THE SYSTEMATICS, ECOLOGY AND PHYSIOLOGY
OF NEW ZEALAND LANDHOPPERS
(CRUSTACEA: AMPHIPODA: TALITRIDAE).

PART II. Ecology and Physiology

A thesis
submitted for the Degree of
Doctor of Philosophy in Zoology
in the University of Canterbury

by
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University of Canterbury
1984
Frontispiece. Living specimens of *Makawe hurleyi*. Above, a healthy, adult female in Stage C of the moult cycle. Below, an individual in a moderately advanced stage of whitey disease.
# CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>i</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1. Respiration</td>
<td>2</td>
</tr>
<tr>
<td>2. Water and osmotic relations</td>
<td>82</td>
</tr>
<tr>
<td>3. Ecology</td>
<td>127</td>
</tr>
<tr>
<td>4. Disease</td>
<td>128</td>
</tr>
<tr>
<td>5. Activity</td>
<td>129</td>
</tr>
<tr>
<td>6. Other aspects of landhopper biology</td>
<td>152</td>
</tr>
<tr>
<td>The breeding season and its control</td>
<td>152</td>
</tr>
<tr>
<td>Ecdysis and copulation</td>
<td>157</td>
</tr>
<tr>
<td>Lethal temperatures</td>
<td>161</td>
</tr>
<tr>
<td>Cuticular structure and microbial inhibition</td>
<td>165</td>
</tr>
<tr>
<td>Habitats</td>
<td>178</td>
</tr>
<tr>
<td>Terrestrial adaptations</td>
<td>188</td>
</tr>
<tr>
<td>Summary</td>
<td>200</td>
</tr>
<tr>
<td>References</td>
<td>217</td>
</tr>
<tr>
<td>FIGURES</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Frontispiece</td>
<td>Makawe hurleyi</td>
</tr>
<tr>
<td>Fig.1.1. Animal chamber used in respirometry</td>
<td>12</td>
</tr>
<tr>
<td>Fig.1.2. Electrolytic respirometer diagram</td>
<td>14</td>
</tr>
<tr>
<td>Fig.1.3. Electrolytic respirometer captions</td>
<td>15</td>
</tr>
<tr>
<td>Fig.1.4. Peraeopod dactyls and the moult cycle</td>
<td>28</td>
</tr>
<tr>
<td>Fig.1.5. Surface area versus weight</td>
<td>30</td>
</tr>
<tr>
<td>Fig.1.6. Gill area versus weight</td>
<td>30</td>
</tr>
<tr>
<td>Fig.1.7. Gill area in four species</td>
<td>35</td>
</tr>
<tr>
<td>Fig.1.8. Weight versus respiration rate</td>
<td>43</td>
</tr>
<tr>
<td>Fig.1.9. Temperature versus respiration rate</td>
<td>45</td>
</tr>
<tr>
<td>Fig.1.10. R.Q. and temperature</td>
<td>46</td>
</tr>
<tr>
<td>Fig.1.11. Arrhenius coefficient and temperature</td>
<td>55</td>
</tr>
<tr>
<td>Fig.1.12. Surface area and respiration</td>
<td>57</td>
</tr>
<tr>
<td>Fig.1.13. Respiration through the moult cycle</td>
<td>59</td>
</tr>
<tr>
<td>Fig.1.14. Respiration while immersed</td>
<td>60</td>
</tr>
<tr>
<td>Fig.1.15. Respiration in normal daylength</td>
<td>66</td>
</tr>
<tr>
<td>Fig.1.16. Respiration in constant light</td>
<td>67</td>
</tr>
<tr>
<td>Plate 1. Transverse section of gill (optical)</td>
<td>Follows 81</td>
</tr>
<tr>
<td>Plate 2. Transverse section of gill (TEM)</td>
<td>Follows 81</td>
</tr>
<tr>
<td>Plate 3. Haemocytes in gill tissue</td>
<td>Follows 81</td>
</tr>
<tr>
<td>Fig.2.1. Underwater maze apparatus</td>
<td>86</td>
</tr>
<tr>
<td>Fig.2.2. Emergence apparatus</td>
<td>86</td>
</tr>
<tr>
<td>Fig.2.3. Calibrations for exosomatic water</td>
<td>89</td>
</tr>
<tr>
<td>Fig.2.4. Contact angles</td>
<td>89</td>
</tr>
<tr>
<td>Fig.2.5. Transpiration in Makawe hurleyi</td>
<td>93</td>
</tr>
<tr>
<td>Fig.2.6. Log transformation of transpiration rates</td>
<td>93</td>
</tr>
</tbody>
</table>
Fig. 2.7. Transpiration rates of various species
Fig. 2.8. Transpiration rate and body weight
Fig. 2.9. V.P.D. and transpiration rates
Fig. 2.10. Wind and transpiration rates
Fig. 2.11. Activity under water
Fig. 2.12. Emergence from water
Fig. 2.13. Speed under water
Fig. 2.14. F.P.D. and exosomatic water
Fig. 2.15. O.P. in drying conditions
Fig. 2.16. Exosomatic water versus weight
Fig. 2.17. Exosomatic water vs weight
Fig. 2.18. Survival etc., Makawe hurleyi
Fig. 5.1. Laboratory actigraph
Fig. 5.2. Activity in the laboratory
Fig. 5.3. Field activity
Fig. 5.4. V.P.D., profiles
Plate and Fig. 6.1. PAS stained cuticle section
Plate 6.2 SEM of cuticle, Makawe hurleyi
Plate 6.3 SEM of antenna 2, Makawe hurleyi
Part II  Abstract  Page 1

ABSTRACT

The terrestrial Talitridae (landhoppers) are dominant members of mesic cryptozoic habitats in Gondwana fragments and other Southern Hemisphere land masses. Their respiratory apparatus consists of simple external gills bathed in fluid (exosomatic water). Respiratory rates are moderately high and vary with species, sex, weight, surface area, temperature, disease status, stage in the moult cycle, breeding status, time of day and season. Season acclimatisation is marked in *Makawe hurleyi* and is largely accomplished by an increase in gill area. Continuous respirometry shows that landhoppers are highly disturbed for a period of hours following their introduction into respirometers. In consequence, they have anomalous rates for some time during an experiment.

Transpiration rates are very high. The time course of water loss is complex, but can be modelled by a series of decaying exponentials. Transpiration rate varies with vapour pressure deficit, body area and wind speed. When immersed landhoppers drown. They cannot swim. Some species can climb and they may possess specific adaptations for finding an escape route and breaking through a meniscus. Survival under water is prolonged if the media is oxygenated, therefore death under water is probably related to respiratory failure. Haemolymph and exosomatic fluid osmotic pressures are lower the more terrestrial the species. *M. hurleyi*, the most terrestrial species investigated, had a mean haemolymph
osmotic pressure 45% that of seawater. Starvation did not affect the haemolymph osmotic pressure of *M. hurleyi*. The supralittoral species, *Transorchestia chilensis*, could regulate in both hypo- and hyperosmotic conditions. If environmental water sources became limited it ceased regulating and became a tolerator. Landhoppers possess extremely effective humidity receptors. Different species and genera favour environments with different degrees of terrestrialism. They are generally not found in regions with less than about 550 mm precipitation per year.

The ecology of two species (*M. hurleyi* and *Talorchestia patersoni*) living in waste grassland is described. The two species exhibit partitioning of their habitat with *T. patersoni* living in dry, flood-free microhabitats immediately around the base of tussocks, and the more abundant *M. hurleyi* living between tussocks. This latter species can escape from flooding by climbing up grass stems. Brood size varies with body size of mother and with species. Eggs are lost from the brood as brooding proceeds. Growth in the field is dependent on temperature but generally there are about 15 instars per year. Mortality is high for young, immature animals, considerably lower for young adults, and high for older adults. Biomass is related to litter thickness.

A whitey disease caused by a strain of *Bacillus subtilis* is affecting indigenous landhoppers. It causes a decline in density, age structure and egg production. It was probably introduced by the adventive *Talitroides topitotum* and is spread around the country by
Part II Abstract Page iii

human agencies.

Landhoppers are nocturnally active with activity peaks after dusk and at dawn. Factors affecting general and climbing activity are discussed based on field and laboratory investigations.

Breeding ceases in winter. The time-setting clue for both cessation and initiation of breeding is daylength. Ecdysis is described in one species. In many species males do not carry females during courtship and copulation. Copulation is described for M.hurleyi. Cuticular structures are described. A mucoid layer covers the body and serves to lubricate and defend the body. The layer has antimicrobial properties. Biotic interactions are important in determining landhopper densities because they vary in density from plant community to plant community even though physical conditions appear to be very similar. They occur in a number of adventive plant communities. They are largely absent from damaged native communities and it is hypothesised that this is due to a nutrient deficiency. The phylogenetic age of landhoppers is considered and it is concluded that they are a relatively ancient group.
INTRODUCTION

This is the second part of the thesis. Part I dealt with the systematics and phylogeny of the New Zealand terrestrial Talitridae (landhoppers). This part deals with their physiology, ecology and aspects of their behaviour.
Chapter 1. RESPIRATION

INTRODUCTION

Of all the animal groups which are terrestrial, the landhoppers are unusual in having external gills, and these are only partially enclosed and protected by coxal plates. The marine ancestors of the landhoppers had a respiratory apparatus adapted for the exchange of dissolved gases in an aquatic environment. Thus, in marine or intertidal species, such as *Hyale grandicornis*, the pleopods beat through a full arc forcing an oxygen-bearing water current to flow forward over the exposed gills on the thoracic appendages. Terrestrial species seem little different, at least in their morphology, from these aquatic amphipods. Their pleopods are functional, even if 1 or more pairs are somewhat reduced and all are more delicate than those of their marine or supralittoral relatives. And external gills are still present, held under the body close to the ventral surface and protected by the coxal plates. In view of this apparent lack of adaptiveness, investigation of the respiratory apparatus and its functioning is of prime importance in a study of the physiological ecology of landhoppers. This is the subject of this chapter.

But a consideration of respiration necessitates a consideration of transpiration (water loss) since the two are intimately interconnected. It appears that a tissue cannot be both permeable
to oxygen and impermeable to water because the size of the two molecules is too similar for such a differentiation to be possible (Hinton, 1969). As a consequence respiratory surfaces of terrestrial animals are wet, and must lose water. Most fully terrestrial animal groups have evolved enclosed respiratory epithelia thus lowering their rate of water loss and often allowing the rate to be controlled (Hoar, 1966). In the isopods, for example, Unwin (1931) showed that a decreased evaporation rate follows greater independence from gills that require moistened surfaces. Carter (1931) considered that the conquest of land in advanced groups, with the possible exception of the gastropods with their mantle, has depended on the evolution of invaginated air sacs and air tubes. Landhoppers are, of course, cryptozoa, not fully terrestrial animals in the sense used by Hoar (1966), but in spite of possessing a respiratory apparatus adapted for life in water rather than air, within the cryptozoic environment they are very abundant and successful. Some explanations for this apparent anomaly is explored in the work reported here.

A common solution to the problem of water loss in terrestrial animals has been to reduce the area of moist respiratory membranes and to make nonrespiratory areas dry and impermeable to water. Many previous studies have found that semi-terrestrial Crustacea have a reduced respiratory area compared to aquatic Crustacea: Pearse (1950) reported such a reduction in crabs and isopods, and Gray (1957) noted a reduction in brachyuran crabs. Hughes (1983) showed that weight specific respiration rate increased and gill area
decreased in a range of decapod crabs from aquatic to above tide habitats.

The simple vascular structure of the landhopper gill is non-plicate and non-lamellate so reasonably accurate estimates of gill area are possible and these can be related directly to rates of oxygen consumption. Furthermore, because some landhoppers are more completely terrestrial than the most terrestrial crabs, the ecological series from aquatic environments to land is more complete.

A variety of internal and external factors are known to affect respiration rate (Prosser, 1973), so a number of factors were investigated experimentally to determine their effect on landhopper respiration. One important factor is body size and this has been well documented by Zeuthen (1953). The relationship between body size and respiration rate can be described for both interspecific comparisons as well as for intraspecific comparisons by a power law of the form:

\[ R = a.W^b \] for respiration rate

and

\[ R/W = a.W^{b-1} \] for weight-specific respiration rate,

where \( R \) is rate of oxygen consumption, \( W \) is body weight, and \( a \) is the regression constant, and \( b \) the exponent or regression coefficient (Rubner, 1993; Voit, 1901). Hemmingsen (1960)
discussed the evolution of the relationship between energy metabolism and body surfaces, while Kleiber (1947) discussed the relationship in general. Kleiber found the value of $b$ was close to 0.75. Subsequently, many workers have obtained identical results in mammals and birds (reviewed by Bartholomew, 1977; Calder, 1981). This value has been so well established that it is often called the 'Brody-Kleiber law' for the body mass exponent.

Heusner (1982) re-examined a large number of carefully chosen data sets and analysed them by analysis of covariance. He found a 'mass exponent' of $\frac{2}{3}$ for intraspecific scaling of basal metabolism and he considered the $\frac{3}{4}$ exponent to be a statistical artifact.

The matter was further examined by Feldman and McMahon (1983) who developed a more complete mathematical model and used this to find the best value of $b$. They showed that the numerical value for $b$ of $\frac{3}{4}$ is a precise estimate of the mass exponent for inter-specific variation, but they consider that there is both experimental and theoretical justification for the use of both exponents in the Brody-Kleiber law.

Other models have been proposed, particularly multivariate models involving weight and temperature. A 'surface rule' model as it has been termed has been considered for Crustacea by Bertalanffy and Krywienczyk (1953). Belehradek (1935, 1957), on the other hand, was scornful of the quest for 'realistic' models and considered that only empirical equations which have been found to be useful in the
past, should be used. In this study a variety of 'realistic' and empirical multivariate models were used in the study of the respiratory uptake of *Makawe hurleyi* and *Talorchestia patersoni* in order to find the most descriptive model.

Temperature is another important factor which influences the rate of respiratory uptake of oxygen, and there have been many studies on the nature of the controlling influences involved. In particular, Crozier (1924-25) proposed that there was a master or controlling reaction in intermediary metabolism that was revealed by plots of the Arrhenius constant with the reciprocal of absolute temperature. According to him, breaks in these plots indicated changes in the master-controlling reaction. However, Kunamoto et al (1971) have shown that such breaks can be the consequence of a phase change.

Many other factors are known to affect respiration so as many were studied as was practical. The specific factors studied were: surface area, sex, species, health, brooding status, stage in the moult cycle, circadian and circannian (seasonal) rhythms, and disturbance artifacts.

Knowledge of the structure of the gill is obviously important in a study of landhopper respiratory ecophysiology. Milne and Ellis (1973) described the gill of *Gammarus oceanicus* as follows (p. 312):

"The gills of *G. oceanicus* are simple oval plates, or
"lamellae". Each gill is attached by a stalk to the posterior corner of the coxopodite of a thoracic leg. The gill is a cuticle-covered, flattened sac. Its walls are formed by a single layer of epithelial cells and, except for wandering haemocytes, all cells in the gill are of this one type. Opposing sides of the gill are held together by direct attachments between the epithelial cells from the opposite side. The nucleus and most of the cytoplasm lie adjacent to this point of attachment. As a result, the cells are 35 to 40 micrometres tall at these sites, but only 5 to 6 micrometres tall between them. Since the attachment sites are arranged in rows, the thickened areas of cytoplasm give the illusion of being cords of cells separated by sinuses when the gill is seen in surface view. At the periphery of the gill, the sinuses fuse to form a large continuous channel which is directly continuous with the blood vessels in the gill stalk.

Some of the aims of the present study were to see if this description fits the landhopper gill and to see how this simple structure functioned as a terrestrial organ of respiration.

After a long period when it was thought that blood oxygen-transporting pigments were not present in amphipods and isopods (Fox and Vevers, 1960), haemocyanin was discovered by Berthet (1963), and subsequently confirmed by Berthet et al (1964), Wieser (1965), and Kerr (1970), in both these groups. It was thought desirable to confirm the presence of haemocyanin in the more terrestrial amphipods, and if possible, to estimate its
concentration relative to other species from the supralittoral habitat.
METHODS

Gill morphology

Animals were killed and fixed by dropping into hot Bouins fixative. They were sectioned in paraffin and stained in Mallory's triple stain. Material for transmission electron microscopy was prepared using Lake's (1973) technique.

Areas of oxygen uptake

Spencer and Edney (1955) developed a technique for the detection of oxygen permeable surface areas using reduced aniline blue, but the technique was not successful when used on landhoppers. Instead, animals were injected with 1% lead nitrate solutions, then washed in water, and exposed to gaseous hydrogen sulphide. Blackened areas (of lead sulphide) showed permeability to hydrogen sulphide and, presumably, other gases including oxygen and carbon dioxide.

Body and gill areas

Body areas of 14 M.hurleyi females were determined by dissecting off each body part from specimens of known weight, boiling these in 10% potassium hydroxide until only chitin was left, then carefully washing, in distilled water, and mounting them between two microscope slides in PVA to which a little lignin pink
had been added. The slides were dried under gentle pressure. The shapes of the body parts were traced using a camera lucida, and their areas measured by the 'cut-out and weigh' method.

The gill areas of 12 M.hurleyi winter males, 7 M.hurleyi summer males, 31 M.hurleyi winter females, 15 M.hurleyi summer females, 33 T.patersoni, 18 Hyale grandicornis and 20 Transorchestia chiliensis were determined using the methods above. As a check on the accuracy of the method the areas of the gills on the two sides of the body were measured separately in 10 specimens. These agreed to within 4%.

Relative density

The relative densities (specific gravities) of 20 M.hurleyi were measured by immersion in a very small (4 ml) relative density bottle with a ground glass socket top into which fitted a cone joined to a capillary tube of about 80 mm length. A mark was scratched near the lower end of this tube. In use, the assembled flask was filled to the mark, then an animal of known weight introduced, the cone replaced and the displacement of the water level up the tube measured. The surplus water was also drawn off with a syringe and weighed separately as a check. Uncontrolled volume changes were avoided as far as possible by minimizing handling and keeping the assembly immersed as far as possible under water at the same temperature as that used for filling. Knowing the internal diameter of the capillary and the height of water displaced, the volume of water displaced could be calculated. This
was the volume of the animal. Its relative density could then be calculated.
Oxygen uptake

Two techniques were used to measure the respiration rate, as oxygen uptake, of landhoppers: Warburg manometry and continuous, electrolytic respirometry.

A non-refrigerated Gallenkamp model of Warburg respirometer was used at Dunedin. This could not be operated at temperatures lower than about ambient +2°C. At Christchurch a Braun model with a refrigeration unit added was used. This respirometer could operate at temperatures from -15°C to 50°C. Talorchestia patersoni and Makawe hurleyi were obtained from a suburban garden in Dunedin and M. hurleyi was collected from the banks of the Okeover Stream in the grounds of the University of Canterbury. The Canterbury populations were prone to a whitey disease of bacterial origin (see the chapter on disease in this volume).

Warburg manometry

Individual animals were placed in a respiration flask, the apparatus assembled and allowed to equilibrate with the chosen temperature for an hour (Dixon, 1951; Umbreit et al, 1964). Then the flasks were closed off from the atmosphere and readings taken over 1/4 hour intervals for one hour. Usually the animals were killed after the experiment was completed, but occasionally, a number of individuals were measured at another temperature. The
FIGURE 1.2. Electrolytic respirometer.

A, glassware. Over-all dimensions 200mm x 200mm x 50mm. Guide and supporting plates not shown:
  a, outgassing tap
  b, waterproof leads to oxygen generating electrodes
  c, electrolysis chamber containing 2 bright platinum electrodes (5mm x 5mm)
  d, plastic base plate
  e, rubber squeeze tube
  f, squeeze plate
  g, animal chamber (see details in C)
  h, threaded brass rod
  i, manometer level adjusting knob
  j, 'closed' arm of manometer
  k, 'open' arm of manometer
  l, open tubing for Warburg mode of operation
  m & n, platinum wire terminals
  o, plastic backing plate.

B. Flexible partition made from spring stainless steel wire with coarse mesh bolting silk sewn on. The diameter may be increased by squeezing the loops together with forceps, thus enabling the partition to be placed inside the animal chamber (C).

C. Animal chamber (shown as g in Fig.1.2A) used in these studies, made from a glass B19 cone and socket joint.
  a, animal space
  b, active charcoal space
  c, empty or filled with sand
  d, space for soda lime
Each space is isolated by a flexible partition.
usual precautions of linearity of response were observed, and anomalous results were discarded.

A number of different flask designs were tried before a satisfactory one was developed. The standard biochemical non-armed type as supplied by Braun was unsatisfactory because the animals frequently came into contact with the potassium hydroxide contained in the wick. To overcome this, small flasks were made in which the animal was carried on a stainless steel gauze platform above the KOH saturated wick (Figure 1.1a). Calibration was by the "short stem" mercury displacement method (Umbreit et al, 1964). These flasks were very sensitive and so were suitable for animals down to 1 or 2 milligrams live weight, but suffered from some disadvantages. The KOH had to be introduced using a hypodermic syringe before the animal was inserted, and when the syringe was withdrawn a minute amount of KOH which may have been left on the tip of the needle could come into contact with the gauze platform and contaminate it with KOH. Animals which came into contact with this KOH showed anomalous respiratory rates and, on removal from the apparatus, necrotic, red body areas could be seen where the body had been in contact with the alkali. The platform was always wiped before the animal was introduced, but this only partly alleviated the problem. A further disadvantage of this design was that faeces would sometimes drop through the gauze into the KOH, whereupon in extreme cases, the volume in the flasks would suddenly expand due to the reaction of the faeces and the KOH with the liberation of gas. This problem could be alleviated by starving the animals for 24 hours
beforehand, but this, in turn, raised storage problems in animals which are very sensitive to storage conditions.

The third type of flask designed and used in this study had the KOH contained in a well fixed on a stainless steel gauze platform above the animal (Figure 1.1b). The assembly consisted of an inner flask made from a glass B19 to 21 adapter with a stainless steel gauze platform on its lower end fixed on by epoxy resin. The platform carried a glass well which contained the KOH saturated wick. This part was placed on the cone of the manometer. On the outside of this was placed the animal chamber, containing a single animal, made from a B21 female socket sealed at its base. In this design the animal was unable to come into contact with the KOH and there was no problem with contamination. However, the volume was larger than with the second type of flask. Calibration was by removal of known volumes of gas (Stauffer, 1964).

In all cases the animals to be measured were placed on small slips of filter paper damped slightly with distilled water. Care had to be taken to avoid over-wetting the paper as this caused elevated respiration rates.

To provide a broader comparison between species, the rates of oxygen uptake in air of 18 adult females of both Hyale longicornis, an intertidal species, and 18 Transorchestia chiliensis, a supralittoral species, were measured.
Continuous respirometry

Electrolytic respirometers for continuous respirometry and the determination of BOD have been developed by Macfadyen (1961), Philipson (1962), Fourche (1964), Fourche et al (1972) and Turner and Stevenson (1974) amongst others. Initially, a Philipson type was employed but was abandoned when it was found that bubbles of oxygen formed on the electrode which allowed electrical conduction, and thus the electrolytic production of oxygen, to continue. Perhaps all those models of electrolytic respirometers in which the same electrode is used both for the detection of manometer levels and for the generation of oxygen would suffer from this fault. So with the assistance of technicians from the Departments of Chemistry and Zoology, a respirometer was constructed more on Macfadyen principles with separate electrodes for sensing the manometer level and for generating oxygen. It was made of glass and could operate in Barcroft mode or Warburg mode. In the Barcroft mode it was less sensitive but more stable. It was operated exclusively in Warburg mode during these experiments in order to maximize sensitivity.

The design is shown in Figure 1.2. The pressure inside the respirometer was detected by a U-shaped manometer containing sealed-in-glass platinum wire electrodes and 1% copper sulphate as the manometer fluid. The level of manometer fluid could be adjusted by means of a mechanical screw which operated onto a plastic squeeze bag. This provided a much more rapid and positive adjustment than the electrical adjustment used in previous designs. One arm of the
manometer opens either to the air just above the level of the surrounding water level in the bath (Warburg mode) or to a closed vessel of about the same volume as the animal chamber (Barcroft mode).

The closed arm of the manometer connects to the animal chamber through glass hemispherical joints. The animal chamber can be of different sizes, but for these experiments it was made from a glass Bl9 'Quick-fit' cone and socket joint. The socket contained granulated soda lime held in place by a flexible expanding partition (Figure 1.2b) constructed from stainless steel wire with a bolting silk partition sewn on. The two ends of the spring could be held together by fine forceps thus narrowing the diameter and allowing the partition to be inserted into the socket. When the spring was released it expanded outwards, holding the partition in place and preventing the animal gaining access to the soda lime; gases could diffuse freely through the coarse-meshed bolting silk of the partition.

The cone contained an animal and a small piece of damp filter paper or cotton wool held by another flexible partition (Figure 2c). The space next to this partition was nearly filled with active carbon, held in place by another flexible partition. This was to reduce the volume inside the flask which made the apparatus more sensitive, and to absorb any toxic, excretory products produced by the animal, thus preventing self-poisoning.
On assembling cone to socket any free space could be diminished with damp sand or coarse glass sand.

The narrow end of the cone connected via a hemispherical joint to the electrolysis chamber and to the atmosphere through a tap (Figure 1.2a). The whole apparatus could be flushed or out-gassed, and then sealed from the atmosphere. The electrolysis chamber contained saturated copper sulphate and shiny platinum plate electrodes sealed in glass at the bottom. It was imperative to keep the level of the copper sulphate above the electrodes. Filling the electrode chamber was easily accomplished using a hypodermic syringe. The assembled manometer-animal chamber-electrolysis chamber part of the respirometer was immersed in a Grant water bath regulated to within $0.05^\circ C$ or better.

The electronic relay and constant current supply circuits are shown in Figure 1.3. In use a very low AC potential exists between the manometer fluid and the searching electrode when the circuit is broken. The use of AC minimizes electrolytic generation of gases in the manometer fluid when the circuit is completed. As an added precaution, when the circuit 'makes' the potential drops to zero. Other circuit details are that a high gain electronic relay trips a mechanical relay which turns on a constant current source to the electrolysis chamber which causes generation of oxygen at one electrode and deposition of copper at the other during the over-voltage electrolysis of copper sulphate. Generation of oxygen continues until the increased gas pressure inside the respirometer
flask causes the level in the closed arm of the manometer to drop sufficiently to break the circuit. Thus, as the animal respires, the oxygen it consumes is exactly replaced by electrolytically produced oxygen. The length of time electrolysis was operating was recorded on a data logger made to my specifications by DAVCO Electronics of Christchurch. This device is a 5-channel self-timer which sends a bit string (at a pre-set baud rate, usually 10) to memory while the mechanical relay is closed. Periodically, it dumps its memory on paper tape together with an elapsed time signal in a special compressed code named Rowe code after its developer. After dumping, the unit clears its memory and starts recording again. When the experiment was completed the paper tape was read and decoded on a Burroughs B6718 computer. Output consisted of hourly respiration rates for each channel (animal) corrected to NTPD as well as integrated rates, and X-Y graphical plots of respiration rate versus time.

Animals could be left in the machine for over 164 hours with no observable ill effects and entirely reproducible daily cycles of metabolic activity.

Copper sulphate deposits in the apparatus could be removed with hot water. The copper deposited on the platinum electrode could be removed with nitric acid. Drying capillaries and other hard-to-dry glassware was facilitated by constructing a manifold which distributed filtered, compressed air through a number of narrow orifices and catheter tubes.
During trials, the respirometer was sensitive enough to measure the oxygen uptake of a single mite, and versatile enough to take animals up to the size of a small mammal.

The landhoppers to be measured in the respirometer were caught the day they were to be used to avoid artifacts due to storage. They had to be weighed and handled quickly so it was a matter of chance as to what sex or even species was used in any particular run. The animals were captured and handled using entomological aspirators. They were weighed to 0.0001 g on an overhead-beam, analytical balance, and inserted into the animal chamber of the respirometer using a broad-necked filter funnel. Usually, the animals were in the flasks within an hour of capture. The number of *Makawe hurleyi* measured and the lighting conditions employed in the continuous respirometer are shown in Table 1.1.

After the experiment, the animals were killed in ethanol and examined to determine species, sex, number of podomeres on antenna 2, stage of breeding, and number and stage of development of eggs if present. Determination of dry weight was not practical since storage in ethanol dissolved out a variable amount of ethanol-soluble body materials and no balance sensitive enough to weigh such small animals accurately was available.
Table 1.1 The number of *Makawee hurleyi* used in the various lighting conditions in the electrolytic respirometer.

<table>
<thead>
<tr>
<th>Lighting condition</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Constant dark</td>
<td>30</td>
</tr>
<tr>
<td>2. Constant light</td>
<td>30</td>
</tr>
<tr>
<td>3. 12 hour dark - 12 hour light</td>
<td>38</td>
</tr>
</tbody>
</table>

**Mathematical analysis**

The data were transformed where appropriate to achieve linearity and Model I regression analyses (Sokal and Rohlf, 1979) were carried out. As a check on the validity of the trends revealed by the regression analysis, the raw data were smoothed using cubic splines (Whitten, 1972).

The importance of temperatures and body mass (as live weight) was estimated by using multivariate models analysed on a Burroughs B6718 computer using IBM Scientific Subroutines. In view of Belehradeck's (1935, 1957) criticisms of functions based on theoretical grounds, his empirical functions (i.e., functions which give good results in most cases) were also estimated and used in the multivariate analyses. Table 1.2 lists the functions used. A factor expressing interaction between live weight and temperature was incorporated in some models by including a derived variable T.W; such equations are here called Newell-Roy (1973) plots. Equations
with \(1/R\) as the dependent variable are Arrhenius plots. Equations 1-20 are based on theoretical models (Hoar, 1966) while equations 21 and 22 are Belehradek plots (Belehradek, 1935, 1957; Johnson et al, 1954) based on 'empirical' relationships. Readings above 27.5\(^\circ\)C were not used for this analysis because they were beyond the temperature optimum for the species, and respiration rate rapidly declined with increasing temperature above the optimum. The monotone equations used could not model this part of the MT curve. The significance of the regressions was tested using standard ANOVA methods (Zar, 1974).

![Circuit diagram for the relay and current supply units of the electrolytic respirometer.](image)

**FIGURE 1.3.** Circuit diagram for the relay and current supply units of the electrolytic respirometer.
Table 1.2. Model equations relating rate of oxygen uptake (R) to live body weight (W), temperature (T), and interaction (R.T.).

<table>
<thead>
<tr>
<th>Equation number</th>
<th>Equation</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$R = a + b.T + c.W$</td>
<td>linear plot</td>
</tr>
<tr>
<td>2</td>
<td>$\log R = a + b.\log T + c.\log W$</td>
<td>linear plot</td>
</tr>
<tr>
<td>3</td>
<td>$\log R = a + b.T + c.\log W$</td>
<td>Q_{10} plot</td>
</tr>
<tr>
<td>4</td>
<td>$\log R = a + b.T + c.W$</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>$R = a + b.\log T + c.\log W$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>$1/R = a + b.T + c.W$</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>$\log (1/R) = a + b.\log T + c.\log W$</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>$\log (1/R) = a + b.T + c.\log W$</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>$\log (1/R) = a + b.T + c.W$</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>$1/R = a + b.\log T + c.\log W$</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>$R = a + b.T + c.W + d.T.W$</td>
<td>interaction added</td>
</tr>
<tr>
<td>12</td>
<td>$\log R = a + b.\log T + c.\log W + d.\log (T.W)$ (Newell plots)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>$\log R = a + b.T + c.\log W + d.\log (T.W)$</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>$\log R = a + b.T + c.W + d.\log (T.W)$</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>$R = a + b.\log T + c.\log W + d.\log (T.W)$</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>$1/R = a + b.T + c.W + d.T.W$</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>$\log (1/R) = a + b.\log T + c.\log W + d.\log (T.W)$</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>$\log (1/R) = a + b.T + c.\log W + d.T.W$</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>$\log (1/R) = a + b.T + c.W + d.\log (T.W)$</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>$1/R = a + b.\log T + c.\log W + d.\log (T.W)$</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>$\log R = a + b.\log (1/(T + 273)) + c.\log W$</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>$\log R = a + 1000.\frac{b}{(T + 273)} + c.\log W$</td>
<td></td>
</tr>
</tbody>
</table>
Stage in the moult cycle, as established by Charniaux-Legrand (1952), Carlisle (1960), and Drach and Tchernigovtzeff (1967), was estimated for each individual by darkness of pigmentation and by examining the dactyl of a peraeopod and assessing the degree of formation of new material in comparison with stages shown in Figure 1.4. But these estimates did not yield a numerical index so a moult index for each specimen of *M. hurleyi* was calculated based on the live body weight vs antenna 2 podomere count relationship as given by:

\[
\text{MIND} = \frac{(W_X - W_i)}{(W_i - W)}
\]

where MIND is moult index, \( W_X \) is live weight of animal \( X \), \( W_i \) is lowest weight recorded for any animal in that instar and \( W_i \) is the heaviest weight of any animal in that instar. The rationale is that a light animal, for a given podomere count, would most probably, be early in that instar, while a heavy animal of the same podomere count would be late in that instar. These moult indices are a proportion, thus they vary numerically from 0, indicating ecdysis to 0.999, which indicates premoult before the next ecdysis. The respiration rate of each animal for which a moult index was calculated, was converted into a percentage of the mean respiration rate for all the animals with the same podomere count. These instar-specific percentage rates (ISPR) were treated as the dependent variable in a stepwise polynomial analysis against moult index (MIND) using the SPSS computer package (Nie et al, 1975) on a Prime 750 computer (release 9.0, version M). The functions tested by the step-up procedure were:

\[
\text{ISPR} = a + b \cdot \text{MIND} 
\]
The presence of haemocyanin in the haemolymph of 10 *M. hurleyi* and 10 *T. chiliensis* intermoult females was investigated using the techniques of Smith (1960). A spectronic reflectometer was used to compare the densities of unstained spots on the paper chromatograms. Total body copper was measured using Weiser's (1965) technique.
FIGURE 1.4. Peraeopod dactyls of *Makawe hurleyi* at different stages of the moult cycle. Captions: cm, central mass; d, dactyl terminal spine; ds, new dactyl spine; ef, epithelial fold; n, new inner spine; nd, new dactyl terminal spine; s, inner spine; sps, strand; st, inner spine strand; t, new cuticle on terminal spine.

The new spines are laid down progressively in strands of tissue. They remain partially invaginated until ecdysis.
RESULTS

The gills in landhoppers are enclosed in an incompletely closed box made up from the ventral surface and the sideplates, and the reflexed abdomen. Observation on live animals shows that in Makawe hurleyi the pleopods are held almost horizontally, close to the ventral surface, so that they point anteriorly, and they oscillate through a small arc. The gills are enclosed in a small volume of fluid and are agitated by the waves in this fluid caused by the oscillation of the pleopods. When fully immersed in water, the pleopods beat through a full arc like their aquatic relatives. Thus the limit to their normal beating must be the surface tension or depth of the water in the branchial chamber.

In the species studied there are 5 pairs of gills, of which the first (that on gnathopod 2) and last (that on peraeopod 4) are the largest. The first gill has a lobe (the subcranial lobe) which protrudes anteriorly underneath the head capsule. The last has a lobe (the pendulous lobe) which projects downward often well below the sideplates in some species. Gill area in more tropical species may be increased by folding and lobulation and some ridging or plication. Filaments have never been observed.

In brooding females, the gills are in intimate proximity with and ventral to the eggs. Post-brooding females show displacement of their gills from their normal horizontal position to a near vertical
FIGURE 1.5. Relationship between cuticular surface area and live body weight in *Makawe hurleyi*.

FIGURE 1.6. Relationship between gill area and live body weight in four talitrid species. Open circles, *Hyale grandicornis* (intertidal); crosses, *Transorchestia chilensis* (supralittoral); dots, *Makawe hurleyi* summer females (terrestrial); pluses, *Talorchestia patersoni* (terrestrial).
orientation. In winter, the oostegites of females of *M. hurleyi* become swollen and blood-filled with the marginal setae reduced to stumps. In this condition the oostegites may possibly function as accessory gills.

Blood flow in the gills is highly directed. Blood enters through the stalk in an afferent vessel which opens into a peripheral sinus which runs around the edge of the gill. Periodically, at points along the afferent course of this channel, some blood leaves the channel to flow through transbranchial channels formed between the 'pillars' which join the upper and lower epidermal surfaces, until it ultimately rejoins the efferent peripheral flow from whence it leaves the gill through the efferent blood vessel in the stalk. The flow in the transbranchial sinuses is from anterior to posterior thus still preserving the counter-current arrangement of the aquatic ancestors even though there is no longer a directed flow of water over the gills. The rate of blood flow, as measured by the rate of passage of haemocytes, is rapid, taking about 2 to 3 seconds from gill entry to exit. A colour change can be observed as the haemocyanin in the haemolymph is oxygenated. This appears to occur very rapidly within a short time of entry to the gill in resting individuals.

Permeable areas
When lead injected animals were exposed to \( \text{H}_2\text{S} \), black sulphide deposits were laid down in gills, on the ventral surface of the thorax and abdomen and in the thin cuticle of joints between body and appendage segments. Less dense deposits occurred over the rest of the cuticle.

**Haemocyanin**

The chromatographic separation of *M. hurleyi* haemolymph yielded 3 major fractions of which haemocyanin was the most mobile. The other two fractions were unidentified yellow and red conjugated proteins (chromoproteins). The densities of the haemocyanin spots from *M. hurleyi* was greater than those from *T. chiliensis* by about 25% on average. Total copper was 10% greater in *M. hurleyi* than in *T. chiliensis*.

**Gill morphology**

The structure of the gill of *M. hurleyi* (plates 1 and 2) is very similar to that of *Gammarus oceanicus* as described by Milne and Ellis (1973) except that it is far less broad and there are fewer pillars linking dorsal and ventral surfaces. The cuticle is thinner being only 0.75 micrometres thick, which is only between 0.25 and 0.1 of the thickness of the cuticle on the general body surface. There is evidence of two cell types being present: the more common one is similar to that described by Milne and Ellis (1973) basal to this in some regions is a more dense cell type which often lines the
haemocoel. The predominant cell type has very pronounced apical foldings to form a structure named the apical labyrinth by Milne and Ellis. At the point where these foldings originate in the cuticle there is a small region of electron dense material probably consisting of microfilaments anchoring the fold to the cuticle. Large (up to 4.8 micrometres) haemocoel channels penetrate between adjacent cells to come to within 1.0 micrometre of the surface. The lateral surfaces of these cells are interdigitated with those of the neighbouring cells and hemidesmosomes occur between them. As noted by Milne and Ellis, the adjacent cells are separated by a space which in _M.hurleyi_ is between 0.1 and 0.25 micrometres wide and is continuous with the basal lamina, though not apparently with the haemocoel as in _G.oceanicus_. Mitochondria are abundant in the predominant cell type and mainly occupy a zone below the apical labyrinth and above the basal foldings. They are intimately associated with the channels that massively penetrate the cytoplasm, and are moderately electron dense, usually spheroid structures with numerous lamellar cristae in the fully differentiated state. Large nuclei occur approximately in the middle of the cells. One of the pillars in Plate 2 shows 2 nuclei present, which is evidence that these pillar structures are multicellular.

