RESPONSES TO LEAD IN THE FRESHWATER ISOPODS *ASELLUS AQUATICUS* AND *A. MERIDIANUS*

BY

ANNE E. MILLMAN

B.Sc. (University of Leicester)

A thesis submitted in candidature for the degree of Master of Philosophy at the University of Leicester.
1984.

CONTENTS

CHAPTER ONE

1.1 INTRODUCTION

There is now an increasing awareness of the deterioration of the environment caused by human activities and much research and debate has centred on this subject in recent years. Current and increasing concern is focused on the release of heavy metals into the environment.

In aquatic environments, the concentrations of heavy metals are generally very low, although higher natural concentrations occur in rivers and estuaries which are associated with outcropping metalliferous lodes. As a result, the concentrations of heavy metals in natural waters can easily increase to levels that aquatic organisms have not previously encountered. The introduction of metals by industrial effluents and domestic sewage (Holdgate 1979) and aerial fallout (Peyton et al. 1976) to aquatic environments can cause heavy metal loads to rise far above natural levels, with subsequent effects on the ecology of the area concerned.

In view of this, it was decided to concentrate on the heavy metal lead, in the investigations, because of its potential role as an environmental pollutant caused by its release into the environment. The freshwater isopods, Asellus aquaticus and A. meridianus were used in the study and experiments concerned with the effects of acute lethal and sublethal concentrations of lead on various aspects of the physiology of the two species were carried out.

Lead is the most abundant of the heavy metals in the earth's crust (Nriagu 1978) and is a characteristic trace constituent in rocks, soil, water, air and plant and animal life. Its physical and chemical characteristics are such that lead is uniquely adapted to many industrial uses, including use in fuels and in the manufacture of paints, batteries and piping. On a global basis, the principal, and by far the greatest, source of lead pollution is from the combustion of lead containing fuel. Coal and fuel oil burned for heating and industrial purposes provide

some input, but one of the major sources is from the combustion of gasoline to which either tetraethyl or tetramethyl lead has been added as an antiknock compound. In local situations, industrial operations associated with the production of lead and its further manufacture into a variety of products, e.g. paint and battery manufacture, may be of primary importance. Mine waste disposal and solution of lead pipes are also sources of lead in surface waters. These inputs from a variety of sources are responsible for its role as a potentially hazardous substance and the particular concern is about the more subtle effects of chronic exposure to lead pollution.

The genus Asellus (Fig. 1) was chosen for this study as it is widely distributed and often abundant in many freshwater habitats - lakes, rivers, canals, ponds and ditches. The two most common species in Britain are A. meridianus Rac. and A. aquaticus L., both of which are used in the present study. A. aquaticus and A. meridianus constitute the food of many species of freshwater fish and invertebrates (Dupey 1967) and they also play an important role in the detritivore cycle of the food chain. The importance of allochthonous material as a major source of energy in aquatic systems has long been recognised (Kormondy 1976) and Asellus plays an important role in converting the energy to a form available to other trophic levels.

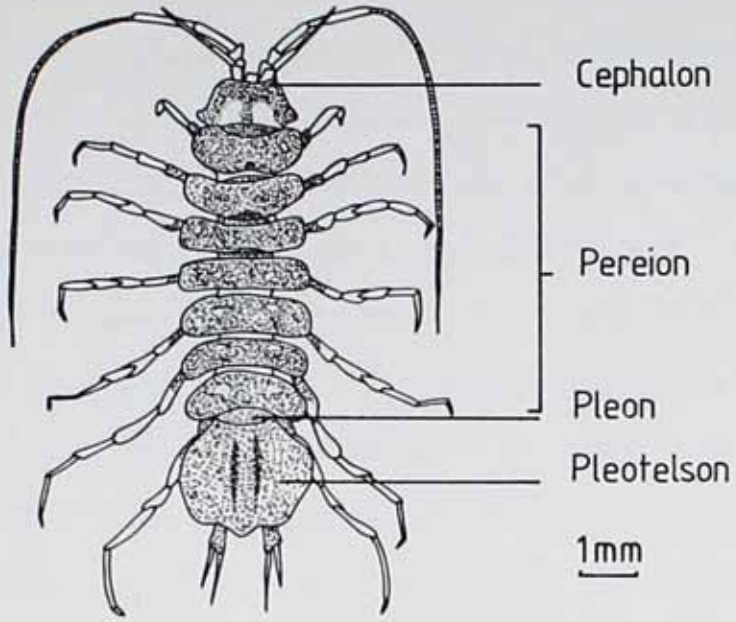


Fig.1a. Asellus in semi-diagrammatic dorsal view
(From Hynes et al., 1960)

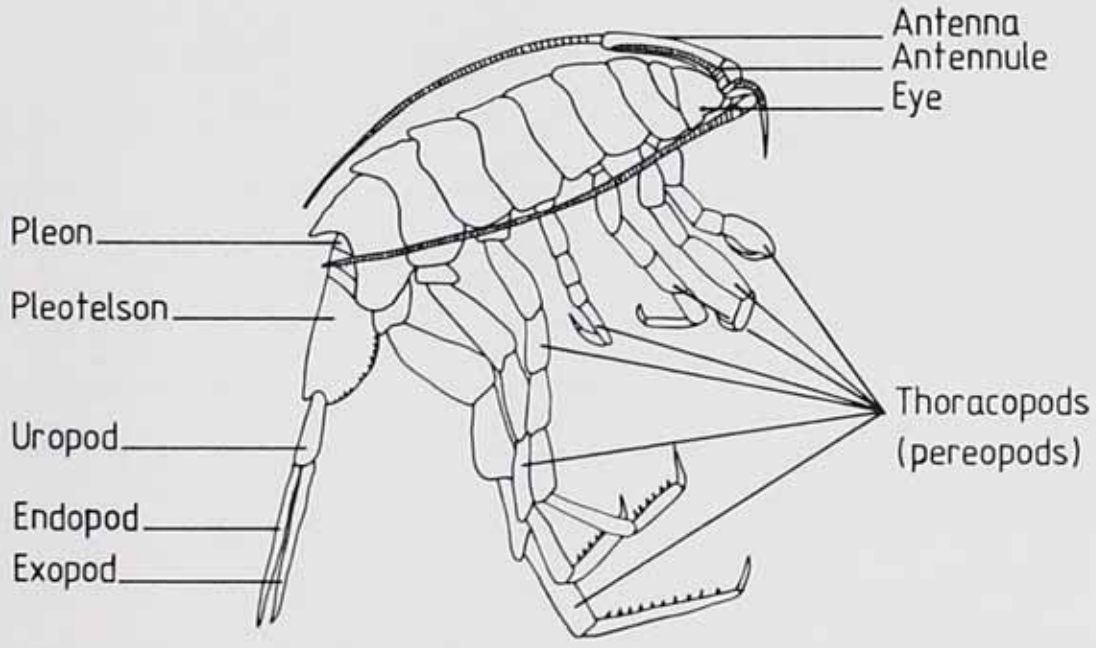


Fig.1b. Adult Asellus indicating morphological features
(From McLaughin, 1980)

1.2 SCOPE OF STUDY

The initial aim was to determine whether any differences are apparent in the effects of lead on different populations of the two species. It was hoped to include two populations of each species in the study, one population of each taken from an unpolluted site and the other from a lead polluted site. The intention was then to compare the responses to lead interspecifically and intraspecifically. Unfortunately, only sufficient numbers of two populations were encountered, one of A. aquaticus and one of A. meridianus. As a result, the effects of lead could only be compared interspecifically.

The A. aquaticus population was obtained at Castle Ashby Lake in Northamptonshire (Grid ref. SP855594) and A. meridianus was collected from a section of the River Lathkill in Derbyshire (Grid ref. SK211659). (A more detailed account of the sites used in given in Chapter 3). A comparison of the lead levels in water samples, sediments and Asellus spp. is included in this investigation, along with a comparison of the formal characteristics of the two sites.

Initially a series of short-term toxicity tests on the two species of Asellus was carried out in order to determine the acute lethal and sublethal concentrations. The acute toxicity tests were used to determine the LC_{50} values (which represent the minimum concentration of the pollutant lethal to 50% of the test organisms in the period of the experiments). Following on from this, the lethal threshold concentration was determined, which is the concentration at which acute toxicity ceases. It is "that level of the environmental entity beyond which 50% of the population cannot live for an indefinite time." (Sprague 1969). Included within this short-term testing section were further experiments attempting to monitor the uptake of lead in both A. aquaticus and A. meridianus. These experiments were designed to include uptake of metals, both from solution and from a food source, to determine whether lead is accumulated.

Further study was then concerned with the sublethal effects of this heavy metal with regard to growth, reproduction and respiration. The growth rates of the two species were monitored in the lethal threshold concentration to investigate whether this concentration would affect growth rate.

The reproduction experiments were designed to detect whether the sublethal concentration had any effect on the breeding biology of the two species, in particular their fecundity. No attempt was made in this study to determine the life history of the two species, since other research workers have provided adequate information on this aspect (Steel 1961; Andersson 1969; Adcock 1975, 1979).

One other possible sublethal effect of a toxicant is that of interference with the general metabolism which could be reflected in the respiration rate of the animal. This would be detectable as a change in the rate of oxygen consumption. Experiments were carried out to test the effects of lead on oxygen uptake in A. aquaticus as a means of measuring the sublethal effects of lead on metabolism.

1.3 LITERATURE REVIEW OF HEAVY METAL TOXICITY IN AQUATIC ENVIRONMENTS.

Attention has been primarily focused on heavy metals as environmental pollutants over the last two decades (Forstner & Prosi 1979) because they are one of the most widespread and toxic groups of pollutants. The term 'heavy metal' is generally held to refer to those metals having a greater density than five (Passow et al. 1961). Two factors contribute to the deleterious effects of heavy metals as environmental pollutants: (1) heavy metals cannot be destroyed through biological degradation as is the case with most organic pollutants and (2) heavy metals tend to accumulate in the environment - especially in the bottom sediments of rivers and lakes. This latter effect results from their association with organic and inorganic matter through process of adsorption, formation of complexes and chemical combination (Förstner et al. op. cit.). The emission of heavy metals has undoubtedly increased in recent decades (Holdgate op. cit.) and there is concern today about the possible hazards to living organisms that might result from the widespread dissemination of metals by man into the environment and particularly about the insidious effects of long term exposure.

Heavy metals in water may be found in a variety of forms - adsorbed on particular matter, absorbed into living and dead organic matter, precipitated as insoluble salts, or in an ionic state - and all may be potentially harmful to the biota. Fish are dependent upon the health of the lower trophic levels for their food supply, with benthic fauna representing a major proportion of the diet for many freshwater species (Murphy, undated). Removal of this component of the food web, due to heavy metals or other pollutants, may indirectly have the same consequences for a fish population as a directly toxic effect. Deleterious effects of heavy metals on the benthic fauna are, therefore, likely to be reflected in the whole ecosystem.

Research into the effects of heavy metals in aquatic environments dates back as far as the 1920s. Carpenter's (1924) observations on streams and rivers in West Wales initiated a phase of research concerning metal toxicity in this region, both in the field and laboratory (Carpenter 1924, 1925, 1927; Jones 1938, 1939, 1940).

Attention has been focused on heavy metals as environmental pollutants more recently, especially since incidents where heavy metal pollution affected the human population. 'Minamata disease' caused by mercury (in the Japanese Minamata Bay area) and 'itai-itai' disease caused by cadmium (in the Jintsu River area) both resulted from a regular diet of fish and shellfish from polluted marine waters. High levels of metal were accumulated by apparently healthy organisms and passed on in the food chain to man. (Bryce-Smith, undated).

Consequently, extensive research has been carried out on heavy metal accumulation by aquatic organisms under both field and laboratory conditions. (e.g. Coombes 1977) along with studies of metal accumulation in aquatic food chains (e.g. Topping 1977).

In the past, emphasis was placed on toxicity tests carried out on sensitive species of fish and this was justified on the grounds that fish represented a resource needing protection. Also, fish were frequently presumed to comprise the most sensitive component of the freshwater ecosystem and standards adequate for their protection, particularly sensitive salmonid species, were regarded as adequate to protect plant and invertebrate life (Murphy op. cit.). However, recent recommended water quality criteria for the protection of freshwater fisheries have recognised that the proposed standards may be inadequate to protect some of the more sensitive invertebrate species (E.I.F.A.C. 1976). As a result of this, extensive research involving toxicity tests with freshwater invertebrates has been carried out and many schemes now give equal weighting to the results of toxicity tests with both macro-invertebrates and fish (Murphy op. cit.)

Initially, studies reported in the literature comprise a series of short-term toxicity carried out to determine relative species sensitivity and lethal concentrations (Warnick & Bell 1969; Biesinger et al. 1972). Acute toxicity tests, however, have often been criticised for their use of high concentrations and short exposure times and, therefore, there have been a number of investigations of sublethal effects of metals, for example on behaviour in rainbow trout (Waiwood & Beamish 1978), on growth and development in plaice and herring (Blaxter 1977). A large number of biochemical effects of heavy metals have also been demonstrated. Copper has been shown to depress the respiration of the crustacean, Artemia salina, by approximately 25% without significantly affecting its motility whereas mercury reduced the motility at a rate much faster than that at which it inhibited its respiration (Corner & Sparrow 1956). In rainbow trout (Salmo gairdnerii), Bilinski and Jonas (1973) thought that the impairment of lactate oxidation in gills, caused by the effects of cadmium and copper, might be due to disruption of the cellular organisation or to inhibition of enzymic activity. The most important mechanism of toxic action by heavy metals is thought to be the inhibition of enzyme systems. Certain metals have a strong affinity for sulphur, especially the thiol (-SH) groups present in many enzymes and proteins, and bonding on to the -SH groups may be an important cause of the toxic effects (Bryce-Smith op. cit.). More experimental evidence is needed to clarify this point.

Various factors affect the toxicity of heavy metals. The importance of modifying factors can scarcely be overestimated. Beyond any doubt they account for much of the variation in toxicity found in the literature (Sprague 1970).

Mineral content of water sometimes acts as a modifying factor, particularly the calcium content. Increased toxicity of heavy metals in soft water has been recorded by many investigators, including Lloyd (1960),

who showed zinc sulphate to be more toxic to rainbow trout in soft water. Hardness can also affect the toxicity of metals depending on the pH of the water (Howarth & Sprague 1978). One general effect observed was that high hardness decreased toxicity to rainbow trout at any pH. Increasing H-ion content (decreasing pH) had a variable effect; there was a decrease, then an increase, and subsequently a decrease in copper toxicity.

Higher temperatures are usually assumed to make a pollutant more toxic. Lloyd (op. cit.) demonstrated that an increase in temperature, in short tests, decreased the survival time of rainbow trout in solutions of zinc sulphate in a hard water, but the threshold concentration was not appreciably affected by changes in temperature. Sprague (1970) states that it is clear that no assumptions should be made about temperature effects on toxicity.

Reduced oxygen content of water may be considered as a modifying factor for toxicity. By reducing the dissolved oxygen concentration of the water, the toxicity of zinc sulphate to rainbow trout was increased, although the effect was reduced when the fish were previously acclimatised to the lower oxygen concentration of the test. (Lloyd, op. cit.).

These are among the environmental entities which may act as modifying factors, but others cannot be ignored. Stumm et al. (1973) showed that the presence of an organic complex forming substance had several consequences. It could reduce the free metal ion concentrations in the solution and such a reduction might increase or decrease the growth of organisms. Also, it increased total soluble metal concentration and, depending on whether the organism can take up or break down the metal chelates, the metal species may become better available to the cells.

The effect of joint toxicity should not be discounted either. Lloyd (1961) indicated that the toxicity of a mixture containing

relatively low concentrations of zinc and copper in either hard or soft water could be calculated from the toxicities of the individual metals by assuming that they exerted a similar joint action.

Most of the research involving heavy metals has concentrated on heavy metal tolerance. Tolerance limits under various conditions have been described for a wide variety of organisms (e.g. Nehring 1976; Warnick et al. op. cit.). A comprehensive review of the literature on tolerance is given by Bryan (1976).

The existence of organisms in a metal-rich water raises the questions as to whether they belong to a species which is generally tolerant of a high level of the metal, or whether the populations have evolved mechanisms capable of dealing with toxic concentrations (Whitton & Say 1975). The mechanisms possessed by organisms for handling natural fluctuation in the availability of heavy metals assume particular importance under contaminated conditions. Different species vary in their natural tolerance and, under polluted conditions, the ecological balance presumably changes to favour more tolerant species. Also, individuals within a population may vary in their tolerance, so that under polluted conditions the more tolerant genotypes will be selected, resulting in a more tolerant population.

In some species, increased tolerance to the toxic effects of some metals can be acquired by previous exposure to sublethal concentrations of the metal. Several examples of this have been given by Sprague (1970) for fish. Many of the reports show acclimation to zinc. For example, minnow fry developed a noticeable degree of tolerance if eggs and embryos were reared in zinc. In rainbow trout, increased survival times were recorded following two weeks acclimation, and a 40% increase in LC_{50} of zinc was reported when the trout were acclimated to 0.5 toxic units. Lloyd (1960) also found that the rainbow trout, Salmo gairdnerii, was more resistant to lethal concentrations of zinc following exposure to sublethal concentrations and, in the same species, Sinley, Goettl and Davies (1974)

showed that more tolerant fish were produced from zinc-treated eggs. In the brine shrimp, Artemia salina, Saliba & Ahsanullah (1973) were able to double the copper tolerance measured as survival time in 1 mg dm^{-3} by pre-treatment with a lower dose of 0.1 mg dm^{-3} of copper for 3 weeks. However, the same authors were unable to adapt the polychaete Ophryotrocha labronica to copper.

During the work of Bryan (1976) and Bryan and Hummerstone (1971, 1973a, 1973b), populations of the polychaete Nereis diversicolor, in the estuaries of Devon and Cornwall, were found which displayed tolerance to certain heavy metals that correlated with the level of the metals in the sediment from which the populations originated. Tolerance in N. diversicolor was observed to silver, zinc, copper and, possibly, lead. The tolerances to the last three metals seemed to have developed separately, but that to silver depended on the presence of tolerance to copper. In N. diversicolor, the adaptations to zinc and copper involved completely different processes. The adaptations to zinc involved a decrease in the permeability of the body surface and probably an improved ability to excrete the metal, whereas in the case of copper, increased resistance appeared to depend on a complexing system which detoxifies the metal and stores it in the epidermis and nephridia (Bryan et al. 1973a). The authors inferred that the tolerance was genetically determined because copper tolerance in N. diversicolor was not easily lost by worms exposed to uncontaminated sediments. The pressure to adapt is probably greatest under conditions of low salinity, since the toxicity of copper to N. diversicolor increases with decreasing salinity (Bryan et al. 1971).

In other work, populations of the genus Asellus were studied for tolerance to lead and copper. Brown (1976, 1977, 1978) studied

A. meridianus Rac. from sites receiving mine drainage in the rivers Hayle and Gannel in Cornwall. Animals from the River Hayle, where there were high concentrations of copper in both water and sediment, but only trace amounts of lead, were tolerant to both copper and lead. Animals from the River Gannel, where there were moderately high concentrations of copper and exceptionally high concentrations of lead were markedly tolerant to only lead (Brown 1976). In her later work, Brown (1978) showed that in the animals showing co-tolerance to copper and lead, tolerance to copper seemed to confer tolerance to lead.

Research by Fraser et al. (1978) and Fraser (1979) concentrated on a population of A. aquaticus from the River Trent, since Brown (reported in Fraser et al. (op.cit) had found that compared to A. meridianus, A. aquaticus as a species was very tolerant to heavy metal pollution. Fraser (op. cit.) reported that in a polluted river, the Calder, A. aquaticus contained higher levels of lead and copper than animals from the unpolluted Woodplumpton Brook. Also, the Calder animals were tolerant to lead, although there was no difference between the populations in their tolerance to copper, zinc, cadmium or arsenic.

The tolerance to lead in A. meridianus was suggested to be genetically determined (Brown 1976) since breeding experiments showed that tolerance to lead in the laboratory raised F_2 generation was maintained. Fraser (op. cit.) attempted to demonstrate whether lead tolerance in A. aquaticus was inherited, but breeding experiments were unsuccessful. However, she demonstrated that tolerance could be achieved by previous exposure to sublethal concentrations. Also, the non-tolerant Woodplumpton animals could be acclimated to lead by exposure to 0.48μM dm^{-3} Pb for 5 or 10 days when they became as tolerant as the Calder population. Fraser concluded that the demonstration of acclimation to lead in A. aquaticus means that there is no need to postulate any genetic basis for tolerance, but that is not to say that genetic adaptation does not occur. Further experiments are needed to clarify this point.

Other experiments by Fraser indicated that 0.48μM dm^{-3} Pb caused the growth rate of A. aquaticus from both populations to increase. In contrast, Brown (1976) found that in A. meridianus 0.48μM dm^{-3} either inhibited growth rate in certain of the populations studied or else caused no significant response. In none of the cases reported by Brown was an increase in the growth rate observed.

Fraser observed that in feeding experiments with lead-loaded Saprolegnia ferax, neither population of A. aquaticus took up lead from their food, although she noted that A. aquaticus could feed on bacteria and that uptake of lead from this source might occur. Brown (1977) found that the tolerant A. meridianus populations accumulated copper and lead from metal-enriched food, but the non-tolerant populations showed no accumulation of either metal.

Further experiments by Fraser indicated that the important storage organ, in both tolerant and non-tolerant A. aquaticus, is the cuticle or its underlying tissues, with a little or no accumulation in the gut or hepatopancreas. In contrast to this, Brown (1977) established that the hepatopancreas served as a principal storage organ for trace metals in A. meridianus and it was apparent in the co-tolerant Hayle population that both copper and lead compete for sites in the hepatopancreas. X-ray microanalysis demonstrated that lead was present only in the 'cuprosomes' and she suggested that lead was more readily bound than copper. However, the Gannel animals appear to have the ability to restrict the uptake of both copper and lead into the hepatopancreas and therefore the high levels of lead which are accumulated in the hepatopancreas of the Hayle animals are not apparent in the Gannel animals. Fraser showed that the action of lead uptake appeared to be a passive process since dead Asellus accumulated similar levels of lead to live Asellus.

In furthering the investigation, Fraser carried out respiration experiments as a means of measuring the sublethal effects of lead on metabolism. Results showed that oxygen uptake was inhibited by lead in both populations and evidence suggested the inhibitory effect was greater in the non-tolerant Woodplumpton animals.

In view of this background of investigation, it was decided to further some of the experiments already carried out by Brown (op. cit.) and Fraser (op. cit.) and also to initiate experiments concerned with the effects of sublethal concentrations of lead on various aspects of the physiology of the genus Asellus.

CHAPTER TWO

MORPHOLOGY AND BIOLOGY OF *ASELLUS*

2.1 Introduction

There are three indigenous species of Asellus in the British Isles - A. aquaticus (L), A. meridianus (Rac) and A. cavaticus Schiodte and one recently introduced North American species A. communis Say, which is not widespread. A. cavaticus is a subterranean animal from wells and caves in Southern England and Southern Wales, which lacks eyes and pigment. The two common species are A. meridianus and A. aquaticus, both of which are used in this present study.

Until 1919, the name Asellus aquaticus covered all the freshwater isopods found in this country, then Racovitza, in 1919 (cited by Steel 1961), after studying A. aquaticus from France, England and various other localities, distinguished two species. Racovitza retained A. aquaticus for the isopod most common in Northern Europe and the other he named A. meridianus.

2.2 Identification

The standard method of identification for A. aquaticus and A. meridianus (Gledhill et al. 1976) depends upon the morphological differences recorded by Racovitza in 1919 when differentiating the two species. Species can be identified by non-sexual features, but the most satisfactory characters are provided by the external reproductive structures. The criteria used to distinguish between the two species are set out in Table I (page 20).

Character used to distinguish between species	*A. aquaticus*	*A. meridianus*
Dorsal surface of head	Two posterio-lateral unpigmented areas separated by a median bar of pigment. (Fig. 2)	Single median posterior unpigmented area. (Fig. 2)
Pleopods (a) Male	Pleopod 1 has a shallow notch on the outer edge. The basal segment has approximately four hooks on the inner margin. (Fig. 4a)	Pleopod 1 is much smaller than in *A. aquaticus*. There is no notch. (Fig. 4a)
	Pleopod 2 has a spur projecting downward from the inner side of the inner distal segment (Fig. 4b)	The distal segment of pleopod 2 is approximately the same length as the basal segment. The inner distal segment has an umbrella-like projection. (Fig. 4b)
Pleopods (b) Female	The first pleopods are absent. The second pleopods are rounded and overlapping. (Fig. 4c)	The first pleopods are absent. The second pleopods are trapezoidal and the setae on the distal borders are about as long as the pleopods themselves. (Fig. 4c)
Walking legs	The first pair of walking legs in the male possesses a large triangular projection on the inner edge of the penultimate segment. (Fig. 5a)	The triangular projection is absent. (Fig. 5b)
Zenker's Organs (a) In the pereion	The Zenker's Organs are restricted to the last three segments (segments 5,6 & 7) (Fig. 7)	The Zenker's Organs are not restricted to the last three segments. (Fig. 7)
(b) In the pleotelson	The distribution of Zenker's Organs does not form a perianal ring. (Fig. 7)	The arrangement of Zenker's organs has a characteristic perianal formation. (Fig. 7)

Table 1. Criteria used to distinguish between *A. aquaticus* and *A. meridianus*.

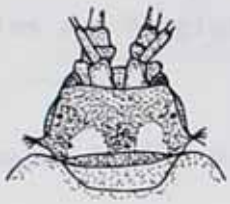
2.2.1. Head Pigmentation

The pattern of head pigmentation may be used to distinguish the two species, but it should be used with caution as there is some variation. Scourfield (1940) first noted that A. aquaticus has two distinct pale patches which lie posterio-laterally on the vertex of the head and are separated by a central band of pigment. In A. meridianus, this central band is absent and the patches coalesce to form a single pale area (Fig. 2). Needham (1942) confirms this distribution and relates it to the insertion of the mandibular abductor-levator muscles. However, as Scourfield (op. cit. P.269) points out, "in A. meridianus the alimentary canal sometimes shows through as a dark band in the middle, thus producing, at first sight, the appearance of two lateral clear spaces as in A. aquaticus." Also, the median band of pigment, characteristic of A. aquaticus, is by no means consistent. Moreover, unpigmented specimens of both species may occur rarely in the field, and preserved specimens tend to lose pigment. So, although the pattern of cephalic pigmentation is specific for the majority of specimens, these exceptions throw some doubt on the reliability of the character, unless one is able to check by some other method.

2.2.2. Pleopods

Sexual features provide the most satisfactory method of identification, the sexes differing mainly in the pleopods. In the female, the first pair of pleopods is missing and the first visible pair of pleopods is morphologically the second. Each member of the pair is of simple

A.aquaticus

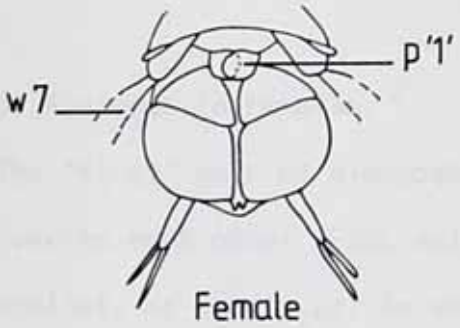


Dorsal surface of head with two posterio-lateral unpigmented areas separated by a median bar of pigment

A.meridianus

Dorsal surface of head with single median posterior unpigmented area

Fig. 2. Head Patterns (Dorsal view)



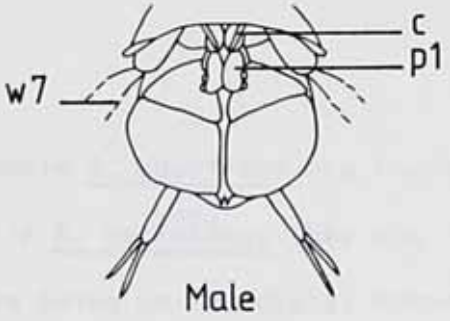


Fig. 3. Pleotelson (ventral view) of A.aquaticus
w7, base of 7th walking leg;
c, copulatory style;
p1, first pleopod of male (with 2nd lying beneath it);
p'1', "first" pleopod of female (actually equivalent to the second of the male, the true first being absent)
(From Hynes et al., 1960)

construction and much smaller than those of the following pairs. In the male all the pleopods are retained, but the first two pairs are small and are modified for use in spermatophore transfer. This difference between the sexes is indicated in Fig. 3

The difference between the pleopod characteristics of the two species in both males and females is shown in Fig. 4.

a) Pleopods in the Males.

In A. aquaticus the distal segment of pleopod I possesses a shallow notch on its outer edge and the basal segment has about four hooks along its inner margin (Fig. 4a). The inner distal segment of the second pleopod has a spur projecting downward from the inner side of its base (Fig. 4b). In the male A. meridianus the first pleopod is smaller than that in A. aquaticus and is as shown in Fig. 4a. The outer distal segment of the second pleopod in A. meridianus is about the same length as the basal segment and over-reaching the tip of the inner distal segment which has an umbrella-like projection (Fig. 4b).

b) Pleopods in Females.

The 'first' pair of pleopods in the female A. aquaticus are rounded and overlap each other (Fig. 4c), whereas in A. meridianus they are trapezoidal, or nearly so, in shape and the setae on the distal borders are about as long as the pleopod itself (Fig. 4c).

2.2.3. Walking Legs

The sexes also differ to a lesser extent in the first pair of walking legs. The differences are shown in Fig. 5. In A. aquaticus the penultimate segment of the first pair of walking legs possesses a large triangular projection on its inner edge which is absent in A. meridianus.

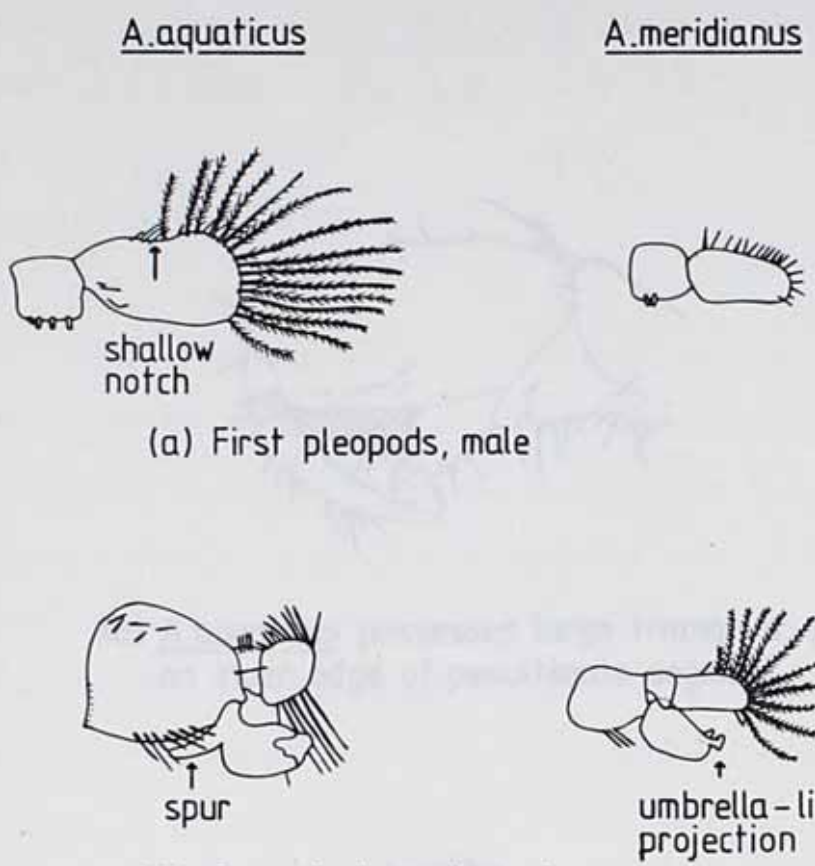
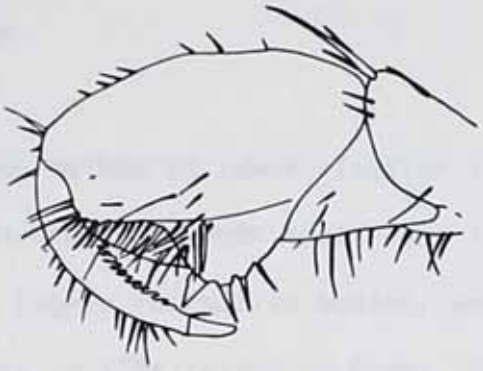


Fig.4. Pleopods (ventral view) of A.aquaticus and A.meridianus (From Hynes et al., 1960)



(a) A.aquaticus possesses large triangular projection on inner edge of penultimate segment

(b) A.meridianus has triangular projection absent

0·5mm

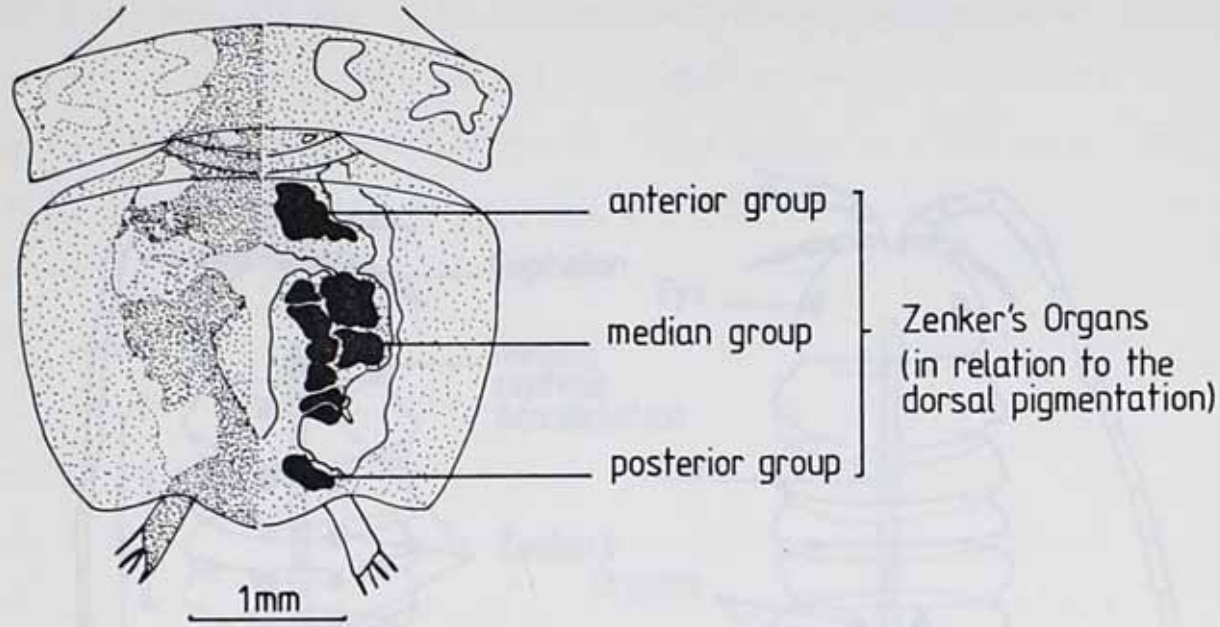
Fig.5. Tips of first walking legs of males
(From Hynes et al., 1960)

A problem in using the sexual characteristics for identification of the two species is encountered in juveniles. The characteristic differences do not become apparent until after sexual maturity (Needham 1942) at a length of about 3.5mm.

