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The use of fluorescent pigments (lipofuscin) for ageing western rock lobster (*Panulirus cygnus*) and scampi (*Metanephrops andamanicus*): a preliminary assessment

C. J. Crossland*, G. Denby*, B. F. Phillips*, and R. Brown†

* CSIRO Division of Fisheries Research, Marine Laboratories
PO Box 20, North Beach, Western Australia 6020

† Western Australian Marine Research Laboratories
PO Box 20, North Beach, Western Australia 6020

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Abstract

Techniques for extracting and analysing fluorescent pigment for determining the age of crustacea are described. Criteria for assessment of fluorescent ageing pigments (FAP) in different tissues are discussed. These pigments in eye and tail muscle tissues from the western rock lobster (*Panulirus cygnus*) and in eye tissues from scampi (*Metanephrops andamanicus*) are considered as a function of carapace length of animals from several geographical areas. The extreme variability in the data probably reflects both differences in the rate of FAP accumulation and the intrinsic problem of using carapace length as a determinant of age. It may be possible to utilise FAP content of eye or tail muscle to determine age groups or annual cohorts of the western rock lobster within a geographical site. However, evaluation of the efficacy of FAP as an age determinant will require long-term assessment (5–7 years) of animals in aquarium studies and tag-recapture experiments. Accumulation of FAP can be affected by variations in diet and other environmental factors influencing metabolic rates. Hence, data from this preliminary study do not provide assurance that FAP will have the resolution needed for age determination in *P. cygnus*.

INTRODUCTION

Determination of the age of juvenile and adult western rock lobsters, *Panulirus cygnus* George, has been confined to morphometric measurements, notably carapace length (see Phillips *et al.*, 1980). Generally, carapace length is used to group lobsters into one-year age classes. This does not allow fine assessments of growth in aquaria or field samples, or of the effects of environmental factors, such as food availability and physico-chemical variations. The establishment of a precise age criterion has wide and important implications for research and for the management of the western rock lobster fishery and, by extension, to lobster fisheries elsewhere.

The term "lipofuscin" originally described age-related brown pigment granules observed in histological sections of animal tissues (see Elleder, 1981). Lipofuscin is thought to accumulate in lysosomes (see Brunk & Collins, 1981) as a result of cellular lipid peroxidation processes. It is characterised by a Schiff-base chromaphore that results from cross-linkage of malonaldehyde or other carbonyl compounds and amino acids or their esters associated with proteins, nucleic acids, phospholipids and other cellular molecules (see Donato, 1981). Lipofuscin is not a single pigment but a class of pigments, with different polymers in different tissues exhibiting characteristic chromaphore fluorescences (Sohal, 1981).

Lipofuscin accumulation in tissues has been determined by histochemical and organic solvent extraction methods (Brizzee & Ordy, 1981; Elleder, 1981). Lipofuscin in chloroform extracts of animal tissues exhibits excitation maxima of 340-370 nm and emission maxima of 420-470 nm (Fletcher *et al.*, 1973). Accumulation is more apparent in non-mitotic tissues or post-mitotic tissues than in mitotic tissues (Sheldahl & Tappel, 1974). However, it is not known whether the fluorescence in chloroform extracts of animal tissues truly reflects lipofuscin *sensu stricto* or lipofuscin and other age-related fluorescent compounds (see Nicol, 1987). This controversy will not be resolved until the chemical nature of the fluorescent materials is fully determined and the biochemical processes of accumulation are elucidated. Hence, we follow the recommendation of Sohal (1984) that fluorescent compounds in chloroform extracts of animal tissues be referred to by a separate term, i.e. fluorescent ageing pigments (FAP).

Fluorescent ageing pigments have been found in the tissues of animals from most phyla (see Ettershank *et al.*, 1983). These pigments, which accumulate as a function of the metabolic activity of the tissues, have been used to determine the physiological age, and in some cases chronological age, in poikilotherms and in homeotherms (see Dapson *et al.*, 1980; Mail *et al.*, 1983; Lehane & Mail, 1985). They have also been used to evaluate the structure of populations of Antarctic krill, *Euphausia superba* (Ettershank, 1983, 1984).

Results are presented from preliminary studies to a) assess the accumulation of FAP in tissues of the western rock lobster and b) determine the efficacy of FAP as a criterion for age determination. A secondary objective was to determine a tissue type that would allow application of the method to specimens used in tag-recapture experiments, to evaluate fine scales of growth and ageing. Eye tissues were considered to be the best choice for this purpose.

Results from opportunistic experiments with scampi (*Metanephrops andamanicus*) are also presented.

MATERIALS AND METHODS

Animal samples

Specimens of the western rock lobster (*Panulirus cygnus*) were collected from the coastal shelf region of Western Australia, near Perth (Garden Island and Yanchep) and Seven Mile Beach, Dongara (Table 1). Animals were held in the aquarium system of the Western Australian Marine Research Laboratory, Watermans, until dissection. Pairs of eyes from the fresh commercial catch received by the Fremantle Fishermans Cooperative (Perth region) were stored frozen (-80°C) for about 8 weeks.

Table 1. Data on experimental specimens of western rock lobster (*Panulirus cygnus*) and scampi (*Metanephrops andamanicus*) collected in 1986.

Western rock lobster	Date collected	Range of carapace lengths (mm)	M	F	Total
Garden Island, Perth	14 Jan	45.6-82.3	12	15	27
Garden Island, Perth	22 Jan	42.4-81.8	11	6	17
Yanchep, Perth	17 Feb	87.6-150.0	10	13	23
Seven Mile Beach, Dongara	20 Feb	35.8-66.3	25	22	47
Fremantle Fishermans Cooperative (eyes only)	30 Jan	77.4-126.9	-	-	58
Total					172
Scampi					
North West Shelf (frozen)	Feb.-Apr.	29.5-57.8	10	22	32
North West Shelf (formalin)	Feb.-Apr.	28.3-60.9	15	19	34
Total					66

Scampi (*Metanephrops andamanicus*) were obtained from the Northwest Shelf fishery (between Dampier and Derby, 350 - 450 m depth) and stored frozen (-20°C, for 4 weeks) or in 5% neutralised marine formalin.

Morphometric characteristics (carapace length, eye fresh weight (FW) and diameter, tail FW, sex) were determined for each live specimen of *P. cygnus* before storage. Generally, eye pairs, tail muscle and the fifth left pereiopod were retained and frozen before FAP extraction. In preliminary experiments, fresh and formalin-stored tissues were assayed for FAP content.

