THE SYSTEMATICS OF THE NEW ZEALAND SPECIES OF *POTAMOPYRGUS* (MOLLUSCA : HYDROBIIDAE), AND STUDIES ON THE BIOLOGY OF *POTAMOPYRGUS ANTIPODUM*

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ABSTRACT

This investigation has shown that only three species of *Potamopyrgus* Stimpson can be recognized from New Zealand, compared with the six species and three subspecies recognized by Suter (1913). The species are, *P. antipodum* Gray 1843, *P. pupoides* Hutton 1882, and *P. estuarinus* n. sp. *P. dawbini* Powell 1955 from the Auckland Islands is probably referable to *P. antipodum*, but the position of (?) *P. melvilli* (Hedley 1916) from the Kermadec Islands has not been determined. The European species *P. jenkinsi* (Smith 1889) cannot be separated from *P. antipodum* on morphological or anatomical grounds and may also be referable to that species. All species now placed in *Fluviopupa* Pilsbry 1911 should probably be referred to *Potamopyrgus*.

P. estuarinus and *P. pupoides* are both smooth-shelled, bisexual, non-ovoviviparous and confined to brackish water. *P. antipodum* is highly variable in shell size, shape and ornamentation, inhabits fresh and brackish water, is ovoviviparous, and populations may consist entirely of parthenogenetic females, or contain variable numbers of sexually functional males. Rearing of snails in the laboratory has shown that snails do not necessarily breed true with respect to shell ornamentation, and that shell shape and ornamentation is not determined primarily by environmental factors.

The shell of *P. estuarinus* cannot be distinguished from that of some *P. antipodum* but *P. pupoides* may be readily identified using shell characters alone. No significant interspecific differences in operculum, external morphology,

body pigmentation or structure of the male reproductive system are found but P. pupoides possesses minor radular differences, and P. antipodum differs in the condition of the female reproductive system. The diploid (2n) chromosome number of all three species is 24.

Qualitative paper chromatography of crude foot muscle and mantle edge extracts, and quantitative ion-exchange chromatography of shell periostracal protein have disclosed no important biochemical differences between species.

P. antipodum is widely distributed in fresh waters and no clear relationship between shell shape and ornamentation, and different kinds of habitat have been found. P. estuarinus has a fairly restricted brackish water habitat and is frequently found near river mouths in harbours where snails may regularly be exposed to the air for part of each tide cycle. P. pupoides is also restricted to brackish water but normally remains fully aquatic at all times. Experimental studies on salinity relationships, habitat selection and the effects of desiccation have demonstrated important differences in the environmental relationships of the three species which can be correlated with their distributions.

Life history and population studies made in three populations of P. antipodum (two ponds and a stream) over a 13-14 month period have shown that reproduction occurs throughout the year with peak activity in spring and summer. Generation time as indicated by laboratory rearing of snails is 9-12 months. Population age structures differed markedly between ponds and stream and reflected differences in the

physical environments of the two habitats. Distribution, occurrence in drift and effects of floods were examined in the stream. Snails were generally most abundant in places sheltered from the main current or among vegetation, and large numbers were present within mats of willow roots. P. antipodum is a regular member of the drift fauna and floods have an important role in regulating population age structure.

The distribution of P. antipodum in thermal waters was also investigated, and experimental work indicates that high water temperature is probably the most important factor limiting distribution. The maximum temperature at which snails were found in the field, 28°C, is also the temperature at which activity ceases and the snails enter a comatose state.

Finally, a study has been made of the parasites of Potamopyrgus. An unidentified protozoan (Sporozoa : Porosporidae), occurring in an encysted state, is the most important internal parasite with infection rates as high as 86% having been recorded. The larvae of 13 species of Trematoda were identified and briefly described and their rates of infection determined. The monostome cercariae are the most important group of parasitic trematodes. The commensal oligochaete Chaetogaster limnaei limnaei was found in association with P. antipodum in Lake Pupuke, Auckland, and was observed to be predacious on embryonic snails.

PREFACE

A thorough investigation of the systematics of the New Zealand species of Potamopyrgus has long been overdue and is the main aim of this thesis. Comparative examinations of morphological, anatomical and biochemical factors, as well as environmental relationships have been made in the search for species differences and in order to describe the extent of inter- and intraspecific variation within the component species. All factors have been examined primarily for any systematic information they may yield rather than to elucidate structure and function for its own sake.

In the systematic section of this thesis the conclusions reached as a result of the study are presented before the detailed account of the investigations leading to their formulation. This has permitted a more cohesive account to be written with the emphasis being placed on interspecific differences.

The secondary aim of this work has been to examine some aspects of the biology of the freshwater species P. antipodum. This has involved studies of the life history and population dynamics in three populations, field and experimental work on thermal relations, and an examination of the snails' parasites and their rates of infection.

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All figures in this thesis were reproduced by the Massey University, Central Photographic Unit.

INDEX TO FIGURES

1.0 THE SYSTEMATICS OF THE NEW ZEALAND SPECIES OF POTAMOPYRGUS

1.1 INTRODUCTION

The Hydrobiidae are a family of the Rissoacea, one of the largest superfamilies of Mesogastropoda (= Taenioglossa). In the past the Hydrobiidae have also been referred to by the family names Amnicolidae, Bulimidae, Bithynidae and Paludestrinidae but according to Stimpson (1865) and Solem (1959) Hydrobiidae has priority. Two genera of Hydrobiidae are at present recognized from New Zealand, Potamopyrgus Stimpson 1865 and Opacuincola Ponder 1966.

The Rissoacea are predominantly marine but are not uncommonly found in brackish and fresh water, they are all small molluscs the majority less than 2-3mm high, and bear a close resemblance to one another. Their major characteristics have been summarized by Fretter and Graham (1962). Typically, they have small, smooth shells lacking an umbilicus, they are gregarious, detritus feeders possessing a crystalline style but no oesophageal glands, a short intestine, a long narrow foot with pedal glands and most males possess a long narrow penis. The female reproductive tract is complex, and in particular the rissoaceans are the first group of monotocardians with the accessory genital structures constricted off the main duct. Considerable variation exists within the group as regards the detailed arrangement of the parts, but all are distinguished by having the receptaculum seminis and bursa copulatrix proximal to the capsule gland on the course of the female duct.

The families Hydrobiidae, Rissoidae, and Assimineidae are closely related but the Hydrobiidae is considered by Fretter and Graham to be the most primitive of the three because of the smooth architecture of the shell, the breadth of the foot, and the possession of long pleuroparietal connectives in the nervous system. Stimpson (1865) and Cooke (1895) suggested that the Hydrobiidae is closely related to and probably originally derived from the marine intertidal family Littorinidae. Johansson (1956) on the other hand assumed the Hydrobiidae, being generally restricted to fresh water, to have originated from some branch of the marine Rissoidae, and considered that the conditions found in this latter family varied enough to justify such an assumption. Johansson rejected Krull's (1935) hypothesis concerning the Pulmonata as the origin of the Hydrobiidae.

Below the family level, classification of the Hydrobiidae has proved most difficult in the past and much confusion in the lower systematics has resulted. Much of this is the result of attempts to produce classifications relying on shell characters, which in many cases have been found to be highly variable (Suter, 1905; Berry, 1943; Hubendick, 1955; Muus, 1963). Conchological similarities are also found between many genera (Stimpson, 1865; Cotton 1943; Iredale, 1943). A parallel situation has been described in the freshwater family Pleuroceridae of which Dazo (1965) has written, "the use of shell characters alone in this group, where intra- and inter-specific variation

is so common, does not seem to provide a good criterion for intrafamilial classification." Stimpson (1865) in his pioneering work on the Hydrobiidae, relied primarily on radular structure to delineate genera but this alone has also proved to be insufficient in producing a reliable classification.

Recent workers (Dazo, 1965; Davis and Lindsay, 1967) have stressed the importance of considering and evaluating a wide range of characters in order to arrive at adequate objective definitions of genera and species. Davis and Lindsay stressed the value of precise anatomical studies involving whole organ systems as well as cytological data, and Davis (1966) suggested that some of the more useful characters for defining generic, specific and subfamilial categories may include external morphology, verge, radula, mode of progression, male gonadal structure, female reproductive system, buccal mass morphology and the nervous system.

Some of the results of the earlier taxonomy have been the superficial application of names to intra-specific variants and the classification of many species in incorrect genera due to systematic decisions being made from inadequate data. Considerable taxonomic and nomenclatural confusion has resulted, clearly exemplified in the Australian Hydrobiidae, four genera being recognized by Cotton (1943), compared with 13 by Iredale the following year. Similarly, variation and plasticity of South American Planorbidae formerly was not recognized, resulting in over 200 names

being applied to probably no more than 25 valid species (Michelson, 1966a).

According to Dazo (1965) all operculate freshwater gastropods from all parts of the world, other than those belonging to the Viviparidae and Pilidae, were initially included in the genus Melania Lamarck 1792. The first hydrobiid named was Amnicola limosa (Say, 1817) originally placed in the viviparid genus Paludina Lamarck 1816. Stimpson (1865) in his important "Researches upon the Hydrobiinae and allied forms" suggested the division of the family Rissoidae (= present day superfamily Rissoacea), already separated from the Littorinidae by Adams (1865), into six subfamilies including the Hydrobiinae. In this family he recognized 15 genera including Potamopyrgus which he erected to contain the New Zealand species Melania corolla Gould 1847. In Thiele's (1931) classification, the family Hydrobiidae (referable to Stimpson's Hydrobiinae) is subdivided into eight subfamilies containing 58 genera, the largest subfamily Hydrobiinae including 38 genera in seven tribes. Potamopyrgus is placed in the Hydrobiinae and species classified in this genus are recognized from New Zealand, Australia including Tasmania, Great Britain, Europe, tropical America, and central Africa.

The New Zealand "species" were initially placed in four genera, Amnicola Gould and Haldeman 1841, Hydrobia Hartmann 1821, Melania Lamarck 1792, and Paludestrina d'Orbigny 1839 on the basis of shell characters, and the European P. jenkinsi has also been classified in Paludestrina and

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its radular structure, form of the female reproductive system and possession of a calcareous smear on the operculum. The most significant difference from Thiele's (1931) diagnosis is that the females are not all ovoviviparous.

1.2 REVISED DIAGNOSIS OF POTAMOPYRGUS

Potamopyrgus Stimpson 1865

Type (Monotypy): Melania corolla Gould, 1847

Shell dextral; height less than 10mm; shape variable ovate-conical-cylindrical; up to eight whorls, ventricose-flat sided, smooth with or without shouldering and/or periostracal spines; body whorl over half height of shell; imperforate; aperture ovoid, continuous (in fully grown shells); operculum ovate thin, corneous, subspiral, usually possessing a calcareous smear. Radula taenioglossan; central tooth trapezoidal, inferior margin nearly straight, faintly trilobate, basal cusps close to lateral margins; lateral tooth denticulate, shank 2-3 times length of subrhomboidal body which possesses no basal peg; marginals finely serrate, long and slender, shanks straight, sharply curved at free ends; cusp formula $\frac{(3-5)1(5-3)}{(2-5)\ (2-5)}$: (7-13) : (14-32) : (21-48). Animal with long pointed tentacles. Bisexual or parthenogenetic, ovoviviparous or non-ovoviviparous. Males with long, narrow non-lobate penis containing a single duct, normally coiled beneath the mantle edge and attached to the head on right of mid-dorsal line; vas deferens strongly coiled; prostate imbedded in visceral mass. Ovoviviparous

females possessing a thin walled brood pouch, with the sperm channel (= ventral channel) incorporated in its floor; non-ovoviviparous females with the sperm duct (= "spermathecal duct" of van der Schalie and Getz, 1962) separated from the accessory glands above, and also functioning as the pallial oviduct. Habitat, fresh and brackish water, occasionally in sea water, and may be semi-terrestrial on high tidal flats.

Synonomy

Until further anatomical information is available the synonomy of other genera with Potamopyrgus must be considered tentative. Such genera may include Austropyrgus Cotton 1942 and Fluviopupa Pilsbry 1911.

1.3 THE NEW ZEALAND SPECIES OF POTAMOPYRGUS

The genus Potamopyrgus was erected by Stimpson (1865) for the New Zealand hydrobiid Melania corolla Gould (1874). Stimpson's diagnosis of the genus was based primarily on radular structure, but also made use of shell characters and external features of the animal. In 1882, Hutton transferred all the New Zealand Hydrobiidae known to him to Potamopyrgus, placing them in four species differing in shell form and ornamentation, as well as radular structure.

Suter (1905) finding Hutton's restriction to four species unsatisfactory, published a second revision of the genus in which he placed the 11 species originally described from New Zealand in five species and three subspecies and also described a further new species. Suter was aware of the great variability found in the shells of New Zealand

species of Potamopyrgus and stated that, "it is difficult to find the same form of a species in more than two or three localities". Nevertheless, he still based his species discrimination on shape, size, colour and spination of the shells, and was apparently convinced in his own mind of the reality of his named species.

Powell (1955) described P. dawbini from the Auckland Islands basing his specific diagnosis solely on shell characters and in the same paper he tentatively transferred Tatea melvilli Hedley 1916, from the Kermadec Islands, to Potamopyrgus. No further revision of the genus has been made since that of Suter although Ponder (1964) has stated that, "clearly there have been too many species named in New Zealand," and he has made suggestions as to likely synonyms. The classifications of Hutton and Suter are given in Table 1.

Suter's revision has remained the definitive work on the New Zealand snails of this genus, but, despite his clear descriptions and awareness of variability within the species, later workers have found that many snails cannot be identified satisfactorily according to his scheme. It is clear that a far greater range of variation is found within this group than was admitted by Suter, and extensive documentation of this has been necessary before the relationships of members of the group could be reasonably determined.

In this study the systematic significance of the shell including protoconch, operculum, radula, anatomy, pigmentation, reproductive status, chromosome numbers, environmental

TABLE 1.

Classification of the New Zealand species of Potamopyrgus as envisaged by Hutton (1882) and Suter (1905).

Suter (All Potamopyrgus)	Original species	Hutton (All Potamopyrgus)
badia	Amnicola badia Gould 1848	corolla*
	Hydrobia fischeri Dunker 1862	
	Hydrobia reevei Frauenfeld 1862	
corolla	Melania corolla Gould 1847	
	Paludestrina cumingiana Fisher 1860	cumingiana
corolla salleana	Paludestrina salleana Fisher 1860	
antipodum	Amnicola antipodanum Gray 1843	antipodum
egenus	Amnicola egena Gould 1848	
spelaeus	Hydrobia spelaea Frauenfeld 1862	
antipodum zelandiae	Amnicola zelandiae Gray 1843	
subterraneus n. sp.	Potamopyrgus subterraneus Suter 1905	
spelaeus pupoides	Potamopyrgus pupoides Hutton 1882	pupoides n. sp.

Footnote to Table 1

1. Suter (1905) gives reasons for omitting three further species described from New Zealand.

(a) Hydrobia crossei Frauenfeld 1865.

This name was proposed in case the name cumingiana of Fischer should not be accepted as there already existed a Paludestrina cumingi d'Orbigny 1840.

(b) (?) Hydrobia ciliata Frauenfeld 1865.

Described as from Liberia but very similar to Hutton's figure of corolla. Suter considered a mistake had been made.

(c) Amnicola gracilis Gould 1852.

The shell first described by Gould as Amnicola egena was inadvertently called gracilis in the final report.

2. *Melania corolla Gould 1847 is not corolla of Hutton but the shell figured by Reeve (1843-78) as corolla. He took the wrong species, A. badia as corolla.

3. Two further species P. dawbini Powell 1955 and (?) P. melvilli Hedley 1916 are not recorded from the two main islands of New Zealand.

relationships and certain biochemical factors have been examined, and it has been concluded that on the main islands of New Zealand only three valid species can be recognized.

The three species can be divided into two groups on the basis of their female reproductive systems, method of reproduction, and habitat preference.

(a) Having normal bisexual reproduction, non-ovoviviparous; inhabiting brackish waters

(b) Ovoviviparous, and either bisexual or parthenogenetic; occupying fresh waters or to a lesser extent brackish water.

The former group contains two species P. pupoides Hutton and P. estuarinus n. sp. and the second a highly variable species P. antipodum (Gray) incorporating all the species recognized by Suter (1905, 1913) from the two main islands of New Zealand with the exception of P. pupoides. Gray's original description of Amnicola antipodanum (= P. antipodum), ("Inhabits New Zealand in fresh water. Shell ovate, acute, subperforated, generally covered with a brown earthy coat; whorls rather rounded, mouth ovate, axis three lines; operculum horny and subspiral; variety spire rather longer; whorls more rounded."), clearly does not apply to P. estuarinus as he states that it inhabits fresh water and is found in rivers with Physa variabilis Gray (= Physa-astra variabilis), a species which occurs only in fresh water.

Important diagnostic characters of the three species are summarised below.

Potamopyrgus pupoides Hutton, 1882

1882 Potamopyrgus pupoides Hutton, Trans. N.Z. Inst. 14:146

1905 Potamopyrgus spelaeus pupoides Suter, Trans. N.Z. Inst. 37:266

Shell, height less than 2.5mm, conic-cylindrical, obtuse in apical region; whorls 5, flat, smooth, never possessing spines or keels, suture often margined below. Reproduction sexual, sexes in approximately equal numbers. Females non-ovoviviparous. Inhabit lower reaches of streams and rivers, and estuaries which are susceptible to tidal influence.

Potamopyrgus antipodum (Gray, 1843)

1843 Amnicola antipodanum Gray, in Dieffenbach, "Travels in N.Z." Vol. 2:241

1905 Potamopyrgus antipodum Suter, Trans. N.Z. Inst. 37:263

1905 Potamopyrgus antipodum zelandiae Suter, " :263

1905 Potamopyrgus badia Suter, " :264

1905 Potamopyrgus corolla Suter, " :260

1905 Potamopyrgus corolla salleana Suter, " :262

1905 Potamopyrgus egenus Suter, " :265

1905 Potamopyrgus svelaeus Suter, " :266

1905 Potamopyrgus subterraneus Suter, " :267

For all earlier synonymy reference should be made to Suter (1913).

Shell ovate-conic, height fully grown 3-10mm; shape highly variable, slender and elongate to ventricose; spire long or short, loosely or tightly coiled, whorls flattened to rounded, with or without shouldering and variable periostracal spination. Females ovoviviparous, the lower oviduct forming a brood pouch. Reproduction sexual or parthenogenetic, sex ratio variable. Inhabit fresh waters of practically every type and also brackish water.

Potamopyrgus estuarinus n. sp.

Shell ovate-conic, height up to 7mm; whorls 6-7, smooth, flattened; never possessing periostracal ornamentation; sutures sometimes margined below; apical whorls frequently eroded. Females non-ovoviviparous, reproduction sexual, sex ratio approximates 1:1. Rostral and mantle

pigmentation always very dark. The ecological niche of this species is restricted and distinctive, the snails inhabiting the lower tidal reaches of rivers, and particularly harbour mud flats adjacent to river mouths, where they are alternately exposed and covered by water of varying salinity. P. estuarinus is indistinguishable from some forms of P. antipodum using shell, radula, and operculum characters and non-reproductive internal anatomy.
Holotype (Fig. 5a): Bell Block, Taranaki, in small, brackish stream -/9/66. M.J. Winterbourn. Deposited in Dominion Museum, Wellington, New Zealand.
Paratypes: Auckland, Dominion and Canterbury Museums.

The shell of P. dawbini Powell from Auckland Islands is similar to that of some populations of P. antipodum from New Zealand and may be referable to that species, but the identity of the Kermadec Islands species Tatea melvilli Hedley (= ? Potamopyrgus melvilli) remains problematical as material was not available for examination.

1.4 DETAILED SYSTEMATIC ACCOUNT

1.41 MORPHOLOGICAL FEATURES

METHODS

(a) SHELL

Examination of shell form and variability was made in two ways:-

(i) Measurement of shell parameters (quantitative)

(ii) Overall comparisons of shape and size (qualitative and quantitative)

(1) Measurements

All shell measurements were made with a linear eye-piece micrometer inserted in a stereoscopic microscope (magnifications X 12.5 and X 32). Three shell parameters, height, width and height of aperture, were measured, with the aperture of the shell facing upwards (Fig. 1). All shells measured were oriented in exactly the same way to ensure uniformity of measurement criteria. Measurements were made to the nearest 0.1mm.

For comparative purposes ratios of shell height to shell width (h/w) and shell height to aperture height (h/ap h) have been employed as well as direct comparisons of initial measurements. Sokal (1965) has pointed out the suitability of ratios when dealing with material heterogeneous for size, although they do have numerical disadvantages by having a greater inherent error than the measurements from which they are calculated. Shells of fully grown snails only were used in comparative studies. This precaution was taken in order to eliminate any errors which might be introduced as a result of allometric growth of shells, although, in those populations examined, growth did not appear to be allometric with respect to the ratios used (Fig. 2). The number of snails taken for measurement from each population was determined partly by numbers available, and in all cases was selected to give a thorough indication of the full range of variation found within the population. Localities of populations from which shell measurement data were obtained are listed in Appendix 2.

FIGURE 1

Measurements made in the study of shell shape variation.

(a) Fully-grown shell

(b) Embryo shell

Key

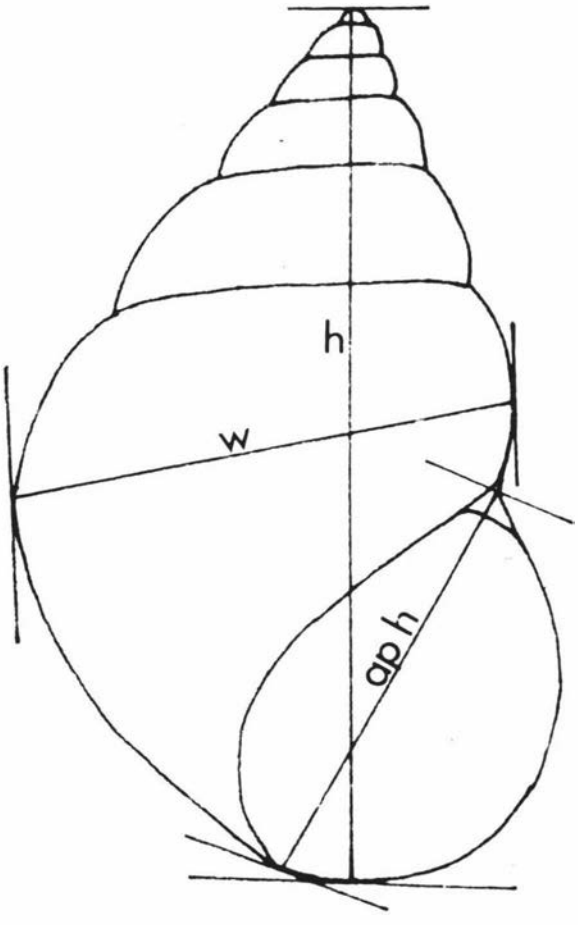
a - diameter of inner half of first whorl

a 2 - diameter of complete first whorl

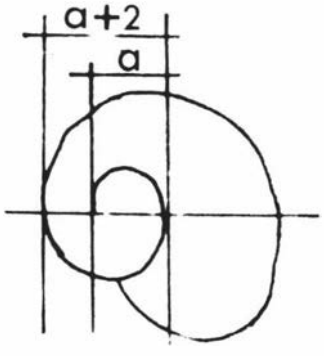
ap h - aperture height

h - shell height

w - shell width



a



b

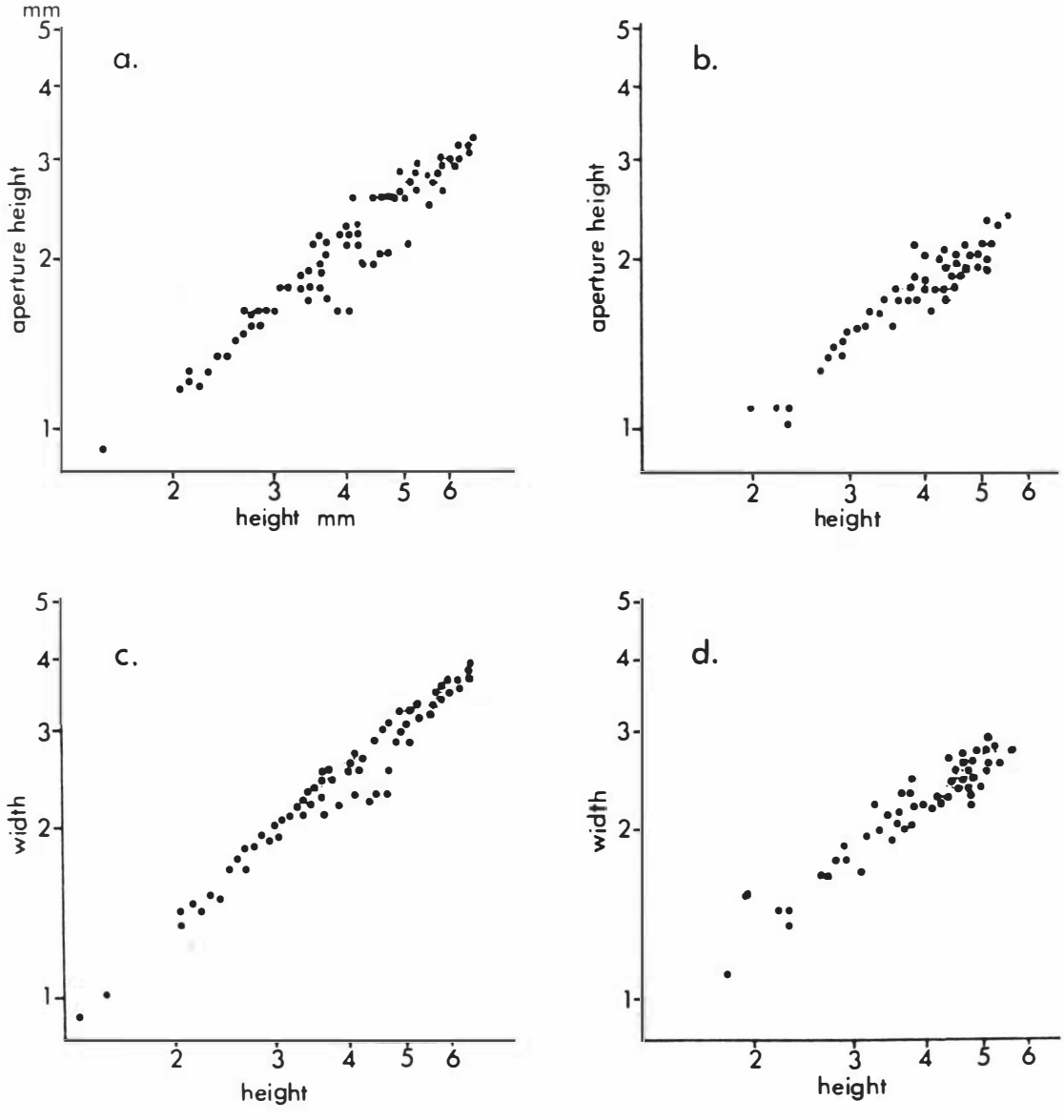
FIGURE 2

Shell height plotted against shell width, and against aperture height, in two populations of *P. antipodum*

Scale: log - log

a and c - Tiritea Stream

b and d - Pond A, Massey University


mm
a.
aperture height
height mm
b.
aperture height
height
c.
width
height
d.
width
height

Whorl counts have been made to the nearest complete whorl. Considerable difficulty in determining whorl numbers was experienced due to erosion of the shell apex and the presence of encrusting material on many shells. Counts have been made, therefore, only when all whorls were clearly visible.

(2) Overall comparisons

Some shell characters cannot be conveniently expressed as measurements, e.g. convexity and shouldering of whorls and degree of ornamentation. These characters do not lend themselves to biometric examination. Comparisons of such characters have, therefore, been made directly and using shell tracings made with the aid of a "Zeiss" drawing apparatus working on the principal of the camera lucida. The three initial shell parameters have also been plotted on triangular graph paper (as percentages of their sum) to facilitate inter- and intra-specific comparisons of overall shell shape.

(b) EMBRYO SHELL

Embryos were taken from brood pouches of female snails, and tracings of shell outlines were made as above at a magnification of X 400. Embryos from 19 populations representing a wide range of shell forms, and having a wide geographical distribution were examined (Appendix 4). Measurements (Fig. 1) made from shell tracings were:-

(i) Diameter of inner half of the apical whorl at right angles to the shell suture.

(ii) Diameter of whole apical whorl as a continuation of the measurement of whorl 1.

(c) OPERCULUM

Opercula were removed from snails and cleaned for a few minutes in a weak solution of oxalic acid. Permanent mounts were made in polyvinyl alcohol (PVA). Examination was made with a binocular microscope using both bottom and top lighting, the slide being placed on a dark background in order to facilitate the observation of any calcification within the operculum. Outline tracings were made at magnifications of X 160 and X 400.

Opercula of snails from 34 different localities throughout New Zealand, representing a wide range of shell types were examined. (Appendix 1).

(d) RADULA

Radulae were extracted from the buccal mass by boiling for several minutes in dilute (about 4%) KOH. Radulae were stained in picric acid and permanently mounted in PVA. Some radulae were mounted intact, whereas the teeth of others were teased apart to facilitate study. Examination was made at magnifications of X 100, X 400 and X 1000. All measurements were made with a linear eyepiece micrometer. Duplicate counts of cusps, denticles and serrations were made on at least three lateral, inner, and outer marginal teeth from each radula. Radulae from 31 populations were examined. (Appendix 3).

Terminology used in describing radulae is that of Ponder (1965).

Teeth

Central - middle (first) tooth of the radula row

Lateral - second tooth of the radula row (paired)

Inner Marginal - third tooth of the radula row (paired)

Outer Marginal - fourth tooth of the radula row (paired)

Minor Terminology

Cusp - a large, distinct cutting process

Denticle - a small less distinct cutting process

Serrations - fine, numerous cutting processes

The term "cusp formula" is used in a general sense to include all three types of cutting processes.

(e) INTERNAL ANATOMY

The anatomy of snails was examined by dissection, serial sections and the use of stained whole mounts.

(1) Dissections

The most successful dissections were carried out on fresh material in transparent watch glasses or cavity slides, using a stereoscopic microscope. Under such conditions maximum definition and contrast between organs was obtained.

(2) Serial sections

Snails were fixed in Bouin's fluid, sections were cut at 5-10μ, stained with Ehrlich's haematoxylin and counterstained with eosin. Material for sectioning was obtained from the following localities.

P. antipodum from Lake Pupuke (Takapuna, Auckland), Whangarei Falls and Tiritea Stream (Palmerston North).

P. estuarinus from Huia (Manukau Harbour) and Havelock (Marlborough Sounds).

P. pupoides from Havelock.

(3) Whole mounts

Some snails were relaxed using magnesium chloride, whereas others were removed from their shells and transferred direct to Bouin's fluid for fixation. They were then stained in borax carmine for 24 hours, differentiated where necessary in acid alcohol, dehydrated, cleared in clove oil and xylol, and mounted in Canada balsam.

Compared with dissections and serial sections, whole mounts were found to be of little value, particularly as the dense pigmentation of many snails obscured much internal structure.

(f) CHROMOSOME NUMBERS

Determination of chromosome numbers was done using squash methods as recommended by Burch (1960) and Patterson (1967) rather than sectioning as employed by Jacob (1957, 1958). Squash techniques permit better observation of individual chromosomes for several reasons; there is no shrinkage of chromosomes as is caused by paraffin embedding; all chromosomes are brought nearly into a single optical plane by pressure of the cover slip; chromosomes are more spread out; the technique is faster and simpler and allows examination of a greater amount of material.

Shells of freshly obtained snails were cracked and examined immediately without prior fixation, or fixed for 24 hours at 4°C in Carnoy's fluid (alcohol: glacial acetic acid: chloroform, 6:1:3, v/v/v). Material was stored in 70% alcohol in a refrigerator until required.

Small pieces of gonad (both testis and ovary were examined) and digestive gland from the apical whorls were removed and stained in acetic-orcein (1% orcein in 45% acetic acid) for 10-15 minutes on a cavity slide. Material was transferred to a plain microscope slide in a minimum of acetic-orcein, gently squashed under a cover slip and examined using a Leitz binocular microscope with oil immersion and magnification of X 1000.

Material examined consisted of:

P. antipodum from Tiritea Stream, Palmerston North (occasional males found in populations but females only examined), Plimmerton (sexes in approximately equal proportions, both examined), and Shannon (parthenogenetic females only).

P. estuarinus from Huia, Manukau Harbour (males and females examined).

P. pupoides from Plimmerton (both sexes examined).

(g) LABORATORY REARING

P. antipodum has been kept successfully in the laboratory in several kinds of small aquaria. Under these conditions growth was normally continuous and fairly rapid (minimum generation time of about 6 months), and embryos

were released by large numbers of adult snails. Mortality of snails of all ages was negligible.

Transparent plastic boxes (14x11x6cm) with loose-fitting lids made suitable aquaria and were adopted as the "standard habitat" in this study. Boxes were half filled with tap water, and contained several grams of finely sieved pond mud and pieces of Elodea canadensis. No artificial aeration of the water was required. After setting up, the only attention necessary was the maintenance of the water level and the addition of small quantities of sieved pond mud at infrequent intervals. Some supplementary rearing was carried out in 9cm diameter petri dishes, which were particularly suitable for obtaining recently released embryos. Van der Schalie and Davis (1964) have also reported on the suitability of petri dishes as growth chambers for the Oriental hydrobiid Oncomelania formosana.

P. estuarinus and P. pupoides have also been kept in the laboratory for shorter periods of time in similar containers containing mud from their original habitats, and water varying in salinity from 0-35$^{o}/_{oo}$.

RESULTS

(a) SHELL

(1) General shell form

The shells of the New Zealand species of Potamopyrgus are small and plain (apart from periostracal ornamentation), and offer few stable or satisfactory taxonomic characters. Because of their small size and simple form they are difficult to describe in any detail. The shells of

P. estuarinus and P. antipodum overlap in size and form but the shell of P. pupoides is distinct in its small size and more cylindrical shape, although some forms of P. antipodum, e.g. shells from Wananaki (Fig. 4), bear some resemblance to them. The range of shell form in the three species is illustrated in Figs. 3-5.

(2) Shell dimensions

An important aspect of this study has been the biometric examination of shell size, shape and variability within and between populations, as shown by measurements of shell height, shell width and aperture height. It was reasoned that by comparing these parameters from a large number of populations, the nature of the shell variation (i.e. whether continuous or discontinuous variation existed) within the Potamopyrgus complex could be determined. Any discontinuities thus found might be indicative of separate lower taxonomic groupings, which could then be further investigated. Results of this examination are summarised and discussed below.

Maximum shell heights in all populations examined are shown in Fig. 6a. The values obtained for P. antipodum form a distribution possessing a single peak and approximating to a normal curve. Maximum shell height of P. pupoides lies outside this range, whereas that of P. estuarinus is within that of P. antipodum.

The relationship between mean height : aperture height ratio and shell height in populations of P. antipodum is shown in Fig. 7a. It is apparent that no clear correlation exists between the two statistics. Similarly, there is no

FIGURE 3

Outline tracings of fully-grown, smooth shells of *Potamopyrgus* spp. from 21 populations, showing variation in size and shape.

e - *P. estuarinus*

u - *P. pupoides*

all others - *P. antipodum*

Localities

a - Whangarei Falls
b - Whatipu (Manukau Harbour)
c - Waitomo
d - Dannevirke
e - Havelock (Marlborough)
f - Khandallah (Wellington)
g - Kahuterawa River (Manawatu)
h - Tirau (Waikato)
i - Whakarongo (Manawatu)
j - Ohakune
k - Pond A, Massey University
l - Kahuterawa River (Manawatu)
m - Lake Rotoiti (Nelson Lakes)
n - Lake Rotoaira (Taupo)
o - Ilam, Christchurch
p - Franz Josef
q - Linton (Manawatu)
r - Tiritea Stream (Manawatu)
s - Te Kaha (Bay of Plenty)
t - Ashhurst (Manawatu)
u - Havelock (Marlborough)

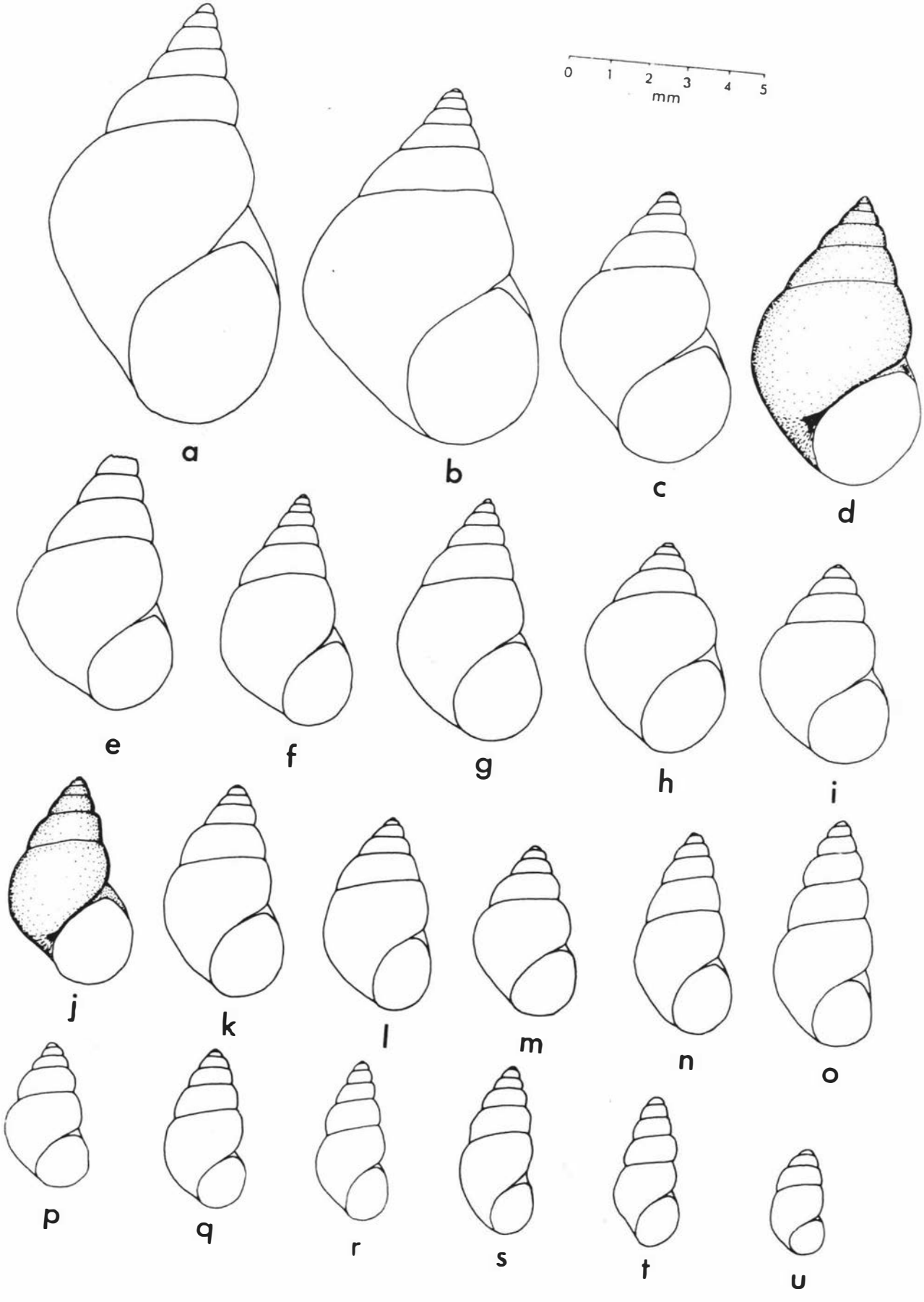

0 1 2 3 4 5
mm
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
u

FIGURE 4

Outline tracings of fully-grown ornamented (spiny) shells of *P. antipodum* from 20 populations, showing variation in size, shape and spine development.

Localities

a - Lake Rupuke, Takapuna
b - Waikato River mouth
c - Auckland Domain
d - Whatipu (Manukau Harbour)
e - Pond A, Massey University
f - Otaki River
g - Pakiri (Northland)
h - Waikato River (Cambridge)
i - Lake Ngahewa (Rotorua)
j - Lake Haringa (Westland)
k - Lake Taupo
l - Lake Ianthe (Westland)
m - Stream, near Otaki
n - Lake Tutira (Hawkes Bay)
o - Lake Tarawera
p - Lake Rotomahana
q - Wenanaki (Northland)
r - Waimangu (Rotorua)
s - Porangahau (Wairarapa)
t - Makamatua Stream (Manukau Harbour)

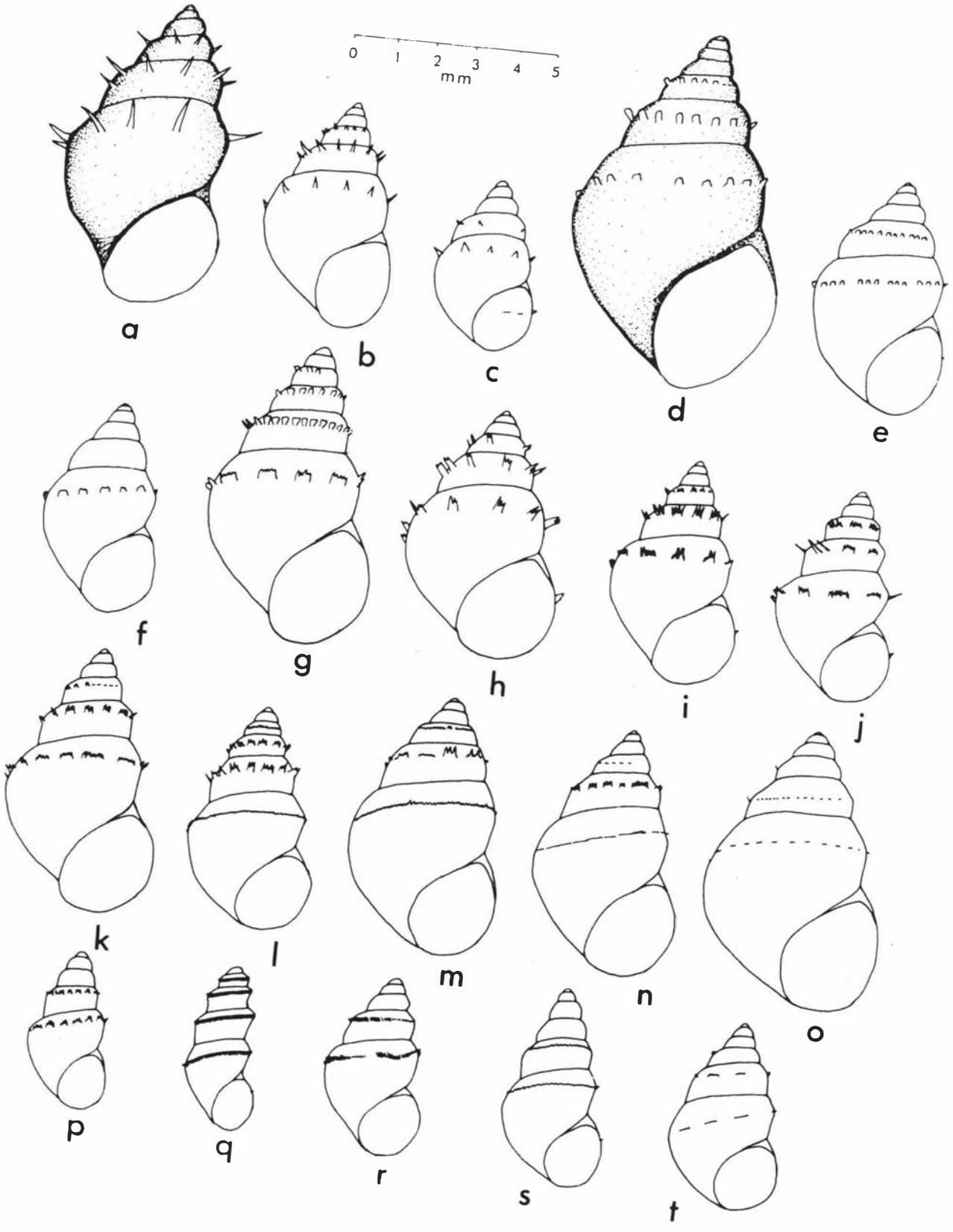
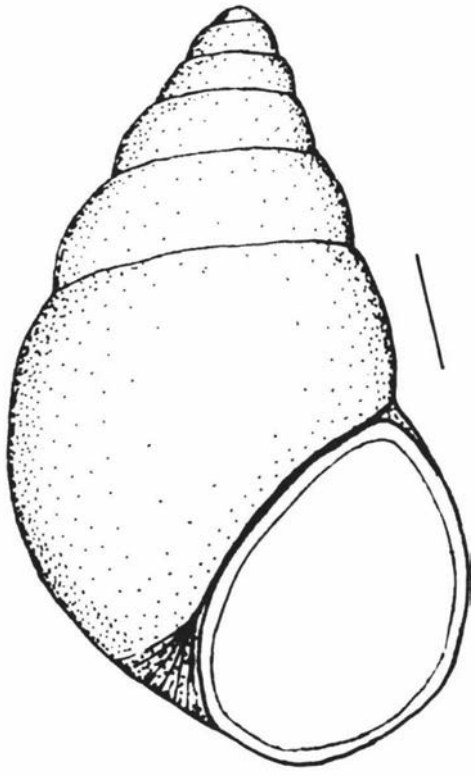

0 1 2 3 4 5
mm
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t

FIGURE 5

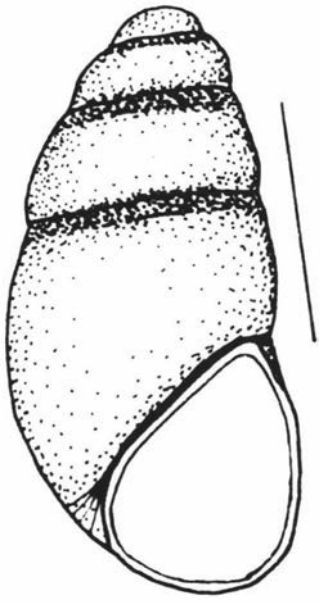
(a) Shell of *P. estuarinus* (Bell Block, New Plymouth)

(b) Shell of *P. pupoides* (Hutt River, Wellington)

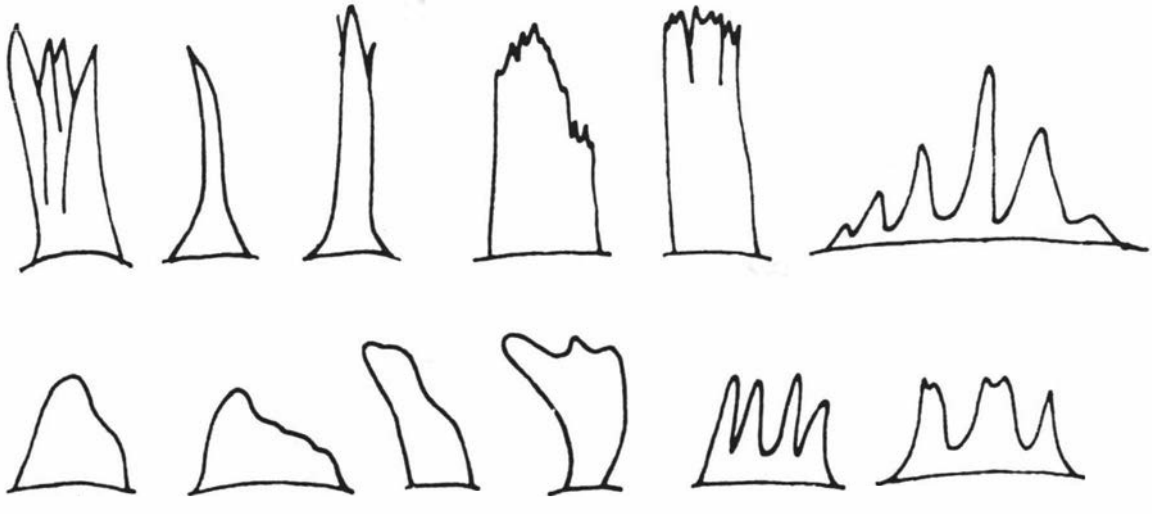
(c) Shapes of periostracal "spines" from shells of *P. antipodum*



a



b



c

FIGURE 6

(a) Maximum shell heights in populations of Potamopyrgus spp.

