Test of Method for Telling Moult Stage of Spiny Lobsters

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THE UNIVERSITY OF MELBOURNE

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Non-technical Summary

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OBJECTIVES:

- to test the feasibility of using the growth of external sense organs as a means of determining the stage and/or age of the rock lobster *Jasus edwardsii*;
- to keep specimens of the rock lobster in individual containers in the aquarium and collect their moult cases so that their growth rate could be recorded;
- to use scanning electron microscopy to record the pattern of growth of selected sensory organs on the legs of the collected moult cases;
- to describe the relationship between size, age and number of elements in the sense organs during development;
- to expose some of the animals to different temperatures to test whether age or size is the prime determinant of sense organ development;
- to assess whether the outcomes indicate that sense organ growth would provide a good indicator of age and to determine whether field trials of a method are warranted.

NON TECHNICAL SUMMARY:
With dramatically escalating demand from the peoples of the world for seafood as a local food source and also as a means of generating export income, it is clear that seafood cannot be treated as an unlimited resource to be harvested at will. Many fisheries are no longer commercially viable because of over exploitation. Governments and their agencies are charged with the responsibility of regulating fisheries which includes setting realistic limits to the number of animals harvested, the period in the year in which it is permissible to harvest, and the age and gender of the animals. In drafting regulations they must be mindful of the immediate livelihoods of people working in the industry as well as the need to provide for a sustainable fishery in the future.

This would be a difficult task even with information about the number and distribution of the animals in the fished population, the seasonal variation, the long-term variation, the age and size structure of the population and other aspects of their biology. Biological systems are complex and variable and the answers, even when we have them, are never simple. As a first step, however, the regulators need accurate information. One of the fundamental pieces of knowledge required for the management of any fishery is the age structure of the population relative to the reproductive maturity of the animals. The traditional means of gauging this has been to collect information on the size and reproductive state of the animals and analyse it for size classes showing each year's recruitment. This works well in known and relatively contained populations but does not generalise outside those populations or allow assessment of individual animals where, as with humans, size is not a good predictor of age. As the need for sound management has become ever more pressing, so the search for accurate age data intensifies and most commercial fisheries have research projects addressing this issue.

The project grew out of an observation by the late Professor Mike Laverack, an expert in the study of crustacean receptors. Crustaceans, grow by shedding their old casing and growing a new one. Laverack noted that the number of sensilla forming a movement detecting organ at the joints of a range of crabs, lobsters and their relatives appeared to increase incrementally with each moult. His preliminary studies suggested that they might do so in an approximately linear way. Since the sensilla have neurons associated with them, Laverack reasoned that their growth might to some extent reflect the growth of the nervous system and so be partially age related. If this were so, then counting the number of sensilla might provide a measure of age independent of size. It was this proposition that the project set out to test.

We used *Jasus edwardsii*, the commercial rock lobster species fished in Southern Australia and New Zealand because it is readily obtainable and large so that if the system appeared to have promise after laboratory studies, it could be field tested by fishing and research personnel using simple magnifying equipment. To assay of the pattern of growth in the receptors over time we needed an absolutely accurate measure of the age of the animals. We achieved this by collecting specimens of the last oceanic larval stages as they came back to shore. So, although we did not know the exact time they had been swimming in the ocean as larvae since hatching, we knew their age exactly from the moult into the first juvenile stage, the stage in which they assume fully the adult body form. The animals were housed individually in a closed aquarium system, fed on mussels and monitored daily. The moult cases were collected and prepared for scanning electron microscope examination. The images obtained from the electron microscope were captured directly onto a computer for measurement and processing of the data obtained. In addition, a set of large adult animals of unknown but approximately the same age were monitored for three years to provide comparative information on receptor changes during the slower growth of large adults. They were large enough that their receptor growth could be monitored with low power, dissecting microscopy.

As the results started to accumulate slowly from the early stages, the correlation between age and the growth of the receptors was remarkable good, but this was not unexpected because a number of factors correlate quite well during early growth. As the animals passed through the 6th or 7th stage however, the rate of change in the number of sensilla in the receptors started to slow, eventually slowing to the point where the number of receptors was giving a less accurate measure of age than size itself. Detailed analysis of the developing receptors showed a remarkable and previously undescribed pattern of development suggesting that, rather than the nervous system proscribing the development of the receptors with a degree of independence of the integument, the integument was regulating the development of the nervous system. To check that this was a real effect, we separated a small group of animals and placed them into colder water and another group into warmer water. Animals grow more slowly in the cold and faster when warmer. We reasoned that if the integument determines the rate of growth of the receptors then receptor growth should change in a way that matches body growth in the altered temperature conditions; and that is exactly what happened.

We therefore showed that counting the sensilla is not a good way to age lobsters except in the rapidly growing early stages. In the process we discovered new crustacean development processes which have been published for the scientific community. The method may still be useful for ageing rapidly growing animals such as prawns. The set of moult cases collected will be used to test a morphometric method that is showing some promise, and the remaining animals, the age of which is accurately known, have been handed on to a group who are studying yet another method that monitors the accumulation of a pigment called lipofuscin.

Background

A knowledge of the size and age structure of any fished population is fundamental for its management. One of the most difficult problems facing the fishing industry and fisheries management authorities is accurate stock assessment. Among the most intractable of the problems standing in the way is that of age determination. Knowing the age of an animal is more important than knowing its size and, although size is obvious and easily determined, it does not necessarily predict age well. The problem of age determination, difficult even in vertebrates which retain many skeletal elements throughout their lifetime, is rendered more so in crustaceans which moult to grow and shed their hard parts each time they moult.

This project grew out of a suggestion by the late Professor Mike Laverack that some parts of the nervous system might develop age dependently rather than size dependently and hence provide an independent measure of age. While some neural elements exhibiting this type of growth might be found within the central nervous system, it would be very difficult to monitor these because the animal would have to be sacrificed to measure them, and growth of that particular specimen would cease. Laverack reasoned, however, that it might be possible to monitor neural elements associated with external receptors by studying their moult cases, thereby leaving the animals that produced them to continue living and growing. With this in mind, he made preliminary observations on the incremental growth of Cuticular Articulated Peg Organs (CAP organs). CAP organs are a type of surface proprioceptor associated with limb joints in decapod crustaceans (Wales *et al.*, 1970; Oakley and Macmillan, 1980). They appear on the surface of the animal as a row or cluster of pegs close to the articulating membrane of the limb joints (Figs. 1 and 2) and when the joint flexes the articulating membrane rolls over them and activates the sensory neuron beneath (Oakley and Macmillan, 1980), presumably signalling the joint angle to the central nervous system, although their role in coordinating position or movement in the animals has not been investigated. Laverack's brief survey covered several different species and suggested that a relationship might exist between the age of the animal and the number of sensilla in the CAP organs (Laverack 1976, 1987; and unpublished data submitted with the FRDC application). He proposed that if the relationship predicted age better than carapace size, then it should be possible for an investigator or fisherman to count the number of sensilla in a given CAP organ in the field by rubbing ink into it and viewing it with a magnifying glass, a procedure he carried out successfully on specimens of *Jasus edwardsii*. The subject of this grant application was a test of Laverack's idea with appropriate scientific controls and manipulations.

