THE NATURE AND FUNCTIONAL SIGNIFICANCE OF MORPHOLOGICAL VARIATION IN FRESHWATER MUSSELS OF THE MURRAY-DARLING RIVER SYSTEM

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Eratum.

The collection site referred to as Callistemon Point has been misnamed. Callistemon Point is in fact on the Ovens River. However, the collection site used in this project is above Lake Mulwala on the River Murray below Albury, and corresponds to Station 10 in Walker, K.F. and T.J. Hillman 1977. Limnological Survey of the River Murray in Relation to Albury-Wodonga 1973-1976. Albury-Wodonga Development Corporation and Gutteridge, Haskins and Davey.

CONTENTS

ABSTRACT

Three species of freshwater mussels in the Murray-Darling river system, *Alathyria jacksoni*, *A. condola*, and *Velesunio ambiguus*, exhibit morphological variations which are systematically associated with the environment. This project is an attempt to examine the variation, particularly that of *Alathyria jacksoni*, and determine whether it may have adaptive significance. Since shell characters used to identify these species are prone to considerable variation within and between populations, they proved an inconclusive means of identifying animals and determining the status of morphological variants. Consequently, electrophoresis was employed to clarify the identity of certain mussel populations and the taxonomic status of morphological variants. The term 'growth form' is applied to these variants, and descriptions of these forms in both qualitative and quantitative terms are established. Qualitative descriptions, while useful, lack the precision and objectivity of quantitative descriptions. Several systems of shell shape measurement are applied to the three mussel species and one system is employed in an experimental analysis of the functional significance of morphological variation in *A. jacksoni*. Shell shape variation while perhaps more obvious than variation in internal organs, is only a portion of the quantifiable variation present. The way in which the relationship between shell and body is regarded is important

since it governs the approach to adaptive significance. One of two viewpoints can be adopted. The first is that shell shape itself is adaptive, and the second that shell shape is a consequence of functional modification of internal organs to meet environmental requirements. The hydrodynamic properties of both arched and winged *A. jacksoni* shells appeared similar, although, orientation and flume experiments indicate that the direction and velocity of water flow are important. Neither arched or winged *A. jacksoni* seemed to be better adapted in terms of hydrodynamics or ability to burrow in different substrates as a consequence of their shell shape, giving no support to the first alternative. Comparative measurements of mechanical strength of the ligament and adductors in *A. jacksoni* are correlated with shell shape. It became evident that shell and body are integrated and that shell shape variation was a response by the body to the environment, mediated through the shell. The outcome of the experimental analysis indicates that for *A. jacksoni*, which has two growth forms, the arched form which occurs in areas of moderate to fast water velocities, requires stronger adductors for anchorage and burrowing than the winged form which occurs in slower flows. Increased adductor strength is achieved by modification of the size, shape and position of the adductors, especially the posterior adductor in arched *A. jacksoni*. A consequence of this modification is that as the mussel grows, the shell assumes an arched form. Hence, the shell shape is a reflection of the environmentally-induced modification of internal organs which adapts a mussel to its environment.

STATEMENT OF ORIGINALITY

I certify that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university and, to the best of my knowledge and belief, contains no material previously published or written by another person, except when due reference is made in the text of the thesis. I also consent to the thesis being made available for photocopying and loan.

Shirley Balla.

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CHAPTER 1

INTRODUCTION

This thesis concerns morphological variation in the freshwater mussels of the Murray-Darling river system. Interest in this topic was stimulated by the observations of Walker (1981), who discussed the ecology of three species of hyriid mussels in the river system, namely Alathyria jacksoni Iredale, Alathyria condola Iredale and Velesunio ambiguus (Philippi). Velesunio ambiguus occurs throughout the Murray-Darling Basin and beyond. Alathyria jacksoni is not recorded from the temporary streams of the upper Darling drainage and is uncommon in the Victorian tributaries of the Murray. Alathyria condola is confined to the middle and upper reaches of the Murrumbidgee and Lachlan Rivers and Mulwala Canal (Walker 1981).

Each of these species exhibit considerable morphological variation; Walker (1981) outlined the shell shape variations in each species. Walker noted two apparently distinct forms of Alathyria jacksoni designated here as the "arched" and the "winged" forms. The arched form has an arched dorsum and an inflected ventral margin, while the winged form has a straight dorsum projected posteriorly into a wing and a convex ventral margin. The arched form is associated with faster flowing habitats in the river while the winged form is found in moderate flows. Alathyria condola was considered briefly and two forms were observed: one with an antero-dorsal excavation anterior to the beak and the other without an excavation, although the extent of excavation was variable. Walker found Velesunio ambiguus to be a variable species with perhaps only one distinctive form, the "evansi" or "billabong" form. The billabong *V. ambiguus* has a rounded shape and a slight wing and was associated with slow or still waters in billabongs, lagoons and swampy areas found along the river.

Morphological variation is a widespread phenomenon in freshwater mussels. Bivalve systematics is largely dependent on shell characters and much confusion has arisen from intraspecific variation (Ortmann 1920, Seed 1968). There is considerable evidence, however, that the variation is systematically associated with the environment (Kauffman 1969, Stanley 1970, Eagar 1978). This phenomenon is also reported in the Gastropoda, especially those inhabiting aquatic environments. For example, the shell of the snail *Pleurocera*, is less globular in headwaters and small tributaries than downstream (Goodrich 1941). Goodrich speculated that the elongate shape would be adaptive in the fast currents encountered in headwaters. However, the marine dog whelk *Nucella lapillus* assumes a tall and narrow form at sheltered sites and a short, wide form at wave exposed sites (Kitching 1977, Seed 1978, Hughes and Elner 1979). Many other aquatic organisms exhibit morphological variation (e.g., Koehl 1982). The stream dwelling larvae of the Tasmanian psephenids, *Sclerocyphon* spp., assume different shapes at low and high altitudes in the same stream (Davis 1982).

These morphological variants are growth forms or ecophenotypes whose form has been influenced by one or more environmental factors. A growth form is therefore defined by a set of morphological characters associated with a particular environment. Transplant experiments with bivalves and environmental modifications through river impoundments (Grier and Mueller 1926, Baker 1928, Walne 1958, Seed 1968, Rhoads and Panella 1970, Clark 1976 and Eagar 1978) provide evidence of the striking phenotypic response of shell growth to environmental change. Within a species, growth forms represent environmentally induced extremes on a spectrum of morphological variation.

Walker (1982) employed the term 'growth forms' to refer to the arched and winged *Alathyria jacksoni*, in view of the distinct shape variation shown in this species. Walker (1981) noted the corresponding trend in shell shape variation between *Alathyria jacksoni* and the Northern Hemisphere pearl mussel *Margaritifera margaritifera* (L.), reported by Eagar (1977). Both species have arched and "oval" (*M. margaritifera*) or winged forms. Eagar hypothesized that the arched form of *M. margaritifera* was better adapted to anchor itself against the rapid water flows common in its environment. This is achieved through a high specific gravity and the possession of a relatively wide pedal gape in the antero-ventral region of the closed shell allowing extension of the foot for prolonged periods. The oval form occurs in slower flows and has a stronger ligament than the arched form as a consequence of its straighter dorsum. Eagar speculated that these mussels had the potential to be more mobile. In addition, oval mussels were more obese than their arched counterparts and, consequently, have higher metabolic potential in relation to their surface area and specific gravity. He concluded that the oval *M. margaritifera* were adapted for mobility while the arched mussels were adapted for strong anchorage. Eagar's hypotheses might be pertinent to *Alathyria jacksoni* considering the similarity of shell shape variations in the two species.

It was against this background that the question arose: Why do these morphological variations occur and are they adaptive? Focus was directed at Alathyria jacksoni because the variation seemed relatively simple and the animals were accessible and abundant in the lower River Muuray. However, all three species were considered to allow a broader comparative approach.

Prior to experimentation, it was crucial to first consider the possibliity that these morphological variants were, in fact, genetically distinct species. Since shell characters used to describe these species are prone to considerable variation within and between populations, they proved an inconclusive means of identifying animals and determining the status of the morphological variants in the Murray-Darling river system. Electrophoresis was therefore employed in an attempt to clarify the status of these morphological variants.

Following taxonomic clarification, it was possible to describe the morpholgical variation and derive a set of characters which would define each growth form. While classical morphological description plays a role in this, a more precise and objective means of description was required for experimental work. Therefore, quantitative descriptions in the form of mathematical models of shape measurement were applied to external shell morphology.

While considerable work has been devoted to morphological variation in the Bivalvia, it is largely descriptive and speculative, with a striking lack of experimental study. However, a review of this literature in relation to the ecology of the River Murray mussels formed an important part of the process of hypothesis formation. It promoted an understanding of how the mussels might be affected by the environment. It also altered the perception of the variation from a "shell" oriented approach toward a consideration of the entire animal. The gradual evolution of perception of the interrelationship between the mussel and its environment and of the shell and the body was an essential prerequisite to elucidating the functional significance of the morphological variation seen in *Alathyria jacksoni*.

CHAPTER 2

SPECIES AND GROWTH FORM DELINEATION USING ELECTROPHORESIS

2.1 Introduction

Small collections of mussels from several locations on the Murray-Darling river system were examined. Uncertainty about the identity of certain populations and individuals made it necessary to clarify the taxonomic status of morphological variants. The taxonomy of mussels of the Murray-Darling relies on morphological characters even though extensive variation exists in many characters between and within populations. Because of the striking differences between populations initially examined, the possibility arose that these might be different species and not merely growth forms.

Collections from the Upper Murray below Lake Mulwala, Mulwala Canal and Callistemon Point appeared very similar. Yet those from Mulwala Canal were thought to be *Alathyria condola* while those from the other two locations were supposed to be *A. jacksoni*. Two questions formed:

1. Are the mussels from below Lake Mulwala and Callistemon Point *A. jacksoni*?
2. Are the "*A. jacksoni*" from below Lake Mulwala the same species as the "*A. condola*" from Mulwala Canal?

None of these populations could be considered as arched or winged, and if they were indeed _A. jacksoni_, this would complicate the picture of variation in this species. Fortunately, there were distinctive populations of _A. jacksoni_ (Lock 3) and _A. condola_ (Darlington Point) which were easily identifiable on the basis of shell characters. These formed an initial basis for comparison with the other populations. Therefore, cellogel electrophoresis was employed to delineate the systematic status of the "species" and "growth forms" and to clarify their distributions in the river system. This chapter reviews the electrophoretic techniques, specifies the methods employed in this study and presents the conclusions drawn from the data.

2.2 Background to electrophoresis

Proteins are composed of amino acids which assume a folded conformation to minimize conflicting electrostatic interactions. They are manufactured in the cytosol from messenger RNA which is directly translated from the DNA. The DNA consists of four bases: adenine, guanine, cytosine and thymine. Three bases code for a single amino acid and a succession of these triplets in the DNA (a gene) codes for a protein. The substitution, deletion or insertion of bases may change the amino acid sequence in a protein. Bases added or deleted in the DNA in anything other than multiples of three leads to the incorporation of a different amino acid with the resultant protein often non-functional and sometimes fatal.

Where substitution of bases has occurred:

a. the same amino acid may be coded for;

b. a different amino acid may be included, but have the same charge as the original, thus while not changing the overall charge of the protein it may change the conformation because the shape of the amino acid differs;

c. a different amino acid with a different charge may be incorporated. This changes both the overall charge of the protein and its conformation. Some amino acid changes will go undetected, only charge and/or conformation changes in the protein can be detected with electrophoresis.

Electrophoresis is the transport of charged proteins through a solvent by an electric field (Freifelder 1976). The velocity of migration (V) of a protein in an electric field depends on the strength of the electric field (E), the net electric charge on the protein (Z) and the frictional resistance (f). The frictional resistance is a function of the size and the shape of the protein. The migration velocity is related to these variables by

$$V = \frac{EZ}{f}$$

(Stryer 1981).

Eukaryotes have at least two sets of chromosomes containing the same genes, but the base sequences in each of the two genes may differ and produce slightly different proteins. Where the protein's charge or conformation differs the proteins will have different rates of migration and separate into two distinct bands on a gel. Such an individual is called a heterozygote for that particular protein. An individual containing proteins identical in charge and conformation has only a single band on a gel and is a homozygote for that protein. This is the simplest case, where single protein chains function alone, and are referred to as monomers. Dimers are proteins which consist of two protein chains bound together to form a single functional unit. Again, homozygotes have a single band, while heterozygotes have three bands.

The slowest migrating band consists of two identical proteins, and the fastest migrating band consists of two identical protein chains which are not the same as the slowest chains. The intermediate band consists of one slow and one fast migrating chain combined to make a functional dimer. Polymeric proteins have an increased number of bands.

2.3 Electrophoretic studies on bivalves

A considerable amount of electrophoretic work, largely taxonomic, has been conducted on bivalves since the early 1970's. Davis and Fuller (1981) used immunoelectrophoretic distances to determine taxonomic relationships among recent Unionacea in North America. Davis *et al*. (1981) looked at molecular genetics and speciation in *Elliptio* and its relationship to other North American unionids. Hornback *et al*. (1980) used electrophoresis to study the genetic and morphological variation in the freshwater clam *Spaerium*. These studies, and others on pulmonates, yeild valuable information about the presence or absence of particular proteins in molluscs. Selander and Kaufman (1973) and Clarke *et al*. (1978) looked at genetic variation and natural selection in pulmonates and Wurzinger and Saliba (1979) compared three populations of the snail *Bullinus*. These studies largely resulted from dissatisfaction with morphologically based species discriminations. This literature played an important part in selecting proteins to be studied here, but most valuable were those studies of the biochemical pathways employed by bivalves. Head and Gabbott (1979) investigated the control of $NADP^+$-dependent isocitrate dehydrogenase acitivity in *Mytilus edulis* L. The contribution of the pentose phosphate cycle to the central pathways of metabolism in *M. edulis* was studied by Zaba and Davies (1979).

Information from Addink and Veenhof (1973), de Zwaan and van Marrewijk (1973), Carrion-Angosto *et al.* (1977) and Rodriguez-Segade *et al.* (1978) concerning the tissue distributions of particular enzymes was also considered.

From these studies, it was possible to construct a list of proteins found in molluscs and collect stain formulae for each protein. Proteins from major biochemical pathways with important metabolic functions were retained. Of these, proteins with stain ingredients in common were selected to minimize the cost of buying chemicals.

2.4 Materials and methods

a. Preparation of samples.

Mussels were removed from their shells and the whole bodies wrapped in aluminium foil and plastic bags and frozen at -20°C. When required, approximately 0.5ml of foot (containing muscle, digestive tissues and gonad) and mantle tissue were cut from each specimen. This tissue was found to be too viscous for mannually operated tissue grinders, and consequently, each sample was macerated with a scaplel for 10 minutes then placed in a 10ml plastic centrifuge tube with 1.0-1.5ml lysing buffer (0.1ml non-ionic detergent TRITON X 100, 0.1ml β-mercaptoethanol, 10mg NAD, 10mg NADP in 100ml 20mM HEPES buffer). The solution was pulverized with a glass rod for two minutes and samples were centrifuged for 25 minutes at 8,000 rpm at 4°C. Samples were usually immediately applied to the cellogel. Some enzymes could tolerate freezing for 24 hours allowing the gel to be run the following day.

b. The electrophoresis tank.

This consisted of a plastic tray divided lengthwise into two compartments by a plastic strip. Two support strips were set on either side of the dividing strip so that a glass plate could rest horizontally inside the tank. This glass plate was evenly smeared with petroleum jelly and the cellogel was rolled flat onto it. Filter-paper wicks connected the long edges of the cellogel to the buffer in the tank. Electrical connections were placed at one end of the tank, and from these, platinum wires ran along the bottom of the tank. The tank contained 400ml buffer (Tris-EDTA-borate, pH 7.8). A glass top was placed on the tank to prevent contamination.

c. Preparation of the gels.

Cellogel 500 (Cellulose acetate on a plastic backing, prepared by Chemetron, Italy) was stored in 30% methanol and soaked in electrode buffer for 20 minutes to wash out the methanol before use. The gel was then blotted and rolled onto the glass plate.

d. Electrophoresis and staining the gel.

Individual samples were applied to the gel in a straight line (the origin) near the cathode, using a draughtsman's pen or a drawn out Pasteur pipette. Individual applications were 4-9mm long. Different samples were separated by gaps of 1-2mm. Three application of each sample produced bands of sufficient density in most cases. The applicator was washed and dried between each sample. One sample was used as a control on each gel to check for warping and to permit comparisons between gels. The control was applied at the centre and each end of the larger gels. After the samples had soaked into the gel (5 minutes) the tank was connected to a DC power supply set at 200V, 10-15 mA (pH 7.8 buffer) for two hours. The power was then disconnected and the gel removed, blotted and placed in 2ml of an enzyme specific stain solution. This solution was prepared from dry ingredients less than 1 minute before use because the ingredients deteriorated rapidly when exposed to light. The bands were recorded as they appeared, and when they had reached maximum resolution, the reaction was terminated by immersing the gel in 10% formalin.

Stain formulae based on Harris and Hopkinson (1976) with modifications developed by Clive Chesson (Department of Genetics, University of Adelaide) were used for most enzyme systems. The original formulae were developed for study of human enzymes so three of the major enzymes examined required modification. For these three enzymes the reaction catalyzed, the method of staining and the modified formula is recorded in Appendix A.

d. Preservation of the gels.

Gels were transferred from the formalin to a solution of 20% glycerol overnight. They could then be dried flat, taking on a white colouration while remaining flexible. Dried gels were stored between sheets of paper toweling. Darkly staining bands could easily be discerned and lighter bands could be readily viewed by holding the gel against a strong light.

e. Specimens.

Specimens were collected from many locations along the Murray-Darling river system (Table 1). Small numbers were donated by various people from sites at Callistemon Point on the River Murray near

Albury (John Hawking, Peter Till Environmental Laboratory,Albury-Wodonga Development Corporation, August 1983), Cooper Creek, Innamincka (Kingsley Turner, Dept. Envrionment, Adelaide, September 1982) and from the South Esk River in Tasmania (Mr. R. Stephens, *fide* K.F. Walker, Univ. Adelaide, November 1982).

TABLE 1

Species	No.	Location
Velesunio ambiguus	10	Albury
	1	Goat Island, Renmark
	12	Lock 2
	11	Mannum
	6	Point Sturt
	7	Pompoota
	9	Swan Reach
	7	Walker Flat
	11	Wongulla
	20	Yatco Lagoon
Velesunio wilsonii	18	Cooper Creek, Innamincka
Velesunio moretonicus	3	South Esk R., Tasmania
Alathyria condola	10	Darlington Point
Alathyria sp?	10	Callistemon Point, R. Murray
	4	Boundary Bend
	6	Mulwala Canal
	6	Below Lake Mulwala
Alathyria jacksoni	10	Blanchetown
	2	Boundary Bend
	11	Lock 2
	25	Lock 3
	7	Morgan
	3	Walker Flat
	18	Wemen

2.5 Results

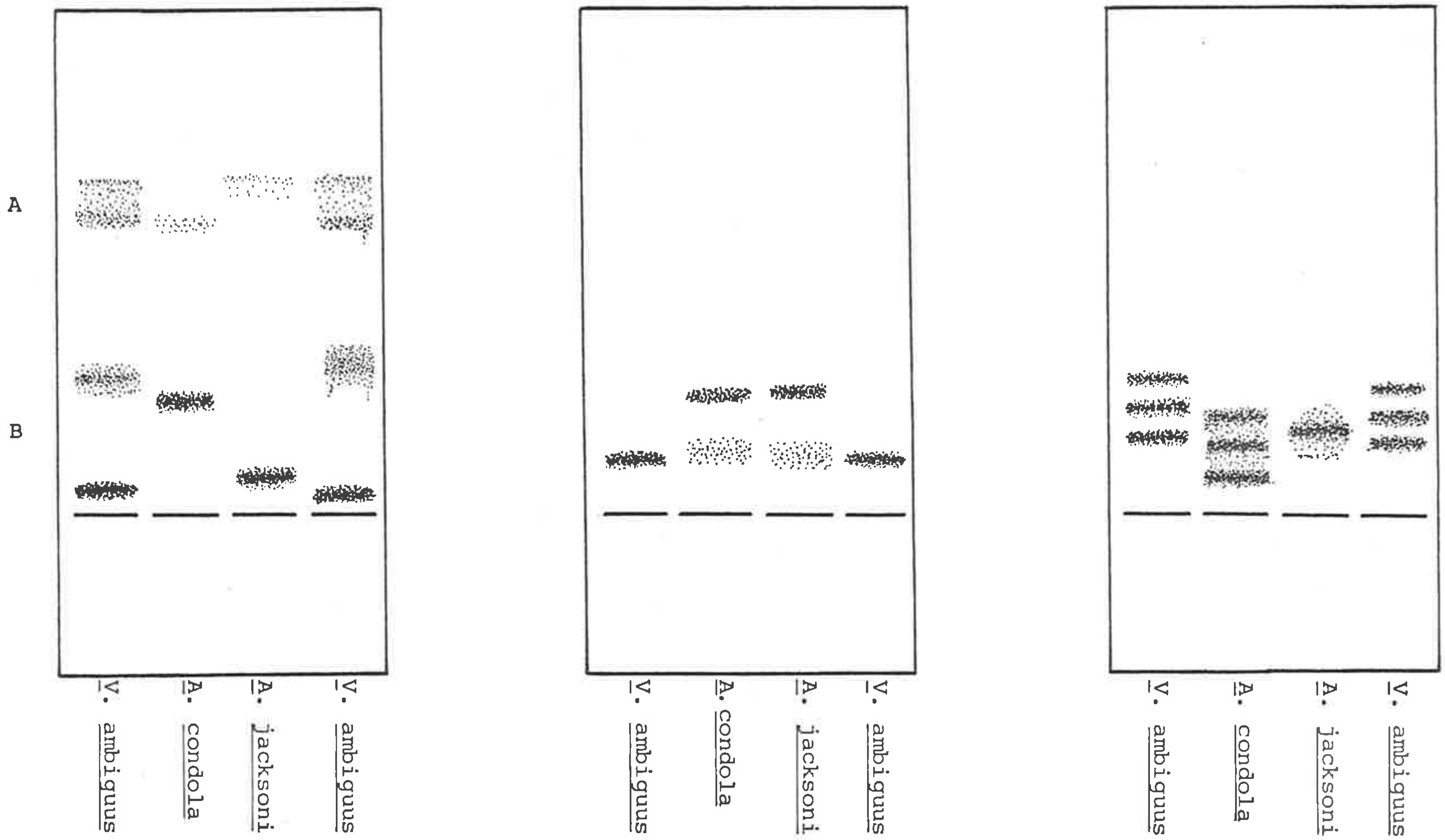
Pilot tests were performed on three specimens of different species in order to select enzymes which would yeild useful information. Species used for pilot experiments: *Alathyria jacksoni*, *A. condola* and *Velesunio ambiguus*. The enzymes tested and the results obtained are summarized in Table 2 and gels are depicted in Figures 1-5.

TABLE 2

Enzymes	Results
1. Acid phosphatase	no bands appeared
2. Adenylate kinase	each species differs, bands very pale
3. Alcohol dehydrogenase	monomer, no variation
4. Alkaline phosphatase	monomer, differs between genera
5. Carbonic anhydrase	monomer, differs between genera
6. Fumarase	no bands appeared
7. Glucose-6-phosph. dehyd.	no bands appeared
8. *Glucose phosph. isomerase	dimer with complex variation
9. Glutamate oxaloacetate transaminase	monomer, *A. condola* and *V. ambiguus* same alleles
10. Lactate dehydrogenase	monomer, no variation
11. *Malate dehydrogenase	monomer, complex variation, pale
12. *Malic enzyme	monomer, differs for each species,pale
13. Mannose phosphate isom.	monomer, *A. condola* differs, pale
14. Nucleoside phosphorylase	no bands appeared
15. Peptidase A	monomer, 2 alleles, no variation
16. Peptidase B	pale, complex variation
17. 6-phosphogluconate dehyd.	pale, complex variation
18. Superoxide dismutase	no bands appeared

* denotes enzymes selected for more detailed study.

FIGURE 1

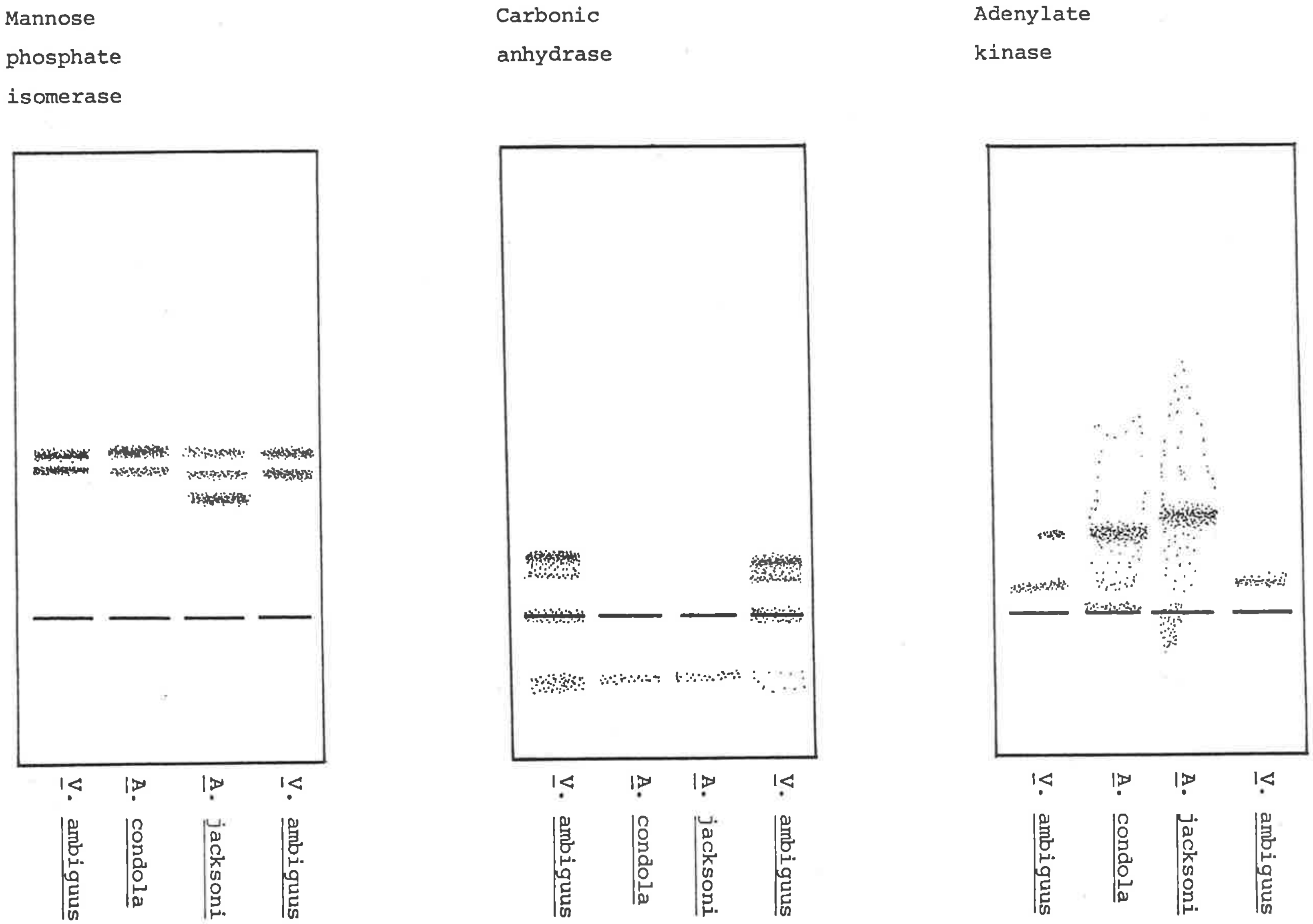


Peptidase A & B

Alkaline phosphatase

Glucose phosphate isomerase

FIGURE 2

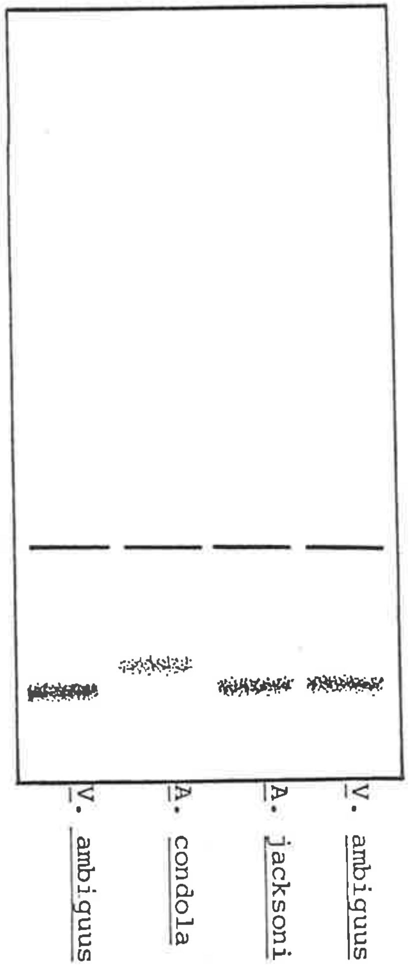


Mannose phosphate isomerase

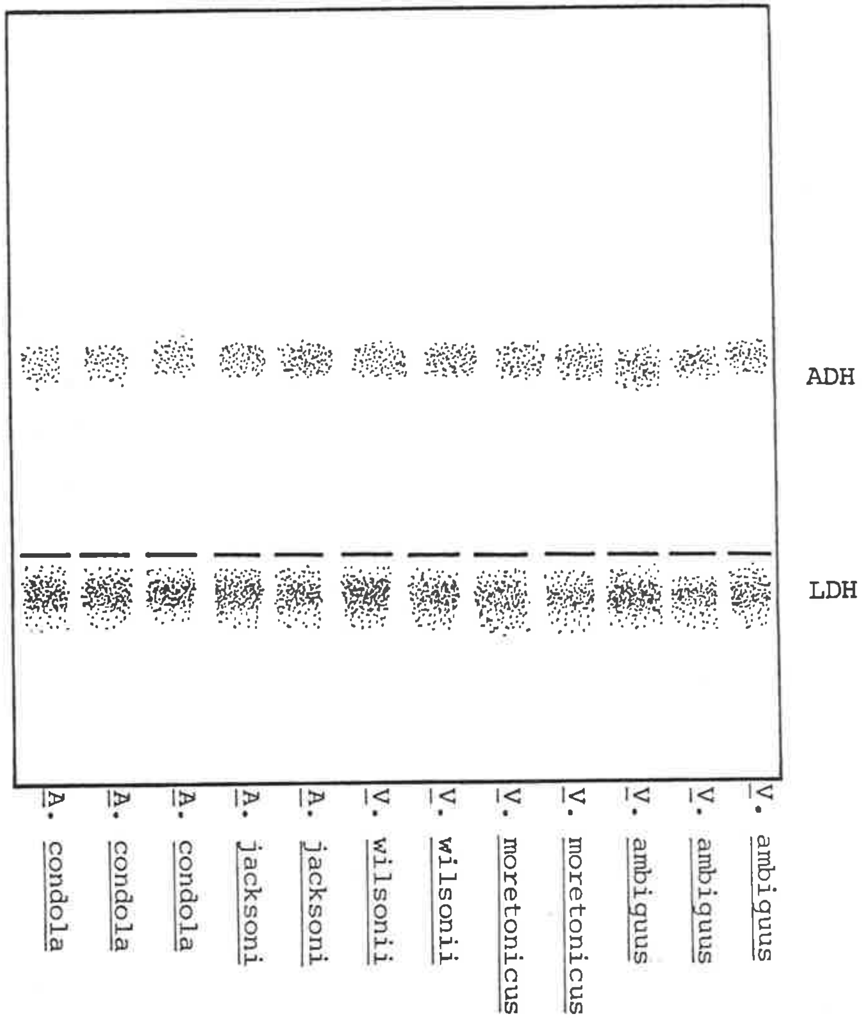
Carbonic anhydrase

Adenylate kinase

FIGURE 3

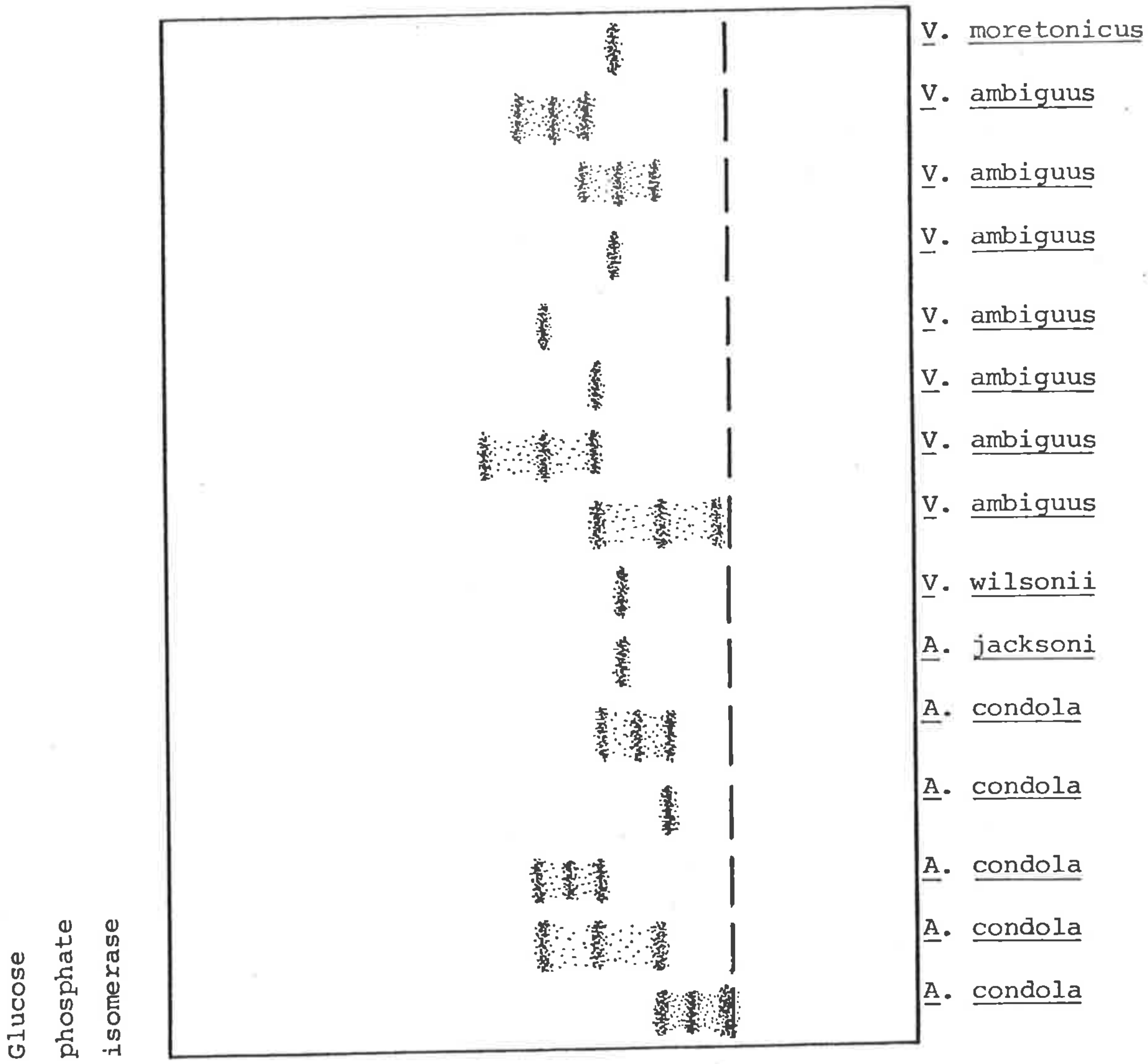


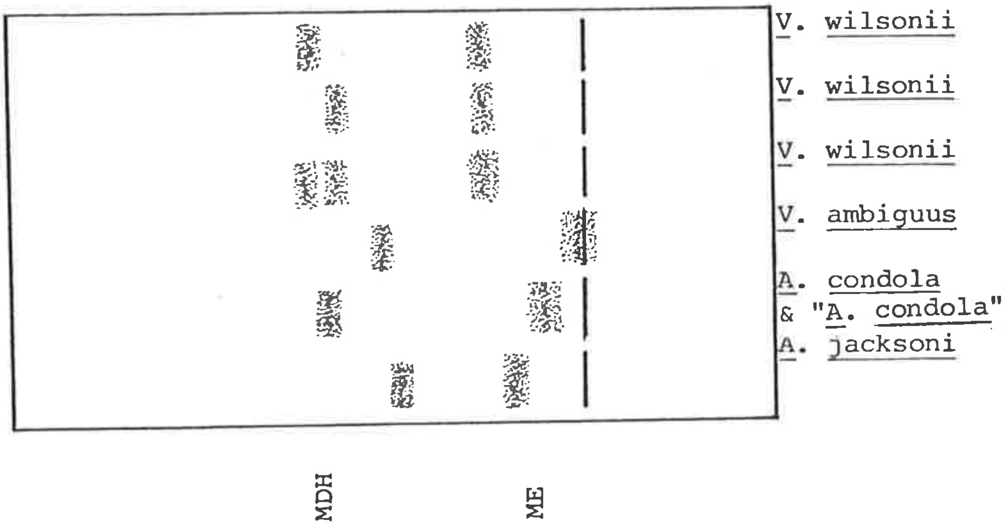
Glutamate oxaloacetate transaminase



Alcohol dehydrogenase ADH
Lactate dehydrogenase LDH

FIGURE 4

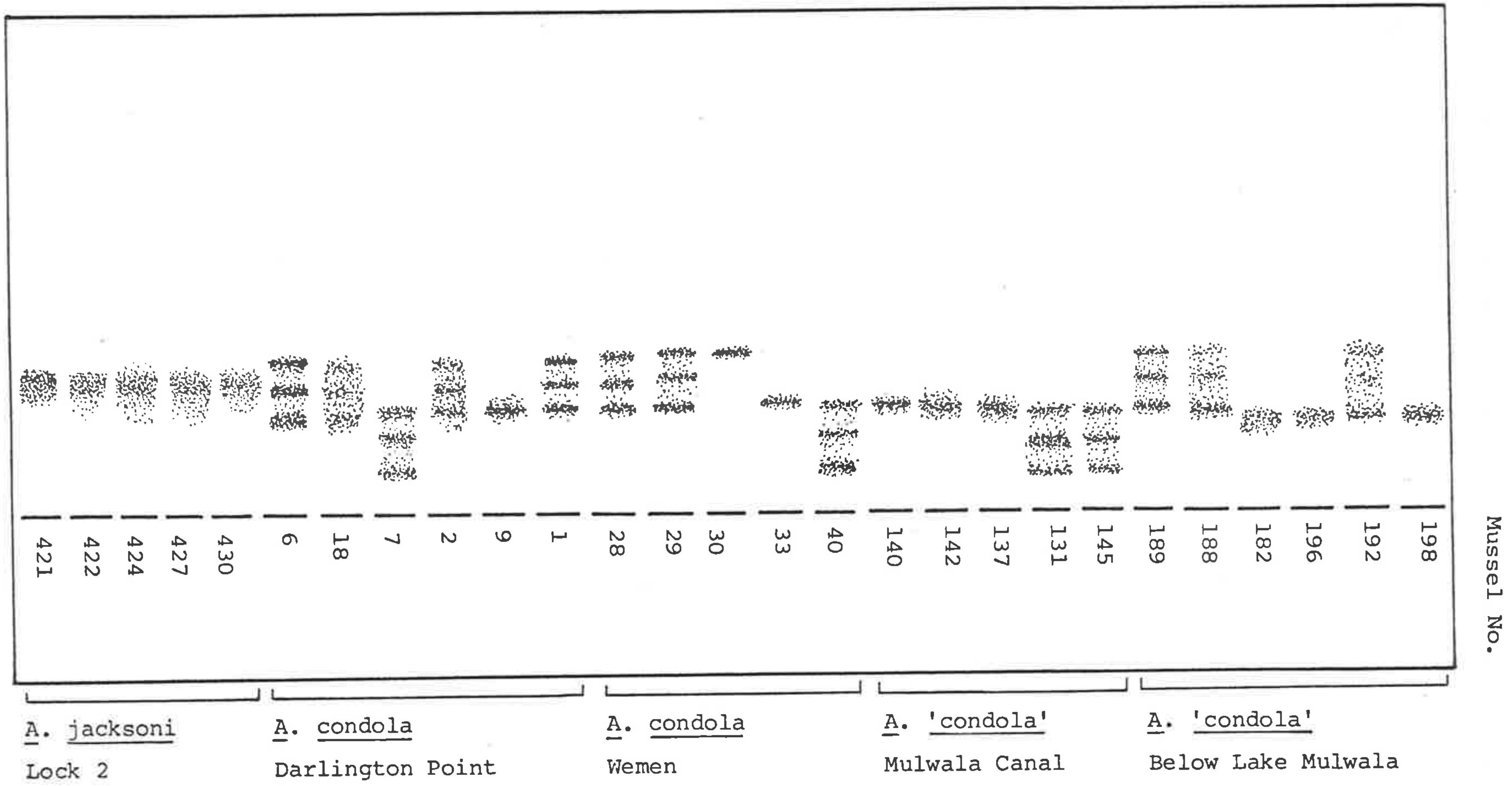

Glucose phosphate isomerase


Malate dehydrogenase MDH

Malic enzyme ME

FIGURE 5

Glucose phosphate isomerase



2.6 Conclusions

The electrphoretic data from these three viable enzyme systems suggests that there is an unrestricted gene flow along the River Murray. Within a species, growth forms did not differ in banding patterns for any of the enzymes tested. It supports the use of the term 'growth form' as opposed to 'ecotype' for these morphological variants.

Alathyria jacksoni can be distinguished from the other two species by its monomeric homozygous allele for glucose phosphate isomerase. (An allele is a gene product and hence each band on a gel is by convention referred to as an allele.) Both *V. ambiguus* and *A. condola* posses multiple alleles for this enzyme (refer to Fig. 4).