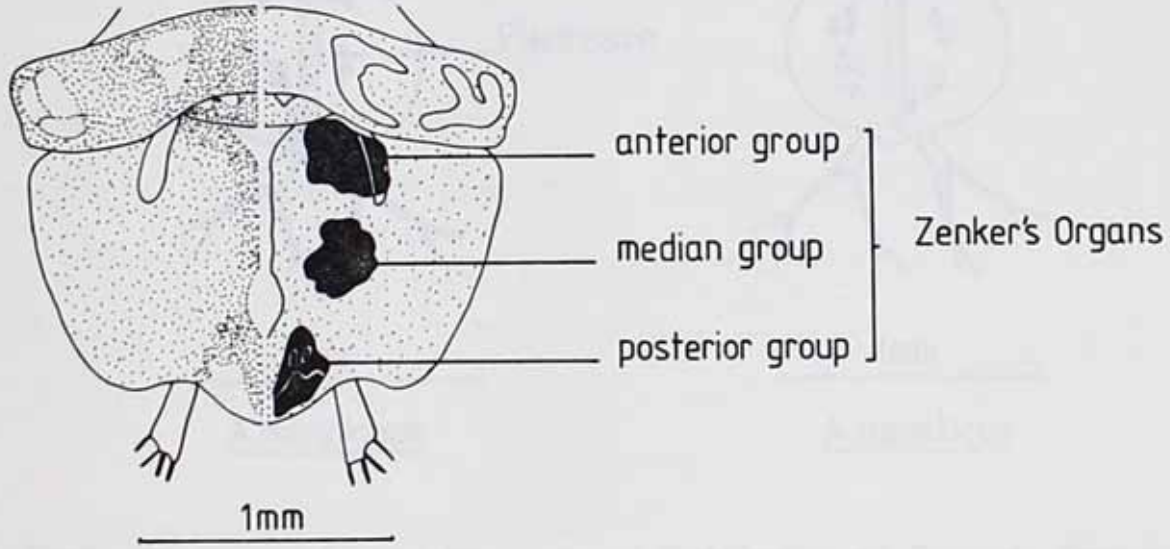
2.2.4. Zenker's Organs

A further, less used, method of identification is to study the distribution of Zenker's Organs in both adults and juveniles. These organs, which appear as highly refractile bodies, were first recorded in A. aquaticus by Zenker in 1854 (cited by Dupey 1967). He was unable to offer a satisfactory explanation as to their function, but Ter-Peghossian in 1909 (cited by Dupey op. cit.) described them as excretory organs of accumulation. Later research on the subject (Husson & Henry 1963) has shown that they are not true organs, but merely accumulations of uric acid. Lockwood (1959) was perhaps the first to realise their potential as a means of distinguishing between the two species.

The distribution patterns of the Zenker's Organs in the two species, in adults and juveniles are shown in Figs. 6 and 7. In the pereion of A. aquaticus, they occur as discrete cylindrical bodies in segments 5, 6 and 7 just beneath the tergal hypodermis. As individuals age, these expand longitudinally to form two more or less continual lines. In the pleotelson of the juvenile A. aquaticus (Fig. 7), the concretions originate as a number of small, yellow bodies scattered on each side of the midline. These also increase in size with age until they fuse to form several (usually 3) irregularly shaped masses occupying nearly all the central part of the pleotelson, with the exception of the posterior tip. Very rarely there is a small median cephalic accumulation under the tergal hypodermis.



A.aquaticus



A.meridianus

Fig.6. Dorsal pigment pattern of pleotelson of adult Asellus spp. (From Dupey, 1967)

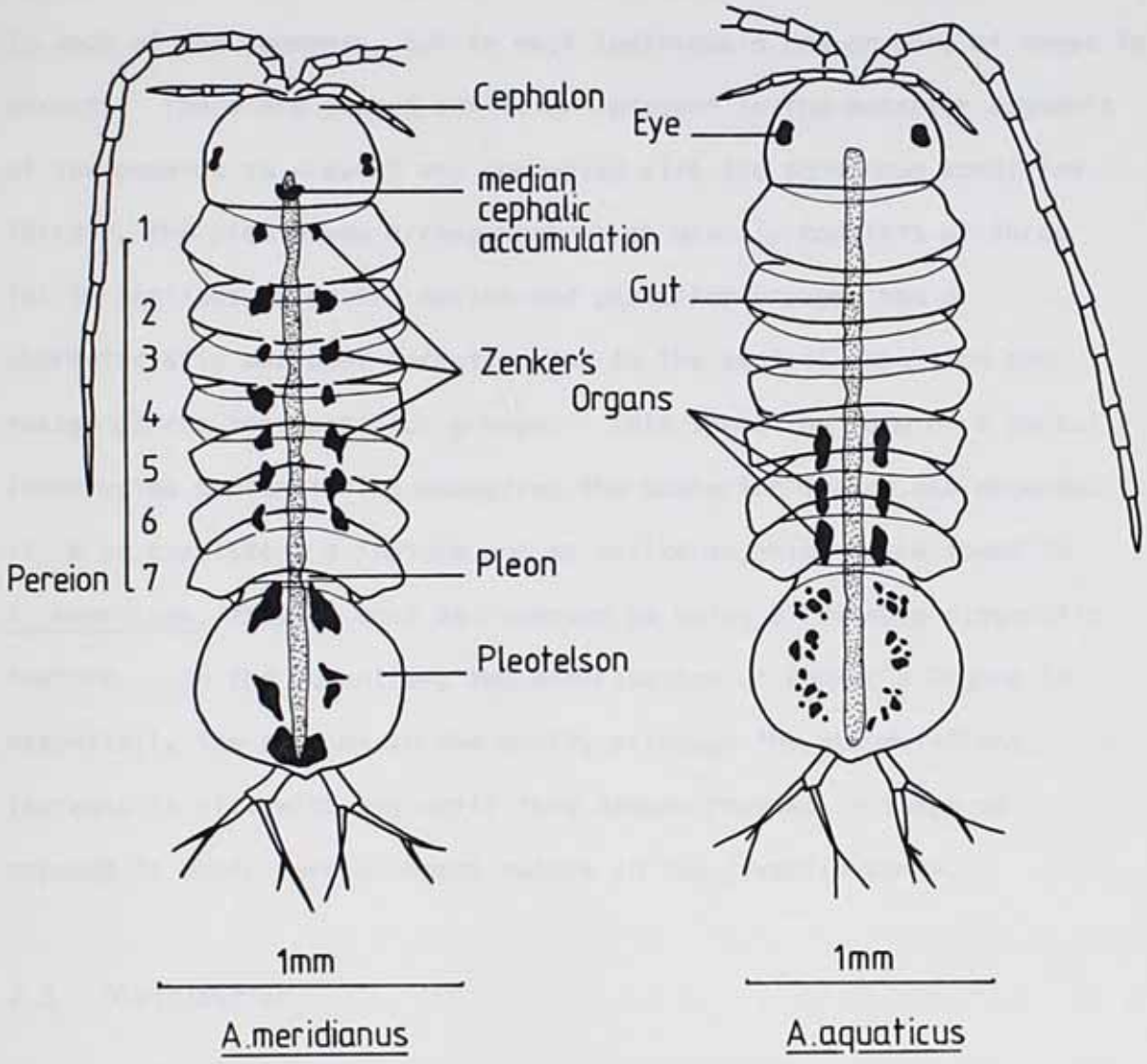


Fig.7. Diagrams showing the size and distribution of Zenker's Organs in the juvenile Asellus spp.
(From Dupey, 1967)

In A. meridianus the distribution of these accumulations is altogether different. Firstly, with the exception of a large median cephalic body, their position is ventro-lateral rather than dorso-lateral. Secondly, they are not restricted to the posterior segments of the pereion and it is possible to find a pair of small accumulations in each of the segments, but in most individuals one or more of these is absent. There are always sufficient present in the anterior segments of the pereion to prevent any confusion with the aquaticus condition. Thirdly, the pleotelson arrangement which usually consists of three fairly distinct anterior, median and posterior groups, has a characteristic perianal formation due to the ventral expansion and fusion of the two posterior groups. This takes the form of a dorsally interrupted perianal ring occupying the posterior tip of the abdomen. It is so consistent a feature and so unlike anything to be found in A. aquaticus, that it must be regarded as being a reliable diagnostic feature. In the juveniles, the distribution of Zenker's Organs is essentially the same as in the adult, although the accumulations increase in size with age until they become rounded in shape as opposed to their more elongate nature in the juvenile stage.

2.3 Distribution

Studies of the ecology of these two species (Williams 1962a, 1962b, 1963) have shown that they are widely distributed in a variety of surface localities - lakes, rivers, canals, ponds and ditches - and exhibit a similar choice in habitats, with the exception of polluted benthic situations and the public water supply mains, both of which appear to be occupied exclusively by A. aquaticus. No

significant factor had been found to control their distribution, both species being present throughout most of the British Isles, although no trace of either population has been found in N. Scotland (Williams 1962a,b).

Moon (1957a,b) thought that A. aquaticus had a more frequent association with man's activities and was, therefore, spread easily by human agencies, a factor which clearly emerged from the work of Dupey (1967) when he found a strong association between A. aquaticus and human populations. Both workers demonstrated a tendency for A. meridianus to inhabit the more isolated situations and Dupey (op. cit.) indicated that it was found more often in ponds than in streams, suggesting this was probably due to the more isolated nature of ponds rather than the fact that streams contained flowing water and ponds did not.

Hynes et al. (1960) noted that the two species displayed differences in their distribution, such that they sometimes co-exist and sometimes occur alone. There was reason to believe that populations of A. meridianus had been, or were being, replaced by A. aquaticus in some areas by competitive exclusion (Williams 1962a,b) and Hynes et al. (1965) demonstrated a strong tendency for A. aquaticus to eliminate A. meridianus in laboratory conditions which suited both species. Dupey (op. cit.) concluded from his work that the replacement of A. meridianus by A. aquaticus, in localities such as Lake Windermere, was due to specific differences in the efficiency with which the habitat was exploited, rather than by any direct effect of one species upon the other. It could not be assumed, however, that the two species could not co-exist for quite considerable periods of time. This present study revealed no co-existing populations.

2.4 Life History

The life histories of the two species of Asellus have been studied by several workers (Steel 1961; Andersson 1969; Adcock 1975, 1979) and similarities in the life cycles in various locations have been noted. Steel (op. cit.) and Adcock (1975) showed that A. aquaticus has two distinct breeding seasons, the spring cohort produced between February and June and the autumn cohort between July and Septemeber. There is no period between them when gravid females are absent, but the peaks of breeding, essentially similar in the two populations, occur in April and July. In both populations, the larger females breed first and progressively smaller females enter the breeding population as each season progresses.

Steel (op. cit.) compared the life histories of A. aquaticus and A. meridianus and analysis revealed that the species are similar in most aspects, although they show slight differences in timing. In A. meridianus there is a complete cessation in breeding between the time the old generation releases its brood and the time these young begin to reproduce. In A. aquaticus there is a transition between the two breeding periods with only a slight drop in the percentage of gravid females. The peak of breeding, as measured by the percentage of ovigerous females, is higher in A. meridianus (85%) than in A. aquaticus (67%) whose spring breeding period is spread over a slightly longer time. A. meridianus lags behind A. aquaticus in releasing its young and, although the young are released over an extended period of time, the majority of young are released from the brood pouch in May (A. aquaticus) and June (A. meridianus). Consequently, the new generation of A. meridianus starts to breed a little later.

2.5 Feeding Habits

Both Asellus species used in this study are capable of feeding on a wide range of plant and animal material (Dupey op. cit.). Their primary food source in lakes appears to consist of decaying tree leaves of which lime, elm and alder are preferentially eaten (Dupey op. cit., Prus 1971). The presence of A. aquaticus in streams containing few tree leaves, but mildly polluted with organic material such as sewage, suggests that it may also feed on other partially decayed organic matter (Adcock 1975).

In the laboratory, A. aquaticus has been grown on a diet of decaying tree leaves (Williams 1962a; Hynes et al. 1965; Prus 1971, 1972) and fine silt containing detritus from lakes (Andersson 1969). Examination of the gut contents of animals from the natural habitat shows that they ingest a variety of materials, including dead and decaying allochthonous tree leaves, aquatic macrophytes, other specimens of Asellus and exuviae, silt and detritus, and algae (Marcus et al. 1978). The general consensus of opinion seems to be that the principal food of A. aquaticus is decaying vegetable matter with its associated flora of fungi, bacteria and micro-organisms and Dupey (op. cit.) indicated that the feeding habits were almost identical in the two species.

Asellus spp. play an important role in the detritivore cycle of the food chain in that, by mechanically breaking down dead plant and animal material, the species accelerates decomposition by organisms such as bacteria (Prus 1972). They are also important in making the energy in allochthonous material available to other trophic levels of the ecosystem when Asellus is itself preyed upon by many species of freshwater fish and invertebrates (Dupey op. cit.).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Animals

From personal undergraduate work it was known that large numbers of *A. aquaticus* were to be found at Castle Ashby Lake in Northamptonshire (Grid reference SP855594) situated within the grounds of Castle Ashby Estate. This area was, therefore, used as the source of all *A. aquaticus* for the study.

Samples of *A. meridianus* were obtained from a section of the River Lathkill in Derbyshire (Grid reference SK211659). The site was chosen primarily because it was the first area where *A. meridianus* was encountered in sufficient numbers. The location of the two sampling areas is shown in Fig. 8.

The two areas warranted different sampling techniques when field collections of the animal species were made. At Castle Ashby Lake, samples of *A. aquaticus* were taken from a dense bed of *Cladophora* sp. and also from the surface of decaying leaf litter. Collections of the latter were made by skimming off the top layer of leaves with a dip net. The *Cladophora* areas were sampled by means of a grappling hook. At the River Lathkill, *A. meridianus* was present in abundance amongst the stems of *Ranunculus penicillatus* and was readily collected with a hand net.

The majority of *A. meridianus* were sorted by hand on site, but in the case of *A. aquaticus* most of the material was taken back to the laboratory for sorting. Attempts were made initially to sort *A. aquaticus* by washing the material through a coarse sieve which retained the plant remains. This was followed by a fine sieve, which retained the animals but discarded the mud. However, this method was soon abandoned because the filaments of *Cladophora* sp. proved too dense for satisfactory separation of animals. The easiest method of sorting, although tedious, was by hand.

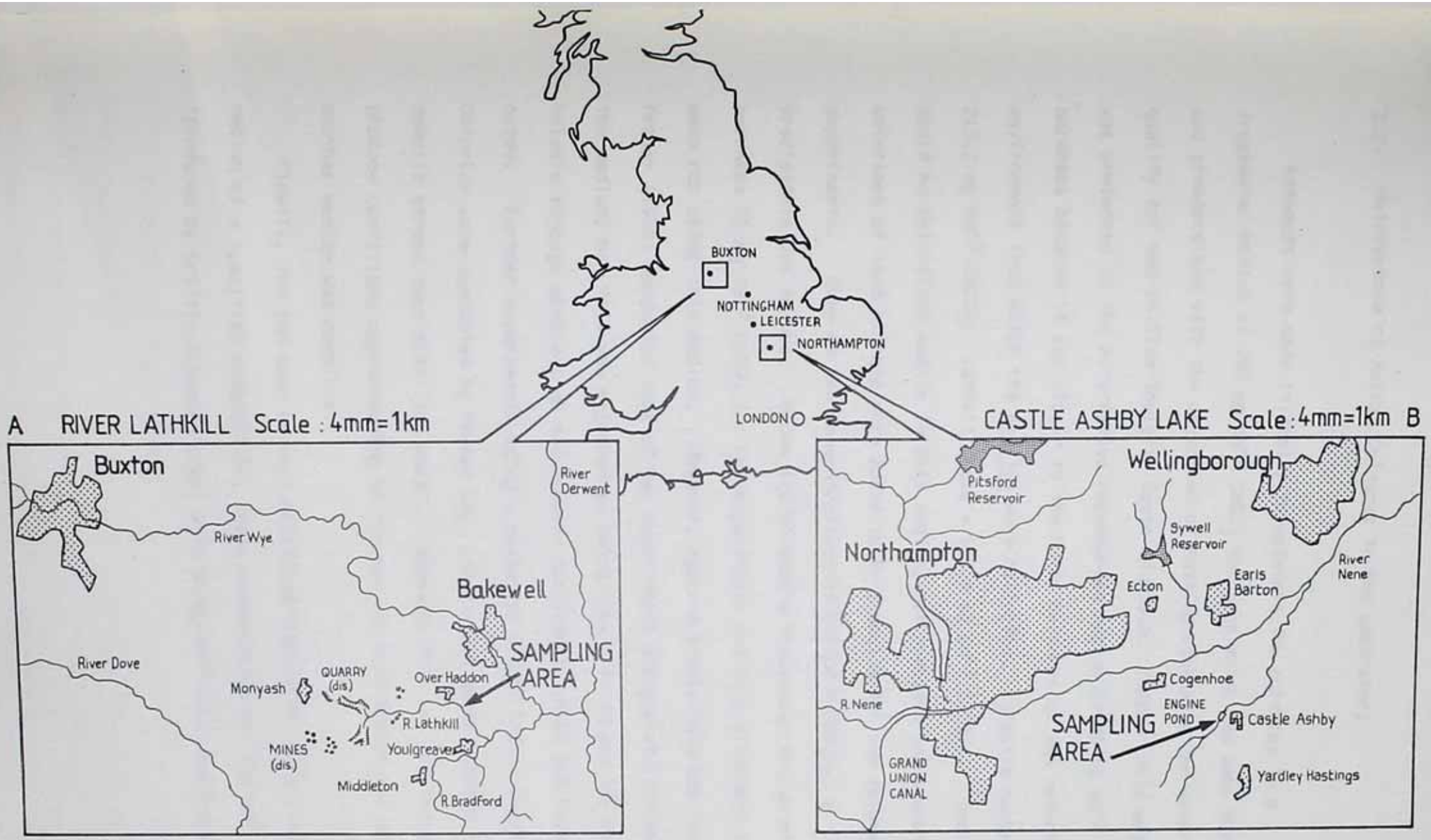


Fig.8. General maps showing the location of the two sampling areas.
(A) The River Lathkill for A. meridianus.
(B) Castle Ashby Lake (Engine Pond) for A. aquaticus.

(from O.S. maps: sheets 119 (A) and 152 (B)

3.2. Maintenance of Animal Cultures in the Laboratory

Attempts were made initially to maintain the cultures in a freshwater medium of 250 mg dm^{-3} $CaCO_3$ hardness which has been developed and standardised with the objective of setting standards for water quality for use in Fish Toxicity Tests (H.M.S.O. 1969). This medium was preferred to the alternative recommended medium of 25 mg dm^{-3} $CaCO_3$ hardness because it was closer to the total hardness of the natural environment from which the Asellus were collected. (Castle Ashby = 213.2 mg dm^{-3} $CaCO_3$: Lathkill Dale = 119.4 mg dm^{-3} $CaCO_3$). The animals could be maintained easily in this medium, but problems arose when stock solutions of lead nitrate were added to this medium for the tolerance experiments. Even at low concentrations of 0.25μM $Pb(NO_3)_2$, a precipitate was formed. Brown (1976) used a freshwater medium of total hardness 25 mg dm^{-3} $CaCO_3$ for her experiments and so preliminary tests were run using this medium. However, again a precipitate was formed. Fraser (1979) conducted some of her experiments using distilled water as the medium, but this was rejected as being likely to stress the test animals through abnormal pH and osmotic conditions, which she herself noted. Further experiments using a medium of 0.025M Analar Calcium Chloride were conducted by Fraser (op. cit.) which would produce less osmotic stress than distilled water. However, this was not thought to produce conditions approximating to the natural environment and so another medium was required.

Finally, the two species were maintained separately in a freshwater medium of a specified composition, known commercially as 'Instant Pond' (produced by Griffin & George Ltd.) with 80 mg dm^{-3} $CaCO_3$ hardness.

'Instant Pond is a mixture of the following composition:

NaCl	-	35.0g per 10 litres of solution
KCl	-	0.5g " " " " "
$CaCl_2$	-	1.0g " " " " "
$NaHCO_3$	-	0.2g " " " " "

The mixture is guaranteed free of metallic impurities.

Experiments conducted with the 'Instant Pond' medium also caused problems in their initial stages. Precipitation was observed with the addition of high concentrations of lead nitrate, though not with the lower concentrations. It was found, eventually, that stirring the 'Instant Pond' medium, prior to the addition of stock solutions of lead nitrate, reduced the amount of precipitate which remained relatively constant over a 72 hr. period. Further experimental procedures continued with the use of this freshwater medium.

Both A. aquaticus and A. meridianus were maintained in the laboratory at a temperature of 15°C and with a daylight regime of 16 hours light and 8 hours dark. The animals were acclimated to these conditions for at least two weeks before exposure to experimental solutions, during which time they were fed on soaked leaves of lime (Tilia europaea) which were preferentially eaten in Castle Ashby Lake.

3.3. General Site Areas

At the two sites chosen, the general characteristics were recorded and, as well as the Asellus spp., all other taxa were collected and identified.

The source of A. aquaticus for this study was Castle Ashby Lake in Northamptonshire, known locally as Engine Pond (Fig. 9). Engine Pond is man-made and was constructed pre 1760 to provide a head of water to drive a double action ram which raised water from a spring to Castle Ashby House (S.J. Price pers. comm.). The Lake itself lies on Upper

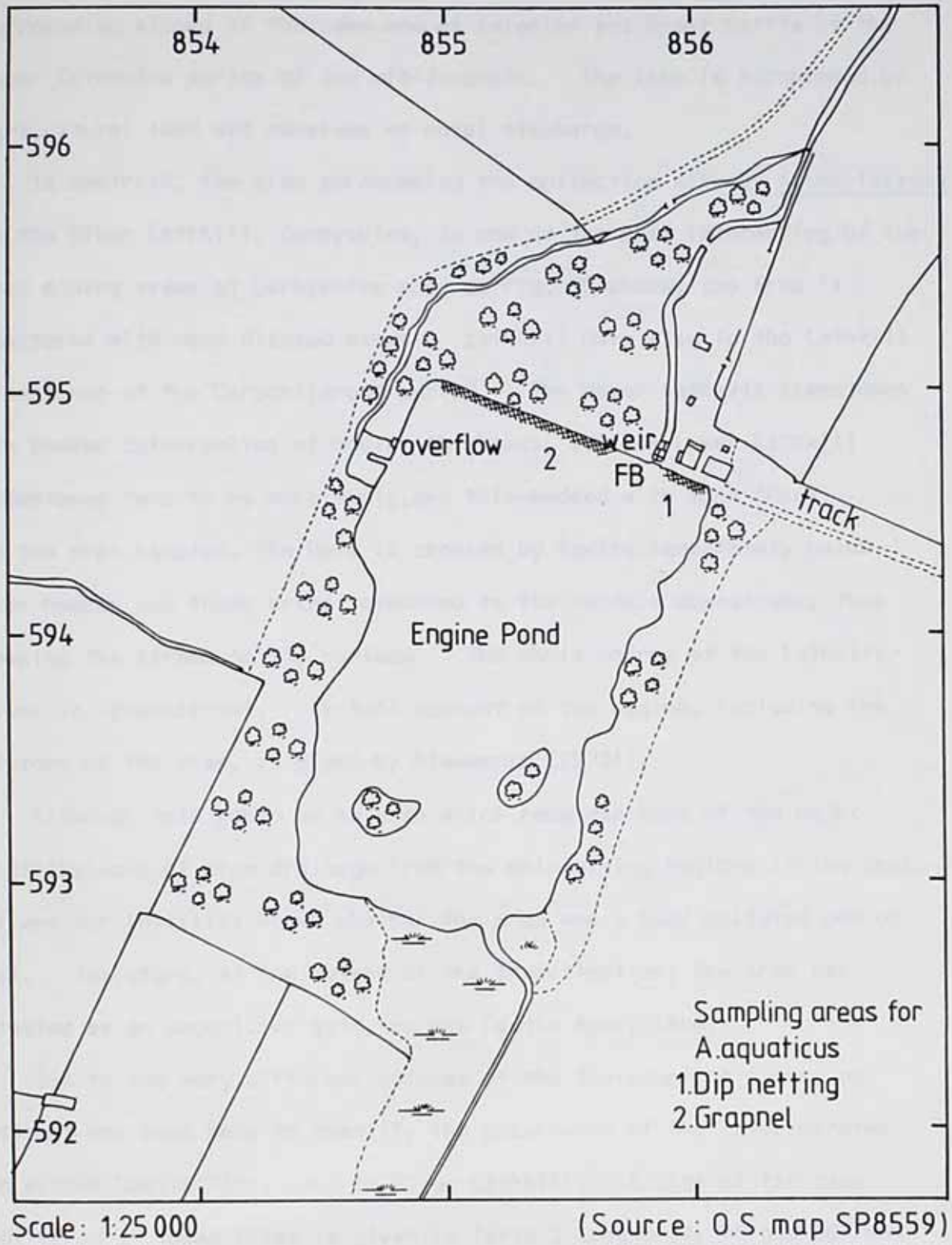


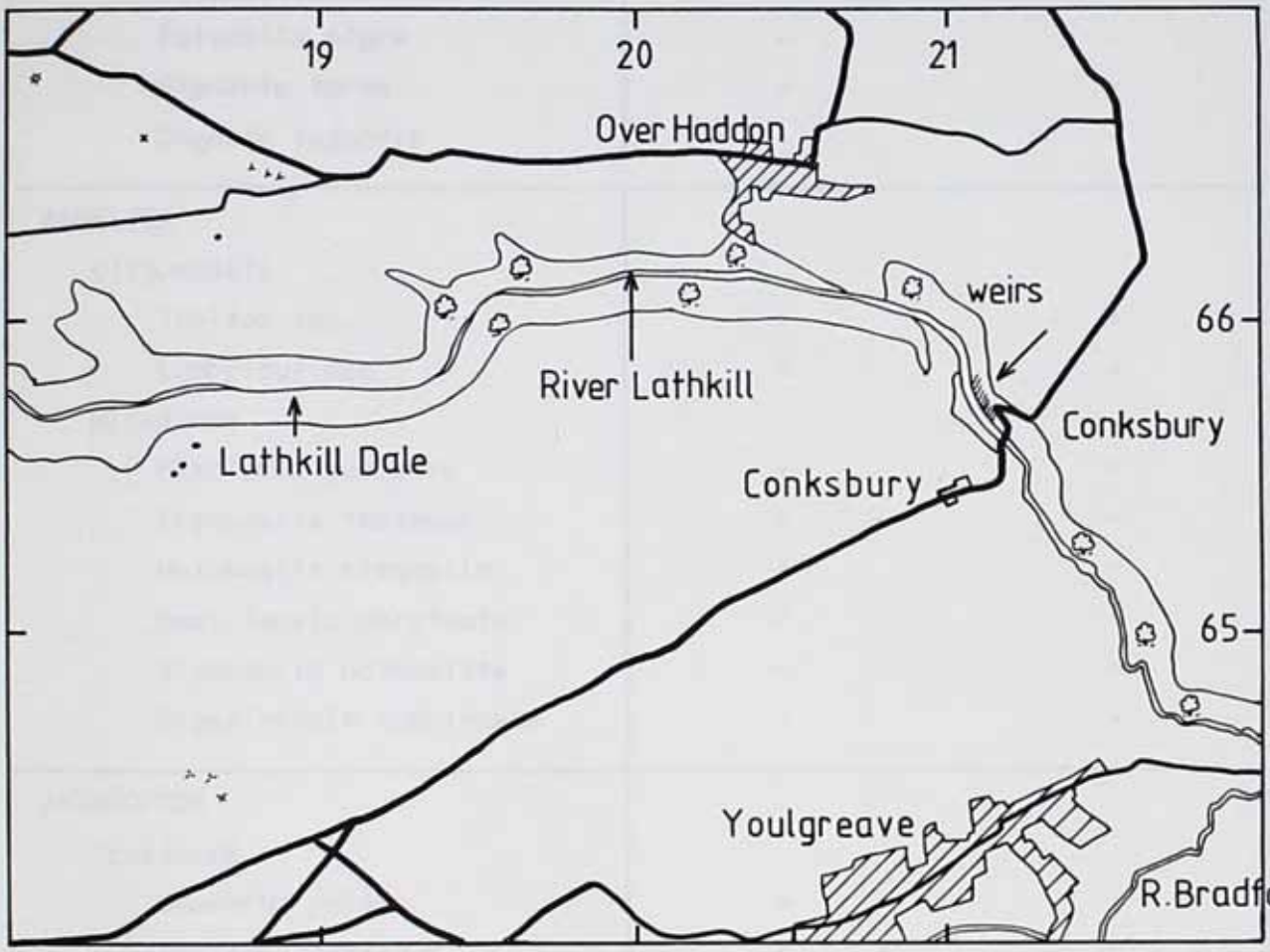
Fig.9. Map of Castle Ashby Lake (Engine Pond) showing the areas sampled for Asellus aquaticus

Lias Clay, this being the uppermost bed of the Lower Jurassic and the surrounding slopes of the Lake are of Inferior and Great Oolite of the Upper Estuarine series of the mid-Jurassic. The lake is surrounded by agricultural land and receives no metal discharge.

In contrast, the area surrounding the collection site of A. meridianus in the River Lathkill, Derbyshire, is one of the most interesting of the lead mining areas of Derbyshire and, as Fig. 10 shows, the area is scattered with many disused mines. Lathkill Dale lies in the Lathkill limestones of the Carboniferous period. The Upper Lathkill limestones are bedded calcarenites of medium thickness, but the Lower Lathkill limestones tend to be more shaly and thin-bedded with much chart. In the area sampled, the Dale is crossed by faults immediately below Over Haddon and these bring toadstone to the surface downstream, thus keeping the stream on the surface. The whole course of the Lathkill River is intermittent. (A full account of the region, including the history of the area, is given by Rieuwerts (1973)).

Although this would be an area which received most of the major contributions of mine drainage from the main mining regions in the past, it was not initially known whether the area was a lead polluted one or not. Therefore, at the outset of the investigation, the area was treated as an unpolluted site, as was Castle Ashby Lake.

Due to the very different natures of the two sampling sites, no attempt has been made to quantify the occurrence of the invertebrates in either Castle Ashby Lake or River Lathkill. A list of the taxa collected at these sites is given in Table 2 (pages 34, 35 and 36) and a comparison between the two areas can then be made. Identification of the invertebrates has been made using the F.B.A. Scientific Publications, except for the Coleoptera, Odonata and Trichoptera which have been identified to family, using Quigley (1977). Confirmation of nomenclature has been made using Maitland (1977).



Scale: 1:25 000 (Source: O.S. maps SK 16 & SK 26/36)

Area sampled

Old Lead Mines

Fig.10. Map of the River Lathkill showing the area sampled for Asellus meridianus

Species	Site	
	Castle Ashby	River Lathkill
PLATYHELMINTHES		
Dendrocoelum lacteum	+	+
Polycelis tenuis	+	+
Polycelis nigra	+	+
Planaria torva	+	-
Dugesia lugubris	+	-
ANNELIDA		
Oligochaeta		
Tubifex spp.	+	+
Lumbriculidae	+	+
Hirudinea		
Piscicola geometra	+	-
Erpobdella testacea	+	-
Helobdella stagnalis	+	-
Hemiclepsis marginata	+	-
Erpobdella octoculata	-	+
Glossiphonia complanata	-	+
ANTHROPODA		
Crustacea		
Gammarus pulex	+	+
Asellus aquaticus	+	-
Asellus meridianus	-	+
Daphniidae	+	-
Cyprididae	+	-
Cyclopidae	+	-
Chydoridae	-	+
Arachnida		
Hydracarina spp.	+	+
Insecta		
Hemiptera		
Corixidae nymphs	+	+
Notonecta glanca	+	-
Corixa punctata	-	+
Coleoptera		
Haliplidae	-	+
Dytiscidae	+	+

Table 2. List of Taxa

Species	Site	
	Castle Ashby	River Lathkill
Insecta (contd)		
Ephemeroptera (nymphs)		
Caenis spp.	+	+
Baetis spp.	+	+
Cloeon spp.	+	+
Centroptilum luteolum	-	+
Procloeon pseudorofulum	-	+
Pleocoptera (nymphs)		
Nemurella picteti	-	+
Isoperia grammatica	-	+
Odonata (nymphs)		
Coenagriidae	+	+
Megaloptera (larvae)		
Sialis lutaria	+	+
Diptera (larvae)		
Chironomidae	+	+
Culex spp.	+	-
Chaoboridae	+	-
Simulium spp.	-	+
Tricoptera (larvae)		
Polycentropodidae	+	+
Limnephilidae	+	+
Phryganea grandis	+	+
Philopotamidae	+	+
Rhyacophila obliterata	-	+

Table 2 (contd) List of Taxa

Species	Site	
	Castle Ashby	River Lathkill
MOLLUSCA		
Gastropoda		
Lymnaea stagnalis	+	-
Physa fontinalis	+	-
Planorbis planorbis	+	-
Lymnaea peregra	+	+
Viviparus viviparus	+	+
Ancylus fluviatilis	-	+
Lamellibranchiata		
Anodonta cygnaea	+	-
Unio pictorum	+	-
Dreissena polymorpha	+	-
Sphaerium spp.	+	+
Pisidium spp.	+	+
TOTAL NUMBER OF TAXA	42	36

Table 2 (contd) List of Taxa

The results indicate that there are rather fewer taxa in the River Lathkill than in Castle Ashby Lake, but this is probably the effect of three factors. Firstly, the Lathkill material was sorted in the field, so that some taxa may have been missed. Secondly, two collecting methods were used at Castle Ashby Lake as opposed to one at Lathkill. Thirdly, the Castle Ashby Lake results have been augmented by records obtained during an undergraduate project.

From the collated list, the dominant taxa recorded in the River Lathkill are insect larvae, with fewer Mollusca, Hirudinea, Oligochaeta, Crustacea and Platyhelminthes. At Castle Ashby Lake, the reverse is true with few insectum species, but more non-insectum species.

3.4. Chemical Analyses

As well as collecting the Asellus spp., samples of the sediment and water were collected from both sites to determine the background levels of lead. Heavy metal levels in sediments (Bryan and Hummerstone 1971) and in waters (Chipman 1966) are often found to reflect the metal levels in invertebrates.

3.4.1. Collection of Sediments

A core of sediment was taken from each site with a 6cm. diameter tube to a depth of approximately 10cm. and this was subsequently placed in an acid-washed plastic container. Lead has a tendency to be relatively immobile in soil which leads to an extreme vertical concentration gradient when the lead is introduced from external sources. In

view of this (since most of the lead, if any, would be within a few centimetres of the surface), considerable care was taken to maintain the vertical integrity of the sample until the sediment was analysed. The sample was separated into sub-samples and air dried prior to analysis by atomic absorption spectrophotometry.

3.4.2. Collection of Water Samples

Waters were sampled in acid-washed polythene bottles and were filtered through 0.45μM Millipore filters. The subsequent analysis of the water samples for lead therefore determined the concentration of the dissolved metal as opposed to the suspended matter. Filtered samples were then acidified to a pH of 1.0 with nitric acid. This acidification of the water samples minimises losses by precipitation or by adsorption on container walls. Boggess et al. (1980) noted that the loss of trace amounts of metal ions through adsorption on container walls is not a trivial problem and such processes account for major changes in the ionic concentrations.

The water samples were also separated into sub-samples for analysis by atomic absorption spectrophotometry.

3.4.3. Atomic Absorption Spectrophotometry (AAS)

Atomic absorption spectrophotometry is essentially a spectroscopic method for determining the concentration of a given element in a sample by measuring the absorption of radiation, at a wavelength characteristic of the element concerned, by atoms in a flame into which the element has been introduced as an aerosol.