The original proposal was submitted jointly by Michael Laverack and David Macmillan but Professor Laverack and his wife lost their lives when a helicopter in which they were travelling to a field site at Heron Island in Queensland lost control and crashed into the sea just as the work was about to start. The grant was transferred to David Macmillan, the author of this report, who carried the project through to completion.

Need

With the exception of some short lived and annual species, determining the age and moult stage of crustaceans is difficult. Crustacean growth occurs as a consequence of moulting which is normally frequent when the animals are young but becomes infrequent, perhaps annual, when they are older. The timing of moults is fairly regular in adults for any given set of conditions, but is subject to important variable environmental factors such as diet and temperature. To complicate matters further, size is also related to those variable factors in complex ways. Some commercial species, such as the rock lobster *Jasus* continue to grow slowly throughout their lives so that the relationship between size and age is likely to become even more unreliable over time. Estimates of population age structure cannot therefore be based on size or weight alone since animals of the same size may differ considerably in age. At present there is no straightforward method of determining moult stage and age in lobsters.

Objectives

The objectives were:

- to test the feasibility of using CAP organ growth as a means of determining the stage and/or age of the rock lobster *Jasus edwardsii*;
- to keep specimens of the rock lobster in individual containers in the aquarium so that their moult cases (exuviae) could be collected and their growth rate recorded;
- to use scanning electron microscopy to record the pattern of growth of selected CAP organs on the legs of exuviae;
- to describe the relationship between size, age and the number of CAP sensilla during development;
- to expose some of the animals to different temperatures to test whether age or size is the prime determinant of CAP organ development;
- to assess whether the outcomes indicate that CAP organ growth would provide a good indicator of age and to determine whether field trials are warranted.

Methods

Animal husbandry

To establish the relationship between the number of CAP sensilla in the CAP organs of the leg and age and size, the factors have to be measured from animals held under conditions in which other factors are constant or the same for all animals. Both juvenile and adult animals were held and studied.

The juvenile specimens were raised from puerulus larvae obtained from crevice collectors set at a variety of sites around the coast of southern Tasmania by collaborators at the Marine Research Laboratory, Department of Primary Industry and Fisheries, Taroona, Tasmania. They were transported in cooled, polystyrene containers to the closed circulation aquarium system in the Department of Zoology at the University of Melbourne, Parkville, Victoria, Australia. The system draws on two 10 000 litre holding tanks situated in a temperature-controlled, sub-basement. One of the tanks is emptied and refilled every six months with Bass Strait water transported in food-quality, stainless steel tankers. The water is pumped to a 4th floor, roof-level, holding tank for reticulation through aquarium rooms in the building. The salinity of the system is checked weekly and adjusted to ~33‰. The water temperature in the tanks where the animals were held is also recorded weekly and varies seasonally from 13°C to 15°C. The aquarium room was subjected to a seasonally adjusted light/dark cycle throughout the year. Because the animals devour their own exuviae and that of other animals, and are also vulnerable to attack by other animals when moulting, they were all held in individual containers. The puerulus larvae were housed individually in cylindrical glass containers (volume 750 ml) with plastic mesh covers through which the tube carrying the inflowing water (approximately 300 ml per minute) was inserted so that it flowed into the bottom section of the jar and there was constant turnover of water. When the animals outgrew the glass containers they were transferred to PVC mesh cylinders suspended in the main sub-basement holding tanks. They were fed every second day on mussels (*Mytilus edulis*) the valves of which were forced apart to expose the soft parts. Providing the size of the mussels was increased as the animals grew, the food provided proved to be more than the animals would eat in the time, and uneaten material was removed at the next feeding session. Some shell was always left to provide additional calcium as required. The animals were checked daily and any moult cases removed and stored in 70% ethanol. The animals thrived on this regime and moulted and grew at rates comparable with those from growth studies on these and other rock lobster species (Aiken, 1980).

Due to a freshwater leak into the aquarium system, all 70 of the animals in the first shipment were lost after they had been successfully reared for six months. The loss was covered by insurance and the animals replaced with new season's stock after a short delay, at no cost to the FRDC grant. The program was, however, set back by about eight months, which is why the final assessment of the outcome and final report are later than was predicted in the initial application.

55 animals of the replacement batch survived the transport and settling-in period and were monitored for 578 days following the moult from the puerulus stage. Of these, there were 51 left by the time they had all moulted to Stage 5. At Stages 5 and 6 we split off two smaller groups of animals to test the effect of temperature on CAP development. These two groups of 16 animals each were placed in water with a either a constantly elevated temperature (19°C) or a constantly reduced temperature (13°C) . Animals remaining at the end of the study were passed on to the Victorian Department of Marine and Fisheries Resources Institute (MAFRI) as a group of animals of known age, to be used in lipofuscin determinations.

The adult lobsters were also obtained from the Marine Research Laboratory, Department of Primary Industry and Fisheries, Taroona, Tasmania, half being collected from faster growing populations in the north of the state and half from slower growing populations in the south. They were airfreighted to Melbourne wrapped in damp sackcloth in polystyrene containers to keep them cool. They were transferred to the open circulation aquarium system at Queenscliff where they were held in individual pvc containers (l=81cm x w=45cm x h=47cm) and fed with mussels, as described for the juveniles. The adult animals were kept and monitored for 728 days from receipt and the animals remaining at the end of the project passed on to the Victorian Department of Marine and Fisheries Resources to be used in their research.

Animals that lost limbs were noted and the way in which the CAP organ re-developed on the regenerating limb was recorded.