The mussels from below Lake Mulwala, Mulwala Canal and Callistemon Point are heterozygous for this enzyme (see Fig. 5), therefore it is concluded that they are not *A. jacksoni*. Examination of the banding patterns for glucose phosphate isomerase, malate dehydrogenase and malic enzyme shows that these mussels have identical patterns to the Darlington Point specimens of *A. condola*. While evidence from only three enzymes cannot be regarded as conclusive, it indicates the likely possibility that these mussels from the Mulwala Canal, below Lake Mulwala and Callistemon Point are *A. condola*. Further electrophoretic examination is required to clarify the identity of these animals, however, this is beyond the scope of this project. Based on the evidence presently available, these mussels will subsequently be referred to as non-excavate "*A. condola*". *A. condola* appears to be numerous in the Upper Murray and also occurs in the Murrumbidgee. Downstream from the Murray-Murrumbidgee confluence only small (and probably young) specimens have been found, suggesting the recent invasion of this area from the Murrumbidgee system. They are found at Wemen (Fig. 5) and probably occur further downstream.

Those mussels inhabiting only riverine environments, i.e., *A. jacksoni* and, so far as is known, *V. moretonicus*, exhibited less heterozygosity at all loci than those capable of inhabiting multiple environments (e.g., river, bilabong, lake, lagoon and irrigation channel) for example, *V. ambiguus* and to a lesser extent *A. condola*. These latter environments exhibit more variability in water flow and temperature. The trend is also reflected in the consistency of length:weight ratios of each population (Table 3).

TABLE 3

Species	Location	No.	x	Minimum	Maximum	Difference
V. ambiguus	Yatco Lagoon	25	4.96	2.51	11.17	10.66
V. ambiguus	Wongulla	18	7.17	2.37	16.83	14.46
V. wilsonii	Innamincka	23	2.89	2.46	3.46	1.00
A. condola	Mulwala Canal	22	1.23	0.75	3.13	2.38
A. condola	Darlington Point	21	0.90	0.69	1.49	0.80
A. condola	Below Lake Mulwala	20	1.48	1.08	2.64	1.56
A. jacksoni	Lock 3	24	1.38	0.93	1.83	0.90
A. jacksoni	Wemen	19	1.03	0.82	1.22	0.40

Enzyme polymorphisms may increase fitness by providing a means of metabolically compensating for a varying environment (c.f. Johnson 1974). One of the most significant factors in variable environments is probably temperature variation. Temperature changes alter metabolic rates, thus slowing or quickening the overall metabolism. Differences between alternative molecular forms of an enzyme with respect to sensitivity of substrate affinity to temperature have been reported for isoenzymes (Hochachka and Somero 1971). Individuals which are heterozygous at a locus (the genes on both chromosomes which code for a particular protein) may be capable of modulating the reaction rate to compensate for variable reaction conditions. For example the fastest moving band of a heterozygous monomeric protein may have the greatest substrate affinity at high temperature, and the slower band may have protein which is more efficient at lower temperatures. That such heterozygosity is associated predominantly with rate limiting reactions in metabolism suggests a selective role for these enzyme polymorphisms (Johnson 1974). Those enzymes which exert acute control on flow through metabolic pathways should be most sensitive to selective forces. Changes at loci whose enzymes regulate flow through pathways could be expected to produce far greater alterations in fitness than changes affecting enzymes which do not regulate metabolic flow. Polymorphic variation at the loci of enzymes governing the ratios of ATP/AMP, NAD^+/NADH or $NADP^+$/NADPH (enzymes such as adenylate kinase, α-glucose phosphate dehydrogenase, glucose phosphate isomerase, lactate dehydrogenase and malic enzyme (Johnson 1974) would make possible a generalized response to metabolic fluctuations. For the present, the consequences of other observed polymorphisms are obscure.

CHAPTER 3

DESCRIPTION OF MORPHOLOGICAL VARIATION IN THE MURRAY-DARLING MUSSELS

3.1 Introduction

Following confirmation of the identity of the mussel populations found in the River Murray, it was appropriate to determine better descriptive criteria for each growth form to allow field identification. The characters used to discriminate growth forms are not necessarily those features which might adapt a growth form to its environment. The identification of adaptive and consequent characters requires extensive knowledge of their interrelationships and significance in the overall functioning of the animal in its environment. This chapter provides a qualitative description of the growth forms of the three Murray species and examines quantitative shape description. The terms used to describe mussel shape are shown in Figure 6.

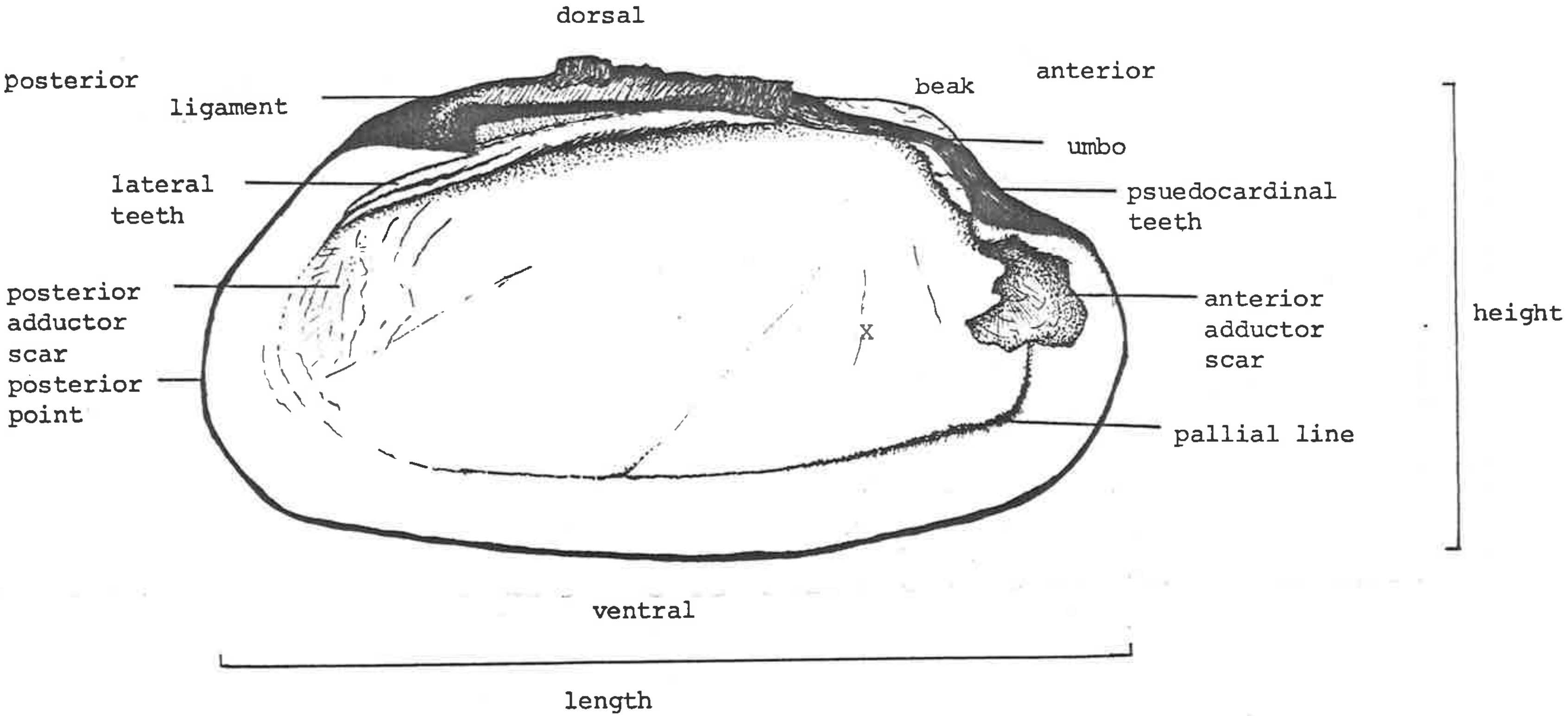
3.2 Qualitative description of growth forms

3.2.1 *Alathyria jacksoni*

This species exhibits two growth forms.

a. The *arched* growth form has an arched dorsum forming a rounded outline extending from the beak, reaching a peak at midlength, and receeding towards the posterior of the mussel in a gradually curved slope. The posterior point falls below the line of midheight and curves sharply around the posterior ventral margin into a concave ventral inflection.

FIGURE 6



The terms used to describe mussel shape. X denotes the point of intersection of the line of maximum length and beak height which is used as an extremal landmark to allow consistent orientation of mussels.

Thus the mussel takes on the appearence of a crescent. Maximum length observed was 140mm; mean adult length was 120mm. The shell is thick ($\bar{x}$=6.2mm, N=23, Wemen population; shell thickness measured at the pallial line on the ventral margin opposite the beak) and they may weigh between 150 and 330g. Internally, the valves have deeply impressed anterior adductor scars ($\bar{x}$=5.6mm deep, N=23, Wemen population; measured from plasticine impressions). The posterior adductors are large and oval, with maximum length dorso-ventrally.

Arched mussels are found in substrates composed of stones, coarse sand and occasionally clay. The water velocity is often greater than 30cm/sec and regularly much faster during floods. This form predominates in the upper and middle stretches of the River Murray (see Fig. 7).

b. The winged growth form has a straight dorsum almost level with the beak, and it extends towards the posterior of the mussel before sloping sharply downwards to the posterior point. The posterior point occurs midway along the point of maximum height, and gradually curves into a blunted and convex ventral margin. The shape is elliptical with an angular distortion of the ellipse caused by the wing in the dorsal-posterior region. These mussels are relatively shorter than the arched growth forms with a maximum observed length of 120mm and a mean length of around 100mm. Shell weight ranges from 130 to 265g and the shells are thinner than the shells of arched mussels ($\bar{x}$=4.6mm, N=27, Lock 3 population). The muscle scars are not as deep as in arched mussels ($\bar{x}$=4.1mm, N=27, Lock 3 population), and the adductors are smaller and rounded in outline, particularly the posterior adductors.

Winged mussels are found in substrates of clay and sand mixtures where the river flows more slowly. The South Australian Murray contains this form (see Fig. 8).

FIGURE 7

Arched *A. jacksoni* from Wemen.

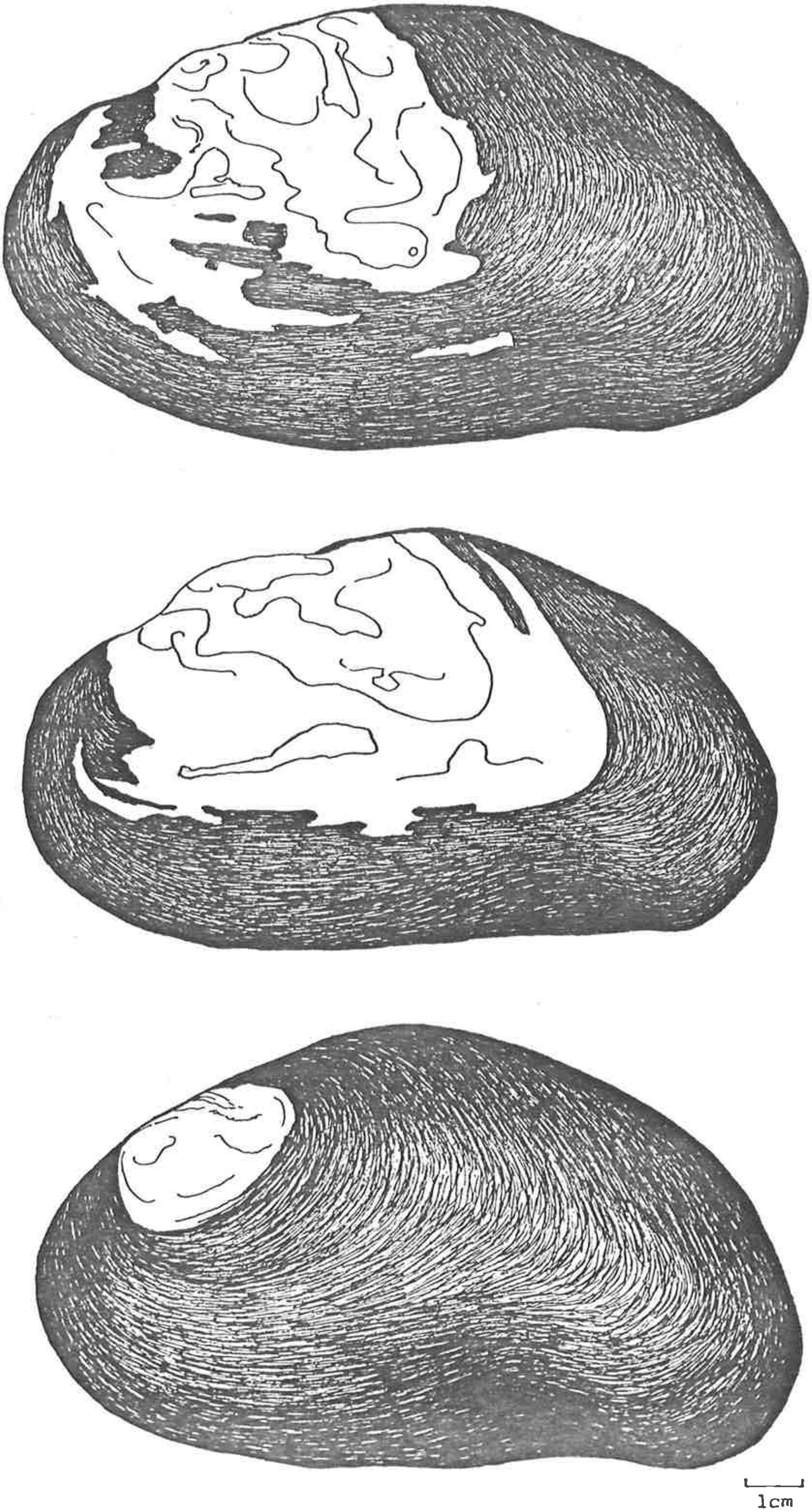
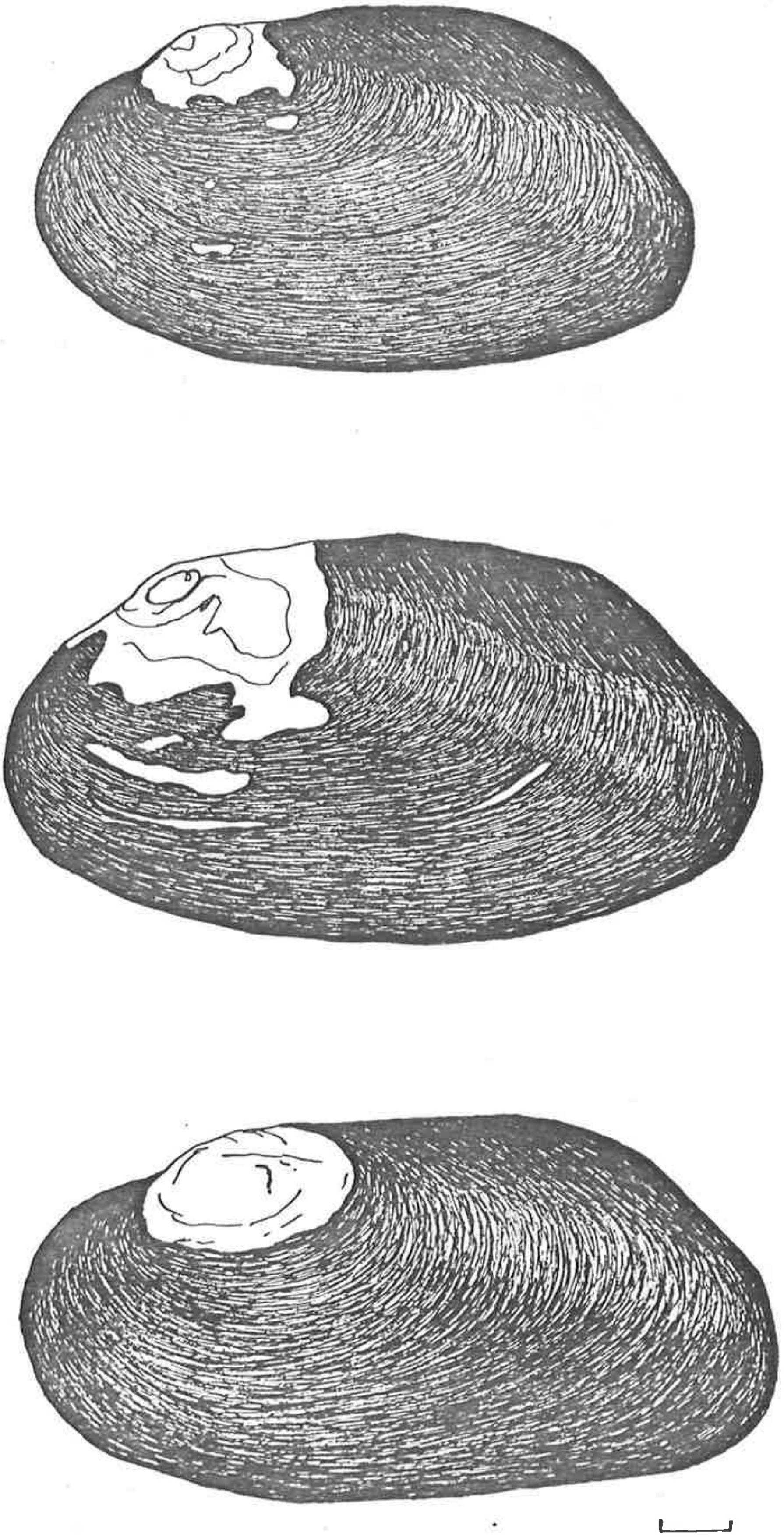


FIGURE 8
Winged *A. jacksoni* from Lock 2.



Morphological characters distinguishing *Alathyria jacksoni* from *A. condola* (including "*A. condola*") are given in Table 4.

TABLE 4

Alathyria condola	*Alathyria jacksoni*
1. The pallial line slightly recessed with raised nacre on the ventral edge.	1. The pallial line less pronounced due to the absence of raised nacre on the ventral edge.
2. Very thick shell producing rounded ventral margins anteriorly. This results in a more obese appearence. ($\bar{x}$=7.7mm, N=20, Darlington Point; $\bar{x}$=7.6mm, N=17, Mulwala Canal).	2. Shell reduced in thickness toward the ventral edge forming a flattened aspect when viewed vertically from the anterior end ($\bar{x}$=6.2mm, N=23, Wemen; $\bar{x}$=4.6mm, N=27, Lock 3).
3. A wide band (about 15mm) between pallial line and ventral margin opposite the beak.	3. The band between the pallial line and the ventral margin very rarely exceeds 10mm.
4. Occasionally has pink nacre.	4. Never has pink nacre.
5. Posterior adductor scar slightly etched into the shell.	5. Posterior adductor scar not etched into the shell.
6. External shell shape generally rounded and globose.	6. External shell shape antero-posteriorly elongate, narrowing width toward the ventral margin.

3.2.2 *Alathyria condola*

This species displays two growth forms here called the excavate and non-excavate forms.

a. The *excavate* growth form has an antero-dorsal excavation anterior to the beak. The anterior of the mussel, consequently, assumes a blunted, upturned appearance. The shell is usually very wide (obese), and the two dimensional outline of the commissure of the valves is circular. They are large mussels with extremely thick and heavy valves. Length maximum = 121mm , mean = 107mm. Height values are greater than all other growth forms in the river. Weight varies between 130 and 170g in adults and the average shell thickness is 7.7mm (N=20, Darlington Point mussels; ventral margin opposite the beak). The adductor scars are deep (anterior $\bar{x}$=7.7mm, S=7.40, posterior $\bar{x}$=1.3mm, S=1.39 N=20, Darlington Point population). The valves are drawn very tightly closed by the adductors. This growth form was collected only from Darlington Point on the Murrumbidgee. Here the water is deep and flows quickly. The substrate is a soft sand-clay mixture (see Fig. 9).

b. The *non-excavate* form possesses no excavation, but maintains the blunt anterior shape. It is more oval in outline and not notably wide. They are consistently smaller than the excavated form with a maximum length of 115mm, mean = 100mm, and between 24 and 153g shell weight. The adductor scars are not as deep as in the excavate form; anterior $\bar{x}$=5.3mm, S=5.15, posterior $\bar{x}$=0.8mm, S=0.89 (N=17, Mulwala Canal population). The adductors are relatively smaller in area and do not draw the valves together as tightly as does the excavated form. This form is found in clay and gravel (T. J. Hillman, pers. comm.), and was obtained from below Lake Mulwala, Mulwala Canal and Callistemon Point on the Murray near Albury (see Fig. 10).

FIGURE 9

Excavate *A. condola* from Darlington Point.

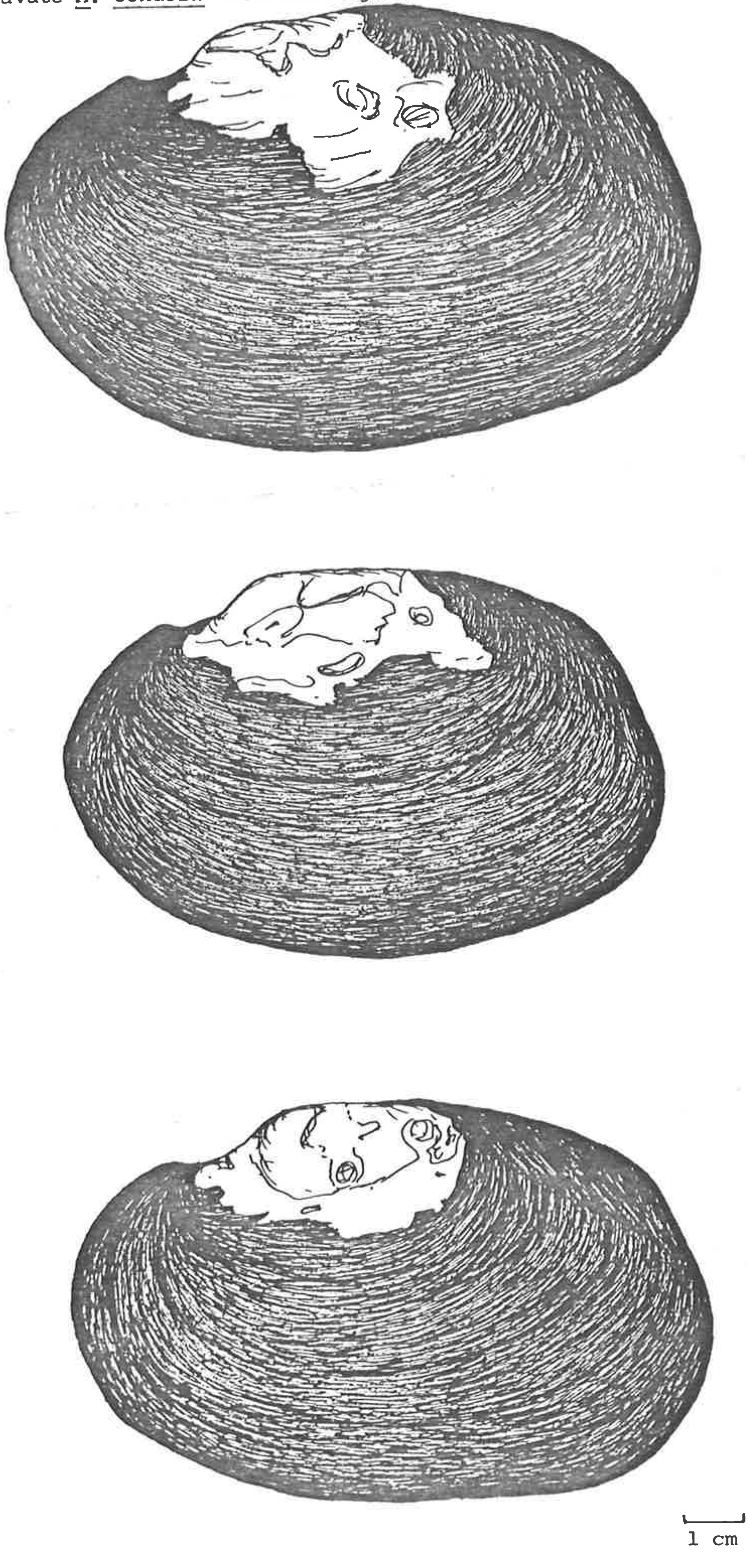
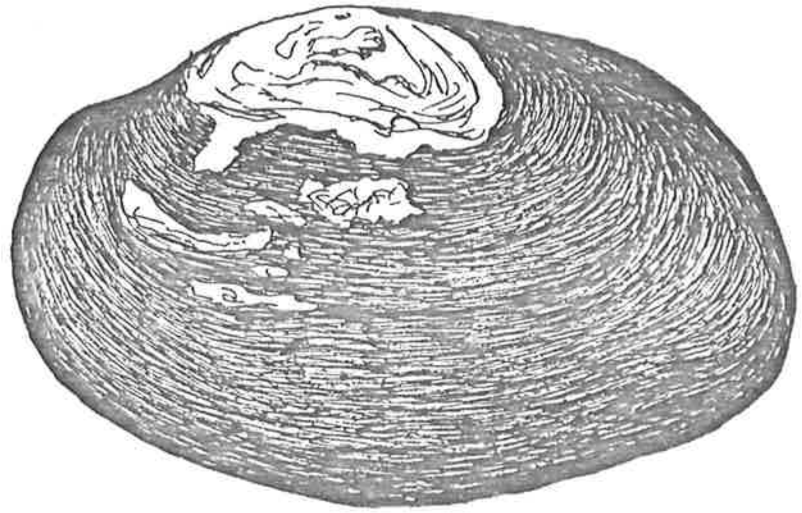
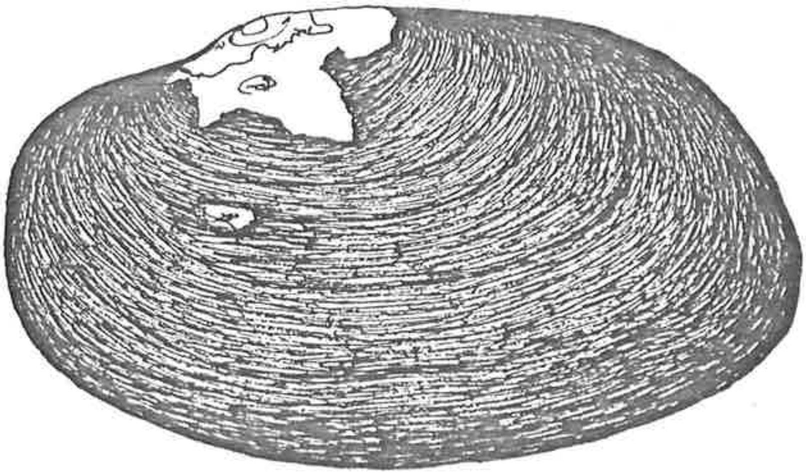


FIGURE 10

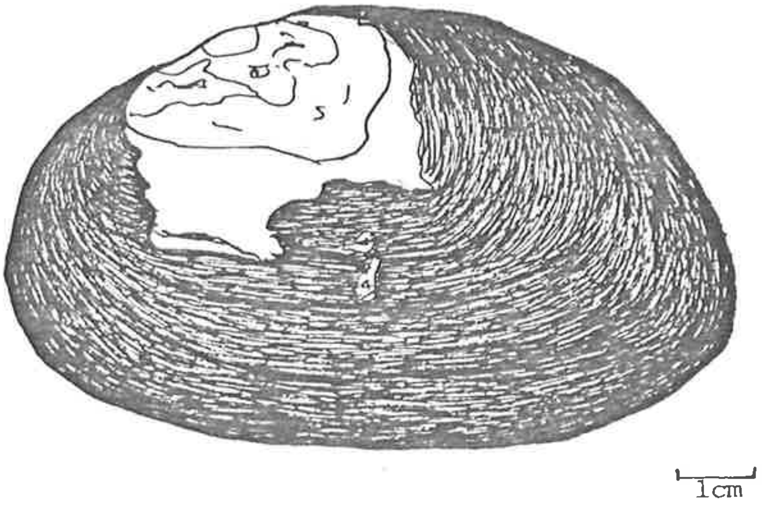
Non-excavate "*A. condola*" from a. Mulwala Canal and b. below Lake Mulwala.

a.





b.



The genus *Alathyria* is easily distinguished from *Velesunio* by the dentition. The pseudocardinal teeth in *Alathyria* are large, rounded and globular, while in *Velesunio* they are narrow, pointed and blade-like.

3.2.3 *Velesunio ambiguus*

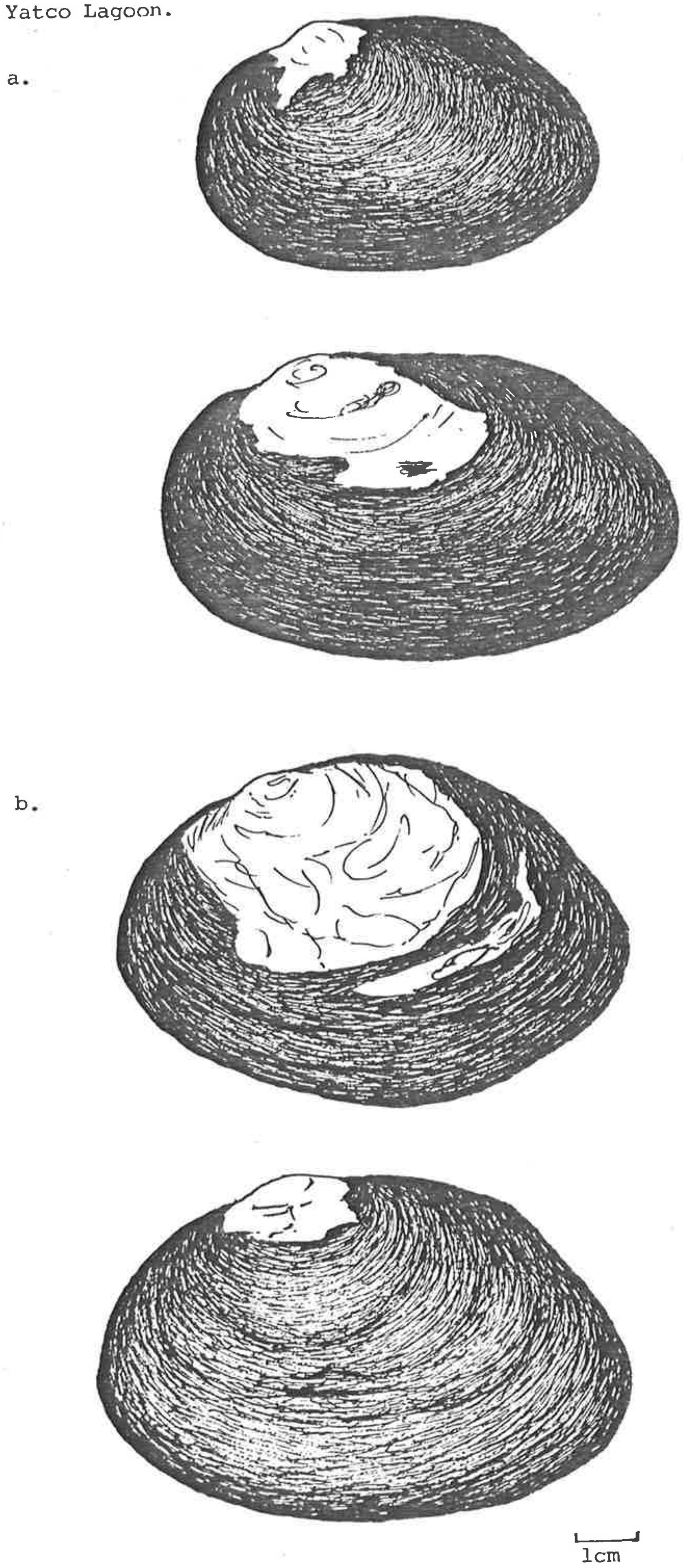
From the specimens collected in the middle and lower sections of the River Murray, it appears that this species also has two growth forms, one rounded and the other elliptical.

a. The *elliptical* growth form has an oval outline where the valves meet. The dorsum is more sloped and the ventral margin considerably more flattened. These mussels have a maximum length of 80mm, average of 76mm, and average height of 45mm. They appear to grow more slowly than the rounded form as indicated by the more closely spaced growth rings. The elliptical form occurs in the river, in slow to moderate flows and may occur with winged *A. jacksoni* in the lower Murray in South Austrailia (see Fig. 11a).

b. The *rounded* growth form has a circular outline where the valves meet. The dorsum is straight and the ventral margin curved. The adults are relatively larger (e.g., maximum length 90mm, $\bar{x}$=82mm, average height of 65mm). This form occurs where the water velocity is normally slow to almost zero, for example in lagoons, wide sheltered bays in the river, billagongs, and creeks. The substrate is often soft ooze or mud, silt or fine sand (see Fig 11b). This growth form encompasses those mussels once described as *Velesunio evansi*, but it is a broader category.

FIGURE 11

a. Elliptical V. ambiguus from Wongulla. b. Rounded V. ambiguus from Yatco Lagoon.



3.2.4 Overview

Qualitative descriptions of shell shape are essentially subjective and therefore prone to certain shortcomings. It is impossible to precisely define morphological variation on the basis of visual assessment because we cannot describe shape in the same terms as we perceive it. This stems from the perception of shape as a complete entity ("Gestalt"). Comparative descriptions can be made only by simplifying the shape to evaluate the individual components of the variation, and this inevitably leads to a loss of potentially important information (through over-simplification and/or fragmentation).

Quantitative description of shape is a more effective means of defining shape variation, primarily because it is more objective. It has potential value as a taxonomic tool since it allows the extent of variation within species to be specifically characterized and consequently, allows unambiguous species identification. Quantification of shape is a primary requirement for experimental analysis of the functional significance of morphological differences within and between species. It enables the extent of expression of a particular morphological character (for example, arching in *A. jacksoni*) to be correlated with other measured anatomical or environmental characters. In addition, comparative experimental results are amenable to statistical analyisis.

3.3 Problems with measuring mussel shape

A general problem in measuring bivalve shape is the selection of absolute "landmarks". Bookstein (1978) divides landmarks into two categories, "anatomical" and "extremal". Anatomical landmarks are defined by some biological reference, and extremal landmarks are inferred from the geometry of the animal. Mussels possess a single anatomical landmark, the umbo, and, in order to obtain an unambiguous orientation for all measurements, a second landmark is required. This is an extremal landmark, arbitrarily defined as the point of intersection of the lines of maximum length and beak height (Fig. 6). Landmarks must be homologous to allow comparisons between individuals.

Once homologous points have been selected and the orientation fixed, it is possible to devise measurements which might play a role in describing shape and allow quantification of systematic shape variation. The selection of simple ratios as measurements for the evaluation of systematic variation is seldom useful. Raup and Stanley (1978) demonstrate the inadequacies of simple height and length ratios in distinguishing between mussels which are obviously different (Fig. 12). The measurements employed must attempt to characterize the component parts of the variation.

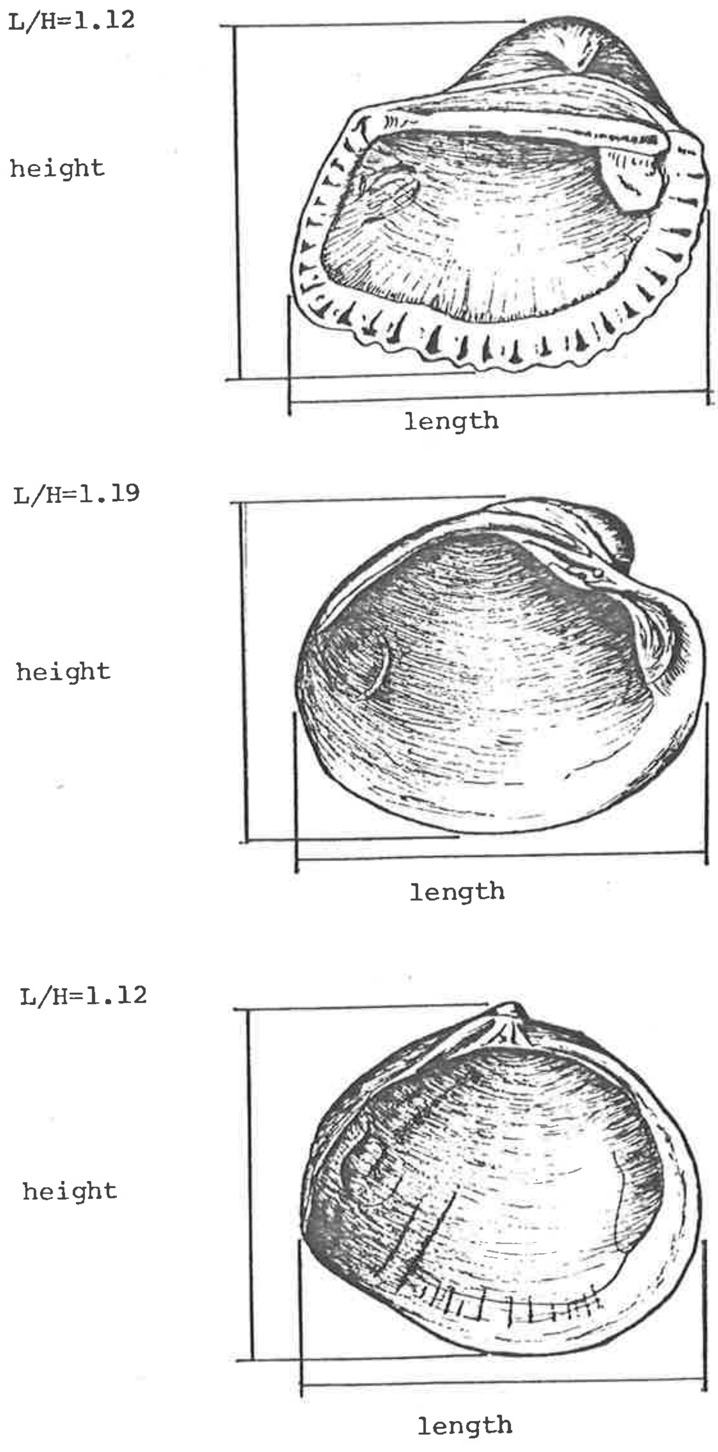
Following data collection, a single individual may be represented by a series of measurements. For the mussels used in this study it was desirable to devise a linear continuum of shape variation from one extreme form to another. Any idividual could then be placed on this continuum and its position compared relative to others.

3.4 Methods of measuring shape

The measurement of shape and shape change has been of concern to many biologists. D'Arcy Thompson was one of the first to consider problems of measuring shape and shape change. In 1917 he developed the

FIGURE 12

The inadequacies of simple height and length ratios.



(From Raup and Stanley 1978)

method of Transformation Grids (Thompson 1942) where the transformation of one shape into another can be expressed using homologous points to map one shape onto another. However, the change in morphological pattern can only be appreciated in terms of a series of diagrams which do not provide the basis for an analytical characterization of the change or for an examination of the factors by which it is determined (Meltzer _et al_. 1967). Avery (1933), Colbert (1935), Olson (1975) and many others explored the possibilities of Transformation Grids and despite the efforts of many biometrists (reviewed in Bookstein 1978), shape change continues to resist quantification.

Ortmann (1920), McMichael and Hiscock (1958), Stanley (1970), Eagar (1977) and others employed simple measurement systems for characterizing bivalve shape. For example, most measured length, width, height, etc., and while statistical comparisons of single parameters could be made between individuals, statistical comparisons of all parameters simultaneously between individuals was not attempted. These measurement systems, with the exception of Eagar's, were general, designed to quantify the variation exhibited by many species. Their intended use is taxonomic and not the elucidation of the functional significance of shape variation.

More advanced techniques for measurement of shape have been available for many years. Geologists interested in the shape of sedimentary particles employed techniques developed in the field of morphometrics (Wadell 1932, Krumbien 1941 and Ehrlich and Weinberg 1970). Resistance to the use of recently developed mathematical techniques was noted by several authors who attempted to make these techniques more accessible to biologists (e.g., Burnaby 1966, Gower 1966, Seal 1966, Meltzer _et al_. 1967, Rohlf 1967, Sprent 1972, Blum 1973, Reyment 1973 and Oxnard 1978). In cases where dimensions cannot be defined on any reasonable biological basis, because the form is inherently featureless, techniques of curve fitting are often useful. The most common is Fourier analysis (e.g., Raup and Stanley 1978). Its utility stems from the fact that any simple curve can be described mathematically as the sum of a series of sine and cosine curves. It may provide a precise mathematical description of shape while making few, if any, biological assumptions.

Benson (1967) employed Fourier series to describe the shape of ostracods using muscle scars as landmarks. Younker (1971) evaluated the utility of Fourier shape analysis for the study of ostracod carapaces. Kaesler and Waters (1972) used Fourier analysis to describe ostracod margins. Several related species described in this way can be compared statistically via either the coefficients of several harmonic terms (Raup and Stanley 1978) or graphically. Prior to computers, such comparisons would have seemed far too large and numerically complex to warrant serious consideration.

3.5 Comparison of measurement systems

Consideration of the purpose for which a measurement system was designed is of the utmost importance when evaluating its effectiveness. General measurement systems designed for taxonomic purposes, usually separation of species, should be robust and detect gross variation. However, to detect the more subtle variations of shape within species, these general systems are less useful. This subtle variation requires more specific measurement systems. Such a specific measurement system would be of little value as a taxonomic tool, but extremely useful in an experimental analysis of adaptive shape variation. Thus measurement systems should fulfil one of the following:

a. distinguish between species,

b. distinguish between proposed growth forms within one or more species.

What follows is a comparison of five measurement systems in their ability to fulfil these general versus specific aims. The conventional measurement systems of McMichael and Hiscock (1958) and Stanley (1970) were selected as taxonomically-oriented general measurement systems. Eagar's (1977) system devised to measure arching *Margaritifera margaritifera* was evaluated and compared with a new system designed to quantify the degree of arch and wing development in *Alathyria jacksoni*. Fourier analysis, previously employed by Benson (1967) and Kaesler and Waters (1972), was applied by Walker (1983, pers. comm.) to measurement of shape in the three River Murray mussels, and was evaluated in its ability to discriminate between speices and growth forms.

For the five systems, the same mussels were used. These were samples from various parts of the Murray and Murrumbidgee Rivers (Table 5), where, for any one location, the majority of mussels assumed the shape of a particular growth form.