The wavelength of the radiation used is specific for, and characteristic of, that element and is produced by an electrical discharge from a lamp with a cathode containing the element to be analysed. The aerosol can be produced from either an aqueous or organical solution, containing salts or compounds of the element. In the flame the end products are free ionic, atomic or molecular species, e.g.

$$NaCl_{(soln)} \longrightarrow NaCl_{(molecules)} \rightleftharpoons Na_{(atoms)} \rightleftharpoons Na_{(ions)}$$

Flame conditions are adjusted so as to produce a maximal, stable, population of atoms with minimal ions or molecules of the metal species being investigated. The flame therefore acts as a 'cell' or atom-reservoir through which the beam of light, of a characteristic wavelength, passes, being reduced in intensity in ± direct proportion to the number of atoms contained in it. The technique therefore requires only dissolution of the sample in an inorganic or organic solvent and no further chemical or physical manipulation. The techniques for the analysis of trace materials, e.g. lead, in biological material and in aqueous samples, by AAS, are fairly standard. Aqueous samples can be used if the concentrations of trace material are high enough, but if they are not, then the sample must be complexed and extracted and so concentrated in an organic solvent.

3.4.3.1. AAS Analysis of Vegetation and Soil

The lead extraction technique used for the leaf meterial and soil involved a wet digestion method. This has the advantage of being fairly rapid, requiring only simple apparatus and being less prone to suffer from volatilization and retention losses because of the liquid conditions and low temperatures involved. Nitric acid is much the most widely used primary oxidant for the destruction of organic matter and

throughout 'Ultrar' grade nitric acid (HNO_3) was used, which contains extremely low levels of Pb (0.01ppm. max).

Approximately 0.02g of the dried leaf material (approx. 0.1g wet weight), which was used in the food experiments, was placed in a $25cm^3$ combustion tube with $10cm^3$ of 25% HNO_3. Tubes were placed in a Block Thermostat (Grant Instrument BT4) and gently boiled for 2 hours. The resultant liquor was cooled and the digest filtered (Whatman No. 541) and made up to $100cm^3$ with distilled, deionised water.

Soil samples were treated similarly after being dried and passed through a 1.5mm sieve. Sub-samples of approximately 0.1g were digested.

Analysis on the Spectrophotometer (Perkin-Elmer 103) used standards of the appropriate concentration of Pb (as $Pb(NO_3)_2$) made up in 2.5% HNO_3 (the final 'background' of the samples). The measurements were made using the 217.0nm line with an air/acetylene flame and a slit width of 0.7mm. Concentrations were read out directly as millimolarities and then subsequently converted to μM.

3.4.3.2. AAS Analysis of Animal Material

The problem of matrix interferences is one of the main barriers to the straightforward analysis of animal tissues. Matter other than Pb produces non-atomic absorption across a range of wavelengths, including the Pb, which interferes by producing false enhancement of the absorption line with both flame and flameless analytical methods. It is assumed that comparison of samples, by taking readings at the same

wavelength with the same sample, using a hydrogen continuum radiation source enables measurement of this component so that results can be corrected. Tests were performed on the Varian Techtron AAS Spectrophotometer and standards covering the ranges of Pb most commonly found in this study showed no such interference.

Specimens of A. aquaticus and A. meridianus were oven dried at 60°C for 12 hours, weighed and placed in a 25cm^3 combustion tube with 10cm^3 of 25% HNO_3. The digestion procedure followed a similar pattern to the vegetation and soil analysis. The cooled digest was filtered and made up to 100cm^3 volume and analysis of lead content was made using a Varian Techtron AAS Spectrophotometer, which is capable of better resolution than the Perkin-Elmer 103 instrument.

3.4.3.3. AAS Analysis of Water Samples

Initially, analysis of the concentration of lead in the samples was attempted directly from the water samples.

The detection limit by flame AA for lead is 0.1μM dm^{-3} and accurate determination of levels approaching 0.24μM dm^{-3} is difficult with the Perkin-Elmer 103. All samples were found to contain Pb below this level. In these circumstances, a concentration step is necessary, as noted earlier. This method was therefore employed in analysis of the water samples from the two sites of interest.

In organic solvent extraction, the metal of interest is reacted with a complexing agent to form a metal complex which can be extracted into an organic solvent. Solvent extraction is a means of improving detection limits, as the element of interest can be concentrated upon being extracted. The use of organic solvents rather than water also

provides a sensitivity increase of 2 to 5-fold and, in addition, possible interferents are retained by the aqueous phase.

When analysing samples in organic solvents, adjustments must be made in the fuel/oxidant flow ratio as the solvent itself is a fuel. The flame is ignited as for aqueous solution, solvent is aspirated and the fuel flow reduced to obtain desired flame conditions. Adjustments to the uptake rate are also necessary. With aqueous solutions, the uptake rate used is normally 3-4cm min^{-1}, but this had to be reduced to $1cm^3$ min^{-1} with organic solvents to minimise its effect as a fuel. The AAS (Varian Techtron AAS) was set at the optimal conditions for use with organic solvents.

The solvent used was 4-methyl-2-pentanone (or methyl isobutyl ketone, MIBK) and the chelating agent was ammonium pyrrolidine dithiocarbamate (APDC). The extractant solution of 1% APDC in water was prepared fresh daily when required.

The following procedure was carried out with both the water samples and the standard solutions.

A $75cm^3$ of aliquot of sample was transferred to a volumetric flask and adjusted to a pH of 3. This pH is required, otherwise the APDC will not chelate the lead. $5cm^3$ of 1% APDC were then added to the mixture and shaken for 30 seconds. $10cm^3$ of MIBK were then added and the flask shaken vigorously for 2 minutes. The layers were then allowed to separate and water was added to bring the aqueous phase to the $100cm^3$ mark, thus standardising the amount of dissolved MIBK. The organic phase was then aspirated directly into the flame.

MIBK's solubility in water is an important consideration to take into account in an analysis (Everson & Parker 1974). The solubility of MIBK in water at $25^{o}C$ is $2cm^3$ $100cm^{3-1}$. The concentration factor

may therefore be increased by decreasing the volume of organic solvent, but the sample volume, extracted solution volume and organic solvent volume, must be kept constant for samples and standards.

3.5 Results of Chemical Analyses

Results of the lead analyses are summarised in Table 3, page 44) The values for total water hardness at each site, expressed as mg dm^{-3}, are also included, since the lethal toxicity of some heavy metals to invertebrates has been shown to be modified by water quality (E.I.F.A.C. 1976). The water hardness was determined using the Hach method (Anon (H.W.A.H.) 1977).

A ratio of mean tissue concentration/mean sediment concentration is also given which is intended as an aid to the examination of the relationship between levels of lead in the animals' tissues and levels in their environment as reflected in substrate concentrations.

The results show that in terms of the amount of lead in the animals there is a variation between the sites, with the higher lead concentrations in A. meridianus from the River Lathkill. In this species there is a significant difference ($p<0.01$) between the sexes, with the females containing significantly higher levels of lead. The females of A. aquaticus similarly have a higher level of lead than the males of the same species ($p<0.05$). The results further show significant differences between the males of the two populations ($p<0.001$) with higher levels in A. meridianus, and significantly higher levels are found in female A. meridianus than in A. aquaticus ($p<0.01$).

Site	Grid Reference	Total Water Hardness	Concentration Lead in Water	Concentration Lead in Sediments	Concentration Lead in Whole Animal Tissue		Ratio mean tissue / mean sediment
		($mg\ dm^{-3}$)	($\mu M\ dm^{-3}$)	($\mu g\ g^{-1}$)	(10 animals/replicate) ($\mu g\ g^{-1}$)		
					MALE	FEMALE	
			n = 5	n = 8	n = 2	n = 7	
CASTLE ASHBY	SP 855594	213.2	0.24 ±0.096	82.0206 ±23.3730	83.1047 ±0.7363	97.2894 ±2.1942	1.0997
RIVER LATHKILL	SK 211659	119.4	0.54 ±0.087	1782.5728 ±100.8409	110.8650 ±0.2496	163.3540 ±1.7070	0.0769
Difference and Significance			0.30 ± 0.13 *	1700.55 ± 103.51 ***			

Table 3a. Results of chemical analysis of waters, sediments and A. aquaticus and A. meridianus from Castle Ashby Lake and River Lathkill.

Species and Sex	A. aquaticus ♀	A. meridianus ♂	A. meridianus ♀
A. aquaticus ♂	*	***	***
A. aquaticus ♀		*	**
A. meridianus ♂			**

Table 3b. Significance levels of difference as indicated by t-tests.
*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Lead concentrations in both water samples are low, but significantly higher levels are recorded in the River Lathkill samples. However, although Chipman (op. cit.) stated that metal levels in the water are often reflected in the levels in invertebrates, levels in water are subject to considerable fluctuations. Thus, samples taken at one time only are probably an unreliable guide to the amount of lead available to an animal.

Sediment levels remain more constant and are probably particularly important to detritus feeders such as Asellus. The concentration of lead found in the River Lathkill sediments is considerably higher than in the Castle Ashby sediments ($p<0.001$ - see Table 3). However, only the Castle Ashby samples are sufficiently similar to suggest that the concentrations of lead in animals and sediments (as indicated by the ratios - Table 3) may be directly related. Concentrations in the River Lathkill samples are not so obviously related to those of the sediment.

As indicated in Table 4 (page46), similar results have been found by other research workers. Eyres et al. (1978) found a clear trend of increasing tissue concentration with increasing substrate levels for lead in A. aquaticus. However, while the 'Index of accumulation' (which they used as determined by mean tissue concentration/mean substrate concentration) approximated to unity for lower substrate concentrations, this fell dramatically at higher sediment levels.

Fraser (op. cit.) also observed increasing tissue concentration with increasing sediment concentration, and higher ratios with the lower sediment concentration, but none of the ratios approximated to unity.

In the work of Brown (op. cit.) levels of lead in A. meridianus showed considerable variation between sites, but only those from the River Gannel exhibited any significant levels of lead, with animal tissue

Source of Reference	Site	County	Concentration of Lead in			Ratio mean tissue / mean sediment
			Waters ($\mu M\ dm^{-3}$)	Sediments ($\mu g\ g^{-1}$)	Animals ($\mu g\ g^{-1}$)	
Eyres and Pugh-Thomas (1978)	Irwell	Lancashire	2.89	236.1 – 13904.5 (range)	*Asellus aquaticus* 221.4 – 595.5 (corresponding range)	1.0664 – 0.0428
Fraser (1979)	Calder	Lancashire	ND	362±80	*Asellus aquaticus* 43±6	0.12
	Woodplumpton	"	ND	84±16	17±3	0.20
Millman (This study)	Castle Ashby	Northamptonshire	0.24± 0.096	82.02±23.37	*Asellus aquaticus* 83.10±0.74 97.29±2.19	1.0132 1.1862
	Lathkill	Derbyshire	0.54± 0.087	1782.57±100.84	*Asellus meridianus* 110.87 ±0.25 163.35±1.71	0.0622 0.0916
Brown (1976)	Hayle(1)	Cornwall	<0.485	112±10	*Asellus meridianus* 0.1	0.0009
	Hayle(2)	"	<0.485	258±31	0.1	0.0004
	Hayle(3)	"	<0.485	180±15	0.1	0.0006
	Gannel	"	0.918	6614±395	466±48	0.071
	Bradwell	Essex	<0.485	56	0.1	0.0018
Bryan & Hummerstone (1971)	Plym	Devon	ND	44	*Nereis diversicolor* 5.9	0.13
	Dart	"	ND	154	4.4	0.03
	Avon	"	ND	35	3.4	0.10
	Camel	Cornwall	ND	21	0.7	0.03
	Tamar	"	ND	299	5.8	0.02
	Restronguet Creek	"	0.097	359	3.5	0.01

ND - No data cited in reference

Table 4. Comparison of lead analyses of waters, sediments and animals obtained by other workers.

concentrations generally reflecting the relative amounts of metal in the sediment and waters at that site.

Various workers have put forward suggestions as to why there may be no obvious correlation between concentrations in the animals and sediments, even if the sediment is the principal source of the metal. Bryan et al. (op. cit.) suggested that the concentration of metal in the sediment which is actually available to the animal need not be related to the total concentration. The animal may also be able to regulate the concentrations of metals in its tissues. Eyres et al. (op. cit.) interpreted the lack of correlation in terms of reduced uptake or improved excretion of metals at high levels.

Although at this stage it is not appropriate to speculate on the mechanisms that might be involved, the results from this chemical analysis seem to suggest that A. meridianus sampled from an area of significantly higher sediment lead levels may have an ability to prevent the accumulation of lead in their tissues.

CHAPTER FOUR

TOXICITY AND UPTAKE OF LEAD IN *ASELLUS*

4.1. Introduction

Water quality criteria for some pollutants or potential pollutants of freshwaters, consistent with the protection of aquatic life, are derived from toxicity test data on representatives of the major components of freshwater food webs, including macroinvertebrates (Murphy, undated).

The deleterious effects of pollutants may result not only from toxicity that directly causes the death of an organism, but also from a variety of sublethal effects. Sublethal impairment of an animal's development, or its capacity to perform and adapt can reduce (1) the chances for survival and (2) the potential for growth and reproduction. In this study, toxicity tests were carried out, using both species of Asellus, to establish the acute lethal concentration (the concentration at which death is caused specifically by direct action) and the sublethal concentration (the concentration below the level which directly causes death) of the heavy metal pollutant lead.

Following on from these experiments, a further study was undertaken to monitor uptake of lead in both species. Many potentially toxic substances possess properties which make them readily available for accumulation by freshwater organisms and this may then lead to the development of tolerance to the pollutant in question. Experiments were therefore designed to include the uptake of lead from both solution and food to determine whether there is accumulation of lead and whether tolerance develops as a result.

4.2 Toxicity Tests.

4.2.1. Methods

The toxicity tests involved exposing groups of animals of each species to a range of different lead concentrations. The tests were carried out using a standard freshwater medium (Instant Pond), under controlled temperature (15°C) and light conditions (16 hours light, 8 hours dark). The animals were acclimated to these conditions for 2 weeks before exposure to the experimental solutions, during which time they were fed on soaked lime (Tilia europaea) leaves. Throughout the toxicity experiments, food was not provided since metals have been shown to be less toxic with food present (Biesinger et al. 1972). In these experiments the concentrations producing an acute toxic effect were required.

A 1 M stock solution of 'Analar' lead nitrate was prepared in double distilled water and stored in a polythene container. Stock solutions were then added, by progressive dilution, to the freshwater medium to give concentrations of 500μM, 250μM, 100μM, 50μM, 40μM, 20μM, 10μM, 7μM, 5μM, 2μM, 1μM, 0.5μM and 0.25μM $Pb(NO_3)_2$. A control treatment was also used. Attempts were made to prepare higher concentrations than 500μM, but in all cases unidentified precipitates were formed. Static tests were employed in this instance, since they require comparatively small volumes of solutions and because equipment to set up constant flow experiments was not available.

400cm^3 of each test solution were placed in separate polythene beakers and 10 animals (4mm - 6mm length) of a single species were

added to each container. This size range of animals was used since it was apparent that in both species it was the most abundant size range available for experimentation.

Metals tend to be adsorbed on to the surfaces of test vessels and are also accumulated by the test animals themselves. Consequently, there is a reduction in the nominal concentrations during the course of the test (Warnick & Bell 1969). To compensate for this, the test solutions were renewed every 72 hours, thereby reducing the dilution of lead by these processes. A further advantage of renewing solutions was that any stress caused by accumulation of excretary products and depletion of oxygen would be reduced.

Appropriate standards were made up and the concentrations at 72 hours were measured against these. The measured concentrations were determined by direct Atomic Absorption Spectrophotometry as described in Section 3.4.3. The results (Table 5 (page53) showed that the lead levels generally fell slightly, but by less than 10%.

The test animals at each concentration were observed every 24 hours when any mortalities were recorded and the dead animals removed. The tests were continued until 100% mortality was recorded in the two or three highest concentrations.

4.2.2. Results

The percentage mortalities for both species for each 24 hour period are presented in Appendix A1. The tests on A. meridianus ceased after 14 days, when the two highest concentrations showed 100% mortality. Those on A. aquaticus continued to day 21, by which time the three

Starting concentration at 0 hours (μM)	Measured concentration at 72 hours (μM)
500	483.0
400	356.0
200	197.5
100	91.0
70	60.5
50	41.0
20	20.0
10	9.5
5	4.5

Table 5. Measured concentrations (μM) of lead in test solutions 0 and 72 hours after renewal of test medium as determined by direct atomic absorption spectrophotometry. (Appropriate standards were made up and the concentrations were measured against these).

highest concentrations showed 100% mortality. The 3-day, 10-day and 14-day percentage mortalities for both species and the 21-day mortalities for A. aquaticus are presented graphically in Fig. 11 and 12. In both species, mortality increases with increasing concentration of lead and eye-fitted lines suggest a more rapid response in A. meridianus.

The percentage mortalities were then converted to probit values, the effect of which is to transform a sigmoid (cumulative normal) curve into a straight line when plotted against the log of the dose (Fig. 13 and 14). For easier graphical representation, the x-axis has been converted to a log (x + 1) scale. (For the method of calculation of probits, see Appendix A2).

The graphs showing the probit regression lines (Fig. 13 and 14) have been corrected for control mortality when necessary and the resulting regression equations are presented in Table 6 (page 55). A 3-day probit line was not obtained for A. aquaticus because the percentage mortality was negligible at the concentrations used and lay below the required range of 2.5 - 7.5 probit values (Finney 1971).

As Fig. 13 shows, the three probit lines for A. aquaticus are roughly parallel indicating a general similarity of slope which is an essential condition for an analytical dilution assay (Finney, op. cit.). t-tests (Table 7, page 56) prove that the slopes are not significantly different from each other, which satisfied this condition , In A. meridianus, the degree of parallelism is still significant, although the 14-day line is only marginally so. The tendency towards deviating from this can be accounted for by the marked increased mortality observed in the six highest concentrations.

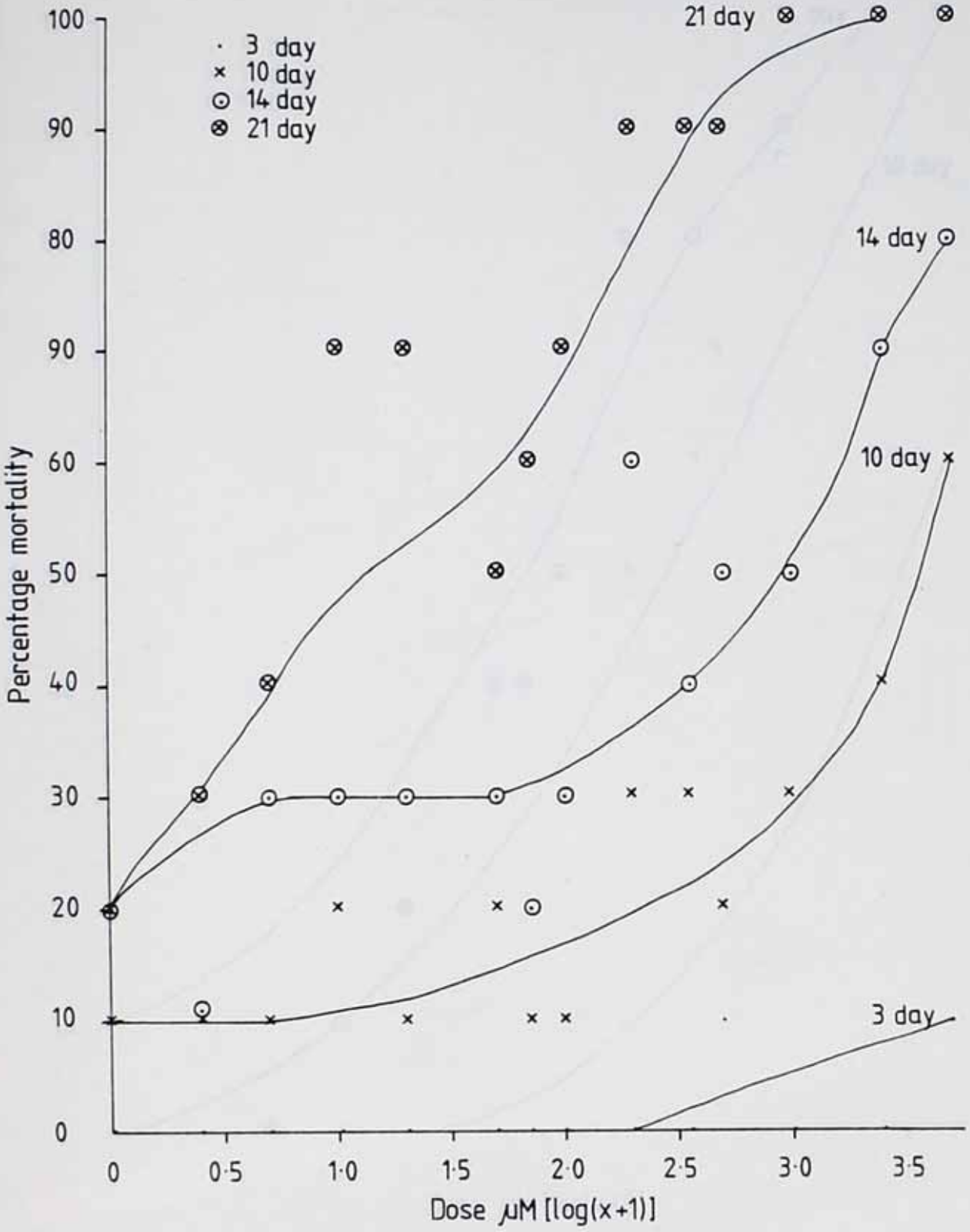


Fig.11. Graph of percentage mortality against concentration of lead over a period of 21 days in A.aquaticus (both sexes)

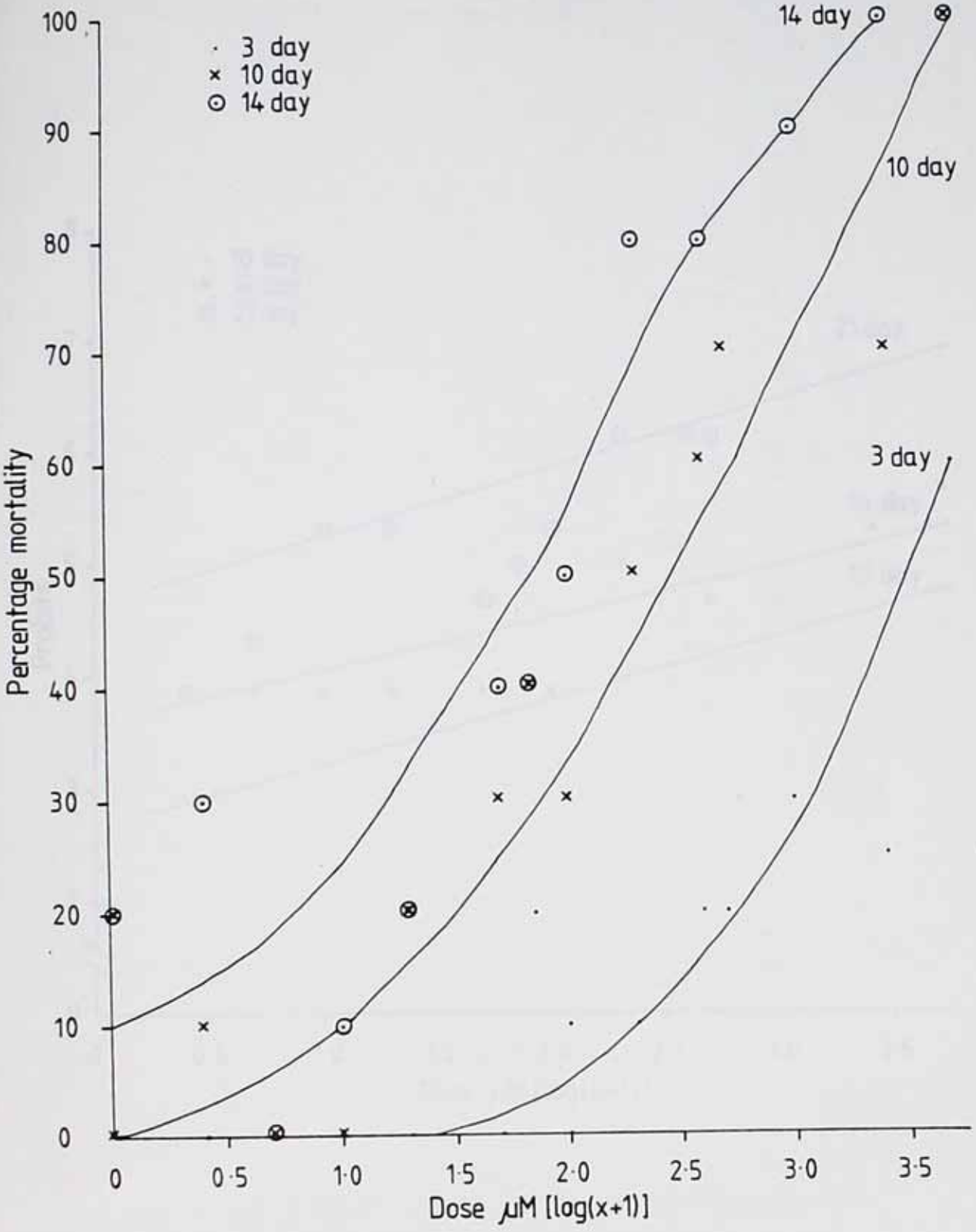


Fig.12. Graph of percentage mortality against concentration of lead over a period of 14 days in A.meridianus (both sexes)

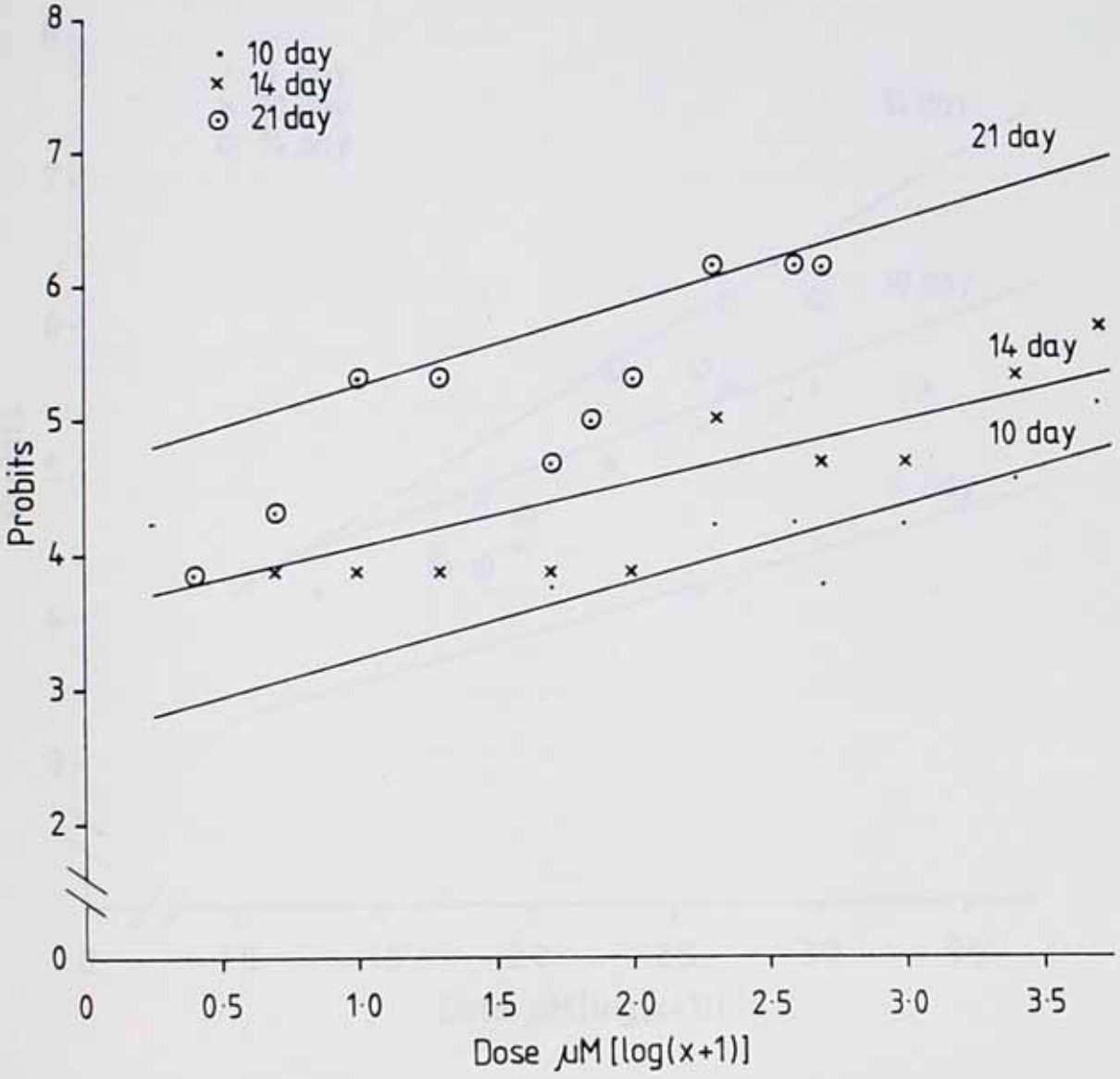


Fig.13. Graph of probits (from percentage mortality) against concentration of lead in A.aquaticus (both sexes)

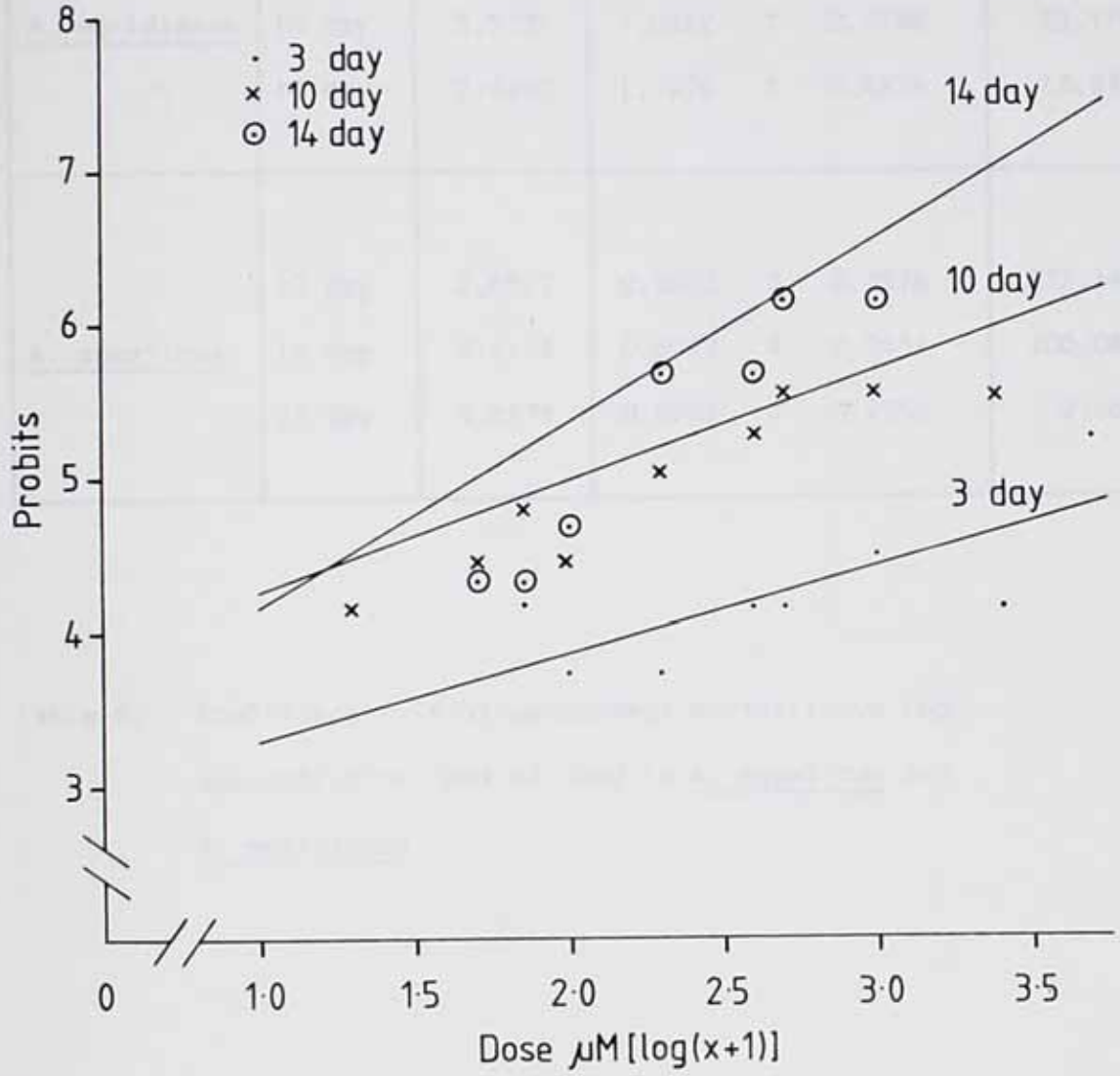


Fig.14. Graph of probits (from percentage mortality) against concentration of lead in A.meridianus (both sexes)

Species	Time	a	b	±	s.e.	LC_{50} (μM)
	3 day	2.7676	0.8199	±	0.1992	528.0103
A. meridianus	10 day	3.5731	1.0462	±	0.1746	23.1102
	14 day	2.9490	1.7976	±	0.4274	13.8352
	10 day	2.6527	0.8603	±	0.3578	535.1490
A. aquaticus	14 day	3.6114	0.6942	±	0.2431	100.0844
	21 day	4.6575	0.8999	±	0.2256	2.4021

Table 6. Probit analysis of percentage mortality on log. concentration (μM) of lead in A. aquaticus and A. meridianus.

Species	Time	b	t	p
A. meridianus	3 day 10 day	0.8199 1.0462	0.8543	NS
	10 day 14 day	1.0462 1.7976	1.6275	NS
	3 day 14 day	0.8199 1.7976	2.0734	NS
A. aquaticus	10 day 14 day	0.8603 0.6942	0.3840	NS
	14 day 21 day	0.6942 0.8999	0.6202	NS
	10 day 21 day	0.8603 0.8999	0.0936	NS

Table 7. Comparison of slopes obtained from Table 6.

LC_{50} values were calculated using the regression lines for each probit and the resultant figures are shown in Table 6. These results show a marked difference between the two species suggesting a greater tolerance to lead in A. aquaticus. The observed lead tolerance in A. aquaticus was approximately 23 times greater than that found in A. meridianus as indicated by the 10-day LC_{50} values, although the 14-day values only indicate a 7-fold increase.

The approximate lethal threshold concentration for both species was also obtained. Sprague (1969) stated this "as the concentration at which acute toxicity ceases" and "is that level of the environmental entity beyond which 50% of the population cannot live for an indefinite time." From the data in Appendix I, approximate values for the lethal threshold concentration have been determined on the basis that, over the whole period of the experiment, less than 50% mortality was recorded in both species at the two lowest concentrations. Hence the lethal threshold concentration was taken as being not far removed from 0.5μM $Pb(NO_3)_2$.

4.3. Sexual Differences in the Toxicity Tests.

4.3.1. Methods

In both species the background levels of lead, determined from the chemical analyses (Section 3.5), have shown clear sexual differences. Consequently this experiment was conducted to investigate the possibility that these differences might be reflected in their response to toxicity testing.

On the basis of the first experiments, using a wide range of doses, LC_{50} values were established. After 14 days an LC_{50} value for A. aquaticus of 100.08μM was obtained with a corresponding value of 13.83μM Pb for A. meridianus. As a consequence, these tests were then conducted with the idea of confirming which concentrations would confer a relatively low mortality over a 14-day time period.