Choice and observation of CAP organs

Although it would have been possible to use the CAP organs from any of the leg joints for this study, the results reported here are based on the CAP organ at the meropodite-carpopodite (M-C) joint of the 3rd and 4th walking legs. The M-C CAP organ is located on the ventral surface of the proximal end of the meropodite, immediately adjacent to the articulating membrane and about two thirds of the way in from the lateral edge. It is composed of a somewhat scattered line of sensilla that curves slightly medially at its distal end (Figs. 1 and 2). We chose it because it is large, accessible even in small animals, has a relatively simple

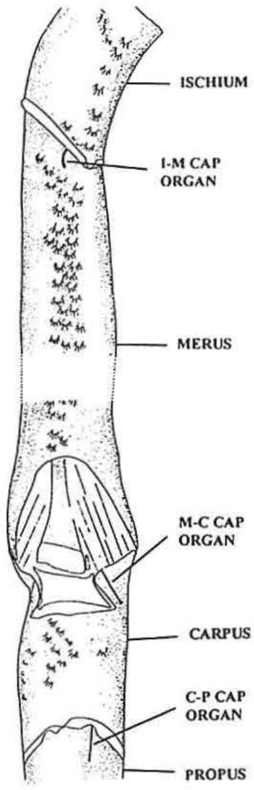

Figure 1. The CAP organs of *Jasus edwardsii*. Diagram of the 3rd walking leg viewed ventrally to show the location of the CAP organs at the Ischiopodite-Meropodite (I-M), Meropodite-Carpopodite (M-C) and Carpopodite-Propopodite (C-P) joints.

linear array of sensilla, and the surface on which the sensilla are located is flatter than that at some of the other joints (Fig. 2). This last feature minimised the possibility of translational errors when taking measurements or constructing maps of the position of the sensilla from two dimensional scanning electron microscope images of a three dimensional surface.

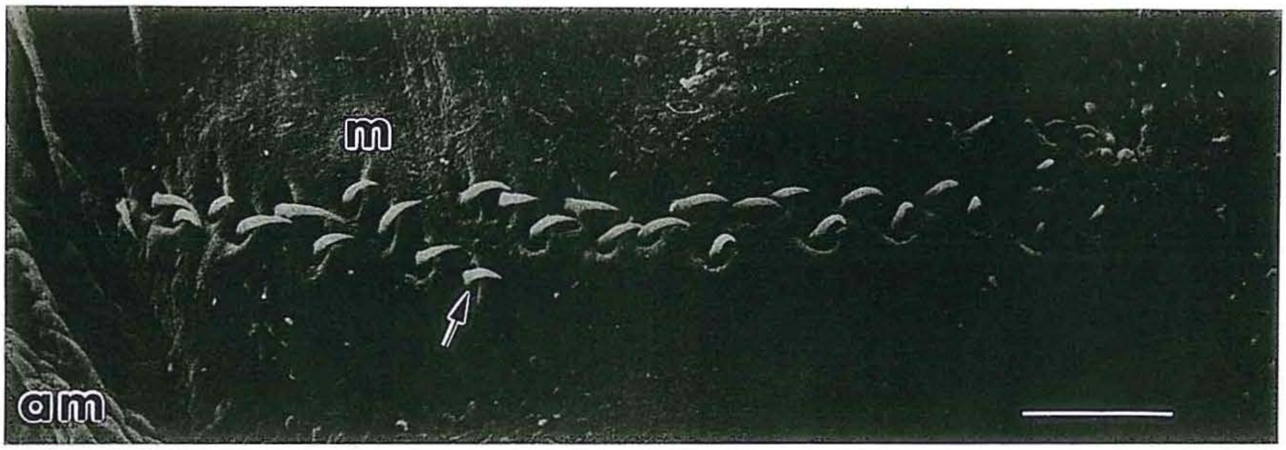

Figure 2. Scanning electron micrograph of the CAP organ at the M-C joint showing the individual CAP sensilla (arrowed) on the merus (m) and the articulating membrane (am). Scale bar: 50μm

The CAP organs from the small animals were examined with the Scanning Electron Microscope (SEM). The selected limbs from exuviae were dehydrated in an alcohol series, sputter coated with gold, and examined with a Phillips 505 SEM. The specimens were oriented so that the CAP organs at the meropodite-carpopodite joints of the 3rd and 4th walking legs were as nearly as possible parallel to the image plane and the view was then captured directly to computer using VideoView software running on a 486 computer with a Microkey VideoView capture board. The images were processed and measured using PaintShop Pro imaging software and the data obtained stored on disk.

With the larger animals the magnification provided by a light microscope was sufficient to make the required observations. The moult cases were taken from the 70% ethanol in which they were stored and the chosen limbs removed, re-hydrated, and sonicated if it was necessary to clear the CAP organs of any encrusting detritus, epizoic organisms, or salt crystals. The limb was then pinned out on Sylgard 184 (Dow-Corning) in a petri dish and dried overnight in a desiccator. When dry they were observed or photographed with a Zeiss microscope fitted with a calibrated graticule and camera mount.

Data and statistical analysis

The animals were checked daily and all moults collected. After the processing and viewing described above, the following data were recorded: date of collection, moult number, water temperature, total carapace length (abbreviated to TCL, the standard measure of size in much of the lobster fishery) and the number of CAP sensilla in the organs on the third and fourth pereiopods.

growth of epibiotic organisms over the CAP organ region sometimes made it impossible to count the CAP compliment for a stage. These small variations are accounted for in all statistical analyses (including variation bars on graphs) but are not indicated stage by stage on the graphs because the numbers involved were always small and the details unnecessary for understanding the graphs. The N value provided is therefore the number of animals in the group when all animals provided a count.

For a small group of animals, details of the structure of the organ were also recorded and analysed. This information was not suitable for age determination and was analysed separately (Macmillan *et. al*, 1998). All data were analysed using Systat (version 1.0).

Detailed Results

Growth and moult pattern in captive juvenile animals

The early moult pattern of *Jasus edwardsii* juveniles is typical of that described for other lobster species (for review see Aiken, 1980). In this group of animals growing under uniform conditions, the duration of the intermoult stages increased only slightly with each stage up to Stage 6 and showed little variability. From Stage 7 on the intermoult interval and the variability between animals increased steadily (Fig. 3 a). The growth during that time showed a similarly typical pattern with the curve sigmoid but already levelling out at around 600 days or Stage 10 (Fig. 3 b). The size of the Southern group of adult animals is also plotted to show the relative size of the adult animals relative to the larval animals.