TABLE 5

No.	Species	Location	Growth form
20	*Alathyria jacksoni*	Lock 3	Winged
20	*Alathyria jacksoni*	Wemen	Arched
20	*Velesunio ambiguus*	Point Sturt	Rounded
20	*Velesunio ambiguus*	Wongulla	Elliptical
20	*Alathyria condola*	Darlington Point	Excavate
20	*Alathyria condola*	Below Lake Mulwala	Non-excavate

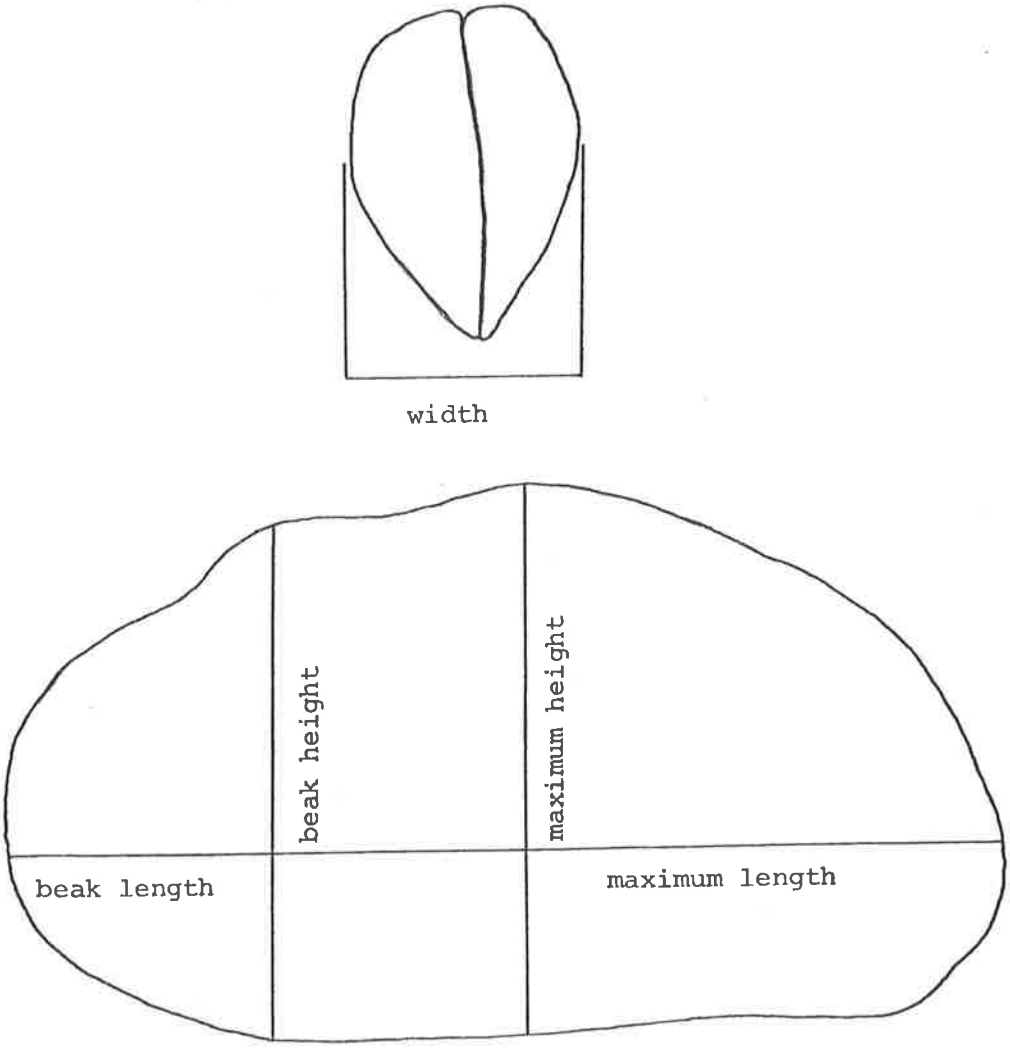
3.5.1 The measurement systems

a. The McMichael and Hiscock measurement system.

This is a general measurement system devised for taxonomic purposes to discriminate between species using ratios. The measurements taken for this system are: maximum height, beak height, total length and width. From these measurements the following indices are calculated: beak length index (beak length/total length expressed as a percentage), maximum height index (maximum height/maximum length as a percentage) and beak height index (beak height/maximum height as a percentage). In the analysis all the measurements and the indices were used. The measurements are illustrated in Fig. 13).

FIGURE 13

The McMichael and Hiscock measurement system.



b. Stanley's measurement system

This is also a general measurement system not designed to detect any particular morphological character. Its primary function is taxonomic and it was employed in a treatise on the relation of shell form to life habits. The main difference between this and McMichael and Hiscock's (1958) system is the measurement of anterior and posterior length along the line of maximum length (see Fig. 14). The measurements used in this system are: maximum length, maximum height, anterior length, posterior length and width.

c. Eagar's measurement system

This is a specific measurement system devised to measure arching in the Northern Hemisphere mussel *Margaritifera margaritifera*. This mussel differs from *Alathyria jacksoni* in some characteristics. The measurements used in Eagar's system (Fig. 15) were the distance from the umbo to the termination of the ligament (UB), the distance from this line to the point where the shell joins the ligament (pq) and the distance from the line UB to the outer edge of the ligament (lm). The ratios lm/UB and pq/UB were calculated from these measurements. In the analysis all measurements and ratios were included.

d. The arch-wing measurement system

This system was developed to quantify the degree of arching and wing development in *Alathyria jacksoni*. Unlike Eagar's system, there is no need to kill the animal to obtain the measurements.

While arching was an important variable in *A. jacksoni*, it

FIGURE 14

Stanley's measurement system.

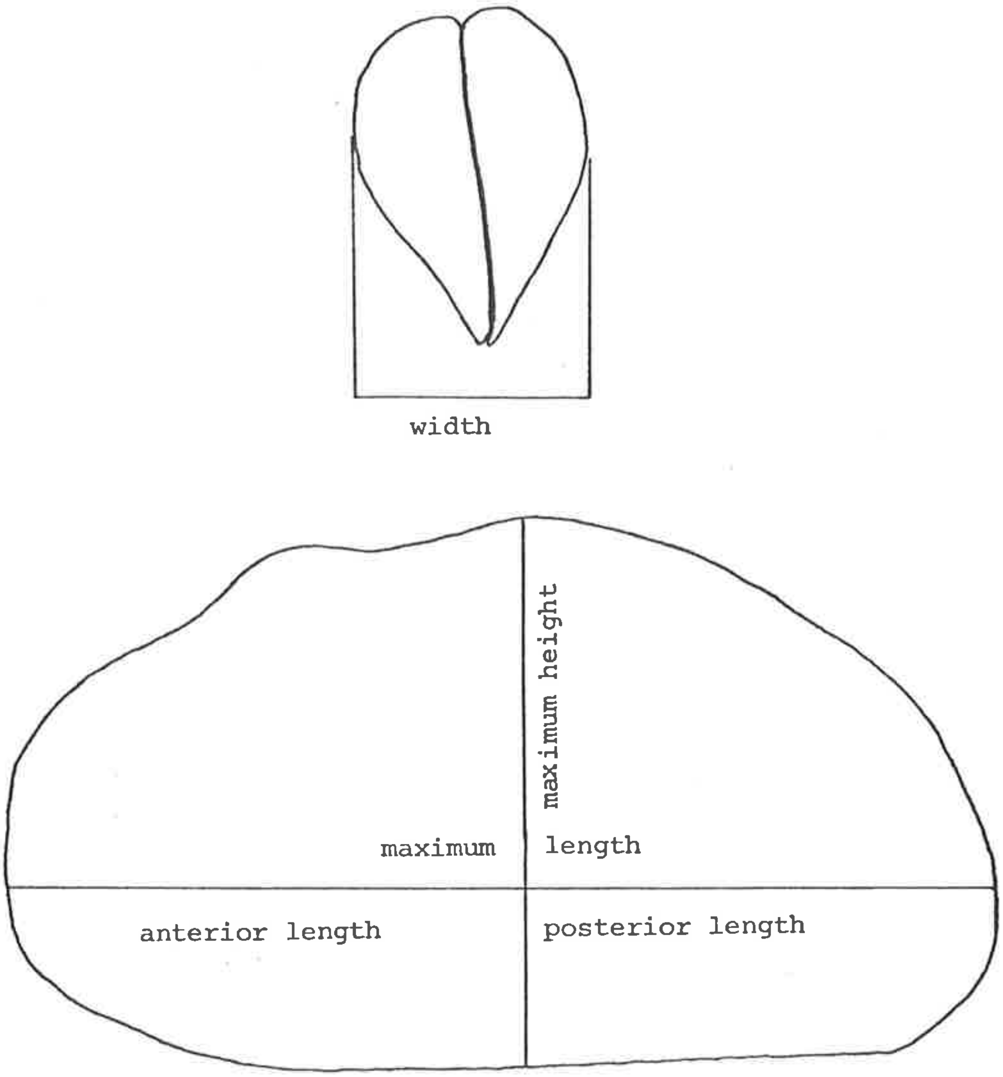
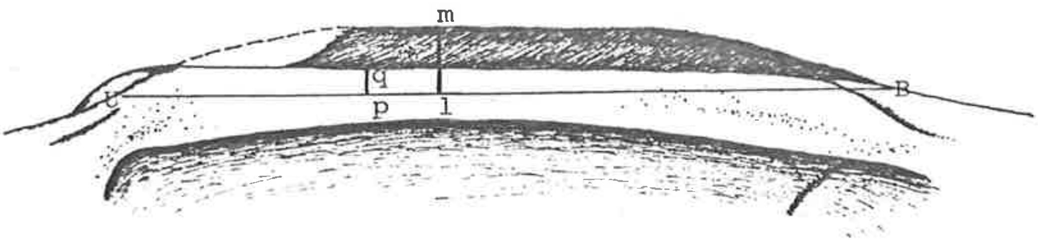


FIGURE 15

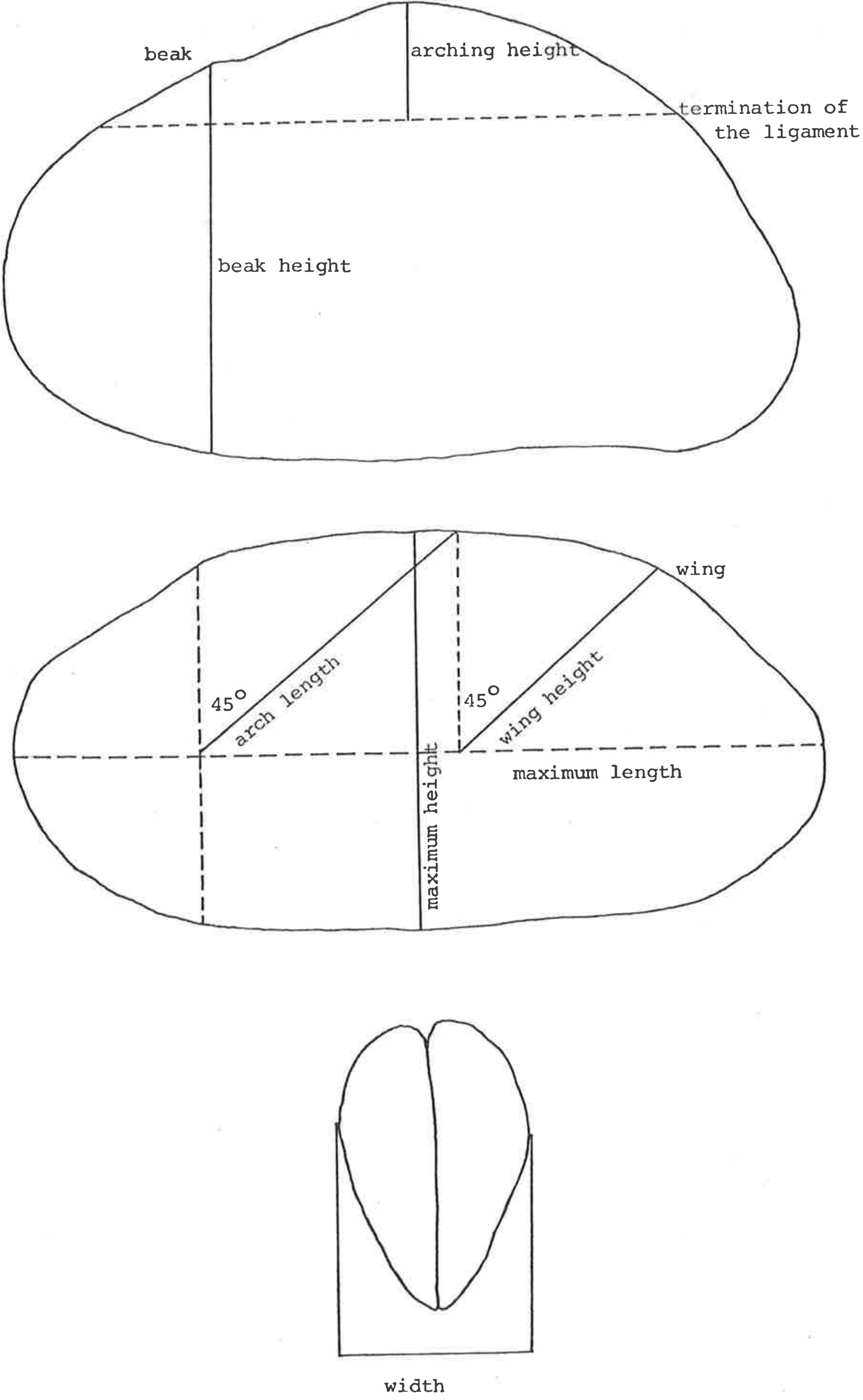
Eagar's measurement system.



UB is the distance between the Umbo (U) and the termination of the ligament B. ml is the distance from the line UB to the outer edge of the ligament. qp is the distance from the line UB to the inner edge of the ligament. (From Eagar 1977a)

FIGURE 16

The arch-wing measurement system.



only represented a portion of the full spectrum of variation. The development of the dorsal wing formed the remaining part of the spectrum and should not be ignored. A measurement system which measured both arching and wing development was needed, preferably one applicable to live animals and suitable for use in the field. The system used the following measurements: beak height, arching height, arch length, wing height, maximum height and maximum width (Fig. 16).

e. The Fourier measurement system

This measurement system is based on the technique of Fourier analysis used by Benson (1967)("Theta Rho" analysis) and Kaesler and Waters (1972) which describes two dimensional shell shape at the plane of intersection of the valves of a mussel. The left valve was placed on a grid (Fig. 17) with the "centre" defined as the homologous point of intersection of the lines of beak height and maximum length (an extremal landmark). Radii from this point were measured at ten degree intervals around the shell. Because the two dimensional outline of a single mussel valve can be described as a periodic function, it can be analyzed in terms of its harmonic components using the technique of Fourier analysis. The radius is a periodic function of angle, and the basic equation from Fourier series is:

$$r(\theta) = \bar{r} + a_1\cos\theta + a_2\cos 2\theta + \ldots\ldots\ldots\ldots + a_n\cos n\theta$$
$$+ b_1\sin\theta + b_2\sin 2\theta + \ldots\ldots\ldots\ldots + b_n\sin n\theta$$

where θ = the angle between consecutive radii.

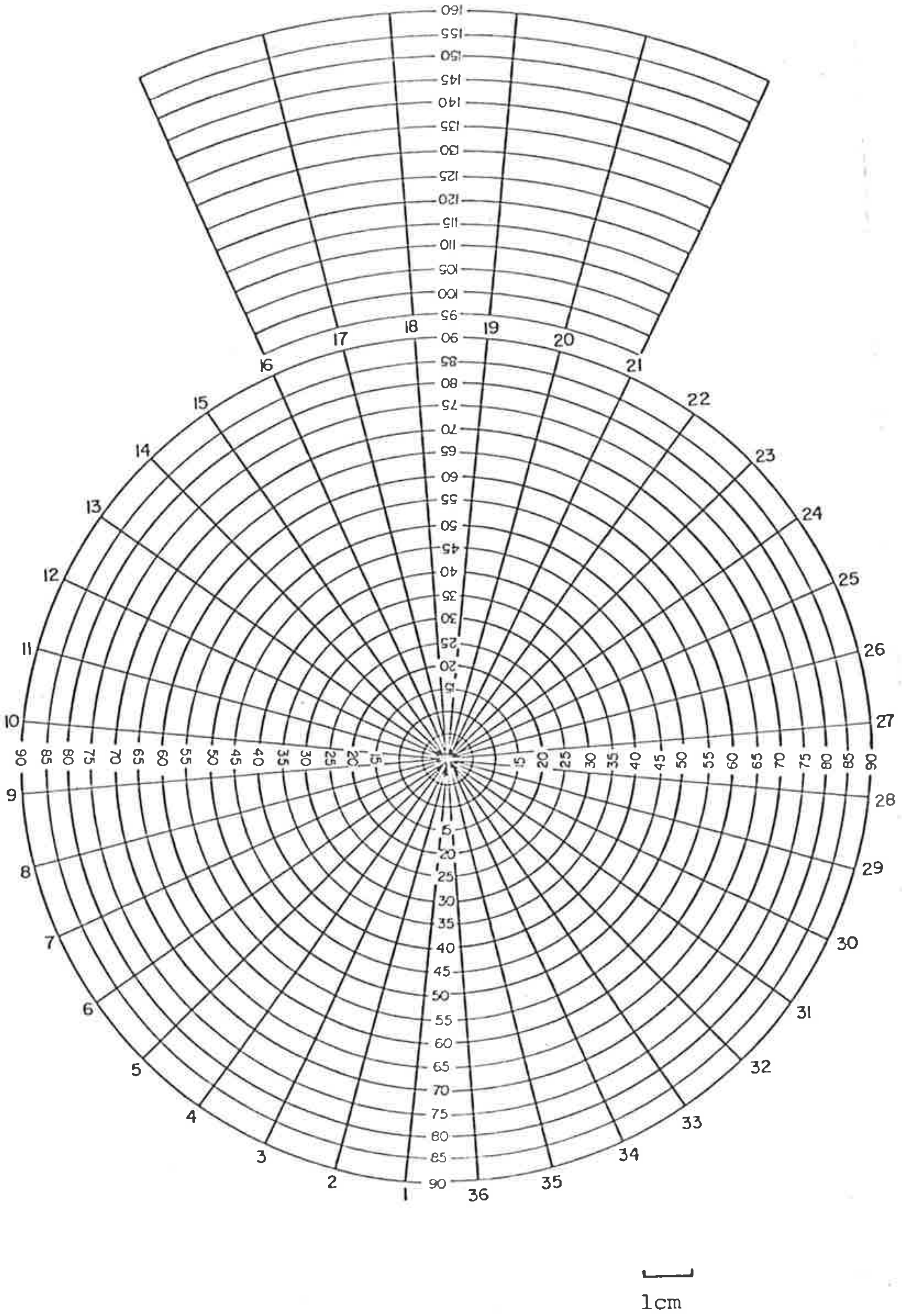
This can be summarized from 1 to ∞

$$r(\theta) = \bar{r} + \sum_{r=1}^{\infty} (a_i\cos\ i\theta + b_i\ \sin\ i\theta)$$

where $a_i = c_i\cos \phi i$

FIGURE 17

The grid used to measure successive radii on mussels for Fourier Analysis (developed by K.F. Walker).



$$b_i = c_i \sin \phi i$$

$$c_i = \sqrt{a_i + b_i}$$

and ϕ is the phase angle associated with the i^{th} harmonic. The summarized form of the equation can be simplified since

$$a_i \cos i\phi + b_i \sin i\phi = a_i \sin (i\theta + \phi i)$$

and the equation becomes

$$r(\theta) = \bar{r} + \sum_{r=1}^{\infty} a_i \sin (i\theta + \phi i)$$

where $i\theta$ = the angle from the reference line (0,10,20....350) (Tolstov 1972).

The Fourier coefficients for each mussel were determined using programs written in Microsoft BASIC and BBC BASIC (K.F. Walker pers. comm.) developed for a micro computer. The parameters for a single mussel are shown in Table 6 and the summed effect of sequential harmonics is illustrated in Figure 18, where:

n = harmonic number

C = harmonic coeffecient (amplitude)

ϕ = phase angle

C_{rel} = ratio of each harmonic coefficient to the 1^{st}

% = percent variance explained by the n^{th} harmonic.

Only the C_{rel} data were incorporated in the analysis. Analysis was conducted using

(a) only the two dimensional harmonic data

(b) the harmonic data and maximum shell width (adding a third dimension).

3.5.2 Comparative analysis

Each measurement system produces a number of measurements which represent a single mussel. Consequently, multivariate statistical analysis is required for comparisons. Multivariate statistics has the advantage of utilizing information in the data contained within patterns

TABLE 6

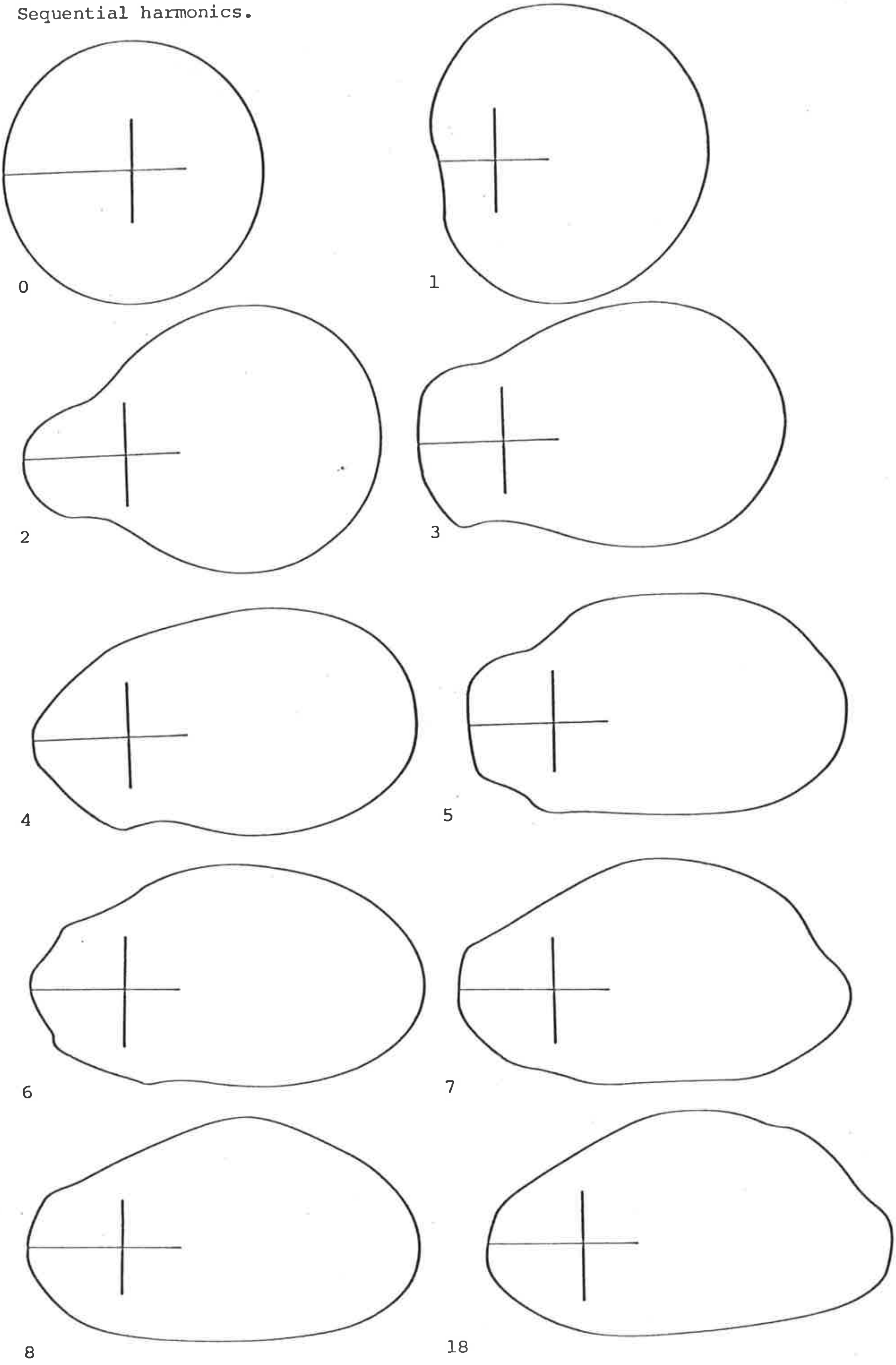
An example of the data collected for a single mussel for Fourier Analysis.

Deg	Rad	Deg	Rad	n	C	C_{rel}	%Var	Ø
10	23.8	190	81.0	0	39.94	1.00	66.19	0.00
20	23.7	200	78.1	1	20.05	0.57	21.78	172.97
30	23.8	210	63.5	2	12.93	0.37	9.06	352 85
40	23.9	220	48.5	3	5.98	0.17	1.94	170.63
50	23.6	230	39.2	4	3.48	0.10	0.66	179.79
60	23.5	240	33.7	5	1.98	0.06	0.21	202.05
70	23.4	250	28.6	6	0.91	0.03	0.05	28.26
80	23.7	260	26.2	7	0.93	0.03	0.05	222.90
90	24.1	270	24.8	8	0.67	0.02	0.02	60.34
100	27.0	280	23.5	9	0.44	0.01	0.01	270.00
110	28.0	290	22.8	10	0.50	0.01	0.01	105.02
120	31.2	300	21.5	11	0.35	0.01	0.01	271.18
130	36.8	310	21.0	12	0.20	0.01	0.00	46.10
140	42.3	320	21.2	13	0.26	0.01	0.00	165.89
150	49.0	330	21.5	14	0.09	0.00	0.00	303.00
160	58.9	340	23.0	15	0.11	0.00	0.00	308.02
170	66.5	350	23.5	16	0.17	0.00	0.00	128.24
180	73.8	360	23.8	17	0.08	0.00	0.00	100.56
cont.	cont.			18	0.11	0.00	0.00	180.01

Total variance is 1844.89

FIGURE 18

Sequential harmonics.



of variances and co-variances (Oxnard 1978). The multivariate method used here was Discriminant Analysis, a subprogram of the Statistical Package for the Social Sciences (SPSS) version 8.3 (Nie et al. 1979). The formal background on Discriminant analysis is described in Blackith and Reyment (1971). Discriminant analysis produces a statistical distinction between two or more groups of cases using a collection of discriminating variables that measure characteristics that are expected to differ between groups. The mathematical objective of discriminant analysis is to linearly combine the discriminating variables so that the groups are as distinct as possible. Discriminant analysis attempts to do this by forming one or more linear combinations (discriminant functions) of the discriminating variables. These discriminant functions are of the form:

$Di = di_1 Z_1 + \ldots\ldots\ldots + di_p Z_p$

where Di is the score on the discriminant function i, d is a weighting coefficient and Z are the standardized (z scores) values of the discriminating variables (p).

Discriminant coefficients are derived by solving the general eigen vector problem

$WA = Ba$

where B and W are between and within group sums of squares and crossproduct matrices. The discriminant function defined by these coefficients is also called a canonical variate, and once derived may be used to analyze and classify the data.

The procedure for classification involves the use of a separate discriminant function for each group. These produce a probability of membership in the respective group and the individual is assigned to the group with the highest probability.

Where growth forms within a species are compared, only two groups are being used in the analysis, and hence, only one discriminant function results. The discriminant score for each individual is computed by multiplying each discriminating variable by its corresponding coefficient and adding together these products. Thus, for each mussel a single discriminant function produces a single discriminant score which can be plotted along with all of the other mussels used in that same analysis. Such a graph visually represents the nature of the distribution of shape variation for a single species.

For each measurement system analysis was conducted to determine the percent of mussels correctly classified as species, growth forms generally, and as growth forms within a particular species.

Results

The computer selects those variables which contribute to the discrimination in the order of their ability to separate the groups. When no additional separation is achieved by adding more variables the remaining variables are not included in the analysis. Results are shown in the following tables and graphs obtained from the computer outputs.

TABLE 7

McMichael and Hiscock's measurement system: Discrimination between species.

Actual group	No. of cases	Predicted group membership		
		A. jacksoni	*V. ambiguus*	*A. condola*
A. jacksoni	40	36 90.0%	3 7.5%	1 2.5%
V. ambiguus	40	0 0%	39 97.5%	1 2.5%
A. condola	40	2 5.0%	4 10.0%	34 85.0%

Percent of grouped cases correctly classified: 90.83%.

TABLE 8

McMichael and Hiscock's measurement system: Discrimination between all growth forms.

Actual group	No. of cases	Predicted group membership. 1	2	3	4	5	6
Group 1	20	17 85.0%	2 10.0%	0 0%	0 0%	0 0%	1 5.0%
Group 2	20	2 10.0%	15 75.0%	0 0%	3 15.0%	0 0%	0 0%
Group 3	20	0 0%	0 0%	14 70.0%	6 30.0%	0 0%	0 0%
Group 4	20	0 0%	0 0%	4 20.0%	15 75.0%	0 0%	1 5.0%
Group 5	20	0 0%	0 0%	0 0%	1 5.0%	18 90.0%	1 5.0%
Group 6	20	1 5.0%	0 0%	0 0%	4 20.0%	1 5.0%	14 70.0%

Percent of cases correctly classified: 77.50%.

1= winged *A. jacksoni*, 2= arched *A. jacksoni*, 3= rounded *V. ambiguus*, 4= elliptical *V. ambiguus*, 5= excavate *A. condola*, 6= non-excavate *A. condola*.

TABLE 9

McMichael and Hiscock's measurement system: Discrimination between arched and winged A. jacksoni.

Actual group	No. of cases	Predicted group membership.	
		winged	arched
winged	20	18 90.0%	2 10.0%
arched	20	2 10.0%	18 90.0%

Percent of grouped cases correctly classified: 90.00%

GRAPH 1

All-groups stacked histogram:

- Canonical discriminant function 1 -

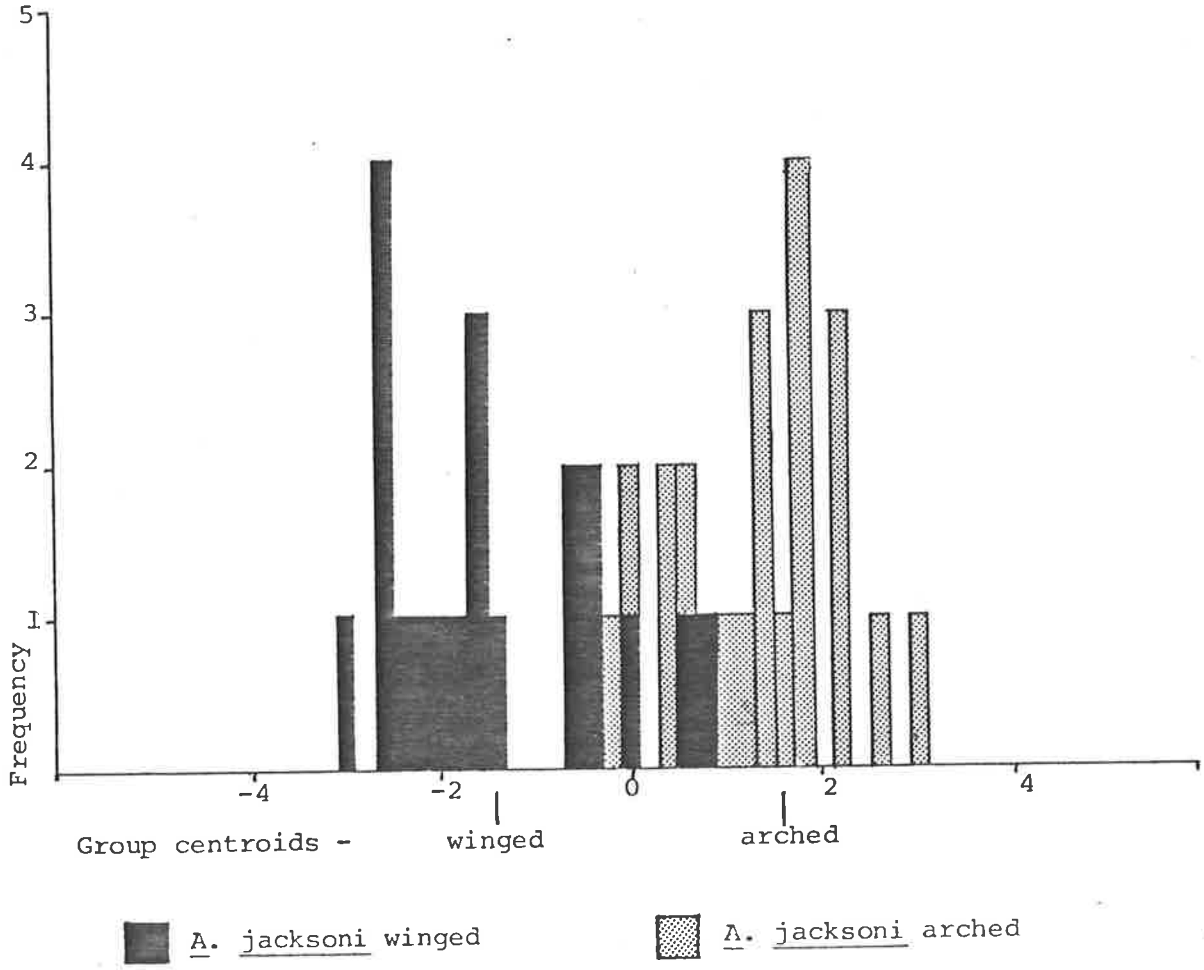


TABLE 10

McMichael and Hiscock's measurement system: Discrimination between growth forms of V. ambiguus.

Actual group	No. of cases	Predicted group membership Rounded	 Elliptical
Rounded	20	18 90.0%	2 10.0%
Elliptical	20	1 5.0%	19 95.0%

Percent of grouped cases correctly classified: 92.50%.

GRAPH 2

All-groups stacked histogram:

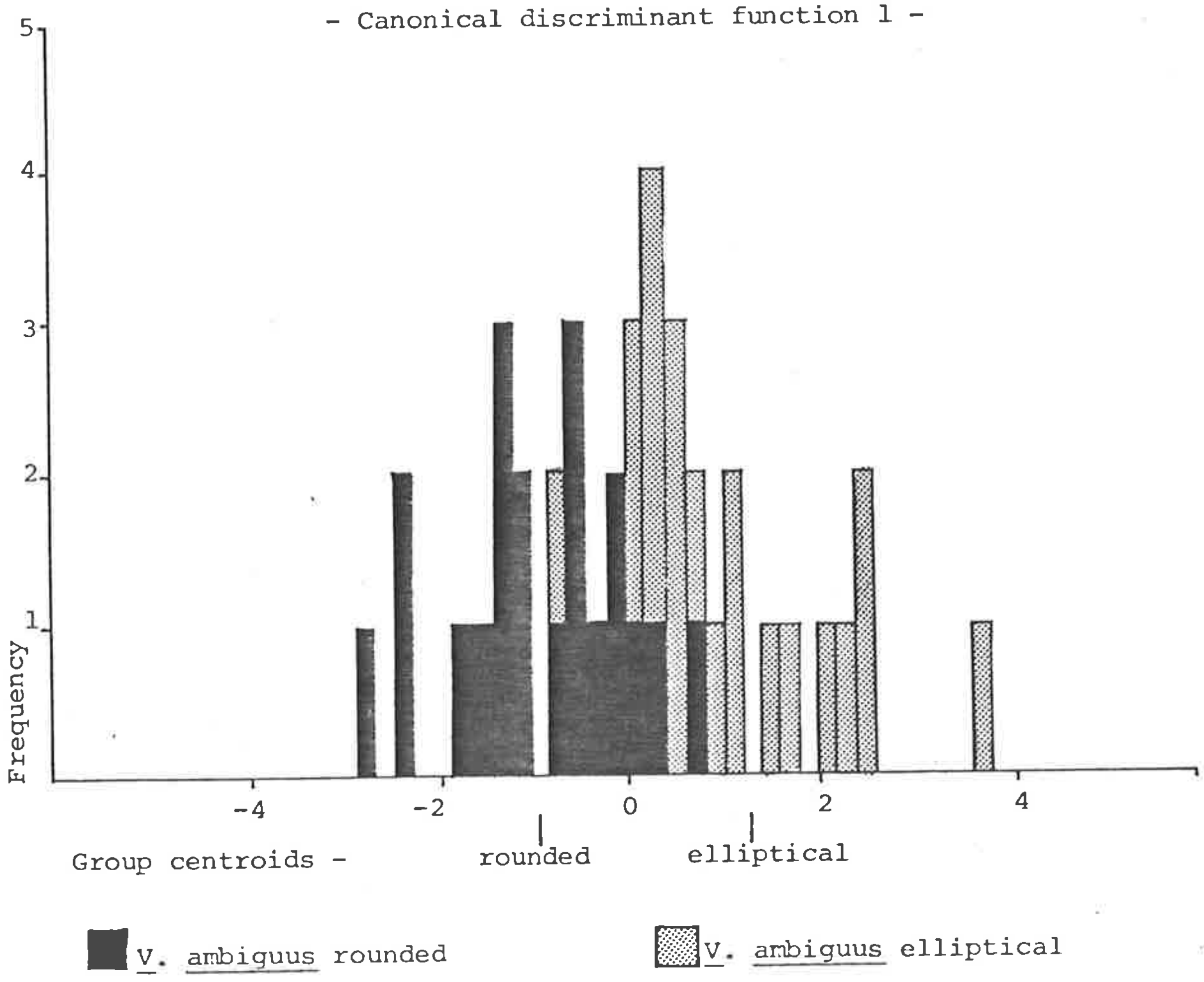


TABLE 11

McMichael and Hiscock's measurement system: Discrimination between growth forms of *A. condola*

Actual group	No. of cases	Predicted group memebership.	
		Excavate	Non-excavate
Excavate	20	20 100.0%	0 0%
Non-excavate	20	1 5.0%	19 95.0%

Percent of grouped cases correctly classified: 97.50%.

GRAPH 3

All-groups stacked histogram:

- Canonical discriminant function 1 -

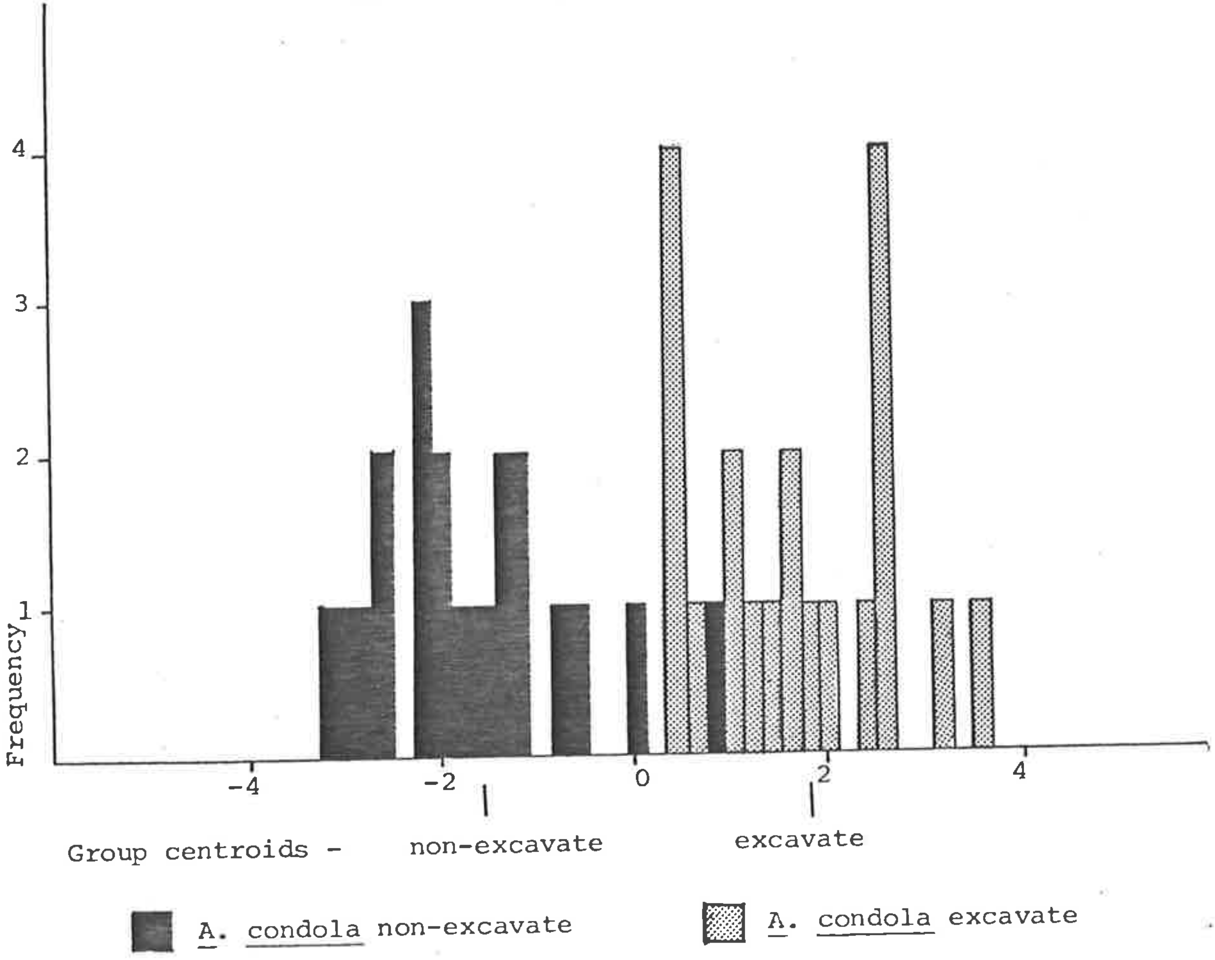


TABLE 12

Stanley's measurement system: Discrimination between species.

Actual group	No. of cases	Predicted group membership		
		A. jacksoni	*V. ambiguus*	*A. condola*
A. jacksoni	40	34 85.0%	3 7.5%	3 7.5%
V. ambiguus	40	0 0%	37 92.5%	3 7.5%
A. condola	40	2 5.0%	4 10.0%	34 85.0%

Percent of grouped cases correctly classified: 87.50%.

TABLE 13

Stanley's measurement system: Discrimination between all growth forms.

Actual group		No. of cases	Predicted group membership 1	2	3	4	5	6
Group	1	20	18 90.0%	1 5.0%	0 0%	0 0%	1 5.0%	0 0%
Group	2	20	2 10.0%	14 70.0%	0 0%	3 15.0%	1 5.0%	0 0%
Group	3	20	0 0%	0 0%	15 75.0%	5 25.0%	0 0%	0 0%
Group	4	20	0 0%	0 0%	8 40.0%	9 45.0%	1 5.0%	2 10.0%
Group	5	20	0 0%	0 0%	0 0%	0 0%	19 95.0%	1 5.0%
Group	6	20	1 5.0%	0 0%	0 0%	4 20.0%	2 10.0%	13 65.0%

Percent of grouped cases correctly classified: 73.33%

1= winged *A. jacksoni*, 2= arched *A. jacksoni*, 3= rounded *V. ambiguus*, 4= elliptical *V. ambiguus*, 5= excavate *A. condola*, 6= non-excavate *A. condola*.