The two sexes of both species were initially separated and acclimated at 15°C for 2 weeks in the freshwater medium before exposure to the experimental solutions. As in the previous experiment, they were fed on soaked T. europaea leaves prior to experimentation, but throughout the experiment food was not provided. Only non-gravid females were used.

This experiment followed the same procedure as in Section 4.3. 400cm^3 of test solution were placed in polythene beakers and 10 animals of a single sex and single species were added to each container, thus giving 4 test conditions - male A. aquaticus, female A. aquaticus, male A. meridianus and female A. meridianus.

Concentrations of 50μM, 10μM, 5μM, 1μM and 0.5μM $Pb(NO_3)_2$ were used in this experiment along with a control treatment. The test solutions were again renewed every 72 hours. After every 24 hour period the test animals at each concentration were examined. Any dead animals were recorded and removed.

4.3.2. Results

The percentage mortalities for the two sexes of both species are presented in Appendix A3. As with the previous experiment, 3-day, 10-day and 14-day percentage mortalities are presented as scatter graphs in Fig. 15 and 16.

Although the mortality tended to increase with time, neither sex reached very high mortalities in any concentration. The resulting probit regression equations, set out in Table 8 (page 59a) also indicate that relatively low mortalities are produced at the lower dosages.

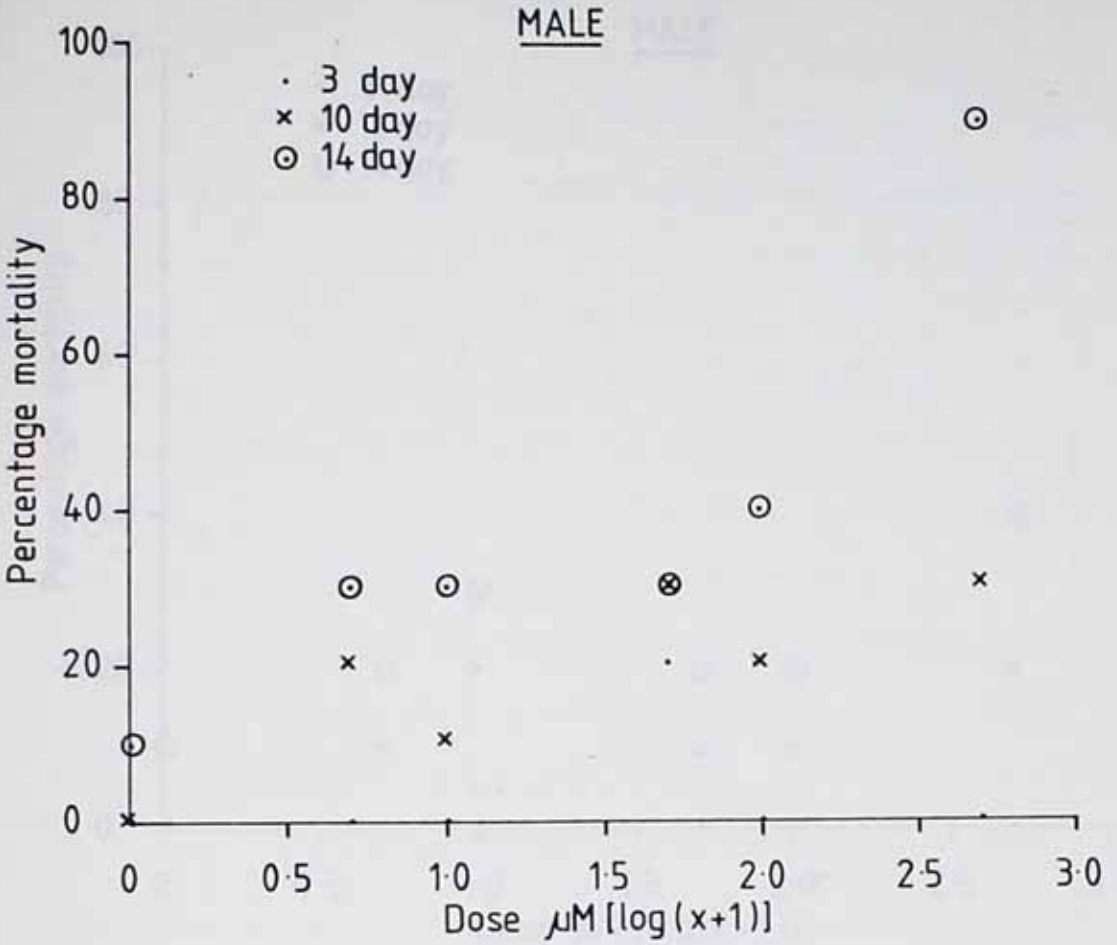
Differences between the sexes are not apparent from these results and, therefore, there is no justification in separating the sexes in future experiments.

In establishing a lead level to be used in metal uptake experiments, the results indicate that up to 70% survival may be expected over a 10-day period in a 5μM $Pb(NO_3)_2$ concentration in A. aquaticus, whilst a similar survival rate can be expected in A. meridianus.

4.4. Metal Uptake from Solution

4.4.1. Methods

This experiment was carried out to investigate whether the two species can accumulate lead from solution. The intention was to ensure that the animals stayed alive, but were exposed to a relatively high dosage. The results from the toxicity tests (Section 4.3.2)



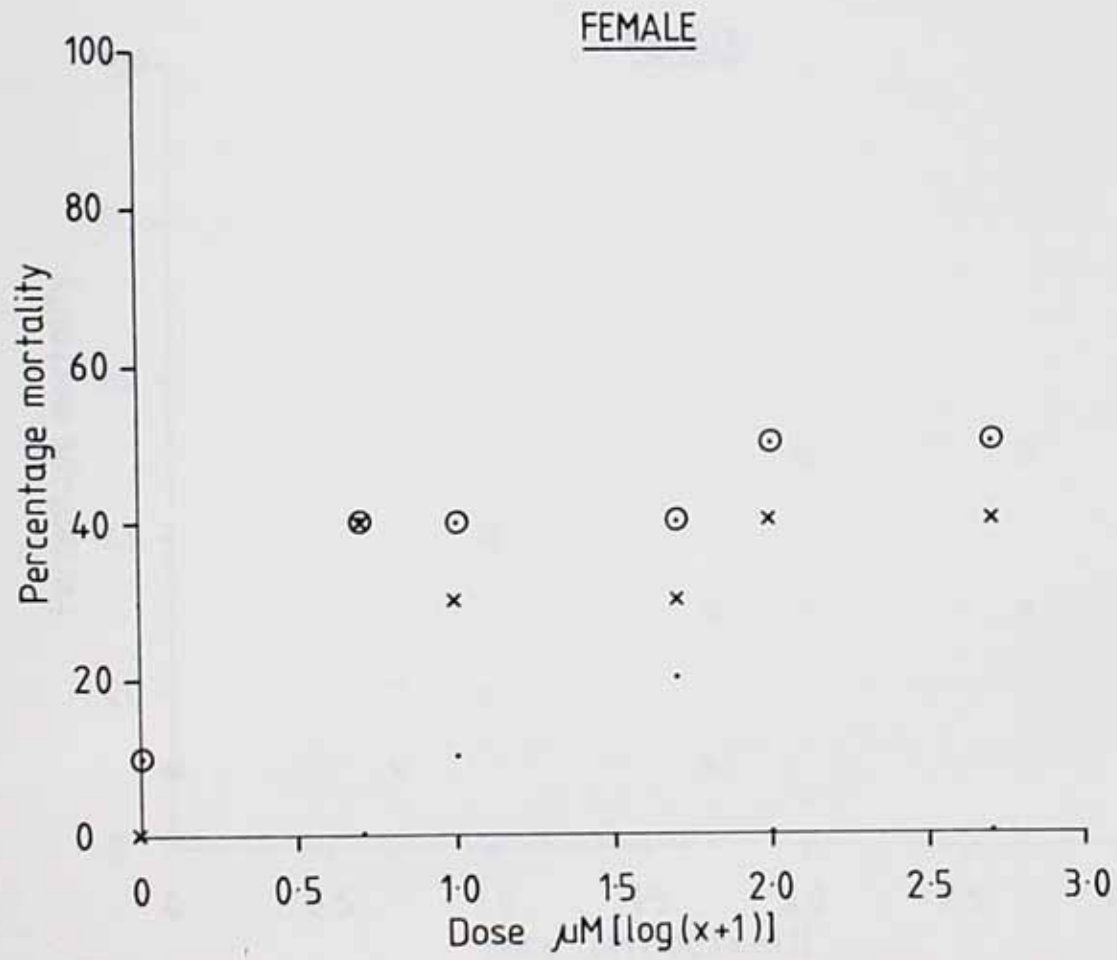
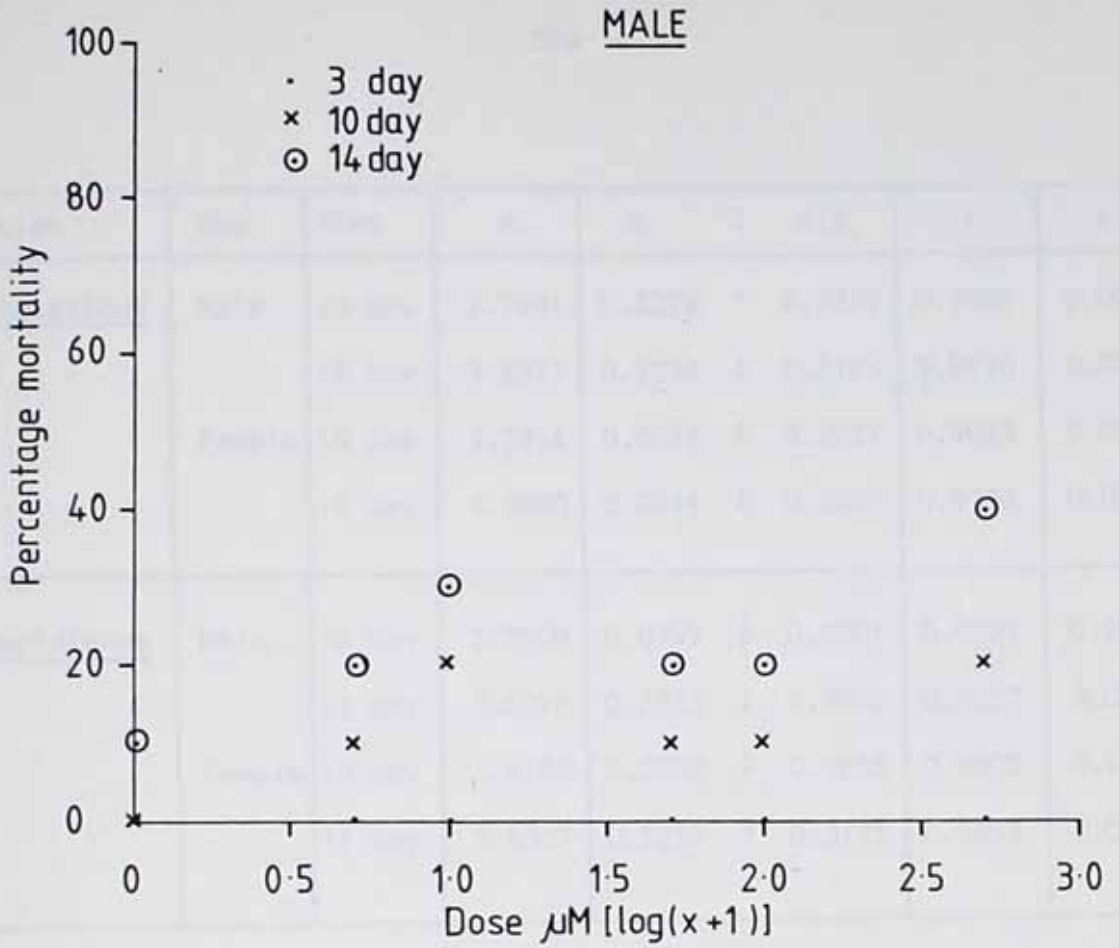


Fig.15. Graphs of percentage mortality against concentration of lead over a period of 14 days in A.aquaticus



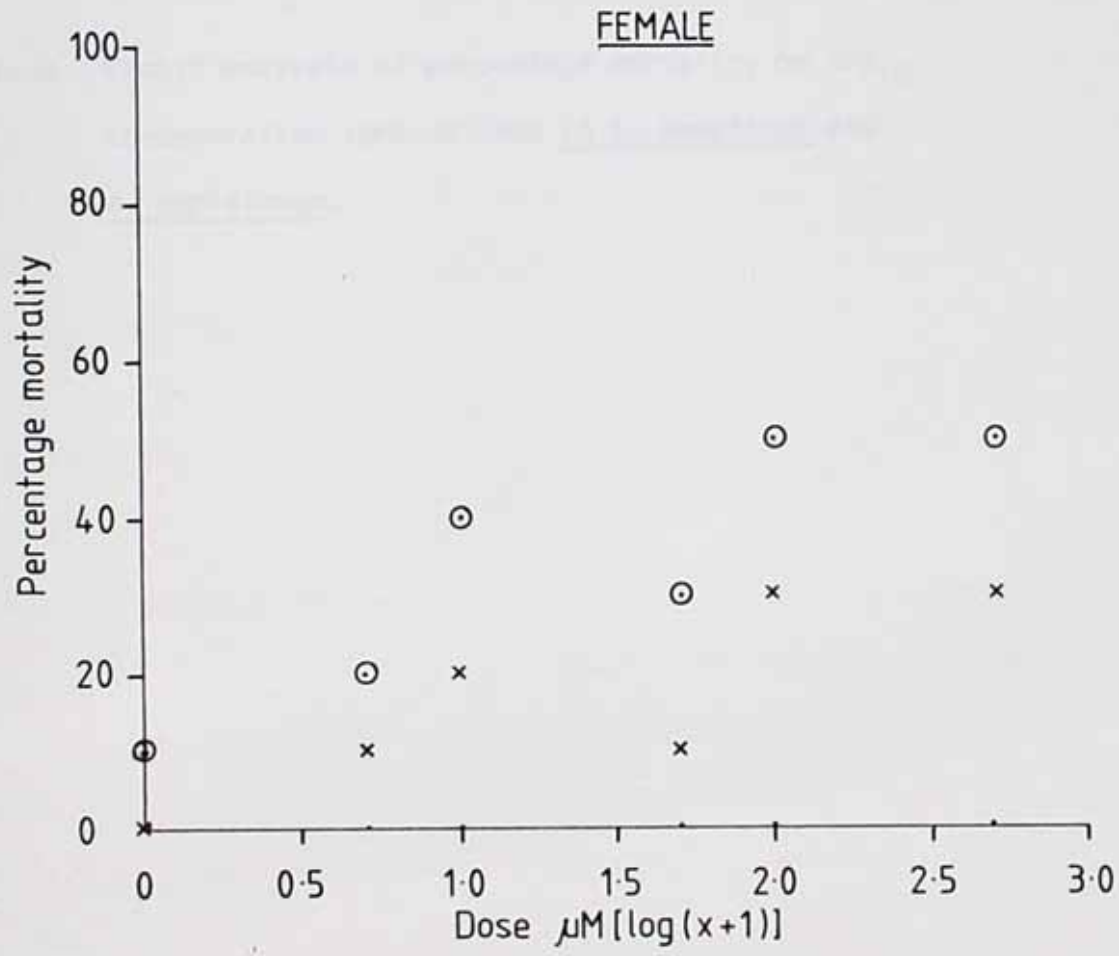


Fig.16. Graphs of percentage mortality against concentration of lead over a period of 14 days in A.meridianus

Species	Sex	Time	a	b ± s.e.	r	p
A. aquaticus	Male	10 day	3.7891	0.2228 ± 0.2837	0.9964	0.005
		14 day	3.8911	0.3274 ± 0.3125	0.9976	0.005
	Female	10 day	4.5914	0.0637 ± 0.2524	0.9023	0.05
		14 day	4.5840	0.0944 ± 0.2855	0.9752	0.025
A. meridianus	Male	10 day	3.7909	0.0799 ± 0.3001	0.9227	0.05
		14 day	3.6076	0.2723 ± 0.3925	0.9527	0.025
	Female	10 day	3.6169	0.3239 ± 0.2853	0.9802	0.01
		14 day	3.8357	0.3235 ± 0.3125	0.9857	0.01

Table 8 Probit analysis of percentage mortality on Log_{10} concentration (μM) of lead in A. aquaticus and A. meridianus.

indicate a relatively high survival rate over 10 days at 5μM $Pb(NO_3)_2$. This concentration was therefore selected to study uptake of lead from solution in the two species.

The test solutions were again renewed every 72 hours to minimise loss of lead by precipitation, adsorption and uptake. $400cm^3$ of 5μM $Pb(NO_3)_2$ and control solutions were made up, as in the preceding section, and were placed in polythene beakers. Each set of solutions was prepared in duplicate for each species and 100 intermoult animals (4mm - 6mm length) were then added to each container. Intermoult animals were selected since the period during a moult is a period of stress which might lead to anomalous results being produced. The animals were acclimated as described in Section 4.2.1, but they were also starved for one week, prior to experimentation, to reduce contamination from the gut contents.

Ten animals were removed each day, from each beaker, to give a single sample for lead analysis from each of the test solutions over a 10-day period. Each group of whole animals was then analysed for lead content as described in Section 3.4.3.2.

4.4.2. Results

The concentration of lead recorded in the whole animal tissue is shown in Fig. 17 for both species. The control animals contained concentrations of lead similar to those recorded in Table 3 and again show that A. meridianus has a higher natural background level of lead than A. aquaticus. Both species had higher levels at the start than

in the initial few days of the experiment. Only in the last 2 days did the lead level in A. meridianus exceed its background level. In contrast, in A. aquaticus, although initially the level dropped, the concentration then increased with time and at the end of the experiment there was a marked accumulation of lead.

Regression lines have been calculated for the concentration of lead accumulated ($\mu g\ g^{-1}$) over time (Fig. 17) and the equations are given in Table 9 (page 62). A. aquaticus shows a significant correlation between the amount accumulated and the time period covered. In A. meridianus the uptake of lead from solution does not attain significance.

4.5. Metal Uptake from Food

4.5.1. Method

Section 4.4. examined uptake from solution. A further method of uptake could be from food and, since Asellus feeds principally on decaying vegetable matter, it was decided to investigate whether the two species can accumulate lead from their food sources.

Tilia europaea leaves, obtained from Castle Ashby Lake, were provided as food and were soaked in 0.1 M lead nitrate solution for 24 hours prior to the experiment. 100 intermoult animals were used for each run of the experiment. 10 animals of each species were placed in individual beakers containing $400cm^3$ of freshwater medium, thus providing 10 beakers for each species. Sub-samples of the soaked leaves, $0.1 \pm 0.0003g$ wet weight, were then removed and placed in the

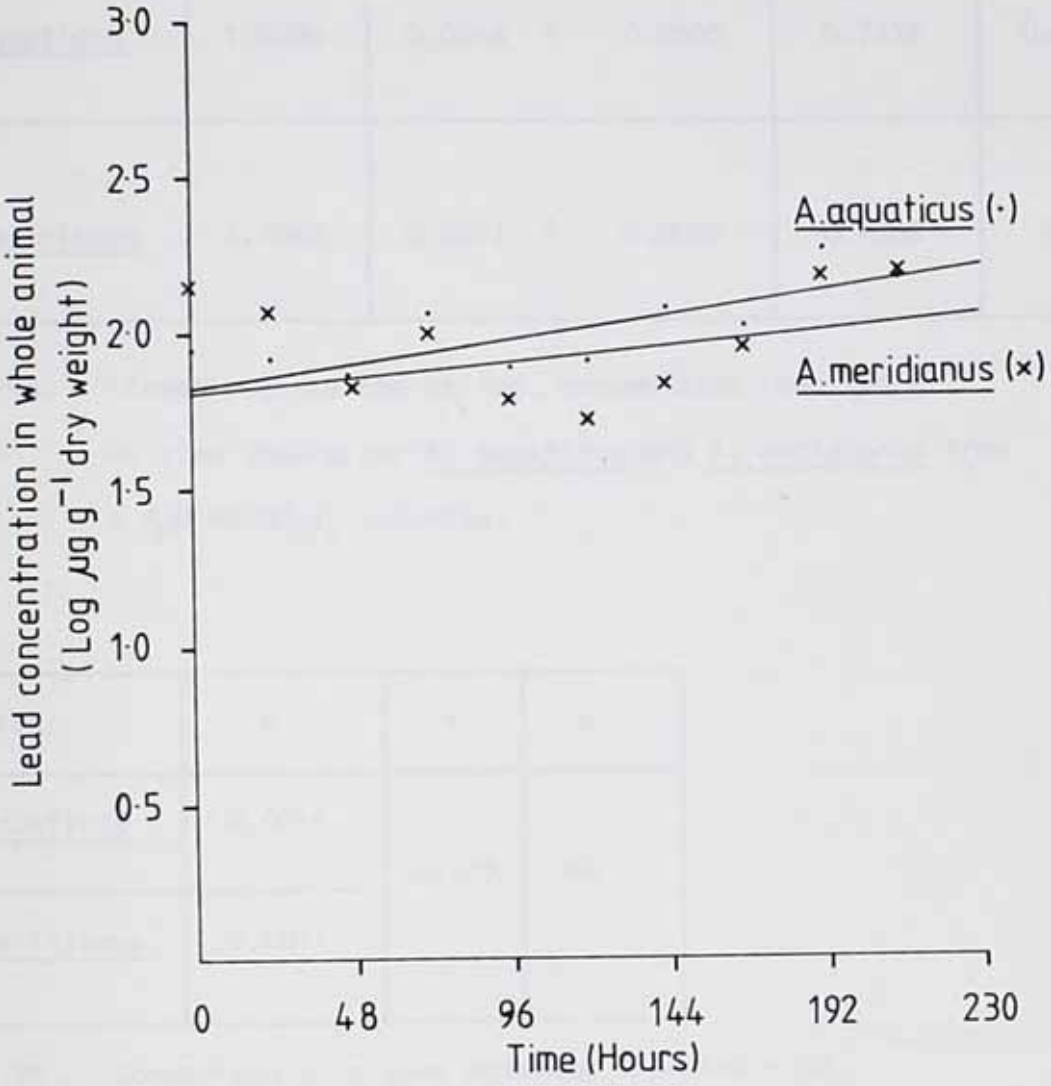


Fig.17. Graph of uptake of lead from a 5µM $Pb(NO_3)_2$ solution (means of two samples) in the whole animal

Species	a	b ± s.e.	r	p
A. aquaticus	1.8296	0.0016 ± 0.0005	0.7538	0.05
A. meridianus	1.7983	0.0011 ± 0.0009	0.4266	NS

Table 9a. Linear regressions of log. accumulated lead ($\mu g\ g^{-1}$) on time (hours) for A. aquaticus and A. meridianus from a 5μM $Pb(NO_3)_2$ solution.

Species	b	t	p
A. aquaticus	0.0016	0.125	NS
A. meridianus	0.0011		

Table 9b. Comparison of slopes obtained from Table 9a.

individual beakers. The leaves were then replaced with fresh supplies every 72 hours during the experiment, when the medium was also renewed. The experiment was maintained for 9 days.

This procedure was similar to that of Brown (1977) in an attempt to compare results directly.

Samples of 10 animals (1 beaker) of each species were then removed every 24 hours for whole tissue analysis. The animals were starved for 24 hours prior to analysis to prevent unassimilated lead being included in the analysis. Samples of leaves and water from each beaker were also analysed on removal of the 10 animals in order to determine the lead content of these. Analyses for lead content were performed using the methods described in Section 3.4.3.

4.5.2. Results

The mean concentrations of lead available to the animals, in this food experiment, are shown in Table 10 (column 4) (page 64). The results show that some lead appeared in the freshwater medium, either by leaching or after passage through the gut of Asellus. On average, this amounted to less than 1% of the lead available to the animals. A comparison with the lead uptake from solution results (Table 10) shows that the concentrations in the freshwater medium were generally similar to those used in the solution experiments. More than 99% of the available lead was, however, provided in the form of food.

The linear regression lines of log. accumulated lead ($\mu g\ g^{-1}$) over time for both species are shown in Fig. 18 with the resultant regression equations shown in Table 11 (page 65). The graphs indicate

Species	Uptake Experiment	Source of Lead	Available Lead *µg g^{-1} +µg dm^{-3}	Accumulated Lead in *Asellus* µg g^{-1}
A. aquaticus	Food	*T. europaea*	36989 *	6996.81
		Freshwater medium	324 +	
	Solution	Freshwater medium	414 +	105.10
A. meridianus	Food	*T. europaea*	30061 *	346.74
		Freshwater medium	255 +	
	Solution	Freshwater medium	414 +	85.17

Table 10. A comparison of the amounts of available lead (µg g^{-1} and µg dm^{-3}) and accumulated lead (µg g^{-1}) in the uptake experiments using *A. aquaticus* and *A. meridianus*.

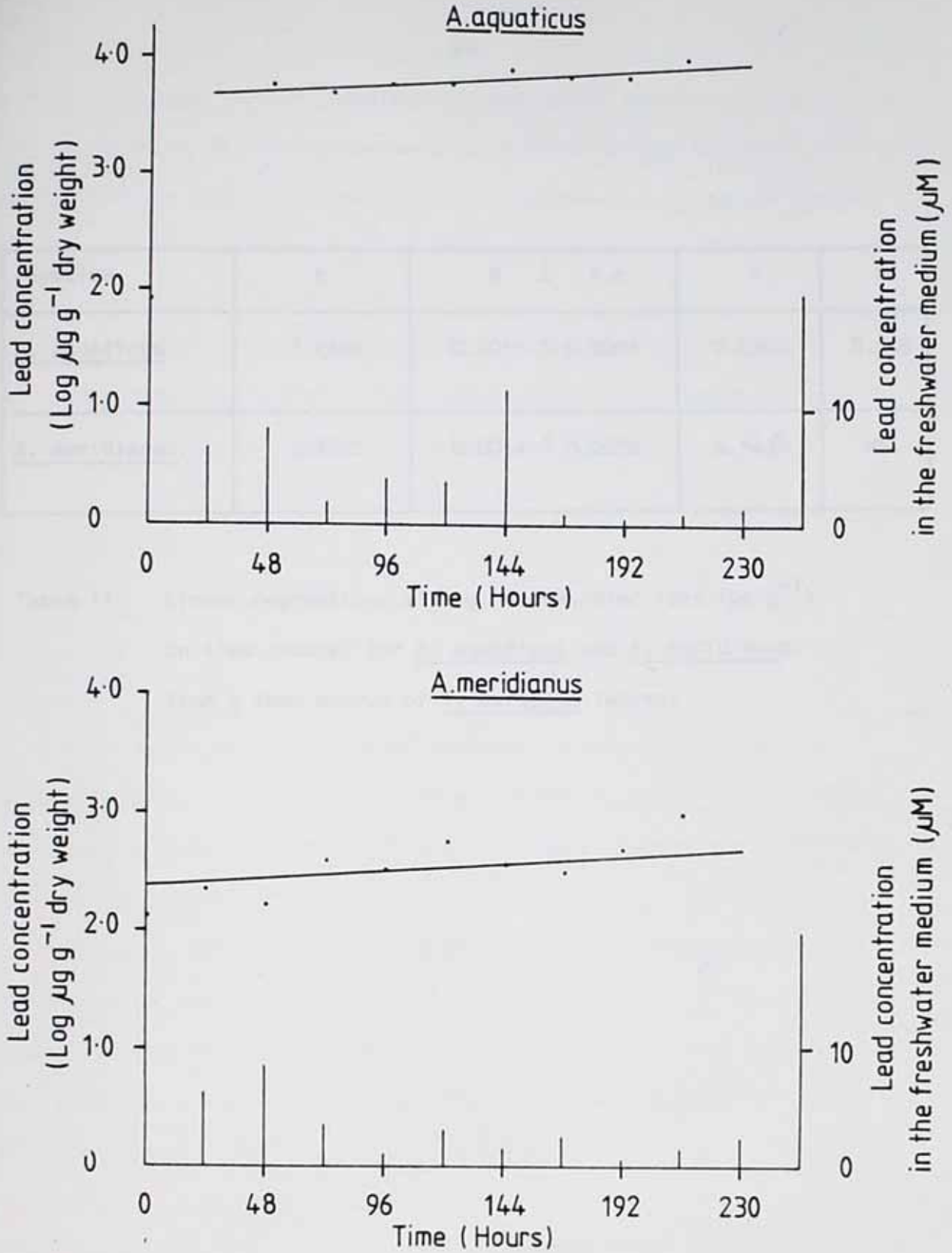


Fig.18. Graph showing uptake of lead from a food source (Tilia europaea) in A.aquaticus
$y = 3{\cdot}6469 + 0{\cdot}0015\ (\pm 0{\cdot}0004)x$
and A.meridianus $y = 2{\cdot}3720 + 0{\cdot}0014\ (\pm 0{\cdot}0008)x$
The lead concentration in the freshwater medium is also shown

Species	a	b ± s.e.	r	p
A. aquaticus	3.6469	0.0015 ± 0.0004	0.8566	0.005
A. meridianus	2.3720	0.0014 ± 0.0008	0.5449	NS

Table II. Linear regressions of log. accumulated lead ($\mu g\ g^{-1}$) on time (hours) for A. aquaticus and A. meridianus from a food source of T. europaea leaves.

that A. aquaticus shows a dramatic increase in the concentrations of lead over the initial 48 hour period and, thereafter, a slight, but significant ($p < 0.005$) accumulation throughout the experiment. A. meridianus did not show the same dramatic rise, although there was an upward trend, but this did not attain significance.

The results do show, however, that all the animals fed on lead enriched leaves accumulated higher levels of lead than those in the previous solution experiments (Table 10, page 64).

4.6 Discussion

4.6.1. Toxicity Test

The overall results obtained from this study suggest that A. aquaticus as a species is more tolerant than A. meridianus in spite of the higher natural background level of lead recorded in A. meridianus (137.11μM v. 90.2μM in A. aquaticus.

The LC_{50} values reported by Brown (1976) for A. meridianus are markedly lower than those reported here. Working with animals of the same size range (4mm - 6mm), she obtained a 48h. LC_{50} of 16.9μM in her most tolerant population and only 1.35μM in the most susceptible. This compares with an LC_{50} (72h) of 528.01μM for the same species in this study. This suggests that there are marked differences in tolerance between populations, although differences in experimental technique may also be important. Those differences lie basically in that Brown used a different freshwater medium (H.M.S.O. 1969) which will clearly differ from the 'Instant Pond' in many respects. For example,

Brown's medium had a total hardness of 25 mg dm^{-3} $CaCO_3$ as opposed to the 'Instant Pond' with a value of 80 mg dm^{-3} $CaCO_3$. This alone could affect results since the E.I.F.A.C. Working Party (1976, p.13) state that "toxicity (of copper) to aquatic organisms is modified by water quality and, in particular, the lethal toxicity to fish, invertebrates and algae is reduced by increase in water hardness."

Fraser's (1979) studies on A. aquaticus similarly present difficulties in comparison. First, she only obtained LC_{50} values for 24h. exposure which ranged from 3579.1µM to 985.51µM for different populations. Secondly, she used distilled water as her basic medium, acidified to a pH of 5.4. Nonetheless, the results of this study and those of Fraser suggest that much higher levels of lead are necessary to obtain the same percentage mortality over the same period of time in A. aquaticus compared with A. meridianus. Hence, it is inferred that A. aquaticus as a species is much more tolerant to lead than is A. meridianus, a point noted by Fraser et al. (1978) who stated that Brown had confirmed this to them by personal communication.

In the chemical analyses in this study, significant differences were observed between the background levels of lead in the males and females of both species, but subsequent toxicity experiments on the separate sexes indicated that there were no significant differences between the survival rates of the sexes in either species. Similar results were found by Fraser (op. cit.) who observed that there were no differences in the survival of males, females and pregnant females in the lead tolerance experiments when the individuals were all 5-7mm long. Fraser (op. cit.) did, however, find an increased tolerance

with increasing size. Since the males are generally larger than females, it may be anticipated that males will have a higher survival rate in the population as a whole.

4.6.2. Metal Uptake Experiments

The results of the study investigating lead uptake from solution indicate that A. aquaticus can accumulate significant concentrations of lead over a period of time, although A. meridianus showed no statistically demonstrable accumulation. Since A. meridianus was shown to have a higher natural background level of lead, it may be that it can only extract lead directly from solution at a concentration exceeding that which was used in this experiment. A. aquaticus has a lower background level and therefore may accumulate lead from a solution source with an initial lower concentration. In this instance, A. aquaticus accumulated a higher final concentration than A. meridianus, which suggests the increased tolerance to lead shown from the toxicity tests.

The results for the food experiments showed that A. aquaticus accumulated lead from its food supply as well and in greater concentrations than from solution alone, although the only dramatic accumulation was over the initial 48 hour period of the experiment. There is a possibility that food containing lead retained in the gut may account for a large amount of the lead concentration, though the likelihood of this is remote since they were starved for 24 hours prior to analysis after experimentation. Alternatively, A. aquaticus may extract lead directly from its food source rapidly, and once a certain concentration is reached, it may then reduce its rate of uptake since

only slight accumulations were observed, or the rate of efflux may balance further uptake.

A. meridianus showed no significant accumulation from a food source.

4.6.3. General Discussion

These results indicate that the principal source of accumulation of lead in A. aquaticus is from a food source rather than from solution. Several authors have reported that food is more important than water as a source of heavy metals, zinc in fish and in crayfish (Bryan 1967), although other workers have found water more important than food for zinc uptake in shrimps (Renfro et al. 1974). Weiser (1967) found that water was a more important source than food in the uptake of copper by marine and intertidal isopods. In a terrestial isopod, the woodlouse Porcellio scaber, Weiser (1966) has shown that the principal source of copper is through its food supply, although it can only extract copper directly from its food source at concentrations in excess of 1000μg g^{-1} Cu, values which Weiser considered unnaturally high. Brown (1977) found that in A. meridianus, food was a more important source of copper and lead than water, especially in the tolerant animals. The same holds for A. aquaticus in this study, this species having been found to be the more tolerant of the two. Brown reported that tolerant animals were capable of accumulating metals from their food and that two possible tolerance mechanisms could be improved storage and/or metal detoxification. In Fraser's work (op. cit.), uptake of lead from food did not seem to be significant in A. aquaticus, at least not over a 16-day period.

In A. meridianus, in this investigation, neither solution nor food appear to produce significant accumulations, even though this species has been shown to be the less tolerant of the two, as determined by the LC_{50} tests where the lead was provided in solution in differing concentrations. It seems likely that A. meridianus is affected by lead in solution, but that it can only extract lead directly from solution at higher concentrations than provided in the solution experiment alone. In the food experiments, A. meridianus did not accumulate lead from food, a result which was similarly recorded in Brown's (1977) non-tolerant animals. In her experiments no live animals remained after 10 days. In this experiment it may be that the animals did not ingest the food. Further investigations on the feeding rates of the two species may provide conclusive evidence.

The investigation has shown that A. aquaticus as a species appears to be more tolerant to higher levels of lead than A. meridianus. The possibility exists here that A. aquaticus employs a mechanism in dealing with the toxic effects of the metal. Basically there are two ways of handling contaminants to avoid their toxic effects, to store them in an inert form or to excrete them. Since A. aquaticus accumulates higher concentrations, it may be that the lead is stored in some form. Further experiments are needed to determine this.

Fraser (op. cit.) observed that in both tolerant and non-tolerant A. aquaticus, lead was stored in the cuticle or its underlying tissues with little or no accumulation in the gut or hepatopancreas. Brown (1977) also noted that in some lead tolerant A. meridianus the cuticle and underlying tissues were important storage areas, although it was established that the hepatopancreas equally served as a storage organ for trace metals in other populations of A. meridianus.