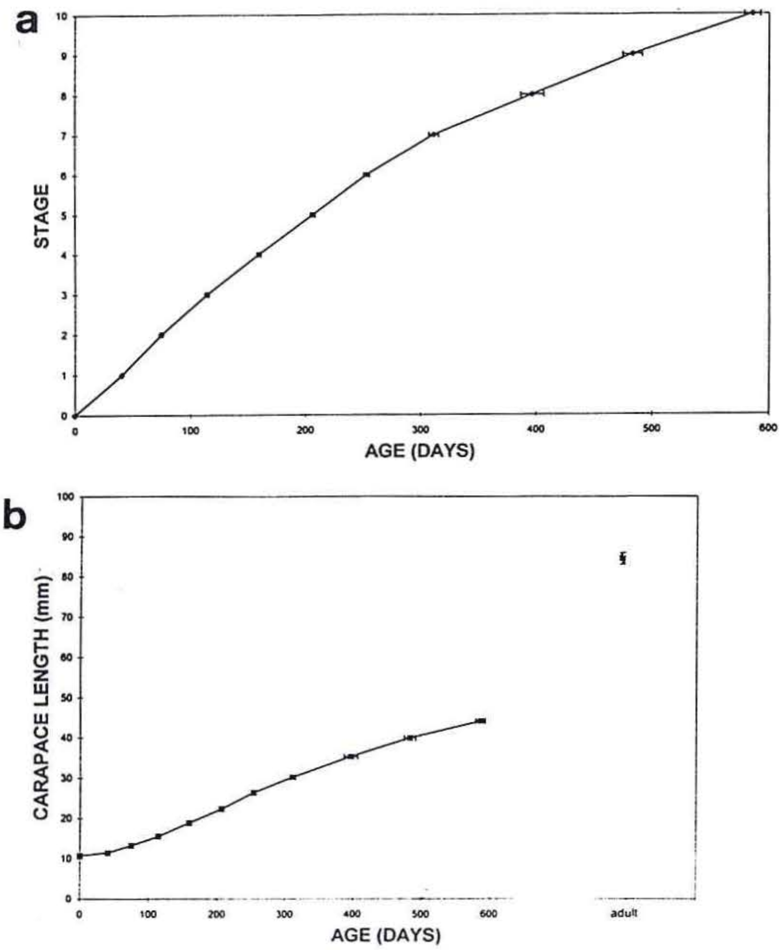

Figure 3. (a) Graph showing the relationship between the age of the juvenile animals in days at the time of moult and the post-puerulus Stage Number for the first ten stages. The age was taken as the number of days post-puerulus because the length of time spent in stages prior to and including the puerulus was not known. This graph shows the pooled results from 24 animals. The error bars show standard error. (b) Graph showing the relationship between the age of the juvenile animals in days and the total carapace length in mm. Note the size of the adult animals from the Southern group is included for comparison. The error bars show standard errors.

Growth of adult animals

The adult animals moulted once per year so that we able to record the size of three stages during the period we held them. The increment of growth of the carapace of these animals, held in uniform conditions, was about 4mm per moult in both the smaller Southern cohort and the larger Northern cohort (Fig. 4).

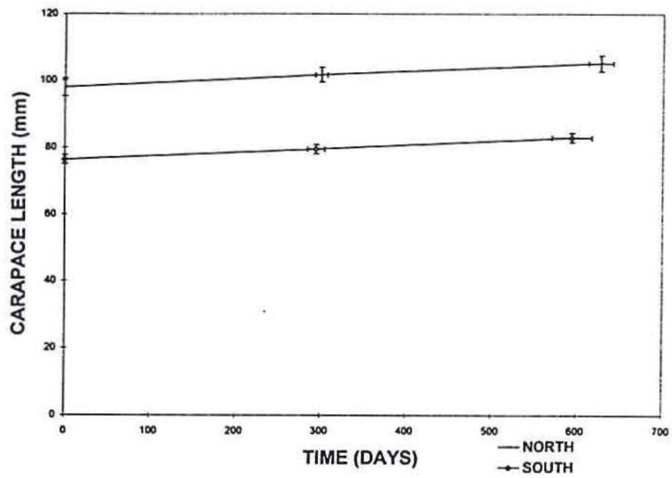

Figure 4. Relationship between time in days since establishment in the holding facility and carapace length in mm for two cohorts of animals, one from the Northern region (N=12) and one from the Southern region (N=12). The error bars show standard errors.

Growth of sensilla in CAP organs

The increase in the number of sensilla in the CAP organs shows a similar relationship whether plotted against age in days (Fig. 5 a), developmental stage (Fig. 5 b) or carapace length (Fig. 5 c). The important point to notice with these three curves is that they all roll off sharply at about the Stage 7 moult when the animals have a carapace length of about 35 mm, so that the number of CAPs added at each moult falls suddenly. Most of the adult animals added one CAP sensillum per moult (Fig. 7).

Effect of temperature on number of sensilla in CAP organs

It is well established that the temperature of the water affects the growth of rock lobsters and other crustaceans (Aiken, 1980). We reasoned that if the growth in the number of sensilla in the CAP organs showed some measure of independence from the size of the animal, we could possibly reveal it by using temperature to change the rate of growth in groups of animals. We therefore removed two groups of 16 animals from the standard cohort, one at Stage 5 and another at Stage 6. We chilled the water temperature of the first group down to 11 °C and warmed the other to 19°C. Four of the animals in the colder water died as they were attempting to moult to the next stage, and another 4 died during the subsequent moult and so the water temperature was increased to 13 °C for the remainder of the experiment. All 16 animals in the warmer water survived. As predicted by the literature (Aiken, 1980), the moult interval increased in the cold water animals and they grew more slowly than the control group; in the warm water animals, the moult interval increased and they grew faster than the control group (Fig. 6 a). The relationship between the age of the animals in days and the number of CAP sensilla in the M-C organ mirrored this change closely (Fig. 6 b).