TABLE 14

Stanley's measurement system: Discrimination between arched and winged *A. jacksoni*.

Actual group	No. of cases	Predicted group membership winged	 arched
winged	20	18 90.0%	2 10.0%
arched	20	6 30.0%	14 70.0%

Percent of grouped cases correctly classified: 80.00%

GRAPH 4

All-groups stacked histogram:

- Canonical discriminant function 1 -

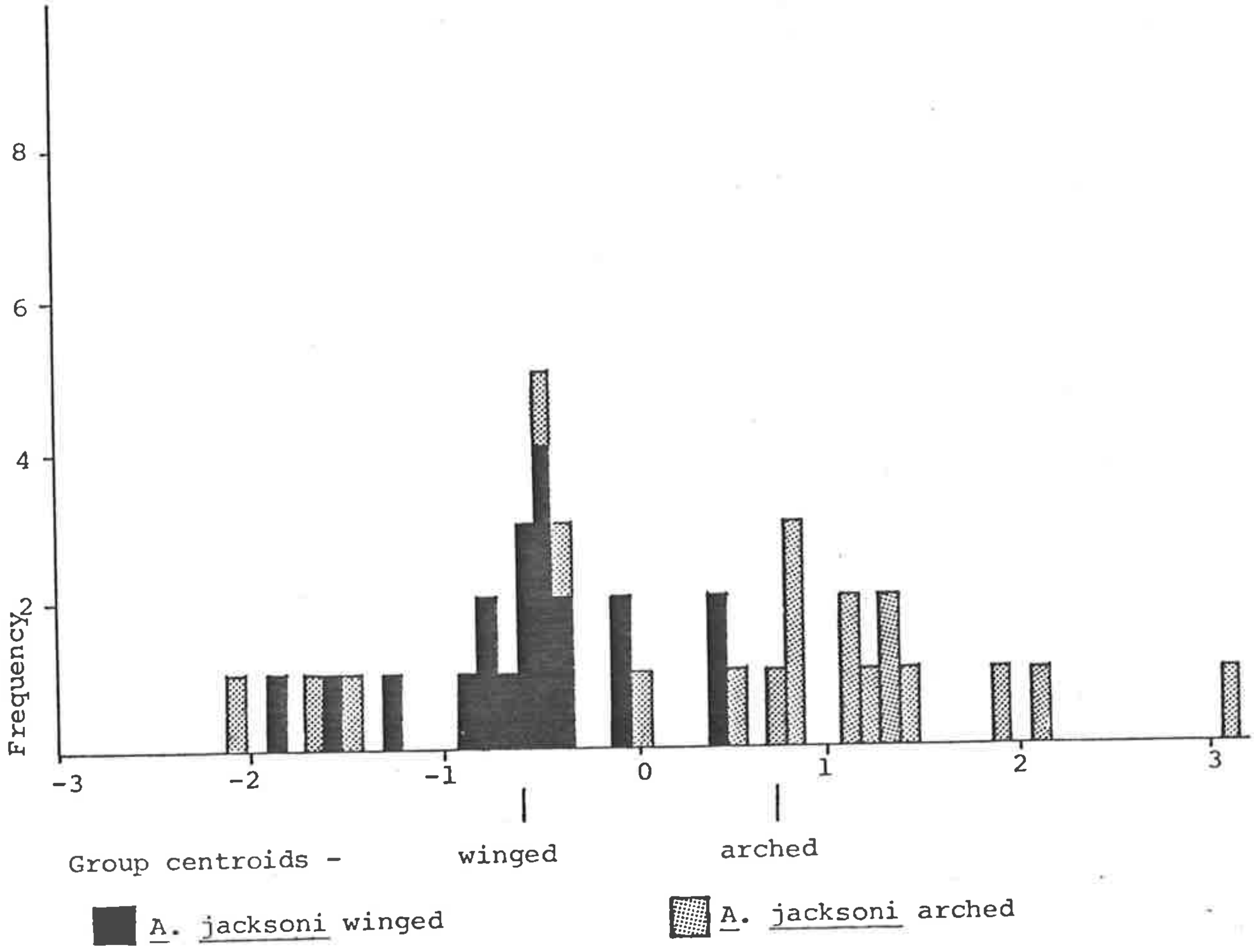


TABLE 15

Stanley's measurement system: Discrimination between rounded and elliptical _V. ambiguus_.

Actual group	No. of cases	Predicted group membership	
		rounded	elliptical
Rounded	20	16 80.0%	4 20.0%
Elliptical	20	3 15.0%	17 85.0%

Percent of grouped cases correctly classified: 82.50%.

GRAPH 5

All-groups stacked histogram:

- Canonical discriminant function 1 -

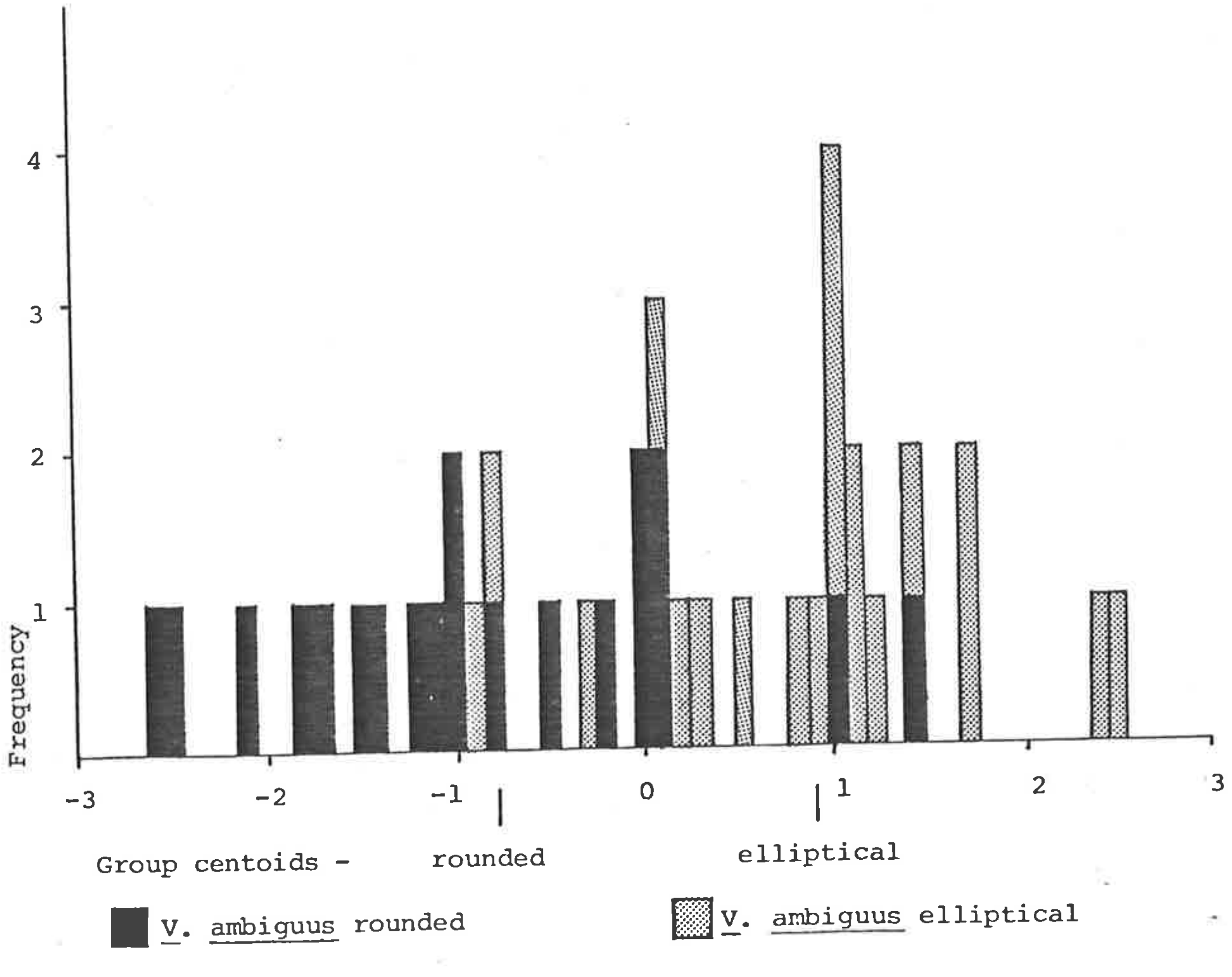


TABLE 16

Stanley's measurement system: Discrimination between excavate and non-excavate A. condola.

Actual group	No. of cases	Predicted group membership	
		Excavate	Non-excavate
Excavate	20	19 95.0%	1 5.0%
Non-excavate	20	1 5.0%	19 95.0%

Percent of grouped cases correctly classified: 95.00%.

GRAPH 6

All-groups stacked histogram:
- Canonical discriminant function 1 -

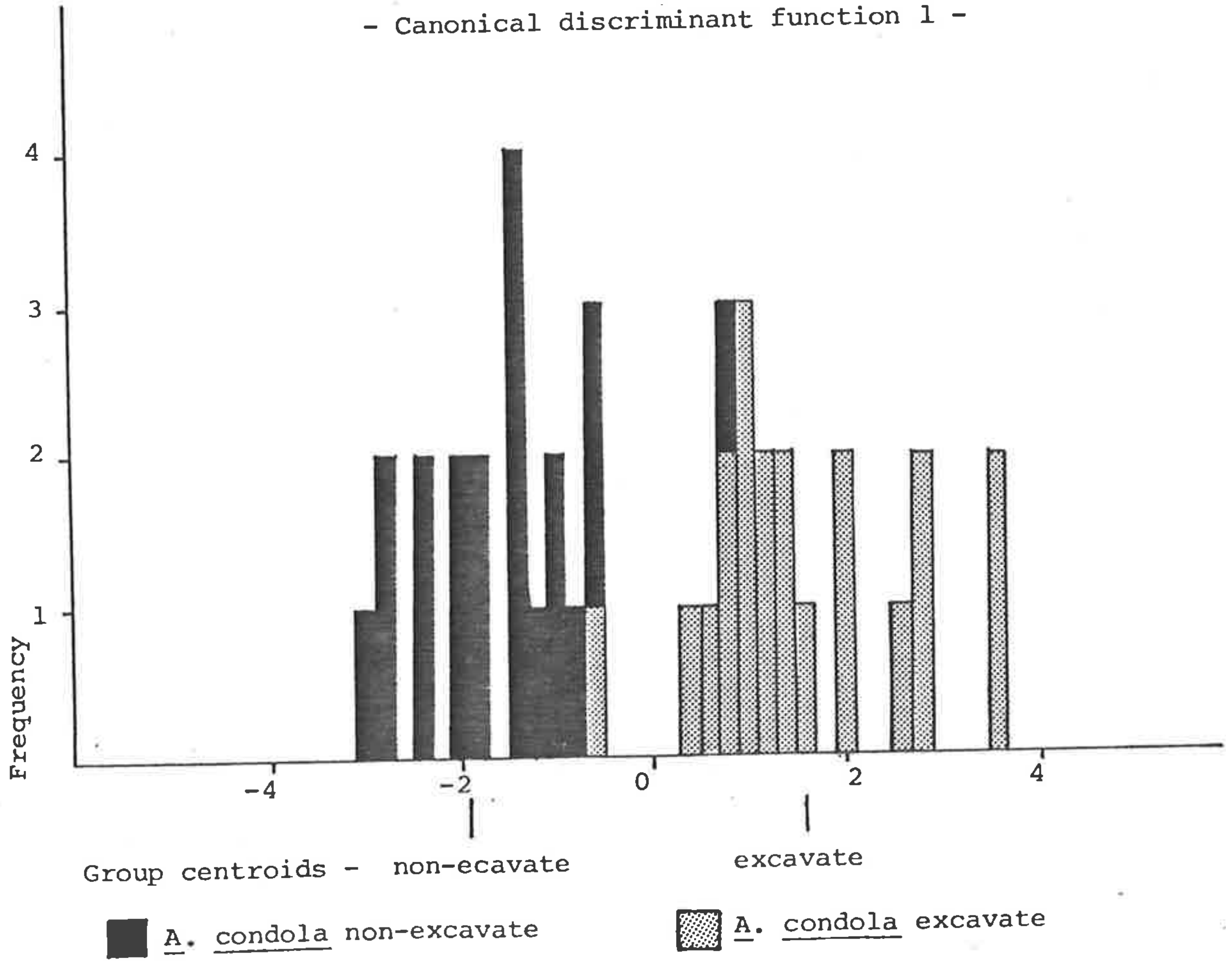


TABLE 17

Eagar's measurement system: Discrimination between species.

Actual group	No. of cases	Predicted group membership *A. jacksoni*	 *V. ambiguus*	 *A. condola*
A. jacksoni	40	31 77.5%	3 7.5%	6 15.0%
V. ambiguus	40	0 0%	38 95.0%	2 5.0%
A. condola	40	7 17.9%	1 2.6%	32 79.5%

Percent of grouped cases correctly classified: 84.03%.

TABLE 18

Eagar's measurement system: Discrimination between all growth forms.

Actual group	No. of cases	Predicted group membership					
		1	2	3	4	5	6
Group 1	20	18 90.0%	0 0%	0 0%	0 0%	1 5.0%	1 5.0%
Group 2	20	2 10.0%	13 65.0%	2 10.0%	0 0%	2 10.0%	1 5.0%
Group 3	20	0 0%	0 0%	17 85.0%	3 15.0%	0 0%	0 0%
Group 4	20	0 0%	0 0%	7 35.0%	11 55.0%	0 0%	2 10.0%
Group 5	20	3 15.0%	2 10.0%	0 0%	0 0%	11 55.0%	4 20.0%
Group 6	20	1 5.3%	1 5.3%	1 5.3%	0 0%	3 15.8%	13 68.4%

Percent of grouped cases correctly classified: 69.75%.

1= winged *A. jacksoni*, 2= arched *A. jacksoni*, 3= rounded *V. ambiguus*, 4= elliptical *V. ambiguus*, 5= excavate *A. condola*, 6= non-excavate *A. condola*.

TABLE 19

Eagar's measurement system: Discrimination between arched and winged *A. jacksoni*.

Actual group	No. of cases	Predicted group membership winged	 arched
winged	20	16 80.0%	4 20.0%
arched	20	2 10.0%	18 90.0%

Percent of grouped cases correctly classified: 85.00%.

GRAPH 7

All-groups stacked histogram:

- Canonical discriminant function 1 -

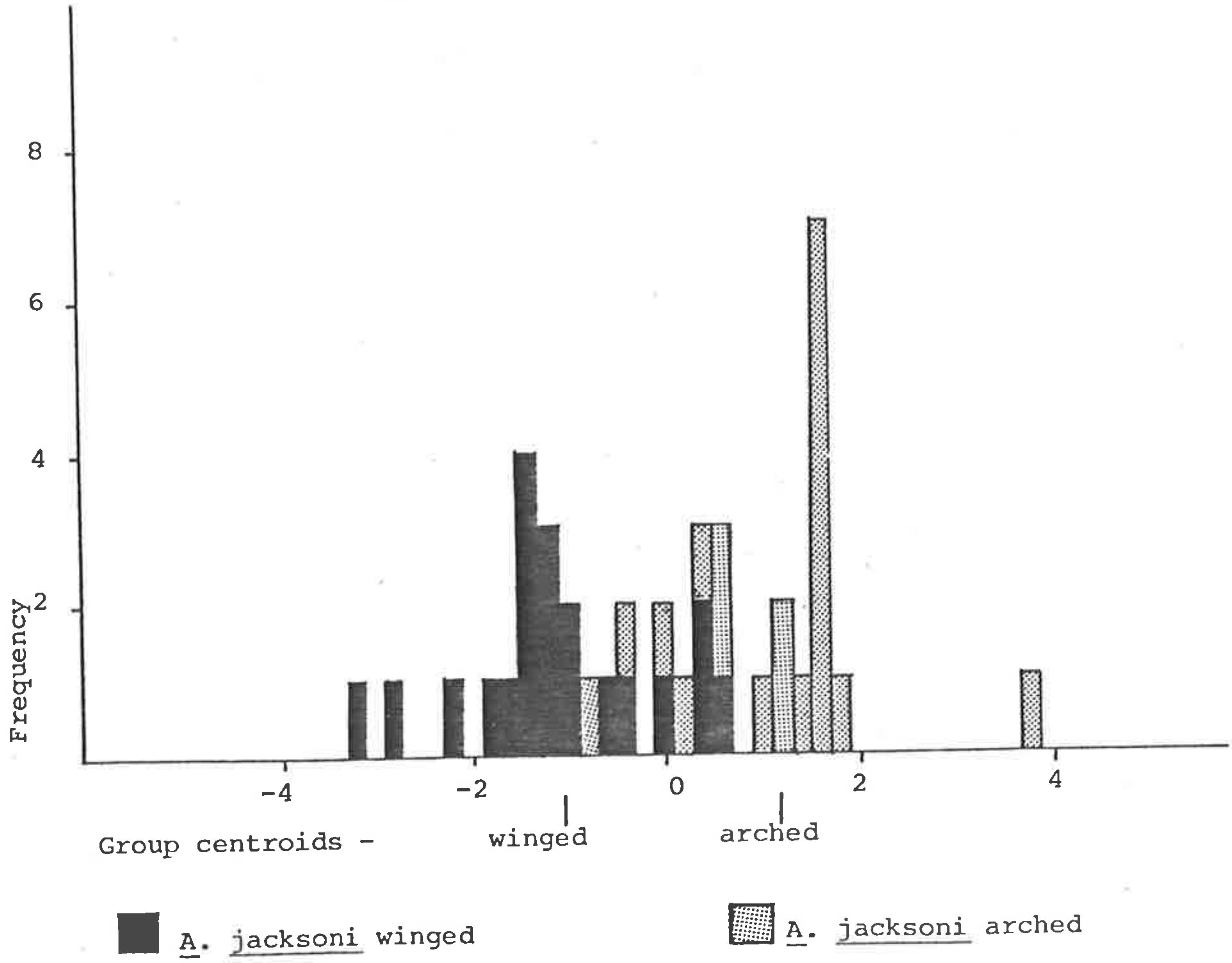


TABLE 20

Eagar's measurement system: Discrimination between rounded and elliptical *V. ambiguus*.

Actual group	No. of cases	Predicted group membership rounded	 elliptical
rounded	20	17 85.0%	3 15.0%
elliptical	20	5 25.0%	15 75.0%

Percent of grouped cases correctly classified: 80.00%.

GRAPH 8

All-groups stacked histogram:

- Canonical discriminant function 1 -

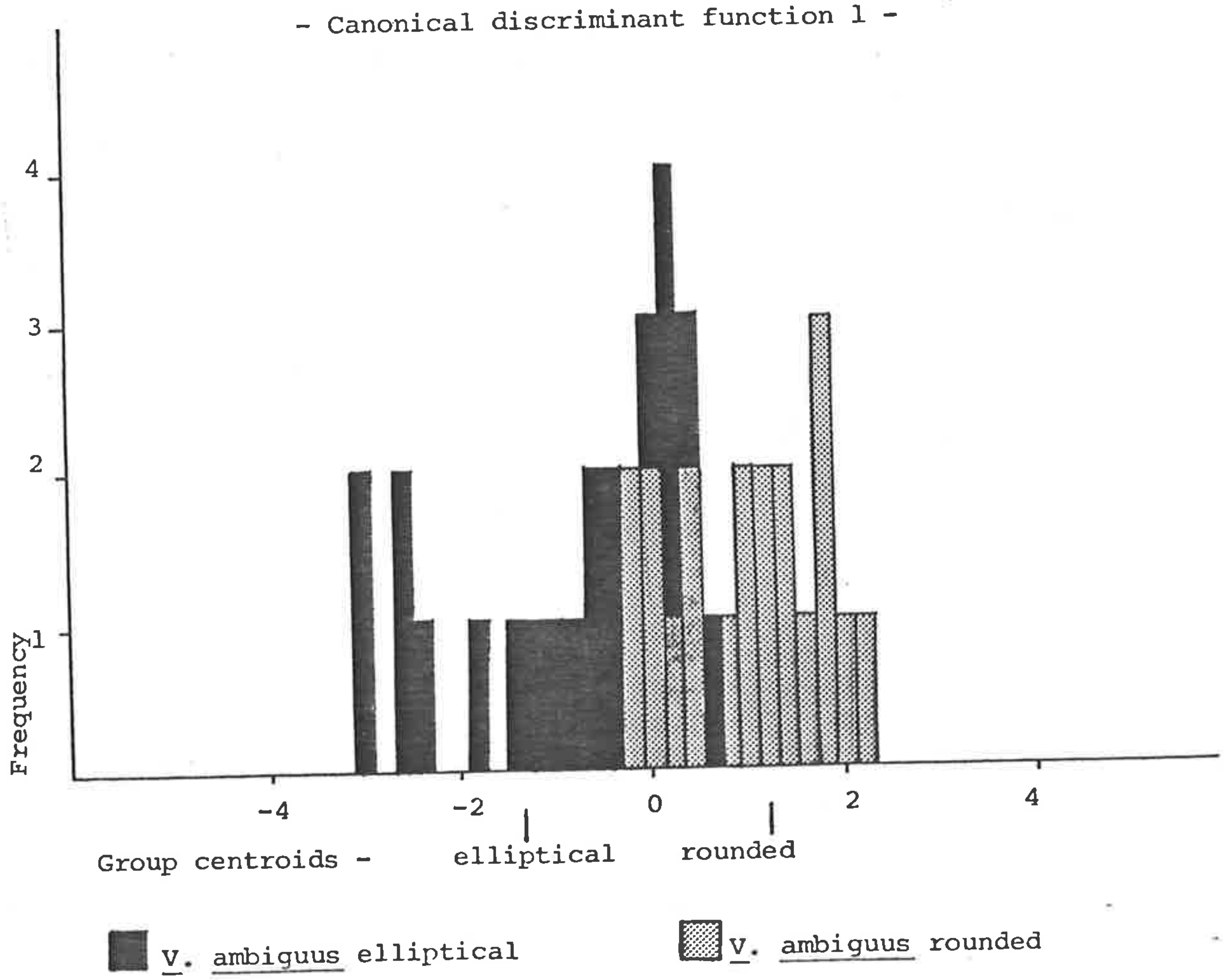


TABLE 21

Eagar's measurement system: Discrimination between excavate and non-excavate *A. condola*.

Actual group	No. of cases	Predicted group membership	
		excavate	non-excavate
excavate	20	15 75.0%	5 25.0%
non-excavate	20	4 20.0%	16 80.0%

Percent of grouped cases correctly classified: 76.92%.

GRAPH 9

All-groups stacked histogram:

- Canonical discriminant function 1 -

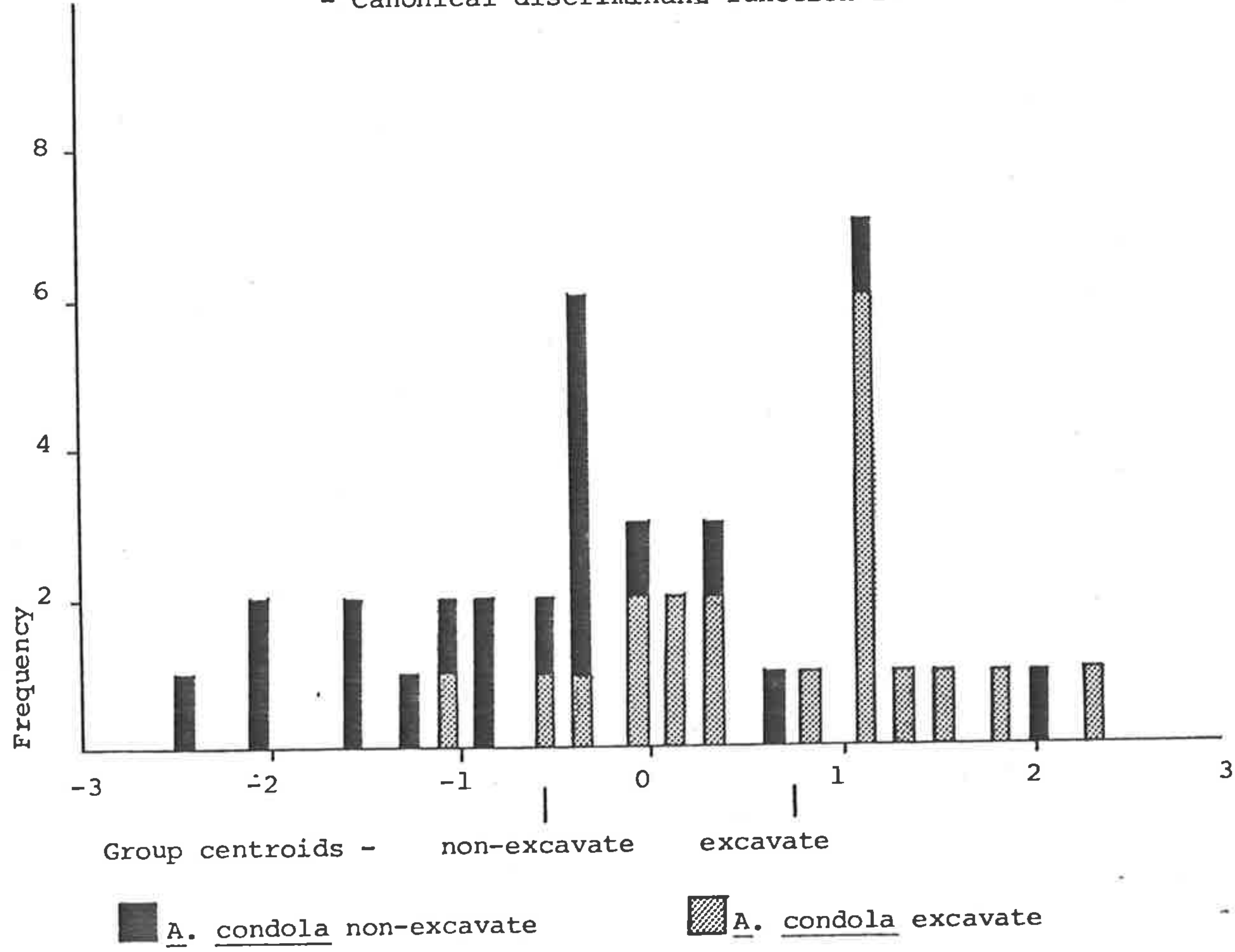


TABLE 22

Arch-wing measurement system: Discrimination between all growth forms.

Actual group	No. of cases	Predicted group membership 1	 2	 3	 4	 5	 6
Group 1	20	7 35.0%	4 20.0%	1 5.0%	4 20.0%	3 15.0%	1 5.0%
Group 2	25	5 20.0%	15 60.0%	0 0%	0 0%	0 0%	5 20.0%
Group 3	24	3 12.5%	0 0%	19 79.2%	0 0%	0 0%	2 8.3%
Group 4	19	0 0%	0 0%	0 0%	17 89.5%	2 10.5%	0 0%
Group 5	27	0 0%	0 0%	0 0%	3 11.1%	23 85.2%	1 3.7%
Group 6	39	2 5.1%	5 12.8%	4 10.3%	1 2.6%	5 12.8%	22 56.4%

Percent of grouped cases correctly classified: 66.88%.

1= arched *A. jacksoni*, 2= winged *A. jacksoni*, 3= excavate *A. condola*, 4= elliptical *V. ambiguus*,

5= rounded *V. ambiguus*, 6= non-excavate *A. condola*.

TABLE 23

Arch-wing measurement system: Discrimination between arched and winged *A. jacksoni*.

Actual group	No. of cases	Predicted group membership	
		arched	winged
arched	11	11 100%	0 0%
winged	11	0 0%	11 100%

Percent of grouped cases correctly classified: 100.00%

GRAPH 10

All-groups stacked histogram:

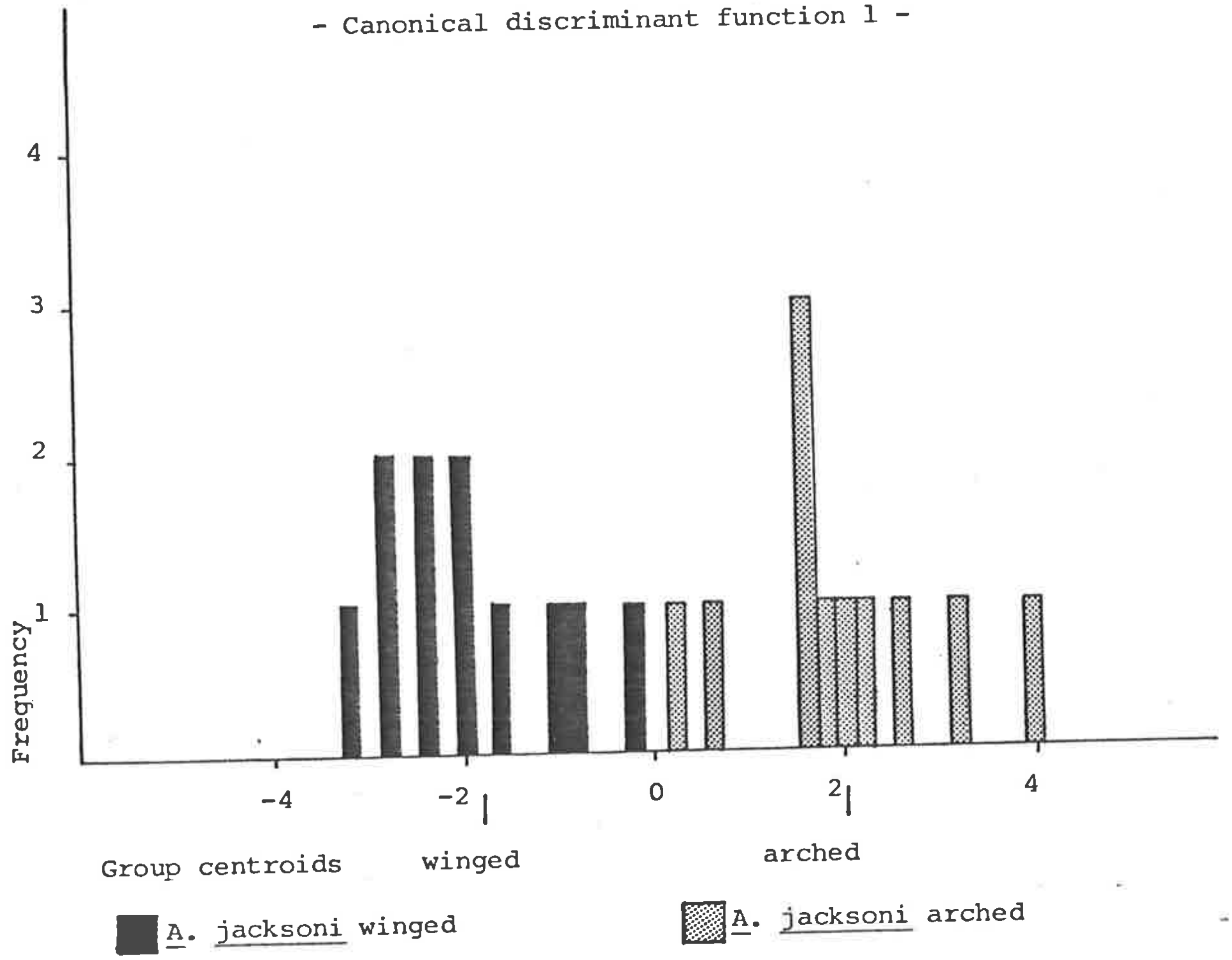


TABLE 24

Fourier analysis measurement system: Discrimination between species.

Actual group	No. of cases	Predicted group membership *V. ambiguus*	*A. jacksoni*	*A. condola*
V. ambiguus	40	36 90.0%	1 2.5%	3 7.5%
A. jacksoni	40	4 10.0%	20 50.0%	16 40.0%
A. condola	58	8 13.8%	21 36.2%	29 50.0%

Percent of grouped cases correctly classified: 61.59%.

TABLE 25

Fourier analysis measurement system: Discrimination between species where those mussels from Mulwala of uncertain identity here called "*A. condola*" are treated as a separate group to *A. condola* from Darlington Point.

Actual group	No. of cases	Predicted group membership			
		V. ambiguus	*A. jacksoni*	"*A. condola*"	*A. condola*
V. ambiguus	40	35 87.5%	0 0%	5 12.5%	0 0%
A. jacksoni	40	3 7.5%	17 42.5%	10 25.0%	10 25.0%
"*A. condola*"	38	6 15.8%	5 13.2%	27 71.1%	0 0%
A. condola	20	1 5.0%	3 15.0%	1 5.0%	15 75.0%

Percent of grouped cases correctly classified: 68.12%.

TABLE 26

Fourier analysis measurement system: Discrimination between all growth forms.

Actual group	No. of cases	Predicted group membership 1	2	3	4	5	6	7
Group 1	20	16 80.0%	0 0%	2 10.0%	1 5.0%	0 0%	0 0%	1 5.0%
Group 2	20	0 0%	14 70.0%	0 0%	0 0%	1 5.0%	4 20.0%	1 5.0%
Group 3	20	1 5.0%	0 0%	12 60.0%	3 15.0%	2 10.0%	0 0%	2 10.0%
Group 4	20	0 0%	1 5.0%	3 15.0%	12 60.0%	3 15.0%	0 0%	1 5.0%
Group 5	18	0 0%	1 5.6%	2 11.1%	6 33.3%	8 44.4%	0 0%	1 5.6%
Group 6	20	0 0%	3 15.0%	1 5.0%	1 5.0%	1 5.0%	14 70.0%	0 0%
Group 7	20	1 5.0%	0 0%	3 15.0%	2 10.0%	3 15.0%	0 0%	11 55.0%

Percent of grouped cases correctly classified: 63,04.

1= rounded *V. ambiguus*, 2= winged *A. jacksoni*, 3= elliptical *V. ambiguus*, 4= non-excavate *A. condola* (Mulwala Canal), 5= non-excavate *A. condola* (below Lake Mulwala), 6= excavate *A. condola*, 7= arched *A. jacksoni*.

TABLE 27

Fourier analysis measurement system: Discrimination of all growth forms with "*A. condola*" populations from Mulwala Canal and below Lake Mulwala combined to form a single group.

Actual group		No. of species	Predicted group membership 1	2	3	4	6	7
Group	1	20	15 74.0%	0 0%	3 15.0%	0 0%	0 0%	2 10.0%
Group	2	20	0 0%	14 70.0%	0 0%	1 5.0%	4 20.0%	1 5.0%
Group	3	20	0 0%	0 0%	16 80.0%	3 15.0%	0 0%	1 5.0%
Group	4	38	1 2.6%	2 5.3%	4 10.5%	29 76.3%	0 0%	2 5.3%
Group	6	20	0 0%	4 20.0%	1 5.0%	2 10.0%	13 65.0%	0 0%
Group	7	20	1 5.0%	0 0%	1 5.0%	2 10.0%	0 0%	16 80.0%

Percent of grouped cases correctly classified: 74.64.

1= rounded *V. ambiguus*, 2= winged *A. jacksoni*, 3= elliptical *V. ambiguus*, 4= non-excavate *A. condola*, 6= excavate *A. condola*, 7= arched *A. jacksoni*.

TABLE 28

Fourier analysis + maximum shell width measurement system: Discrimination between species.

Actual group	No. of cases	Predicted group membership *V. ambiguus*	 *A. jacksoni*	 *A. condola*
V. ambiguus	40	39 97.5%	0 0%	1 2.5%
A. jacksoni	40	3 7.5%	36 90.0%	1 2.5%
A. condola	58	2 3.4%	2 3.4%	54 93.1%

Percent of grouped cases correctly classified: 93.48%.

TABLE 29

Fourier analysis + maximum shell width measurement system: Discrimination between all growth forms.

Actual group	No. of cases	Predicted group membership. 1	2	3	4	6	7
Group 1	20	16 80.0%	0 0%	4 20.0%	0 0%	0 0%	0 0%
Group 2	19	0 0%	15 78.9%	0 0%	1 5.3%	2 10.5%	1 5.3%
Group 3	21	1 4.8%	1 4.8%	18 85.7%	1 4.8%	0 0%	0 0%
Group 4	38	0 0%	2 5.3%	3 7.9%	26 68.4%	0 0%	7 18.4%
Group 6	20	1 5.0%	2 10.0%	2 10.0%	0 0%	15 75.0%	0 0%
Group 7	20	0 0%	0 0%	2 10.0%	2 10.0%	0 0%	16 80.0%

Percent of grouped cases correctly classified: 76.81.

1= round *V. ambiguus*, 2= winged *A. jacksoni*, 3= elliptical *V. ambiguus*, 4= non-excavate *A condola*, 6= excavate *A. condola*, 7= arched *A. jacksoni*.

TABLE 30

Fourier analysis + maximum shell width measurement system: Discrimination between arched and winged *A. jacksoni*.

Actual group	No. of cases	Predicted group membership	
		winged	arched
winged	20	19 95.0%	1 5.0%
arched	20	0 0%	20 100%

Percent of grouped cases correctly classified: 97.50%.

GRAPH 11

All-groups stacked histogram:

- Canonical discriminant function 1 -

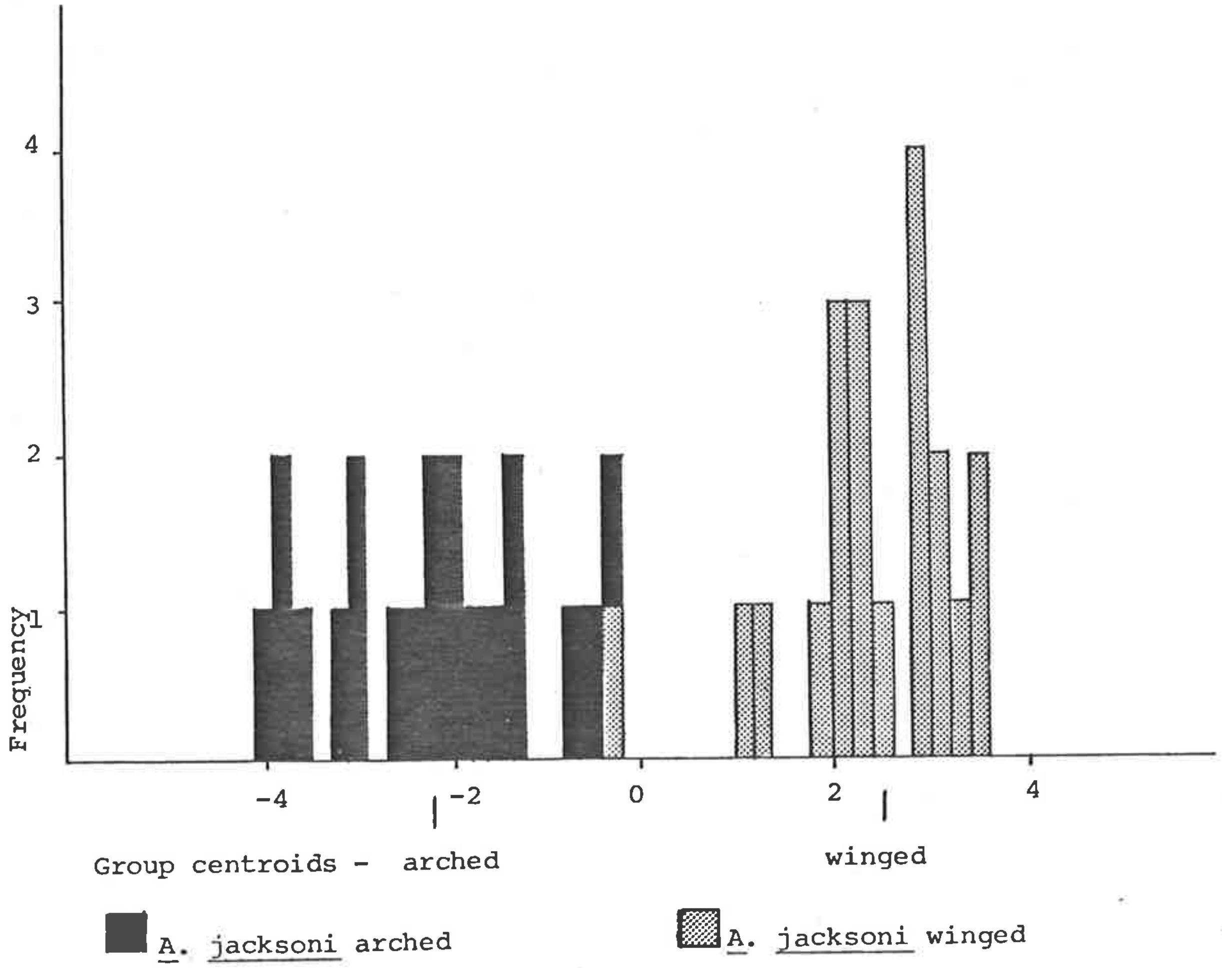


TABLE 31

Fourier analysis + maximum shell width measurement system: Discrimination between rounded and elliptical *V. ambiguus*.

Actual group	No. of cases	Predicted group membership	
		rounded	elliptical
rounded	20	17 85.0%	3 15.0%
elliptical	20	0 0%	20 100%

Percent of grouped cases correctly classified: 92.50%.