Solid line - P. antipodum (100 populations)

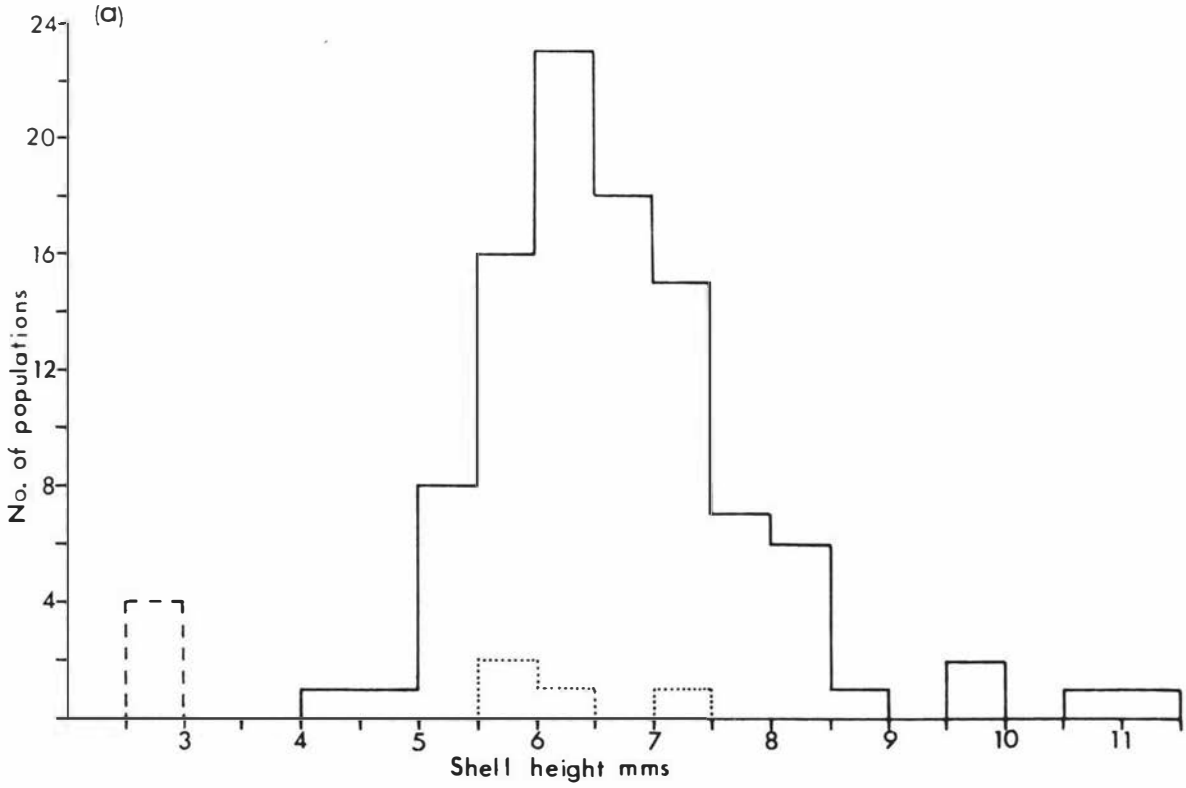
Broken line - P. pupoides (4 populations)

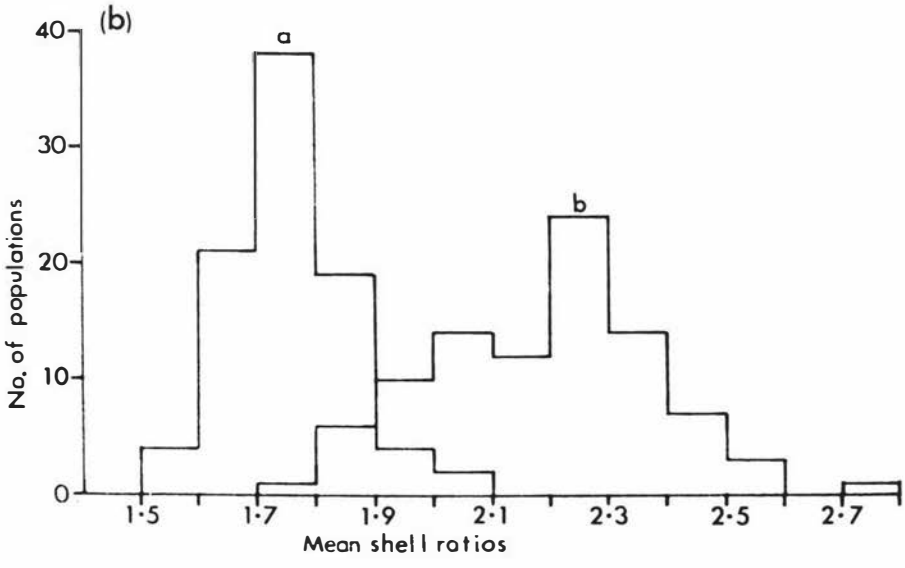
Dotted line - P. estuarinus (4 populations)

(b) Mean height:width (h:w) ratios, and mean height:aperture height (h:ap h) ratios, in 100 populations of P. antipodum

a - h:w ratio

b - h:ap h ratio


(a)
24
20
16
12
8
4
No. of populations
3
4
5
6
7
8
9
10
11
Shell height mms


(b)
40
30
20
10
0
No. of populations
a
b
1·5
1·7
1·9
2·1
2·3
2·5
2·7
Mean shell ratios

clear relationship between the height : width ratio and shell height (Fig. 7b).

When the frequency of occurrence of the mean h/ap h and h/w ratios are plotted for all populations of P. antipodum (Fig. 6b) the distributions are approximately normal, although a wider range of variation is exhibited by the h/ap h ratio. Range of variation of shell ratios within populations is compared in Fig. 8b. The distribution curves have a single peak, but in both cases also have a moderate degree of positive skew. This is probably not significant, however, as some degree of skew, normally to the right (i.e. positive), is to be expected with many zoological variates according to Simpson, Roe and Lewontin (1960), and may generally be ignored.

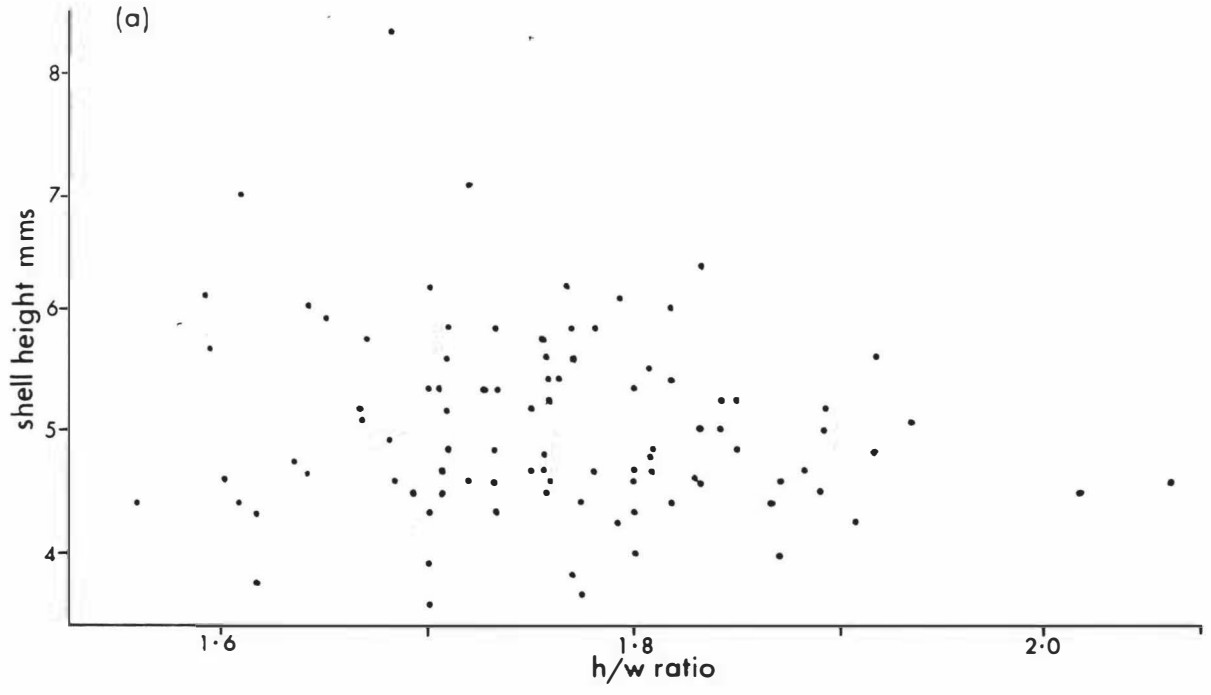
Shell ratios of selected populations, presented as "dice grams" are compared in Figs. 9 and 10. Although some populations of P. antipodum are so unlike that they could be considered at least subspecifically different (Mayr, Linsley and Usinger, 1953), it is clear that continuous variation in shell shape is found within this species. In Figs. 9 and 10 the populations are arranged in order of increasing mean h/ap h ratios, and little correlation between aperture height and shell width is apparent.

Fig. 11 compares shell ratios in three populations at 12 month intervals. Smooth and spiny shelled snails are considered separately. Although shell form has remained relatively constant in some populations, in others a shift in the mean ratios and a change in the extent of variation

FIGURE 7

(a) Mean shell height plotted against mean height:aperture height (h:ap h) ratio, in 92 populations of *P. antipodum*

(b) Mean shell height plotted against mean height:width (h:w) ratio, in 92 populations of *P. antipodum*


(a)
shell height mms
8
7
6
5
4
1·6
1·8
2·0
h/w ratio

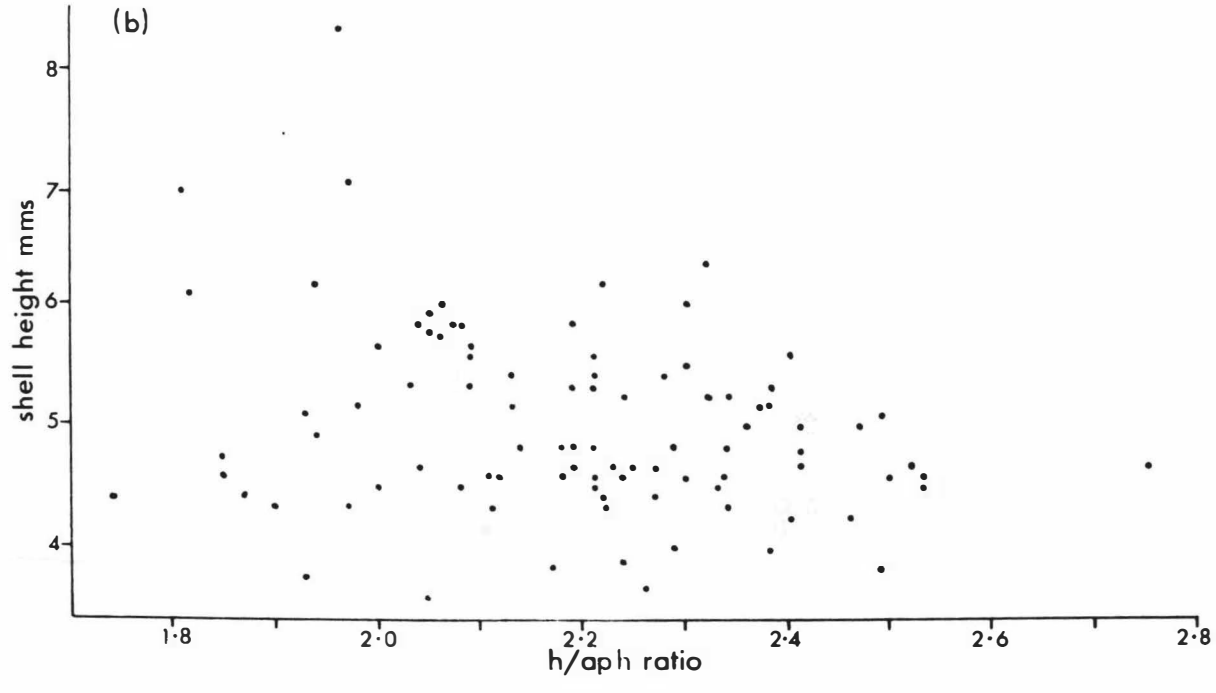

(b)
shell height mms
8
7
6
5
4
1·8
2·0
2·2
2·4
2·6
2·8
h/aph ratio

FIGURE 8

(a) Whorl diameters of embryo shells of *P. antipodum*

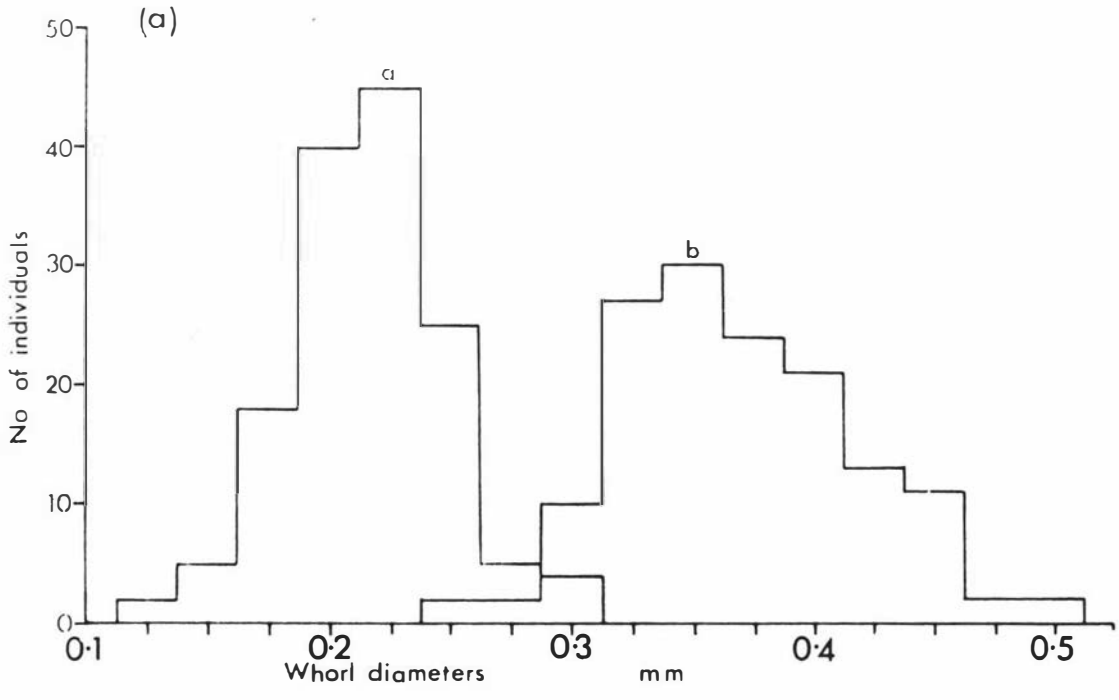
a - inner half of first whorl

b - complete first whorl

(139 shells from 19 populations measured)

(b) Range of variation in shell height:shell width (h:w) ratio, and shell height:aperture height (h:ap h) ratio, in 95 populations of *P. antipodum*

(A minimum of 10 shells from each population measured)


(a)
50
40
30
20
10
0
No of individuals
a
b
0·1
0·2
0·3
0·4
0·5
Whorl diameters
mm

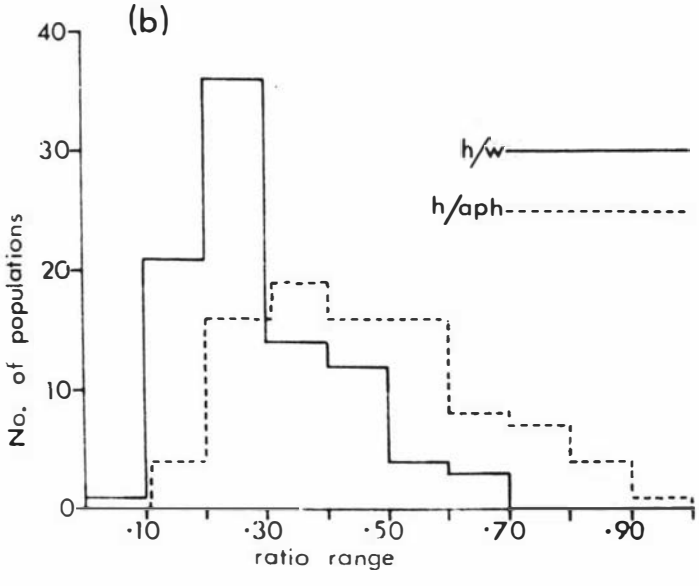

(b)
40
30
20
10
0
No. of populations
h/w
h/aph
·10
·30
·50
·70
·90
ratio range

FIGURE 9

"Dice-grams" showing variation in shell height:shell width (h:w) ratios, and shell height:aperture height (h:ap h) ratios, in 18 selected populations of *P. antipodum*. (Numbers at right are sample sizes.)

Key

Horizontal line - range

Open rectangle - 1 SD

Black rectangle - 1 SE

Vertical bar - mean

Localities

a - Tokomaaru River
b - Whatipu
c - Waiomio
d - Lake Tarawera
e - Waiwakawa River
f - Ohakune
g - Whakarongo
h - Apiti
i - Khandallah
j - Lake Rupuke
k - Dannevirke
l - Bunnythorpe
m - Mount Wharite
n - Pakiri
o - Woodville
p - Makara River
q - Lake Paringa
r - Lake Rotoaira

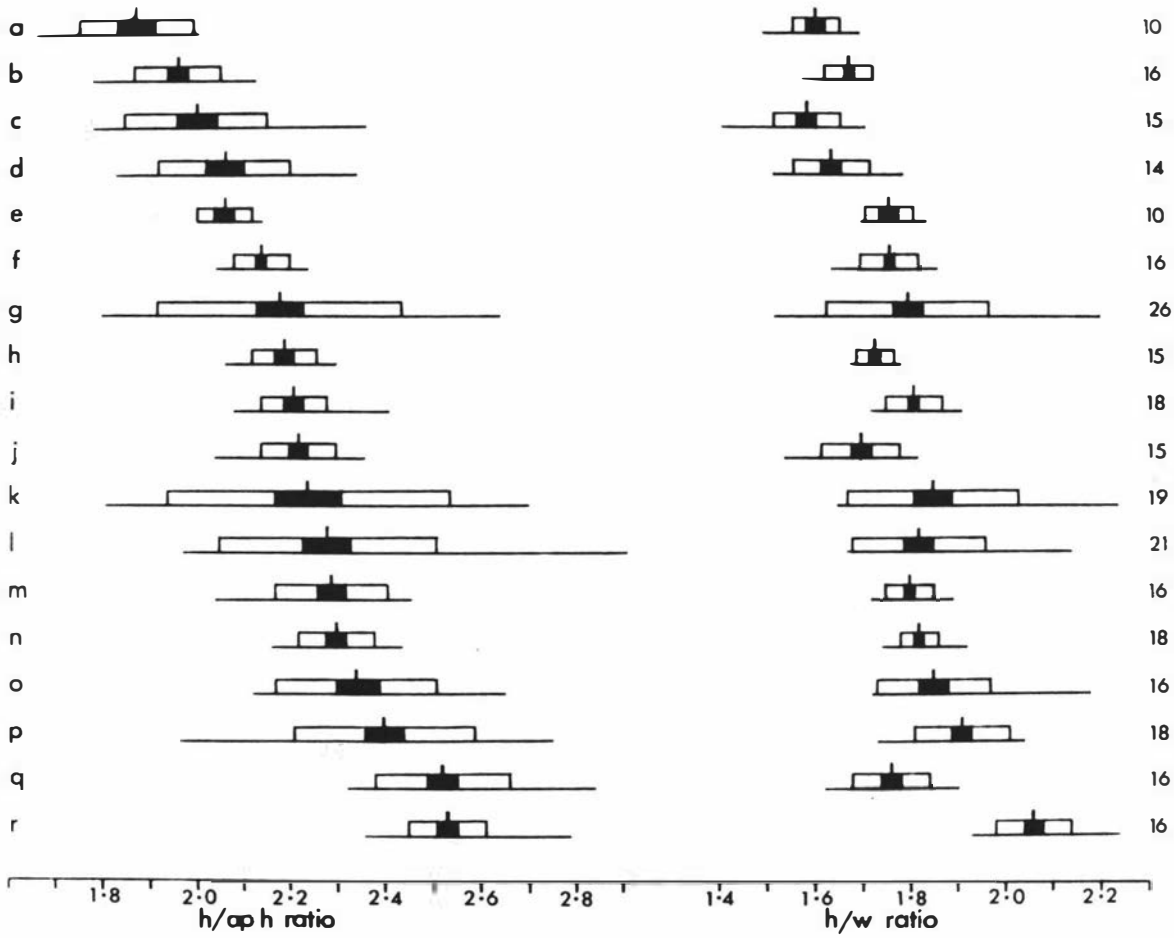

a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
10
16
15
14
10
16
26
15
18
15
19
21
16
18
16
18
16
16
1·8
2·0
2·2
2·4
2·6
2·8
h/ap h ratio
1·4
1·6
1·8
2·0
2·2
h/w ratio

FIGURE 10

"Dice-grams" showing variation in shell height:shell width (h:w) ratios, and shell height:aperture height (h:ap h) ratios, in populations of *P. pupoides* and *P. estuarinus*. (Numbers at right are sample sizes.)

a - d - *P. pupoides*

e - h - *P. estuarinus*

Localities

a - Havelock (Marlborough)
b - Heathcote
c - Wananaki
d - Porirua
e - Heathcote
f - Havelock
g - Huia
h - Kaikato Heads

For Key to "Dice-grams" see Figure 9

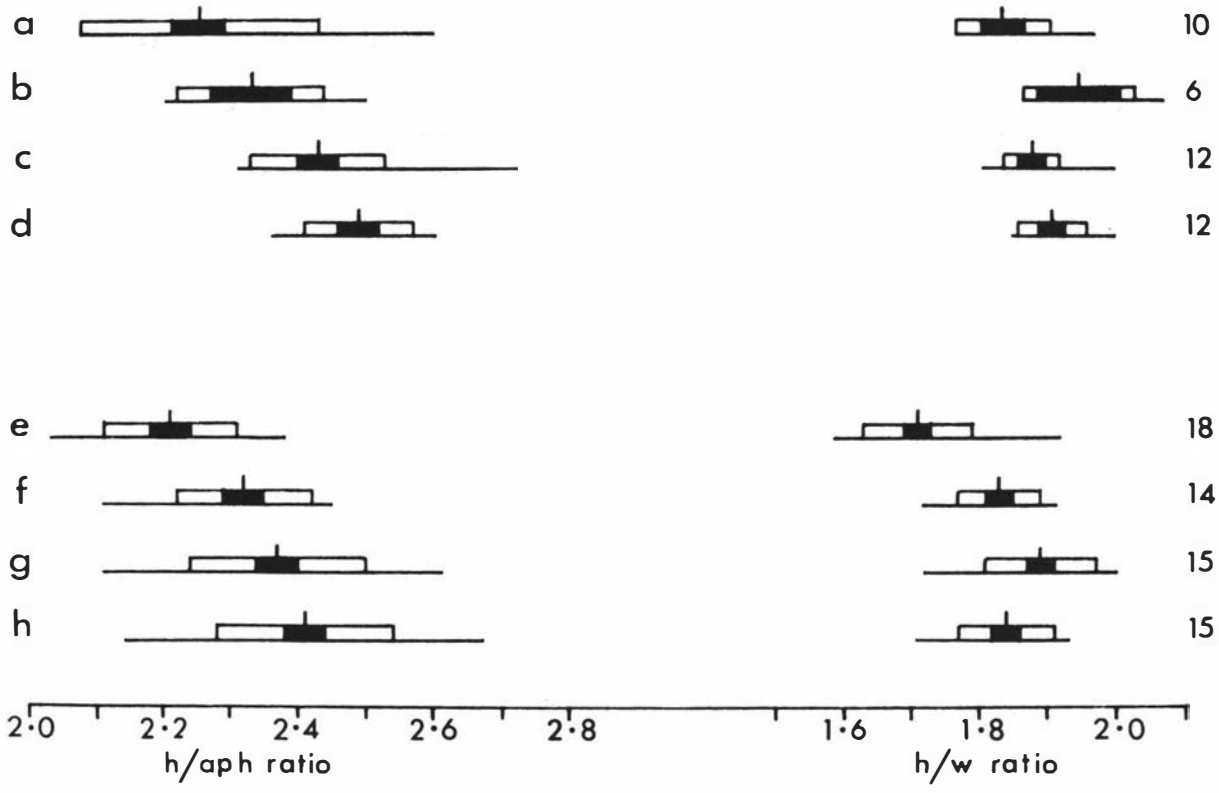

a
b
c
d
e
f
g
h
10
6
12
12
18
14
15
15
2·0
2·2
2·4
2·6
2·8
h/aph ratio
1·6
1·8
2·0
h/w ratio

FIGURE 11

"Dice-grams" showing variation in shell height:shell width (h:w) ratios, and shell height:aperture height (h:ap h) ratios in *P. antipodum* from three populations at 12 monthly intervals (April 1965, above; April 1966, below). Numbers at right show sample sizes.

a - Tiritea Stream (smooth shells)

b - " " (spiny shells)

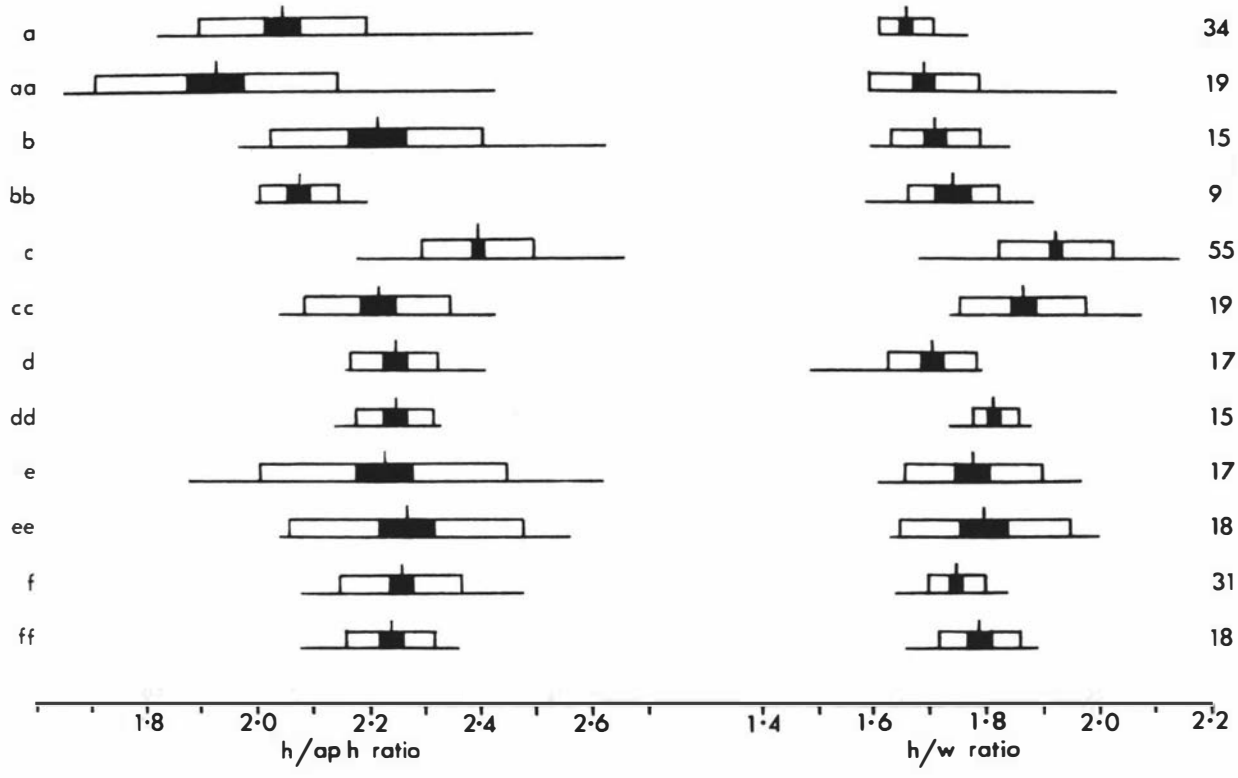
c - Pond A, Massey University (smooth)

d - " " " (spiny)

e - Pond B, " " (smooth)

f - " " " (spiny)

For Key to "Dice-grams" see Figure 9


a
aa
b
bb
c
cc
d
dd
e
ee
f
ff
34
19
15
9
55
19
17
15
17
18
31
18
1·8
2·0
2·2
2·4
2·6
h/aph ratio
1·4
1·6
1·8
2·0
2·2
h/w ratio

present has been found. The total range of variation in shell shape in the three species, as indicated by the three shell measurements is summarised graphically in Fig. 12a.

Finally, the results of an examination of whorl numbers from full-grown shells in 100 populations of P. antipodum are presented in Table 2. Considerable variation is found between populations but no clearcut divisions into discrete groups is apparent, although as a general rule the taller the shell the greater the number of whorls developed.

TABLE 2.

Numbers of complete shell whorls compared with shell heights in 100 populations of P. antipodum.

Shell height mm	Whorls					Totals
	4	5	6	7	8	
3-3.9	1	13	1			15
4-4.9		23	30	1		54
5-5.9	1	4	15	2		22
6-6.9		1	5	1		7
7-7.9			1			1
8-8.9					1	1
Totals	2	41	52	4	1	100

To conclude, measurement of shell parameters has not provided evidence of clearcut morphological groups existing within the P. antipodum complex but rather has shown the existence of continuous variation of size and shape throughout the group. P. pupoides is easily distinguished by its small more "cylindrical" shell, but the shell of P. estuarinus is indistinguishable from some forms of P. antipodum.

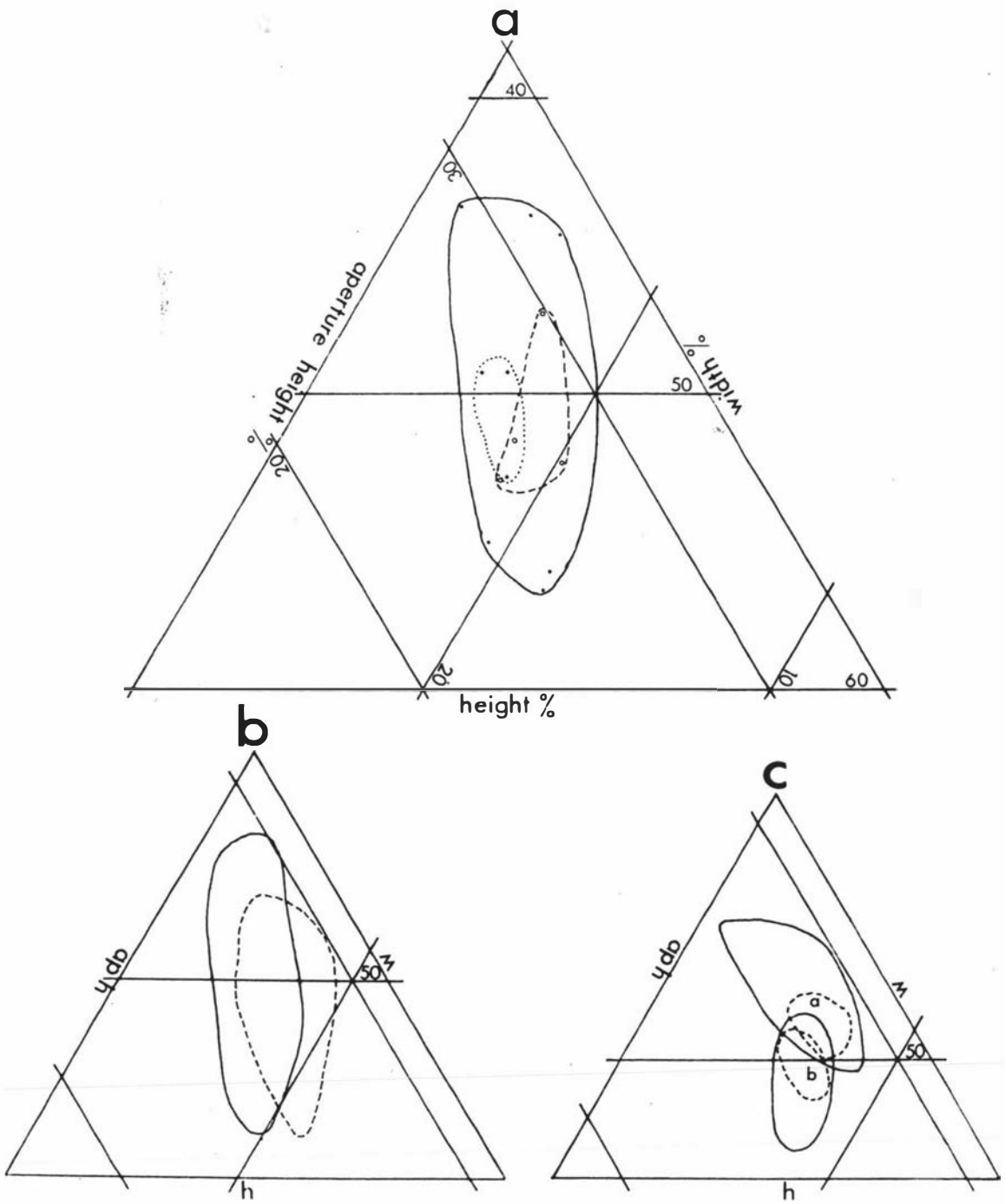

a
40
30
20
aperture height %
width %
50
20
10
60
height %
b
apth
w
50
h
c
apth
w
a
b
50
h

FIGURE 12

(a) Comparative graphical representation of variation in shell shape, as indicated by relative proportions of shell height, shell width and aperture height

Solid line - *P. antipodum*

Broken line - *P. estuarinus*

Dotted line - *P. pupoides*

(b) Maximum variation in shell shape found in single populations of *P. antipodum*

Solid line - Whakarongo (26 shells measured)

Broken line - Bunnythorpe (21 shells measured)

(c) Variation in shell shape in a field sample, and in the progeny of a single individual from that population, reared in the laboratory

a - Shannon (17 field and 17 laboratory reared individuals measured)

b - Pond A, Massey University (19 field and 19 laboratory reared individuals measured)

Solid lines - field

Broken lines - laboratory reared

All three graphs are drawn to the same scale

(3) Shell ornamentation

The presence or absence of spines has been considered important in the separation and identification of some species of Potamopyrgus (Suter, 1905, 1913), but field observations and rearing experiments made in the course of this study have shown that within the P. antipodum complex, considerable variation in degree and nature of shell ornamentation is found, even, in many cases, within a single population (Fig. 13). Also, the development of spines may or may not be associated with shouldering of the whorls. The shape of individual spines is shown in Fig. 5c. In some cases each spine is separate but in others a number of projections have a common base, culminating in the totally fused condition found in some shells such as those from Wananaki (Fig. 4q).

Ornamentation is purely periostracal as in P. jenkinsi (Boycott, 1929), and no calcium is found in the spines since they remain intact and continuous with the periostracum when the shell is decalcified with HCl. It has been suggested that the periostracum is a quinone-tanned protein (Brown, 1952; Degens, Spencer and Parker, 1967).

(4) Laboratory rearing programme

P. antipodum was reared in the laboratory in order that shell form and ornamentation of progeny of known parent snails could be examined. Other investigators have considered that much shell variation is the result of exposure to different environmental conditions (Dell, 1953, 1956; Hunter, 1961), and this has been observed in Simlimnaea tomentosa reared under various conditions in the

FIGURE 13

Outline tracings of shells showing the variation in shape and ornamentation in five populations of P. antipodum

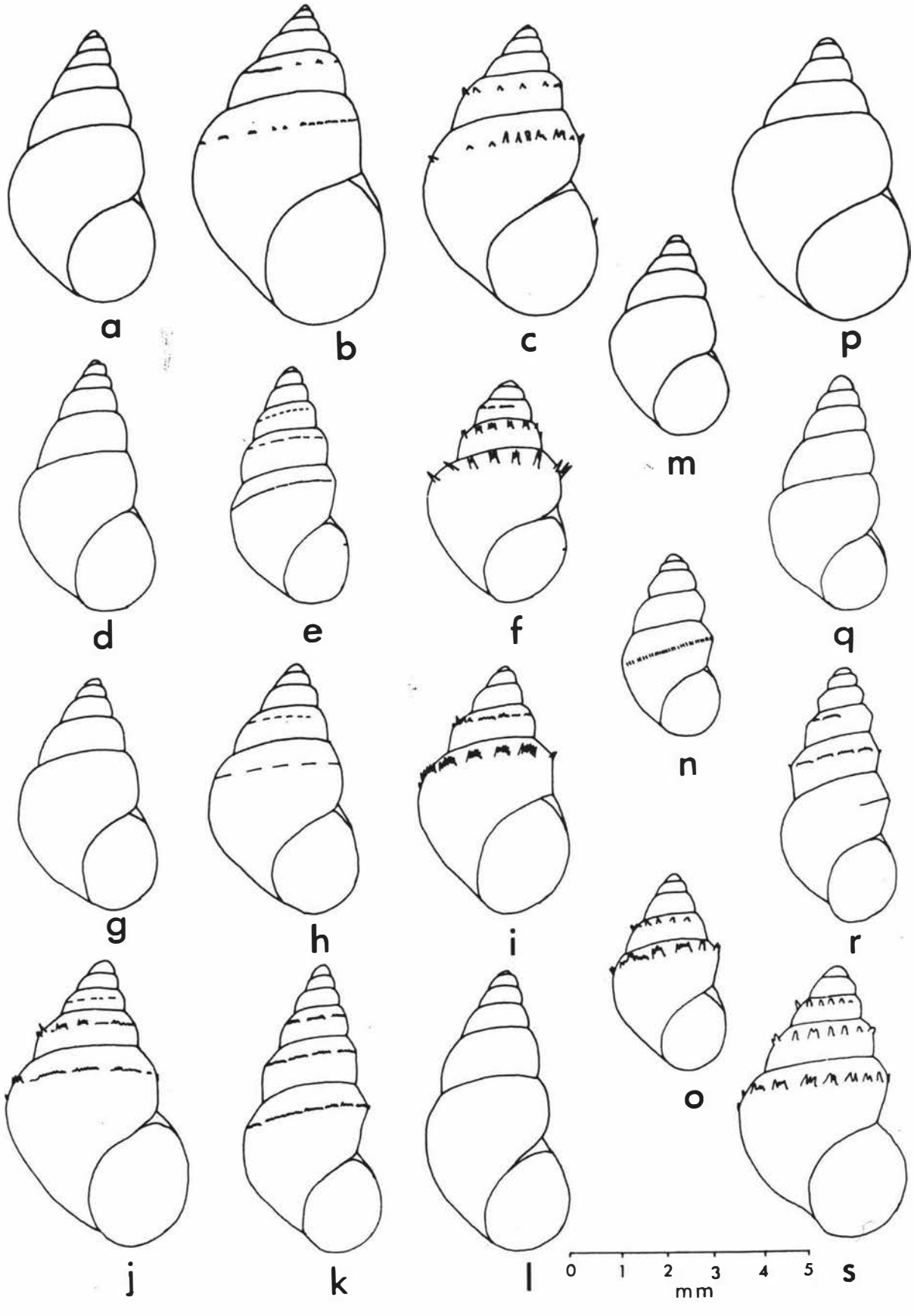
a-c - Piha Stream

d-f - Kahuterawa River tributary

g-i - Stream by Lake Rotowhero

j-l - Bunnythorpe (stream)

m-s - Makara River


a
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c
p
d
e
f
m
q
g
h
i
n
r
j
k
l
o
s
0 1 2 3 4 5
mm

laboratory by Boray and McMichael (1961). In this study the experimental situation has been reversed and snails taken from differing environments have been reared in the laboratory under identical conditions. Experimental populations were maintained for a period of three years.

(i) Ornamentation

Table 3 summarises the type of shell ornamentation developed by progeny of 32 snails from 12 populations. Of 14 smooth parent snails, nine produced totally smooth young and five both smooth and spiny young. No smooth parent was found to produce only spiny juveniles. Of 18 spiny adult snails, however, only three produced all spiny young, three produced both spiny and smooth young, and 12 produced smooth young. In all cases snails from natural populations consisting solely of smooth snails bred true in the laboratory. A contrasting situation existed when comparing snails from Lakes Paringa and Eltawater, both of which are predominantly spiny in the field. Whereas under laboratory conditions progeny of the former were predominantly spiny, three out of four snails from the latter produced only smooth shelled young.

As snails from different populations were reared under identical laboratory conditions it is impossible to infer environmental influences as the only factors determining shell ornamentation. This must therefore have a genetic basis (see p. 92). Results of a more comprehensive rearing experiment are summarised in Table 4. All of the P_1 generation were taken from Massey Pond which consists solely

TABLE 3.

Results of laboratory rearing of snails showing development of shell ornamentation.

Population	Shell form of Parent	Juveniles Smooth	Juveniles Spiny	Shell form in Natural Habitat	Habitat*
Shannon	Spiny	6	-	All extremes from strongly spiny to smooth	Ditch
"	"	49	-		
"	"	25	-		
"	"	46	-		
"	"	40	-		
"	"	25	-		
"	"	6	-		
"	"	7	-		
"	Smooth	30	-		
"	"	28	-		
Porangahau	Smooth	20	-	Mixed	Ditch
Tiritea	Smooth	79	2	Smooth dominant	Stream
"	"	25	-		
Makara	Smooth	22	1	Mixed shells	Stream
"	"	10	1		
"	"	3	3		
"	Spiny	5	2		
Tokomaaru	Smooth	6	1	Mixed	Lowland river
"	Spiny	-	14		
Khandallah	Smooth	10	-	All smooth	Brook
Paringa	Spiny	-	18	Almost all spiny	Lake
"	"	2	35		
Eltawater	Spiny	-	17	Almost all spiny	Lake
"	Slight spiny	25	-		
"	Spiny	56	-		
"	"	15	-		
Otaki	Spiny	16	-	Mixed	Stream
"	"	16	6		
Eastbourne	Smooth	5	-	All smooth	Trickle
Franz Josef	Smooth	13	-	All smooth	Stream
" "	"	9	-		
Ohau	Smooth	12	-	Mixed	Lowland river

*Habitat classification after Elton and Miller (1954).

of parthenogenetic females, and in which smooth and spiny shelled snails are present in approximately equal numbers.

All generations were kept under identical experimental conditions and a considerable amount of variation in shell ornamentation was found between the progeny of siblings, and between generations.

TABLE 4.

Results of laboratory rearing experiments using partheno-genetic females taken from Pond A, Massey University Campus.

P_1	F_1	F_2	F_3
Smooth shelled Adult	Smooth	35 smooth	
	Smooth	37 spiny	a sp → 3 spiny, 2 smooth
			b sp → 10 spiny
			c sp → 22 spiny
	Smooth	43 smooth	
	Smooth	-	
	Spiny	20 spiny	a sp → 33 spiny
		4 smooth	b sp → 10 spiny
			c sp → 16 spiny
Spiny shelled Adult	21 smooth	a sm → 43 smooth	
	20 spiny	b sm → 12 smooth	
		c sm → 5 smooth	
		d sm → 6 smooth	
		e sm → 20 smooth	
Spiny shelled Adult	56 smooth		
	3 spiny		
4 smooth shelled Adults	92 smooth	a sm → 25 smooth	a sm → 11 smooth
		b sm → 24 smooth	
		c sm → 35 smooth	
		d sm → 27 smooth	
		e sm → 20 smooth	
		f sm → 8 smooth	

(ii) Shape

In contrast to results obtained in ornamentation inheritance experiments, it was found that overall shell form (size, whorl convexity and proportions of shell parameters

but not shouldering of whorls, which is apparently linked with spine development), of young snails closely resembled that of the parent. This held for snails from 12 widely differing habitats, reared under the same environmental conditions. Range of shell variation between daughter snails is slight and less than that found in samples of randomly selected adult snails from the original habitats (Fig. 12c). It therefore seems unlikely that environmental changes which may occur in the habitat will result in immediate, large scale phenotypic changes in shell shape in the immediately following generations.

(b) EMBRYO SHELL

The embryo shell is the protoconch of the free-living snail, and is frequently of different design, texture and colour from the adult snail. Often molluscs with similar adult shells have different protoconchs which can therefore be of considerable assistance in determining relationships (Powell, 1957). Even in closely related species the shell apices may often be different, probably due to different modes of development (Dall, 1924). Berry (1943), discussing the Michigan Hydrobiidae, considered the shape and position of the protoconch to be one of the best taxonomic characters of the shell.

In Potamopyrgus the shell apex is often broken, severely eroded or encrusted in older shells, but as an embryo the shell is semi-transparent and the young mollusc is visible in outline within it. There is no ornamentation

on the shell but transverse growth rings are visible. The embryo shell possesses one and a half whorls when released from the parent's brood pouch and in older snails is not differentiated from later developed shell.

When the diameters of the internal half of the apical whorl and the whole first whorl of embryo shells of P. antipodum are plotted in histogram form (Fig. 8a), a single peak is obtained in each case, and the distributions are approximately normal. More variation is found in the first whorl, as by this time more growth has occurred and there is more room for individual variation. The range of measurements found in four populations is given below.

Population	Nos. of shells measured	1st half whorl microns	1st whorl microns
Lake Rotoiti (Nelson)	10	15 - 20	33 - 35
Lake Pupuke	10	12 - 25	25 - 35
Mt. Wharite	10	12 - 25	28 - 40
Lindis Pass	10	20 - 25	38 - 50

It is clear that almost as much variation in size can occur within a single population as between all populations, and that neither qualitative characters nor whorl measurements are of value in distinguishing taxonomically distinct forms.

(c) OPERCULUM

The structure of the operculum has been widely used as a taxonomic character in gastropod classification, but it appears to have been of little value in separating members

of the Hydrobiidae below the generic level. Berry (1943), considering the Michigan Hydrobiidae, regarded variation in the form of the operculum within a species as being too great for it to be a reliable character. However, it does help to separate subfamilies, and Cotton (1943) produced a key to what Hubendick (1955) calls "the 13 so-called genera of Australian Paludestrinidae", largely based on opercular characters.

Hydrobiid opercula were described by Stimpson (1865) as like those of the Rissoinae (= Rissoidae), in being subspiral, and not provided with a process. He describes the operculum of Potamopyrgus simply as corneous. Suter (1913) did not elaborate on this statement, but Ponder (1967) has provided a clear figure of the operculum of P. antipodum and has noted the presence of a calcareous smear close to the columella edge.

The following more detailed description is based on all material examined in this study. Terminology used is that of Ponder (1965).

The operculum (Fig. 14b) is flat, thin, semi-transparent, colour range yellowish - golden - brown; oval to ear shaped; the shape and size dependent on that of the shell aperture. Nucleus subcentral; subspiral growth lines clearly visible; no distinct marginal area; left end more pointed than right; outer margin more strongly curved than inner; muscle insertion area indistinct; a narrow, clear, quasi crescentic area extending over half the length of the operculum is present close to the inner margin. The clarity

FIGURE 14

(a) Radular teeth

(b) Operculum (outer side)

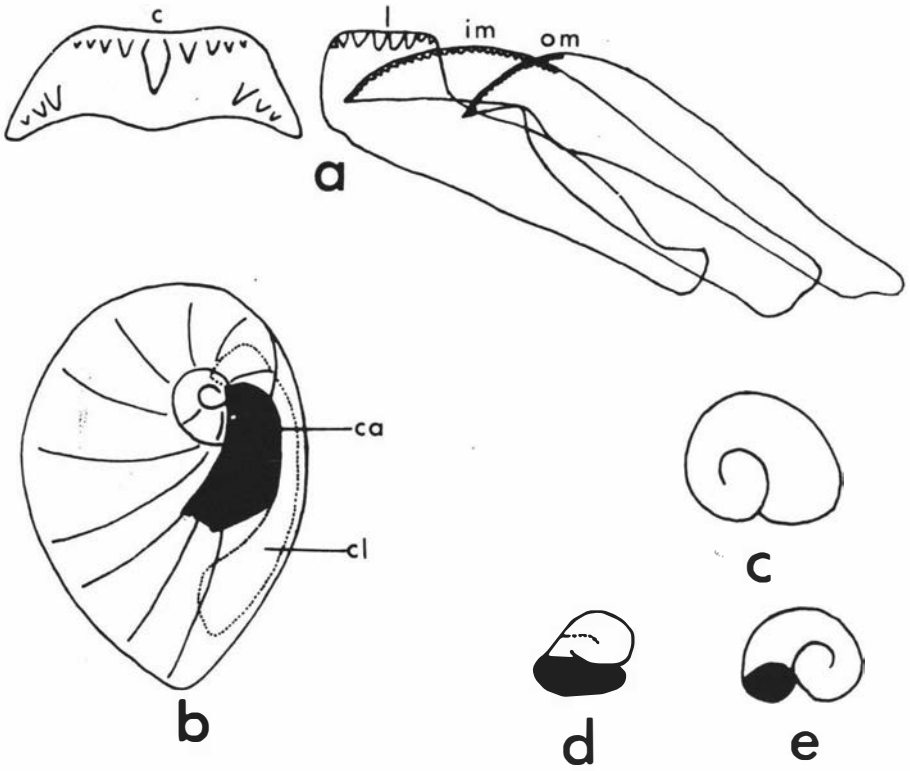
(c-e) Embryo shells

(f) Animal extended (ventral)

(g) Head pigmentation (dorsal)

Abbreviations

c - central	t - tentacle
l - lateral	m - mouth lobe
im - inner marginal	mg - mucous groove
om - outer marginal	ap - aperture
ca - calcareous smear	g - granule
cl - clear area	op - position of operculum


c
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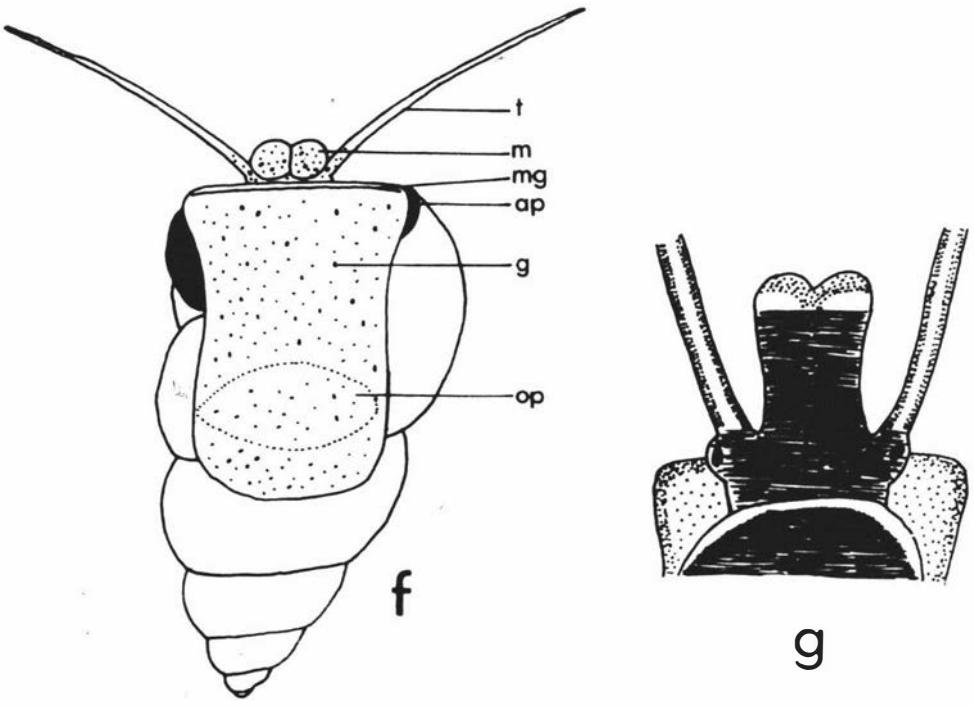

t
m
mg
ap
g
op
f

g

of this area is somewhat variable. A small, irregularly shaped, calcareous smear is usually present to the right of the nucleus. The extent and degree of calcification is also variable but it is clearly visible when the operculum is viewed with top lighting against a dark background.

The operculum is of no value in distinguishing the New Zealand species of Potamopyrgus.

(d) RADULA

The structure of the radula has been of major importance in the classification of Hydrobiidae particularly at the generic level (Thiele, 1928, 1931; Wenz, 1938-44; Stimpson, 1865; Pilsbry, 1911; Berry, 1943). However, it has been shown that considerable intraspecific variation in tooth morphology may occur (Krull, 1935; Berry, 1943; Solem, 1955; Muus, 1963; Davis, 1966), and therefore differences found must be evaluated with extreme caution before their taxonomic value can be determined.

The radula of Potamopyrgus is taenioglossan, possessing the typical row formula 2:1:1:1:2. No important differences in general tooth morphology have been found between species, and representative teeth are illustrated in Fig. 14a. Slight variations are found in the positions of teeth on the radular ribbon with respect to one another. Some individuals have a clear space between the central and lateral teeth, whereas in others no gap is found. This may vary, even within populations.