Different mechanisms for controlling the effects of the metals also appeared to be operating in the different populations (Brown 1977). High levels of lead accumulated in the hepatopancreas of the Hayle animals, but the Gannel animals appear to have the ability to restrict the uptake of both copper and lead and thus the high levels are not apparent in the Gannel animals. These same mechanisms may be operating in the two populations in this investigation, since A. aquaticus accumulates higher concentrations of lead, whereas A. meridianus appears to restrict the uptake of lead, although no increased resistance is shown in this species.

The relation between tolerance and uptake is difficult to understand. Bryan et al. (1971) have shown that tolerance is not necessarily related to high uptake, even when tolerant animals do accumulate higher levels of the metal. They found that copper-tolerant Nereis accumulated more Cu than non-tolerant animals, but also found that tolerant animals raised from juvenile size in the laboratory (without Cu in the environment), accumulated lower levels than the non-tolerant animals, but retained their tolerant status. They suggested that previous exposure to copper increased the numbers of binding sites for the metal. Under normal circumstances, high copper animals almost certainly absorb and excrete much more copper than those from low-copper sediment and this ability is reflected in their greater resistance to the toxic effects of copper solutions.

In attempting to relate this to the present investigation, it may be said that A. meridianus could absorb and excrete more lead, but no increased resistance has been shown to the toxic effects of lead solutions, whereas in A. aquaticus, greater resistance is shown, with higher lead concentrations accumulated.

The results from this investigation do not provide clear evidence relating to the effects of uptake and tolerance. It may not be the overall rate of uptake that is important, but its physiological effects or the way it is bound in different tissues. Clearly, further work related to this study is essential.

CHAPTER FIVE

GROWTH STUDIES

5.1. Introduction

The purpose of this section of the study was to compare the growth of the two species and to determine whether sublethal levels of lead affected it.

Both Brown (1976) and Fraser (1979) have included growth rate studies in their initial investigation of sublethal effects of lead on A. meridianus and A. aquaticus respectively. In both instances the growth rates of the individuals were measured at regular intervals in 0.48μM Pb (0.1mg dm^{-3} as stated in their studies) and in a freshwater medium which was used as the control solution. The food source provided throughout by Brown consisted of $2cm^3$ of concentrated spinach extract which was fed to A. meridianus on alternate days. Fraser provided A. aquaticus with approximately 0.2g of the fungus Saprolegnia ferax weekly.

Some experimental work on the feeding and growth of A. aquaticus had already been reported (Marcus, Sutcliffe & Willoughby 1978; Marcus & Willoughby 1978; Willoughby & Marcus 1979). In these works, feeding activity was examined and the animal proved to be widely omnivorous in the laboratory when presented with various vascular plants, algae, fungi and bacteria, which were all obtained from its habitats. The larger and more obvious food materials in the littoral of a lake system, such as decaying leaves, conferred high growth rates, whilst microbial foods, with the exception of the fungus Saprolegnia proved to be poor foods. A reservation about the poor feeding and growth performance on the bacterial foods has been that Asellus is not obviously equipped to work as a filter feeder; its biting and chewing mouth parts seem more

suited to solid macroscopic food materials (Willoughby et al. (op. cit.). Although A. aquaticus attained similar lengths over a 49 day period when fed on decaying oak leaves and Saprolegnia separately (initial size 2.5mm; 49 days 5.7mm) (Marcus & Willoughby 1978), it was preferred to test the growth performance of A. aquaticus and A. meridianus in this study on decaying leaves, since it has been widely reported that these are an effective food source. In this instance leaves of T. europaea were an abundant and preferred source of food in the natural habitat of A. aquaticus at Castle Ashby, and it was noted in the laboratory that A. meridianus fed readily on T. europaea. The lime leaves were collected from the littoral of Castle Ashby Lake. They were soft, denoting fairly lengthy residence in the lake, but not skeletonised.

5.2. Method

Twenty-five juveniles of each species were selected from stock cultures and were placed in specimen trays containing 125cm^3 of test solutions. The trays were partitioned into 25 sections so the growth of each individual could be monitored. A lid was placed on each tray to prevent evaporation of the solutions and concentration of the lead solutions. Increase in length, as a measure of growth, was then measured every 7 days in a 0.5μM Pb $(NO_3)_2$ solution and in the freshwater medium serving as the control. The food, per individual, consisted of a 0.5cm diameter disc of T. europaea placed in each chamber of the specimen tray. The experiment was carried out over a 21 day period and the animals were kept under constant temperature and light conditions.

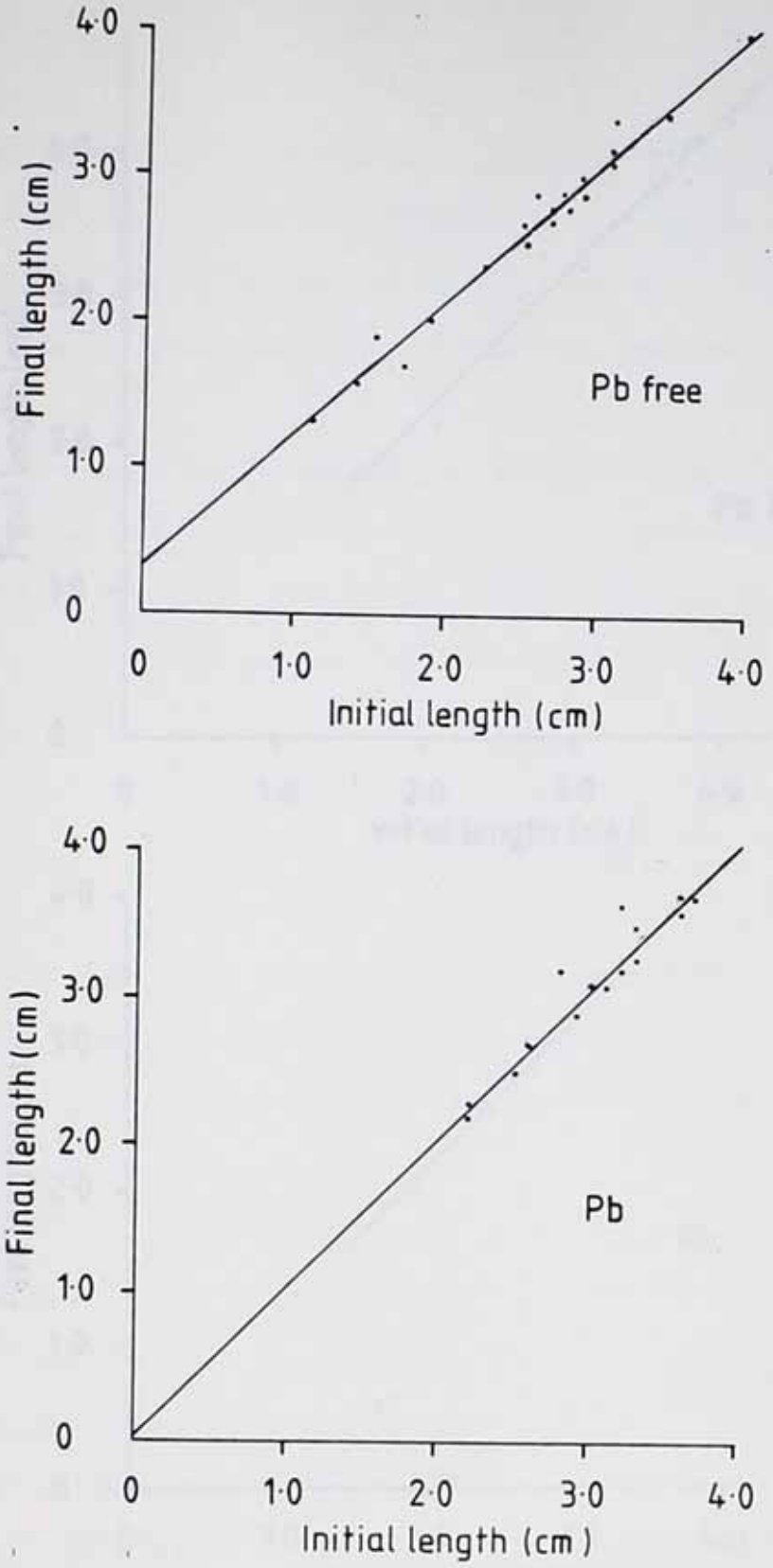
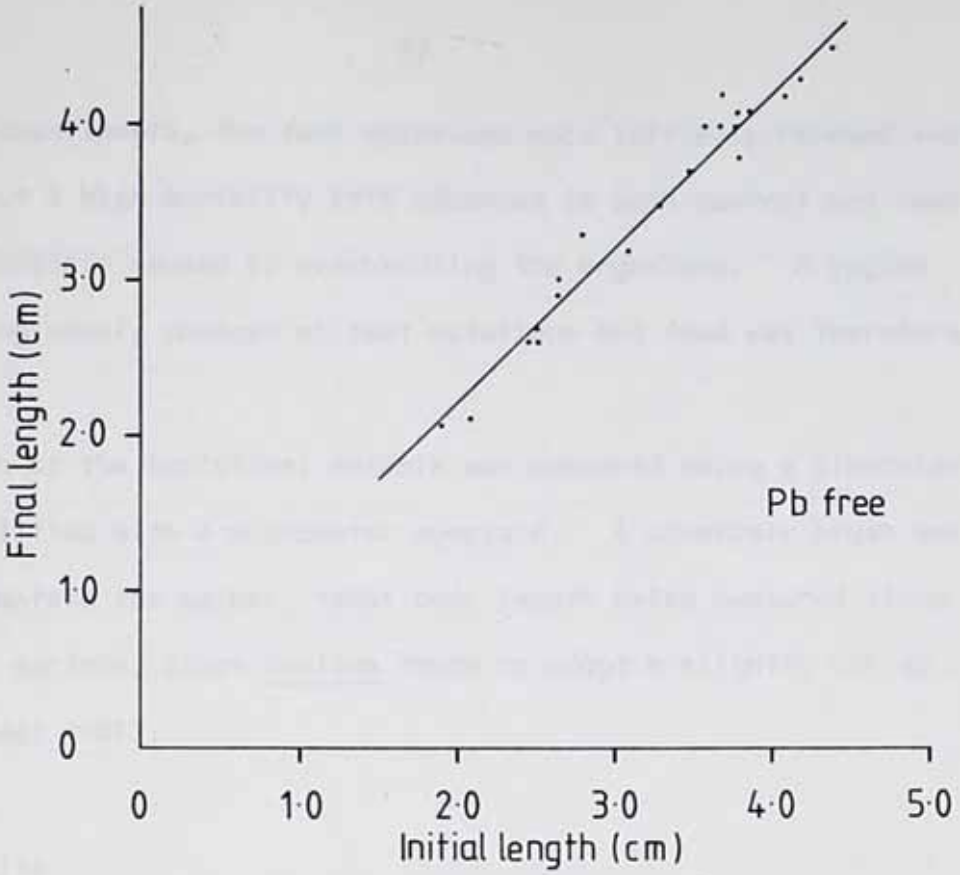


Fig.19. Graph of final length against initial length in A.aquaticus

Pb free $y = 0{\cdot}3227 + 0{\cdot}9179\ (\pm 0{\cdot}0315)x$

Pb $y = 0{\cdot}0483 + 1{\cdot}0147\ (\pm 0{\cdot}0667)x$



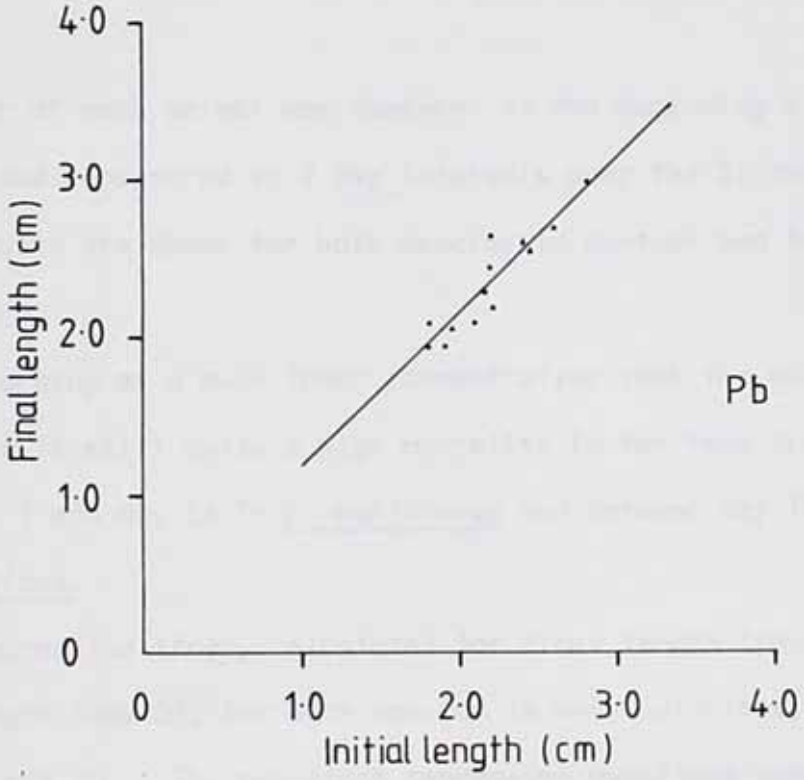


Fig.20. Graph of final length against initial length in A.meridianus

Pb free $y = 0{\cdot}1936 + 1{\cdot}0060\ (\pm 0{\cdot}0365)x$

Pb $y = 0{\cdot}1336 + 1{\cdot}0100\ (\pm 0{\cdot}1458)x$

In trial experiments, the test solutions were initially renewed every 72 hours, but a high mortality rate occurred in both control and lead solution, possibly caused by overhandling the organisms. A regime incorporating weekly changes of test solutions and food was therefore used.

The length of the individual animals was measured using a binocular microscope fitted with a micrometer eyepiece. A camelhair brush was used to orientate the animal, total body length being measured along the ventral surface, since Asellus tends to adopt a slightly curved posture (Steel 1961).

5.3. Results

The length of each animal was measured at the beginning of the experiment and remeasured at 7 day intervals over the 21 days of the test. Results are shown for both species in control and lead solutions in Appendix B.

Despite working at a much lower concentration than the obtained LC_{50} value, there is still quite a high mortality in the lead treatment between day 7 and day 14 in A. meridianus and between day 14 and day 21 in A. aquaticus.

Linear regression lines, calculated for final length (day 21) on initial length (day 0), for both species in both solutions, are shown in Fig. 19 and 20. The resultant regression equations are shown in Table 12a (page 78). Over the period of the experiment, the exposure to the sublethal dose of lead produced no significant effect on the growth in A. aquaticus and A. meridianus as indicated by comparison of the regression slopes (Table 12b, page 78).

Species	Treatment	a	b	±	s.e.
A aquaticus	Control	0.3227	0.9179	±	0.0315
	Lead	0.0483	1.0147	±	0.0667
A. meridianus	Control	0.1936	1.0060	±	0.0365
	Lead	0.1336	1.0100	±	0.1458

Table 12a. Linear regressions of final length (cm) on initial length (cm) for A. aquaticus and A. meridianus in control and lead (0.5μM $Pb(NO_3)_2$ solutions.

Species	Treatment	b	t	p
A. aquaticus	Control vs Lead	0.9179 / 1.0147	1.3123	NS
A. meridianus	Control vs Lead	1.0060 / 1.0100	0.0259	NS

Table 12b. Comparison of slopes obtained from Table 12a.

5.4. Discussion

A high degree of mortality was observed in the lead treatment in both species, 52% in A. meridianus and 28% in A. aquaticus at day 21, but in those surviving, lead was shown to cause no significant inhibition or increase in growth in either species. The increased mortality was observed earlier in A. meridianus at day 14 as compared to day 21 in A. aquaticus and this may be due to the increased tolerance in A. aquaticus shown by the previous toxicity tests. Although the lead was presented to the animals at a concentration well below the obtained LC_{50} values, it may be that these smaller animals are more susceptible to the toxic effects of lead since the LC_{50} values were obtained using animals 4-6mm. in length. In experiments by Fraser (op. cit.), small animals were shown to be less tolerant. Also, since the animals would already be stressed during measurement, by then placing them in a further stressful situation, i.e. lead, they could be more susceptible than the control animals.

In this study, a sublethal dose of lead produced no significant effect on growth. Brown (op. cit.) observed considerable variation in the mean growth rates of A. meridianus from different sites. Growth was inhibited by lead in the non-tolerant animals but not in the tolerant ones. Compared to A. meridianus used in her study, both species here could be categorised as 'tolerant' (using the LC_{50} values) and therefore the results were comparable. Growth was not inhibited by lead in A. aquaticus or A. meridianus in this study. Fraser (op. cit.), in contrast, showed that in A. aquaticus growth was stimulated by lead in both the tolerant and non-tolerant animals, although the

non-tolerant animals did not have such a high basic growth rate as the tolerant A. aquaticus. Fraser thought it possible that very low levels of lead stimulated the metabolic rate. Unlike in this study, Fraser found that lead had no discernible toxic effects during the period of the experiment since initial deaths were similar in all treatments.

No statistical tests have been performed on the growth rates in these experiments, because the animals selected were not all of the same size. The initial sizes varied from 1.13mm to 4.40mm, depending on the availability of each species at the time of experimentation. Any difference in growth over time would not necessarily be related to the treatments used. Differences could simply be due to the species' natural growth. In Asellus the early (juvenile) phase of growth, leading to sexual maturity, is marked by the fastest relative rates of growth as distinct from the largest growth increments. Sexual maturity is reached at a length of approximately 3mm (Steel op. cit.). Since, in this experiment, several of the animals' initial size were over 3mm., then growth would be more linear rather than the curvilinear growth of the smaller animals. Further investigation would be required before any conclusive evidence could be produced relating to the growth over time.

As a result of the procedure carried out here, other experimental techniques could be employed in future investigations. Although unlikely, since the control animals showed no mortality, the high mortality observed in the lead solution could possibly be a result of overhandling of the animals or otherwise as a result of poor nutrition. Discs of decaying T. europaea were used as the food source here.

Dupey (1967) noted that the presence or absence of an intact epidermis or cuticle on the leaf is a most important factor in the survival of juveniles of both species. Evidently the mouth parts of the juveniles are not adequate for the task of penetrating the cuticle of freshly soaked leaves. Further observations by Dupey showed that the barrier presented by the cuticle becomes progressively less effective as decomposition of the leaves proceeds. In future work, therefore, the food source will have to be carefully chosen. Highly decayed leaves will be necessary in order that the juvenile Asellus are assured of a rich supply of nutrition. Alternatively, a fungal source, Saprolegnia, could be utilised, which has been used successfully for feeding by other authors (Marcus & Willoughby op. cit; Fraser op. cit.). In this investigation, decaying leaves of T. europaea were used so that a uniform food source was used throughout the whole study.

Further investigations should take these factors into account to ensure that future work provides further conclusive evidence and also that comparative studies with other work could be made.

CHAPTER SIX

REPRODUCTION STUDIES

6.1. Introduction

The aim of these studies was to determine whether levels of lead affect the reproductive potential of the two species. Generally, these effects might be considered to apply to the fecundity, this being referred to as the egg production per female, and to the successful fertilisation of the egg. Lead could affect Asellus numbers within their habitat through impairing their fecundity, which would then affect the number of live offspring produced.

All Asellus carry live young in a brood pouch on the pereion between the legs. Following copulation, the female moults, producing the pouch as a series of extended oostegites (that overlap, but are not sealed) into which the eggs are shed. Gravid females can be identified by the presence of the brood pouch. (Steel 1961; Andersson 1969; Adcock 1975, 1979).

6.2 Method

To determine whether sublethal doses of lead affect the breeding biology of the two species, experiments were set up to establish whether any correlation between the size of the female, the length of the brood pouch at varying stages of the incubation period and the number of young released, existed. Regressions on the data for control and lead situations could then be compared to assess whether lead affects the breeding activity of Asellus.

Animals were selected for the reproduction studies by choosing pairs which were already in the precopulatory clasp, a position they

may maintain for up to 2 weeks (Steel op. cit.). Individual pairs of each species were placed in 7.5cm x 2.5cm specimen tubes with $10cm^3$ of test solutions added to each tube. The test solutions comprised 0.5μM $Pb(NO_3)_2$ and Instant Pond, and to each tube slightly decomposed leaves of T. europaea were added as food. Discs of leaf were cut from the leaf lamina, avoiding the central midrib, using a 2cm cork borer. These were washed with distilled water, cut in half and then each half was placed in individual tubes. This procedure was repeated with both species, in both the lead and control solutions. Solutions were changed and new food added at 72 hour intervals. The tubes were maintained under controlled conditions of 15°C and with a 16 hour light and 8 hour dark regime.

All pairs were inspected daily. As soon as the precopulatory clasp had been relinquished and the female was observed to moult, implying that sperm transfer had taken place, the male was removed. The length of the brood pouch was then measured and the number of eggs counted. The females were inspected every day thereafter and the number of days before releasing the juveniles was recorded from the time the brood pouch was noted. Thus the number of days in the test solution varied from animal to animal. During the incubation period, any change in the length of the brood pouch was recorded, along with any loss of brood in the form of eggs or embryos. At the end of the incubation period. the number of fully developed young was recorded.

6.3. Results

In the experiments with A. meridianus no 'successful' pregnancies were recorded in either control or lead treated animals. In many instances precopula was frequent and prolonged, but no broods were produced. Also, in some A. meridianus, copulation was easily broken when transferring the copula pairs to the specimen tubes and repairing of the animals proved unsuccessful. The A. meridianus experiments were also carried out at a later date than the A. aquaticus experiments when reproductive activity was ceasing in the culture and no further gravid females were available for experimentation.

The results for each treatment with A. aquaticus are given in Tables 13 and 14 (pages 87 and 88). Correlation and regression analyses were carried out, the correlation coefficients being indicated in Table 15 (page 89) and the graphs showing the regression lines are presented in Fig. 21 to 25. For the lead treatment, only the points have been plotted on Fig. 22 to 25 because of insufficient data for meaningful regression analysis.

The results for the control experiments indicate that in all the analyses carried out there is a significant correlation between:

a) the length of the female and the length of the brood pouch (in early and in late incubation)

b) the length of the brood pouch (in early incubation) and the length of the brood pouch (in late incubation)

c) the length of the female and the number of young released, and

d) the length of the brood pouch (in late incubation) and the number of young released.

The incubation period was fairly uniform throughout, regardless of the length of the female, ranging from 18 - 24 days.

LENGTH OF A. AQUATICUS (mm)		LENGTH OF BROOD POUCH DURING EARLY AND LATE DEVELOPMENT (mm)		INCUBATION PERIOD - NUMBER OF DAYS	NUMBER OF FULLY DEVELOPED YOUNG RELEASED*
MALE	FEMALE	EARLY	LATE		
9.50	6.00	2.10	3.00	19	50
8.75	6.40	2.20	2.40	20	51
9.50	7.00	2.56	3.00	19	77
9.50	7.50	2.86	3.40	20	69
9.25	7.00	2.63	3.13	24	60
9.50	6.00	2.33	2.53	20	41
8.75	6.50	2.20	2.73	19	42
10.00	7.00	2.50	3.10	20	38
9.50	6.20	2.13	2.73	18	52
10.00	6.00	2.00	2.36	18	24
9.00	6.10	2.20	2.76	18	34
9.00	6.20	2.23	2.56	22	41
8.00	5.40	1.30	1.73	21	16

*Equals number released into brood pouch.

Table 13. Fecundity data for A. aquaticus under control conditions

LENGTH OF A. AQUATICUS (mm)		LENGTH OF BROOD POUCH DURING EARLY AND LATE DEVELOPMENT (mm)		INCUBATION PERIOD - NUMBER OF DAYS	NUMBER OF EGGS RELEASED AND SUCCESSFULLY RETAINED EGGS	NUMBER OF FULLY DEVELOPED YOUNG RELEASED	NUMBER OF EGGS RELEASED DURING INCUBATION	TIME OF EGG RELEASE FROM BROOD POUCH AFTER LAYING - NUMBER OF DAYS
MALE	FEMALE	EARLY	LATE					
6.40	5.00	1.83	-	-	7	-	7	2
8.10	6.10	1.80	-	-	25	-	25	2
7.50	4.90	1.93	-	-	15	-	15	3
9.30	5.00	1.56	-	-	12	-	12	2
6.70	5.10	1.90	2.30	16	32	32	-	-
7.90	5.60	1.80	-	-	14	-	14	1
7.20	5 30	2.13	2.30	18	19	1	18	2
7.40	6.00	2.23	-	-	16	-	16	2
9.30	4.90	1.83	2.10	21	5	3	2	9
8.10	5.10	1.83	-	-	3	-	3	1
7.60	5.30	2.10	-	-	3	-	3	9
7.50	5.50	2.13	-	-	13	-	13	6
7.90	5.40	1.93	2.20	13	4	4	-	-
7.80	5.62	2.00	2.33	13	25	25	-	-
7.90	5.90	2.05	2.13	13	1	1	-	-
7.90	5.50	1.93	2.30	12	15	15	-	-

Table 14. Fecundity data for A. aquaticus under lead (0.5μM $Pb(NO_3)_2$) conditions.

		Control		Lead	
		Female Size(mm)	Brood Pouch Size (Late)(mm)	Female Size(mm)	Brood Pouch Size (Late)(mm)
Brood Pouch	Early	0.8899 *	0.9006 * *	0.5423 *	0.3957 NS
	Late	0.8526 * *		0.1059 NS	
Number of Young released		0.7714 * *	0.7522 * *	0.0123 NS	0.4342 NS

Table 15. Correlation coefficients for linear regressions on the fecundity data for A. aquaticus.

* * $p < 0.01$; * $p < 0.05$; NS - not significant

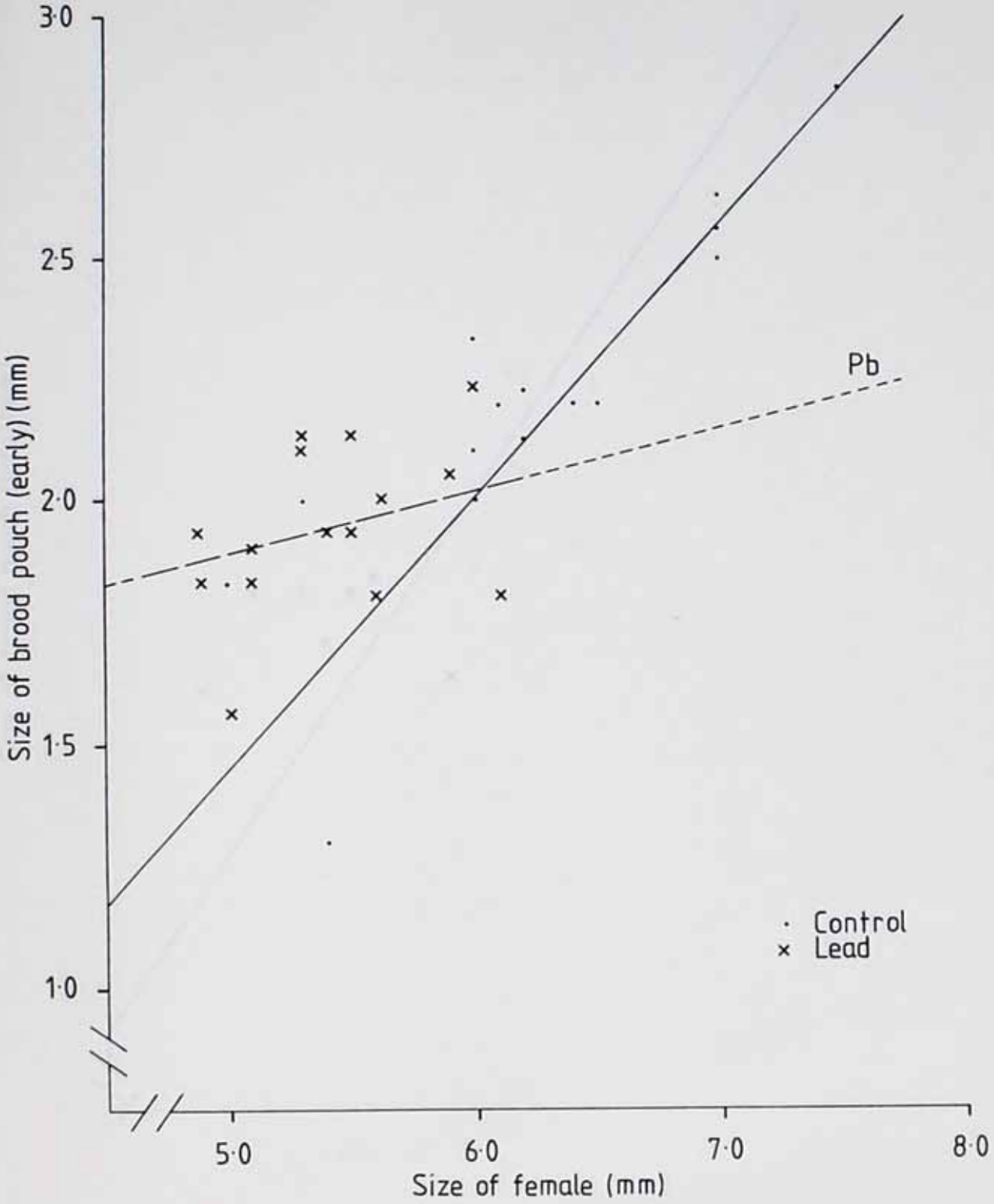


Fig.21. Graph of brood pouch size (early) against female size, under control conditions ($y = 0{\cdot}56(\pm 0{\cdot}0824)x - 1{\cdot}352$) (·——·) and under lead stress (×----×) ($y = 0{\cdot}22(\pm 0{\cdot}1027)x + 0{\cdot}792$) in A.aquaticus

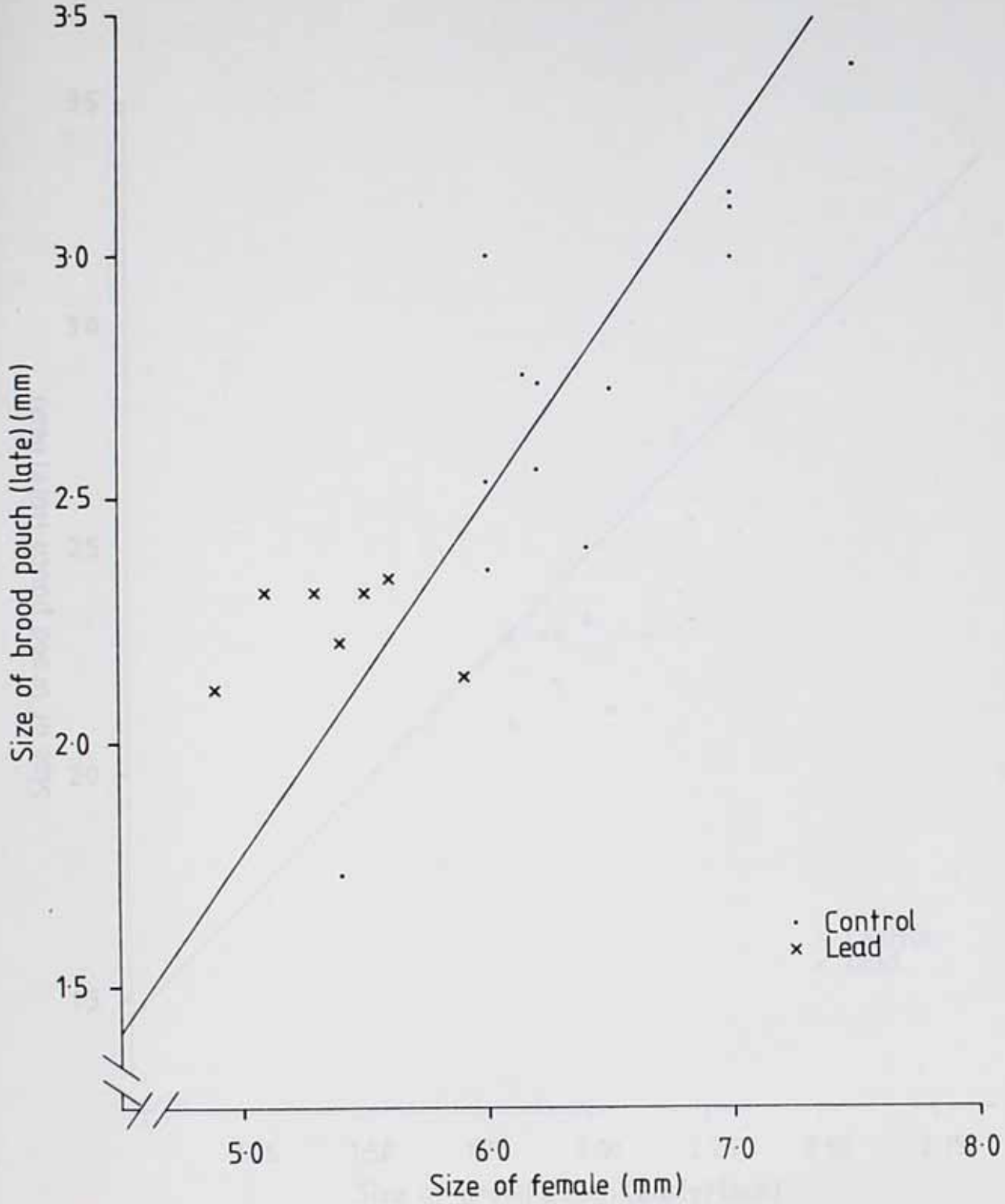


Fig.22. Graph of brood pouch size (late) against female size under control conditions ($y = 0{\cdot}64(\pm 0{\cdot}1180)x - 1{\cdot}372$) and under lead stress (only points plotted) in A.aquaticus

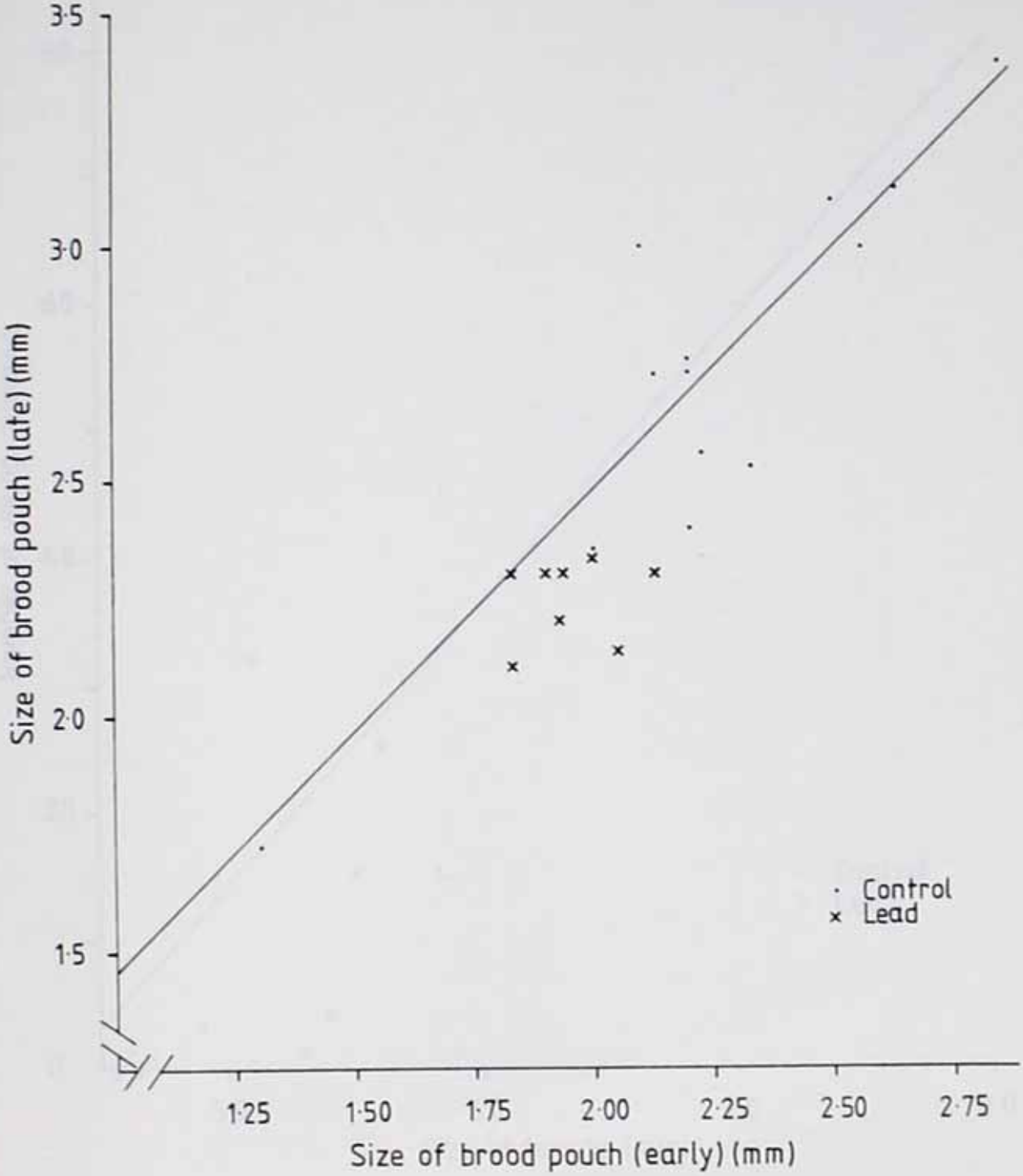


Fig.23. Graph of brood pouch size (late) against brood pouch size (early) under control conditions ($y = 0{\cdot}425 + 1{\cdot}03(\pm 0{\cdot}1502)x$) and under lead stress (only points plotted) in A.aquaticus