GRAPH 12

All-groups stacked histogram:

- Canonical discriminant function 1 -

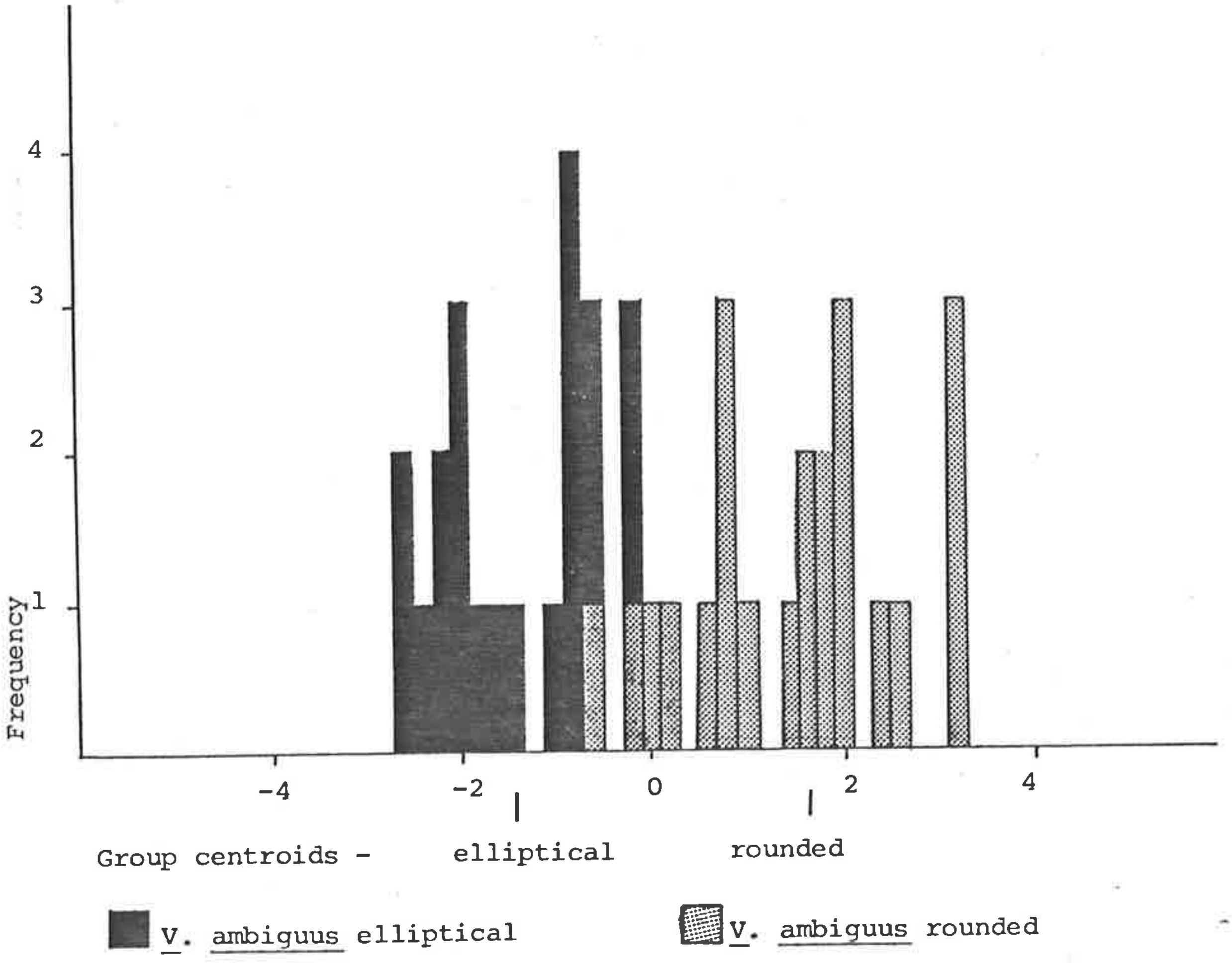


TABLE 32

Fourier analysis + maximum shell width: Discrimination between non-excavate and excavate *A. condola*.

Actual group	No. of cases	Predicted group membership	
		non-excavate	excavate
non-excavate	38	38 100%	0 0%
excavate	20	1 5.0%	19 95.0%

Percent of grouped cases correctly classified: 98.28%.

GRAPH 13

All-groups stacked histogram:

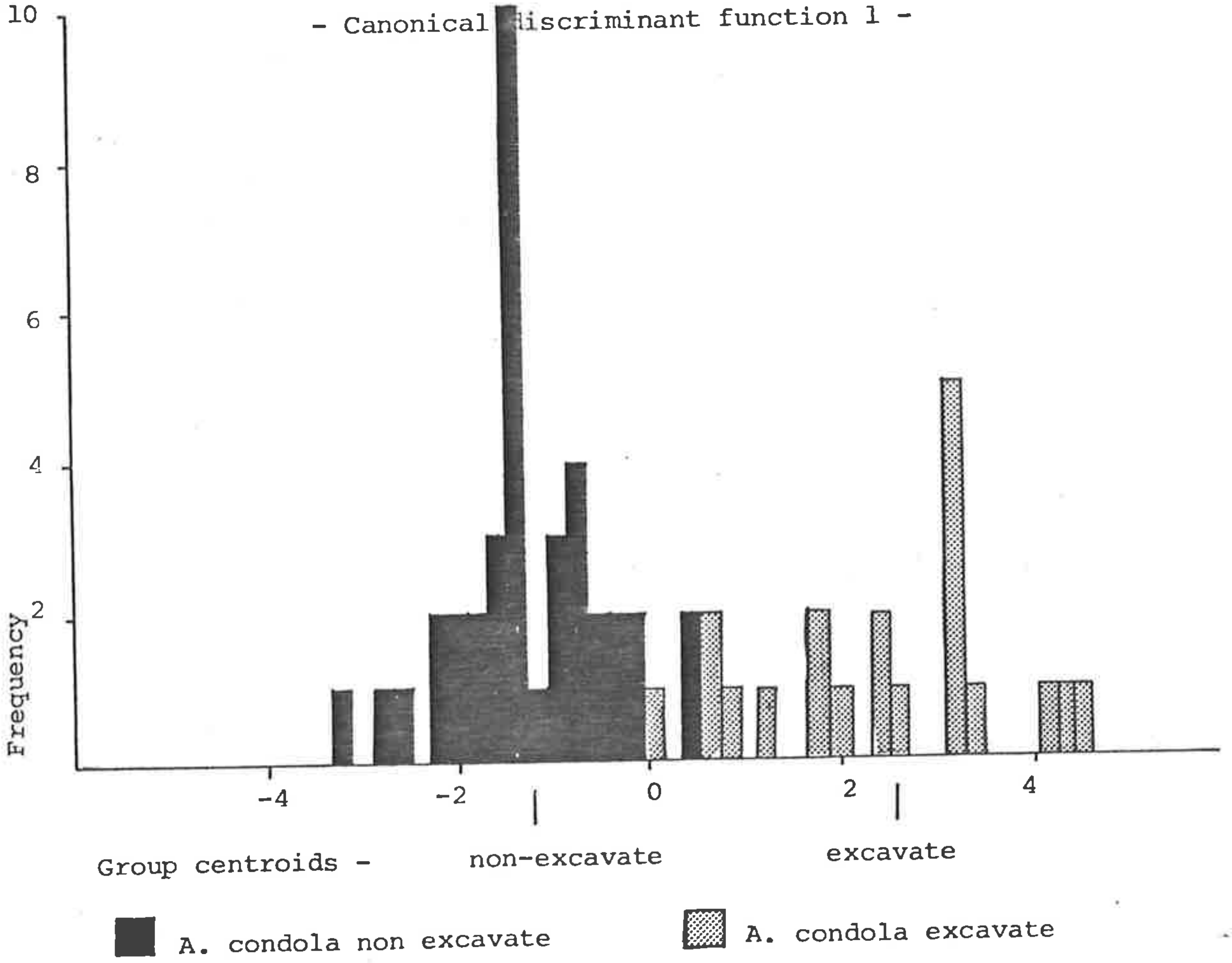


TABLE 33

Summary of the results from all the measurment systems.

System	Discrimination	Percent correct
McMichael and Hiscock	Species	90.83%
	All growth forms	77.50%
	A. jacksoni forms	90.00%
	V. ambiguus forms	92.50%
	A. condola forms	97.50%
Stanley	Species	87.50%
	All growth forms	73.33%
	A. jacksoni forms	80.00%
	V. ambiguus forms	82.50%
	A. condola forms	95.00%
Eagar	Species	84.03%
	All growth forms	69.75%
	A. jacksoni .forms	85.00%
	V. ambiguus forms	80.00%
	A. condola forms	76.92%
Arch-wing	Species	66.50%
	All growth forms	66.88%
	A. jacksoni forms	100.00%
	V. ambiguus forms	60.25%
	A. condola forms	72.50%
Fourier analysis	Species	61.59%
	All growth forms	63.04%
Fourier analysis + maximum width	Species	93.48%
	All growth forms	74.64%
	A. jacksoni forms	97.50%
	V. ambiguus forms	92.50%
	A. condola forms	98.28%

3.6 Evaluation of measurement systems

3.6.1 General systems.

McMichael and Hiscock's (1958) measurement system falls into the category of general systems because it was devised to distinguish between species for taxonomic purposes. McMichael and Hiscock used three indices to distinguish between mussel species, but the analysis performed here show that all the measurements including these indices are valuable in distinguishing between species. This system is very good at distinguishing between species, and consequently fulfills the function for which it was created. In addition, this measurement system is also very good at distinguishing between the growth forms of each species. This indicates that the entire shell is involved in the morphological variation exhibited by these growth forms.

Stanley's (1970) system is also a general system, but apparently does not fulfil its function well for these mussels. Separation of species is poor for the River Murray mussels. However, this system was designed to distinguish between species of different life habits, for example swimming, bysally attached, epifaunal and infaunal bivalves. Thus, it was designed to detect gross morphological differences. The three species used here all have the same type of life habit and are relatively similar in shape. Consequently, Stanley's measurement system was not sensitive to the smaller morphological variations seen in these mussels. Stanley's system, however, was able to successfully distinguish between the excavate and non-excavate forms of A. condola on the basis of height and width measurements alone. The measurements are not unique to this system, but could possibly be of some value in elucidating the functional significance of these differences in this species.

The Fourier analysis system falls into this category because it does not seek to quantify a specific morphological feature. Rather, it gives a complete description of the two dimensional outline of mussels. If the variable characters cannot be detected in this outline, then the system will not distinguish between species or growth forms. This seems to be the case using only the Fourier analysis with a periodicity of 10 degrees. However, if a third dimension is represented using the maximum width of each mussel, the discriminant analysis can separate species with success. Separation between growth forms of each species is also very good although maximum width is not always included as a useful discriminating variable. It should be noted that only C_{rel} (the ratio of each harmonic coefficient to the first) was used in these anlayses. This eliminated the size factor involved in the measurements, but could possibly result in loss of information. Further experimentation with reduced phase angles might have produced interesting results, however, the magnitude of the data for each mussel to be analyzed would have been extremely large (e.g., 72 measurements per shell at 5° phase angle).

3.6.2 Specific measurement systems.

Eagar's (1977) measurement of arching is not particularly successful at distinguishing between the growth forms of _A. jacksoni_, nor is it useful for separating species of growth forms of other species. It is possible that _A. jacksoni_ is significantly different to _M. margaritifera_, the mussel for which the system was devised. This is particularly the case with regard to the ligament which appears to be neater and thinner in _M. margaritifera_. In _A. jacksoni_ the ligament is often thick and uneven due to repeated depositions for strengthening and repair.

The arch-wing measurement system fulfils its purpose extremely well in that it separates the growth forms of *A. jacksoni* completely. It is of no use in distinguishing between species or between the growth forms of the other two species.

3.6.3 Evaluating measurement systems with respect to elucidation of adaptive significance of morphological variation.

Any measurement system which can distinguish between the growth forms of a species can be employed to elucidate the functional significance of morphologcial variation. The general systems of McMichael and Hiscock (1958) and Fourier analysis could be employed for all the River Murray species, while Stanley's (1970) system would be of use only for *A. condola*. These general systems are useful for preliminary work on functional significance because they are using non-specific measurements to separate growth forms. However, in order to begin correlative work between shape and internal anatomical and mechanical features, specific measurement systems must be employed. A specific measurement system that measures the extent to which a particular morphological feature is manifest produces a linear spectrum of variation which is ideally suited to quantitative correlations with internal anatomical and mechanical features. It is only at this stage that hypotheses about the functional significance of morphologcial variation can be constructed and tested.

CHAPTER 4

THE NATURE OF MORPHOLOGICAL VARIATION AND ITS ADAPTIVE POTENTIAL

4.1 Introduction

With the identity of morpholgical variants established and the description of growth forms achieved in qualitative and quantitative terms, attention could then be directed towards the significance of the variation. Much of what follows is based on the assumption that the morphological variation exhibited by these mussels is adaptive in some way. This assumption is implicit in all the work reviewed on variation in bivalves. Interest in morphological variation developed out of necessity for taxonomists. This chapter examines how approaches to morphological variation have evolved over time.

4.2 Historical perspective

The occurrence of morphological variation in bivalves was apparent to the early systematists. Some (e.g., Lea) disregarded the possibility of intraspecific variation and formed a new species for virtually every local variation. Later investigators synonymized many described species. Variations were occasionally noted, but observations on the variations themselves and their probable link to the environment were not numerous until the early twentieth century. However, Wetherby (1882, in Ball 1922) noted that some species varied more than others according to the nature of the local environment. Several European investigators observed that the same species takes different forms in rapid and quiet waters. Israel (1910) found that Unio crassus varied with the substrate and water velocity such that mussels from swift streams were shorter and rounder than mussels from quiet pools. The different forms were regarded as a single species because the glochidia were alike (Israel 1910). Isley (1911, 1913) believed that the environmental effects exerted on young shells determined the form of the adults. Meek and Clark (1912) noted that shell size and thickness decreased in riverine Rotundarai tuberculara as one moves further downstream. Wilson and Clark (1914) found that in a river system, mussels became more obese downstream where the current was slower and the bottom muddier. Utterback (1916) observed a similar trend and Danglade (1914) observed that mussels became smaller with distance downstream.

The first work solely devoted to shell variation was Grier (1920) who compared shells from Lake Erie with corresponding shells of the Upper Ohio. Grier found several correlations between various morphological features and the environemnt. The Lake Erie shells tended to be smaller, have thinner valves, greater obesity, a greater posterior development and a better developed posterior hinge-length than the forms in the Ohio. Ortmann (1909, 1918) made similar observations on mussels of the Tennessee River drainage and noted that for some species, a flat compressed form was found in the headwaters, an obese form in the larger rivers and an intermediate form in between. In 1920 Ortmann attempted to substantiate the apparent correlation of obesity and position in a stream using measurements on several species groups. He also synonymized species connected by intergrades into single species with several varieties defined by their relation to an arbitrary obesity measurement. In addition to confirming his former conclusions on obesity, he also found that young shells were more obese than older ones and that less obese shells were relatively longer, and a tuberculated form frequently lost its ornamentation in the headwaters. However, many species did not show this trend and were equally swollen throughout the river system.

With the exception of Grier (1920) and Ortmann (1920), the conclusions of those who reported morphological variation in bivalves (e.g., Wetherby 1882, Israel 1910, Utterback 1916) were based on qualitative observations, rather than on quantitative measurements. Even in these two studies, only maxima, minima and mean shell dimensions were considered. Ball (1922) was the first to attempt correlations of shell shape and environmental factors. He examined a multitude of species, using very general measurements. Despite great effort, he could only confirm previous observations on decreasing obesity towards the headwaters. It appears that before studies of this type could yeild useful information, the mode of shell growth and the role of genetics needed to be clarified.

4.3 Origin of morphological variation

Morphological variation in bivalves can be attributed to genetic and environmental sources. Interspecific variation, of course, is predominantly genetic, whereas intraspecific variation is due mostly to environmental factors (Stanley 1970). Where marked variation in form exists within a single population occupying a particular environment, genetic factors are likely to make a significant contribution.

Although a combination of genetic and environmental factors are likely to influence shell shape, environmental factors are more easily characterized while genetic components have been largely neglected. Genetic variations in structural proteins may be identified by electrophoresis (Wurzinger and Saliba 1979, Hornbach *et al*. 1980, Davis *et al*. 1981). To discover what this variation entails in biochemical terms would require time and knowledge not commonly available.

Environmental factors may be grouped into physical, chemical and biological elements (Hamai 1934b, Stanczykowska 1977). Physical factors implicated in previous studies (Grier and Mueller 1926, Baker 1928, Hamai 1935, Clark 1976) are temperature, depth, water velocity and substrate quality. Among chemical factors, calcium and oxygen availability, and salinity have been found to affect growth (Hamai 1934a, 1935; Stanczykowska 1977). Biological factors include nutrition and crowding (Coker *et al*. 1922, Agrell 1948, Brown *et al*. 1976, Seed 1980).

Transplant experiments often lead to the conclusion that environmental factors influence shell growth. Grier and Mueller (1926) and Baker (1928) found evidence of a change in shell shape in some anodontid river species following environmental modification by impoundment; the changes in shape were toward lacustrine forms of these river species. Walne (1958) also found that influx of mud and salinity decrease changed the shape of *O. edulis*. Experiments with the cockle *Venerupis rhomboides* (Eagar 1978), the mussel *Mercenaria mercenaria* (Rhoads and Panella 1970), the marine clam *Argopecten gibbus* (Clark 1976) and the mussel *Mytilus edulis* (Seed 1968), showed that the shells of transplanted animals grow like those of animals native to the new environment. These experiments indicate that each individual in a population is responding to environmental stimuli by producing a particular growth form.

Eagar (1948, 1977a) proposed that the observed variation between populations was due to natural selection, where individuals genetically determined to become one growth form could not survive in the environment of the other growth form. This view is not easily reconciled with the results of the afore-mentioned transplant experiments.

It should be noted, however, that some transplant experiments are unsuccessful. Transplantation of a bivalve with high specific gravity to a soft, muddy bottom will result in the animal sinking into the mud, thus occluding the siphons, and killing the animal. Morphological variation, then, is not usually a result of natural selection, but is the response of a growing bivalve to its environment.

Morton and Yonge (1964) and Owen (1953a) considered that changes in growth gradients around the mantle margin produced shape changes. This implied that variations in the rate of shell formation determine variation in shell form. According to Wilbur (1964), the rate of shell formation may be governed by the following factors:

1. the supply to the mantle of calcium by the blood or external medium,
2. the rate of conversion of carbon dioxide and bicarbonate to carbonate,
3. the rate of synthesis of the organic matrix in which calcium carbonate crystals become embedded,

4. the rate of secretion of calcium and components of the matrix by the mantle, and
5. a sufficiently alkaline pH of the extrapallial fluid to permit deposition of calcium carbonate.

The nature of the stimuli which produce directional, differential shell growth remains unknown. However, regeneration studies indicate that mechanical irritation of the cells of a small portion of the mantle stimulates secretion of shell material over a considerable area (Wilbur 1964). This suggests that selective secretion is possible and may be related to the formation of different shell shapes. Identification of the elements involved in inducing selective activity of mantle cells could provide a link between environmental stimuli and shell secretion.

4.4 Toward an understanding of morphological variation

The early studies of morphological variation were primarily descriptive, lacking any statistical analyses and in ignorance of river ecology and mussel physiology. Consequently, the adaptive significance of morphologcial variation eluded these workers. Descriptive ecological surveys of the mussel faunas in North American and European rivers continued to reveal morphological variations (Yonge 1946, 1953, Macan 1950, Hendelberg 1960).

An understanding of behavioural, physiological and mechanical aspects of mussels was slowly acheived through the experimental studies of many authors. Studies on the ligament structure, composition and mode of action were initiated by Trueman

(1950, 1951, 1953, 1954), and studied in many species (Owen et al. 1953, Owen 1958, Russell-Hunter and Grant 1962, Thomas 1976 and Kahler et al. 1976). Experiments on the mode of burrowing and substrate preferences were again initiated by Trueman (Trueman 1954, Ansell 1962, Trueman 1966c, Trueman et al. 1966a,b, Ansell 1967, Ansell and Trueman 1967, Trueman 1968a,b, Stanley 1975). More recent physiological studies, especially those on shell formation and regeneration have been valuable for understanding the growth process in bivalves (Owen 1953a, Wilbur 1964, Wilbur and Owen 1964, Beedham 1964, Kelley 1967, Saleuddin 1979, Palmer 1980, Weiner and Traub 1981). The success of these experimental studies was partly due to an approch of focusing on small problems and asking specific questions.

Almost simultaneously, paleoecologists became interested in morphological variation in bivalves (Menard and Boucot 1951, Ladd 1957, van Haaften and Verwey 1959, Kauffman 1965, 1967, 1969, Cox 1969, Haas 1969, Rhoads and Panella 1970). The body form of most living bivalves is reflected in the morphology of the valves (e.g., shell form and inflation, muscle insertion areas, characteristics of the pallial line, marginal gapes, dentition, ligamenture and a variety of internal structures such as platforms, ribs and furrows). This close relationship between body and shell means that not only are bivalves suited to studies of functional morphology, but that the general characteristics of the body in fossils can be accurately deciphered from shell morphology to allow interpretation of adaptive trends. The body and shell came to be observed as a single unit which evolved as an integrated whole. Previously, (e.g., Yonge 1953-1958) the body and shell had been regarded as separate, but interacting, evolutionary phenomena, independently adapting to the same set of environmental conditions. Kauffman (1969) maintained that relating the body to features of the shell is probably the most critical and basic step in interpreting functional morphology and adaptive evolution in the Bivalvia. It allows partial reconstruction of the body and subsequently, the life habit of fossil forms and provides the spectrum of data needed for evaluation of evolutionary change.

Development of a functional adaptive trait rarely involves only a single structure, but rather, encompasses a suite of functionally integrated structures. It follows that functional interpretation of single structures is not as meaningful as interpretation based on whole systems of integrated features, both anatomical and morphological (Kauffman 1969).

Carter (1967) believed that many of the morphological features of the bivalve shell were protective adaptations against predation. Kauffman (1969) also beleived that the primary function of the shell is to protect and that any accessory structure shares in this protective function. However, Stanley (1970) concluded that relatively little variation had evolved specifically for protection against predation. Stanley maintained that bivalves have fundamental adaptations which serve the need for protection and that shell morphology was directly influenced by food supply, substratum character and water movement.

Morphological variation adapts a bivalve to its environment and it is the summation of all features interacting that achieves this end. While individual morphological traits may not be adaptive, Carter (1967) pointed out that selectively neutral charcters are very rare in living animals. Most workers have concluded that non-functional structures are too rare and too hard to define to warrant close consideration. The lack of an obvious function for a bivalve structure probably reflects a lack of basic data concerning form, function, behaviour and habitat (Kauffman 1969).

Usually a structure has more than one known function and closely interacts with other structures (Thomas 1976). Thus, each structure is likely to have a primary function and one or more secondary functions. <u>Primary</u> structural adaptations develop with growth to better adapt the animal, at the time of their formation, to cope with the environment. <u>Secondary</u> adaptations follow the formation of a primary structure. They are not necessarily related to the moment of formation, but better adapt the entire animal to the environment in more subtle ways than a primary adaptation (Kauffman 1969).

The identification of primary and secondary adaptations is not essential to an understanding of the significance of morphological variation. It is not usually possible to subdivide features into primary and secondary adaptations because changes in one feature necessitate compensatory changes in another to balance the forces acting on the shell. In addition, it is possible for one feature (for example, ornamentation) to have several functions. What follows is a brief account of the mechanical and behavioural forces which influence shell morphology, with emphasis on the interrelationships between these features.

4.4.1 Stresses on the bivalve shell.

The material and structure of the valves are physically tested every time the animal closes its shell. Contraction of the adductors is sufficient to break the shell in some bivalves if a narrow strip of shell is removed from the ventral margin of one valve, or if they are artificially prevented from closing (Wainwright 1969). The position of the adductors is affected by the form of the shell (Owen 1958), and closely interacts with the strength and position of the ligament. The positioning of the ligament on the dorsal margin of the valves in many bivalves is correlated with the symmetry of the shell, so that stresses placed on the ligamental material in opening the valves are as evenly distributed as possible (Kauffman 1969). Failure to achieve this leads to splitting of the ligament, which substantially reduces its strength. When the adductor muscles close the shell, the elastic inner layer of the ligament is compressed. Release by the adductors allows the elastic energy stored in the compressed ligament to expand and force the valves apart (Wainwright 1969). The inner layer of the ligament is always under compressive stress, and the adductors and outer layer of the ligament are under constant tensile stress. In addition, the part of each valve between the adductor muscles and the ligament is under shear (bending) stress (Wainwright 1969). The opposing forces of the adductors and the ligament must be balanced to minimize the strain on the shell, and therefore, change in position of the ligament would require a change in the position of the adductors.

4.4.2 Ornamentation.

Ridges radiating from the oldest point of the shell strengthen it against adducting forces, and the domed shape characteristic of most shells strengthens it against forces impinging from the outside (e.g., crowding in mytiliids; Seed 1968, Brown et al. 1976). Thickened valves and certain types of plicate sculpture may confer resistance to shell damage (Thomas 1975, Vermeij 1978). Coarse surface ornamentation strengthens the valves and stabilizes the burrowing bivalve in the substrate (Kauffman 1969) by increasing the area of contact between shell and substrate and by creating resistance to being dislodged (Trueman et al. 1966a,b, Stanley 1978 and Vermeij 1978). For less efficient burrowers, a certain amount of ornamentation is advantageous. In the marine mussel Glycymeris pectinata, ribs assist a weak ligament in bracing the shell against the sediment; thus more than offsetting the disadvantage of resistance to the pull of the securely anchored foot (Thomas 1975). Ribs are seldom specialized for any one function, but have several different roles.

The development of spines, coarse radial and concentric ribs, and thick shells among many tropic and warm temperate marine bivalves may be an adaptive response to the increased importance of crushing predators (Vermeij 1978). Raised ribs may make it more difficult for starfishes and boring snails to grasp potential prey (Thomas 1975).

In a study of the effect of the environment on the marine mussel Cardium edule Purchron (1939) has shown that shells from sheltered estuarine mud are more lightly built and possess fewer ribs than those from wavebeaten sand. Burowing in estuarine mud would probably not require strong probing of the foot as penetration is easily achieved and ribs would not appear advantageous in such a soft substrate. On the other hand, ribs may have adaptive value in wave-washed sand, increasing stability and aiding pedal protrusion.

4.4.3 Ligament strength.

Elongation of the ligament is essential so that it maintains its effectiveness as the bivalve grows (Cox 1969, Thomas 1975). Often the elastic properties of the ligament decrease as the animal grows, due to splitting of the exposed anterior portion of the ligament (Owen 1953b). Bivalves which are rapid burrowers require strong ligaments because they must brace their shells against the sediment during probing (Thomas 1976). This is especially true of heavy-shelled burowers (Trueman 1966a, 1968 a,b) and obese bivalves (Roberts 1981). Trueman et al. (1966a) found that weak ligaments are typical of sluggish burrowers, and the most powerful ligaments occur in active, deep burrowers. This is because the greater the force with which the valves gape, the more firmly is the shell held in the burrow during downward probing of the foot. For a rapid digging cycle, rapid opening of the valves must occur after adduction and retraction.

4.4.4 Factors influencing burrowing ability.

Many factors besides shell form determine the depth of burial. For example:

1. Ability to rock the shell during burrowing, which aids the speed of penetration.

2. Size and nature of the foot and its ability to probe and anchor in the substrate.
3. Presence or absence of external ornamentation.
4. Gape of the shell during burrowing.
5. Ability to produce powerful water jets to clear sediment ahead of the burrowing animal.
6. Shell size (larger shells are harder to pull into the sediment than smaller ones of the same shape).
7. Available energy used in burrowing (especially once the shell has penetrated below the substrate and burrowing becomes more difficult owing to increased drag). (Trueman _et al_. 1966b, Kauffman 1969).

Trueman _et al_. (1966a) observed that bivalves with broader shells exhibited a pronounced rocking movement that presumably counteracted the increased resistance from the substrate due to their width. Burrowing in these species would necessitate a powerful foot and their burrowing ability may be limited by the holding power of the foot, rather than the strength of the retractor muscles. Valve inflation appears to be the major factor in determining resistance to shell penetration (Kauffman 1969).

4.4.5 Pedal gape.

Various bivalves have elongated shell gapes in the area of the foot and siphonal extension. In deep burrowing forms, such as the small marine _Ensis_ and _Mya_, these may become greatly enlarged and may even allow extrusion of the foot and siphons without broad opening of the valves. This is adaptive for rapid burrowing, as the gaping shell in most bivalves hinders the burrowing process by offsetting shell streamlining (Kauffman 1969). In some mussels, pedal gape may be associated with extension of the foot for long periods, as in those animals anchored in fast flowing waters (Trueman 1968a). Streamlining reduces turbulence and lift, and therefore, would be advantageous in fast currents. Shells in strong currents tend to be long and narrow, or compressed and straight (Hargar 1972, Baker 1922, Coker _et al_. 1922, Hendelberg 1960) with reduction of projecting surfaces, an increase in the relative symmetry of the shell and a smooth shell surface (Kauffman 1965, 1967).

4.4.6 Obesity, specific gravity and substrate interactions.

In some environments, bivalves have no need to burrow because the substrate is soft; indeed there may be a tendency for the shell to sink (Tevesz and McCall 1979, Roberts 1981). Such environments occur in lakes and slow-flowing rivers, The ideal shell would be light weight and have a large suface area relative to specific gravity. Shell thickness is the primary determinant of specific gravity (Eagar 1978), and comparison of species which occur in both rivers and lakes often reveals the lake forms as more obese and of lower specific gravity than the river forms (Ortmann 1920, Ball 1922, Baker 1928, Agrell 1948 and Haas 1969). Baker (1927) attributed the thinness of lake shells of _Anadonta_ to a lack (or unavailability) of calcium (c.f. Coker _et al_. 1922).

Eagar (1977b) suggested that increased obesity provided increased bulk of soft tissues against surface area and, therefore, a higher energy or metabolic potential in conditions of comparative tranquility. Obese bivalves burrow slowly and expend large amounts of energy, but in suitable environments a round shell form is of adaptive value becuse it allows optimum free spatial distribution of internal organs. In addition, it provides for a greater number of sensory receptors at the mantle margin, so that the animal is effectively forewarned of danger.

4.4.7 Influence of the internal organs on shell shape.

Internal features help determine the shape of the shell. For example, in oysters the gills determine the outline of the shell because they are relatively large in the absence of a foot (Stenzel 1969). In the marine mussel Thyasiria the foot is slender and up to ten times the length of the shell. It serves three functions: burrowing, locomotion and formation of an elongate siphon. When retracted the foot is loosely coiled in the mantle cavity, which is somewhat expanded. In addition, the gills are reduced in size and the adductors compressed providing more space for the foot (Kauffman 1967).

4.4.8 Overview.

The shape of the bivalve shell is constrained by the stresses and strains imposed on it by the ligament, adductor muscles, and external forces. Changes in ligament strength must be accompanied by compensatory changes in other internal organs, principally the adductor muscles, and changes in the shell. Ornamentation has many functions: it strengthens the shell, aids in burrowing (in moderation) and is protective. Ligament strength is determined by its length: strong burrowers require a strong ligament for the same reasons as weak burrowers, namely, for anchorage ability and mobility. Anchorage and burrowing ability require strong ligaments and adductors, and a well-developed foot, and so, to a lesser extent, does mobility. Often bivalves in strong currents need to burrow and anchor in the sediment to prevent being washed away. Therefore, an obese mussel may be highly mobile with strong ligament and adductors, but be a poor burrower because of its width. There are many subtle differences in shape which suit each bivalve to its environment.

4.5 Elucidation of functional significance

Experimental investigation is the only direct means of elucidating the functional significance of morphologically variable characteristics. Simple descriptions of shape variation are not sufficient and many non-specific, unsubstantiated claims are scattered throughout the literature. Much of the work described in this chapter consists of assertions about the functions of particular characters with no attempt to substantiate them experimentally.

Rudwick (1961) was among the first to recognize the need for an orderly process of functional analysis. Previous studies (Ortmann 1920, Ball 1922, Baker 1922, 1927 and Hamai 1934a) described the variation exhibited and attempted to correlate it with location or the physical and chemical nature of a water body. Few definite conclusions were drawn from these studies because many authors found that they were unable to interpret the variation in relation to environmental factors (e.g., Hamai 1935). Rudwick

(1961,1964) proposed the 'paradigmatic' (or 'mechanistic') method to elucidate the functional significance of morphological characters:

1. Perception.

A structure is examined in detail and compared with related living and fossil animals and with unrelated bivalves having similar structures. As a result, more than one reasonably plausible function for the structure is proposed.

2. Specification.

The 'ideal' structure is conceived to fulfil the postulated function with maximal efficiency, under the limitations imposed by the available material. There are paradigms for each postulated function.

3. Evaluation.

The correspondence between any paradigm and an observed structure is a measure of the efficiency with which the structure would have fulfilled a postulated function. Of course, this cannot establish the probability that the structure did fulfil its postulated role.

4. Interpretation.

Assuming the structure will never completely meet its paradigm, it is necessary to make comparisons with the structural paradigms of several possible functions. Whichever paradigm is most closely approached by the structure is taken to be the dominant function. The other postulated functions may then (subjectively) be considered as secondary functions or, alternatively, may be discarded. Rudwick's method was devised primarily for fossil bivalves but could equally apply to extant mussels. There are several problems with the paradigmatic method. From the outset, Rudwick assumes that it is possible to identify a structure and to immediately propose a function for it. This is not always possible where the 'structure' is merely a subtle variation in shell outline. Indeed, proposing a function for a structure is probably the most difficult aspect of elucidating functional significance. Rudwick's method therefore, is most appropriate to obvious structural differences which are less common than subtle variations.

It is at the interpretative stage that the paradigmatic analysis is weakest because there is no way of quantitatively determining how closely a structure approaches several alternative paradigms. Hence, the choice between such paradigms is subjective. Rudwick's method was used by Carter (1967) to postulate a function for spines on shells of the marine clams Hysteroconcha and Hecuba using shells alone. He concluded that although valuable analyses could be performed on shells alone, a satisfactory interpretation of many bivalve structures can be reached only in conjunction with studies of the living animal. Carter proposed that although it was not always possible to maintain the rigour of paradigmatic methodology, it was an essential tool in any closely reasoned functional analysis.

Structural similarities between fossil and extant bivalves were used by Kauffman (1969) as a means to compare and interpret the form and function of a variety of bivalves without regard for age or phylogeny. He believed that structural similarity between even unrelated forms should have priority in interpretation, whether the comparison involves similar whole shells or like structures on otherwise dissimilar shells. However, Kauffman (1969) recognized that development of a functionally adaptive trait rarely involves a single structure, but rather, encompasses a suite of functionally integrated structures. Therefore, a functional interpretation of such structures as separate entities is less meaningful than interpretation based on whole systems of integrated anatomical and morphological features. Kauffman's approach involved:

1. Documentation of form,
2. Proposal of functions for major shell features, and for interacting structures of the shell and the inner body, as discrete entities,
3. Integrated analysis of complex, functionally interacting systems or suites of body and shell morphology.

Kauffman asserted that the shell and body of a bivalve should be treated as an integrated system. Therefore, it is more meaningful to study suites of characters rather than single structures. Despite this, he approached functional morphology as Rudwick had, by considering individual structures. Nor does Kauffman propose a method for arriving at a possible function for a structure and makes no provision for testing hypothesized functions.

Stanley (1970) critically examined Rudwick's method and produced a modified method for elucidating the function of a structure.

1. Hypothetical functions are proposed for a 'problematical' structure,
2. Life-habit and habitat data are gathered for species possessing this structure,
3. These data, with mechanical considerations, provide critical tests for evaluation of the working hypotheses, and

4. Usually one hypothesis emerges as the most reasonable, the others being inconsistent with the life-habit and habitat data.

Stanley recongized the existence of multiple effects that make compromises necessary in evolution. Thus, the ultimate form of a bivalve is a compromise solution for selection pressures in two or more directions. Stanley implies that subjective selection of the most plausible hypothesis is sufficient, without testing the validity of the conclusion. Where fossils are concerned, he proposes that a living species with the same structure should be studied and a function for the structure proposed. This proposed function would subsequently be applied to the fossil and the likelihood of homology evaluated. Thus, one is unable to conclusively demonstrate a function for the fossil structure, since the hypothesis can never be tested. Stanley does not explain how the evaluation of homology between the structure of living and fossil bivalves is achieved. Again, the final analysis is subjective.

Each of the above methodologies have qualities in common. First, a comparison between bivalves is made. This is essential, as it is only by comparison that the differences are revealed. Secondly, a structure is identified and functions proposed. Thirdly, the proposed functions are evaluated, and one (subjectively) selected as the most probable.

The second and third steps concern single attributes and attempt to attribute functional significance to the differences in the bivalves being compared. As Kauffman (1969) emphasized, the morphological and anatomical features of a bivalve are integrated and it is unrealistic to consider them individually. Consideration of the more spectacular features of a bivalve shell, therefore, may be quite misleading, since more subtle variations are unlikely to be revealed as significant factors.

There is a need for a more realistic way of elucidating the functional significance of variation. It must avoid the subjectivity associated with previous methods, and approach the problem by developing ideas amenable to experimental analysis. It must not assume that a 'structure' is involved and its probable functional significance obvious. This would imply that the probable functional significance can be arrived at by induction. However, an inductive approach is unrealistic as induction seldom plays a part in hypothesis formation, rather, it is as Popper maintained, an intuitive process. A revised method is presented here. It is in accordance with Popper's assertions that theory cannot be fabricated from observations, it can only be invented. Observations are interpretations of facts observed in the light of theories already present (Magee 1975).

4.5.1 Proposed method.

The first step in attributing functional significance to morphological variation is to delineate differences by comparisons between individuals. This is best achieved by a measurement system which quantifies the variation. Only by comparison will the differences become evident. The observed variations are probably adaptations to environments which differ in substrate or water velocity. Behavioural comparisons of burrowing activity (e.g., comparisons of uprighting behaviour, the speed and depth of burrowing and probing force of the foot) could reveal differences between forms. Similarly, comparisons of physiological tolerances (e.g., tolerance to low oxygen, salinity and dehydration) could indicate differences related to specific environments. These environmental, physiological and mechanical factors should be quantified.

The second step is to propose hypotheses based on the differences between bivalves, to test them, and so obtain an indication of what is important to the growth forms in their respective environments. The creation of these hypotheses is partly intuitive, and dependent on the skill and insight of the investigator. They will provide tentative answers to questions raised by the observed differences and must be tested experimentally. The results should be amenable to statistical analysis.

The third step requires assimilation of the test results in order to perceive the morphological variation as an integration of form and function. The assimilated information should present a total picture from which one can formulate a testable theory of how the different growth forms are adapted to their respective environments.

4.6 Conclusions

The early descriptive studies of morphological variation in mussel faunas in Northern Hemisphere rivers were primarily concerned with systematics. The few studies which concentrated on morphological variation (e.g., Grier 1920, Ball 1922) attempted to correlated shape with environmental character such as water velocity and substrate type. They were largely unsuccessful because the problems they tackled were too large (e.g., entire faunas of river systems), measurements were too crude and general to accurately quantify the variation, and ignorance of the role of genetics and the mode of shell growth hindered interpretation. It was not until the experimental analyses of the mechanical, behavioural and physiological characteristics were conducted that it became evident that the bivalve shell and body were intimately related. Consequently, the interpretation of morphological variation became the study of functionally adaptive traits. Investigators focused on smaller problems and formulated specific questions about features of mussel shells in relation to behaviour, mechanics and anatomy. Paleoecologists introduced systematic approaches to the elucidation of functional significance of shape variation, and it became clear that the quantification of morphological variation was a prerequisite for statistical analysis of experimental work on adaptive variation.

CHAPTER 5

THE RELATIONSHIP BETWEEN THE MUSSEL AND ITS ENVIRONMENT

5.1 Introduction

This project began with a perception of shell shape variation in *Alathyria jacksoni* that was somewhat volatile and subjective. The wide array of variation in the three River Murray mussels was confusing. While much of this confusion was alleviated by electrophoretic analysis, devising and testing measurement systems also made an important contribution. The measurement systems prompted objective consideration of shape variation and occasionally revealed differences not previously perceived. Particular morphological characteristics of the growth forms of the three species became more clearly defined.

Perceptions of the interrelationship between the body and shell of a mussel influence the approach to the elucidation of functional significance of mophological variation. In order to understand this relationship, it is important to know how the shell is adapted to the environment. Eagar (1977, 1978) formulated ideas on how the shell shape of *Margaritifera margaritifera* was adapted to the environment. In this chapter Eagar's ideas are examined and consideration is given to their applicability to *Alathyria jacksoni*.

5.2 The work of Eagar on *Margaritifera margaritifera*

Eagar (1977) examined shell shape variation in the freshwater pearl mussel *Margaritifera margaritifera*. As noted in Chapter 1, there is a superficial resemblance in the shell shape variation in both *M. margaritifera* and *A. jacksoni*. Eagar's 1977 and 1978 papers contain ideas which, although not drawn into a concise summary, can be interpreted in the following way.

The arched form of *M. margaritifera* was found in areas of rapid water flow and the oval form (equivalent to winged *A. jacksoni*) in slower flows. Eagar notes that the arched form has a "pronounced" pedal gape in the antero-ventral region of the shell and a mid-ventral inflection. Eagar suggested to Trueman that feeding behaviour of oval and arched *M. margaritifera* be compared, but no differences were found. Trueman (1968) also examined burrowing behaviour and ligament strengths of the pearl mussel. He found that in both forms the foot never extended beyond the mid-ventral region of the shell and suggested that the pedal gape in arched mussels may be associated with extension of the foot for long periods to ensure a better anchorage in fast currents. Ligament strength (specifically opening moment) in arched mussels was little more than half that of the oval variety. Eagar speculated that the ventral inflection in the arched mussels might facilitate entry into burrowing position. Shell thickness in *M. margaritifera* reaches a maximum on the dorsal margin and tails off to a minimum on the ventral periphery. Eagar asserted that arching of the dorsum and straightening and reflection of the ventral margin together add to that part of the shell margin which is heaviest and reduce that part that is thinnest and lightest. Two equal length (99 and 100mm) mussles were weighed with the arched mussel being 35g and the oval 28g. Eagar concluded that the arched mussel had the availability of 7g or 25% more probing force of the foot than the oval mussel, and therefore, had an "unquestionable" advantage in the initial stage of burrowing.

Eagar regarded the two patterns of growth as a reflection of two functional activities. The arched mussels had the provision of weight and probing power for the foot at the commencement of burrowing. The oval mussels maintained ligament strength for active surface movement in a more optimal water flow.

To evaluate the applicability of Eagar's ideas to Alathyria jacksoni two things must be considered:

a. how valid are Eagar's conclusions, based on the data available to him, and

b. are the two species similar enough to allow valid comparisons?

The first can be addressed immediately, however, to consider the second, an intensive investigation of the characteristics of A. jacksoni is needed.

5.2.1 The validity of Eagar's conclusions.

Both of Eagar's (1977, 1978) papers contain very little experimental work or quantification of statements (e.g., How pronounced is the pedal gape?). This results in unverified speculations. For example, there is no evidence to support Eagar's assertion that the ventral inflection might facilitate entry into burrowing position, nor support for the conclusion that oval mussels are more mobile than arched mussels.

Trueman reported that pedal gape may result from extension of the foot for long periods in the arched mussels. Eagar apparently interprets this to mean that the pedal gape improves anchorage.