Radular length generally increases with an increase in snail size (Fig. 15), radulae of full-sized P. pupoides measuring up to 0.6mm, whereas those of the other two species may be up to 1.4mm long.

In all three species radulae of fully grown individuals examined possessed 62 to 93 rows of teeth (Appendix 3). The rows of teeth are closer together in P. pupoides than in P. estuarinus or P. antipodum (Fig. 16b).

Cusp formulae for the three species are given below.

P. pupoides

$$\frac{(4\text{-}5)\text{-}1\text{-}(4\text{-}5)}{(4\text{-}5)\ \text{-}\ (4\text{-}5)} : 9\text{-}11 : 21\text{-}25 : 29\text{-}30$$

P. estuarinus

$$\frac{(3\text{-}4)\text{-}1\text{-}(3\text{-}4)}{3\ \text{-}\ 3} : 8\text{-}9 : 14\text{-}19 : 21\text{-}35$$

P. antipodum

$$\frac{(3\text{-}5)\text{-}1\text{-}(3\text{-}5)}{(3\text{-}5)\ \text{-}\ (3\text{-}5)} : 7\text{-}13 : 15\text{-}32 : 24\text{-}48.$$

Results of a detailed study of cusp variation in three populations of P. antipodum are presented in Table 5 and Figure 16a.

Variations in cusp formulae are considerable, particularly in P. antipodum. Within this species similar shell forms may have very different formulae, whereas conversely, very different shell forms sometimes have similar radulae. Some of these relationships can be examined by reference to Appendix 3 and Figs. 3 and 4. Muus (1963) and Davis (1966) both report that cusp variation is also commonly found within individual hydrobiid radulae, although variants are normally a small minority (often 2% or less). Davis gives

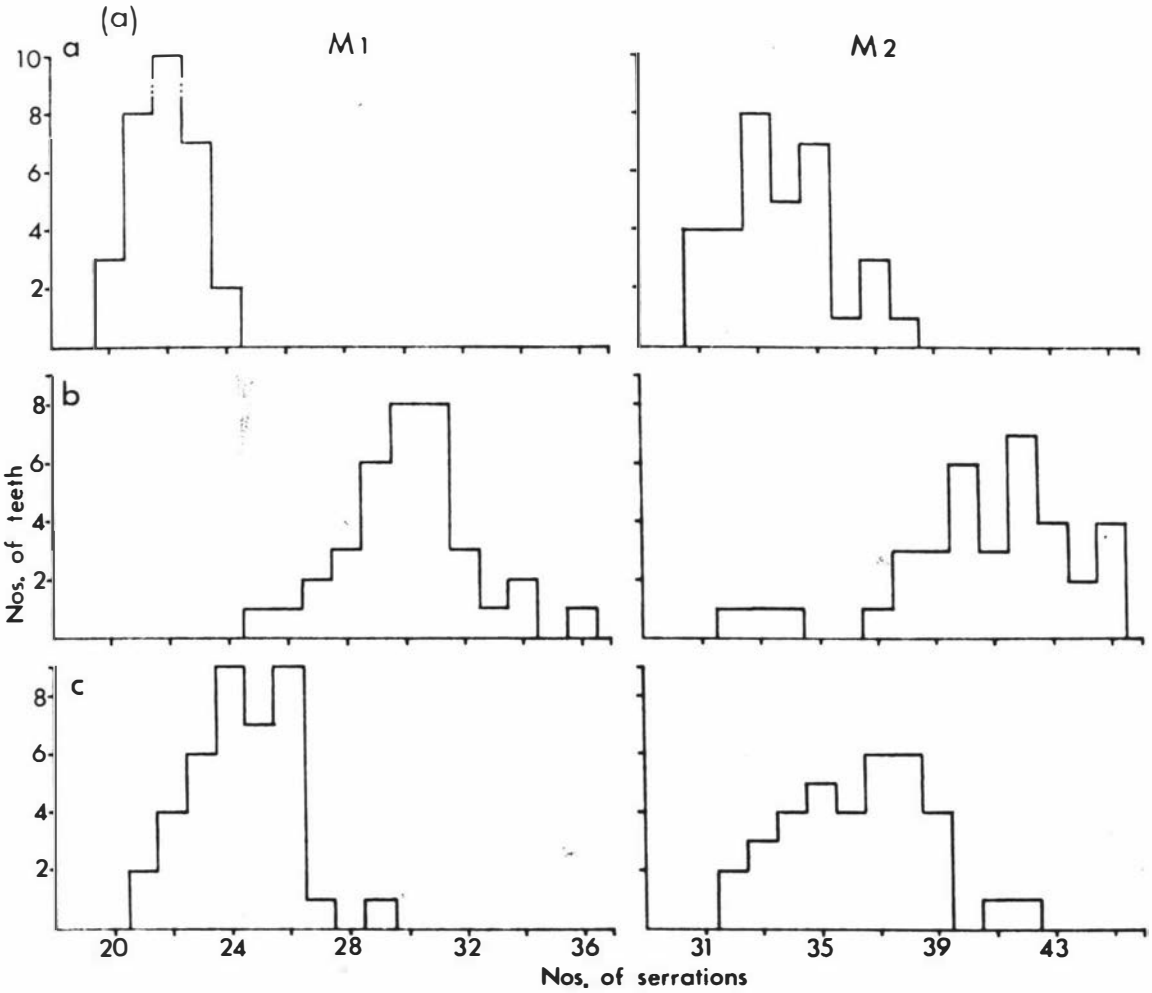
FIGURE 15

Radula length plotted against shell height in 14 populations of *Potamopyrgus* spp.

p - *P. pupoides*

e - *P. estuarinus*

all other points - *P. antipodum*


(a)
a
M1
M2
10
8
6
4
2
b
8
6
4
2
Nos. of teeth
c
8
6
4
2
20
24
28
32
36
31
35
39
43
Nos. of serrations

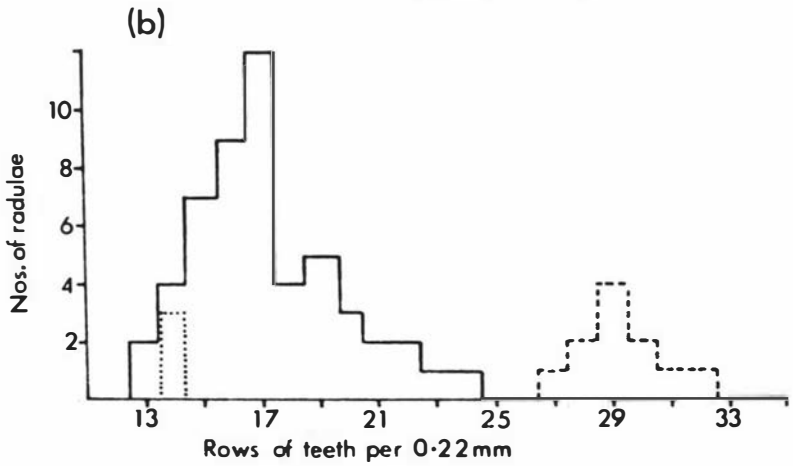

(b)
10
8
6
4
2
Nos. of radulae
13
17
21
25
29
33
Rows of teeth per 0·22mm

FIGURE 16

(a) Numbers of serrations on inner and outer marginal teeth in three populations of *P. antipodum*. (Twelve radulae examined from each population; counts made from three teeth in each row of each radula.)

M1 - inner marginal

M2 - outer marginal

a - Pond A, Massey University

b - Tiritea Stream

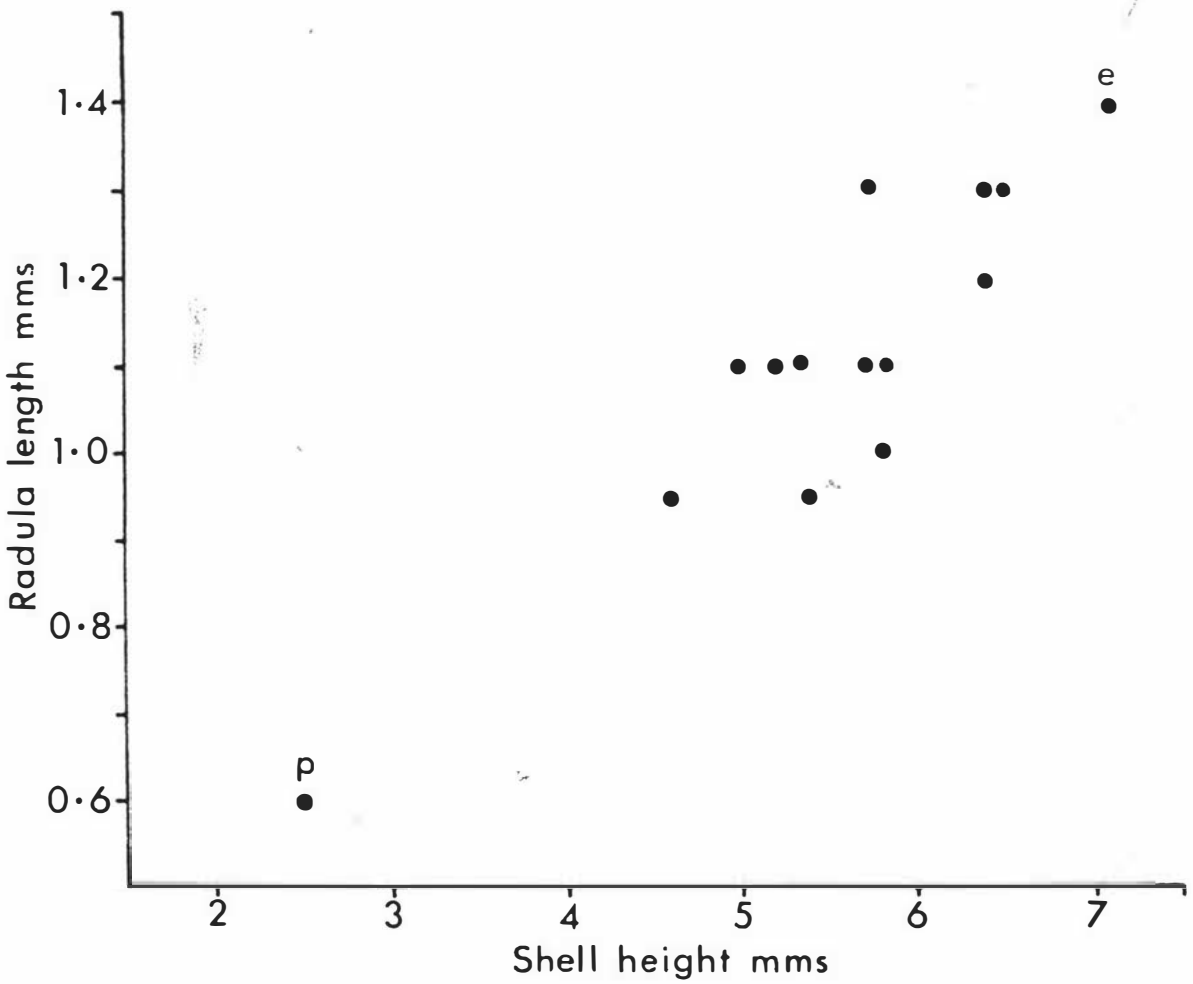
c - Makara River

(b) Number of rows of teeth per unit length of radula (0.22mm, in middle of ribbon) in *Potamopyrgus* spp.

Solid line - *P. antipodum* (52 radulae)

Broken line - *P. pupoides* (11 radulae)

Dotted line - *P. estuarinus* (3 radulae)


Radula length mms
1·4
1·2
1·0
0·8
0·6
p
e
2
3
4
5
6
7
Shell height mms

of this area is somewhat variable. A small, irregularly shaped, calcareous smear is usually present to the right of the nucleus. The extent and degree of calcification is also variable but it is clearly visible when the operculum is viewed with top lighting against a dark background.

The operculum is of no value in distinguishing the New Zealand species of Potamopyrgus.

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Cusp formulae for the three species are given below.

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$$\frac{(4\text{-}5)\text{-}1\text{-}(4\text{-}5)}{(4\text{-}5)\ \text{-}\ (4\text{-}5)} : 9\text{-}11 : 21\text{-}25 : 29\text{-}30$$

P. estuarinus

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P. antipodum

$$\frac{(3\text{-}5)\text{-}1\text{-}(3\text{-}5)}{(3\text{-}5)\ \text{-}\ (3\text{-}5)} : 7\text{-}13 : 15\text{-}32 : 24\text{-}48.$$

Results of a detailed study of cusp variation in three populations of P. antipodum are presented in Table 5 and Figure 16a.

Variations in cusp formulae are considerable, particularly in P. antipodum. Within this species similar shell forms may have very different formulae, whereas conversely, very different shell forms sometimes have similar radulae. Some of these relationships can be examined by reference to Appendix 3 and Figs. 3 and 4. Muus (1963) and Davis (1966) both report that cusp variation is also commonly found within individual hydrobiid radulae, although variants are normally a small minority (often 2% or less). Davis gives

TABLE 5.

Summary of variation in numbers of cusps, denticles and serrations on the radula teeth of P. antipodum from three populations*

Population	Central	Lateral	Inner Marginal	Outer Marginal
Massey Pond	$\frac{(4-5)-1-(4-5)}{(3-4)-(3-4)}$	9 - 11	20 - 25	31 - 47
Tiritea Stream	$\frac{5-1-5}{(3-4)-(3-4)}$	9 - 11	25 - 35	32 - 45
Makara River	$\frac{(4-5)-1-(4-5)}{(3-4)-(3-4)}$	9 - 11	21 - 29	32 - 42

*Examination of (1) Twelve snails per population
(2) Duplicate counts of cusps, denticles and serrations on at least three teeth per row per radula.

the extent of known variation within Hydrobia totteni as follows:

$$\frac{(2-5)-1-(2-5)}{1-1} : 6\text{-}9 : 10\text{-}13 : 8\text{-}12$$

When this is compared with species of Potamopyrgus, the variation in cusp number on the central tooth is found to exceed that of any of the New Zealand species. Cusp variation on the lateral lies between that found in P. pupoides and P. antipodum, and that of the marginals can probably be considered comparable to that found in P. pupoides and P. estuarinus but less than in P. antipodum. Comparison is difficult here because of the overall reduction in cusp number in H. totteni.

To conclude, P. pupoides can be separated from P. estuarinus and P. antipodum using radular characters (it is smaller, and the rows of teeth are closer together), but within P. estuarinus and P. antipodum there is sufficient variability in shape, cusp formulae and radula-shell ratios to prevent specific differences being defined.

Hutton's (1882) cusp formulae for four New Zealand "species" cannot, therefore, be given the diagnostic importance he gave them. The minor variations in tooth shape shown in his figures appear to have been produced by orientation of the radula for illustration rather than by true structural differences, and the radula dimensions he provided are all too large. Ponder's (1967) figure of the radula of P. antipodum is also inaccurate. The central tooth is somewhat distorted with excessively long and narrow basal processes, and misplaced basal cusps, and the lateral shows too few denticles on the cutting edge.

When radulae of P. antipodum and P. estuarinus are compared with those of the European P. jenkinsi described by Woodward (1892) and Krull (1935) (new material also examined) shape of teeth, cusp formulae, radula length and numbers of rows of teeth are all found to lie within the same range. Woodward states that the laterals do not project beyond the centrals but are clear of them, although in the New Zealand species both conditions occur. It seems likely that further studies of the radula of P. jenkinsi would show the existence of more variation than has been recorded to date.

The Venezuelan species placed in Potamopyrgus by Baker (1930) differ from the New Zealand species in the shape of the outer marginal tooth and by the possession of a peg on the lateral tooth of the radula. These, and other anatomical differences suggest that they do not in fact belong in Potamopyrgus.

(e) EXTERNALS OF ANIMAL (Figs. 14 f and g)

The external morphology of the three species is identical, except for differences in size, and intensity of head and mantle pigmentation. The following description applies to all three species.

Tentacles long and slender, clear, with black pigment distributed as in Fig. 14g. Eyes with prominent pigment cups, in bulges at bases of tentacles. Rostral pigment distributed in fine transverse bands, dark and evenly dispersed in P. pupoides and P. estuarinus but often lighter and more variable in P. antipodum. Mouth lobes white, normally with grey crescentic markings dorsally. Pigmentation of head behind level of the eyes always dark. Buccal mass often visible dorsally near the base of the rostrum. Foot broad, grey, with a stippled appearance, rounded posteriorly, truncated anteriorly, the anterior margin nearly straight, the antero-lateral angles somewhat auriculated. The anterior mucus slit is prominent and extends the width of the foot. Mantle skirt black, with a well defined, pale, anterior margin. Large numbers of shining white "granules" are found in the foot and pale

mantle edge, and frequently in the mouth lobes and tentacles close to the eyes. They do not appear to be distributed in an organized manner. Similar "granules" have been described by Davis (1966) in the tissues of H. totteni.

Robson (1920) has noted considerable variability in colour and pigmentation of P. jenkinsi, and Warwick (1952) has suggested that it may exist in three strains, differing in mantle pigmentation as well as shell shape. Heywood and Edwards (1962), however, consider that lack of pigmentation in some populations of P. jenkinsi is probably a phenotypic response to environmental conditions. Both Fretter and Graham (1962) and Muus (1963) state that head pigmentation is distinctive in different European species of Hydrobia, and is a useful aid in identification. Their respective figures are contradictory, however, and do not convincingly support this view. Davis (1966) has also noted that head pigmentation of H. totteni is distributed in a distinctive manner, although it may vary in intensity. No consistent specific differences in pigment distribution have been found between New Zealand species of Potamopyrgus and within P. antipodum no correlation has been found between pigment intensity and shell form.

(f) REPRODUCTION

(1) Sex ratio

In Europe, populations of P. jenkinsi normally exist as parthenogenetic females and only a solitary male has been recorded (Patil, 1958), from the River Thames in

England. Similarly, the New Zealand "species" of Potamopyrgus have been assumed to be parthenogenetic (Ponder, 1964), apart from P. pupoides which Ponder found to have normal bisexual reproduction.

The present investigation has shown that in New Zealand, Potamopyrgus by no means consists solely of parthenogenetic females, and in fact males are relatively common and present in all three species. Why males have not previously been recognized is no doubt partly because of a lack of interest in the living animal by earlier workers, and also because the penis of the male is not immediately visible when the animal is withdrawn from its shell. It is hidden beneath the mantle edge which must be folded back before it can be seen.

A preliminary investigation of sex ratios was made by examining six to 10 individuals from 63 populations of P. antipodum, five populations of P. estuarinus and three populations of P. pupoides. Males were found in all populations of the two latter species and in 24% of the P. antipodum populations.

Results of a more comprehensive investigation are presented in Table 6. Males were found in nine of the 24 populations of P. antipodum examined, and in seven of these they constituted less than half of the total sample. In P. estuarinus the sex ratio approximated 1:1 and in P. pupoides females predominated in a ratio of about 3:1.

Considerable variations in sex ratio have also been recorded in Hydrobia ulvae by Rothschild (1938). In the

hydrobiids Pomatiopsis cincinnatiensis and P. lapidaria, of which extensive collections have been examined, females outnumber males by 1.7:1 and 2.9:1 respectively (van der Schalie and Dundee, 1955; Dundee, 1957).

In some populations of P. antipodum from West Coast lakes sexual dimorphism with regard to shell height has been found, females attaining a greater size than males. This is clearly indicated in Table 6 by comparing the sex ratios for large and medium-sized snails in these populations. Sexual dimorphism in hydrobiids has also been noted by Dundee (1957) in Pomatiopsis lapidaria and by van der Schalie and Davis (1965) in Oncomelania formosana.

(2) Male reproductive system (Fig. 17)

The gross anatomy of the male reproductive system is identical in all three species, and closely resembles that of P. jenkinsi described by Patil (1958). The testis lies in the upper whorls of the shell on the columella side, and from it arises the vas deferens, a narrow, highly convoluted tube with a thin, muscular wall. It passes through a large prostate gland embedded in the tissues of the visceral mass at the posterior end of the body whorl, and finally runs forward on the head, close to the skin, to the penis, opening at its tip.

No proximal dilation of the vas deferens, as described by Patil, and suggested as a seminal vesicle, has been found. The vas deferens of mature individuals in all three species is normally crammed with living sperm throughout its length. This gives it a conspicuous white appearance.

FIGURE 17

(a) Diagrammatic representation of male reproductive system

(b) Sperm

(c-d) Position of penis on head

(e) Penis extended

(f) Penis contracted

(g-s) Penis shape in preserved specimens

(t) Section of vas deferens containing sperm

Abbreviations

p - penis

pr - prostate

t - testis

v - vas deferens

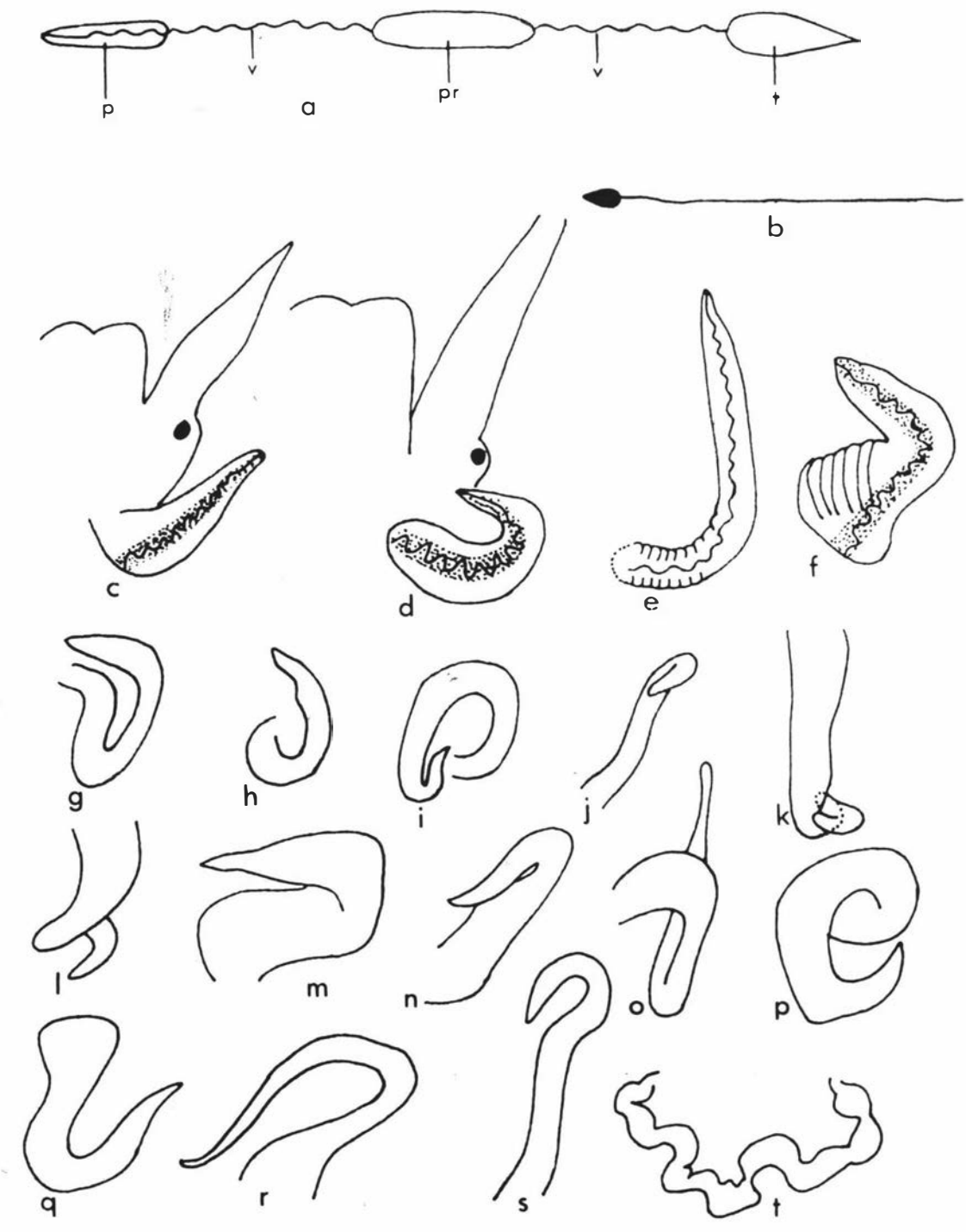
p
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TABLE 6.

Sex ratios determined in samples of *Potamopyrgus* spp. from 30 localities

Population	Number in Sample	Female	Male	% Male	Notes
(a) *P. antipodum*					
Waitomo Stream	100	59	41	41	
Lake Pupuke	89	61	28	31	Large snails. No sexual dimorphism.
" "	57	36	21	37	Small snails.
Lake Taupo	100	100	0	0	
Lake Pukaki	100	100	0	0	
Avon River	100	100	0	0	
Makara River	100	100	0	0	
Lake Paringa	100	91	9	9	Large snails only. Sexual dimorphism found.
" "	31	15	16	52	Medium sized snails.
Green Lake	100	100	0	0	
Lake Wairarapa	50	50	0	0	
Hokowhitu Lagoon	100	100	0	0	
Lake Tutira	50	50	0	0	
Lake Wahapo	100	70	30	30	Large snails. Sexual dimorphism found.
Lake Ianthe	20	19	1	5	Large snails. Sexual dimorphism found.
" "	40	19	21	52	Medium sized snails.
Waikato River (Cambridge)	30	27	3	10	Large snails. No sexual dimorphism.
" "	30	27	3	10	Medium sized snails.
Linton Stream	200	200	0	0	
Ohau Pond	50	50	0	0	
Kahuterawa River					
(tributary 1)	100	100	0	0	
(tributary 2)	84	84	0	0	
Pond A, Massey University	> 500	All	0	0	
Pond B, Massey University	> 500	All	0	0	
Dannevirke (ditch)	50	49	1	2	
Tiritea Stream	40	37	3	7.5*	
Waiotapu (stream)	50	50	0	0	
Piha River E	50	33	17	34	
(b) *P. estuarinus*					
Waikato River E	50	27	23	46	
Havelock (river) E	50	32	18	36	
Heathcote River E	50	29	21	42	
Huia Stream E					
(Dec.)	40	18	22	55	
(Aug.)	60	25	35	58	
(c) *P. pupoides*					
Plimmerton E	40	36	4	10	
Havelock (river) E	50	36	14	28	
Wananaki E	75	57	18	24	

E exposed to brackish water

* Maximum % males in 15 monthly samples.

Sperms have a slender conical head and a long, lash-like tail (Fig. 17b), and are all of the one kind. Their dimensions (living) are given in Table 7 in which comparison is made with the sperm of P. jenkinsi and H. ulvae.

TABLE 7.

Dimensions of sperms of New Zealand species of Potamopyrgus compared with those of two species of European Hydrobiidae

Species	Total Length (microns)	Head Length (microns)	Reference
P. antipodum	110	3	This study
P. estuarinus	140	"	" "
P. pupoides	110-120	"	" "
P. jenkinsi	40	4-6	Patil (1958)
H. ulvae	100	?	" "

The sperms of the New Zealand species are comparable in length with those of H. ulvae but two to four times the size of those described for P. jenkinsi. As the sperm of P. jenkinsi was observed in sectioned material, however, it is possible that the dimensions given are not a good indication of their length in life, as it is possible that wax embedding contracts the sperm tails in a similar way that it contracts chromosomes as indicated by Burch (1960).

The penis (verge) is situated on the right side of the head beneath the mantle edge. It is simple in form, tapering at its tip and bears no accessory lobes. It thus closely resembles the penis of P. jenkinsi figured by Patil

(1958). In life the penis is colourless and semi-translucent, the vas deferens being visible within. It is capable of considerable contraction and expansion, and when contracted the walls near its base have a somewhat telescopic appearance, and the vas deferens becomes strongly convoluted. In preserved specimens the shape and orientation of the penis tend to vary considerably, and usually it becomes somewhat coiled, especially at the tip. Fig. 17g-s illustrates the condition of the penis in a number of preserved snails. Histologically, it is similar to that of P. jenkinsi.

As a taxonomic character, the male copulatory organ has been considered by Berry (1943) to be most reliable, and he found that different species of Michigan Hydrobiidae had characteristic penis structures, which paralleled differences in their radulae. By contrast, New Zealand species of Potamopyrgus have simple non-lobate copulatory organs which are of no value as specific characters.

(3) Female reproductive system (Fig. 18)

The structure of the female reproductive system divides the New Zealand species of Potamopyrgus into two distinct groups which possess major differences in the form of the lower section of the oviduct, and its associated glands.

(i) P. antipodum

The ovary is situated on the columellar side of the digestive gland in the apical whorls and reaches almost to the tip of the spire. It has a white, rather lumpy appearance when mature, and contrasts strongly in colour with the brownish digestive gland which has a stippled

FIGURE 18

(a) Diagrammatic representation of the female reproductive system of *P. estuarinus* and *P. pupoides*

(b) Diagrammatic representation of the female reproductive system of *P. antipodum*

(c) *P. antipodum* - animal removed from shell

(d) T.S. empty brood pouch of *P. antipodum* showing position of sperm groove

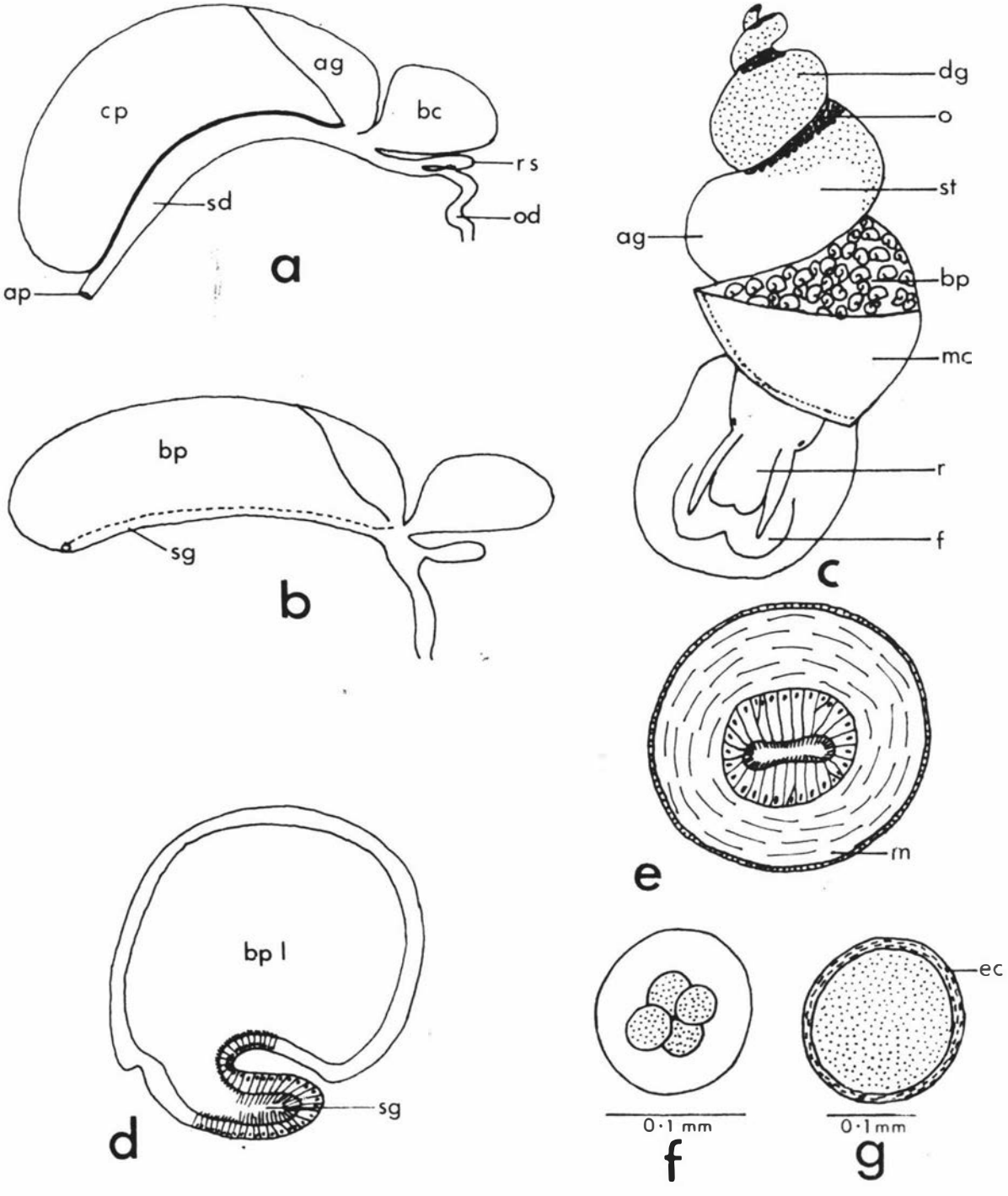
(e) T.S. "spermathecal duct" of *P. estuarinus*

(f) Developing egg of *P. estuarinus* at the 4-cell stage, taken from the lumen of the capsule gland

(g) Egg capsule of *P. estuarinus*

Abbreviations

ap - female opening to pallial cavity
cp - capsule gland
ag - albumen gland
sd - "spermathecal duct" (= pallial oviduct)
bc - bursa copulatrix
rs - receptaculum seminis
od - oviduct
bp - brood pouch
sg - sperm groove
dg - digestive gland
o - ovary
st - stomach
mc - mantle cavity
r - rostrum
f - foot
m - muscle
ec - egg capsule
bpl - brood pouch lumen


ag
cp
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appearance. The oviduct leading from it is slender and thin walled, but its walls become greatly thickened in the region of the bursa copulatrix and receptaculum seminis. Anteriorly, the reproductive system consists of the pallial oviduct which has a prominent, clearly demarkated groove, the sperm channel, on its ventral surface. In immature individuals the thin walled lower oviduct is circular in cross section but in mature snails it becomes greatly enlarged and distended to form a brood pouch within which over 100 embryos in various stages of development may be found. The sperm channel leads directly to the very large bursa copulatrix and the smaller receptaculum seminis. Both normally function to store sperm (Fretter and Graham, 1962), but must have lost this function in parthenogenetic individuals. Fretter and Graham suggest that the well developed bursa copulatrix of P. jenkinsi may act as a waste dump for excess egg capsule secretions. Surrounding the posterior wall of the brood pouch are a prominent albumen gland and a mucus (shell) gland. The single opening of the pallial oviduct is situated close to its anterior extremity. The condition found in P. antipodum agrees with that found in P. jenkinsi by Patil (1958) and Fretter and Graham (1962), but contradicts the claim of Krull (1935) that in P. jenkinsi there are two openings to the exterior, one from the brood pouch and one from a physically separated sperm channel. Reproduction occurs throughout the year.

(ii) P. estuarinus and P. pupoides

In these two species the form of the female system is identical and differs markedly from that of P. antipodum in

the structure and function of the lower section which is dominated by the strongly developed capsule gland. The ovary, positioned as in P. antipodum communicates with the oviduct, at the lower end of which the bursa copulatrix and receptaculum seminis are situated. These are of similar size and shape to those of P. antipodum but in fertilized individuals the receptaculum seminis has a vivid, white appearance, given to it by masses of sperm packed inside. Both these diverticulae are outgrowths of the "spermathecal duct", a straight tube with a muscular wall, similar in cross section to that of Pomatiopsis lapidaria figured by Dundee (1957). It opens to the anterior of the mantle cavity. The "spermathecal duct" is completely separate from the capsule gland above. This is unlike the condition found in Hydrobia, where the capsule gland forms the pallial oviduct, with the "spermathecal duct" running along its ventral surface only partially separated by longitudinal folds of tissue. At the posterior end of the capsule gland, and immediately in front of the bursa copulatrix is the smaller albumen gland whose lumen is continuous with that of the capsule gland, and also in communication with the "spermathecal duct" below. Although the exact course of the eggs through the system has not been firmly established it seems certain that the capsule gland does not function as a pallial oviduct. Evidence from serial sections indicates that it has no anterior opening to the mantle cavity nor any connection with the "spermathecal duct" below. Also, developing eggs have never

been found in its lumen. Eggs (up to the eight cell stage of development) have, however, been found in the lumen of the albumen gland from which it is assumed they pass down into the "spermathecal duct", which also acts therefore as the pallial oviduct. This situation is similar to that found by van der Schalie and Getz (1962) in Pomatiopsis cincinnatiensis, in which the "spermathecal duct" is separated from the accessory glands and functions as the pallial oviduct.

The eggs of P. estuarinus and P. pupoides resemble those of the terrestrial hydrobiid Pomatias elegans figured by Fretter and Graham (1962). They are sperical with a granular appearance, possess a thick (15μ), striated, fibrous-looking shell, have no organs of attachment, and are laid singly. Eggs of P. estuarinus have a diameter of about 200μ, whereas those of P. pupoides are larger, and about 370μ.

Gametogenesis has been observed in collections of P. estuarinus made in January, May, August, September and December, and it therefore seems probable that it occurs throughout the year. Less is known regarding P. pupoides but females containing developing ova have been observed in spring and summer.

(g) CHROMOSOME NUMBER

Squashes of male and female gonads from all three species were examined to determine chromosome numbers. Interpretation of ovarian material was not easy as the

majority of nuclei were in interphase, but testis squashes included numerous cells at various stages of spermatogenesis and could be readily interpreted. Chromosomes could be distinguished with some difficulty in early prophase and were most clearly counted in late prophase and metaphase. In all three species the diploid number 2n=24 was found and male gametes possessed the haploid complement n=12.

Rhein (1935) reported the diploid chromosome number of P. jenkinsi from Europe to be 20-22 and Sanderson (1940) found British specimens to have 36-44 chromosomes and suggested that they may be a tetraploid "race" derived from the Continental diploid "race". Both these observations were made on sectioned material, and Patterson (1967) has indicated the need for more exact studies using squash techniques before these figures can be confirmed.

In his review of chromosome numbers in the Streptoneura (=Prosobranchia), Patterson (1967) lists haploid numbers for 10 species of Hydrobiidae in the genera Pomatiopsis, Oncomelania and Hydrobia. These all lie within the range 16 to 18. He also states that in families or genera where there is chromosomal number variation, this variation is usually not more than $\pm$ two bivalents, although a maximum variation of 12 has been found in the pleurocerid genus Semisulcospira, and even a variation of six within a single species of the muricid Purpura. As a rule, chromosome numbers in the Streptoneura tend to be conservative, as they are in the Euthyneura (=Opisthobranchia + Pulmonata)

(Burch, 1965) and this is borne out in the New Zealand species of Potamopyrgus which all possess the same chromosome number. Conservativeness in this genus is also indicated by Rhein's (1935) figure of 2n=20-22 for P. jenkinsi. It seems unlikely that much, if any, significance can be given to the fact that the chromosome number possessed by species of Potamopyrgus is the lowest of all the hydrobiids examined to date, as, although Burch (1965) found that the lower groups of Authyneura possessed lower numbers, Patterson (1967) has found that in many cases it is impossible to correlate low numbers with "primitiveness" in Streptoneura.

(h) INTERNAL ANATOMY (non-reproductive)

The gross internal anatomy, excluding the reproductive system, of all three species is essentially the same and shows no significant differences from the situation described for P. jenkinsi by Robson (1920), Krull (1935) and Fretter and Graham (1962). The most obvious difference between the species is in the number of gill filaments, 35 being found in specimens of P. antipodum and P. estuarinus examined, but only 15 in P. pupoides.

1.42 BIOCHEMICAL FACTORS

INTRODUCTION

In recent years attempts to use biochemical characters in taxonomic studies have been made with varying degrees of success (Alston and Turner, 1963). The advantages of using biochemical, as opposed to morphological characters is

presumed to lie in the fact that they are generally affected only in a quantitative way by modifiers, whereas many morphological characters may be influenced qualitatively by numerous modifiers, many of which exert their effect in a cryptic way. It should be realized, however, that biochemical factors by themselves are not necessarily any more objective than precise anatomical data when it comes to comparing taxa in terms of genetic, or phylogenetic relationship, unless the factors under consideration are known to be homologous (Davis and Lindsay, 1967). By themselves biochemical differences do not define taxonomic categories, but when considered along with anatomical and cytological data, biochemical studies may make a useful contribution. Among the biochemical substances which have been examined for taxonomic information in animals and plants are amino acids, proteins, fatty acids, carbohydrates, alkaloids, cyanogenetic substances, phenolic substances, quinones, and terpenoids as well as serological and immunological data (Alston and Turner, 1963). In taxonomic studies on Mollusca, the techniques of paper- and ion-exchange chromatography, disc- and immunoelectrophoresis, spectrophotometry, and serology and immunology, have been employed in examinations of blood, egg, and foot proteins, blood and shell amino acids, and body mucus. The value of these techniques has been reviewed by Michelson (1966a, 1966b), Davis and Lindsay (1967), and Ghiselin, Degens and Spencer (1967). In this study, body mucus, free amino acids in foot and mantle tissue, and the amino acids of periostracal protein have been examined for any taxonomic information they may contain.

Taxonomic studies of molluscs involving body mucus were first made by Kirk, Cain and Beyer (1954). They applied paper chromatography to fresh tissue extracts of the foot muscle of seven species of land snails and obtained characteristic ultra-violet fluorescence and absorption patterns for each species. These patterns were all clearly distinguishable from each other, and showed neither geographic variations, nor differences when the snails were fed different diets. Michejda (1958) also obtained species specific fluorescent patterns from snail mucus and foot tissue preparations, and Wright (1959) in a study of the chromatographic patterns of fluorescent substances in species of Limnaea found that it was the body mucus, not the slime trail mucus which gave the pattern. He also obtained constant patterns for different species, although in a later study (Wright, 1964), he found a marked degree of variation in pattern between different populations of the morphologically variable species Limnaea peregeri. Wright (1959) speculated that chromatographic patterns may represent more fundamental taxonomic characters than many morphological characters in general use. The nature of the fluorescing matter in mucus is not known (Wright, 1964), although Robertson (1957) has suggested that some fluorescing substances may be amino-sugars, and Pasamune and Yosizawa (1950) quoted by Wright (1959) have identified glucosamine and galactose in Helix foot mucus, and glucosamine, mannose and galacturonic acid from snail mucus mucin.

Chromatographic analyses of free amino acids have been made by a number of workers using tissue squashes from

several arthropod groups, and qualitative differences interpreted as having taxonomic significance have been found in a number of chem. Lewis (1957) found that muscles of various species of Crustacea appear to contain different amino acids some of which seem to be species specific. Muzzati-Taverso (1953) obtained constant and distinctive patterns of amino acids and fluorescent substances from tissues of genetically known strains of Drosophila melanogaster, although the interpretation of his results has been queried since by Alston and Turner (1963). Ball and Clark (1953) obtained free amino acid differences from the bodies of the mosquitoes Culex quinque fasciatus, C. tarsalis and C. stigmatosoma, and concluded that paper chromatography of amino acids might offer an additional and useful method of delineating species differences, or of indicating phylogenetic relationships. Wicks (1954) also applied amino acid chromatography to a study of certain mosquito species which were difficult to separate on morphological bases and he obtained convincing differences. Later Wicks (1956) obtained distinctive differences between certain Hemiptera, Diptera and Orthoptera, at the ordinal level, and also could distinguish between three genera of cockroaches but not between species within a single genus. Robertson (1957) obtained differences in chromatogram patterns between 17 species of Coleoptera, Lepidoptera, Diptera and Hymenoptera, although he found that the analyses were complicated by pattern differences evident in larval, pupal and adult stages of some species. He concluded that as

differences are found at the specific level, paper chromatography of this kind is a valuable taxonomic tool.

In this study, free amino acid patterns from molluscan foot muscle and mantle skirt tissue have been examined. The foot was chosen as the primary source of material as it is a relatively large and homogeneous structure, easily separable from the visceral mass, and it is an organ characteristic of the phylum Mollusca.

The use of amino acids from molluscan shell protein for phylogenetic and taxonomic purposes is a very recent development, and preliminary studies have indicated that it could be a useful taxonomic technique (Degens, 1967; Ghiselin et al., 1967). The molluscan shell is produced by secretion of precursors from the epithelial tissue in specialized areas of the mantle and may consist of several layers. The outer layer or periostracum is not calcified and over 95 % is composed of protein (Degens, 1967). It acts as a protective outer covering. The inner layers of the shell are calcareous and include a proteinaceous matrix which serves as the sitefor the nucleation of the mineral phase. The uncalcified matrix is laid down extracellularly, and only after it is formed is inorganic material from the underlying tissue incorporated into the shell (Ghiselin et al., 1967). In Mytilus californianus the organic matrix represents about 1% of each mineralized shell layer (Hare, 1963). As a species is defined, in part, by its distinct genetic composition differing from that of other species, and as proteins are genetically determined structural elements of the body, it is reasonable to expect that genetic

divergence between species would be displayed in a divergence of protein composition. Proteins are characterized by their individual amino acid sequences, and by employing ion-exchange chromatography quantitative estimates of the amino acids present can be obtained, differences in values thus obtained being indicative of differences in protein composition.

In this investigation, amino acid analyses of periostracal protein have been made but the matrix of the calcified shell has not been examined. Periostracum was chosen for two main reasons;

(a) It is easy to obtain relatively large quantities of material compared with minimal amounts of matrix protein.

(b) Shell ornamentation is periostracal, and comparisons of amino acid composition of smooth and spiny shells is of interest.

METHODS

(a) Mucus fluorescence

Fresh foot tissue was crushed on a sheet of Whatman no. 1 filter paper with a glass rod, making a concentrated spot (diameter up to 1.0cm), 1-2cm from one edge. A number of spots were prepared on each paper, up to 20 snails contributing to each spot, and were dried thoroughly in a current of warm air. Butanol-acetic acid-water (100:22:50 v/v/v) was used as solvent, as recommended by Kirk et al. (1954) and Wright (1964). Papers were developed by ascending chromatography in a saturated atmosphere. At the

end of a run, the paper was dried, and examined for fluorescence patterns using an ultraviolet lamp, with a Mazda 125w mercury vapour bulb. Examination of papers prepared for free amino acid analysis (Methods b) were also examined for fluorescence patterns. The following species were examined.

Potamopyrgus antipodum, P. estuarinus, P. pupoides, Melanopsis trifasciata, Latia neritoides, Simlimnaea tomentosa, Physa fontinalis, Helix aspersa and an unidentified limacid slug.

(b) Free amino acids

Free amino acids in foot muscle and mantle tissue were examined qualitatively by descending paper chromatography. The visceral mass was first separated mechanically from the foot and mantle skirt of each snail, and the foot and mantle tissue was then placed, separately, or together, in a centrifuge tube containing a few drops of distilled water. Up to 20 freshly killed snails were used in each preparation. It was important that only a minimal amount of water was added to the snail tissue so that the preparation remained relatively viscous and concentrated, yet was fluid enough to allow easy application to the paper. Snails were pulverized thoroughly in the centrifuge tube with a clean glass rod, and were then centrifuged for 5-10 minutes. The somewhat viscous supernatant was spotted onto a sheet of Whatman no. 1 chromatography paper about 10cm from one end. Several spots as well as standard reference amino acids were run on each sheet. Spotting was carried out

FIGURE 19

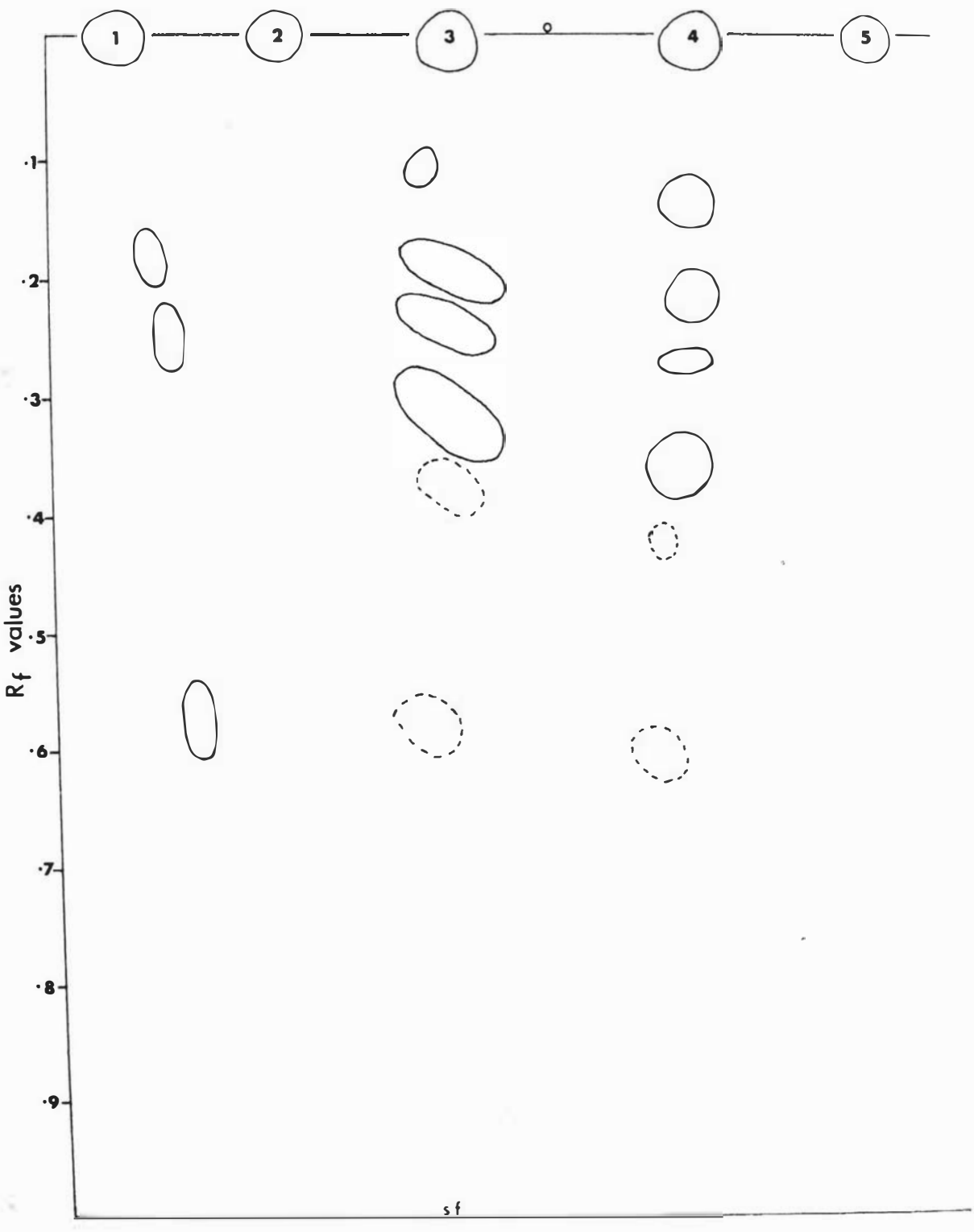
Tracings of ultra-violet fluorescence and absorption patterns obtained by chromatographing squashes of fresh foot muscle from four species of Gastropoda. Solvent; butanol:acetic acid: water (100:22:50, v/v/v). All spots yellow; those with broken outlines faint.