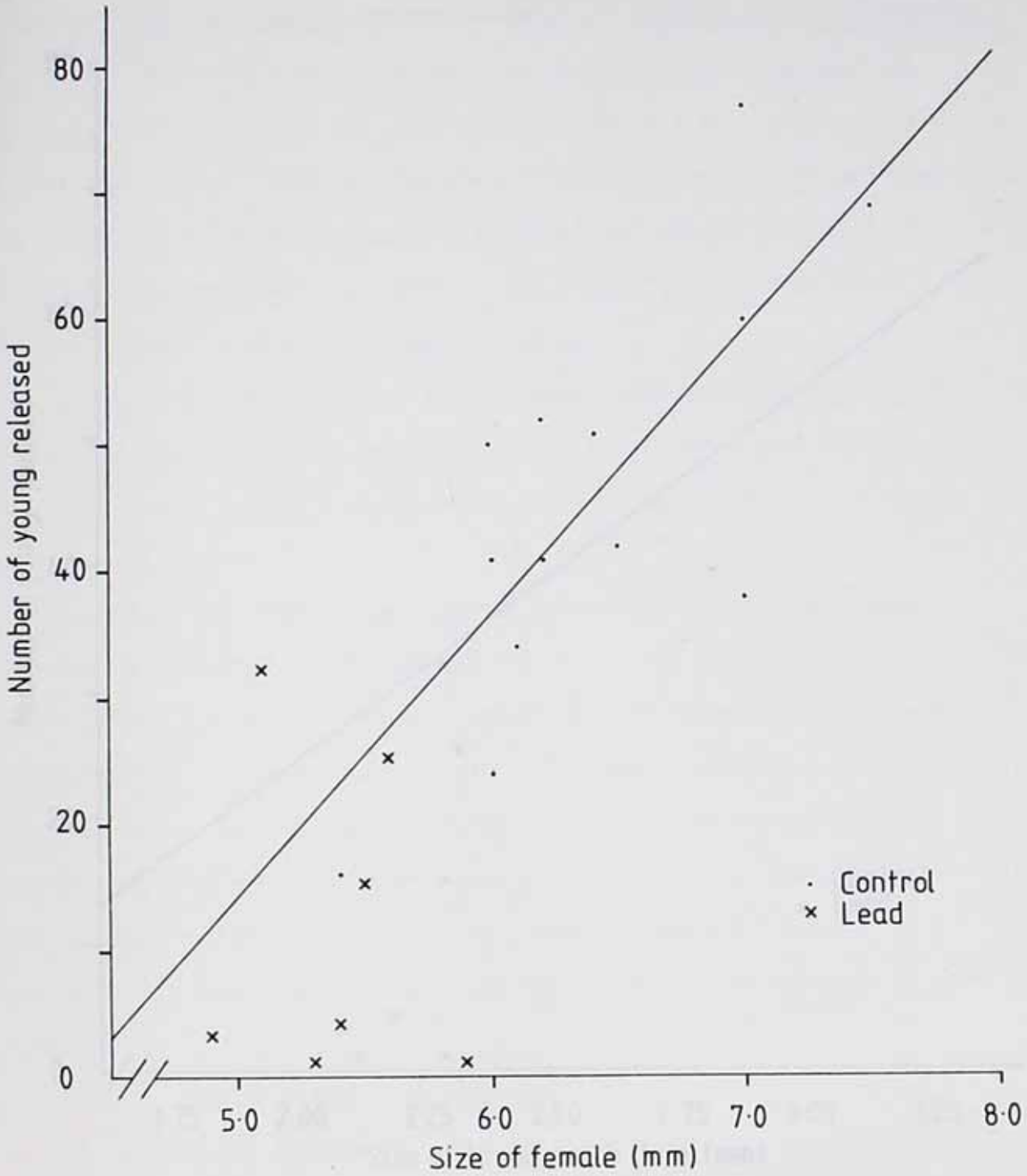


Fig.24. Graph of number of young released against female size under control conditions ($y=22{\cdot}71(\pm 5{\cdot}6414)x-99{\cdot}80$) and under lead stress (only points plotted) in A.aquaticus

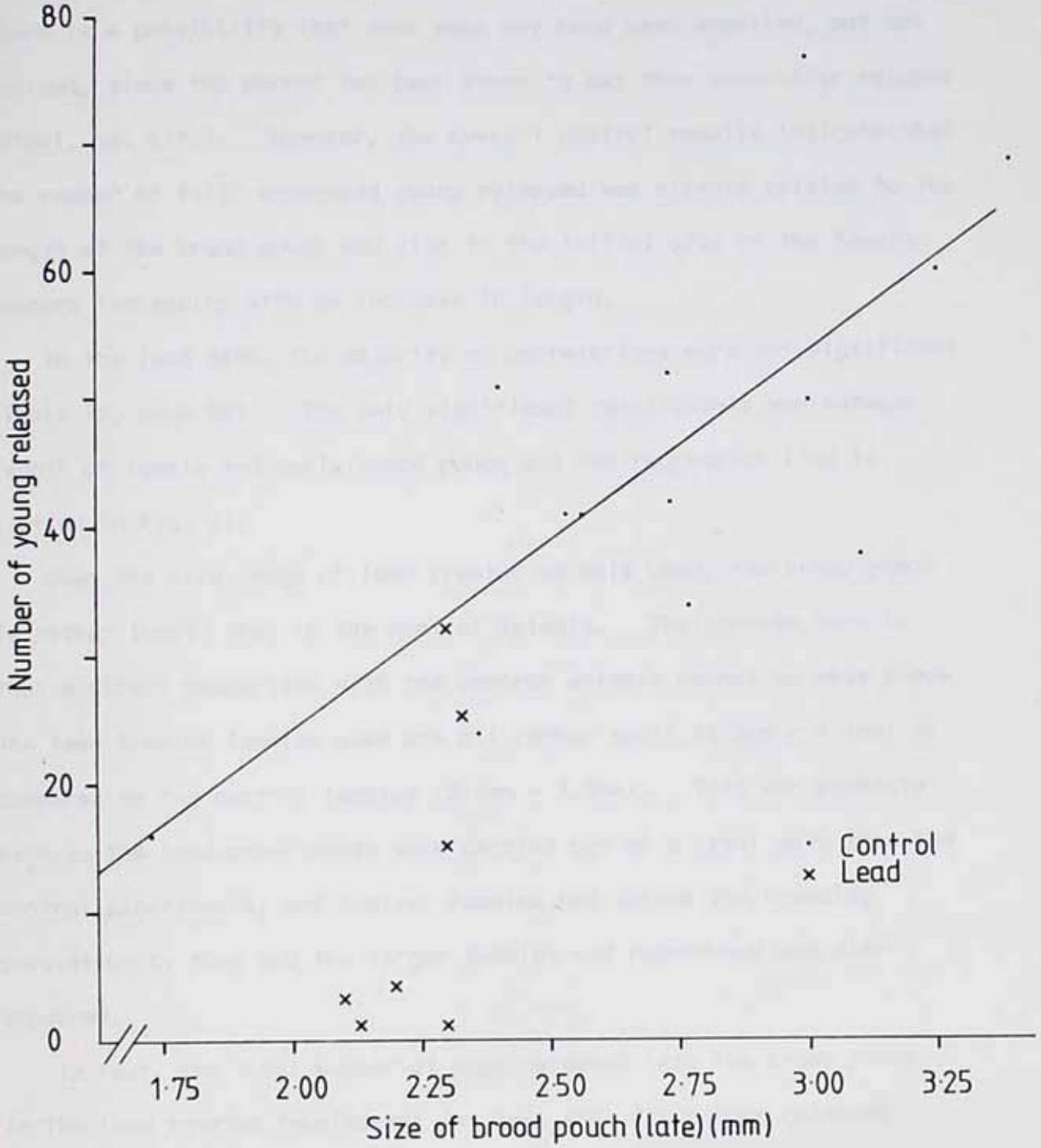


Fig.25. Graph of number of young released against brood pouch size (late) under control conditions ($y = 29{\cdot}62 (\pm 7{\cdot}8093) x - 35{\cdot}09$) and under lead stress (only points plotted) in A.aquaticus

From the data in Table 13 (page 87), it can be seen that there was a variation in the numbers of young released. In the larger A. aquaticus, there is a possibility that some eggs may have been expelled, but not noticed, since the parent has been known to eat them soon after release (Steel, op. cit.). However, the overall control results indicate that the number of fully developed young released was closely related to the length of the brood pouch and also to the initial size of the female, numbers increasing with an increase in length.

In the lead data, the majority of correlations were not significant (Table 15, page 89). The only significant relationship was between length of female and early brood pouch and the regression line is plotted in Fig. 21.

Over the size range of lead treated animals used, the brood pouch is rather longer than in the control animals. The problem here is that a direct comparison with the control animals cannot be made since the lead treated females used are all rather small (4.9mm - 6.1mm) as compared to the control females (5.4mm - 7.5mm). This was probably because the lead experiments were carried out at a later date than the control experiments, and smaller females had joined the breeding population by then and the larger females had reproduced and disappeared.

In fact, the total number of eggs released into the brood pouch in the lead treated females was far less than the number released into the brood pouch in the control animals (Tables 13 and 14, pages 87 and 88). A comparison between these was made using a Mann-Whitney U test which indicates that the observed difference is highly significant ($U = 200.5$; $p < 0.001$).

Of the 16 lead treated females which produced a brood pouch, ten of them released the majority, if not all, of their eggs prematurely, principally within the first 3 days of the incubation period (Table 14, page 88). These 'unsuccessful' pregnancies resulted in the brood pouch breaking antero-posteriorly during the incubation period. The number of eggs released prematurely may, in fact, be higher than that recorded since some may have been eaten by the adults. Whether the brood pouch breaks as a direct effect of the lead on the animals is not clear, but no similar occurrence was recorded in the control animals. In two of the lead treated females it was noted that whilst some eggs were released during the incubation period, fully developed young were also produced at the end of incubation. In these instances, the brood pouch remained intact.

Following on from this, of those eggs which did survive so that fully developed young were released from the lead treated females, they were, in general, released earlier than from the control females (Tables 13 and 14, pages 87 and 88). The incubation period ranged from 13 - 21 days in the lead treated as opposed to 18 - 24 days in the control females. A Mann-Whitney U test indicated that there was a significant difference between the incubation periods ($U = 79$; $p < 0.005$).

6.4. Discussion

The majority of reproductive programmes using Asellus spp. have so far concentrated on the life histories of the species and their general breeding biology (Steel, op. cit; Andersson, op. cit; Adcock op. cit.). An attempt was made in this study to determine whether their breeding biology, as defined by these workers, was affected by

sublethal doses of lead. Rather than finding the level most toxic to the animal, it was thought more informative to note the possible effects of the sublethal levels in Asellus on its basic functions.

A comparison between the two species of Asellus could not be made since no successful results for A. meridianus were obtained. In many of the A. meridianus precopula was frequent and prolonged, but no broods were produced and no obvious cause for this could be found. It is possible that they were in the precopulatory passive phase which has been defined by Parker (cited in Manning, 1975) as "a stage of the male's reproductive behaviour during which he remains mounted on or otherwise attached to the female but without true genital contact." (Manning (op. cit.) indicated that the duration of the passive phase can be substantial, although this is modified by intermittent pairing. The latter occurrence was also observed in A. meridianus.

Many of the pairs of A. meridianus which were selected, separated on transfer to the experimental container and repairing of them proved unsuccessful. Also, of those animals which were successfully transferred as pairs, many of the females were later abandoned by the males and no repairing was observed. It was noted that the female size of A. meridianus ranged from 4.4mm - 5.6mm, whilst those of A. aquaticus ranged from 4.9mm - 7.5mm. In A. meridianus it is possible that the males were exhibiting some form of discrimination. Manning (op. cit.) stated that the discriminating mechanism may consist of an assessment of female size, although other cues such as those of an olfactory nature could not be precluded. In addition, smaller females were more likely to be discarded in the absence of copulation. In A. meridianus, since the females were generally smaller, it may be that the males were

"selecting for passive phase association those females exhibiting a cue which correlates with imminent oviposition and large brood size. This cue may be female size or another characteristic which is correlated to size. This behaviour pattern yields an increase in offspring number to those males exhibiting it." (Manning op. cit.).

Dupey (1967) reported that precopula is far more easily broken in A. meridianus than in A. aquaticus, though there was no direct evidence of this affecting the reproductive potential of A. meridianus. Short-term comparative breeding programmes, run by Dupey, did not reveal any significant differences between the numbers of juveniles produced by similar size females of either species. Further investigations relating to this present study are therefore required before any comparisons of this nature can be made.

In the case of A. aquaticus, the results indicate that sublethal levels of lead affect the fecundity of this species. Although over the size range of females tested the length of the brood pouch is significantly longer on the lead treated females than on the controls, the total number of eggs released into the brood pouch was, in fact, significantly smaller in the lead treated females.

Another notable observation was that in a large proportion of the lead treated gravid females, the brood pouches split antero-posteriorly, thus releasing the eggs prematurely. Whether this was a direct consequence of the effect of the lead is unclear, but no control animals showed any such response. Research into the effects of zinc on Gammarus has revealed that high levels of zinc cause moulting in the animals (M. Hardwick, pers. comm.). Whether this is

true in the case of lead would have to be further investigated, but this may be connected with the early release of the eggs.

In a small proportion of lead treated females, some eggs were released during the incubation period, but fully developed young were also produced. It is possible that the eggs may have been expelled by the oostegites being withdrawn from the body and then being forced out of the brood pouch (Steel op. cit.). Although no expulsion of eggs was noted in the control females, it is known that the parent Asellus often eat them soon after release.

Other causes of variation in the numbers of young released may be due to eggs dying and decaying whilst in the brood pouch. Opaque eggs, presumably dead ones, were noted on occasions, though not in sufficient numbers to cause the total variation. The results suggest that the variation is caused by the presence of a sublethal dose of lead, i.e. there are smaller numbers of eggs and therefore smaller numbers of fully developed young in the lead treated females.

The incubation period was also observed to be significantly reduced in length in females maintained in a sublethal dose of lead. Thus, overall, it would appear that a sublethal dose of lead impairs the fecundity of Asellus.

One factor which might lead to a misconstruction of the results is that the majority of the females used in the lead dose were smaller than those in the control. As mentioned, the lead experiments were carried out at a later date than the controls and smaller females were present in the breeding population then. It may be that the smaller numbers of eggs released into the brood pouch and the reduced length of the incubation period were not due to the effect of lead but were

a result, or partial result, of the initial size of the female. A study including a wider size range of females over the whole breeding season is necessary before further conclusions could be drawn. The length of the incubation period, for instance, may shorten as the breeding season continues, although this is unlikely to be the cause in this study since controlled temperature and light conditions were used throughout.

Breeding experiments set up by Fraser (op. cit.) in an attempt to investigate whether lead tolerance in A. aquaticus is inherited were disappointing. The fault appeared to be not in the mating, but in the production of fertile eggs or viable young. In her investigation it was thought that low pH, poor nutrition, or a combination of both, were the most likely reasons for the failure of the breeding experiments.

It is possible that the pH factor may be one contributory cause of the unsuccessful breeding in A. meridianus. The pH of the freshwater medium used in these experiments was quite low (6.0) and, although Asellus survive well in the freshwater medium, the low pH may be a reason for the failure of the experiment. This factor should be taken into account with further experiments.

Although the results were disappointing in that no comparison could be made between the species, they were encouraging with respect to A. aquaticus. Overall it appears that sublethal doses of lead would affect the reproductive potential of A. aquaticus by reducing the numbers of viable young released into the population.

CHAPTER SEVEN

RESPIRATION STUDIES

7.1. Introduction

The effects of heavy metals on respiration in fish have been extensively studied. Usually the effect is to reduce the rate of oxygen uptake, e.g. silver on gill tissues of sticklebacks, Gasterosteus aculeatus (Thurberg & Collier 1977), zinc on G. aculeatus (Jones 1947; Brafield & Mathiesen 1976).

In invertebrates, heavy metals have usually been found to depress oxygen consumption, e.g. mercury, copper, zinc and lead in the shrimp Caridina rajadhari, (Chinnaya 1971). Brkovic-Popović and Popović (1977) found that metals had varying effects depending on the metal under study. Cadmium and mercury could increase or decrease oxygen consumption in tubificid worms according to the concentration of the metals. Copper always inhibited oxygen consumption, but zinc, nickel and chromium caused uptake to increase.

Very little work has been reported on the physiological effects of lead on arthropods and so it was decided to test the effects of lead on oxygen uptake in Asellus as a means of measuring the sublethal effects of lead on metabolism.

7.2 Measurement of Respiration Rate

The Asellus used in these experiments were taken from the stock cultures. The sexes were separated and only non-gravid females were used along with the males. Only one size class of both sexes was examined, 4-6mm, this being the most abundant size class available and, after sorting, the groups were placed in control and lead solution

(0.5μM $Pb(NO_3)_2$) for each set of experiments. The animals were maintained in these solutions, without food, for 24 hours prior to respirometry.

The following procedure was carried out using a Gilson Differential Respirometer to determine oxygen uptake. Initially only A. aquaticus, both male and female, were used in the two solutions, the control experiments being carried out completely separately from the lead experiments.

The Gilson water bath was equilibrated to 15°C. Fourteen 7.5cm^3 reaction flasks were used for the respiration studies, while the remaining six flasks were retained as thermo blanks ('controls'). A 1.5cm x 1.0cm piece of filter paper was placed in the central well of each flask (reaction and thermo blanks) and a few drops of 5% potassium hydroxide solution were added to absorb carbon dioxide. 1 cm^3 of Instant Pond water at the experimental temperature was added to each of the flasks. The total air volume of the 20 flasks was therefore 130cm^3 (6.5cm^3 x 20) and the air space in the reference vessel was reduced to this volume by introduction of deionised water.

A. aquaticus were then added to the reaction flasks. Five Asellus were added to each flask with seven flasks containing males and seven containing females. Each flask was then immediately placed on the Gilson apparatus and the flasks were allowed to equilibrate for one hour before readings commenced.

Micrometer readings of oxygen uptake were taken at 30 minute intervals for the 5 hours between 1100h and 1600h. The flasks were then removed from the Gilson respirometer and the animals anaesthetised in 70% alcohol. They were then washed in distilled water, blotted dry on tissue paper and transferred to numbered foil pans. They were then dried in a vacuum oven at 60°C overnight, cooled in a dessicator and

weighed to obtain the dry weight to within ±0.01mg. Because of the problem of removing external moisture from an aquatic animal, it is considered that dry weight is a much better measure of an animal's size than live weight.

This test was then repeated using the lead solution. Both the control and lead experiments were duplicated.

7.3. Results

The mean readings of the thermo blanks were used to correct the readings of the reaction flasks. The raw data for both the control and lead experiments are given in tables in Appendix C. The values for the reaction flasks are the corrected gas uptake readings (in microlitres). In order to obtain an estimate of oxygen uptake the result of each flask of oxygen utilisation was regressed against time, and oxygen uptake was calculated as (1) oxygen consumption per individual per hour and (2) oxygen consumption per mg. per hour. The oxygen consumption per individual was calculated as a regression coefficient of oxygen per minute x 60. Flasks which showed correlations of < 0.9 for oxygen on time have been omitted from these and from all subsequent computations.

Tables 16 and 17 (pages 101 and 102) show the results of the linear regressions of oxygen uptake on time giving O_2 ind^{-1} h^{-1} and Wt.Sp.O_2 mg^{-1} h^{-1}. For each sex and treatment, oxygen uptake (μl O_2 ind^{-1} h^{-1}) (Log (x + 1)) was plotted against the mean dry weight (mg) (Log (x + 1)) of the individual in the group (Figures 26 and 27). Linear regression lines for each are shown on the graphs. In Fig. 27, the regression equation for the lead treatment

Sex	Dry weight 5 individuals (mg)	Mean dry weight(mg)	O_2 $ind^{-1}h^{-1}$ (μl)	Wt.Sp.O_2 mg^{-1} h^{-1}	r
♀	7.46	1.492	1.0325	0.6920	0.99548
♀	7.88	1.576	1.0056	0.6381	0.99312
♀	7.44	1.488	1.4642	0.9840	0.99940
♀	9.24	1.884	1.2752	0.6900	0.99132
♀	6.90	1.380	0.8272	0.5994	0.98538
♀	7.42	1.484	1.0032	0.6760	0.99259
♀	6.36	1.272	1.3004	1.0223	0.99371
♀	7.54	1.508	1.2352	0.8191	0.99915
♀	7.38	1.476	1.6058	1.0879	0.99885
♀	6.38	1.276	0.9246	0.7246	0.99668
♀	6.78	1.356	0.7290	0.5376	0.99662
♀	7.40	1.480	1.0218	0.6904	0.99920
♀	8.21	1.642	1.3081	0.7967	0.99734
♀	6.24	1.248	0.8982	0.7197	0.99663
♀	7.06	1.412	1.0347	0.7328	0.99807
♂	7.66	1.532	1.2061	0.7872	0.99361
♂	7.92	1.584	1.3999	0.8838	0.99808
♂	9.52	1.904	1.3576	0.7130	0.99693
♂	8.35	1.670	1.2385	0.7416	0.99088
♂	7.15	1.430	1.7503	1.2240	0.99704
♂	6.35	1.270	1.1185	0.8809	0.99798
♂	7.78	1.556	1.0943	0.7033	0.99209
♂	7.19	1.438	1.2822	0.8916	0.99763
♂	7.30	1.460	0.9088	0.6225	0.99957
♂	7.63	1.526	1.2102	0.7930	0.99821
♂	6.90	1.380	1.3808	1.0006	0.99955
♂	8.35	1.670	1.3304	0.7967	0.99644
♂	8.27	1.654	1.6381	0.9904	0.99668

Table 16. Computed data for oxygen uptake (μl O_2 30 $mins^{-1}$) by A. aquaticus under control conditions. (Raw data in Appendix C).

Sex	Dry weight 5 individuals (mg)	Mean dry weight(mg)	O_2 $ind^{-1} h^{-1}$ (μl)	Wt.Sp.O_2 $mg^{-1} h^{-1}$	r
♀	9.16	1.832	1.1459	0.6255	0.99812
♀	7.84	1.568	1.1762	0.7502	0.99759
♀	6.95	1.390	1.0007	0.7199	0.99552
♀	7.02	1.404	0.9440	0.6724	0.98930
♀	9.60	1.920	1.1644	0.6064	0.99873
♀	12.00	2.40	1.5813	0.6588	0.99832
♀	12.20	2.440	0.6783	0.2780	0.99425
♀	8.60	1.720	1.0691	0.6216	0.99832
♀	8.40	1.680	1.3745	0.8182	0.9980
♀	8.80	1.760	1.0747	0.6106	0.99865
♀	11.70	2.340	1.3702	0.5855	0.99797
♂	9.16	1.832	1.2175	0.6645	0.99649
♂	7.88	1.576	0.6490	0.4118	0.99718
♂	11.00	2.20	0.9779	0.4445	0.98860
♂	7.90	1.580	0.9576	0.6061	0.98899
♂	9.56	1.912	0.7704	0.4029	0.95785
♂	10.55	2.110	1.4604	0.6921	0.9478
♂	11.60	2.320	1.3287	0.5727	0.98694
♂	10.70	2.140	1.5559	0.7270	0.97879
♂	9.50	1.90	1.4817	0.7798	0.99032
♂	13.20	2.640	0.6601	0.2500	0.98065
♂	10.25	2.050	1.3811	0.6737	0.99796
♂	9.90	1.980	1.0272	0.5188	0.98451
♂	13.60	2.720	1.1547	0.4245	0.98668

Table 17. Computed data for oxygen uptake (μl O_2 $ind^{-1} hr^{-1}$) by *A. aquaticus* under lead ($0.5 \mu M$ $Pb(NO_3)_2$) conditions. (Raw data in Appendix C).

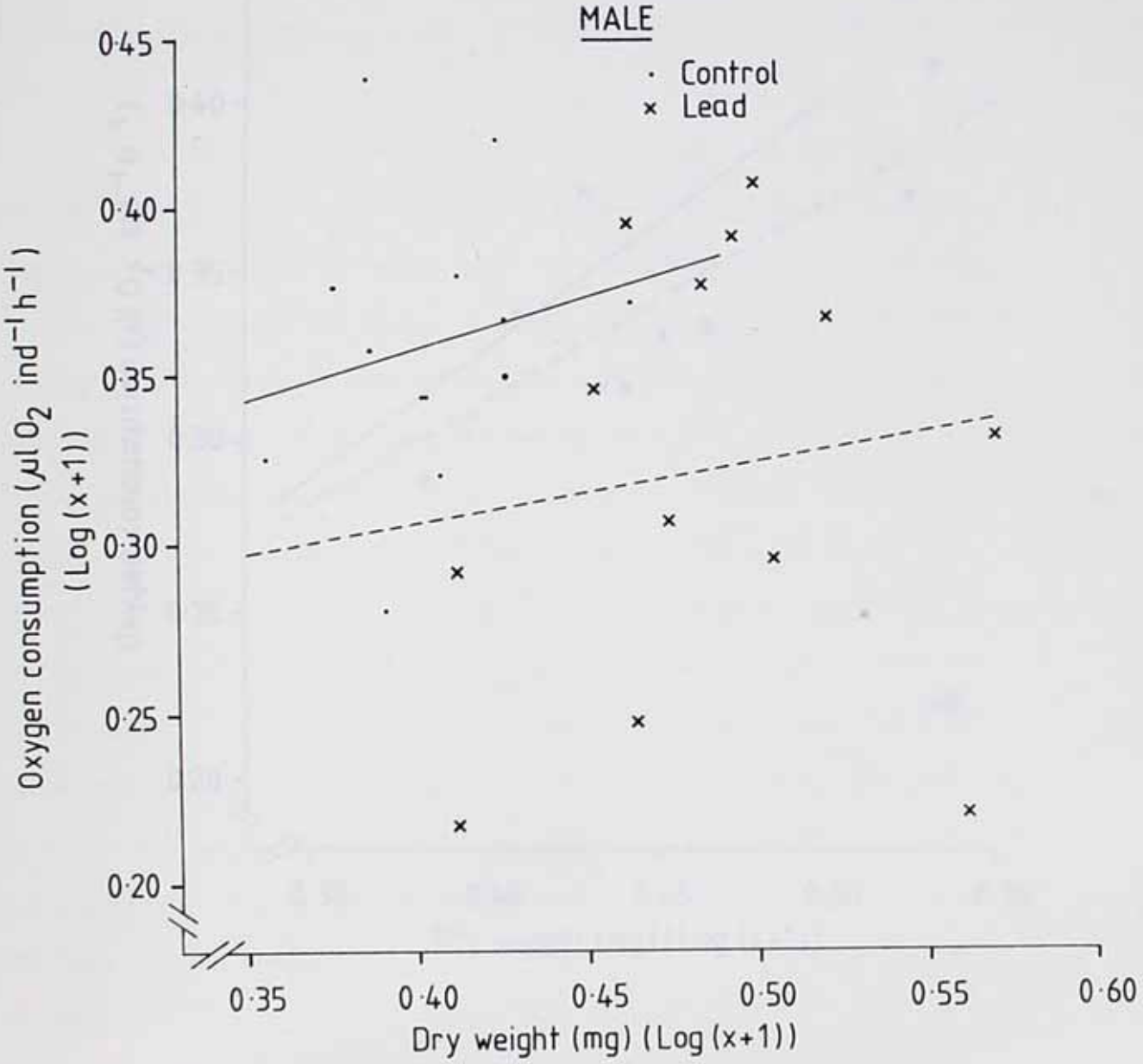


Fig.26. Graph of oxygen consumption (µl O_2 ind^{-1} h^{-1}) as a function of dry weight (mg) in control conditions
(———— $y = 0{\cdot}2439 + 0{\cdot}3094(\pm 0{\cdot}4503)x$)
and under lead stress in A.aquaticus
(- - - - - - - $y = 0{\cdot}2345 + 0{\cdot}1819(\pm 0{\cdot}4059)x$)

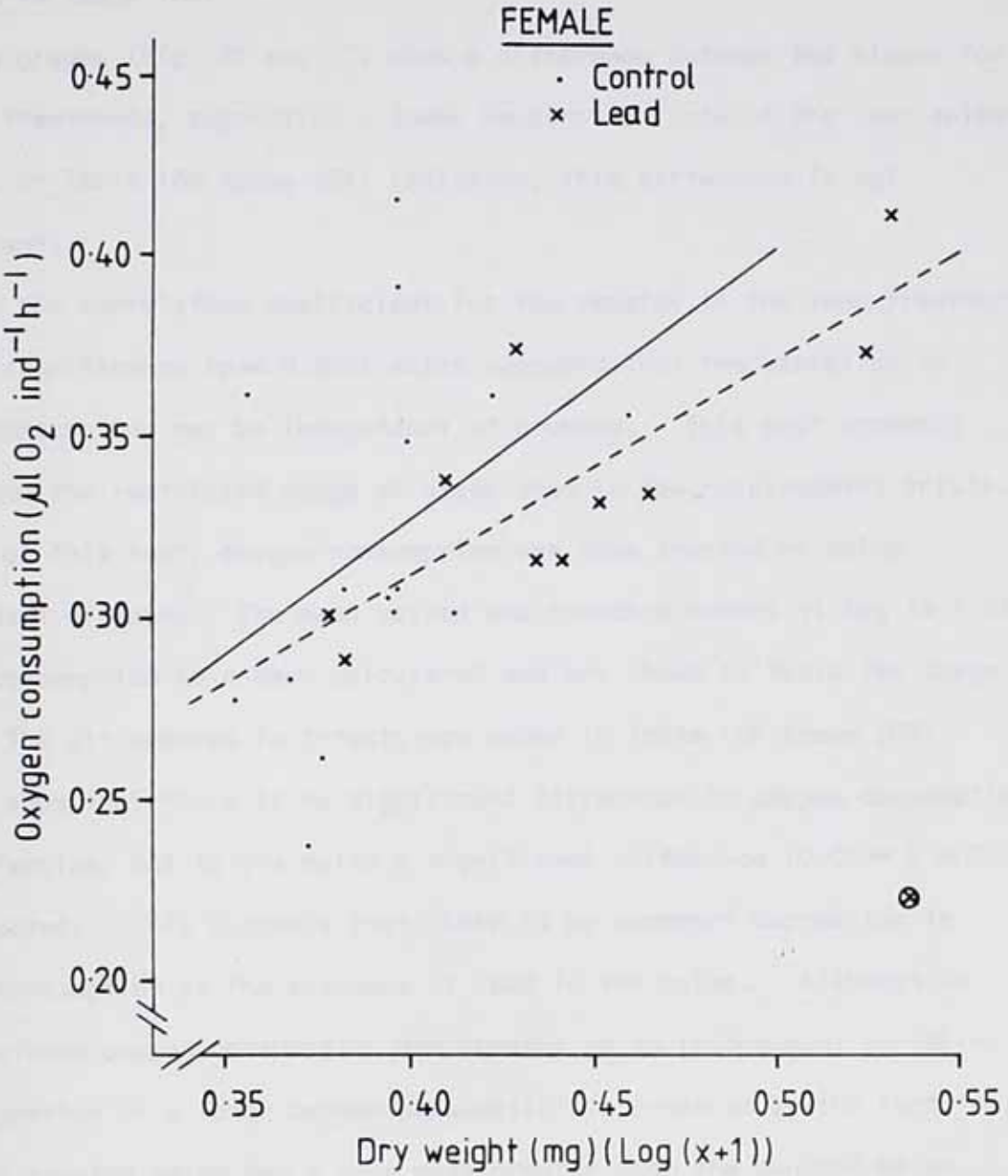


Fig. 27. Graph of oxygen consumption (µl O_2 $ind^{-1}h^{-1}$) as a function of dry weight (mg) in control conditions (——— $y = 0{\cdot}0289 + 0{\cdot}74091(\pm 0{\cdot}4497)x$) and under lead stress in A.aquaticus (----- $y = 0{\cdot}0743 + 0{\cdot}5952\ (\pm 0{\cdot}1495)x$)

has been calculated omitting the anomalous point at 0.536, 0.225 (circled on the graph). The resulting regression equations are shown in Table 18a (page 104).

Both graphs (Fig. 26 and 27) show a difference between the slopes for the two treatments, suggesting a lower respiration rate in the lead animals. However, as Table 18b (page 104) indicates, this difference is not significant.

Only the correlation coefficient for the females in the lead treatment attains significance ($p \leq 0.005$) which suggests that the variation in oxygen consumption may be independent of biomass. This most probably stems from the restricted range of sizes used in the respirometry trials. In view of this fact, oxygen consumption was then treated as being independent of mass. The mean values and standard errors of log $(x + 1)$ oxygen consumption have been calculated and are shown in Table 19a (page 105). The differences in t-tests are shown in Table 19b (page 105). Results show that there is no significant difference in oxygen consumption in the females, but in the males a significant difference ($0.05 \leq p \leq 0.1$) is indicated. This suggests that there is an apparent depression in oxygen consumption in the presence of lead in the males. Although in this instance oxygen consumption was treated as an independent variable, the suggestion of a lower oxygen consumption is enhanced by the fact that the lead treated males had a mean mass greater than the control males ($\bar{x}$ Control = 1.5392; $\bar{x}$ Lead = 2.0556).

Treatment	Sex	n	a	b ±	s.e.	p
Control	Male	13	0.2349	0.3094 ±	0.4503	NS
	Female	15	0.0289	0.7491 ±	0.4497	NS
Control	Male	13	0.2345	0.1819 ±	0.4059	NS
	Female	12	0.0734	0.5952 ±	0.1495	0.005

Table 18a. Linear regressions of Log (x + 1) respiration rates ($\mu l\ O_2\ ind^{-1}\ h^{-1}$) on Log (x + 1) dry weight (mg) for A. aquaticus.