Fluorescent age pigment (FAP)

Preliminary experiments with *P. cygnus* showed that tissues must be finely macerated to maximize FAP extraction by organic solvents and achieve acceptable repeatability of FAP yields. Less finely macerated tissues required relatively long periods (5 to 8 min) of ultrasonic disruption of both tail muscle (100-500 mg FW) and eye tissues (70-400 mg FW). This treatment heated the extraction solvent and increased evaporation of chloroform from the extraction solution. Consequently, FAP extraction was carried out in two stages: first, aqueous maceration and lyophilisation of tissues, and second, extraction by organic solvents. In addition, the aqueous maceration allowed replicate subsampling of a tissue during preliminary experiments to assess the effects of different disruption techniques on FAP yield. Repeated freezing and thawing of tissues, mechanical grinding, chemical (1-5% Triton X-100) and ultrasonic disruption of cellular membranes were evaluated for their efficacy in the extraction process. The effect of storage (frozen and formalin-treated tissues) on FAP yield was also evaluated.

Generally, 0.5-1.0 g FW of tail muscle (anterior oblique 1; Paterson, 1968), a single eye (> 150 mg FW per eye) or an eye pair (< 100 mg FW per eye) from *P. cygnus* was thawed, weighed, and homogenised in 4 ml distilled water with a glass mortar and pestle. The empty exoskeleton of eye samples was discarded. The tissue slurry was lyophilised to dryness and the dry weight determined.

Fluorescent ageing pigments were then extracted into chloroform by standard organic solvent methods (see Ettershank, 1983). A known dry weight of lyophilised tissue was placed in a glass centrifuge tube and 5 ml spectroscopic grade chloroform-methanol (2:1, v:v) was added. The tube was transferred to a beaker of ice and water and the sample was ultrasonically disrupted (Dawe Soniprobe, Model 753A with microprobe) at 125 watts for 2 min. Chloroform (1.0 ml) was added to replace evaporative loss during sonication, and the sample was held in an ice bath for 10 min. The solution was phase-separated by addition of 2.5 ml distilled water and centrifugation (3000 *g*, 10 min). The lower chloroform layer containing FAP was transferred to a stoppered measuring cylinder for volume determination prior to spectrofluorometry at 20°C. In some cases, the volume of the residual methanol-water homogenate was noted and a subsample frozen for protein determination.

Spectrofluorometry

The fluorophore content of extracts was evaluated with a Zeiss spectrofluorometer (Model PMQ3) fitted with an automatic amplification unit, using manufacturer's settings and standard 1 cm^2 square-section spectrofluorometer cuvettes. Fluorescence emission values were recorded at 10 nm intervals between 390 and 480 nm with an excitation wavelength of 350 nm. The spectrofluorometer was standardised with quinine sulphate solution (1 mg l N H SO excitation 350 nm, emission 445 nm) and fluorescence intensities were determined in 1/1000th units of the full-scale deflection of the standard solution.

Spectrofluorometric (excitation 350 nm, emission spectra 360-600 nm) and spectrophotometric (380-600 nm) scans were made of the FAP extracts for each tissue type from a range of specimen sizes (carapace lengths) to evaluate any interfering pigments.

Protein determination

A sample (eye, 1.0 ml; other tissues, 0.5 ml) of the methanol-water fraction was assayed for protein (Lowry *et al.*, 1951), using bovine serum albumen as a standard.

Data analysis

Fluorescence intensity at the appropriate spectral maximum for each tissue (eye, 420 nm; tail and pereiopod muscle, 460 nm) was corrected for the chloroform blank emission, and the result expressed as a percentage of the quinine sulphate standard in Relative Fluorescence Units (RFU). The fluorescence intensity for extracts from eye tissues of both *P. cygnus* and *M. andamanicus* was diminished by an emission-absorbing pigment at a concentration determined by specimen size (see Results). The RFU for each eye sample was calculated after the fluorescence intensity value was corrected for sample self-absorption. Allowing for use of a constant sample volume and the geometry of the fluorescence emission unit and cuvette, mean self-absorption in the sample would be represented by (100-%T)/2, where %T is the spectrophotometric percentage transmission of the chloroform extract at the spectrofluorometric spectral maximum of the tissue type. Hence for the eye extracts the corrected fluorescence intensity = observed fluorescence intensity x $100(100 - (100 - \%T)/2)^{-1}$. The %T for eye tissue extracts was sometimes determined directly, but was generally calculated from linear functions for %T versus carapace length determined experimentally for *P. cygnus* and *M. andamanicus*.

Generally, FAP content was expressed as RFU, g-1 FW tissue or RFU, eye-1 and considered as a function of animal size (carapace length). Curve functions were determined by iterative fitting with the Simulation, Analysis and Modelling program (Berman & Wiess, 1967) revised to SAAM-31 adapted for an IBM-compatible Olivetti M24 PC (R. Boston, pers. comm.).

RESULTS

The relationship between eye and tail weights and carapace length in both *Panulirus cygnus* and *Metanephrops andamanicus* are best described by non-linear functions (Figs. 1, 2 & 3). However, the animals used in the FAP determinations all had carapace lengths within a nearly linear phase of the curve; in *P. cygnus* this represents about 90% of the known size range of the benthic phase of the life cycle.

Protein concentration was considered as an alternative to fresh weight for normalising FAP content between tissue samples, but was rejected because of its greater variability (Fig. 4). The non-systematic errors in protein colorimetry probably resulted from inhomogeneity of finely macerated tissues in the methanol-water fraction. The variability in protein values did not indicate that penetration of tissues and extraction of FAP by the chloroform-methanol solution was either variable or inadequate, as a close correlation was demonstrated for *P. cygnus* between extracted FAP and weight of tail muscle (Fig. 5).

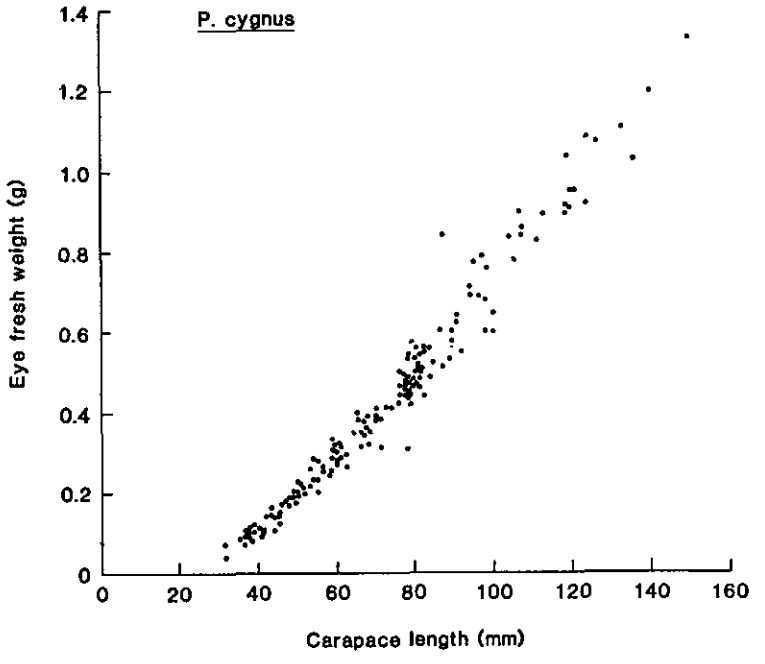

FIG. 1. *Panulirus cygnus*. Fresh weight per eye as a function of carapace length. ($y = 0.00015 + 0.000002\ x^{1.83 \pm 0.03}$; where y = gFW, eye^{-1} and x = carapace length (mm), estimate ± standard deviation).