The second cell type noted here are possibly undifferentiated replacement cells. These have a more granular cytoplasm with fewer mitochondria which are less differentiated with fewer cristae and a less dense matrix. These cells are basal to the epithelial cells.
Wandering haemocytes penetrate deep into the respiratory epithelium. In one moulting individual they were found outside the new cuticle in the space between the old and new cuticles which is filled with moulting fluid (Plate 3). The old epithelial tissue had been autolysed leaving only the cuticle fibres and cuticle. It is of interest that these fibres apparently penetrate deeply (up to 0.53 micrometres) into the epithelium tissue judging by the length exposed by autolysis.

The whole structure is more similar to the gills of G.oceanicus acclimated to 100% 'instant ocean' than those of animals acclimated to 20%.

Body and gill areas.

The well-known 2/3rds power law relating volume (or weight) to surface area in such equations as

\[(\text{surface area}) = \text{constant.} \ (\text{weight})^{2/3}\]  \hspace{1cm} (1)

(Kleiber, 1947; Hemmingsen, 1960) fits the M.hurleyi data shown in Figure 5 very well. Gaussian least squares regression was used to find the parameters, thus giving the function:

\[A = 2.72.W^{2/3} + 1.82\]  \hspace{1cm} (2)

where \(A\) is surface area in \(\text{mm}^2\) and \(W\) is live weight in g.10^{-4}. Amphipod growth in area is not continuous since a growth push during and shortly after ecdysis is followed by a prolonged period of stasis, but the continuous function fits well because of the paucity of data and the experimental error.
FIGURE 1.7. Relationship between gill area and live body weight in *Makame hurleyi* males (A) and females (B) in winter (open circles) and summer (filled-in circles).
The relative density of *Makawe hurleyi* is $1.09 \pm 0.04$, therefore the relationship between surface area and volume ($V$) can be found by multiplying through the right hand side of equation 2 to yield:

$$A = 2.96 \cdot V^{2/3}$$

Thus, the surface area of an amphipod is nearly three times greater than a cube of the same volume.
Table 1.3. Gill areas of amphipods from an ecological series of habitats ranging from intertidal to terrestrial. The areas quoted are for an individual female of 20 mg weight. The percentages given are of *Hyale* gill area.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>HABITAT</th>
<th>GILL AREA % of Hyale area</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hyale grandicornis</em></td>
<td>intertidal</td>
<td>36 (100%)</td>
</tr>
<tr>
<td><em>Transorchestia chilliensis</em></td>
<td>supralittoral</td>
<td>19 (53%)</td>
</tr>
<tr>
<td><em>Makawe hurleyi</em></td>
<td>terrestrial</td>
<td>(28 (winter) (78%) )</td>
</tr>
<tr>
<td></td>
<td>(temperate grassland)</td>
<td>(14 (summer) (39%) )</td>
</tr>
<tr>
<td><em>Talorchestia patersoni</em></td>
<td>terrestrial</td>
<td>12 (33%)</td>
</tr>
<tr>
<td></td>
<td>(cool temperate)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(coastal-strand)</td>
<td></td>
</tr>
</tbody>
</table>

The gill areas in all sexes and species may be related to body weight by functions of the form shown by equation 1, but linearised by taking logs:

\[
\log(A_g) = a + b \log(W)
\]

where \( A_g \) is gill area, expressed in \( \text{mm}^2 \), and \( W \) is live body weight. These relationships are shown in Figure 1.6 and the regression statistics are given in Table 1.4a. For the same body weight, the greatest gill area is found in the intertidal *H. grandicornis*, followed by the supralittoral *T. chilliensis*, then *M. hurleyi*. 
Table 1.4a. Regression equations of gill area on live body weight for four talitrid species. Each is of the form \( \log(\text{area}) = a + b \cdot \log(\text{weight}) \).

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Sex</th>
<th>Season</th>
<th>N</th>
<th>F</th>
<th>( r )</th>
<th>( b )</th>
<th>( \text{SE}_b )</th>
<th>( a )</th>
<th>( \text{SE}_a )</th>
<th>Gill Area (mm(^2)) of a 20 mg specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. grandicornis</strong></td>
<td></td>
<td></td>
<td>29</td>
<td>79.55</td>
<td>0.86</td>
<td>0.653</td>
<td>0.073</td>
<td>1.626</td>
<td>0.227</td>
<td>36.04</td>
</tr>
<tr>
<td><strong>T. chiliensis</strong></td>
<td></td>
<td></td>
<td>18</td>
<td>88.57</td>
<td>0.92</td>
<td>0.739</td>
<td>0.079</td>
<td>0.830</td>
<td>0.187</td>
<td>21.07</td>
</tr>
<tr>
<td><strong>M. hurleyi, m</strong></td>
<td>W</td>
<td></td>
<td>11</td>
<td>13.76</td>
<td>0.78</td>
<td>1.046</td>
<td>0.444</td>
<td>-0.954</td>
<td>0.427</td>
<td>53.68</td>
</tr>
<tr>
<td><strong>M. hurleyi, m</strong></td>
<td>S</td>
<td></td>
<td>8</td>
<td>2.95</td>
<td>0.58</td>
<td>0.727</td>
<td>0.422</td>
<td>0.696</td>
<td>0.351</td>
<td>17.74</td>
</tr>
<tr>
<td><strong>M. hurleyi, f</strong></td>
<td>W</td>
<td></td>
<td>39</td>
<td>128.1</td>
<td>0.88</td>
<td>1.045</td>
<td>0.092</td>
<td>0.040</td>
<td>0.331</td>
<td>23.81</td>
</tr>
<tr>
<td><strong>M. hurleyi, f</strong></td>
<td>S</td>
<td></td>
<td>26</td>
<td>13.96</td>
<td>0.61</td>
<td>0.605</td>
<td>0.162</td>
<td>0.679</td>
<td>0.337</td>
<td>12.12</td>
</tr>
<tr>
<td><strong>T. patersoni</strong></td>
<td></td>
<td></td>
<td>30</td>
<td>88.38</td>
<td>0.87</td>
<td>0.685</td>
<td>0.073</td>
<td>0.036</td>
<td>0.279</td>
<td>8.08</td>
</tr>
</tbody>
</table>

\( f = \text{female}, \ m = \text{male}, \ S = \text{summer}, \ W = \text{winter} \)
(terrestrial), while the terrestrial *T.patersoni* has the smallest gill area (Table 1.3). The males of *M.hurleyi* have larger gill areas than females of the same species and weight, while both sexes show a seasonal acclimatisation in that, for the same body weight, the gill areas are larger in winter than in summer. Note, however, that the regression for *M.hurleyi* males summer was not significantly different from that of winter males at 5%, probably because of the small sample size, but it is included for the sake of completeness.

Analysis of covariance of these regression (Table 1.4b) indicates that there is a significant difference (at 5%) between the females of the four species (summer data only), and between seasons within sexes in *M.hurleyi*, but not between sexes in *M.hurleyi*. 
Table 1.4b. Analysis of covariance.

1. for all species, females, winter only.

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. hurleyi</td>
<td>14</td>
<td>12</td>
<td>151.69</td>
<td>12.64</td>
</tr>
<tr>
<td>T. patersoni</td>
<td>28</td>
<td>26</td>
<td>34.77</td>
<td>1.34</td>
</tr>
<tr>
<td>T. chilensis</td>
<td>18</td>
<td>16</td>
<td>146.66</td>
<td>9.16</td>
</tr>
<tr>
<td>H. grandicornis</td>
<td>29</td>
<td>27</td>
<td>952.06</td>
<td>35.26</td>
</tr>
<tr>
<td></td>
<td>81</td>
<td></td>
<td>1285.18</td>
<td>15.86</td>
</tr>
<tr>
<td>Pooled</td>
<td>84</td>
<td></td>
<td>1988.84</td>
<td>23.67</td>
</tr>
<tr>
<td>Difference between slopes</td>
<td>3</td>
<td></td>
<td>703.66</td>
<td>234.55</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td></td>
<td>9526.48</td>
<td>108.25</td>
</tr>
<tr>
<td>Between adjusted means</td>
<td>3</td>
<td></td>
<td>7537.64</td>
<td>2512.55</td>
</tr>
<tr>
<td>Chi-square for heterogeneity</td>
<td></td>
<td></td>
<td>103.33 for 3 df**</td>
<td></td>
</tr>
<tr>
<td>F for slopes</td>
<td></td>
<td></td>
<td>14.78 for 3 and 81 df**</td>
<td></td>
</tr>
<tr>
<td>F for constants</td>
<td></td>
<td></td>
<td>14.70 for 3 and 82 df**</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.4b continued

2. Seasonal differences within *Makawe hurleyi* females.

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>26</td>
<td>24</td>
<td>3.57</td>
<td>0.148</td>
</tr>
<tr>
<td>Winter</td>
<td>40</td>
<td>38</td>
<td>13.03</td>
<td>0.343</td>
</tr>
<tr>
<td>Pooled</td>
<td>62</td>
<td>62</td>
<td>16.60</td>
<td>0.267</td>
</tr>
<tr>
<td>Difference between slopes</td>
<td>1</td>
<td>0.014</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>64</td>
<td>20.13</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Chi-squared for heterogeneity 10.66 for 1 df**

F for slopes 0.052 for 1 and 62 df, ns.

F for constants 13.34 for 1 and 63 df**


<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>39</td>
<td>37</td>
<td>4.05</td>
<td>0.109</td>
</tr>
<tr>
<td>Males</td>
<td>11</td>
<td>9</td>
<td>1.64</td>
<td>0.182</td>
</tr>
<tr>
<td>Pooled</td>
<td>46</td>
<td>46</td>
<td>5.70</td>
<td>0.12</td>
</tr>
<tr>
<td>Difference between slopes</td>
<td>1</td>
<td>0.31</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>48</td>
<td>0.468</td>
<td>0.468</td>
</tr>
</tbody>
</table>

Chi-squared for heterogeneity 2.30 for 1 df ns.

F for slopes 2.52 for 1 and 46 df, ns.

F for constants 3.66 for 1 and 47 df, ns.

Nb. ns = not significant
* = significant at 5%
** = significant at 1%
Table 1.5. Percentages of total body surface area made up of gill and general body surface in different sized *Makawe hurleyi* summer females.

<table>
<thead>
<tr>
<th>Live weight (mg)</th>
<th>Percent body surface area as gill</th>
<th>Percent of total surface area which is not gill</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20.0</td>
<td>80.0</td>
</tr>
<tr>
<td>5</td>
<td>14.1</td>
<td>85.9</td>
</tr>
<tr>
<td>10</td>
<td>13.5</td>
<td>86.5</td>
</tr>
<tr>
<td>15</td>
<td>13.2</td>
<td>86.8</td>
</tr>
<tr>
<td>20</td>
<td>13.0</td>
<td>87.0</td>
</tr>
</tbody>
</table>

The mature summer female of *M. hurleyi* has thin, flat broodplates with long setae. In winter, however, the broodplates become blood filled with setae reduced to basal stumps only. The winter form in the male is far less easily detected, but the enlarged gills make it reasonably obvious.

The relationship between gill area and total surface area is allometric (Table 1.5) which has important consequences for the form of the equation describing the relationship between mass and metabolic rate.
FIGURE 1.8. Effect of body weight and species on the rate of oxygen consumption of Stage C, winter, non-breeding females of Makawe hurleyi (open circles) and Talorchestia patersoni (solid circles). A and B are functions (for M.hurleyi and T.patersoni respectively) derived from the relative contributions of gill and general body surface to respiratory rate.
Respiration

Animals used

The respiration rate (oxygen consumption) of 633 *M. hurleyi* and 166 *Talorchestia patersoni* was determined in the Warburg respirometer at a range of temperatures ranging from 0°C to 35°C. A further 98 *M. hurleyi* were used in the electrolytic respirometer with each animal being contained in the apparatus for a minimum of three days and nights.

Table 1.6 shows the size distribution of the animals used in the experiments. Very small animals are under-represented because they were very difficult to handle and measure.
FIGURE 1.9. Effect of temperature on the respiration rate of Makawe hurleyi and Talorchestia patersoni. The respiration rates of Hyale grandicornis (intertidal) and Transorchestia chiliensis (supralittoral) at 20 degrees Celsius are included for comparison.
FIGURE 1.10. Relationship between respiratory quotient and temperature for Makawe hurleyi.
### Table 1.6. The number of landhoppers used in Warburg manometry (excluding those that suffered damage during the experiment) and their frequency distribution by podomere number.

<table>
<thead>
<tr>
<th>Podomere number</th>
<th>M. hurleyi Male Summer</th>
<th>M. hurleyi Female Summer</th>
<th>M. hurleyi Winter</th>
<th>T. patersoni Male Summer</th>
<th>T. patersoni Female Summer</th>
<th>T. patersoni Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>1</td>
<td></td>
<td>10</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>10</td>
<td></td>
<td></td>
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<td></td>
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<tr>
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<td>10</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>15</td>
<td>12</td>
<td>28</td>
<td>59</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>17</td>
<td>39</td>
<td>26</td>
<td>15</td>
<td></td>
<td></td>
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<tr>
<td>17</td>
<td>25</td>
<td>32</td>
<td>44</td>
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<td></td>
</tr>
<tr>
<td>19</td>
<td>14</td>
<td>48</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>9</td>
<td>2</td>
<td>13</td>
<td>6</td>
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<td></td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Number</td>
<td>129</td>
<td>65</td>
<td>243</td>
<td>196</td>
<td>166 (inc. 34 males)</td>
<td></td>
</tr>
<tr>
<td>Mean podomere number</td>
<td>17.1</td>
<td>16.3</td>
<td>17.1</td>
<td>18.5</td>
<td>14.4</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity in all sexes and species increases in summer compared with winter. For example, in *M. hurleyi* females the sum of squares for residuals as a percentage of the total sum of squares are: summer, 39.7%; winter, 34.6%. Large, brooding females show a heightened respiration rate which distorts the form of the equations and increases heterogeneity. No compensation for this increased heterogeneity was undertaken in the mathematical analyses.
Table 1.7. F-ratios [(MS attributable to regression)/ (MS of deviations from regression)] for the equations listed Table 1.2.

<table>
<thead>
<tr>
<th></th>
<th>T. patersoni males</th>
<th>T. patersoni females</th>
<th>M. hurleyi males, summer</th>
<th>M. hurleyi males, winter</th>
<th>M. hurleyi females, summer</th>
<th>M. hurleyi females, winter</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>45.8</td>
<td>55.5</td>
<td>8.2</td>
<td>17.1</td>
<td>181.3</td>
<td>308.3</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>50.1</td>
<td>43.6</td>
<td>13.1</td>
<td>115.1</td>
<td>226.4</td>
<td>448.7</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>46.6</td>
<td>55.1</td>
<td>11.8</td>
<td>115.0</td>
<td>242.5</td>
<td>471.4</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>40.7</td>
<td>48.1</td>
<td>11.2</td>
<td>106.8</td>
<td>229.7</td>
<td>436.9</td>
</tr>
<tr>
<td>5</td>
<td>0.4</td>
<td>48.6</td>
<td>37.1</td>
<td>8.4</td>
<td>15.9</td>
<td>141.5</td>
<td>251.9</td>
</tr>
<tr>
<td>6</td>
<td>0.4</td>
<td>19.9</td>
<td>27.4</td>
<td>9.4</td>
<td>35.4</td>
<td>111.8</td>
<td>204.3</td>
</tr>
<tr>
<td>7</td>
<td>0.4</td>
<td>50.1</td>
<td>43.6</td>
<td>13.1</td>
<td>115.1</td>
<td>226.4</td>
<td>448.7</td>
</tr>
<tr>
<td>8</td>
<td>0.4</td>
<td>46.6</td>
<td>55.1</td>
<td>11.8</td>
<td>115.0</td>
<td>242.5</td>
<td>471.4</td>
</tr>
<tr>
<td>9</td>
<td>0.4</td>
<td>40.7</td>
<td>48.1</td>
<td>11.2</td>
<td>106.8</td>
<td>229.7</td>
<td>436.9</td>
</tr>
<tr>
<td>10</td>
<td>0.4</td>
<td>26.8</td>
<td>41.4</td>
<td>12.3</td>
<td>46.5</td>
<td>148.4</td>
<td>275.8</td>
</tr>
<tr>
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<td>37.2</td>
<td>5.5</td>
<td>11.9</td>
<td>145.4</td>
<td>231.3</td>
</tr>
<tr>
<td>12</td>
<td>0.0</td>
<td>20.7</td>
<td>38.4</td>
<td>6.0</td>
<td>75.3</td>
<td>148.6</td>
<td>289.0</td>
</tr>
<tr>
<td>13</td>
<td>0.3</td>
<td>34.9</td>
<td>37.8</td>
<td>8.8</td>
<td>77.6</td>
<td>166.6</td>
<td>326.0</td>
</tr>
<tr>
<td>14</td>
<td>0.3</td>
<td>32.9</td>
<td>31.8</td>
<td>9.3</td>
<td>76.4</td>
<td>162.5</td>
<td>313.2</td>
</tr>
<tr>
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<td>0.0</td>
<td>7.0</td>
<td>29.2</td>
<td>6.2</td>
<td>12.6</td>
<td>91.8</td>
<td>146.8</td>
</tr>
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<td>1.0</td>
<td>15.5</td>
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<td>26.2</td>
<td>79.9</td>
<td>148.7</td>
</tr>
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<td>9.4</td>
<td>27.2</td>
<td>6.4</td>
<td>75.3</td>
<td>121.7</td>
<td>240.1</td>
</tr>
<tr>
<td>18</td>
<td>0.3</td>
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<td>8.8</td>
<td>77.6</td>
<td>166.6</td>
<td>326.0</td>
</tr>
<tr>
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<td>32.9</td>
<td>31.8</td>
<td>9.3</td>
<td>76.4</td>
<td>162.5</td>
<td>313.2</td>
</tr>
<tr>
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<td>0.7</td>
<td>26.2</td>
<td>4.4</td>
<td>31.0</td>
<td>96.1</td>
<td>158.4</td>
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<td>47.1</td>
<td>54.2</td>
<td>11.9</td>
<td>115.7</td>
<td>246.4</td>
<td>475.8</td>
</tr>
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</table>
Table 1.8. Multiple correlation coefficients for the respiratory multiple regression analysis.

<table>
<thead>
<tr>
<th>EQUATION</th>
<th>Patersoni males</th>
<th>Patersoni females</th>
<th>M. hurleyi males summer</th>
<th>M. hurleyi males winter</th>
<th>M. hurleyi females summer</th>
<th>M. hurleyi females winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.27</td>
<td>0.67</td>
<td>0.77</td>
<td>0.58</td>
<td>0.43</td>
<td>0.77</td>
</tr>
<tr>
<td>2</td>
<td>0.28</td>
<td>0.68</td>
<td>0.73</td>
<td>0.66</td>
<td>0.78</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>0.28</td>
<td>0.67</td>
<td>0.77</td>
<td>0.65</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>4</td>
<td>0.28</td>
<td>0.64</td>
<td>0.74</td>
<td>0.63</td>
<td>0.76</td>
<td>0.80</td>
</tr>
<tr>
<td>5</td>
<td>0.27</td>
<td>0.68</td>
<td>0.70</td>
<td>0.58</td>
<td>0.42</td>
<td>0.72</td>
</tr>
<tr>
<td>6</td>
<td>0.28</td>
<td>0.51</td>
<td>0.64</td>
<td>0.60</td>
<td>0.56</td>
<td>0.68</td>
</tr>
<tr>
<td>7</td>
<td>0.28</td>
<td>0.68</td>
<td>0.73</td>
<td>0.66</td>
<td>0.78</td>
<td>0.80</td>
</tr>
<tr>
<td>8</td>
<td>0.28</td>
<td>0.67</td>
<td>0.76</td>
<td>0.65</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>9</td>
<td>0.28</td>
<td>0.64</td>
<td>0.74</td>
<td>0.64</td>
<td>0.76</td>
<td>0.80</td>
</tr>
<tr>
<td>10</td>
<td>0.28</td>
<td>0.56</td>
<td>0.72</td>
<td>0.65</td>
<td>0.62</td>
<td>0.73</td>
</tr>
<tr>
<td>11</td>
<td>0.48</td>
<td>0.67</td>
<td>0.58</td>
<td>0.77</td>
<td>0.44</td>
<td>0.79</td>
</tr>
<tr>
<td>12</td>
<td>0.11</td>
<td>0.60</td>
<td>0.60</td>
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<td>0.77</td>
<td>0.80</td>
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<td>0.29</td>
<td>0.69</td>
<td>0.67</td>
<td>0.77</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>14</td>
<td>0.28</td>
<td>0.68</td>
<td>0.68</td>
<td>0.74</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>15</td>
<td>0.14</td>
<td>0.40</td>
<td>0.61</td>
<td>0.73</td>
<td>0.45</td>
<td>0.72</td>
</tr>
<tr>
<td>16</td>
<td>0.50</td>
<td>0.54</td>
<td>0.64</td>
<td>0.66</td>
<td>0.58</td>
<td>0.70</td>
</tr>
<tr>
<td>17</td>
<td>0.14</td>
<td>0.45</td>
<td>0.72</td>
<td>0.61</td>
<td>0.77</td>
<td>0.77</td>
</tr>
<tr>
<td>18</td>
<td>0.29</td>
<td>0.69</td>
<td>0.77</td>
<td>0.67</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>19</td>
<td>0.28</td>
<td>0.68</td>
<td>0.74</td>
<td>0.68</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>20</td>
<td>0.22</td>
<td>0.67</td>
<td>0.76</td>
<td>0.65</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>21</td>
<td>0.28</td>
<td>0.67</td>
<td>0.76</td>
<td>0.65</td>
<td>0.78</td>
<td>0.81</td>
</tr>
</tbody>
</table>
Model equations

The program used in the multivariate analysis of the models in Table 1.1 produced multiple regression analyses, a multiple correlation analysis, and an analysis of variance of the sources of variation. Table 1.7 gives the F ratios (variance ratios of the mean squares attributable to the regression versus mean squares of deviations from regressions) which may be used to assess the goodness of fit for each of the species and models. Table 1.8 gives the multiple correlation coefficients for each model.

It was somewhat surprising that no significant regressions were found for T. patersoni males with all models, implying no effect of body weight and temperature on the rate of respiratory oxygen uptake. But this is possibly an artifact resulting from the relatively small numbers of males of this species measured.

In all other cases, except for one with Equation 20, the regressions were significant, often highly so. Thus the procedure does not reject any model as inappropriate with the possible exception of Equation 20. This is a reflection of the heterogeneity of the data. Oxygen uptake rates are affected by many more factors than weight or temperature and these added significantly to the heterogeneity. An attempt was made to decrease heterogeneity by selecting only intermoult, healthy individuals. But many diseased individuals could not be detected without being sacrificied. And the visual intermoult criterion (colour and darkness of colouration)
turned out to be quite unreliable. And, as shown by the electrolytic respirometer, different individuals had a differing response to the disturbance of being introduced into the respirometer. All these factors significantly increased heterogeneity/

However, the analysis does suggest that some models are better than others. Newell models have little additional advantage over simpler models. Linear models were surprisingly good, almost as good as any, although the standard error of estimate was high. The Arrhenius models were universally good, but the best models were Equations 3 and 21. The values of the statistics for these two equations and the various standard errors are given in Table 1.9 for the different species, sexes and seasons investigated.
Table 1.9. Values for the statistics of the multiple regression analysis of the respiratory data for Equation 3 and 21.

A. EQUATION 3.
M. hurleyi, females, summer
Equation:
\[ \log(R) = -1.068 + 0.0325 \cdot T + 1.034 \cdot \log(W) \]
Standard errors:
for T: 0.0028, for W: 0.0965, for estimate: 0.189
Multiple correlation coefficient: 0.776

M. hurleyi, females, winter
Equation:
\[ \log(R) = -0.525 + 0.0220 \cdot T + 0.739 \cdot \log(W) \]
Standard errors:
for T: 0.0011, for W: 0.0615, for estimate: 0.142
Multiple correlation coefficient: 0.808

M. hurleyi, males, summer
Equation:
\[ \log(R) = -0.370 + 0.0186 \cdot T + 0.655 \cdot \log(W) \]
Standard errors:
for T: 0.0024, for W: 0.111, for estimate: 0.168
Multiple correlation coefficient: 0.765

M. hurleyi, males, winter
Equation:
\[ \log(R) = -0.0483 + 0.0177 \cdot T + 0.0330 \cdot \log(W) \]
Standard errors:
for T: 0.00253, for W: 0.00647, for estimate: 0.175
Multiple correlation coefficient: 0.743

T. patersoni, females
Equation:
\[ \log(R) = -0.444 + 0.0176 \cdot T + 0.700 \cdot \log(W) \]
Standard errors:
for T: 0.00321, for W: 0.0917, for estimate: 0.180
Multiple correlation coefficient: 0.671

T. patersoni, males
Equation:
\[ \log(R) = 0.00378 + 0.0125 \cdot T + 0.272 \cdot \log(W) \]
Standard errors:
for T: 0.0146, for W: 0.423, for estimate: 0.211
Table 1.9 continued

B. EQUATION 21

M. hurleyi, females, summer
\[ \log(R) = 8.667 - 2661/(T + 273) + 1.031 \log(W) \]

M. hurleyi, females, winter
\[ \log(R) = 6.272 - 1861/(T + 273) + 0.7394 \log(W) \]

M. hurleyi, males, summer
\[ \log(R) = 5.295 - 1552/(T + 273) + 0.6642 \log(W) \]

M. hurleyi, males, winter
\[ \log(R) = 10.98 - 3092/(T + 273) + 0.4137 \log(W) \]

T. patersoni, females
\[ \log(R) = 5.180 - 1544/(T + 273) + 0.7011 \log(W) \]

T. patersoni, males
\[ \log(R) = 3.951 - 1083/(T + 273) + 0.2718 \log(W) \]

The results from the Dunedin populations were analysed separately because the animals from this area did not suffer from whitey disease, and it was hoped that the analysis of the effect of weight, temperature, and season would be more accurate than the analysis given earlier. Table 1.10 gives, for the Dunedin population of M. hurleyi and T. patersoni, the regression parameters for the weight-specific respiration rate (R/W) as related to live weight (W) by the relationship

\[ \log(R/W) = \log(a) + b \log(W) \].

The parallel regression analyses used by Heusner (1982) and Feldman and McMahon (1983) were not used here because such analyses obscure weight – temperature interactions which are likely to be important in landhoppers. These interactions are due to one size group showing a different metabolic response to the other size groups at a
particular range of temperatures, and they show up as a change in the numerical value for \( b \), the slope. Interactions of this kind are especially important at the extremes of the temperature range.

Factors affecting respiration

The factors which can affect respiration rate include the extrinsic factors of temperature, season, and disturbance, and the intrinsic factors of species, size, sex, stage of the moult cycle, state of brooding, state of health, and circadian rhythms. Each of these will be considered in turn.

Species

Under the same conditions, the respiration rate of healthy \( M.\text{hurleyi} \) is higher than for \( T.\text{patersoni} \). Figure 1.8 shows the respiration rate of intermoult females of both species at 20°C. The multivariate analysis shows that the difference between species is not constant but depends on conditions. Figure 1.8 also gives interspecific comparisons of the rate of oxygen uptake from air at 20°C for adult females of four species, ranging from the intertidal \( H.\text{grandicornis} \), to the supralittoral \( T.\text{chiliensis} \), to the terrestrial \( M.\text{hurleyi} \) and \( T.\text{patersoni} \). The results parallel those for relative gill areas in that the intertidal species has the greatest rate, the supralittoral species is next, and the two terrestrial species have the smallest rate.
FIGURE 1.11. Relationship between the log of the Arrhenius constant, \( A \), and the reciprocal of the absolute temperature, \( T \) (degrees Kelvin). Closed circles, *M. kawe hurleyi*; open circles *Talorchestia patersoni*. 
Temperature

Figure 1.9 shows the effect of temperature on the rate of oxygen uptake of *M. hurleyi* and *T. patersoni* for specimens of 20 mg live weight. The temperature optimum for *M. hurleyi* is 27.5°C which is slightly higher than that for *T. patersoni* (25°C). Both sexes of *M. hurleyi* show a seasonal shift of the MT curve which is especially apparent in small individuals. This phenomenon will be considered in more detail under 'season'. The relationship between $Q_{10}$ and temperature in *M. hurleyi* is shown in Figure 1.10, while the relationship between the Arrhenius coefficient, $A$, and temperature is shown in Figure 1.11 for *M. hurleyi* and *T. patersoni*. The Arrhenius coefficient, $A$, was calculated according to the equation

$$A = \frac{8.31917[\ln(R_2/R_1)]}{[1/T - 1/T_0]}$$

where $R_1$ and $R_2$ are rates of respiratory uptake of oxygen at temperatures $T_1$ and $T_2$ °K respectively (Hoar, 1966).

Interaction between temperature and weight

The interaction term in model equations 11 to 20 in Table 1.2 did not significantly improve the fit so it can be concluded that there is no significant interaction between weight and temperature. However, at temperatures of 30°C and above smaller animals often died within a couple of hours, and before they died their respiratory rate began declining much more rapidly than did those of
FIGURE 1.12. Relationship between total surface area and respiration rate for *Makawe hurleyi* (females, summer) at 25 degrees Celsius. The line is unit slope and zero constant.
larger animals. This phenomenon of age specific susceptibility to temperature did not affect the analysis presented here since the results from the higher temperatures were not used in the multivariate analyses.

**Surface area**

It is commonly assumed that the surface area to volume relationship follows a simple relationship of the form

\[ \text{area} = a \cdot \text{volume}^{\text{exponent}} \]

While this is true for *M. hurleyi* (Figure 1.5), the logical conclusion that respiration rate follows exactly the same relationship is not true because total surface area is made up of two types of respiratory area: the gills, and the rest of the cuticle. Since the growth relationship between these two is allometric, as has already been noted, a simple 2-parameter equation must be an approximation only. Figure 1.12 shows the relationship between respiration rate and surface area for *M. hurleyi* females, summer at 25°C. In the figure the line: \( \text{area} = \text{respiration rate} \) is shown.

Because the areas of gill and cuticle and the respiration rates are known, it is possible to find the diffusion coefficients for oxygen for gill surface tissue \((x)\) and cuticle \((y)\) by solving series of simultaneous equations of the form:
FIGURE 1.13. Respiration rate through the moult cycle in Makawe hurleyi. The stage in the moult cycle is indicated by the moult index.
FIGURE 1.14. Mean rates of respiratory uptake of oxygen during immersion in distilled water of 10 Makawe hurleyi and 5 Talorchestia patersoni. The mean live body weights were: $M.\text{hurleyi}$ 24.5 mg; $T.\text{patersoni}$ 20.5 mg.
\[ a \cdot x + b \cdot y = R_1 \]
\[ c \cdot x + d \cdot y = R_2 \]
The method is illustrated for *T. patersoni*. An animal of 10 mg live weight has a gill area of 5.0 \(\text{mm}^2\) and a cuticle area of 53.5 \(\text{mm}^2\) with a respiration rate at 20°C of 4.3 \(\text{ul.hr}^{-1}\), while an animal of 15 mg live weight has a gill area of 7.1 \(\text{mm}^2\) and a cuticle area of 70.9 \(\text{mm}^2\), and a respiration rate of 6.0 \(\text{ul.h}^{-1}\). Thus there are two simultaneous equations:

\[ 5.0X + 53.5Y = 4.3 \]
\[ 7.1X + 70.9Y = 6.0 \]

Solving by using the usual methods, we get \(X = 0.54 \text{ ul.mm}^{-2} \cdot \text{h}^{-1}\), and \(Y = 0.030 \text{ ul.mm}^{-2} \cdot \text{h}^{-1}\). Thus diffusion through the gill is 18 times greater than through the general body surface. The equivalent values for *M. hurleyi* are: gill, 0.38 \(\text{ul.mm}^{-2} \cdot \text{h}^{-1}\), cuticle, 0.082 \(\text{ul.mm}^{-2} \cdot \text{h}^{-1}\). The permeability of the cuticle appears to diminish with increasing size as shown by the following:

<table>
<thead>
<tr>
<th>weight (mg)</th>
<th>Permeability (ul.mm(^{-2}).h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.082</td>
</tr>
<tr>
<td>10</td>
<td>0.071</td>
</tr>
<tr>
<td>15</td>
<td>0.070</td>
</tr>
<tr>
<td>20</td>
<td>0.066</td>
</tr>
<tr>
<td>25</td>
<td>0.058</td>
</tr>
</tbody>
</table>

The decrease in permeability with size may be related to increasing cuticle thickness, or a change in cuticle composition as the animal grows older. Using the relations and constants given in Prosser (1973), it is possible to recast these as diffusivity. Thus: gill
The allometry between gill area and cuticle implies that the simple exponential relationship between respiration rate and body weight is only an approximation. If respiratory uptake of oxygen is surface limited in landhoppers, then an exact relationship for the rate of respiratory uptake of oxygen would be:

\[ R = p_1 A_g + p_2 A_c \]

where \( R \) is respiration rate, \( p_1 \) is permeability of gill to oxygen, \( A_g \) is area of gill, \( p_2 \) is permeability of cuticle (general body surface other than gill), and \( A_c \) is surface area of cuticle. This relationship is tested in Figure 1.8. In this figure \( T. \) patersoni has a lower \( R \) mainly because of its smaller surface area, particularly of gills, for a given live weight.

A more complete model (not tested here) should take into account the decline in cuticular permeability with increasing size of animal.

**Sex**

*M. hurleyi* males generally have a higher metabolic rate than do females of the same species, sex, season, and live body weight. The exception seems to be large females in summer at higher temperatures. This is probably a consequence of brooding.
The male metabolic rate is not a constant proportion of female metabolic rate, but varies with live body weight, season, and temperature.

The data from male *T. patersoni* are not very reliable because of the small sample size, but the male metabolic rate is generally less than that of females of the same body weight, season, and temperature.

**Moult cycle**

The polynomial analysis of ISPR (respiration index) and MIND (moult index) indices showed that respiration rate varied throughout the moult cycle according to the equation:

\[
ISPR = 27.98 - 267.35MIND + 476.21MIND^2 - 208.64MIND^3 + 40.80.
\]

The F-ratio for the ratio [MS explained by regression]/[MS residual] was 3.56 for 3 and 48 d.f which is significant at 5% but not at 1%. Using this equation to solve for the numerical values of ISPR for particular values of MIND gives:
Part II Respiration

<table>
<thead>
<tr>
<th>moult index,</th>
<th>respiration index</th>
</tr>
</thead>
<tbody>
<tr>
<td>(MIND)</td>
<td>(ISPR)</td>
</tr>
<tr>
<td>early</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>27.98</td>
</tr>
<tr>
<td>0.1</td>
<td>5.80</td>
</tr>
<tr>
<td>0.2</td>
<td>-8.11</td>
</tr>
<tr>
<td>0.3</td>
<td>-15.0</td>
</tr>
<tr>
<td>0.37</td>
<td>-16.31</td>
</tr>
<tr>
<td>0.4</td>
<td>-16.12</td>
</tr>
<tr>
<td>0.5</td>
<td>-12.72</td>
</tr>
<tr>
<td>0.6</td>
<td>-6.06</td>
</tr>
<tr>
<td>0.7</td>
<td>2.61</td>
</tr>
<tr>
<td>0.8</td>
<td>12.05</td>
</tr>
<tr>
<td>0.9</td>
<td>21.00</td>
</tr>
<tr>
<td>late</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>28.2</td>
</tr>
</tbody>
</table>

On three separate occasions individual animals moulted in the electrolytic respirometer. The time record of one of these is illustrated in Figure 1.15. The peak rate achieved was over three times that normal for an animal of that size. This rate was maintained for only a short period, and was followed by a very rapid decline to a day-time minimum much lower than normal. Thus the pattern of respiration throughout a 24-hour period is different during the actual ecdysis with a higher peak rate, almost no dawn peak, and a lower minimum rate.

Disease

As discussed in Chapter 5, *M. hurleyi* suffers from a bacterial disease caused by *Bacillus subtilis*, which turns the haemolymph white (Frontispiece). The effect of this disease is to depress the respiration rate. Thus the heterogeneity of the diseased population of *M. hurleyi* at Christchurch is much greater than that at Dunedin.
Immersion

The rate of oxygen uptake as measured in a Warburg respirometer, while the animals were immersed in distilled water is shown in Figure 1.14 for 10 M.hurleyi and 5 T.patersoni. Both species invariably died under water: T.patersoni did so within 2 hours and M.hurleyi died within six hours.

Disturbance

After the animals were introduced into the electrolytic respirometer they showed a heightened respiration rate which varied in intensity and duration from individual to individual. This disturbance period could last between 2 and 12 hours and the intensification could be between 1.2 and 3 times the rate normal for that period. The cause of this variability is unknown, but its existence must cast doubts on the validity of short-term measurements, of respiration rate. Certainly, it is a major source of the heterogeneity seen in the earlier Warburg respirometry presented here.

Daily rhythms - light

A bimodal pattern of oxygen uptake was seen when the respiration rate of animals was measured continuously for some days in the electrolytic respirometer (Figures 1.15 and 1.16). The peaks
FIGURE 1.15. Respiration rate of *Makawe hurleyi* measured continuously at 20 degrees Celsius and 'normal' day-night conditions.
FIGURE 1.16. Respiration rate of Makawe hurleyi measured continuously at 20 degrees Celsius in constant light.
of oxygen consumption occurred just after dusk and at dawn while the minima were at midnight and at mid-day. This pattern persisted for at least 7 day-night cycles in constant dark or light, although the peaks and troughs were smaller in the constant light conditions, and the individual levels of activity more variable.

Seasonal acclimatisation

In view of the marked seasonal acclimatisation in *M. hurleyi* gill area, it is of considerable interest to observe clear indications of acclimatisation in the MT curve of this species equivalent to a shift of a number of degrees Celsius. The effect is clearly seen in the Dunedin specimens and in male and young females from Christchurch. Larger summer females in the Christchurch population tend to have very variable respiratory rates because of their frequent molts, the presence of broods, and the presence of disease.

The evidence with regard to *T. patersoni* is less certain (Table 1.10), but there does seem to be a shift by a small amount, particularly in young specimens.