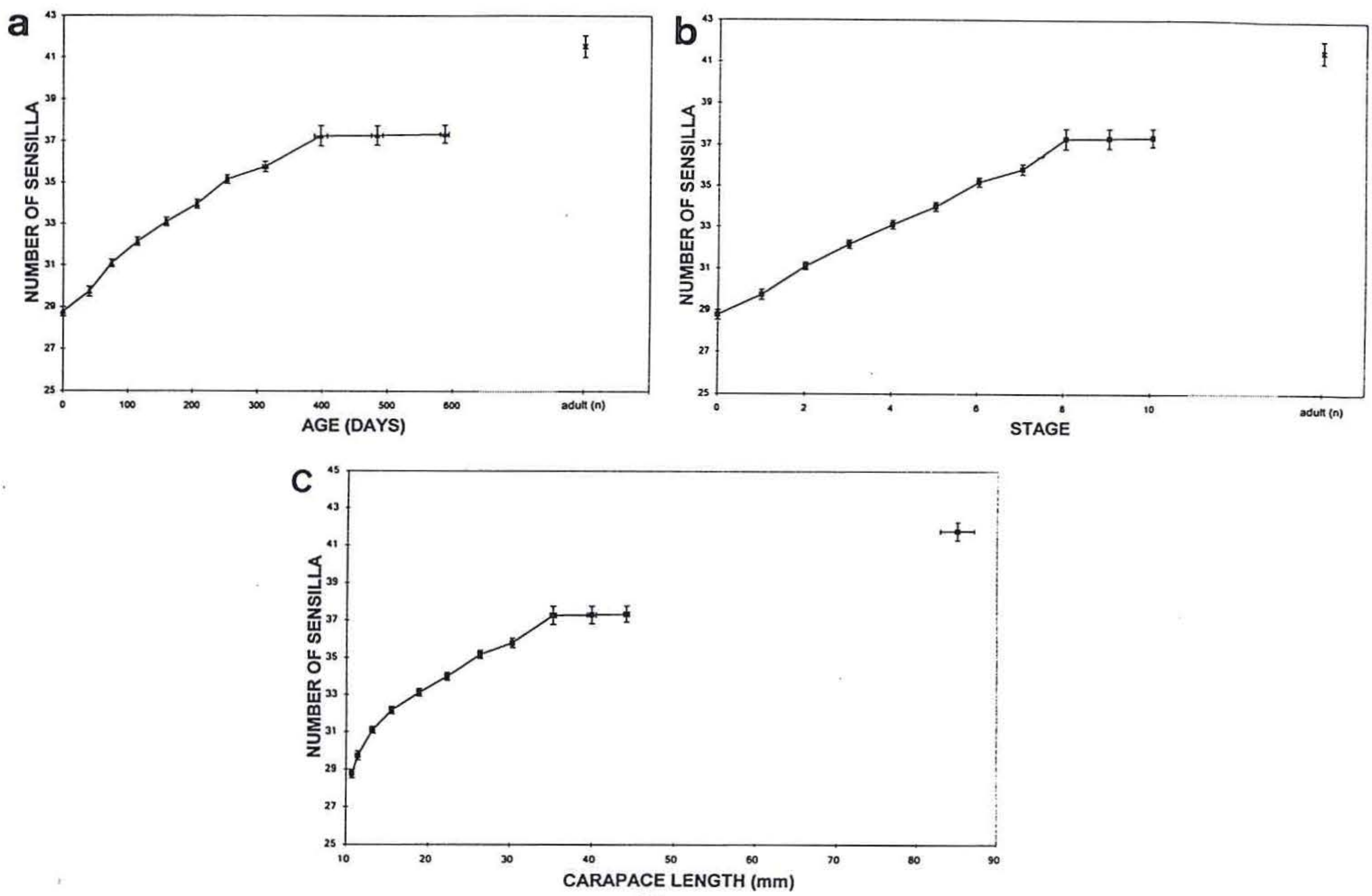

Figure 5. Graphs showing the relationship between age and growth of the juvenile animals. This graphs shows the pooled results from 24 animals. (a) Relationship between age in days and number of CAP sensilla in the M-C Cap organ. (b) Relationship between developmental stage post puerulus and number of CAP sensilla in the M-C Cap organ. (c) Relationship between carapace length in mm and number of CAP sensilla in the M-C Cap organ. Note that the the parameters for the adult animals from the Southern group are included for comparison.The error bars show standard errors.

CAP sensilla numbers in a regenerating limb

Those specimens which lost limbs normally had a regenerating limb bud following the next moult. New CAP sensilla were usually detected by the second moult following the loss, and the rate of addition was faster than the rate for uninjured animals of the same size. A curious observation was that the number of CAP sensilla appeared to overshoot that predicted by the control provided by the animal's other limb. Because we observed this serendipitously when animals lost limbs, there were too few observations for the conclusion that this is a real phenomenon. It is, however, of sufficient developmental interest to warrant a properly designed series of experiments to test it.

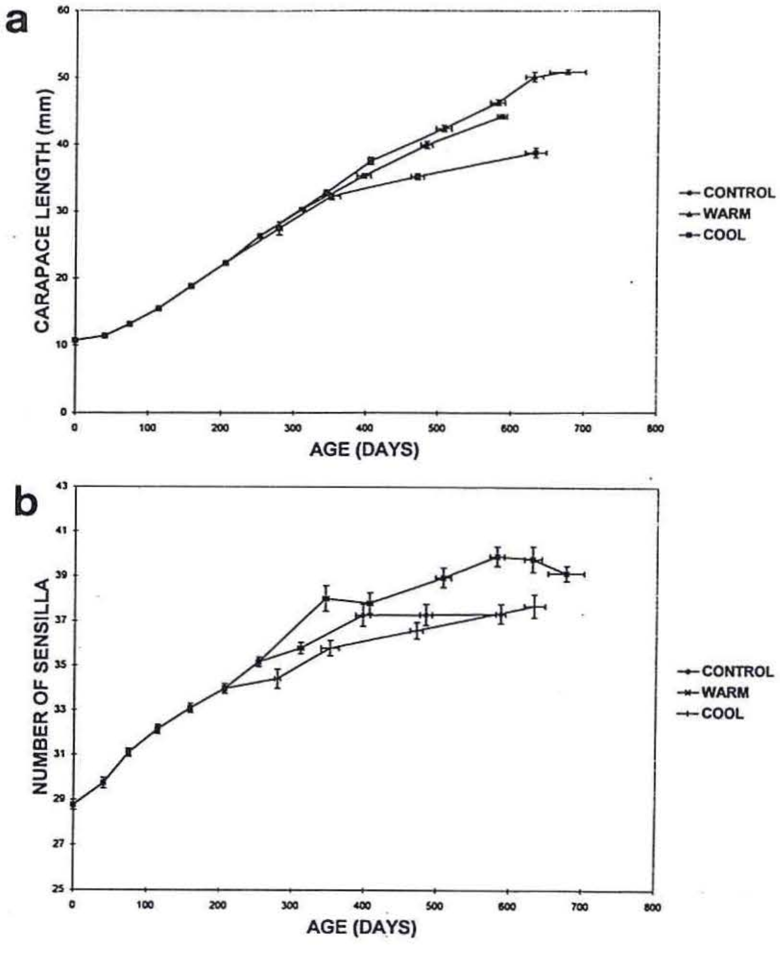

Figure 6. The effect of changing temperature on growth and the number of CAP sensilla in the M-C organ. (a) Graph showing the relationship between age in days and the carapace length. Up to Stage 5 post puerulus there were 51 control animals. 16 animals were split off from the group and placed in water at 11 °C. At Stage 6 another 16 animals were removed and placed in water at 19 °C. From Stage 7 on there were 19 control animals. There were 19 control animals (ambient temperature), 16 warm water animals, and 8 cold water animals at the end of the experiment. (b) The relationship between carapace length and the number of sensilla in the M-C CAP organ in the three temperature cohorts. The error bars show standard errors.