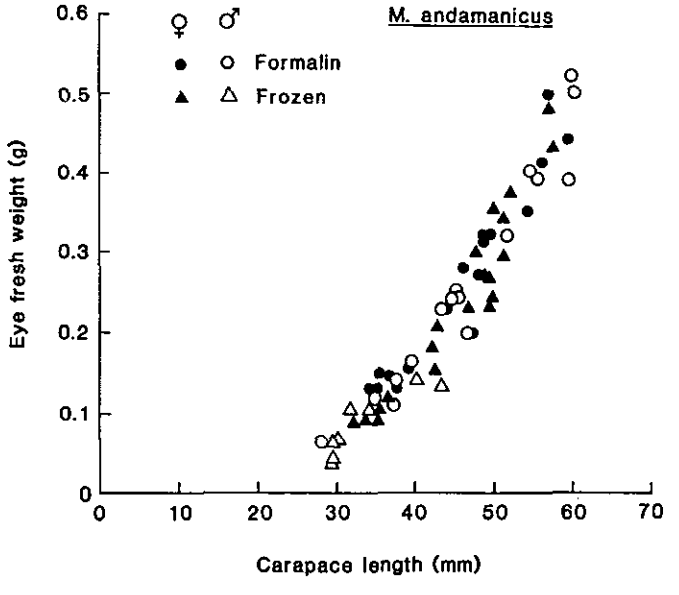

FIG. 2. *Metanephrops andamanicus*. Fresh weight per eye as a function of carapace length. ($y = 0.00008 + 0.000003\ x^{2.66 \pm 0.13}$; where y = gFW, eye^{-1} and x = carapace length (mm), estimate ± standard deviation).

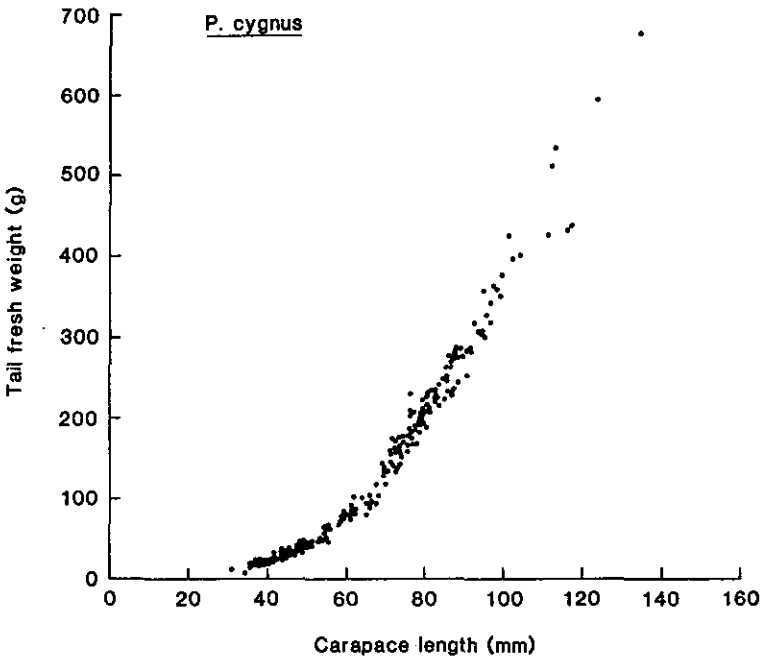

FIG. 3. *Panulirus cygnus*. Fresh weight per tail as a function of carapace length. ($y = 0.00059 + 0.00007\ x^{2.87 \pm 0.02}$; where y = g FW $tail^{-1}$ and x = carapace length (mm), estimate ± standard deviation).

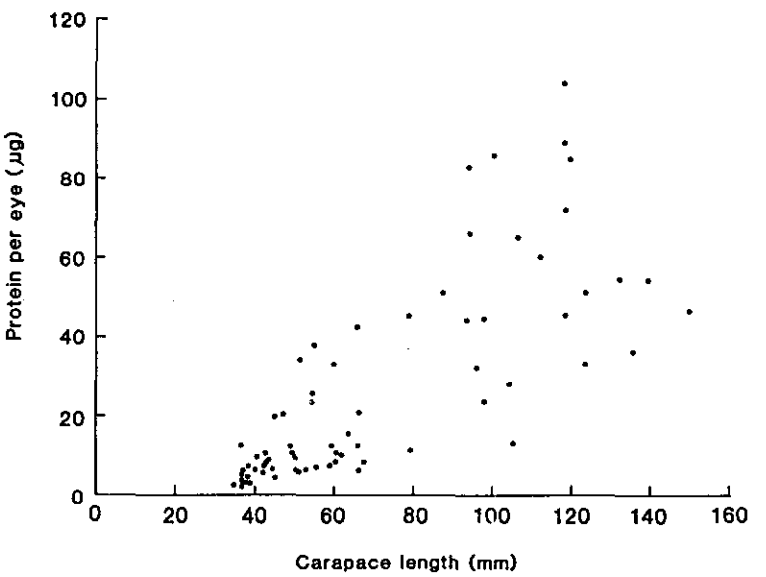

FIG. 4. *Panulirus cygnus*. Protein content per eye as a function of carapace length.

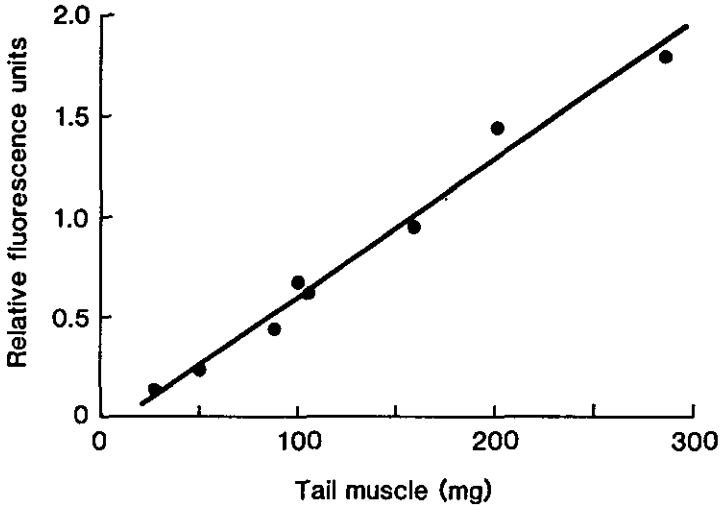

FIG. 5. *Panulirus cygnus*. Effect of quantity of tail muscle extracted on fluorescence intensity. ($y = 0.007x - 0.08$, where y = relative fluorescence units and x = mg FW of tail muscle; $r^2 = 0.98$).

