cms apart. The average distance of cocoons from the waterline was 76.5 cms and these were found at an average depth of 5.7 cm. Despite a thorough search of the small pond, no cocoons were found at this site.

Figure 5.2 shows the approximate location of cocoons found in the general survey of the northern end of Lade gravel pit and the two specific areas selected for a detailed study of cocoon distribution (see large arrows).

All cocoons were restricted to Type 2 profile (i.e. associated specifically with the root system of the Great Willowherb, E. hirsutum) and were retained for laboratory hatching for the study of leech fecundity (see Chapter 6 for more details). Plate 5.4 shows the pair of cocoons found in zone 2 at a distance of approximately 3-4 cms from each other (note: ro t system of E. hirsutum).

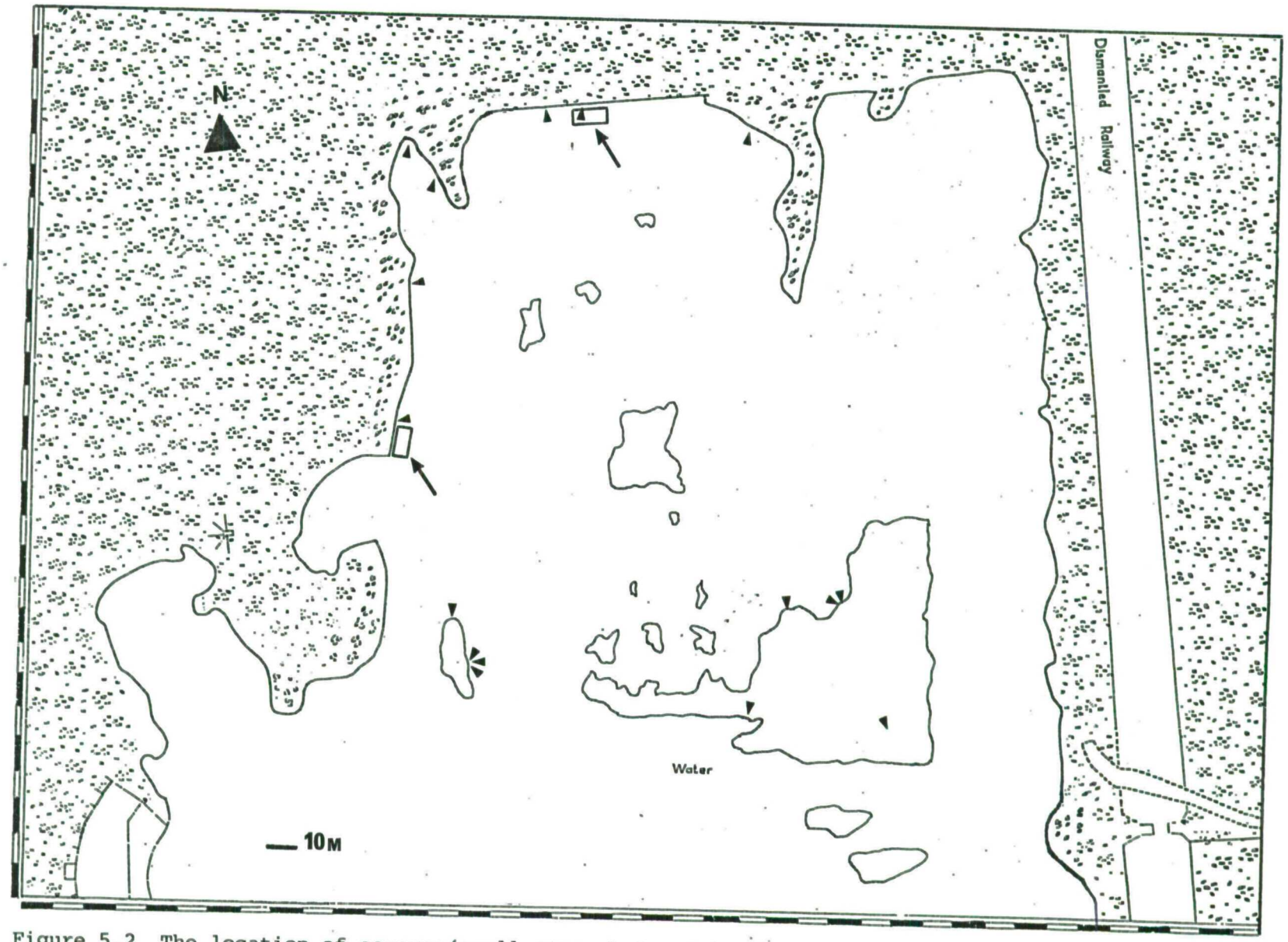


Figure 5.2 The location of cocoons(small arrows) found in the general survey of the Northern end of Lade gravel pit during July-August 1983. The two specific areas selected for a detailed study of cocoon distribution are also shown(boxes)



Plate 5.3 Type 2 shoreline; composed of shingle without associated emergent vegetation but with a dense stand of Great Willowherb, E.hirsutum



Plate 5.4 A pair of Medicinal leech cocoons embedded in the root system of Great Willowherb, E.hirsutum

Scale shown

The initial results of quadrat sampling in the two specific areas were also disappointing, with a total of four, single, cocoons obtained from 200 x 20 cm^2 quadrats (a total sampling area of 8 m^2). It was hoped that data obtained from this detailed quadrat sampling method would be sufficient to statistically test the preliminary observation, in the general survey, that there may be a tendency towards a contagious or clumped distribution of leech cocoons in the wild,(Elliott, 1971, gives a full account of the statistical use of χ^2 tests (goodness-of-fit) for agreement with a negative binomial distribution). The result of finding three cocoons from zone 4 and one from zone 3/1 was insufficient data for any statistical analysis.

A further 100 x 20 cm^2 quadrats were randomly sampled in each area after which a further 10 cocoons were found. This gave a total of eight cocoons from zone 4 and six from zone 3/1. From this total of 14 cocoons only a single pair were found in zone 3/1 approximately 5 cms apart. These cocoons were returned to the laboratory for fecundity studies.

Discussion

The small number of cocoons found in the general survey, while giving little indication of any preferred laying areas, was nevertheless a significant finding.

An important consideration for this study was the viability of the leech population at Lade gravel pit. Its continued survival depends on a proportion of the population attaining sexual maturity, successfully laying cocoons and generating recruits for successive seasons. As the results of population structure show (see Chapter 1 for details), there is recruitment into the population, but the finding of relatively few cocoons in the wild (i.e. approximately one cocoon per 13.5 m^2) may indicate that this recruitment is slow. It was not possible to assess accurately, the proportion of the leech population which attains sexual maturity each year but the few cocoons found may indicate that this proportion is small.

Although it was not possible to describe statistically the distribution of cocoons at the two specific sites, several general conclusions were possible.

The cocoons of the leech were never found just above the waterline where the microhabitat immediately below the surface shingle remained waterlogged. Similarly, cocoons were not found at the other end of the shoreline profile in much drier, sub-surface conditions. All the cocoons were found just below the ground surface and in a band 60-95 cms from the waterline. There was no assessment made of the physical characteristics of this microhabitat band, although moisture/humidity may have been the important factors.

The cocoons of the Medicinal Leech were always associated with the root system of the Great Willowherb, Epilobium hirsutum. There was evidence that the leech had burrowed into the root mass. The leech secretes a soft, proteinaceous 'froth' which surrounds the inner egg capsule but after a short time this outer layer partially hardens to form a spongy, protective coat. It appears that part of the Willowherb root system becomes embedded while the secretion is soft and as this dries, firmly anchors the cocoon in place.

It was interesting to observe that the act of cocoon laying appeared to have involved the gravid leech moving over ground above the waterline to a selected point where the animal had then burrowed below the ground surface. During the whole of this survey, no leeches were found above the waterline in the act of laying cocoons so it was not possible to assess how long the animal remains in the vulnerable position out of the water.

It was interesting to find several cocoons in close proximity to each other. It was not possible, however, to attribute any of the cocoons to any individual leech. Therefore, finding these clustered cocoons could either have represented more than one leech coincidentally selecting the same local, microhabitat or, less likely, the results of cocoon laying by a single leech. The results obtained from the laboratory study of leech breeding (see Chapter 6 for more details) suggested a period of several days between successive cocoon laying by an individual leech. It is unlikely that, in the wild, a leech will remain out of the water for more than a few hours (the Medicinal Leech is unable to retain body water in air and will dessicate after several hours). Therefore the incidence of cocoons clustered together may indicate that the leech is highly specific in its choice of cocoon laying sites and the general description of a site "just above the waterline" (Elliott & Mann, 1979) is an over-simplification.

CHAPTER SIX

A LABORATORY STUDY OF REPRODUCTIVE BEHAVIOUR, CAPTIVE BREEDING AND COCOON CULTURE IN THE MEDICINAL LEECH, HIRUDO MEDICINALIS (L) WITH A CONSIDERATION OF ITS LIFE-HISTORY AND FECUNDITY IN THE WILD.

Introduction

In comparison with the growing literature describing the anatomy and physiology of the leech nervous system, relatively little work has been done on the reproductive biology of H. medicinalis. The neuro-secretory control of spermatogenesis in the leech and the main environmental factors of light and temperature affecting this control has been studied by Nekhayev (1957), Hagadorn (1966a, 1966b) and Malecha (1970a, 1970b, 1970c). Their results are particularly interesting in relation to the leech in the wild and are discussed in the context of life-history and seasonal effects in the discussion below.

Several other references cover related areas of reproductive biology and embryology, for example Zick (1933) examined the chemical nature of the leech cocoon and concluded that the main constituent was a scleroprotein closely related to fibroin, a component of insect silk. Pastisson (1968) examined the ultrastructure and histochemistry of the leech clitellum, although attention was paid to the mature gland, rather than a developmental approach. Wissocq and Malecha (1975) made a broad study of the ultrastructure of spermatozoa from several leech species including H. medicinalis. They found that all the species possessed a cork-screw shaped acrosome and nucleus. A recent study of the embryological development of H. medicinalis was made by Fernandez & Stent (1982). Their work included the maintenance of a viable breeding population in the laboratory based on earlier work by Sineva (1944, 1949).

There have been many attempts at captively breeding H. medicinalis and during the height of its use in medicine in the 19th century, all the major treatises on the animal c ntained detailed accounts of 'Hirudoculture' (Fermond, 1854). The laboratory rearing of medicinally useful leeches has not been restricted to H. medicinalis and several authors have described work with its close relatives in North America

(Macrobdella decora), Asia (Hirudinaria sp) and Japan (Hirudo nipponia). These authors include Khan (1912), Matthai (1920), Gouck, Taylor and Barnhart (1967), Keegan et al. (1968) and Toshioka (1977). The results from these studies provide valuable comparative information on the fecundity or the capacity for producing viable offspring, of laboratory reared relatives of H. medicinalis and are used further in the discussion below.

More recent experimental studies indicated that food, temperature and light are important factors for successfully breeding H. medicinalis in captivity. This research has, however, been largely confined to the Russian literature and I am indebted to Dr. R. T. Sawyer for providing translations of the work of Sineva (1944, 1949), Schchegolev, Shchegoleva and Sidorenka (1964) and Zapkuviene (1972a, 1972b). These studies provided the basic experimental methods used in this work and their results, describing the fecundity of the leech in the laboratory, are used in a comparative way in the discussion.

The main aims of attempting to breed the leech in captivity were twofold. Firstly, to bring the techniques developed by the Russian workers up to date and to examine whether similar methods could be used successfully to breed and rear the leech, and secondly to obtain hatchlings for the controlled growth experiments described in Chapter 4.

As described in Chapter 5, cocoons were obtained from the littoral areas of Lade gravel pit but it was not possible to link these with the activity of individual animals. It was important, therefore, to assess the fecundity of individual leeches in the laboratory in order to compare with the findings by other authors (for example Sineva (1944, 1949)).

Recently, Calow and Riley (1980, 1982) discussed the reproductive effort (measured in terms of offspring production per parent) invested by British erpobdellid and glossiphoniid leeches with different life-cycles. It was felt important that a discussion of H. medicinalis in this context of reproductive effort would be important in any consideration of the animal's life-history tactics, potential survival and conservation in the wild.

The observations, in this study, of the reproductive behaviour of the Medicinal Leech in the laboratory, were a consequence of the work on captive breeding. Sineva (1944) provided a description of mating behaviour, duration and observation on whether insemination was one-sided, or reciprocal. She also provided line drawings of mating leeches. It was decided to add to her work by producing a photographic record illustrating the mating behaviour of the leech.

Methods

(i) Coco ns Collected from Lade Gravel Pit

A total of 30 Medicinal Leech cocoons were collected, during July and August 1983, from Lade gravel pit, using methods described in Chapter 5, and returned to the laboratory.

Each cocoon was placed on a piece of moist cotton wool in a round plastic, clip-top container, 6.5 cm in diameter x 4 cm deep. The lid carried small perforations to allow ventilation. The walls and base of the containers were kept moist to prevent any possible desiccation of emerging hatchlings between checks. Each container was maintained in dim light conditions at a temperature of 24 $\pm$ 2^{o} C and checked daily to ensure that moist, humid conditions were maintained. This was the basic experimental method used for culturing all subsequent cocoons laid in the laboratory with the aim of simulating moist, humid and warm condtions present in natural cocoon-laying sites (see Chapter 5 for a description of these sites).

(ii) Establishing a Basic Stock Laboratory Population of the Medicinal Leech

All the Medicinal Leeches used in these experiments were obtained from two sources. A small group of 15 leeches (mean $\pm$ SD body weight 2.76 $\pm$ 0.83 g) were supplied from Germany by a commercial leech supplier (see Appendix 6.1, while a sample of 100 leeches was obtained directly from Lade gravel pit using sampling methods described in Chapter 1. Within this sample, 52 leeches were captured, during September 1983, none of which were in a gravid state (the identification of this state is

described below). The mean ± SD body weight of this sample of 52 leeches was 1.86 ± 1.25 g.

A further 48 leeches were captured during late June and mid-July 1984, all in a clearly gravid state. (Note that individuals in a gravid state might have been expected to remain sessile in preparation for cocoon deposition but clearly the potential opportunity for feeding and response to water disturbance was an overriding stimulus). The mean ± SD body weight of this sample of 48 leeches was 4.33 ± 0.92 g. It was impossible to assess whether any one of these leeches had laid any cocoons prior to their capture, particularly those individuals captured in the middle of July. Therefore the discussion of fecundity or the number of cocoons produced per leech is limited by this fact. It was hoped to rear individuals in the laboratory from hatching to sexual maturity in order to gain a better idea of fecundity but as Chapter 4 describes this was largely unsuccessful.

The assessment of whether Medicinal Leeches are in a gravid state can be made in two ways. There is a clear discoloration or yellowing of a section of the anterior body segments as the clitellum develops and a slight swelling in this area can be detected by very gently rolling an individual between the fingers. Plate 6.1 shows the typical appearance of a gravid Medicinal Leech; note the swelling of anterior segments (see arrow). These criteria were used throughout this study to assess whether an individual was in a gravid state and the attainment of this state was found to be the only clear, external evidence that a leech had reached sexual maturity. The presence of a clitellum has also been used to assess sexual maturity in another leech, Nephelopsis obscura (Collins and Holmstrand, 1984).

The 115 leeches formed the basic stock laboratory population. They were all housed individually in clear perspex containers, 17 x 11 x 6 cm, with secure lids. Each container was partly filled with aerated tap water and several shingle pebbles added to provide shelter and to allow the leech to rest partially out of the water. This was the standard housing used for all 'resting'leeches while not involved in any breeding experiments. These leeches were maintained at a room temperature of 15 ± 2°C and natural daylength (or a minimum of 10 hours light).

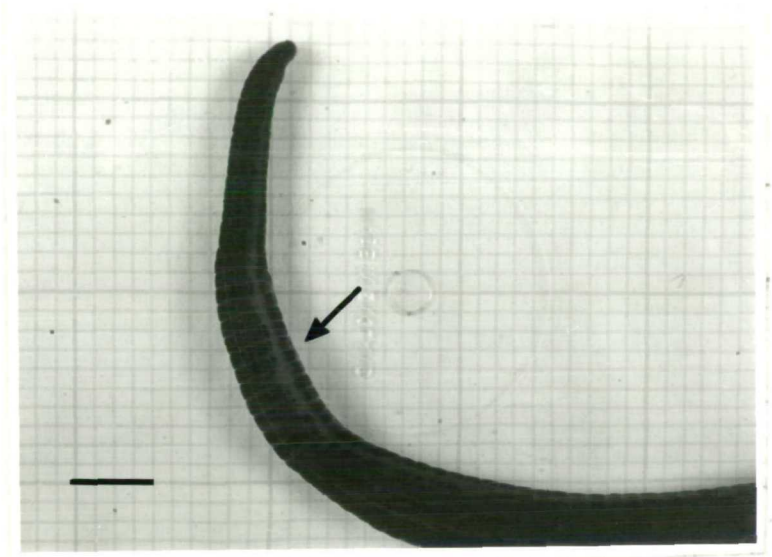


Plate 6.1 The typical appearance of a gravid state in the Medicinal leech; note the swelling,(and discolouration), of anterior segments,(arrowed)

Scale bar - 1cm

(iii) Establishing a Cocoon-Laying Chamber

In her work on breeding the Medicinal Leech, Sineva (1944, 1949) experimented with various covered and uncovered soil types. She found that an aquarium filled with a shallow layer of moist peat, covered with a layer of turf, provided a favourable environment for cocoon laying and the hatching of young leeches. In her experiments she placed groups of five leeches together in an aquarium 30 x 10 x 25 cm and expressed her results as the mean number of cocoons per leech. In this study it was decided to design cocoon-laying chambers for each gravid leech so that cocoons and subsequent hatchlings could be attributed to individuals.

The same type of perspex container described above was used as a cocoon-laying chamber. Each was partly filled with a layer of moist moss peat and covered with a small piece of turf. It was important to ensure that the lid remained secure on the container as even gravid leeches were highly mobile. Each container was prepared two days before its use and a soil temperature of 24 $\pm$ 2°C was maintained in the same experimental room used for the breeding and cocoon culturing experiments. All cocoon-laying chambers were checked daily to ensure that moist, humid conditions prevailed. It was important to handle these chambers with great care while occupied by a gravid leech since any slight disturbance during the act of cocoon deposition often resulted in the leech withdrawing, leaving a deformed, indented cocoon which did not develop further. Plate 6.2 shows a view through the side of a cocoon-laying chamber. The leech can be seen between the upper turf layer and bottom peat layers in the early stages of cocoon deposition. The 'frothy' outer protective coat soon hardens to a spongy structure and it is only after this had occurred and the leech had withdrawn that it was possible to remove safely the cocoon to a cocoon-culturing chamber as described above. It was not generally possible to measure the time period between the clitellum secretory stage and the final withdrawal of the leech fr m the cocoon as this would have involved disturbance resulting in an aborted cocoon. An approximate time period for this process, based on the individual in plate 6.2 and three other observations, was 30-60 minutes.



Plate 6.2 A view through the side of a perspex cocoon laying chamber; note the Medicinal leech is visible,between the upper turf layer and lower peat layer, in the early stages of cocoon deposition

Scale - 1cm (linear)

(iv) Cocoon Laying by Gravid L eches Obtained from Lade Gravel Pit

This sample of 48 leeches was obtained during June and July 1984. In order to maintain summer field water temperature and daylength they were kept in individual containers partly filled with aerated tap water and maintained at a temperature of 24 $\pm$ 2°C and a daylength of 14 hours.