Adult animals and the Northern cohort vs. the Southern cohort

We included adult animals in the study because we needed to be sure that any observations on early stage animals could be related to later stages, to obtain some measure of the range in sensilla number in mature lobsters, and to obtain an extrapolation point for our graphs. We found that the adult animals reflected all our juvenile observations. Because adult animals are only adding one or two sensilla per moult, there is little variability in a sample of adult animals of similar but unknown age (Fig. 7). Animals in samples from the South of Tasmania are smaller than those from the north and, as our study would predict, have fewer CAP sensilla. This difference has been attributed largely to the difference in temperature of the growing conditions. We held the two groups of animals in identical temperature conditions (ambient Queenscliff, Victoria) and might therefore have expected the average size of the two groups to converge. Interestingly, they appeared to keep growing at the same rate (Fig. 4), although a longer sampling period would be required to establish this observation because the animals only moulted once a year. There was, however, a suggestion of convergence in the CAP sensilla measure (Fig. 7) and it might therefore provide a more sensitive measure of change in rate of growth than carapace length.

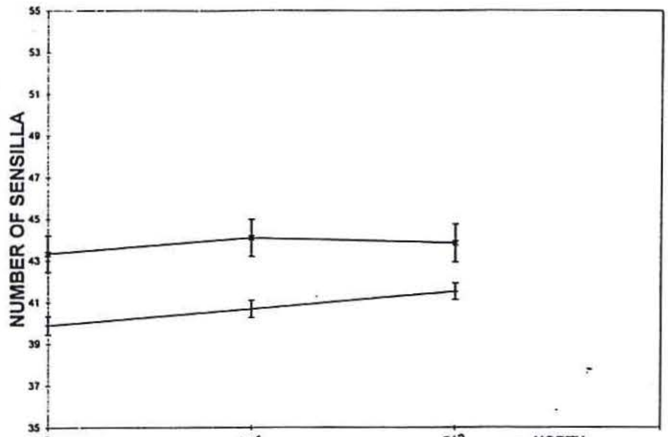

Figure 7. CAP sensilla growth in adult animals. Number of CAP sensilla at the M-C joint over the time in which they were held. The error bars show standard errors.

Discussion of Results

The objective of this study was to investigate a novel method for determining the moult stage in spiny lobsters. This was done and it was concluded that the method does not provide any substantial advantage over methods, such as carapace length, that are already available and in use. The information upon which this conclusion is based will be published in scientific journals following the submission of this report. A considerable body of related information on growth and development in this important commercial species was obtained during the study and most of this has already been published (see Publications Resulting from the Study, below).

The detailed purpose of this study was to test the hypothesis that CAP organ numbers would provide an estimate of the age of rock lobsters. More specifically, it was designed to test the practicality of using the number of sensilla in the CAP organ as a field tool for estimating age and to determine whether this would provide a more accurate estimate than size. The important issue is whether the CAP sensilla development curve is independent of the size or stage curves for that part of the animal's life when it becomes a reproductive individual, which is about the same time that it becomes a legally fished animal. For it to be a useful tool in the industry, part of the CAP sensilla growth curve must be independent of the carapace growth curve or to roll off more slowly with growth, so that a count of the CAP sensilla gives a better estimate of growth (Fig. 8).

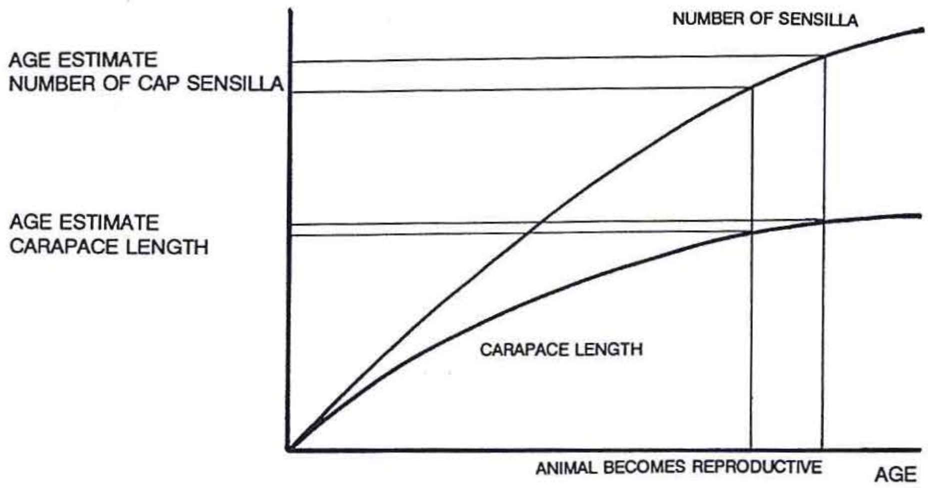

Figure 8. Graph showing ideal outcome of CAP sensilla growth relative to growth in the body measured by carapace length if the CAP sensilla growth is to provide a better estimate of age.

In fact, up until Stage 7, the CAP count can give a more accurate estimate of age and stage in these animals (compare Fig. 3b, 5a and 5b) in these animals grown under uniform conditions. The increment in the numbers of CAP sensilla per moult falls off rapidly after Stage 7 however (Fig. 5a, b), so that it is unlikely to prove more reliable than the present methods of estimating age. Extrapolating the growth curves to the points we have for the adult (also shown on each of the graphs) we have an estimate of maturity between 4 and 6 years which accords well with estimates from population studies (Phillips *et al.*, 1980). By that time the CAP organs are only incrementing by about one sensillum per moult and would need to add only one or two per moult to make up the difference between Stage 10 and the adult (Fig. 5). This provides an index with a low level of discrimination for either Stage or Age.

A plot of the relationship between the carapace length and CAP sensilla number against age on equivalent scales suggests that after about stage 5-7 carapace length should provide a better prediction of age in these animals (Fig. 9). To check this we conducted a covariate analysis of the relationship between number of CAP sensilla, carapace length and age. The analysis confirmed that both carapace length and number of CAP sensilla predict the age of the animal but that carapace length has the stronger relationship (Table 1a). We then compared the strength of the relationship between the number of CAP sensilla and age and the number of CAP sensilla and carapace length and found that carapace length predicts the number of CAP sensilla better than age does (Table 1b, c).