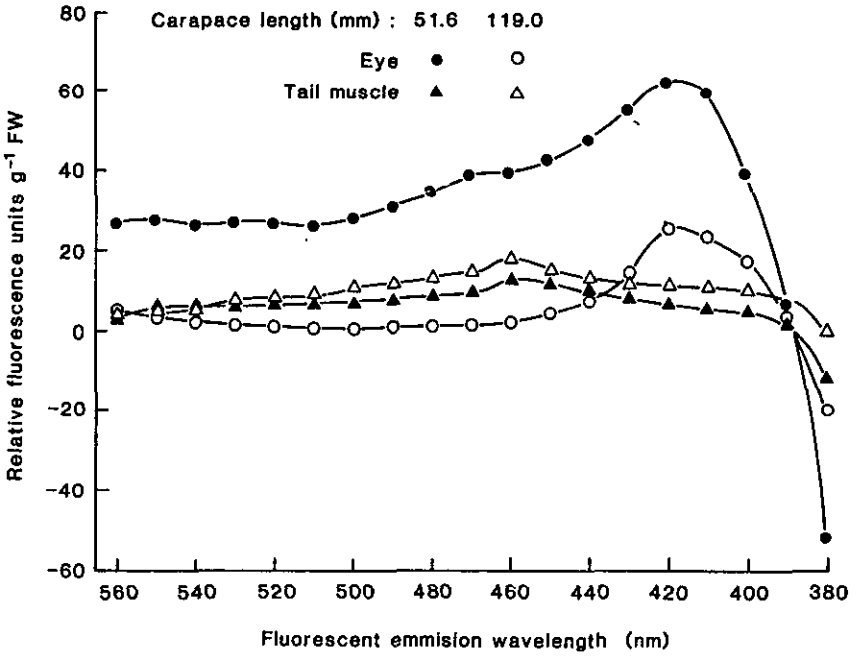

FIG. 6. *Panulirus cygnus*. Fluorescence emission spectra for extracts of eye and tail tissues from small and large animals. (Fluorescence intensity corrected for chloroform blank but uncorrected for "interference" compounds).

Aqueous homogenisation and lyophilisation of tissues yielded more FAP than did direct organic extraction techniques (Table 2). The lower yield from direct extraction could have resulted from limited solvent penetration of the relatively coarse sample fragments, high evaporative loss of chloroform and heat generated during the extended ultrasonic treatment (> 3 min) required to obtain consistent results between replicates. The aqueous extraction sequence yielded a finely dispersed tissue sample, which may have enhanced organic solvent penetration and necessitated a shorter ultrasonic period for maximum FAP yield. Separate experiments showed that mechanical disruption of tissues by freeze-thawing or grinding in a hand-homogeniser during direct organic solvent extraction slightly improved the FAP yield but did not reduce the variability between tissue replicates. Ultrasonic disruption of tissues was necessary in both extraction methods to achieve maximum FAP yield. Addition of Triton X-100 during aqueous extraction resulted in FAP values 60-80% those of ultrasonic treatment alone; combining these two membrane disruption techniques did not produce a synergistic increase in FAP yield.

Tissues stored in 5% neutralised marine formalin yielded higher FAP levels than did frozen tissues (Table 3). This yield enhancement was most marked in smaller specimens of *P. cygnus*. Fresh and frozen tissues of eye and tail muscle of P. cygnus had similar FAP yield.

Three different fluorescence emission maxima were found for the seven tissue types assessed: 440-470 nm for tail muscle (anterior oblique 1), antennal peduncle muscle, uropod muscle, and cerebral ganglia; 420-450 nm for heart, and the mesopodite of the fifth pereiopod; and 410-440 nm for eye tissue. The variation in wavelength of maximum emission within a tissue type may reflect the complexity and interaction of different fluorophores and absorbing molecules in the crude extracts (M. Sheehy, pers. comm). The spectrum of fluorescence emission from tail muscle extracts of *P. cygnus* (Fig. 6) typifies those of the other muscle tissues and cerebral ganglia in showing a small but distinct and replicable peak in emission intensity (415 mm for eye tissue and 460 mm for tail muscle). The spectra of heart and pereiopod extracts were similar to those of tail muscle, but generally with peak emission at 445 nm. Fluorescence emission spectra for eye tissue extracts from *M. andamanicus* were similar to those of *P. cygnus* (Fig. 6).

Chloroform extracts of eye tissues from both species had a characteristic yellow-orange colour, which intensified with increased size of the specimens (Fig. 7). Similarly, the orange-red colour of the chloroform extract from pereiopod tissues intensified with animal size. However, the intensity varied between specimens of similar size, probably as a result of differences in the intermoult phases of growth and associated development of membrane epicuticle (Dall & Barclay, 1977).

The visible pigmentation in extracts of eye and pereiopod tissues from *P. cygnus* and eye tissues from *M. andamanicus* acted to "quench" the fluorescence emission. The degree of absorption was a function of both the size of the specimen and the emission wavelength (Fig. 8). For eye tissue extracts, correction factors for sample self-absorption were calculated from percentage transmission (%T) spectra determined with a chloroform reference solution: *P. cygnus*, $y = 0.87 - 0.0047x$; *M. andamanicus*, $y = 1.66 - 0.024x$, where y = %T and x = carapace length (mm). Tail muscle extracts showed no significant "quench" i.e. mean %T (standard deviation) = 98.3 ± 1.60 between 380 nm and 480 nm emission wavelength.

Table 2. Effect of ultrasonic treatment on FAP yield from eye and tail muscle tissues of western rock lobster (*Panulirus cygnus*) during aqueous and organic extraction periods (One eye, aqueous extraction and one eye, organic extraction for each animal.
Carapace lengths: Eye (#1) 77.9-78.6 mm,
(#2) 79.7-80.9 mm; tail muscle (#3) 68.2 mm, (#4) 82.3 mm).