After a resting period of 24 hours each gravid leech was transferred to an individual cocoon-laying chamber where it was kept until a cocoon had been laid. This period varied between 1-4 days for the initial cocoon. After this initial cocoon had been laid the leech was replaced into its water filled container and the cocoon transferred to a cocoon-culturing chamber. Subsequently, an alternating regime of 36 hours in the cocoon-laying chamber followed by 24 hours in water was established for each leech. The aim of alternating between the aquatic and terrestrial environments was to give each leech the maximum opportunity to lay cocoons as well as to simulate the probable situation in the wild where the animal may leave and return to the water several times during the cocoon laying period. It was felt that this arrangement was preferable to waiting for visual signs of a gravid state to become apparent before transferring the leech to a cocoon-laying chamber.

Throughout the cocoon laying period of each leech regular body weighings were taken, particularly after laying, but no food was offered. After this period had ceased, as indicated by a loss of gravid state, each leech was returned to its individual water-filled container and moved to a room temperature of 15 $\pm$ 2°C and reduced daylength (10 hours fluorescent light). They were maintained in this 'resting state' until required for further breeding experiments.

(v) Breeding Experiments using the Basic Stock Laboratory Population of the Medicinal Leech

The basic stock population of 115 leeches was regarded as two sub-groups for the purpose of these breeding experiments.

One group consisted of those leeches obtained from Germany and Lade gravel pit in 1983, none of which were in a gravid state and having no external signs indicating that sexual maturity had been

attained. The other group of leeches, obtained from Lade gravel pit during July and August 1984, were in a gravid state indicating that sexual maturity and successful mating had occurred in the wild.

In all recent studies on breeding the Medicinal Leech (see above for references), several important factors were identified which stimulated sexually mature leeches to breed. Sineva,(1944, 1949), found that the leech could be reared to sexual maturity in 8-10 months on a diet of four meals of rabbit blood . These factors included a recent bloodmeal, isolation before pairing, a change of water and being transferred to a higher water temperature. Although these factors are known to be significant for inducing mating in the leech, the optimum sequence and duration appears less clear. Schchegolev et al. (1984) suggested the following regime for mating and multiple cocoon deposition.

Stage 1: 10 days isolation at a water temperature of 25 - 27°C with a mammalian bloodmeal feed.

Stage 2: Paired for 30 days at a water temperature of 25-27°C

Stage 3: Isolated and transferred to a terrestrial chamber for 30-45 days.

For the breeding experiments in this study all leeches, while not involved in mating pairs, were maintained individually in water filled containers. All were maintained in a continually satiated state on a series of heparinized whole bovine bloodmeal rather than being offered a specific bloodmeal immediately prior to pairing.

The group of leeches received from Germany and those captured from Lade gravel pit in September 1983, were maintained in individual containers partly filled with aerated tap water at 15 $\pm$ 2°C and a natural daylength of 8-10 hours for 2-3 months. After this period a bloodmeal was offered and immediately after feeding all gorged leeches were placed, still in individual containers, at a higher temperature of 24 $\pm$ 2°C and at an extended daylength (artificial light) of 14 hours for 20-30 days). During this preparation period before pairing, this sample of leeches began to show signs of 'stress' soon after being moved to the higher temperature. (A 'stressed' state in the laborat ry leech

was shown by irregular, body contortions and a tendency to disgorge the bloodmeal. Indeed mortality was significant and 41 leeches died during this period. It was found that leeches maintained at the lower temperature of 15 $\pm$ 2°C for 7-10 days after feeding behaved normally and could subsequently be maintained without obvious stress at the higher temperature. This practice was adopted for all post-fed leeches in these experiments. Gorged leeches maintained indefinitely at 15 $\pm$ 2°C show no signs of stress so it is likely that the rise in temperature increases the metabolic rate of the animal and possibly the demand on dissolved oxygen in the water. In fact Bialaszewicz (1924) and more recently Zebe, Roters and Kaiping (1986) noted that metabolic rate, measured as oxygen consumption, was greatest in the hours immediately following a bloodmeal.

After the mortalities which occurred during the preliminary trials at 25 $\pm$ 2°C, the remaining 36 leeches were replaced at the 'resting' temperature of 15 $\pm$ 2°C for 10 days. After this period, the sample was replaced at the raised temperature for a further 10 days, after which pairings were made between leeches of similar body size/weight. These pairs were observed for 1-2 hours immediately after pairing and daily for about an hour during the mating period. The mating behaviour of the leech which is described in the results section below is based on these observations. These pairings were maintained for 35 days, at the raised temperature. An important factor at the raised water temperature was maintaining clean water conditions. Certainly the defaecation rate was greater during the post-fed period at 25 $\pm$ 2°C than at the lower temperature of 15 $\pm$ 2°C and as a result changes of water (always aerated) were made daily at this raised temperature. At the end of the mating period each leech was maintained in individual containers at 25 $\pm$ 2°C and observed daily for signs of clitellum development. Gravid leeches were transferred to a cocoon-laying chamber and the same alternating aquatic/treestrial regimes were used as described above, until cocoon laying had ceased.

All the leeches in this sample, whether they developed a gravid state or not, were returned to the 'resting' temperature of 15 $\pm$ 2°C after a total of 4½ months after initial pairing at the raised temperature of 24 $\pm$ 2°C, i.e. during the period February-May 1984. After

25-30 days at the resting temperature, a further heparinized, whole bovine bloodmeal was offered. All 36 leeches readily fed on this occasion, a period of 7 months after the previous meal. As before all leeches were maintained for 7-10 days at 15 $\pm$ 2°C post-feeding before again being transferred to a raised temperature of 24 $\pm$ 2°C. The same pairing regime and observations of mating behaviour were adopted except that all leeches, after separation, were maintained at 24 $\pm$ 2°C for the rest of the experiment, i.e. June 1985.

During this period a series of four bloodmeals were offered and five leeches exceeded a body weight of 30 g, one of which gained a maximum post-fed body weight of 61 g (see Plate 4.4 (f) in Chapter 4.) The ability of the Medicinal Leech to reach such a size and body weight does not appear to have been previously recorded and the scale is comparable to the giant Glossiphoniid Leech, Haementeria ghillanii (Sawyer, pers.comm.). Certainly Medicinal Leeches of this order of magnitude were never found in the wild at Lade gravel pit.

The group of 48 gravid leeches captured in June - July 1984 were offered a heparinised, whole bovine bloodmeal 2-3 months after the end of the cocoon laying period (October-November 1984). A similar post-feeding 'resting' period of 7-10 days at 15 $\pm$ 2°C was allowed before being transferred to the raised temperature of 24 $\pm$ 2°C where they were maintained in individual containers for 3 months. After this period (March 1985), a series of pairings were made lasting 30 days. Individuals were then parted and replaced in separate containers, still at 24 $\pm$ 2°C where they were maintained for a further 2½ months (June 1985). During this period a bloodmeal was offered. As in the previous breeding experiments observations were made immediately after pairing and on a daily basis.

Results

From the total of 30 cocoons collected at Lade gravel pit during July-August 1983, 11 failed to develop fully mature hatchling leeches. These failed coco ns were carefully opened and examined for any obvious development of embryos. Of this group of 11 cocoons, 8 contained embryos with clearly developed eyes, suckers and segmentation. The

other 3 cocoons appeared to contain an undifferentiated egg mass and could have represented either failure during very early embryonic development or else a cocoon which was originally sterile when laid. Unfortunately no definite evidence was obtained to suggest that an unfertilised leech will, nevertheless, lay sterile cocoons. These 8 failed cocoons contained a mean $\pm$ SD of 7.2 $\pm$ 1.5 embryos per cocoon (range 5-10 embryos per cocoon) but these were not included in any estimates or discussion of fecundity (a convention used with other results described below).

Most of the remaining 19 cocoons were maintained until hatchlings emerged naturally (it was not possible to estimate the development time of the hatchlings since the time of actual cocoon deposition was unknown). In several cocoons, fully developed hatchlings remained in the cocoon and on one occasion mortality occurred although the cause was unclear. It was possible to observe fully developed hatchlings in the cocoon by their movements and using enhanced background light. A maximum period of 24 hrs was allowed for hatchlings to emerge naturally after which the cocoon 'plug' (originally created by the parent leech in the final stages of cocoon deposition) was carefully cut thus releasing the hatchlings.

From this total of 19 viable cocoons, 159 hatchlings were successfully obtained (mean $\pm$ SD = 8.3 $\pm$ 3.5 hatchlings per cocoon, range 2-13 hatchlings per cocoon). Figure 6.1 shows a scatter diagram of the average weight of hatchlings and the number of hatchlings obtained from each cocoon. Although there appears to be a slight trend in the cloud of points suggesting that the body weight of hatchlings decreases with an increase in the number of hatchlings per cocoon, it is not possible to clearly define the relationship with a regression line and a lot of variance still needs to be accounted for.

From the initial cocoon-laying period of 48 gravid leeches captured during June and July 1984, a total of 97 cocoons were laid. From this number, 64 viable cocoons were successfully cultured in the laboratory to produce 480 fully developed hatchlings (mean $\pm$ SD = 7.5 $\pm$ 2.1 hatchlings per cocoon, range 1-12 hatchlings per cocoon). Figure 6.2 shows a scatter diagram, similar to that shown in Figure 6.1,

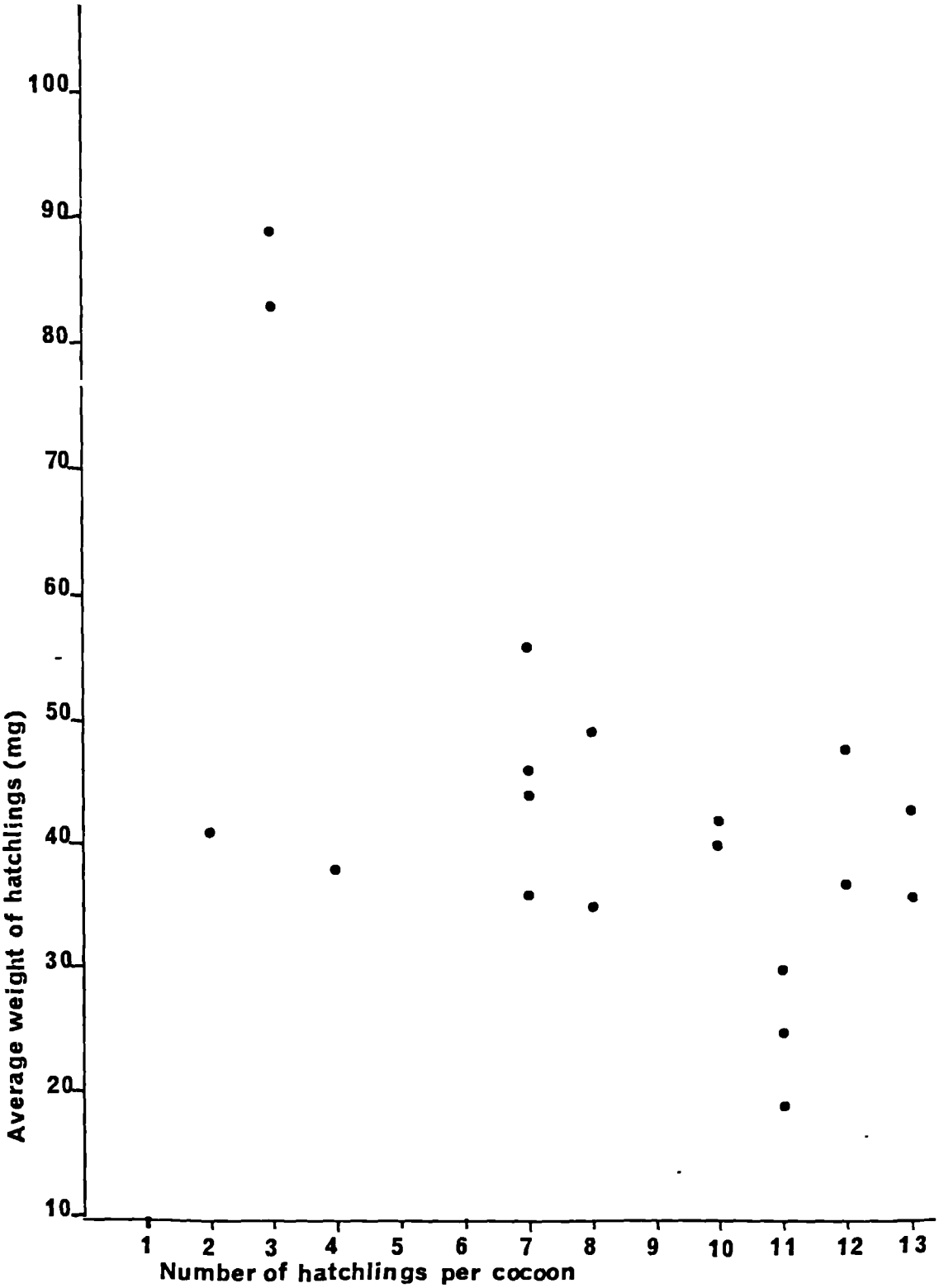


Figure 6.1 A Scatter diagram showing the relationship between the number of hatchlings per cocoon and the average weight of hatchlings in 19 viable cocoons colle ted from Lade gravel pit during July-August 1983

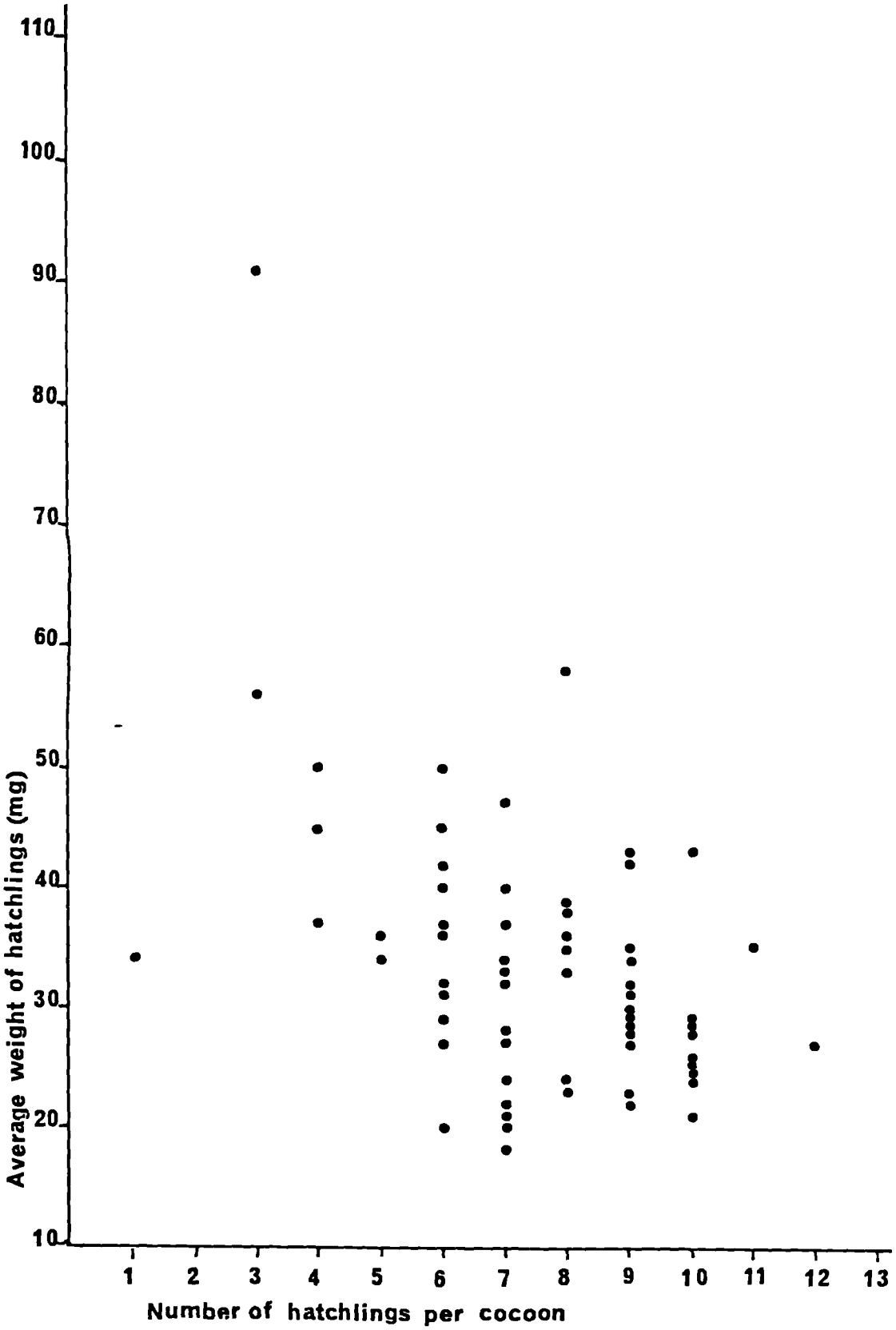


Figure 6.2 A Scatter diagram showing the relationship between the number of hatchlings per cocoon and the average weight of hatchlings from 64 viable cocoons laid by gravid leeches obtained from Lade gravel pit in June-July 1984

for the 64 viable cocoons. A similar trend is apparent although there still appears to be a lot of variance; for example in the total of 11 cocoons with 6 hatchlings the range of average bodyweight was 20-50 mg.

From the remaining 33 cocoons which failed to produce fully developed hatchlings, 20 contained a total of 129 (mean $\pm$ SD = 6.4 $\pm$ 1.7 embryos per cocoon, range 2-14 embryos per cocoon) well developed embryos (i.e. clearly visible eyes, suckers and segmentation).

The remaining 11 cocoons showed no evidence of differentiation within the jelly mass.

It was more difficult to interpret the distribution of cocoons, per leech, since these captured leeches, particularly in mid-July, may have already laid cocoons in the wild. Table 6.1 below shows the number of cocoons produced by the sample of 48 leeches maintained in the laboratory.