1. Physa fontinalis
2. Potamopyrgus antipodum
3 and 4. Helix aspersa
5. Latia neritoides

Abbreviations

o - origin

sf - solvent front


1
2
3
4
5
·1
·2
·3
·4
·5
·6
·7
·8
·9
Rf values
sf

the three pulmonates *P. fontinalis*, *L. neritoides* and *H. aspersa* from which identical chromatograms were obtained. Six spots identified as lysine, glycine, alanine, proline, valine and leucine were obtained, the last two sometimes being fairly faint. A typical chromatogram is shown in Fig. 20.

(c) Amino acids of periostracal protein

The species could not be distinguished by quantitative examination of their periostracal protein amino acids which are similar in all species and possess a high degree of intra-specific variation.

Results of analyses are presented in Table 8 (p.57). Localities and shell forms of snails examined are shown below.

Sample no.	Species	Collection locality	Shell form
1	*P. pupoides*	Wananaki	Smooth
2a	*P. estuarinus*	Huia	"
2b	"	"	"
2c	"	"	"
3	"	Heathcote	"
4	*P. antipodum*	Dannevirke	"
5	"	Lake Pupuke	Spiny
6	"	Whangarei Falls	Mixed smooth and spiny
7	"	Lake Tutira	Smooth
8	"	" "	Spiny

Reproducibility of results was tested on two samples of *P. estuarinus* from Huia (Table 8, Nos. 2a,2b,2c; p.57). When two identical runs were made on the same sample (2b and 2c), the mean variation between amino acid values was

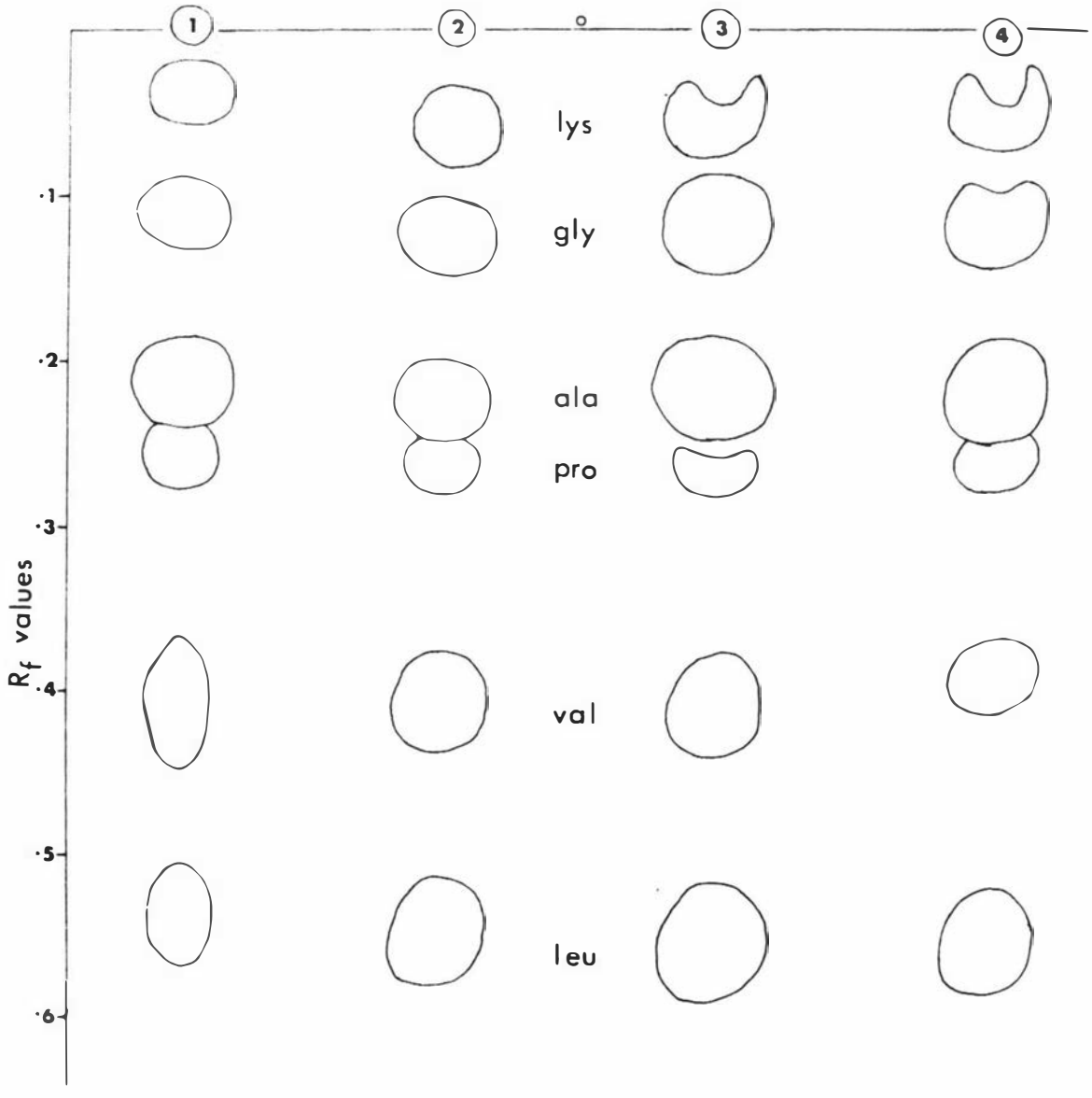
FIGURE 20

Free amino acid patterns obtained on chromatographing foot muscle and mantle-edge tissues from four species of Gastropoda. Solvent; butanol:acetic acid: water (3:1:4, v/v/v).

1. Physa fontinalis (foot)
2. Latia neritoides (foot)
3. Helix aspersa (mantle)
4. Potamopyrgus antipodum (foot)

Abbreviations

ala - alanine	lys - lysine
gly - glycine	pro - proline
leu - leucine	val - valine


1
2
3
4
lys
gly
ala
pro
val
leu
·1
·2
·3
·4
·5
·6
R_f values

2.6°/oo (range 0.1-6.3°/oo). The mean variation between the same amino acids from two different samples from the same locality (2a and 2b) was 7.1°/oo (range 1.1-28.0°/oo). This variation incorporates differences between specimens, and errors introduced by decalcification, chromatography and data handling.

Marked differences in amino acid concentrations were found between Huia and Heathcote samples of P. estuarinus, glycine, proline, tyrosine and phenylalanine being greatly reduced in the latter, whereas most others showed corresponding increases in proportions. Values for P. pupoides corresponded closely to those of P. estuarinus from Huia, although tyrosine was markedly low. A wide range of variation was also found in P. antipodum, and no relationship between amino acid concentration, and shell ornamentation was apparent. This is clearly demonstrated by comparing the extreme shell forms represented by Lake Pupuke (5) and Dannevirke (4) samples. In these, with the exception of tyrosine (highest in spiny Lake Pupuke sample) relative proportions of amino acids are of a similar order. This similarity is clearly demonstrated in Table 9 (p.58) where amino acid ratios in all samples have been adjusted for ease of comparison, glycine being given a value of 100. The presence of increased tyrosine in spiny shells is probably of no significance, however, as high concentrations are also found in the smooth shelled P. estuarinus.

The finding of considerable variation in concentrations of amino acids in the periostracum of Potamopyrgus spp.

TABLE 8.

Shell periostracum amino acids in three species of Potamopyrgus. Expressed in parts per 1000 (°/oo) total amino acids.

Species	pupoides	estuarinus				antipodum				
Sample no.	1	2a	2b	2c	3	4	5	6	7	8
Locality	Wananaki		Huia		Heathcote	Dannevirke	L. Pupuke	Whangarei Falls	L. Tutira (smooth)	L. Tutira (spiny)
Amino acid										
Aspartic Acid	121.4	129.3	114.8	109.4	149.3	133.4	126.6	114.8	117.5	98.7
Threonine	43.9	35.1	32.0	32.1	44.8	45.8	44.5	31.6	48.2	55.0
Serine	53.4	44.3	40.6	40.1	53.0	47.2	43.7	35.4	50.8	67.0
Glutamic Acid	70.1	68.2	61.5	63.7	86.2	73.7	75.9	52.1	74.4	85.3
Proline	47.8	47.3	54.0	58.1	21.7	23.9	22.6	36.2	44.8	53.6
Glycine	329.3	357.0	342.2	342.3	265.9	318.8	288.9	400.9	268.9	205.4
Alanine	69.8	57.4	54.5	51.6	76.8	43.9	70.6	40.9	87.1	109.6
Half Cystine	T	T	T	T	T	T	T	T	T	T
Valine	45.0	41.4	44.2	40.1	60.2	56.4	49.8	34.2	44.3	62.5
Methionine	6.9	7.0	4.9	5.2	8.3	8.0	5.8	1.7	5.2	10.2
Isoleucine	24.9	22.8	21.1	20.7	27.8	26.7	28.3	17.6	29.8	36.8
Leucine	46.0	43.4	40.1	40.2	54.5	57.8	57.5	39.7	55.5	60.2
Tyrosine	23.7	62.4	69.2	71.3	37.1	40.9	63.9	47.8	32.0	32.0
Phenylalanine	54.9	45.3	41.2	47.5	43.1	54.3	51.2	64.1	54.9	45.9
Lysine	27.0	21.2	49.2	43.1	34.1	32.3	29.3	33.8	44.2	37.3
Histidine	3.9	1.8	2.9	2.1	1.3	5.9	2.3	11.3	7.3	3.4
Arginine	31.8	16.2	27.4	32.7	35.7	30.9	39.0	37.8	35.3	37.3

T = Trace

TABLE 9.

Ratios of periostracal protein amino acids where glycine = 100, in three species of Potamopyrgus.

Species	pupoides	estuarinus			antipodum				
Sample no.	1	2a	2b	2c	3	4	5	6	7	8
Amino acid										
Asp	37.0	36.2	33.6	32.1	56.2	41.8	43.8	28.6	43.6	48.0
Thr	13.3	9.9	9.4	9.4	16.9	14.4	15.4	7.9	17.9	26.7
Ser	16.2	12.4	11.7	11.7	20.0	14.9	15.1	8.8	19.2	32.6
Glu	21.3	19.1	18.0	18.6	32.5	23.1	26.2	13.0	27.6	41.5
Pro	14.5	13.3	15.8	17.0	8.2	7.5	7.8	9.1	16.7	26.0
Gly	100	100	100	100	100	100	100	100	100	100
Ala	21.2	16.1	15.9	18.6	28.9	13.8	24.6	10.2	32.4	53.2
½ Cys	T	T	T	T	T	T	T	T	T	T
Val	13.7	11.6	12.9	11.7	22.6	17.7	17.3	8.5	16.5	30.4
Met	2.1	2.0	1.4	1.5	3.1	2.5	2.0	0.4	1.9	5.0
Isoleu	7.6	6.4	6.2	6.1	10.4	8.4	9.8	3.9	11.1	17.9
Leu	14.0	12.2	11.7	11.7	20.5	13.1	19.9	4.4	20.6	29.3
Tyr	7.2	17.5	20.2	20.9	14.0	12.3	22.1	11.9	11.9	15.5
Phe	16.7	12.7	12.0	13.9	16.2	17.0	17.7	16.0	20.4	22.3
Lys	8.2	5.9	14.4	12.6	12.3	10.2	10.1	8.4	16.4	18.1
His	1.2	0.5	0.8	0.6	0.5	1.9	0.8	2.8	2.7	1.7
Arg	9.7	4.5	8.0	9.6	13.4	9.3	13.5	9.5	13.1	18.1

T = Trace

TABLE 10.

Ratios of amino acids of periostracum in various Mollusca and Brachiopoda selected from the literature for comparison with Potamopyrgus. All amino acid values are in parts per 1000. Numbers in parentheses are 1σ ranges.

References	Present Study Potamopyrgus spp. Mean of 9 indivs.	Degens (1967) Gastropoda Mean of 12 spp.	Piez (1961) Australorbis glabratus	Degens (1967) Bivalvia Mean of 12 spp.	Hare (1963) Mytilus californianus	Degens (1967) Cephalopoda 1 sp.	Degens (1967) Brachiopoda 1 sp.	Jope (19 Brachiop 1 sp.
Amino Acids								
OH-Pro	-	0.4 (0-26)	3	-	-	65	-	-
Asp	121	144 (131-158)	183	31 (13-72)	26	224	89	135
Thr	41	(37-70)	22	(8-35)	9	48	31	16
Ser	47	101 (81-126)	91	35 (21-59)	69	67	48	75
Glu	71	(119-142)	81	(10-38)	25	101	50	47
Pro	41	37 (32-43)	47	38 (28-54)	24	74	49	52
Gly	312	77 (68-87)	265	512 (391-671)	503	132	171	324
Ala	66	89 (81-99)	83	37 (23-62)	8	46	304	37
½ Cys	T	2 (1-5)	-	5 (1-14)	14	38	1	13
Val	48	69 (66-72)	32	30 (16-55)	32	49	39	26
Met	6	6 (4-9)	1	18 (6-56)	4	3	5	1
Isoleu	26	57 (49-66)	15	12 (6-26)	13	28	11	12
Leu	49	97 (91-103)	34	18 (9-35)	9	41	26	20
Tyr	48	11 (9-14)	35	30 (12-74)	155	12	31	59
Phe	50	37 (30-46)	21	43 (24-80)	10	19	16	61
Lys	35	28 (20-39)	52	20. (14-29)	22	17	25	28
His	4	1 (0-48)	4	5 (2-11)	17	-	6	32
Arg	32	32 (23-45)	15	27 (13-55)	55	35	98	64

* Whole shell protein analysed of this freshwater species.

reinforces similar findings obtained in other studies. Hare (1963) found that periostracum showed more variation in amino acid composition than any other structural unit of the shell, and showed that samples from the growing edge, around the perifery of a single specimen of Mytilus californianus, may vary from 10-15% in numbers of residues of many amino acids. Clearly defined differences in amino acid composition of periostracum between individuals of Laqueus californianus (a brachiopod) have also been demonstrated by Jope (1967), and Ghiselin et al. (1967) have found environmentally controlled variation in the amino acid composition of periostracum sufficient to mask genetic differences in many cases. Another important source of amino acid variation may be protein heterogeneity, i.e. more than one protein may contribute to the periostracum, as has been suggested by Hare (1963), Degens et al. (1967) and Jope (1967).

Although comparisons between proteins from different shell layers are not strictly valid, the amino acid composition of Potamopyrgus periostracal protein shows a closer affinity to the shell matrix proteins of the freshwater pulmonates Australorbis glabratus (Table 10; p.59) and Helisoma sp. (Ghiselin et al., 1967) than to any marine or terrestrial molluscs examined. All the freshwater species are characterized by high concentrations of glycine, glutamic acid and aspartic acid. Ghiselin et al. (1967) have found that a marked change in amino acid composition of shell matrix protein occurs with a change from a marine to a freshwater environment, the concentrations of a number of the amino acids changing in the direction of concentrations

found in more primitive (marine) molluscs, e.g. Haliotis and Nucula.

In this study, determinations of amino acid composition of periostracal protein have not successfully distinguished between species of Potamopyrgus, nor shown that characteristic differences exist between shells having different degrees of shell ornamentation. Rather, it has shown the existence of shell variability at the molecular level, paralleling the wide range of variation found in overall shell shape and form.

1.43 ENVIRONMENTAL RELATIONSHIPS

(a) DISTRIBUTION AND GENERAL ECOLOGY

Populations of Potamopyrgus spp. are found throughout the two main islands of New Zealand, and P. antipodum also occurs on a number of islands including Stewart, Hen, Great and Little Barrier and Chatham. The Auckland Islands species P. dawbini may also be referable to P. antipodum. The known distributions of P. estuarinus and P. pupoides, and the localities from which P. antipodum has been collected are given in Appendix 1.

(1) P. estuarinus

P. estuarinus has a clearly circumscribed habitat, and is confined to brackish water. Commonly it is found near the mouths of streams and rivers entering harbours, where the water is of fluctuating salinity. Frequently, the snails live a semi-terrestrial existence on mud flats or muddy banks adjacent to river channels, or in harbour backwaters and salt swamps. In these situations they may lie

exposed to the air for over half a tide cycle, and for the other half live in water of high salinity. The snails are inactive when exposed on mud flats, and may lie on the surface of the mud, be partially buried, or be grouped gregariously alongside or under stones, wood, etc. Williams (1960) noted that in the Heathcote estuary snails tended to bury themselves in the mud when the tide was out, and also to occupy cracks and crab burrows. Rosenberg (1963) recorded up to 884,000 snails per square metre in the Heathcote estuary.

Other snails remain immersed throughout the tide cycle, and may occupy various substrates including sand, mud, the upper and lower surfaces of stones, and clumps of weed. In river estuaries, snails are normally most abundant towards the seaward end, where salinities remain high.

Some past reports of the finding of P. antipodum in brackish water (Suter, 1913; Oliver, 1923; Powell, 1933; Bruce, 1958; Ponder, 1964) undoubtedly refer to P. estuarinus. All of Oliver's* observations probably refer to that species which he noted living a semi-terrestrial existence near the land edge of mangrove (Avicennia resinifera) in Shoal Bay, Auckland Harbour, on mud near high water at Rangitoto Island, and along the landward border of tidal mudflats in Tauranga Harbour. Among the animals he found associated with P. estuarinus were the mud snail Amphibola crenata, the crab Helice crassa, and species of

* Shells deposited in the Dominion Museum collection are all typical of P. estuarinus.

amphipod. Subsequent field observations made during this investigation, and by Bruce (1958), have shown that these species are almost invariably present on mudflats where P. estuarinus is found.

(2) P. pupoides

P. pupoides is confined to brackish water, and is frequently found in association with P. estuarinus in river estuaries, but is less frequently found on mud flats where it would be exposed to the air for regular periods of time. P. pupoides exhibits no marked substrate preferences and is found on stones, mud, and among living and decaying vegetation. Frequently, it is abundant in river estuaries on a substrate of smooth, clean sand.

(3) P. antipodum

P. antipodum occupies a wide variety of habitats, including lowland rivers, stony streams, creeks, ditches, estuaries, ponds, lakes (as deep as 420 feet, according to Suter, 1905b), coastal dune lakes, springs, wells and permanent seepage. One of the few freshwater habitats it seems unable to colonize is the temporary pond (Barclay, 1966), as the snails lack resistant stages capable of carrying them over long dry seasons (see p.78). Within the P. antipodum complex a number of relationships between particular shell forms and geographical or ecological distribution are evident but none of these relationships appears to be so well circumscribed, or clearly defined, as to warrant taxonomic recognition of the populations concerned. The main trends noted are:-

(i) Many snails at high altitudes, and/or in relatively oligotrophic waters, e.g. Lake Rotoiti, Nelson Lakes National Park, have a much smaller adult size than do most lowland populations. These snails are predominantly smooth shelled.

(ii) Snails in many, but not all, populations north of Auckland attain a very large size, their shells sometimes exceeding 10mm in height.

(iii) There is a tendency for spiny-shelled snails in lake, and river populations, to have relatively slender, strongly shouldered shells in the south, whereas they become broader and less strongly shouldered further north.

Although laboratory rearing work has indicated that shell form is not a simple phenotypic response to environment, small size may be partly a result of decreased growth at low temperatures, or where the quality of food available is poor. Conversely, attainment of large size may be partly attributable to exposure to higher temperatures for a longer period, permitting an increase in the rate and amount of growth. The North Auckland snails larger size is produced by an increase in whorl number, rather than by an overall increase in size of the whole shell.

Snails of this species are also able to tolerate waters of markedly different calcium content. On the one hand they have been taken in streams flowing from limestone caves at Waitomo and Waiomio (Northland), and on the other from a stream at Waiotapu where calcium incorporation into the shells is so poor that it is almost impossible to pick up snails without crushing their shells.

Maximum shell height, degree of bacterial encrustment on shells, and ornamentation of shells have been compared in populations representing five major habitat types (Fig. 21).

In all five habitats the mean maximum shell height lay between 5 and 6 millimetres, and the greatest range of sizes was found in ditches and stony streams, from which, however, the largest numbers of samples had been drawn.

All degrees of encrustment, light, medium and heavy (see key to Fig. 21), were encountered in all five habitats, and were fairly evenly represented in ditches, stony streams and ponds. In lakes and lowland rivers, however, over 60% of populations consisted of snails with only slightly encrusted shells.

Populations possessing all five degrees of shell ornamentation were found in four out of five habitats, and only in ponds were populations dominated by spiny individuals not found. Only six ponds were represented in the survey, however. As a general rule lakes tended to consist predominantly of spiny individuals, whereas smooth-shelled snails were more abundant in running water. Further discussion on the ecology of P. antipodum in running waters is given in Section 2.1.

This examination has shown that no clearly defined relationship between shell form and geographical or ecological distribution can be demonstrated. This serves further to emphasize the heterogeneous nature of P. antipodum.

FIGURE 21

Relationships between shell height, shell form, shell encrustment, and type of habitat, in 97 populations of P. antipodum

Habitat types (based on the classification of Elton and Miller, 1954)

1. Ditch - A small body of water containing slowly moving or stagnant water. Generally in open farmland. Muddy and weed infested.
2. Stony stream - A small body of water with medium water speed. Bottom of stones or gravel rather than mud. Usually lacking much vegetation. Often upland or in bush.
3. Graded River (or stream) - Lower reaches of a lotic habitat where substrate is mainly mud and silt not stones or gravel. Vegetation has often reappeared.
4. Pond - A small medium sized body of still water (<1 acre), usually with a muddy bottom and considerable weed.
5. Lake - A large body of still water, more permanent than a pond. Vegetation often confined to the margins.

The 97 populations consisted of: 28 ditches, 40 stony streams, 7 graded rivers, 6 ponds and 16 lakes.

Shell encrustment

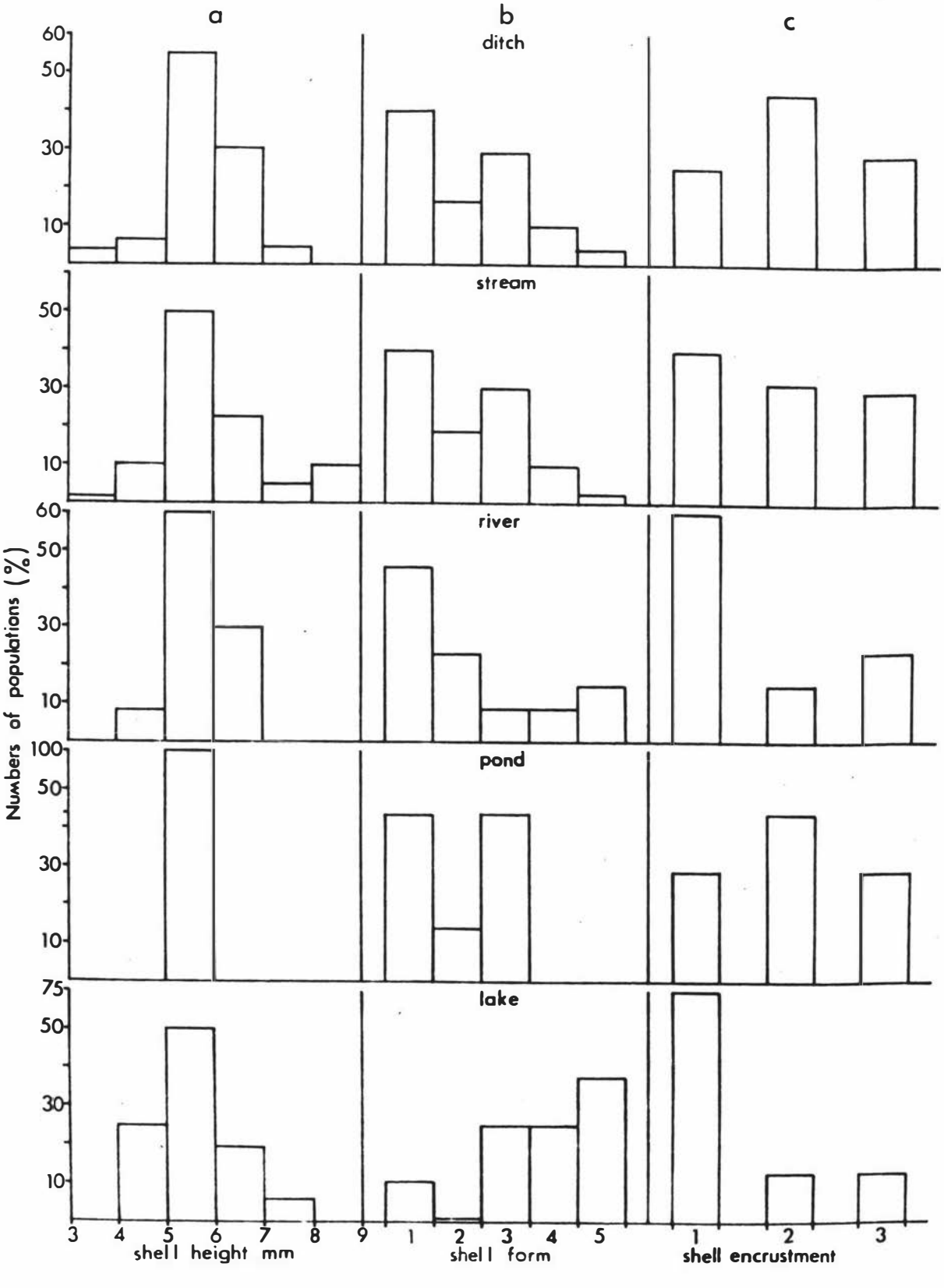
Light - Shells clean or lightly flecked with encrusting material.

Medium - Shells lightly but fairly completely covered.

Heavy - A thick coating of encrusting material begins to obscure shell structures and distorts the shell apex.

Shell form

1. All (or almost all) snails smooth.
2. Predominantly smooth.
3. Half smooth, half spiny.
4. Predominantly spiny.
5. All (or almost all) spiny.


a
b
c
ditch
stream
river
pond
lake
Numbers of populations (%)
shell height mm
shell form
shell encrustment

(b) SALINITY RELATIONS

(1) Salinity range

All three species of *Potamopyrgus* are found over a wide range of salinities, but only *P. antipodum* is found in fresh water. The range of salinities at which the three species have been observed is shown in Table 11, and results of salinity determinations made in a series of habitats are given in Table 12 (p.67). In some cases, salinities were obtained from a single station at high and low tide, in order to determine the range of salinities to which individual snails were exposed.

TABLE 11.

Salinity ranges within which the three species of *Potamopyrgus* have been found living.

Species	Salinity °/oo		Maximum Diurnal Range
	Maximum	Minimum	
P. antipodum	26.4	0	17.7
P. pupoides	32.3	2.7	"
P. estuarinus	34.8	2.7	"

TABLE 12.

Water salinities recorded in habitats of *Potamopyrgus* spp.

Localities	Salinity °/oo	Species present		
		P. antipodum	*P. pupoides*	*P. estuarinus*
Portland Road estuary (Auckland)				
Station A	<1	X		
B	<1	X		
C	16.3-4.7	X	X	X
D	19.0-2.7	X	X	X
E	21.0-3.3	X	X	X
F	26.4-12.9	X	X	X
G	26.4-27.0		X	X
Hobson Bay (Auckland) (mangrove swamp)	30.6		X	X
Otama Bay (Coromandel Pen.)				
Stream - Upper Station	27.2-14.7	X	X	X
" - Lower Station	29.4-19.7		X	X
Littoral pond	32.3		X	X
Supralittoral trickle	<1	X		
Waikato River (Tuakau)	<1	X		
Kaotuna River (Coromandel Pen.)	10.6		X	X
Waihou River (Hauraki Plains)	16.3			X
Porirua Harbour area				
Kahao Stream	6.2		X	X
Kahao Stream mouth (pool)	18.6		X	X
Porirua Harbour mud flats	34.8			X
Horokiwi Stream mouth	19.7			X
Horokiwi Stream	1.0	X		
Small unnamed tributary	12.1			X
Hutt River (Wellington)	22.1-13.4		X	X
Waikanae (stream)	<1	X		
Lake Ellersmere*	9.5-6.0	X		

*data supplied by G.W. Yeates (pers. comm.)

(2) Survey of snail distribution within a single estuary

Introduction

The three species of *Potamopyrgus* are rarely found living together, but they do coexist in a narrow inlet of Hobson Bay, Auckland, which runs parallel to Portland Road. A small, permanent, freshwater stream runs into the upper end of the inlet, and the mixing of fresh and salt water results in the formation of a salinity gradient (Fig. 22).

The substrate of the inlet is mainly foul smelling, highly reduced black mud on which are scattered stones, bricks, broken branches and pieces of scrap metal. Filamentous algae grow on the upper surfaces of the more stable stones, and occasional patches of weed also grow in the upper reaches. The estuary is bordered by grassy banks and rushes, some of which are submerged for much of the time. The firmer substrates and vegetation provide habitats for all three species of *Potamopyrgus* which exhibit no clear substrate preferences, although the mud forming the bulk of the estuary bed is not colonized. During high, spring tides, the influence of salt water may be felt as high as Station B (Fig. 22). When the tide is out, water recedes down the inlet, and above Station F most of the bottom is exposed, except for pools of water trapped in small depressions. The invertebrate fauna associated with *Potamopyrgus* spp. in the brackish water section includes the mud snail *Amphibola crenata*, *Tenagomysis novae-zealandiae*, Amphipoda, Polychaeta, and dipterous larvae, including *Chironomus zealandicus*, and representatives of the Tipulidae, Ephydridae, Stratiomyidae and Syrphidae.

Methods

Because of the irregular distribution of the snails, quantitative sampling procedures were not used, and samples were taken by sweeping a hand net through vegetation, and by scraping snails from the surfaces of hard objects into the net. Water samples were taken in mid-stream close to the stream bed at Stations A-E, and at the side of the stream among vegetation where snails were found at Stations F and G. Water salinities were determined by titration with silver nitrate (Harvey, 1963). A single set of water samples was taken at high and low water during one tide cycle.

Results

Results are shown in Fig. 22. P. antipodum inhabited fresh water and the upper estuary, whereas the other two species occupied the lower estuary. In the section of widely fluctuating salt content all three species were present together, P. antipodum being numerically dominant at the upper station. Maximum salinity to which P. antipodum was found exposed was $26.4^{o}/_{oo}$ and the minimum at which P. pupoides and P. estuarinus were found was $2.7^{o}/_{oo}$.

(3) Experimental studies

In order to determine the full range of salinities tolerated by each species, a number of laboratory tests were carried out. Eleven salinities were selected, 0, 10, 20, 30 --- 100% sea water, made up by diluting freshly collected sea water with distilled water. Sodium chloride solutions were not satisfactory as in preliminary experiments using them, snails were inactivated at concentrations

FIGURE 22

Distribution of *Potamopyrgus* spp. in the Portland Road arm of Hobson Bay, Auckland, in relation to water salinity.

Key

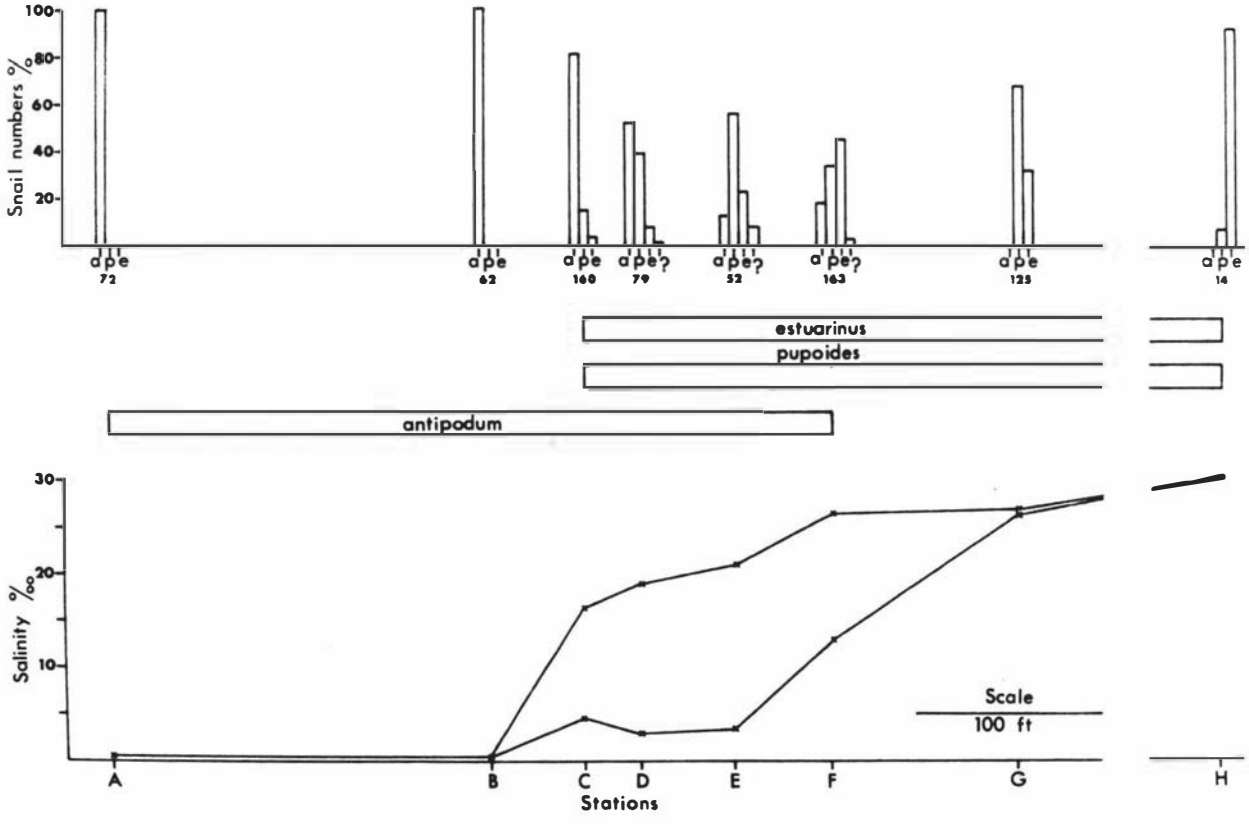
Graph shows salinity readings at high and low water during a single tide cycle.

A-H. Sampling stations.

Horizontal bars show distribution of each species.

Vertical histograms show numbers of each species at each station as a percentage of the total snail numbers. (Values below histograms are sample sizes.)

a - *antipodum* p - *pupoides* e - *estuarinus*


Snail numbers %
100
80
60
40
20
a p e
72
a p e
62
a p e
160
a p e?
79
a p e?
52
a p e?
163
a p e
123
a p e
14
estuarinus
pupoides
antipodum
Salinity ‰
30
20
10
Scale
100 ft
A
B
C
D
E
F
G
H
Stations

of less than 10% sea water (3.5°/oo salinity). Salinities of all experimental waters were checked by titration with silver nitrate. Ten fully-grown individuals of P. estuarinus, 10 of P. pupoides, and 20 of P. antipodum, half from a fresh-water habitat and half from brackish water, were placed in glass finger bowls containing 200ml of water, at each salinity. Localities from which the experimental snails were taken are listed below.

P. estuarinus - Porirua Harbour tributaries

P. pupoides - Porirua Harbour tributaries

P. antipodum (fresh water) - Tiritea Stream

(brackish water) - Waikanae

Snails were transferred direct to experimental salinities from water from their natural habitats. All experiments were run at room temperature (18-20°C) for a standard time of 24 hours. On terminating an experiment all inactivated snails were transferred to water with a salinity of 3.5°/oo and then examined again after 24 hours. All experiments were run in duplicate.

Results and discussion

After 24 hours, all individuals of P. estuarinus and P. pupoides exhibited normal activity at all experimental salinities, 0-100% sea water. P. antipodum, from both populations, was active up to 50% sea water (17.5°/oo salinity). Some reduced movement of P. antipodum was found at 60% sea water (21°/oo salinity), but at higher salinities all snails remained completely withdrawn into their shells, their opercula acting as physical barriers isolating the snails from the surrounding water. After a further 24 hours

in water of 3.5°/oo salinity, all previously inactivated snails resumed normal activity.

In the field, the highest salinity at which P. antipodum has been found is 26.4°/oo (75% sea water), slightly higher than the greatest salinity at which activity occurred under experimental conditions. It is possible, therefore, that some intraspecific variation is found in P. antipodum with respect to salinity tolerance as has been found in P. jenkinsi by Duncan and Klekowski (1967). Although P. estuarinus and P. pupoides have not been observed living in fresh water in the field, they seem able to live in it, and have been kept in fresh water in the laboratory for several months. This ability to tolerate a wide range of salinities is clearly advantageous, as rapid changes in salinity are regularly encountered in the estuarine reaches of rivers inhabited by populations of all three species.

By comparison with the New Zealand species, salinity relationships of the European P. jenkinsi have been well documented over the years (Johansen, 1918; Quick, 1920; Ellis, 1932; Nicol, 1936; Adam, 1942; Bondeson and Kaiser, 1949; Lumbye, 1958; Bryan, 1963; Todd, 1964; Duncan and Klekowski, 1967). Adult snails from both fresh water and brackish water have been found to be tolerant of salinities from fresh water to full sea water, in all of which they actively move and feed, and establish new levels of internal concentrations very rapidly. Young, however, are born only in salinities from fresh water to 12°/oo (34% sea water) in freshwater populations, and up to 18°/oo (51% sea water) in

brackish-water populations (Duncan and Klekowski, 1967). These findings seem to suggest that like P. antipodum, P. jenkinsi is gradually losing its euryhaline character, and becoming a more closely adapted freshwater form.

(c) HABITAT PREFERENCE

Introduction and methods

Apart from inhabiting waters of different salinities, P. antipodum and P. estuarinus are frequently found in contrasting physical environments. P. estuarinus is often abundant on high tidal mud flats bordering streams, where snails may be exposed to the air for an appreciable period of each tide cycle, whereas P. antipodum always remains in the water and is never found on semi-exposed flats.

Laboratory experiments were carried out to compare the behaviour of the two species when a choice between submerged and exposed substrates was offered. The experimental apparatus consisted of a rectangular plastic box (20x10x7cm) with a cardboard floor covered in a layer of river mud forming a sloping "ramp". The floor was subdivided into three zones, a lower submerged section, an upper zone of slightly damp, exposed mud, and a middle zone of saturated mud also exposed to the air. Snails used were obtained from Huia (P. estuarinus), and Piha (P. antipodum), 100 snails being used in each experimental run. Tap water was used in experiments on both species and sea water was also used with P. estuarinus. The different salinities did not affect the responses of P. estuarinus in the experimental situation. All experiments were carried out at room temperature, 18-20°C.

Results and discussion

Results of typical experiments are shown in Fig. 23. In (a) the snails were distributed evenly throughout the box at the start of the experimental period and a single examination of their subsequent distribution was made after 17 hours. In (b) all snails were placed in the submerged section of the box on commencing the experiment, and their distribution was examined after 1, 2, 24 and 72 hours. Similar results were obtained in (a) and (b). A clear behavioural difference between the two species was apparent, the majority of P. estuarinus finally selecting the driest substrate, whereas almost all P. antipodum remained in the water, or were buried in the water-saturated mud of the middle zone. Movement of P. estuarinus from the water to the dry upper zone is clearly shown in Fig. 23b1-3.

The results obtained for P. antipodum agree well with the situation found in the field, the snails remaining almost entirely aquatic. The relatively large numbers occupying the middle zone of water-saturated mud, is probably explained by the presence of favourable respiratory conditions at the air-water interface in this zone. A similar situation is regularly found in still water laboratory cultures lacking vegetation, in which the majority of snails move up the sides of the containers and settle immediately beneath the surface film.

Although in the experimental situation most P. estuarinus remained permanently in the dry zone and exhibited no active movement back to the water, in their natural habitat they do not normally remain exposed to

FIGURE 23

Selection of submerged and exposed substrata by *P. estuarinus* and *P. antipodum* in laboratory experiments.

(a) Snails initially distributed throughout box. Examined after 17 hours.

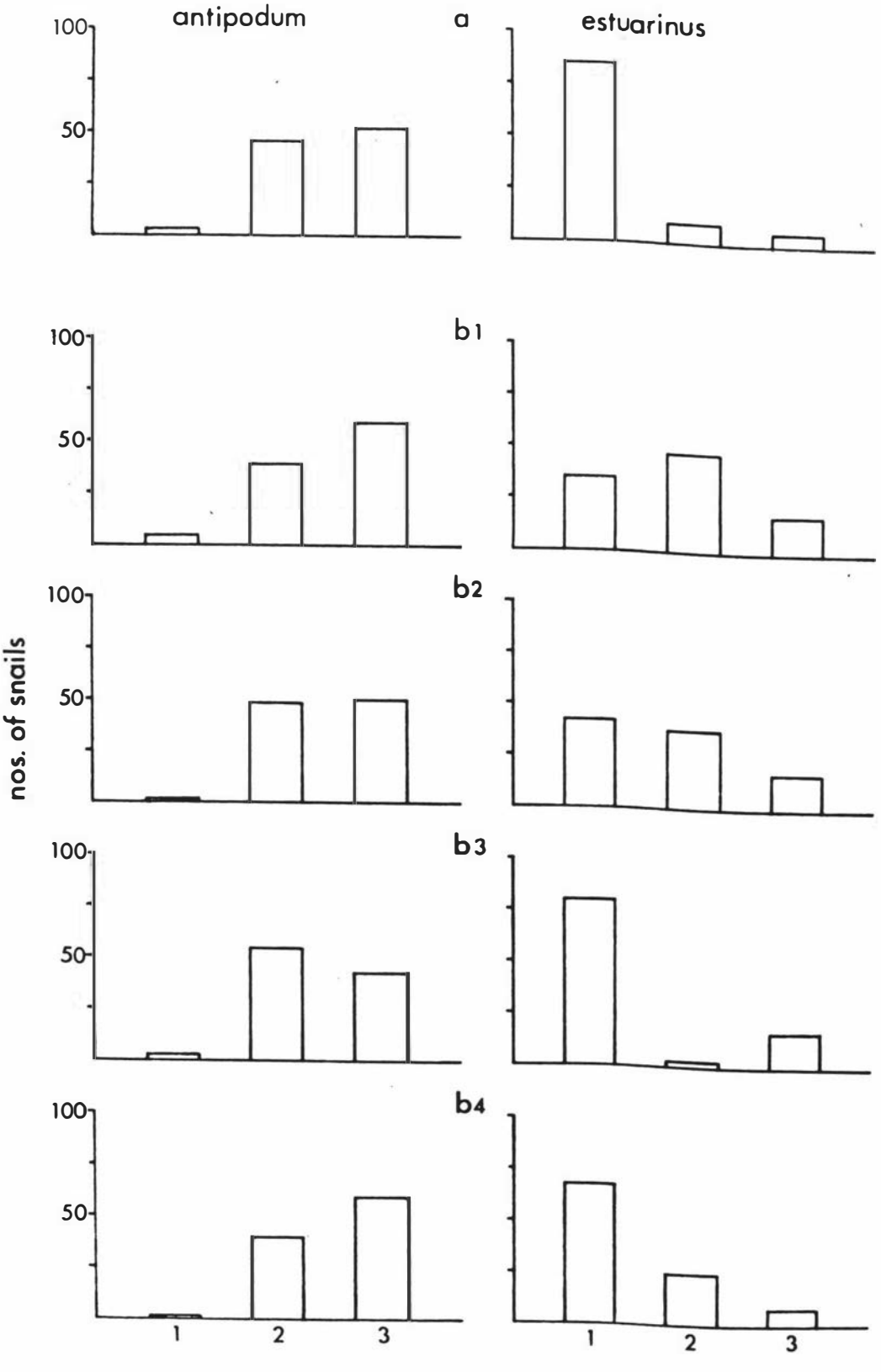
(b) All snails initially in water.

b1 - examined after 1 hour

b2 - " " 2 hours

b3 - " " 24 "

b4 - " " 72 "


antipodum
a
estuarinus
100
50
b1
b2
b3
b4
nos. of snails
1
2
3

the air for more than a few hours at a time, as tidal movements ensure they will be covered at regular intervals. It is essential that the habitat should be submerged regularly as snails cannot move about and feed when the substrate is dry. One consequence of this positive movement out of water could be to prevent colonization of permanent river channels where salinities are lower than those experienced on adjacent mud flats. This would also effectively isolate populations of P. estuarinus and P. antipodum in many areas where their ranges overlap.

(d) DESICCATION

Associated with the colonization of a non-aquatic habitat is the problem of desiccation. This is a problem potentially faced by all terrestrial animals and is likely to be of considerable importance to aquatic species such as P. estuarinus which find themselves periodically exposed to the air. P. antipodum although strictly aquatic sometimes inhabits small bodies of fresh water which have fluctuating water levels, or which are susceptible to draining by natural or artificial means. In such situations, if the snails are unable to withstand exposure to air, whole populations may be quickly destroyed. An example of the effect of artificial drainage of a pond on a population of P. antipodum is described in Section 2.1.

Laboratory tests were designed to examine the tolerance of the three species of Potamopyrgus to desiccation, firstly under totally artificial conditions in perfectly dry air and

on a dry substrate, and secondly under more natural conditions, on a damp substrate and in moisture-saturated air.

Methods

(1) To determine the time snails can exist in a dry atmosphere before death occurs, experiments similar to those of van der Schalie and Getz (1961, 1963) were carried out. Shells of experimental snails were dried thoroughly with filter paper and placed in dry, 9cm diameter, open, petri dishes which were kept in a desiccator containing calcium chloride as desiccant. The apparatus was maintained at room temperature, 20-22°C.

In a first series of experiments 50 snails each of P. estuarinus and P. antipodum, and 20 of P. pupoides were used. All P. pupoides were fully grown individuals, whereas the other two species included snails of various sizes. Five individuals of each species were removed from the desiccator every hour for the first three hours, and then every six hours after the first six hours until all were dead. A snail was considered dead if it showed no sign of movement within an hour of being placed in a shallow container of water.

In a second series of experiments 10 large (shell height > 5mm), and 10 small (< 2.5mm) individuals of P. estuarinus and P. antipodum were examined after 16 hours.

(2) A permanently saturated (100% RH) atmosphere was produced in 9cm petri dishes, by placing six thicknesses of water-soaked filter paper on the floor of each dish which was covered with a lid. As petri dish lids are loose

fitting, they permit adequate gaseous exchange with the outside atmosphere (Davis, 1964). Dishes were kept at room temperature, 20-26°C. Forty individuals of each species were used in the experiment, and all were examined daily to determine whether they were dead or alive, until all snails had died. Death was not easy to determine towards the end of the experiment, as with an increase in time the snails gradually withdrew further into their shells until in many cases the operculum could no longer be seen. A snail was considered dead when no withdrawal reaction was elicited upon prodding the operculum firmly with a needle, or when signs of putrefying tissue were visible around the aperture of strongly withdrawn individuals. Because of the nature of the experiment it was not possible to immerse snails in water to determine whether they were alive or dead.

Results and discussion

(1) Results of exposing snails to a still, dry atmosphere are shown in Tables 13 and 14(p.77).

TABLE 13.

Survival time of Potamopyrgus spp. in a still, dry atmosphere, and on a dry substrate.

Species	Time in hours		
	All alive	Some dead	All dead
P. antipodum	0-6	6-30	30
P. estuarinus	0-6	6-42	42
P. pupoides	0-6	6-24	24

TABLE 14.

Numbers of large and small individuals of P. antipodum and P. estuarinus alive after 16 hours at 0% relative humidity.

Species	Numbers of snails			
	Large > 5mm		Small < 2.5mm	
	Alive	Dead	Alive	Dead
P. antipodum	8	2	0	10
P. estuarinus	4	6	0	10

In all three species, the first individuals died after 6-12 hours in a dry atmosphere, and the longest exposure before death was between 36 and 42 hours, by some individuals of P. estuarinus. It is clear from results of the subsidiary experiment (Table 14), that large individuals were able to tolerate longer periods of desiccation than small ones. This is probably to be expected, as more water can be held inside the shells of large, withdrawn snails than within the shells of smaller ones. This water should take longer to evaporate and so protect the snail tissues against desiccation for a longer time. A similar result was obtained by van der Schalie and Getz (1961) for the amphibious North American hydrobiid Pomatiopsis cincinnatiensis, small individuals (<2mm) being less tolerant to desiccation than adult snails (4mm). Maximum survival time for P. cincinnatiensis at 25% RH and 27°C was 43 hours for young, and 75 hours for adult snails.

(2) Survival of snails in a moisture-saturated atmosphere, and on a damp substratum is shown in Fig. 24. Direct observations indicated that snail tissues did not become rapidly desiccated under these conditions, and that at all times moisture was maintained within the shells of the snails. On a damp, but non-submerged substrate, however, movement, and consequently feeding, cannot occur and therefore death may result from starvation combined with desiccation, rather than by desiccation alone. Deaths of P. antipodum and P. pupoides are attributed to the combined effects of desiccation and starvation, therefore, but the situation found in P. estuarinus was very different.

P. estuarinus apparently entered a state of dormancy or aestivation and had a high survival rate over a long period, under the experimental conditions. Individuals which had remained dormant up to 50 days resumed activity when transferred to a vessel of water. Hibernation, aestivation and tolerance of desiccation are considered to be primarily achievements of the higher Stylommatophora (Morton, 1958), although short term aestivation does occur in some Pomatiasidae (Hunter, 1964) and Hydrobiidae (Dundee, 1957). Little is known about aestivation in most operculate genera, although it has been suggested that the operculum could be a preadaptation permitting successful sealing of the shell (Hunter, 1964). It would therefore correspond in function to the epiphragm, or the dried mucus film, across the aperture of aestivating or hibernating land pulmonates.