	Fluorescence Yield (RFU, g tissue^{-1})							
Tissue:	Eye				Tail			
Extraction:	Aqueous		Organic		Aqueous		Organic	
Sonic treatment (minutes)	(#1)	(#2)	(#1)	(#2)	(#3)	(#4)	(#3)	(#4)
0	9.6	-	0.8	-	2.8	2.0	4.3	8.3
0.5	24.6	-	0.4	-	4.2	8.0	-	-
1.0	35.0,12.3,13.3	38.9,36.2,56.1	2.3,3.9,2.0	2.7,3.8,5.5	5.7	13.2	5.6	9.5
1.5	-	-	-	-	7.9	26.2	-	-
2.0	98.8,92.8,95.2	66.6,62.3,56.2	4.3,2.1,1.8	7.2,5.3,3.4	7.0	27.6	5.2	9.2
3.0	79.2,112.0,68.7	-	1.9,6.4,3.0	-	8.2	22.2	-	-

Table 3. Effect of freezing and formalin storage on FAP yield from eyes of western rock lobster (*Panulirus cygnus*) and scampi (*Metanephrops andamanicus*). (For each lobster, one eye was frozen and one eye was stored for 11 days in 5% buffered marine formalin prior to aqueous extraction. Separate specimens of scampi eyes with similar carapace length were selected from freezer or formalin storage conditions for aqueous extraction).

Treatment	Fluorescence Yield (RFU, g tissue^{-1})									
Western rock lobster										
Carapace length (mm)	49.1	60.4	70.1	80.2	89.3	101.4	113	120.8	140	150
Frozen	64.3	54.6	70.6	41.0	14.6	48.4	26.8	14.7	20.2	18.7
Formalin	602.8	354.2	344.1	307.2	108.3	156.6	43.8	48.2	40.9	30.3
% Yield,Frozen/formalin	11	15	20	13	13	31	61	31	49	95
Scampi										
Carapace length (mm)		53.4	54.3			57.0	56.9			
Frozen		10.2	-			18.8	-			
Formalin		-	24.4			-	34.4			
% Yield Frozen/formalin		42					55			

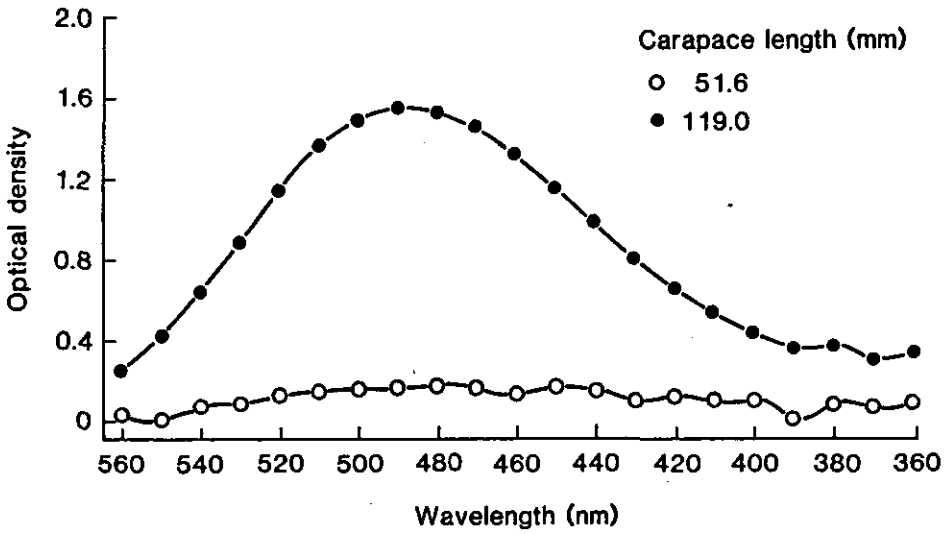

FIG. 7. *Panulirus cygnus*. Absorption spectra for extracts of eye tissues from small and large animals.

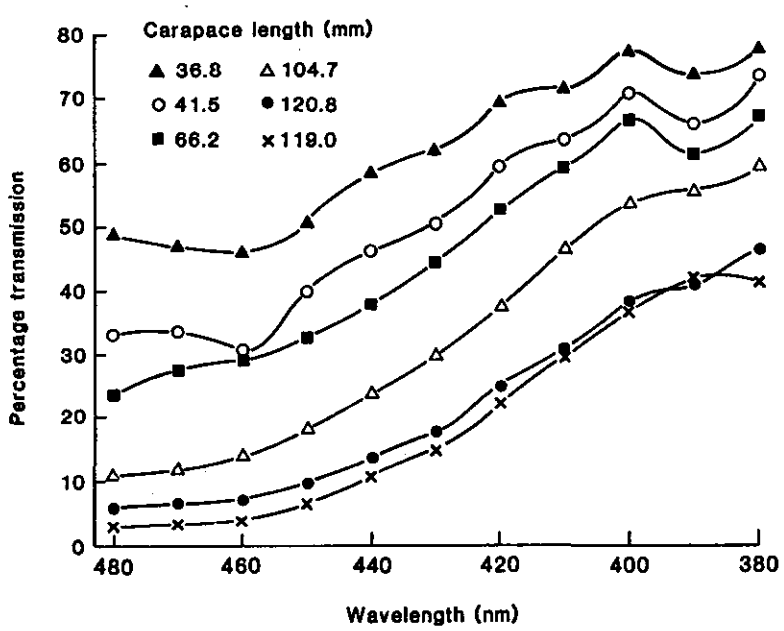

FIG. 8. *Panulirus cygnus*. Percentage transmission spectra for extracts of eye tissues from animals of different size.

The fluorescence intensity at the appropriate emission maximum was used to estimate the relative FAP content of eye and tail muscle tissues from *P. cygnus* and eye tissues from *M. andamanicus*. There was no systematic relationship between the apparent FAP concentration in the anterior oblique 1 tail muscle and the size of *P. cygnus* (Fig. 9). There was marked variability in FAP concentration between animals in each sample group, with no trend apparent within a sample group. The wide spread of data is reduced when FAP is expressed on a whole tail basis (Fig. 10), assuming the FAP concentration of the anterior oblique 1 muscle is representative of the entire tail tissue. However, high variability remains between and within the specimen sample groups. The difference between sample groups is marked (see 50-70 mm carapace length, Fig. 10). Within sample groups, the FAP content for similar sized specimens ranges from two-fold (70 mm and 80 mm carapace length) to five-fold (120 mm carapace length), while the carapace length for similar FAP content covers more than a two-fold range (see 400, 2500, 4500 RFU $tail^{-1}$).

The FAP concentration in eye tissues from *P. cygnus* decreased with carapace length (Fig. 11) and showed high variability. Expressed on an entire eye basis (Fig. 12), FAP content was sustained with animal size but showed a wide variation between and within each sample group. The spread pattern of data was not attributable to sex or site of collection of the specimens. Similarly, the FAP content of entire eyes from *M. andamanicus* was independent of specimen size (Fig. 13). No data were available on moult stages of the experimental animals.