Table 6.1 The distribution of cocoons laid in the laboratory by 48 gravid leeches captured during June-July 1984 at Lade gravel pit

No. of cocoons laid	No. of leeches
1	16
2	21
3	6
4	4
5	1

In the work of Calow and Riley (1982), a positive, linear relationship was found between the number of offspring being brooded by 3 species of British Glossiphoniid leeches and the fresh weight of the parent. The data obtained from the sample of 48 gravid Medicinal Leeches in this study were insufficient to derive any definite

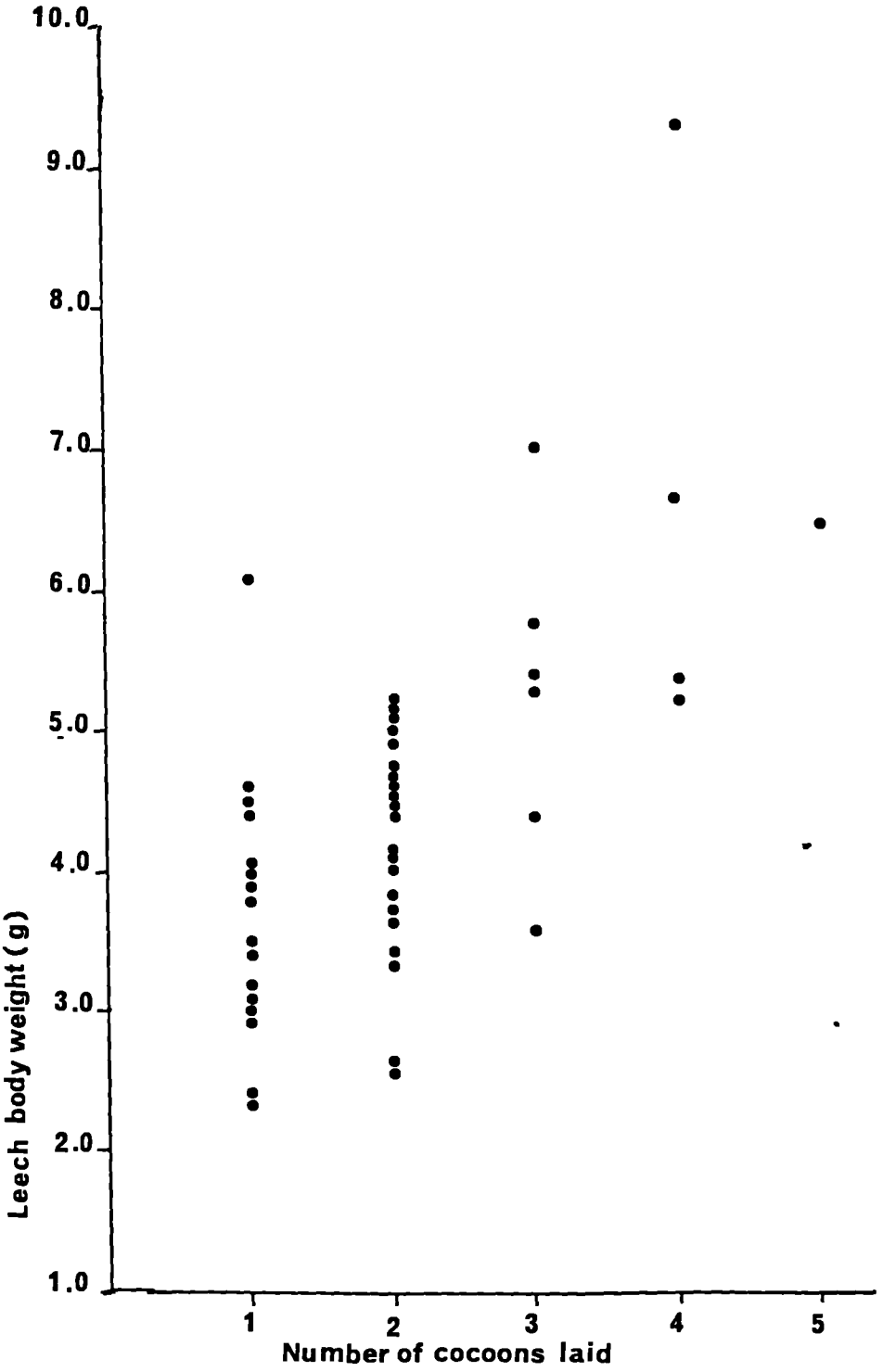


Figure 6.3 A Scatter diagram showing the relationship between parent body weight (immediately after capture), and the number of cocoons subsequently laid by 48 gravid leeches captured during June-July 1984 at Lade gravel pit

correlation between parent body weight and cocoon/hatchling production, although Fig. 6.3 does suggest a trend towards heavier leeches laying more cocoons. Note that parent leech bodyweight was measured immediately after capture.

An interesting finding related to the bodyweight of the parent leech was a significant overall decrease in bodyweight by the end of the cocoon-laying period. This was best illustrated in those leeches which laid multiple cocoons. Figure 6.4 shows the change in bodyweight of 2 leeches which laid multiple cocoons in a period of 42 days in the laboratory. The upper graph represents the individual which laid a series of 5 cocoons, while the lower graph is for one of the leeches which laid 4 cocoons. The most surprising feature were the successive gains in bodyweight reaching peaks immediately before cocoon deposition. Since no feeding occurred during the cocoon-laying period the gradual increase in body weight may have been due to water absorption, possibly associated with cocoon production. The sharp losses in bodyweight coincided with cocoon deposition.

A comparison of the total production of viable hatchlings and embryos in failed cocoons, by 10 individual leeches is shown in Table 6.2. It was possible, with all laboratory laid cocoons, to estimate the development time of H. medicinalis between cocoon laying and the emergence of fully developed hatchlings. In the group of 64 viable cocoons, laid by the gravid leeches captured in 1984, the mean $\pm$ SD developmental time was 27.5 $\pm$ 0.5 days (range 20-33 days) at 24 $\pm$ 2°C. Individual leeches frequently laid both viable and sterile cocoons. The results indicate that the total production of viable hatchlings may be highly variable and the number of cocoons laid by an individual leech may not be a good indication of total production.

The results of the breeding experiments were generally disappointing with significant mortality in the total laboratory population and relatively few cocoons laid (despite a total of 16 mating pairs observed).

Within the sub-group of 67 leeches, obtained from Germany and Lade gravel pit in 1983, only 22 individuals survived until the end of the

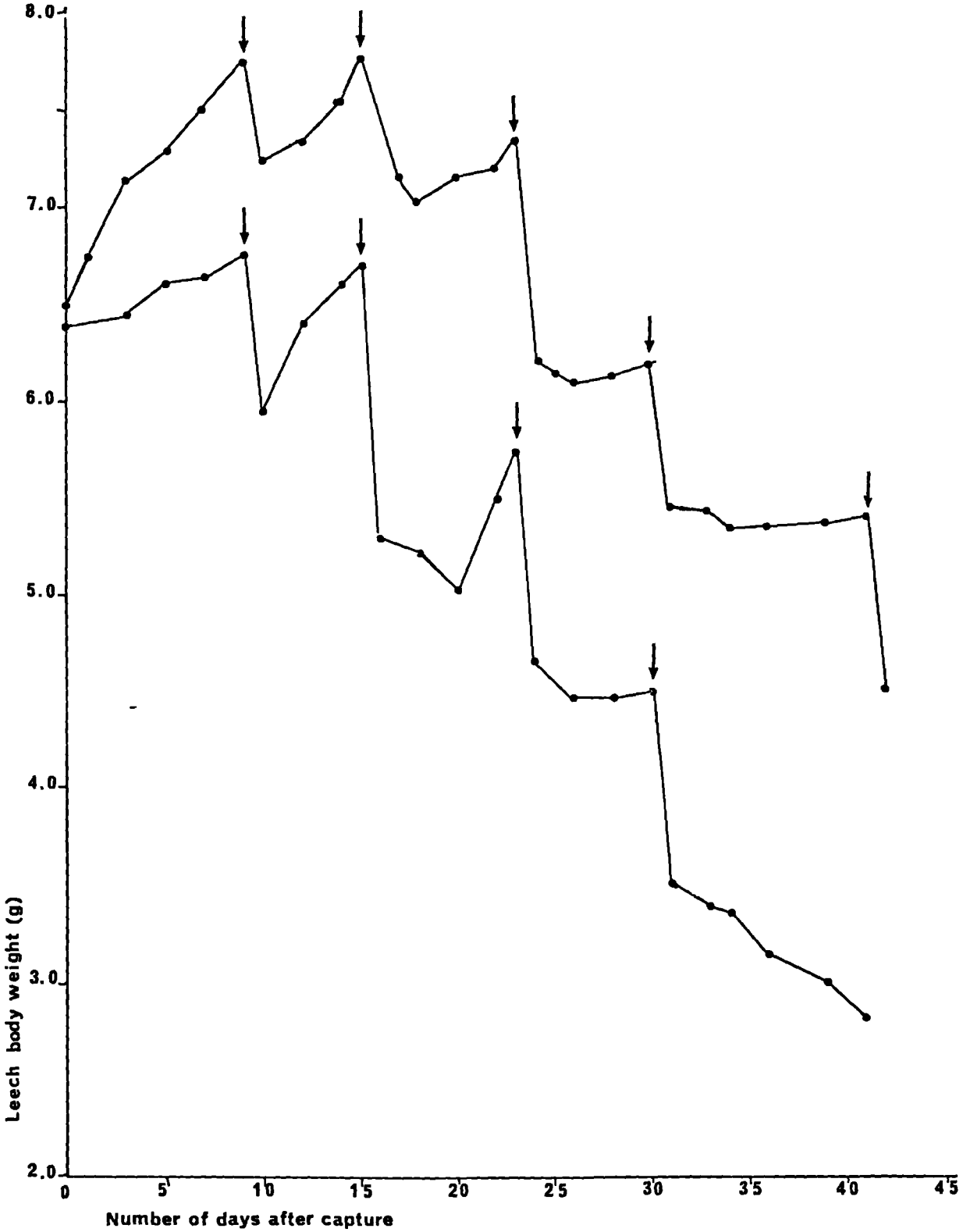


Figure 6.4 The change in bodyweight of two gravid Medicinal leeches during the cocoon laying period in the laboratory; note the arrows indicate bodyweight just before cocoon deposition

Table 6.2 A comparison of the total production of viable hatchlings, and of embryos in failed cocoons*, by 10 Medicinal Leeches which laid more than two cocoons. (From the group of 48 gravid leeches captured during June-July 1984 at Lade gravel pit)

Leech	No. of viable cocoons	No. of failed cocoons	Total no. of viable hatchlings	Total wt of viable hatchlings (mg)	Total No. of embryos in failed cocoons
1	3	2	24	1199	15
2	4	-	32	1087	-
3	3	1	26	534	7
4	4	-	27	788	-
5	4	-	31	1125	-
6	-	4	-	-	31
7	3	-	25	603	-
8	1	2	9	384	15
9	1	2	9	321	15
10	2	1	17	482	9

* Note: Fernandez and Stent (1982) described the embryonic development of the Medicinal Leech. During days 5-9 of development, larval structures become fully developed.

experiment (June 1985). The survival rate within the sub-group of 48 originally gravid leeches, captured in 1984, was better with 41 individuals surviving to the end of the experiment. It remained unclear throughout the experiment why this level of mortality occurred but the factors of bloodmeal disgorgement and maintenance for long periods at a temperature of 24 $\pm$ 2°C may well be involved. It is possible that metabolic stress resulting from a decrease in the availability of dissolved oxygen at the raised temperature was involved although each individual was maintained with daily changes of aerated tap water. It is worth noting that Sawyer (pers. comm.) found that chlorination of tap water caused gradual stress in laboratory maintained leeches although in this study individuals maintained in tap water at 15 $\pm$ 2°C remained apparently unstressed. Another factor involved in moving leeches to the raised temperature of 24 $\pm$ 2°C was an increase in the frequency of defaecation which made daily inspections of containers essential. A short period of several hours in water fouled with the characteristically dark brown, haem defaecatory by-product, caused rapid body contortions by the leech, indicating stress.

The Medicinal Leech should be regarded as a species prone to stress under laboratory conditions and requires very careful monitoring. This point is particularly relevant to any consideration of large-scale farming, a subject discussed in detail in the conservation strategy below.

Of 63 leeches surviving from the original laboratory population of 115, 12 became gravid and laid a total of 28 viable cocoons (mean $\pm$ SD, 2.3 $\pm$ 1.3 cocoons per leech, range 1-5). Although much more information is required for any firm conclusion on the fecundity of the Medicinal Leech, especially under captive conditions, the behaviour of several of these individuals, and others which failed to lay cocoons during the breeding experiment, provided some interesting findings.

A general observation was that, given optimum laboratory conditions of raised water temperature (24 $\pm$ 2° C) and extended daylength (14 hours artificial light), breeding and cocoon deposition can be induced at any time of the year. General activity throughout the year was also maintained at temperatures above 10-12°C, even in shorter,

winter daylengths (8-10 hours). In these experiments, 7 out of the 28 viable cocoons were laid during January and February under optimum laboratory conditions.

A common occurrence, in at least 21 individuals,was the clear development of a gravid state, but despite careful monitoring and alternating the regime of aquatic and terrestrial environments, designed to induce cocoon deposition (see above), no cocoons were laid by this group. After a period of 30-40 days the gravid state disappeared. This group included 16 individuals which were observed mating.

From the group of 12 leeches which successfully mated and laid viable cocoons in the laboratory, 7 individuals were from the sub-group originally captured from Lade gravel pit in September 1983. Of particular interest was one individual which, having been brought into the laboratory without signs of being in a gravid state, developed a clitellum 40 days after receiving a bloodmeal but before any laboratory pairing had occurred. This individual proceeded to lay a series of 5 viable cocoons (mean no. of hatchlings per cocoon = 7.0, range 5-9) over a period of 42 days. It is possible that the stimulus of a bloodmeal, on this previously fertilised individual, caused cocoon development and may support the idea that feeding is closely associated with successful reproduction in the Medicinal Leech. This individual was captured at the end of September, several weeks after the known field cocoon laying period. It is possible that the Medicinal Leech may overwinter in a fertile state and so support the previous suggestion by Mann (1962) that there may be a delay of 1-9 months between copulation and cocoon deposition.

Within the group of 12 leeches which laid viable cocoons, 7 individuals were involved in two separate cocoon laying periods. Within this group 5 were originally captured in a gravid state in June-July 1984 and four of them became gravid again after laboratory pairing in March 1985. The ability of the leech to produce two broods per year in the laboratory was also found by Zapkuviene (1972). One leech in this group of five originally laid a series of 4 cocoons over a period of 43 days after capture from the wild. It was subsequently fed and maintained in an individual water-filled container at 24 $\pm$ 2 C^{o}but was

not paired. After a period of 175 days this individual became gravid again (without any intervening copulation) and laid a further 4 cocoons, although only a single cocoon proved viable. This result was both surprising and difficult to interpret. The production of the first batch of cocoons must have resulted from a successful mating in the wild at a previously unknown time. However, the production of a single viable cocoon, after an apparent 'resting' period of 175 days, appeared to result from fertilisation with sperm from the original mating. It is believed that the delay between copulation and cocoon production is due to the leech's ability to store the donor sperm (Mann, 1962). In the case of the above leech, sperm may have only been partially released, possibly stimulated by summer environmental conditions, to fertilise the first batch of eggs followed by a further storage period until optimum conditions were again established (on this occasion represented by laboratory feeding and an equivalent summer water temperature of $24 \pm 2^{o}C$), when the cocoon in the second batch was produced. This ability to store sperm for long periods is certainly an area requiring further investigation and is referred to again in survival terms in the conservation strategy.

During these breeding experiments clear episodes of mating behaviour and actual copulation were observed. As mentioned above, the sequence of mating behaviour was observed in only 16 mating pairs (4 pairs were observed on two separate occasions in the pairing period).

Each of the 20 observed mating episodes appeared to follow a basic pattern, involving a series of three distinct stages preceding actual copulation.

Stage 1: Both leeches cease vermiform crawling or swimming and each individual becomes anchored by the posterior sucker. This is followed by a period of mutual examination along the body surface of both leeches using anterior mouth parts. Plate 6.3 shows this preliminary examination phase.

Stage 2: After mutual examination, one of the pair becomes attached by the posterior sucker and anterior sucker and mouth to the partner leech. This was not always one-sided and this action usually involved an alternating 'passive' and 'active' member of the pair. During this stage

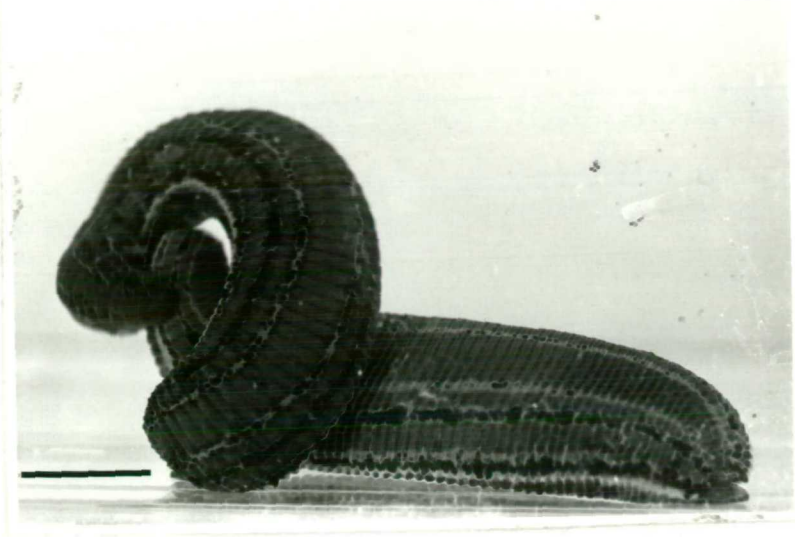
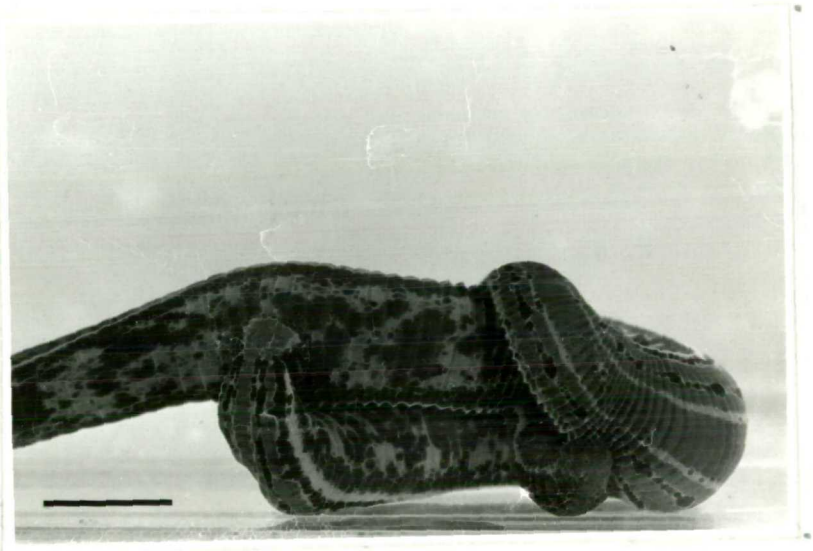


Plate 6.3 Stage 1 of th mating repertoire of the Medicinal leech showing inspection of the body surface by one of the mating pair

Scale bar - 1cm

Plate 6.4 Stage 2 of the mating repertoire of the Medicinal leech showing full attachment by one of the mating pair to the other.

Scale bar - 1cm



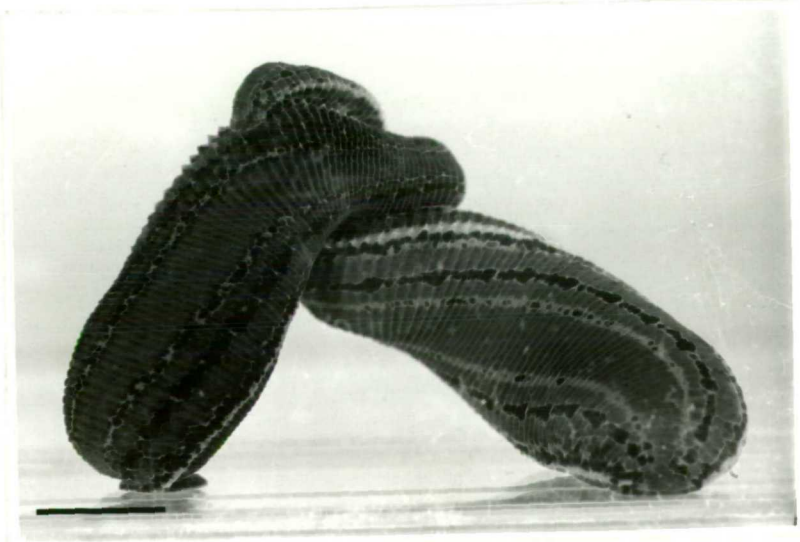


Plate 6.5 Stage 3 of the mating repertoire of the Medicinal leech showing both partners firmly anchored by posterior suckers and anterior segments, carrying the genital apertures, in close contact.