The arched mussels with their heavier shells (especially where the weight increase is along the dorsum), will have more difficulty uprighting the shell to commence burrowing than oval mussels with lighter shells. This may invalidate Eagar's belief that arched mussels have an "unquestionable" advantage at the initial stage of burrowing. However, it seems reasonable that the added weight might provide more probing power for the foot once upright. There does not appear to be a very rigorous justification for Eagar's conclusion that the two contrasting patterns of growth are consistently related to two functional activities. However, valid conclusions can be drawn from this work. Eagar is evidently convinced that water velocity is the determining factor of shell shape and there is no evidence to contradict this. It may be retained as a plausible hypothesis. Other pieces of information may also be of use, for example, Trueman's proposal that the foot anchors the mussels and that oval mussels have stronger ligaments than arched mussels. Eagar's ideas seem to indicate that he views the shell as separate from the body, and indeed his work does not consider the body at all. Perhaps the problem should be approached another way.

5.3 Functional significance and the environment

The basic approach to elucidation of functional significance is governed by the way the bivalve is viewed; as shell separate from body or totally integrated. This is inextricably related to whether the shape and position of internal organs determines the shape of the shell, whether the environment affects the shell shape or whether both contribute. Yonge (1953, 1976) maintained that the body and shell of the bivalve should be treated as separate entities. However, Yonge seems to be in the minority. Gunter (1938) approached shell shape variation by studying shell growth, attributing the different shell forms to the deposition of the mantle and other body organs. He was hindered by lack of knowledge about shell secretion, but was among the first to recognize that the form of the shell was directly related to internal organs. Stasek (1963) and Kauffman (1969) claimed that the shell reflected the positions of certain internal organs.

In studying the mussels of the River Murray this view of the interaction of shell and body has been adopted. Quantitative measurements of shell shape are of considerable value in connecting shell and body and are important as the first step of elucidating functional significance of morphological variation. The construction of a measurement system played a major role in discovering objectively what characters differ between growth forms.

Together with an understanding of the environment from which the growth forms originate, hypotheses can be formed as the second step in elucidating functional significance. For *Alathyria jacksoni* the morphological variation may be related to an interplay of water velocity and substrate type. Burrowing and ability to remain firmly wedged in the substrate may be important for survival and maintenance of position in the river during periods of rapid flow. A multitude of studies on burrowing (e.g., Ansell 1962, Trueman 1954, 1968a,b, Treuman *et al*. 1966a,b, Stanley 1975, Roberts 1981) indicate that the strength of the ligament and adductors could be important.

What follows is an experimental investigation of the possible environmental factors that may be related to arching and wing development in *Alathyria jacksoni*.

5.4 The importance of substrate and water velocity

5.4.1 Substrate experiments.

Substrate type is often held responsible for certain aspects of mussel morphology (Harman 1972, Tevesz and McCall 1979). Mussel habitats in the River Murray from Lake Alexandrina to Hume Dam were surveyed and described. In the sections of the Murray sampled, the substrate ranged from clay to coarse sand and mussels were found in all substrate types. *V. ambiguus* tend to be found more frequently in areas where the particle size is small and *Alathyria* in sand-clay to coarse sand. Is this due to each species being unable to burrow into substrate types in which they are not normally found, or is it a function of water velocity (which determines particle sizes at a given location)? To test burrowing ability and substrate preferences, two experiments were designed.

Materials and methods.

Sand collected from various locations was washed repeatedly and seived into the following size classes: >4mm, > 2mm, > 1mm, > 0.5mm, > 0.25mm, <0.25mm, using Endecotts Laboratory Sieves.

a. Burrowing ability

Ten small aquaria were filled with 10cm of sand of each size class, submerged in dechlorinated water and aerated. Mussels were placed in the tanks and left undisturbed for 48 hours at 20°C, with a 12 hour light/dark regime. The amount of mussel body buried was recorded as a percentage of the total body. Arched *A. jacksoni* from Wemen, winged *A. jacksoni* from Lock 3 and *V. ambiguus* from Morgan were tested,

(N=12, N=12, N=10 respectively).

b. Substrate preference

A large tank with a graduation of particle sizes from >4mm to <0.25mm was filled with dechlorinated water circulated with a magnetic pump to oxygenate the water. Mussels were placed in rows so that they covered the surface of the sand at equidistant intervals. Positions of the mussels were recorded every 24 hours for 5 days.

No's used: _A. jacksoni_ 11 arched, 13 winged & 16 _V. ambiguus_.

Results.

a. Burrowing ability

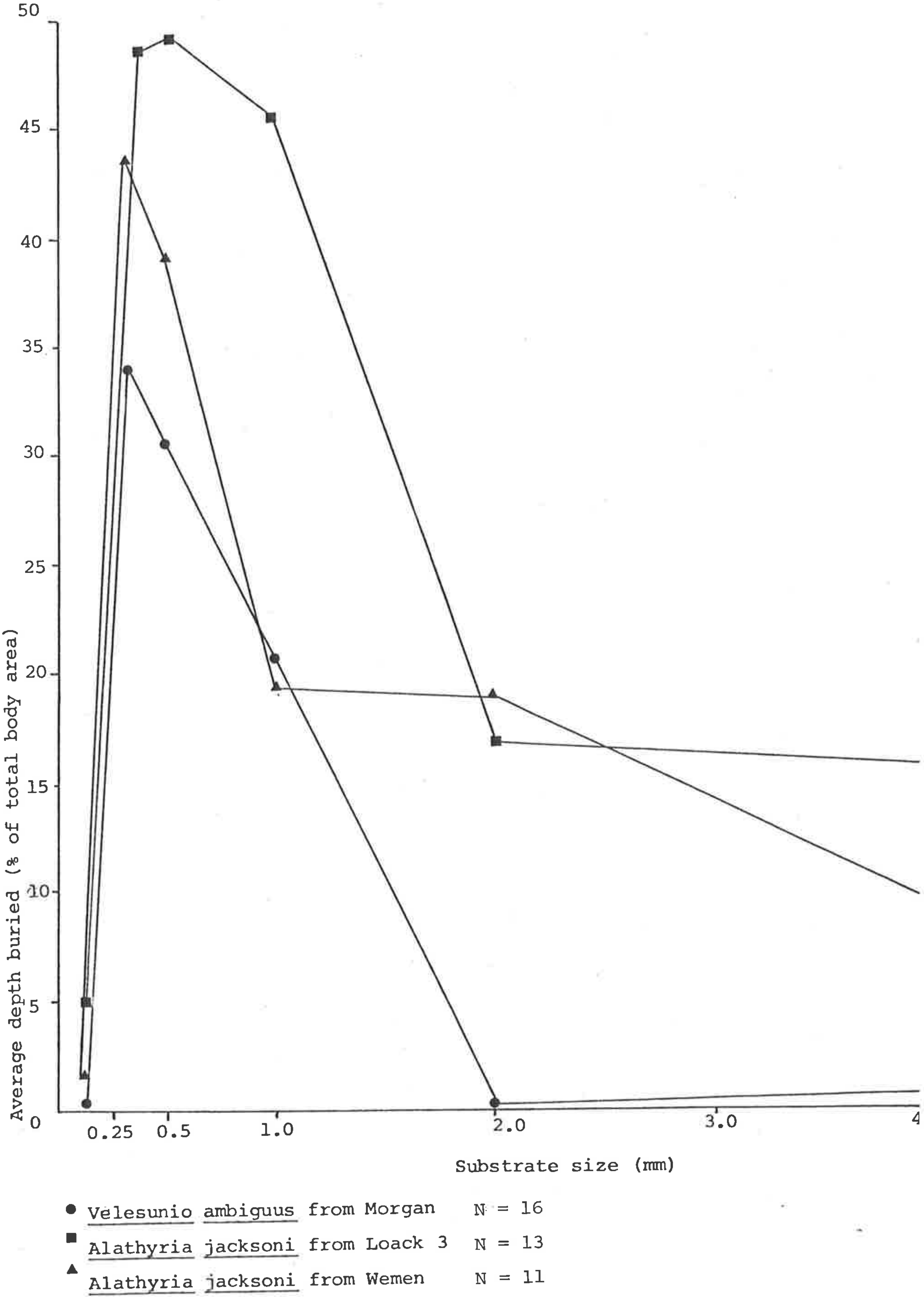
Mussels burrowed more deeply into substrates between >0.25mm to >0.5mm than in other substrate sizes (Graph 14). More mussels attempted to bury in the >0.25 to >0.5mm size classes. If the percent of mussels that attemped to bury versus the sand particle size is plotted (Graph 15)(see also Table 34), it is evident that _V. ambiguus_ and _A. jacksoni_ differ markedly. Arched _A. jacksoni_ are more proficient burrowers than winged _A. jacksoni_, and both are more proficient than _V. ambiguus_.

b. Substrate preference

Mussels tended to bury where they were placed and once buried, moved no further. No preferences were obvious, although mussels in the largest and smallest particle size classes tended to burrow up to 24 hours later than mussels on the other size classes.

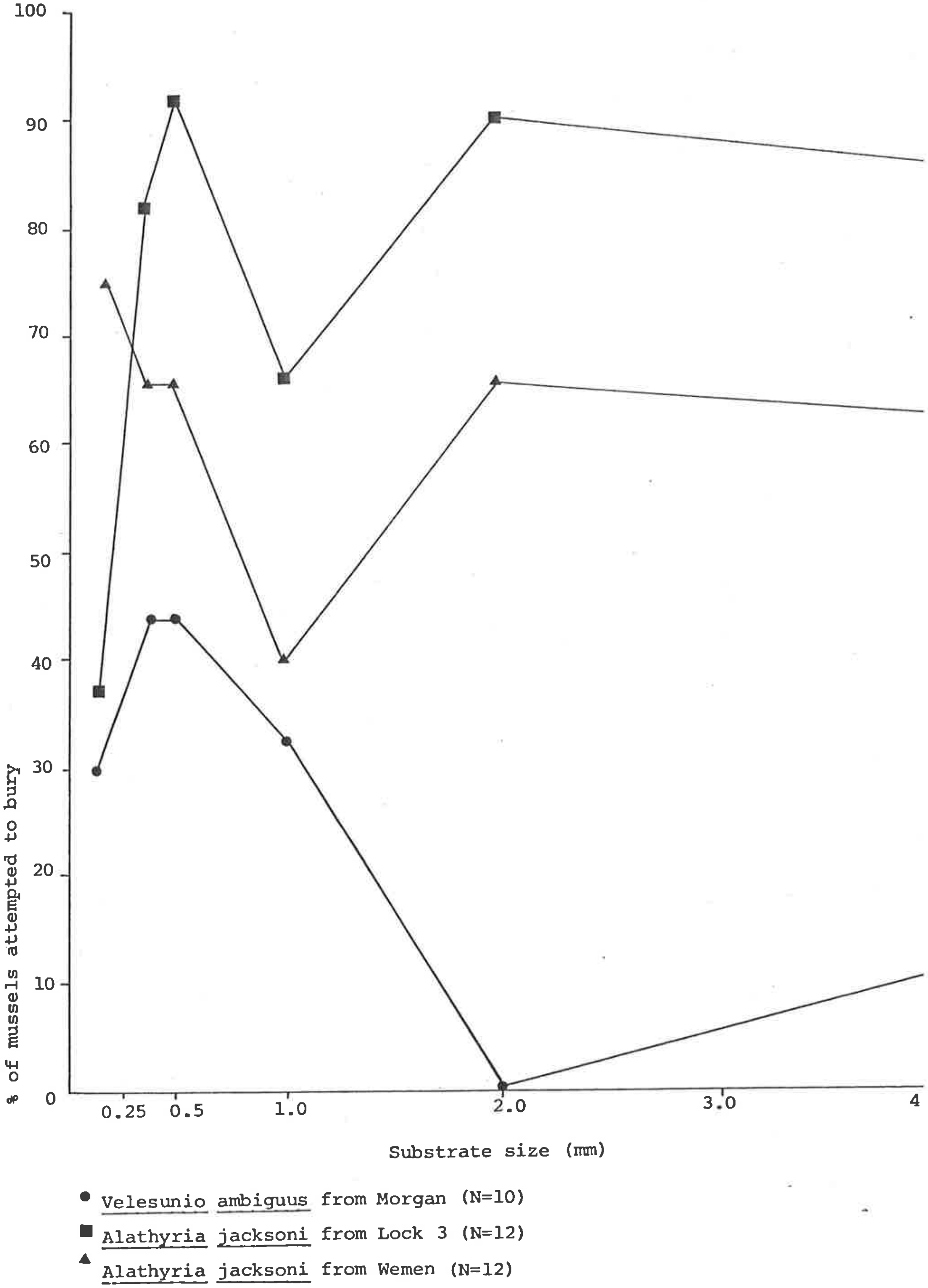
GRAPH 14.

Burrowing experiment: Substrate size vs depth buried.



GRAPH 15

Burrowing experiment: Substrate size vs percent of mussels attempted to bury.



Discussion.

Both species were able to completely bury themselves in all size classes of sand. However, burrowing was more quickly initiated in the >0.25mm to >0.5mm size classes. They were able to overcome the difficulties encountered in burrowing at each extreme of substrate size. The larger particles are difficult to penetrate because the grains

TABLE 34

Substrate	Species	Locality	N	Ave. % buried	% attempted
>4	*A. jacksoni*	Wemen	12	8.13	62.5
	A. jacksoni	Lock 3	12	15.25	87.5
	V. ambiguus	Morgan	10	0.22	11.1
>2	*A. jacksoni*	Wemen	12	18.83	66.7
	A. jacksoni	Lock 3	12	0.00	0.0
>1	*A. jacksoni*	Wemen	12	19.40	40.0
	A. jacksoni	Lock 3	12	45.42	66.7
	V. ambiguus	Morgan	10	20.56	33.3
>0.5	*A. jacksoni*	Wemen	12	39.58	66.7
	A. jacksoni	Lock 3	12	48.75	91.7
	V. ambiguus	Morgan	10	31.67	44.4
>0.25	*A. jacksoni*	Wemen	12	43.33	66.7
	A. jacksoni	Lock 3	12	48.08	83.3
	V. ambiguus	Morgan	10	34.44	44.4
<0.25	*A. jacksoni*	Wemen	12	0.50	75.0
	A. jacksoni	Lock 3	12	5.00	37.5
	V. ambiguus	Morgan	10	5.50	30.0

resisted sliding past each other. The smaller sized particles tend to pack closely and resist penetration, however they are more easily blown away by the water current produced by the contraction of the mussels valves during burrowing.

5.4.2 Water flow experiments.

a. Maximum water velocity.

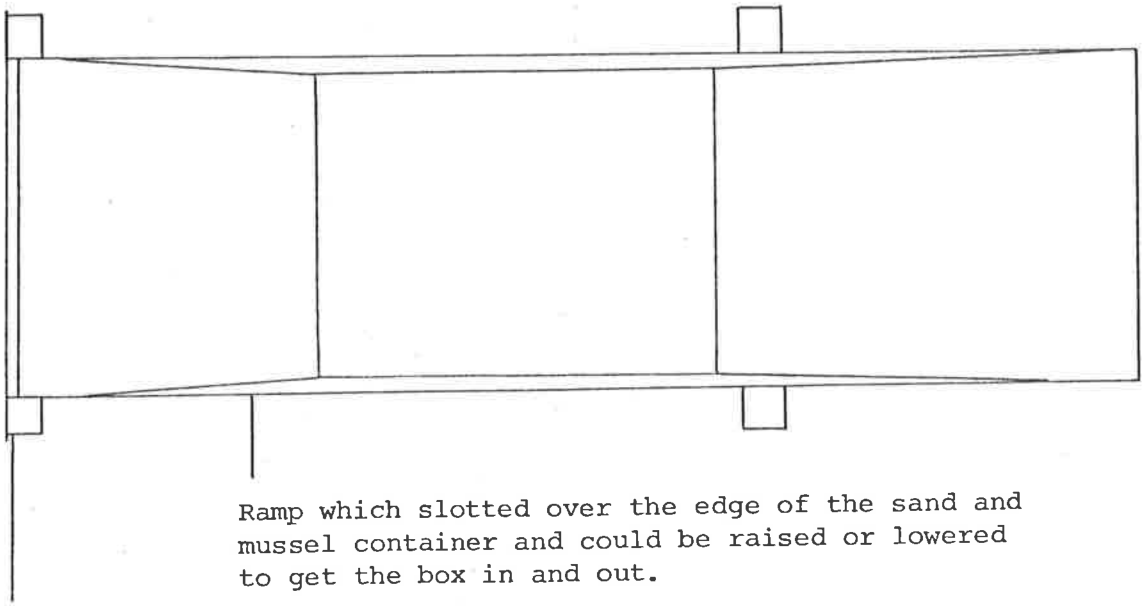
An attempt to determine the maximum water velocity needed to remove winged and arched A. jacksoni from sand was conducted using a flume in the Department of Civil Engineering, University of Adelaide. An understandable desire to keep sand out of the flume created some problems. Mussels need to burrow into sand underwater, and unless the sand was level with the top of the container in the flume, the resultant turbulence removed it. Thus, a container filled with sand was immersed in a container of water. A directional water flow to allow mussles to assume a natural orientation was created using a magnetic pump. When the container of sand and mussels was in the flume, it became necessary to minimize turbulence which scoured the mussels and sand out of the container. Turbulence was reduced by building a metal ramp in which the mussels and sand sat. The result is illustrated overleaf (Figure 19).

Results.

A current of 25cm/sec would cause a mussel lying on the surface of the sand to pivot on the swollen anterior end so that the posterior end came to lie downstream. A current of 30cm/sec was sufficient to move mussels along the surface of the sand if they were not buried. Buried mussels remained stationary while the sand downstream of them was scoured away in the turbulent wake of the mussel.

FIGURE 19

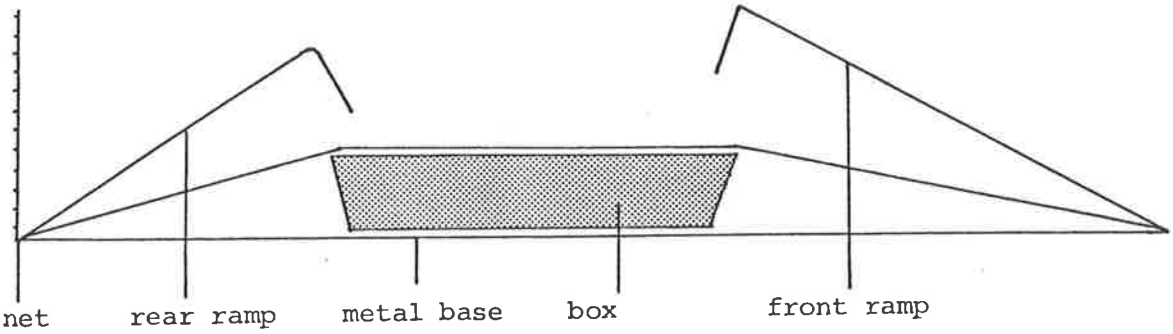
Metal ramps and supportive base from above.



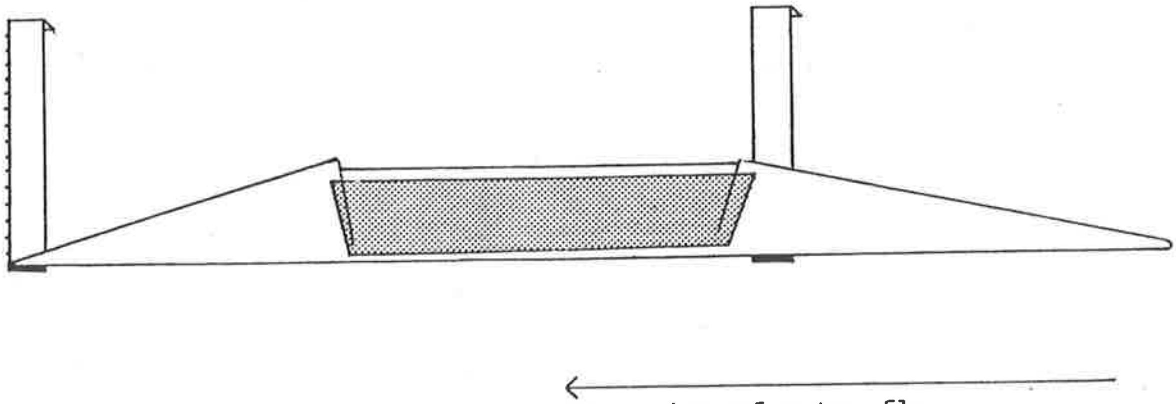
Ramp which slotted over the edge of the sand and mussel container and could be raised or lowered to get the box in and out.

Net for catching dislodged mussels with metal strips on either side to help keep the structure stationary in the flume.

Side view cross section.



The sand container was fitted in as indicated with stippling, and the ramps lowered to the height of the container as indicated below.



direction of water flow.

Discussion.

Certain problems were associated with this system. The regulation of water velocity was crude and it was not possible to increment velocity in uniform steps. In addition, the flume was 20m long, 0.5m wide with walls 0.75m high. Thus, the size of the flume and the rusty water, made it difficult to see the mussels. However, it is evident from this experiment that mussels which are buried will not be washed downstream by a current of 30 cm/sec whereas unburied mussels will. Consequently, it is important for the mussels to remain constantly buried in areas where the flow is rapid.

An examination of the patterns of dye traces injected into the water in front of mussel shells was attempted using both winged and arched A. jacksoni. A water current was created using a magnetic pump and a smooth sand substrate was used to partially bury the mussels in. Milk was stained blue with Naphthalene black and released from tapered Pasteur pipettes upcurrent from the mussels. Unfortunately, the maximum water velocity attainable with this system was only 2 cm/sec. Using this water velocity, mussels buried in their normal state (with a small protrusion of the posterior end downstream) created little turbulence. In addition, the flow pattern did not differ between growth forms. Mussels placed on the surface created maximum turbulence when placed perpendicular to the direction of flow with the dorsum facing downstream. Once again there was no discernible difference in the pattern of flow between arched and winged mussels. This system lacked sensitivity to small changes in flow patterns mainly because of its primitive nature. A more sophisticated experimental apparatus was required to allow better and more extensive investigation of turbulence patterns created by different forms.

b. Orientation experiments.

When collecting mussels it became evident that they would orient themselves to the current so that the anterior end would bury into the substrate facing the oncoming current. What could be the value of this to the mussels?

Materials and methods

A tub containing 15cm of sand and filled with dechlorinated water circulated at 15 cm/sec with a magnetic pump was filled with mussels placed about 10 cm apart. Winged and arched A. jacksoni and rounded and elliptical V. ambiguus were used. They were left undisturbed for 48 hours so that they could move and bury themselves. No's of animals; A. jacksoni 10 arched, 10 winged, V. ambiguus 10 rounded, 10 elliptical.

Results.

Between 80 and 90% of the A. jacksoni oriented themselves so that the posterior end with the siphons pointed downstream. Only 20% of the V. ambiguus oriented this way with the remainder showing no orientation preference.

Discussion.

The posterior end of the A. jacksoni is more tapered than the anterior end, and as it projects above the substrate surface it causes a small turbulent current around the siphons. This seems to aid the feeding activity of the mussels since drawing water from a rapid laminar flow requires more effort than drawing water from a turbulent flow because the lateral transport of momentum greatly augments exchange at the water-surface interface (Vogel 1981). However, there is a limit to how much turbulence can be created before scouring of the sand begins. As water velocity increases, less projection of the posterior end is needed to create the turbulence. The *A. jacksoni* usually occupy areas of the river where the current is noticeable, whereas *V. ambiguus* usually occupy areas that have no noticeable current. Consequently, *V. ambiguus* show less inclination to orient in a particular direction.

5.5 Conclusions

V. ambiguus and *A. jacksoni* appear to be proficient burrowers and are not limited in their distribution by burrowing ability although *V. ambiguus* were less inclined to attempt to burrow and usually burrowed less deeply than *A. jacksoni*. These mussels could, theoretically, bury anywhere along the river. Water velocity appears to be more important since *A. jacksoni* orient themselves in such a way as to minimze drag but still enable feeding to take place.

It should be noted that Eagar (1977) reports the orientation of *Margaritifera margaritifera* and freshwater Unionidae generally, to be opposite to that seen in *A. jacksoni*. Eagar states that these animals have their posterior ends facing upstream, or approximately so, and cites Zhadin 1939; Max B. Batteson, personal communication; and Stein in Eagar 1974, p235 in support. Additional support for Eagar came from a study of *M. margaritifera* by Roscoe and Redelings 1964:

> "Most of the clams in the bed had the siphons directed upstream, but among the transplants the clams assumed every angle fron the siphon directed toward the current to siphons directed away from the current. In eddies there is a tendency to orient the siphons toward the current"
>
> (Roscoe and Redelings 1964, p27).

Alathyria jacksoni is not unique in orienting its posterior end downstream. Illustrations of *Modiolus* and *Arca* in Kauffman (1969) show inhalant and exhalant siphons down-current. If *A. jacksoni* assumed the reverse orientation with the posterior end facing upstream, it would be hydrodynamically disadvantageous due to the creation of greater turbulence upstream of the mussel and result in scouring of the sand from beneath the mussel.

Completely buried mussels create no turbulence and would only become exposed to the current when surrounding sand was scoured away by an increase in current velocity. Presumably burrowing further into the substrate could prevent them being washed away with the surface sand.

Eagar's work does not seem to be applicable to *Alathyria jacksoni*. Perhaps this is not surprising considering the differences between the two species revealed in the experiments described in this chapter (see Table 35).

TABLE 35

Comparison of characteristics of *A. jacksoni* and *M. margaritifera*

A. jacksoni	*M. margaritifera*
1. Shell heavy 100-350g.	1. Shell light 25g.
2. Maximum shell thickness along ventral margin at anterior end.	2. Maximum shell thickness on dorsum.
3. Foot extends posteriorly of mid-ventral region.	3. Foot never extends posteriorly of mid-ventral inflection.
4. No significant difference in obesity between forms.	4. Oval mussels more obese.
5. No pedal gape.	5. "Pronounced" pedal gape.
6. Orient posterior downstream.	6. Orient posterior upstream.

Given that the shells that Eagar chose to weigh may not be representative, because of the differing distributions of weight, the differences in the way the foot extends, the pedal gape in arched *M. margaritifera* and the different orientations toward the current, these mussels are anatomically and behaviourally different to such an extent that to compare them is inappropriate.

However, the most useful aspect of examining Eagar's work is that it encourages consideration of other ways of regarding shape variation in mussels. The lack of results from Eagar's work suggests that another approach might be more productive. It might be possible that shell shape is not itself adapted to the environment, but is in fact, a consequence of anatomically mediated behavioural adaptations. In order to pursue this train of thought it is obvious that internal anatomy and the functional and behavioural aspects of these mussels must be examined.

CHAPTER 6

ELUCIDATING THE ADAPTIVE SIGNIFICANCE OF MORPHOLOGICAL VARATION

6.1 Introduction

From Chapter 5 it is evident that the direction and velocity of water flow is important to Alathyria jacksoni. Failure to remain buried during periods of rapid flow is likely to result in the downstream transportation of mussels when water velocity exceeds 30cm/sec. Such transportation would involve significant risk of possible shell damage and deposition on unsuitable substrates or burial under substantial quantities of debris. On average, it is probably better to remain in the original location.

How, then, can a mussel maintain its position in the face of rapid water velocities? Strong anchorage and an ability to rebury as the sand is scoured away from around the mussel would be distinct advantages. Burial requires strong adductors and a functional ligament. Adduction puts the ligament under strain, and subjects the fluids contained within the valves to pressure resulting in ejection of water from the mantle cavity and blood into the pedal haemocoel. Trueman (1966a) has filmed records showing that ejection of water liquifies the sand adjacent to the shell immediately prior to retraction, allowing easier penetration. In addition, the increased pressure in the foot, together with the relaxation of the transverse muscles distally, causes dilation. Dilation must occur before retraction in order to give a firm pedal anchorage so that the shell can be pulled down. Similarly, anchorage is achieved by wedging the foot in the substrate using contraction of the adductors to maintain the hydraulic pressure in the water filled foot (i.e. pedal dilation) (Trueman 1968b).

One would expect that since arched A. jacksoni occupy environments with moderate to fast water velocities they would have stronger ligaments and adductors than winged mussels which occupy environments with slower water flows. Consequently, this chapter compares ligament and adductor strength in arched and winged A. jacksoni, using V. ambiguus as a reference with which to guage the magnitude of any differences encountered.

6.2 The function of the ligament in the River Murray mussels

Ligaments in bivalves can be external, internal or both. The River Murray mussels have external ligaments which are considered to be "primitive" (Kahler et al. 1976). An external ligament is under tensile stress when the valves are closed and serves two functions. The first is to maintain the juxtaposition of the valves at the point of articulation, and the second, to open the valves during burrowing. The valves are pressed outward during the static period to produce a shell anchor against the downwards probing of the foot. The more powerful the ligament, the stronger the anchorage and consequently, the more effective the probing (Trueman 1968a). However, unnecessarily strong ligaments require a larger continuous expenditure of energy by the adductors to keep the valves closed and could be almost as disadvantageous as a ligament too weak to fulfil its function (Thomas 1976). Bivalves with weak ligaments often use additional hydraulic forces generated by pedal and siphonal retraction to open the valves when the shell is more than one third buried (Trueman 1968b). In Mya arenaria, siphonal retraction causes stretching of the adductors as the valves gape (Trueman 1966b). Although this implies that the ligament is not strong enough to open the valves fully, the ligament is still able to press the valves outwards sufficiently to effect a shell anchor. The ligament is acting as a mechanism which stores part of the energy of the adductors for use during later stages of the digging cycle (Trueman 1968a).

The strength (opening moment) and resilience (resistance to compression) of ligaments has been measured by several authors. Trueman (1951, 1953) was the first to develop techniques for measuring torque and obtaining hysteresis loops to quantify ligament efficency. Russel-Hunter and Grant (1962) studied the ligament of the burrowing bivalve *Spisula solidissima* and found that the ligament was the main mechanism of opening the shell. Kahler *et al*. (1976) found that ligament strength (opening moment per gram of shell) was distinct from resilience. There was no obvious trend relating life style to ligament strength or resilience in several species examined. Trueman (1950) found that in adult *Mytilus edulis* the ligament gradually split and ceased to function. This does not occur in mussels of the River Murray, in which a considerable amount of ligament maintenance and repair is done. This suggests that the ligament is important to these animals. Simple measurements of the ligament were recorded and examined in relation to other anatomical characters in an attempt to determine the role of the ligament in anchorage and burial in *A. jacksoni*.

6.2.1 Methods.

The anterior and posterior adductors of both *Alathyria jacksoni* and *Velesunio ambiguus* were severed with a Stanley knife by slipping it between the valves and pulling it towards the ligament. A scalpel was used to cut any remaining connections. Mussels were placed on one valve in a tray of water for 30 minutes to allow the ligament to maximally expand. The gape between the valves and the ligament length were then measured using calipers. Other characters measured were weight, shell width, height, length, anterior adductor area, posterior adductor area, separation between the adductors, adductor gape, degree of arching (using the arch-wing measurement system Chap. 3), circularity (Harmonic analysis Chap. 3), exposure of the lateral tooth, size of the cardinal tooth, depth of muscle scars and ligament thickness.

6.2.2 Results.

Measurement of arching using the arch-wing system resulted in the selection by the computer of three variables for the discrimination. These were crest length (CRSTLN), the distance from the ligament to a line drawn dorsoventrally connecting the ends of the ligament (MAXDIS) and height. Discriminant scores were used as a measure of arch and wing development in the correlations. It should be noted that MAXDIS measures the size of the dorsal arch, while CRSTLN measures the size of the wing (Refer to Figure 16 in Chapter 3). Thus the individual effects of these two characters are examined separately when appropriate in the analysis in addition to the discriminant scores themselves. Both arched and winged populations of Alathyria jacksoni showed similar ligament lengths (arched $\bar{x}$=82.77, max 96.1mm, min 66.1mm, winged $\bar{x}$=80.32, max 94.9mm, min 65.8mm) and ligament gapes (arched $\bar{x}$=15.49, max 21.0mm, min 10.0mm, winged $\bar{x}$=14.75, max 25.0mm, min 9.8mm). These differed significantly from the V. ambiguus population (ligament length; $\bar{x}$=55.39, max 65.7mm, min 44.6mm, ligament gape; $\bar{x}$=11.86, max 16.5mm, min 9.2mm). While V. ambiguus had a range of gapes that encompassed most of the A. jacksoni gapes, the ligament lengths were smaller because the *V. ambiguus* have smaller shells.

Velesunio ambiguus from Lock 2.

As mussels graded from small ligament gapes to larger gapes, the mussels became more circular in outline, the size of the cardinal tooth increased and the ligaments became thinner and shorter (Graph 16).

Alathyria jacksoni from Lock 2.

As the length of ligaments increased, ligament gapes decreased (Graph 17), mussels showed a tendency to become more arched and more circular and the amount of lateral tooth exposed decreased. There was also a deepening of the muscle scars. As gape decreased the ligament became thicker.

Alathyria jacksoni from Wemen.

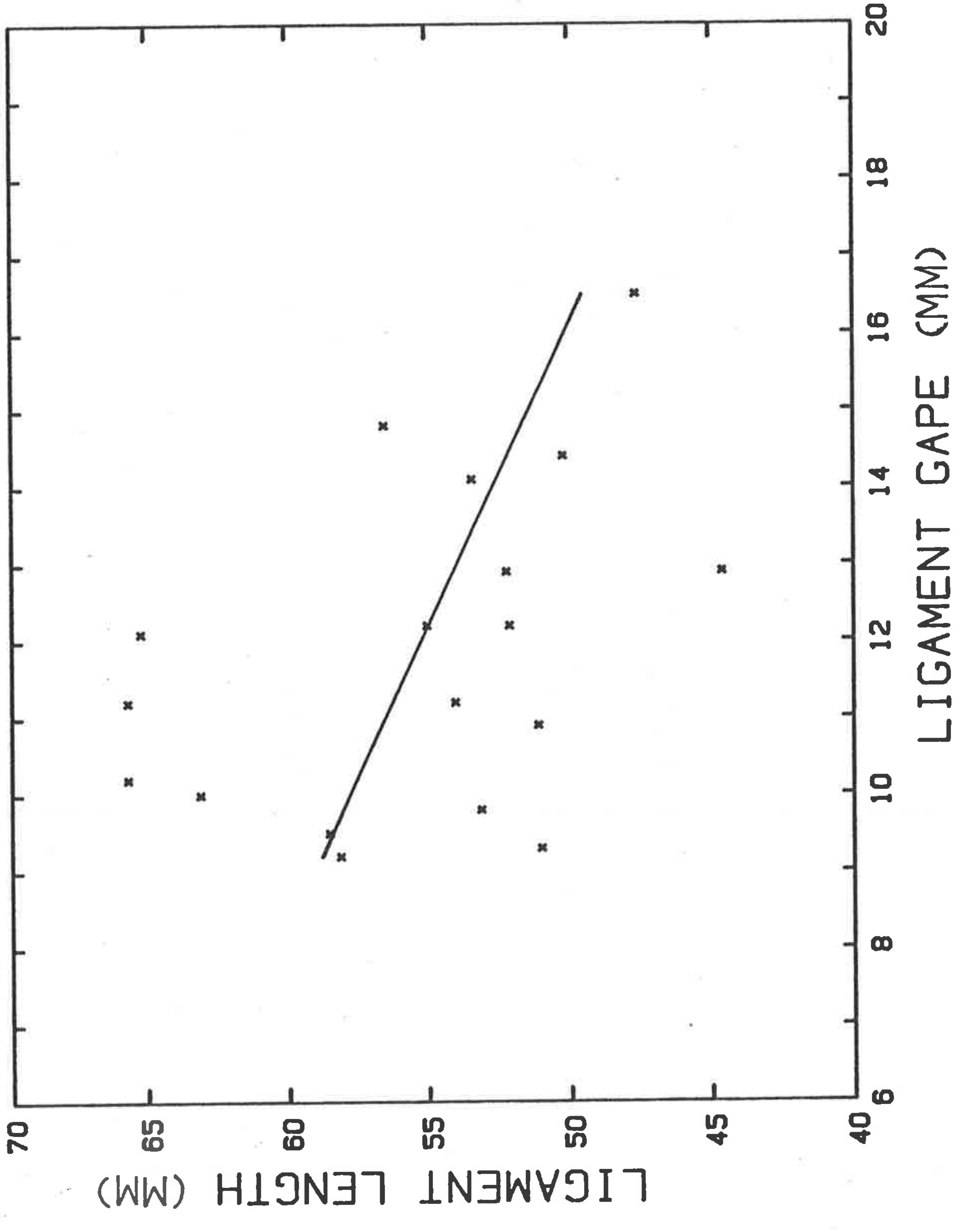
As ligament length increased, gapes decreased (Graph 17), arching (MAXDIS) became more pronounced (Graph 18), and the lateral tooth became more exposed. As gape increased, the animals tended to become less arched (MAXDIS) (Graph 19) and the thickness of the ligament increased.

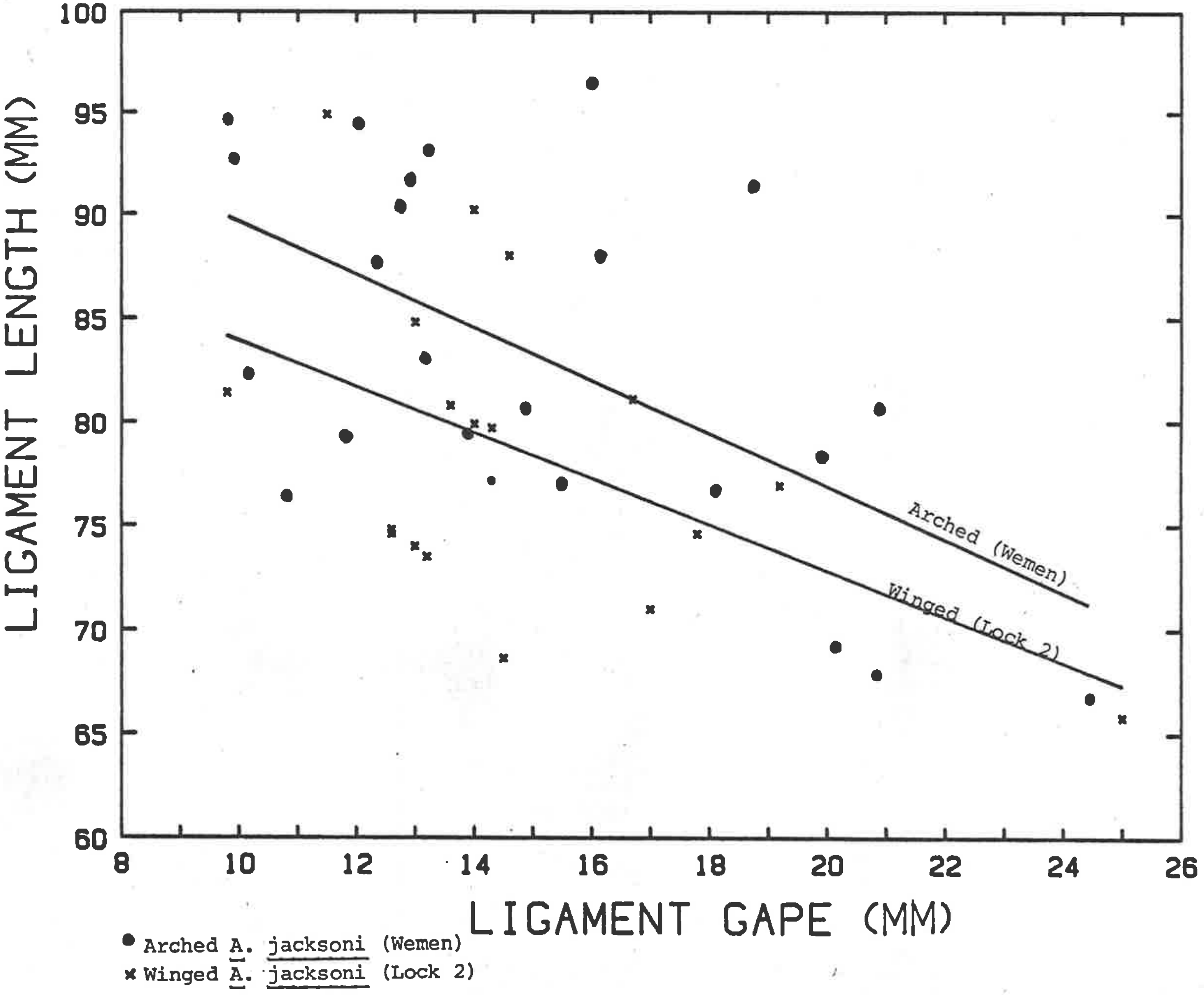
Discussion.

The ligament operates most efficiently in a straight line and a longer ligament should be stronger than a shorter one. A larger gape means a stronger ligament. In *V. ambiguus*, as ligament length decreases, gape increases, indicating that shorter ligaments are more efficient for this species. As gape increases the mussels also become more circular. The circular mussels have a straighter dorsal margin with a relatively abrupt tapering towards the posterior end, compared with more elliptical

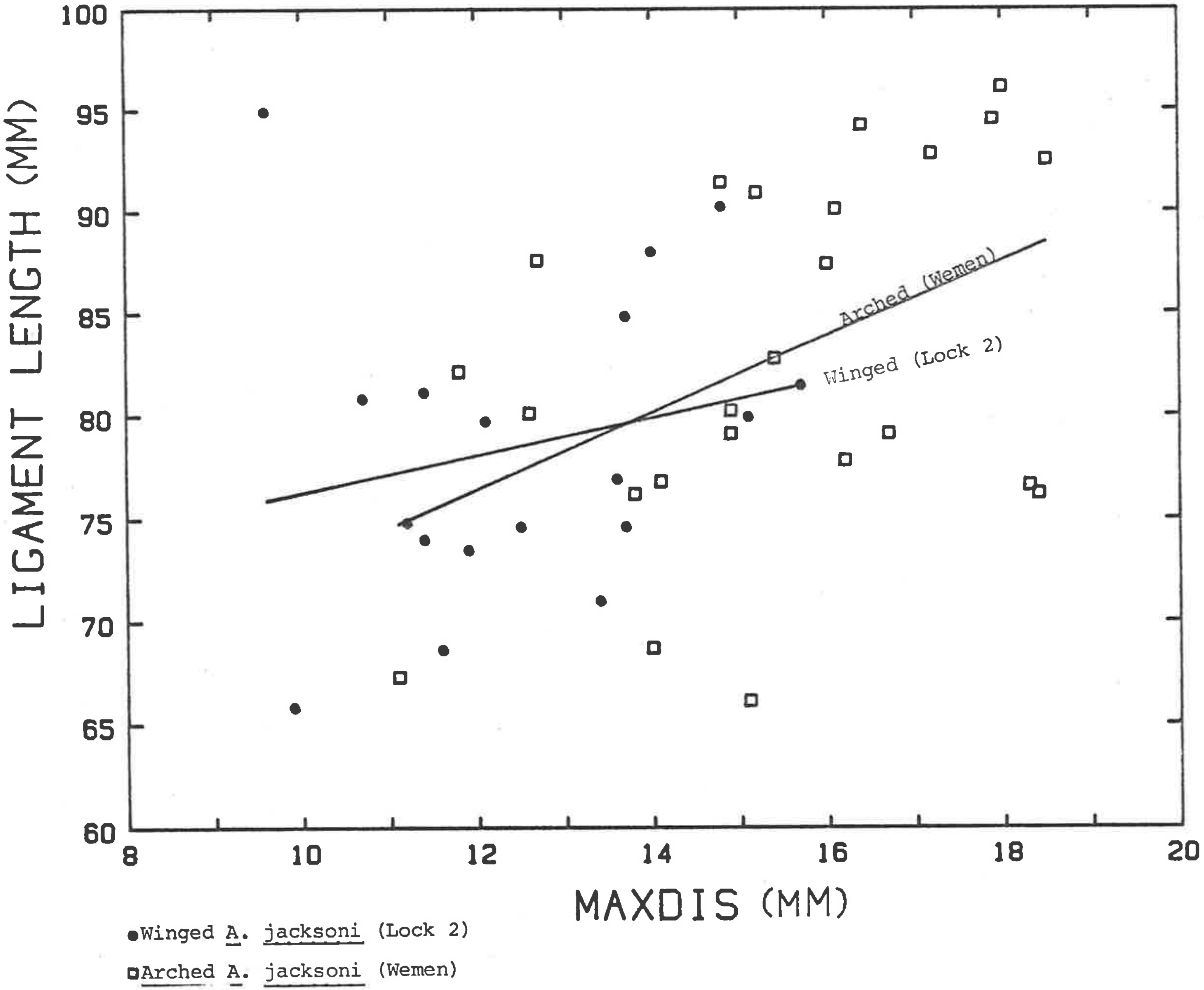
GRAPH 16

<u>Velesunio</u> <u>ambiguus</u>: Ligament gape vs ligament length.