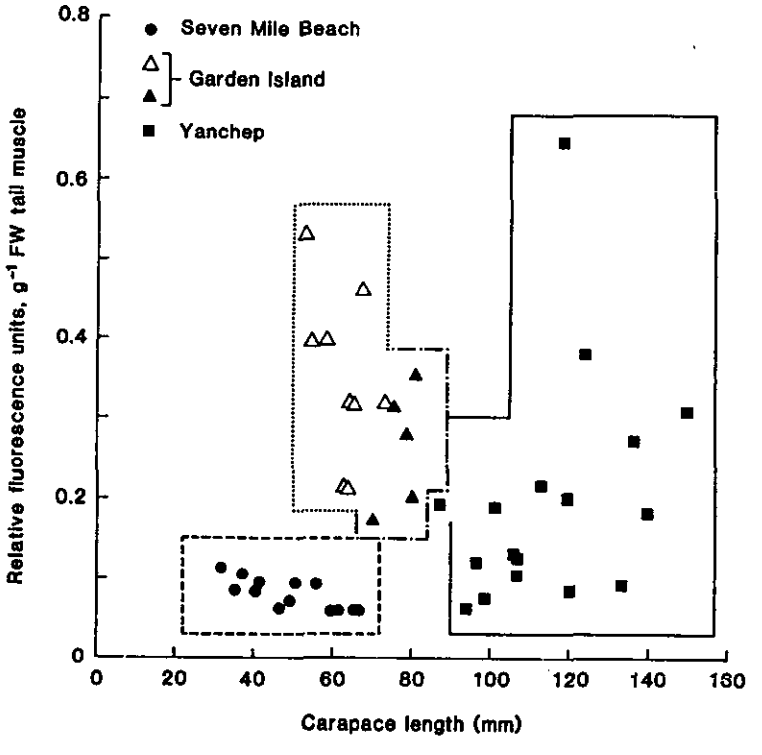

FIG. 9. *Panulirus cygnus*. FAP concentration in extracts of tail muscle from animals collected at Seven Mile Beach, Garden Island and Yanchep.

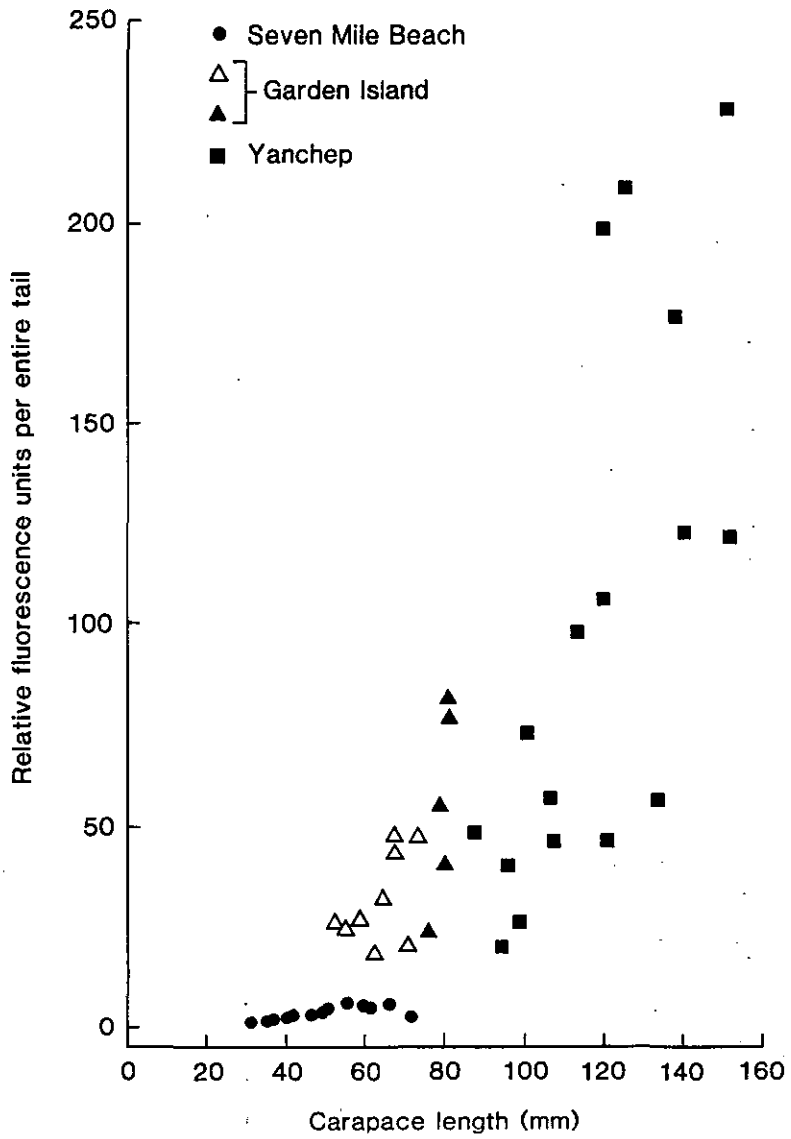

FIG. 10. *Panulirus cygnus*. FAP content per entire tail of animals from Seven Mile Beach, Garden Island and Yanchep.