Many land prosobranchs desiccate and die extremely readily, and most are restricted to habitats where the

FIGURE 24

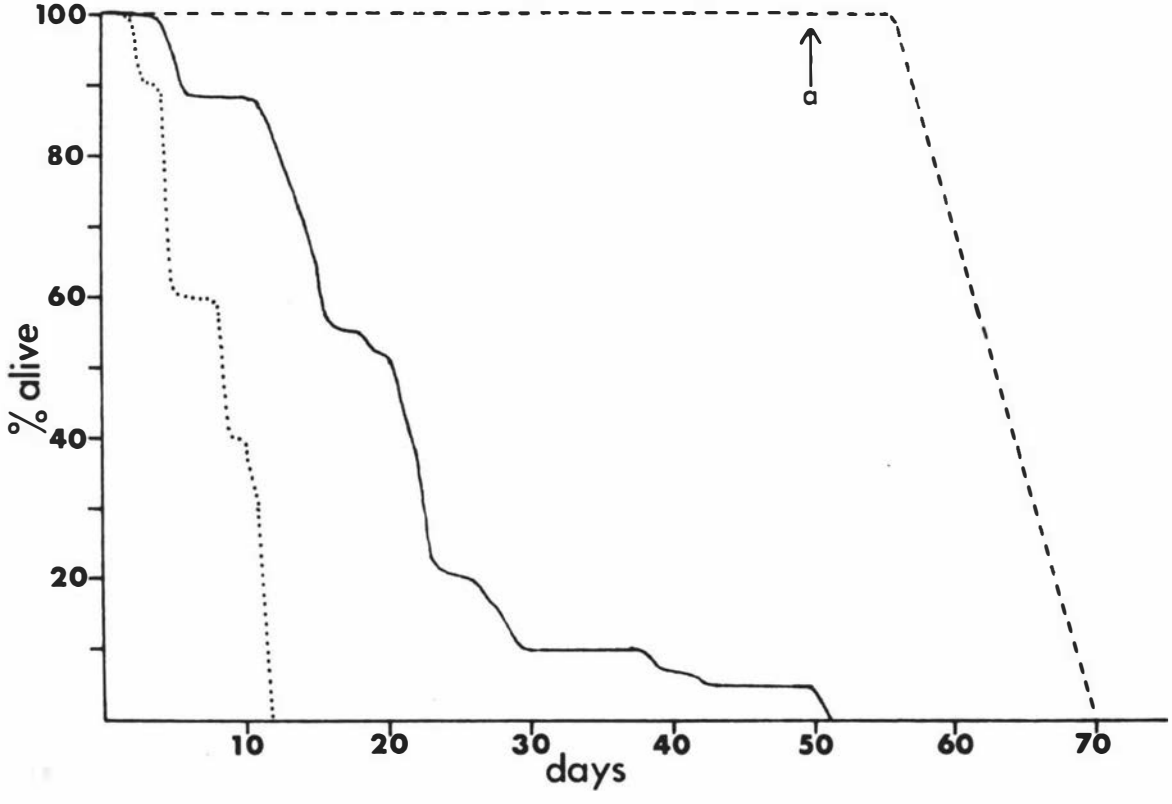
Survival time of *Potamopyrgus* spp. at 100% relative humidity, on a damp substratum, at room temperature (20-26°C).

Solid line - *P. antipodum*

Broken line - *P. estuarinus*

Dotted line - *P. pupoides*

a - subsample of 10 *P. estuarinus* removed and placed in water. All resumed activity.


100
80
60
40
20
% alive
a
10
20
30
40
50
60
70
days

atmosphere is moist (Morton, 1958). Quick (1920) found that Hydrobia spp. could withstand long periods of exposure and survive in an apparently desiccated state, and Dundee (1957) has noted that dormancy occurs in the amphibious Pomatiopsis lapidaria in very cold or hot and dry weather. This evidently ensues when there is a lack of sufficient available moisture, the snails lying with their opercula inserted well into the shell apertures during the inactive period, and becoming reactivated with the onset of rain. Clearly the ability to withstand long periods of exposure out of water is advantageous to snails such as P. lapidaria and P. estuarinus which possess an amphibious way of life, and may suffer prolonged periods of exposure out of the water.

1.44 DISCUSSION

(a) THE SPECIES PROBLEM

As now envisaged, three species of Potamopyrgus are recognized in New Zealand. Two of these, P. pupoides and P. estuarinus, are clearly distinguished using morphological, reproductive, and ecological evidence, but P. antipodum contains a heterogeneous assemblage of forms embracing all the purely freshwater populations. It includes a wide range of morphological variants, as well as differing reproductive forms, and is found under diverse environmental conditions. In the past, many of the forms included in this species have been considered morphologically distinct enough to be recognized as separate species, or to have had restricted geographical distributions allowing them subspecific recognition. This study has

shown that continuous morphological variation exists within the complex and that discrete geographical distributions of taxonomic subgroups, consistent with the definition of the subspecies (Mayr et al., 1953), are difficult to find. A gradation in reproductive types from normal bisexual forms, through populations with few males to total parthenogenesis is also found, and the possession of these different states, apparently unassociated with particular morphological forms, or the occupation of particular habitats, adds further to the difficulty of discriminating distinct taxonomic units within the complex.

The possession of a parthenogenetic mode of reproduction by a large proportion of the populations of P. antipodum is perhaps the major factor responsible for so much of the taxonomic uncertainty within the genus, as it has permitted the formation of many reproductively isolated clones in which divergent evolution has been able to occur. A comparable situation is found in the viviparid genus Campeloma in which both bisexual reproduction and parthenogenesis occur, and in which "the systematics of the species are in a chaotic state" (van der Schalie, 1965).

The biological species definition (e.g. Mayr, 1963: "Species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups"), applies only to bisexual organisms, and is not applicable where asexual or apomictic reproduction occurs. Asexual and parthenogenetic organisms therefore present problems in nomenclature, and it is recognized that

in general the taxonomy of obligatory parthenogens must be arbitrary. In the past it has been based primarily on morphological evidence (Buchanan, 1947; White, 1954; Mayr et al., 1953; Brown, 1959; Dobzhansky, 1961; Sneath, 1961; Mayr, 1963). In practice, the majority of bisexual species have also been recognized by criteria other than reproductive isolation and morphological characters which are often the only evidence available, have been the most widely used criteria for species discrimination.

Unfortunately, in freshwater invertebrates, marked intraspecific variability is characteristically found (Rensch, 1959), and nowhere is this more marked than in the Mollusca (Berry, 1943; Hubendick, 1951, 1955; Dell, 1953, 1956; Hunter, 1957; Berrie, 1959). Shell size, shape, thickness and markings, vary within many species, and much nomenclatural confusion has arisen because of descriptions based on few shells from few localities. In the Hydrobiidae, Berry (1943) considered the shell to be the least reliable character for species determination because of its minute size, generalized shape and lack of constant characters. Variation of the operculum is also too great to allow its use as a reliable character, and variation is also found in the radula.

The problems introduced by variation in defining the molluscan "morphospecies" have been discussed by Schilder (1963) and Solem (1959). The former concluded that "groups of similar shells should be treated as different species if they can be separated by at least one well-recognizable

character showing no intermediates even in extreme specimens". Solem in his studies on New Hebrides land snails emphasized the importance of morphological intergradation as proof of specific identity, and considered that variations in size, colour, height of spire and thickness of shell were by themselves of little or no importance in separating species. Solem was also disinclined to use "subspecies" as a means of indicating variation obviously correlated with physical conditions of local areas. In contrast, earlier workers, e.g. Powell (1949) and Iredale (1943), were in favour of naming such forms if only to "keep them under notice".

Continuous morphological variation is found in the shell, radula and operculum of P. antipodum and similar morphological variation has been described by Dell (1956) in the New Zealand freshwater pulmonates Simlimnaea and Physastra, both of which he concluded consisted of a single, highly variable species. Dell (1953) also considered that there were strong grounds for employing a single name to cover all the nominal species of the freshwater mussel Hyridella because of their extreme morphological plasticity. However, because a few forms could be defined geographically, as well as morphologically, he retained names for them in the belief that they were subspecies in the making.

The occurrence of sexual reproduction and parthenogenesis in P. antipodum poses further problems. White (1954) and Mayr (1963) have pointed out that it is illogical to recognize parthenogenetic and bisexual "races" of the same

species, irrespective of the morphological resemblances between the genotypes, and they considered that such forms were better recognized as sibling species, if they were indistinguishable by ordinary taxonomic criteria. On the other hand, Mayr et al. (1953) have agreed that it is unjustifiable to give nomenclatural recognition to forms with temporary or facultative parthenogenesis. In P. antipodum, sexually reproducing and parthenogenetic forms are connected by intermediates possessing limited numbers of males, and it seems likely that in such populations both parthenogenesis and sexual reproduction may occur.

In view of this lack of a sharp division between reproductive types, and the presence of continuous morphological variation within the complex, it is most sensible to consider the whole range of intergrading populations as a single species as recommended by Huxley (1942), Hecht and Tandon (1953), Lewis (1957), and Alston and Turner (1963).

The inclusion of all the New Zealand freshwater forms of Potamopyrgus in a single variable species, P. antipodum, also supports the claims of Hubendick (1954) and Hunter (1957) that the freshwater gastropods in general consist of a comparatively small number of widely distributed species possessing a high degree of intraspecific variation, characteristics which reflect the small size and transitory state of the freshwater environment, and the high degree of small-scale, short-term isolation which can occur within them compared to a terrestrial or marine population.

(b) THE SIGNIFICANCE OF PARTHENOGENESIS

Parthenogenesis was first discovered in molluscs by Boycott (1919) in P. jenkinsi and was confirmed by Quick (1920) and Robson (1923), and studied cytologically by Rhein (1935) and Sanderson (1940). Parthenogenesis was later discovered in the American viviparids Campeloma rufum and C. decisum by van Cleave and Altringer (1937) and Medcof (1940), and the former was studied cytologically by Mattox (1937). Parthenogenesis in Potamopyrgus and Campeloma is thelytokous (female diploid parthenogenesis) of the apomictic type, (i.e. it is ameiotic and neither chromosome reduction nor fusion of nuclei takes place in the egg), and a single maturation division is found. Parthenogenesis has also been found in four species of Melaniidae (Jacob, 1957), and again it is ameiotic but involves two maturation divisions. In many animals, parthenogenesis is frequently accompanied by polyploidy (Suomalainen, 1950), and of the molluscs examined three species of Melanoides are polyploid and one species is diploid (Jacob, 1957). It has been stated that P. jenkinsi exists as two distinct genotypes, a diploid race in Europe (2n=20-22) and a tetraploid race in Great Britain (2n=36-44) (Sanderson, 1940), but Suomalainen (1950) and Patterson (1967) consider, however, that these results need re-investigation.

In the Melanoides species, parthenogenesis is obligatory, although small numbers of sexually non-functional males are found in two species (0.01-3.0% of populations).

Obligatory parthenogenesis has been considered the rule in P. jenkinsi also, although the finding of a male by Patil (1958) provides possible evidence to the contrary. Males occur sporadically in populations of C. rufum (about 1%) and are scarce or rare in three other species of Campeloma about whose reproduction little is known (Mattox, 1938; van der Schalie, 1965).

In P. antipodum parthenogenesis is apomictic (2n=24) and polyploidy has not been observed in any snails examined. In populations where males are present, they are always sexually functional and male gametes possess the haploid chromosome number (n=12). Circumstantial evidence therefore suggests that parthenogenesis is not necessarily obligatory in all populations of P. antipodum, and that where it is not, and fertilization occurs, a reduction in the chromosome number of ova must occur so as to maintain the diploid number and not produce a triploid race. Perhaps the stimulus bringing about meiosis in the developing egg is the occurrence of copulation, or the presence of sperm in the female system. A situation closely paralleling that found in P. antipodum has been described by Robertson (1966) in species of the chrysomelid beetle Calligrapha. These possess extremely variable sex ratios, ranging from 1:1 to all female populations, and parthenogenesis in at least one species, Calligrapha scalaris, is facultative.

The origin of parthenogenesis in all cases examined is considered to be from sexually reproducing forms, i.e. it is a secondarily derived condition (Mayr, 1963; Suomalainen,

1961), and Mayr has stated further that, with the apparent exception of the bdelloid Rotifera, virtually every case of parthenogenesis in the animal kingdom is probably of very recent origin. A recent origin for parthenogenesis in P. antipodum is indicated by the continued presence today of bisexual as well as parthenogenetic populations, and by the retention of the sperm channel, bursa copulatrix and receptaculum seminis in the reproductive system of parthenogenetic females. A parallel situation is found in parthenogenetic species of Calligrapha which retain a non-functioning spermatheca (Robertson, 1966).

The advantages parthenogenesis gives to a species have been discussed by several workers (White, 1954; Mayr, 1963; Tomlinson, 1966), who have concluded that it is particularly advantageous to animals inhabiting temporary or marginally suitable habitats where population densities are often low. In these situations it permits a single individual to commence breeding without requiring a mate as a new clone may be started immediately, and the reproductive capacity of its members is doubled as all individuals will be egg producing females. Parthenogenesis is also likely to be advantageous to sessile or sluggish animals whose gametes are not widely distributed. In short, parthenogenesis increases productivity by allowing rapid build up of populations, and therefore it can be of definite, short term advantage to forms possessing it.

Contrasting with these short term advantages, however, are probable long term disadvantages imposed by the genetic

limitations of this form of reproduction in which genetic interchange with other organisms has been lost. Apomictic (diploid) parthenogenesis is normally associated with great genetic stability, as genotypically new forms can arise only through mutations. This stability must be expected to lead to a lack of adaptability in obligatory parthenogens, followed by eventual extinction, or perhaps a return to sexuality, according to White (1954). Because no exchange of genes is possible, a parthenogenetic species will consist of an indefinite number of biotypes which will continue to diverge as different mutations establish in different lines of descent. Thus, a high degree of variability will ultimately result within many parthenogenetic species, variability which will not necessarily be correlated with geographic distribution in the same way as in a bisexual form. The attainment of such great variability is not universally found, however, and some parthenogenetic species seem to have no more variation than sexually reproducing species. Perhaps this is because the vast majority of mutations are recessive, and not able to become homozygous in the absence of recombination (Mayr, 1963).

In P. antipodum, a large amount of variation is found both within and between populations, variation which in most cases is not clearly correlated with geographical or ecological distribution. Also, the evidence available suggests that totally parthenogenetic populations are most common in the more temporary bodies of water such as ponds and ditches, rather than in the relatively more permanent

and stable conditions provided by large rivers and lakes.

Compared with P. antipodum, a far smaller amount of variability is found in P. jenkinsi in Great Britain (T. Warwick, pers. comm.), and if Sanderson's (1940) finding is correct that in Great Britain P. jenkinsi is a polyploid, then this may help to explain this condition. Evolution certainly does not come to a complete standstill in polyploid, parthenogenetic populations (Suomalainen, 1961) as has been claimed on theoretical grounds (White, 1954), but polyploids do differ from diploid organisms in that it is very difficult for a mutation to affect the phenotype. This is because each new allele has to compete with a number of wild-type alleles and the only gene mutations that can be expressed in the phenotype of polyploid apomictic parthenogens are those dominants which exert an effect strong enough to overshadow that of two or more alleles at the same locus. Such mutations are rare (Suomalainen, 1961; Mayr, 1963).

Further speculations regarding the expression of mutations in apomictic parthenogenetic animals, which may be relevant to P. antipodum have been made by Suomalainen (1961, 1962). He argues that increasing heterozygosity will occur between more and more gene pairs (because elimination of recessive mutations by natural selection is impossible) until the two chromosome sets can no longer be considered diploid or polyploid in a genetic sense. This will reduce obstacles to the expression of the mutations present in them, and may thus, in part, even allow the formation of

morphologically divergent biotypes. Further, with a continuous increase in the degree of heterozygosity, an apomictically parthenogenetic form gets an ever increasing chance to benefit from heterosis (hybrid vigour). This may therefore provide the basis for the great adaptiveness and dispersive ability of many parthenogenetic forms (Suomalainen, 1962), although it is in direct contrast with the widely held view alluded to previously, that parthenogenesis leads to a lack of adaptability, and long term disadvantage (White, 1954).

(c) SHELL ORNAMENTATION

Studies into the factors causing spininess in shells of P. jenkinsi have been the concern of several workers (Robson, 1926; Boycott, 1929; Boettger, 1931; Steusloff, 1939; Adam, 1942; Bondeson and Kaiser, 1949; Warwick, 1944, 1952), and a number of hypotheses have been put forward to account for this condition. None, however, has answered this difficult question satisfactorily, and research is being continued by Mr. T. Warwick of the University of Edinburgh (pers. comm.) who has been concerned with this problem for over 24 years. Mr. Warwick is also examining ornamentation in the New Zealand species P. antipodum, and therefore experimental studies in this field have not been made during the course of the present investigation. Nevertheless, because considerable variation in the degree of ornamentation is found in P. antipodum as in P. jenkinsi, a discussion of the situation pertaining in the latter is of considerable interest and relevance.

Ornamentation in P. jenkinsi exists in many degrees of strength, from a faint line to a well marked spinous keel, and in addition accessory keels may also exist (Warwick, 1944; Fretter and Graham, 1962). The ornamentation is purely periostracal (Boycott, 1929; Boettger, 1931), and Boycott found that internally, no irregularity of the mantle edge corresponding to the ornamentation could be found. Warwick (1952), however, concluded that the keel was produced by a small, blunt lobe of the mantle edge, and Steusloff (1939) has stated that in certain cases the whole shell participates in ornamentation. In P. antipodum ornamentation is purely periostracal and no sign of a mantle lobe has been found.

Since P. jenkinsi was first discovered in 1889 in brackish waters in the British Isles, its subsequent dispersal through the brackish and fresh waters of Britain and Europe has been followed with considerable interest. The keeled form was the first found, both in the British Isles (Marshall, 1889) and in Belgium (Adam, 1942), in both cases in brackish water. Adam stated that in Belgium, shells ornamented by a keel or spines were found exclusively in saline water, whereas fresh water was inhabited by animals with smooth shells. Bondeson and Kaiser (1949) reviewing the situation in Denmark noted a similar situation, the older records mentioning the keel as typical of the shell whereas more recent records from inland localities showed an increase in the numbers of individuals without keels. Siefert (1935) stated that the more saline the water the more ornamentation

was found, and Steusloff (1939) found that water of high salinity caused by industrial waste pollution harboured spiny snails, whereas lower down the river where the water was purer smooth shelled snails predominated. As a result of these observations, water salinity was considered to be the most important keel inducing factor. Results of experimental work, however, have not upheld this view. Bondeson and Kaiser (1949) were unable to find any real relationship between shell structure and salt content, and Robson (1926) and Boycott (1929) both bred the spined form in salt and fresh water. After 15 years' work, Boycott tentatively concluded that ornamented individuals were the result of breeding under "bad" conditions such as small containers or dirty water. Boettger (1949) on the other hand propounded the view that keel development was due to optimal conditions provided by a combination of food, temperature, pH, and especially a surplus of oxygen in fresh or brackish water, and Warwick (1944, 1952) has suggested that algal metabolites carried in the water may be the keel inducing factors. He considered that the keel inducing influence need only act during the first few weeks of life, and that it probably acted in a quantitative manner to produce keels of varying strengths. Warwick's work has also indicated that both common and trace inorganic constituents of natural waters have no direct effect on keel production, that excess plant organic matter neither causes nor inhibits keel formation, and that good aeration from birth has no keeling effect in either brackish or fresh water. He has also

suggested that in Britain P. jenkinsi may exist in three strains which differ phenotypically and genetically, although they may live side by side, and that keel production is partly genetically determined by the strain to which the parent snail belongs. Bondeson and Kaiser (1949) have found it difficult to accept Warwick's 'algal-metabolite-keel-inducer' proposal and suggest that his results might be better explained as the result of optimal environmental conditions as concluded by Boettger (1949).

In P. antipodum all degrees of keel production may occur in a great variety of habitats, and within populations, or even between the progeny of a single individual there may be marked differences in shell form. Where P. antipodum is living in brackish water, both smooth and spiny shells are regularly found. The two solely estuarine species P. estuarinus and P. pupoides always have smooth shells, however, as have the three hydrobiid species, Hydrobia ulvae, H. ventrosa, and H. neglecta, which represent their nearest ecological counterparts in Europe. It seems reasonable to conclude, therefore, that shell ornamentation developed along with ovoviviparity and parthenogenesis, concurrently with the invasion of fresh water from a former marine and/or estuarine environment (Fig. 25).

A possible genetic basis for shell polymorphism in P. jenkinsi and P. antipodum is suggested as follows. Ornamentation may be under polygenic control rather than determined by a single pair of alleles, and the expression of different degrees of shell ornamentation could result from interaction

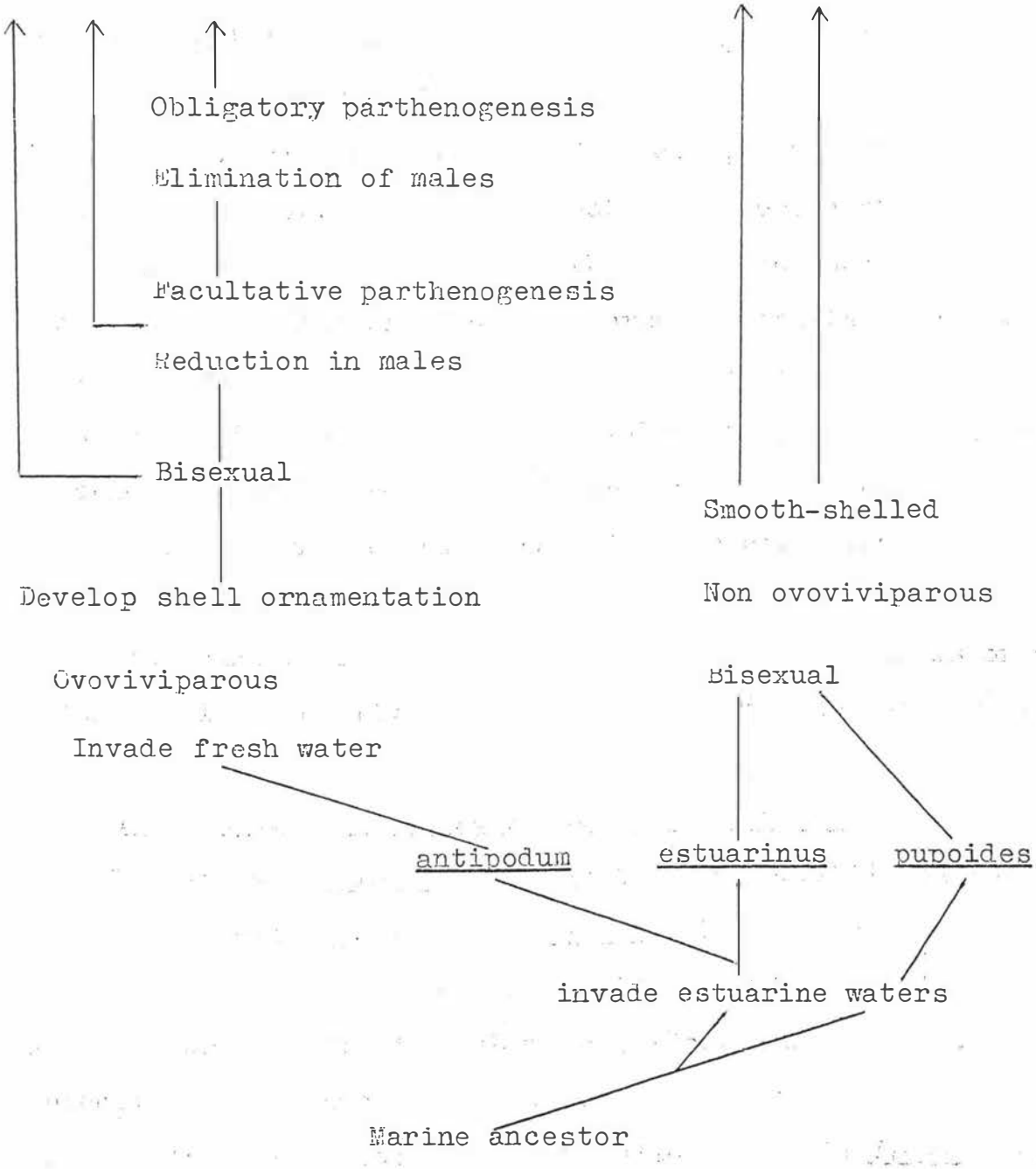


FIGURE 25.

Suggested steps in the evolution of the New Zealand species of *Potamopyrgus*.

between environmental factors and the genomes of shell secreting cells in the mantle. Characteristically, only a part of a cell's genome is manifest at any one time (Markert, 1965), and environmental changes would modify and direct gene function producing phenotypic differences, e.g. inducing spine development, when the correct genes were active. This mechanism is in accordance with the situation found in the production of different isozymic forms of lactate dehydrogenase in vertebrates, in which environmental changes (possibly differences in oxygen availability) regulate gene function (Markert, 1965). A similar mechanism could reasonably account for the apparently random, intra-specific variation in shell ornamentation which is frequently found, and which cannot be explained in simple Mendelian terms or as solely environmentally controlled changes of the phenotype.

(d) THE RELATIONSHIP OF FLUVIOPUPA TO POTAMOPYRGUS

The genus Fluviopupa Pilsbry 1911 was established for a Fijian species Fluviopupa pupoides Pilsbry, and now contains species from the New Hebrides, Lord Howe Island and the Austral Islands (Hubendick, 1952; Solem, 1959). Hubendick (1952) has suggested that a number of other Pacific Islands Hydrobiidae might also belong in Fluviopupa, including the New Zealand species Potamopyrgus spelaeus. In fact the species referred to by Hubendick was almost certainly Suter's (1905) P. spelaeus pupoides which possesses a pupiform shell unlike his P. spelaeus spelaeus. As a result of the present study P. pupoides has been reinstated

as a full species, whereas P. spelaeus has been synonymysed with P. antipodum.

Solem's (1959) revised diagnosis of Fluviopupa is given below.

Fluviopupa Pilsbry, 1911

Shell minute, pupiform, with obtuse apex and only slightly rounded whorls. Aperture ovate, vertical or sloping forward below, peristome completely or almost completely free from under portion of body whorl. Operculum thin, horny, paucispiral, with sub-central nucleus. Radula typically amnicolid, central $\frac{(4-5)-1-(4-5)}{(2-4)-(2-4)}$, lateral with 7-12 cusps, marginals with about 30 minute cusps. Penis with single duct, tip simple or bilobed, some species with a median bulb.

Type species: (monotypy) Fluviopupa pupoides Pilsbry.

This diagnosis could equally well refer to Potamopyrgus with respect to radula, operculum and penis structure, and with some reservation to the form of the shell. Males of the three New Zealand species of Potamopyrgus, and the male of P. jenkinsi all possess simple, non-lobate penes similar to those of some species of Fluviopupa, and the radula structure including cusp formulae of the two genera fall within the same range of variation. Typically, shells of Fluviopupa species are smaller and more pupiform than those of Potamopyrgus species but that of F. brevior (Ancey)(formerly known as Potamopyrgus brevior) is less pupiform, and more conical than that of P. pupoides, and falls within the

range of shell shape found in P. antipodum.

As this study and others have shown that the shells of Hydrobiidae are generally unreliable as taxonomic characters, P. pupoides should, in our present state of knowledge, be retained in Potamopyrgus. The validity of Fluviopupa as a distinct genus must be considered open to question, unless anatomical studies, particularly of the reproductive system, show important differences from Potamopyrgus.

(e) THE RELATIONSHIP OF P. ANTIPODUM TO THE EUROPEAN SPECIES P. JENKINSI

P. jenkinsi made a sudden appearance in Europe, being first described by E.A. Smith in 1889, although it may have been present as early as 1859 (Frömming, 1956). Its origin is uncertain and has been the subject of considerable speculation which has been reviewed by Adam (1942), Bondeson and Kaiser (1949), and Fretter and Graham (1962). The subsequent distribution of P. jenkinsi through Europe has also been discussed by these authors as well as Hubendick (1950), Hunter and Warwick (1957), Lucas (1960), and Heuss (1961). Unconfirmed fossil evidence which indicates that it was living in England in Roman times has been presented by Kennard (1941), but Bondeson and Kaiser (1949) have stated that these records "must be much doubted". Attempts to explain the sudden appearance of P. jenkinsi in Europe have been made by various authors, and two possible explanations have been suggested, (a) that it arose by mutation, and (b) that it had been introduced from elsewhere. Steusloff (1927) suggested that P. jenkinsi arose by mutation from

Hydrobia ventrosa, but there has been no further support for this theory. Boettger (1949) suggested that it may be a mutant developed from the West Indian species Potamopyrgus crystallinus introduced into Europe. Bondeson and Kaiser (1949) have hypothesized a possible Australian origin on account of the close resemblance to the Australian species Austropyrgus pattisoni, and Boettger (1951) has more recently suggested a New Zealand origin for P. jenkinsi, as he considered its shell characters identical with those of the South Island species P. badia (= P. antipodum). As a result of this present investigation, a closer comparison can be made between P. jenkinsi and P. antipodum.

Both P. jenkinsi and P. antipodum show a high degree of morphological variation with respect to the shell, radula and body pigmentation although this variation is greatest in P. antipodum (Warwick, pers. comm.). The two species cannot be differentiated between using these three characters, the operculum, or the structure of the reproductive system. The chromosome number 2n=24 found in P. antipodum corresponds closely to Rhein's (1935) unconfirmed value of 2n=20-22 in P. jenkinsi from Europe. Both species are ovoviviparous, and P. jenkinsi is parthenogenetic as are many populations of P. antipodum. A single male of P. jenkinsi has been described, however, (Patil, 1958), and it is possible that a similar situation to that found in P. antipodum in which variable numbers of males occur in some populations, also exists in P. jenkinsi.

Both species reproduce throughout the year, an unusual

condition in freshwater Mollusca (Fretter and Graham, 1962), and although a maximum of only 35-40 embryos have been recorded in the brood pouch of P. jenkinsi, compared with over 100 in some individuals of P. antipodum, this is unlikely to be of systematic significa ce. Rather, it is probably a function of the snails' (and therefore the brood pouch) size, as P. jenkinsi rarely exceeds about 5mm in shell height, whereas P. antipodum may attain a size of 10mm. The colder northern European climate experienced by P. jenkinsi may help to explain its smaller maximum size compared with the New Zealand species.

As in morphological and reproductive features, considerable variation in ecology is found within the two species. P. jenkinsi was initially found in brackish water (1889) and has since colonized inland waters throughout Europe and the British Isles, first having been recorded in fresh water in England in 1893 (Hunter and Warwick, 1957). P. antipodum similarly is found in fresh and brackish water although it is primarily a freshwater species and has certainly been established in that environment for a much longer period than has P. jenkinsi. Salinity records and experimental work have shown that both species possess a high degree of euryhalinity and can tolerate considerable and rapid changes in salinity. Maximum salinities at which P. jenkinsi can live and reproduce (12-18$^{o}/_{oo}$) correspond closely to the value of 17.5$^{o}/_{oo}$ obtained in this study at which normal activity of P. antipodum ceases and the snails withdraw into their shells. Both species tolerate waters

with high and low calcium content, and live in a variety of still, and running water habitats, on hard and soft substrates, and amongst vegetation.

Because reproduction is primarily by parthenogenesis, the species criterion of interfertility between groups cannot be applied. Consequently, determination of species limits must be based on the morphological, cytological and biological evidence discussed above. The possession of parthenogenesis by these species has led to the establishment of many reproductively isolated populations between which no gene exchange can occur, and it has been postulated that this genetic isolation of populations has permitted the production of such a high degree of phenotypic and genotypic variation within P. antipodum. Presumably a similar situation is occurring in P. jenkinsi, although in the short time that it has been established in Europe only a limited amount of divergence between populations (and gene pools) has been able to occur. P. jenkinsi has been described as genetically unstable by Fretter and Graham (1962), as occurring in a number of strains some showing substrains by Warwick (1952), and by Bondeson and Kaiser (1949) as a species in evolution.

To conclude, no significant difference between the two nominal species have been found, and the evidence available suggests they are therefore the same. In this case, P. jenkinsi (E.A. Smith, 1889) should be synonomyzed with P. antipodum (Gray, 1843), the latter being the first described and having priority.

The systematics of the Australian freshwater hydrobiids, some of which are, or have been placed in Potamopyrgus, are not clear at present and their relationship to the New Zealand species of Potamopyrgus cannot be clarified until after comprehensive morphological and biological studies have been made. The presence of several related genera and species in Australia, including Tatea, which Ponder (1967) has shown to be a hydrobiid genus closely allied to Potamopyrgus, and the widespread occurrence of Fluviopupa (= Potamopyrgus?) species and others possibly referable to this group in the South Pacific (Hubendick, 1952) strongly suggests that New Zealand is near the geographical centre of Potamopyrgus evolution. It seems likely, therefore, that the European snails have been introduced from the Australasian region, thus supporting Boettger's (1951) hypothesis.

2.0 BIOLOGICAL STUDIES ON P. ANTIPODUM

2.1 POPULATION STUDIES

INTRODUCTION

No studies have been made specifically to examine aspects of the ecology or life histories of Potamopyrgus spp. in New Zealand, but some observations on their distribution and ecology have been made during the course of other investigations. The most comprehensive population data available are those provided by Allen (1951) who made quantitative assessments of snail numbers and biomass in Horokiwi Stream for a period of over two years (1939-41). He found that P. antipodum was an important member of the invertebrate bottom fauna and was freely eaten by trout. Allen (1958) also discussed variations in snail numbers in riffle samples as part of a general consideration of bottom fauna distribution. Scott (1965) has carried out experimental studies on the distribution of P. badia (= P. antipodum) in relation to substrate particle size.

Information on Potamopyrgus spp. gathered during the course of synecological studies has been recorded by Suter (1905b), lakes; Oliver (1923) marine littoral zone; Armstrong (1955), Lake Taupo; Cunningham et al. (1953), dune lakes; Hirsch (1958), polluted rivers; Bruce (1958), Williams (1960) and Rosenberg (1963), estuary pollution; Barclay (1966), ponds; and Hopkins et al. (1966), streams and effect of DDT. The importance of Potamopyrgus as trout food has been examined by Phillips (1931), Smith (1959), Lane (1964) and Fish (1966), and as food of other fish and

birds by Cairns (1942), Burnet (1952) and Hopkins (1965), eels; Parrott (1929) and McDowall (1965), bullies; Dickinson (1951), shags (probably secondarily via fish); and Carroll (1967), white faced heron.

In this study, population structure and reproductive activity in three populations of P. antipodum have been examined over a period of 13-14 months. Supplementary information on growth rate and generation time has been obtained by laboratory rearing of snails, and some aspects of the ecology of a stream population have been investigated.

STUDY AREAS

(a) Tiritea Stream

Tiritea Stream has its origins at an altitude of 1450 feet in the northern Tararua Ranges and flows into the Manawatu River twelve miles to the west. The catchment area of Tiritea Stream and its tributaries is clad partly in regenerating bush, with steep grassland comprising the remainder. The lower section of the stream meanders through gently undulating, terraced grassland. Observations were made in the lower half of the stream where the generally unstable bed consists of silt, gravel and small stones forming alternating pool and riffle areas. It is subject to frequent and rapid changes in water level throughout the year although in dry summers extensive weed beds of charophytes, Potamogeton ochreatus, and filamentous green algae may develop in many places. The stream is subject to a minor degree of organic pollution derived from farms, Massey

University and D.S.I.R. laboratories situated in the Tiritea valley.

(b) Ponds A and B

The two ponds, situated about 300 feet apart, had surface areas of approximately 500 ft^2 and maximum depths of 3 feet. Both were filled with water pumped from an artesian bore, and although situated about 120 feet from Tiritea Stream and normally isolated from it, in times of severe flooding (such as occurred in April 1965) they could be inundated by water from the stream when the latter overflowed its banks. There was, therefore, a limited opportunity for mixing of snails between the three populations. The pond beds were covered by a shallow (up to 2 inches) coating of fine mud, and during most of the year their waters were choked by a dense growth of the oxygen weed Elodea canadensis. In August-September 1965, the ponds were emptied and cleaned, and in May 1966, Pond B was permanently filled in.

CLIMATE

Andrewartha and Birch's (1954) environmental component "weather" is in many ways less complex in aquatic than terrestrial environments because humidity is not involved, and also because extremes of temperature are less and change is not so rapid. Temperature is the main variant (Reynoldson, 1966) and its importance has been stressed by numerous workers (e.g. Muirhead-Thomson, 1958; Macan, 1963; WHO Report 120, 1957). A second climatic factor often of considerable importance to freshwater molluscs is rainfall, which affects

water level, flooding, current speed, temperature and water chemistry in the snails' environment.

(a) Temperature

Local air temperatures normally provide a reliable guide to temperatures found in small bodies of water, and have been utilized by ecological workers, e.g. Duncan (1959) in a study of the pulmonate mollusc, Physa fontinalis (L.). In New Zealand, Winterbourn (1966) found a close relationship between air and water temperatures in Swanson Stream, and Barclay (1966) reported a similar close correlation between water temperature of a temporary pond and the surrounding air temperature.

Air temperatures recorded at Grasslands Division, D.S.I.R., in the lower valley of the Tiritea Stream during the period of regular monthly sampling are summarized in Fig. 26a.

Mean monthly temperatures ranged from 8°C to 19°C, with mean maximum temperatures of 23°C, and mean minimum temperatures of 4.5°C. The diurnal temperature range of the stream water and the air above it was recorded in September, 12 readings being taken over a 24 hour period (Fig. 26b). As expected, temperature fluctuations were far greater in the air, 21-5.7°C (range 15.3°C) than in the water, 16.1-10.9°C (range 5.2°C). Changes in water temperature followed those in the air, although there was a time lag of about one hour. Mean air and water temperatures recorded over this 24 hour period were almost identical (12.4 and 12.8°C respectively). This evidence supports the

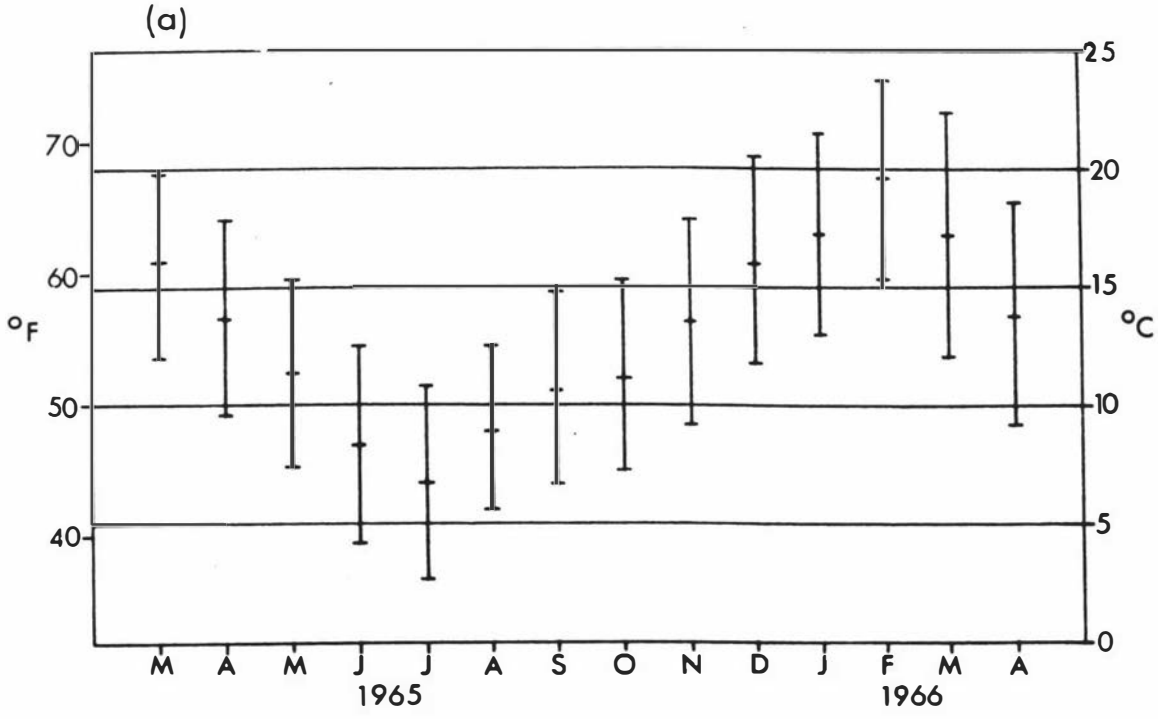
FIGURE 26

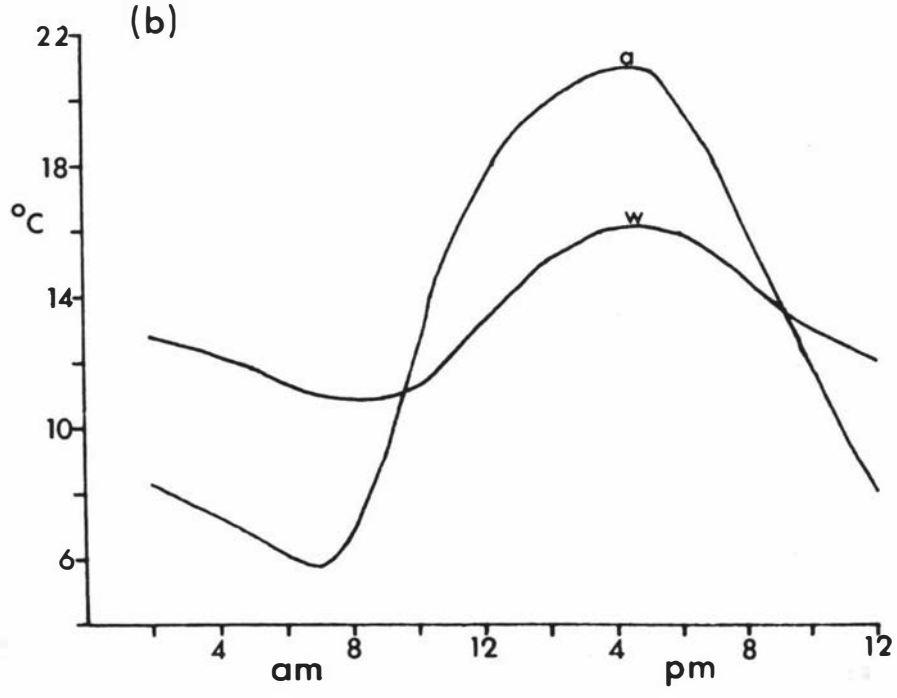
(a) Air temperatures recorded at Grasslands Division, D.S.I.R., Palmerston North, in the lower valley of Tiritea Stream, during the period of monthly sampling (March 1965 - April 1966).

Key

Vertical bars show temperature range each month.
Horizontal bars show mean minimum, mean maximum, and mean monthly ($\frac{1}{2}$[max+min]) temperatures.

(b) Diurnal air and water temperatures recorded in Tiritea valley at Massey University on 16-17 September, 1966.


(a)
70
60
50
40
°F
25
20
15
10
5
0
°C
M A M J J A S O N D J F M A
1965
1966


(b)
22
18
14
10
6
°C
a
w
4 8 12 4 8 12
am
pm

contention that local air temperatures provide a reliable indication of mean water temperatures.

(b) Rainfall

Allen (1951) found that an unexpectedly close relationship existed between flood conditions in the Horokiwi Stream, and rainfall figures recorded at Plimmerton three to four miles away between the lower valley of the stream and the sea coast. The nature of this relationship was that a given rainfall at Plimmerton normally produced a rise about 12 times as great in the stream level in the following 24 hours. A similar relationship exists between rainfall and level of the Tiritea Stream as indicated by recordings taken at Grasslands Division, D.S.I.R. (Table 15). An increase in stream level of the order of 10 to 12 times the rainfall recorded in 24 hours was found to occur.

TABLE 15.

Relationship between rainfall recorded at Grasslands Division, D.S.I.R., Palmerston North, and rises in level of Tiritea Stream in May 1967.

Date	Rainfall (inches)	Water level (inches above normal)	Date	Rainfall (inches)	Water level (inches above normal)
8 May	0.2	0	22	0.0	0
9	0.1	3	23	0.0	0
10	0.1	2	24	0.1	0
11	0.2	5.5	25	0.6	6
12	0.0	3.5	26	0.2	8
15	0.1	2	27	0.5	14
16	0.3	8			

Allen (1951) classified floods into three categories according to their severity, and his scheme shown below has proved suitable in evaluating flood conditions in Tiritea Stream.

Classification of floods (after Allen, 1951).

Type	Rise in level	Average rainfall (24 hours*)	Disturbance of bed
Slight	12-23"	1.0-1.99"	Some movement of surface gravel.
Moderate	24-35"	2.0-2.99"	Extensive movement of surface gravel. Some bank erosion.
Severe	>36"	>3.0"	Widespread, deep gravel disturbance. Overflowing banks.

*Consistent rainfall over several days has a cumulative effect on water level of a similar order to the 24 hour values given.

Employing rainfall data obtained by Grasslands Division, occurrence and severity of floods during 1965-66 were determined (Table 16).

TABLE 16.

The occurrence of slight, moderate and severe floods as determined by rainfall data obtained at Grasslands Division, D.S.I.R., Palmerston North, during 1965-66.

Year	Type	Months												
		J	F	M	A	M	J	J	A	S	O	N	D	Totals
1965	Slight	1	1	2							1	1		6
	Moderate													0
	Severe			1									1	2
1966	Slight	2		1					1				2	6
	Moderate								1				2	3
	Severe		1					1						2

METHODS

(a) Life history and population dynamics

(1) Sampling

Observations were made over a 14 month period (March 1965-April 1966) in Pond A, and for 13 months (April 1965-April 1966) in Pond B and Tiritea Stream. Samples were collected monthly, except in the period after the ponds had been drained when additional sampling was carried out. All samples were taken with a long-handled dip net (18 meshes/cm) which was swept through vegetation and bottom mud in the ponds, and along a submerged shelf and beneath the banks in the stream. Sampling was non-quantitative with respect to substrate area but embryo counts were utilized to provide a quantitative aspect to life history data. About 200 individuals, randomly selected from field samples were examined each month (Appendix 6). Samples half this size have been used successfully in comparable studies by Duncan (1959) and Hunter (1961). During the period when Pond A was dry, 2ft^2 samples of bottom mud were collected at weekly intervals and examined for snails.

(2) Size determination

Maximum shell height was used as the sole indicator of size. This is a widely accepted practice as the use of linear shell measurements is considered to provide a general index of the whole growth of the snail (Wilbur and Owen, 1964). Measurements were made as in Section 1.4.

(3) Embryo examination

Embryos were removed from adult snails by two methods:

(i) Snails were squashed between two plates of glass. This was a satisfactory method of obtaining embryos but the soft parts of snails were invariably damaged when using it. As other observations were being made on the snails simultaneously, it was generally unsuitable.

(ii) Shells were dissolved in a weak (approximately 2N) solution of hydrochloric acid. Soft parts of preserved snails were not affected when this technique was used, and embryos were easily removed by making an incision in the brood pouch wall. This method was adopted on most occasions.

(4) Sex determination

Sex could not be determined while the snail remained in its shell. Mature females with shells removed were easily recognizable by their brood pouches containing embryos while males of all ages possess a penis which is found on the right side of the head behind the right tentacle. The penis is normally hidden beneath the anterior mantle edge which must be turned back to render it visible.

(5) Determination of growth rate and generation time

Because of the lack of a clearly defined reproductive period, it was not possible to follow the growth of snails using population data obtained in the field. Therefore snails were reared experimentally in the laboratory to obtain an estimate of the growth rate and generation time.

One quart "Agee" jars which had been immersed in Pond A for a month to allow encrusting green algae to settle on

their sides were used as rearing containers. Jars were filled with tap water, and several strands of Elodea canadensis and about 10 grams of finely sieved pond mud were added. No artificial aeration was necessary. Further additions of pond mud were made at intervals throughout the growth period and the decomposing organic matter within it provided sufficient food material to sustain continuous growth.

P. jenkinsi is listed as a detritus feeder by Fretter and Graham (1962), Newell (1965) has shown that the related H. ulvae digests mainly the bacterial coating on organic debris and silt, and van der Schalie and Davis (1965) were able to rear the Oriental hydrobiid Oncomelania successfully on a substrate of unsterilized mud supporting a rich microflora of diatoms which when decaying formed the snails' main food supply.

During the growth period water temperature was recorded continuously with a maximum-minimum thermometer suspended in a separate container alongside the four experimental aquaria. Shell height was employed as the sole indicator of growth.

(b) Stream ecology

(1) Sampling

Difficulties encountered in obtaining quantitatively meangingful samples from freshwater habitats, particularly running waters are many, and have been expressed by innumerable workers (for reviews see Cummins, 1962, and Southwood, 1966). The tremendous variability of microhabitats within a small area of stream bottom normally

leads to the variance of a series of samples being very large, e.g. Needham and Usinger (1956) found that they required 73 $1ft^2$ Surber samples from a single riffle, to obtain an accurate estimate (95% confidence level) of the numbers of bottom fauna organisms present. It is not feasible for such enormous sampling programmes to be carried out in most investigations which therefore have to rely on limited quantitative, or semi-quantitative methods, a number of which have been discussed recently by Morgan and Egglishaw (1965).

To investigate the distribution of P. antipodum in Tiritea Stream two surveys were carried out, in October 1967 and March 1968. All samples were taken with a $1ft^2$ ($9.21dm^2$) Surber sampler. In October, distribution was examined at three stations (Stations 1,3,6, Table 24, p.128), 15 samples being taken from a riffle at each station, three samples from adjacent pools, and at Station 3, four samples from within willow roots along the stream margins, and two from mud in still water beneath the banks. In March, three extra stations were incorporated in the survey (Stations 2,4,5, Table 24, p.128). On this occasion 10 samples were taken from a riffle at each of the six stations.

In conjunction with bottom fauna sampling the following physical and chemical measurements were made.

Measurements	Methods
1. Surface water velocity	- measurement of pressure waves.
2. Water temperature	- mercury thermometer.
3. Dissolved oxygen concentration and 5-day BOD	- Winkler method.
4. pH	- battery pH meter.
5. Total alkalinity (at pH 4.5)	- titration with 0.02N HCl, methyl orange as indicator.

(2) Drift

Drift sampling was carried out using the method employed by Waters (1962). A Terylene net (18 meshes/cm), 3 feet long, its mouth 3 feet wide and 2 feet high, was enclosed in a protective outer bag having a strong canvas base. The net was set up on the stream bed in a current, two long brass poles driven into the substratum holding it firmly in place. The lower edge of the mouth of the net was held firmly against the stream bed by a heavy chain enclosed within the front hem of the canvas base and attached to the two brass poles by metal rings. Drift samples could not be taken when the river was in flood because large amounts of silt accumulated in the net and the increased weight resulted in it being wrenched from its original position on the stream bed.

(3) Effects of flooding

Because of the irregular distribution of snails on similar substrata within the stream bed,(Table 24, p.128), estimates of numbers per unit area of stream bed provided by limited sampling programmes are susceptible to a high degree of sampling error. Also, as snails were frequently most abundant in still water and on an uneven substrate, e.g. among willow roots or beneath banks, quantitative stream samplers such as the Surber sampler could not always be successfully employed. Because of these limitations, estimates of snail numbers before and after flooding were made from samples representing "one standard sweep", taken with a fine meshed net (40 meshes/cm), from a carefully selected and clearly defined area of stream bed.