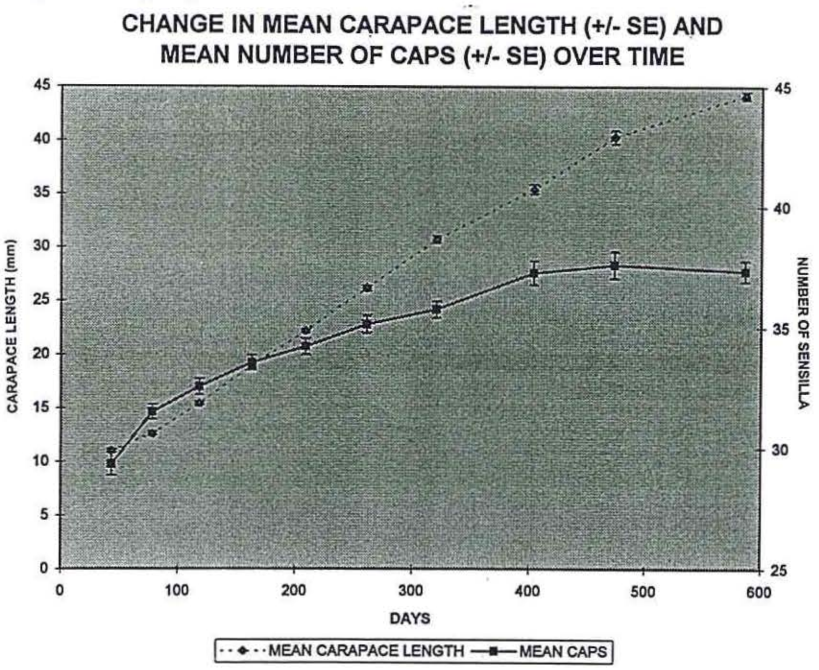

Figure 9. Graph comparing the change in mean carapace length and the number of CAP sensilla as a function of age.

These analyses suggest that the growth of the CAP organs is determined by the surface area or some other parameter associated with the growth of the integument, a conclusion that is supported by the results of the temperature manipulation. It appears that if the animal grows larger it will have more CAP sensilla. In hindsight this might appear to be an almost intuitive outcome, but it could not have been predicted from the literature. For example, the way in which ocelli and ommatidia, which are also related to the cuticle, are added to the eyes of some myriapods and insects, predicts the developmental stage closely (Vachon, 1947; Meinertzhagen, 1973). Some external hairs are also good predictors of developmental stage in the early stages of crayfish development (Letourneau, 1976; Schmitz, 1992, 1993). Shelton and co-workers looked to see whether the ommatidial structure could be used to determine the age of the European lobster *Homarus gammarus* but concluded that the growth of the eye is also more closely related to carapace length (Shelton *et al.*, 1991). They suggested, however, that other integumental structures should be examined to test the generality of their result further since the eye could be a special case.

Table 1. (a) Covariate analysis of the relationship between number of CAP sensilla, carapace length and age. Both carapace length and number of CAP sensilla predict the age of the animal but that carapace length has a stronger relationship. (b) Covariate analysis of the relationship between the number of CAP sensilla and age. (c) Covariate analysis of the number of CAP sensilla and carapace length.

a

VARIABLE	COEFFICIENT	T-VALUE	P-VALUE
constant	-114.59	-6.665	0.000
cl	15.46	70.892	0.000
nocaps	-0.709	-1.14	0.255

R squared=0.96

b

VARIABLE	COEFFICIENT	T-VALUE	P-VALUE
constant	29.862	222.017	0.000
days	0.017	29.202	0.000

R-squared=0.603

c

VARIABLE	COEFFICIENT	T-VALUE	P-VALUE
constant	26.989	141.905	0.000
CL	0.289	33.039	0.000

R-squared=0.645

To look at the generality of the CAP finding, we used the moult cases to study the development of sensory hairs on the telson. What we found there was one mechanisms linked to carapace size and one completely new way of adjusting the density of a sensory structure. The number of spines on the telson is determined by the size of the animal, just like the CAPs, but the hairs on the spines are always added at the same rate at all times (Stuart and Macmillan, 1997). This type of addition cannot provide an ageing method but demonstrates that there are a number of different ways in which development of integumental structures can be programmed and that this should be borne in mind when generalising about this process.

As the data slowly accumulated in the present study, we started to suspect that the outcome would be as reported here and so we examined the relationship between the development of the CAPs and the integument more closely. The result of that study is reported in Macmillan *et al.*, 1998). In it we report a phenomenon in which the growth of the CAP sensilla appears not only be tied closely to the development of the cuticle but also affects the way the cuticular nervous system develops to match the central nervous system. This is an important discovery as it has not been described previously in such a system and coincides with a similar discovery in the chemosensory system (Sandeman and Sandeman, 1996).

With Shelton's (1991) result and the present outcome, it now seems unlikely that any cuticular structure will provide a simple indicator of age. It is still possible that some internal parts of the nervous system could provide a more accurate measure of age than carapace length or size. Such an indicator would be more akin to the biochemical methods presently under investigation, such as lipofuscin assay (Sheehy and Wickins, 1994; Sheehy *et al.*, 1995). Like those methods, central neural development could provide some improvement in age estimation over carapace length, but a method dependent on the internal structure of the nervous system would be likely to share a number of their disadvantages (Crossland *et al.*, 1987).

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Publications Resulting from the Study

Macmillan, D.L., Sandow, S.L., Wikeley, D.M. and Frusher, S. (1997) Feeding activity and the morphology of the digestive tract of the rock lobster *Jasus edwardsii*. *Marine and Freshwater Research* **48**:19-26.

Macmillan, D.L., Stuart, T. and Thomas, M. (1998) Development of a proprioceptive organ on the walking legs of the rock lobster *Jasus edwardsii* (Decapoda:Palinuridae) by ordered addition and loss of receptor elements. *Journal of Crustacean Biology*. In press.

Stuart, T. and Macmillan, D.L. (1997) Development of Spines and Sensory Setae on the Tailfan of the Rock Lobster *Jasus edwardsii* Hutton, 1875 (Crustacea, Decapoda). *Australian Journal of Zoology*, in press.

Stuart, T., Macmillan, D.L. and Thomas, M. (1996) The effect of background colour on the colour of developing juvenile Rock Lobsters, *Jasus edwardsii* (Crustacea:Decapoda). *Marine and Freshwater Behaviour and Physiology* **27**:269-273.