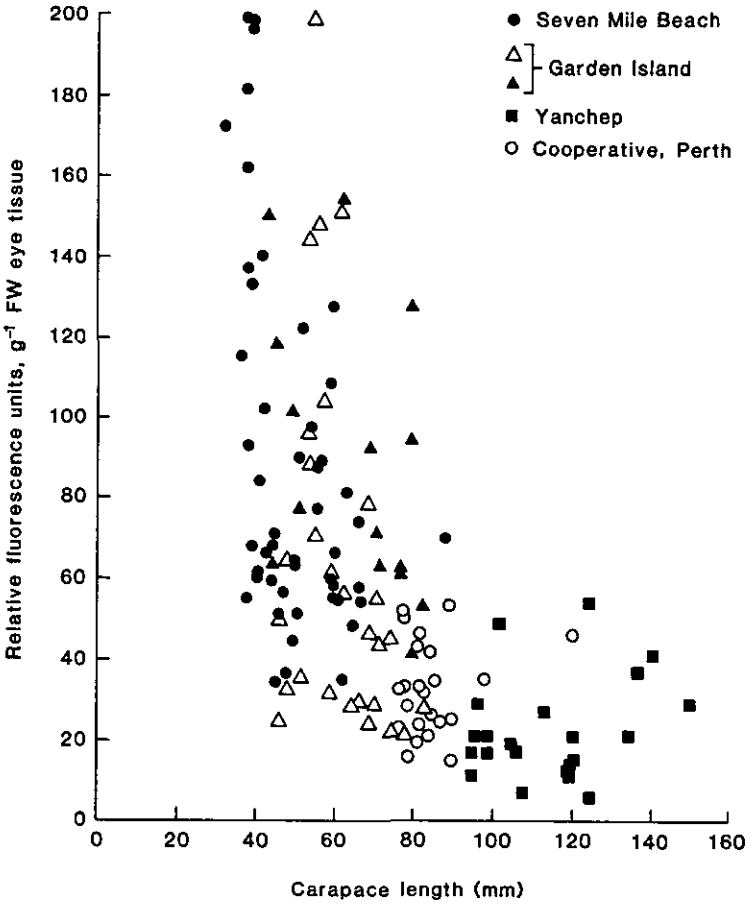

FIG. 11. *Panulirus cygnus*. FAP concentration in extracts of eye tissues from animals collected at Seven Mile Beach, Garden Island, Yanchep and from the Fremantle Fishermans Cooperative.

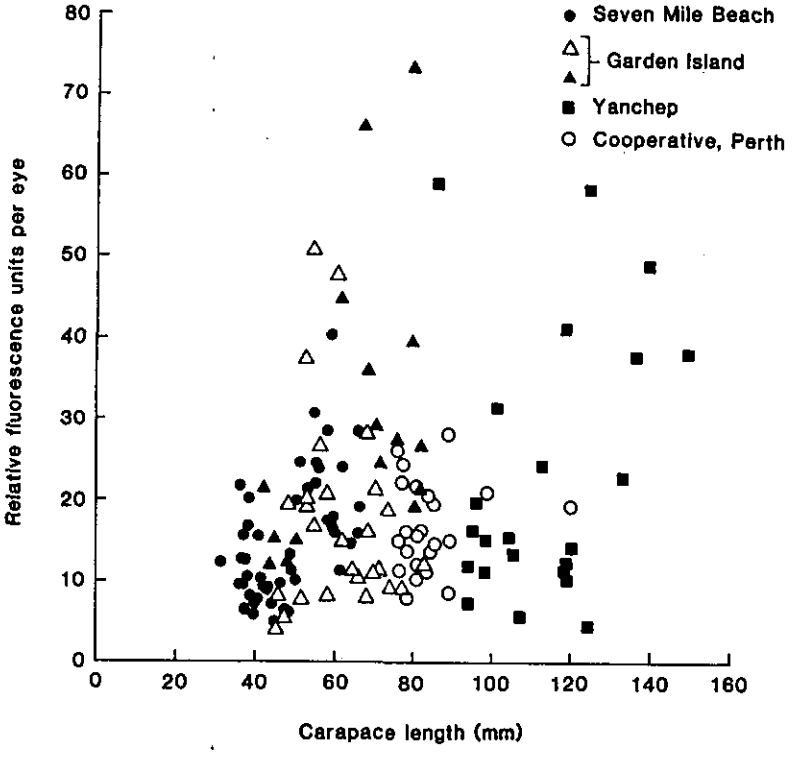

FIG. 12. *Panulirus cygnus*. FAP content per eye of animals collected from Seven Mile Beach, Garden Island, Yanchep and from the Fremantle Fishermans Cooperative.

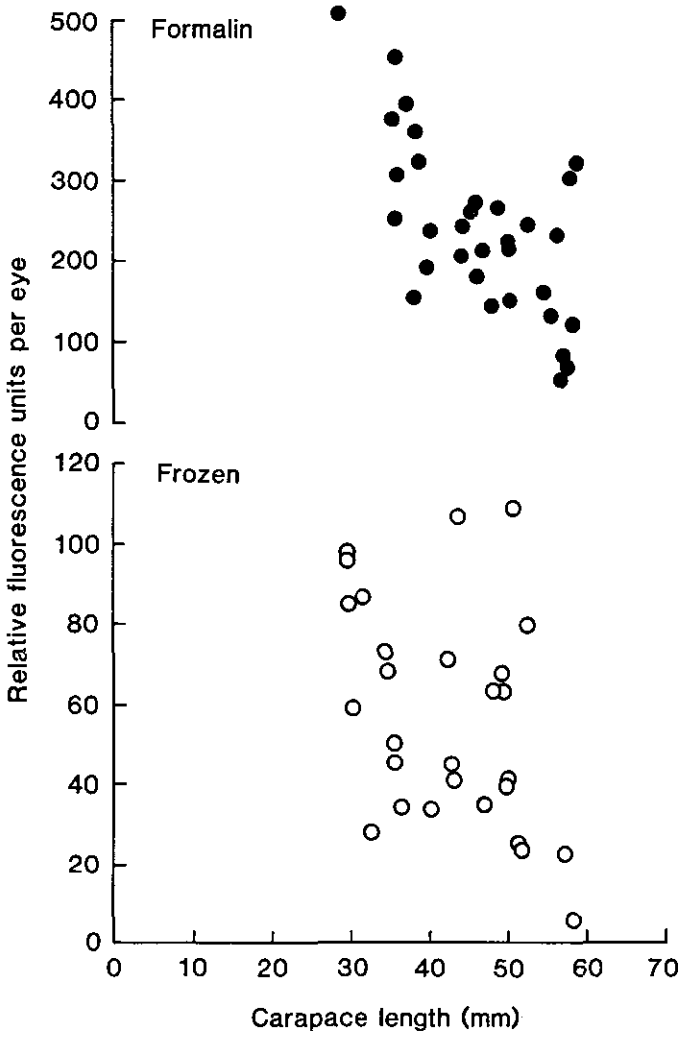

FIG. 13. *Metanephrops andamanicus*. FAP content per eye of animals stored frozen or in 5% buffered marine formalin.

DISCUSSION

Chloroform extracts of eyes, tail muscle and other tissues from *Panulirus cygnus* and eyes from Metanephrops andamanicus yielded fluorescence emission spectra similar to those described for fluorescent ageing pigments (lipofuscin) in other organisms (Fletcher *et al.*, 1973; Czallany & Azaz, 1976; Donata, 1981; Siakotos & Munkres, 1981). The requirement for ultrasonic treatment during extraction of tissues, and the lesser but elevated yield from Triton X-100 treatment alone, suggest that fluorescent materials are associated with cellular membranes or organelles.