Calculating the averages of the parameters of the equations in Table 1.9, but excluding the anomalous results for *M. hurleyi* summer at 30°C, we obtain:

- *M. hurleyi* summer:  \( R = 655W^{-0.17} + 1.76 \)
- winter:  \( R = 1603.W^{-0.34} + 1.73 \)
For an animal of unit weight the winter respiration in *M. hurleyi* is 2.45 times that of summer, while in *T. patersoni* the winter rate is only 1.24 times the summer rate. Over the range of environmental temperatures the $Q_{10}$ is 2.7 (Figure 1.10), so by solving the equation

$$\frac{10/(t_1 - (t_1 - 10))}{2.45} = \frac{(R_1 / R_2)}{(R_1 / R_2)^{10/(t_1 - t_x)}}$$

for $t_x$ we obtain a measure of the seasonal acclimatisation shift of the MT curve in degrees Celsius. This relationship simplifies to

$$D = \frac{10 \cdot \log(X)}{\log(Q_{10})}$$

where $D$ is the shift in degrees Celsius and $X$ is the increase in rates given above (2.45 for *M. hurleyi* and 1.24 for *T. patersoni*). Thus for *M. hurleyi* females of 20 mg live weight the seasonal acclimatisation is equivalent to a shift in the MT curve of 9.0°C,
while for *T. patersoni* females of the same weight it is equivalent to 2.2°C.

There is a seasonal shift in the values of the regression constant and regression coefficient (Table 1.10) for weight-specific respiration rate. If the regression lines for any one species in any one season are considered to be parallel, the average slopes are: for *M. hurleyi* in winter, $b = 0.34$, whereas in summer $b = -0.102$, and for *T. patersoni* in winter, $b = -0.227$, while in summer, $b = -0.13$. This indicates that the degree of acclimatisation depends on the size of the organism.
DISCUSSION

In the evolutionary conquest of land by terrestrial animals the ancestral aquatic respiratory apparatus has been modified or replaced by a more suitable terrestrial apparatus to a greater or lesser extent in different groups (Carter, 1931; Hoar, 1966). The major differences between the aquatic and terrestrial environments are that water is abundant in one and scarce in the other, while at 20°C saturated water contains only $\frac{1}{40}$ the oxygen in the same volume of air. Landhoppers have made minimal progress in this regard since they still possess the same basic respiratory apparatus, that of external gills, as do their aquatic relatives such as Hyale. In consequence, it is not possible for them to live in advanced terrestrial conditions where desiccation is likely to be severe. In spite of this obvious limitation, however, their body structure and functioning, of which the respiratory apparatus is a major part, must possess some advantages to have enabled them to become such dominant members of the mesic cryptozoic environment. These advantages may not be those which traditionally have been regarded as important for terrestrial animals, but may be advantageous enough to compensate for the very severe limitations and disadvantages which ensue from the possession of such a primitive respiratory apparatus.

Traditionally, it has been thought that the major respiratory problems faced by terrestrial animals have been the prevention of excessive body water loss through moist respiratory surfaces, and
the physical support and protection of delicate respiratory tissues. Regarding the first of these, any land animal has the problem of water loss from the respiratory surfaces because membranes permeable to oxygen are also permeable to water (Hinton, 1953). Edney (1977) points out that membranes permeable to oxygen but not to water are unknown. As Hinton (1969) has stated:

"... any relatively dry environment presents the animal with contradictory demands: on the one hand, it must have an extensive surface permeable to oxygen but, on the other hand, it must not lose too much water through such a surface."

The solution adopted by most terrestrial animals is to enclose their respiratory membranes in sacs or invaginations within the body, and make the non-respiratory surfaces relatively impermeable to oxygen and water. Many go further and control the openings of the respiratory sacs. Even without this control, invaginated respiratory membranes lose less water than evaginated membranes. Fick's law of diffusion (a special state of Laplace's equation) states that the rate of diffusion of a substance through a column \( \frac{dx}{dt} \) is proportional to the cross-sectional surface area of the column \( A \) multiplied by the concentration gradient of the diffusing substance \( \frac{dc}{dl} \). Therefore,

\[
\frac{dx}{dt} = -D.A.\frac{dc}{dl}
\]

where \( D \) is Fick's constant (Radford, 1964).
Consider only the last term; if, in a simple example, the air in the general atmosphere is perfectly dry while the air in the immediate vicinity of the respiratory membrane is saturated (due to transpiration occurring) then, in the case of completely exposed evaginated membrane, the distance along which a water molecule diffuses from the membrane surface before reaching 'zero concentration' is extremely short. Since the permeability of animal tissue to water is lower than the 'permeability' of dry air, water is evaporated away from the boundary layer next to the membrane as fast as it diffuses across the membrane. Thus the rate of water loss in completely exposed tissues is very high and is limited only by the permeability of the tissue.

In an evaginated sac, however, the distance over which the gradient in air extends is much greater, therefore the rate of diffusion from the surface to a zone of dry air will be much slower. The effective cross-sectional area of an invaginated sac is much less than that of an evaginated sac and this, too, will reduce the rate of evaporation. Even in situations where the surrounding air approaches saturation, the invaginated sac will have an advantage over the evaginated sac in conserving water. Naturally, the simple invaginated sac (i.e., one without ventilatory mechanisms) will be much less efficient at taking up oxygen since modifications which are effective in reducing water loss are also effective in reducing the rate of oxygen uptake. But, in terrestrial situations, this is not very serious since oxygen is an abundant resource while water tends to be limited. Furthermore, it is unlikely that an animal
with evaginated membranes such as exposed gills would be able to maintain an equitable balance between the rate of water loss and the rate of oxygen uptake in dry air whereas the invaginated system does allow an equitable balance to be achieved. Edney (1977) points out that this analysis only holds if the phase of the diffusing substance is the same throughout its diffusing path.

The two cases considered so far, the evaginated (such as polychaete gills) and invaginated (such as the book lungs of many arachnids) systems represent the two ends of a graded series. It is possible to have a greater or lesser degree of invagination. The location of the gills within a branchial cavity (crayfish) or mantle cavity (molluscs) represents incomplete invagination. In a marine environment enclosure of gills within a chamber represents a device whereby the delicate respiratory membranes are afforded some degree of physical protection and ventilatory currents can be properly directed. But it also represents a preadaptation to terrestrial life since any impediment to the rate of diffusion will assist in conserving water in a terrestrial environment. Such impediments can be structural (the branchial cavity in semi-terrestrial decapods) or, perhaps, even behavioural. This latter can be achieved by the animal keeping its respiratory apparatus close to, but not touching, the substratum. In this case diffusion would be impeded. A typical example is the slater (Porcellio scaber) which has its respiratory membranes ventral and close to the substratum. If a slater is caught out in the open during the daytime, it will initially become very active and seek refuge. If it becomes too weak before finding
a suitable refuge, it frequently clamps down onto the substrate so that diffusion to and from the respiratory membranes is greatly slowed down.

Landhoppers have evaginated gills enclosed in an incomplete box held close to the substratum. Thus their rate of water loss is not as great as would be expected if their gills were completely exposed. They show a reduction in the area of gill tissue as they become more terrestrial but their respiratory rate also drops compared with their supralittoral and intertidal relatives. These latter species, however, have the problem of obtaining oxygen in two media: water and air, thus their respiratory apparatus must compromise between the demands of the two environments. The respiratory rate of the semi-aquatic species in air is high, hence the reduction in gill area and respiration rate in terrestrial species may not have anything to do with water conservation but may be a reflection of their adaptation toward obtaining oxygen from air alone.

Mechanical support for the delicate respiratory surfaces is another problem faced by terrestrial animals since they are no longer supported by water. In aquatic environments oxygen is limited so the respiratory areas are often increased to maximize the rate of uptake. Many aquatic organisms have delicate filamentous gills with a large surface area - the gills being supported by the surrounding water. But when such highly evaginated gills are surrounded by air, the surface tension of the film of water on their
surface tends to cause the filaments to stick together so reducing surface area (Carter, 1931, 1957). Furthermore, in air such gills are now much heavier than the surrounding medium and can collapse under their own weight and become adfixed to the general body surface so reducing their effectiveness. Gills adapted to an aquatic environment are, on the whole, unsuited to a terrestrial environment.

The highly spongy nature of the amphipod gill, however, frees them from the necessity of having such delicate structures. Since haemolymph is brought very close to the surface in such gills, the mean diffusion path of oxygen into or carbon dioxide out of the body is very short. Amphipod gills can function very successfully with a relatively robust structure which helps explain their success in such physically demanding environments as the intertidal and the supralittoral. Support in the terrestrial environment is achieved by surrounding the gills in a small volume of fluid agitated by the pleopods. The importance of this agitation is such that although the second and third pairs may be reduced in a great many species, the first pair of pleopods are greatly reduced in only two species—Talorchestia patersoni from New Zealand, and Spelaeorchestia koloana from limestone caves on Kauai I., Hawaiian Islands (Bousfield and Howarth, 1976). It is probably significant that T. patersoni is restricted to relatively equitable, though cool, oxygen-rich environments where stagnation in the branchial fluid would be less of a problem. S. koloana also lives in highly stable conditions.
Part II Respiration

The simple, but effective, structure of talitrid gills also enables them to be easily cleaned, an important pre-adaptation to the saprophyte-rich cryptozoic environment.

Possibly, oxygen taken up by the gills is targeted for tissues lying deep inside the body, such as the brain and the central muscle masses. More superficial tissues may be supplied by uptake through the general body surface, particularly through the thin cuticle at the joints between the segments on the body and the appendages.

In brooding females, the gills may supply oxygen to the eggs which lie morphologically dorsal to them.

Respiration rates in landhoppers are high for Crustaceans and approach values more typical of insects (Prosser, 1973). Clark (1955) found respiration rates ranging from 0.3 to 0.5 cc.g\(^{-1}\).h\(^{-1}\) for 'Talitrus sylvaticus' which are very similar to the rates reported here. The rates for the supralittoral species, *T.chiliensis*, is much higher than this, about twice the rate, while those for the intertidal *H.grandicornis* in air are up to 10 times this rate. Such high rates may reflect the fact that the supralittoral species is slightly and the intertidal species is highly, mal-adapted for air breathing since they must compromise between two environments and, therefore may be less well-adapted for any one. If oxygen supply rate is a rate-limiting factor of intermediary metabolism then the possession of high respiration rates is indeed a mal-adaptation, since the respiratory loss of
energy and material would be high. In the landhoppers, gill area is reduced, probably to help conserve water loss, but also to reduce metabolic rates to ecologically advantageous levels in an oxygen-rich environment. This conclusion is contrary to that found by Hughes (1982) who showed that in an ecological series of crabs from aquatic to above tide habitats, the gill area decreased with increasing terrestrialism, but respiration rate per unit gill area increased dramatically. In the present study for a 20 mg body weight animal, respiration rate per unit gill area decreased from 829 ml/m$^2$/hr for *H.grandicornis*, (intertidal), 293 ml/m$^2$/hr for *T.chiliensis* (supralittoral), 105 ml/m$^2$/hr for *M.hurleyi*, (terrestrial), to 104.5 ml/m$^2$/hr for *T.patersoni*. But Hughes was quoting Vernbergis (1956) work on the oxygen consumption of excised gills in aquatic media, so is not comparable to the work reported here. The uptake by excised gills probably reflects the amount of osmotic work done by the gills themselves, and so is not related to the total body oxygen demand. Nor can respiration in water be related necessarily to respiration in air.

The respiratory quotient in both landhopper species tested of 0.98 indicates a carbohydrate-rich diet which is in accord with their diet of leaf litter.

The seasonal acclimatisation in the metabolism-temperature curves is of Type IIA (translation) in Prosser's (1973) classification, and it is of considerable importance for species living in a temperate climate with a marked seasonality in
environmental temperatures, since it enables activity to continue through winter. *M. hurleyi*, in particular, is very active during winter even at temperatures below zero degrees Celsius (see section on ecology in this volume). Seasonal acclimatisation compensates to a considerable extent for the decline in environmental temperatures, but it is interesting that the more southerly *T. patersoni* shows only a small amount of acclimatisation. It should be noted, however, that the inland *M. hurleyi* occupies a region of great climatic extremes with high summer temperatures and very low winter temperatures. The upland part of its range is covered in snow for long periods in winter. In contrast, the southern coastal region occupied by *T. patersoni* is cool but equable without prolonged periods of snow or cold temperatures. Both species give up brooding in winter, although an occasional individual of *T. patersoni* will be seen with eggs far into winter at Dunedin, which is near the northern limit of its range. In *M. hurleyi* the mechanism partly responsible for seasonal acclimatisation is an increase in gill area in winter acclimated animals.

The results from the electrolytic respirometer are important in view of the existence and variability of the disturbance factor which they show. Short-term measures of respiration of terrestrial cryptozoa are probably only of value for first-order approximations, and the existence of a variable disturbance factor probably explains the widely disparate measures which exist in the literature for a wide range of species belonging to a variety of invertebrate groups, not only Crustacea. In the medical field it is well appreciated
that extraordinary steps have to be taken to obtain reliable and repeatable measures of standard oxygen consumption, but few studies of invertebrate respiration have controlled the experimental conditions as well as this, because of practical difficulties in invertebrate whole-animal respirometry. I believe the best solution is to adopt continuous respirometry techniques and thereby the variable disturbance factors, at least, can be overcome.

In the continuous respirometer a bimodal activity pattern of metabolism was found consistently in the three lighting conditions. These patterns are, therefore, free-running circadian rhythms (Halberg, 1959; De Coursey, 1961). No attempt was made to see if they could be reset by providing environmental clues as this lay beyond the scope of this study although Muller (1966) has shown that activity rhythms of Gammarus pulex can be changed by providing new zeitgebers. The presence of a dawn peak may be thought to be artificial, resulting from the design of the apparatus with the animal wandering around at dawn to find a non-existent refugium. But in the section on ecology in this volume, evidence from pitfall trapping is presented to show that such a dawn peak does occur in the activity of field populations. Visual observations on animals in the respirometer indicates that the increased metabolic rate is caused by locomotory activity while the metabolic minima occur when the animals are quiescent during the day while sheltering in refugia. At dusk they locomote to forage and feed and their metabolic rate is elevated. In the middle of the night they are relatively quiescent and their metabolic rate falls. But at dawn
locomotor activity increases again possibly because they are searching for refugia, and so their metabolic rate rises to another peak.
Plate 1. Transverse section of *Makawe hurleyi* gill showing: b, basement membrane; c, peripheral haemocoel channels; e, respiratory epithelium; ei, epithelium forming pillars; n, nuclei; t, transbranchia channels. See diagram below for captions.
Plate 2. Transverse sections of gill tissue of *Makawe hurleyi*. Print magnification 5850 times. Captions: cu, cuticle; ch, haemocoel channel; h, haemocoel; cl, predominant cell type; c2, cell type 2.
Plate 3. Haemocyte between old and new cuticle in a specimen of Makawe hurleyi about to undergo ecdysis. Print magnification 9450 times. Note undifferentiated state of apical labyrinth (a) in new tissue and its regular mitochondria (m). The haemocyte has dense post-lysosomes (p) and a large nucleus (n). The smaller formed bodies are probably primary lysosomes, vacuoles and Golgi bodies.
The problems faced by terrestrial animals of controlling the amount of water in their bodies is much more acute than for aquatic animals since water is generally a scarce resource in terrestrial conditions, and the air surrounding terrestrial animals is rarely saturated. Potentially at least, most terrestrial animals face a very high negative concentration gradient, which if uncontrolled, would cause rapid desiccation. Transpiration losses, as evidenced by isopods (Lindqvist, 1971), can occur via respiratory surfaces, the general body surface, and by means of oral and anal discharges. Many animals show specific adaptations toward controlling the rate of water loss (Carter, 1931, 1957, Hoar, 1966), and animals with such adaptations show lowered transpiration rates (Edney, 1957).

The transpiration rates of landhoppers may help explain features of their ecology, such as their nocturnalism and their activity responses to different environmental factors such as temperature, humidity, and wind speed. Perversely, free water may prove a real threat to land dwelling animals because their respiratory apparatus may not have the capacity to take up oxygen from water, and they may suffer hydration problems because they do not have the osmotic apparatus to cope with osmotic stress. When immersed in water an animal must detect that it is under water, it
must give up normal activity, become mobile, and, if it is well adapted, actively seek a way out if it is not to drown.

Furthermore, a small animal, such as a landhopper, has the problem of physically breaking through the meniscus if it is to escape when immersed. The attraction of a surface for water is measured by its contact angle (Wenzel, 1936; Holland, 1964) so the measurement of these angles for cuticle throws light on adaptations of importance for terrestrial life (Holdgate, 1955; Noble-Nesbitt, 1963).

Terrestrial animals can take up water from their food, from the substrate, or from the atmosphere (Babcock, 1912; Leclercq, 1946; Govaerts & Leclercq, 1946; Fraenkel & Blewett, 1944; Wharton & Devine, 1968). They may also possess means of selecting suitable microenvironments. So the responses of landhoppers in humidity gradients were investigated along the lines pioneered by Shelford (1913) and since followed by many workers for different groups. Another problem faced by terrestrial animals, closely related to that of water relations, is that of ionic (osmotic) control. The loss of diffusible solutes from the body into low conductivity environmental water pools is probably a major problem. Solutes lost this way may, however, be replaced from food or other sources.
METHODS

Transpiration

At Dunedin two kinds of apparatus were used to measure the rate of water loss from the body of whole animals: a wind-tunnel transpirometer, and randomly agitated transpirometer.

The wind-tunnel transpirometer was constructed from a round drum, 20 cm in diameter, into which was placed an electric-motor driven impeller. The speed of the impeller, and hence air speed, could be varied by varying the voltage supplied to the motor through a rheostat. Air velocity in the tunnel was measured using a standard meteorological anemometer. Humidity and temperature control was achieved by controlling the humidity and temperature in the small room in which the apparatus was run. Trays of anhydrous calcium chloride lowered the humidity until the desired relative humidity was reached while wet cloths hung around the room successfully raised it when necessary. An electric room heater was used to raise the temperature. Usually, these adjustments took a morning before a run could be made in the afternoon. To operate the machine, the room temperature and relative humidity were set (20°C and 65% RH were used throughout these experiments) the trays of CaCl₂ or wet towels were removed and the heater turned to low. The air speed in the wind tunnel was set to the desired velocity. Then up to 10 pre-weighed animals were introduced into the air stream, each in an individual coarse-meshed stainless steel gauze cage. The animals were weighed at periodic intervals. The operator had to
leave the room whenever possible as human respired breath was a major source of humidity disturbance.

The other kind of respirometer were made from 0.568 litre glass preserving jars with tops drilled to take glass tubes which had stainless steel cages at the end. An impeller was driven by a shaft through the lid to a small electric motor. The air speed in the chambers was intended to be well above 3 m.s\(^{-1}\), so that the plateau in the relationship between wind, speed and transpiration rate was reached. The bottom of the jar held a solution of potassium hydroxide (Solomon, 1951) to control humidity. Temperature was controlled by immersing the jar to its top in a thermostatically controlled water bath. At Christchurch, the same kind of apparatus was used, but with a series of salt solutions (Winston and Bates, 1960) to control humidity in the bottom.

Since the surface area-live weight relationship was known for *M. hurleyi* transpiration rates were converted to rates per unit surface area. For some, comparisons this was plotted against vapour pressure deficit (Buxton, 1931).

Transpiration rates were determined for *M. hurleyi*, *T. patersoni*, and a few *Porcellio scaber*, as well as a few 2% agar blocks cut to a cube with 1 cm sides.
FIGURE 2.1. Underwater maze for testing landhopper climbing responses. Animals were released at A or B. On arriving at X or Y they faced a choice between paths leading up or across or down. Water level is indicated by the dashed lines on the 'success' (upward) branches.

FIGURE 2.2. Apparatus used to test the speed of emergence from water of different salinities.
Escape from water

An under-water maze was constructed (Figure 2.1) from rough-textured cardboard. It was immersed up to the mark shown in the figure in 30% sea water. The maze had a number of points which offered a choice to animals running along the various paths. Thus the animal could choose between 'up' 'down' or 'along' at point A. Two paths provided escape routes from the water. In another test, a tussock of cocksfoot grass was cut out from a field in which M.hurleyi and T.patersoni were abundant, place in a large bucket and carefully flooded. The responses of the landhoppers in the tussock was noted.

The animal's escape responses under water of different salinities were further tested on an inclined plane (Figure 2.2) which was immersed in a water bath. Controlled temperature water was passed through the heat-exchange coil made of chromium plated copper tubing. This maintained the temperature at 23°C. The inclined plane trough was made from perspex, the floors of which were sanded by coating them in a slurry of clean sand in ether. In use, the trough was filled with water of known salinity to the mark shown in the figure. The water was allowed to come to temperature then batches of 20 to 30 animals a time were introduced into the centre of the trough through a broad-necked funnel. The time that each one took to escape was noted by using a kymograph as a recording device. No animal was used twice in this apparatus because of the possibility of adverse osmotic effects from the
earlier trial prejudicing the performance in subsequent trials. The animals were collected from nearby suburban gardens and used within minutes of capture. In between times they were kept in dampened paper in saturated air.

**Freezing point depression**

Freezing point depressions of blood and branchial chamber water (exosomatic water) were measured by the Ramsay and Brown (1955) technique in a modified cryoscope which used a refrigeration unit instead of dry ice to provide refrigeration.

Samples of blood and exosomatic fluid were drawn off specimens anaesthetised in CO₂ and placed under liquid paraffin. A suction micropipette with a flame-polished tip was used to draw fluid from the branchial chamber, and the second antennae were ablated (both had to be cut otherwise haemolymph would not flow) and the haemolymph which exuded was drawn up in the micropipette. Care had to be taken with *T.chiliensis* to avoid contamination with antennal gland discharge fluid.

**Volume of exosomatic water**

The volume of exosomatic fluid was determined by the decrease in optical density of a dye stuff when an animal was immersed. The fluid surrounding the body (exosomatic water) mixed with a known volume of dye solution when the animal was immersed for a short time.
FIGURE 2.3. Calibration of exosomatic fluid volume. The figure illustrates the relationship between fluid volume and Spekker absorptiometer drum reading.

FIGURE 2.4. Contact angles between the body of a landhopper and water.
(no more than 10 seconds) and the resulting dilution could be measured in a sensitive spectrophotometer. Most modern spectrophotometers do not have the sensitivity required but an old Spekker absorptiometer proved ideal. The dye used was edicol supra carmoisine WS, a common food colourant which had no surface absorption nor was it taken up by the animal into its body. It was also pH stable. A 20 g/l stock solution was diluted with 24 parts of distilled water to make the working solution. To a weighed Kahn tube 0.05 ml of the working solution was added. The tube was then weighed. An animal of known weight was immersed in the fluid in the tube and gently agitated for 10 seconds. The animal was then removed and the tube weighed again. The volume was calculated and diluted exactly 20 times with distilled water. Then its absorption was measured in the Spekker absorptiometer at maximum sensitivity at a wavelength of 510 micrometres. The volume of exosomatic fluid cold be read off from a calibration graph relating volume of exosomatic water to decline in optical density. This technique could measure down to 1 microlitre with an accuracy of about ± 0.35 microlitre. A typical calibration graph is shown in Figure 2.3.

Contact angles

Surface contact angles were determined using a horizontally mounted microscope. The eyepiece of the microscope had a pointer attached running over a circular scale mounted on the barrel of the microscope. As the eyepiece was rotated a reading could be taken on the circular scale. A quartz optical cell which had been cleaned
with chromic acid and filled with distilled water was mounted in the optical path and the microscope focussed through the meniscus. Animals were attached to a Palmer adjustment arm which allowed them to be lowered slowly into the water while being observed through the microscope. The angle of contact made by the water with the cuticle could then be measured easily for both advancing and retreating angles (Figure 2.4).

**Survival under water**

The survival of the two species under water was tested against a range of salinities from 0% to 100% seawater at 20°C. Survival and Na⁺ and K⁺ content was monitored while individuals were immersed in pure isotonic NaCl, isotonic NaCl + 5% sea water, pure isotonic sucrose, and isotonic sucrose + 5% sea water. Ten adult females were placed in 400 ml of each solution. Two complete sets were started: one was terminated after 5 hours immersion; the other after 26 hours. The isotonic point was taken to be the mean blood osmotic pressure for *M. hurleyi* as determined by the technique below. Survival rates in these four solutions were tested without aeration and with aeration using pure oxygen. The concentrations of Na⁺ and K⁺ ions was measured on a Gallenkamp flame photometer.

**Humidity gradient apparatus**
This consisted of a horizontal glass tube 300 mm long and 40 mm in diameter with holes drilled in the middle and at 50 mm intervals through its upper surface. Anhydrous calcium chloride was placed in a bag at one end, while cotton wool saturated with water was placed at the other end. Both ends were stoppered. Cobalt thiocyanate paper was pinned to small corks which stoppered the holes down the length of the tube. When a humidity gradient was established, as indicated by the colours of the humidity-sensitive thiocyanate paper, animals were introduced individually by removing the centre stopper and dropping them in to the middle of the gradient.

RESULTS

Transpiration

1. Time course.

Figure 2.5 shows the weight of three animals at succeeding times when exposed to a relative humidity of 95% and a temperature of 21°C. The decline in weight as water is lost from the body seems to follow a complex, non-linear time course made up of two phases: initially, an exponential decay at a certain rate until about 19% of the body weight has been lost, then another, more rapid exponential decay. This two-phase decay pattern occurred in all cases bar three. Animals would survive if they were taken from the apparatus while still in the first phase (I), but they died if removed when in the second phase (II). If the animals were left in the apparatus, death did not occur until the weight lost was 39.7 ± 1.22%. Phase I
FIGURE 2.5. Time course of body weight of three specimens of *Makawe hurleyi* in drying conditions (95% R.H., and 21 degrees Celsius).

FIGURE 2.6. Relationship between the log of body weight and time for two specimens of *Makawe hurleyi* exposed to drying conditions (95% R.H., 21 degrees Celsius). Specimen A had an initial live body weight of 41.06 mg and specimen B had an initial body weight of 30.21 mg.
is a recoverable phase while phase II is an irrecoverable phase.

The first phase is the one of major interest here, and so only those readings up to the inflexion point were used in the calculations below and the calculation of rates. For these values a linearizing transformation is obtained as follows: If transpiration rate follows the Laplace equation and occurs in one direction (dimension) only, and the characteristics of the cuticle do not change with time then

\[ \frac{dy}{dt} = -(Y_t - Y_0) \]

where \( y \) is amount of water lost, \( Y_t \) is amount of water left inside the body at time \( t \), and \( Y_0 \) is the amount of water outside the body. This is to say that the rate of water loss is proportional to the concentration gradient across the transpiring membrane. But for all practical purposes, \( Y_0 \) is zero, thus

\[ \frac{dy}{dt} = -Y_t \]

and, therefore,

\[ Y = e^{-kt} \]

Body weight is made up of two components: body water (\( Y \)) and non-transpirable matter, \( X \). Thus the weight at any time \( t \), \( W_t \), is given by

\[ W_t = Y_t + X \]
Thus, \( \frac{dy}{dt} = W - X \)

\[ W_t - X = (W_0 - X)e^{-kt} \]

which can be tested by graphing the log weight of a transpiring animal against time as is done in Figure 2.6. The two results illustrated in Figure 2.6 were picked at random. The linearity of the transformation in every other case was tested by the analysis of variance in regression technique. All were linear within a very low error.

There is an initial rapid drop in weight before the long steady exponential decay of Phase I occurs, as is illustrated in Figure 2.6. This is attributed to the rapid loss of water on the outside of the body, which does not have to pass through the cuticle and so is lost very much more readily than is internal water. By fitting a regression line to the second and subsequent points in a time course experiment, the theoretical initial weight of the body minus this exosomatic water can be calculated. By subtracting this value from the actual initial weight, the weight (or volume) of this exosomatic fluid can be calculated. This is termed back-calculation. It is only an approximate indication and the volume can be found by other methods, but it does serve as a check on these other methods. Not all conditions allowed the back-calculation method to be applied, particularly those with a high vapour pressure deficit (high temperature or low humidity or both). For the animals tested in low vapour pressure deficits, the mean amount of exosomatic water (as a percentage of body weight) was 4.84% ± 1.08.
FIGURE 2.7. Rate of water loss as a percentage of initial live body weight with time of two species of landhoppers (Makawe hurleyi and Talorchestia patersoni), a terrestrial isopod (Porcellio scaber) and an agar block.

FIGURE 2.8. Scattergram of transpiration rate per unit area (R) against initial live body weight for Makawe hurleyi. The low value of the Pearson product-moment correlation coefficient (r) shows there is no statistically significant relationship between transpiration rate with size of animal.
2. Species

Figure 2.7 shows the transpiration weight loss, as a percentage of body weight, for a 2% agar block, 20 *M. hurleyi*, 15 *T. patersoni*, and 2 *Porcellio scaber*, all in 65% relative humidity and at 20°C. The 95% confidence belts are shown for the two landhopper species, while the line for *P. scaber* is the regression of the pooled values from the two very similar sized individuals. Thus *M. hurleyi* has a higher transpiration rate than *T. patersoni*, but both transpire much more rapidly than does the isopod.

3. Effect of temperature and humidity

The individual transpiration rates were determined for 200 specimens of *M. hurleyi* exposed to humidities of 60%, 65%, 70%, 80%, 85%, 90% and 95% at temperatures of 15, 20, 25, and 30°C. A regression line was fitted to the log of the body weights as in Figure 2.6 and the rate of water loss calculated. This was expressed as rate per unit area since the surface area of this species is known (Figure 1.5). For any set of conditions (i.e., constant temperature and humidity) the rate per unit area is constant. There was no significant correlation with size either measured as total area or live body weight. Figure 2.8 shows this for one of these conditions. Here the correlation coefficient between transpiration rate and body weight was 0.176 which is not significant. So the mean rate for each experimental condition was
FIGURE 2.9. Relationship between hourly transpiration rate per unit area of body surface and vapour pressure deficit. Two linear relationships are evident: one between 15 and 25 degrees Celsius, and one between 25 and 30 degrees Celsius.

FIGURE 2.10. Relationship between hourly transpiration rate per unit area of body surface and wind speed in Makave hurleyi. The vertical bars indicate 95% confidence intervals.
calculated and plotted against the vapour pressure deficit of that condition. This is shown in Figure 2.9 which indicates that there is a linear relationship up to a temperature of 25°C, but at higher temperatures a new relationship, possibly also linear, holds. This new relationship at high temperatures may be caused by the behaviour of the animals. At high temperatures they roll up, exposing less body surface as they experience heat shock. Another explanation is that the cuticle becomes less permeable at these high temperatures. As is well known, temperature and relative humidity, at least over the environmental range of temperatures, are not primary variables determining transpiration rate, but instead determine between them the vapour pressure deficit which is the primary variable. The rate of water loss from the body in drying conditions over the range of environmental temperatures is linearly related to vapour pressure deficit, which is a measure of the drying power of the air (Buxton, 1931).

4. Wind velocity

Figure 2.10 shows the relationship between wind velocity and transpiration rate at 20°C and 65% relative humidity. The relationship shows a declining rate of increase with wind speed, but no plateau is reached over the range measured.
FIGURE 2.11. Time course of activity under water shown by 18 individuals of *Makawe hurleyi*. Period of locomotory activity are shown by thick bars, periods of rest by thin bars.

FIGURE 2.12. Log probability plot of the cumulative frequency (%F) of the time taken by *Makawe hurleyi* (h) and *Talorchestia patersoni* (p) to emerge from distilled water in the inclined plane apparatus illustrated in Figure 2.2.
When immersed, the initial reaction of both \textit{M.hurleyi} and \textit{T.patersoni} is to run rapidly in a near straight line or an arc of about 300 mm radius. This initial burst of activity is followed by a period of rest when the animal remains motionless. Figure 2.11 shows these activity and rest periods for 18 \textit{M.hurleyi} while immersed until they escaped from the water. Of the 1191 animals tested only 1 voluntarily re-entered the water once out.

The pattern in Figure 2.11 is very variable, but all animals show an initial rapid rush for 10 to 60 seconds followed by a comparatively long rest period. There was usually a middle period of short bursts of activity and short rests, followed by a period in which the rests tend, on the average, to become longer. The animal's haemolymph changes to a blue colour during this period.

When the cocksfoot tussock was flooded, the landhoppers which had been living in the litter at its base immediately left the litter and walked about the litter surface at high speed. There were 30 \textit{M.hurleyi} present, and they soon started climbing the grass stems (within 20 seconds). If a grass stem did not lead out of the water, but stopped some distance short of the surface, then the individual waited for about 60 seconds at the top of the stem before kicking off and 'kick-sinking' to the bottom or to another stem lower down. This was the closest to swimming behaviour observed, but it was a very ineffective means of lateral locomotion, resulting in a displacement of only 30 to 40 mm in a fall of 100 to 200 mm.
Once out of the water the individuals rested close to, but above, the water surface. No attempt was made to re-enter.

The 5 T. patersoni in the sward did not climb, but showed a tendency to lie on the surface of the litter and kick until they eventually drowned.

In the immersed laboratory maze M. hurleyi moved in a relatively straight line along the paths offered them, sweeping the substratum before them with their second antennae in an arc in front of the body. None of the 30 individuals tested took the 'down' path when at the junction of 'along' and 'down', but none distinguished between 'up' and 'along'. If, while at the junction of 'along' and 'down', they did not discover the 'along' path, they hesitated for about 60 seconds before 'kick-jumping' off the maze. All eventually found their way out of water. Most hesitated for about 30 seconds below the meniscus before forcing their way through and escaping. T. patersoni was ineffective at the maze since it never climbed the vertical paths. If placed on these it invariably fell off.

A total of 1173 individuals of M. hurleyi and 57 T. patersoni were tested on the immersed inclined plane apparatus. The M. hurleyi specimens were tested in salinities of 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% seawater. Because so few T. patersoni were available, all available were tested in distilled water. The percent cumulative frequency of emergence time was plotted for each species and condition on log probability paper. The two worst fits
are shown in Figure 2.12, and these are for *M. hurleyi* and *T. patersoni* in distilled water. The rationale for the use of log probability paper is that the probability of successful emergence of a landhopper from water is not constant, but declines the longer the animal is immersed, since the animal becomes weaker the longer it is under water. The fit for the *M. hurleyi* data is excellent up to about F% = 80 then, for the remaining 20% of animals, the fitted line under-estimates their actual emergence time. The fit is less good for *T. patersoni* in that only the frequencies up to 37% are fitted well, but in fact only 53% of the animals found their way out in any case, the remaining 47% died while still under water.

The advantages of the log-normal plots are considerable. They allow the unbiased geometric mean and standard deviation to be found even though the distribution may tail, and they allow the inflexion point (if present) to be found easily.

The mean emergence time varies with salinity (Table 2.1), being greatest at about 30% seawater and least at 0% and 100% seawater. The mean emergence time can be converted to average speed since the path length in the apparatus is known.
Table 2.1. Emergence time and speed under water in the inclined plane apparatus for *Makawe hurleyi*.

<table>
<thead>
<tr>
<th>% seawater</th>
<th>N tested</th>
<th>Mean emergence time (min.)*</th>
<th>Speed (mm/minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>172</td>
<td>6.45 +/- 0.25</td>
<td>31.01</td>
</tr>
<tr>
<td>10</td>
<td>94</td>
<td>11.95 +/- 0.20</td>
<td>16.74</td>
</tr>
<tr>
<td>20</td>
<td>91</td>
<td>13.70 +/- 0.26</td>
<td>14.60</td>
</tr>
<tr>
<td>30</td>
<td>94</td>
<td>15.50 +/- 0.24</td>
<td>12.90</td>
</tr>
<tr>
<td>40</td>
<td>74</td>
<td>10.72 +/- 0.22</td>
<td>18.69</td>
</tr>
<tr>
<td>50</td>
<td>143</td>
<td>8.34 +/- 0.23</td>
<td>24.10</td>
</tr>
<tr>
<td>60</td>
<td>89</td>
<td>5.07 +/- 0.37</td>
<td>4.00</td>
</tr>
<tr>
<td>70</td>
<td>90</td>
<td>5.72 +/- 0.43</td>
<td>3.51</td>
</tr>
<tr>
<td>80</td>
<td>112</td>
<td>3.72 +/- 0.27</td>
<td>5.38</td>
</tr>
<tr>
<td>90</td>
<td>102</td>
<td>3.60 +/- 0.26</td>
<td>5.55</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>2.83 +/- 0.38</td>
<td>7.07</td>
</tr>
</tbody>
</table>

* The mean quoted is the antilog of the log normal mean, but the standard error is in logs.

Plotting these speeds against osmotic pressure of the external medium gives the relationship shown in Figure 2.13. Both above and below the osmotic pressure (indicated by an arrow in the figure),
Table 2.2. The effect of starvation on the freezing point depression of exosomatic fluid and blood from *Orchestia hurleyi*.

<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ blood</td>
<td>.881</td>
<td>.916</td>
<td>.876</td>
<td>.823</td>
<td>1.026</td>
<td>.995</td>
<td>.826</td>
<td>.925</td>
<td>.912</td>
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<tr>
<td></td>
<td>.851</td>
<td>.911</td>
<td>.869</td>
<td>.964</td>
<td>1.003</td>
<td>1.029</td>
<td>.960</td>
<td>1.017</td>
<td>.950</td>
</tr>
<tr>
<td>Δ fluid</td>
<td>.109</td>
<td>.116</td>
<td>.086</td>
<td>.099</td>
<td>.072</td>
<td>.042</td>
<td>.081</td>
<td>.256</td>
<td>.096</td>
</tr>
<tr>
<td></td>
<td>.068</td>
<td>.111</td>
<td>.151</td>
<td>0.017</td>
<td>.086</td>
<td>.078</td>
<td>.172</td>
<td>.105</td>
<td>.121</td>
</tr>
</tbody>
</table>

**ANOVA** of these data:

a) Source | df | SS | MS | F | Source | df | SS | MS | F | Source | df | SS | MS | F |
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<thead>
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<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Among days</td>
<td>8</td>
<td>0.02700</td>
<td>0.003374</td>
<td>0.6066 not significant</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within days</td>
<td>9</td>
<td>0.05006</td>
<td>0.005562</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>0.07706</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

\[ F_{0.05[8,9]} = 3.072 \]

b) Exosomatic fluid

| Source | df | SS | MS | F | Source | df | SS | MS | F | Source | df | SS | MS | F |
|---|----|----|----|---|---|----|----|----|----|---|---|----|----|----|----|---|
| Among days | 8  | 0.06065 | 0.007581 | 1.3694 not significant |
| Within days | 8  | 0.04429 | 0.00536 |
| Total | 16  | 0.10494 |

\[ F_{0.05[8,8]} = 3.438 \]
the relationship is linear. Thus speed of movement under water appears to be related to the osmotic pressure difference between the medium and the body.