Treatment	Sex	b	t	p
Control vs Lead	Male	0.3094 0.1819	0.2103	NS
Control vs Lead	Female	0.7491 0.5952	0.3247	NS

Table 18b. Comparison of slopes obtained from Table 18a.

Treatment	Sex	Mean oxygen consumption (Log. (x + 1))	±	s.e.
Control	Male	0.3601	±	0.0115
	Female	0.3218	±	0.0128
Lead	Male	0.3228	±	0.0182
	Female	0.3389	±	0.0120

Table 19a. Values for oxygen consumption as an independent variable. (A. aquaticus).

Treatment	Sex	t	p
Control vs Lead	Male	1.7326	$0.05 < p < 0.1$
Control vs Lead	Female	-0.9746	NS

Table 19b. t-tests on the data in Table 19a.

7.4. Discussion

Ecologists in the past have used respiration studies for two primary purposes. Firstly, respiration is measured in order to assess one of the parameters in the formulation of an energy budget. Secondly, it has been used as a convenient measure of metabolic activity in order to assess or compare the role of populations in ecosystem processes (Petrusewicz and Macfayden 1970). With the increasing interest in heavy metal pollution however, attention has been focused on the sublethal effects of these metals on the general metabolism of aquatic organisms, with specific interest on respiration studies.

The effects of heavy metals on respiration in fish have been extensively studied as mentioned earlier (Section 7.1.), and in invertebrates, respiration studies, as a means of detecting the sublethal effects of toxicants, have increased. In this present study, lead, when applied at a sublethal concentration, causes a slight (though not significant) depression in the oxygen consumption in both male and female A. aquaticus. However, apart from the lead treated females, there is a lack of significant correlations between biomass and oxygen consumption which is probably due to the narrow range of sizes used in this study. Further investigations, using a wider size range, may provide conclusive evidence that oxygen consumption is dependent on biomass. Results using oxygen consumption as an independent variable show that there is a depression in the oxygen consumption in the lead treated male A. aquaticus.

The depression in oxygen consumption generally observed might be enhanced if further work was done using animals which had been maintained

for longer periods of time in the test solutions prior to respirometry. The animals here were maintained for only 24 hours, since time proved to be the limiting factor with this series of experiments.

Unfortunately, insufficient material was available for experimentation using A. meridianus so no interspecific comparisons were made. The results with A. aquaticus do suggest that experiments using A. meridianus would prove worthwhile.

Other workers have found that heavy metals depress oxygen consumption. Fraser (1979) studied the effects of lead on the oxygen uptake in tolerant and non-tolerant A. aquaticus. In these experiments lead was found to cause a decrease in the oxygen consumption when applied at concentrations similar to those used in her toxicity tests (6.04 - 15.10 mM dm^{-3} Pb). There was possibly a greater susceptibility to the effects of lead on respiration in the non-tolerant animals. These experiments were only carried out over a period of 20 minutes and the levels were too high for any real conclusions to be drawn about the effects of lead in the environment. Differences in the basic respiration rates observed between the non-tolerant and tolerant animals were not necessarily connected with lead tolerance, since lead had similar inhibitory effects on both populations. Fraser put forward the hypothesis that this was possibly due to an adaptation to differing oxygen levels or flow rates, since Fox and Simmonds (1933) had found that Asellus from fast flowing streams have a higher respiration rate than animals from slow-flowing streams. Results from a further experiment (Fraser, op. cit.) to test this showed no significant difference in the rate of oxygen uptake. A further investigation by Fraser (op. cit.) in an attempt to associate the inhibiting effects of lead (19.32 mM dm^{-3} Pb) with differences in the

ventilation rates of the respiratory pleopods, also showed no significant results. The rate of oxygen uptake was therefore concluded not to be dependent on the ventilation rate. Lockwood (1968) suggests that the body surface may also be permeable to oxygen.

Whitely and Sikora (1970), investigating the effect of lead, nickel and pentachlorophenate on the respiration rate of tubificid worms, found that the respiration function varied according to the substance present. It was affected little by nickel, inhibited by lead and stimulated by pentachlorophenate. In a further study using tubificid worms, Brkovic-Popović and Popović (1977) showed that the direction of the change in respiration rate. caused by various metals, does not have to be the same and depends upon the range of concentrations tested. Cadmium, mercury and copper, at concentrations which were lethal during 24 and 48 exposure, had a depressive effect on the respiration rate. Copper also had a depressive effect at very low concentrations, but cadmium and mercury increased oxygen consumption at concentrations lower that the acute lethal range. Zinc, nickel and chromium, at lethal concentrations, considerably increased the respiration rate of tubificids, but in the range of concentrations under the 48h LC_{50} the respiration rate did not differ significantly from the control.

The results from this present study suggest that at a sublethal level there may be a slight reduction in the oxygen consumption in A. aquaticus but further studies need to be undertaken to establish whether, as in the tubificid worms, studied by Brkovic-Popović et al. (op. cit.), the direction of change in the respiration rate is dependent upon the concentration used. Other investigations are also necessary to provide data to substantiate the nature of oxygen uptake in this instance. The

results here follow a relatively short period in the lead conditions and these cannot be readily extrapolated to the long term. A longer dosing period is therefore necessary before any attempt could be made to relate these respiratory measurements to field conditions.

Respiratory measurements are a useful measurement of an animal's activity and importance in a community. The results here suggest that when lead is present at a sublethal concentration the oxygen consumption may well be reduced which may reduce the effectiveness of Asellus as a detritivore in that community.

CHAPTER EIGHT

CONCLUSIONS AND GENERAL DISCUSSION

The main conclusions from this study can be summarised as follows:

(a) A. meridianus from the River Lathkill contain significantly higher levels of lead than A. aquaticus from Castle Ashby Lake. The higher concentrations correspond with higher lead concentrations found in the River Lathkill sediments. However, although the mean tissue concentration/mean sediment concentration in the A. aquaticus samples is in a 1:1 ratio, in A. meridianus the ratio is 1:14 which suggests that these animals have an ability to prevent the accumulation of lead in their tissues.

(b) In spite of this, A. aquaticus is shown to be more tolerant to lead in short-term acute toxicity tests with a 10 day LC_{50} of 535.15μM $Pb(NO_3)_2$ compared to a 10 day LC_{50} of 23.11μM $Pb(NO_3)_2$ in A. meridianus.

(c) No significant differences in tolerance were observed between the sexes of either species in spite of significant differences observed between the sexes in the background lead concentration in both species.

(d) Significant concentrations of lead are shown to be accumulated from a solution of 5μM $Pb(NO_3)_2$ only by A. aquaticus. A. meridianus showed no such accumulation.

(e) A. aquaticus accumulated lead from a food source of lead-soaked Tilia europaea leaves. Higher levels of lead were accumulated than from a solution alone and the results indicate that food was the principal source of accumulation. No significant accumulation of lead from a food source was recorded in A. meridianus.

(f) A sublethal dose of lead 0.5μM $Pb(NO_3)_2$ produced no significant effect on the growth of either species.

(g) In the controls in the reproduction experiments using A. aquaticus there is a significant correlation between:

(i) the length of the female and the length of the brood pouch (in early and late incubation);

(ii) the length of the brood pouches in early and late incubation;

(iii) the length of the female and the number of young released and

(iv) the length of the brood pouch (in late incubation) and the number of young released.

(h) In the lead treated A. aquaticus only the correlation between length of female and length of brood pouch (in early incubation) proved significant.

(i) Over the size range of animals studied, lead at a concentration of 0.5uM significantly increased the size of the brood pouch.

(j) Significantly fewer eggs were released into the brood pouches of lead treated A. aquaticus.

(k) In the presence of lead more than 50% of the females released the majority, if not all, of their eggs prematurely. This resulted from the brood pouch breaking antero-posteriorly during the incubation period. No evidence of this occurrence was recorded in the control animals.

(l) Of the eggs that survived so that fully developed young were released, a significantly shorter incubation period was observed in the lead females.

(m) No conclusive results were obtained of the effect of lead on reproduction in A. meridianus.

(n) In the respiration studies, regressions of oxygen consumption on dry weight showed that there was an apparent, though not significant, depression in oxygen consumption in the lead treated A. aquaticus.

(o) A significant depression in oxygen consumption (as an independent variable) was observed in the male A. aquaticus.

At the outset, it was intended to study the effects of lead on two populations of each species, one drawn from a lead polluted site and the other from an unpolluted site. Unfortunately, only one population of each species was encountered and so the results could only be compared interspecifically.

Initially problems arose with the heavy metal used. Lead is the least favoured base metal of the experimentalist and the complexity and low solubility of many of its most common compounds in aqueous solutions make it a relatively unattractive target for laboratory research (Rickard & Nriagu 1978). Problems of this nature were experienced during this series of experiments, when even at low concentrations of $Pb(NO_3)_2$, unidentified precipitates formed in the first medium used. This medium had been developed and standardised with the objective of setting standards for water quality in Fish Toxicity tests (HMSO 1969). After testing various media, the one finally used was Griffin's Instant Pond which even then caused problems in the initial stages, but which proved to be the most satisfactory of the solutions tried.

The experimental techniques used in the various sections were not completely satisfactory and improvement of the techniques is necessary before further experiments are carried out. In many instances the procedure followed with A. aquaticus did not always appear to suit A. meridianus as exemplified in the reproduction studies. In future work, where the effects of lead on Asellus were tested (Brown 1976, 1977, 1978; Fraser 1979) only one species was used in each case, though

differing populations were studied enabling intraspecific comparisons. In each instance different experimental techniques were used and it may be that this is necessary in such studies, although a direct interspecific comparison could not then be made.

In this study only animals of one size class were used, 4mm-6mm length. This was because this was the most abundant size group in the A. meridianus population and there were insufficient numbers of other size groups for experimentation. Although several size classes were available in the A. aquaticus population, only the 4mm-6mm group were used so that direct comparison with the A. meridianus population could be made. In future work various size groupings should be utilised. Fraser et al. (1978) found that larger animals were more tolerant to lead than smaller animals, which they suggested was because the smaller animals have a larger surface area/volume ratio and therefore might accumulate proportionately larger concentrations of lead. This, they assumed, was because lead was absorbed through the integument.

From the results obtained in this present study, several points have emerged which compare favourably with previously reported work on the same subject. A. aquaticus was found to be more tolerant to lead than A. meridianus which was similarly noted by Fraser et al. (op. cit.) although the actual mechanism of tolerance was not indicated here. Fraser (op. cit.) postulated that A. aquaticus in her experiments developed tolerance to lead through acclimation, whereas Brown (1976) suggested that tolerance in A. meridianus developed as a result of selection pressure.

The development of tolerance can be achieved by dealing with the toxic effects of the metal in two ways: by storing it in an inert

form or by excreting it. Fraser found that the tolerant A. aquaticus accumulated higher concentrations than the non-tolerant animals and attributed this to the more efficient binding of lead in the body, presumably in an inactive form. The development of tolerance in A. meridianus noted by Brown appeared to be achieved by reduced uptake or by increased excretion as compared to the non-tolerant animals since greater accumulations were recorded in A. aquaticus as compared to the less tolerant A. meridianus.

There are a number of possible routes for lead uptake in Asellus. Lead may enter the body across the body surface, either across the semi-permeable cuticle or whole cuticle, or across particularly permeable areas such as the gills. It may also be taken up through the gut, either from food, or from occasional gulping of water, noted by Waterman (1961). The principal source of accumulation in A. aquaticus was noted to be from a food source. This was also the main route of uptake by the A. meridianus populations used by Brown (1977). Fraser (op. cit.) did not find this to be the case with A. aquaticus, at least not over the 16-day period covered in her experiments. Studies over longer periods might reveal differences in the populations which are not apparent in these short term tests and so further studies should be carried out.

No significant accumulations of lead were observed in A. meridianus in this study from either solution or food. Since A. meridianus has been shown to be less tolerant it may be that this species can only extract lead directly from solution at higher concentrations than were provided in the solution experiment alone. When higher concentrations were available to A. meridianus in the form of food, the low levels of lead recorded may be explained by the animals not ingesting it. The possibility of this species having an ability to employ a mechanism

enabling it to excrete the metal is unlikely, since no tolerance developed as a result.

Many factors influence the rates of uptake and absorption and thus have an important influence on the acute toxicity of pollutants. The chemical or sometimes the physical form of a contaminant in the water or food is a very important control on the rate of uptake. Studies on the uptake of mercury from food by the plaice, Pleuronectes platessa, showed that the retention of methyl mercury was 80-93% with Nereis as food, but only 4-42% with Mytilus (Pentreath 1976). Competition between chemically similar ions can influence rates of uptake and Bryan and Hummerstone (1973a) showed that the rate of uptake of zinc by N. diversicolor was reduced by increasing the level of zinc. Fraser suggested that differences in ionic regulation may influence the uptake and toxicity of lead in the two species of Asellus since Sutcliffe (1974) found that A. meridianus was much less able than A. aquaticus to maintain its sodium concentration in ion-poor water and concluded that A. aquaticus was better adapted to freshwater.

Other important factors are those relating to the state of an organism such as its age, size and stage in life history. For example, small organisms having a large surface area to volume ratio would be expected to absorb contaminants more rapidly than large organisms if the rate of uptake was through the cuticle. Rate of feeding is also related to size and in the Coho Salmon, Oncorhynchus tshawytscha, it was shown that, because the smaller fish consumed proportionately more food, they received the highest dose of DDT (Buhler and Shanks 1970). In crustaceans the moulting phase is very important since the permeability of the surface increases. Thus, in Carcinus maenas, the rate of absorption of ^{134}Cs

increases during the moult, and this is also the time when ^{90}Sr can be readily incorporated during the deposition of calcium in the new shell (Bryan 1961).

Environmental factors, including temperature, hardness, salinity, dissolved oxygen, pH and light can influence both the form of the contaminant in the environment and the physiology of the exposed organisms. These factors have been extensively studied in toxicity experiments and often have a considerable influence (Sprague 1970; Bryan 1976). On the other hand, they may have a smaller influence on the concentrations of contaminants ultimately accumulated, since rates of loss may equally be affected.

In this investigation no attempt has been made to determine the factors affecting rates of absorption of lead in Asellus, although it is important to realise that they exist and therefore could present further areas for consideration in future work.

Neither was any attempt made to determine whether there was direct proportionality between uptake and the external concentration, as only one concentration of solution was used in this series of experiments. Since direct proportionality was observed between the tissue concentration and sediment concentration in the Castle Ashby samples, though not in the River Lathkill samples, it may prove a worthwhile investigation.

In other work, a direct proportionality has been observed for manganese in polychaetes (Bryan & Hummerstone 1973b), for cadmium in shrimps and mussels (Fowler and Benayoun 1974) and for chromate and lead in the oyster, Crassostrea virginica (Schuster and Pringle 1969). In other cases uptake is not directly proportional, e.g. for mercury, copper, silver and zinc in Nereis (Bryan 1976) and for lead and copper

in A. meridianus (Brown 1978). Further work is necessary to determine the effect of concentrations on A. aquaticus and A. meridianus from this study.

The growth studies reported here produced results which further enhanced the view that A. aquaticus is the more tolerant of the two species. A high degree of mortality was observed in the lead treatment in both species, but this occurred earlier in A. meridianus (at day 14) and by day 21 52% of A. meridianus were killed whereas only 28% of A. aquaticus were affected. This suggests that, even at the sub-lethal level used (0.5μM $Pb(NO_3)_2$), these small animals (2mm-4mm) of the population are susceptible to the toxic effects of lead. (Animals of 4mm-6mm length were used to obtain the LC_{50} values). These smaller animals may possibly be more susceptible due to their larger surface area to volume ratio which may enable them to accumulate proportionately larger concentrations of lead if the passage of lead is through the cuticle.

Of those animals that did survive, however, lead produced no discernible effect on the growth of either species. Other workers showed varying results. In Brown's work (1976) lead was shown to inhibit growth in non-tolerant A. meridianus but not in tolerant animals. The tolerant animals produced similar results in this study. Fraser (op. cit.) in contrast, showed that in A. aquaticus growth was stimulated in lead in both tolerant and non-tolerant animals.

The differences in the results may be due to differences in the experimental procedures. In all three series of experiments, different food materials were used which could produce different growth patterns in the species used. Brown used concentrated spinach extract, Fraser

provided the fungus Saprolegnia ferax as food, whereas in this study discs of T. europaea leaves were used. Before any real comparisons can be made, greater standardisation of the experimental techniques is needed.

The most important sublethal effect of a pollutant is the significance to reproduction. Lead could affect Asellus numbers within their habitat through impairing their fecundity, this being the egg production per female, which would then affect the number of live offspring produced. In fact, the total number of eggs released into the brood pouch was significantly smaller in the lead treated females, and therefore the number of live offspring was reduced. The incubation period was also significantly reduced in females maintained in a sublethal dose of lead.

Beeby (1978), in a study on the effects of lead on the fecundity of Porcellio scaber, found that the lead levels assimilated by this species did not affect the gestation period. Extremely high levels of lead were used in the experiment - 1000μM and 10,000μM $Pb(NO_3)_2$ as opposed to the 0.5μM $Pb(NO_3)_2$ used in the present study. Beeby's work concluded that the lead level of the gravid female is related to the animal's calcium level and the number of days feeding on the dosed food. The level of lead in the young animals was not directly related to the parental lead, though the higher the adult lead content the greater was the tendency for lead to be present in the young. This aspect of the study was not looked into in this present investigation, but it may provide a further area of research with regard to Asellus.

In a study using the mussel Mytilus edulis (Bayne et al. 1976), the effect of a pollutant was to cause a decline in fecundity which, in part, was caused by the resorption of eggs as a result of the release of lysosomal enzymes into the cytoplasm of the developing oocytes. Oocytes that did develop were smaller than normal which produced reduced chances of survival. Whether similar events occur in A. aquaticus is unknown.

Overall in this study it appears that sublethal doses of lead affect the reproductive potential of A. aquaticus by reducing the numbers of viable young released into the population. Unfortunately, no similar conclusions could be made with regard to A. meridianus since no broods were produced, but no obvious cause for this has been found. Further investigations are clearly essential before any interspecific comparisons can be made.

Asellus is an important constituent of the benthic community and any pollutant that may impair the animal's activity, by reducing its metabolic activity, could reduce its effectiveness as a detritivore in that community. The results from this study indicate that exposure to a sublethal dose of lead does not significantly reduce oxygen consumption of A. aquaticus when this is dependent on biomass, but there is a slight depression thereby implying a reduction in the animal's metabolism. When oxygen consumption is treated as an independent variable, it is shown that there is a significant depression in the oxygen consumption in the lead treated males. Studies on whole organism respiration and on isolated tissues and homogenates could provide other evidence as to the effect of lead on animals' activity.

Table 20 (pages 121,122) shows a comparison of results of respiration experiments with various freshwater invertebrates using sublethal doses of heavy metals. As the results show, the effect of the heavy metal varied according to the metal used and the species involved.

In Fraser's (op. cit.) experiments using A. aquaticus, she found that lead had an inhibitory effect on oxygen uptake. Her experiments were carried out using distilled water and a solution containing 0.025M $CaCl_2$, but she felt that further investigations were necessary to test the effects of lead using a freshwater medium. Similar problems to those encountered in this study were identified by her since she stated that future investigations would require long-term exposure at low levels in order to avoid precipitation of lead salts.

Species	Source of reference	Metal	Metal salt	Concentration mg l^{-1} of metal	Experimental apparatus	Test Conditions			Response Criteria
						Temp.°C	Hardness	pH	
Gammarus pulex	Jones(1937)	Copper	$CuSO_4$	1.3-6.4	Closed bottle respirometer	-	-	6.2-6.4	Respiration initially stimulated then depressed
Polycelis nigra	Jones(1937)	Copper	$CuSO_4$	12.7-31.75	Closed bottle respirometer	-	-	6.2-6.4	Respiration initially stimulated then depressed.
Polycelis nigra	Jones(1937)	Copper	$CuSO_4$	1.27	Closed bottle respirometer	-	-	6.2-6.4	Respiration depressed.
Tubifex tubifex	Whitely & Sikora (1970)	Lead	$Pb(NO_3)_2$	10-60	Static test	-	-	6.5,7.5 or 8.5	Reduction in oxygen consumption.
T. tubifex	Whitely & Sikora (1970)	Nickel	$NiCl_2$	0-60	Static test	-	-	7.5,8.5	No significant effect on respiration.
Phasganophora capitata (plecoptera)	Kapoor & Griffiths(1976)	Copper	$CuSO_4.5H_2O$	0.8-4.0	Flow through respirometer	20°C	≃0.33	-	24-55% increase in oxygen consumption.
T. tubifex	Brkovic-Popović & Popović(1977)	Cadmium	$3CdSO_4.8H_2O$	0.01	Respirometer	20°C	34.2	7.2	Increase in respiration 6h exposure.
T. tubifex	Brkovic-Popović & Popović(1977)	Cadmium	$3CdSO_4.8H_2O$	0.045	Respirometer	20°C	34.2	7.2	Reduction in respiration 6h exposure.

Table 20. Comparison of respiration experiments using sublethal does of heavy metals and the results obtained for various freshwater invertebrates.

Species	Source of reference	Metal	Metal salt	Concentration mg l^{-1} of metal	Experimental apparatus	Test Conditions			Response Criteria
						Temp. °C	Hardness	pH	
T. tubifex	Brkovic-Popović + Popović(1977)	Chromium	$KrCr_2O_7$	1.53	Respirometer	20°C	34.2	7.2	Increase in respiration 6h exposure.
T. tubifex	Brkovic-Popović + Popović(1977)	Copper	$CuSO_4.5H_2O$	0.21	Respirometer	20°C	34.2	7.2	Significant increase in respiration 6h exposure.
T. tubifex	Brkovic-Popović + Popović(1977)	Mercury	$HgCl_2$	0.082	Respirometer	20°C	34.2	7.2	Reduction in oxygen consumption at this concentration and above
T. tubifex	Brkovic-Popović + Popović(1977)	Nickel	$NiSO_4.7H_2O$	7.0	Respirometer	20°C	34.2	7.2	Increase in respiration at this concentration and above
T. tubifex	Brkovic-Popović + Popović(1977)	Zinc	$ZnSO_4.7H_2O$	0-3.64	Respirometer	20°C	34.2	7.2	Increase in respiration 6h exposure
Asellus aquaticus	Fraser(1979)	Lead	$Pb(NO_3)_2$	0-3125	Oxygen electrode in distilled water	20°C	-	-	Reduction in respiration as concentration increases
A. aquaticus	Fraser(1979)	Lead	$Pb(NO_3)_2$	0.80	Oxygen electrode (0.025m calcium chloride sol^n)	20°C	-	-	Reduction in respiration.
A. aquaticus	Millman (this study)	Lead	$Pb(NO_3)_2$	0.5μM (≏0.1mg l^{-1})	Respirometer	15°C	80	6.0	Apparent reduction in oxygen consumption 5h exposure.

Table 20 (Contd). Comparison of respiration experiments using sublethal doses of heavy metals, and the results obtained for various freshwater invertebrates.

Lead may act by destroying the structure of the gills, thereby causing changes in the oxygen uptake. This has been recorded for a number of metals, e.g. zinc in the stickleback Gasterosteus aculeatus which progressively causes gill abnormalities, presumably reducing the efficiency of oxygen uptake (Brafield and Matthiessen 1976), and lead and cadmium in the marine isopod Jaera nordmanni (Bubel 1976). The gills may not be the only surfaces for gas exchange in Asellus, and Lockwood (1968), suggests that the body surface may also be permeable to oxygen. The surface area across which gaseous exchange occurs in A. aquaticus is, in fact, the limiting factor governing metabolic rate (Bertalanffy 1957). Lead could in some way reduce that surface area, thereby causing a reduction in oxygen uptake, although if this is the case then it was not apparent in this study. Whitely and Sikora, (1970) (see Table 20, pages 121,122) in their investigation on the effect of lead, nickel and pentacholorophenate on the respiration rate of tubificid worms concluded that the effect of metal on the respiratory process might be of a mechanical nature, i.e. a reduction or interruption of respiration due to precipitation of the mucus secreted by epidermal cells.

Other workers have found heavy metal effects on biochemical processes involved in respiration, e.g. copper and cadmium on the oxidation of lactate by trout gills (Bilinski and Jonas 1973), and cadmium and lead on electron and energy transfer in isolated corn mitochondria (Miller et al. 1973; Koeppe and Miller 1970). The works of O'Hara (1971) on acute copper poisoning in bluegills (Lepomus machrochirus) and Hiltibran (1971) on zinc poisoning in tissue homogenates of the same species, also suggest that heavy metals inhibit oxygen consumption at the mitochondrial level. It has been suggested that lead may exert toxic effects on mitochondria by being taken up instead of other divalent cations such as calcium or

magnesium (e.g. Koeppe and Miller, op. cit.).

Clearly the results presented here in this study do not produce conclusive evidence as to the mechanism causing the apparent reduction in oxygen consumption in A. aquaticus. Much more detailed work is necessary involving many of the parameters discussed here. Other factors, such as time of day (Lang and Ruzickova-Langova 1951), time of year (Mann 1958) and temperature (Macan 1974; Adcock 1975) should also be considered.

CHAPTER NINE

SUMMARY

Populations of A. aquaticus from Castle Ashby Lake, Northamptonshire, amd of A. meridianus from the River Lathkill, Derbyshire, were studied under laboratory conditions to determine their responses to lead.

The introductory chapter describes the reason for the study and briefly states the scope of the work. A literature review of heavy metal toxicity in aquatic environments is provided. Methods of identifying the two species are included in Chapter Two, along with an outline of their distribution, life histories and feeding habits.

The two species were maintained separately in the laboratory in a freshwater medium, known as 'Instant Pond', of total hardness 80 mg dm^{-3} $CaCO_3$. A temperature of 15°C and a light regime of 16 hours light and 8 hours dark were provided. The animals were acclimated to these conditions for at least two weeks before exposure to any experimental conditions. Soaked Tilia europaea leaves were provided as food during the acclimation period.

At the two site areas the general characteristics were recorded and, as well as the Asellus spp., all other taxa were collected and identified. The dominant taxa recorded in the River Lathkill were insect larvae, with fewer Mollusca, Hirudinea, Oligochaeta, Crustacea and Playhelminthes. At Castle Ashby Lake, the reverse was true, with few insectum species, but more non-insectum species.

Samples of the sediment and water were also collected to determine the background levels of lead. Analyses were made using the atomic absorption spectrophotometry methods described in Chapter Three. The Asellus spp. were also analysed. In both species the females contained significantly higher concentrations of lead than did the males. Both sexes of A. meridianus contained significantly higher levels than either sex of A. aquaticus. The higher concentrations of lead in A. meridianus from the River Lathkill corresponded with higher concentrations in the

water and sediment at this site. The ratio of mean tissue to sediment concentration in the Castle Ashby samples was approximately 1:1. Concentrations in the River Lathkill A. meridianus were not related to those of the sediment.

The initial series of short-term toxicity tests was carried out on both species to determine the acute lethal and sublethal concentrations of lead. These tests revealed a 10 day LC_{50} value for A. aquaticus of 535.15μM $Pb(NO_3)_2$) with a corresponding value of 23.11μM $Pb(NO_3)_2$ for A. meridianus. It was concluded from this that A. aquaticus was the more tolerant of the two species. A sublethal concentration of 0.5uM $Pb(NO_3)_2$ was inferred from these tests. Toxicity experiments on the separate sexes of the two species revealed no significant differences in their survival rates, so the subsequent uptake experiments did not separate the sexes.

In establishing a lead level to be used in the metal uptake experiments, the results from the toxicity experiments on the separate sexes indicated that a 5μM $Pb(NO_3)_2$ concentration produced a good survival rate. Two different approaches were used to determine the mode of uptake of lead, (1) from solution (5μM $Pb(NO_3)_2$) and (2) from a lead soaked food source. In both sets of experiments, A. aquaticus showed a clear response, the principal source of accumulation of lead being from a food source. A. meridianus showed no significant accumulation from either source.

Further study was then concerned with the sublethal effects of lead on growth, reproduction and respiration of the two species.

In the growth experiments, juveniles (2mm-4mm length) were measured every 7 days over a period of 21 days in the experimental conditions, increase in length being taken as a measure of growth. A high level of

mortality was observed in the lead conditions (52% in A. meridianus and and 28% in A. aquaticus) suggesting that these smaller animals were more susceptible to the toxic effects of lead. (The sublethal level has been obtained using animals 4mm-6mm in length). Of those surviving, a sublethal dose of lead produced no significant effect on growth in either species.

In the reproduction experiments, no conclusive results were obtained of the effect of lead in A. meridianus. In both the lead and control, precopula was frequent and prolonged in many of the pairs, but no broods were produced. Also, other pairs separated on transfer to the experimental container and in others some females were later abandoned by the males and no repairing occurred.

In A. aquaticus the results indicate that sublethal levels of lead affect the fecundity of the species. Over the size range of animals tested (4.9mm-6.0mm) the length of the brood pouch was significantly longer on the lead treated females. The total number of eggs released into the brood pouch was significantly smaller in the lead treated females. In the presence of lead more than 50% of the females released the majority, if not all, of their eggs prematurely, with an associated breaking of the brood pouch antero-posteriorly during the incubation period. Whether this was a direct consequence of the effect of lead is unclear, but no control animals showed any such response. Of the eggs that did survive so that fully developed young were released, a significantly shorter incubation period was observed in the lead treated females. Overall, it appears that a sublethal dose of lead affects the reproductive potential of A. aquaticus by reducing the numbers of viable young released into the population.

The respiratory studies were not fully conclusive. Insufficient material was available for experimentation using A. meridianus and A. aquaticus could only be maintained in the test solution for 24 hours prior to respirometry. The majority of results, using A. aquaticus, indicate an apparent, though not significant, depression in oxygen consumption (when dependent on biomass) in both sexes, in the presence of lead. Further results show that, in the lead treated males, there is a significant depression in oxygen consumption when this is taken as an independent variable. Experiments covering a longer dosing period and using a wider size range of animals are needed before full conclusive evidence can be reported.

It has long been known that heavy metals, such as lead, when present in abnormal concentrations, can stress aquatic communities to the extent that quantitative and qualitative changes occur. Results from this present study confirm this. Using acute lethal concentrations of lead, it has been shown that both species of Asellus are affected by the toxic properties of the metal, but A. meridianus is less tolerant than A. aquaticus.

Sublethal concentrations of lead have also been shown to affect the physiology of Asellus. Results here show that the reproductive potential of Asellus will be affected by a reduction in the numbers of viable young released into the population. Also the effectiveness of Asellus, as a detritivore in the community, may be reduced, caused by a depression in the oxygen consumption which is used as a measure of the animal's activity and importance in that community.

These effects, although observed at much lower levels of lead than those which actually cause death, show that these levels will still have dramatic effects on population density and biology.

APPENDICES

APPENDIX A1

Days \ Concentration (μM)	Control	0.25	0.5	1.0	2.0	5.0	7.0	10.0	20.0	40.0	50.0	100.0	250.0	500.0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	10	0	0	10
4	0	0	0	0	0	0	0	0	0	10	10	10	0	10
5	0	10	0	0	0	10	0	10	10	20	10	10	10	20
6	0	10	10	10	0	10	0	10	10	20	10	10	20	30
7	10	10	10	10	10	20	10	10	10	20	10	30	20	40
8	10	10	10	10	10	20	10	10	30	30	20	30	30	50
9	10	10	10	10	10	20	10	10	30	30	20	30	30	60
10	10	10	10	20	10	20	10	10	30	30	20	30	40	60
11	10	10	20	20	20	20	10	10	40	40	30	30	50	70
12	20	10	30	30	20	20	10	20	40	40	40	40	50	80
13	20	10	30	30	30	20	10	20	60	40	50	40	70	80
14	20	10	30	30	30	30	20	30	60	40	50	50	70	80
15	20	30	30	40	40	30	20	40	70	70	50	50	80	80
16	20	30	40	40	40	40	20	40	70	70	60	60	80	90
17	20	30	40	50	50	40	20	40	70	70	60	70	90	90
18	20	30	40	50	50	40	30	50	80	80	70	80	100	100
19	20	30	40	60	50	40	30	50	80	80	70	90	100	100
20	20	30	40	60	60	50	50	60	90	90	80	100	100	100
21	20	30	40	70	70	50	60	70	90	90	90	100	100	100

Percentage mortality of A. aquaticus (both sexes) in varying concentrations of lead nitrate ($Pb(NO_3)_2$

Concentration (μM) / Days	Control	0.25	0.5	1.0	2.0	5.0	7.0	10.0	20.0	40.0	50.0	100.0	250.0	500.0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0	0	30
2	0	0	0	0	0	0	0	10	10	0	10	10	10	60
3	0	0	0	0	0	0	20	10	10	20	20	30	20	80
4	0	0	0	0	0	10	20	20	20	40	30	40	40	100
5	0	0	0	0	0	10	20	20	20	50	40	50	60	100
6	0	10	0	0	20	20	30	20	30	50	60	50	60	100
7	0	10	0	0	20	20	30	20	30	60	70	50	70	100
8	0	10	0	0	20	20	30	30	40	60	70	60	70	100
9	0	10	0	0	20	30	40	30	50	60	70	60	70	100
10	0	10	0	0	20	30	40	30	50	60	70	70	70	100
11	10	20	0	0	20	30	40	30	50	70	80	70	90	100
12	10	30	0	0	20	40	40	30	50	70	80	80	90	100
13	20	30	0	0	20	40	40	40	80	70	90	90	100	100
14	20	30	0	10	20	40	40	50	80	80	90	90	100	100

Percentage mortality of A. meridianus (both sexes) in varying concentration of lead nitrate ($Pb(NO_3)_2$

APPENDIX A2

PROBIT METHOD (without natural mortality on controls)

1. Tabulate log. dosage. x
2. Tabulate number of animals tested. n
3. Tabulate number responding to dosage. r
4. Tabulate percentage responding to dosage. p
5. Obtain empirical probits from Table - Transformation of percentages to probits. (see Finney 1971).
6. Plot empirical probits on graph and draw best fit line.
7. Obtain Y values from graph. These are an approximation to the expected probits at each dosage.
8. For each Y, read the weighting coefficient, W, from the Table - The Weighting Coefficient and Q/Z. (Finney op. cit.) Multiply W by n and tabulate as nw.
9. For each Y and p, determine the working probit, y, from the Table - Working Probits. (Finney op. cit.).
10. Form columns of products nwx and nwy.
11. Sum the columns nw, nwx, nwy, for each preparation and form:

$$\bar{x} = \frac{Snwx}{Snw} \qquad \text{and} \qquad \bar{y} = \frac{Snwy}{Snw}$$

12. Obtain:

$$Sxx = Snwx^2 - \frac{(Snwx)^2}{Snw}$$

$$Sxy = Snwxy - \frac{(Snwx)(Snwy)}{Snw}$$

13. Use these weighted means and weighted sums of squares and products to give linear regression equations:

$$b = \frac{Sxy}{Sxx} \qquad \text{and} \qquad Y = \bar{y} + b(x - \bar{x})$$

14. Evaluate Y for each x. If these differ much from the expected probits in the first cycle, repeat the computations with the new Y values, substituted for the original Y column. Continue to iterate until good agreement is obtained.
(A reasonable standard to adopt is that no value of Y at the end of the last cycle should differ from the corresponding Y at the beginning by as much 0.2).

PROBIT METHOD (with natural mortality in controls.