RESULTS AND DISCUSSIONS

(a) LIFE HISTORY AND POPULATION DYNAMICS

(1) Size structures of populations

Monthly height distributions of snails in the three populations are shown in Fig. 27 in which numbers of individuals in each millimetre size class are plotted as a percentage of the total sample. It is clear that small individuals were being recruited into all three populations throughout the year, and that growth of any particular age group could not be followed from month to month by inspection of histograms.

(i) Tiritea Stream

Population structure in Tiritea Stream remained almost constant in all months. At all times a high percentage of the snails were small individuals in the 0.5-3.5mm classes, and in January and February 1966 individuals of the smallest class (0.5-1.5mm) constituted 70% of the total population. There must be an increasingly high rate of mortality as snails grow older, as numbers in the large size classes remained consistently small throughout the year.

(ii) Pond A

From March to August 1965, the population was predominantly an old one, the two largest size classes constituting up to 81% of the total snails. These large snails almost certainly represented a number of generations. Throughout this period small numbers of young snails were being recruited into the population. In November a marked increase in numbers of young snails was found. This high

FIGURE 27

Monthly size structures of three populations of *P. antipodum* from April 1965 to April 1966.

a - Pond B

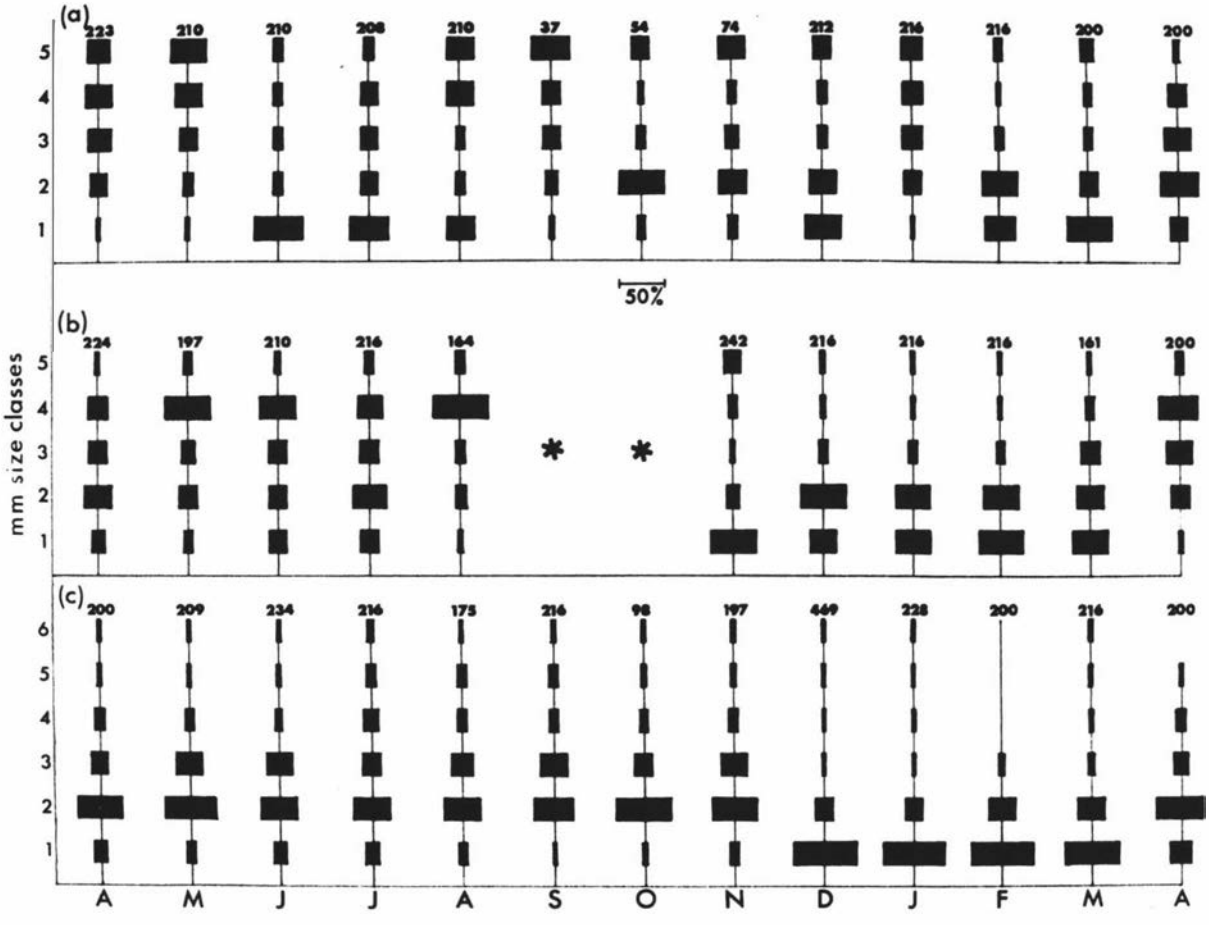
b - Pond A

c - Tiritea Stream

* Period when the pond was drained. See Figure 28.

The width of each bar is proportional to the percentage of the total population in that size class.

The number at the head of each column shows sample size.


(a)
223
210
210
208
210
37
54
74
212
216
216
200
200
5
4
3
2
1
50%
(b)
224
197
210
216
164
242
216
216
216
161
200
*
*
mm size classes
(c)
200
209
234
216
175
216
98
197
449
228
200
216
200
6
A
M
J
J
A
S
O
N
D
J
F
M
A

production rate was maintained until February 1966, but was beginning to decline in March, and had fallen to a fairly low level by April. The population structure from November 1965 to March 1966 was therefore almost the reverse of that found from March to August 1965.

Draining of the pond did not appear to result in selective mortality of particular size groups (Fig. 28b). Reduction in population size each week after draining is shown in Fig. 28a. Following refilling there was a gradual increase in numbers from 45 snails per $2ft^2$ to 150 per $2ft^2$ in the first five weeks. This was accomplished by the release of young, and redispersal through the pond of snails which had accumulated in small damp depressions during the draining period. Nine weeks after refilling a $2ft^2$ sample of mud contained 240 snails, the majority being young individuals.

(iii) Pond B

Population structure, as indicated by histograms, varied considerably from month to month. In May and September 1965, the population was predominantly old, numbers of snails increasing in each successive size class. By contrast, large numbers of young individuals were found in June and July 1965, and February and March 1966. From late September to December and to a lesser extent in early 1966 the population assumed a bimodal form, but by April the larger peak was no longer evident and recruitment of young snails into the population had also declined.

Before draining of the pond the population was a fairly old one, 54% of the snails being in the two largest size

FIGURE 28

Population structure of P. antipodum in Pond A during the period between draining and refilling.

(a) Numbers of snails per $2ft^2$ of pond bed

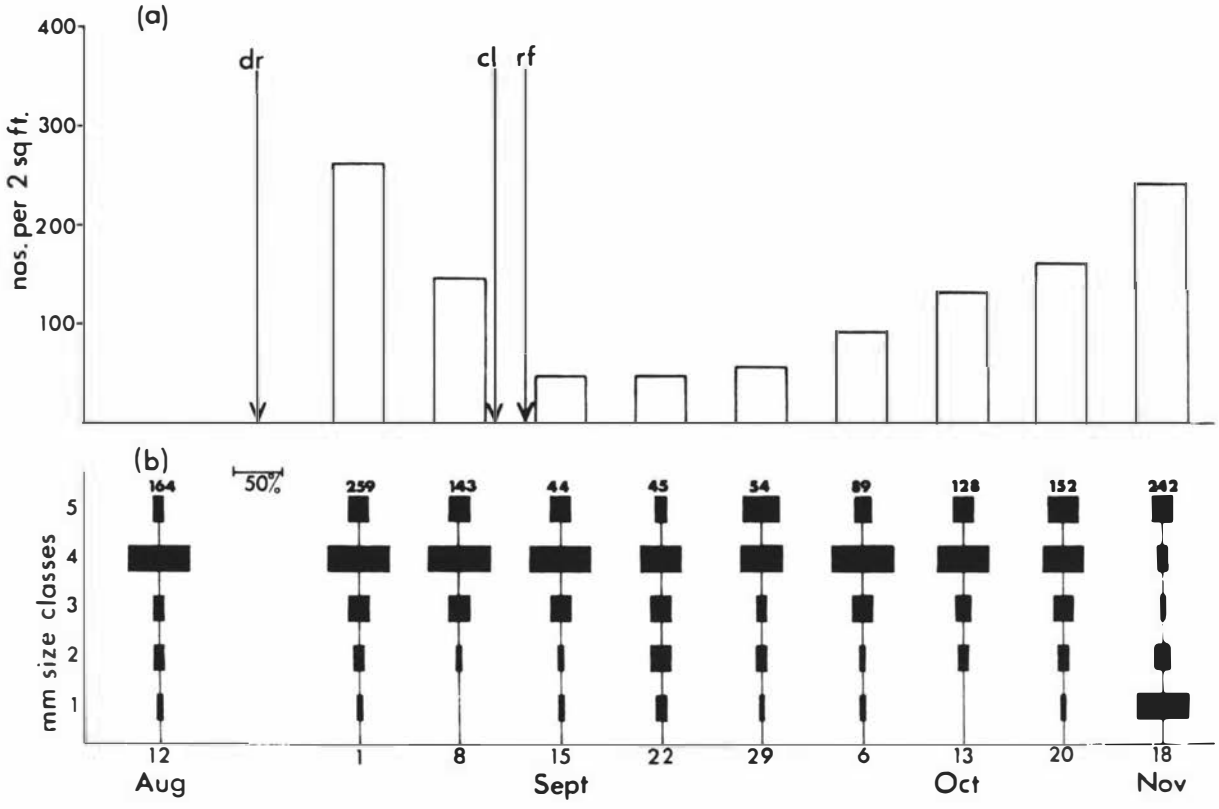
(b) Size structure

Key

cl - pond cleaned out, dead plant material and a considerable amount of surface mud removed.

dr - water drained from pond.

rf - pond refilled.


(a)
400
300
200
100
nos. per 2 sq ft.
dr
cl
rf
(b)
50%
164
259
143
44
45
54
89
128
152
242
5
4
3
2
1
mm size classes
12
Aug
1
8
15
22
29
6
13
20
18
Sept
Oct
Nov

"Students" t-test with Bessel's correction (Moroney, 1963) was applied to the pooled monthly data.

This is adequately explained when it is realized that shells may take several months to develop spines, so that some smooth-shelled juveniles are potentially spiny adults. Percentages of smooth-shelled snails, adults and juveniles, between Ponds A and B did not differ significantly from each other ($P>0.40$), and similarly no significant differences were found between Pond A and Tiritea, or Pond B and Tiritea populations when adult and juvenile snails were considered separately. Significant values ($P<0.05$ and $P<0.01$ respectively) were obtained when total populations were compared however. These results can be explained when size-structures of the populations are taken into account (Fig. 27). In Tiritea Stream, adult snails constituted a very small part of the total population compared with the situation found in the ponds. Therefore, when all individuals were lumped together, the larger snails did not greatly affect the proportion of smooth-shelled individuals in the whole population. As the juvenile section of the population necessarily produces a high percentage of smooth-shelled snails, as explained earlier, the percentage of smooth individuals in the whole population will consequently be high. This underlines the necessity of comparing directly comparable groups in a situation like this where morphological characters change with age, and age-structures of the populations being compared are different.

TABLE 17.

Percentages of smooth-shelled snails each month, in Tiritea Stream, Pond A and Pond B populations: March 1965 - April 1966.

	Months													
	M	A	M	J	J	A	S	O	N	D	J	F	M	A
Tiritea Stream														
Total snails	-	86	82.3	93	91	90.5	96	90	90	98.3	99.2	99.7	96.4	90.6
> 3.5mm	-	72	45.5	96.5	93	87	83.6	73.4	78.8	80	92.4	-	89	58.8
< 3.5mm	-	88	86.5	92.3	90	91.5	100	92.8	92	98.8	99.5	99.7	96.6	93.5
Pond A														
Total snails	90.0	98.6	81.2	93.4	94.5	90.3	*	*	75.6	78	79.1	72.7	66	90
> 3.5mm	89.4	98.5	78	88.5	89.6	91.5	*	*	41.7	50.2	46.2	45.5	33.3	96
< 3.5mm	93.5	98.9	86	97	96.7	86.4	*	*	90	80.3	81.2	74	70	83.6
Pond B														
Total snails	-	72.6	75.5	94.2	92.6	92.5	*	83.4	84	74	71	83.2	91.5	86.5
> 3.5mm	-	66	74.4	83.6	81.3	89.5	*	86.7	75.7	78.5	64	61.5	80.5	78
< 3.5mm	-	81.5	78	97.5	96.2	96	*	82	90	72.7	77.7	86.2	94.5	88.8

* see Table 20.

TABLE 18.

Percentages of smooth-shelled snails in Ponds A and B during the period of draining and cleaning: September-October 1965.

	September					October		
	1	8	15	22	29	6	12	20
Pond A								
Total snails	88.5	80.5	95.5	98	86.4	87.6	77.3	77.6
> 3.5mm	86.2	77	93.6	100	81.5	86	75.6	80.2
< 3.5mm	94.4	93.6	100	95.8	100	100	82.4	71.2
Pond B								
Total snails				75.7	82.2			
> 3.5mm				71	80			
< 3.5mm				84.5	85			

TABLE 19.

Mean percentages of smooth-shelled snails in the three populations, over the whole period of the investigation.

	Tiritea Stream	Pond A	Pond B
Total snails	91.3	85.2	82.8
> 3.5mm	79.3	74.1	76.5
< 3.5mm	91.8	88.7	86.5

(5) Seasonal changes in embryo production

In order to examine seasonality of breeding, numbers of gravid snails (>3.5mm), and counts of embryos present in brood pouches of gravid snails were made each month. As well as seasonal changes in embryo production, differences in numbers of embryos present in individual snails will be caused by their immediate reproductive state, but errors introduced in this way should cancel each other out when samples of adequate size are taken. Percentages of adult snails containing embryos each month, in the three populations are given in Table 20 (p.118).

At all times a far higher percentage of pond than stream snails contained embryos. The comparatively small percentage of gravid snails occurring in the stream can probably be explained, in part, by the more rigorous living conditions provided by the stream habitat. It is likely that, in the stream, snails require a higher proportion of their energy budget for growth and maintenance activities, whereas in the ponds the sheltered habitat is highly favourable to snail growth and energy can more readily be utilized for reproduction.

Numbers of embryos present in brood pouches of gravid snails each month, in each population, are shown in Fig. 29. In Tiritea Stream peak embryo production occurred in summer and reproductive activity was least in winter. Spring was a period of increasing activity, but in autumn numbers did not greatly exceed those obtained in winter. In both ponds, spring, rather than summer, was the season of greatest

FIGURE 30

Mean growth of four laboratory populations of *P. antipodum*.

Key

_ Upper graph shows water temperatures recorded in the laboratory each month. Vertical bars show temperature ranges. Means are joined by a continuous line.

m - shell height at which snails may first become reproductively active

r - first release of embryos

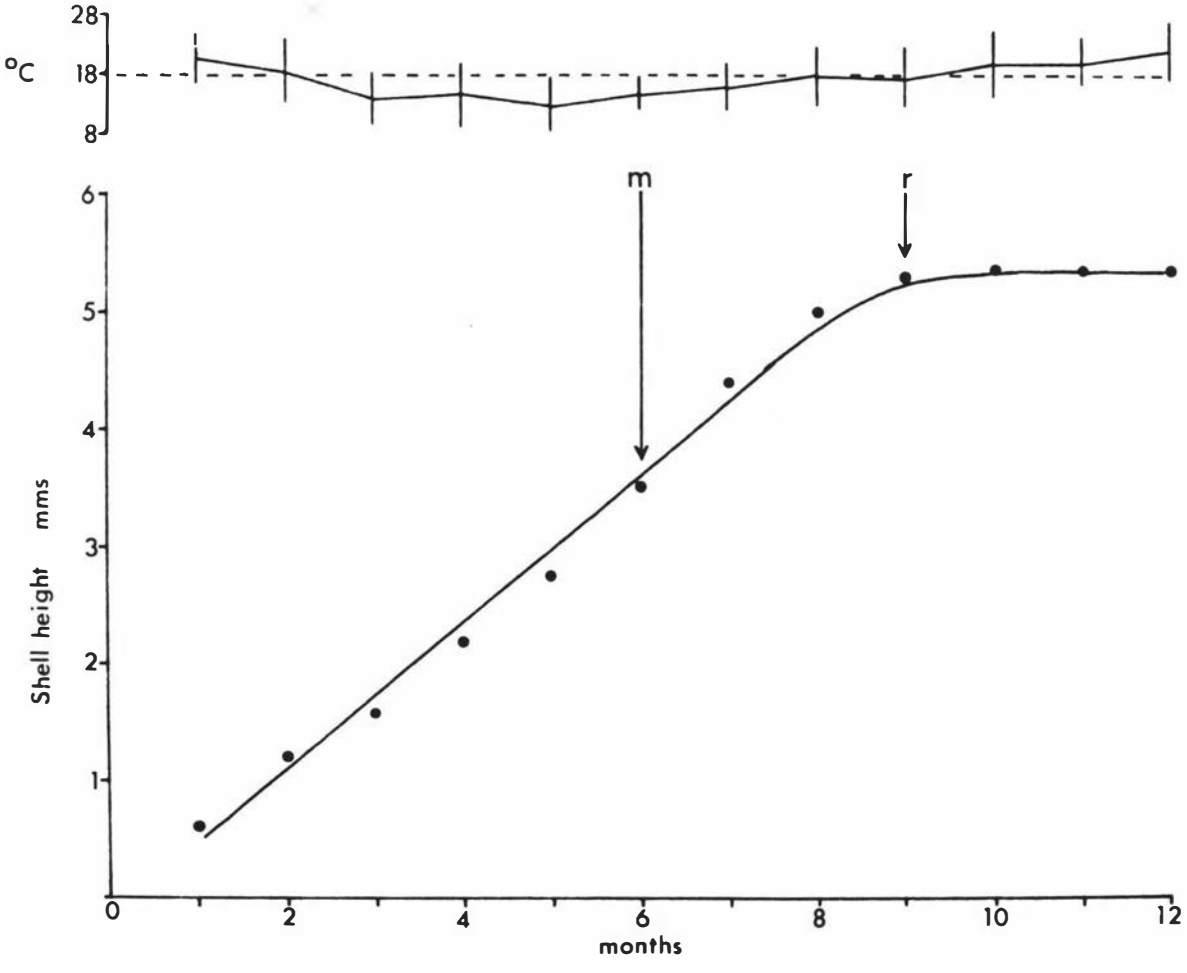

°C
28
18
8
m
r
6
5
4
3
2
1
Shell height mms
0
2
4
6
8
10
12
months

TABLE 20.

Percentages of adult snails (shell length >3.5mm) with embryos each month and for the whole study period (March 1965 - April 1966), in Tiritea Stream, Pond A and Pond B. Overall percentages are the means of the monthly values.

	Months														
	M	A	M	J	J	A	S	O	N	D	J	F	M	A	Over-all
Tiritea Stream	31.3	6.2	12.5	9.4	10	31	27.5	26.7	37	43	30.8	-	27	9.3	23.2
Pond A	93.5	76.6	66.6	80	93.5	90	97	96.8	90	86.7	100	-	100	33.4	88.8
Pond B	-	81.6	93.4	93.4	80	100	87	93.5	84.6	87	90	80	83.4	70	86.5

FIGURE 29

Numbers of embryos per gravid female each month, in three populations of *P. antipodum*.

a - Tiritea Stream

b - Pond A

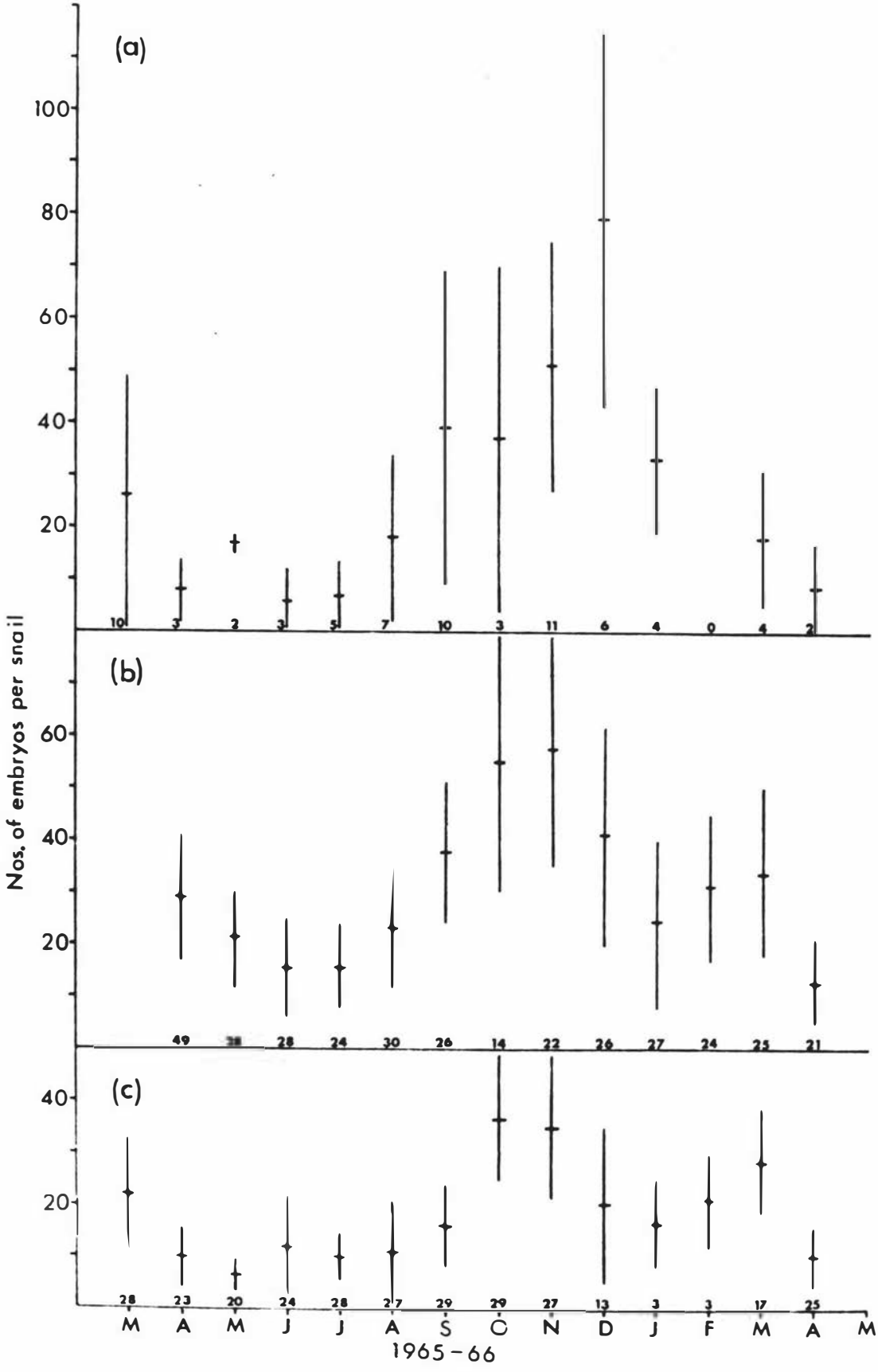
c - Pond B

Key

Horizontal lines show mean numbers.

Vertical bars show one standard deviation either side of the mean.

Numbers are sample sizes


(a)
(b)
(c)
Nos. of embryos per snail
100
80
60
40
20
10 3 2 3 5 7 10 3 11 6 4 0 4 2
49 28 24 30 26 14 22 26 27 24 25 21
28 23 20 24 28 27 29 29 27 13 3 3 17 25
M A M J J A S O N D J F M A M
1965-66

reproductive activity. When mean production per snail in the three populations is compared (Table 21), snails from Pond B are found to have been most productive, mean numbers of embryos produced per snail being significantly higher than was found in Pond A ($P<0.01$), but not significantly greater than was found in Tiritea Stream ($P>0.05$).

TABLE 21.

Mean numbers of embryos per gravid snail in the three populations over the full study period. (Means derived from means of all monthly values.)

Populations	Mean numbers of embryos per snail
Tiritea Stream	26.8
Pond A	18.3
Pond B	30.7

(4) Sex ratios

Both pond populations consisted solely of parthenogenetic females, but limited numbers of males were present in Tiritea Stream. These were assumed to be sexually functional as living sperm was obtained from the testes and vas deferens of those snails examined. Seasonal occurrence of males in Tiritea Stream is shown in Table 22.

TABLE 22.

Numbers of male, female, and gregarine infected snails (shell height > 3.5mm) in monthly samples from Tiritea Stream.

	Months													
	M	A	M	J	J	A	S	O	N	D	J	F	M	A
Females	64	65	24	32	50	45	40	15	35	21	13	12	37	43
Males	1	0	1	0	0	0	0	1	2	0	1	1	3	0
Gregarine infected	1	0	0	0	1	0	1	5	5	2	2	0	7	2

(5) Incidence of parasitism

P. antipodum can be severely infected with the cysts of an unidentified sporogoan protozoan (Gregarinida; Porosporidae) and to a lesser extent by the larval stages of a number of trematode species. (See Section 4.0 where parasitism is discussed in greater detail.) In the three populations studied, no infection of snails by trematode larvae was found, although eels (Anguilla spp.) examined from Tiritea Stream contained gut trematodes whose larval stages infect P. antipodum. Sporozoan cysts were found in snails in the stream population, and numbers of infected snails found each month are shown in Table 22. Snails harbouring cysts are rendered infertile, and in some males the penis becomes greatly reduced. During the course of the study only two snails in Pond B were found infected and none were discovered in Pond A. Heavy infection by cysts must be of considerable

importance in actively reducing reproductive potential in natural populations of *P. antipodum*, but in Kirites Stream its effect is probably slight.

(6) Growth rate and generation time

Twenty, month old snails, the young of individuals obtained from Pond A were used in the laboratory studies, five being reared in each experimental container. The young snails had a mean shell height of 1.2mm (standard deviation 0.14mm) when the study began. Measurements were made monthly for 11 months and are recorded in Table 23 (p.122).

Growth data is presented graphically in Fig. 30 where results from all four containers have been pooled. Water temperatures recorded in the laboratory throughout the growth period are also given. There was an increase in the amount of linear shell growth each month for the first six months until a mean height of 4.3mm had been attained. In the following two months the amount of shell growth declined rapidly and full size was reached after eight months. Minimum size of sexually mature snails, as indicated by the initial appearance of embryos in the brood pouch, occurs when shell height is about 3.5mm. This size was reached after six months. It is probable that most individuals did not commence embryo production until about a month later, however, this time coinciding with the decrease in shell growth. The first juveniles were released after nine months.

FIGURE 30

Mean growth of four laboratory populations of *P. antipodum*.

Key

_ Upper graph shows water temperatures recorded in the laboratory each month. Vertical bars show temperature ranges. Means are joined by a continuous line.

m - shell height at which snails may first become reproductively active

r - first release of embryos

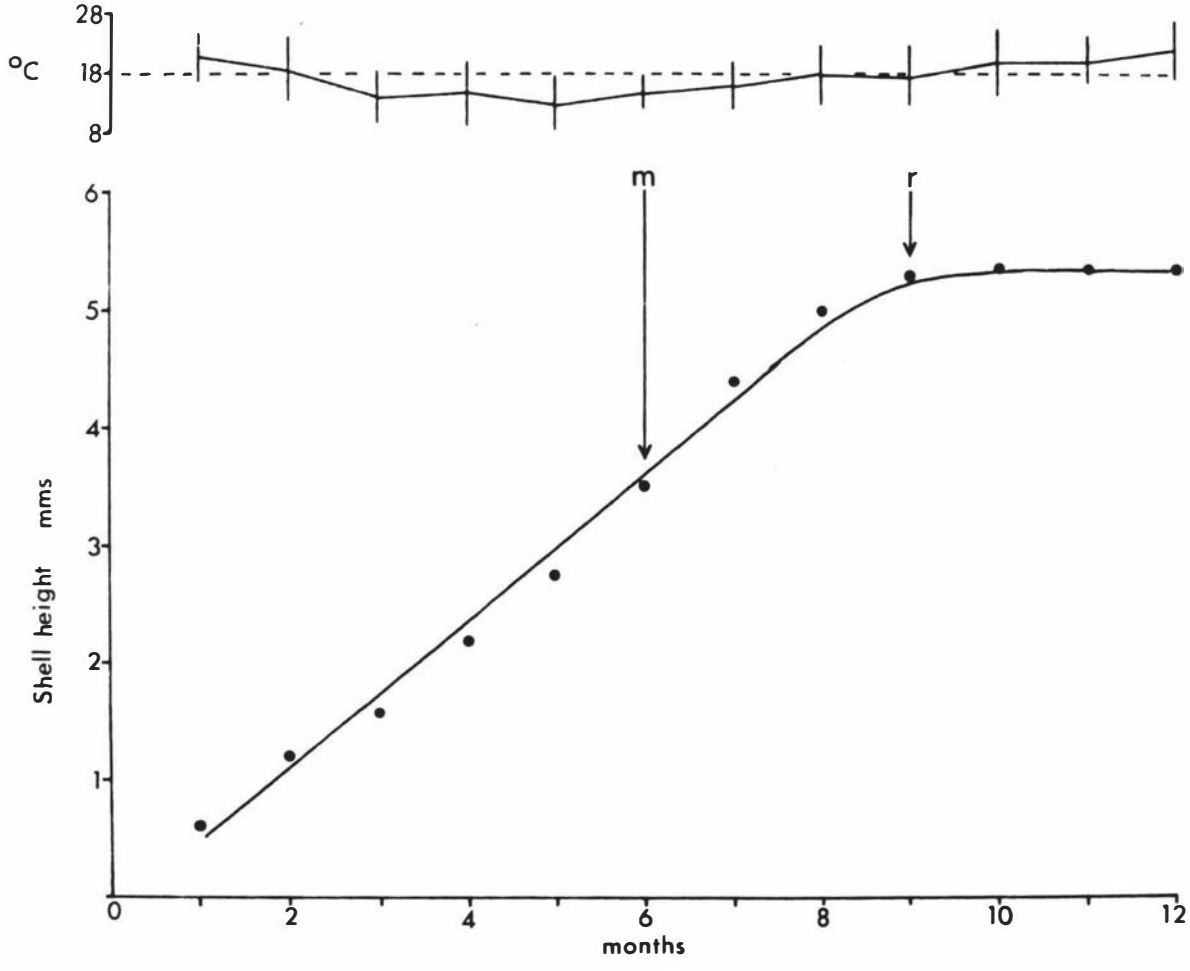

°C
28
18
8
m
r
Shell height mms
6
5
4
3
2
1
0
2
4
6
8
10
12
months

TABLE 23.

Mean monthly growth of P. antipodum in four laboratory populations.

	Months										
	A	M	J	J	A	S	O	N	D	J	F
Mean shell length (mm)											
Population A	1.2	1.7	2.5	3.2	4.2	4.8	5.3	5.4	5.4	5.4	5.4
Population B	1.1	1.4	2.0	2.5	3.0	4.1	4.9	5.3	5.4	5.4	5.4
Population C	1.2	1.4	1.7	2.1	3.1	4.4	4.8	5.3	5.3	5.3	5.3
Population D	1.1	1.6	2.1	2.8	3.4	4.0	4.8	4.9	4.9	4.9	4.9
Mean monthly growth in populations combined (mm)	-	0.35	0.6	0.55	0.75	0.9	0.65	0.65	0.25	0	0

(7) Summary of life history and population data

Three populations of I. antipodum were studied, two in small man-made ponds, and a third in a lowland (Tiritea) stream. In all populations snails existed in two shell forms,

(a) with spines and keeled whorls,

(b) with smooth unornamented shells.

Considerable variation in shell size and shape was found between and within populations. Small numbers of males were present in the stream population, but all pond snails were parthenogenetic females.

Proportions of smooth and spiny shelled snails were determined each month. Over 82% in Tiritea Stream, 66% in Pond A and 71% in Pond B had smooth shells throughout the sampling period, and when all monthly samples were pooled it was found that no significant differences in proportions of smooth and spiny shells existed between populations. It was also found that a larger percentage of juveniles (<3.5mm) than older snails possessed smooth shells, the result of late spine development occurring in some individuals.

Population size structures were examined each month, but because reproduction occurred throughout the year, growth of particular age groups could not be followed from month to month. Throughout the year the stream population was dominated by large numbers of small individuals, whereas both ponds contained a predominance of larger snails, in most months. These larger snails probably represented more than one generation. Differences in population structure probably

reflect a higher mortality rate brought about by the less favourable environment of the stream compared with the sheltered eutrophic conditions, and absence of predators and parasites in the ponds. Draining and cleaning of the ponds was carried out during winter, and although this seriously reduced the numbers of snails present it did not eliminate the populations.

Numbers of mature snails (>3.5mm) containing embryos were determined each month. A lower overall percentage of snails in the stream contained embryos than in the ponds. Seasonal fluctuations in numbers of reproductively active snails within each population were slight, but numbers of embryos produced varied seasonally, maximum production being in spring in the ponds, and in summer in the stream. These findings confirmed histogram data which suggested that the greatest numbers of juveniles were liberated in the warmer months. Rearing of snails in the laboratory showed that adult size (>3.5mm) may be attained after six months growth, and that release of the first young snails may occur after nine months.

(3) Discussion: growth and reproduction

Laboratory growth studies have not always provided an accurate guide to growth in the field (Dundee, 1957; Risenberg, 1966) and the generation length (release from brood pouch to production of young) of nine to 12 months obtained in the present laboratory study should not, therefore, be regarded as an absolute, or unvarying value. It is probable that growth varies considerably in the field, as found in Hydrobia ulvae by Rothschild (1938).

According to Frömming (1956) the generation time in P. jenkinsi is four to five months, with few individuals living longer than seven months, and Boycott (1936) has given one year as the life span of that species. Compared with these values, P. antipodum has been found living for up to nine months after parturition in the favourable conditions provided by laboratory cultures, although it has not been found breeding a second time. It is suspected that similar longevity occurs in Ponds A and B.

Reproduction throughout the year is unusual in freshwater molluscs which generally possess a restricted and clearly defined breeding period (Fretter and Graham, 1962). The European P. jenkinsi, however, like P. antipodum can reproduce throughout the year (Patil, 1958), although it does not always do so. Lumbye and Lumbye (1965) who studied populations of P. jenkinsi at three localities in Denmark, found that snails did not grow, or reproduce in winter during which water temperatures below 2°C were recorded. They found that spring and summer were the most important times for reproduction and growth. Parturition occurs only in summer in Campeloma rufum, a North American, parthenogenetic, ovoviviparous, freshwater gastropod, although ovulation and embryonic development occur in all seasons (Van Cleave and Altringer, 1937; Medcof, 1940). In Northern Hemisphere temperate regions, considerable intraspecific variation is known to occur in growth rates, reproduction, and life cycles of freshwater pulmonates (Hunter, 1964), but the most common pattern is a simple, annual life cycle with breeding in late

spring or early summer. Hunter (1961) considered that the onset of the breeding season was dependent on the interaction of both endogenous (genetically determined) and external (environmental) factors, and this view was also held by Duncan (1959) who concluded that the most important environmental factor stimulating onset of breeding and influencing growth rate was water temperature.

In New Zealand, the only published account of the life cycle of a freshwater mollusc is that by Percival (1931) of the mussel Diplodon lutulentus (= Hyridella menzesi) which was found to have a restricted breeding season, the ripe glochidia being produced from the end of November to the end of January. In contrast to this, other New Zealand freshwater invertebrates, including some Plecoptera (Winterbourn, 1966), and the crayfish Paranephrops planifrons (Hopkins, 1966), are able to reproduce throughout the year. Similarly, in the three populations of P. antipodum examined in this study, neither environmental, nor endogenous factors restricted the period of reproductive activity to part of the year. Nevertheless embryo production did not occur evenly throughout the year, and the peak periods of embryo production found in spring and summer may be related to the higher water temperatures experienced at those times.

The capacity to reproduce at any time of the year is obviously of considerable benefit to a species such as P. antipodum which has limited active powers of dispersal, especially as it often inhabits impermanent waters where without the ability to reproduce immediately upon

colonization the building up of populations would be severely curtailed.

(b) STREAM ECOLOGY

(1) Distribution

Results of the two surveys in Tiritea Stream are given in Table 24 (p.128). In October, the stream bed was clean, and weed was absent except at Station 6 where charophytes and Potanogeton ochreatus formed a permanent weed bed. By contrast, the March observations were made when the water level was very low, current velocity had fallen, and luxurious growths of weed were present in many places.

P. antipodum is not an important member of the fauna in shallow, swiftly flowing water where the bottom is stony and unstable, and the water turbulent (Stations 1 and 2), but where vegetation has become established on the stream bed, a more permanent and stable environment is produced and colonized by P. antipodum. Where water velocity was low and flow non-turbulent as at Station 5 and in parts of Stations 3 and 4 in March, snails could also colonize the main stream channel even where vegetation was absent. The most important microhabitats in Tiritea Stream are provided by regions of relatively still water at the sides of the stream out of the main current, where vegetation (including willow roots) provides a comparatively protected niche, and where the substratum of fine mud containing decomposing organic matter provides an abundant and accessible food supply. Conditions of this kind are abundant in the lower reaches of the stream.

The reduction of dissolved oxygen found in the water at

TABLE 24

Distribution of *L. amblodum* in Whitea Stream in relation to physical and chemical factors, and vegetation.

Stations	1	2	3	4	5	6
Distance from stream mouth (miles)	4	2.5	1.7	1.5	0.7	0.5
Water temperature °C: (Oct. '67)	7.5		7.0			9.0
(Mar. '68)	18.5	21.0	19.5	18.0	17.5	17.5
Dissolved oxygen % sat. (Oct.)	96		88			80
(Mar.)	95	100	112	82	53	46
5-day BOD (Oct.)	1.4		2.3			2.5
(Mar.)	1.7	1.9	1.8	2.2	1.3	1.8
pH (Oct.)	7.6		7.6			8.0
(Mar.)	7.4	7.5	7.5	7.3	7.2	7.2
Total alkalinity ppm (at pH 4.5) (Mar.)	34	35	29	35	37	38
Mean surface current velocity cm/sec (Oct.)	180		69			44
(Mar.)	33	36	31	44	22	43
Substratum	Large stones	Gravel	Gravel and silt	Gravel and silt	Small gravel and silt	Small gravel and silt
Aquatic vegetation (Oct.)	Absent		Absent			Charophyte bed
(Mar.)	Absent	Sparse algae	*Chara*; abundant algae	*Chara*; sparse algae	Algae at margins	Dense *Chara*, *Potamogeton*
Snail distribution (Nos. per $1ft^2$)						
(a) Riffles						
Means (15 samples) (Oct.)	<1		3.3			51.5
SD	-		2.6			74
Ranges	0-1		0-10			3-271
Means (10 samples) (Mar.)	1.3	2.7	31.6	14.0	64.8	45.7
SD	-	3.2	8.0	11.2	54.0	52.5
Ranges	0-5	0-9	24-40	6-32	11-160	3-140
(b) Pools						
Substratum (3 samples) (Oct.)	Silt 0-1		Silt 0			Mud and silt 25-59
(c) Within willow roots (4 samples) (Oct.)			182-794			
(d) Mud beneath banks (2 samples) (Oct.)			295-490			

To examine the distribution of different size classes, snails were subdivided into three groups on the basis of shell height:

small 0.5-2.4mm;

medium 2.5-4.4mm;

large 4.5mm.

Samples taken from places having different substrata and current velocities were found to possess similar population structures as shown in Table 25.

TABLE 25.

Size structures of five samples of *P. antipodum* taken from different conditions in Piritea Stream.

Sample	Nos. of snails in sample	% Large	% Medium	% Small	Description of station
1	716	3	18	79	Weed bed in strong current
2	490	3.5	22	74.5	Pool; mud substrate; current negligible
3	118	3.5	28	68.5	Grass covered shelf; little current
4	424	4	10	86	Willow roots; fairly strong current
5	295	5	17	78	Willow roots; no current

Within the important willow root habitat, the three size classes did not exhibit a uniform vertical distribution, however, a higher proportion of large snails being found near the free ends of roots than towards their bases or within the sediment trapped between them (Table 26).

Table 26.

Distribution of three size classes of snails at different vertical levels within willow roots.

Substratum	Number of snails in sample	% Large	Medium	Small
Complete roots sediment	794	2	3	95
Matted upper roots; no sediment	402	6	23	71
Free untangled roots	624	14	49	37

(2) Occurrence in stream drift

Eight, 24 hour drift samples were taken in May during a period of intermittent rain during which a number of fluctuations in stream level occurred. The net was set up as shown in Fig. 31 immediately downstream from a concentration of willow roots within which a substantial population of snails was living. No snails occupied the section of stony stream bed between the willow roots and the net. Numbers of snails taken per 24 hour period ranged from 45 to 246 (mean 93.5) but no clear relationship between total numbers and stream level was found (Table 27). During severe flooding drift samples could not be taken. Proportions of different size classes present in the drift were similar to those in the population as a whole, so it can be inferred that no one size of snail was more susceptible to drifting than any other.

FIGURE 31

Diagram of a section of Tiritea Stream showing position of drift net.

Abbreviations

r - riffle (snails absent)

sb - shingle bank

wr - region of willow roots

wt - willow tree

Arrows indicate main current

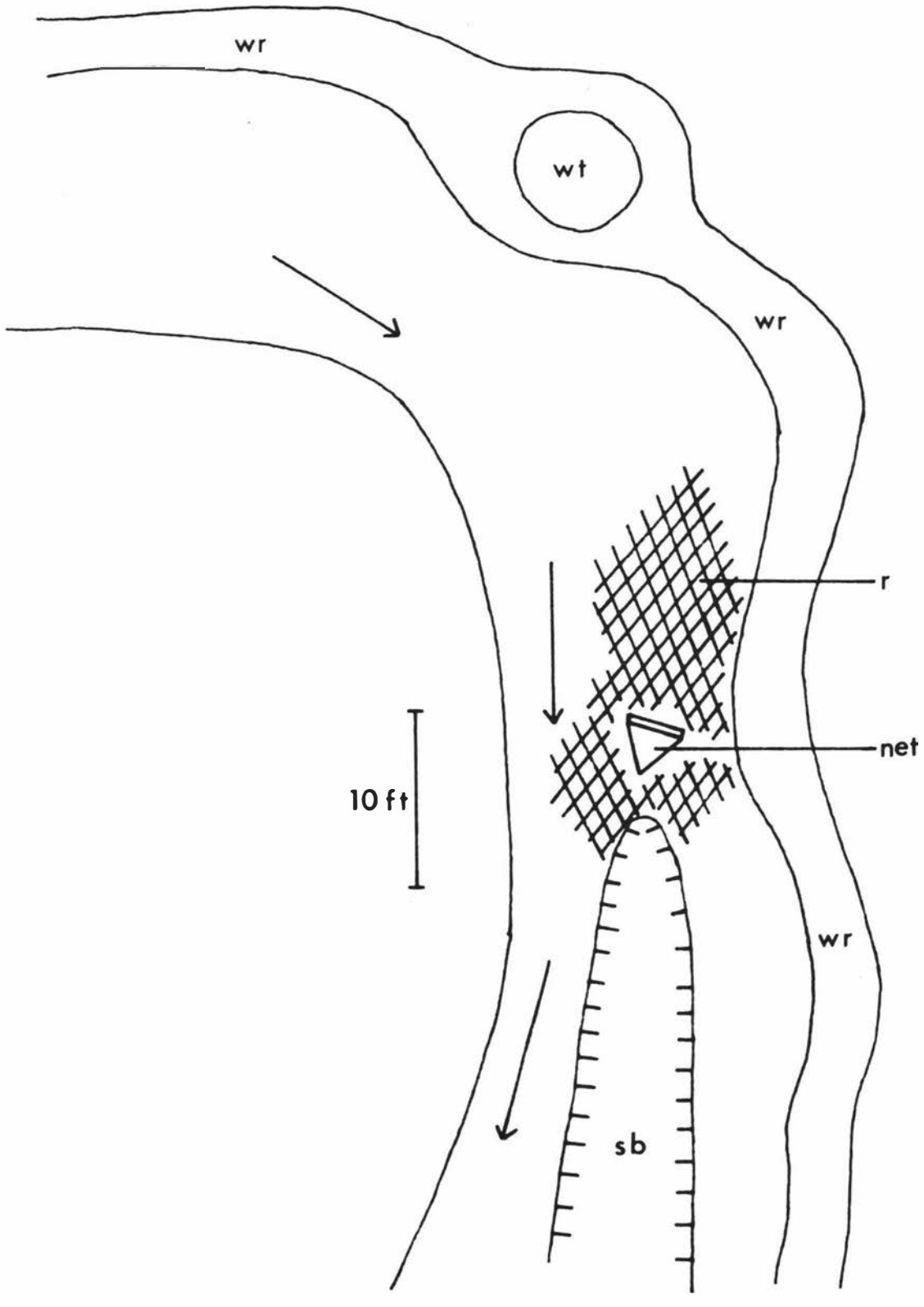

wr
wt
wr
r
net
10 ft
wr
sb

TABLE 27.

Analysis of 24 hour drift samples taken in Piritoa Stream in May 1967.

Date	8	9	10	11	12	15	16	22	23	24	25	
Water level (Inches above normal level)	0	3	2	5.5	3.5	2	8	0	0	0	6	Means
Nos. of snails taken	*	50	86	53	246	*	109	*	70	89	45	93.5
% large		6	5	8	4		14		9	3	9	7
% medium		17	17	15	11		22		27	19	35	20.5
% small		76	78	77	85		64		64	78	56	72.5
* Setting up net - no sample obtained.												

(3) Effect of floods

In conjunction with drift sampling, observations were made on the effect of flooding on population numbers. It was found that no appreciable increase in numbers of drifting snails occurred following a raising of the stream level eight inches above normal, and similarly a light flood (14 inches above normal) had no destructive effect on snails living along the stream margins, as the water there still remained relatively undisturbed. On 22 June, however, the water level rose 24 inches, and standard sweep samples taken when the level had fallen to 10 inches above normal showed that a substantial reduction in snail numbers had occurred. After flooding an average 160 snails per standard sweep (mean of four samples) was obtained compared with an average of 596 (mean of four samples) before flooding; i.e. the population

had been reduced to 27% of its previous size. The population had the same size structure before and after flooding, however, so it appears that snails of all sizes are equally susceptible to the effects of floods of this magnitude.

(4) Discussion

Results obtained in the stream distribution surveys agree well with those of Hirsch (1958) and Allen (1951). Hirsch found that snails were abundant on aquatic weeds as well as on the stream bed itself particularly where the current was not especially rapid, and Allen found that Potamopyrgus (= P. antipodum) occurred predominantly in quiet water in Horokiwi Stream. The discovery of P. antipodum as an important member of the stream drift is particularly interesting in view of Waters' (1961, 1962) findings that molluscs did not appear to drift at all in the stream he had under observation. P. antipodum has, however, been recorded in drift samples by Hopkins (1966). Although the functions of drift are rather obscure (Waters, 1965; Elliott, 1967), its prime importance to P. antipodum could well be as a dispersal mechanism.

The effects of floods on a stream bottom fauna in New Zealand have been examined by Allen (1951), who found that in Horokiwi Stream some floods destroyed half or more of the total invertebrate bottom fauna. In particular, he found that P. antipodum and the other outstanding quiet water groups, the Elmidae (Coleoptera) and Oligochaeta, were seriously reduced in numbers, density of Potamopyrgus being estimated at as little as 10% of that before flooding.

Moderate or severe floods were shown to have occurred in Piritea Stream on at least seven occasions in 1965-66 (Table 16; p.105) and it is postulated that such floods actively control population size and structure, their frequency preventing large numbers of adult snails accumulating. The population therefore retains a structure dominated by young individuals. Because large numbers of small snails live in the relative shelter of sediments lodged among thick mats of willow roots, it is likely that a nucleus of young snails would always be retained, regardless of the severity of the flooding.

The importance of floods as regulators of population density in stream invertebrates has also been noted by numerous other workers, including Badcock (1949), Zahar (1951), and Maitland and Penney (1966). Although it is postulated that flooding is the most active agent regulating size and structure of the snail population in Piritea Stream, other factors which must have an effect include availability of suitable microhabitats, parasitism, and predation by fish. As the bottom fauna consists of only a relatively few abundant species, P. antipodum is probably an important component of the diet of eels (Anguilla spp.), trout (Salmo sp.), and bullies (Gobiomorphus spp.), all of which are abundant in Piritea Stream, and known to feed on Potamopyrgus. (See references p.100 and also confirmed by examination of gut contents of all three fish species from Piritea Stream.)

3.0 THERMAL RELATIONSHIPS OF P. ANTIPODUM

INTRODUCTION

Surveys of thermal waters in the central North Island (Winterbourn and Brown, 1967; Winterbourn, 1968) have shown that the distribution of P. antipodum is restricted to waters of relatively low temperature. The maximum temperature at which it has been found is 28°C, in a small stream in Waimangu Valley, and it has also been recorded at 26°C in Lake Rotomahana, and in a stream at Waiotapu. It is absent from Lake Rotowhero (32-34°C) but is present in a cold stream less than 100 feet away, and is abundant in the littoral region of all non-thermal lakes examined in the Rotorua-Taupo region. These observations suggest that distribution of P. antipodum may be limited by high water temperatures, either directly or indirectly, and experimental studies have been undertaken to test this hypothesis.

MATERIALS AND METHODS

The importance of settling on a standard rate of heating in all lethal temperature experiments has been emphasized by Evans (1948), as rate of heating affects the time of death. A rate of increase of 1°C/5 minutes, assumed to be sufficiently slow to make any lag between body temperature and the surrounding water temperature small enough to be neglected, was adopted by Cowanloch and Hayes (1926), Broekhuysen (1940), and Evans (1948), and was recommended by Muirhead-Thomson (1958) for studies on bilharzia-snails in WHO research programmes. Gunter (1957) also considered that useful comparisons could be made in this way. When carrying out experimental

work using this rate of temperature increase, the importance of acclimatization of the organisms to different temperatures is largely overlooked or neglected, however. The importance of acclimatization has been discussed and emphasized by Heilbrunn (1952), Giese (1963), Macan (1963), and Shirmunsky and Vashkova (1963), and other workers have used longer periods of exposure often incorporating short term acclimatization, e.g. Fry, Brett and Clawson (1942), and Fraenkel (1968).