The small but consistent fluorescence emission maxima and the shape of the spectral curve for tail muscle of *P. cygnus* indicate an interference from other fluorescent compounds in the crude extracts. The initial aqueous extraction and the water separation of chloroform-methanol minimised interferences from water-soluble compounds (e.g., flavins and reduced pyridine nucleotides; Fletcher *et al*, 1973). Czallany & Azaz (1976) have shown that retinol and an unidentified, low molecular weight compound contribute to the fluorescence spectra in chloroform extracts of mice tissues; the degree of interference may vary among tissue types. Such interference can result in a broadening and shift in the fluorescence emission maxima of lipofuscin polymers (see Elleder, 1981). The shift in the fluorescence emission maximum for tail muscle (450 nm to 460 nm) with increased size of *P. cygnus* specimens is consistent with retinol interference, as is the difference in wavelength of the fluorescence emission maxima of muscle tissue types, although each tissue may have its own form of FAP (Sohal, 1981). The presence of a fluorescence emission maximum between 450 nm and 460 nm and the absence of an emission peak around 495 nm in tail muscle indicate that retinol interference does not dominate the FAP signature.

The emission data and the higher FAP values determined with increasing quantity of tail muscle support the use of the chloroform-methanol extraction technique and the validity of using data from crude extracts for comparing FAP content in different-sized lobster specimens, at least in these preliminary experiments. Partial purification of FAP by gel permeation and other chromatographic methods, the degree of interference by other fluorescent compounds, and further characterisation of the pigment and functional chromatophore (Taubold *et al.*, 1975; Czallany & Azaz, 1976; Siakolos & Munkres, 1981) would be necessary for fine measurement of ageing pigments. Here, histochemical methods should be used in association with solvent extraction techniques to validate variations in FAP, lipofuscin concentration and processes of accumulation (Elleder, 1981; Nicol, 1987).

The fluorescence emission spectra of the crude chloroform extracts from the eye tissues of *P. cygnus* and *M. andamanicus* are apparently free of any major interference by other fluorescence compounds. However, an unidentified pigment differentially absorbed emission wavelengths but showed a systematic relationship with the size of lobster or scampi specimens. A correction ("quench") factor calculated for the appropriate wavelength was applied to each FAP value determined for the eye tissues. The spectrophotometric absorption spectra of chloroform extracts from eye tissue were similar to that of the visual pigment, rhodopsin (Cummins *et al.*, 1984).

The increased yield of FAP from formalin-stored eye tissues is consistent with the demonstration of elevated yields from alcohol-stored Diptera tissues (Ettershank *et al.*, 1983). Similarly, increased fluorescence with formalin and alcohol treatments has been shown for euphausid and squid tissue (Nicol, 1987) and for *Daphnia carinata* (using fluorescence microscopy)(Sheehy & Ettershank, in prep.). There is controversy about the relative extractability of fixed and non-fixed tissues and the validity of data based on histochemical as against solvent extraction methods (see Elleder, 1981). The chloroform-methanol extraction technique may reflect free intracellular, and only a portion of membrane-bound, FAP. Histochemical methods may demonstrate only membrane-bound (lysosomal) FAP. While the numerical values for FAP concentration in frozen and formalin-fixed eye tissues of *M. andamanicus* differed, the relative values with animal size were similar for both tissue preparations. Lipid polymers become stabilised by aldehyde fixation and, while the membrane material is possibly more readily extracted in this state, the probability of a formaldehyde-generated artefact has not been determined. The FAP yield in response to tissue fixation types and methods and the processes of tissue change during fixation require further evaluation.

The FAP concentration in eye tissues from *P. cygnus* and *M. andamanicus* diminished with animal size. There was no size-related pattern in FAP concentration for anterior oblique 1 tail muscle from the lobster. However, the FAP content of both tissue types, expressed as a function of the entire organ (i.e. per eye, per tail), tended to increase with the size of the lobster specimen. No similar trend was apparent in scampi, perhaps because of the smaller size range of the experimental sample. The difference in the trends of FAP concentration and content per entire organ in *P. cygnus* probably relates to different mitotic activity in the two tissue types. Generally, lipofuscin pigments accumulate more rapidly in post-mitotic cells, though significant amounts have been found in tissues of young vertebrates (Brizzee & Ordy, 1981). The nerve-related eye tissue would be relatively less mitotic than the tail muscle. However, there is little information available on the cellular turn-over of eye tissues and the data for FAP in the entire tail assumes that concentration in the anterior oblique 1 muscle is representative of all tail muscle. There is no information on the degree of cellular damage resulting from ecdysis; such damage may influence FAP content of tissues.

The accumulation of FAP is considered to be a function of the metabolic activity of a tissue and hence reflects physiological age rather than chronological age (see Dapson *et al.*, 1980; Sohal, 1981; Zuckerman & Geist, 1981). However, FAP has been used to describe chronological age in some animals (see Dapson *et al.*, 1980; Sheehy & Ettershank, in prep.). While there was a broad trend in FAP accumulation in the eye and tail muscle of *P. cygnus*, the variability among specimens is too extreme to assert that there is a predictable relationship between FAP and the morphometric characteristic of carapace length, which is traditionally used to approximate chronological age in the lobster. Some of the variability is a result of the inadequacy of carapace length as a criterion for chronological age, as demonstrated by the differential growth of *P. cygnus* in aquarium conditions, under ambient and controlled temperatures (Chittleborough, 1975; Phillips *et al.*, 1977; Phillips *et al.*, 1983) and tag-recapture experiments in the field (Joll & Phillips, 1984; R. Brown, unpublished). Differences between geographical sites in water temperatures and the quality and quantity of the diet may also contribute to the variability in FAP found within and between each sample group of *P. cygnus*. For example, in Fig. 9, similar values were determined for the FAP content per tail, in the 13 specimens from the Seven Mile Beach sample, which had a chronological age range of approximately 2-4 years, based on carapace length (Chittleborough, 1976).

In summary, these preliminary experiments indicate that the FAP content of eye and tail tissue from *P. cygnus* and *M. andamanicus* does not provide a suitable criterion for determination of chronological age. It may be useful in evaluating physiological age of population groups within a locality, but within the limits of current knowledge, FAP content of tissue is no more effective than the traditional morphometric measure of chronological age, carapace length. Further study of population dynamics in western rock lobsters by tag-recapture techniques, especially utilising microtagging systems in early juvenile stages (B.F. Phillips, unpublished), will undoubtedly improve understanding of growth characteristics. Evaluating FAP content of selected tissues in conjunction with these and associated aquarium experiments may provide understanding of the wide variability determined here between specimens and perhaps yield a criterion of value for description of age in *P. cygnus*.

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Marine Laboratories
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Division of Oceanography
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HEADQUARTERS
Castray Esplanade, Hobart, Tas
GPO Box 1538, Hobart, Tas 7001,
Australia

QUEENSLAND LABORATORY
233 Middle Street, Cleveland, Qld
PO Box 120, Cleveland, Qld 4163

WESTERN AUSTRALIAN LABORATORY
Leach Street, Marmion, WA
PO Box 20, North Beach, WA 6020

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