Freezing point depressions

The freezing point depressions (FPD) of haemolymph and exosomatic fluid was measured for specimens of *T. patersoni* from a suburban garden in Dunedin and the Town Belt, Dunedin; and for specimens of *M. hurleyi* from the same suburban garden in Dunedin and from the campus of the University of Canterbury; and for specimens of *T. chiliensis* collected from the supralittoral at the Avon-Heathcote Estuary near Estuary Road. *T. chiliensis* were taken at intervals during a period of prolonged drought in summer. During this time the sea-wrack became progressively drier and salt crystals more common.

The mean FPD of exosomatic fluid for *M. hurleyi* from the University of Canterbury campus population is $0.120^{\circ}C \pm 0.0358$, with a coefficient of variation of 29.9%. The mean FPD of the blood for the same animals was $0.914^{\circ}C \pm 0.020$ with a coefficient of variation of 2.2%. The effect of starvation on FPD was investigated by starving 20 *M. hurleyi* while being kept in saturated air. Each day 2 individuals were removed, sacrificed, and their FPD of their haemolymph and exosomatic fluid determined. The results, and the analysis of variance of these results, given in Table 2.2, show that starvation had no effect on the FPD of either haemolymph or
Figure 2.14 shows the relationship between the FPDs of haemolymph and exosomatic water for the three species. The haemolymph osmotic pressure of the supralittoral species (T.chiliensis) is greater than either of the terrestrial species; while, of the terrestrial species, the haemolymph of T.patersoni has a higher osmotic pressure than that of M.hurleyi. The FPD of the exosomatic water shows a similar trend.

The results for T.chiliensis have special interest. In hypo-osmotic (to haemolymph) conditions the animal can obviously regulate. The animals showing this were sampled at varying intervals following rain. As the environment dried out during a prolonged period of drought, during which the dry nor'wester (a type of foehn wind) blew, the environment became progressively more dry and the free water present presumably became more concentrated. During this time it was noticed that salt crystals were very common in the sea-wrack. However, blood samples taken from the animals during this time showed that they were still regulating their haemolymph osmotic pressure even though the exosomatic fluid was now hyperosmotic to haemolymph. But when the environment became very dry and free water was, presumably, very limited, the animals tended to become tolerators rather than regulators - the osmotic pressure of their haemolymph tended to increase above the 1.6°C depression, which is the normal limit, towards a more iso-osmotic condition. Great difficulty was experienced in obtaining samples of exosomatic
FIGURE 2.13. Relationship between speed of locomotion under water and salinity (as osmotic pressure, OP). The arrow indicates a minimum which is close to, but not identical with the osmotic pressure of the haemolymph (about 45% seawater).

FIGURE 2.14. Relationship between freezing point depression (F.P.D.) of haemolymph and F.P.D. of the fluid in the branchial chamber (exosomatic fluid) for three species of talitrids: open circles, Transorchestia chiliensis; closed circles, Talorchestia patersoni; dots, Makawe hurleyi.
water during this latter period - in only 10 specimens was it possible to measure both blood and exosomatic fluid freezing points. The haemolymph osmotic pressure was measured for another 41 individuals, but none of these had enough exosomatic water for an FPD determination to be made.

A culture of *T.chiliensis* with a sand substratum and a wrack cover was desiccated in the laboratory for 15 days. Seven specimens were taken each day and the freezing point depression of their blood and exosomatic water determined. The results are shown in Figure 2.15. The osmotic pressure of the exosomatic water is initially low but rose as the culture was desiccated until it became hyperosmotic to the haemolymph. However, the haemolymph did not deviate from its normal value. At the end of 15 days there were no animals left.

![Figure 2.15](image)

**FIGURE 2.15.** Time course of osmotic pressure (as freezing point depressions) of haemolymph and exosomatic fluid in *Transorchestia chiliensis* exposed to drying conditions.
Table 2.3. Mean contact angles and standard errors for various talitrid species.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>condition</th>
<th>Dorsal</th>
<th>Ventral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$A^*$</td>
<td>$R^*$</td>
</tr>
<tr>
<td>M. hurleyi</td>
<td>59</td>
<td>alive</td>
<td>61.99</td>
<td>23.69</td>
</tr>
<tr>
<td>(terrestrial)</td>
<td></td>
<td></td>
<td>+1.02</td>
<td>+1.07</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>desiccated</td>
<td>54.58</td>
<td>30.82</td>
</tr>
<tr>
<td>T. patersoni</td>
<td>30</td>
<td>alive</td>
<td>79.83</td>
<td>47.12</td>
</tr>
<tr>
<td>(terrestrial)</td>
<td></td>
<td></td>
<td>+1.55</td>
<td>+2.12</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>desiccated</td>
<td>62.08</td>
<td>34.60</td>
</tr>
<tr>
<td>T. chiliensis</td>
<td>12</td>
<td>alive</td>
<td>80.25</td>
<td>57.25</td>
</tr>
<tr>
<td>(supralittoral)</td>
<td></td>
<td></td>
<td>+4.18</td>
<td>+2.90</td>
</tr>
<tr>
<td>T. quoyana</td>
<td>9</td>
<td>alive</td>
<td>63.11</td>
<td>18.00</td>
</tr>
<tr>
<td>(supralittoral)</td>
<td></td>
<td></td>
<td>+5.53</td>
<td>+1.67</td>
</tr>
</tbody>
</table>

* $A$ = advancing, $R$ = retreating, $M$ = median
FIGURE 2.16. Relationship between volume of exosomatic fluid and body weight for Makawe hurleyi from wet and dry habitats (not differentiated in the figure).

FIGURE 2.17. Relationship between volume of exosomatic water and body weight for Talorchestia patersoni from wet (open circles) and dry (closed circles) habitats.
Volume of exosomatic water

The results of the dye-dilution method for the determination of exosomatic water are given in Figures 2.16 and 2.17 which show the relationship between volume of exosomatic water and live body weight for both species. The relationship is non-linear and is very variable although it is more constant for *M. hurleyi* than for *T. patersoni*. For specimens of *T. patersoni* collected from the forest habitat, the volume of exosomatic water is very small compared with the volume carried by specimens from the waste grassland habitat.

Survival under water

Figure 2.18A shows the percentage of *M. hurleyi* surviving when immersed in water of different salinities. Peak survival was in 60% seawater, although even in this salinity most animals were moribund after 24 hours. Survivorship in low salinities was poor, even though the animals in these low salinities were initially more active than those in higher salinities (Figure 2.18A).

After immersion for more than a few minutes the animal's haemolymph turns blue, evidence of respiratory malfunction. Figure 2.18B shows that the respiratory apparatus does exhibit severe osmotic effects, especially at lower salinities where the gills may be distended (labelled 'expanded' in the figure) by the osmotic entry of water to such an extent that the pillars holding the two surfaces of the gills together are torn and the gill swells from its
FIGURE 2.18. Activity, percentage survival and gill condition following immersion in water of different salinities in Makawe hurleyi.

A. The dotted line shows the percent active after immersion for 1 hour. The solid line shows the percent active after 24 hours.

B. The dotted line indicates the percentage of animals with collapsed gills due to bursting and loss of haemolymph and tissue fluid, following 24 hours immersion. The dashed line indicates the percentage of animals with swollen gills due to osmotic entry of water causing rupture of the gill pillars. The solid line indicates the percentage of animals with gills of normal appearance. Note the haemolymph osmotic pressure in this species is about 45% that of seawater.
normal discoidal shape to become spheroidal. In very low salinities this process may be so severe that rupture of one or other of the surfaces occurs and the gills collapse.

Survival rate is enhanced when the medium is oxygenated. All animals were alive after 24 hours of immersion in oxygenated 50% seawater.

The concentration of Na\(^+\) and K\(^+\) after immersion in isotonic glucose, or isotonic NaCl, or mixtures of these with seawater, are given in Table 2.4. Ionic concentrations are comparable in all media except the pure glucose where survivorship was poor (although still far better than unoxygenated seawater of the same tonicity), and the concentration of both Na\(^+\) and K\(^+\) was low.
Table 2.4. Survivorship and concentration of Na\(^+\) and K\(^+\) in *Makawehurleyi* immersed in oxygenated isotonic glucose or NaCl.

<table>
<thead>
<tr>
<th></th>
<th>% alive after 24 hours</th>
<th>mg K(^+)/g</th>
<th>mg Na(^+)/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic glucose</td>
<td>10</td>
<td>3.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Isotonic glucose + 5% seawater</td>
<td>90</td>
<td>6.3</td>
<td>13.0</td>
</tr>
<tr>
<td>Isotonic NaCl</td>
<td>77</td>
<td>6.0</td>
<td>22.2</td>
</tr>
<tr>
<td>Isotonic NaCl + 5% seawater</td>
<td>100</td>
<td>5.6</td>
<td>21.6</td>
</tr>
</tbody>
</table>

Environmental water conductivity

Table 2.5 shows that the osmotic pressure (as measured by electrical conductivity) of the environmental water in the immediate vicinity of various terrestrial species varies from about that of seawater for the oceanic supralittoral *Talorchestia quoyana*, to about 50% for the estuarine supralittoral species *Transorchestia chilensis*, to water of very great purity in the environments of the terrestrial species *Makawehurleyi* and *Parorchestia ihurawhao*. The values for water wrung from the litter may be elevated by the mobilisation of conductive particles because of the physical
disruption of litter due to wringing. These additional particles may not be present in the water normally in contact with the landhopper's body, but may be locked away in plant cell remains or sedimented out of the aqueous phase. So the water in streams may be a better indication of the osmotic pressure of litter water than that wrung out of the litter.

Table 2.3 gives the mean contact angles, standard errors of the means and number of determinations for two terrestrial species of terrestrial and two supralittoral species. The standard errors of the contact angles were calculated using the additivity property of variances (Mack, 1968). The number of determinations on the supralittoral species is low so the reliability of the data is poor. They are included merely to serve as a comparison. The ventral surface wetting angles are presented only for desiccated, terrestrial animals because the presence of exosomatic water in the brood chamber of living animals made the determinations of wetting angles on the ventral surface impossible. Again the reliability of these data is poor since great difficulty was experienced in making meaningful measurements.

In desiccated specimens (dried for one day at 20°C at a relative humidity of 50%) it was noted that when the specimens were first brought in contact with the water, water rapidly entered the brood chamber filling up all the space occupied by the exosomatic water in normal, non-desiccated specimens. It took less than one
Table 2.5. Electrical conductivity of environmental water found in the habitats of an ecological series of terrestrial talitrids.

<table>
<thead>
<tr>
<th>SPECIES AND HABITAT</th>
<th>SOURCE OF ENVIRONMENTAL WATER</th>
<th>CONDUCTIVITY ($10^3$ μS)</th>
<th>APPROXIMATE EQUIVALENT SALINITY (°/oo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talorchestia quoyana</td>
<td>Water in pit</td>
<td>35.3</td>
<td>31</td>
</tr>
<tr>
<td>(Oceanic, sandy beach</td>
<td>dug in sand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>supralittoral. Armers Beach,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaikoura)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transorchestia chiliensis</td>
<td>Estuary water</td>
<td>2.97</td>
<td>2</td>
</tr>
<tr>
<td>(Estuarine supralittoral.</td>
<td>Water wrung out of litter</td>
<td>16.8</td>
<td>14</td>
</tr>
<tr>
<td>Armers Beach, Kaikoura)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Makawe hurleyi</td>
<td>Rainwater puddle</td>
<td>0.228</td>
<td>0.2</td>
</tr>
<tr>
<td>(Coastal-inland terrestrial.</td>
<td>Wrung out of Carpodetus litter</td>
<td>1.207</td>
<td>1.1</td>
</tr>
<tr>
<td>Riccarton Bush, Christchurch.</td>
<td>Runoff stream</td>
<td>0.406</td>
<td>0.38</td>
</tr>
<tr>
<td>11.25 km from sea)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parorchestia tenuis</td>
<td>Wrung out of moss</td>
<td>0.228</td>
<td>0.2</td>
</tr>
<tr>
<td>(Inland terrestrial</td>
<td>Wrung out of Nothofagus litter</td>
<td>0.311</td>
<td>0.3</td>
</tr>
<tr>
<td>Arthurs Pass, Canterbury.</td>
<td>Drainage pool</td>
<td>0.287</td>
<td>0.25</td>
</tr>
<tr>
<td>5.6 km from sea)</td>
<td>Small stream</td>
<td>0.280</td>
<td>0.25</td>
</tr>
</tbody>
</table>
second for complete filling after contact. The same rapid filling of the branchial chamber occurred in living animals when these were tested in the same way.

The setae on the second antennae were found to be hydrofuge with wetting angles in excess of $80^\circ$. This low wettability prevented the second antenna from breaking the surface when it was brought in contact with it. Yet the cuticle surface of the second antennae was quite wettable with a contact angle much lower than that of the antennae 2 setae.

The inner surface of peraeopods 3, 4 and 5 dissected from non-desiccated specimens had contact angles of less than $14^\circ$.

**Humidity gradient**

When placed in the middle of a humidity gradient, individual landhoppers (*M. hurleyi*) took between 2 and 15 (average 6.2) seconds to reach the humid end. This compares with the 1.34 minutes that *Porcellio scaber* took on average. The responses by the landhoppers were quite dramatic. After being dropped in the apparatus, the animals stood up, perhaps turned around once, and set off down the correct arm of the apparatus to reach the humid end. Of the 100 animals tested, some repeatedly, not one made a false choice. In contrast, the isopod took the wrong choice almost as often as it took the right choice. In contrast to the amphipod, it often found the right end after repeatedly wandering up and down almost the
entire length of the apparatus for minutes at a time.

Once the landhopper came into contact with the damp cotton wool it never moved until nightfall. The isopod, on the other hand, periodically made excursions down the apparatus even after it had found the damp end.

Covering the antennae, or the peraeopods or both with petroleum jelly did not impair the animal's ability to find the humid end. Only when the whole dorsal surface and the second antennae were covered in petroleum jelly was there a significant diminution in the ability of the animal to find the humid end. However, animals so treated still found the damp end, but they took much longer to do so (4.5 minutes on average).

DISCUSSION

Landhoppers are terrestrial animals. They can neither swim nor survive under water unlike their supralittoral relatives which can survive prolonged immersion in seawater and which can swim. The landhoppers equivalent to swimming is a sinking 'jump-kick', a poor means of lateral locomotion which is used only as a last resort by submerged animals.
When immersed *M. hurleyi* possesses responses which enable it to select an escape route leading upwards out of the water. The speed of locomotion of this species is faster than *T. patersoni* in equivalent conditions, but is dependent on the osmotic pressure of the external environment. *T. patersoni* in contrast, is a poor climber and has difficulty in finding an escape route. This conclusion is confirmed by field observations presented in the chapter on ecology.

Avoidance of free water has been described by Bulesheva (1957) who noted that supralittoral species ('terrestrial' species in her terminology) climbed grass stems to avoid the incoming tide. The same kind of response was seen for *M. hurleyi*, but not *T. patersoni*, when a grass tussock was flooded.

In the animal's natural environment, soil flooding can occur occasionally, especially in the grasslands inhabited by *M. hurleyi*. So an escape response is advantageous. But *T. patersoni* occurs predominantly in coastal or strand forest where flooding would be infrequent. Thus the presence or absence of a climbing response is probably related to the frequency of flooding in the species' natural environment. Some other species also must have good climbing responses since they are frequently taken during wet weather by sweeping the aerial parts of plants. In the collections I have studied, *Parorchestia tenuis*, in particular, must be a climber. Indeed, in Fiordland, where rainfall may exceed 9000 mm per annum and it rains heavily nearly every day, landhoppers are not
found in the leaf litter, but are to be found under bark, in the frass in crotches, on epiphytes, and particular in the epiphytic moss and liverwort beards that cover the trunks and branches of the larger trees. These aerial parts, though wet, are free draining and do not suffer the water-logging that the litter and soil does.

The high contact angles on the dorsal surface of the body of landhoppers enables them to break through the meniscus if they ever become trapped in a pool of water, while the low angles on the peraeopods and ventral surface enable water from the substratum to be taken up by capillary attraction to replenish the fluid bathing the eggs and gills. The functions of this fluid (exosomatic water) is to keep the respiratory membranes, and the eggs if present, moist. The volume of fluid is quite large, particularly in *M. hurleyi*.

Survivorship under water is related to both the osmotic pressure exerted by the water and to the oxygen tension of the water. The fact that animals die when immersed does not necessarily mean that osmotic phenomena are responsible. Since the gills of terrestrial species are relatively small and are adapted to the uptake of oxygen from air, respiratory mechanisms may fail on prolonged immersion because the quantity of oxygen in saturated water at 20 °C is only 1/40 th of the amount of oxygen in the same amount of air. Following respiratory failure, active transport mechanisms may fail, and an osmotic imbalance may cause the structural changes in the gill tissue that were observed.
Certainly, the colour change of the haemolymph to blue on immersion suggests that respiratory failure has occurred. The prolonged survivorship of immersed specimens when the external medium was oxygenated supports this conclusion. The structure of the gill tissue reported in Chapter 1 suggests that active transport may be a major function of the gills.

Landhoppers face considerable osmotic stress when it is raining or during heavy dew when they may come into contact with water of extreme purity. They control their osmotic conditions largely by behavioural means, avoiding free water if necessary: M. hurleyi by climbing and T. patersoni by avoidance.

The conductivity of upland waters in New Zealand is very low (Table 2.5). This water has, of course, run off through the soil and litter picking up solutes and ions as it goes. So the conductivity of water in the landhoppers' environment must be even lower than this. Yet landhoppers are abundant in such environments, so the osmotic and ionic problems set by such low conductivity waters can not be limiting. No doubt ion losses are made up from food, and the animal can select suitable micro-environments in which to live that minimize the danger of drowning, which seems the most important limitation on their occupation of certain microenvironments.
In Otago and Canterbury provinces, landhoppers occur widely in regions with an annual rainfall in excess of 550 mm. In regions with less rainfall than this, such as Central Otago, they occur on the banks of rivers, swamps and irrigation channels if the litter is thick enough. The eastern side of the South Island is subjected to periodic droughts (Garnier, 1958), so in regions with a more equable rainfall, landhoppers may occur in places with even less annual precipitation than the 550 mm limit seen in the South Island.

Death through desiccation is a major problem facing landhoppers. They have reduced gill areas compared with supralittoral species, but this may be more an adaptation to respiration in terrestrial conditions than to reduced transpiration (discussed in Chapter 1). While they rely on moist external surfaces for respiration then transpiration is going to be a problem. They certainly have far higher transpiration rates than those recorded for isopods (reviews in Edney, 1977). But the simple gills of landhoppers are efficient, comparatively robust, and are easily cleaned, which is an important consideration in animals inhabiting such a saprophyte-rich environment as litter. Body size affects transpiration rate only with respect to the fact that smaller animals will survive for a lesser time than will larger animals in the same desiccating conditions because they have a larger relative surface area; the rate of water lost per unit body surface area is constant regardless of size. Vapour pressure deficit is a major determinant of the rate of water loss. Thus it is the interaction of temperature and humidity which is important,
not these factors in isolation. Wind speed, too, is an important determinant of transpiration rate. Evidence will be presented later than landhoppers are strictly nocturnal with their greatest activity on calm nights of low vapour pressure deficit. As was reported for other terrestrial arthropods by Cloudsley-Thompson (1958a,b, 1959), emergence activity is less on windy nights or on nights with high vapour pressure deficits.

During desiccation, three pools of water are involved sequentially. Initially, the exosomatic water (and, perhaps, oral and anal discharges) is rapidly evaporated off. This is followed by internal water, probably of haemolymph origin. Finally, when about 19% of the body weight has been lost, a new pool of water becomes available for transpiration. The source of this water is unknown but is probably cellular following massive disruption of cell membranes due to desiccation.

The cuticular pores illustrated in Part I of this work have the function of coating the body in mucus. A dry cuticle would be disadvantageous to a cryptozoic animal for reasons which will be discussed later, even though this secretion material apparently adds to their transpiration problems.

The landhopper's success in this cryptozoic environment is due to their ability to survive the physical and biotic influences which this environment contains. The biotic interactions, particularly those due to micro-organisms, have not been stressed before but may
loom large as evolutionary determinants. There may be two goals of terrestrial life toward which evolution bends the organisms which are becoming terrestrial: that of dry-land dwelling which involves massive efforts toward water conservation; and that of mesic cryptozoic living which entails massive effort toward controlling biotic interactions, particularly those with the saprophytic and pathogenic micro-organisms which abound in this environment. Water loss is not such a problem in the cryptozoic environment, and so strict water economy may be sacrificed if comensurate gains are made in other ways. As the terrestrial isopods prove, however, water conservation is still advantageous for some cryptozoa at least.

Since landhoppers are able to inhabit litters of very low conductivities, osmotic relations are probably not as important as are other aspects of their ecological physiology. Talitrids, as a group, inhabit a wide variety of osmotic conditions from sea water to fresh water, so their physiological capacities are potentially well able to meet the challenge of the osmotic problems presented by cryptozoic living. One of the main mechanisms adopted by landhoppers has been to lower the haemolymph osmotic pressure to 45% that of seawater. However, different species within the landhopper group do seem to have different osmotic preferenda because different groups inhabit soils of different conductivities. The genus Kanikania lives in strand forest with high conductivity soils, Makawe lives in coastal situations with a lower salt load than those inhabited by Kanikania, while Parorchestia lives in very low conductivity soils remote from the sea. Thus the different
ecological groups - which largely correspond to their taxonomic grouping - are adapted to different osmotic regimes.

Water relations are a different matter. The limits to distribution seem to be set by the amount of precipitation and its evenness throughout the year. Landhoppers are generally absent from regions with less than about 550 mm of rain per annum. Although even in comparatively dry areas they may occur in small patches wherever a suitable microenvironment is found. River banks and irrigated regions provide suitable environments and landhoppers can be quite common in these localised microenvironments. Usually there is no absolute cut-off boundary between suitably mesic areas and unsuitable xeric areas, but microenvironments suitable for landhoppers become patchier, smaller and scarcer the drier the area becomes.

It is this aspect of their physiology that seems the most important limitation to their conquest of the terrestrial environment. But their remarkable ability at detecting and responding to humidity gradients enables them to find and exploit suitably humid microenvironments even when these may be only patchily distributed throughout the region.
Chapter 3. ECOLOGY

Prefatory Note

The following paper is included as a chapter of this thesis. It describes the ecology of the two landhoppers studied most intensively in this work. In Part I of this thesis I changed the name of the species studied to Makawe hurleyi and Talorchestia patersoni.
The ecology of two species of terrestrial Amphipoda
[Crustacea: Family Talitridae] living in waste grassland**)

By Kelvin W. Duncan

With 9 Figures

(Received 1. 11. 1968)

1. Introduction

The typical habitat of terrestrial amphipods is the litter layer of forests, but a few species such as *Talurus sylvaticus* Haswell, 1880; Hurley, 1955 and *Orchestia patersoni* (Stephenson) 1938; Hurley, 1957, invade the litter of the long grass habitat.

These species are not widespread throughout grassland, but tend to be localized around patches of forest from which they have dispersed. The range of *O. patersoni* in grassland is up to 15 km from forest in the Dunedin district. Only *Orchestia hurleyi* Duncan, 1968 is known to inhabit the long grass habitat extensively.

Except for a few natural history notes in the taxonomic literature, little is known of the biology of terrestrial amphipods. Grimmett (1925) gave some very approximate density estimates for soil and litter animals (including amphipods) in a New Zealand forest. Lawrence (1953) gave some information on the ecology of South African amphipods. Clark (1955, 1956) worked on the external respiration and ecology of *Talurus sylvaticus* but much of his work remains unpublished. Hurley (1959) discussed the zoogeography, ecology and morphological adaptations of the terrestrial Talitridae.

2. Materials and methods

2.1. Description of site

The site selected for intensive study was a flat, ¼ ha waste grassland in the grounds of the Zoology Department of the University of Otago. The flora of the site consisted of common temperate wasteland grasses and herbs, including *Dactylus glomerata* Graebn. (cocksfoot), *Bromus mollis* L. (brome), *Festuca arundinacea* Schreb. (tall fescue), *Agropyron repens* (L.) (couch), *Poa pratensis* L. (meadow grass), *Holcus lanatus* L. (Yorkshire fog), *Agristis stolonifera* L. (creeping bent), *A. teniis* Sibth. (brown top), *Taraxacum officinale* Wiggers (dandelion), *Plantago lanceolata* L. (plantain), and *Rumex* spp. (dock). Also found in the area were a few cultivated plants, such as an apple tree, two gooseberry bushes, and a small patch of dahlias, all remnants from the time it was a cultivated, suburban garden. The only native plants were a few invading shrub seedlings (*Coprosma* sp. J. R. et G. Forst., and *Pittosporum eugenioides* A. Gunn.).

Two species of amphipods occurred in the area — *O. hurleyi* and *O. patersoni*. The latter species is typically a forest dweller. Its grassland population was probably derived from second-growth native forest (Town Belt, Dunedin) which lies about one km from the study site.

*) Present address see p. 341.
**) This study was carried out while the author was the recipient of N.Z. University Grants Committee Research Grant.
2.2. Climate

The meteorological data recorded included daily maximum and minimum temperatures for both air and litter, soil temperatures (at 900 hours), grass minimum temperatures, relative humidity (at screen height), rainfall and dewfall. The latter was measured by weighing at sunrise three tared Petri dishes set out the previous night. Rainfall interception values under *Dactylis glomerata* canopies were measured by placing collecting vessels at different distances from the plant centres. On a number of occasions the relative humidity at different heights above the soil was measured at two-hourly intervals throughout a 24 hour period by using slips of cobalt thiocyanate paper (Solomon 1941) inserted into small cages (2 cm by 0.5 cm) made of 30 gauge stainless-steel gauze and fastened at 2.6 cm intervals on to a board placed vertical to the soil surface. Soil water content was measured weekly by drying soil samples at 105 °C to constant weight.

2.3. Population sampling

The study area was divided into two sections — the first was used for systematic and daily sampling and for trapping; the second for a randomized sampling programme. In the first area six pitfall traps were set level with the soil layer. Three of these were placed under the canopies of *Dactylus glomerata* and three in shorter grasses. Four wide-mouthed glass vessels (4 cm diameter) set on the litter surface served as another type of trap, capturing animals falling from the overhanging grass canopies. Two of these traps were placed under *Dactylus glomerata* canopies and two in shorter grasses.

Core borers (13 cm long, 10 cm in diameter) were made from galvanized iron pipe. Preliminary experiments showed that 10 cm diameter borers were easier to use in long grass than larger diameter borers.

The samples were extracted in a Tullgren funnel. The cores were left in the borers and placed upside down in the extractor. The extraction efficiency varied according to litter thickness and type, but in all cases it exceeded 95% (excluding those animals killed by the edge of the core). As amphipods turn pink after death, the animals which were not extracted were quickly sorted out from the litter. To minimize the errors due to edge mortality the cores were inspected for animals killed by the edge — animals with heads present were counted, while those without heads were not.

Two sampling programmes were carried out. One was fully randomized involving 20 samples on each of nine occasions. The other consisted of systematic sampling along radii from the centre of *Dactylus glomerata* plants.

Features noted about each sample site were height and composition of canopy, plant species in immediate environs, litter thickness, and soil structure. After extraction, the animals were identified, sexed and counted; the podomeres on the second antenna were counted for each individual and the presence (and stage of development) or absence of eggs was noted. The eggs carried by the females were not counted because a variable number were lost from the brood chamber on the death of the mother. Examination of the gills showed if the females had been carrying eggs. Normally the gills are held more or less horizontally, but the presence of eggs displaces them to a more vertical position. They appear to remain in this displaced state until the next moult.

A number of arbitrary daily samples were taken, using a modified insect pooter, to set more accurate limits to the breeding season and to obtain specimens for studies on relative growth and brooding.

All computer programs used in this work were run on an I.B.M. 360, model 44.

3. Results

3.1. Climate

Figure 1 shows the rainfall, means (weekly) of the daily median litter temperatures, means (weekly) of the daily minimum grass temperatures, the most extreme grass minimum temperature in each week, day-length, and the extent of the breeding season.

3.2. Systematic core sampling

Twenty four core samples were taken systematically along eight radii from the centres of six *Dactylus glomerata* plants. These plants were all well grown with umbrella-like canopies. Distances along the radii were expressed as percentages of the canopy radius.
Fig. 1. Climate of the study area during 1966.
A $\chi^2$-test showed that both intra- and inter-species distributions were significantly different along the radii (Table 1), with *O. hurleyi* concentrated beyond the canopy limit and *O. patersoni* concentrated under canopies. *O. hurleyi*, however, is less influenced by canopy than *O. patersoni*.

Table 1  The distribution of amphipods along radii from *Dactylus glomerata* plants

<table>
<thead>
<tr>
<th>Position of samples</th>
<th>Mean number per core</th>
<th>$\chi^2$ values</th>
<th>Association between species</th>
<th>Differences between classes (O. hurleyi)</th>
<th>Differences between classes (O. patersoni)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under canopy</td>
<td>1.43</td>
<td>3.86</td>
<td>Association between species</td>
<td>33.5**</td>
<td></td>
</tr>
<tr>
<td>Edge of canopy</td>
<td>4.43</td>
<td>2.3</td>
<td>Differences between classes (O. hurleyi)</td>
<td>11.0*</td>
<td></td>
</tr>
<tr>
<td>Outside canopy</td>
<td></td>
<td></td>
<td>Differences between classes (O. patersoni)</td>
<td>30.6**</td>
<td></td>
</tr>
</tbody>
</table>

*) Significant at 5%  
**) Significant at 1%

This distribution pattern appears to be related to the amount of water present, which varies according to the amount of rain intercepted by the canopy. Intercepted water is either held on the leaves and eventually evaporated off, or run off to the canopy edge or centre. In consequence, the water regime in litter under canopies differs from the regime in litter outside canopies. However, the variation in amount of water present may be more significant to amphipods than the mean water content. Table 2 shows the interception values, and mean and range of soil moisture at points under, at the edge of, and beyond *Dactylus glomerata* canopies.

Table 2  Interception and soil moisture values along a radius from the centre of cocksfoot tussocks

<table>
<thead>
<tr>
<th>Position of samples</th>
<th>Average interception*</th>
<th>Soil moisture**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>Under canopy</td>
<td>78%</td>
<td>36.97</td>
</tr>
<tr>
<td>Edge of canopy</td>
<td>24%</td>
<td>49.51</td>
</tr>
<tr>
<td>Beyond canopy</td>
<td>--</td>
<td>44.39</td>
</tr>
</tbody>
</table>

*) Expressed as the percentage of the precipitation beyond the canopy edge.  
**) Expressed as a percentage of the dry weight.

3.3. Trapping

Table 3 shows the capture frequency of *O. hurleyi* in pot traps during different meteorological conditions. Captures increased when rain fell or dew formed, showing that climbing activity is affected by these conditions.

Climbing activity in the canopy enables *O. hurleyi* to feed on dead material still attached to the plant. It also enables the species to avoid osmotic stress when the soil is waterlogged. *O. patersoni* was never caught in pot traps nor was it ever observed climbing grass stems at night.
Table 3 Captures of *O. hurleyi* in pot traps during different meteorological conditions

<table>
<thead>
<tr>
<th>Meteorological conditions</th>
<th>Percentage frequency of occurrence of these conditions</th>
<th>Percentage frequency of amphipod capture days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days when rain fell</td>
<td>48</td>
<td>78</td>
</tr>
<tr>
<td>Days when dew formed</td>
<td>27</td>
<td>79</td>
</tr>
<tr>
<td>Days with no rain or dew</td>
<td>25</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 (≈ 351 days)</td>
</tr>
</tbody>
</table>

3.4. Relative growth

The relations between live weight and second antenna podomere number (*A*) are given in Figure 2. Observation of many individuals larger than *A* = 13, showed that these add one podomere to the flagellum of the second antenna at each moult. Because the relationship between *A* and body weight is the same for both small and large individuals, the assumption was made that animals smaller than *A* = 14 also add one podomere per moult. Both species hatch with six podomeres and reach maturity with either 15 podomeres (*O. hurleyi*) or 13 podomeres (*O. patersoni*). Wilder (1940) and Geilser (1944) both noted the increase in podomeres with age in *Hyalella azteca* (Saussure) but discounted its effectiveness as an age criterion because of individual variation. However, Cooper (1965)
considered that it is a reasonable method for ageing *H. azteca* as, though there is individual variation, most animals lie very close to the mean. Because the deviations from the lines in Figure 2 are not large and the different instars are relatively discrete, the number of podomeres on the second antenna is considered a useful and reasonably accurate age criterion for *Orchestia*.

### 3.5. Brooding

The relationship between the size of mature female and brood size is shown in Figure 3. The actual relationship depends on the age of the eggs as, late in the brooding period, there are 30% fewer eggs in the brood than early in the period. The relationships in Figure 3 do not change during the breeding season — females which breed early in the season carry the same number of eggs as females of the same size which breed late.

Fig. 3. The relationships between body weight of mother and size of brood. The top pair of curves are drawn from females carrying 'early' eggs while the bottom pair are drawn from females carrying 'late' eggs. For the criteria of 'early' and 'late' see the footnote to Table 4. Standard errors of estimate (Sy.x) are shown by vertical lines through the mean of each curve. The regression equation used \[ e^{\beta_1 x + \beta_2} \] is the most efficient of the exponential family (tested by an analysis of variance of the deviation from the regression).

Measurements were made on the size and dry weight of eggs at different developmental stages. The increase in egg dimensions for *O. hurleyi* are shown in Table 4. *O. patersoni* eggs are 150% larger in all dimensions than *O. hurleyi*. Egg weights (dry) decline during development as wastes are excreted. The weight change in *O. hurleyi* is from $8.7 \times 10^{-5}$ g (early) to $6.5 \times 10^{-5}$ g (late). The percentage of infertile eggs was low, being 2.83% of all eggs for *O. hurleyi* and 1.32% for *O. patersoni*. Infertility was judged by loss of turgor in the egg and failure to develop to the superficial cleavage stage. Some eggs, though still apparently developing normally, had not developed as far as the other eggs in the brood; however, these were not considered to be infertile. The frequency distribution of infertile eggs in the broods is given in Table 5. Both species are significantly different from each other ($P(x^2) < 1\%$) but are not different (at a probability level of 5%) from Poisson series with the same means.
Table 4  Changes in the dimensions of *O. hurleyi* eggs during development

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>Width (mm)</th>
<th>Length (mm)</th>
<th>Depth (mm)</th>
<th>Number examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early *)</td>
<td>0.573</td>
<td>0.805</td>
<td>0.633</td>
<td>75</td>
</tr>
<tr>
<td>Middle +)</td>
<td>0.595</td>
<td>0.974</td>
<td>0.747</td>
<td>36</td>
</tr>
<tr>
<td>Late ++)</td>
<td>0.71</td>
<td>1.099</td>
<td>0.854</td>
<td>56</td>
</tr>
</tbody>
</table>

All standard errors less than 1.5% of the mean.

*) Morula and blastula stage — colour disseminated throughout egg.

+ ) Gastrula stage — colour localized but extra-embryonic spaces yolky.

++) Colour localized, extra-embryonic space clear — animal in process of limb formation.

Table 5  Percentage frequency distribution of the number of infertile eggs per brood. Theoretical values based on Poisson series with the same means are given below the observed values

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of eggs infertile</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. hurleyi</em></td>
<td>0 1 2 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>81 (76.2) 13 (21.9) 5 (6.3) 2 (1.8)</td>
<td>0.287</td>
</tr>
<tr>
<td><em>O. patersoni</em></td>
<td>96 (89.3) 16 (12.0) 2 (1.8)</td>
<td>0.135</td>
</tr>
</tbody>
</table>

Number of broods examined: *O. hurleyi* — 562, *O. patersoni* — 376.

3.6. Randomized sampling programme

3.6.1. Prefatory note

Difficulty was encountered in taking adequate cores as ungrazed grassland is not an easy environment in which to work. The most frequent cause of failure was deformation of the core inside the borer. In all, 35% of all attempts to take cores were failures.

Only 78% of the study area could be sampled by coring and of the unsampled area 3/4 was uninhabited by amphipods. However, no corrections have been used in the population data given below — these data refer to the area sampled, not to the total area.

The area sampled or affected by sampling was about 0.2% of the site area.

3.6.2. Annual cycle of numbers

The density of each instar throughout the year is shown in Figure 4 for both species. The average error of sampling (for a sample size of 20 cores) was ±35% of mean (95% confidence limits) assuming that the distributions of the samples were normal. In fact they were log normal so that the error in the estimation of the mean was ±42% of the mean value (95% confidence limits). The data quoted below have been normalized by a log (x + 1) transformation so that the “means”, as quoted, are the anti-logs of the means, minus one.

By using the regression of live weight against the number of podomeres in the second antenna it is possible to calculate the amphipod biomass in the field. The biomasses of both species are plotted against “physiological time” in Figure 5. Because the rates of most metabolic processes in poikilotherms are temperature dependent, physiological or temperature dependent time has been used as a base rather than absolute time. Estimates
Fig. 4. Density of each instar throughout the year for both species. The number of podomeres on the second antenna (equals instar number plus five) is given on both sides of the 'kites'. The horizontal axis represents time (months).

of population mortality, natality and growth are erroneous unless this temperature dependence is considered. Physiological time is taken as the product of time, $T$, and the ratio of the rate of metabolism $R_t$ at the mean field temperature $t$ (over the period $T$) to the rate of metabolism $R_0$, at some convenient temperature $t_0$. That is

$$pT = T\left[\frac{R_t}{R_0}\right]$$

(1)

where $pT$ is the physiological time. $R_0$ was taken as the rate of oxygen uptake at $5 \degree C$, while the other metabolic rates were derived from oxygen consumption data for both species (DUNCAN, in prep.). With the reference point chosen (a temperature of $5 \degree C$) equation (1) resolves to

$$pT = T \cdot (Q_{10}) \left(\frac{t-t_0}{10}\right)$$

since $Q_{10} = \left(\frac{R_t}{R_0}\right) \left(\frac{10}{t-t_0}\right)$ by definition.
This equation is valid provided the $Q_{10}$ is constant over the range of environmental temperatures.

Population growth in the field can be traced by plotting the maximum (or minimum) density of all instars against the corresponding time (on a $pT$ scale). Figure 6 shows this relation for both species. The percentage of mature males in the adult populations are: $O. \text{ hurleyi}$, 52.8; $O. \text{ patersoni}$, 48.1. A $\chi^2$-test shows that, in both species, these sex ratios are not significantly different from ratios of 1:1 ($P_{(O. \text{ hurleyi})} = 0.2$; $P_{(O. \text{ patersoni})} = 0.6$).