In this study it has been possible to make some comparisons between snails living in thermal and cold waters so that long term acclimatization has been built into the experimental procedure. Three series of experiments were carried out using different rates of temperature increase, 1^{o}C/5 minutes, 1^{o}C/hour, and 1^{o}C/24 hours. All snails were kept in the laboratory for at least 24 hours before experimentation, and were used within three days of collection to eliminate any possible effects of starvation (Dumbye and Dumbye, 1965).

Series 1.

Snails were obtained from three localities:

1. Waipuwerawera Stream (warm), Taupo; 22-24^{o}C.
2. Lake Taupo; 10^{o}C at time of collection.
3. Pond A, Massey University campus; 16^{o}C at time of collection.

The general method of Evans (1948) was employed. Snails were placed in 400ml beakers of tap water, and the water was slowly heated over a bunsen flame at a rate of increase of 1^{o}C/5 minutes. Water was aerated continuously

using a small aquarium pump. Twenty-four snails were used in each experimental run.

The end point was reached when snails ceased activity, and the operculum was withdrawn well inside the shell aperture. Comatose snails characteristically assumed this position, but with a lowering of water temperature resumed activity.

Series 2.

Beakers containing tap water were placed in a water bath with mechanical stirrer, and thermostat control of temperature accurate to $\pm$ 0.2°C. Water temperature was increased at a rate of 1°C/24 hours. All beakers were aerated continuously. Four hundred snails taken from Pond A, Massey University, were used in this series of experiments.

Death was the experimental end point. At all experimental temperatures employed, the snails were inactive, but on being transferred to water at air temperature at the end of each 24 hour period, living snails quickly showed some degree of activity. Snails which showed no sign of movement one hour after transference to water at air temperature were considered dead.

Series 3.

Experiments were carried out under the same conditions as Series 2 but the temperature was increased at a rate of 1°C/1 hour. In duplicate experiments, each employing 100 snails obtained from Pond A, Massey University, half the snails were kept in normally aerated water, and half in water through which pure oxygen was continuously bubbled

(Fig. 32). This increased the concentration of oxygen dissolved in the water about five times.

The number of snails showing any perceptible movement was determined at the end of each hourly period. Before observations were made, flasks were shaken gently to activate snails which were merely at rest and not in a comatose state.

RESULTS

Series 1.

Results are summarized in Table 28. LD50s of individual experiments ranged between 29 and 32°C and LD100s between 32 and 34°C. No significant differences between snails from populations living at different environmental temperatures, or between snails acclimatized in the laboratory at different temperatures were found.

TABLE 28.

Experimental temperatures at which snails from waters having different environmental temperatures ceased activity. Rate of temperature increase 1°C/5 minutes.

Expt. no.	Source of material	Environmental temperature °C*	LD50	LD100
1	Waipuwerawera Stream	24°C	31	34
2	" "	"	31	33
3	" "	"	32	34
4	" "	"	30	32
5	Pond A, Massey University	16°C	30	32
6	" "	"	30	32
7	Lake Taupo	10°C	31	33
8	"	"	31	33

* Water temperature at time of collecting.

FIGURE 32

Experimental apparatus used to provide super-oxygenated conditions at above-ambient temperatures.

Abbreviations

ab - airtight rubber bung

br - bubbler

o - atmosphere of pure oxygen

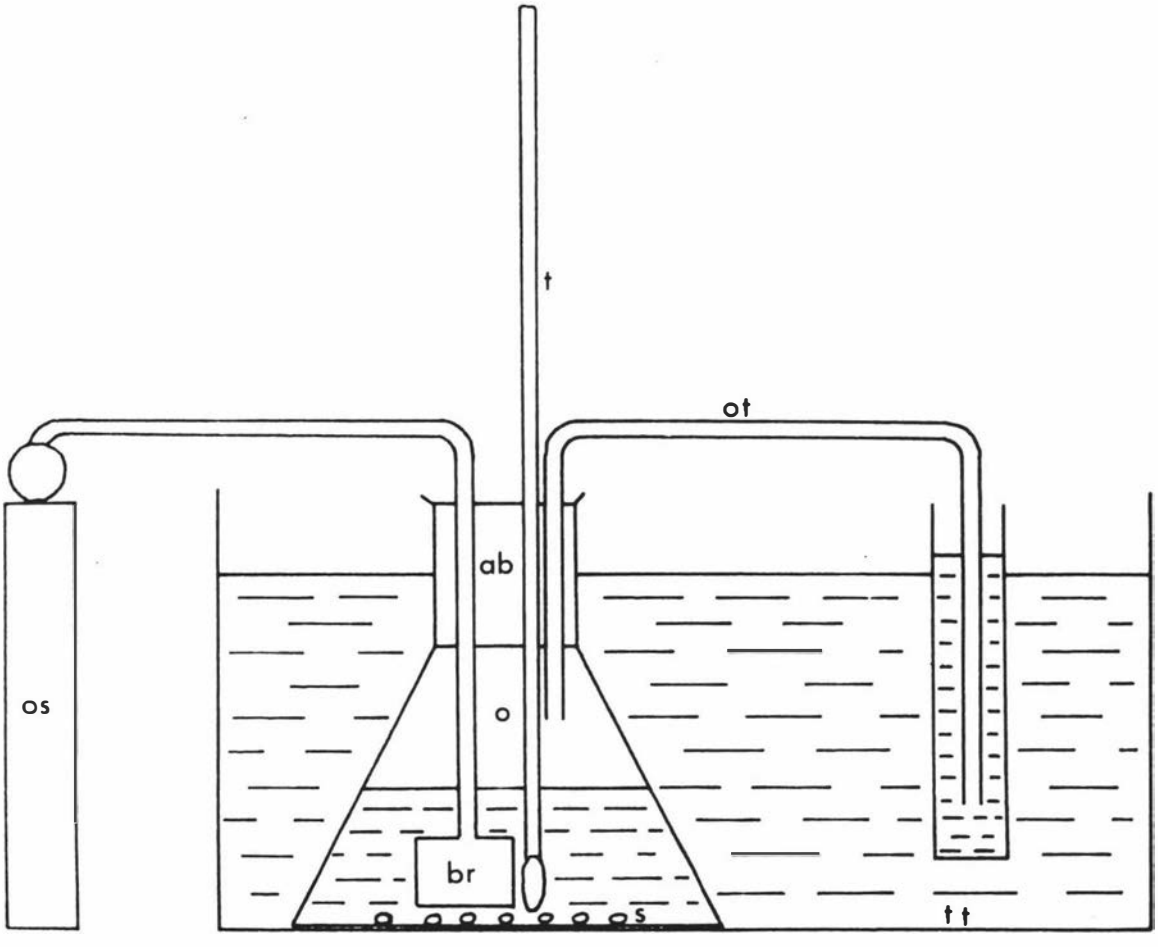
os - oxygen source

ot - outlet tube

s - snails

t - thermometer

tb - thermostatically controlled water bath


t
ot
ab
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s
tt

Series 2.

Results obtained by increasing water temperature 1°C/24 hours are given in Table 29. The upper lethal limit and LD50 were 32°C.

TABLE 29.

Survival of snails exposed to high temperatures. Rate of increase 1°C/24 hours. Results expressed as percentages of snails alive.

Experiment number	Experimental temperatures °C			
	29	30	31	32
1	100	98	82	0
2	100	94	53	0

Series 3.

A reduction in snail activity occurred with an increase in water temperature from 26 to 30°C, in both normally aerated and superoxygenated water (Fig. 33). This suggests that the increase in temperature itself was the major factor depressing activity. However, the fall in activity was less rapid under superoxygenated conditions than in air saturated water, and this indicates that the reduction in available oxygen found at higher temperatures was also a contributing factor.

When the water temperature was lowered again from 30°C, snail activity gradually increased, but never reached the same high levels as in the first half of the experiments,

FIGURE 33

Numbers of snails active at successive experimental temperatures, increased at a rate of 1°C/hour.

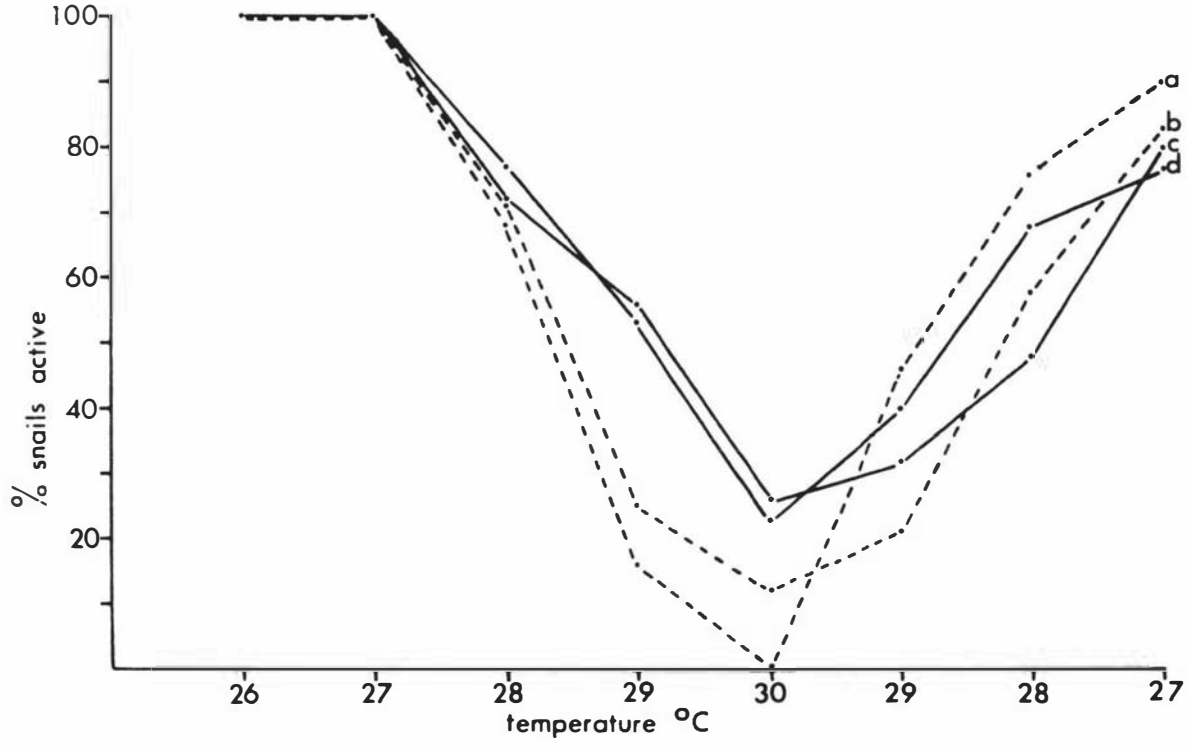
a - c - Experiment 1

b - d - Experiment 2

Key

broken lines - normal aeration

solid lines - super-oxygenation


100
80
60
40
20
% snails active
26
27
28
29
30
29
28
27
temperature °C
a
b

showing that recovery from exposure to higher temperatures was not complete in all individuals.

DISCUSSION

The experimental results obtained in this study show that a close correlation exists between the temperature at which snail activity ceases and heat stupor ensues, and the maximum temperature at which snails have been found in the field (28°C). Clearly, therefore, water temperature is important in limiting the distribution of P. antipodum in New Zealand's thermal region.

The temperature relationships of P. antipodum also closely resemble those described by Lumbye (1958) for P. jenkinsi, in which a rapid fall in standard metabolic rate was found at 29°C in brackish water and 32°C in fresh water.

4.0 PARASITES OF POTAMOPYRGUS

INTRODUCTION

In conjunction with systematic and ecological studies, an examination of the parasites associated with Potamopyrgus spp. was carried out, emphasis being placed on the degree and incidence of infection of snail populations rather than on the parasites themselves. The parasites belonged to two major groups, porosporid Protozoa and larval Trematoda, and a commensal oligochaete exhibiting possible parasitic tendencies has also been found. Organisms associated with the outside of the shell, including filamentous and encrusting Algae, Bacteria and stalked peritrichous Protozoa were not considered in this study.

METHODS

Living snails were squashed between two sheets of glass, and on microscopic examination any parasites could be readily removed from the snail tissue. Close examination of parasites was made using a binocular, compound microscope (up to X 400 magnification) and an inverted phase-contrast microscope with similar magnifications. Methylene blue and methyl green (1% in acetic acid) were used to stain specimens, and photographs were also taken to aid direct observation. All measurements were made with a linear or squared eyepiece micrometer, and unless stated otherwise are of fully grown larvae (Trematoda) in death attitude. As the primary aim of this study was not to make an anatomical study of the parasites themselves, but to investigate the extent of parasitism in populations, only brief descriptions of the parasites are given to provide a taxonomic basis to this work.

LARVAL TREMATODA

Interest in cercariae infecting Potamopyrgus spp. was first engendered by applied workers who wanted to know whether snails of this genus acted as intermediate hosts of liver fluke, Fasciola hepatica. Hopkirk (1927) believed he had found the larva of F. hepatica, as well as the cercariae of three other species parasitizing Potamopyrgus but Macfarlane (1937) showed that it was definitely not the intermediate host although it was infected by, "at least 14 other flukes whose adults live in fish, birds and mammals". In later years, Macfarlane (1939, 1945, 1951, 1952) described the life histories of three of these species, Coitocaecum anaspidis, Telogaster opisthorchis, and Stegadexamene anguillae (Fig. 34). All larval trematodes recorded from New Zealand freshwater molluscs are listed in Table 30(p.141a).

(1) Monostome Cercariae

Cercaria M1 (Fig. 35)

Body white, granular in appearance, a pair of dark longitudinal bands extending down the body, following the courses of the main anterolateral excretory canals; oral sucker inconspicuous; no eye spots present; posterior locomotor pockets (adhesive papillae) not strongly developed; posterior angles of body smoothly rounded; excretory vesicle circular; tail less than half length of body. Cercariae continually change shape by muscular body contractions, but the tail is little affected by such movements. Locomotion by a looping, rather than swimming action.

Body length : 0.43 - 0.56mm
Body width : 0.09 - 0.14mm
Tail length : 0.11 - 0.15mm

FIGURE 34

Trematode parasites of P. antipodum

- the species recorded by Macfarlane.

a-c Stegodexamene anguillae Macfarlane

a - cercaria (dorsal)

b - cercaria (lateral)

c - redia

d-g Coitocaecum anaspidis Hickman

d,e - cercaria (dorsal)

f - cercaria (lateral)

g - sporocyst

h-j Telogaster opisthorchis Macfarlane

h - cercaria (dorsal)

i - cercaria (lateral)

j - redia

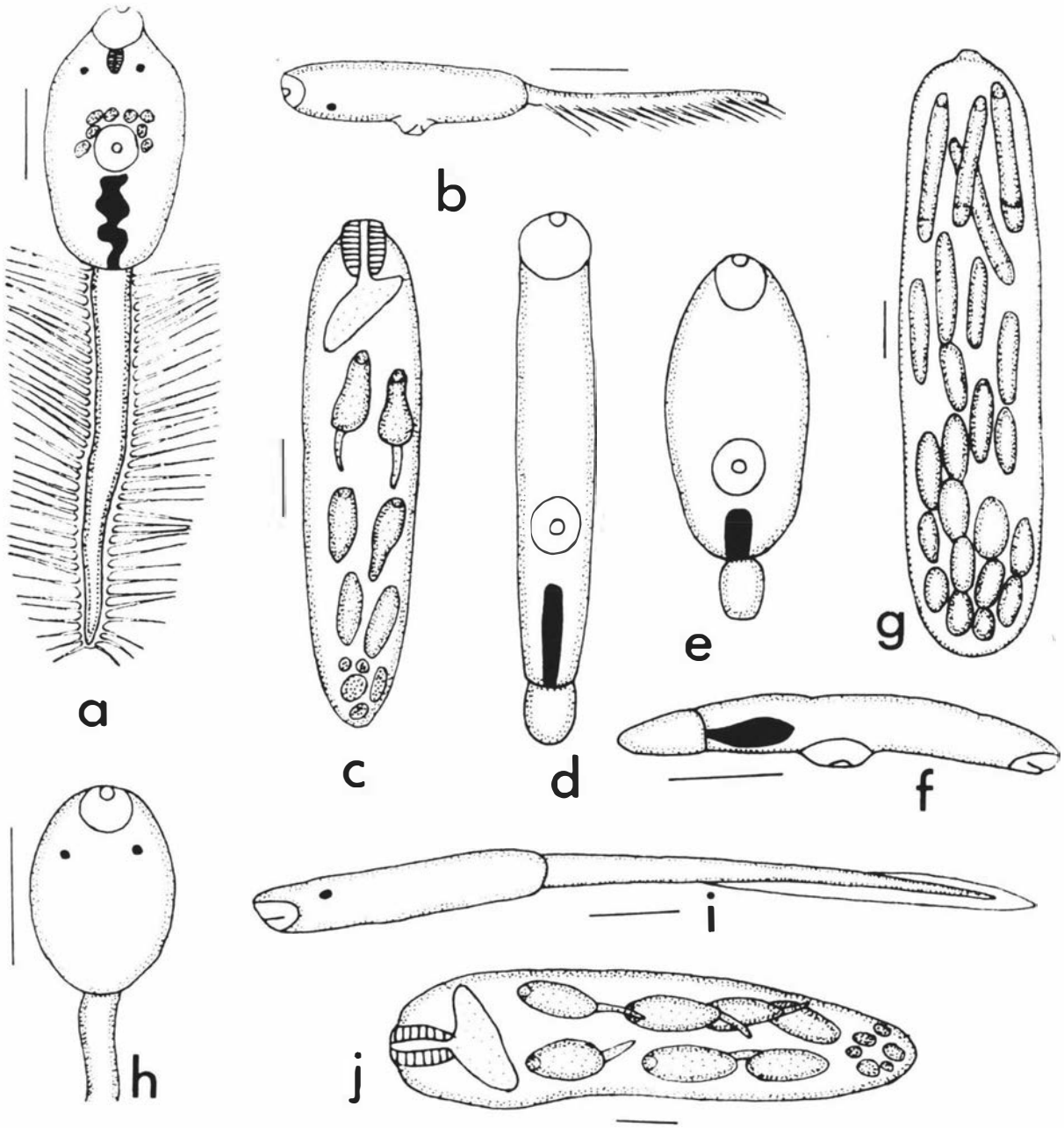

a
b
c
d
e
f
g
h
i
j

FIGURE 35

Trematode parasites of Potamopyrgus spp.
- Monostome cercariae

a-e Cercaria M1

a - cercaria (dorsal)
b,c - changes in shape of cercaria
d - young redia
e - metacercaria removed from cyst

f-l Cercaria M2

f - full sized cercaria (dorsal)
g - young cercaria
h - older cercaria developing pigmentation
i - redia
j,k - metacercarial cysts on shells
l - lateral view of cyst

m-n Cercaria M3

m - cercaria (dorsal)
n - redia

o-t Cercaria M4

o - cercaria (dorsal)
p,q - changes in shape of cercaria
r - cercaria (lateral)
s - redia
t - cercaria encysting

Abbreviations

ap - adhesive papilla
bg - gut bifurcation
dc - developing cercaria
es - eye spot
ev - excretory vesicle
f - fin
fa - filamentous alga
gc - germ cells
i - intestine
il - inner layer
mc - metacercaria
ol - outer layer
op - operculum
os - oral sucker
pg - penetration gland
ph - pharynx
po - protrusible organ
st - stylet
stc - striated cuticle
t - tail
v - virgula organ
vs - ventral sucker

Length of linear scales, 0.1mm.

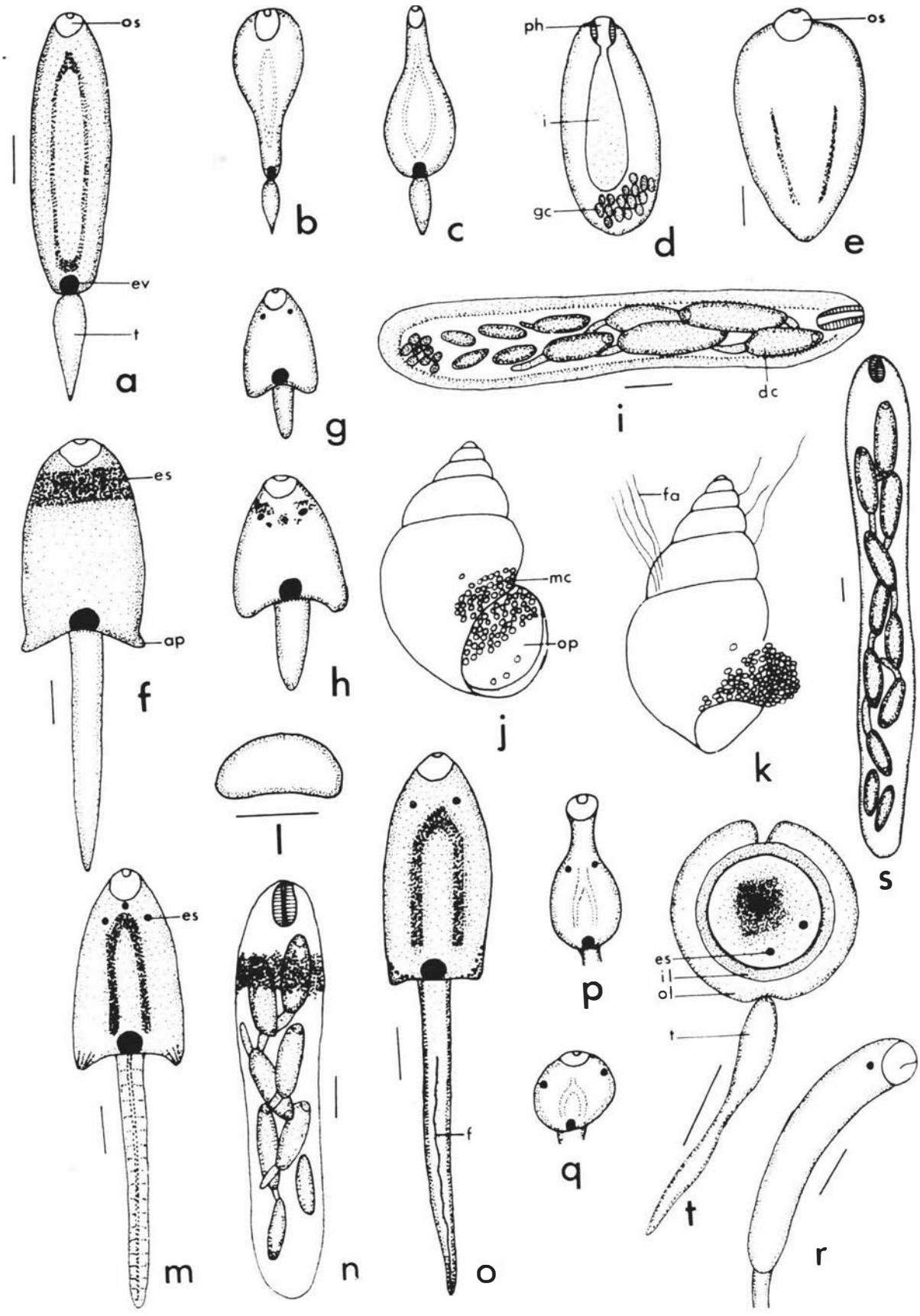

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TABLE 30.

Larval trematodes recorded from New Zealand freshwater Mollusca

Trematode sp.	Primary Host	Molluscan Host	2° Intermediate Host	Authority
Coitocaecum anaspidis Hickmann	Gobiomorphus gobioides Salmo fario Galaxias brevipennis G. attenuatus (all fish)	P. antipodum	Paracalliope fluviatilis (Amphipoda)	Macfarlane (1939)
Telogaster opisthorchis Macf.	Anguilla australis (eel)	P. antipodum	G. gobioides G. brevipennis Philypnodon spp.	Macfarlane (1945)
Stegadexamene anguillae Macf.	A. australis A. dieffenbachii	P. antipodum	Philypnodon spp. G. gobioides G. brevipennis	Macfarlane (1951)
Calicophoron ijimai (Fukui)	Bovis domesticus (domestic cattle)	Planorbis kahuika	-	Jonathan (1952)
Echinostome cercaria	?	P. kahuika	-	Jonathan (1952)
Cercaria longicauda	Fuligula novaezealandiae (black teal)	Simlimnaea tomentosa	-	Macfarlane (1949)
Fasciola hepatica L.	Ovis aries (sheep)	Simlimnaea tomentosa	-	Macfarlane (1937)
Gorgodera australiensis Johnston	Hyla aurea (frog)	Sphaerium spp. Pisidium spp.	Xanthocnemis zelandica (damselfly)	Dale (1967)
Bifid-tailed cercaria	?	P. antipodum	?	Hopkirk (1927)

Redia

White; active; capable of limited contraction; pharynx prominent.

Length : 1.1mm

Greatest width : 0.29mm

Metacercaria and Cyst

Metacercarial cysts are roughly spherical; diameter, 0.11 - 0.18mm. Cysts are attached to the operculum of P. antipodum or on the shell close to the aperture. The surface of the cyst attached to the snail (or other cysts) is flattened to fit the curvature of the surface. All shells examined having cysts attached, also contained active cercariae. As the incidence of infection found within any snail population was never high, it seems probable that cercariae encyst on the shell of the snail they have just left. In some instances cysts were found to be infected by a fungal growth on their outer surfaces.

The metacercaria resembles the cercaria when relaxed. Its body tapers posteriorly, and a prominent oral sucker is present. When in the cyst the metacercaria lies curled up, its anterior and posterior ends meeting. It is capable of revolving movements. No morphological structures can be distinguished through the cyst wall.

Host snail: P. antipodum

Localities found

Lake Pupuke, Takapuna, Auckland; stream near Bunnythorpe, Manawatu.

Cercaria C2 (Fig. 35)

Body contractile, oval when relaxed, its posterior margin shallowly concave; opaque, white, granular in appearance; oral sucker not conspicuous; a clear, circular, excretory vesicle visible posteriorly; adhesive papillae very prominent; a pair of dark eye spots present anteriorly behind the oral sucker, in some individuals each "eye" appearing to be made up of two adjoining spots; between and around the eyespots is a band of very conspicuous dark pigmentation; tail simple and contractile. Inactive.

Newly emerged cercariae differ from fully grown cercariae by not having the anterior pigment band developed, and by having the tail less than half the length of the body when at rest.

Body length : 0.34 - 0.44mm

Body width : 0.19 - 0.21mm

Tail length : 0.22 - 0.39mm

Redia

Similar to that of Cercaria C1; white, granular in appearance; pharynx prominent; active, contractile.

Length : 0.95mm

Maximum width : 0.38mm

Metacercaria and Cyst

The cercaria encysts on the operculum and shell of the host snail as does Cercaria C1. Snails taken from Lake Pukaki had over 300 cysts attached to their shells. Cyst diameter, 0.17 - 0.23mm.

Host snails: P. antipodum, P. pupoides

Localities found

Stream near Linton, Manawatu; Hokowhitu Lagoon, Palmerston North; Avon River, Christchurch; Lake Ohinaki, Canterbury; Makara River, Wellington; Lake Tutira, Hawkes Bay; Kahao Stream, Porirua Harbour.

Cercaria #3 (Fig. 35)

Body grey-brown, tail more transparent; two opaque, longitudinal bands on body as in Cercaria #1; three eyespots present, two lateral, one median immediately posterior to the oral sucker; two pronounced adhesive papillae at the posterior body angles; tail with a segmented appearance in both life and death. Cercariae very active compared with other monostome cercariae found, swimming being accompanied by strong contractions of both body and tail. When attached to a flat surface the two posterior adhesive papillae are in contact with the substratum and appear to act as substitutes for a ventral sucker.

Body length : 0.37 - 0.44mm

Body width : 0.11 - 0.19mm

Tail length : 0.33 - 0.55mm

Redia

Indistinguishable from those of Cercariae #1 and #2.

Encystment of Cercaria

Cercariae of this species encysted on the bottom of a glass dish within minutes of being removed from the snail host. During encystment the tail thrashed frantically while attached to the outside of the cyst, and the body of the cercaria rolled around continuously within the developing

cyst. At this stage the three eyespots were clearly visible through the cyst wall but later the wall became more translucent and the eyespots could no longer be seen.

The cyst consists of two layers, an inner layer initially having a golden appearance, which is laid down first, and a thicker outer layer which at first is entirely clear. On completion of encystment, the inner layer assumes a grey, somewhat fibrous appearance, and the outer layer appears more golden when viewed with transmitted light. A narrow, clear space exists between the animal and the cyst wall and allows for movement of the developing metacercaria.

Cysts are normally found attached to shells and opercula of host snails as in the other monostome cercariae.

Dimensions of the completed cyst and its components:

Maximum diameter of cyst	: 0.17 - 0.21mm
Thickness of outer cyst wall	: 0.015 - 0.023mm
Thickness of inner cyst wall	: 0.007 - 0.011mm
Diameter of encysted cercaria	: 0.15 - 0.18mm

Host snail: P. antipodum

Localities found

Hokowhitu Lagoon, Palmerston North; tributary to Kahuterawa River, south of Palmerston North.

Cercaria M4 (Fig. 35)

Body white, opaque; tail more transparent, with a fin running the length of the tail ventrally and three-quarters of its length dorsally; two prominent, dark eyespots present; longitudinal bands of pigment extend down the body to the clear, circular, excretory vesicle; oral sucker not clearly

seen; posterior margins of body "flanged" forming adhesive papillae. The body, but not the tail, exhibits considerable contractility, and can change from a spherical to a narrow, elongated form. In life the tail is normally about twice as long as the body, but at death it contracts, and approximates the body length. Cercariae swim actively by thrashing movements of the tail which is held vertically above the body. If the tail is detached from the body it continues to thrash about vigorously for several minutes.

Body length : 0.29 - 0.43mm

Body width : 0.11 - 0.21mm

Tail length : 0.29 - 0.56mm

Redia

Sac-like, cylindrical; translucent white with an almost circular pharynx; inactive. Over a dozen cercariae may clearly be seen within the redia.

Length : 2.0mm

Greatest width : 0.19mm

Encystment

Cercariae encyst on shells of Potamopyrgus species and encystment has also been observed in a glass dish soon after liberation from the host snail. The process is identical to that described for Cercaria M3.

Host snails: P. antipodum, P. estuarinus

Localities found

Stream near Linton, Manawatu; Avon River, Christchurch; Lake Pupuke, Takapuna, Auckland; Huia, Manukau Harbour; Waitomo.

Adult flukes and their definitive hosts

Adult flukes corresponding to the larvae described above are not known, but they are assumed to be parasites of birds, possibly ducks. Cercaria L3 closely resembles the cercaria of Notocotylus seineti Fuhrmann (= Cercaria monostomi Linstow) a trematode parasite of the domestic duck in Europe (Dawes, 1946). No published accounts of the trematode parasites of ducks have been made in New Zealand, and the Department of Agriculture's Wallaceville Animal Research Centre had no knowledge of any bird flukes in New Zealand (pers. comm., Nov. 1966). However, an examination has been made of two slides of monostome flukes, held in the collection of the Canterbury University Zoology Department. Flukes on both slides were collected from ducks at Lake Wairarapa in May 1951. One has been identified as Catatropis verrucosa (Frölich), and the other as a second species of Catatropis. It is possible that they are the adult stages of two of the larval monostomes reported on above.

(2) Furcocercous Cercariae

Cercaria F1 (Fig. 36)

White, translucent, shape rigidly defined; suckers not easily seen, the ventral sucker posterior to the mid-point of the body; excretory vesicle small, V-shaped; tail stem half the length of the body and containing two rows of caudal bodies clearly visible within it; furcae slightly longer than tail stem, tapering towards their apices; active swimmer.

Body length : 0.16mm

FIGURE 36

Trematode parasites of *P. antipodum*

- Furcocercariae, Cercariaea, Xiphidiocercariae

a-b Cercaria F2

a - cercaria (dorsal)

b - sporocyst

c-d Cercaria F1

c - cercaria (dorsal)

d - sporocyst

e-i Cercaria C1

e - cercaria (dorsal)

f,g - young cercariae showing changes in shape

h - cercaria (lateral)

i - sporocyst

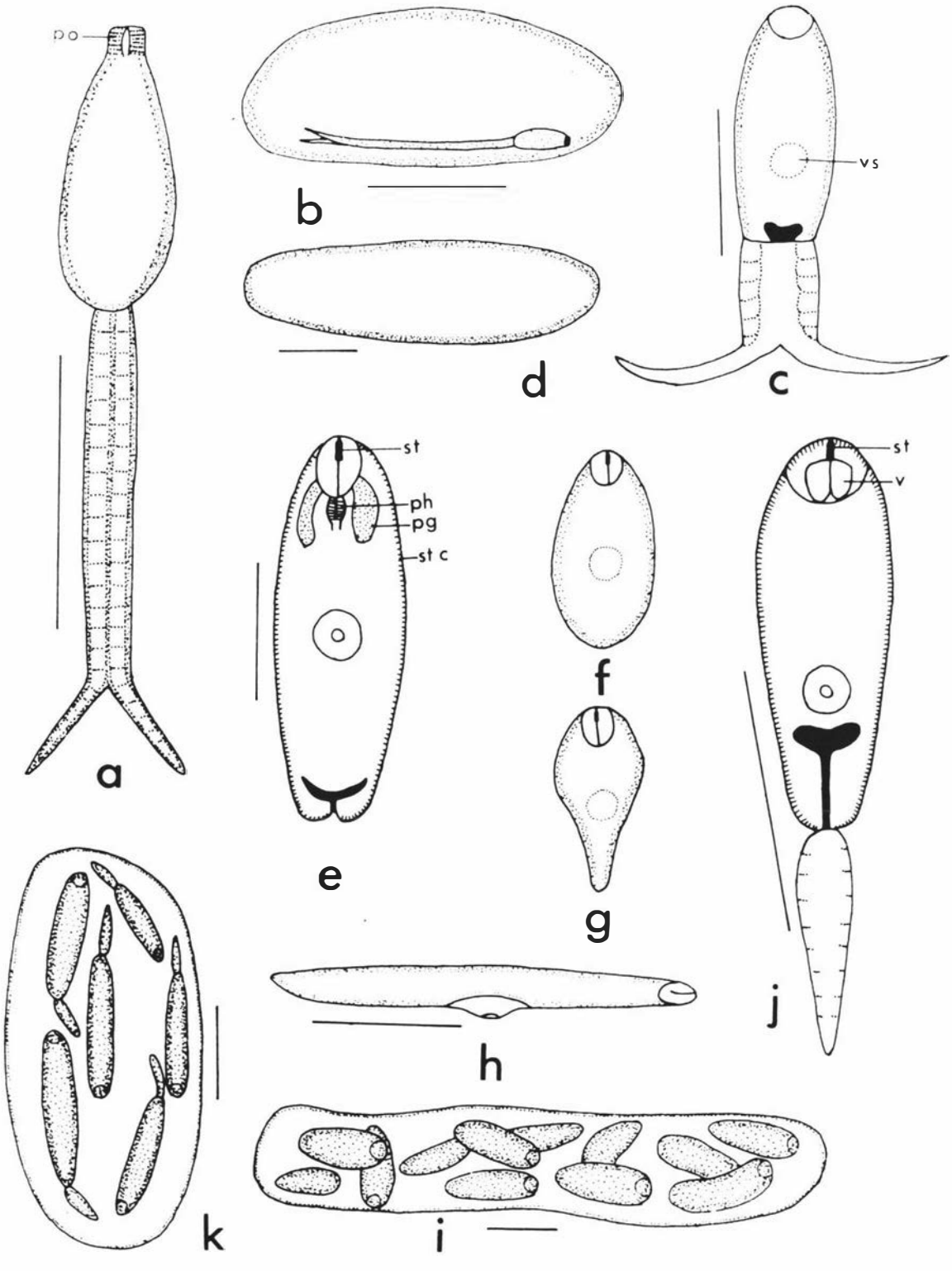
j-k Cercaria X1

j - cercaria (dorsal)

k - sporocyst

Linear scales, 0.1mm

For meanings of abbreviations see Figure 35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Sporocyst

White; cylindrical with rounded ends.

Length : 0.44mm

Width : 0.12mm

Snail host: L. antipodum

Locality found

Lake Wairarapa.

Remarks

Furcocercous cercariae similar to this are known to be the juvenile forms of flukes of the family Strigeidae whose hosts are birds, including domestic ducks, geese and swan (Dawes, 1946). Two slides in the Canterbury University, Zoology Department collection contain strigeid flukes from the small intestine and duodenum of ducks at Lake Wairarapa. These may represent the adult of Cercaria #1.

Cercaria #2 (Fig. 36)

A member of the "Lophocerca" group (apharyngeal, brevifurcate, monostome cercariae) of Furcocercariae. No normal suckers present, but possesses a muscular protrusible, anterior organ which exhibits strong, telescopic movements; a narrow dorsal fin present on body; tail stem divides into two furcae at about three quarters of its length; body undergoes continual contraction and expansion, and the tail exhibits active thrashing movements.

Body length : 0.06 - 0.10mm

Maximum body width : 0.04mm

Tail stem length : 0.15mm

Length of furcae : 0.03mm

Sporocyst

Ovoid, cylindrical. Cercariae about to leave the sporocyst are almost as long as the sporocyst itself.

Length : 0.26 - 0.28mm

Width : 0.12 - 0.14mm

Snail host: P. antipodum

Localities found

Stream near Linton, Manawatu; Lake Tutira, Hawkes Bay.

Remarks

A number of lophocercous cercariae are known to develop into parasites of the blood vascular system of Cyprinid fishes (carp). At least four species of cyprinid are found in New Zealand (Woods, 1963) and could therefore provide a definitive host for this fluke.

(3) Xiphidiocercariae

Cercaria X1 (Fig. 36)

A member of the "Cercariae virgulae" group of Lühe (1909). White, translucent; considerable contraction and expansion of the body and short tail is found; oral sucker larger than ventral sucker, stylet and virgula organ very prominent; excretory vesicle a V-shaped sac with a long, narrow posterior stem; cuticle striated; movement by muscular contractions and by side to side lashing of the tail.

Body length : 0.15mm

Body width : 0.06mm

Tail length : 0.07mm

Sporocyst

White, oval, the sides somewhat flattened. About six developing cercariae may be visible inside.

Length : 0.44mm

Width : 0.19mm

Snail host: P. antipodum

Locality found

Lake Tutira, Hawkes Bay.

(4) Cercariaea

Cercaria C1 (Fig. 36)

Translucent, white; cuticle strongly striated; oral sucker and stylet prominent; large penetration glands present, one group either side of the oral sucker and extending some distance behind it; pharynx small, inconspicuous; ventral sucker not obvious from above, centrally placed, almost as large as oral sucker; posterior margin of the body drawn in medially in the form of a shallow V at the excretory pore; excretory vesicle a narrow, two branched Y-shaped structure; tail absent. Cercariae move by a looping action similar to that of a caterpillar. They are very active but have not been observed to swim.

Body length : 0.28mm

Maximum body width : 0.09mm

Sporocyst

Tubular, semi-transparent, inactive; containing large numbers of clearly visible cercariae.

Length : 0.81mm

Width : 0.25mm

Snail host: P. antipodum

Locality found

Stream at Waitomo.

(5) Gymnocephalus Cercariae

Cercaria G1 (Fig. 37)

Body opaque, white, flattened, oval, non contractile; tail strongly developed, no fin present; oral sucker and pharynx clearly visible; a pair of dark eyespots present behind the level of the pharynx; ventral sucker not visible from above, the same diameter as the oral sucker and placed slightly posterior to the midpoint of the body; inactive.

Body length : 0.20 - 0.26mm

Body width : 0.07 - 0.09mm

Tail length : 0.17 - 0.20mm

Redia

Large, sac-like, narrowing anteriorly; pharynx small and inconspicuous. Fully grown rediae contain about 12 mature cercariae which completely fill the body cavity.

Length : 1.44mm

Maximum width : 0.38mm

Snail host: P. antipodum

Localities found

Stream near Linton, Manawatu; Lake Tutira, Hawkes Bay.

Cercaria G2 (Fig. 37)

The largest cercaria found parasitizing P. antipodum. Body white, elliptical, greatest width at the midpoint; ventral sucker extremely protrusible, larger than oral sucker, and placed slightly posterior to the middle of the

FIGURE 37

Trematode parasites of P. antipodum

- Gymnocephalous cercariae

a-d Cercaria G1

a - cercaria (lateral)

b - cercaria (dorsal)

c - cercaria in swimming position

d - redia

e-i Cercaria G2

e - cercaria (dorsal)

f - cercaria (lateral)

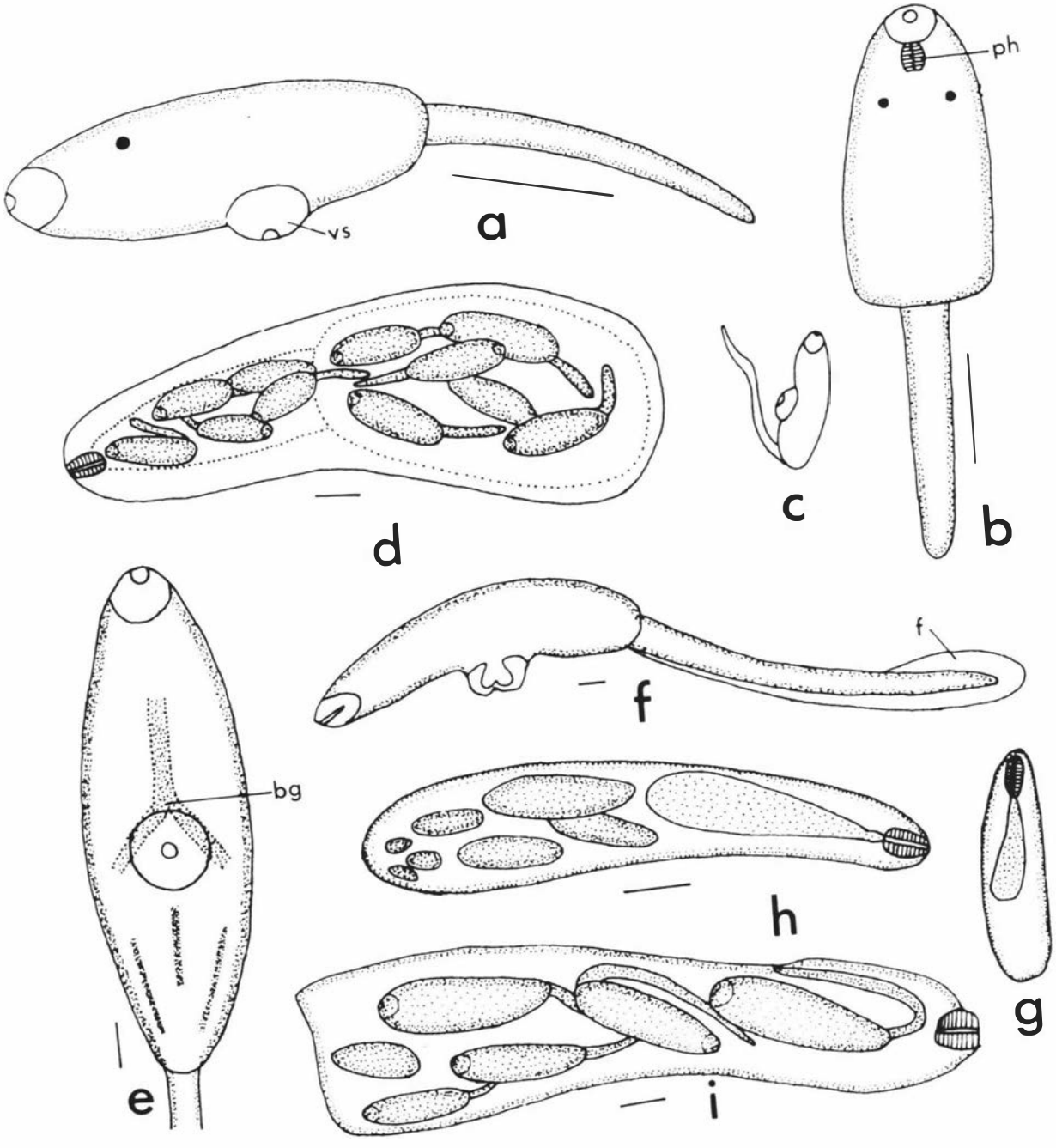
g - young redia

h - middle aged redia

i - fully grown redia

Linear scales, 0.1mm

For meanings of abbreviations
see Figure 35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body; bifurcation of the gut clearly visible at the anterior margin of the ventral sucker; tail almost as long as body, a well developed fin runs the length of the tail ventrally and along the posterior third dorsally; poor swimmer. When released from the snail host many cercariae assume a still, curved attitude, lying on their dorsal surfaces.

Body length : 1.0 - 1.1mm

Body width : 0.3mm

Tail length : 0.75 - 0.87mm

Redia

White; tubular; exhibit slight wriggling movements; pharynx prominent; developing cercariae (generally about six in mature rediae) clearly visible. In young rediae the gut posterior to the pharynx is clearly visible as a simple sac extending almost half the body length.

Length : 1.5mm

Maximum width : 0.25mm

Snail host: P. antipodum

Locality found

Lake Tutira, Hawkes Bay.

(6) Incidence of infection of Potamopyrgus species by larval trematodes

Infection by all trematode species renders the host snail infertile. Rates of infection in all snail populations examined are shown in Table 31. Cercariae were encountered in 13 of the 17 populations of P. antipodum examined, and in 12 of these monostome species were present, making them clearly the most important group of trematodes parasitizing this species. Highest infection rates within

TABLE 31.

Incidence of infection of Potamopyrgus spp. by porosporid Protozoa and Trematoda

Snail Host	Locality	Date of Collection	Number of snails examined	Porosporidae % infection	Trematoda Number of Cercaria spp. found	Trematoda % snails infected
P. antipodum						
	L. Pupuke	Dec. '66	100	86	0	0
	L. Pukaki	Jan. '66	100	66	1	4
	Makara R.	Apr. '66	100	38	1	3
	L. Pupuke	Aug. '66	100	28	1	1
	Avon R.	Jan. '66	100	27	2	2
	L. Tutira	Jan. '67	260	12.8	6	4.6
	L. Wahapo	Jan. '66	100	10	0	0
	Green L.	Jan. '66	100	10	0	0
	Kahuterawa R.(1)	Oct. '66	100	8	0	0
	Hokowhitu Lagoon	May '66	100	8	2	8
	Kahuterawa R.(2)	Oct. '66	84	6	1	1.2
	L. Taupo	Apr. '66	35	2.9	2	8.6
	Bunnythorpe (Stm.)	Oct. '66	150	1.3	3	2
	L. Paringa	Jan. '66	100	1	1	2
	Bunnythorpe (pond)	Oct. '66	200	0	0	0
	Linton (stm.)	Oct. '66	200	0	4	2.5
	L. Wairarapa	Jan. '67	200	0	2	5.5
	Waitomo (stm.)	Dec. '66	100	0	2	3
P. estuarinus						
	Huia	Aug. '67	200	0	1	0.5
P. pupoides						
	Kahao Stm.	Oct. '67	150	0	1	0.66
P. estuarinus and P. pupoides						
	Wananaki	Dec. '66	100	0	0	0
	Heathcote R.	Jan. '66	200	0	0	0
	Havelock R.	Jan. '66	150	0	0	0
	Avon R.	Jan. '66	200	0	0	0
	Hutt R.	Jan. '67	100	0	0	0

individual populations, 8 and 8.6% of adult snails, were also produced by monostome larvae alone. Maximum infection rates for all species are shown in Table 32 (p.155).

These may be compared with the figures given by Macfarlane (1939, 1952). He found that in Heathcote River 0.5-1.0% of adult Potamopyrgus were infected with cercariae of Coitocaecum anaspidis, 2.7% by Telogaster opisthorchis and Stegadexamene anguillae combined, and 15.8% by all trematode species (number and identity not given), and in Hut Stream (Canterbury) he recorded 0.7% of snails infected by S. anguillae.

Numbers of snails infected by trematode larvae in New Zealand populations are low compared with many infection rates recorded elsewhere. In the United Kingdom Rothschild (1941) found that up to 70% of Hydrobia ulvae over 3.75mm high were parasitized, and in a study of a Welsh lake Probert (1966) found 51% of all molluscs examined were infected, including 64% of the prosobranch Bithynia tentaculata. These rates are much higher than the maximum of 8.6% found in this study, or the 15.8% recorded by Macfarlane, but sporozoan infections of P. antipodum have been found to attain comparable high levels (Table 31; p.153).

Most species of mollusc that have been examined act as intermediate host for less than 10 trematode species (Ewers, 1964), although there are a number of exceptions including the pulmonate Limnaea natalensis, the host of at least 43 known species of cercariae. At least 13 species are now known to use P. antipodum as an intermediate host, and this

TABLE 32.

Maximum incidence of infection by trematode cercariae in populations of Potamopyrgus spp.

Cercariae	Maximum % infection
Coitocaecum anaspidis	4.5
Telogaster opisthorchis	0.7
Stegadexamene anguillae	2.0
Cercaria M1	1.15
Cercaria M2	6.0
Cercaria M3	2.0
Cercaria M4	1.2
Cercaria F1	1.0
Cercaria F2	0.8
Cercaria G1	1.2
Cercaria G2	0.4
Cercaria X1	1.2
Cercaria C1	1.0

concentration of trematode larvae in P. antipodum may have developed because suitable alternative snail hosts are lacking in New Zealand. Probert (1966) found that trematodes which have a prosobranch snail as intermediate host never parasitize pulmonate snails, and P. antipodum is the only prosobranch widely distributed in New Zealand fresh waters.

The development of a large number of host-parasite relationships also provides further strong evidence that P. antipodum has been established in New Zealand fresh waters

for a long time. This contrasts strikingly with the situation found in Europe and Britain where P. jenkinsi is parasite free (Fretter and Graham, 1962). Little information is available on parasitism of freshwater snails in Australia, although in a literature survey Johnston and Cleland (1957) have quoted a single instance of unidentified trematode larvae being found in a Potamopyrgus species from Ballarat, Victoria.

PROTOZOA

Many individuals of P. antipodum have been found containing cysts of a porosporid protozoan, which completely pack the spire of the shell and replace much of the digestive gland and gonad, as well as occupying spaces between the visceral organs. Parasitism of P. estuarinus and P. pupoides has not been found. Infected snails are always rendered infertile by the presence of cysts but non-reproductive behaviour does not appear to be affected.

Life cycles of members of the Porosporidae nearly always involve a crustacean primary host and a molluscan secondary host (Vogiel, 1965). Sporozoites in the gut of a crustacean encyst and produce huge numbers of naked gymnospores (gametes). These are then transferred to the secondary molluscan host where copulation occurs, usually in the blood lacunae of the gills. Zygotes form oocysts in each of which a single sporozoite develops. The crustacean is usually reinfected by eating infected molluscs.