Scale bar - 1cm

it was usual for the penis to be everted by both leeches. Plate 6.4 shows clearly the attachment of one of the leeches of the pair to the other; the arrow shows a typical arching movement of the anterior part of the body which served to bring the genital apertures in close contact. This particular movement was increased in the next stage.

Stage 3: This stage involves both leeches being firmly anchored by the posterior sucker while gripping each other by the mouth and anterior sucker. The arching of the anterior part of the body often results in a pushing movement causing the animals to "stand up". This is clearly shown in Plate 6.5.

The final act of copulation was never clearly visible as each leech came very close together along the anterior segments which included the genital apertures. Although it was usual for both leeches to evert a penis it was not possible, without excessive disturbance, to observe whether copulation was one way or reciprocal. There was no way of detecting, externally, whether eggs had been fertilised. Plate 6.6 shows the typical appearance of a pair of leeches immediately before copulation; note the arching and pushing together of anterior segments. Although it is not clear on this plate, the arrow shows the everted penis of the individual to the left. From the total of 20 observed mating episodes the total time between the initiation of Stage 1 and copulation ranged between 20-30 minutes. Once copulation had been achieved, both leeches often remained locked together for a further 1-2 hours.

There was usually a delay of 10-30 minutes between the actual movement of pairing of previously isolated leeches and the initiation of Stage 1 mating behaviour. Often, both leeches came into body contact but did not cease independent vermiform crawling or swimming. The initiation of mating behaviour was, therefore, rarely automatic. Rather, it appeared that some form of trigger might be involved, for often a pair of leeches, which had for a period of time appeared to "ignore" each other, suddenly became attracted. It does pose the question of whether a tactile stimulus is involved or possible some form of sexual pheromone which attracts sexually mature adults. It is believed, by this author, that the proportion of sexually mature adults in the leech population at Lade gravel pit is small and the probability of two such

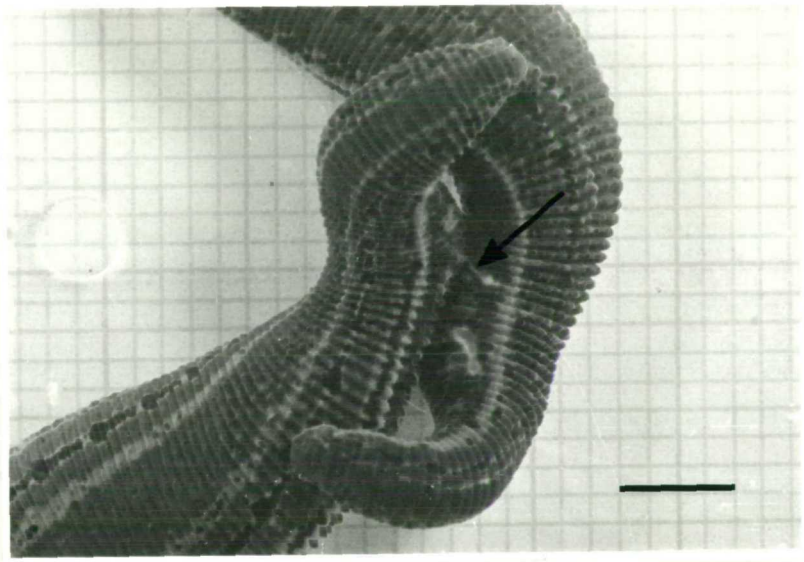


Plate 6.6 The same pair of Medicinal leeches as plates 6.3-6.5,from above, showing the extruded penis of the left-hand leech just before insertion into its partner, (arrowed)

Scale bar - 1cm

leeches randomly encountering each other in the wild may be similarly small. The production of a sexual pheromone in the water would be an obvious advantage and might increase the probability of successful encounters. This is a highly successful strategy used in several species of marine polychaete worms. This is certainly another area of work which should benefit from further investigation.

Discussion

The British freshwater leeches vary considerably in their reproductive and life-cycle patterns (Elliot and Mann, 1979). Two main types of life-cycle can be distinguished; one in which animals breed and then die (semelparous) and the other in which they may breed repeatedly on several, separate occasions (iteroparous), (Calow and Riley, 1980). These are broad categories and within the leech families a range of strategies have been adopted. For example, in the Erpobdellid leeches, a family which lays cocoons on submerged stones and vegetation and which deserts them immediately, both semelparous and iteroparous populations of, for example, Erpobdella octoculata, have been found (Calow and Riley, 1980). Within the family of Glossiphoniid leeches, a group which broods eggs and carries young, Calow and Riley (1982) found that all populations of Helobdella stagnalis and Glossiphonia heteroclita studied were semelparous while Glossiphonia complanata was iteroparous.

The British Gnathobdellid leeches, which include H. medicinalis and the Horse Leech, H. sanguisuga, lay unprotected cocoons out of water and show a perennial, iteroparous strategy. However, in comparison with the other British Leech families (see Learner and Potter, 1974; and Calow and Riley, 1982), there is little information on reproductive effort in the gnathobdellid leeches, particularly H. sanguisuga. Table 6.3 has been compiled from the results of this study and other available references to H. medicinalis, together with a comparison with its relatives. The data refer mainly to laborat ry reared animals under different regimes of feeding, light and temperature and are not strictly comparable. The production of H. medicinalis in this study, in terms of number of young, was well below that of the other Russian workers. It could have been due to the experimental methods being less well developed in this study. The general trend

Table 6.3. A comparison of the reproductive output of the Medicinal Leech, H. medicinalis (L) and its relatives; ranges only are given and unless otherwise stated all data refer to laboratory rearing under optimum feeding and temperature conditions.

Species	Reference	No. of cocoons per adult	No. of young per cocoon	Average weight of young per cocoon (mg)
Hirudo medicinalis	This study	1-5	3-12 (2-13)[1]	18-91
	Sineva (1944, 1949)	1-8	18-25 (10-15)[1]	20-60
	Shchegolev et al. (1964)	1-5	12-16[2]	-
	Zapkuviene (1972)	1-7	3-30	28
Hirudinaria granulosa	Khan (1912) Matthai (1920) Keegan et al. (1968)	1-4		
Hirudinaria manillensis	Keegan et al. (1968)	1-4	4-14	-
Hirudo nipp nia	Toshioka et al. (1977)	Group of 10 cocoons	5-14	2-5
Macrobdella decora	Gouck et al. (1967)	Group of 6 cocoons	average 16	-

1. Cocoons obtained from the wild

2. Shchegolev et al. (1964) reported are individual which deposited 5 cocoons (112 young) in November, 1954, 8 cocoons (173 young) in April 1955, and 8 cocoons (132 young) in August 1955 for a total of 21 cocoons and 413 young in one year.

shown in Table 6.3 is that in the gnathobdellid leeches, reproductive effort seems to be restricted to comparatively few cocoons and offspring.

An important review of life-history tactics was produced by Stearns (1976), later followed by Calow (1979a) who discussed theoretical aspects of the selective forces which mould life-cycle patterns. Several important premises are relevant to the apparent reproductive effort and life-history strategy adopted by H. medicinalis.

A central premise is that reproduction stresses parents and thereby impairs future survivorship and/or reproductive potential. In the case of H. medicinalis the behaviour of gravid leeches leaving the water to lay cocoons must also represent an added risk to survival. In a species where, as a result of extrinsic factors, parents are more valuable than offspring (from the point of view of future fitness) restraint should be made towards reproductive effort. Otherwise, parents should maximise reproductive output even if by doing so they reduce their own survival chances. It has been discussed elsewhere that the Medicinal Leech probably feeds relatively infrequently in the wild and may take a long time to reach sexual maturity. This, together with the relatively small proportion of gravid leeches found in the Lade gravel pit population, suggests that the adult leech is far more valuable than its offspring. The evidence does suggest that the adult leech minimises reproductive effort by spreading reproductive capability over several seasons and thereby maximises survivorship.

Stearns (1976) predicted that only particular combinations of traits should occur in specified circumstances. In stable environments late maturity, multiple broods, a few, large young, parental care and small reproductive efforts should be favoured (K-selection). In fluctuating environments, early maturity, many small young, reduced parental care and large reproductive efforts should be favoured (r-selection) It would appear that H. medicinalis follows a strategy of k-selection. It should be noted that the traditional habitat of the leech is a well established pond or lake representing a relatively stable environment.

Finally, an important reproductive strategy in H. medicinalis is its ability to store sperm and thereby delay the act of cocoon production from the time of copulation and transfer of donor sperm.

It is likely that, for most of the individuals which attain sexual maturity, mating and consequent laying of fertile cocoons occurs in a single season. However, the known capacity of the leech to store sperm could mean that the results of mating in one breeding season could take effect in the following season. The extent to which the leech uses this strategy in the wild or whether it affects our interpretation of the leech breeding season requires more investigation.

The Hirudinea are protandric hermaphrodites (i.e. the production of sperm precedes that of eggs) and both oogenesis and spermatogenesis are mutually exclusive processes. The work of Schegolev et al. (1964) showed that feeding and a raised temperature (20-25°C) speeded oogenesis and that superimposed periods of extended illumination had a synergistic effect. But the combination or protandry and sperm storage by a recipient leech means that the attainment of sexual maturity should be equated more with the process of spermatogenesis. For this reason it has been researched in more detail, mainly by two workers. Malecha (1970a, 1970b) identified four distinct stages in sperm maturation in H. medicinalis. He found that a temperature rise from 7°C to 20°C speeded maturation of sperm to stage 4. Of more relevance to this study was the work of Hagadorn (1966) who discussed spermatogenesis in a seasonal context. He measured the percentage of total sperm clusters in each of the four stages of maturation. He found that under natural conditions mature stage 4 sperm were absent between November and April but gradually developed to a peak (25%) in August (no details of environmental condition were given). These results appear to conflict with the widely held view that mating occurs in June when, according to Hagadorn (1966), the proportion of mature sperm clusters is only 9%.

A significant observation was made by this author concerning two large leeches ($>$3.0 g) captured (in a sample) from Lade gravel pit in early April 1984. This sample was placed in the laboratory at a temperature of 15 $\pm$ 2°C. It was noted that the ambient water temperature at the site was 10-12°C during this period and removal to the laboratory temperature was therefore unlikely to be a significant rise. After a period of 24 hours in the laboratory the two leeches were observed copulating. It was felt that the behaviour of these two leeches was independent of any laboratory effects since no feeding or

extended daylength was involved. It appeared to represent an instance of opportunistic mating well in advance of the accepted period of mid-Summer (although it was the only instance observed of mating at this time of year despite other equally sized leeches in the samples). It is interesting that Hagadorn (1966) noted no mature sperm clusters in April. There is at present, no evidence of the transfer of immature stage 2 or 3 sperm which subsequently mature in the recipient leech, a capability which might explain instances of early season mating as described above.

There appear to be two main contradictions which still need to be reconciled by further investigation. One is the probably opportunistic element in the mating of H. medicinalis in the wild. It is likely that the opportunity of two mature, adult leeches meeting is small and, in survival terms, with the capacity of storing donor sperm it would seem advantageous to mate as a result of an opportunistic encounter. This is possibly what was observed in the pair of leeches described above. The contradiction lies in the results of Hagadorn (1966) which indicate that the peak (i.e. $>$ 9% of total sperm clusters) availability of mature sperm is restricted to the months of June, July and August.

The second contradiction lies in the leech's capacity to store mature sperm from a donor, for apparently long periods of time under laboratory conditions. Again Hagadorn's results indicate that sperm maturation is suspended at the early stage 1 between November and April with remaining mature, stage 4 sperm apparently undergoing atrophy from September to November. Hagadorn (1966) suggested that low winter temperature is the major factor which suppresses sperm maturation. How is it possible, therefore, for mature, donor sperm to be maintained in a viable state in an overwintering recipient ? Certainly there is no reliable evidence of overwinter sperm storage in the wild although this capacity would give the Medicinal Leech greater survival opportunity than storage within a single breeding season. It would mean that the leech could take advantage of favourable environmental conditions f r cocoon development without the need to find a mate in the same breeding season.

Again these ideas remain speculative and indicate that many areas of the reproductive strategy of H. medicinalis in the wild need to be resolved.

APPENDIX 6.1

The source of imported Medicinal leeches used in the captive breeding experiments was:

Frau E. Nell,
Blutegelimport und Versand,
4350 Recklinghausen-Sud,
Elisabethstrasse 16a, West Germany.

Telephone 62632.

CHAPTER 7

THE OCCURRENCE OF THE MEDICINAL LEECH AT DUNGENESS, WITH A PROPOSAL FOR RESTOCKING AND A REPORT OF SURVEYS UNDERTAKEN IN THREE AREAS WHERE THE LEECH HAS BEEN PREVIOUSLY RECORDED; NEW FOREST, HANTS, BOOKHAM COMMON, SURREY AND LA SANGSURIERE, NORMANDY, FRANCE

Introduction

The discovery of a population of the Medicinal Leech, H. medicinalis at Lade gravel pit, Dungeness, Kent (TR077217) was the first record of the animal in the county (Philp, pers. comm.). This is surprising for much of the county lies in the Weald where a great number of small ponds were dug as marl pits on grazing land; the pond habitat with access by grazing animals was typically associated with the leech. Indeed Furley (1871), in his 'History of the Weald of Kent', remarked that in the 17th century it was common 'to open a pit in every field'. 'In Bethersden alone', he says, 'there are no less than two hundred ponds, hassocks and swampy places. Yet in a recent survey by Neve (1976), of 125 Wealden ponds, there was no record of the leech, despite apparently favourable habitats. A wider survey of ponds in Kent, to record the occurrence of the Great Crested Newt, Triturus cristatus, did not find any evidence of H. medicinalis (Frazer, pers. comm.).

The distribution of the Medicinal Leech in Britain and Europe has been recnetly reviewed by Elliott and Tullet (1982, 1984). The British records give almost no clue to the leech's present distribution since many are now dated and all geographically widespread. This lack of information on distribution was tackled in two ways in this study. Firstly, a thorough search was made of all accessible water bodies on Dungeness and sections of the Romney Marsh dyke system adjacent to Lade gravel pit. Secondly, surveys were carried out at three sites where the leech has been recorded previously. They included two British sites, the New Forest, Hants and Bookham Common, Surrey, where records are now dated and which represent very different habitat types to that of an oligotrophic gravel pit. The third site was La Sangsuriere, Normandy, France, one of only two published records of the leech in France; it is probably more widespread than the records suggest, e.g. Ricarimpex of Bordeaux, which is a company supplying leeches to the pharmaceutical industry, almost certainly obtains them locally.

The New Forest in Hampshire, represents the largest expanse of unenclosed land in Southern England, covering an area of approximately 37,500 hectares. It was at one time a Royal Hunting Forest and today continues to provide rough grazing for horses, sheep and cattle. Under 'common' grazing rights these animals roam freely and have access to the great number of ponds to be found in the area. It was beleived that this access to wet areas in the 'forest' served to sustain leech populations.

The last published record of the Medicinal Leech in the New Forest was described by Blair (1927) who, reporting on survey work carried out nearly twenty years previously, 'found it in most ponds and streams'. A record also exists, dating from 1919, of its occurrence in the large Hatchet pond (TR 365015). Recently Dr. Deryck Frazer (pers. comm.) mentioned that the leech had occurred in large numbers in a pond near Brockenhurst, but survey work at this site in Spring 1984 failed to detect any leech activity. Dr. Colin Tubbs, of the Nature Conservancy Council at Lyndhurst, believes that some local naturalists still regard the leech as occurring widely although in small numbers in the Forest. A record of the leech, found in Spring 1985 in a pond near Brockenhurst (SU 314036), was kindly supplied by Mr. P. Toynton (in litt.). It was decided to visit this locality and others suggest by Dr. Tubbs.

Bookham Common in Surrey, covers an area of approximately 203 hectares and comprises mainly Pedunculate Oak woodland (Quercus robur) with smaller areas of grassland and scrub. The common forms a bowl shape and as a consequence there are many wet areas including ponds, streams and marshy areas. These habitats support all three British species of newt and very high numbers of toads, Bufo bufo. At one time cattle and sheep were allowed access for 'common' grazing but this ceased in the 1940s. Today the common is owned and managed by the National Trust and it has been extensively researched by the London Natural History Society (Castell, 1965; Harvey, 1943).

The occurrence of the Medicinal Leech at Bookham Common was recorded by Le Gros (1974) who referred to individuals found in the large Isle of Wight pond in 1938 and 1947 (see Figure 7.4). Groves reported two further records of individual leeches from the same site in 1958 and the

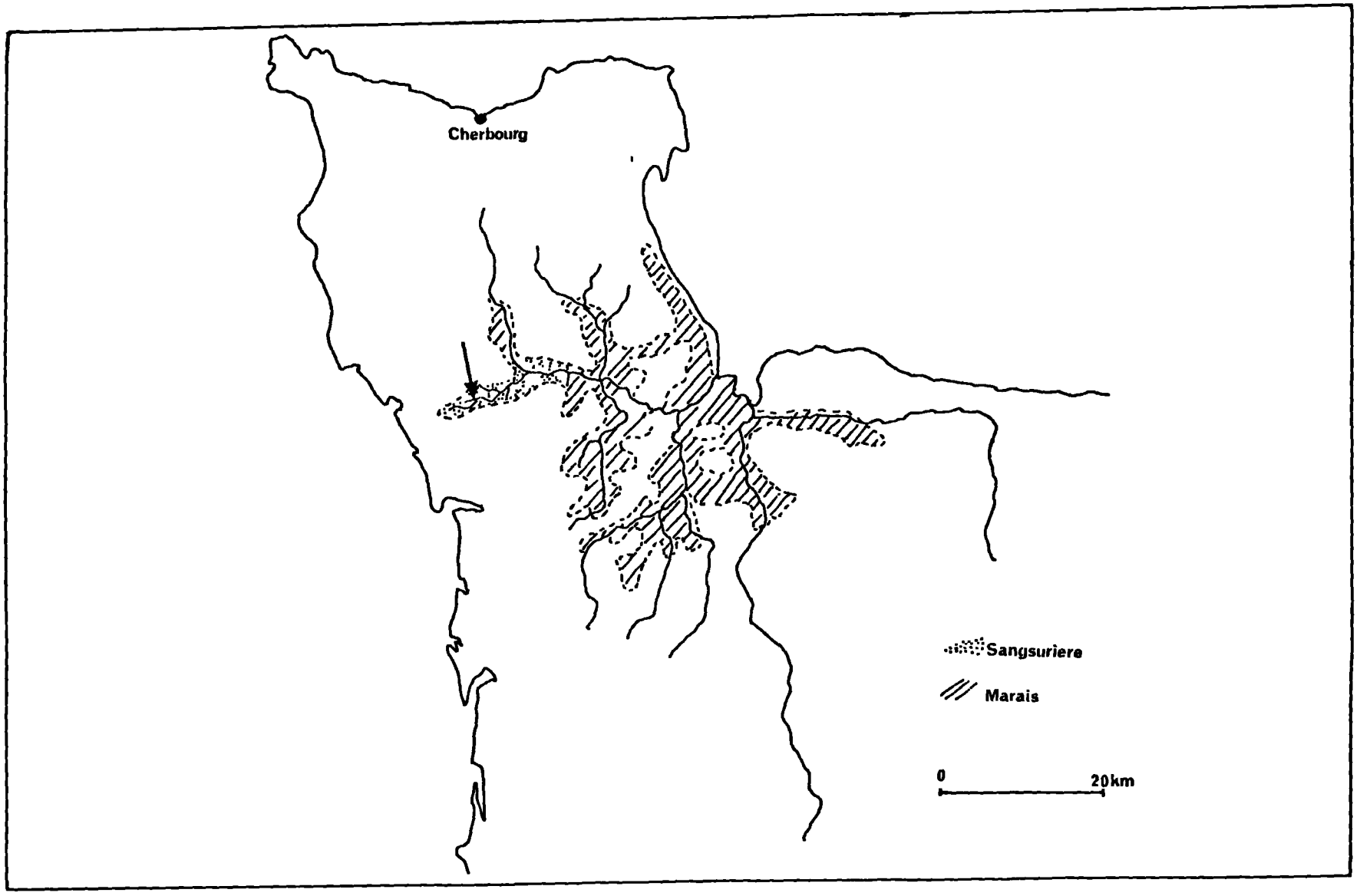


Figure 7.1 A record of the Medicinal leech at La Sangsurière, Normandy, France; note the arrow indicates the general area ,(after Debout and Provost,(1981))

last in 1961. Important changes in the management of the common have occurred in recent years and it was thought timely to provide an update of the leech's presence or absence on the common.