3.6.3. Mortality

If survival rate does not vary with age, the numbers of animals in successive instars of a stable population form a decaying series of the form

$$N = n_0 + s \cdot n_0 + s^2 \cdot n_0 + \cdots + s^m \cdot n_0$$

since $n_i = s \cdot n_{i-1}$

and where $N$ is the total number of living animals, $n_i$ is the number in instar $i$ ($i = 0$ to $m$), and $s$ is the survival rate per moult. Obviously

$$N = n_0 + s \cdot n_0 + s^2 \cdot n_0 + \cdots + s^m \cdot n_0$$

$$= n_0 \sum_{i=0}^{m} s^i$$

and $s$ can be estimated by averaging the estimates given by

$$s_{i+1} = n_{i+1}/n_i \quad (2)$$
where \( i \) is an integer taking the values \( 0 \leq i \leq m - 1 \). That is, the survival rate can be estimated by dividing the number of individuals in a certain stage by the number in the preceding stage. These estimates of \( s \) can be further extended by averaging the successive estimates given by

\[
\hat{s}_{i+u} = \left[ \frac{n_{i+u}}{n_i} \right]^u
\]

(3)

where \( u \) is a positive integer taking values between 1 and \( m - 1 \), providing \( i + u \leq m \).

This survival rate (\( \hat{s} \)) is determined not only by the true mortality, but also by changes in the duration of successive instars. Providing these changes are regular, then \( \hat{s} \) can be as useful as the true survival rate in erecting life tables. If, however, there is a fundamental change in the growth rate at some stage in the life cycle, then \( \hat{s} \) will be biased. A means of detecting bias is to plot the successive estimates of \( \hat{s} \) given by equation (2) against the corresponding instar \((i + 1)\).

For the amphipod populations, the number in each instar over a year is given by integrating the curves of density against time (Figure 5). These integrals can be either

![Graphs showing growth rate estimates for O. patersoni and O. hurleyi](image)

Fig. 6. Estimates of growth rate in the field.
calculated mathematically or measured empirically (by fitting curves or straight lines to the points, cutting out the areas and weighing). With the second method, smooth curves are theoretically preferable, but are more subjective and less reproducible than fitting straight lines (as has been done in this study).

The estimates of $s$ using equation (3) can be expressed in a symmetrical, triangular two-dimensional array (Table 6). The leading diagonal (entries enclosed in brackets) in Table 6 has values rather lower than those in the rest of the Table. This results from the higher number of animals present in the first instar as many newly hatched individuals do not leave the mother immediately, but cling to her until she mouls. First instar animals do not moult until they spend some time in free life, independent of the mother.

Furthermore, there is the possibility that the prepubertal moult ($i = 9$) marks a change in the growth rate, with less energy expended on growth and more on reproductive products. The entries affected by this have been marked with an asterisk in Table 6.

Table 6  Estimates of the annual survival rate ($s$) for each instar of *O. hurleyi*. For explanation see text

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>(.4857)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>.9184</td>
<td>(.662)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>.9822</td>
<td>.950</td>
<td>(.761)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>.7934</td>
<td>.882</td>
<td>.891</td>
<td>(.768)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>1.2965</td>
<td>1.016</td>
<td>1.002</td>
<td>.981</td>
<td>(.854)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7</td>
<td>.6448</td>
<td>.916</td>
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<td>.952</td>
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<td>.938</td>
<td>.936</td>
<td>(.858)</td>
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<td></td>
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<tr>
<td>8</td>
<td>.9021</td>
<td>.846</td>
<td>.976</td>
<td>.925</td>
<td>.938</td>
<td>.936</td>
<td>(.858)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.4542*</td>
<td>1.27*</td>
<td>1.013*</td>
<td>1.078*</td>
<td>1.016*</td>
<td>1.008*</td>
<td>.995*</td>
<td>(.910)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>.9231</td>
<td>1.163*</td>
<td>1.142*</td>
<td>.990*</td>
<td>1.046*</td>
<td>.999*</td>
<td>.996*</td>
<td>(.985)*</td>
<td>(.911)*</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>.7210</td>
<td>.815</td>
<td>.993*</td>
<td>1.017*</td>
<td>.929*</td>
<td>.990*</td>
<td>.953</td>
<td>.961*</td>
<td>.950*</td>
<td>(.890)*</td>
</tr>
<tr>
<td>12</td>
<td>1.0219</td>
<td>.856</td>
<td>.825</td>
<td>1.000*</td>
<td>1.019*</td>
<td>.946*</td>
<td>.988*</td>
<td>.971*</td>
<td>.964*</td>
<td>.959*</td>
</tr>
<tr>
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<td>.797</td>
<td>.875</td>
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<td>.991*</td>
<td>.932*</td>
<td>.902*</td>
<td>.850*</td>
<td>.953*</td>
</tr>
<tr>
<td>14</td>
<td>.7191</td>
<td>.816</td>
<td>.796</td>
<td>.822</td>
<td>.841</td>
<td>.922*</td>
<td>.948*</td>
<td>.881*</td>
<td>.939*</td>
<td>.923*</td>
</tr>
<tr>
<td>15</td>
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<td>.394</td>
<td>.697</td>
<td>.768</td>
<td>.758</td>
<td>.777</td>
<td>.856*</td>
<td>.772*</td>
<td>.853*</td>
<td>.890*</td>
</tr>
<tr>
<td>16</td>
<td>.3765</td>
<td>.296</td>
<td>.530</td>
<td>.609</td>
<td>.419</td>
<td>.674</td>
<td>.705</td>
<td>.780*</td>
<td>.802*</td>
<td>.787*</td>
</tr>
<tr>
<td>17</td>
<td>.0638</td>
<td>.141</td>
<td>.236</td>
<td>.312</td>
<td>.381</td>
<td>.427</td>
<td>.436</td>
<td>.522</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Another group of abnormal data is that corresponding to $i = 14$ to $17$, u (larger adults). These, when plotted against $i$, show a great decrease in survival rate (Figure 7). For the remaining groups (juveniles and adults) the estimate of $s$ is 87.0% per instar with a standard error 3.3%. The complete analysis for both species, separating adults from juveniles, is shown in Figure 7. Inspection of this figure shows that the estimates based on physiological time give smaller deviations than those based on absolute time.

A mortality analysis of the data in Fig. 7, based on the method of Chapman and Robson (1960) for catch curves, gives values of 84.5% ($O. hurleyi$) and 83.9% ($O. patersonl$) for the survival rate per instar. A $\chi^2$-test shows that these estimates are not significantly different from each other ($P = 0.99$; 8 d.f.). Theoretical curves fitted to either species using either estimate, are not significantly different (by a $\chi^2$-test) from the observed values. Therefore, for situations like this, mortality analyses which use smoothing techniques or calculate estimates based on means are not as useful as methods using untreated data.

The relation between biomass per core and litter thickness is shown in Figure 8. The total regression (for both species) is the sum of the individual species regressions so that litter thickness (or better, some attribute of litter) is acting on the total amphipod population and not on the individual species. The distribution of litter thickness is log normal, as shown by a plot of percentage cumulative frequency against litter thickness on log
probability paper (Figure 9). The distribution of animals is also contagious. There are two hypotheses which could explain this. Firstly, underdispersion could be brought about by gregarious behaviour. This hypothesis is not supported by any observations made during this study. Amphipods in culture (except mature males responding to receptive females) avoid actual physical contact with each other — one individual may even repel another by lying on its side and kicking with its abdomen. Individuals in the field are usually separated from all others by a small space even when the population density is high. Mutual avoidance of physical contact is possibly advantageous in reducing cross-infection of diseases, ectoparasites and epizoites. There is the possibility, however, that the animals may still be attracted to each other and yet avoid physical contact.

The second hypothesis is that the distribution of animals follows the distribution of some essential commodity which is in short supply. Litter is an important commodity to amphipods, since it serves as both food and shelter. If it is in short supply then
the amphipod populations would be forced to follow its distribution pattern. This hypothesis is more consistent with the spacing behaviour of the animals. Furthermore, the close relations between litter thickness and biomass per core (Figure 8), total amphipod density (Figure 5 and Table 8) and mean size per core (Table 7) suggest that some attribute of litter is a limiting factor on the amphipod populations (see Section 4.2.).

The relation between the size of animal and litter thickness (Table 7) shows that the mean size of animal per core increases with litter thickness. This distribution could result from larger animals excluding the smaller animals from favoured sites, but in all samples,
even those with thick litter, there are always a large number of small animals. The increase in mean size with increasing litter thickness is due to the relative increase in the number of larger animals — not the exclusion of smaller ones (Table 7).

Table 7  Mean number per core of each instar (*O. hurleyi*) in different litter thicknesses. Data drawn from samples taken during the breeding season and excluding all cores with *O. patersoni* present.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Litter thickness (mm)</th>
<th>0.0–5.0</th>
<th>5.0–10.0</th>
<th>10.0–15.0</th>
<th>15.0 +</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.45</td>
<td>1.52</td>
<td>2.30</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.54</td>
<td>0.52</td>
<td>0.90</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.18</td>
<td>0.24</td>
<td>0.60</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.27</td>
<td>0.33</td>
<td>0.45</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.18</td>
<td>0.52</td>
<td>0.25</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.18</td>
<td>0.76</td>
<td>0.40</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.09</td>
<td>0.43</td>
<td>0.55</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.00</td>
<td>0.52</td>
<td>0.50</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.18</td>
<td>0.81</td>
<td>0.90</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.27</td>
<td>0.95</td>
<td>1.05</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>—</td>
<td>0.48</td>
<td>1.40</td>
<td>1.80</td>
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<td>12</td>
<td>—</td>
<td>0.62</td>
<td>1.55</td>
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<td>13</td>
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<td>1.00</td>
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<td>0.50</td>
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<td>16</td>
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<td>0.30</td>
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<td>17</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.30</td>
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</table>

Number of cores  11  21  20  10

Fig. 9. The percentage cumulative frequency distribution of litter thickness plotted on log probability paper. The 'winter' distribution is from the August samples while the 'summer' distribution is from the December samples.

336
Table 8  Correlation of amphipod biomass and dry matter production for grassland. Data on grass production drawn from Hudson, Doak and McPherson (1933). The production during certain periods (measured in weeks preceding the date of amphipod sampling) has been correlated with amphipod biomass (live weight). The correlation between litter thickness and biomass is also given.

<table>
<thead>
<tr>
<th>Production period*</th>
<th>Correlation coefficient (r)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 2</td>
<td>0.265</td>
<td>—</td>
</tr>
<tr>
<td>2 to 4</td>
<td>0.829</td>
<td>*</td>
</tr>
<tr>
<td>4 to 6</td>
<td>0.740</td>
<td>*</td>
</tr>
<tr>
<td>6 to 8</td>
<td>0.589</td>
<td>—</td>
</tr>
<tr>
<td>8 to 10</td>
<td>0.604</td>
<td>—</td>
</tr>
<tr>
<td>10 to 12</td>
<td>0.679</td>
<td>—</td>
</tr>
<tr>
<td>12 to 14</td>
<td>0.417</td>
<td>—</td>
</tr>
<tr>
<td>Litter thickness</td>
<td>0.887</td>
<td>**</td>
</tr>
</tbody>
</table>

*) Less than 5% probability
**) Less than 1% probability

4. Discussion

4.1. Value of microclimate records

The microclimate records given earlier show only the average climate conditions. It is difficult to measure the climate experienced by nocturnally active litter animals in a meaningful way. Daytime litter temperatures and night surface temperatures are perhaps the best measures of the temperatures experienced by amphipods. Soil and air temperatures, however, vary according to the distance from the soil surface (Geiger 1959; Aslying and Stendal 1965) and since various elements of the amphipod populations inhabit a variety of soil depths during the day and a variety of heights above the soil at night, it is obviously inaccurate to use one or two temperature measurements to describe the temperature regime experienced by the amphipod populations. This error may seem trivial when the differences experienced by the different components of the populations appear small (between 1°C and 5°C). But, if an estimate of the respiratory budget of the population is contemplated then serious errors could arise. For example, assuming a Q₁₀ of 2.5 and a temperature difference of 5°C, the higher rate of metabolism is 158% of the lower.

A further disadvantage to the present study is that the recorded temperatures are not related linearly to the animals' metabolic rate. The geometric (logarithmic) mean of the diurnal temperature cycle would be a more meaningful measure provided that the Q₁₀s of the study species were known.

4.2. Reproduction and recruitment

The loss of eggs during brooding (30%) can be attributed to a decrease in the available space in the brood chamber as development proceeds. It appears that the female packs as many eggs as possible into the brood chamber during laying. The eggs absorb water and swell as they develop. Space becomes limited, the mother's gills are displaced and, by accident, one or two of the posterior eggs, which are not held as tightly as the anterior eggs, are lost from the chamber. From the data in Table 4, the increase in egg volume (assuming no change in shape) is approximately 220% which would result in a loss of
56% of the original brood if space is the limiting factor. The discrepancy between the observed loss (30%) and the calculated loss (56%) probably arises because no allowance has been made for more efficient packing during the latter stages of development due to the change in egg shape from a spheroid to an ellipsoid.

4.3. Growth of populations in the field

The data in Figure 6 suggests that the complete life cycle takes about 12 months, although the 'minimum density' curve for O. patersoni gives a shorter period (nine months). The linearity of the data in this figure is anomalous as most reported growth curves are curvilinear (Von Bertalanffy 1957). Linearity in podomere growth rate implies that the intermoult period is constant, and independent of age. This result could be fortuitous since so few samples were taken and further, mortality could be selective, depending on density, season and age. Selective mortality of this type is not likely to be elucidated in the mortality analyses carried out in section 3.6.3., as these give only average conditions for a whole year.

There is no evidence that winter temperatures fall below a growth threshold. Growth (and moulting) continue throughout the winter, though at a much slower rate than summer. A similar situation has been reported by Mills (1967) in Ampelisca spp.

4.4. Fluctuations in population density

During the study period the populations of both species were considerably reduced during autumn. This phenomenon has been observed in a semi-quantitative fashion over three years at various localities. The population decline is concurrent with a reduction in litter thickness which diminishes in spite of rapid autumnal replacement by dead grass material. In the late summers of 1966 and 1967, the litter had an open, skeletal appearance with only the more resistant plant material remaining. Even these remains had been eaten in part, and were covered in faeces.

To explain the population decline it is hypothesized that, in late summer and autumn, the amphipods are eating far more food than is being produced. This results in a population crash after the standing crop of litter has been utilized. The population increase from August to December occurs while average field temperatures are relatively low. With the higher temperatures in late summer and autumn, daily energy requirements are greatly increased. At the temperatures prevailing during this season, the amphipod populations respire about $4.43 \times 10^{-3}$ l of $O_2/\text{m}^2/\text{hour}$ and they have an R.Q. of about 0.85 (Duncan, in prep.). This corresponds to the rate of energy uptake of 0.523 kcal/m$^2$/day [using Kleiber's (1961) formula and assuming that the R.Q. is 'nitrogen free']. Making the further assumption that their energy is derived from the complete utilization of dead grass, the rate of energy uptake (summing both species) corresponds to a utilization rate of 0.125 g of dry grass/m$^2$/day (grass caloric equivalent from Lambourne 1957).

This respiratory demand represents between 2% (minimum) and 10% (maximum) of the January dry matter production per day as given by Hudson, Doak and McPherson (1933). The amount eaten per day will be greater than this as no allowance has been made for incomplete utilization, or for the energy used in growth and reproduction.

Changes in population density can be correlated with grass production even though this was not measured at the study site. There is a general growth pattern in temperate Festucoid grasses (Evans, Wardlaw and Williams 1964) with (1) and early spring acceleration in leaf growth, (2) a decline in late summer after the initiation of flowering, and (3) a partial recovery in autumn after flowering.

Dry matter production data for perennial pasture land was drawn from Hudson, Doak and McPherson (1933) and averaged to give weekly production values (stems and leaves
The data used did not include any from plots that had fertilizer added during the study period. It was hoped that this data would reveal the pattern of grass production for perennial Festucoid grasses in New Zealand since their study covered a number of situations, grass species and years. The correlations between amphipod biomass (live weight) and grass production (dry matter), given in Table 8, suggest that there is a four to eight week period between production and utilization. Amphipod winter populations appear to be maintained by the grass produced in autumn. As this autumn growth varies from year to year (Hudson, Doak and McPherson 1933) it may be expected that the size of the over-wintering amphipod population will also vary.

Another hypothesis which could explain the population crash in autumn is that mortality is principally due to desiccation. This hypothesis could well account for population fluctuations in amphipods as they have high transpiration rates. However, in the area studied, the relative humidity of the air contained in litter never fell below 100%. The litter and soil moisture levels were relatively constant, being buffered by the rank and continuous grass canopy. Furthermore, Dunedin's climate is typically cooler and wetter than at most other places in New Zealand, with only one or two months of the year when potential evapotranspiration is higher than precipitation and even then the differences are slight (Garnier 1958).

Desiccation, therefore, is unlikely to be a significant cause of mortality in cool, southern areas. Desiccated specimens were rarely found in the study site but they were quite common in drier areas such as Central Otago and Canterbury.

4.5. Mortality

Observations made at night suggest that the major causes of death in amphipods are predation, osmotic stress, desiccation, and disease.

(a) Predation. This occurs mainly during ecdysis. Intermoult animals can hop in an erratic fashion when disturbed — an escape mechanism which can be considered as protean behaviour (Humphries and Driver 1967). It appears to be effective as, in both field and laboratory situations, lycosid spiders were unable to catch amphipods until the latter were weakened through either starvation or desiccation. In a similar test, peripatus (Peripatodes nova-zealandiae Hutton) were able to catch intermoult amphipods. Peripatus, however, did not occur in the study area.

Another escape mechanism is their ability to squeeze through confined spaces as their body shape enables them to fit into small crevices. Furthermore, although they appear unstable, their agility is remarkable. They are able to move through litter on their sides or upside down, parting the leaves as they go.

During ecdysis, however, the animals are comparatively immobile. They are only able to execute weak scuffling movements by flexing their abdomen. At this stage they are liable to heavy predation. Even slow-moving flatworms (Geoplana spp.) have been observed feeding on moulting amphipods.

(b) Osmotic stress. Specimens of O. patersoni were frequently found dead in small pools of water. These had a swollen appearance and the gill ligaments had ruptured. The gills are normally flattened structures with the two planes held together with ligaments but these specimens had gills which were swollen and turgid. The cause of death in these individuals was attributed to osmotic stress.

(c) Desiccation. This was extremely uncommon in the study site, but was common in drier areas with thin litter.

(d) Disease. In the more northern part of its range, O. hurleyi suffers from a disease which gives the body a creamy-white appearance. In culture, diseased animals are less active than, and die before, normal animals. The Dunedin population of O. hurleyi was virtually free from this disease.
The major causes of mortality in the study area appear to be predation on the animals undergoing ecdysis and, possibly, starvation — although the latter factor would not be a proximate mortality factor.

The delay in intense mortality until the third adult instar (Figure 7) could be a means of increasing biotic potential (Cole 1954) but it is more likely to be the result of selective predation by larger predators such as the hedgehog (Erinaceus europaeus Linn.).

4.6. Comparison of the two species

Reproduction in *O. hurleyi* is less efficient than *O. patersoni* because it carries a larger number of eggs, it has a higher mortality rate and relatively longer immature stage. Therefore, the ecological or production efficiencies of the populations must differ. An index of ecological efficiency may be made by comparing the total biomass in the immature stages with the total adult biomass. That is, \[ I = \frac{W_a}{W_{im}} \] where \( I \) is the index, \( W_{im} \) is the biomass of immature animals and \( W_a \) is the adult biomass. Low values for \( I \) reflect low net production because, in this case, many animals die before they reproduce. This index can only be used for comparing very similar species.

The index for *O. hurleyi* is 1.17 while for *O. patersoni* it is 2.12.

In situations where inter-specific competition is intense, *O. hurleyi* would be a poorer competitor than *O. patersoni*. However, the latter species is limited to areas with a relatively constant water supply, such as under *Dactylus glornerata* canopies, where the chance of death from osmotic stress is low. In these areas *O. patersoni* would probably displace *O. hurleyi* as it has a higher ecological efficiency. In southern mixed podocarp-hardwood forests, the typical habitat of *O. patersoni*, *O. hurleyi* is absent even though it is abundant in the surrounding grassland and ecotones (forest-grassland).

The ability of *O. hurleyi* to climb grass stems enables it to avoid osmotic stress and utilize additional food, but climbing does have the disadvantage that the species is liable to predation from a wider range of predators than a strictly ground-dwelling amphipod. The ecological inefficiency of *O. hurleyi* is therefore advantageous in grassland situations as it enables the population to maintain itself in spite of high mortality rates. Where predation rates are lower, however, ecological inefficiency would lead to higher rates of intra-specific competition.

Another interpretation of their ecology is to regard *O. patersoni* as a more advanced animal in the sense that it is more highly adapted to terrestrial life. Its vestigial pleopods, shorter antennae, smaller brood size, and larger eggs can all be cited as evidence for this view. Yet the males of *O. hurleyi* have very reduced second gnathopods and have given up the carrying habit during copulation. These points, together with the ecological evidence that it is more successful in a rigorous terrestrial habitat, suggest that *O. hurleyi* is more 'advanced'.

The viewpoint advanced earlier gives a more consistent explanation of their ecology. In summary, this view is that the two species are adapted to different habitats. These habits, however, overlap to a greater or lesser extent in some features, and so *O. patersoni* is able to live in certain restricted parts of grassland with varying degrees of success.

Further evidence for this view will be presented on a future occasion when the physiological adaptations of both species will be considered.

5. Acknowledgements

I am grateful to Dr. W. C. Clark and Prof. R. L. C. Pilgrim for reading and criticising this paper.
6. Literature


Address of the author: Mr. K. W. DUNCAN, Zoology Department, University of Canterbury, Private Bag, Christchurch, New Zealand.
Les coléoptères du sol dans les champs de betterave sucrière sur chernozem et sol noir des prairies dans le nord-est de Yougoslavie

Par P. VouKassovitch, D. Čamprag, J. Djurkić, R. Sekulić

(Reçu le 10.11.1968)

1. Introduction

Ce n'est que depuis les dix dernières années qu'un nombre restreint de chercheurs ont entrepris l'étude de l'entomofaune du sol de Yougoslavie.

Les recherches ont été orientées vers la connaissance de la macroentomofaune du sol des principales plantes cultivées (blé, maïs, betterave sucrière et luzerne) et des paturages se trouvant sur différents types de sol.

Nous exposons dans le présent travail les résultats de deux années de recherches portant sur les Coléoptères; ces recherches ont été poursuivies dans des parcelles emblavées en betterave sucrière. L'étude a été financée par le Fond de Recherche scientifique de la République socialiste de Serbie.

2. Territoire prospecté, matériel et méthode de travail

Les recherches ont été effectuées dans le rayon de la Bačka (territoire de la Vojvodina), dans le nord-est de la Yougoslavie. Cette région, située entre le Danube et la Theiss (Tisa), se caractérise au point de vue du climat par une variété particulière (sud-ouest) de climat semi-aride steppique. La moyenne de plusieurs années de précipitations et la moyenne mensuelle des températures (en °C) sont les suivantes:

- Janvier 34 mm (-0,1 °); février 47 mm (0,6 °); mars 36 mm (4,6 °); avril 50 mm (11,4 °);
- mai 63 mm (16,6 °); juin 82 mm (19,9 °); juillet 57 mm (21,8 °); août 48 mm (21,3 °); septembre 36 mm (17,3 °); octobre 42 mm (11,1 °); novembre 58 mm (5,6 °); décembre 57 mm (1,8 °).

Le total des précipitations annuelles est de 610 mm et la température moyenne de 11,0 °C.

Les recherches ont été poursuivies sur chernozem et sol noir des prairies. Dans le rayon de la Bačka, ces types de sols sont les plus fréquents; ils constituent en outre les meilleures terres, aux propriétés physiques exceptionnelles.

L'examen du sol a été poursuivi pendant 2 années, dans un total de 30 localités. En 1966, 193 parcelles représentant 8.600 ha de betterave sucrière, ont été examinées; 4.556 échantillons de sol y furent prélevés. En 1967, les recherches portèrent sur 164 parcelles, totalisant 8.200 ha; au cours de cette seconde année, 4.072 échantillons furent recueillis. Les recherches des deux années portèrent donc au total sur 357 parcelles, représentant 16.800 ha de culture de betterave sucrière.

Les échantillons ont été recueillis en septembre et en octobre, en utilisant la méthode de prélevement manuel. L'échantillon de sol mesure 50 x 50 cm (= 0,25 m²), et le creusage atteint 50 cm de profondeur. On a prélevé dans deux ans, 8.628 échantillons de 0,25 m³, soit en moyenne, un échantillon pour deux hectares. La superficie totale d'échantillonnage est de 2.157 m². Seuls ont été étudiés les coléoptères appartenant aux six plus importantes familles, du point de vue économique, soit les Elateridae, Tenebrionidae, Alleculidae, Scarabaeidae, Curculionidae et Carabidae.

Un total de 7.256 spécimens ont été déterminés.

1) Remarque: dans le "summary" il est écrit "south-western variety", ce qui signifie sud-ouest occidental a d'être contrôlé.

342
Chapter 4. DISEASE

Prefatory Note

The following paper has been included as a chapter of the thesis. I have changed the name of the species involved to *Makawe hurleyi* in the first part of this thesis, which, of course, was after the paper was published.

Observations made after the paper was written suggest a route by which the disease-causing organism entered New Zealand. Many indigenous species are infected with the disease, and one adventive landhopper, *Talitroides topitotum*, also carries it. In 1981 this adventive species was found in and near a compost heap on the campus of the University of Canterbury near where some university botanists had thrown out some potted plants collected in North Island. The landhopper thrived and greatly increased in numbers during spring, but died out in the drought of summer leaving the indigenous *Makawe hurleyi* as the sole landhopper present.

Similar episodes to this would result in the spread of the disease carrying species to areas where, even if it persists for only a time, it could spread the disease to indigenous species. *Talitroides topitotum* does not seem to be as susceptible as the indigenous landhoppers because it has a high biotic potential.
The effect on *Orchestia hurleyi* (Amphipoda: Talitridae) of a whitey disease caused by *Bacillus subtilis*

K. W. Duncan

Department of Zoology, University of Canterbury, Private Bag, Christchurch, New Zealand

Field observations made over 10 years suggested that a bacterial disease of adults of the terrestrial amphipod *Orchestia hurleyi* Duncan, caused by *Bacillus subtilis*, is progressing southwards down the eastern side of New Zealand’s South Island. As the disease spread, amphipod density appeared to decline and population age structure became truncated. In the vicinity of Dunedin and further south the amphipods are still disease-free. Signs of the disease are a progressive weakening and wasting. The animal cannot jump, and its speed of walking is reduced. Its body becomes opaque white instead of the normal translucent reddish-brown. Disease females do not brood. There is no evidence that diseased animals moult. Death is caused by general wasting or by predators. The disease-causing organism was isolated, and healthy amphipods were re-infected from the isolate. Signs of the disease were apparent within 7 days of inoculation. The presence of the disease-causing organism in the haemocoel causes host defences to be mobilised, as shown by elevated haemocyte counts (4512 mm⁻³, cf. 300 mm⁻³ in healthy, disease-free adults), but as the disease progresses the animal’s defences are overcome, and haemocyte counts fall to an average of 784 mm⁻³ during the later stages of disease. The blood of terminally diseased amphipods is thick and creamy white, packed with motile bacterial cells, and few (if any) haemocytes are present in the circulation. Two populations were studied, one disease-free (at Dunedin) and the other heavily diseased (at Christchurch). The incidence of disease (as measured by a performance test) was about 30% in Christchurch adults. The disease-causing strain of *B. subtilis* was found on the body surface of almost all adults in the diseased population. It is possible that the bacterium gains entry to the haemocoel through wounds suffered during ecdysis, conflict, or predator attack. The main differences shown by the diseased population relative to the disease-free population were: lower average density (992 m⁻³, cf. 1677 m⁻³); lower maximum density (3104 m⁻³, cf. 9971 m⁻³); smaller average size, with fewer adult instars; a smaller proportion of females brooding in each instar; and much lower egg production. The brood size/mother age relationship was the same for both populations: number of eggs in brood = -4.9 + 0.64 (instar number of mother)—because in the diseased population only healthy females breed. Lower egg production in the diseased population reflects the smaller proportion of healthy females, and the number of broods per female is lower since life expectancy is much less. A computer model based on Leslie matrices was used to simulate the ecological effects of the disease. It gave predictions which conformed with the observed population features with respect to age structure and density.

**Keywords:** Amphipoda; Talitridae; *Orchestia hurleyi*; whitey disease; *Bacillus subtilis*; pathology; population structure; computer model; disease spread

**INTRODUCTION**

Terrestrial amphipods of the family Talitridae are widely distributed in the Indo-Pacific region, where they are extremely abundant in leaf litter (Hurley 1968). Most species live in forest litter, but a few are found in waste grassland and scrubland, where they may be locally abundant. Birch & Clark (1953) recorded *Talitrus sylvaticus* at densities of up to 4000 m⁻³ in Australian rain forests, and Duncan (1969) recorded over 3000 m⁻³ in waste grassland.

One grassland species, *Orchestia hurleyi* Duncan, is widespread and abundant on the eastern side of New Zealand's South Island, where it occurs in scrubland, waste grassland, and suburban gardens.

In the southern part of its range it is sympatric with *O. patersoni*, but Duncan (1969) has demonstrated considerable niche differentiation between them. *O. patersoni* lives mainly in rain forest, ranging into scrubland and waste grassland only where it has protection from local flooding. Unlike *patersoni*, *hurleyi* can escape from flooding by climbing into the aerial vegetation. It inhabits all kinds of grassland with the exception of sheep-cropped pasture, where it is restricted to living under the larger deposits of sheep dung or in the longer vegetation under fence lines or hedgerows.

A disease of *O. hurleyi* was first detected in 1968. Infected animals had an appearance similar to

__Received 12 March 1981__
insects affected by milky disease, as described by Dutky (1963), in that they were an opaque, milky white instead of the normal translucent reddish-brown. The diseased condition was first detected in individuals collected in 1968 from around Christchurch, but the disease seems to have subsequently spread about 330 km south-westwards down the coast towards Dunedin. As the disease spread, the density of the species appeared to decline in recently infected areas. In certain places this decline was very marked. Once the disease was prevalent in the population, substantially fewer large individuals were found. This suggests that one of the main ecological effects of the disease is a truncation of the population age structure and a reduction in breeding potential. Around Dunedin and further south O. hurleyi is still disease-free, and population densities are still high.

Because terrestrial amphipods are such important litter comminutors (Birch & Clark 1953), and because so little is known of the ecological effects of naturally occurring pandemic diseases in invertebrates, I undertook this study to compare a disease-free population from a grassland area at Dunedin with a diseased population living in long grass on the banks of the Avon River in the University of Canterbury grounds at Ilam, Christchurch.

METHODS

SPREAD OF THE DISEASE

The spread of the disease was monitored by visiting a number of sites (Fig. 1) throughout the known range of O. hurleyi in 1968, 1970, 1974, and 1978. Animals at each site were inspected for signs of the disease.

ISOLATION OF THE PATHOGEN

Freshly collected diseased individuals were anaesthetised in carbon dioxide. Their second antennae were ablated, and the first two drops of haemolymph were discarded. The remaining drops were allowed to fall on sterile Petri dishes containing Sabouraud's medium, nutrient agar, or blood agar.

To confirm that the bacterium isolated by this procedure was the disease-causing agent, 25 apparently healthy adult individuals from an apparently disease-free population were each inoculated while under carbon dioxide anaesthesia by jabbing a new, sterile, 27-gauge hypodermic needle into the bacterial culture and then inserting it into the dorsum, at the junction of the thorax and abdomen. Twenty-five controls were similarly treated, but the needles were jabbed into sterile nutrient agar before insertion into the animal. Each animal was kept unfed in individual plastic lunch boxes with a floor of 'Vermiculite' (basic expanded mica) dampened with distilled water. By maintaining sterility and avoiding cross-contamination, outbreaks of the disease due to airborne or foodborne spores—which had been troublesome in earlier cultures—were avoided.

Animals that died during the experiment were removed, and their haemolymph was plated out on nutrient agar. After 7 days, haemolymph samples were taken from every surviving animal (a procedure which killed them) and plated out on nutrient agar.

TOXICITY

To investigate the incidence of the bacterium on or in the animals in a field population, 100 adults were collected by hand-sorting litter samples.

Each animal was closely examined for signs of milky disease, then plunged into boiling water for about 1 min. The body was then squashed on to a nutrient agar plate. No attempt was made to distinguish between bacteria from the haemolymph and the body surface. The likelihood of cross-contamination was so great that no consistent results would have been obtained had haemolymph samples been collected and plated out individually.
MAMMALIAN TOXICITY
The toxicity of the bacterium to mammals was tested by injecting five mice interperitoneally with 0.1 ml sterile saline in which a small amount of bacterial isolate had been suspended. Five control mice were each injected with the same volume of sterile saline.

HAEMOCYTE COUNTS
It had been observed that diseased amphipods progressively declined over a period of weeks, becoming whiter and whiter until they died. This suggested that they might have no effective defence reactions. To investigate this, haemolymph samples were taken from a series of amphipods, each at a different stage of the disease. Attempts to take serial samples from single individuals failed because the animals were invariably killed by the extraction procedure.

The cell counting methods used by Yaeger & Taeber (1935) and Sawyer et al. (1970) could not be employed because diseased amphipods yielded too little haemolymph. Therefore, samples of haemolymph were taken by cutting off the second antenna of animals immersed in liquid paraffin, and taking up the haemolymph droplets which exuded from the stump into a micro-pipette. The haemolymph sample was then placed in a drop of liquid paraffin on the counting grid of a haemocytometer. With the coverslip in place, the preparation remained in good condition for a number of hours. Phagocyte movements could be observed, and the haemocytes could be counted readily. The results obtained by this method were checked against Yaeger & Taeber's (1935) by using their method on healthy animals.

The haemolymph cell count was determined for 25 healthy, non-brooding adults, 13 healthy, brooding adults, 3 healthy animals undergoing ecdysis, and 34 diseased animals of which 10 had the black or dark brown areas indicative of recent cuticle damage (Fontaine & Lightner 1973, Fontaine 1975).

PROPORTION OF DISEASED ADULTS
The proportion of disease-affected adults in the Christchurch population was estimated by a performance test. Field observations showed that diseased individuals move more slowly than disease-free individuals, and they do not jump at all. As a consequence, they were much easier to collect than healthy animals and were thus over-represented in collections taken using an entomological aspirator. Escape from predators by terrestrial amphipods depends very largely on their locomotion. Their ability to jump, walk rapidly over the soil surface, and scuttle sideways through loose leaf litter confuses and outpaces most invertebrate predators. Thus, any impairment of their locomotory ability would seem to be of considerable consequence to their survival, in that diseased animals would be more likely to be caught by predators.

To investigate the diminution in locomotion caused by the disease, the pattern and rate of walking of individuals immersed in 25% sea water (preliminary work had shown that O. hurleyi survives longest at this salinity) was determined for 25 healthy and 25 diseased animals. Since the animals could not jump while immersed, locomotion in the two groups could be compared more meaningfully than if the same experiment had been carried out in air. Furthermore, immersion in water avoided the problem of desiccation.

The pattern of movement under water was a short burst of walking in a relatively straight line for about 10 s, followed by a longer period of rest (about 60 s). Both kinds of activity were timed, and the speed at which the individuals moved over the surface was estimated.

To estimate the proportion of the diseased population affected by the disease, another kind of test was devised. It was based on the observation (Duncan 1969) that O. hurleyi will climb out of water if an escape route is available. A shallow, V-shaped, perspex trough (Fig. 2) was constructed. The floor was lightly sanded by suspending clean, sieved sand in chloroform and pouring this slurry over the two floors of the trough. It was then 90% filled with distilled water at 23°C, and the time taken for at least 100 individuals, randomly collected from the diseased population, to emerge was noted. The experiment was repeated using fresh animals immersed in 10%, 20%, 30%, etc. to 100% sea water. Polymodal analysis (Cassie 1954, Aitchison & Brown 1957) of the emergence time-frequency data was used to separate the diseased and disease-free groups and to estimate their size.

ECOLOGICAL METHODS
The effect of the disease on amphipod populations was studied on a disease-free population living in long grass in a suburban garden at Dunedin (NZMSI S 73178) and on a diseased population living in long grass on the banks of the Avon River, Christchurch (NZMSI S 573957). The effect of the disease on population growth, natality, and density in the two populations was investigated by taking 78 cm² soil/litter core samples using a heavy metal corer and extracting them for 4 days in Tuligren funnels following the methods of Duncan (1969). All cores were inspected for recently dead amphipods. Very few were found, although they were very obvious since they turned pink after death. These animals were considered to have died during extraction, and so were included in the subsequent analysis.
All samples were taken in mid summer (January-February). The animals from each core were counted, their sex was determined, the number and stage of development of eggs were noted for brooding females, and the podomeres on the second antenna flagellum were counted (this gave a measure of the number of instars that the animal had been through). To check on egg loss during extraction, a number of brooding females were carried by each one were counted.

A time-specific mortality analysis was carried out using the methods in my earlier study (Duncan 1969). The estimates of mortality and brood size were analysed using a population growth model described by Emlen (1973, pp. 240-260), based on Leslie matrices (Leslie 1945). The mathematical analysis was carried out on a Burroughs B6718 computer using a program written in BASIC.

RESULTS

SPREAD OF THE DISEASE

Fig. 1 shows that the disease has spread rapidly from the Christchurch urban area in which it was first detected in 1968, towards Dunedin. Populations in the wetter foothills flanking the drought-prone Canterbury Plains still seem to be disease-free. Whitey disease does occur in other species, because at Karamea on the west coast of the South Island it was detected in another, undescribed Orchestia species.

PATHOLOGY

Diseased animals changed progressively from pale translucent white to dense creamy white. They were unable to jump, and moved more slowly than healthy individuals. Examination of live, diseased amphipods under a light microscope showed that their haemolymph, normally from translucent pale blue to clear, was packed with motile bacteria interspersed with a few haemocytes. The gradual change in colour to creamy white was due to an increase in the numbers of bacteria in the haemolymph, since the colour of the bacterial cultures on agar was much the same.