Presentations of aspects of the work in public seminars and forums

Victorian Rock Lobster Meeting, Queenscliff, Victoria
Southern Rock Lobster Meeting, Adelaide South Australia
The Department of Chemistry and Biology, Deakin University
The Department of Zoology, University of Melbourne
The Neuroethology Society, University of Cambridge, U.K.
The Marine Biological Laboratory, Woods Hole Massachusetts, U.S.A.
Quantum, ABC General Interest Science Program

Benefits

The most important direct benefit to the lobster industry will probably come from the finding concerning feeding and the structure of the gut in larval lobsters (Macmillan *et al.*, 1997), carried out in collaboration with the Tasmanian Fisheries group at Taroona, even though this was a finding ancillary to the main thrust of the study. This is because of its likely impact on the culture of rock lobster species which has been hampered by knowledge to develop suitable diets for the early stages (Kittaka, 1994).

The rejection of Laverack's hypothesis concerning the possibilities of the CAP sensilla as an age indication was disappointing but adds to our understanding of integumental growth so that we have the tools to be more critical of any future proposal to use integumental structures. The data set that we have will now be used to test the feasibility of using a modification of Rohlf's Geometric Measure Theory to develop a morphometric method of ageing rock lobsters and other animals. The surviving animals, the age of which is known, have been passed to the group studying the feasibility of using lipofuscin as an aging method at MAFRI, Queenscliff.

The publicity obtained by the featuring of this program on the ABC science program Quantum helped to bring the importance of the ageing issue in fisheries management before a wide general public so that support for programs to solve it are more likely to be well received.

There are a number of other scientific outcomes in which we describe several previously undescribed methods of development of integumental structures and these are of benefit to the knowledge base in these areas of developmental and marine biology.

Intellectual Property

There is no intellectual property from this project that should be protected. All the information will benefit the general community, the fishery, and the scientific community best if it is made freely available.

Further Development

Our finding that most external cuticular structures are unlikely to provide a new ageing tool should not be taken to mean that the proportions of the developing exoskeleton might not provide one. The size and possibly the shape of the exocuticular elements apparently determine the development of many of the external structures, but some aspects of their development may be age related. Morphometric analysis has always been an attractive candidate for ageing studies because it is non-invasive and requires little equipment. The results have, in general, been disappointing, however (Aiken, 1980) because no one feature gives a very good estimate alone and suitable mathematical methods for combining a number of parameters have not been available. A new method for considering multi-factor measurements of 2 dimensional spatial shapes, based on Kendell projections, has been developed in the US (Rohlf's Geometric Measure Theory). We have obtained a copy of Professor Rohlfs programs and he has offered advice on ways to develop them to plot two and three dimensional shape change against time. We believe that this may be a way forward for ageing crustaceans and it is certainly worth further study. There was some suggestion in the telson hair study (Stuart and Macmillan, 1987) that different parameters of the telson change at different times so that the combined outcome might predict age. On this basis we have started a pilot project to test the potential of the Rohlf theory using the telson data obtained in the present study. The findings of this preliminary study will be the subject of later reports and, depending on the outcome, may also form the basis of further grant applications.

One interesting outcome of the study is that during the early, fast growing stages of development, the CAP sensilla do provide a better indicator of age than carapace length. This finding has proved to be of interest to CSIRO researchers working in support of the prawn culture industry who also have an age assessment problem. They have sent us material to assess and if it looks promising, we will do more extensive trials.

Staff

Director of Research:
David L. Macmillan

Assistants employed directly by the grant to work on the project:
Mark Thomas
Tobi Stuart

Assistants employed part-time as required to assist with various aspects of the grant:
Bram Bakker

Other personnel who contributed technical expertise or other assistance:
John Ahern (Department of Zoology, University of Melbourne)
Terry Beattie (Department of Zoology, University of Melbourne)
Rob Day (Department of Zoology, University of Melbourne)
Stewart Frusher (Tasmanian Department of Primary Industry)
June Hook (Department of Zoology, University of Melbourne)
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Michael Stevenson (Department of Zoology, University of Melbourne)
David Paul (Department of Zoology, University of Melbourne)
Rod Watson (Queenscliff Marine Station)
David Wikeley (Tasmanian Department of Primary Industry)

Final Cost

Fisheries Research and Development Corporation

Statement of Receipts and Expenditure for the period ending 30 June 199–

Name of Research Organisation (include organisation reference)	FRDC Project Number	Title of Project
UNIVERSITY OF MELB	93/085 (421085)	TEST OF METHOD FOR TELLING MOULT STAGE OF SPINY LOBSTERS

Budget Summary	199–-9–	199–-9–	199–-9–	199–-9–(1)
Original Budget				
Current Budget(2)				

Summary Receipts and Expenditure for the Project since commencement

	1993-94	1994-95	1995-96	1996-97(1)	
B/F	N/A	5434.69	(19990.00)	(8683.24)	
FRDC Funds (Plus)	41736.00	19990.00	42376.00	–	104,102.00
Expenditure (Minus)	36301.31	45414.69	31069.24	13703.76	126,489.00
Refunds (3)	N/A	N/A	N/A		
Balance C/F	5434.69	(19990.00)	(8683.24)	(22387.00)	(22,387.00)

Details Financial Year to 30 June 19–

Funds Available

	Balance bought forward from previous year		
TOTAL	Total funds received from FRDC during Financial Year 199–-9–		104,102.00
PROJ. ALLOC.	Funds Available for FY 199–-9– (TOTAL PROJECT FUNDS RECEIVED)		104,102.00
Allocation FY(5)	*Less Expenditure(4)*		
$82,351	Salaries	89,140.19	
$–	Travel	–	
$39,138	Operating	31,416.81	
$5,000	Capital (TOTAL PROJ EXPS)	5,932.00	126,489.00
Total $126489	Balance as at 30 June		(22,387.00)

Notes
(1) Use this column for the final year ONLY regardless of the length of the project.
(2) Total current budget shall not exceed Total original budget without approval, in writing, from the FRDC.
(3) Refunds should only be paid at completion of the Project together with the final audited statement.
(4) ACTUAL EXPENDITURE (whether cash or accrual) ONLY. Commitments shall **NOT** be included.
(5) Show allocation for the current financial year. Transfers between budget heads allowed under 9(f) of the Project Agreement, or approved, in writing by the FRDC, shall be listed in the comments.

Comments:

Certified by: Geza Horvanyi

(Signature)

FOR: R A RICKARD (DEPUTY DIRECTOR OF FINANCIAL OPERATIONS)

(Print Name)

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