The occurrence of the Medicinal Leech has been recorded in an area known as La Sangsuriere in Normandy, France, by Debout and Provost (1981) (see Figure 7.1). It represents an old wetland pasture in a tributary valley of the River Douve . The pasture is intersected by a system of slow flowing streams, and is grazed during the summer months. In Winter this flat area undergoes considerable flooding. It forms part of a larger area known as La Marais de Carentan. The name, La Sangsuriere is derived from its past fame as a locality for the supply of leeches to the medical profession, and indeed the record of Debout and Provost (1981) is only an historical note and does not mention its recent occurrence. Today La Sangsuriere is an important wetland site but is threatened by land use changes involving a major drainage proposal. It was decided to sample along a section of the stream system while on a visit to the area in September 1984.

The idea for an experimental introduction or restocking of the Medicinal Leech to other sites on Dungeness arose partly from the threats posed to the main Lade gravel pit population (see the Management Plan) and as a result of survey work on its occurrence in the area.

Where a habitat is threatened or where an animal or plant is at the edge of its distribution, particularly if that distribution is undergoing contraction, a careful programme of introduction can be a positive management tool. The rationale for species introduction, including many examples, is given by Rose (1979) and Pinder (1981). There are many examples where deliberate or accidental introductions have had dramatic effects on community balance (Elton, 1958; Lever, 1979).

The recommendations in Green (1979) were particularly useful for deciding on the specific aims of the proposed restocking. The formal restocking proposal is presented in its complete form in the discussion section.

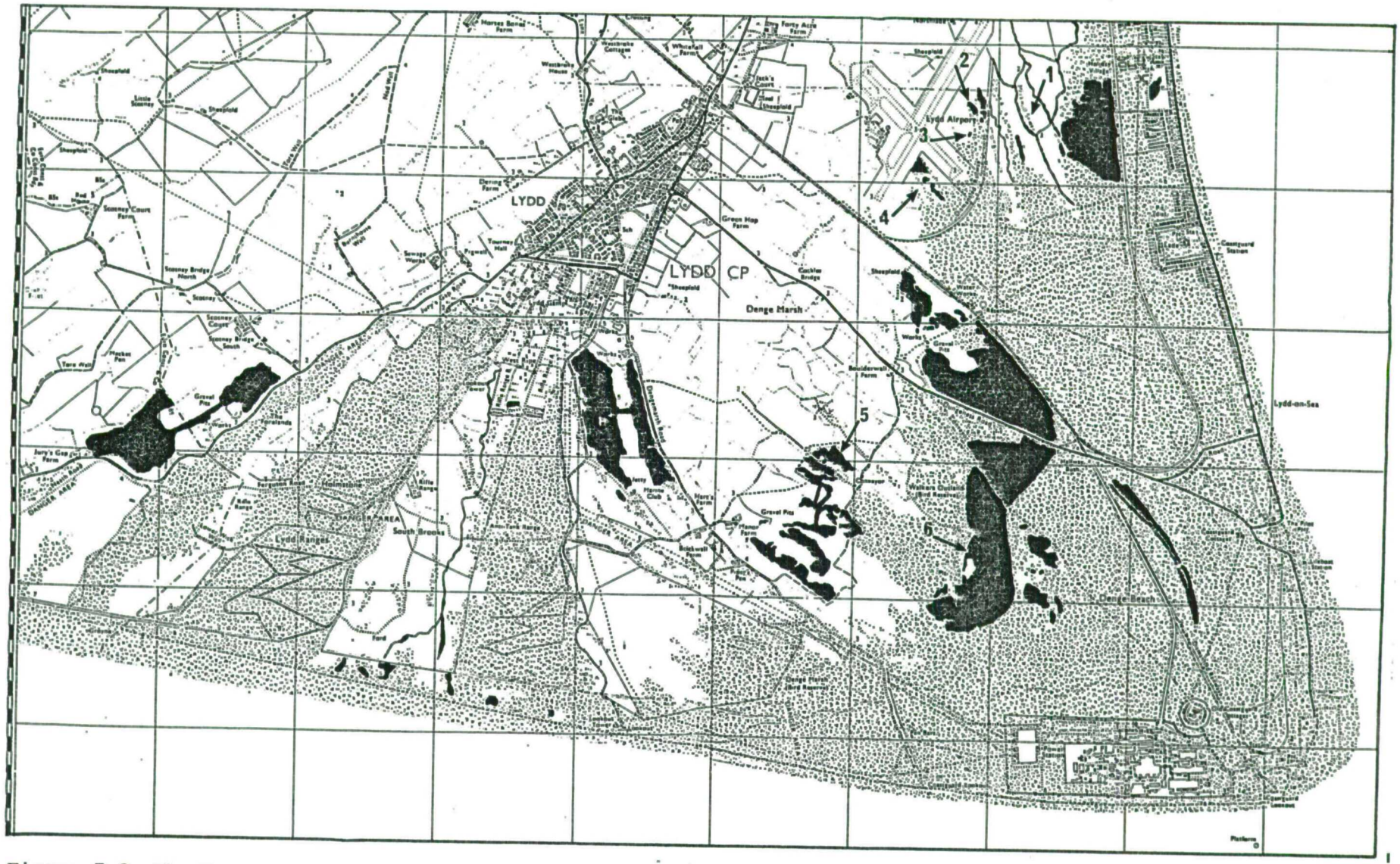


Figure 7.2 The Dungeness foreland showing all the areas of open water and sections of dyke sampled for the presence of the Medicinal leech,(shaded black); note the specific numbered sites 1-6 show those areas where the leech was found,(details in text);based on Ord.nance Survey

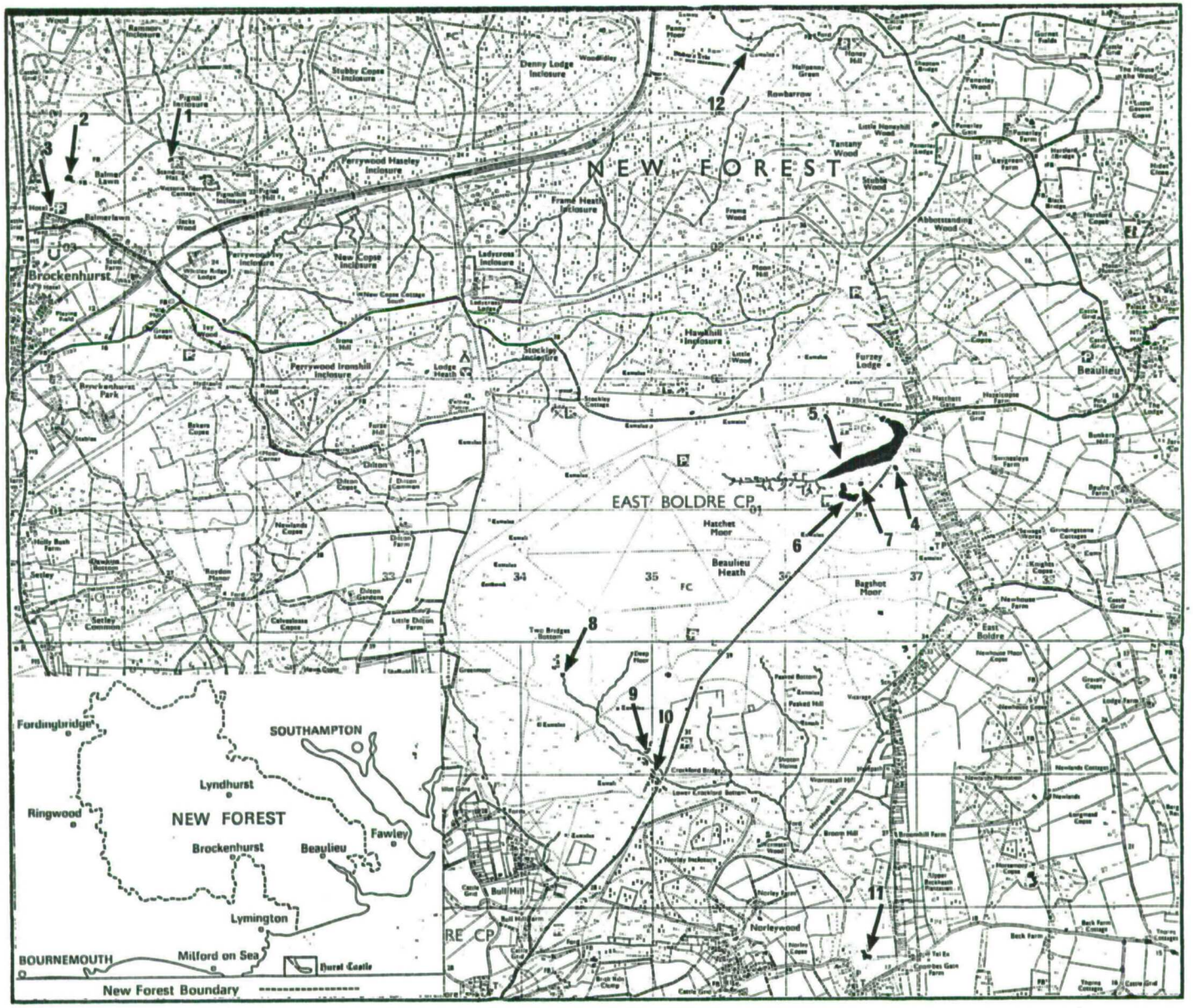


Figure 7.3 A map of the S.E. corner of the New Forest, Hampshire, showing 12 ponds sampled in July 1985 for the presence of the Medicinal leech, (based on Ord nance Survey)

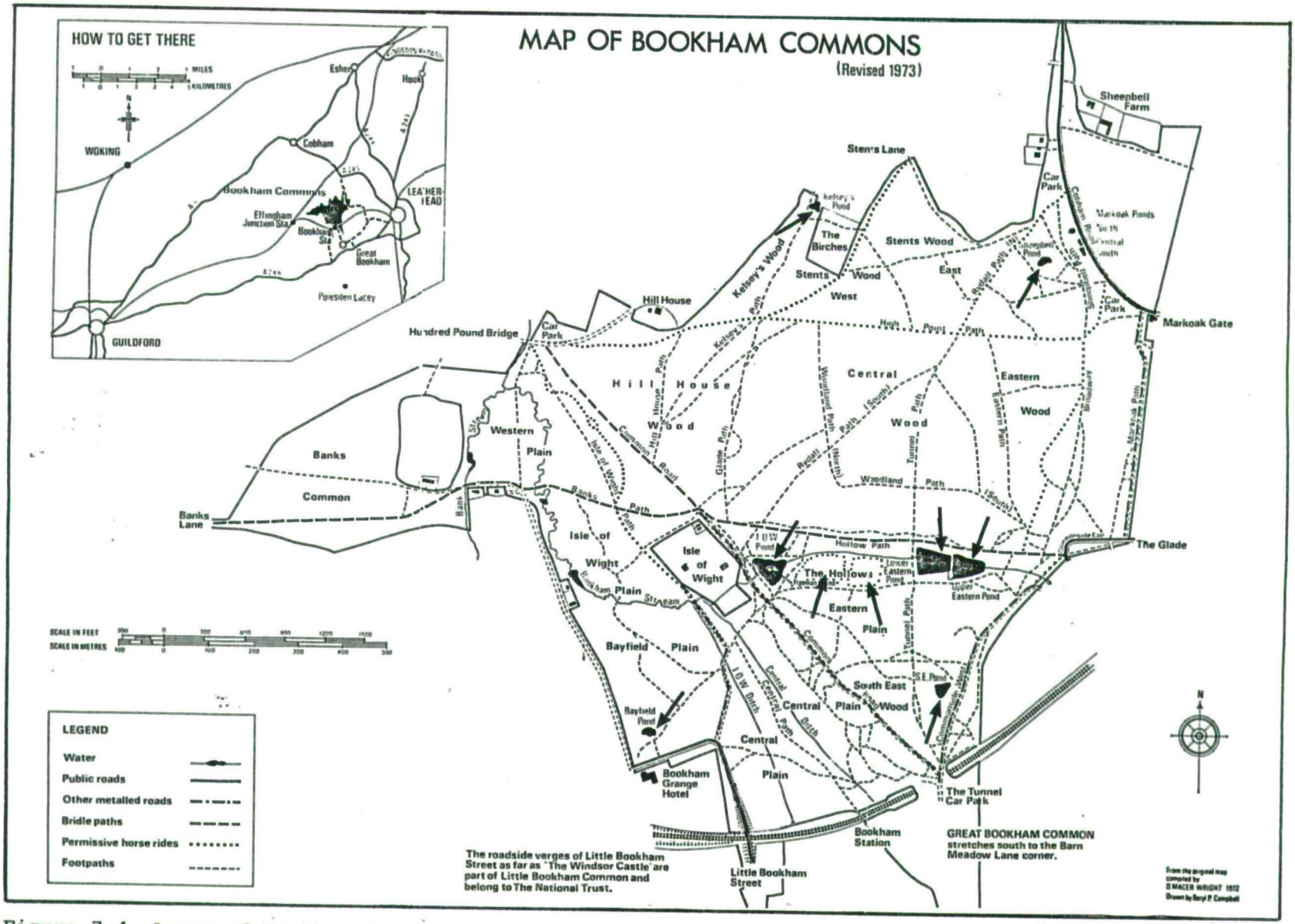


Figure 7.4 A map of Bookham Common,Surrey showing 9 ponds sampled for the presence of the Medicinal leech in August 1985, (based on 'Bookham Common' by the Bookham local management committee of the National Trust)

Methods

Sampling, on a non-quantitative presence/absence basis, was carried out at 37 separate water bodies on Dungeness and approximately 3 km of dyke system (see Figure 7.2). Sampling was carried out during April-July 1984/85 using methods described in Chapter One. Although the time and intensity (i.e. degree of water disturbance) of sampling was not standardised it was considered, on each occasion, to be sufficient to attract leeches if present. Obviously the presence or absence recorded referred to that sampling occasion and it was possible that small numbers of leeches could have remained undetected. To reduce this pssibility sampling points were selected so as to have overlapping fields of water disturbance. All water bodies were visited on two occasions during the sampling period.

A series of 12 ponds were surveyed, using a similar sampling method, mainly in the South East of the New Forest between Brockenhurst and Beaulieu over two days in July 1985; Figure 7.3 shows their distribution.

A series of 9 ponds were surveyed at Bookham Common on a single visit in August 1985 using similar sampling methods; Figure 7.4 shows their distribution.

A section of stream, approximately 100 metres in length was sampled at Neuville-en-Beaumont in La Sangsuriere, Normandy on a single visit in September 1984 (see Figure 7.1 (arrows) and Plate 7.1).

Results

In additi n to Lade gravel pit, the Medicinal Leech was found at six other sites on Dungeness:

(1) Mockmill sewer TR073214 (see Plate 7.2)
(2) Lydd airport pond TR068216 (see Plate 7.3)
(3) Lydd airport temporary pond TR068213
(4) Lydd airport pond/wetland TR065210 (see Plate 7.4)
(5) Burrowes gravel pit TR069185
(6) Hamilton Farm gravel pit TR058190

Their distribution is shown in Figure 7.2 (arrowed 1-6)

The most significant record was the pond/wetland site at Lydd airport (TR065210). This area is covered by a pond of approximately 2 ha created from gravel extraction, and to the South West an area of wetland with open shallow water (<0.5 m depth) created in a depression of the shingle beach. It was found to support a viable population of H. medicinalis and its size and structure was studied during June-August 1985. The methods used were similar to those described in Chapter 1, except that only leeches weighing more than 1.0 g were marked (with a pair or parallel, diagonal lines on the dorsal surface). A single Mark and Recapture Petersen estimate was used (see Chapter 1 for formula). An estimate of 409 was obtained for the population fraction weighing more than 1.0 g. This is a conservative estimate of the total population size especially looking at the proportion of leeches weighing less than 1.0 g in the population structure histograms (see Figure 7.5 (a) and (b).

The other important record was at Burrowes gravel pit (TR069185). This recently excavated pit supports very little littoral vegetation and reaches depths of 3-4 m. The large area of open water attracts great numbers of birds and is managed by the Royal Society for the Protection of Birds. In June 1984 the reserve warden observed four large specimens of the Medicinal Leech attached to the underside of a timber raft in a shallow inlet. A single, gorged specimen was recovered, weighing 15.8 g, a weight in excess of any other wild specimen obtained elsewhere on Dungeness (see Plate 7.5). Serological analysis of a sample of gut contents indicated that the specimen had recently gorged on a bird bloodmeal (see Chapter 3 for details of methods).

The other records on Dungeness together with numbers and weights of leeches obtained are shown in Table 7.1

All these individuals are below the weight classes found to contain sexually mature individuals in the wild.