Colony growth of the bacteria isolated from the haemolymph was maximal on nutrient agar plates incubated at 25°C. In 24 h a gelatinous, white, spreading bacterial colony developed, composed of motile, Gram-positive, aerobic, sporulating rods. The bacteria were identified as *Bacillus subtilis*; the diagnosis and identification were confirmed by the Laboratoire de Lutte Biologique, Centre d'Identification de *Bacillus thuringiensis*, of the Institut Pasteur, Paris, and by the Pathology Laboratory, Colston House, Christchurch, New Zealand.

Fig. 2. Apparatus used to find the proportion of diseased *Orchestia hurleyi* in a sample. A number of animals are introduced via the funnel, and the time each takes to emerge from the water is noted. (Diseased animals have impaired locomotion, and so take longer to escape.)

<table>
<thead>
<tr>
<th>Table 1. Experimental infection of <em>Orchestia hurleyi</em> and re-isolation of the disease-causing organism, <em>Bacillus subtilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Effect of whitey disease on the number of haemocytes in the circulation of <em>Orchestia hurleyi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy, non-brooding, inter-moult adults</td>
</tr>
<tr>
<td>Healthy, brooding females</td>
</tr>
<tr>
<td>Diseased animals at an early stage of the disease</td>
</tr>
<tr>
<td>Diseased animals at a late stage of the disease</td>
</tr>
<tr>
<td>Healthy animals undergoing ecdysis</td>
</tr>
</tbody>
</table>

Inoculation of healthy amphipods with the cultured *B. subtilis* caused the same signs of disease as were seen in the naturally infected amphipods. During this experiment, six control animals and five experimental died within 24 h of inoculation, presumably from inoculation trauma. Fisher's exact test (Sokal & Rohlf 1969) showed no significant difference in the proportions killed by the inoculation procedure between the control and the experimental group ($P = 0.5$). The animals which recovered appeared to behave normally.
Nineteen of the experimental group and one of the controls developed signs of the disease (Table 1); the bacterium was re-isolated from 17 of the 19 and from one control.

The suspensions of *B. subtilis* injected into mice caused no apparent long-term ill effects up to 30 days later. Immediately following the injection the experimental mice did sit hunched and quivering, with closed eyes, for a few hours, but their subsequent behaviour was normal.

**HAEMOCYTE RESPONSES**

The effect of the disease on haemocyte counts is shown in Table 2. The average count for non-breeding adults between moult is about half that for healthy, brooding females and individuals undergoing ecdysis. High haemocyte counts are also seen in animals with signs of recent cuticle damage (wounds, or damaged or missing appendages). Such high counts could be a response to wounding and infection.

Inspection of Table 2 suggests that the initial response to *B. subtilis* infection is a massive increase in the number of haemocytes in the circulation. As the disease progresses, however, the number of circulating haemocytes declines, until in very diseased individuals none can be seen.

A Kruskal-Wallis test (Siegel 1956) on the data in Table 2 indicated significant heterogeneity (*H* = 16.244; 5 d.f.; *P* < 0.01). An *a posteriori* test on the inter-group differences showed that, at the 5% significance level, animals in the early stage of the disease had significantly elevated haemocyte counts relative to healthy non-breeding adults (*z* = 5.485, *P* < 10⁻⁶), but animals in the late stage of the disease had significantly lower counts (*z* = -2.152; *P* < 0.05) than those in the earlier stage of the disease.

When stained with Giemsa, thionin, or 0.05% aqueous methylene blue, most of the haemocytes of diseased animals proved to be dense 'granulocytes', in Bauchau & De Brouwer's (1974) terminology. Electron microscopy showed them to be phagocytic cells packed with post-lysosomes, which presumably contain bacterial remains, as has been reported for insects by Racliffe & Rowley (1974). The haemocytes from disease-free animals were of the customary 'hyaline' or 'granulocyte' types (Arvy 1952, Cornick & Stewart 1978).

**EFFECTS ON LOCOMOTION**

The locomotory ability of diseased animals was markedly impaired relative to healthy individuals. Fig. 3 shows that diseased animals take longer rests between their short bursts of activity, so that their chances of escaping from water in a given time are proportionately less. This difference between diseased and healthy individuals provides a means of investigating the incidence of disease in a population. Fig. 4 illustrates the polymodal analysis of log emergence time plotted against cumulative percent frequency of emergence from distilled water. The log normal is thought to be more appropriate than the normal distribution here, since the average speed of locomotion of immersed amphipods declines with increasing submersion time (Fig. 3); presumably, the longer the animals are immersed the weaker they become, thus the probability of emergence, per unit time, diminishes in a regular manner with immersion time.

The usual techniques of polymodal analysis (Cassie 1954) can be used on log normal distribution, but the means given by the analysis are geometric means, not arithmetic ones, and the standard deviations are also logarithmic (Aitchison & Brown 1957).

The overall mean emergence time from distilled water is ln(5.7) min, with a standard deviation of ln(6.2) min. An inflexion at *F* = 67% separates two groups. The first is a fast-emerging group, consisting of only healthy individuals, with a mean emergence time of ln(4.2) min and a standard deviation of ln(3.8); 6.7% of the sample belong to this group. The second group have a mean emergence time of ln(20.0) min and a standard deviation of ln(13.5). Only 52% of this slow-emerging group escaped; the rest died under the water. Most of this group were visibly diseased.

Kolmogorov-Smirnov tests (Sokal & Rohlf 1969) showed the departures from the two log-normal distributions fitted to the observed data (Fig. 4) to be non-significant.

Similar results were obtained using fresh animals in serial dilutions of sea water. Mean emergence time varied with salinity (it was greatest in the 30% dilution), but two groups could always be isolated by polymodal analysis. The slow-emerging group varied from 20% to 35% of the total sample, with a grand mean of 29.8%.

**ECOLOGICAL ASPECTS**

The life cycle of *O. hurleyi* involves nine pre-reproductive (juvenile) instars and up to nine reproductive (adult) instars. As in other amphipods, the eggs are held ventrally outside the body in a kind of box (the marsupium) comprising the ventral surface of the thorax, the flat, plate-like oostegites, and the thoracic side-plates. A small volume of water fills the marsupium, surrounding the gills and eggs; its surface tension possibly helps hold the eggs up against the ventral surface of the body. The abdomen is usually tucked under the thorax, and this too assists in holding the eggs in place.

The brood size of older females is greater because their marsupium is larger; indeed, the relationship
between number of eggs and maternal age is adequately described by a linear equation (Fig. 5). It should be stressed, however, that only females carrying eggs which were about to hatch (embryo fully developed and surrounded by clear—not yolky—fluid) were used in these computations, since Duncan (1969) showed that a significant proportion of eggs are lost as brooding proceeds. The eggs swell as they develop, and since marsupium volume is fixed, a number of eggs are forced out of the marsupium and lost.

In the diseased population at Christchurch, 66 apparently disease-free brooding females were collected by hand. Of these, 33 were brooding ‘late’ eggs. Of the 138 brooding females found at Dunedin, 56 were brooding late eggs. A few infertile or fungus-attacked eggs were found in recently laid broods. Their absence from older broods is probably due to selective ejection by the parent, which turns and cleans the eggs with the brush-like setae and blood-filled ‘pellucid lobes’ of the abdominal limbs, especially the gnathopods.

Fig. 3. Duration of rest intervals as a function of immersion time for 100 diseased and 100 healthy Orchestia hurleyi. Vertical bars indicate standard errors.

Fig. 4. Cassie plot on log probability paper of the cumulative frequency distributions of log emergence time (from water) of 100 Orchestia hurleyi taken from the Christchurch (diseased) population (●), raw data; ○, transformed data.)
Presumably, eggs which lack turgor owing to disease or infertility, and which are shrunken and misshapen, either slip from the parent's grasp and fall out of the brood or are actively ejected.

The average age of brooding females is lower in the diseased population (3.6 adult instars, cf. 5.5 in the disease-free population), and there are no large brooding females in the diseased population (Fig. 5).

Analysis of covariance on the data in Fig. 5 shows that there is no significant difference between the slopes and the intercepts for the two samples, i.e., both lie on the same regression line (F slopes = 0.539, 1:12 d.f.; F intercepts = 0.0005, 1:13 d.f.). A Model 1 regression using Gaussian least-squares of the data in Fig. 4 gives $E = 4.90 + 0.64M$, where $E$ is the number of eggs in the brood and $M$ is the instar number of the mother. The 95% confidence limits for the slope are 0.984 and 0.297; the regression was significant at 0.1%. There were significantly fewer brooding females per adult instar of the diseased population relative to the healthy population (Table 3). Thus, natality in the diseased population must have been lower because fewer eggs would have been produced. Diseased adult

![Diagram](image_url)
females were never seen brooding eggs, nor did any have the dark uterine streak in the posterior thorax which indicates incipient ovulation. The proportion of adult females not brooding because of disease was estimated at about 30%. This is based on the size of the 'slow-emerging' group in the performance test, and assumes equal susceptibility to disease between the sexes.

The 27 brooding females found in the Christchurch core samples carried an average of 5.30 eggs each (s = 1.84) for the 48 from Dunedin the average was 6.92 (s = 3.20). Thus 36.8% of all adult females in the diseased population carried eggs as against 53.3% in the healthy population. The average number of eggs per female was 2.04 in the diseased population and 3.69 in the healthy population. Therefore, egg production per mature female in the diseased population was only 55.3% that of the healthy population.

The observed population densities given in Table 4 reveal a mortality pattern similar to that reported in my earlier study (Duncan 1969). Young adults (stages 2, 3, and 4) have a low mortality, whereas older adults have a higher mortality which increases with age. The diseased population has a truncated age structure (Fig. 6), presumably because of induced mortality. In the life table analysis (Table 4) the mean brood sizes are those given by regression analysis of the data for hand-caught brooding females, not those taken in the core samples, because it was suspected that many females in the core samples lost their eggs during the extraction process.

Table 4. Life tables for diseased (D) and disease-free (D-f) populations of Orchestia hurleyi, based on an age-frequency analysis in a series of core samples (Obs., observed; Exp., expected).

<table>
<thead>
<tr>
<th>Stage(*)</th>
<th>Survivorship(*)</th>
<th>Brood size(*)</th>
<th>Density (n/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-f</td>
<td>D</td>
<td>D-f</td>
</tr>
<tr>
<td>1 (juv.)</td>
<td>0.13</td>
<td>0.13</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>0.99</td>
<td>0.88</td>
<td>3.35</td>
</tr>
<tr>
<td>3</td>
<td>0.95</td>
<td>0.95</td>
<td>4.47</td>
</tr>
<tr>
<td>4</td>
<td>0.83</td>
<td>0.65</td>
<td>5.59</td>
</tr>
<tr>
<td>5</td>
<td>0.64</td>
<td>0.51</td>
<td>6.70</td>
</tr>
<tr>
<td>6</td>
<td>0.59</td>
<td>0.01</td>
<td>7.81</td>
</tr>
<tr>
<td>7</td>
<td>0.47</td>
<td>—</td>
<td>8.93</td>
</tr>
<tr>
<td>8</td>
<td>0.13</td>
<td>—</td>
<td>10.04</td>
</tr>
<tr>
<td>9</td>
<td>0.02</td>
<td>—</td>
<td>11.16</td>
</tr>
</tbody>
</table>

Mean: 7.32 | 6.30  
S: 2.746 | 3.066

Theoretical rate of population growth: 1.175 | 1.063
Average n/m²: 1677 | 1861
992 | 795

(*)Based on instars, but note that the 8 juvenile instars have been pooled as Stage 1  
()As given by Duncan's (1969) method  
Based on least-squares fitted linear regressions of mother size against egg numbers for 154 ovigerous females from the disease-free population and 140 from the diseased population  
Based on 100 cores each of 7.6 cm diameter taken in January-February  
Based on 90 cores each of 9,5 cm diameter taken in January-February.

Table 5. Size frequency distribution of disease-free (D-f) and diseased (D) Orchestia hurleyi in a sample collected by hand from the diseased population at Christchurch.

<table>
<thead>
<tr>
<th>Instar</th>
<th>Observed frequency D-f</th>
<th>Observed frequency D</th>
<th>Proportion diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1</td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>1</td>
<td>0.33</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1</td>
<td>0.33</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td></td>
<td>0.33</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>2</td>
<td>0.33</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>3</td>
<td>0.33</td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>14</td>
<td>0.66</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>5</td>
<td>0.45</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>7</td>
<td>0.88</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>2</td>
<td>0.66</td>
</tr>
<tr>
<td>S.E.</td>
<td>0.421</td>
<td>17.200</td>
<td></td>
</tr>
<tr>
<td>Mean:</td>
<td>13.847</td>
<td>0.320</td>
<td></td>
</tr>
<tr>
<td>Coefficient of skewness:</td>
<td>-0.293 NS</td>
<td>-1.468*</td>
<td></td>
</tr>
<tr>
<td>Coefficient of kurtosis:</td>
<td>-0.829 NS</td>
<td>3.466*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at 1%

To have used results based on Tullgren-extracted females, as in Table 3, would have led to gross errors. Using estimates based on hand-caught females lessens this error.

The observed densities in Table 4 are very high. In the healthy population the average density for
Fig. 6. Observed (boxes) and predicted (bars) age pyramids for the Christchurch (diseased) and Dunedin (disease-free) populations of *Orchestia hurleyi*. Predictions based on Leslie matrix techniques.

100 cores was equivalent to 1677 animals per m²; for the diseased population it was 992 per m² for 90 cores. The highest density found in any one core was equivalent to 9971 per m² for the healthy population and 3104 per m² for the diseased population. The average densities predicted by the computer model were reasonably close to the observed densities. For the healthy population the prediction was 1861 individuals per m² (observed 1677), and for the diseased population it was 795 per m² (observed 992).

For the diseased population, age structure predicted by the model (Fig. 6) was very close to the observed structure, in that the age pyramid was severely truncated. For the healthy population the agreement was not as good, especially for instars 11, 12, and 13 (equivalent to stages 4, 5, and 6 in the life table; see Table 4).

Table 5 gives the size frequency distribution of the healthy and diseased animals in a sample from 11m collected by hand (so that diseased animals could be recognised). Only larger animals are represented; there is a sampling bias against the smaller animals, which are more difficult to see and catch. Even so, the results clearly demonstrate that the disease mainly affects larger animals. The frequency distribution of healthy animals was normal. The mean age was 13.847 instars (standard error 0.421 instars), and the distribution was not significantly kurtotic or skewed. The frequency distribution of diseased animals, on the other hand, showed a much higher mean age (17.2 instars; S.E. 0.320 instars), negative skew, and positive kurtosis. The two distributions in Table 5 have a significantly different location at 1% (Mann-Whitney U test – U = 1679.5, \(U_c = 1362\) at 1%; Sokal & Rohlf 1969), and the proportion of diseased animals in each instar increases with age.

DISCUSSION AND CONCLUSIONS
The rapid spread of the disease (Fig. 1) may be assisted or caused by human agency. The habit of some gardeners of taking seedlings and shrubs from native forest remnants for transplanting in gardens would disperse the soil and litter animals taken accidentally together with the plants. Probably this is the way that terrestrial amphipods were accidentally introduced into Europe and North America (Chevreux 1896, Calman 1912, Bousfield & Carlton 1967, Richardson 1980).
The commonness of the disease in urban areas and its absence from the bush remnants flanking the Canterbury Plains (where density of human settlement is low) gives support to the idea that human activities were important in spreading the disease. But confirmation of the disease organism as a strain of the extremely common aerobic soil bacterium *Bacillus subtilis* poses a number of problems. If it is so common, why is this particular strain so virulent? Why are the amphipod's defence mechanisms ineffective, especially since they are activated by the disease? And how is the disease organism transmitted?

*B. subtilis* has been recorded as a disease-causing organism in certain insects (Fekl 1956, Gilliam & Valentine 1976), but its infectivity is low, and most organisms have such effective defences against it that it is generally considered to be non-pathogenic (Parish 1972).

In *Orchestia hurleyi* the bacterium probably gains entrance into the haemocoel when the animal is wounded. The bacterium is common on the surface of the animal, where it possibly forms part of the normal epiphrora. The mechanisms used by *Bacillus thuringiensis* to enter the haemocoel of susceptible insects (Dutky 1963, Salt 1970) is unlikely to be used here because of the absence of parasporal bodies in *B. subtilis*.

If the 'wound entry' route hypothesis is correct, then at least 30% of the adult population must have suffered wounding during their life. Such wounding could come about either by predator attack or during intraspecific conflict. It is possible for these amphipods to escape from a predator, even though firmly grasped (and wounded), because their escape mechanism of abdominal reflexure is particularly powerful.

Wounding may also occur during intraspecific conflicts. Large individuals have been observed to repel others attempting to occupy the same refuge. They do this by lying on their side and 'kicking' with their abdomen (abdominal reflexure). The large spines on the telson and uropods may be strong enough to damage the softer parts of the attacked amphipod. Amphipods are particularly vulnerable during and immediately after ecdisis, when the cuticle is soft. The only defence mounting animals have against attackers is a sideways shuffling movement while lying on their side; this has been sufficient to effect escape from a predator in two instances that I have observed.

Once in the haemocoel, the bacterium multiplies in spite of activation of the host's defence mechanisms. The pathogen becomes so numerous that the haemolymph appears translucent white, and the animal becomes weak. Possibly there are no direct ill effects on the host, such as the bacteria attacking host tissues or the host reacting unfavourably to the end products of bacterial metabolism. Instead, the host may become weaker merely because the bacteria pre-empt nutrients and oxygen for their own use. Also, circulation of haemolymph may be impaired by increased viscosity, decreased haemolymph pressure, and increased circulation time caused by the presence of so many bacterial cells. Thus, the host tissues are deprived of nutrients and oxygen, and the animal becomes so weak that it is unable to breed. Nor can it escape from predators as effectively as can healthy individuals. It cannot jump at all, and when heavily diseased it can move only slowly, and then only for short distances. Thus, it is much more prone to capture. Not all diseased animals ultimately fall victim to predators, however; a few cadavers have been found that appear to have died from general wasting.

Once the host dies and decays, bacterial spores and vegetative cells would be liberated into the soil, thus completing the life cycle. It seems likely that, in addition to infesting *O. hurleyi*, this strain occupies the normal niche for *B. subtilis* in the soil community. If it is a facultative pathogen, then its population density may be independent of the amphipod's.

The effect of this highly infectious, rapidly spreading disease on the amphipod is of interest in view of the host's importance in the litter community. It is tempting to ascribe the decline in population density and the truncation in population structure, which I observed as the disease spread southwards, to the influence of the disease. Although this sounds logical enough, in the absence of satisfactory confirmation it would be dangerous to make such a conclusion. Population densities and age structures are subject to many influences, any one of which could produce the effects described.

### Table 6. Effect of increased mortality load on a theoretical adult population of *Orchestia hurleyi*

<table>
<thead>
<tr>
<th>Instar</th>
<th>Population ('disease-free') with 25% mortality per instar</th>
<th>Population ('diseased') with 25 ± 30% mortality per instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>16</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>17</td>
<td>56</td>
<td>25</td>
</tr>
<tr>
<td>18</td>
<td>42</td>
<td>12</td>
</tr>
<tr>
<td>19</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>23</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>
The most satisfactory confirmation involves widespread experimentation, but this was not possible in the present study. Instead, the validity of the assumptions made were checked using computer modelling. The life table analysis (Table 4) tests whether or not the known effects of the disease could account for the observed differences in population density and structure between the healthy and diseased populations. If the predictions of a realistic mathematical model are in reasonable harmony with the observed demographic effects, then the disease could be held to have the potential to cause the effects.

The model predicts the respective age structures and densities surprisingly well, considering the relative paucity of the data. We can conclude that the effects of the disease are of sufficient magnitude to have caused the observed decline in density and the truncation of population structure in the diseased population.

This conclusion may be independently checked by assuming that the estimate of a 30% incidence of the disease in adults is correct, and that the diseased animals die in the same instar as that in which they contracted the disease. The effect of this additional mortality load on the population structure is shown in Table 6. We start off with 100 young adults in each of two populations, one of which (the diseased) has an additional 30% loading on the usual 25% mortality. Inspection of the table shows that the additional mortality does truncate the population's age structure to approximately that seen in the diseased field population. Yet the survivorship values given in the third column of Table 4 show that the additional 30% (or greater) mortality due to disease could occur only in larger adults. This is confirmed by the size distribution of diseased animals shown in Table 5. Young adults have very low mortality rates, whereas older adults have mortality rates which increase with age, even in the healthy population. The disease affects older adults much more than the young adults (Table 5), so, in spite of the high adult mortality due to the disease, egg production in the diseased population is maintained at a reasonably high level, though only about half that of the healthy population.

This difference in mortality between the younger and older adults may reflect a size-related difference in the predator spectrum. Hedgehogs and birds may be preying on the larger adults only, and these homeothermic vertebrate predators may be more efficient at catching amphipods than are the invertebrate predators—such as terrestrial flatworms—that prey on the smaller amphipods.

An interesting result from this study was the low haemocyte counts obtained for *O. hurleyi*—an order of magnitude less than counts reported for other crustaceans by Yeager & Tauber (1935). These results are not an artefact of method. When the same method was used on the supralittoral amphipod *O. chilensis* and the intertidal crab *Hemigrapsus edwardsi*, values comparable to those reported in the literature were obtained. Presumably, low numbers of circulating haemocytes are a phenomenon associated with the fully terrestrial habit of *O. hurleyi*. The significance of this has yet to be investigated.

ACKNOWLEDGMENTS

I am very grateful to Dr A. Cole for permission to use the microbiology facilities of the Botany Department, University of Canterbury, and for his encouragement, advice, and criticism. I thank the staffs of Colston House and Institut Pasteur, Laboratoire de Lutte Biologique, Centre d'Identification de Bacillus thuringiensis, for identifying the disease-causing organism; and Rob Stephenson for criticizing the manuscript.

REFERENCES


Chapter 5. ACTIVITY

INTRODUCTION

Supralittoral talitrids are largely nocturnal animals whose night-time activity pattern may be modified by tidal cycles and other environmental factors (Ruppell, 1967; Wildish, 1970; Bregazzi, 1972; Bregazzi and Naylor, 1972; Jaramillo et al., 1980; Williams, 1980 a,b,c). Terrestrial landhoppers are also nocturnal, and since activity is an important feature of their biology, it was thought worth-while to investigate their daily activity pattern and the factors responsible.

METHODS

Pattern of activity in the laboratory

A rocker-type actigraph was used to investigate the locomotory activity patterns of Makawe hurleyi throughout a day-night cycle. An animal was enclosed within a balanced, perforated tube (Figure 5.1). Each time the animal moved to one end it rocked the tube slightly which set off a micro-switch and activated a circuit to a relay which marked a kymograph. The rocker was contained inside a large battery jar with the atmosphere inside the jar maintained at the highest humidity possible by draping the sides with damp cheese cloth.
FIGURE 5.1. Rocker-type actigraph used to investigate the locomotory activity of *Makawe hurleyi* in the laboratory. The terminals lead to an electronic relay and marker unit (not shown).

FIGURE 5.2. Typical results for *Makawe hurleyi* from the actigraph illustrated in Figure 5.1. Activity is measured as the number of times the relay was activated per hour. A bimodal pattern is evident.
Laboratory experiments are open to the criticism that they are artificial; the animal is removed from its natural environment and is subjected to unnatural influences. Hence the results obtained from experiments in artificial conditions may be themselves artificial, and may not show the natural behaviour of the animal. To check on this, pitfall trapping in the field was used to monitor the emergence activity of a natural population of Makawe hurleyi.

Description of study sites

The study was conducted on two sites on Mr B. Parkinson's land at the head of Kaituna Valley, which is a southward facing valley on the south side of Mt. Herbert, Banks Peninsula. One site was forested with regenerating subcanopy trees and shrubs such as tree ferns, mahoe, broadleaf, five-finger, wineberry, milk tree, fuchsia, and marble leaf. A number of lianes were present, and the soil was an upland yellow brown with a rich organic A-level overlaid by a moderately thick litter. The litter surface was of dry Schefflera leaves and fern fronds. Few understorey shrubs and herbs were present. There were some areas which were completely covered in ground ferns. This site had been milled in the last decades of last century, and judging by the tree ferns, a slip had occurred some time ago in the upper reaches of the bush above the region sampled. The 10 m high canopy was thick and continuous even though no
pre-milling 'climax' species were present. The fencing around the bush was in good condition, but was old. Probably stock had grazed the vegetation at times in the past thus reducing the lower storey of vegetation. Regeneration of sub-canopy species was moderately good although there were signs of moderate to heavy opossum (Trichosurus vulpecula) damage. The area was maintained in bush by Mr Parkinson to prevent slips blocking the road below and the fields bordering the stream in the valley floor.

The second site was on the valley floor on the east side of the stream in a field of long grass and isolated fuchsia or mahoe trees. The field was grazed by sheep and cattle. There was no regeneration of trees or shrubs in this area. The soil and litter under the trees was trampled and thin due to the activities of the stock.

Both sites were in the shade of Mt Herbert for much of the day, and since precipitation in the area is high, the soil-litter conditions were damp and cool thus favouring the development of a rich cryptozoic fauna. Table 5.1 gives a more detailed description of each pitfall site.

Equipment

Pitfall traps were constructed from the bottom halves of 2 l capacity plastic ice-cream containers which had a number of small holes drilled in the base for drainage. They were placed in the ground so that their lip was level with the H-horizon of the soil.
Table 5.1. Pitfall site details

<table>
<thead>
<tr>
<th>Site</th>
<th>Canopy</th>
<th>Nearby shrub</th>
<th>Soil</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IN</td>
<td>FOREST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Mahoe</td>
<td>Astelia nervosa</td>
<td>Organic</td>
<td>Fuchsia &amp; Pittosporum</td>
<td></td>
</tr>
<tr>
<td>2 Mahoe</td>
<td>Prince of Wales fern &amp; Pseudopanax seedling</td>
<td>Organic</td>
<td>Open understory, ground layer continuous continuous</td>
<td></td>
</tr>
<tr>
<td>3 Marble-leaf</td>
<td>Coprosma</td>
<td>Stoney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Mahoe</td>
<td>Ferns</td>
<td>Large stones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Mahoe</td>
<td>Pseudopanax &amp; Coprosma seedlings Tree ferns</td>
<td>Organic</td>
<td>Bush lawyer, fern overhang trap</td>
<td></td>
</tr>
<tr>
<td>6 Mahoe</td>
<td>Coprosma</td>
<td>Organic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Kanuka/Fuchsia/Schefflera</td>
<td>Coprosma</td>
<td>Organic</td>
<td>On steep bank</td>
<td></td>
</tr>
<tr>
<td>8 Mahoe/kanuka</td>
<td>Ferns</td>
<td>Organic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Nothopanax/mahoe</td>
<td>Coprosma</td>
<td>Organic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 Fuchsia</td>
<td>Dichondra, grass</td>
<td>Organic</td>
<td>Under old trees</td>
<td></td>
</tr>
<tr>
<td>11 Pseudopanax</td>
<td>Muhlenbeckia</td>
<td>Organic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Bracken</td>
<td>California thistle</td>
<td>Organic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 Fuchsia</td>
<td>Thistle/nettle</td>
<td>Large rocks Sheep excluded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 Pasture grass</td>
<td>Organic</td>
<td>Bidibidi abundant</td>
<td>Schefflera tree nearby</td>
<td></td>
</tr>
</tbody>
</table>

Nine were set out in the bush, and five in the grazed field. They were inspected at 3-hourly intervals and the animals captured were released. Lights were used as little as possible; string guide lines were set up as guides to the traps at night.
Light intensity was measured by an LDR light meter. Humidity was measured with a Zeal hygrometer, a Lovibond humidity test kit, and a Duratherm hygrometer. The latter was found to be unreliable. A continuously recording thermohygrograph was also used to monitor humidity and temperature. Dew was measured by placing a glass Petri dish of known weight in open sites during dry periods. The amount of dew was determined by weighting on a 'Dial-o-gram' balance.

'Surface wetness' was measured by placing a filter paper of known weight on the surface of the ground and covering with a Petri dish. After three hours exposure the paper was removed and weight to determine the amount of water it had absorbed. Other climatic features were measured using the usual meteorological equipment.

At the end of the period, core samples were taken in the vicinity of each trap and extracted in a Tullgren apparatus for three days.

Factors affecting activity

Pitfall trapping was carried out in the Zoology garden at Dunedin (for description of site see chapter on ecology in this volume) using two kinds of traps:

(1) A pitfall trap was constructed from two plastic pipes of about 100 mm diameter and 200 mm length. One of the tubes (the liner) fitted neatly inside the other. A stainless steel gauze platform was glued into place 1/3 up the inner tube. The pitfall
was placed with its lip level with the A horizon of the ground. In use, the liner tube, which captured the animals, could be slipped out of the outer tube without disturbing the soil. Drainage was provided by the gauze platform, thus the captured animals were not drowned as in many other simpler designs. These traps caught any animals moving on or through the litter.

(2) 'Climbing' traps were simple glass 500ml preserving jars placed under the grass canopy on the surface of the litter. Any animals falling off the canopy immediately above the jar would be trapped. Damp, crumpled paper was put in the bottom to give trapped animals some protection.

Ten traps of both kinds were set out. The traps were inspected daily and the animals they contained released.

Climatic conditions measured were: grass minimum temperature, night air maximum temperature, litter minimum temperature, litter maximum temperature, litter median temperature, soil temperature (10 cm depth), maximum relative humidity, mean relative humidity (night), rainfall, and dew. Dew was measured by exposing a glass Petri dish of known weight and re-weighing at dawn. These climate factors were regressed with the number caught each night (the dependent variable, y) using a stepwise regression analysis (IBM Scientific Subroutines Package) which used a step-up procedure. The regression equations tested were the linear
$$y = a + b \cdot X_1 + c \cdot X_2 + \ldots + z \cdot X_n$$

where $a$ is the regression constant, $b, c, d, \ldots, z$ are the regression coefficients and $X_1, X_2, \ldots, X_n$ are the independent variables. and the log-linear relationship

$$\log(y) = a + b(\text{temperature}) + c \cdot \log(X_1) + \ldots + z \cdot \log(X_n)$$

In this latter regression all variables other than temperature were transformed by taking logs (base 10). Since the relationship of metabolic rate with temperature is more or less exponential (see chapter on respiration in this volume) then activity may also be exponentially related to temperature. In some analyses interaction terms between temperature and humidity were included by including 'derived' interaction variables obtained by multiplying the temperature and humidity variables together. Thus:

$$\log(y) = a + b(\text{temperature}) + c \cdot \log(X_1) + \ldots + a_a(\text{temperature} \cdot \text{humidity}).$$

The experiment was carried out for 60 days during spring starting on August 4.

24-hour VPD profiles

To obtain field profiles of the drying power of the air — as measured by vapour pressure deficits of water (VPD) — at different points above the ground, slips of cobalt thiocyanate paper were
mounted in stainless steel gauze envelopes fastened 2.5 cm apart on a stick fixed vertically in a sward of grass, the canopy of which was 25 cm above the ground. At 2-hour intervals the slips were removed and the humidity determined using a Lovibond humidity test kit. The air temperatures in the shade at each point were also measured. Thus the vapour pressure deficit profile from ground level to above the canopy was determined for a 24-hour cycle during which time no rain fell.
FIGURE 5.3. A composite graph showing activity of Makawe hurleyi in the field as measured by pitfall trapping (lower graph) and the following climatic variables: w1, surface wetness at the field site; w2, surface wetness at the forest site; dew; rainfall (field site); air and litter temperatures. Night and day times are also shown. A bimodal pattern of activity is evident.
RESULTS

Laboratory activity

Fifteen animals were tested in the laboratory actigraph. Two showed a unimodal locomotory activity pattern, while all the rest showed a bimodal locomotory activity pattern with a large dusk and dawn peak, a mid-night minimum, and a total cessation of activity during daylight hours. Figure 5.2 shows the typical results for a single animal over a 24-hour period.

FIGURE 5.4. A. Time course over 24 hours of vapour pressure deficits (VPD) at different heights above the ground in a grass sward.

B. Vapour pressure deficit profiles (VPD) with height above ground at 0115 hours (NZST) and 1315 hours (NZST).
Table 5.2. Meteorological readings from the station in the grass field and in the forest.

<table>
<thead>
<tr>
<th>TIME (NZMS)</th>
<th>DRY</th>
<th>WET</th>
<th>WIND SPEED</th>
<th>RAINFALL (mm)</th>
<th>DEW (g/m²)</th>
<th>SURFACE WETNESS (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500</td>
<td>6.8</td>
<td>6.8</td>
<td>0</td>
<td>trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Overcast, light mist, damp)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1800</td>
<td>4.5</td>
<td>4.5</td>
<td>0</td>
<td>0</td>
<td>0.38</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(no rain, 10/10 cloud, clearing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2100</td>
<td>4.2</td>
<td>4.2</td>
<td>slight 0</td>
<td>0</td>
<td>0.13</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(rain started at 0150)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2400</td>
<td>4.2</td>
<td>4.2</td>
<td>0</td>
<td>0.9</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(rain persistent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0300</td>
<td>4.0</td>
<td>4.0</td>
<td>0</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(rain lessening)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0600</td>
<td>3.9</td>
<td>3.9</td>
<td>0</td>
<td>0.2</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(misty, 10/10 cloud)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0900</td>
<td>4.6</td>
<td>4.6</td>
<td>0</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>5.9</td>
<td>5.9</td>
<td>0</td>
<td>0.9</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>5.2</td>
<td>5.2</td>
<td>0</td>
<td>0.7</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>1800</td>
<td>4.2</td>
<td>4.2</td>
<td>0</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(clearing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2100</td>
<td>4.8</td>
<td>4.8</td>
<td>0</td>
<td>0.6</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>2400</td>
<td>4.4</td>
<td>4.4</td>
<td>0</td>
<td>0</td>
<td>1.02</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(no rain, light cloud, dew)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0300</td>
<td>4.1</td>
<td>4.1</td>
<td>0</td>
<td>0</td>
<td>1.78</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(no rain, light overcast, moonlit, exposed rocks dry)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0600</td>
<td>4.8</td>
<td>4.8</td>
<td>slight 0</td>
<td>2.42</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(ground drying)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0900</td>
<td>5.9</td>
<td>5.9</td>
<td>slight 0</td>
<td>4.58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.2 continued

<table>
<thead>
<tr>
<th>TIME</th>
<th>HUMIDITY (%)</th>
<th>LITTER TEMP. (°C)</th>
<th>WIND SPEED</th>
<th>SURFACE WETNESS (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1800</td>
<td>100</td>
<td>6.5</td>
<td>0</td>
<td>0.14</td>
</tr>
<tr>
<td>2100</td>
<td>100</td>
<td>5.0</td>
<td>0</td>
<td>0.10</td>
</tr>
<tr>
<td>2400</td>
<td>100</td>
<td>5.0</td>
<td>0</td>
<td>0.13</td>
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<td>0300</td>
<td>100</td>
<td>5.0</td>
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<td>0.13</td>
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<tr>
<td>0600</td>
<td>100</td>
<td>4.9</td>
<td>0</td>
<td>0.75</td>
</tr>
<tr>
<td>0900</td>
<td>100</td>
<td>5.0</td>
<td>0</td>
<td>1.27</td>
</tr>
<tr>
<td>1200</td>
<td>100</td>
<td>5.1</td>
<td>0</td>
<td>0.95</td>
</tr>
<tr>
<td>1500</td>
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<td>0.54</td>
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<tr>
<td>1800</td>
<td>100</td>
<td>5.1</td>
<td>0</td>
<td>0.36</td>
</tr>
<tr>
<td>2100</td>
<td>100</td>
<td>5.0</td>
<td>0</td>
<td>0.26</td>
</tr>
<tr>
<td>2400</td>
<td>100</td>
<td>5.1</td>
<td>0</td>
<td>0.22</td>
</tr>
<tr>
<td>0300</td>
<td>100</td>
<td>4.5</td>
<td>slight 0.34</td>
<td></td>
</tr>
<tr>
<td>0600</td>
<td>100</td>
<td>4.8</td>
<td>slight 0.09</td>
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<tr>
<td>0900</td>
<td>100</td>
<td>5.2</td>
<td>0</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Activity in the field

The meteorological conditions for the two meteorological stations at the Kaituna Valley site are given in Table 5.2 and Figure 5.3. Over the 48 hours of the study the general conditions were damp, cold, and calm, with persistent rain falling for much of the period. When rain was not falling dew formed so the ground surface was continually wet. The temperature-buffering effect of the canopy and litter can be seen in the figure as evidenced by the far more constant temperature in litter compared with air. The surface of the open field was consistently wetter than the forest floor because the canopy of the forest intercepted much rainfall, and dew formed on the canopy rather than the litter surface.

In spite of the low temperatures landhoppers were very active (Table 5.3). They were about the only group of poikilotherms to be active, since the only other animals seen or captured were 3 lepidopteran larvae, 1 millipede, 2 carab beetles, 2 Thysanura, 16 spiders, and 3 dipteran larvae. This compares with the 69 landhoppers caught.

The number of animals caught varied from trap to trap. The 9 traps in the forest caught an average of 5.56 animals per trap with a standard deviation of 2.19, while the 4 in the grazed field (excluding site 13 which was in an ungrazed place) caught an average of 1.75 animals per trap.
Table 5.3. The number of Makawe *hurleyi* caught in pitfall traps at each site and sampling occasion.

<table>
<thead>
<tr>
<th>TIME (NZMS)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<td></td>
</tr>
<tr>
<td>1800</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2100</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2400</td>
<td></td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
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<td>1</td>
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<td></td>
</tr>
<tr>
<td>0600</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1200</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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The pattern of activity shown by the pooled results in Figure 5.3 is for a dusk peak, a slight midnight lull, then a large dawn peak. This pattern was repeated on both nights of the study and is very similar to the pattern of respiratory uptake of oxygen reported in the respiration section of the present work. There was a little daytime activity during the wet morning of the second day, but the animals were predominantly nocturnal.

The dawn peak seen in the laboratory studies is, therefore, natural and not an artifact of method or laboratory conditions.
The Tullgren extractions of cores from the forest yielded an average of 99 ± 28.9 amphipods per m$^{-2}$, whereas in the grass field the density was 12.7 ± 4.0 amphipods per m$^{-2}$ which indicates that there are nearly 8 times the density of landhoppers in the forest than in the grass field. And in the forest they are distributed reasonably evenly, whereas in the grass field they tend to be clumped under logs, and down the side of rocks, protected from trampling by stock.