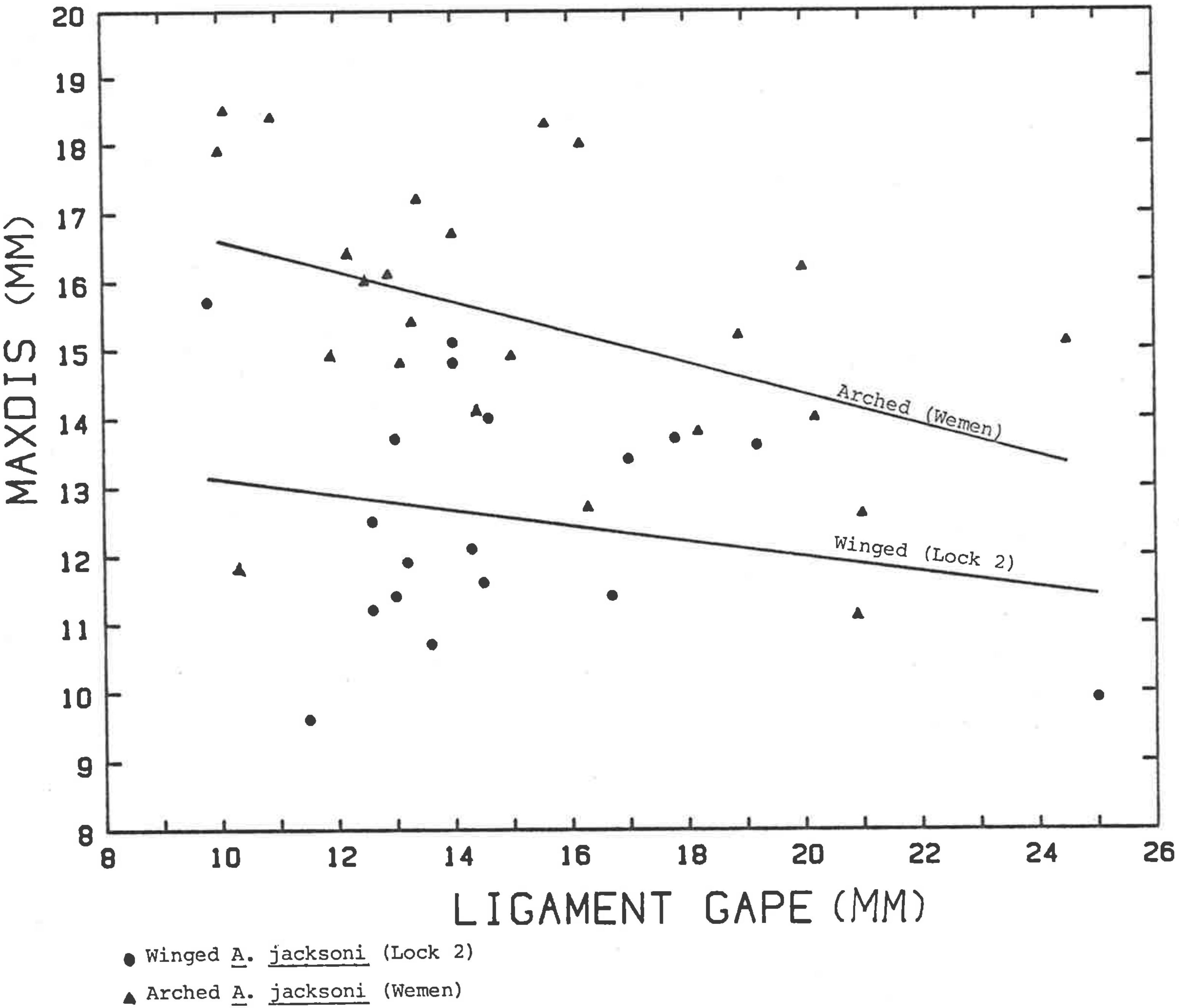


GRAPH 17
Alathyria jacksoni: Lock 2 and Wemen populations, Ligament gape vs ligament length.



GRAPH 18

Alathyria jacksoni: Lock 2 and Wemen populations, MAXDIS vs ligament length.



GRAPH 19

Alathyria jacksoni: Lock 2 and Wemen populations, ligament gape vs MAXDIS.

V. ambiguus. Therefore, these rounded mussels have straighter and stronger ligaments than elliptical mussels. The ligament in the elliptical mussels binds the valves together at the outer edges and thus, restricts gape.

Both populations of Alathyria jacksoni showed similar tendencies. Once again, mussels with longer ligaments had smaller gapes. This was a consequence of the tendency of the mussels with longer ligaments to be more arched. This trend was most striking in the Wemen population, since they exhibit extremes of arching. The Wemen mussels had thicker ligaments due to repeated deposition and repair in response to the tensile stress on the curved ligament. It is evident that the ligament is actively maintained and is important in burrowing. Neither V. ambiguus or A. jacksoni is a particularly rapid burrower, but they are by no means weak burrowers. Some hydraulic mechanisms (e.g. pedal retraction) are probably employed to assist in burrowing.

6.3 The function of the adductors in River Murray mussels

The mussels of the River Murray have dimyarian adductor muscles - that is, muscles that are equal or almost so (Kauffman 1969). The adductors function primarily to close the valves, and through prolonged contraction, to keep them closed against the tensional and compressive forces of the ligament. The muscles are divided into two parts called the catch and quick portions which are composed of non-striated and striated muscle components repectively. The quick portion is adapted for rapid contraction and closing of the valves and functions as a defense mechanism, as a means of cleaning the mantle cavity and valve surface and in burrowing. The catch portion is designed to keep the valves closed against the force of the ligament and is capable of sustained contraction (Wilkie 1968).

The placement of the adductor muscles is critical. They function most efficiently when they are situated to directly oppose forces placed on the shell by the ligament. The size of the adductor muscles and the relative distribution of striate and non-striate tissues are general indications of their strength and can be interpreted to varying degrees from the nature of the muscle insertion area (Kauffman 1969).

Examination of the adductors of *V. ambiguus* and *A. jacksoni* reveals several similarities and differences, the consequences of which can be elucidated by experimentation. For both species, the posterior adductor is composed of approximately equal proportions of striated and non-striated muscle delineated by a vertical line through the longest axis. The adductors of *A. jacksoni* are larger than those of *V. ambiguus* in accordance with greater shell size. However, the posterior adductor is disproportionately elongate in *A. jacksoni*. Within these species there is a considerable amount of variation in size, shape and positioning of adductors. Quantification of this variation along with a measure of arching (using the arch-wing measurement system Chap. 3) may reveal a relationship between shell shape and the mechanics of these muscles.

6.3.1 Methods.

As a measure of the strength of the adductors, a piece of plastic tubing was inserted between the valves of individual *A. jacksoni* and *V. ambiguus*. This tubing was hollow with a diameter of 16.0mm which could be compressed to a minimum diameter of 4mm. The compressive force was correlated to the diameter using a spring balance (Graph 20). The gape between the valves was measured at intervals of 15 minutes for one hour and then at 30 minute intervals to record the change in gape over time. The entire experiment was conducted out of water so that the mussels would be more inclined to close the shell to prevent dehyration.

The area of the adductors was then measured by severing them with a Stanley knife, opening the valves and recording the longitudingal and horizontal diameters of the anterior and posterior adducors. The measurements were converted to radii and incorporated into the formula for obtaining the area of an oval πab where a and b are radii.

The separation between the adductors was measured with calipers from the middle of the inner edge of each muscle. Arching was measured by taking the six measurements used in the arch-wing measurement system described in Chap. 3. The arch-wing measurement system was selected for this experimental work because it specifically focused on arching and wing developments, required relatively few measurements and could be used on live animals. While the Fourier analysis showed promise, it would have required further modification and involved many measurements for each mussel. The arch-wing measurements were then analysed using a discriminant analysis program to obtain discriminant scores.

Each of these measurements, along with records of weight, shell width, height and length were taken simultaneously with measurements on the ligament described earlier.

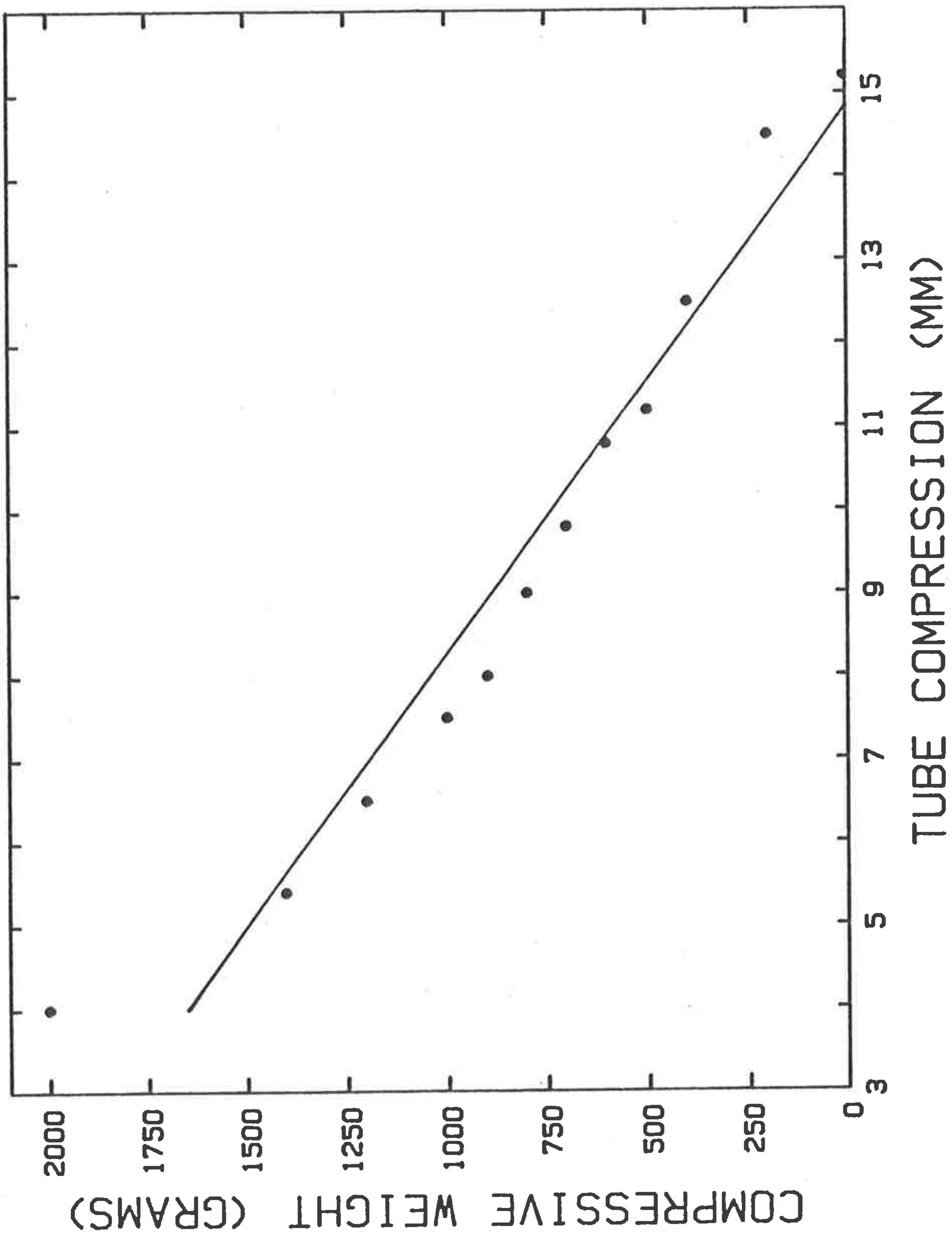
Results.

Velesunio ambiguus from Lock 2

To avoid confusion, the gape between the valves produced by the adductors closing on the tubing is called the adductor gape. The gape between the valves produced by expansion of the ligament after the

GRAPH 20

Mechanical compression of plastic tube from which pressure values can be calculated.



adductors are severed is called ligament gape. No apparent correlation existed between adductor gape and height, length and width of the shell. However, adductor gape versus weight showed a negative correlation (N=20, r_s=-0.4289, critical value -0.45, p 0.05) which was not statistically significant (Graph 21). The combination of all four characters to give the "size" of the animal showed a smaller negative correlation, hence weight must play an important role with lighter mussels having relatively stronger adductors. Adductor gape was also negatively correlated with adductor area (Graph 22) and, in fact, the anterior adductor area made a greater contribution than the posterior area (N=20, r_s=-0.4613 critical value -0.48) (Graph 23). Anterior adductors are etched into the shell, while the posterior adductors are not. There was no correlation betwen adductor gape and the separation between adductors, shell size or circularity.

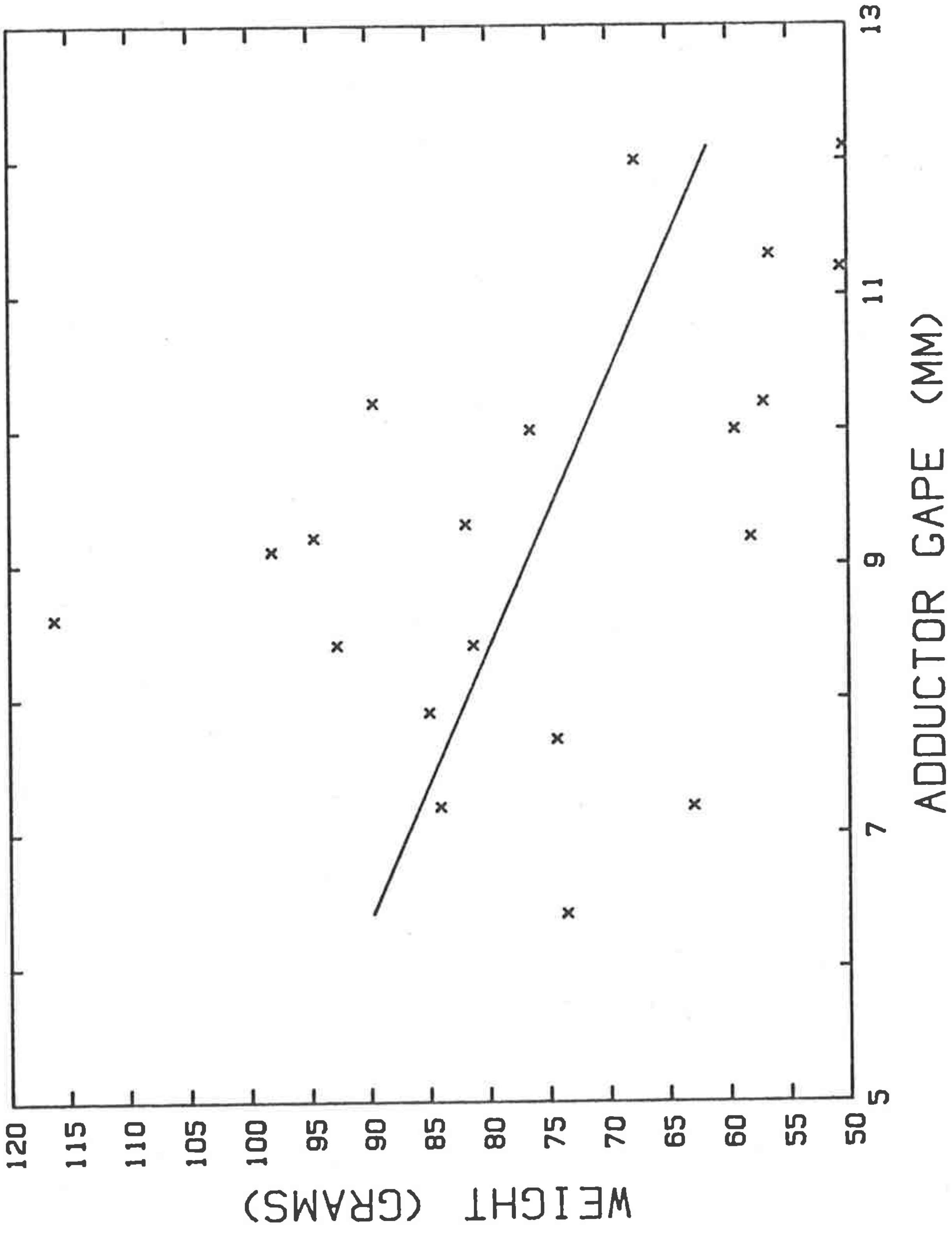
Adductor area was positively correlated with the size of the muscle scar, but was not related to shell size or circularity. Separation between adductors was positively correlated with shell size.

Alathyria jacksoni from Lock 2

Adductor gape was not correlated with height, width, length, weight or size. However, there was a statistically significant correlation (N=18, r_s=-0.7028, p 0.01) with combined adductor area (Graph 24) so that mussels with smaller gapes had larger adductors. Both anterior and posterior adductors were contributing equally and when the effect of each muscle was considered individually, correlations were not statistically significant. There was no correlation between adductor gape and the separation between the adductors. Adductor gape and arching (MAXDIS) were positively correlated (Graph 25; combines arched and winged populations) so that larger gapes were seen in more arched mussels. Adductor area was positively correlated with the size of the

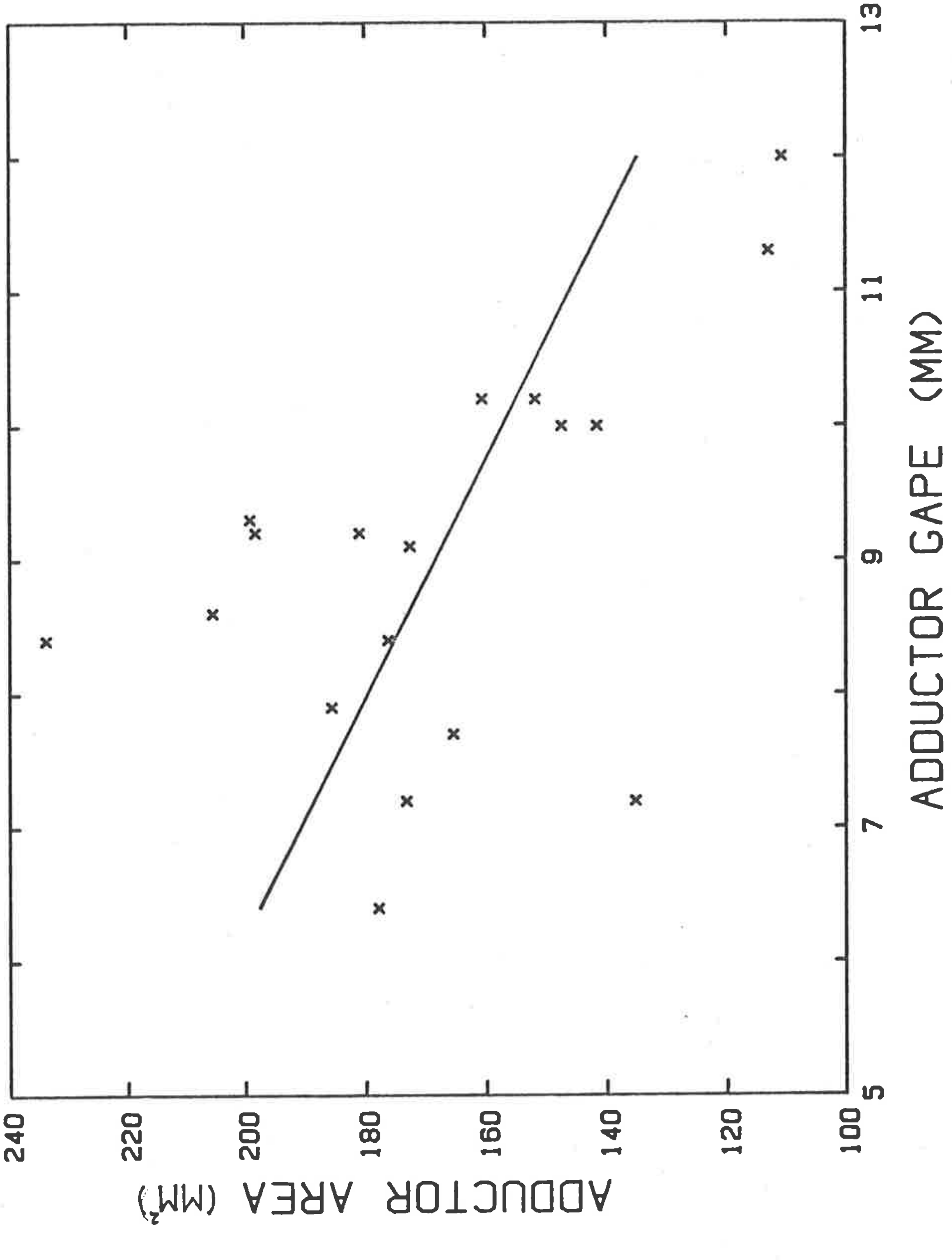
GRAPH 21

Velesunio ambiguus: Adductor gape vs weight.



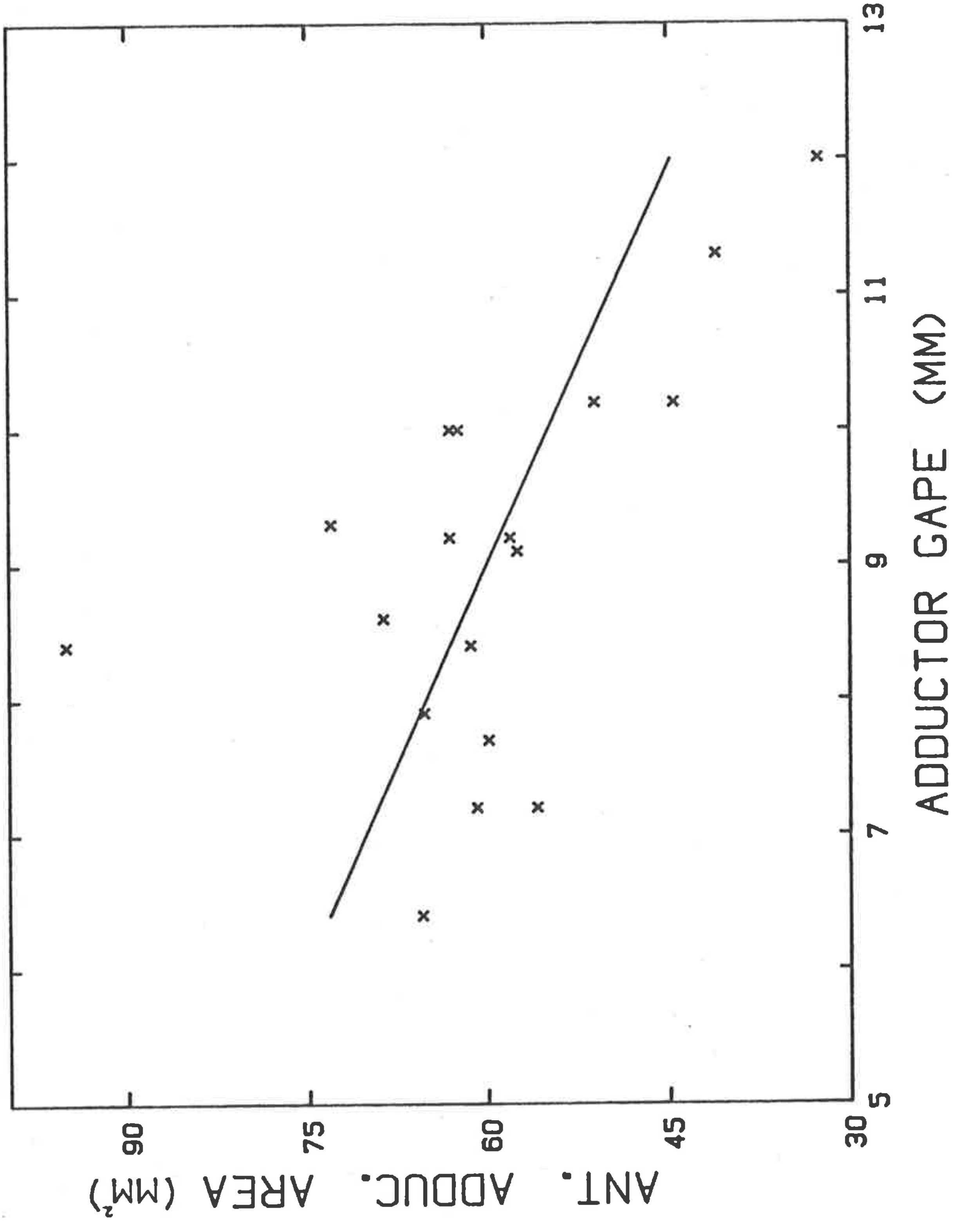
GRAPH 22

Velesunio ambiguus: Adductor gape vs total adductor area.



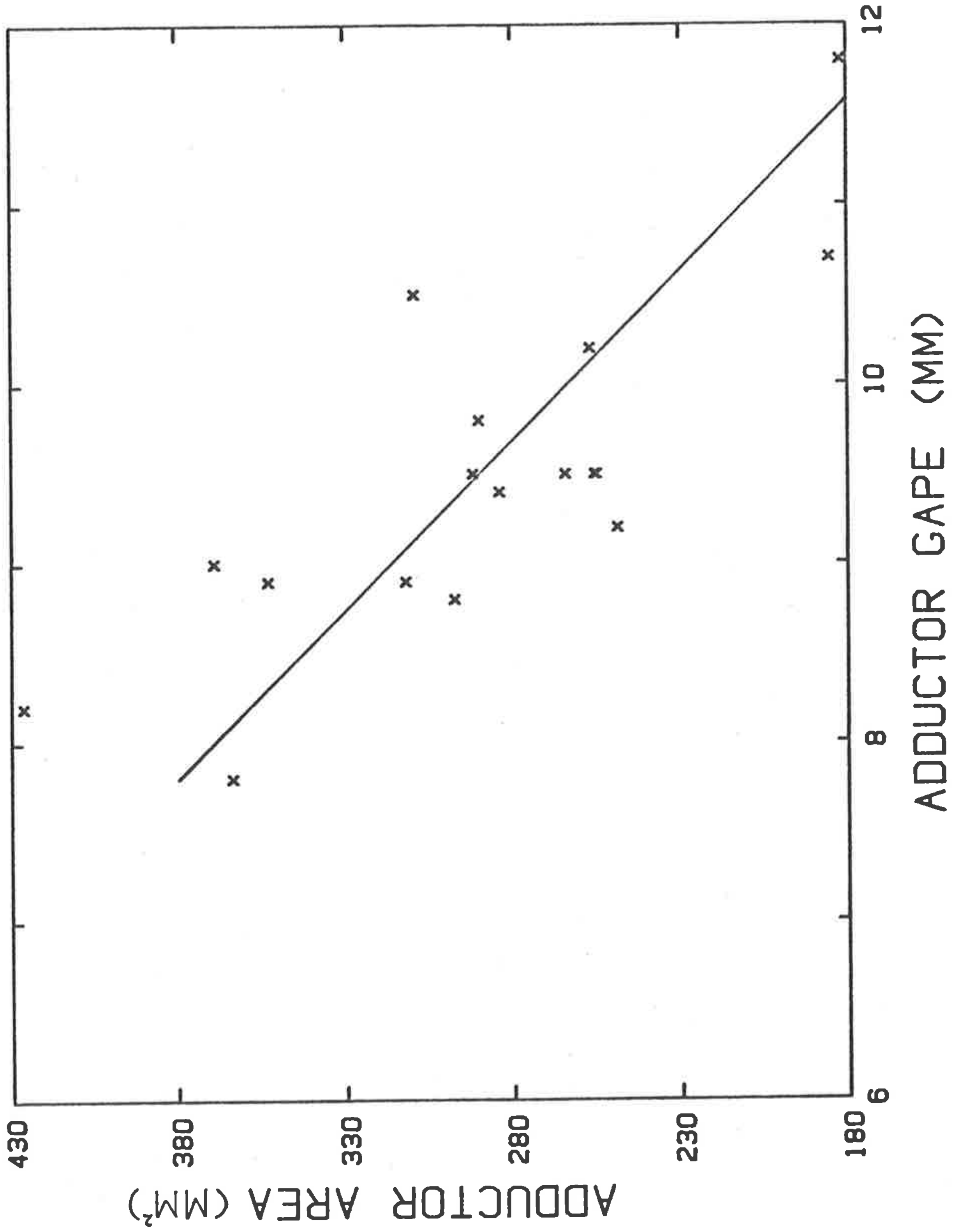
GRAPH 23

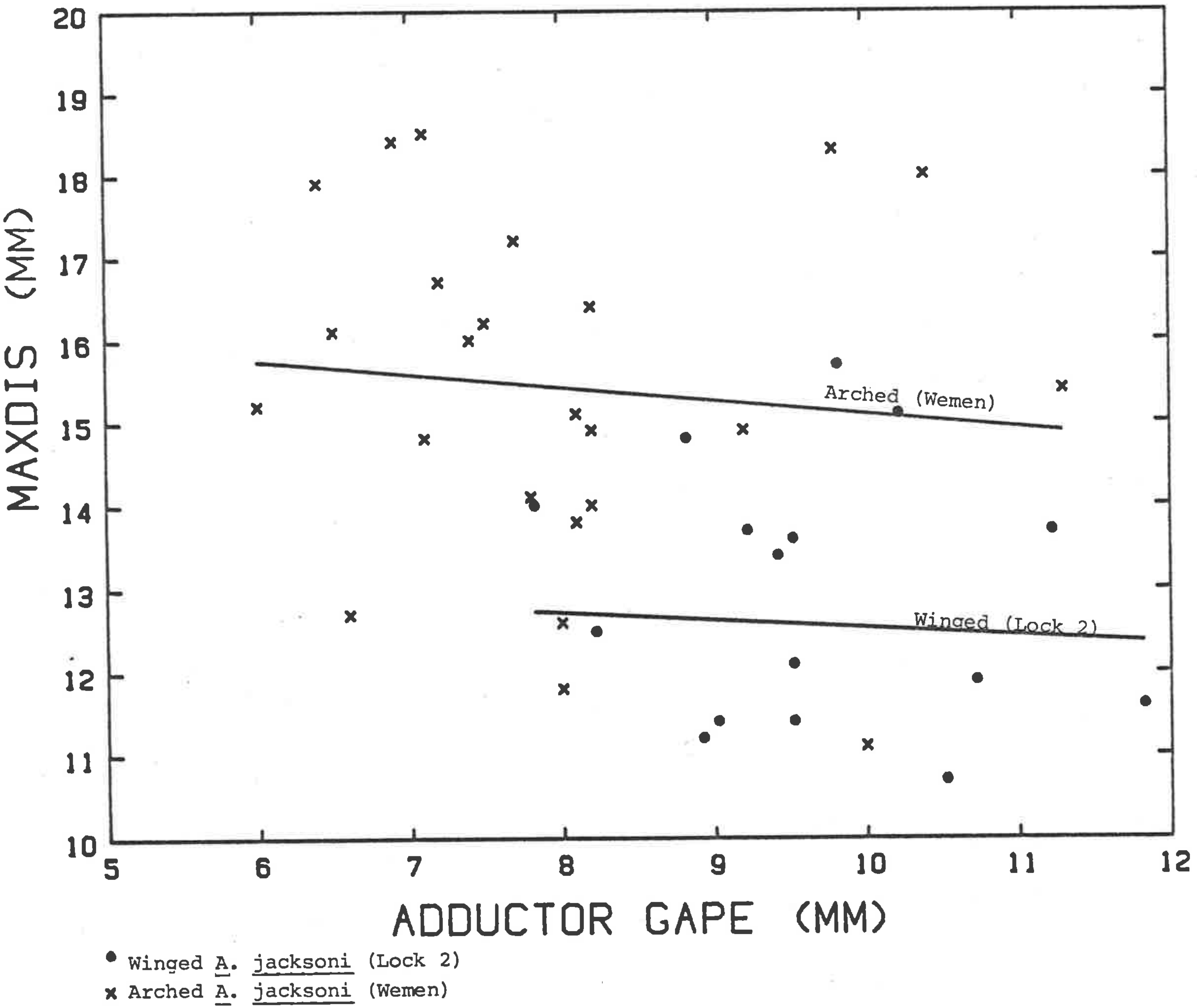
Velesunio ambiguus: Adductor gape vs anterior adductor area.



GRAPH 24

Alathyria jacksoni: Winged from Lock 2, adductor gape vs total adductor area.





GRAPH 25
Alathyria jacksoni: Lock 2 and Wemen populations, Adductor gape vs MAXDIS.

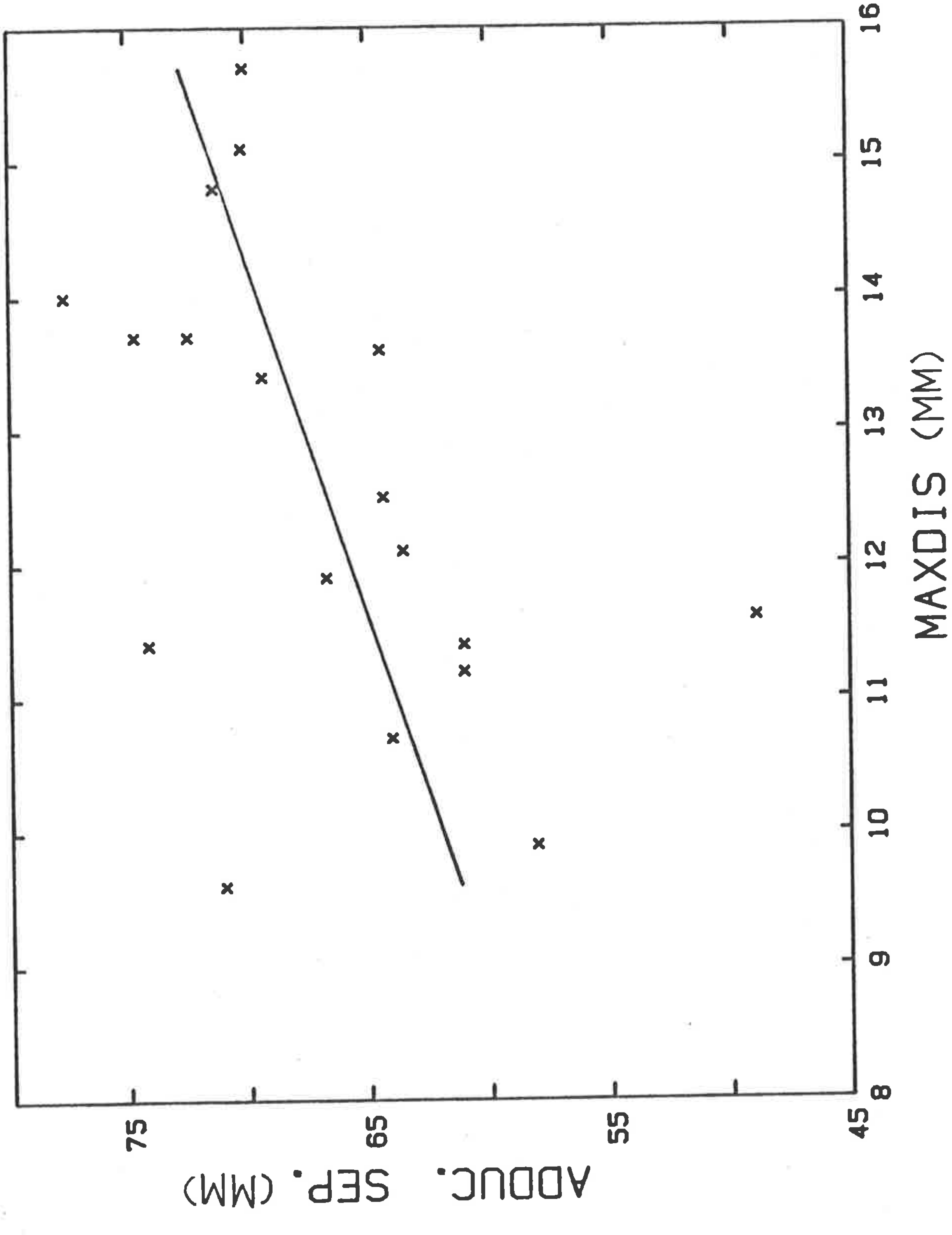
muscle scars and those with small areas had more deeply etched muscles. Adductor separation showed a non-significant postive correlation with size, but it was sometimes obvious that the shell length was not in proportion with position of the adductors which were placed in from the edge of the shell. There was no correlation between adductor separation and depth of muscle scars. A non-significant positive correlation of adductor separation and arching (MAXDIS) existed (Graph 26), thus there was greater separation in the arched mussels.

Alathyria jacksoni from Wemen.

Adductor gape was not correlated with shell height, but with length, weight and size there was a minor negative correlation. Width and adductor gape were negatively correlated, but it was not statistically significant. There was no apparent relationship between adductor gape and adductor area for anterior, posterior or both combined adductors (Graph 27). However, adductor gape and separation were negatively correlated (N=24, r_s=-0.3698 critical value -0.41) (Graph 28) where mussels with smaller gapes had adductors further apart and length of shell played some role. On average, the Wemen population had smaller gapes than the other populations. Mussels with the smallest gapes had flattened ventral margins or ventral inflections. Adductor gape was negatively correlated with arching (MAXDIS, Graph 25), so animals with smaller gapes were more arched. Mussels with larger gapes tended to have more deeply etched adductor scars. The adductor area was reflected in the size of the scars, but was unrelated to their depth. Mussels with smaller adductor areas were more elongate, but adductor area was not related to the size of the shells and there was a non-significant correlation of separation with arching (Graph 29), mussels with larger separations were more arched.

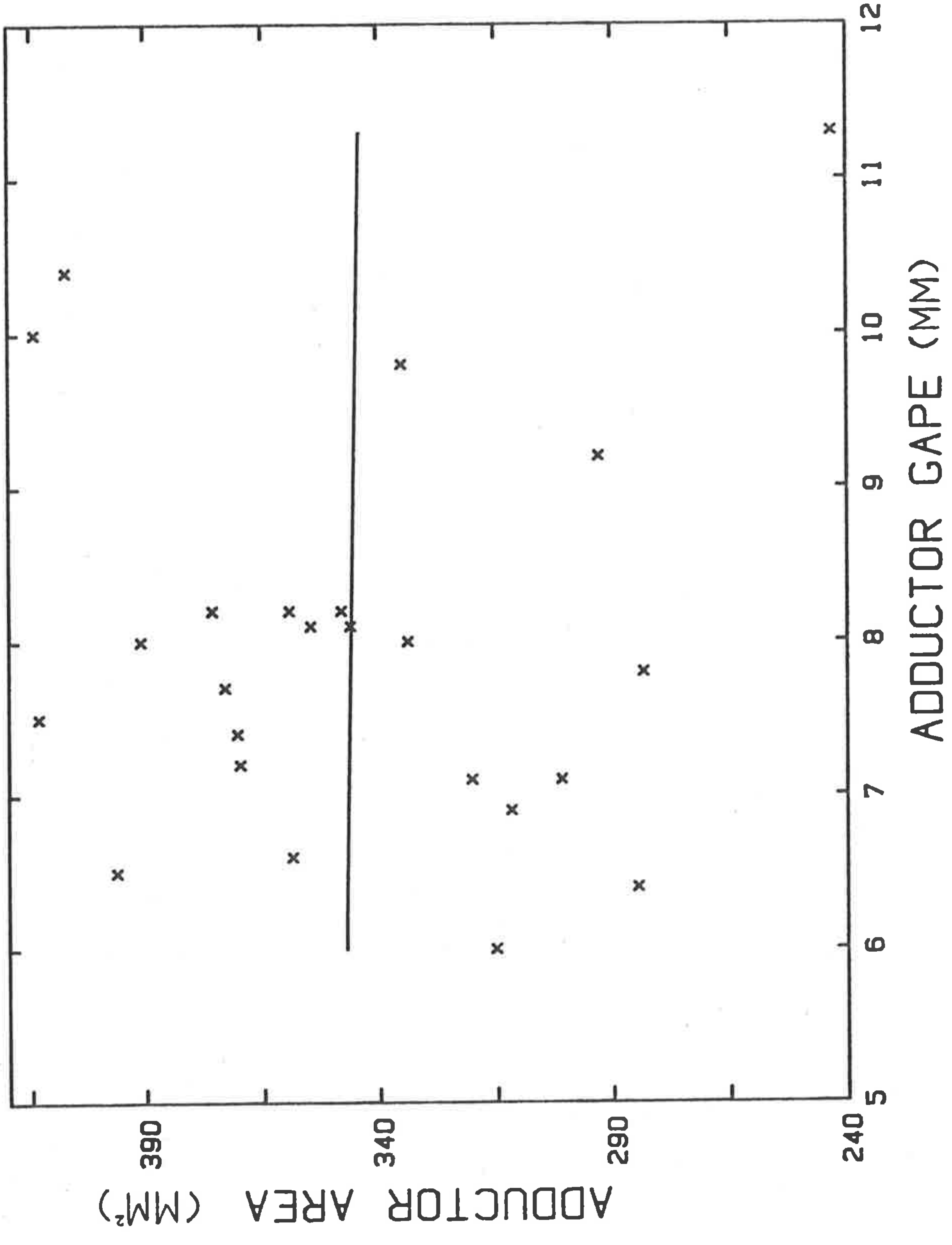
GRAPH 26

<u>Alathyria jacksoni</u>: Lock 2, MAXDIS vs adductor separation.