Plate 7.1 A view of part of La Sangsurière, Normandy, France, showing wetland,grazing pastures with a network of 'dykes'



Plate 7.2 Mockmill sewer in the vacinity of Lade gravel pit, Dungeness- the only section of dyke system sampled where the Medicinal leech was located; note the adjacent sheep-grazed pastures



Plate 7.3 A view of Lydd Airport pond, (TR068216), Dungeness, where a single specimen of the Medicinal leech was found



Plate 7.4 A view of Lydd Airport Pond/wetland, (TR065210), the site of another significant population of the Medicinal leech on Dungeness

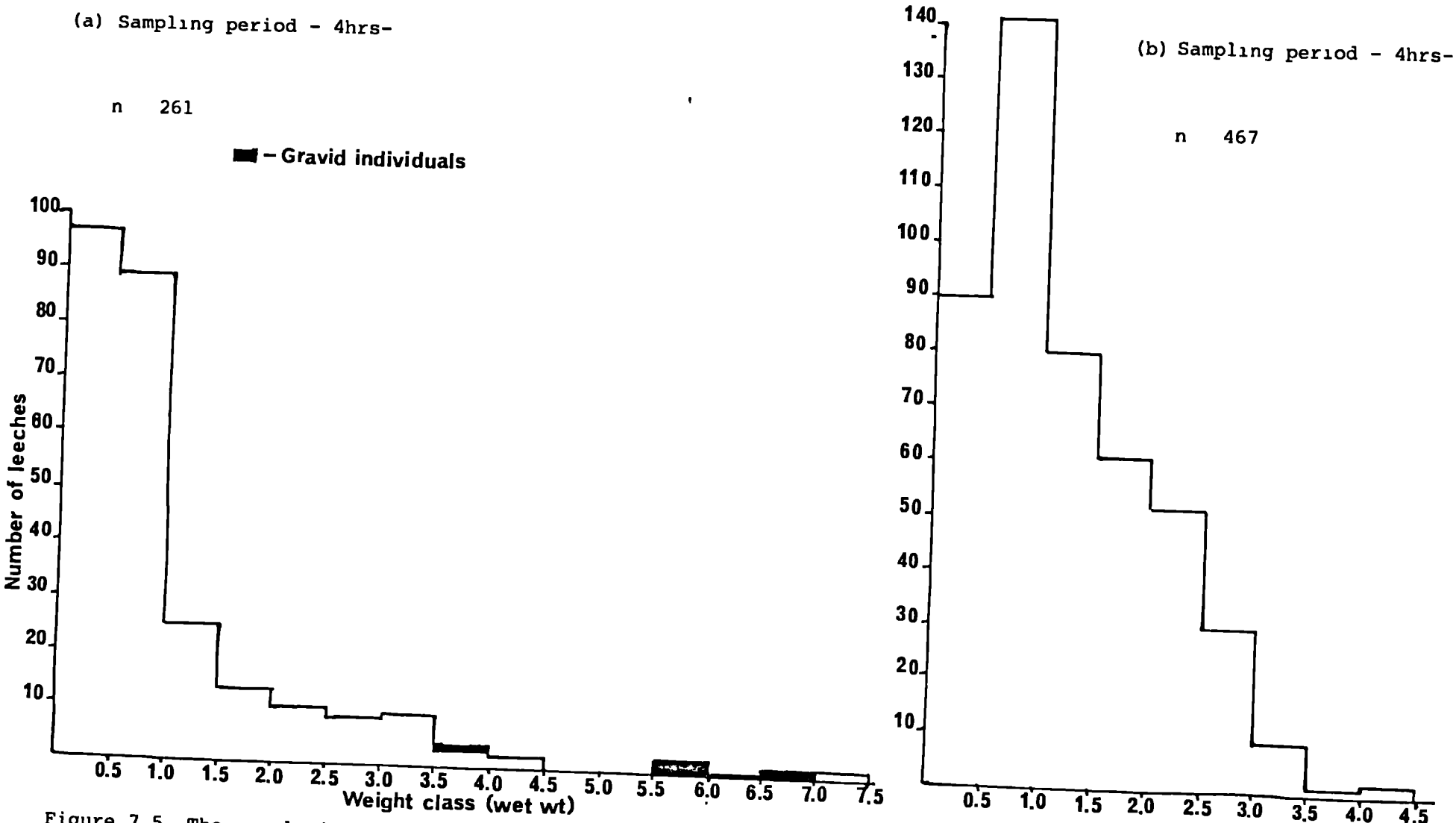


Figure 7.5 The p pulation structure of the Medicinal leech at Lydd Airport pond wetland in (a) June and (b) August 1985

Table 7.1 The occurrence of the Medicinal Leech at other sites on Dungeness, Kent.

Site	No. of leeches	Weights (g)
Mockmill sewer	15	0.3-1.2
Lydd Airport-Pond	1	1.2
Lydd Airport Temporary Pond	1	0.45
Hamilton Farm gravel pit	2	0.15, 0.86

The 1985 record of the Medicinal Leech at a pond near Brockenhurst (SU 314036) was confirmed (see Figure 7.3, pond 1). A single specimen was recovered weighing approximately 0.5 g from a small area of water-logged ground (see Plate 7.6). It appeared that much drying out had occurred since Spring when several leeches were found in shallow water near the pond margin, (P. Toynton, pers. comm.). No other record of the leech was found in the other ponds surveyed, although ponds 4, 6 and 7 (Figure 7.3) supported good numbers of larval Smooth and Great Crested Newts (Triturus vulgaris and T. cristatus). All ponds surveyed had access by grazing animals. All the ponds sampled were slightly acidic (pH 6.6) and humic in colour.

No record of the Medicinal Leech was found in the nine ponds sampled at Bookham Common, Surrey (see Figure 7.4). Some of the ponds represent typical small woodland ponds being heavily choked with leaves, humic in colour and slightly acidic (e.g. Sheepbell, Bayfield). A series of ponds between Isle of Wight pond and Upper Eastern pond were larger in size and differing in the extent of management. The Isle of Wight pond, the site of previous leech records, was cleared in 1972/73 and stocked with carp. There is little established emergent vegetation and erosion by anglers is a problem. It is interesting to compare a section of Isle of Wight pond after management (Plate 7.7) and Lower Eastern pond (Plate 7.8) which has been suggested for similar future management. The effects on aquatic vegetation such as Hornwort, (Ceratophyllum) by clearing and grazing by fish is obvious in the former pond. The latter pond at present represents a potentially favourable habitat for the leech due to less disturbance, high numbers of Smooth Newt (T. vulgaris) and dabbling birds such as Coot, Moorhen and Duck. It would be interesting to carry out a



Plate 7.6 An area of waterlogged ground near Pond 1 in the New Forest, (see Figure 7.3), where a small Medicinal leech, (<1g) was found in July 1985; note wild, grazing ponies and cattle were believed to be the main hosts for the leech when it was more common in the area.



Plate 7.7 The Isle-of-Wight pond, Bookham Common, (the site of previous records of the Medicinal leech), showing the combined effects of clearance and the introduction of Carp on surface vegetation.



Plate 7.8 Lower Eastern pond, Bookham Common before planned 'improvement' by clearance showing a potential site for re-introducing the Medicinal leech to the locality

reintroduction to this pond in an attempt to re-establish the Medicinal Leech at Bookham Common.

Although the Medicinal Leech was not found at La Sangsurière the unimproved wetland pastures provided another habitat type for comparison. It would be valuable to make a more extensive survey of this area.

Discussion

There is evidence to suggest that the Medicinal Leech is extending its di tribution in the Dungeness area. It seems likely, from results on population size, that Lade gravel pit represents the nucleus from which dispersion may be taking place. Certainly the population at Lydd Airport pond/wetland (TR065210) appears self-sustaining with large gravid leeches present and a high proportion of individuals in the weight class less than 0.5 g which represent previous year's hatchlings. Habitat conditions at this site are similar to Lade gravel pit with a richer vegetation structure.

The record at Burrowes gravel pit (TR069185) could represent the nucleus of a founder population with individuals being in a similar weight class to gravid leeches found elsewhere. Certainly the results of laboratory studies on captive breeding (see Chapter 6) confirm the idea that it may be several months after copulation before cocoon laying occurs, implying sperm storage. It is possible that these individuals, observed in June, could develop into a gravid state later in the season. However, a major disadvantage for the establishment of a population is the extent of bare shingle margin representing an inhospitable site for cocoon deposition. The record at Burrowes pit also invites the idea that birds may be an important dispersal agent for the Medicinal Leech. An important piece of evidence for this idea is the finding that the gorged individual had recently fed on bird blood. Apart from deliberate introduction by man, which is unlikely, the only other possible dispersal mechanism could be the Marsh Frog (Rana ridibunda) which is extending its range from the dykes of Romney Marsh slowly across the shingle of Dungeness. They are certainly more evident at the Oppen pits as compared with 2-3 years ago (P. Makepiece, pers. comm.). The idea of frogs as dispersal agents could explain the number of leeches found in

Mockmill sewer which forms part of the dyke system and is very close to Lade gravel pit. It is, however, unlikely that frogs caused the Burrowes gravel pit introduction since it is separated from the dyke system by a large expanse of shingle.

The numbers of leeches found at other sites on Dungeness (see Table 7.1) were all small, being in a weight range below that of gravid leeches found elsewhere. It is unlikely, at present, that these introductions could develop into viable populations. They may, however, represent examples of a continuous process of dispersal resulting from attachment to birds which have been shown to move regularly between the open water areas on Dungeness. The results from laboratory studies on feeding (described in Chapter 4) indicated that, depending on pre-fed body weight, a leech may spend between 10-70 minutes gorging on a host, which could be sufficient time to be carried by birds between sites.

The proposal for restocking the Medicinal Leech at two sites on Dungeness was designed to examine whether, long-term, an introduced population would be viable and to offset the threats posed to the Lade gravel pit population by extending the leech's distribution in the area. The Lydd Airport pond (TR068216) was selected to consolidate the single individual found at the site during the survey, while ARC pond (TR064202) represented a new site with similar habitat characteristics. It was necessary to contact interested persons with a consultation document and this is presented below:

A Proposal for Restocking the Medicinalis Leech, *H. medicinalis* to Sites at Dungeness, Kent.

The Medicinal Leech has been recorded in Britain on only 18 occasions in the last 100 years despite being previously widespread and abundant. It is generally regarded as being rare throughout its European range and is listed in the Invertebrate Red Data Book, produced by the International Union for the Conservation of Nature. The dramatic decline in numbers can probably be explained by a combination of over-collecting for past medicinal use, the loss of wetland

habitat and grazing practice and the decline of amphibian populations. In the wild, the leech is an opportunistic feeder on a range of hosts including birds, frogs and fish. It may take several seasons to reach adult size and therefore any disturbance of a population by removal or habitat destruction could result in a very slow process of recruitment or even local extinction.

In 1978 a population of the Medicinal leech was discovered at Lade pit (TR077217). It represents the first record of the leech in Kent and the most significant record in Britain, in terms of population size. It has been the subject of a research project undertaken by Wye College during the last three years. During this period individual leeches have been found at other sites but they do not represent viable populations:

Mockmill Sewer	TR073214
Lydd Airport Pond	TR068216
Lydd Airport-temporary Pond	TR068213
Burrowes Pit (RSPB)	TR069185
Hamilton Farm Pit	TR058190

This year, however, an important population was discovered in the Pond/Wetland area at Lydd Airport (TR065210).

The owners of the Lade pit site, Hall Aggregates Ltd., have been very co-operative towards the research work and have agreed to preserve the present nature of the habitat, despite further developments in the future. However, due to the proximity of the site to local housing and holiday centres disturbance is a continuous problem. The most potentially serious problem is the dumping of rubbish, including petroleum and chemical containers, but damage to vegetation and wildlife is also common, despite warning signs and fencing. Therefore, a proposal for restocking, using laboratory reared leeches together with a small supplement from Lade pit, would be a positive management tool to lessen the effects of a possible extinction at the Lade pit site.

The rationale behind this proposal is based on the policy guidelines set out in the report "Wildlife Introductions to Great Britain" prepared by Dr. Bryn Green, Senior Lecturer in C nservation at Wye College. In

this report, restocking is defined as:

"The deliberate or accid ntal release of a species or race into an area in which it is already present".

It is proposed that a population of 200 Medicinal Leeches be released to each of the following sites:

	Lydd Airport Pond	TR068216
and	ARC Pond	TR064202

These sites were selected on the following criteria:

(i) They represent similar habitat types to the main Lade pit site and the numbers of leeches would not exceed their carrying capacity.

(ii) They would provide a similar host range in sufficient numbers.

(iii) There is restricted human access resulting in less disturbance.

(iv) They are at the boundary of the Dungeness shingle/Romney Marsh pasture and are possibly lower risk sites for future gravel extraction within the Site of Special Scientific Interest.

(v) They represent gaps in a distribution from Lade pit to Burrowes pit.

In making an environmental impact assessment of the restocking proposal, several factors were considered:

(i) The leech would not cause any imbalance to other invertebrate populations at the sites.

(ii) Mortality to the Smooth Newt and Marsh Frog populations would be negligible given the numbers involved. The use of birds as hosts would be non-fatal.

(iii) The leech is not a carrier of disease and d es not present a health risk.

(iv) It is unlikely that the leech could become a pest.

(v) The removal of a small number of leeches from the Lade pit site would not prejudice the survival of that population.

and (vi) Future monitoring of the restocked populations could be undertaken by Wye College or the Kent Field Club to assess the success or failure of the release.

Proposal circulated to:

Dr. B. Green	Dept. Countryside Planning, Wye College
Mr. R. Findon	ARO Nature Conservancy Council, Wye
Mr. E. Philp	Maidstone Museum/Kent Field Club
Ms P. Evans	Conservation Officer, Kent Trust for Nature Conservation
Mr. D. Bush	Production Manager, Hall Aggregates
Mr. Santer	ARC Unit Manager, Denge Beach Works
Mr. Heritage	Lydd Airport Authority
Mr. P. Makepiece	RSPB Dungeness Reserve Warden
Dr. D. Frazer	Warren Farm, Boxley, Maidstone

The proposal was received favourably by the conservation parties, providing adequate future monitoring was organised. After consultation with the Nature Conservancy Council's Chief Scientist's Team it was agreed to allow restocking at Lydd Airport pond but not at ARC pond where the leech had not been previously recorded. The Lydd Airport owners responded positively with permission to restock the leech with the proviso that the company retained the right to use the site as required in the future.

The restocked population used at Lydd Airport pond was entirely composed of individuals from a laboratory population and previously maintained for feeding experiments. There was no need to supplement numbers from Lade gravel pit as originally proposed. All individuals were fed on heparinized whole bovine blood to satiation before release. The population structure of 200 leeches introduced to Lydd Airport pond is shown in Figure 7.6.

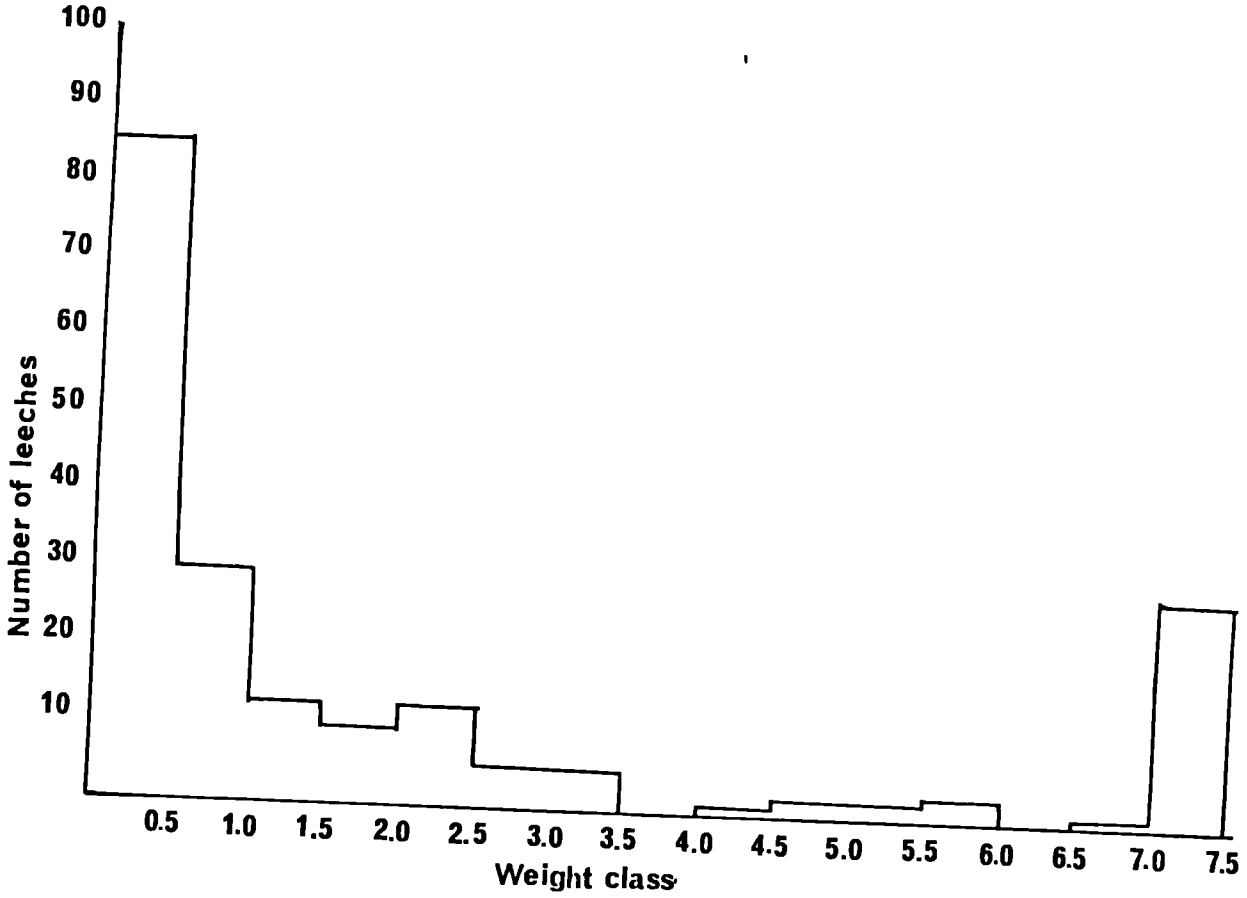


Figure 7.6 The population structure of 200 Medicinal leeches introduced to Lydd Airport pond(TR 68216) in September 1985

The most significant fraction of the introduced population was the weight class 7.0 g and over. These individuals were used in captive breeding experiments, having been optimally fed over a period of 1-2 years. The average weight in this class was 10.5 g with one individual weighing 22.5 g. These weights were not found in the wild and may be a consequence of optimum laboratory feeding. In 32 individuals weighing more than 2.5 g (16% of the population) recent copulation and cocoon laying had been observed in the laboratory. This sexually mature proportion of the restocked population is far higher than the number of gravid leeches obtained in wild samples elsewhere and it is hoped that this will enhance the survival of this population. It is planned to visit this site to monitor progress in the future.

The survey results have shown that the leech occurs at sites on Dungeness separated by up to 2.3 km. This dispersal could represent a series of separate, natural introductions from unrecorded populations beyond the immediate Dungeness area. It is more likely that emigration is occurring from the largest populations at Lade gravel pit (and possibly Lydd Airport pond/wetland). It appears clear that transport by birds is probably the basis of a successful introduction, but one cannot exclude the possibility that the leech was introduced to Lade gravel pit by man. As discussed in the Management Plan, this population cannot be older than initial excavation of the site in 1947, and the population of over 400 uniquely marked individuals inhabiting the small pond (see Chapter 1) must have been established since 1972.

It must also be remembered that the hermaphrodite state, found in the Medicinal Leech, could be a valuable attribute for the establishment of a population since a single, sexually mature, previously cross-fertilised leech could with an optimum fecundity, yield up to 50 hatchlings, and be the basis of a founder population. The source from which the Lade gravel pit population was founded, can be speculated from an examination of the variation in dorsal and ventral body patterning. For example, Kaczmarek (1968) noted seven basic types of dorsal colour and pattern within a population of the Medicinal Leech. This author, however, found a much greater variation ranging from bright olive green ground colour with bold orange longitudinal stripes, to individuals with a brown

or even black ground colour. Certainly dorsal colour and patterning could be regarded taxonomically as racial differences from an examination of specimens obtained from Eastern Europe and France and those in the U.K. It is however the colour and extent of patterning on the ventral surface which may provide the real clue to the origin of the Lade gravel pit population. On Dungeness, and from the specimen obtained in the New Forest, the ventral surface has a black ground colour with a mottling of yellow marks. This bears a close resemblance to markings observed on specimens from populations studied in Holland (Drescher and Higler, 1982) and on the Baltic coast of Norway (Forselius, 1952). It contrasts sharply with a plain, olive green ventral body colour without patterning in the Eastern European and French specimens.

It is interesting to speculate further by suggesting that the Lade gravel pit population of the Medicinal Leech originated from a single or series of post-1947 introductions resulting from the transport of gorged, sexually mature leeches by passage migrant birds.

DISCUSSION

TOWARDS A CONSERVATION STRATEGY FOR THE MEDICINAL LEECH, HIRUDO MEDICINALIS.