**Day-to-day activity**

The stepwise regression analysis of number of amphipods caught in pitfall traps on environmental factors is presented in Table 5.4. This analysis shows that the most appropriate model is the log-linear without interactions. Of those tested, the most important environmental factors determining number of animals trapped are litter median temperature and maximum relative humidity. Rainfall and dew are relatively unimportant. The equation which gives the best fit is:

$$\log(X_{12}) = 0.048 \times X_5 + 2.422 \times \log(X_7) - 4.36 \pm 0.182$$

where $X_{12}$ is the number of amphipods caught in pitfall traps, $X_5$ is litter median temperature, and $X_7$ is maximum relative humidity. However, many other factors not investigated here must be responsible since the percentage explained by regression of the total mean square is only 46.8%. Suprisingly, the interaction
variable does not contribute significantly to the reduction of MS. It was hoped that this interaction variable would express the vapour pressure deficit, which had been found in the chapter on water relations to be so important for determining the rate of transpiration loss. But, presumably, the direct effect of temperature on activity is far more important than its indirect effect on determining vapour pressure deficit. Thus relative humidity in these circumstances is an appropriate measure without the need for a more direct measure of the drying power of the air.
Table 5.4. Stepwise regression analysis of number of amphipods caught in pitfall traps.

1. Variables

<table>
<thead>
<tr>
<th>Number</th>
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<tr>
<td>1</td>
<td>Grass min. temp.</td>
</tr>
<tr>
<td>2</td>
<td>Air max. temp.</td>
</tr>
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<td>3</td>
<td>Litter min. temp.</td>
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<td>Litter max. temp.</td>
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<td>5</td>
<td>Litter median temp.</td>
</tr>
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<td>6</td>
<td>Soil temp.</td>
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<td>7</td>
<td>Max. rel. humidity</td>
</tr>
<tr>
<td>8</td>
<td>Mean temp.</td>
</tr>
<tr>
<td>9</td>
<td>Mean rel. humidity</td>
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<td>Rainfall</td>
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<td>11</td>
<td>Dew</td>
</tr>
<tr>
<td>12</td>
<td>Number of animals caught (dependent var.)</td>
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<td>19</td>
<td>Interaction (derived variable, see text)</td>
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2. Analyses
   a. Linear regression

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<tr>
<td>Percentage reduced</td>
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<td>4.6</td>
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<td>Multiple corr. coef. (adj. for deg. freedom)</td>
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<td>0.49</td>
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<tr>
<td>F (Variance ratio)</td>
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<td>4.35</td>
<td>3.52</td>
<td>3.00</td>
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   b. Log-linear

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<tr>
<td>F</td>
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Proportion 'explained' = 0.46

   c. Log-linear with interaction

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<tbody>
<tr>
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<td>0.53</td>
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<tr>
<td>F</td>
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<td>4.27</td>
<td>3.53</td>
<td>3.10</td>
<td>2.80</td>
<td>2.73</td>
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</table>
Table 5.5. Stepwise regression analysis of number of amphipods caught in 'climbing' traps. See Table 6.4 for meaning of variables and symbols.

<table>
<thead>
<tr>
<th>Variable</th>
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<th>8</th>
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<th>10</th>
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<tbody>
<tr>
<td>% reduced</td>
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<tr>
<td>F</td>
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<td>2.85</td>
<td>2.59</td>
<td>2.99</td>
<td>2.40</td>
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</table>

The number of amphipods of both species caught per pitfall trap per night was: *Talorchestia patersoni* 1.175, *Makawe hurleyi* 1.027.

The stepwise regression of the number of *Makawe hurleyi* out of the grass canopy into the 'climbing' traps on environmental conditions is shown in Table 5.5. While the regression is still significant, there is a high error term. The most important factor was mean relative humidity, followed by grass minimum temperature, then mean temperature, and so on as shown in the table. These factors are not the same as those that determine field locomotor activity. *Makawe hurleyi* will climb when the humidity is high and the temperature in the grass is high. It emerges and locomotes, however, when the litter temperature is high. The two temperatures, litter and grass minimum, are obviously related, but the correlation coefficient between them is only 0.66 showing that, at times, one can be high and the other low, and vice versa. For example, on clear, still nights with no cloud, following a warm day, the litter temperature can stay high while the grass canopy temperature can fall quite low due to radiant energy losses to the clear sky. In
such conditions amphipods will be active in and on the litter, but will not climb very much.

In the work referred to in the ecology section, climbing traps were left out for nearly a full year and inspected daily. Meteorological conditions were not measured except to record whether or not rain or dew fell. This work showed that climbing is more common during wet weather especially when field saturation capacity is exceeded.

The 24 hour vapour pressure deficit profiles are shown in Figure 5.4. There is a considerable increase in the drying power of the air with height above ground. At or close to the ground the vapour pressure deficit is maintained at a low value because of evaporation from the soil and transpiration from ground plants. In addition, the canopy shades the litter and subcanopy beneath it from the direct heating effect of the sun, and also gives protection from drying winds.

There is a marked change from day to night in these profiles. Even at the ground surface there is still some drying in the daytime, whereas at night there is a marked general decline in the drying power at all heights of the profile.
DISCUSSION

Landhoppers are nocturnal although there may be a little daytime activity when the vapour pressure deficit is low. The usual pattern of locomotor activity is bimodal with dusk and dawn peaks. *Makawe hurleyi* is active at low temperatures and has been seen walking over frozen vegetation during $3^\circ$C frosts. As has been shown in the chapter on respiration, this species has a marked seasonal acclimatisation which must enable it to maintain its activity at low temperatures. Food is not a problem for landhoppers in winter even though all bar two native woody plant species in New Zealand are evergreen and leaf fall in most species occurs either semi-continuously throughout the year or in spring rather than in autumn as in northern hemisphere temperate forests. Food is available in winter because, although there is no great autumn leaf fall, breakdown is slower at the lower temperatures of winter and, therefore, more litter accumulates.

There are more amphipods active on warm, humid nights than on cold and/or dry nights. The most important factors determining activity are litter temperature and humidity. The relationship between emergence activity, as measured by daily pitfall trapping, and temperature is exponential, not linear. This follows the same relationship as the metabolism temperature curve discussed in the section on respiration. *T. patersoni* are evidently more prone to being captured in the pitfall traps than are *M. hurleyi* since a higher number were caught per trap even though their density at this
site is much lower than *M. hurleyi* (see ecology section). As shown by the maze experiments discussed in the section on water and osmotic relations, *M. hurleyi* can detect edges and can distinguish between 'down' and 'along', which *T. patersoni* cannot do. Thus *M. hurleyi* is better able to avoid pitfall traps.

In the ecology section it was shown that more *M. hurleyi* would climb into the canopy during wet weather. Climbing is a normal activity for *M. hurleyi* which enables individuals to find and exploit dead plant material before it falls to the ground. Thus this species has an advantage over those species which do not climb. Some individuals of tree climbing species do not return to the litter during the daytime but remain up in the trees sheltering under bark or in the frass beneath epiphytes or in the crotches of branches. They may enrich these aerial refugia by depositing faeces and urine. Epiphytes and trees may take advantage of this enrichment because Nadkarni (1981) reported that canopy roots of rainforest trees exploited the nutrient resource in aerial frass. I have observed canopy roots in *Fuchsia exorticata* growing in the crotches of branches many metres above the ground. Climbing activity is not directly related to general activity, however, since the environmental conditions which favour one kind of activity are not the same as those which favour the other. At times general activity can be high and climbing activity low, and vice versa depending on the values of the specific environmental conditions determining each kind of activity.
The survival value of nocturnal activity in talitrids has been discussed by Jaramillo et al (1980) who point out that nocturnal activity does reduce predation from birds which, as a group, use sight rather than the other senses. Nocturnalism also means that landhoppers are active during a time, when the drying power of the air is regularly at a minimum (Figure 5.4). Thus they minimize the risk of death due to desiccation.
Chapter 6. OTHER ASPECTS OF LANDHOPPER BIOLOGY

In this chapter are reported a variety of topics dealing with somewhat unrelated aspects of landhopper biology including: the control of the breeding season, ecdysis and copulation, lethal temperatures, integumental structure and function in the defence against micro-organisms, landhopper habitats, and adaptation to terrestrial life.

6.1 THE BREEDING SEASON AND ITS CONTROL

IN Makawe hurleyi

In the chapter on ecology it was observed that Makawe hurleyi began breeding in spring at a certain daylength, and ceased breeding in autumn at the same daylength. Thus the breeding season is symmetrical about the shortest day unlike the mean environmental temperature cycle which, because of thermal lag effects is asymmetrical about the shortest day with the early part of winter being warmer than the latter part. Breeding in the Dunedin population of M.hurleyi begins on or shortly after August 4 when the mean environmental temperatures are still low but daylength has reached or exceeded a critical value, the same value at which breeding ceased in autumn.
This suggests that photoperiod, and not temperature, is the factor controlling both the onset and the termination of breeding. In *M. hurleyi* cessation of breeding is marked by a particular moult in which the gills are increased in area and the oostegites lose their long terminal brood spines, while the initiation of breeding is associated with a moult in which the above features are reversed.

Photoperiod is known to play an important part in the timing of the onset of breeding (or nonbreeding) seasons in decapod Crustacea (Paris and Jenner, 1952; G.J. Stephens, 1952; G.C. Stephens, 1955; Lowe, 1961; Aitken, 1969; Perryman, 1969; Rice and Armitage, 1974), and in isopods (Paris and Pitelka, 1962; Weiser, 1963; McQueen and Steel, 1980; Madhavan and Shribbs, 1981), but there have been no previous studies reported on terrestrial amphipods.

**BREEDING SEASON – METHODS**

The effects of daylength and temperature were tested by a simple factorial experiment in which about 400 *Makawe hurleyi* were cultured in 4 large culture boxes (about 100 in each). One box was exposed to one of following conditions for a period of 60 days:

- Box A, 6 hours daylength, 15°C
- Box B, 16 hours daylength, 15°C
- Box C, 6 hours daylength, 5°C
- Box D, 16 hours daylength, 5°C

The experiment was run at both Otago and Canterbury Universities in controlled environment chambers. At Otago the experiment started on
23 June using animals collected locally from suburban gardens. At Canterbury the animals were collected from a field of long grass on campus and the experiment was carried out twice, one beginning on 18 November and one beginning on 5 May. The animals were fed when necessary on steam sterilised leaf litter and straw.

**BREEDING SEASON - RESULTS**

Survival of the animals in the experiment carried out at Otago was good; over 90% were able to be recovered at the end of the experiment. At Canterbury whitey disease caused the death of over 40% of the animals in both experiments.

In all trials the animals exposed to short day conditions with or without warmth, did not breed. The adult females in short day conditions had oostegites of the winter type indicating that the winter moult had occurred. In contrast, the animals exposed to long day conditions breed irrespective of temperature. In cold, long day conditions, however, only between 29% and 35% of the females carried eggs, and the broods were small (an average of 1.8 per brooding female) with many infertile and even fungus attacked eggs present. In the warm, long day conditions breeding was normal with about 65% of the females carrying eggs.
BREEDING SEASON - DISCUSSION

The onset and cessation of brooding seems to be controlled by photoperiod in *Makawe hurleyi* and not by temperature. The animal uses a more reliable "date-giver" than temperature because, in New Zealand, temperatures over a day or a period of days within a season can be quite atypical of the average for that season. As an illustration, it has snowed on Christmas Day at Clinton while June at Christchurch can have many days with temperatures well over 18°C. A species using temperature as its date giving clue for the onset or cessation of breeding is likely to be very confused in the overall mild but very variable climate of New Zealand. Many species of adventive plants which use temperature as their environmental date-giving clue show this confusion in some years by breaking into flower during the mild and prolonged autumns which are reasonably frequent in Christchurch. These indian summers or 'false springs' as they are called locally are marked by the flowering of spring bulbs, blossom on fruit and flowering deciduous trees, and the bursting of new buds. Presumably, these organisms have adapted to a native environment which has more reliable seasonal temperatures than does New Zealand. Landhoppers, however, have adapted to local conditions and are not confused by these common seasonal perturbations.

The cessation of breeding during the cold winter season is advantageous in preventing unsuccessful brooding. From the experiments in the cold temperature-long day conditions it appears
that at low temperatures the number of eggs laid is greatly diminished and brooded eggs are much more likely to be infertile or diseased. Attack by micro-organisms on eggs in the brood presents obvious dangers to the mother. These risks and the wasteful expenditure by the mother of energy, matter and time on relatively unsuccessful reproduction are avoided by the cessation of breeding the during winter season.

Madhavan and Shribbs (1981) showed for Armadillium vulgare that both photoperiod and temperature were important in the timing of the reproductive season: 90% of females exposed to low temperatures for two months underwent ovigerous moulting when returned to high temperatures and short photoperiod. This possibility has not been investigated in M. hurleyi.
Moulting, or ecdysis, is not easily observed in landhoppers. Their habit of moulting in secretive places at night makes it almost impossible to observe them in the field. I have attempted to do so many times using a red light, but they appear to be able to detect red light and so even when I was able to observe the rare occurrence of an animal undergoing ecdysis in the open it was able to detect my presence and scuttle away. However, ecdysis was observed on three separate occasions in laboratory reared specimens of Parorchestia ihurawao, one of the most easily cultured of the landhopper species.

Individuals in stage D of the moult cycle, as defined by Drach (1944) and Charniaux-Cotton (1952), could be detected by their dark-brown body colour. Early events in the ecdysis of P.ihurawao are virtually identical to those described for other amphipods by Le Roux (1957) but are repeated here for completeness. Ecdysis begins with the animal lying on its side. Splits develop transversely between the first and second thoracic segments and laterally down the sides of the abdomen and posterior part of the thorax when the animal vigorously flexes its body. Continued violent flexing removes the exuviae from the posterior part of the body. This removal is assisted by the animal bringing its uropods into contact with the ventral parts of the thorax whereupon the dorsal and terminal spines on the uropods, especially those of uropod 1, grip the exuviae and violently rip it off as the abdomen is suddenly
reflexed. When a substantial gap has been made between the old and new ventral cuticles, the abdomen is passed into the gap and complete the removal of the ventral exuviae by violent abdominal reflexure. Once the animal is freed of the ventral exuviae it can free itself from the dorsal exuviae by making vigorous body movements.

Sometimes hatched young had a tendency to remain clinging on to the mother until the violent exertions made by the mother as she freed herself from the exuviae during the early stages of ecdysis finally made these young individuals leave the brood chamber and become fully independent.

The remaining exuviae, that on the cephalon and first thoracic segment, is removed as follows: the face mask, which covers the head and first thoracic segment, is removed by the gnathopods gripping its posterior edge and easing it forward off the head. The second antennae, which now are the only body parts left encased, are freed by being passed between the rami of the first uropods where they are gripped by the inter-ramal spur. Violent reflexion of the uropods then pulls off the antennal exuviae leaving the animal completely free.

The whole process of ecdysis took as little as 30 minutes in one specimen, 46 minutes in another and about 2 hours in a third.
After ecdysis the newly moulted animal is in stage $A_0$ of the moult cycle. In this stage it is incapable of standing because the new cuticle is soft and flexible. It can move, however, by a scuffling action while lying on its side and flexing its body rapidly and repeatedly. It also uses this action as a defence, although with little success against a determined predator since even terrestrial flatworms were able to prey successfully on landhoppers caught at this stage.

Copious quantities of moulting fluid are present during the actual ecdysis. This probably lubricates the body as well as separating the old and new cuticles. To investigate the antimicrobial properties of this fluid samples of about 1 microlitre were taken from a moulting specimen of *Makawe hurleyi* and plated on nutrient agar and cultured at $30^\circ C$. After three days a diverse and abundant microflora had developed on the surface of the plates showing that the moulting fluid from this specimen had no antimicrobial properties.

Ecdysis commonly occurred after rain especially at Christchurch, which is subject to periodic droughts, because moulting may cease altogether during periods of drought.

In cultures animals which have moulted eat their exuviae and subsequently produce pink faeces rather than the usual blackish-brown coloured faeces. By eating exuviae landhoppers reclaim useful materials from the cast skins. However, eating
exuviae may not be as common in the field since unconsumed exuviae are commonly found in the litter. These remains show the typical lateral tearing and face masks of exuviae so cannot be the remains of dead animals.

Recently moulted mature females evidently produce an air-borne pheromone which causes the males in the vicinity to become very active and to attempt copulation with any stationary object they encounter. Copulation has been observed only in M. hurleyi. In this species the male does not carry the female during courtship and copulation. A receptive, recently moulted female lies prone her side and is mounted by one, two or even three males. Courtship is brief or nonexistent and the female is mounted shortly after her ecdysis and before she can stand. Males are considerably smaller than females in this species and during copulation they position themselves at right angles to the long axis of the female's body, lying between her peraeopods with their heads pointing toward her dorsal surface, their ventral surface in contact with her lateral surface and their abdomen tucked into her brood chamber. In the few times it has been observed copulation lasted only a few minutes. It was observed only once during daylight hours and three times at night.
6.3. LETHAL TEMPERATURES

INTRODUCTION

In the chapter on respiration it was shown that the maximum rate of respiratory uptake of oxygen, as shown by the maxima in the MT curves, occurred at a temperature below 30°C in both Makawe hurleyi and Talorchestia patersoni. Furthermore, both species died at temperatures within the range of 30°C to 35°C. These temperatures are not particularly high and are well below the maximum air temperatures recorded at Christchurch (42°C). Thus the temperature tolerance of landhoppers might be an important factor in limiting their distribution, and occasionally it may also be an important factor in causing widespread mortality during periods of high ambient temperature.

LETHAL TEMPERATURES—MATERIALS AND METHODS

Specimens of four species of talitrid amphipods were collected as follows: Talorchestia quoyana from the sandy beach supralittoral at Brighton Beach, Christchurch; Transorchestia chiliensis from the estuarine supralittoral of the Avon-Heathcote Estuary; Talorchestia patersoni from scrub and toi-toi litter at Warren Street Reserve, Oamaru; and Parorchestia ihurawao also from litter at Warren Street Reserve. The collections were made during August. Immediately they were collected the animals were transferred to the laboratory. They were immersed in 50% seawater at 32°C and heated up in one degree
Celsius intervals. They were maintained at each temperature for a period of 2 minutes and the number dead noted. No difficulty was experienced in deciding whether or not an individual was alive or dead since dead animals held their second antennae at a characteristic oblique \((120^\circ)\) angle to the head capsule. The animals died within seconds of being transferred to the temperature that caused death. In every species the range of lethal temperatures spanned only two degrees Celsius, so the median lethal temperature \(LT_{50}\) can be calculated by linear interpolation as follows:

Let \(p_1\) be the proportion dying at the lower temperature, \(t_1\), and \(p_u\) be the proportion dying at the upper temperature, \(t_u\), then the median lethal temperature, \(LT_{50}\), for that species is given by

\[
LT_{50} = \frac{(p_1 - (p_u - p_1)/(t_u - t_1)) - 0.5}{(p_u - p_1)/(t_u - t_1)}
\]

LETHAL TEMPERATURES - RESULTS

The median lethal temperatures \(LT_{50}\) for the four species of talitrids are given in Table 6.1. Death was very sudden in every individual tested and the range of temperatures over which individuals of a particular species died spanned no more than \(2^\circ\)C. The sandy beach supralittoral species, \textit{Talorchestia quoyana}, had the highest \(LT_{50}\) while \textit{Talorchestia patersoni}, which is a southern terrestrial species, had the lowest. The other two species tested had \(LT_{50}\)'s slightly above that for \textit{T.patersoni}. 
Table 6.1. Median lethal temperatures \((LT_{50})\) in an ecological series of talitrid amphipods.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>HABITAT &amp; RANGE</th>
<th>(LT_{50})</th>
<th>N TESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talorchestia quoyana</td>
<td>Oceanic sandy beach supralittoral</td>
<td>37.5</td>
<td>55</td>
</tr>
<tr>
<td>Transorchestia chilensis</td>
<td>Estuarine supralittoral</td>
<td>34.5</td>
<td>75</td>
</tr>
<tr>
<td>Parorchestia ihurawao</td>
<td>Inland terrestrial (South Island, N.Z.)</td>
<td>34.0</td>
<td>101</td>
</tr>
<tr>
<td>Talorchestia patersoni</td>
<td>Coastal terrestrial (southern South Island, Stewart Island and Snares Island)</td>
<td>33.1</td>
<td>10</td>
</tr>
</tbody>
</table>

LETHAL TEMPERATURES - DISCUSSION

The high median lethal temperature \((LT_{50})\) of Talorchestia quoyana is probably an adaptation to the high temperatures in its environment which can occur on days with intense solar radiation. Individuals of this species are found in sand burrows and under sea wrack; both of these habitats can become very hot on bright sunny summer days.
The estuarine supralittoral species, *Transorchestia chilensis*, had an \( LT_{50} \) more akin to those of the terrestrial species tested, presumably because its habitat of mud and wrack is similar in its temperature characteristics to soil and litter because of albedo similarities.

Of the two terrestrial species tested, the one with the northern distribution, *Parorchestia ihurawao*, had a slightly higher \( LT_{50} \) presumably correlated with the higher average environmental temperatures experienced in its more northerly distribution zone. The difference between the two species is not as marked as might be expected since *P. ihurawao* lives mainly inland on the foothills which form the inland margin to the Canterbury Plains. Here, because of the elevation and the distance from the moderating influence of the sea, the average temperature is less than would prevail at the sea coast.
6.4. CUTICULAR STRUCTURE AND MICROBIAL INHIBITION

INTRODUCTION

Except for the case of the whitey disease reported earlier in this volume, living hatched landhoppers have never been observed to be attacked by the micro-organisms that are so abundant in their immediate environment. Yet when the animals die they decompose rapidly. The living organisms must have particularly effective defences against micro-organisms which enable them to live in an environment rich in decomposing organic matter and saprophytes. Internal defence mechanisms are known in landhoppers (see the chapter on disease in this work) but the front-line defences, those of the integument, are less well understood.

The invasion routes of micro-organisms into arthropods are: through the integument, the tracheal system (if present), the gut, the rectum, or by transovarian transmission (Benz, 1963; Brock, 1966). Of these, only the integumentary, gut and rectal routes are likely to be of importance for micro-organisms invading the body of landhoppers. The chitinous epidermis is an effective barrier against penetration by protozoa, bacteria and viruses in insects, although some fungi produce chitinases (Brock, 1966). In the gut the peritrophic membrane usually bars most organisms.
The cuticular microstructure of landhoppers has been described recently by Meyer-Rochow (1981) and Cuadras (1982). Meyer-Rochow was unable to detect any 'cuticular microscales' in the unidentified Orchestia species he collected from a Hamilton suburban garden, North Island, New Zealand. The species he studied is probably Talitroides topitotum, an adventive tramp species of Indian origin, since this is the only species I have found in Hamilton City. Examination of his micrographs shows that the cuticular microstructures are obscured by a thick, superficial layer of mucus. If the specimens had been cleaned then the microstructure would have been revealed.

Cuadras found peg-like 'microtrichs' - a term used earlier by Needham (1942) and Fish (1972) to describe similar structures in isopods - which emerged from cuticular pits of less than 1 micrometre diameter in Gammarus sp., Liljeborgia sp., and Orchromene sp. In these species single units faced distally and were arranged in rows with several rows occupying a single integumental polygon. He presumed that the microtrichs were sensory in function perhaps for the detection of water currents in aquatic species and the detection of humidity in semiterrestrial and terrestrial species. Particular 'sensillae' had a specific distribution pattern, which had been reported earlier for other Crustacea by Fleminger (1973), and Mauchline and Nemato (1977). Cuadras showed that in the supralittoral Orchestia sp., cuticular pits of 0.1 micrometre diameter were borne on the segments, antennae and appendages. He found no evidence of protruding structures and he presumed the pits
were the openings of pore canals. Very short conic, microtrichs (sensilla coelonica) of 0.2 micrometres width and 0.25 micrometres length issued from pits of 0.3 micrometres diameter at the periphery of integumental polygons, especially in the mesosoma, antennae, gnathopods, and peraeopods. There was no apparent directionality to these structures. At the vertices of the polygons were cuticular and non-rimmed sockets of hair-like sensilla (trichodea) of 10-20 micrometres in length separated apart by a distance of more than 50 micrometres. He detected no apparent relationship between coeloconic microtrichs and sensilla trichodea. He suggested that these structures are sensory in function detecting humidity and perhaps wind currents. In the present work the three pore-based structures described by Cuadras are termed 'micropores' (= peg-like microtrichs or pore canals), 'mesopores' (= Cuadras' coeloconic microtrichs), and macropores (= Cuadras' sensilla trichodea). These names are used in this work since the functions of the structures are not yet determined.

**CUTICULAR STRUCTURE - METHODS**

Large specimens of *Makawe hurleyi* were dropped into liquid nitrogen and mounted while still frozen on to the stub of a freezing microtome. Sections were cut at between 12 and 18 micrometres and stained according to the methods referred to by Shyamasundari and Hanumantha Rao (1974). Mucopolysaccharides were detected using PAS (Galigher and Kozloff, 1971).
Plate and Figure 6.1. PAS stained section of *Makawe hurleyi* cuticle. Captions: c, cuticular polygons; di, duct of dermal gland opening; dp, duct of dermal glands at posterior margins of polygons; en, ecdocuticle; ep, epithelial cells; ex, exocuticle; m, mucus layer; mg, mucus gland.
Investigation of the inhibitory properties of the fluid on the outside of the body was accomplished by making extracts in distilled, sterile water by immersing live animals in 1ml volumes for one minute. Lawns of Penecillium sp., Mucor sp., and an unidentified fungus, all taken from the body of a dead landhopper in the field, were made on Sabouraud's medium (Davis et al, 1974). When the medium was set, wells were cut of 6mm diameter using sterile borers, and the bottom of the wells were lined with a little of the culture medium. Three drops of the extract were put into each alternate well, and the remaining wells had three drops of sterile water introduced to act as controls. Ten replicates were made, and the cultures were incubated at 27°C for three days.

The inhibition of fungal germination was investigated as follows: a fungal lawn was grown on medium in the top of a large Petri dish. When fruiting bodies formed on this lawn it was inverted over a Petri dish containing uncontaminated medium with the extract in wells as before. As many controls as experimental wells were made. In this arrangement, the fungal spores from the inverted lawn were free to drift down onto the culture medium below and germinate. If any germination inhibition occurred it could be detected in the zone around the experimental wells. The cultures were incubated at 27°C as before.
Plate 6.2. The surface of the integument of *Makawe hurleyi*. In the centre is a large microtrich emerging from a macropore.

Figure 6.3. The cuticle surface of antenna 2 of *Makawe hurleyi*. 
Figure 6.1 shows a section of *Makawe hurleyi* integument which has been cut in a freezing microtome and stained in PAS. In this section mucopolysaccharides are pinkish red or rose/purple. The section shows a thick layer of mucus over the cuticle. The thickness of this mucus layer varies from 15 micrometres to 17.5 micrometres. Mucopolysaccharide density is not uniform within this layer but is greater close to the cuticle surface and close to the dermal gland pores. The surface microstructures, including the verandas which overhang the dermal gland pores and the structures termed microtrichs by Cuadras (1982), are embedded deep within the mucus layer. The cuticle is secreted by epidermal cells which are stained a brownish colour in Figure 7.1. Each epidermal cell secretes a polygon of cuticle. These polygons can be seen in surface view in the SEM micrographs in Part I and in Figure 6.2. They are usually equal-sided pentagons with relatively straight margins. The boundaries between adjacent cuticular polygons is marked in most species by special structures, usually a small ridge or groove in the exocuticle. The exocuticle is about 1.8 micrometres thick in the section illustrated. The endocuticular blocks corresponding to the cuticular polygons are between 11 and 18 micrometres thick on average and are slightly shorter than the cuticular polygons. Presumably, one exocuticular polygon and the corresponding endocuticular block are secreted by one epidermal cell. The epidermis is about 5 micrometres thick. There is a considerable gap (an average of 5 micrometres) between adjacent
endocuticular blocks. The gap appears to be occupied by exocuticle and dermal gland ducts. The adjacent faces of the endocuticular blocks are generally oblique and directed posteriorly at an angle to the body surface. Thus the blocks appear to be rhomboidal in section. The exocuticle seems to be a more continuous layer than the endocuticle and is interrupted or penetrated only by the dermal gland pores. As shown in Figure 6.2 these dermal gland pores are arranged in single rows near or at the posterior margin of the two posterior sides of the cuticular polygon and are directed posteriorly. In some species, *Makawe hurleyi* for example, dermal gland pores open at other places within the general area of the cuticular polygons. Ducts leading to these pores can be seen penetrating the endocuticular blocks in Figure 6.1. The mucus-producing dermal glands can be seen in Figure 6.1 as deeply rose-coloured bodies lying between epidermal cells at the junction of two or more cuticular polygons, and 38 micrometres deep in the tissue below the epidermal layer. The duct from the deep gland cell clearly leads to a duct cut obliquely in the endocuticular block. Thus the pores which open in non-marginal positions appear to originate in mucus-producing glands located beneath the epidermis deep in the underlying tissue, unlike those which open along the posterior margins which seem to be located within the epidermal layer between epidermal cells. The dermal gland pores have a species-specific distribution pattern over the integument, but are generally more dense on the dorsal body surface, and on the ventral surface of the uropods. In live animals the mucus layer appears as a shining, iridescent layer due to interference colours.
Microtrichs on antennae 2 are much longer than those on the general body surface (Figure 6.3). These structures would probably project beyond the superficial layer and be exposed to the general atmosphere.

Cleaning

In dirty or dusty conditions landhoppers clean themselves with a frequency which may reach one cleaning action every 10 seconds. Cleaning the dorsal surface is carried out by the posterior three pairs of peraeopods, especially peraeopod 4, while the head capsule, the antennae and ventral surface are groomed by the gnathopods. The appendages used for cleaning are well provided with cleaning spines and combs (Holmquist, 1982). Cleaning activity is less frequent in cleaner situations. Ovigerous females use their gnathopods to clean and move the eggs in the brood, which may help to prevent infection and adhesions. Infertile or diseased eggs are removed by the mother from the brood. Epizooites are frequently found attached to the gills and less often to the undersurface of the body and appendages. Obviously, the host is unable to remove them by cleaning.

Defence mechanisms

The growth of all fungal species tested was inhibited by aqueous extracts from live Makawe hurleyi. The inhibition was most clearly seen during the first three days of mycelium growth when the most concentrated of the extracts made from live animals caused a
zone of clearance extending throughout the well and on the surface of the medium for a distance of about 3 mm from the lip of the well. The surface of the medium was usually overgrown by fungal mycelium after about 3 days at the incubation temperatures used, but the bottom of the well, where presumably the inhibition factor was more concentrated, continued to show inhibitory action when compared with the controls even up to 1 week after inoculation, when the fungal mycelium was many millimetres thick on the surface of the medium.

Germination of fungal spores was clearly inhibited in a zone within 1 mm of the experimental wells in the 'germination' series of experiments. In the control wells the spores germinated in the wells and on the surface of the medium.

CUTICULAR STRUCTURE - DISCUSSION

The ever-present threat of attack by micro-organisms is a problem of major proportions to cryptozoa living in the saprophyte rich environment of the soil/litter community. Thus the specific and effective mechanisms possessed by landhoppers to defend themselves against attack by micro-organisms are adaptations of major significance to the animals. These defensive adaptations involve both internal and external mechanisms.

External defence mechanisms include cleaning activities and production of a surface mucus layer containing fungal growth and germination inhibitors. Bacteriostatic or bacteriocidal substances
may also be present, but these have not been tested for in this work. The mucus secreted on the surface probably has the additional function of lubricating the body to assist the animal's passage through the soil and litter. The mucus layer must profoundly affect the surface properties of the cuticle and is likely to be the major cause of the very low wetting angles reported in the chapter on water relations. It may also lower the rate of transpiration of water from the body in some animal groups, but in landhoppers it appears from the work reported in the section on water loss in this volume, that water from the external mucoid layer is lost more rapidly than that from the body.

Integumentary protective mechanisms against attacks by micro-organisms are well known in mammals. A fungal spore or bacterium settling on the integument of a mammal will be swept off with the sloughed-off epidermal cells as these are shed. Lysozyme and perhaps other enzymes are present on the mammalian skin which, together with certain saturated fatty acids, affect the growth or survival of micro-organisms (Jawetz et al, 1974). Further protection is afforded by the activities of the normal flora present on the mammalian integument (Marples, 1965). If, however, the invading micro-organism does penetrate the integumentary barrier it will be faced by the body's second line of defence, the internal mechanisms.
If the secretion of mucus by the landhopper integument is continuous then it would act as the functional equivalent of mammalian sloughing. Invading micro-organisms would be swept off before they could attach to the surface of the body. Any disadvantage to the landhopper caused by the high transpirational water loss from the moist external mucus layer would be more than compensated for by the protective advantage such a film confers. The presence of inhibitory factors in this mucus would assist in the general defence of the body. These mechanisms are, therefore, very important for the success of the landhoppers in the cryptozoic environment. Mucus may also be a permeability barrier to viruses.

The mucus of other slimy-bodied cryptozoa, such as oligochetes, may also prove to be protective as well as acting as a lubricant. For these slimy bodied animals, as well as for landhoppers, water relations are more complex than previously thought because they are faced by conflicting demands: the need for a flow of aqueous mucus to keep the integument surface washed clean, and the need to conserve water. Hence the view that animals with a low transpiration rate are more advanced is a little simplistic. It is probably valid for animals living in environments with little risk of attack from micro-organisms, but it may not be true of the cryptozoa who are under a constant and massive challenge from the myriads of micro-organisms in their immediate environment. Perhaps it is possible to distinguish two tracks in the evolution of terrestrial organisms. The first track is toward dry land dwelling where water is such a scarce resource that water conservation
mechanisms represent major terrestrial adaptations. For animals in this track dry integuments are possible and these, if present, provide extremely effective protection against attack by micro-organisms. The other track is toward mesic cryptozoic dwelling where dry integuments are not possible because of the moistness of the environment, and where the constant threat from the micro-organisms living on the rich, moist organic matter in the soil and litter makes adoption of protective mechanisms so important that water conservation may be partially sacrificed to achieve protective efficiency. Animals in the second track probably become 'locked in' because successful exploitation of the cryptozoic environment makes it impossible for them to reverse course, give up mucus production in favour of water conservation, and become dry land dwellers. Terrestrial talitrids, therefore, should not be regarded as 'being on the starting line of an evolutionary line leading to dry land dwelling'; their adaptations are not appropriate for this path but are adaptive for cryptozoic living. In particular, it is most unlikely that further evolution will enable them to invade dry land.
6.5. HABITATS

The earlier section on ecology was concerned with variations in density within a single habitat. In this present section we consider the very great differences that occur in mean or apparent density between habitats.

The fundamental requirement for landhopper populations to successfully maintain themselves is that the environment be wet enough. Wetness is not only a matter of having sufficient precipitation. Dry litter can occur even in regions of abundant rainfall if the dominant plant species produces open, water-proof, free-draining litter. Gorse (*Ulex europaeus*) and manuka/kanuka (*Leptospermum* spp.) are examples of plants producing this kind of dry litter which supports very few landhoppers.

Low temperature seems relatively unimportant in New Zealand as a limiting factor since landhoppers are abundant in mountain conditions and in the subantarctic islands. Perhaps seasonal acclimatisation, of the type reported for the two species studied intensively in the chapter on respiration in this volume, may be common in landhoppers. If so, it would make them relatively free of the effects of low temperature. However, they do die when frozen, so low temperature may be the limiting factor to their altitudinal and southern latitudinal distribution.
High temperatures may be important for southern species in
limiting their northerly latitudinal distribution, since their
maximum lethal temperature is low.

Biotic interactions, especially those induced by the dominant
plant, seem to be of great importance in determining habitat
suitability. The relative abundance of landhoppers, of all species
and as assessed subjectively by me, is summarised in Table 6.2. In
the kauri Agathis australis community the predisposing cause for the
absence of landhoppers may the vast accumulation of plant exudates
and products of secondary plant metabolism which are produced by the
canopy dominant, the kauri, as a defence compound (Bloomfield, 1953;
Enzell and Thomas, 1965, 1966 a and b). This material accumulates
in the litter and on the surface of the soil as a thick layer of
partly decomposed and granular matter with a very low mineral
content. Under the foot of the giant kauri tree Tane Mahuta, in
Northland, I determined by thin layer chromatography on silica gel
plates and by loss on extraction using organic solvents that
nonpolar compounds originating from secondary metabolic processes in
kauri trees made up 14% of the dry weight of the litter, which at
that point was 2 m thick. Most of the components normally present
in exudates could be found in the litter. The litter was toxic to
amphipods when they were exposed to it in laboratory conditions.
Steam volatilisation of kauri litter produced a vapour which was
toxic within two minutes to amphipods even when cooled. The soil
consisted of only two principal horizons: the dark brown, nutty
litter comprised of large (up to 20mm diameter) aggregates of humus
at pH of 3.5 or less; and a grey leached horizon (termed sugar sand by gum prospectors, and $A_1$ by Bloomfield), which had little organic matter present. The only animals present in 30 Tullgren and 15 Baermann samples of the litter were a few ciliates and one nematode. Seedlings of lettuce and tomato failed to grow in the kauri soil and litter, but when the non-polar fraction was extracted from the soil/litter using petrol ether-acetone (50:50) fungi flourished, lettuce and tomato germinated and grew satisfactorily, and amphipods survived and reproduced on it.

Thus as suggested by Bloomfield it is the products of the dominant tree kauri, which profoundly influences the composition of this very well defined community. The inland forests of Northland are a mosaic of the kauri community (whose members usually are to be found under the canopy of the kauri) and the floristically and faunistically richer taraire (Beilschmiedia tarairi) community (Cockayne, 1908) or hard beech (Nothofagus truncata) community (Sexton, 1940-41; Rennison, 1964). Landhoppers are abundant in the taraire community, sparse to common in the hard beech community, and absent from the kauri community.

Some species of landhoppers have made incursions into the adventive plant communities that now dominate New Zealand. The most aggressive of these indigenous invaders is Makawe hurleyi whose native habitat seems to have been the grasslands on the eastern South Island, particularly the Canterbury Plains. $M.\,hurleyi$ is happily at home in suburban gardens in southern urban centres, a
niche occupied by the adventive *Talitroides topitotum* in northern urban centres.