In this case it is not known what crustacean host is involved but it seems likely that it could be the amphipod

Paracalliope fluviatilis which is frequently found in association with P. antipodum. In the three habitats studied for over a year on the Massey University campus, cyst infected snails were found in the two in which P. fluviatilis was also found, but not in the third where the crustacean was absent.

Sporozoite (Fig. 38)

Sporozoites have not been discovered free within the body of an infected snail, but have been removed from cysts by applying gentle pressure to them, thereby breaking the cyst walls. The sporozoite is clubshaped, and granular in appearance. Its protoplasm contains numerous vacuoles but no obvious nucleus, although somewhat diffuse sections of protoplasm take up the nuclear stain, methyl green. The outer membrane of the sporozoite has a striated appearance.

Length : 0.21mm

Maximum width : 0.05mm

Cyst (Fig. 39)

Cysts are spherical and bounded by a smooth clear wall. Inside the cyst, the sporozoite is coiled with its anterior and posterior ends meeting. There is a small space left between the sporozoite and the cyst wall and the sporozoite is able to move within the cyst. Most encysted sporozoites appear to have two dark patches present in their cytoplasm, but these have not been observed as definite structures after the sporozoite has been removed from its cyst.

Diameter of cyst : 0.10 - 0.12mm

Thickness of cyst wall : 0.005mm

FIGURE 38

The protozoan (Sporozoa : Porosporidae) parasite of Potamopyrgus spp.

a - Sporozoite removed from cyst

b - Sporozoite being squeezed from cyst and showing the striated appearance of the body surface

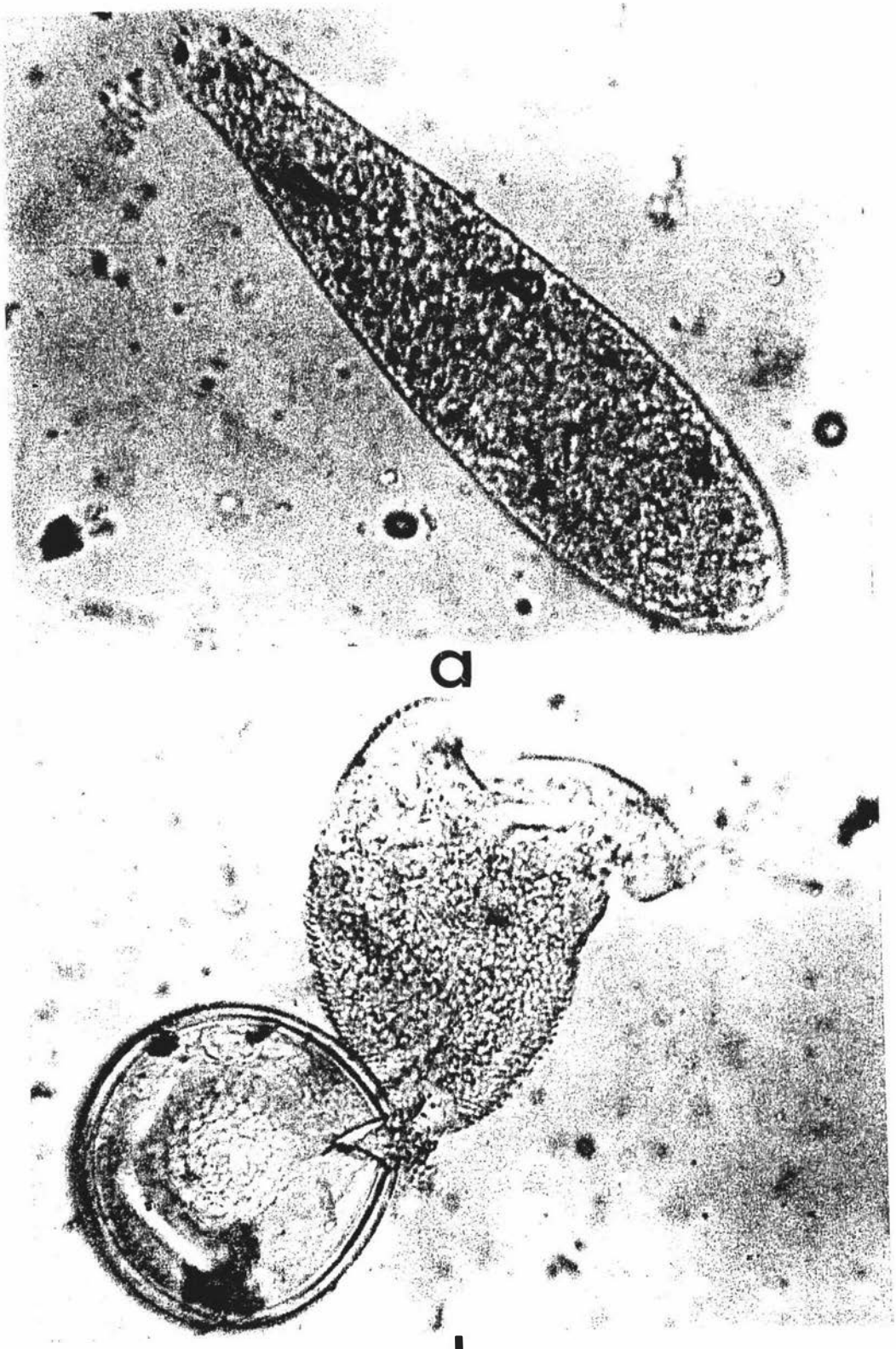
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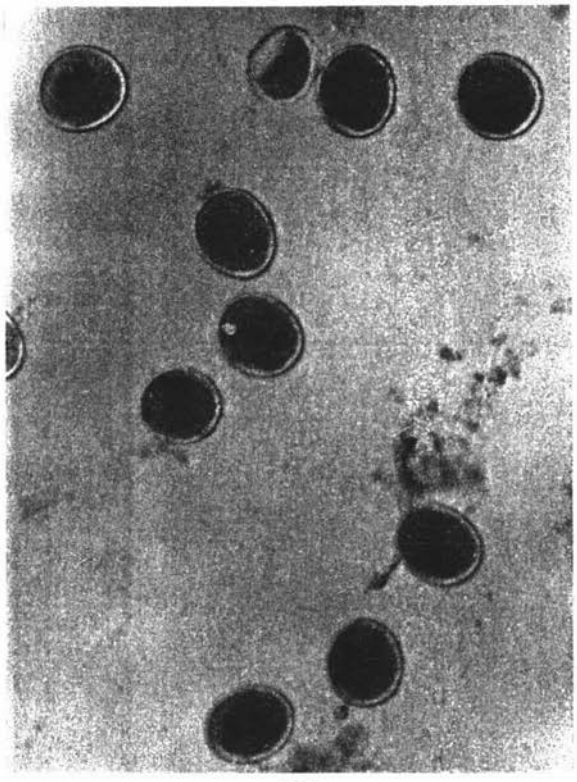
FIGURE 39

The protozoan parasite of Potamopyrgus spp.

a - cysts removed from snail tissue

b - cyst with outline of sporozoite seen coiled inside

c - T.S. cyst infect snail visceral mass



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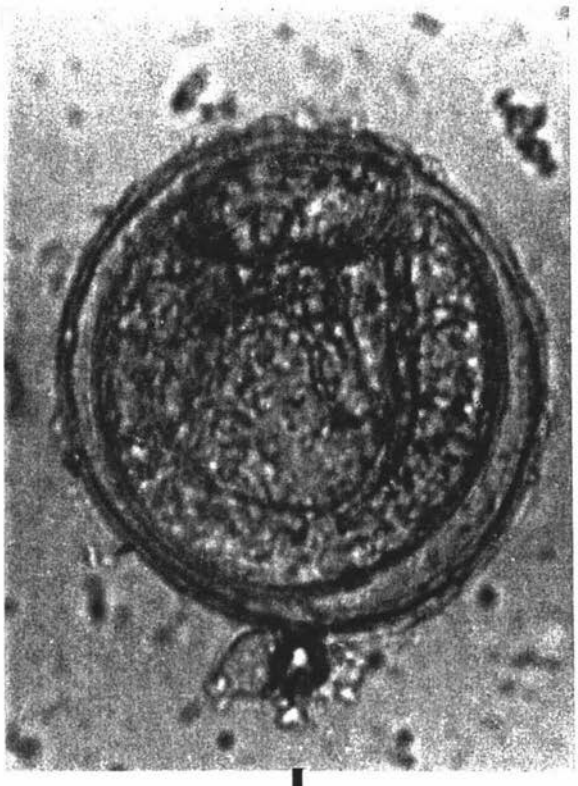

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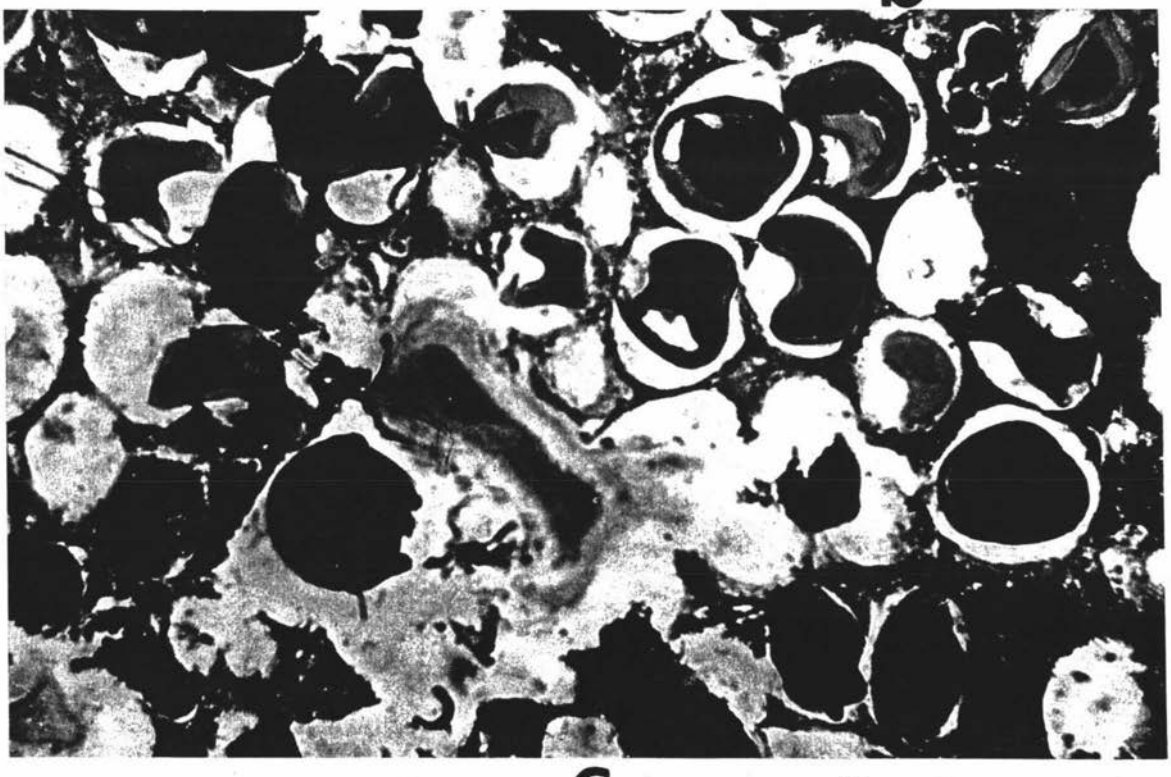

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Incidence of infection

Incidence of infection found in populations of Potamopyrgus spp. from throughout New Zealand is shown in Table 31 (p.153). All snails examined were over half grown and showed no external sign of infection. Infection is not restricted to older snails, however, and in two collections (August and December, 1966) of juvenile snails (<2.5mm high) from Lake Pupuke, Takapuna, 10 and 30% respectively were found to be infected.

Monthly infection rates of P. antipodum in Tiritea Stream from March 1965 to April 1966 are shown in Table 22 (p.120).

OLIGOCHAETA

Chaetogaster limnaei

The naidid, Chaetogaster limnaei, was found in close physical association with P. antipodum in Lake Pupuke, Takapuna, Auckland, but was not found in any other population. Ch. limnaei exists in two forms, considered by Gruffydd (1965a) to be subspecies, which differ in setal number, habitat and diet. Only the outer form, Ch. limnaei limnaei has been recovered from P. antipodum, the worms being found on the head and anterior region of the foot, and around the mantle edge.

Previously, Ch. limnaei seems to have been recorded from pulmonate snails only, principally limnaeids and planorbids. In New Zealand, Marples (1962) records it as being common in the mantle cavities of Planorbis sp. in the vicinity of Dunedin, and the writer has seen one specimen

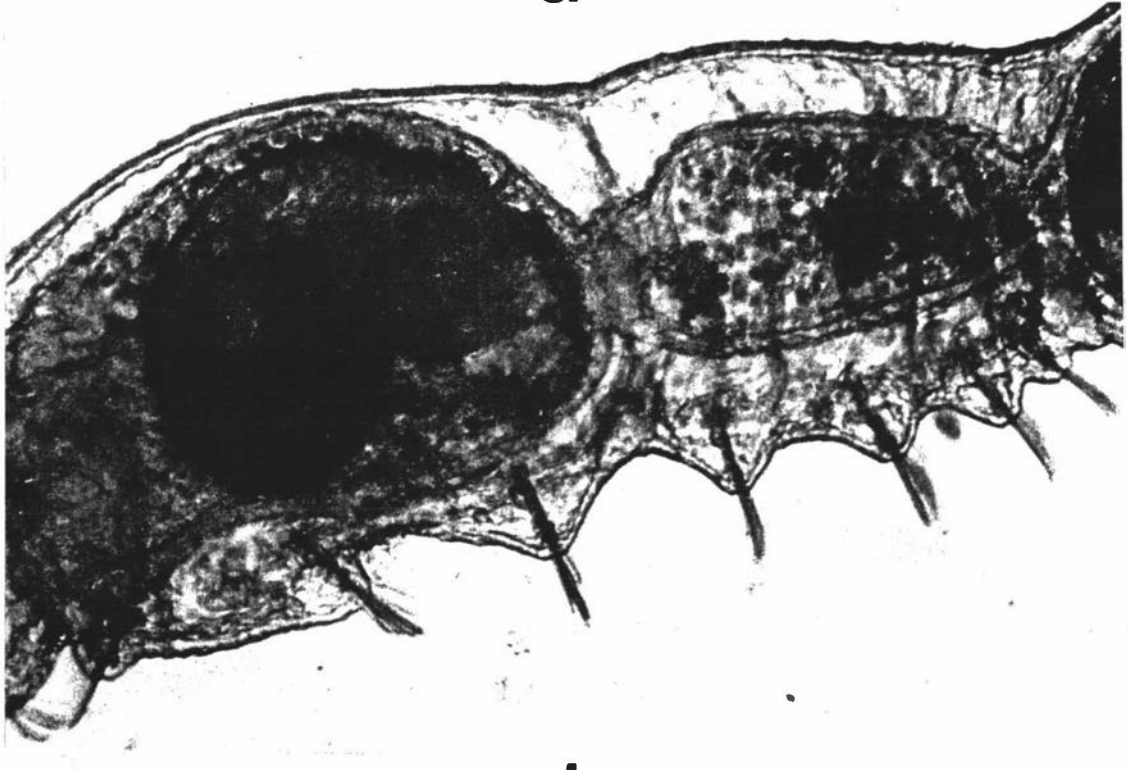
FIGURE 40

Chaetogaster limnaei limnaei (Oligochaeta : Naididae)

a,b - embryo shell of P. antipodum

in gut of Ch. l. limnaei

a



b

of Lymnaea stagnalis from a home aquarium infested with over 90 worms.

Incidence of infection

Six percent of snails in a sample of 100 individuals taken from Lake Pupuke on 26 September, 1966, carried Ch. l. limnaei, one to three worms per snail.

Food, and the worm-snail relationship

Gut contents were examined by squashing worms beneath a cover slip on a microscope slide. Dominant food organisms were diatoms. This is in accordance with Gruffydd's findings and confirms the identification of this worm as Ch. l. limnaei, as the subspecies vaghini feeds only on molluscan kidney cells and concretions. In one individual, however, a most interesting condition was found, the pharynx and crop each containing a single embryonic shell of P. antipodum (Fig. 40). Ch. l. limnaei is known to be predatory on Protozoa, rotifers, and trematode cercariae (Gruffydd, 1965b) living within the tissues of the host snail, but a situation such as this in which the worm feeds upon the young of its host may well indicate an advance from pure commensalism towards a parasitic relationship with the host. Whether the embryonic snails concerned were removed from the brood pouch of the adult or were preyed upon after they had assumed a free-living existence is not known, but if the former were the case then the attribution of parasitic tendencies would certainly seem justified.

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APPENDICES

APPENDIX 1.

Index to localities from which snails have been collected during this study.

Localities are grouped under land districts (McLintock, 1959).

Key

(a) Shell measurements)
Radula)
Operculum) X = populations examined
Embryo shell)

(b) Sex determination

m = males found

f = females only found

x = detailed sex ratio examination made

(c) Habitat types

Classification modified after Elton and Miller (1954)

1. Ditch
2. Flowing "upland" stream
3. Graded "lowland" river or estuary
4. Pond
5. Lake
6. Trickle

a Tidal influence at times

Land District 1 North Auckland

No.	Locality	Shell	Radula	Operculum	Embryo Shell	Sex (a) Prelim.	Sex (b) Detailed
P. antipodum							
1	Whatipu	X	X	X	X	m	
2	Auckland Domain	X				f	
3	Portland Rd., Auckland						
4	Northcote	X			X		
5	Swanson						
6	Takapuna	X	X	X	X		
7	Lake Pupuke	X	X	X	X	m	x
8	Kakamatua Stm.	X		X		m	
9	Waiomio Stm.	X	X	X	X	f	
10	Little Huia	X					
11	Pakiri Stm. 1	X				m	
12	" " 2	X					
13	Piha Stm.					m	x
14	Whangarei Falls					f	
15	Wananaki						
P. pupoides							
A	Wananaki	X	X	X		m	x
B	Hobson Bay						
C	Portland Rd., Auckland						
P. estuarinus							
a	Waikato River mouth	X	X	X		m	x
b	Huia	X				m	x
c	Wananaki						
d	Hobson Bay						
e	Portland Rd.						

Land District 2 South Auckland

P. antipodum							
16	Tirau, Waimakariri R.	X				f	
17	Lake Tarawera	X	X	X	X	f	
18	Lake Taupo	X	X	X		f	x
19	Green Lake, Rotorua	X		X	X	f	x

(Cont.)

Land District 2 South Auckland (Cont.)

No.	Locality	Shell	Radula	Operculum	Embryo Shell	Sex (a) Prelim.	Sex (b) Detailed	Habitat
P. antipodum (Cont.)								
20	Waikaretu No. 1	X				f		1
21	No. 2	X						1
22	Waitomo	X				m	x	2
23	Waipuwerawera Stm., Taupo	X						2
24	Waimangu							2
25	Stream, L. Rotowhero							2
26	Lake Rotorua							5
27	Lake Ngahewa							5
28	Waiotapu Stm.							2
29	L. Rotomahana							5
30	Waikato R., Cambridge	X		X		f	x	3
31	Waiotapu No.2							1
32	Otama Bay (Coromandel Pen.)							3a
33	Waikato R., Tuakau							3
P. pupoides								
E	Otama Bay (Coromandel Pen.)							3a
F	Kaotunu R. (Coromandel Pen.)							3a
P. estuarinus								
f	Otama Bay							3a
g	Kaotunu R.							3a
h	Waihou R. (Hauraki Plains)							3a
	Land District 3 Taranaki							
P. antipodum								
34	Kaponga	X	X	X		f		2
35	Dawson Falls	X				m		2
36	Opunake	X	X	X		m		2
37	Warea	X				f		2
P. estuarinus								
i	Bell Block					m		1a

Land District 4 Gisborne

No.	Locality	Shell	Radula	Operculum	Embryo Shell	Sex (a) Prelim.	Sex (b) Detailed	Habitat
P. antipodum								
38	Te Kaha							2

Land District 5 Hawkes Bay

No.	Locality	Shell	Radula	Operculum	Embryo Shell	Sex (a) Prelim.	Sex (b) Detailed	Habitat
P. antipodum								
39	Wallingford	X				f		3
40	Wimbledon					f		1
41	Woodville	X				f		1
42	Dannevirke No. 3	X				f		1
43	No. 1	X						3
44	Manawatu R.	X				f		2
45	Porangahau	X						1
46	Dannevirke No. 2	X	X			m		1
47	Te Aute							4
48	Lake Tutira							5
49	Mt. Wharite	X	X	X	X	f		6

Land District 6 Wellington

No.	Locality	Shell	Radula	Operculum	Embryo Shell	Sex (a) Prelim.	Sex (b) Detailed	Habitat
P. antipodum								
50	Whakarongo No. 2	X						1
51	Apiti-Utuwai	X			X	f		1
52	Karioi	X						4
53	Hokowhitu	X				f	x	1
54	Whakarongo No. 1	X						1
55	Shannon	X				f		1
56	Pahiatua	X						2
57	Bunnythorpe No. 1	X	X					4
58	Levin No. 1	X						1
59	Bunnythorpe No. 2	X						1
60	Maxwell	X						3
61	Utuwai	X		X		f		1
62	Aokoutere	X				f		4
63	Levin No. 2	X				f		1
64	Kahuterawa R., trib. 3	X						6
65	Otaki	X	X			f		2
66	Khandallah	X	X	X		m		2

(Cont.)

Land District 6 Wellington (Cont.)

No.	Locality	Shell	Radula	Operculum	Embryo Shell	Sex (a) Prelim.	Sex (b) Detailed	Habitat
P. antipodum (Cont.)								
67	Kahuterawa R.	X				f		2
68	Mt. Bruce	X				f		2
69	Waiwakawa R.	X			X	f		1
70	Konewa	X				f		1
71	Lake Rotoaira	X	X	X		f		5
72	Tokomaaru R.	X		X		f		3
73	Waikawa R.	X				f		2
74	North of Mt. Bruce	X				f		2
75	Ohau River	X				m		2
76	Tiritea Stm.	X	X	X		f	x	2
77	Massey University No. 1	X	X	X		f	x	4
78	No. 2	X		X		f	x	4
79	No. 3	X					x	2
80	Pond, Ohau R.	X			X			4
81	Tokaanu	X				f		5
82	Kahuterawa R., trib. 1	X				f	x	1
83	" 2	X					x	2
84	Bunnythorpe Stm.	X				f		2
85	Ohakune	X						2
86	Tongariro							2
87	L. Wairarapa							5
88	Eastbourne					m		2
89	Paekakariki		X			m		1
90	Waikanae							2a
91	Horokiwi Stm.							3a
92	Kahao Stm.							3a
93	Makara River	X	X	X	X	f	x	2,3
94	Linton						x	1
P. pupoides								
G	Hutt River							3a
H	Horokiwi Stm.							3a
I	Kahao Stm.	X						3a
P. estuarinus								
j	Hutt River							3a
k	Horokiwi Stm.							3a
l	Kahao Stm.		X					3a
m	Porirua Hbr.							3a
n	Porirua Hbr. trib.							3a

Land District 7 Nelson

No. Locality	Shell	Radula	Operculum	Embryo Shell	Sex (a) Prelim.	Sex (b) Detailed	Habitat
P. antipodum							
95 Lake Rotoiti	X			X	f		5
96 Doughboy Ck., Murchison	X				f		2
97 Karamea	X				f		1
98 Lake Rotoroa	X	X	X	X			5
99 Waimea Creek, Westport							6
100 Birchfield, Westport							1
101 Waterworks, Westport							1
102 McKennas Ck., Westport							2
103 Piu Piu Springs							4
	Land District 8 Marlborough						
P. antipodum							
104 Pelorus Bridge	X	X	X		f		2
105 L. Eltawater		X	X				5
P. pupoides							
J Havelock R.	X	X	X		m	x	3a
P. estuarinus							
o Havelock R.	X	X	X		m	x	3a
	Land District 9 Westland						
P. antipodum							
106 Lake Wahapo	X	X	X			x	5
107 Lake Ianthe	X		X	X		x	5
108 Lake Paringa	X	X	X	X	m	x	5
109 Punakaiki							2
110 Franz Josef	X	X	X	X	f		2

Land District 10 Canterbury

No.	Locality	Shell	Radula	Operculum	Embryo Shell	Sex (a) Prelim.	Sex (b) Detailed	Habitat
P. antipodum								
111	Avon R. mouth	X				m		3a
112	Avon R. Chch.	X	X	X	X	f	x	3
113	Ilam, Chch.	X	X			f		1
114	Lake Pukaki	X	X	X		f	x	1
P. pupoides								
K	Avon R. mouth	X	X	X		m		3a
P. estuarinus								
p	Heathcote R. mouth	X	X	X		m	x	3a

Land District 11 Otago and Southland

P. antipodum								
115	Lindis Pass	X	X	X	X	f		2
116	Lake Wanaka							5

APPENDIX 2.

Population	Shell measurement data									
	Mean height/aperture height	Range height/aperture height	Standard deviation height/aperture height	Standard error height/aperture height	Mean height/width	Range height/width	Standard deviation height/width	Standard error height/width	Maximum height millimetres	Number in sample
P. antipodum										
1	1.96	2.12-1.78	0.09	0.022	1.68	1.73-1.58	0.06	0.015	9.1	16
2	2.19	2.57-2.00	0.13	0.029	1.75	2.08-1.65	0.12	0.027	5.3	20
4	2.40	2.74-2.21	0.15	0.040	1.92	2.24-1.77	0.11	0.030	6.1	14
6	2.11	2.40-1.85	0.13	0.032	1.73	1.85-1.56	0.08	0.020	5.2	16
7	2.22	2.36-2.04	0.08	0.020	1.70	1.82-1.54	0.08	0.020	6.8	15
8	1.82	2.02-1.69	0.10	0.027	1.59	1.71-1.49	0.07	0.019	7.4	14
9	2.00	2.36-1.78	0.15	0.038	1.59	1.71-1.41	0.07	0.018	7.2	15
10	1.81	2.00-1.65	0.09	0.024	1.61	1.76-1.53	0.07	0.019	8.5	14
11	2.30	2.44-2.16	0.08	0.019	1.82	1.92-1.74	0.04	0.010	6.4	18
12	1.97	2.08-1.85	0.06	0.016	1.72	1.96-1.64	0.09	0.024	8.5	14
16	1.85	1.96-1.70	0.10	0.042	1.60	1.68-1.53	0.05	0.020	5.2	6
17	2.06	2.34-1.83	0.14	0.038	1.64	1.79-1.52	0.08	0.022	6.9	14
18	2.34	2.59-2.18	0.13	0.035	1.76	1.90-1.65	0.08	0.022	6.2	14
19	2.05	2.34-1.83	0.13	0.032	1.65	1.75-1.52	0.07	0.017	6.9	17
20	2.38	2.64-2.14	0.13	0.032	1.80	1.89-1.67	0.06	0.015	5.9	16
21	2.21	2.36-2.07	0.08	0.022	1.76	1.87-1.67	0.07	0.019	6.3	13
22	1.94	2.08-1.80	0.08	0.020	1.68	1.77-1.61	0.05	0.012	6.1	17
23	2.38	2.53-2.22	0.08	0.018	1.87	2.00-1.67	0.08	0.018	4.3	20
30	2.07	2.27-1.77	0.12	0.028	1.71	1.83-1.59	0.07	0.016	6.6	19
34	2.22	2.60-2.00	0.23	0.057	1.82	2.08-1.61	0.16	0.040	4.8	16
35	2.13	2.29-1.96	0.12	0.030	1.71	1.82-1.57	0.05	0.012	6.2	16
36	2.08	2.22-1.94	0.08	0.025	1.77	1.89-1.67	0.06	0.019	6.4	10
37	2.09	2.22-1.97	0.06	0.016	1.70	1.75-1.63	0.04	0.011	5.9	14
49	2.29	2.46-2.04	0.12	0.030	1.80	1.89-1.72	0.05	0.012	4.6	16
39	2.27	2.77-1.96	0.26	0.081	1.80	2.04-1.63	0.15	0.047	5.4	10
41	2.34	2.65-2.12	0.17	0.042	1.85	2.18-1.72	0.12	0.030	5.3	16
42	2.32	2.82-1.94	0.40	0.125	1.84	2.18-1.66	0.20	0.062	5.9	10
43	1.93	2.48-1.72	0.32	0.160	1.62	1.92-1.48	0.21	0.105	4.4	4
44	1.93	2.08-1.73	0.11	0.028	1.67	1.78-1.58	0.05	0.013	5.9	15
45	2.53	2.74-2.25	0.16	0.050	2.02	2.08-1.86	0.08	0.025	4.8	10
46	2.04	2.19-1.89	0.10	0.030	1.78	1.84-1.66	0.07	0.021	6.8	11
93	2.33	2.84-2.17	0.15	0.036	1.89	2.07-1.70	0.10	0.024	5.0	18
50	2.21	2.61-1.96	0.24	0.073	1.72	1.90-1.61	0.11	0.033	5.5	11
51	2.19	2.30-2.06	0.07	0.018	1.73	1.78-1.68	0.04	0.010	6.2	15
52	2.49	2.56-2.42	0.07	0.041	1.77	1.81-1.71	0.05	0.029	4.0	3
53	2.34	2.68-2.20	0.12	0.032	1.87	2.04-1.80	0.06	0.016	5.1	14
54	2.18	2.64-1.80	0.26	0.051	1.80	2.20-1.52	0.17	0.033	6.2	26

Pop.	h/aph Mean	h/aph Range	h/aph SD	h/aph SE	h/w Mean	h/w Range	h/w SD	h/w SE	Max. ht. mm	No. in sample
P. antipodum (Cont.)										
55	2.08	2.36-1.71	0.19	0.051	1.69	1.78-1.55	0.08	0.022	5.3	14
56	2.00	2.11-1.88	0.09	0.045	1.71	1.77-1.62	0.07	0.035	5.3	4
57	2.18	2.28-2.00	0.09	0.022	1.81	1.94-1.63	0.10	0.025	5.2	16
58	2.09	2.52-1.92	0.16	0.043	1.76	2.00-1.61	0.09	0.024	6.1	14
59	2.22	2.36-1.96	0.10	0.026	1.87	2.04-1.61	0.12	0.031	5.0	15
60	2.11	2.22-2.04	0.09	0.037	1.68	1.76-1.62	0.06	0.027	5.1	5
61	2.21	2.56-2.03	0.12	0.030	1.73	1.90-1.64	0.07	0.017	5.9	16
62	2.36	2.54-2.08	0.14	0.035	1.89	2.04-1.77	0.12	0.030	5.3	16
63	2.13	2.28-1.91	0.10	0.026	1.76	1.89-1.65	0.06	0.015	6.0	15
64	2.34	2.54-2.16	0.12	0.030	1.80	1.96-1.68	0.08	0.020	5.7	16
65	2.24	2.45-2.00	0.13	0.032	1.76	1.89-1.65	0.08	0.020	6.0	16
66	2.21	2.41-2.08	0.07	0.017	1.81	1.91-1.72	0.06	0.014	5.6	18
67	1.98	2.10-1.83	0.09	0.028	1.67	1.76-1.62	0.05	0.016	5.8	10
68	2.03	2.35-1.91	0.17	0.071	1.70	1.78-1.63	0.05	0.021	6.0	6
69	2.06	2.14-2.00	0.06	0.019	1.76	1.84-1.70	0.05	0.016	6.9	10
70	2.04	2.36-1.80	0.18	0.056	1.64	1.80-1.50	0.09	0.028	6.4	10
71	2.53	2.79-2.36	0.08	0.020	2.06	2.24-1.93	0.08	0.020	5.0	16
72	1.87	2.00-1.66	0.12	0.037	1.61	1.70-1.50	0.05	0.016	5.3	10
73	1.97	2.17-1.74	0.15	0.053	1.62	1.75-1.52	0.08	0.028	5.3	8
74	1.93	2.18-1.65	0.18	0.075	1.64	1.78-1.53	0.09	0.037	4.6	6
75	1.74	1.92-1.62	0.13	0.059	1.56	1.67-1.49	0.07	0.032	4.7	5
80	2.19	2.39-2.08	0.09	0.020	1.73	1.81-1.66	0.04	0.008	5.4	20
81	2.30	2.54-2.09	0.13	0.032	1.81	1.94-1.71	0.08	0.020	6.4	16
82	2.30	2.67-1.90	0.24	0.065	1.83	2.11-1.56	0.13	0.035	5.1	14
83	2.09	2.24-2.00	0.06	0.013	1.77	1.83-1.72	0.04	0.009	5.9	20
84	2.28	2.91-1.97	0.23	0.050	1.82	2.14-1.67	0.14	0.030	5.9	21
85	2.14	2.24-2.04	0.06	0.015	1.76	1.86-1.64	0.06	0.015	5.3	16
95	2.24	2.56-2.09	0.13	0.037	1.70	1.80-1.58	0.08	0.023	4.3	12
96	2.29	2.65-2.08	0.15	0.038	1.71	1.83-1.57	0.07	0.018	5.4	15
97	2.27	2.46-2.18	0.10	0.042	1.77	1.86-1.66	0.08	0.032	4.8	6
98	2.46	2.82-2.23	0.15	0.038	1.79	1.97-1.69	0.07	0.018	4.7	15
104	2.05	2.32-1.90	0.10	0.027	1.70	2.18-1.56	0.15	0.026	5.0	15
110	2.26	2.45-2.11	0.27	0.069	1.77	1.87-1.61	0.07	0.018	3.8	15
106	2.75	2.94-2.55	0.12	0.029	1.88	2.00-1.70	0.06	0.015	5.3	17
107	2.49	2.73-2.26	0.14	0.038	1.93	2.11-1.79	0.09	0.024	5.6	14
108	2.52	2.84-2.32	0.14	0.035	1.76	1.90-1.62	0.08	0.020	5.0	16
114	2.37	2.48-2.11	0.10	0.025	1.75	1.82-1.56	0.07	0.017	5.9	16
111	2.19	2.26-2.11	0.06	0.021	1.73	1.85-1.67	0.06	0.021	6.3	8
112	2.47	2.80-2.24	0.15	0.037	1.83	1.93-1.74	0.05	0.012	5.8	16
113	2.50	2.92-2.22	0.17	0.036	1.83	2.16-1.62	0.12	0.025	5.9	22
115	2.41	2.90-2.26	0.16	0.041	1.81	1.93-1.63	0.08	0.020	5.1	15

Pop.	h/aph Mean	h/aph Range	h/aph SD	h/aph SE	h/w Mean	h/w Range	h/w SD	h/w SE	Max. ht. mm	No. in sample
P. Dubois										
I	2.49	2.60-2.36	0.08	0.026	1.91	2.00-1.85	0.05	0.020	2.5	12
A	2.43	2.72-2.31	0.10	0.029	1.88	2.00-1.81	0.04	0.018	2.55	12
J	2.25	2.60-2.07	0.18	0.047	1.84	1.97-1.77	0.07	0.029	2.2	10
K	2.33	2.50-2.20	0.11	0.066	1.95	2.07-1.87	0.08	0.056	2.2	6
P. estuarinus										
o	2.32	2.45-2.11	0.10	0.027	1.83	1.91-1.72	0.06	0.016	7.1	14
b	2.37	2.61-2.11	0.13	0.033	1.89	2.00-1.72	0.08	0.020	5.9	15
p	2.21	2.38-2.03	0.10	0.024	1.71	1.92-1.59	0.08	0.019	6.3	18
a	2.41	2.67-2.14	0.13	0.033	1.84	1.93-1.71	0.07	0.018	5.9	15

APPENDIX 3.

Radula measurements

Population No.	Cusp nos. on teeth				
	Median dorsal	Median lateral	Lateral	M1	M2
1	9(11)	3-3	13	24-26	40-42
a	7	3-3	8	15	33-35
6	9	3-3	9	28-29	35-37
7	9	3-3	8	20	32-36
9	11	4-4	11	24-25	38-41
A	9	4-4	9	21-22	-
17	11	3-3	12	29-32	31-34
18	9(11)	3-3	11	22-23	32-35
35	9(11)	4-4	11	25	29-31
37	7(9)	4-4	11	22	38-39
49	9(11)	3-3	10	27	46-48
46	9(11)	(5)4-4(5)	11	26-29	33-43
49	9	4-4	9(11)	21	34-38
57	9(11)	4-4	11	22-24	38-40
65	9	3-3	9	21-23	37-38
66	9	4-4	9(11)	20-24	26-29
71	9	3-3	11	17-20	27
76	9(11)	3-3	11	See text	
77	9(11)	3-3	9	See text	
89	9	4-4	9	24-26	33
98	9	3-3	9	15-16	24-25
104	9	4-4	12	22-23	31-34
o	7	3-3	8	18-19	24-25
105	11	4-4	11	29-31	37-39
110	9	3-3	9	27	44-48
106	7(9)	4-4	10	21	31-33
108	9	4-4	9	18	33
114	11	4-4	9	20-22	24-26
p	7(9)	3-3	8(9)	14-17	21-25
112	9	3-3	11	27-28	41-44
113	9	3-3	10	26-27	32-34
115	11	4-4	11	21-22	30-34
J	11	5-5	11	24-25	29-30

APPENDIX 4.

Embryo shell measurements

Outlines of embryos were drawn on paper with the aid of a camera lucida at 400 times magnification using a Zeiss binocular microscope.

Measurements given are the tracing diameters (400X) of the innermost whorls taken by drawing a pencil line at right angles to the beginning of the suture (see Fig. 1).

(a) First half whorl
(b) First whorl

Measurements in mms.

Locality	First half whorl	First whorl
L. Rotoiti (Nelson) (10)	12	26
	14	25
	14	25
	11	25
	13	26
	16	26
	16	27
	15	27
	13	26
	15	26
Utuwai-Apiti (5)	14	25
	12	24
	16	26
	14	27
	17	29
Pupuke Pond (3)	15	27
	16	31
	16	32
L. Pupuke (10)	14	26
	9	20
	19	27
	16	25
	18	28
	18	28
	13	28
	13	27
	14	20
	14	21
Ohau Pond (8)	18	30
	18	27
	19	32
	16	31
	15	31
	17	32
	16	30
	16	36
Franz Josef (8)	18	30
	15	26
	18	32
	20	35
	20	32
	20	30
	16	30
	20	32
L. Ianthe (7)	14	23
	17	24
	17	25
	18	25
	14	26
	16	27
	16	26
L. Paringa (7)	23	31
	18	30
	17	31
	23	32
	19	24
	18	27
	17	28

Locality	First half whorl	First whorl
LeRoys Bush (4)	18 18 16 18	29 31 23 27
Makara (8)	16 16 19 17 19 18 15 21	27 24 24 29 26 33 26 30
Avon R. (9)	16 17 19 18 17 13 17 14 15	27 29 31 33 25 24 25 23 27
Green L. (7)	17 18 20 20 23 19 22	31 31 39 35 36 25 28
Wharite (10)	18 16 14 17 19 9 15 13 11 11	26 28 29 32 30 21 24 25 27 27
Waiomio (6)	17 17 15 15 15 14	33 35 28 28 35 33
Whatipu (7)	20 21 17 17 19 18 16	28 25 34 33 30 29 35
Waiwakawa R. (8)	17 16 20 22 15 15 16 16	32 33 32 36 33 33 29 30
Lindis Pass (10)	19 17 19 19 19 19 18 16 19 19	33 30 39 38 35 36 33 29 32 31
L. Rotoroa (8)	16 18 18 18 19 18 23 21	28 28 30 27 30 30 33 27
L. Tarawera (8)	18 18 17 16 16 15 18 18	28 27 38 41 36 34 25 26

Total Populations = 19
Total Individuals = 143

APPENDIX 5.

Raw data obtained in ecological studies on P. antipodum

Key to Tables

Column

a. Population number as in Appendix 1.

b. Habitat classification as in Appendix 1.

c. Ornamentation of shells

1. All (or almost all) snails smooth
2. Predominantly smooth
3. Half smooth, half spiny
4. Predominantly spiny
5. All (or almost all) spiny

d. Bacterial shell coating

1. Light
2. Medium
3. Heavy

e. Substrate (= snail habitat)

1. Stones
2. Weed
3. Mud
4. Earth banks
5. Grass

** Sample too small for analysis

f. Maximum shell height (mm)

a	b	c	d	e	f	a	b	c	d	e	f
1	2	3	2	2,4	9	22	2	1	3	4	6
2	2	3	1	4	5	23	2	1	3	2	4
3	3a	1	2	2	5	24	2	2	1	1	5
4	2	1	3	3,2	6	25	2	3	1	2	5
6	4	1	2	3	5	26	5	5	1	1	5
7	5	4	1	2,4	7	27	5	4	1	2,3	5
8	2	3	1	1	7	28	2	1	3	1	5
9	2	4	1	1	7	29	5	4	3	2,3	4
10	2	3	2	4,5	8	30	3	5	1	2,3	6
11	2	5	3	4	6	31	1	1	3	5	3
12	2	4	2	4	8	34	2	1	2	4	4
13	2a	3	3	1,3	6	35	2	1	3	4	6
14	2	2	3	2	9	36	2	2	3	1	6
15	3a	4	3	1,2,3	5	37	2	3	2	1	5
16	3	1	1	4	5	38	2	2	2	2	4
17	5	4	1	2	6	49	6	1	1	2	4
18	5	3	1	2	6	39	3	5	1	4	5
19	2	2	1	2	6	40	1	3	2	2	4
20	1	5	2	2	5	41	1	1	2	2,3	5
21	1	4	1	2,3	7	42	1	1	1	2,3	6

a	b	c	d	e	f
43	3	1	1	4	4
44	2	1	2	4	5
45	1	3	2	5	5
46	1	1	2	2	6
47**	4	-	-	-	-
48	5	3	2	2,4	5
93	2	3	3	2	5
50	1	2	2	2,3	5
51**	1	3	2	2	6
52**	4	-	-	-	-
53	1	2	3	3	5
54	1	3	2	2	6
55**	1	2	3	2,3	5
56**	2	-	-	-	-
57	4	1	3	2,3	5
58	1	2	3	2,3	6
59	1	1	3	2	5
60	3	1	1	3	5
61	1	1	2	5	5
62	4	2	3	2,3	5
63	1	4	3	2,3	6
64	6	1	2	1	5
65	2	4	1	1	6
66	2	1	1	1,2	5
67	2	1	1	4	5
68	2	3	2	1	6
69	1	3	1	2,3	6
70	1	1	2	5	6
71	5	1	3	1	5
72	3	1	2	2	5
73	2	3	1	1,2	5
74	2	2	1	1	5
75	2	3	1	1	5
76	2	2	2	2,4	6
77	4	3	1	2,3	5
78	4	3	1	2,3	5
79	2	3	1	2	5
80	4	3	2	2,3	5
81	5	3	1	2	6
82	1	3	1	2,4	5
83	2	1	3	2	5
84	2	3	2	1	5
85	2	1	1	4	5
86**	2	-	-	-	-
87	5	5	2	2	5
88	2	1	2	1	4
89	1	1	1	2	5
92	3a	2	2	3	5
94	1	3	2	2	5

a	b	c	d	e	f
95	5	1	1	1	4
96	2	1	2	1	5
97	1	4	2	2,5	4
98	5	5	1	2	4
99	6	1	1	2,5	2(i)
100	1	3	3	1,2	5
101	1	1	1	2,3	5
102	2	4	3	2,3	5
103	4	1	2	2	5
104	2	1	1	1	5
105**	5	3	1	2	4
109**	2	-	-	-	-
110	2	1	1	1,2	3
106	5	5	1	2,4	5
107	5	5	1	2	5
108	5	5	1	2	5
114	1	1	2	2,3	5
111	3a	3	1	3	6
112	3	2	1	4	5
113	1	2	1	2	5
115**	2	1	2	1,2,3	5
116**	5	-	-	-	-

(i) juvenile specimens only

APPENDIX 6.

Raw population data

(a) Tiritea Stream

mm groups	Numbers of snails in monthly samples 1965-66											
	A	M	J	J	A	S	O	N	D	J	F	M
0.6-1.0	2	1	7	4	0	0	0	1	339	34	50	22
1.1-1.5	29 (1)	9 (1)	26	31	12	6	1	12		126	89	111
1.6-2.0	63 (6)	47 (6)	41	53 (5)	37 (3)	35	24 (1)	36 (5)		46 (1)	37	47 (4)
2.1-2.5	40 (5)	71 (8)	61 (12)	35 (2)	39 (5)	58	36 (3)	60 (3)	96	8	19 (1)	17 (2)
2.6-3.0	33 (6)	37 (6)	49 (2)	19 (4)	26 (1)	44	16 (2)	41 (3)	14	0	5	7 (1)
3.1-3.5	8 (3)	22 (4)	23 (2)	18 (5)	15 (2)	18	6	14 (2)	(3)	1	0	3
3.6-4.0	10 (4)	15 (8)	9	18 (2)	14 (2)	13 (5)	4 (1)	13 (4)	12	1		4 (1)
4.1-4.5	3	2 (1)	5	11 (1)	6 (2)	11 (3)	4 (1)	8 (1)	(2)	2		1
4.6-5.0	5 (2)	3 (3)	4 (1)	7 (1)	8 (1)	5 (1)	5 (2)	6 (2)	6	2 2		1 1
5.1-5.5	1 (1)	1	4	8	11 (1)	11	0	3	(2)	5 (1)		2
5.6-6.0	2		5	12	7	14	2	1	2	3		1
6.1-6.5	4					1		2				
Totals	200 (28)	209 (37)	234 (17)	216 (20)	175 (17)	216 (9)	98 (10)	197 (20)	469 (7)	228 (2)	200 (1)	216 (8)

Figures in brackets are numbers of spiny shelled snails.

(b) Pond A

mm groups	Numbers of snails in monthly samples 1965-66												
	M	A	M	J	J	A	S*	O*	N	D	J	F	M
0.6-1.0	1	0	0	15	11	1			14	9	10	39	16
1.1-1.5	0	37	19	27	32	5			114	59	81	63	43
			(1)			(1)				(3)	(3)	(1)	(1)
1.6-2.0	4	45	20	28	51	5			25	61	61	54	26
	(2)	(1)	(2)		(1)	(1)			(2)	(20)	(15)	(17)	(4)
2.1-2.5	6	27	14	17	22	15			9	43	29	28	21
			(4)	(3)	(2)				(7)	(10)	(11)	(17)	(12)
2.6-3.0	16	23	9	18	17	9			5	20	18	12	26
	(1)		(3)	(1)	(1)	(3)			(5)	(6)	(9)	(7)	(19)
3.1-3.5	18	26	16	19	16	9			3	5	4	9	11
		(1)	(1)		(1)	(1)			(3)			(8)	(7)
3.6-4.0	36	26	37	31	22	37			10	6	3	3	6
	(3)	(1)	(9)	(6)	(2)	(5)			(8)		(2)	(3)	(5)
4.1-4.5	34	32	67	45	34	61			18	3	3	4	7
	(7)		(15)	(3)	(3)	(4)			(8)		(1)	(3)	(4)
4.6-5.0	91	7	13	10	9	16			29	9	6	3	4
	(9)		(2)	(1)	(2)	(1)			(15)	(1)	(4)		(2)
5.1-5.5	35	1	2		2	6			15	1	1	1	1
	(2)								(11)				(1)
5.6-6.0	1												
Totals	241	224	197	210	216	164			242	216	216	216	161
	(24)	(3)	(37)	(14)	(12)	(16)			(59)	(40)	(45)	(59)	(55)

(c) Pond B

0.6-1.0	1	1	42	53	27	1	1	0	21	1	35	55
1.1-1.5	2	6	75	40	28	1	5	7	64	8	42	49
									(10)		(3)	(1)
1.6-2.0	13	15	16	19	10	5	13	11	34	22	49	27
	(1)	(3)	(1)	(1)			(1)	(1)	(21)	(2)	(9)	(2)
2.1-2.5	24	14	7	11	5	0	13	12	22	23	37	14
	(6)	(5)	(1)	(3)			(2)	(1)	(9)	(7)	(7)	(3)
2.6-3.0	33	17	11	22	14	2	4	5	12	26	23	11
	(4)	(5)	(1)	(2)	(3)	(1)	(2)	(1)	(1)	(5)	(5)	(2)
3.1-3.5	24	21	10	15	12	4	3	6	8	28	4	3
	(7)	(1)	(1)		(1)	(1)	(2)	(1)	(3)	(10)	(2)	(1)
3.6-4.0	31	22	14	11	23	1	1	4	3	32	3	7
	(11)	(1)		(1)	(1)			(2)		(10)	(3)	
4.1-4.5	35	34	14	18	36	7	3	4	13	22	5	6
	(11)	(13)	(2)	(6)	(6)	(1)		(1)	(3)	(9)	(3)	(4)
4.6-5.0	40	66	16	15	45	11	7	19	13	37	16	24
	(16)	(17)	(6)	(2)	(5)	(5)		(4)	(2)	(16)	(4)	(4)
5.1-5.5	20	14	5	4	10	5	4	6	18	16	2	4
	(5)	(4)				(1)	(2)	(1)	(6)	(3)		
5.6-6.0									4	1		
										(1)		
Totals	223	210	210	208	210	37ø	54ø	74ø	212	216	216	200
	(61)	(49)	(12)	(15)	(16)	(9)	(9)	(12)	(55)	(63)	(36)	(17)

Figures in brackets are numbers of spiny shelled snails.
* See Appendix 6(d).
ø 2ft^2 samples during dry period.

(d) Pond A Period of draining and cleaning 1965

mm groups	Numbers of snails in 2ft^2 samples							
	Sept.1	8	15	22	29	Oct.6	13	20
0.6-1.0	0	0	0	0	0	0	0	0
1.1-1.5	2	0	1	4	3	1	0	3
1.6-2.0	9 (1)	3	2	5	3	3	5	5 (2)
2.1-2.5	15 (1)	2	0	6	4	1	9 (4)	5 (2)
2.6-3.0	27 (1)	15 (2)	6	5	3	10	8 (1)	19 (4)
3.1-3.5	17 (1)	11	3	3 (1)	3	8	12 (1)	13 (5)
3.6-4.0	34 (4)	15 (3)	2	4	4 (1)	8 (2)	23 (4)	25 (3)
4.1-4.5	102 (19)	73 (21)	23 (1)	12	16 (3)	46 (6)	45 (14)	39 (9)
4.6-5.0	43 (3)	23 (2)	5 (1)	4	16 (3)	11 (3)	23 (4)	35 (3)
5.1-5.5	10	2	2	1	2	1	2 (1)	8 (6)
5.6-6.0				1			1	
Totals	259 (30)	143 (28)	44 (2)	45 (1)	54 (7)	89 (11)	128 (29)	152 (34)