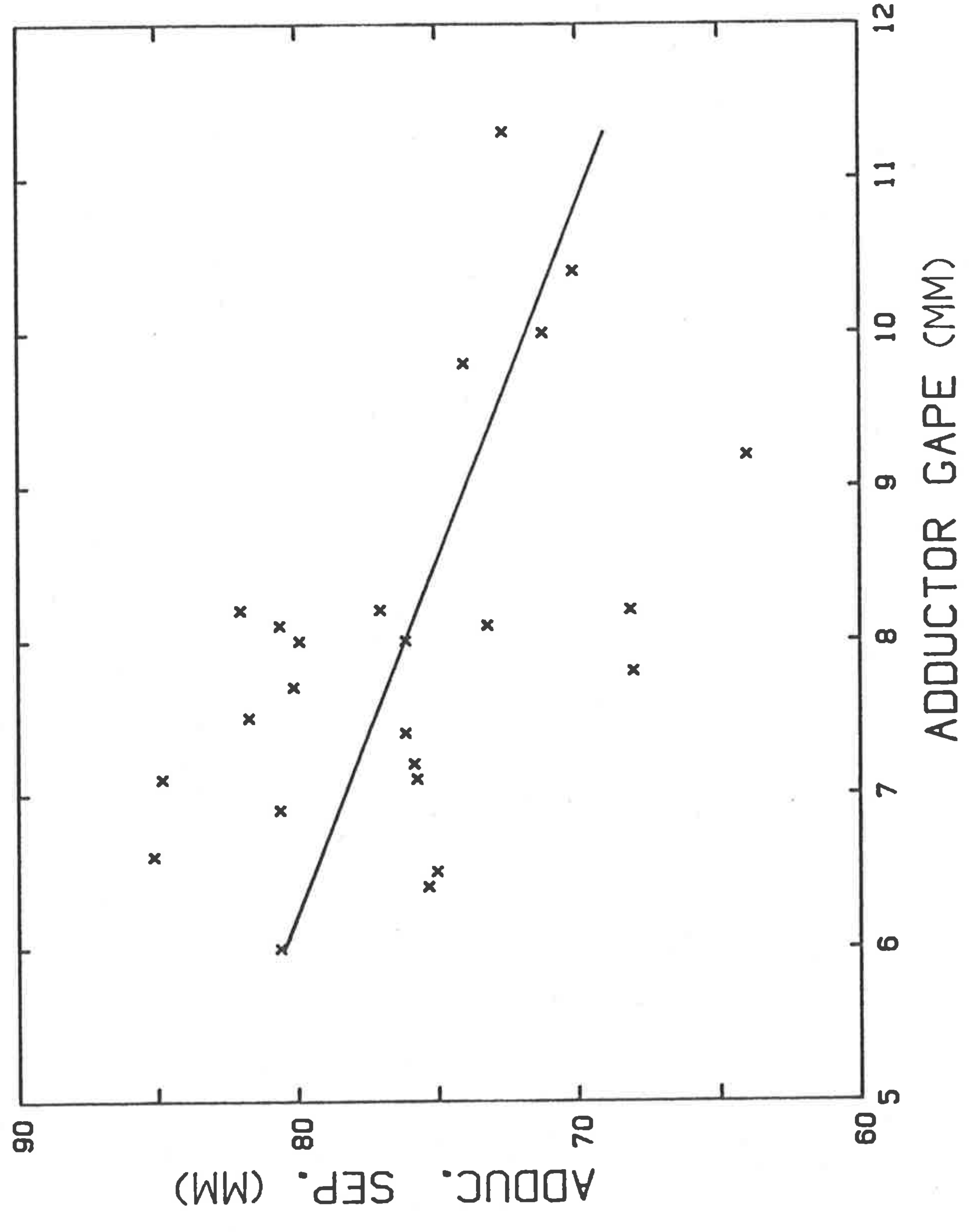
GRAPH 27

Alathyria jacksoni: Wemen, adductor gape vs total adductor area.



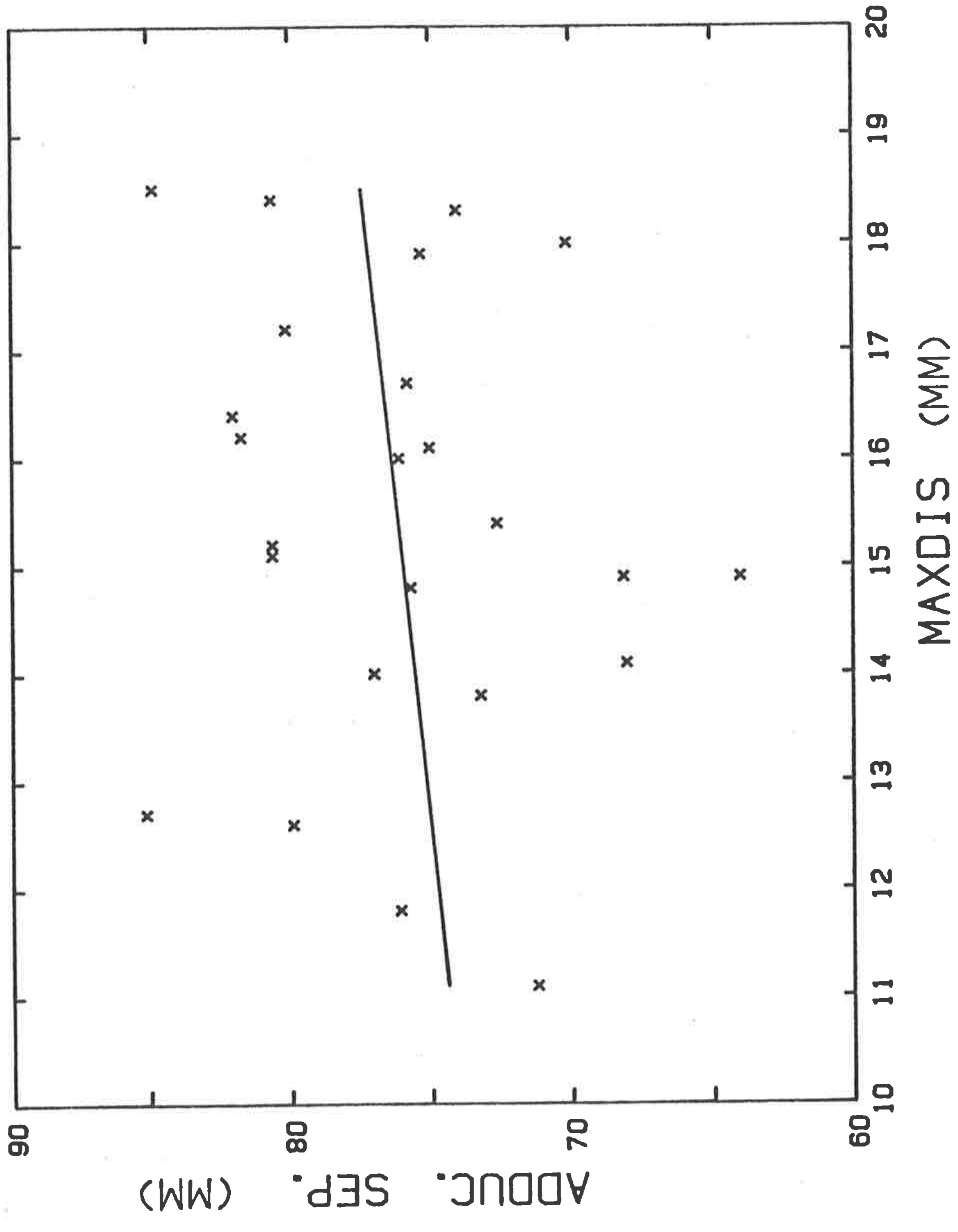
GRAPH 28

Alathyria jacksoni: Wemen, adductor gape vs adductor separation.



GRAPH 29

Alathyria jacksoni: Wemen, MAXDIS vs adductor separation.



Discussion.

The range of pressures recorded were from 9.81×10^3 Pa (adductor gape 14.5mm) to 1.26×10^5 Pa (6.0mm) with the maximum possible pressure where the tube is fully compressed to 4.0mm 1.96×10^5 Pa not attained. The pressures obtained have been treated as a relative index of the strengths of the adductors. The pressures exerted by each mussel were almost constant over long periods of time. There was no indication of change after 4.5 hours, and no observed decrease due to fatigue. Longer periods were avoided to prevent excessive dehydration.

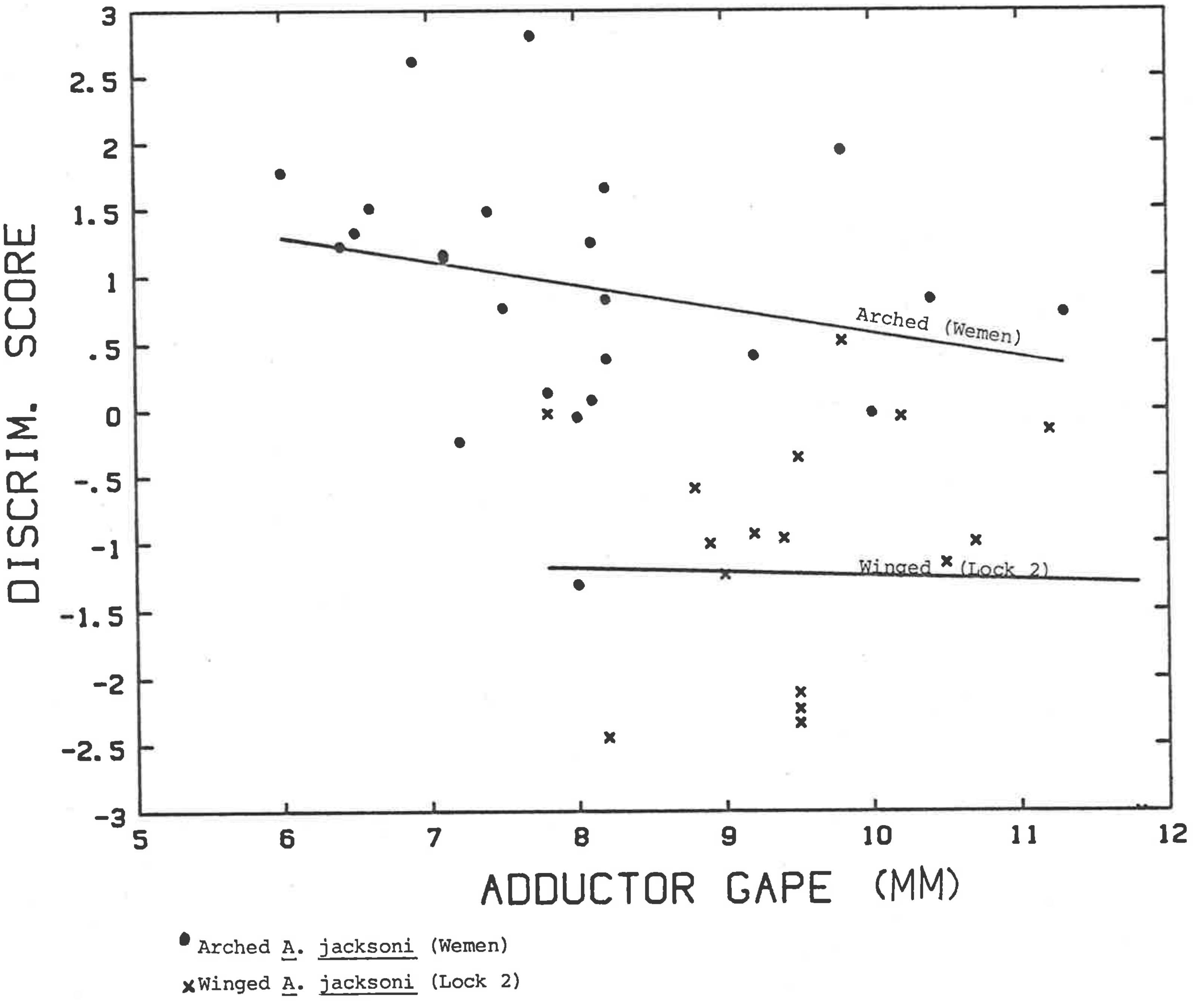
The Lock 2 *V. ambiguus* showed a fairly even distribution over widely ranging pressures (3.92×10^4 Pa to 1.18×10^5 Pa). The heavier mussels had stronger adductors and the larger the anterior adductor, the more pressure was exerted. The posterior adductor seemed to play only a minor role.

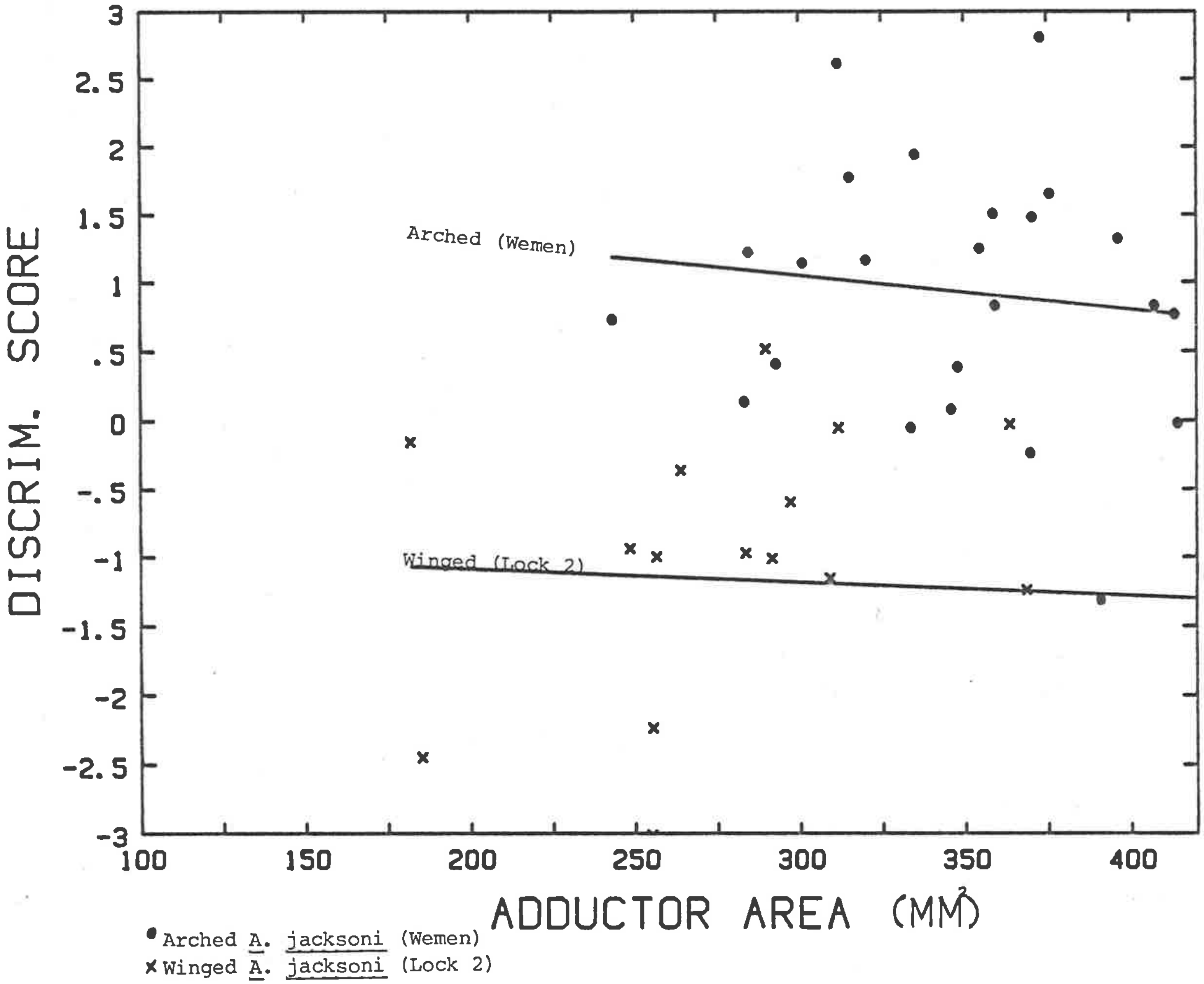
A. jacksoni from Lock 2 had a minimum pressure of 4.0×10^4 Pa and a maximum of 9.81×10^4 Pa with the median of 4.41×10^4 Pa. For this population the primary means of increasing adductor strength was to increase the size of the adductors.

The *A. jacksoni* from Wemen has pressures which ranged from a minimum of 4.02×10^4 Pa to a maximum of 1.27×10^5 Pa with the median around 9.41×10^4 Pa; on average, twice the strength of the Lock 2 population. Graph 30 shows that while no significant trend between discriminant scores and adductor strength occurs in either population, combination of the two populations shows a distinct increase in adductor strength as arching increases (negative discriminant scores indicating winged mussels and positive discriminant scores arched mussels). The same is true of the trend displayed in Graph 31. As mussels change from winged to arched, the adductor area increases. In the Wemen population,

GRAPH 30

Alathyria jacksoni: Lock 2 and Wemen populations, adductor gape vs discriminant score.





GRAPH 31

Alathyria jacksoni: Lock 2 and Wemen populations. Total adductor area vs discriminant score.

adductor strength was not related to the area of the adductors. Animals with stronger adductors had greater separation of the adductors and the length of the mussels was independent of adductor separation. Mussels with greater arching had stronger adductors and greater separation between the adductors. The posterior adductor is also elongated ventrally, further from the ligament. The adductors are performing two important functions, one is to oppose the ligament and close the shell, the other is to expand the extended foot by closing on it, thus increasing the hydraulic pressure in the foot and wedging the mussel in the substrate. For both functions, separation of the adductors and shifting them towards the ventral edge increases the muscles' power without increasing the work done. Whereas the Lock 2 *A. jacksoni* are subject to slower flows, and can attain sufficient adductor power by expanding the area of the adductors, this is not sufficient for those mussels which inhabit faster flowing parts of the river. The Wemen mussels have already expanded their adductors to the maximum possible area given the room available to them, and have moved the adductors ventrally to attain the necessary power required to anchor the mussel in the substrate (see Figure 20). Graph 32 shows that while the degree of wing development (measured as CRSTLN) is positively correlated with the area of the posterior adductor in winged *A. jacksoni*, there is no such correlation in arched *A. jacksoni*. Thus, winged mussels accommodate the posterior adductor with the wing and achieve sufficient power by expanding the posterior adductor area.

How is it that adductors are so plastic in their positioning? It may be a consequence of the mode of growth of mussels. Shell growth occurs at the edge of the mantle with the successive secretion of the periostracum and nacre (Wilbur 1964). In *A. jacksoni*, shell growth

GRAPH 32

Alathyria jacksoni: Lock 2 and Wemen populations, posterior adductor area vs CRSTLN.

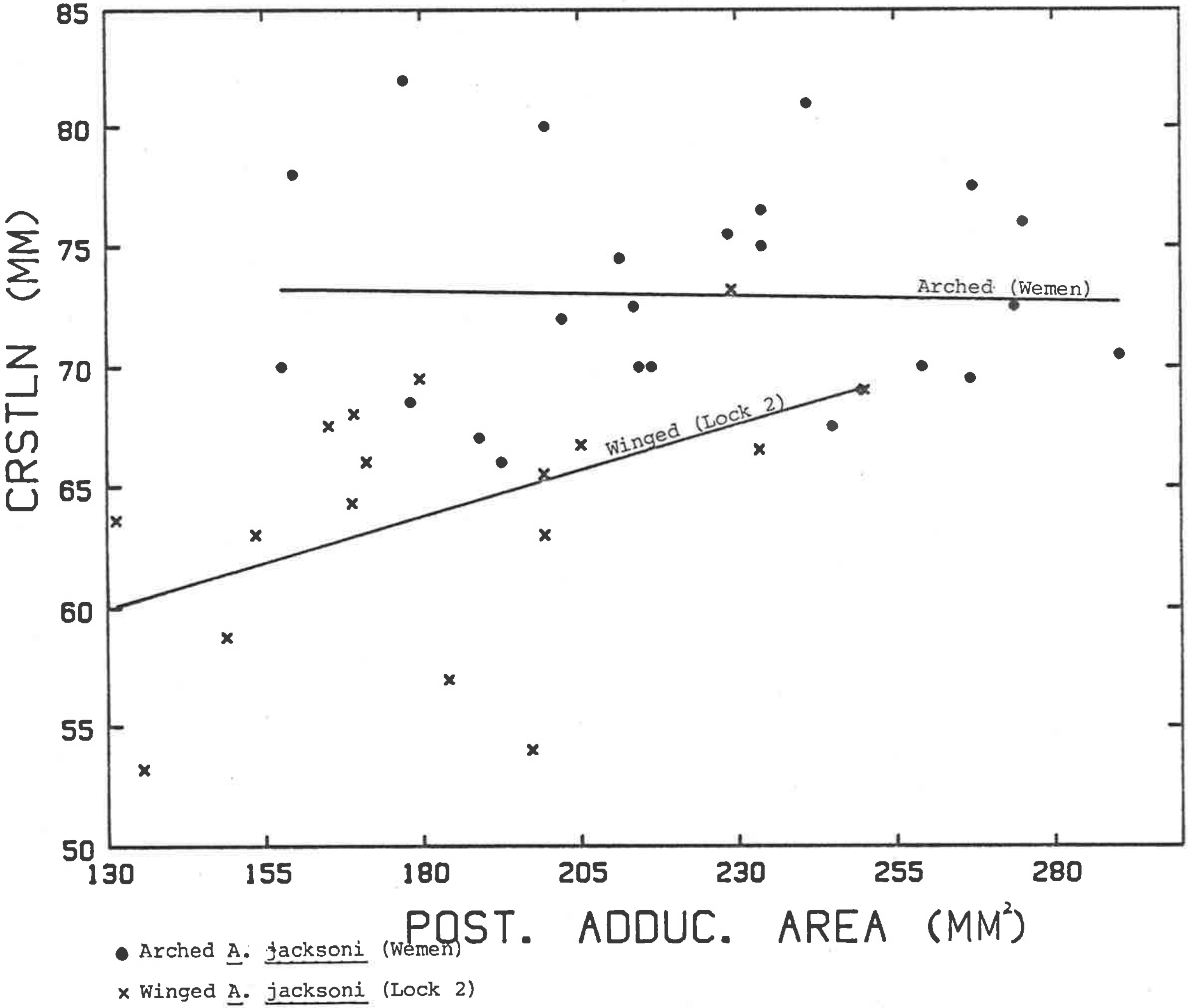
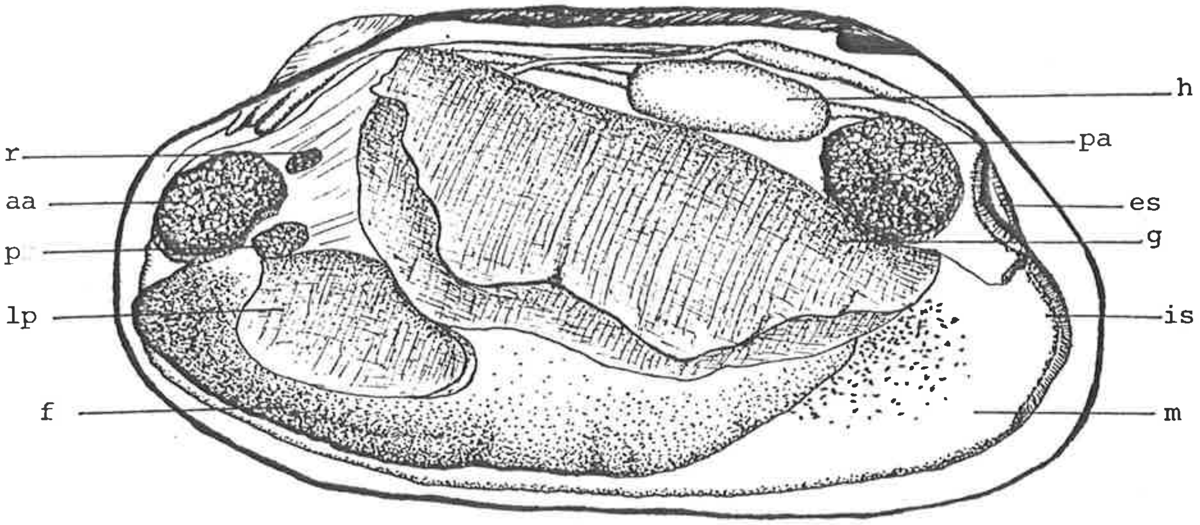


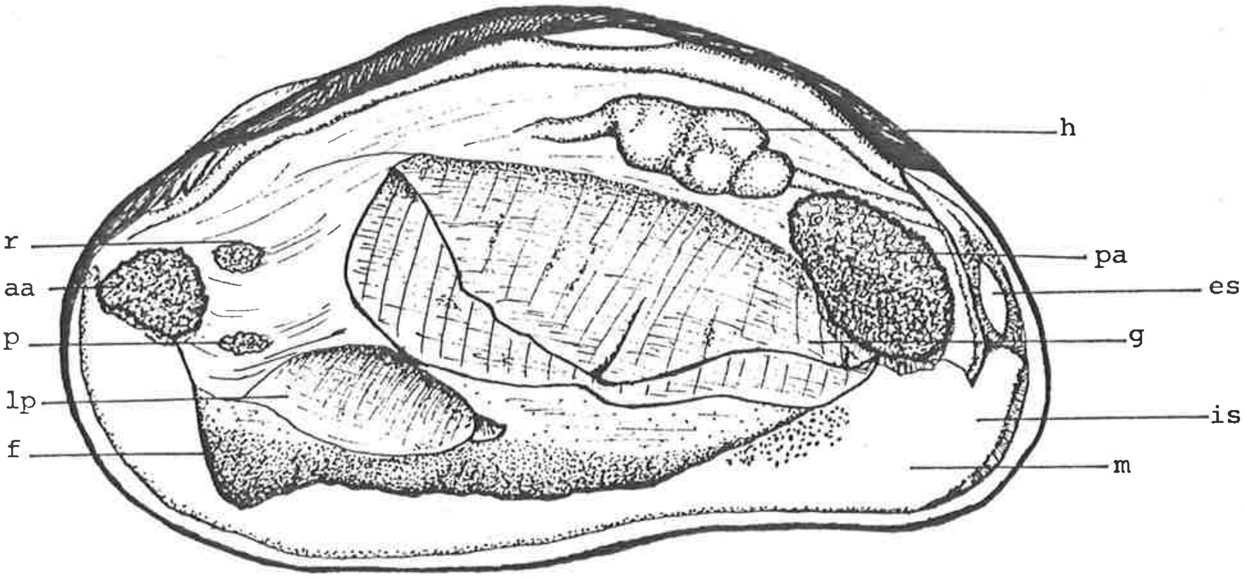
FIGURE 20

Dissection (mantle removed) showing the difference in the size and position of posterior adductors in arched and winged *A. jacksoni*



Winged *A. jacksoni*

Female (closely spaced water tubules in gill), aa=anterior adductor, pa= posterior adductor, r=retractor, p=protractor, es=exhalent siphon, is= inhalent siphon, m=mantle, g=gills, h=heart, lp=labial palps, f=foot.



Arched *A. jacksoni*

Male (widely spaced water tubules in the gill). Abreviations as above. Note the enlarged posterior adductor in the arched form.

occurs when the mantle is fused to the edge of the shell and if pulled loose, has a fringe of black periostracum attached to it. In addition a substantial quantity of water is found between the mantle and shell while growth is occurring. As the shell size increases the internal organs enlarge. The pallial muscles of the mantle and the adductors, retractors and protractors are inserted on the shell and are gradually moved in concert with shell growth. The pallial muscles maintain their position near the edge of the shell by moving toward the edge as successive rings of shell are deposited. The adductors (particularly the posterior) must move from their juvenile positions near the umbo to the anterior and posterior ends of the shell. This is achieved by building on muscle fibres at the leading edge and allowing muscle fibres on the inside edge to degenerate, thus allowing continuous function of the adductors during growth. In view of this manner of adductor growth, the placement of the adductors is already quite plastic and will be governed by mechanical requirements dictated by the environment. Thus, where the adductors must be strong, muscle fibre becomes more abundant and the deposition of new fibre occurs ventrally to increase contractive power.

6.4 Conclusions

In the arched *A. jacksoni*, the comparatively ventral positioning of the adductors has consequences for the shape of the shell. The mantle lies directly over the internal organs and secretes the shell. Thus the form of the body is reflected in the shape of the shell. The size and postioning of the posterior adductors in *A. jacksoni* influences the shape of the posterior part of the shell. A mussel from a slow flowing environment has a relatively small rounded posterior adductor positioned close to the hinge-ligament. The mantle lying over this adductor secretes a shell that assumes a winged shape. A mussel from a moderate to high water velocity environment has maximized adductor strength by enlarging it, elongating it ventrally, and locating it further from the hinge-ligament. These features are achieved during the growth process of a mussel. The mantle overlying this posterior area of the mussel consequently secretes the shell to assume an arched shape.

CHAPTER 7

CONCLUSIONS

This study originated from the observations of Walker (1981) on shell shape variation in the mussels of the Murray-Darling river system. It was assumed that these variations had some adaptive significance, and the primary aim of this project was to understand the adaptive significance of this morphological variation, particularly in *Alathyria jacksoni*.

It was inappropriate merely to assume that these 'growth forms' (and those of *V. ambiguus* and *A. condola*) were variants of each species. Hence, electrophoretic investigations were undertaken to elucidate the roles of genetic and environmental influences on shell shape. Within each species, the apparent 'growth forms' were not electrophoretically distinct. It appeared that the environment was responsible for inducing gross shell shape variation such as arch and wing development, while genetics was involved in the more subtle irregularities of shape. This confirmed that the various 'types' were indeed variants of the nominate species. The electrophoretic data for both *V. ambiguus* and *A. jacksoni* in the lower River Murray indicated that there was an unrestricted gene flow between populations despite the numerous locks and weirs. In addition electrophoresis yeilded valuable information on the distributions of speices. Mussels from below Lake Mulwala, Mulwala Canal and Callistemon Point were all one species, based on the three major enzymes selected for detailed examination. They were not, however, identical to *A. jacksoni* from the middle and lower Murray. Banding patterns and certain morphological characters were most similar to *A. condola* from Darlington Point. The tentative conclusion that they were "non-excavate *A. condola*" was drawn, subject to further investigation. These animals are easily isolated throughout this thesis and feature prominently only in chapter 3. If they are found not to be *A. condola*, the conclusions drawn for *A. jacksoni*, *A. condola* from Darlington Point and *V. ambiguus* remain independent. The electrophoretic data also suggested that *A. condola* was extending its distribution downstream from the Murray-Murrumbidgee cofluence at least as far as Wemen. All of these individuals were young and may have originated from the Murrumbidgee. This also illustrates the potential of electrophoresis as a taxonomic tool.

Measurement of shape played an important role in familiarization with the extent of variability. Initial perceptions of shell shape variation were quite volatile. Comparing measurement systems identified consistent trends in shell shape: those environmentally induced as compared with those genetically induced. In addition it is evident that a measurement system should be designed to suit a particular purpose. The Fourier measurement system has potential for use as a general taxonomic tool with which bivalve species can be separated. However, the arch-wing measurement system, while useful for functional analysis of *A. jacksoni*, is not likely to be of use in a similar investigation of other species because of its specific nature. The arch-wing measurement system demonstrates that subtle variations in shell shape can be quantified with simple linear measurements when they are specifically designed to quantify the differences between forms. This system is economical and potentially useful for work in the field. The Fourier measurement system has a wider applicability to other species which exhibit shell shape varaition. However, further development of its potential is required. Its investigation in relation to variation of A. jacksoni here is merely preliminary. However, its value in examining variation in V. ambiguus (with rounded and elliptical forms) could be significant. Examinations of ways of measuring shape and shape variation could occupy an entire research project.

The major problems with previous investigations of morphological variation are the lack of experimentation and the inability to quantify shell shape variation. The measurement of shape is satisfactorily achieved in this study and the availability of computer facilities made it possible to analyse large amounts of data.

From examination of studies on other species (e.g., studies by Eagar, Stanley, Kauffman and Thomas) it became evident that the way one perceives a bivalve is important in determining the approach to the significance of variation. Early workers had treated the body and shell separately (e.g. Owen 1953), while more recent studies advocated viewing shell and body as an integrated whole (e.g. Kauffman 1969). There are two alternative viewpoints associated with the different approaches to the extent of interrelationship between shell and body. One is that shell shape itself is adaptive, and the second is that shape is a consequence of some functional adaptation. Flow experiments were designed to address the first viewpoint. It became evident that a buried mussel is almost unaffected by water flow other than protrusion of the siphons from a small area of the posterior of the shell. In addition, the mussels remain buried virtually constantly. Consequently, the attitude was adopted that shell shape variation is merely a component of the total variation, and while perhaps more obvious than variation in the size, shape and position of internal organs, it cannot be considered as a separate entity. It can only be understood as a component of the total variation.

The lack of positive results from pursuing experimental work associated with the view that shell shape itself had adaptive significance (water velocity studies, flow and burrowing experiments), led to consideration of the second hypothesis that shell shape itself was not adaptive, but was instead a consequence of some functional adaptation. While shell shape may well be adaptive for a riverine environment with a hydrodynamically appropriate shape compatible with a burrowing life habit, the particular variable characters with which this project was concerned were not modifications of these features.

Dissections of many mussels revealed a considerable amount of internal variation. However, visual attempts to identify and separate components of this variation failed because it was too complicated. In order to examine the hypothesis that shell shape variation was a consequence of some functional adaptation, measurements of functionally important characters were needed.

At this stage in the project, it was evident from flow experiments that water flow was important since the oriented in a consistent direction that enable the creation of a small turbulent current at the siphons from which they can draw water. Thus, burrowing and anchorage in fast currents would be essential to these mussels. From the work of Trueman and others, the adductors, ligament and the foot were principal components in burrowing and anchoring. The foot, because of its flexible shape was difficult to readily quantify and hence disregarded. The adductor strength and ligament strength was more easily measured. Correlations between these and measurements of many other characters including shell shape were made. The results incorporated a statistically significant correlation between arching and adductor strength with evidence of non-significant trends in related characters.

Re-examination of anatomical drawings and further dissections confirmed these experimental results and revealed the mechanism whereby an increase in adductor strength was achieved in arched and winged A. jacksoni.

In essence, arched A. jacksoni have stronger adductor muscles than winged A. jacksoni and it is hypothesized that the stronger adductors are needed for burrowing and anchorage due to the faster water flow in areas occupied by arched mussels. Stronger adductors allow the arched mussels to achieve the necessary strength to maintain anchorage in areas of rapid water flow by closing on the fluid-filled foot and hence, expanding it in the substrate to form a wedge. In addition, the necessity of reburrowing once excavated in extremely rapid flows (for example floods caused by heavy rain), requires frequent use of adductors. The increased adductor strength in arched A. jacksoni is achieved by increasing the size of the adductors, elongating the posterior adductor ventrally and moving the adductors away from the hinge towards the ventral margin to increase leverage power without increasing the work done. The modification of the shape, size and position of the posterior (and to a lesser extent the anterior) adductor to achieve the necessary strength causes the shell to assume an arched shape as the mussel grows. Thus, the shell may be viewed as the mediator between the environment and the internal organs. The behaviourally induced response of the internal organs to the environment is reflected in the shape of the shell.

From the less intensive examinations of the morphological variations exhibited by Velesunio ambiguus and Alathyria condola, certain speculations are possible on the features of these species which produce the variations. Velesunio ambiguus seems to employ similar tactics to increase adductor strength as winged *A. jacksoni*. That is, they increase the adductor strength by increasing the size of the adductors. However, there are other shell features which are very intriguing in *V. ambiguus*. It is reasonable to hypothesize that the rounded swollen mussels are adapted to soft substrates and have a lower specific gravity than the mussels living in the river where the substrate is firmer. But what is the significance of having a narrow shell that is rounded in two-dimensional outline is elusive.

Excavate *Alathyria condola* are able to close the valves extremely tightly, and although not tested, probably have stronger adductors than arched *A. jacksoni*. The anterior adductor might have a more important role in increasing adductor strength in this species. In order to increase strength in this anterior adductor, it must be enlarged and it seems reasonable that this leads to the formation of the excavated appearence of the shell. It is more of an anterior protrusion of the shell beyond the normal anterior antero-dorsal margin to allow for the expansion of this adductor, rather than an "excavation" into the shell. In view of the possibility that the "non-excavate *A. condola*" may not be *A. condola*, it seems inappropriate to speculate on the possible fucntional relationships between these two forms.

This thesis provides a systematic, experimental approach with which to attempt to understand the morphological variation displayed by many bivalves. It follows the proposed method of elucidating the functional significance of mophological variation in Chapter 4. Although Kauffman (1969) maintained that features of the body of a bivalve were often reflected on the inside of the valves and Trueman (1950-1968b) initiated studies on the ligament and burrowing activity, there have been no previous experimental attempts to connect the two concepts. It is conceivable that virtually all bivalve variation has a functional basis, and the significance of variation can be elucidated by experimentation based on functional analysis of the interrelationships between internal analtomy, the shell and the environment.

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APPENDIX A

Enzymes used in electrophoresis with modified staining recipes.

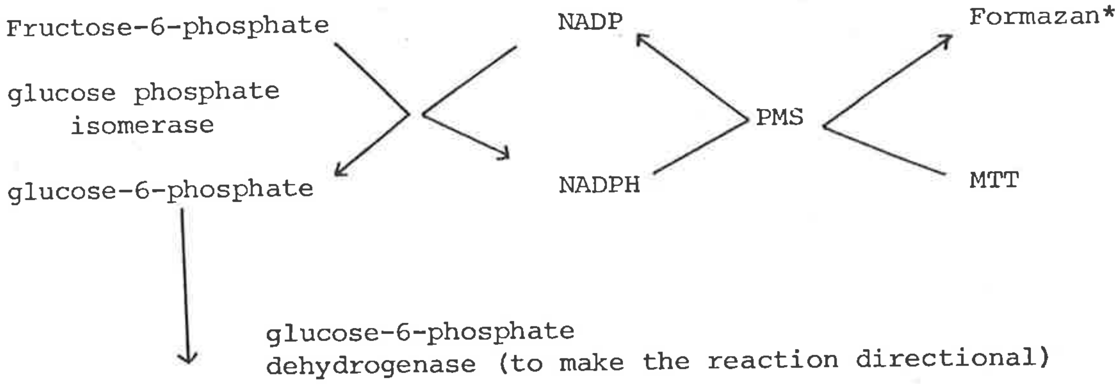
1. Glucose phosphate isomerase.

This is a regulatory enzyme since the substrate/product ratio is sometimes displaced from equilibrium.

The reaction catalyzed:

Fructose-6-phosphate   glucose-6-phosphate.

Stain reaction



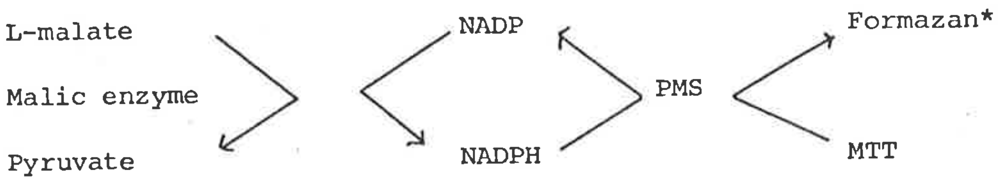
Stain:
.004g MTT
.002g PMS
.002g NADP
.004g Fructose-6-phosphate
.1ml .2M $MgCl_2$
.4ml .1M TRIS_HCl pH8
12ul glucose-6-phosphate dehydrogenase.

2.Malic enzyme.

Reaction catalyzed:

malate + $NADP^+$ pyruvate + CO_2 + NADPH

This is a regulatory enzyme. Malic enzyme in the cytoplasm produces NADP which limits the rates of synthetic reactions. The enzymes activity is responsive to dietary conditions and it limits the rate of fat synthesis in adipose tissue (Johnson 1974). Malic enzyme in the mitochondrion produces NADP and in a kinetically irreversible reaction.



Stain:

1ml .1M TRIS-HCl pH8
.2ml L-malic acid 2M pH8 (NaOH)
.2ml .3 $MgCl_2$
.2ml 10mg/ml (.01g) NADP
.2ml .5mg/ml (.005g) PMS
.2ml 2mg/ml (.02g) MTT

In Mytilus edulis malic enzyme has been found only in very low activities (de Zwaan and Van Marrewijk 1973). Isocitrate dehydrogenase is another source of NADPH and has seven times greater activity than malic enzyme (Addink and Veenhof 1973) so malic enzyme may not be a major source of NADPH and therefore in low concentrations in samples.

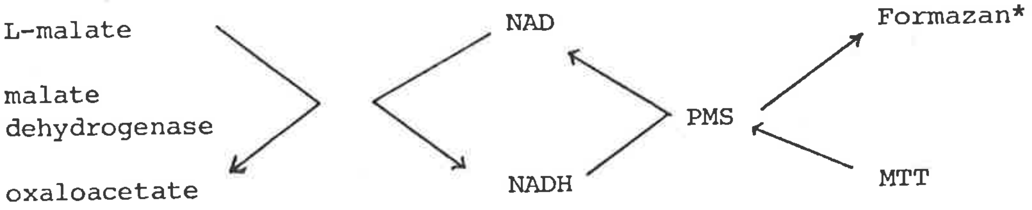
3. Malate dehydrogenase.

Reaction catalyzed:

Malate + NAD^+ oxaloacetate + NADH

This enzyme is involved in the NAD^+/NADP shuttle in the cytoplasm and the tricarboxcylic acid cycle in the mitochondria.

Stain reaction:



This is a non-regulatory enzyme since the substrate/product ratio is at equilibrium (Johnson 1974).

Stain:

1ml .1M TRIS-HCl pH8
.2ml L-malic acid 2M pH8
.2ml .3M $MgCl_2$
.2ml 10mg/ml (.01g) NAD
.2ml 2mg/ml (.02g) MTT
.2ml .5mg/ml (.005) PMS