1. Tabulate log dosage. x
2. Tabulate number of animals tested. n
3. Tabulate number responding to dosage. r
4. Tabulate percentage responding to dosage. p'
5. Tabulate percentage allowing for natural mortality in controls. P

i.e. $P = \frac{(p' - C)}{(1 - C)}$

C = mortality numbers in control (as a decimal)

p' = mortality in test solutions (as a decimal)

6. Obtain empirical probit from Table - Transformation of percentages to probits - Using P value as %. (Finney 1971)
7. Plot empirical probits against x, and draw best fit line.
8. Obtain y values from graph. These are the expected probits from the regression line.
9. Obtain weighting coefficients (W) from Table - The Weighting Coefficient and Q/Z. (Finney op. cit.).
   Multiply W by n and tabulate as nw.
10. Obtain auxillary variate, x', from Table in 9. (x' = Q/Z).
11. Obtain working probits, y, from Table - Working Probits. (Finney op. cit.).
    Use the P value as % kill.
12. Form columns nwx, nwx' and nwy.
13. Sum columns nw, nwx, nwx' and nwy.
14. Calculate weighted means:

$$\bar{x} = \frac{Snwx}{Snw} \quad , \quad \bar{x}' = \frac{Snwx'}{Snw} \quad , \quad \bar{y} = \frac{Snwy}{Snw.}$$

15. Calculate:

$$Sxx = Snwx^2 - \frac{(Snwx)^2}{Snw}$$

$$Sxx' = Snwxx' - \frac{(Snwx)(Snwx')}{Snw}$$

$$Sx'x' = Snwx'^2 - \frac{(Snwx')^2}{Snw}$$

$$Sxy = Snwxy - \frac{(Snwx)(Snwy)}{Snw}$$

$$Sx'y = Snwx'y - \frac{(Snwx')(Snwy)}{Snw}$$

$$Syy = Snwy^2 - \frac{(Snwy)^2}{Snw}$$

16. To calculations Sx'x, add:

$$\text{number of control animals} \times \frac{\% \text{ surviving}}{\% \text{ dead}} \qquad \text{(as decimal)}$$

and to Sx'y' add:

$$\text{number of control animals} \times \frac{\text{observed no. dead - expected no.}}{\text{expected number}}$$

17. Calculate maximum likelihood equations:

$$\text{(a)} \quad bSxx + \frac{\delta C}{1-C} Sxx' = Sxy$$

$$\text{and (b)} \quad bSxx' + \frac{\delta C}{1-C} Sx'x' = Sx'y$$

18. In equation (a), substitute b with v_{11} and $\frac{\delta C}{1-C}$ with v_{12},

and in (b), substitute b with v_{12} and $\frac{\delta C}{1-C}$ with v_{22},

and replace right-hand sides of euqations 17(a) and 17(b) above by 1.0 and solving. Substitute in equations to solve equation (b).

i.e. $Sxx v_{11} + Sxx' v_{12} = 1$

$Sxx' v_{11} + Sx'y v_{12} = 0$

and $Sxx' v_{12} + Sx'x' v_{22} = 1$

$$\begin{pmatrix} v_{11} & v_{12} \\ v_{12} & v_{22} \end{pmatrix} = \text{Inverse matrix of coefficients.}$$

19. Calculate: $b = Sxy \times v_{11} + Sx'y \times v_{12}$

and $\frac{\delta C}{1-C} = Sxy \times v_{12} + Sx'y \times v_{22}$

20. By substitution of the provisional value of C (i.e. % surviving) (as a decimal)

$$\delta C = \frac{\delta C}{1-C} \times (\% \text{ surviving})$$

and the revised estimate of the natural response rate is

$$C = \% \text{ dead (as a decimal)} + \delta C$$

21. Calculate a for regression equation:

$$a = \bar{y} - b\bar{x} - \frac{6C}{1-C}\bar{x}'$$

22. Substitute values in regression equation:

$$y = a + bx$$

23. Evaluate Y for each x. If these differ much from the expected probits obtained initially, then repeat with a second cycle of calculations. If the revised estimate of C is almost identical with the provisional value, and the expected probits calculated from equation 22 agree closely with those obtained initially, then no second cycle of calculations is needed.

APPENDIX A3

	A. aquaticus											
	Male						Female					
Concentration (μM) / Days	Control	0.5	1.0	5.0	10.0	50.0	Control	0.5	1.0	5.0	10.0	50.0
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	10	0	0	0	0	0	10	0.	0
3	0	0	0	20	0	0	0	0	10	20	0	0
4	0	0	0	20	0	0	0	0	10	30	0	0
5	0	10	10	20	0	0	0	0	10	30	0	20
6	0	10	10	30	0	10	0	10	10	30	10	30
7	0	10	10	30	0	20	0	20	10	30	10	30
8	0	10	10	30	10	20	0	30	10	30	30	30
9	0	20	10	30	20	20	0	30	20	30	30	40
10	0	20	10	30	20	30	0	40	30	30	40	40
11	0	20	10	30	30	30	10	40	40	40	40	40
12	10	20	10	30	30	40	10	40	40	40	40	40
13	10	30	20	30	40	60	10	40	50	40	50	40
14	10	30	30	30	40	90	10	40	40	40	50	50

	A. meridianus											
	Male						Female					
Concentration (μM) / Days	Control	0.5	1.0	5.0	10.0	50.0	Control	0.5	1.0	5.0	10.0	50.0
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	10	0	0	0	0
3	0	0	0	0	0	0	0	10	0	0	0	0
4	0	0	0	0	0	0	0	10	0	10	10	0
5	0	0	10	0	10	10	0	10	0	10	20	0
6	0	0	10	0	10	10	0	10	10	10	20	10
7	0	0	20	0	10	10	0	10	10	10	30	20
8	0	0	20	0	10	10	0	10	10	10	30	30
9	0	0	20	10	10	10	0	10	20	10	30	30
10	0	10	20	10	10	20	0	10	20	10	30	30
11	0	10	20	10	10	30	0	20	40	20	40	30
12	0	10	30	10	10	40	10	20	40	20	40	40
13	10	10	30	10	10	40	10	20	40	20	50	40
14	10	20	30	30	20	40	10	20	40	30	50	50

Percentage mortality of A. aquaticus (male and female) and A. meridianus (male and female) in varying concentrations of lead nitrate ($Pb(NO_3)_2$)

APPENDIX B

CONTROL					LEAD				
	END OF					END OF			
	DAY 0	DAY 7	DAY 14	DAY 21		DAY 0	DAY 7	DAY 14	DAY 21
	1.13	1.20	1.30	1.33		3.00	3.10	3.10	3.10
	1.90	2.00	2.00	2.03		2.50	2.50	2.50	2.50
	1.60	1.63	1.73	1.73		2.20	2.20	2.20	2.20
	1.43	1.53	1.53	1.56		2.20	2.20	2.20	2.20
	1.53	1.60	1.66	1.90		2.20	2.30	2.30	2.30
	2.80	2.80	2.85	2.90		2.90	2.90	2.90	2.90
	3.10	3.10	3.10	3.10		2.80	3.10	3.10	3.20
	2.70	2.70	2.70	2.70		3.30	3.30	3.30	3.30
	2.80	2.80	2.80	2.90		3.30	3.40	3.50	3.50
	3.40	3.40	3.40	3.40		3.20	3.20	3.20	3.20
	3.10	3.40	3.40	3.40		3.20	3.20	3.40	3.65
	2.80	2.85	2.90	2.90		3.10	3.10	3.10	3.10
	2.55	2.57	2.60	2.60		3.50	3.50	3.60	3.60
	2.25	2.30	2.35	2.40		3.60	3.60	3.60	3.60
	2.50	2.60	2.70	2.70		3.50	3.60	3.60	3.60
	2.90	3.00	3.00	3.00		3.60	3.60	3.70	3.70
	2.80	2.80	2.80	2.90		3.70	3.70	3.70	3.70
	2.70	2.72	2.72	2.80		2.58	2.60	2.70	2.70
	2.85	2.85	2.90	2.90		2.30	2.30	2.40	-
	3.10	3.15	3.20	3.20		2.50	2.60	2.60	-
	2.80	2.80	2.80	2.80		3.10	3.40	3.40	-
	3.10	3.10	3.10	3.10		3.35	3.40	3.42	-
	2.90	2.90	2.90	2.90		3.10	3.20	3.20	-
	2.60	2.70	2.90	2.90		3.75	3.80	3.80	-
	2.30	2.35	2.40	-		3.00	3.10	3.10	-

Growth (mm) of A. aquaticus in Control and Lead (0.5μM $Pb(NO_3)_2$) conditions.

CONTROL	END OF				LEAD	END OF			
	DAY 0	DAY 7	DAY 14	DAY 21		DAY 0	DAY 7	DAY 14	DAY 21
	3.30	3.30	3.40	3.50		2.60	2.60	2.60	2.60
	3.70	4.00	4.00	4.00		2.20	2.40	2.45	2.45
	3.70	4.00	4.00	4.20		1.80	1.90	2.10	2.10
	2.10	2.10	2.10	2.10		2.45	2.50	2.50	2.55
	3.60	3.90	4.00	4.00		2.40	2.40	2.50	2.60
	3.80	4.00	4.00	4.00		2.10	2.10	2.10	2.10
	3.50	3.50	3.60	3.70		2.80	3.00	3.00	3.00
	2.00	2.00	2.00	2.20		1.95	2.00	2.05	2.05
	3.80	3.80	3.80	3.80		2.15	2.20	2.30	2.30
	3.10	3.10	3.10	3.20		2.20	2.20	2.40	2.65
	3.80	4.00	4.00	4.10		1.92	1.94	1.94	1.94
	4.40	4.60	4.60	4.60		1.97	2.10	-	-
	4.40	4.40	4.50	4.50		1.90	2.02	-	-
	3.90	4.00	4.00	4.10		2.00	2.00	-	-
	4.10	4.10	4.10	4.20		1.80	1.80	-	-
	3.70	3.90	3.90	4.00		1.90	1.90	-	-
	4.20	4.20	4.30	4.30		2.80	3.00	3.00	-
	2.50	2.60	2.60	2.70		1.87	1.90	-	-
	2.45	2.50	2.60	2.60		1.35	1.35	-	-
	2.50	2.60	2.60	2.60		1.92	1.95	-	-
	2.80	3.05	3.10	3.30		2.60	2.60	2.70	-
	2.65	3.00	3.00	3.00		1.97	2.00	-	-
	2.65	2.80	2.80	2.90		2.18	2.20	-	-
	1.90	2.00	2.00	2.07		1.90	1.93	-	-
	2.05	2.10	2.15	-		2.20	2.20	2.20	2.20

Growth (mm) of A. meridianus in Control and Lead (0.5μM $Pb(NO_3)_2$) conditions.

APPENDIX C

Sex	Dry Weight per 5 Individuals -mg.	µl Oxygen									
		1100h	1130	1200	1230	1300	1330	1400	1430	1500	1530
♀	7.46	0.3	2.9	6.9	10.4	11.3	14.5	15.9	19.2	21.8	23.9
♀	7.88	3.4	6.1	9.4	12.8	15.7	17.5	20.3	22.5	23.6	25.9
♀	7.44	5.9	9.7	13.7	17.9	21.1	24.9	28.6	32.2	35.6	38.6
♀	9.24	0.8	1.3	3.2	6.6	9.6	12.9	16.9	20.7	24.6	27.6
♀	6.90	4.2	5.7	7.4	8.0	10.9	12.7	17.8	18.3	20.6	21.0
♀	7.42	-2.0	-0.9	0.9	3.0	6.5	8.5	13.0	15.3	17.0	18.5
♀	6.36	-0.2	0.9	3.2	6.4	10.8	12.9	17.8	19.4	24.9	27.7
♀	7.54	2.0	4.9	7.5	10.6	13.3	16.6	19.7	23.3	26.8	29.4
♀	7.38	4.5	8.7	13.3	17.5	20.7	25.9	29.2	33.5	37.5	40.0
♀	6.38	3.7	6.8	9.2	11.8	14.3	17.0	18.3	21.1	23.0	24.4
♀	6.78	1.1	1.9	3.8	5.8	7.7	9.7	11.1	13.4	15.9	16.3
♀	7.40	3.9	6.0	9.6	12.1	14.3	17.0	19.5	21.8	24.5	26.8
♀	8.21	-1.1	0.9	3.5	6.5	9.7	13.3	17.1	20.9	23.6	27.6
♀	6.24	4.8	7.9	11.1	12.8	15.2	17.7	19.3	21.8	23.3	25.6
♀	7.06	4.3	7.8	10.6	13.4	15.3	18.0	20.9	23.4	26.2	27.5
♂	7.66	3.8	8.1	10.7	15.7	19.1	21.1	23.7	26.0	28.4	31.9
♂	7.92	6.1	9.5	14.9	18.0	21.3	24.9	27.5	31.4	35.0	37.7
♂	9.52	3.4	6.5	12.4	14.9	18.7	21.9	24.3	28.2	31.0	3.43
♂	8.35	0.4	1.5	4.5	6.5	9.7	12.9	18.8	21.7	24.2	25.5
♂	7.15	-1.2	3.5	-0.9	1.4	7.0	12.1	19.1	25.4	31.2	36.4
♂	6.35	-0.9	0.6	2.8	6.1	9.1	11.4	14.9	17.3	20.7	23.5
♂	7.78	-0.4	0.7	4.7	8.5	12.3	12.6	16.5	17.0	21.3	24.2
♂	7.19	4.1	7.5	10.7	13.8	17.3	20.0	23.5	24.9	30.7	33.4
♂	7.30	-0.7	1.9	3.7	6.3	8.3	10.6	13.3	15.5	17.6	19.6
♂	7.63	2.2	4.4	7.9	10.5	12.9	16.9	18.9	22.8	26.7	28.8
♂	6.90	2.9	6.0	9.8	13.1	16.9	19.8	24.0	27.4	30.5	33.4
♂	8.35	4.9	9.6	13.0	16.8	20.7	23.4	27.1	30.1	31.9	35.3
♂	8.27	1.2	2.2	4.6	7.1	11.1	16.4	21.2	26.6	31.6	35.9

Raw data for oxygen uptake (µl O_2 30 $mins^{-1}$) under control conditions for A. aquaticus (corrected gas uptake readings).

Sex	Dry weight per 5 individuals -mg	µl Oxygen									
		1100h	1130	1200	1230	1300	1330	1400	1430	1500	1530
♀	9.16	0.4	4.0	7.1	10.4	12.7	15.4	17.5	21.8	24.3	26.3
♀	7.84	2.4	6.1	9.7	13.1	16.0	18.1	21.0	24.4	26.9	29.1
♀	6.95	0.9	5.1	7.5	10.9	13.1	14.5	17.1	19.8	22.6	24.1
♀	7.02	-1.1	-1.7	3.2	6.0	8.9	11.1	12.1	15.1	17.3	18.5
♀	9.6	0.0	3.5	6.2	10.3	12.5	15.3	18.1	20.4	23.9	26.7
♀	12.0	3.4	9.3	12.7	16.6	21.3	25.3	27.8	32.4	35.6	40.3
♀	12.2	-0.6	2.1	2.9	4.6	7.3	8.0	9.6	10.8	13.1	15.8
♀	8.6	3.6	6.2	8.5	11.1	13.9	17.9	19.5	21.6	25.2	27.3
♀	8.4	2.7	7.0	10.4	14.4	17.3	21.5	22.6	27.6	31.1	34.2
♀	8.8	1.2	3.6	6.0	8.7	12.2	15.3	16.9	19.4	22.8	25.0
♀	11.7	-0.4	3.1	6.2	10.1	14.5	18.2	21.2	23.9	26.7	30.1
♂	9.16	4.4	8.4	11.1	14.9	19.0	21.1	24.1	27.2	29.3	31.7
♂	7.88	-0.4	1.1	1.9	3.5	5.6	6.9	8.5	10.4	12.3	14.1
♂	11.0	0.4	1.5	2.7	3.5	8.7	11.1	13.2	15.8	19.3	20.6
♂	10.25	1.3	4.6	7.2	10.6	14.1	17.1	21.3	24.0	29.5	32.0
♂	7.9	-0.5	-0.5	1.7	3.9	6.9	8.9	10.1	14.6	16.9	20.4
♂	9.56	-4.7	-5.9	-6.0	-4,6	-1.9	0.6	2.1	5.7	8.5	10.4
♂	10.55	9.8	19.7	24.9	29.2	34.7	39.1	41.0	42.8	43.7	43.7
♂	11.6	8.1	14.7	19.8	23.1	26.2	28.6	29.6	35.2	37.6	40.2
♂	10.7	9.1	18.5	24.5	28.6	32.2	36.0	37.3	41.4	44.6	47.4
♂	9.5	3.7	10.6	15.2	19.6	23.7	26.8	28.2	32.8	35.7	39.1
♂	13.2	1.8	4.3	5.0	5.4	6.6	5.1	10.2	12.5	15.3	17.5
♂	9.9	1.7	5.4	7.2	9.5	11.8	13.4	13.9	21.2	23.4	25.3
♂	13.6	-4.1	-3.2	-2.4	0.2	4.2	7.9	8.8	14.2	17.4	20.3

Raw data for oxygen uptake (µl O_2 30 $mins^{-1}$) under lead (0.5µM $Pb(NO_3)_2$ conditions for A. aquaticus (corrected gas uptake readings).

REFERENCES

ADCOCK J.A. (1975) The bioenergetics of a population of Asellus aquaticus (L.) (Crustacea, Isopoda). M. Phil. Thesis. University of Leicester.

ADCOCK J.A. (1979) Energetics of a population of the isopod A. aquaticus: Life history and production. Freshwater Biology, 9, 343-355.

ANDERSSON E. (1969) Life cycle and growth of A. aquaticus (L.) with special reference to the effects of temperature. Rep. Institute of Freshwater Research, Drottingholm, 49, 5-26.

ANON. (1977) Hach Water Analysis Handbook. Hach Chemical Company.

BAYNE B.L., LIVINGSTONE D.R., MOORE M.N. and WIDDOWS J. (1976) A cytochemical and biochemical index of stress in Mytilus edulis L. Marine Pollution Bulletin, 7, 221-224.

BEEBY A. (1978) Lead in the ecology of roadside verges. Ph.D. Thesis University of Leicester.

BERTALANFFY L. von. (1957) Quantitative laws in metabolism and growth. Quarterly Review of Biology, 52, 217-231.

BIESINGER K.E. and CHRISTENSEN G.M. (1972) Effects of various metals on survival, growth, reproduction and metabolism of Daphnia magna. Journal of the Fisheries Research Board of Canada, 31, 486-490.

BILINSKI E. and JONAS R.E.E. (1973) Effects of cadmium and copper on the oxidation of lactate by rainbow trout (Salmo gairdnerii) gills. Journal of the Fisheries Board of Canada, 30, 1553-1558.

BLAXTER J.H.S. (1977) The effects of copper on the eggs and larvae of plaice and herring. Journal of the Marine Biological Association, U.K., 57, 849-858.

BOGGESS W.R. and WIXSON B.S. (1980) Lead in the environment. Castle House Publications Ltd.

BRAFIELD A.E. and MATTHIESSEN P. (1976) Oxygen consumption of sticklebacks (Gasterosteus aculeatus L.) exposed to zinc. Journal of Fish Biology, 9, 359-370.

BRKOVIC-POPOVIĆ I. and POPOVIĆ M. (1977) Effects of heavy metals on survival and respiration rate of tubificid works. Part II: Effects on respiration rate. Environmental Pollution, 13(2), 93-98.

BROWN B.E. (1976) Observations on the tolerance of the isopod Asellus meridianus Rac. to copper and lead. Water Research, 10, 555-559.

BROWN B.E. (1977) Uptake of copper and lead by a metal tolerant isopod Asellus meridianus Rac. Freshwater Biology, 7, 235-244.

BROWN B.E. (1978) Lead detoxification by a copper tolerant isopod. Nature, 276, 388-390.

de BRUIN A. (1971) Certain biological effects of lead upon the animal organism. Archives of Environmental Health, 23, 249 -254.

BRYAN G.W. (1961) The accumulation of radioactive caesium in crabs. Journal of the Marine Biological Association, U.K., 41, 551-575.

BRYAN G.W. (1967) Zinc regulation in the freshwater crayfish (including some comparative copper analyses). Journal of Experimental Biology, 46, 281-296.

BRYAN G.W. (1976) Some aspects of heavy metal tolerance in aquatic organisms. In : Effects of Pollutants on Aquatic Organisms (ed. P.M. Lockwood).

BRYAN G.W. and HUMMERSTONE L.G. (1971) Adaptation of the polychaete Nereis diversicolor to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptations to copper. Journal of the Marine Biological Association, U.K., 51, 845-863.

BRYAN G.W. and HUMMERSTONE L.G. (1973a) Adaptation of the polychaete Nereis diversicolor to estuarine sediments containing high concentrations of zinc and cadmium. Journal of the Marine Biological Association, U.K., 53, 839-857.

BRYAN G.W. and HUMMERSTONE L.G. (1973b) Adaptation of the polychaete Nereis diversicolor to manganese in estuarine sediments. Journal of the Marine Biological Association, U.K., 53, 859-872.

BRYCE-SMITH E. (undated) Heavy metals as contaminants of the human environment. Chemistry cassette ETSG.

BUBEL A. (1976) Histological and electron microscopical observations on the effects of different salinities and heavy metal ions on the gills of Jaera nordmanni (Rathke) (Crustacea, Isopoda). Cell and Tissue Research, 167, 65-95.

CARPENTER K.E. (1924) A study of the fauna of rivers polluted by lead mining in the Aberystwyth district of Cardiganshire. Annals of Applied Biology, 11, 1-13.

CARPENTER K.E. (1925) On the biological factors involved in the destruction of river fisheries by pollution due to lead mining. Annals of Applied Biology, 12, 1-13.

CARPENTER K.E. (1927) The lethal action of soluble metal salts on fishes. Journal of Experimental Biology, 4, 378-390.

CHINNAYA B. (1971) Effect of heavy metals on the oxygen consumption of Gammarus pulex and Polycelis nigra. Journal of Experimental Biology, 18, 153-161.

CHIPMAN W.A. (1966) Uptake and accumulation of chromium by the clam Tapes decussatus in relation to physical and chemical form. In : Disposal of Radioactive Wastes into Seas, Oceans and Surface Waters (p.571-582).
International Atomic Energy Agency, Vienna.

COOMBES T.L. (1977) Uptake and storage mechanisms of heavy metals in marine organisms. Proceedings of Analytical Division Chemical Society, 14(8), 219-222 .

CORNER E.D.S. and SPARROW B.W. (1956) The modes of action of toxic agents. I. Observations on the poisoning of certain crustaceans by copper and mercury. Journal of the Marine Biological Association, U.K., 35, 531-548.

DUPEY J.R. (1967) An experimental investigation into the ecology of two freshwater isopods, A. aquaticus (Linnaeus) and A. meridianus (Racovitza). Ph.D. Thesis. University of Leicester.

E.I.F.A.C. (1976) Water quality for European freshwater fish. Report on copper and freshwater fish. Technical Paper No. 27. F.A.O. Rome.

EVERSON R.J. and PARKER H.E. (1974) 3-Hepatone versus 4-Methyl 2-Pentanone as extracting solvents in atomic absorption spectrophotometry. Analytical Chemistry, 46, 2040-2042.

EYRES J.P. and PUGH-THOMAS M. (1978) Heavy metal pollution of the River Irwell (Lancashire, U.K.) demonstrated by analysis of substrate materials and macroinvertebrate tissue. Environmental Pollution, 16, 129-136.

FINNEY D.J. (1971) Probit Analysis, 3rd edition, Cambridge University Press.

FÖRSTNER U. and PROSI F. (1979) Heavy metal pollution in freshwater ecosystems. In : Biological Aspects of Freshwater Pollution (ed. O. Ravera) p.129-161.

FOWLER S.W. and BENAYOUN G. (1974) Experimental studies on cadmium flux through marine biota. Radioactivity in the Sea, No. 44. International Atomic Energy Agency, Vienna.

FOX H.M. and SIMMONDS B.G. (1933) Metabolic rates of aquatic arthropods from different habitats. Journal of Experimental Biology, 18, 153-161.

FRASER J. (1979) Lead tolerance in the freshwater isopod Asellus aquaticus. Ph.D. Thesis. Preston Polytechnic.

FRASER J., PARKIN D.T. and VERSPOOR E. (1978) Tolerance to lead in the freshwater isopod Asellus aquaticus. Water Research, 12, 637-641.

GLEDHILL T., SUTCLIFFE D.W. and WILLIAMS W.D. (1976) A revised key to the British Species of Crustacea : Malacostraca occurring in freshwater. F.B.A. Scientific Publication No. 32.

HILTIBRAN R.C. (1971) Effects of cadmium, zinc, manganese and calcium on oxygen and phosphate metabolism of bluegill liver mitochondria. Water Pollution Control Federation Journal, 43, 818-823.

H.M.S.O. (1969) Fish Toxicity Tests. Report of the Technical Committee. Leaflet No. Dd 139779 K36 12/69.

HOLDGATE M.W. (1979) A Perspective of Environmental Pollution. Cambridge University Press.

HOWARTH R.S. and SPRAGUE J.B. (1978) Copper lethality to rainbow trout in waters of various hardness and pH. Water Research, 12, 455-462.

HUSSON R. and HENRY J.P. (1963) "Etude chez trois espèces de genre Asellus des concrétions des organes de Zenker." Societe Zoologique de France.Bulletin, 88, (2-3) 274-77.

HYNES H.B.N., MACAN T.T. and WILLIAMS W.D. (1960) A key to the British species of Crustacea : Malacostraca occurring in freshwater. F.B.A. Scientific Publication, No. 19.

HYNES H.B.N. and WILLIAMS W.D. (1965) Experiments on competition between two Asellus species (Isopoda, Crustacea). Hydrobiologia, 26, 203-210.

JONES J.R.E. (1937) The toxicity of dissolved metallic salts to Polycelis nigra (Muller) and Gammarus pulex (L.). Journal of Experimental Biology, 14, 351-365.

JONES J.R.E. (1938) The relative toxicity of salts of lead, zinc and copper to the stickleback (Gasterosteus aculeatus L.) and the effect of calcium on the toxicity of lead and zinc salts. Journal of Experimental Biology, 15, 394-407.

JONES J.R.E. (1939) The relation between the electrolytic solution pressures of the metals and their toxicity to the stickleback (Gasterosteus aculeatus L.) Journal of Experimental Biology, 16, 425-437.

JONES J.R.E. (1940) The toxicity of the double chlorides of mercury and sodium. I. Experiments with the minnow (Phoxinus phoxinus L.) 17, 325-330.

JONES J.R.E. (1947) The oxygen consumption of Gasterosteus aculeatus L. in toxic solutions. Journal of Experimental Biology, 23, 298-331.

KAPOOR N.N. and GRIFFITHS N. (1976) The effect of copper on the oxygen consumption rates of the stonefly nymph, Phasganophora capitata (Pictet, Plecoptera). Zoological Journal of the Linnean Society, 59, 209-215

KOEPPE D.E. and MILLER R.J. (1970) Lead effects on corn mitochondrial respiration. Science, 167, 1376-1378.

KORMONDY E.J. (1976) Concepts of Ecology. Prentice Hall.

LANG J. and RUZICKOVA-LANGOVA M. (1951) Periodicity in oxygen consumption of Asellus aquaticus. Mém. Soc. Zool. Tchécosl, 15, 89-98.

LLOYD R. (1960) The toxicity of zinc sulphate to rainbow trout. Annals of Applied Biology, 48, 84-94.

LLOYD R. (1961) The toxicity of mixtures of zinc and copper sulphates to rainbow trout (Salmo gairdnerii Richardson). Annals of Applied Biology, 49, 535-538.

LOCKWOOD A.P.M. (1959) The osmostic and ionic regulation of Asellus aquaticus (L). Journal of Experimental Biology, 36, 546-555.

LOCKWOOD A.P.M. (1968) Aspects of the Physiology of Crustacea. Oliver and Boyd, London.

MACAN T.T. (1974) Freshwater Ecology. Longman, London.

MAITLAND P.S. (1977) A coded checklist of animals occurring in freshwater in the British Isles. Institute of Terrestrial Ecology N.E.R.C.

MANN K.H. (1958) Seasonal variation in the respiratory acclimitisation of the leech Erpobdella testacea (Sar.). Journal of Experimental Biology, 35, 314-323.

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

MARCUS J.H., SUTCLIFFE D.W. and WILLOUGHBY L.G. (1978) Feeding and growth of Asellus aquaticus (Isopoda) on food items from the littoral of Windermere including green leaves of Elodea canadensis. Freshwater Biology, 8, 505-519.

MARCUS J.H. and WILLOUGHBY L.G. (1978) Fungi as food for the aquatic invertebrate Asellus aquaticus. Transactions of the British Mycological Society, 70(1), 143-146.

McLAUGHLIN P.A. (1980) Comparative Morphology of Recent Crustacea. W.H. Freeman & Co.

MILLER R.J., BITTELL J.E. and KOEPPE D.E. (1973) The effects of cadmium on electron and energy transfer reactions in corn mitochondria. Physiologia Plantarum, 28, 166-171.

MOON H.P. (1957a) The distribution of Asellus in Windermere. Journal of Animal Ecology, 26, 113-123.

MOON H.P. (1957b) The distribution of Asellus in the English Lake District and adjoining areas. Journal of Animal Ecology, 26, 403-409.

MURPHY P.M. (undated) A manual for toxicity tests with freshwater macroinvertebrates and a review of the effects of specific toxicants. The University of Wales Institute of Science and Technology.

NEEDHAM A.E. (1942) Microanatomical studies on Asellus. Quarterly Journal of Microscopical Science, 84, 49-72.

NEHRING R.B. (1976) Aquatic insects as biological monitors of heavy metal pollution. Bulletin of Environmental Contamination and Toxicology, 15, 147-154.

NRIAGU J.O. (1978) The Biogeochemistry of Lead in the Environment. Part A. Elsevier/North Holland Biomedical Press.

O'HARA J. (1971) Alterations in oxygen consumption by bluegills exposed to sublethal treatment with copper. Water Research, 5, 321-327.

PASSOW H., ROTHSTEIN A. and CLARKSON T.W. (1961) The general pharmacology of the heavy metals. Pharmacological Review, 13, 185.

PENTREATH R.J. (1976) Monitoring of Radionuclides. FAO Fisheries Technical Paper 150, p.8-26.

PETRUSEWICZ K. and MACFAYDEN A. (1970) Productivity of terrestrial animals : principles and methods. IBP Handbook No. 13. Blackwell, Oxford.

PEYTON T., McINTOSH A., ANDERSON V. and YOST K. (1976) Aerial input of heavy metals into an aquatic ecosystem. Water, Air and Soil Pollution, 5(4), 443-451.

PRUS T. (1971) The assimilation efficiency of Asellus squaticus. L. (Crustacea, Isopoda). Freshwater Biology, 1, 287-305.

PRUS T. (1972) Energy requirements, expenditure and transformation during development of Asellus aquaticus. Polish Archives of Hydrobiology, 9, 97-112.

QUIGLEY M.V.J. (1977) Invertebrates of Streams and Rivers. A key to identification. Edward Arnold.

RENFRO W.C., FOWLER S.W., HEYRAND M. and LAROSA J. (1974) Relative importance of food and water pathways in the bioaccumulation of zinc. Technical Report I.A.E.A. 163, 11-20. International Atomic Agency, Vienna.

RICKARD D.T. and NRIAGU J.O. (1978) Aqueous environmental chemistry of lead. In: The Biogeochemistry of Lead in the Environment. Part A. Elsevier/North-Holland Biomedical Press.

RIEUWERTS J.H. (1973) Lathkill Dale : its mines and miners. Moorland Publishing Co.

SALIBA L.J. and AHSANULLAH L.M. (1973) Acclimation and tolerance of Artemia salina and Ophryotrocha labronica to copper sulphate. Marine Biology, 23, 297-302.

SCHUSTER C.N. and PRINGLE B.H. (1969) Trace metal accumulation by the American Eastern oyster, Crassostrea virginica. Proceedings of the National Shellfish Association, 59, 91-103.

SCOURFIELD D.J. (1940) Note on the difference in the colouration of the head in A. aquaticus and A. meridianus. Essex Naturalist, 26, 268-270.

SINLEY J.R., GOETTL J.P. and DAVIES P.H. (1974) The effects of zinc on rainbow trout (Salmo gairdnerii) in hard and soft water. Bulletin of Environmental Contamination and Toxicology, 12, 193-201.

SPRAGUE J.B. (1969) Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Research, 3, 793-821.

SPRAGUE J.B. (1970) Measurement of pollutant toxicity to fish. II. Utilising and applying bioassay results. Water Research, 4, 3-32.

STEEL E.A. (1961) Some observations on the life history of Asellus aquaticus (L.) and A. meridianus Racovitza (Crustacea : Isopoda). Zoological Society of London. Proceedings, 137, 71-87.

STUMM W. and BILINSKI H. (1973) Trace metals in natural waters - difficulties of interpretation arising from our ignorance of their speciation. In: Advances in Water Pollution Research. Proceedings 6th International Conference. Jenkins S.H. (Ed.) p.39-52.

SUTCLIFFE D.W. (1974) Sodium regulation and adaptation to freshwater in the isopod genus Asellus. Journal of Experimental Biology, 61, 719-736.

THURBERG F.P. and COLLIER R.S. (1977) Respiratory response of cunners to silver. Marine Pollution Bulletin, 8(2), 40-41.

TOPPING G. (1977) Measurement and effects of sublethal levels of some heavy metals on marine life in simulated marine ecosystems. Proceedings of Analytical Division Chemical Society, 14(8), 222-224.

WAIWOOD L.G. and BEAMISH F.W.H. (1978) Effects of copper, pH and hardness on the critical swimming performance of rainbow trout. (Salmo gairdnerii Richardson). Water Research, 12, 611-619.

WARNICK S.L. and BELL H.L. (1969) The acute toxicity of some heavy metals to different species of aquatic insects. Water Pollution Control Federation Journal, 41, 280-284.

WATERMAN W. (1961) Physiology of Crustacea. Volume II. Academic Press, London.

WHITELY L.S. and SIKORA R.A. (1970) The effects of three common pollutants on the respiration rate of tubificid worms. Water Pollution Control Federation Journal, 42, 57-66.

WHITTON B.A. and SAY P.J. (1975) Heavy Metals. In: River Ecology Ed. by Whitton B.A. p.286-311. Blackwell Scientific Publications. Oxford.

WIESER W. (1966) Copper and the role of isopods in degradation of organic matter. Science, 153, 67-69.

WIESER W. (1967) Conquering terra firma : the copper problem from the Isopod's point of view. Helgolaender Wissenschaffliche Meeresuntersuchungen, 15, 282-293.

WILLIAMS W.D. (1962a) The ecology of A. aquaticus (L.) and A. meridianus (Rac). Ph.D. thesis. University of Liverpool.

WILLIAMS W.D. (1962b) Notes on the ecological similarities of A. aquaticus (L.) and A. meridianus Rac. (Crustacea, Isopoda). Hydrobiologia, 20, 1-30.

WILLIAMS W.D. (1963) The ecological relationships of the Isopod crustaceans A. aquaticus (L.) and A. meridianus (Rac.) Zoological Society of London. Proceedings, 140, 661-679.

WILLOUGHBY L.G. and MARCUS J.H. (1979) Feeding and growth of the Isopod Asellus aquaticus on actinomycetes, considered as model filamentous algae. Freshwater Biology, 9, 441-449.

ACKNOWLEDGMENTS

I should like to thank all those people at Nene College, Northampton, where the majority of research was undertaken, who provided assistance, including accommodation, during this work. Thanks are due to Michael Quigley and Norman Copland for their supervision and advice and to Helen Stilliard for her technical assistance.

At the University of Leicester, my thanks go to Dr. John Bullock for his advice and for reading and criticizing the writing of this work. Thanks also to Steve Iveson for his technical assistance and to Mary Hardwick and E. Thomas for allowing me to use their Probit Analysis computer programme.

Finally, to my parents, who I cannot thank enough, not only for their financial support, but also for their encouragement during this work.