A conservation strategy for any species must extend beyond a consideration of physiological and behavioural adaptations which enhance survival, to include the dimension of habitat conservation. This seems particularly relevant in the case of the Medicinal Leech which shows, both at the individual and population levels, several important adaptations which are clearly advantageous to survival. Nevertheless, it has suffered a dramatic decline in numbers, undoubtedly due to the destruction of pond and wetland habitats.

It is only relatively recently that more precedence has been given to the conservation of habitats and whole ecosystems, regarding species conservation as an inevitable consequence of these initiatives. It is, for this reason, that this conservation strategy builds on a series of levels from the individual and population through to a consideration of the primary leech sites on Dungeness, the wider issues of wetland conservation and the future role of conservation organization and legislation. The discussion concludes with an assessment of the world's first commercial leech farm which may have a vital role to play in the future conservation of the Medicinal Leech.

There seem to be five key adaptations which enhance the survival of *H. medicinalis* at the individual level.

In comparison with most of the other British species of leech, the Medicinal Leech is able to survive in prolonged conditions of low dissolved oxygen concentrations and can even withstand periods of anoxia. This appears to be a significant adaptation, especially in the typical habitat of ponds which are usually subject to summer stratification of oxygen concentrations with anoxic lower levels.

The seasonal behaviour of the leech appears to be an adaptation in energy investment terms and clearly contrasts with other British leeches, particularly the sanguivorous species, which are active throughout the year. The controlling factor of water temperature on

the activity of the leech still remains a puzzle and it is likely that a synchronisation with maximum host activity, during the summer months, has evolved.

The various adaptations to an haematophagous habit were described in the literature review. Despite this apparently precarious, highly specialised vertebrate bloodfeeding, the adaptations which have evolved appear to maximise the effectiveness of this feeding strategy and enhance survival. The design and efficienty of the biting mouthparts, the array of salivary biochemicals and the internal gut defences to protect against attack from white blood cells in the bloodmeal are good examples of these adaptations.

A reproductive strategy which appears to minimise reproductive effort, by the production of relatively few cocoons each season, but which spreads this effort over several seasons must be considered to enhance the long term survival of the parent. This is an important strategy for it is possible that an adult body size and attainment of sexual maturity may take several seasons.

Arguably, the most important adaptation which enhances the survival of the leech is its feeding and digestive strategies. The saltatory feeding pattern which the leech adopts under optimum conditions may have developed from maximising the use of a host on each feeding occasion by gaining a large bloodmeal. It is likely that, in the wild, the leech never establishes a true saltatory feeding pattern and invests much energy in locating a host and, more often than not, fails to make a successful contact. Once contact has been made, however, it would be a selective advantage to maximise host use rather than part-feed. The overall saltatory pattern may also be a function of a very slow digestive breakdown of the bloodmeal. Many authors have found that the leech is able to survive very long periods of time between meals. This capacity has, in the past, only been ascribed to large adult leeches, but, as the results in this study show, this capacity is also shown by hatchling leeches. This certainly allows a proportion of hatchlings to survive overwintering without feed after emerging from the cocoon in late Summer.

Sibly (1981) provided an interesting theoretical base to digestive strategies and it is useful in order to emphasise the survival potential of the Medicinal Leech, to quote three key processes:

(i) Retention time: the time from starting digestion that digesta are retained in the absorptive region of the gut.

(ii) Throughput time: the time from ingestion of food to defecation of undigested residue.

(iii) Throughput rate: the rate of flow of digesta past any specified point in the gut.

The Medicinal Leech appears to maximise processes (i) and (ii) while the slow release of the bloodmeal from the crop region into absorptive areas minimises process (iii). It would be an interesting area for further study to quantify these processes.

The involvement of a gut bacterial symbiont in the digestive process of the leech is not a unique adaptation but the apparent antibiotic action of this single species of bacterium, which eliminates other putrifying gut symbionts, does reduce the speed of breakdown of the bloodmeal and is an unusual adaptation.

The conclusion on the survival of the Medicinal leech, at the individual level, is that the range of adaptations confer robust selective advantages.

The most important adaptation illustrated at the population level is the range of host utilisation, and, at Lade gravel pit, the important use of birds as hosts and as dispersal agents. The evidence suggests that the population (and individual) is not host specific but is rather opportunistic. To emphasise this, no evidence was obtained from the field, that hatchling leeches are obligate feeders on amphibia for their first feed. Large hatchlings ($>$ 100 mg) which were hatched in the laboratory were able to bite and feed from a chicken and small fish fry suggesting that opportunistic feeding may be a characteristic of all weight classes in the population.

A significant characteristic of the leech population at Lade gravel pit was the relatively small proportion of gravid leeches obtained in samples. This may suggest that the leech is slow to mature in the wild and could be a serious disadvantage to survival. Feeding has been found to be closely associated with the attainment of sexual maturity in the leech and the recommendation in the Management Plan, to modify the present Lade gravel pit site to secure and increase the availability of hosts could sustain an increasing proportion of sexually mature leeches in the population. This would be an important long term conservation strategy.

Apart from the disadvantage of a small sexually mature proportion of the population and relatively few cocoons found in the wild, the general survival potential at the individual and population levels appear good. It is at the level of the primary leech sites on Dungeness, the future management of the local area and the wider issues of wetland conservation within a legislative framework that the main thrust of the conservation strategy must lie.

The landscape of Dungeness has been changing rapidly in the past 10-15 years as more and more gravel has been extracted. An account of this and a detailed plan for the conservation and improvement of Lade gravel pit is given in the Management Plan. The future successful management of this primary leech site, and the other at Lydd Airport, depends on a basic non-consumptive approach by the owners (Reinhold, Hardisky and Phillips, 1980), which does not involve, primarily, commercial exploitation. This will involve giving top priority to the intrinsic scientific interest at these sites in any discussion of further landscape changes or multipurpose amenity use. There is certainly no shortage of expertise either in the literature (Duffey and Watt, 1971; Usher, 1973; Good, Wm igmam and Simpson, 1978) or in the area of impact assessment and land use planning at Wye College, Kent (Matthews, 1978; Barton and Buckley, 1983).

Any site of Special Scientific Interest cannot be regarded as an 'island' and changes in the surrounding landscape may have a damaging impact and therefore must be considered in a conservation

strategy. Increasing agricultural production in recent years and damage to the environment as a result of careless land management and pesticide use has been well documented (Muirhead-Thompson, 1971; Green, 1973; Newbold, 1977; Pye-Smith and Rose, 1984). An important conservation strategy in the immediate area of Lade gravel pit is to retain the present rough, grazing pasture which receives no spray or fertiliser application.

The most potentially damaging operation which is planned for the Dungeness area is the Channel Tunnel project. If the plan goes ahead it will create a problem of disposing of millions of tons of spoil. The open water gravel pits on Dungeness have been suggested as possible dumping sites for this spoil with an eventual reclamation back to agriculture. Therefore the conservation strategy must include a strong opposition to this particular option of disposing of the tunnel spoil.

Developments in certain areas could actually serve to underpin a conservation strategy for the Medicinal Leech by increasing the profile of ecological evaluation, environmental impact assessment and the necessity for a long-term conservationist philosophy. They could also strengthen the conservationist's voice on occasions of major environmental impacts such as the Channel Tunnel.

There must be an increase in the employment of professional ecologists at County Hall level and a greater awareness by the local planning authorities, whose responsibility it is to implement the County Structure Plan system, to the need for careful ecological evaluations and environmental impact analysis of potentially damaging operations (POD), (Tubbs and Blackwood, 1971; Selman, 1976, 1981, 1982).

The thrust of conservation activity must extend beyond the present vital work of habitat management, public involvement and educ tion (e.g. Hoskins, 1981 on the ev lution of our landscape and Sheail, 1976, on conservation) to devel p a greater say at County planning level (Beynon and Wetton, 1978; King and Clifford, 1985) and even at national level in the formulation of conservation legislation.

The effective 'corporate plan' produced by the Nature Conservancy Council (Wilkinson, 1984) was an important step in this direction.

The most important piece of conservation legislation in this country is the Wildlife and Countryside Act 1981. The appendices in the act list those species which have been identified as requiring protection but, to date, they do not include the Medicinal Leech despite other notable invertebrate rarities such as the Swallowtail Butterfly (Papilio machaon). It is important to the conservation strategy that the Nature Conservancy Council recognises the rarity and importance of the Medicinal Leech and promotes its inclusion into the act through the Secretary of State for the Environment.

On the international scene, the degree of co-operation in formulating major conventions protecting ecosystems (e.g. the Ramsar Convention on the Conservation of Wetlands) and species (e.g. The Convention on International Trade in Endangered Species, CITES) has increased in recent years. To support these various inter-governmental initiatives have been the development of truly international research projects such as the International Biological Programme (IBP) (Boardman, 1981). The Conservation Monitoring Centre of the IUCN (International Union for the Conservation of Nature and Natural Resources) has been promoting the inclusion of the Medicinal Leech, H. medicinalis, in Appendix II of CITES which would restrict the trade in leeches and commercial exploitation of wild populations. A draft proposal, prepared by the Conservation Monitoring Centre, is included at the end of this discussion for reference. So far, efforts to have H. medicinalis listed have failed, but it is hoped that it will finally be included after a review of its case later in 1987. It is also hoped to obtain a measure of international protection for H. medicinalis by including it in the Berne Convention for the Protection of Habitats of Major Importance. Obviously initiatives such as these are of vital importance to a conservation strategy for the leech.

Conservationists rarely find a smooth pathway and most major initiatives involve a conflict of interests, often with a political undercurrent. It is vital that conservationists develop an 'edge'

through a full understanding of how the political and legislative framework operates. Certainly during the last 2-3 years significant developments have been made towards a 'kinder' approach to lowland agriculture culminating in the recent EEC directive to adopt less intensive methods and the removal of land from production. It is a vital opportunity for the environmental organizations to rally and effect real, long-lasting changes in the area of rational rural planning. Even as I write a Parliamentary Bill is proceeding as a direct result of the persistence of the conservation lobby, which will resolve the present conflicts in that unique wetland landscape of Broadlands.

Finally in this discussion, the establishment by Dr. R. T. Sawyer of the world's first commercial leech farm at Biopharm Ltd., Swansea, South Wales, could provide the most important long-term contribution to a conservation strategy for H. medicinalis.

Biopharm's primary aim is to captively breed the leech in sufficient numbers to supply live material for research and occasional medical application and to synthesise a range of potentially valuable biochemicals, using the leech as raw material. To date, a spreading factor known as Orgelase has been patented which has potential in the tissue culture market. What is still possibly in doubt is how well the medically related anticoagulant and potential anaesthetic and antibiotic (produced by the leech gut bacterium) could successfully compete with the present, well established drugs on the market. A potentially valuable commercial area might be developed with the leech protein Eglin, which could have applications in the treatment of lung emphysema.

It is hoped that the successful breeding techniques developed at Biopharm will expand to such an extent that, with the restrictions embodied in CITES which would prohibit the trade of wild leeches, captive breeding sources become the only sources of Medicinal Leeches for research purposes. A vital area which, at present, Biopharm has not been involved in, is as a reservoir of leeches for restocking programmes at potentially favourable sites in the wild. This is perhaps where the concept of commercial leech farming might have its greatest contribution to conserving this quite remarkable animal.

CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES
OF WILD FAUNA AND FLORA

A. PROPOSAL

Inclusion of Hirudo medicinalis in Appendix II.

B. PROPONENT

C. SUPPORTING STATEMENT

1. Taxonomy

1.1. Class: Hirudinea
1.2. Order: Arhynchobdelliformes
1.3. Family: Hirudinidae
1.4. Species: Hirudo medicinalis Linnaeus 1758
1.5. Common Names:
English: Medicinal leech
French: Sangsue médicinale
Spanish: Sanguijuela
1.6. Code Numbers:

2. Biological Data

2.1. Distribution: Was once abundant from western and southern Europe to the Ural Mountains and countries bordering the eastern Mediterranean. Known from 26 countries, but no records have been obtained recently for Portugal or European Turkey. Elliott and Tullet (1984) provide a distribution map and references to records listed below (although details of the localities are not given); additional references in Wells et al. (1983) and Jazdzewska (1983).

Albania
2 records (Elliott and Tullett, 1984).

Austria
2 records (Elliott and Tullett, 1984); according to Kuhnelt (in litt., 30.09.86), only known population is in Lake Neusiedlersee.

Belgium
2 records: 1 near Arlon (Maquet, 1985) and 1 near Tournai (d'Huart in litt., 10.09.84); previously not found since 1938. A possible third locality is being checked (Decraemer in litt., 5.8.86).

Bulgaria
2 recent records (Elliot and Tullett, 1984); formerly widespread but declining (Deltshev in litt., 5.11.82) and considered vulnerable

Czechoslovakia
4 records (Elliot and Tullett, 1984).

Denmark
Known from 31 localities on the mainland; observed at 11 of these localities from 1960-1986 and at 20 of the known 37 localities on the island of Bornholm (Baagoe and Jensen, 1985; Baagoe in litt., 24.7.86). Elliott and Tullett (1984) mention 15 records.

Spain
8 records (6 recorded by Elliot and Tullett, 1984); including the Aiguamolls de l'Empordà National Park (Castello d'Empuries, Girona) (Molinas in litt., 21.7.86) and the Coto Donana (Mountfort, 1958).

Sweden
1 record (Elliot and Tullett, 1984); however a few localities have been recorded in the province of Scania, and on the Baltic islands of Öland and Gotland (Wells et al., 1983); in the 18th and 19th centuries, populations were boosted by importing leeches for the purpose of rearing them in ponds: such attempts were reportedly unsuccessful (Dahm in litt., 15.9.82).

Switzerland
4 records (Elliot and Tullett, 1984).

Turkey
No scientific records but reported to be collected for commercial purposes here (Wells et al., 1983; Fink, 1984)

UK
19 records scattered throughout the country; once common but became less frequent by 1816 and was declared extinct in 1910. Rediscovered in isolated populations in Kent, New Forest, Lake District, Anglesey, Yorkshire, Islay, Argyll, and Sutherland (Anglesey population may have been destroyed as the lake has been drained and refilled since 1954). Five new localities have been reported since 1982, including Kenfig Pool west of Port Talbot. It is probable that the leech occurs at more localities than those currently known.

USSR
33 records (Elliot and Tullett, 1984); mainly southern USSR, Ukraine and Moldavia. Northern limit of range runs from just north of Baltic provinces to Yaroslavl district, south of the Kama basin and Tyumen district, and to Barabinsk lakes and Altai (Lukin, 1957). It does not occur in western and eastern Siberia and in the easternmost parts of the USSR.

Yugoslavia
3 records (Elliott and Tullett, 1984).

2.2. Population: Unknown. Believed to be rare in its western range but possibly more abundant in the eastern Mediterranean part of its range. Listed in the IUCN Invertebrate Red Data Book (Wells et al., 1983) as "Indeterminate" i.e. known to be 'endangered', 'vulnerable' or 'rare', but there is not enough information to say which of the three categories is appropriate. It is considered not threatened in three countries (Hungary, Norway, Sweden), extinct in one (Ireland), threatened or apparently threatened in twelve (Austria, Belgium, Bulgaria, Czechoslovakia, Denmark, Finland, France, Italy, Luxembourg, Poland, Romania, Spain) and its status is unknown in ten countries.

Albania
No information on status.

Norway
Not considered threatened (Solem in litt., 10.9.86).

Poland
Intensive collecting caused a decline in populations by the 19th century. The species is now rare particularly in the Podlasie region of eastern Poland, due to marsh drainage and collecting (Jazdzewska, 1983).

Romania
Threatened; although still abundant in many areas but it is continuously declining and being replaced by Haemopis sanguisuga (Bacescu in litt., 6.8.86).

Spain
Rare; considered not threatened by Molinas (in litt., 21.7.86) but considered seriously threatened as a result of pollution, by Alvarado (in litt., 15.9.86).

Sweden
Common in the 19th century; has declined since but is not considered threatened (Wells et al., 1983).

Switzerland
No information.

Turkey
No information.

UK
Less common than it used to be.

USSR
Rare [.....)

Yugoslavia
No information.

2.3. Habitat: Occurs in freshwater ponds, streams and marshes. In Poland it is also found in peat bogs, swamps and marshes, and in Denmark it is found in small lakes and ponds on farm land as well as in forest, apparently irrespective of the degree of eutophication) Baagoe in litt., 17.11.86). It is more tolerant of anoxic water than other species and is often found in stagnant waters. Often associated with areas where farm animals graze and with populations of toads and frogs upon which the young feed. The leech is reported to bury itself during hot periods when the marshes dry out and to hibernate in cold weather (Elliott & Mann, 1979).

3. Trade Data

3.1. National Utilisation: The medicinal leech was used several centuries BC in ancient Rome, Greece, India and China. In the 19th century enormous numbers were used by European countries for blood-letting therapy. Before the war, leeches were used in the UK in the treatment of haematomas and inflamed areas, especially in the reduction of swellings close to the eye (Evered in litt. to Stewart, 10.3.82). The largest current users are major pharmaceutical companies; uses are described under the section on international trade.

3.2. Legal International Trade: Enormous numbers of leeches were in trade in the 19th century for medicinal purposes. Records of tax imposition on leeches imported into France allow an estimate of over 1 000 000 000 leeches imported during the last century. Similar unrecorded trade occurred in other European countries. Recently the harvest is said to be in the order of hundreds of thousands of leeches a year (Fink, 1984).

The Medicinal leech is now of particular interest for the large number of medically useful substances which have been isolated (Seemüller et al., 1980; Snider et al., 198?; Dodt et al., 1985; Petersen et al., 1976; Bode et al., 1986). These include:

1. Hirudin: a non-enzymatic polypeptide which prevents the coagulation of blood via specific anti-thrombic activity.
2. Histamine: a vasodilator which increases blood flow during feeding.
3. Hyaluronidase: a B-endoglucuronidase spreading factor which hydrolyzes the internal glucuronidic bonds of hyaluronic acid. Its mechanism of action is different from existing hyaluronidases from animal (bovine or ovine testicular tissue) and bacterial sources.
4. Bdellins: proteolytic inhibitors with anti-typsin/plasmin/ acrosin activity.
5. Eglins: proteolytic inhibitors with anti-chymotrypsin/ elastase activity. Both bdellins and eglins are the basis of the anti-inflammatory response following a leech bite. Eglins have now been genetically engineered and patented by Ciba-Geigy A.G. and Plantorgan.
6. Anaesthetic: the substance has never been isolated nor characterized but the leech bite is absolutely painless.
7. Plasminogen-activator: prevents the coagulation of blood by activating the body's natural plasminogen system. This property is common to a number of bloodsucking animals and is the basis of the currently used anti-coagulants urokinase and streptokinase.

The largest present-day users are major pharmaceutical companies. Many of these supply purified hirudin for use in preparations or as a research tool, each purification requiring thousands of leech heads although the amount of hirudin available from a leech is very variable. World sales of hirudin in the year to June 1984, were £3.5 million or US$5.1 million MSP (Manufacturers' Selling Price). Of these sales, almost £3.2 million (over 90%) were in F.R. Germany; the second major consumer is the USA. Annual world sales of hyaluronidase at June 1984 were £8.8 million (but this includes some hyaluronidase derived from mammalian testicular tissue) (Perry in litt. to Wachtel, 5.11.84). The leech is also used in micro-surgery involving the replacement of severed appendages; the anaesthetic and vasodilator are used to aid the rejoining of severed limbs, and the leech may reduce associated swelling (Henderson et al., 1983; Whitlock et al., 1983.