Exotic hardwood forests are not extensive in New Zealand but make excellent habitat for native landhoppers. Exotic softwood forests, however, have been planted throughout the country and now occupy extensive areas. In these forests landhopper densities are low when few plant species are present, and are particularly low when ground vegetation is absent. The forest litter when little ground vegetation is present is a thick dry mat held together by a dry fungal mycelium with few animals present. Nutrient return processes are impaired as evidenced by the accumulation of litter. On the other hand, if the conifer forest is 'weedy' with native plants forming a subcanopy and ground layer, then amphipods may be relatively common to abundant. The litter in these weedy forests is far thinner, and more moist, and it is darker in colour showing that decomposition is more rapid and complete. The soil in such situations is also darker with more incorporated organic matter. It is no coincidence that bird life in weedy forests is greater too. Unfortunately, it is considered bad forestry practice to allow silvicultural weeds to proliferate since they add to the fire danger and make access for thinning operation more difficult. So it is common practice to slash or spray weed plants within production softwood forests to control weed species which has the unfortunate side-effect of making the forest far less suitable for native fauna.
In native communities, other than those dominated by beech (Nothofagus spp.) or manuka/kanuka (Leptospermum spp.), landhoppers are abundant. The litter in these communities is a thin mull-mor and the soil is dark brown to black in colour. The upper few millimetres of the litter may be thick in landhopper faeces, and this layer is frequently invaded by plant feeding roots, root hairs and mycorhiza. Native plants tend to have very shallow roots with root hairs and feeding rootlets growing very near to the surface and penetrating the litter. This habit makes them very susceptible to wind throw and flood damage, but in the fiercely competitive, floristically rich evergreen forests of New Zealand and the tropics, it is an obvious adaptation to enable immediate uptake of minerals as soon as they are released by decomposer action.

However, when stock or deer become too abundant these delicate feeding roots can be damaged by trampling or can be consumed when litter is eaten during times of food shortage. This profoundly affects the dynamics of the forest, impairing nutrient uptake and consequently plant growth. Landhoppers, too, suffer from reduced production in such damaged forests. And if all the understory and ground cover plants are eaten out by adventive mammals, as occurs in a great number of forests when stock or deer are abundant, landhoppers become extremely scarce or absent. Eventually, plant litter accumulates to form a thick, dry layer. The dominant trees show unmistakable signs of ill thrift and senescent decay. No regeneration is apparent so the forest will die, even though it may look reasonably healthy to the unskilled eye for another 50 or 100
years.

The cause of this phenomenon (other than the role played by mammals) is not known, but the one characteristic in all these forests I have visited is that the green vegetation is restricted to the canopy many metres above the ground. As no green matter is present on or near the ground I hypothesised that it may be the quality of the litter which was causing the decline in landhopper density. Even though litter was abundant, there may be some factor which renders it much less suitable for exploitation by landhoppers. To test the susceptibility of landhoppers to food of low quality thirty M. hurleyi were cultured in laboratory conditions and fed damp filter paper only. They took two weeks before they started eating the paper by which time it was stained by the microflora growing on it. After one month they began losing body pigmentation, and after three months they were transparent with the blue colour of their haemolymph showing through the transparent cuticle vividly. By this stage they had stopped breeding and the young hatched from earlier broods had died. The adults survived with additional filter paper being supplied as needed for six months in all when they were sacrificed and their blood proteins analysed using paper electrophoresis as described earlier. The same three bands were found as in normal specimens, but the two least mobile bands had lost their colour suggesting that the associated hydrocarbons in these conjugated proteins had been lost or had been severely modified. Presumably therefore, the landhoppers were suffering from a nutrient or vitamin deficiency when fed pure cellulose which
caused changes in their conjugated haemolymph proteins and a consequent cessation of breeding and moulting and a loss of body colour. Conjugated proteins, such as those conjugated with astaxanthin, have been shown to have a role in breeding and other metabolic processes (Goodwin, 1960).

The litter in severely damaged communities appears to be of low quality and may induce this or similar deficiency diseases in landhoppers and other cryptozoa. It is perhaps idle to speculate which deficiency, if any, is responsible, but since chromoproteins are concerned and the part of the molecule affected is hydrocarbon then perhaps Vitamin A or some essential precursor of this vitamin (Fisher, 1960) or astaxanthin or its carotenoid precursors (Goodwin, 1960) are the most likely candidate. Access to green vegetation may be a necessity for landhoppers since this is their supply of Vitamin A or astaxanthin or their precursors (animals cannot synthesise these compounds), and where all the accessible green vegetation is removed they are unable to persist. It is true that landhoppers can climb, but journeys of 20 to 30 m just to obtain green matter probably are not possible. Other cryptozoa, such as earth worms, may be affected even more seriously because they cannot climb. If the important litter communiters die out there would be serious effects on litter break-down and a flow-on effect causing ill thrift and premature senescence in the plants in the community because essential minerals would be locked up in the undecayed litter. Germination of seedlings would still be possible, even though germination would be impaired by the thick, dry litter mats
that accumulate on the surface of the ground, but the growth of seedlings would be poor and they would be subjected to strong herbivory from the herbivores still present - mainly the adventive mammals. Furthermore, the weakened mature plants would not produce good quality seed, nor would there be many seeds produced per plant. In such circumstances it is entirely possible that regeneration would be poor or lacking altogether.

This hypothesis is admittedly speculative and based on few facts, but it is worth further investigation if New Zealand is not to lose vast areas of remaining native forests. And if the hypothesis is valid then it has important consequences for the management of native plant and animal communities. The hypothesis suggests that it is not sufficient to merely control adventive mammals, as is done at present: more active steps must be taken to ensure the development of complete, structured plant communities with their attendant, and highly necessary, animal communities before the long-term survival of endangered terrestrial communities can be assured.
Table 6.2. Relative abundance of landhoppers in various communities.

<table>
<thead>
<tr>
<th>Community</th>
<th>Landhopper abundance</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kauri (Agathis australis) forest</td>
<td>absent</td>
<td>Waipoua Forest</td>
</tr>
<tr>
<td>Taraire (Beilschmiedia tarairi)</td>
<td>abundant</td>
<td>Waipoua</td>
</tr>
<tr>
<td>Pohutukawa (Metrosideros excelsa) or ngaio (Myoporum laetum) strand forest</td>
<td>common unless damaged</td>
<td>Whangaparoa Peninsula</td>
</tr>
<tr>
<td>Podocarp-hardwood forest</td>
<td>abundant unless ground vegetation destroyed by mammals when absent</td>
<td>Catlins, Kirks Bush</td>
</tr>
<tr>
<td>Upland beech (Nothofagus) forest</td>
<td>sparse to moderate</td>
<td>Catlins Ra. Southern Alps upland forests</td>
</tr>
<tr>
<td>Flax (Phormium) stands</td>
<td>abundant</td>
<td>Curio Bay</td>
</tr>
<tr>
<td>Kanuka/manuka (Leptospermum) catastrophe forest</td>
<td>sparse to rare if pure kanuka, more abundant if other plants present.</td>
<td>Kowai Bush Hapuku Reserve Opoho Bush</td>
</tr>
<tr>
<td>Native tussock grassland</td>
<td>moderate if lightly grazed</td>
<td>Pigroot, Rock and Pillar Ra.</td>
</tr>
<tr>
<td>Gorse (Ulex europaeus) stands</td>
<td>absent to rare</td>
<td>Circle Hill</td>
</tr>
<tr>
<td>Bracken (Pteridium aquilinum)</td>
<td>rare to moderate</td>
<td>Maungatua</td>
</tr>
</tbody>
</table>
Table 6.2 continued.

<table>
<thead>
<tr>
<th>Community</th>
<th>Landhopper abundance</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmland</td>
<td>depends on intensity of grazing (absent to moderate)</td>
<td>Tokomairiro Plain</td>
</tr>
<tr>
<td>Exotic hardwoods</td>
<td>generally moderate to abundant</td>
<td>Nelson Forests</td>
</tr>
<tr>
<td>Exotic softwoods</td>
<td>absent to sparse in pure exotic forests sparse to moderate in 'weedy' exotic forests</td>
<td>Tokoroa, Heriot Forests</td>
</tr>
</tbody>
</table>
6.6 TERRESTRIAL ADAPTATIONS

Some aspects of the evolutionary transition of landhoppers from the supralittoral to the terrestrial environments were discussed by Hurley in 1959 and in 1968 (where he presented some of my early thoughts) and by Wildish (1979). Some of their general conclusions are listed below and discussed in the context of the work presented in this thesis.

(1) Landhoppers have a reduced body size. This conclusion assumes evolution from large-bodied supralittoral species and not from the smaller bodied, and more plesiomorphic supralittoral or littoral species. Since the development of a large, robust body in supralittoral species is an adaptation to the physical rigours of the supralittoral environment, it is not necessarily true that large bodies preceded evolution to land, but large, robust bodies may have evolved in supralittoral species as a specific adaptation after the terrestrial stock had diverged off. Indeed, compared with amphipods from other environments, landhoppers are comparatively large.

(2) In a comparison of four supralittoral species Wildish concluded that the growth rate of terrestrial species is lower than that of their supralittoral relatives. Little evidence for is available to support this conclusion. The only attempt to measure growth rates in the field is reported here and concerns two rather plesiomorphic species reported in this work living in temperate
grasslands (not, as misreported, in tropical grasslands).

(3) The number of eggs per brood is smaller in terrestrial species and the average size of the eggs is larger. However, there are species of landhoppers with large broods of small eggs, so more work is needed before firm conclusions can be made.

(4) Sex ratios may be biased. This was first mentioned by Thompson in 1881, and in some species it does appear to be a real phenomenon, but in others - mainly the sexually similar group of the Parorchestia assemblage - it may be more apparent than real because the males are so similar to the females that they are easily misidentified unless every specimen is carefully searched for penial organs.

(5) Females are larger than males in some species, but not others. Makawe hurleyi males are much smaller than are the females but the males of Talorchestia patersoni are the same size as are females at the same stage.

(6) There has been a reduction in the gill area in the two species investigated. Presumably, this is an adaptation to the oxygen rich terrestrial environment and may affect transpiration rate.
(7) Seasonal acclimatisation of respiratory rate may be a common feature in temperate and subantarctic species, but it is most unlikely to be found in tropical species.

(8) Haemolymph osmotic pressure is reduced markedly in the two terrestrial species investigated.

(9) Behavioural patterns are adapted to circadian rhythms so that the animals are strictly nocturnal and their activity is regulated by terrestrial environmental factors.

(10) There is a trend toward simpler, less spiny appendages with loss or reduction of certain body parts e.g., pleopods. This has been explained earlier in this work as being a consequence of the landhoppers' invasion of an environment which is less demanding physically than is the supralittoral environment inhabited by their ancestors. Yet it is difficult to explain the reduction of unused parts of the body without lapsing into teleology. Parts which are no longer used are only selected against if they diminish fitness. Yet many appear to be neutral in a selection sense. Perhaps Vincent (1982) has provided a clue when he states (p.185):

"In deciding how much 'care' the organism should put into designing and constructing its supportive system, the organism presumably has done - in evolutionary terms - a cost-benefit analysis such that it produces materials which are no better designed than they have to be yet fulfill their function
This cost-benefit analysis of evolutionary processes is a very powerful concept which predicts that selection favours individuals which minimize expenditure (i.e., costs) of time, material and energy and maximize benefit (i.e., fitness). Thus individuals which have the tendency toward reduced unused parts will be able to expend more effort on more advantageous parts or activities, and thereby gain a selective advantage. Applying this concept to landhoppers, it is apparent that if robust bodies are no longer advantageous, the reduction of the body and unused body parts is advantageous.

Other terrestrial adaptations are mentioned under specific topics throughout this work and will not be considered again here, but most are based on investigations of very few species and the whole topic needs much more investigation on a much larger number of species than has been carried out to date before valid generalisations can be made about the evolution of the group and its adaptation to the terrestrial environment.

Recently, Matsuda (1981) considered that the evolution of terrestrial talitridae is explained by genetic assimilation (= Baldwin effect) whereby environmentally induced changes are converted by natural selection into inherited characters. Ignoring the obvious riposte that there can be no differential selection if genotype expression is already flexible enough to respond adaptively, his argument can be shown to rest on faulty assumptions.
and deductions about landhopper ecology and physiology. He seeks to show that the neotenous evolution in landhoppers was induced by a reduction in moult frequency caused by the lower light intensity under litter compared to sea wrack. He envisaged that the proto-landhoppers had an ecdysial system controlled by light intensity, and when they invaded land and substituted litter for seaweed as their refugia, they received less light and moult frequency diminished. Gradually this became a genetic attribute fixed by 'natural selection'.

This argument assumes that landhoppers evolved from large-bodied supralittoral ancestors, yet such an ancestry involves a number of problems, and it is by no means certain that landhoppers evolved by this route. Landhoppers probably originated in the Mesozoic, since Schram (1982) considers that the Amphipoda as a whole originated then or even earlier, and the present day distribution of the landhoppers makes such a date for their origin very likely. Landhoppers may have evolved into the terrestrial environment directly from aquatic ancestors at about the same time that the supralittoral groups were evolving. This view holds that the upper littoral talitrids, exemplified by Hyale, are closer to the direct evolutionary line leading to landhoppers than are the supralittoral species. Since they first evolved, supralittoral species have made massive and highly successful adaptations to the supralittoral environment. These adaptations are not shown by their terrestrial relatives. Thus supralittoral species are highly apomorphic compared with the presumed stem talitrids. Evolution
directly from intertidal species to the terrestrial environment without an intervening supralittoral stage, could have taken place on sheltered shores where the terrestrial environment abuts the high tide. Such situations are common throughout the region now occupied by landhoppers. There is effectively no supralittoral in such places, and so any organism evolving to the terrestrial condition could not pass through a supralittoral stage. Intertidal talitrids are, in many respects, much better candidates as the ancestors of landhoppers than are advanced supralittoral species since they are smaller, they breathe air, and they have a less specialised body. Any adaptations to the supralittoral made by a proto-landhopper, would have to be "unmade" once it "progressed" to the terrestrial situation. There is evidence that some of the more terrestrial of the supralittoral species do show this to a small extent, but these should not be confused with the truly terrestrial species.

While allowing for the possibility of this direct route, I still consider that the evolution of the truly terrestrial species was via a proto-supralittoral stage which was possibly estuarine or subject to freshwater influences, and not directly from the marine littoral. But these proto-landhoppers were probably much more similar to intertidal species than to the highly evolved sandhoppers which now exist.

Matsuda's argument also assumes that there has been a diminution in ecdysis frequency in terrestrial species, but this has yet to be demonstrated. Smaller body size does not, by itself,
indicate decreased moult frequency. Primitive insects such as the Collembola, have many more mouls than do their more advanced relatives, yet they have a much smaller body size (Agrell 1941, 1948).

Matsuda does give any measurements of light values to support his contention that there is less light under litter than in the 'exposed littoral zone'. To test his hypothesis a series of measurements were taken within 30 minutes of each other at mid-day of the light intensity under sea wrack and gravel in the Avon-Heathcote Estuary - where the supralittoral Transorchestia chiliensis lives - and in the litter layer at Riccarton Bush which is inhabited by the terrestrial species Makawe hurleyi. In both situations the light intensity varied considerably from place to place, but the range at each site was similar. In the supralittoral the light intensity varied from unmeasureably close to zero up to 50 lux, whereas in the terrestrial environment the intensity ranged from 1 to 40 lux. There was, therefore, no evidence for a diminution in light intensity in the litter compared with the supralittoral habitat.

Matsuda's thinking is probably influenced by his experience of the deep, thick mor litters than occur in cool-temperate forests. But in the southern hemisphere litters tend to be thin and the soils mulls or mull-mors, possibly because landhoppers (and other cryptozoa) are such good litter reducers. And the climate is non-continental and therefore much more equable. In mesic
tropical forests inhabited by landhoppers there is effectively no litter. In these forests there may be only a few indigestible twigs lying on the surface of the ground. The only leaf remains would be a few skeletonized remnants. Landhoppers seek refuge during the daylight hours under twigs, fallen trees, amongst the stems of ground vegetation or in the leaf bases of bromeliads and similar plants. In these microenvironments the light intensity they experience during the day would be as bright as in an average room; very much brighter than under sea wrack. Cover in order to conserve water is not so much of a problem in mesic tropical forests since rain falls abundantly and frequently and the forest is almost always soaking wet.

Another weak point in Matsuda's theory is the lack of evidence that the amount of androgenic hormone released in talitrids diminishes with diminishing light intensity. Yet this is crucial to his theory which states that the diminished light levels in the terrestrial environment diminishes the amount of androgenic hormone released which, in consequence, causes a diminution in moult frequency and a small, neotenous body form to evolve. Zeleny (1905) did find that ablation of the eyestalk shortened the intermoult interval, but as shown by many subsequent workers this is due to ablation of the X-organ or the sinus gland or both and not the optical receptors of the eye (Cooke and Sullivan, 1982). In fact, Hartnoll (1982) concludes that neither duration nor intensity of light affects the growth increment.
It is difficult to see how Matsuda's model allows evolution to proceed at all since he assigns to natural selection only the role of eliminating those individuals which failed to respond adaptively enough to the new environment. This would leave a phenotypically plastic population potentially capable of inhabiting both the supralittoral and the coastal terrestrial environments with equal facility. There would be no selective advantage according to his model since the plastic phenotype already fits the species for life on land as well as on the shore. How then do phenotypic changes get fixed into the genotype? Any such fixing is likely to result in a less plastic phenotype and hence a less fit individual since the population spans two habitat types.

Furthermore, Matsuda confused the use organisms make of time-giving clues (zeitgebers) in their environment for timing the release of genetically determined cell products at times optimal for their action with the possibility that the environment determines the amount of product released. The formation of hormone is a result of gene expression, not environmental expression. It is a long established principle of evolutionary genetics that the environment may modify and even control the results of gene expression but it does not modify the genetic information directly except in the event of mutagenic action, and these mutational events are random and are not directed.
Matsuda also assumes that evolution has occurred by a kind of sympatric cladogenesis when he states (P.736):

"......, invasion of the terrestrial zone might well have been done by many individuals, simultaneously or in rapid succession, since in many lands the forest reaches right down to the sea's edge or borders streams running into the sea." It is difficult to imagine genetic changes occurring in the face of the massive gene flow which must have occurred between those individuals invading land and those remaining on the shore. Perhaps saltatory changes (White, 1978; Merrell, 1981) in the structure of the chromosomes of one or other the groups (the terrestrial or the supralittoral) would serve to isolate the proto-landhoppers from the swamping effects of the gene flow from their supralittoral neighbours. If these chromosomal changes have occurred then there may be clear karyotopic differences between supralittoral species and terrestrial species. However, there have been no investigations of the karyotypes of terrestrial species.

In view of the fact that the supralittoral talitrid species are much more cosmopolitan than are terrestrial talitrid species then the traditional allopatric cladogenic or reticulate models of speciation employing established genetical theory seem to account for the known facts better than does the sympatric Baldwin-effect model proposed by Matsuda.
The localised distribution of many terrestrial species as compared with the more cosmopolitan distribution of supralittoral species (Hurley, 1959, 1968) can be accounted for by their ancient origins, their poorer chances for dispersal, and the far greater geological activity in terrestrial environments which would have provided very many more vicariance events for speciation to occur.

The phylogenetic age of landhoppers was discussed by Meyer-Rochow (1981) who considers that they are a recently evolved group because of the absence of cuticular microscales on an unidentified *Orchestia* species collected from a suburban garden in Hamilton, North Island. I have found only *Talitroides topitotum* there so this is probably the species he investigated. The micrograph reproduced in his paper on which he based his conclusion clearly shows the presence of an obscuring layer of mucus. Therefore, the absence of microstructures is not established. In Part I of this work cuticular microstructures were shown to be present on the surface of every species examined, including *T.topitotum*. Occasionally, mucus may obscure the details but it may be removed by cleaning or enzymatic action. Indeed, the complexity and variety of the microstructures present adds weight to the proposal advanced in Part I that the landhoppers are a relatively ancient group, originating in the Mesozoic.

The phylogeny of the New Zealand landhoppers has been discussed in Part I of this thesis, and various terrestrial adaptations have been discussed throughout the work and are drawn together in the
summary, so they will not be discussed further here. But it should be emphasized that the conclusions mentioned in this work apply only to the species investigated and may not necessarily indicate general trends of 'terrestrialism' present throughout the whole landhopper group. Landhoppers are, most probably, an ancient group, and much of their adaptation is at the physiological level of organisation which requires careful and exhaustive work before evolutionary trends can be commented on. Until a reasonable amount of work has been done on a representative range of species it would be unwise to extend what may be species specific adaptations to cover all landhoppers. Certainly, there are trends present in the evolution of landhoppers since they have been canalized by the potentialities of their genotypes and the restrictive nature of the terrestrial environment, but at the present these trends are perceived only dimly because so little work has been done on this interesting and unusual group.
Respiration

The external respiratory apparatus of terrestrial talitrid amphipods (landhoppers) consists of 5 pairs of external endites on each of the thoracic appendages except for gnathopod 1 and pereopod 5. They are simple blood-filled sacs attached to the base of the pereopods by a stalk. The external gills are enclosed in and supported by a small pool of water (exosomatic fluid) lying external to the body and within the branchial chamber. The pool is agitated by the pleopods beating through a small arc. The rate of respiratory loss of water is reduced by the gills being partially enclosed in the branchial chamber with the ventral surface held close to the substratum and closed off posteriorly by the reflexed abdomen.

Respiratory uptake is mainly through the gills, but a significant amount occurs through the thinner parts of the general body surface. Oxygen taken up by the gills is probably targeted for deep tissues, while that taken up through the general body surface probably supplies superficial tissues.

The gills are very thin plates which are relatively smooth in temperate species. In tropical species they may have their surface area increased by folding and plication. The gills vary in size from body segment to body segment, the first and the last pairs
being the largest. In brooding females they lie ventral to the eggs and are possibly important for the supply of oxygen to the eggs. Two cell types are present in the gills: the predominant type has an apical labyrinth, a plicate base and lateral interdigitations with its neighbours. Mesosomes are present and it has many mitochondria of moderate density and large nuclei. The tissue formed by these cells is massively penetrated by haemolymph channels which may approach the surface closely. The second cell type is generally basal to the main cell type and is evidently a replacement cell judging by its electron density and undifferentiated mitochondria. Wandering haemocytes penetrate deep into the gill tissue and may be found between old and new cuticle during the pre-moult.

The two surfaces of the gill are held together by multinucleate pillars which leave haemosinuses between them. There is a major circumferential (peripheral) sinus around the margin of the gill. The haemolymph enters the gill through an afferent vessel in the stalk and flows in a counter-current manner around and across the face of the gill in a highly directed manner, then leaves the gill by an efferent vessel in the stalk. At 20°C it takes only a second or two for the haemolymph to traverse the gill, and during this time the colour change as it becomes oxygenated is marked and rapid.

Total body area is related to body mass by an exponential equation with an exponent of 2/3. The relative density of *Makawe hurleyi* is 1.09, thus the surface area of this amphipod is nearly
three times greater than a cube of the same volume.

Gill area decreases in an ecological series of talitrids consisting of *Hylae grandicornis* (intertidal), *Transorchestia chiliensis* (estuarine supralittoral), *Makawe hurleyi* (terrestrial) and *Talorchestia patersoni* (terrestrial). The ratios of the gill areas were: *grandicornis* 1; *chiliensis* 0.53; *hurleyi* winter 0.78; *hurleyi* summer 0.39; *patersoni* 0.33. The smaller gill areas of the terrestrial species may be to conserve water but also probably represent an adaptation to life in an oxygen rich environment. The males of *M.hurleyi* have larger gills than females of the same body weight. In this species there is an increase in gill area during winter following the cessation of breeding. The exponent of the power law relating gill area to body mass is between 0.605 and 0.739 for all species and seasons except for *M.hurleyi* males and females in winter when it is close to 1. The relationship of gill area with general body surface is allometric; larger specimens have gills which comprise a smaller proportion of total surface area. This has important implications for the form of the relationship between respiration rate and body mass.

In winter the brood plates of adult female *M.hurleyi* become haemolymph filled and the brood setae are reduced to terminal stumps. In this condition these plates possibly function as accessory gills.
The respiratory uptake of oxygen was measured in Warburg respirometer and in a newly developed electrolytic continuous respirometer. The species studied intensively were Makawe hurleyi and Talorchestia patersoni. The areas from which they were collected were Dunedin (M.hurleyi and T.patersoni) and Christchurch (M.hurleyi). The latter population was affected by a whitey disease of bacterial origin. In addition, a few readings were made of H.grandicornis and T.chiliensis for comparative purposes.

A variety of equations were used to model the multivariate relationship between respiration rate \((R)\) and temperature \((T)\) and body mass \((W)\). No interactions between temperature and body mass were detected. The best fits were given by equations of the form

\[
\log(R) = a + b.T + c.\log(W)
\]

and

\[
\log(R) = a + b/(T+273) + c.\log(W)
\] .

The respiration rate of the Christchurch population showed greater heterogeneity due to a proportion of the measured animals having respiration rates depressed well below normal values by the whitey disease. In addition, female landhoppers show a higher heterogeneity in summer because of frequent mouls and broods. M.hurleyi males have higher metabolic rates than females of the same weight, whereas T.patersoni males have lower rates than females.

M.hurleyi has a higher metabolic rate than T.patersoni. Both of these terrestrial species have much lower rates than the supralittoral and littoral species breathing air. This is thought to be the result of the terrestrial species being adapted to
breathing in one medium, air, while the supralittoral and littoral species must be able to breath in both water and air and so they must compromise between the need for a large respiratory area in water and a small respiratory area in the oxygen-rich, but dry, terrestrial environment. Their larger gills (and metabolic rate) is possibly a maladaptation for the terrestrial environment and is a result of this compromise.

During moulting the respiration rate is elevated to 2 to 3 times normal values during the actual period of ecdysis, but falls to a lower than usual value immediately following the casting off of the exuviae. Over the whole period of of a moult cycle the day-time respiration rate steadily declines from a peak during ecdysis to a minimum at late stage C, then rises steadily again until the next ecdysis.

Both terrestrial species die within a few hours if immersed in water, and during the period of immersion they show an initial elevation of metabolic rate followed by a steady decline to death.

In the series of continuous respiration measurements, a disturbance factor was detected which varied in intensity (amplitude) and duration between individuals. This phenomenon explains a considerable proportion of the otherwise unaccounted for heterogeneity in non-continuous determinations of respiration rate.
Once the disturbance phase had passed *M. hurleyi* revealed a bimodal, free-running, circadian rhythm of metabolic activity with peaks at dusk and at dawn, a moderate minimum at midnight, and a profound minimum during the day. This rhythm was persistent even after a week in constant dark or light.

There is a marked seasonal acclimatisation of type IIA in Prosser's scheme equivalent to a shift in the MT curve of 9.0°C for *M. hurleyi* and 2.2°C for *T. patersoni*. In the former species this shift is largely accomplished by a seasonal change in gill area. Although *T. patersoni* lives in more southerly regions than *M. hurleyi*, the climate it experiences is less extreme. The greater acclimatisation ability of the inland dwelling *M. hurleyi* is advantageous in a species which inhabits regions with extremes of temperature, including upland and alpine areas.

**Water and osmotic relations**

Landhopper transpiration rates are very high: much higher than those of terrestrial isopods. Of those landhoppers tested, *Talorchestia patersoni* had the lowest rate of water loss, presumably because it had a smaller gill area for a given body weight.

The time course of water loss was complex, consisting of a rapid initial loss of the water on the outside of the body, which included the fluid bathing eggs and gills and the mucoid layer over the whole body, followed by two successive phases of exponential
decay. The first phase was at a relatively slow rate and persisted until about 19% of the initial weight was lost. Animals removed from drying conditions while still in this phase recovered completely. The source of the water transpired during Phase I was presumably free internal water, particularly the aqueous phase of the haemolymph. This phase was succeeded by a second exponential decay - Phase II - at a higher rate of transpiration. Animals removed during this phase did not usually recover. The higher rate is possibly due a massive disruption of internal membranes due to desiccation. Death in drying conditions occurred when about 40% of the body weight had been lost.

Smaller animals lost weight at a higher relative rate (per unit area or weight) because they had a greater surface area relative to their body mass. There was no statistically significant relationship between the rate of water loss per unit area and body mass, so the permeability (to water) of the cuticle does not appear to change as the animal grows larger.

In *M. hurleyi* the transpiration rate is linearly related to water vapour pressure deficit up to a temperature of 25°C. Above this temperature there is a new relationship, also linear, but at a lower rate of water loss which is accounted for by the relative immobility of the animals at these higher temperatures and their habit of partially conglobulating, exposing less surface area for evaporation.
Wind has an important effect on transpiration rate; the relationship between these two variables is exponential with the rate of transpiration increasing with increasing wind speed but at a declining rate.

When immersed in water neither species swam. *M. hurleyi* did perform a kind of sink-swim, but this was a very ineffective form of lateral locomotion. *T. patersoni* was even worse at swimming. Both species drowned under water although *M. hurleyi* survived longer than *T. patersoni*. Survivorship is prolonged in oxygenated media suggesting that respiratory failure precedes the breakdown of ionic/osmotic control. Submerged survival time is also related to the tonicity of the medium, and is greatest at tonicities closest to the osmotic pressure of haemolymph and shortest at 0% and 100% seawater.

*M. hurleyi* was found to be proficient at climbing. On an underwater maze it could distinguish between paths leading up or along and between paths leading along or down, but it could not distinguish between paths leading up or along. Its ability to break through the meniscus was assisted by the relative hydrofuge nature of its dorsal surface. Its ventral surface was highly hydrophilic which assisted the replenishment and retention of brood chamber water (exosomatic water). *T. patersoni* could not climb either in air or submersed. In water it locomoted horizontally until it finally succumbed and drowned.
One of the adaptations shown by landhoppers for reducing osmotic stress has been the reduction of haemolymph osmotic pressure (OP). The supralittoral *Transorchestia chiliensis* was found to have haemolymph osmotic pressures slightly below seawater. In hyposomotic conditions this species maintained its haemolymph OP at a relatively constant level; it is a powerful hyposmotic regulator. In hyperosmotic conditions its haemolymph OP was constant while environmental water was still abundant, but when water became scarce the animal ceased regulating and became a conformer so that its haemolymph OP increased to match that of the environmental water.

*T. patersoni* had haemolymph osmotic pressures well below that of *T. chiliensis* while *M. hurleyi* was lower again, at about 45% that of seawater. The exosomatic water of *T. patersoni* had a low OP while *M. hurleyi* had exosomatic water with an OP almost as low as stream water.

The reactions of *M. hurleyi* in a humidity gradient showed that it possessed extremely efficient humidity detecting mechanisms (hygrotaxes) which appear to be located on the general body surface. Hygrotaxes enable landhoppers to quickly select and move to suitably mesic refugia.

Different genera of landhoppers appear to favour soils of different tonicities (or conductivities, since this was how they were measured in this study). Strand species, such those of the genus *Kanikania*, favour high tonicity soils including soils rich in
bird guanao. Coastal species, such as *T. patersoni*, are limited to a range of medium to low tonicity soils, while inland and upland species, such as those of the genus *Parorchestia*, favour very low tonicity soils.

The most important factor limiting the distribution of landhoppers is water. Too much free water and they are prone to drowning. In very wet environments where the soils may be saturated for long periods of time the local species may be found in the canopy, in moss beards and aerial parts of trees, which, though wet, are free draining. Too little environmental water and talitrids are unable to maintain a positive water balance. In progressively drier environments landhoppers are to be found in smaller and smaller patches in decreasing numbers and with a decreasing average size as suitable refugia become scarcer and smaller. In general, landhoppers are not found widely distributed in regions with less than about 550 mm precipitation, although in regions drier than this limit they may be found in suitably mesic patches such as the edges of swamps and streams.

The ecology of two species, *Talorchestia patersoni* and *Makawe hurleyi*, living in waster grassland, is described. They were found to partition their habitat with *T. patersoni* living close to the bases of the tussocks where it is protected from drowning, and the other, *M. hurleyi*, lives between the tussocks. The latter species escapes from flooding by climbing. Climbing activity is more common.
on wet days. The two species could be aged to instars by counting
the podomeres on their second antennae. Field growth rates depended
on temperature but generally averaged about 15 instars per year for
both species. Generations overlapped considerably. Brood size
depends on mother size, stage of development of the eggs (eggs are
lost from the brood as development proceeds), and species
(\textit{T. patersoni} has fewer eggs). Mortality was high in young
individuals, moderately low in young adults and high for old adults.
Their distribution is patchy and density depends upon litter
thickness - litter provides refuge as well as food. The population
crash in winter is due to cessation of breeding and scarcity of
food.

\textbf{Disease}

Field observations made over 10 years suggested that a
bacterial disease of the adults of the terrestrial landhopper \textit{Makawe
hurleyi} is progressing southward down the eastern side of New
Zealand's South Island. As the disease spreads, amphipod density
appeared to decline and the population structure became truncated.
In the vicinity of Dunedin and further south the amphipods are still
disease-free. Signs of the disease are a progressive weakening and
wasting. The animal cannot jump, and its speed of walking is
reduced. Its body becomes opaque white instead of the normal
reddish-brown. There is no evidence that diseased animals moult.
Death is caused by general wasting or by predators. The
disease-causing organism was isolated, and healthy amphipods were
re-infected from the isolate. Signs of the disease were apparent within 7 days of inoculation. The presence of the disease-causing organism in the haemocoel causes host defences to be mobilised, as shown by elevated haemocyte counts (4512 mm\(^{-3}\) cf. 300 mm\(^{-3}\) in healthy, disease-free adults), but as the disease progresses the animal's defences are overcome, and haemocyte counts fall to an average of 784 mm\(^{-3}\) during the later stages of disease. The haemolymph of terminally diseased amphipods is thick and creamy-white, packed with motile bacterial cells and few haemocytes are present in the circulation.

Two populations were studied: one disease-free (at Dunedin) and the other heavily diseased (at Christchurch). The incidence of disease (as measured by a performance test) was about 30% in Christchurch adults. The disease causing strain of *Bacillus subtilis* was found on the body surface of almost all adults in the diseased population. It is possible that the bacterium gains entry to the haemocoel through wounds suffered during ecdysis, conflict, or predator attack.

The main differences shown by the diseased population relative to the disease-free population were: lower average density (992 m\(^{-1}\), cf. 1677 m\(^{-1}\)); lower maximum density (3104 m\(^{-1}\), cf. 9971 m\(^{-1}\)); smaller average size, with fewer adult instars; and much lower egg production. The brood size/mother age relationship was the same for both populations - number of eggs in brood = -4.9 + 0.64(instar of mother) - because in the diseased population only
healthy females breed. Lower egg production in the diseased population reflects the smaller proportion of healthy females, and the number of broods per female is lower since life expectancy is much less.

A computer model based on Leslie matrices was used to simulate the ecological effects of the disease. It gave predictions which conformed with the observed population features with respect to age structure and density. The disease has been detected in other species of landhoppers particularly Parorchestia tenuis and the adventive Talitroides topitotum. Possibly the disease was introduced into New Zealand by this adventive landhopper which is relatively immune from the ecological effects of the disease because of its high biotic potential. The disease may be spread around the country by human agencies. As an example of this, recently T.topitotum was found in high numbers on a compost heap in the grounds of the University of Canterbury having escaped from some potted North Island plants thrown out by university botanists. The colony only survived winter and spring as they were killed by a drought during summer. But during the time it did survive it co-existed with the indigenous M.hurleyi, and this latter species could have contracted the disease.

Activity
Landhoppers are nocturnally active with peaks of activity just after dusk and at dawn. This pattern was shown both in a laboratory actigraph and by pitfall trapping in the field. Measurement of relative humidity profiles above the surface of the ground showed that nocturnalism is advantageous in minimising transpiration.

Factors affecting the number of emergent landhoppers captured by pitfall trapping include litter temperature and humidity whereas climbing is affected mainly by relative humidity, mean air temperature and rainfall. More landhoppers are active on warm, humid and calm nights than on cold, dry and windy nights.

Other aspects

Breeding

The winter form of *M. hurleyi* can be induced in the laboratory by exposure to a regime of short day lengths irrespective of ambient temperature. At low temperatures, however, broods are small and contain a number of infertile and diseased eggs. The cessation of breeding in winter is triggered by short days and not by low temperatures. This is seen as adaptation to the variable temperatures in New Zealand. The winter non-breeding season avoids the poor development of eggs in low temperatures and allows the adoption of larger gills and in females the use of broodplates as accessory gills, for the seasonal acclimatisation of metabolic rate. Such acclimatisation would not be possible if eggs had to be accommodated in the brood chamber as well as enlarged gills.
Ecdysis and copulation

Ecdysis in landhoppers is very similar to that in supralittoral species, but the long antennae are freed by the use of the inter-ramal spur on uropod 1. In culture animals eat their own exuviae, but this may not occur in the field. During the period of ecdysis they are virtually defenceless and can only move by abdominal flexure while lying on their side. Moulting fluid has no detectable antimicrobial activity.

Having moulted, mature females apparently release an air-borne pheromone which cause males in the vicinity to become agitated an attempt copulation with any stationary object. In M. hurleyi the male does not carry the female during courtship and copulation. Courtship is extremely brief and copulation is achieved by the much bigger female lying on its side and one, two or even three males mounting her transversely.

Cuticular antimicrobial activity

The landhopper cuticle is penetrated by three kinds of pores. The larger two kinds appear to excrete a mucoid substance which coats the body with a glistening, iridescent, slightly sticky layer. This layer presumably functions to lubricate the body so easing the passage of the animal through the litter or the soil, and to carry off any bacterial or fungal spores that might otherwise
germinate on the surface of the animal. In addition, this mucoid layer is active against some common saprophytic fungi, and especially against spore germination.

Habitats

Biotic interactions seem to be important in determining landhopper distribution and density since different plant communities differ in their suitability in spite of physical conditions being reasonably similar. In particular, communities with an extensive ground layer and understory of plants seem to support the highest landhopper density. Landhoppers are also found in adventive plant communities.

In some highly damaged native communities understory plants may be absent having been eaten out or trampled by adventive mammals. The litter layer in such damaged communities is usually thick showing impaired nutrient recycling, and the canopy trees show ill-thrift and premature senescence. Landhoppers are absent from these damaged communities in spite of the thick litter. Their absence may be due to a nutritional factor since animals maintained on a diet of pure cellulose in the laboratory failed to reproduce and lost all body pigmentation. It is hypothesised that green vegetable matter is a vital component of their diet.
Terrestrial adaptations

In the evolution toward more terrestrial conditions a number of trends may be detected including: smaller body size, simpler, less spiny body, reduction of certain body parts especially the pleopods, lower growth rate, smaller broods and larger eggs and biased sex ratios in favour of the female. However, all of these conclusions are based on incomplete knowledge and much more work needs to be carried out on a wider range of species before firm pronouncements can be made.

Phylogenetic mechanisms are considered and Matsuda's (1981) proposed evolution by genetic assimilation is rejected in favour of a more traditional model.

The phylogenetic age of landhoppers is somewhat controversial. The present work indicates that they are older than the period of New Zealand's isolation from Gondwana which is at least 80 M years. On the other hand, Meyer-Rochow (1982) lends support to the view that they are of recent origin, but his conclusion is based on his failure to detect microstructure on the cuticle of an unidentified landhopper (probably Talitroides topitotum). In Part I cuticular structures are shown to be complex and very different in different species. Unfortunately, the structures in Meyer-Rochow's specimen were obscured by mucus.
Part II

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