Medicinal leeches are supplied by the biological supply market for education and research purposes. The main zoological interest is in the neuromuscular co-ordination of swimming and in neuropharmacology (with special reference to neurotransmitter receptors). US universities use about 2000 leeches a year for neurophysiological research (at about $5-10 each), although total US university demand for live leeches may be as high as 1 500 000 a year (Goldsmith

'Haemo-exhirud' for haemorrhoids (£683 000 MSP June 1984); and 'Dolo-exhirud' for rheumatism (£356 000 MSP June 1984). In 1984 sales of hirudin in Germany rose by 17% compared with a world market growth of 7% (Perry in litt. to Wachtel, 5.11.84). In 1984 Plantorgan Werk's main sources were reported to be Greece, Hungary, Turkey and Yugoslavia (Fink, 1984).

Greece
Protected in 1980, but reportedly exported to F.R. Germany (Nell in litt., 6.10.81) and believed to be a major source for Plantorgan Werk (Fink, 1984).

Hungary
Large numbers were exported to F.R. Germany in 1981 (Nell in litt., 6.10.81), and Hungary was a major source for Plantorgan Werk in 1984 (Fink, 1984). According to dealers in Austria, practically all of the leeches available there come from the Plattensee in Hungary (Kuhnelt in litt., 30.9.86). Two companies are known. According to the main one, they used to export to F.R. Germany and Switzerland (Molnar (in litt., 13.8.86), but exports have declined to only 6 kg in 1986 (Molnar in litt., Sept. 1986).

Italy
4 suppliers known. Feliciano once imported from Switzerland and Poland, but now supplies only locally caught leeches (in litt., 18.8.86). Grassi Oriano used to import from Yugoslavia (in litt., 10.10.81) but now imports from Hungary and sells about 20 000 leeches a year to France, Switzerland, UK and USA. In 1981 the leeches were sold at L1500 each but now they cost L1000 each (Oriano in litt., 10.10.1981 and 4.11.86).R. Brookes & Co., UK, import some of their 5000 live leeches from Italy.

Poland
Leeches were once exported to France (Jazdzewska, 1983); and more recently to Italy (Feliciano in litt., 18.8.86).

Portugal
Believed to be a source of leeches sold in Canada, although there are no recent records of this species in the country.

Romania
Not exploited according to Bacescu (in litt., 6.8.86) but is exploited according to Fink (1984).

Spain
Hirudin, not of Spanish origin, is available in chemist shops. Live specimens are known in one instance to be imported from F.R. Germany for use in "phlebotherapy" (Molinas in litt., 21.7.86).

Sweden
No commercial trade (Dahm in litt., 27.8.86).

Switzerland
Feliciano, Italy, used to import from Switzerland (in litt., 18.8.86). The pharmaceutical company Pentapharm (Basle) imports 30-50 litres of hirudin extract from France annually. In 1984, 150 specimens were imported from F.R. Germany (Nell) for use in a university; total expected imports were 400 leeches (Dollinger in litt., 27.3.84). One product containing hirudin has been registered with the Intercantonal Office for the Registration of Medicaments (Dollinger in litt. to Rudge, 20.6.83).

4.3. Additional Protection Needs: Surveys are needed in order to assess the distribution and status of major populations. Effective protection and management programmes should follow.

Changes in farming methods have contributed to the decline in H. medicinalis through habitat destruction. Loss of marsh land is a major threat since this is also causing a decline in the marsh frog, which is the host of young Medicinal leeches (Wells et al, 1983; Fink, 1984). Assessment of changes in land use on population levels are needed to decide what other measures should be taken to safeguard the species.

Eventually, genetic engineering and captive breeding may obviate the need for wild animals. Both eglins and bdellins have been genetically engineered by Plantorgan and Ciba-Geigy A.G.

5. Information on Similar Species

Species such as Haemopis sanguisuga and the Giant Amazonian leech Haementeria ghilianii are increasingly used for educational purposes in schools and universities instead of Hirudo medicinalis. Haementeria ghilianii is being successfully bred in captivity. In Australia, the five-striped leech, Hirudo quinquestriata is used instead of the European variety, and in Canada the American medicinal leech, Macrobdella decora is often used (Davies in litt., 29.7.86). There is a large market for live and dry leeches in Oriental medicine, but Asian species are mainly used. Studies of the anti-coagulants of Haemodipsa zeylanica, collected in the wild in Borneo, are also under way (Sawyer in litt., 21.3.82).

Substitution of other Hirundinaria spp. such as the "Buffalo" leeches, abundant in Asia, may be possible. An Australian subspecies Hirudo australis bosisto and the European trout leech Hirudo trocincta (sometimes regarded as a subspecies of H. medicinalis) were imported into the United Kingdom instead of H. medicinalis in the 19th century. Some of these species were released in the UK and some populations of trout leech may have survived and be taken for Hirudo medicinalis.

6. Comments from Countries of Origin

This proposal was sent in draft to all countries of origin for comment. Only the following responses have been received:

Italy

The CITES Management Authority in Italy affirms that H. medicinalis requires protection from the threat of depletion by commercial trade and supports this proposal (Bortolotti in litt., 10.11.86).

Ireland

Ireland supports this proposal as H. medicinalis now seems to be extinct there and there is a need to control trade (T. O'Shaughnessy, Department of Tourism, Fisheries and Forestry, in litt., 22.10.86).

7. Additional Remarks

In the past, the leech was reared in shallow, muddy, vegetated ponds in Poland (Jazdzewska, 1983), France (Gironde), Belgium and Holland (Lameere, 1895; Van der Eijk, 1977), and breeding programmes were attempted in France, Germany and the USA in the 19th century (Wells et al., 1983). Research into captive breeding of H. medicinalis has also been undertaken in the USSR (Wells et al., 1983).

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Sawyer, R.T. (1976). The medicinal leech Hirudo medicinalis L., an endangered species. In: Forsythe, D.M. and Bruce Ezell, W. Jr. (Eds), Proc. First South Carolina Endangered Species Symp.

Seemüller, U., Eulitz, M., Fritz, H. and Strobl, A. (1980). Structure of the Elastase-Cathepsin G Inhibitor of the Leech Hirudo medicinalis. Hoppe Seyler's Z. Physiol. Chem 361: 1841-1846.

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Van der Eijk, R. (1977). Blodezuiger tabel. Jeugdbandsuitgeverij. 20 pp.

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References cited in Wells et al. (1983) are not repeated here.

Known suppliers

1. Mr J. Desbarax
   RICARIMPEX
   33 980 Audenge (Gde)
   Bordeaux
   France
   (1981) Supplies hospitals (skin grafting), universities (research), pharmaceutical industry (hirudin extract).

2. Frau E. Nell
   Blutegel import und Versand
   4350 Recklinghausen-Sud
   Elisabethstrasse 16a
   F.R. Germany
   (1982)

3. Dr Theodor Schuchardt & Co.
   Chemische Fabrik
   8011 Hohenbruun/Industriesiedlung
   Postfach 80 15 49
   8000 Munchen 80
   F.R. Germany
   (supplies hirudin)

4. Carl Roth
   Pharmaceutical and Chemical Supplier
   Karlsruhe
   F.R. Germany

5. VEB Arzneimittelwerk Dresden
   Radebeul 1
   Postfach 89-90
   Dresden
   F.R. Germany

6. MAVAD
   Hungarian Cooperative Enterprise
   for Game Trading
   1014, Budapest
   Uri Utca 39
   Hungary
   (Mrs. Korosmezey Miklosne, 1982)

7. Reamal
   Pharmaceutical and Chemical Suppliers
   Budapest
   Hungary

8. Mr Grassi Oriano
   Vendito Sanguisughe
   San Sisto di Pesaro 25
   61020 Piandimeleto (PS)
   Italy
   (1981)

9. Mr Aversano Feliciano
   Via Bologna 153
   5007 Prato
   Florence
   Italy
   (Sawyer, 1981)

10. Mr G. Pozzi
    Via Tommaso Salsa, 42 bis
    31100 Treviso
    Italy
    (Sawyer, 1981)

Known users

1. Prof. R.W. Payton (research)
   Memorial University of
   Newfoundland
   St. John's
   Newfoundland
   Canada

2. Dr. T.E. Petersen (paper on hirudin in:
   Dept. of Molecular Biol. "Protides of the Biological Fluids",
   University of Aarhus 1976).
   DK-8000, Aarhus C
   Denmark

3. Prof. Baudet (skin grafting 1981)
   Hopital Pellegrin de Bordeaux
   France

4. Dr. Hans Fritz (used for research into hirudin etc.)
   Abteilung fur Klinische Chemie und
   Klinische Biochemie
   in der Chirurgischen Klinik Innenstadt
   der Universitat Munchen
   Nussbaumstrasse 20
   Munchen 2
   West Germany

5. Dr. T. Dietl (1979, worked on hirudin)
   Anatomisches Institut
   Universitat Erlangen-Nurnberg
   852 Erlangen
   Krankenhaussstrasse 9
   West Germany

6. Zoologischen Institut der (600 leeches a year used for excretion
   Freien Universitat tests)
   1000 Berlin 41 (supplied by E. Nell, FRG.)
   Grunewaldstr. 34
   West Germany

7. Dr. F. Markwardt (paper on Hirudin in journal
   Institut fur Pharmacologie und Die Pharmazie, 1981)
   Toxikologie der Medizinischen
   Akademie Erfurt
   50 Erfurt
   East Germany

8. Dr. D. Bagdy (co-author with Petersen)
   Research Institute for
   Pharmaceutical Chemistry
   H-1325
   Budapest 4
   Hungary

9. Swiss University Institute (Dollinger, 1984)

10. Pentapharm Ltd. (were unable to disclose trade data
    Basle (pharmaceutical product) 27.5.82)
    Engelgasse 109
    PO Box, CH-4002 Basle
    Switzerland

SUMMARY

1. A Management Plan for Lade gravel pit, Dungeness, Kent provides the most detailed account of the habitat of a known population of the Medicinal Leech, Hirudo medicinalis.

   A series of recommendations suggest improvements at the site to enhance the survival of the leech population. The immediate priority is to reconcile recent public and development pressures with the conservation value of the area. There is an urgent need to negotiate the future of the site with the owners following a recently granted planning permission allowing the development of canoeing and angling activities. This author is currently assisting the Nature Conservancy Council in opposing these potentially damaging activities, which would undoubtedly affect what could be an internationally important Medicinal Leech population.

2. A Mark-Release-Recapture experiment estimated the population size of the Medicinal Leech at Lade gravel pit to be 6-12,000. The analysis of population structure histograms showed a consistent skew towards a high proportion of individuals of less than 1g bodyweight (note: most wild, gravid leeches were in weight classes > 2.5g). The proportion of gravid leeches in the samples was very small.

   The movement of marks showed that the leech was not restricted to a particular niche and probably moves throughout the site in response to host activity.

   The growth of 10, uniquely marked leeches in the wild was very restricted over a two year period. A true saltatory growth pattern, which was developed by laboratory specimens under optimum conditions, was not observed in these wild leeches.

   Future sampling at the site should be undertaken to examine the survival of marks which would measure the longevity of the leech in the wild, as well as monitoring changes in population size.

3. Observations indicated that the Medicinal Leech is seasonal in its activity and appears to be linked with a water temperature of 8-10^{o}C, below which the population remains inactive. The Medicinal Leech population at Lade gravel pit is active between early April and mid October.

The Medicinal Leech does not show any significant diurnal rhythm in its activity.

The Medicinal Leech was observed utilising the Smooth Newt, Triturus vulgaris, the Marsh Frog, Rana ridibunda and fish as hosts. There was no evidence of mammals acting as hosts. Evidence was obtained indicating that the Medicinal Leech will feed as a scavenger on dead, decaying animal material. The Horse Leech, Haemopis sanguisuga was also found associated with this activity.

The leech, Helobdella stagnalis was found associated with the Medicinal Leech during a restricted period in June of each field season. Insufficient evidence was obtained to conclude whether it represented hyperparasitism on the Medicinal Leech, or a temporary commensal relationship possibly providing a dispersal mechanism for H. stagnalis.

No information of predation on the Medicinal Leech was obtained in the wild although laboratory experiments showed that the Grass Snake, Natrix natrix, would not predate on the leech and took its usual prey of Smooth Newt, T. vulgaris in preference. Hatchling leeches were attacked by Libellulid dragonfly nymphs and the Water Boatman, Notonecta viridis.

The cocoons of the Medicinal Leech were not consumed by a range of leaf litter invertebrates. Predation remains an important area of the leech's ecology which requires further, quantitative investigation.

A series of preliminary laboratory experiments examined the foraging behaviour of the Medicinal Leech which responded to the courtship repertoire of the Smooth Newt, T. vulgaris. Further work in this area might be to use this interaction as a model to test Optimal foraging theory. The repertoire of distinct host-locating movements used by the Medicinal Leech could be described in quantitative, behavioural terms.

The results of preliminary scanning electron microscope observations of the jaws and teeth of 3 Medicinal Leeches were inconclusive and it was not possible to comment on the idea that hatchling leeches are limited by jaw efficiency to initial bloodmeals on the relatively thin

amphibian integument. This could be another area for further investigation, particularly quantifying the number of teeth per jaw linked with biting trials on different membranes.

4. Observations of bloodmeal smears obtained from the guts of wild Medicinal Leeches showed a large amount of stained, nuclear material from fragmented blood cells. This indicated a non-mammalian blood source and this was supported by several samples showing intact nucleated erythrocytes indicating a bird or fish origin.

   Serological analyses showed that the Marsh Frog, R. ridibunda was a significant host and confirmed the importance of birds as hosts. Several results indicated that two or more host types had provided a bloodmeal; notably one individual with fish, bird and frog antigens in a single bloodmeal sample.

   It was concluded that the Lade gravel pit population of the Medicinal Leech is being sustained on non-mammalian blood sources and the results throw into question the long held view that mammals are important hosts and that a prerequisite for attaining sexual maturity in the wild is a mammalian bloodmeal. The significance of birds as hosts requires further investigation and could involve raising species-specific antisera for more detailed, serological analyses. It would be interesting to see whether the Medicinal Leech utilises a 'behavioural group' of birds such as dabbling duck, waders or gulls.

5. Hatchling Medicinal Leeches, raised in the laboratory, established a saltatory growth pattern when provided with an optimum frequency of whole, or part-whole, bovine bloodmeals although growth rate and final body weight at the end of the experiment was significantly reduced in leeches fed on Diet D (blood plasma fraction). Several hatchlings fed on whole bovine blood attained a final body weight of over 20g, which far exceeded the maximum body weight of 8-9g found in samples of wild leeches.

   Feeding hatchlings on a bloodmeal of washed erythrocytes in Leibovitz culture medium was unsuccessful. Several hatchling leeches were fed infrequently over a 2 year period and did not establish a saltatory growth pattern. This may reflect a more realistic growth pattern in the wild.

A sample of hatchling Medicinal Leeches withstood long periods of starvation over an overwintering period. It indicated that a proportion of hatchlings, which appear in the wild in late July-August, may survive until the following active season (early April), without feeding. This finding extends present ideas about the ability of the Medicinal Leech to survive very long periods, of up to a year in large specimens, between feeds. Certainly the feeding and digestive strategies of the Medicinal Leech are important areas for further study, particularly quantifying retention time in absorptive areas of the gut and throughput time. This could possibly be accomplished using labelled bloodmeals.

6. The microhabitat of the cocooning sites of the Medicinal Leech at Lade gravel pit is described. Quantitative sampling of the shoreline produced a total of 30 cocoons which represented a very restricted distribution of 1 cocoon per $13.5m^2$. On two occasions, pairs of cocoons were found close together which probably indicated microhabitat preference by two leeches rather than the deposition of both cocoons by an individual leech.

7. Laboratory conditions were developed which stimulated large, optimally fed Medicinal Leeches to mate and lay cocoons. Efforts to rear laboratory hatched leeches to sexual maturity were largely unsuccessful.

   Analysis of 30 cocoons obtained from the wild found 19 viable. These yielded a total of 159 hatchlings (mean $\pm$ S.D. $\simeq$ 8.3 $\pm$ 3.5 hatchlings per cocoon, range 2-13 hatchlings per cocoon). A scatter diagram of the average weight of hatchlings and the number of hatchlings per cocoon showed a trend suggesting that the body weight of hatchlings decreased with an increase in the number of hatchlings per cocoon.

   From 48 gravid leeches sampled from Lade gravel pit, a total of 97 cocoons were laid of which 64 were viable. These viable cocoons yielded 491 hatchlings (mean $\pm$ S.D. = 7.6 $\pm$ 2.1 hatchlings per cocoon). A scatter diagram showed a similar trend of a decrease in the average weight of hatchlings with an increase in the number of hatchlings per cocoon. A further scatter diagram of leech body weight against the the number of cocoons laid showed a trend towards the heavier leeches laying more cocoons.

The change in bodyweight of leeches laying multiple cocoons was monitored. Although there was an overall decrease in bodyweight after the cocoon laying period, bodyweight increased to a peak just before each successive cocoon deposited. This may have been due to water absorption, possibly associated with cocoon production.

The development time of the Medicinal Leech between cocoon laying and the emergence of fully developed hatchlings was 27.5 $\pm$ 0.5 days. (range 20-33 days) for 64 viable cocoons at 24° $\pm$ 2°C.

Leeches which attained sexual maturity in the laboratory laid cocoons at any time of the year at a water temperature of 24° $\pm$ 2°C and a daylength of 14 hours artificial light.

A group of 21 leeches became gravid in the laboratory but failed to lay any cocoons. Evidence was obtained suggesting that the Medicinal Leech stores sperm, causing a delay between mating and cocoon deposition. One individual produced a viable cocoon 175 days after a first batch of 4 cocoons, without an intervening opportunity to mate.

Certainly this strategy of sperm storage could have important advantages to survival in the wild and is an important area for further study to examine the extent to which it is used, and whether environmental conditions influence it.

The mating behaviour of the Medicinal Leech was observed and photographed in the laboratory. Three distinct stages were identified and described.

The location of mating partners in the wild may be assisted by an, as yet, undetected pheromone and this possibility could be an interesting area for further study.

8. A survey of the Dungeness area discovered another important, viable population in a wetland area at Lydd aiport (TRO65210). Isolated individuals were also found at five other locations. The most significant was an individual Medicinal Leech from Burrowes gravel pit (TR069185) which was in a gorged state. Serological analysis indicated a bloodmeal of bird origin suggesting that the bird host, in addition

to providing a bloodmeal, acted as a dispersal agent possibly from the larger Lade gravel pit or Lydd airport populations.

A survey of 12 ponds in the S.E. corner of the New Forest, Hants, where leeches had been recorded in the past, found only a single small specimen.

The Medicinal Leech was not found in a survey of 9 ponds at Bookham Common, Surrey, the site of an earlier record. A small survey of dykes at La Sangsurière, Normandy, France, a previously recorded site, did not find any sign of the leech.

A proposal for restocking the Medicinal Leech at Dungeness is presented and the population structure of 200 Medicinal Leeches introduced to Lydd Airport pond (TR068216) is described.

Speculation is made as to the origin of the Lade gravel pit population of the Medicinal Leech and general body colouration and patterning was noted as being similar to north European specimens.

9. A multilevel conservation strategy is presented for the Medicinal Leech, and the potential of the world's first successfully commercial leech farm is discussed.

An important draft proposal for the inclusion of the Medicinal Leech to Appendix II of CITES (produced by the IUCN monitoring centre, Cambridge) is included for reference.

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