FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Potential of Snapper, *Pagrus auratus*, for Mariculture

Project No. DAN13Z

FINAL REPORT

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FINAL REPORT TO THE FISHERIES RESEARCH & DEVELOPMENT CORPORATION

PROJECT TITLE

Potential of snapper, *Pagrus auratus*, for Mariculture.

PROJECT NO.

DAN13Z

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NON-TECHNICAL SUMMARY

The potential of snapper, *Pagrus auratus*, for mariculture in Australian temperate waters was assessed by growth trials. Juvenile snapper were taken from the wild and held in tanks and a saltwater pool for up to three years. The snapper were fed dry pelletised diets. We showed that captive snapper reared on pellets grew at approximately twice the rate of wild snapper. We further increased the growth rate of captive snapper by feeding a pellet diet we formulated specifically for snapper. As the cost of food is the major operating cost, we tried to reduce the cost of the diet we developed. The most expensive component of the diet was fish meal; the protein source. We lowered the cost by replacing some of the fish meal with cheaper sources of protein such as soybean meal and poultry by-product meal. We found we could replace about half the fish meal and not have a significant effect on growth rate. We also examined the effect of water temperature on the growth of juvenile captive snapper. Over winter we doubled the growth rate of juvenile snapper by rearing them in seawater heated to between 3 and 5°C above its ambient temperature.

Another objective of the project was to find a suitable means of identifying individual juvenile snapper. Electronic tags were found to be the most suitable. Most importantly we showed that they did not affect the growth of juvenile snapper.

One method of ageing fish is to examine the ear bones or otoliths. The age is estimated from the number of concentric

bands seen when the otolith is sectioned. We examined the otoliths of juvenile snapper held captive for different lengths of time and validated the method.

BACKGROUND

Australia has very modest natural fisheries resources compared to most other countries. Most commercially exploited wild fisheries are in danger of over exploitation and yields are generally declining. In addition, the import of white portion fish has been increasing; nationally we import about 40,000 t pa, valued at $150 million. Factors such as the decline in our natural fish stocks and the need to address our net trade deficit has stimulated interest in marine fish farming or mariculture. Few species of fish indigenous to south-eastern Australia have potential for farming. The market price of many of the abundant in-shore species (e.g. luderick, sea mullet, bream) is not high enough to attract commercial enterprise . The snapper, *Pagrus auratus*, is an exception. It is a highly sought after table fish and, importantly, the highest prices are paid for the smallest marketable fish. The attraction in farming this species would become even greater with the development of a market for live fish in restaurants.

Snapper have been farmed in Japan for about 20 years and current production exceeds 50,000 t pa. The artificial propagation of snapper is well established and documented. Locally, the production of juveniles for grow-out could done using similar technology. The economic viability of snapper farming then partly depends on: (1) the time it takes captive fish to get to

market size and (2) the cost of food. This project looked at both aspects by doing growth/nutrition trials on wild caught juvenile snapper.

OBJECTIVES

1. To determine whether the growth rate of snapper can be increased by varying water temperature and feeding regimes.
2. To assess the suitability of the Visible Implant tag for marking juvenile snapper.
3. To validate estimates of the age of juvenile snapper based on growth rings deposited in otoliths.

INTRODUCTORY TECHNICAL INFORMATION

Fish meal is the protein source of choice for fish feeds although increasing demand , reduced supply and increasing cost have led to a major research effort to replace fish meal with alternative sources of protein. In Australia, the production of large quantities fish meal is not feasible because bait fish are scarce. Therefore, a pre-requisite for the large scale development of marine fish farming is the identification of a local supply of high quality protein to replace fish meal. We substituted a combination of poultry by-product meal and soybean meal for fish meal. We compared the growth of juvenile snapper fed a reference diet (64% fish meal) to that of those fed diets with less fish meal.

It is well known that growth rate depends on water temperature. We examined the effect of water temperature on the growth of

juvenile snapper and also looked for an interaction of diet and temperature.

Passive Implantable Transponder (PIT) tags were used to identify individual fish in the growth/nutrition experiments. PIT tags have only become commercially available recently and were tried because they have advantages over other methods of identification.

RESEARCH METHODOLOGY

Growth/nutrition experiments were designed as factorial experiments with replicate tanks nested within each factor combination. The design was justified because of the need to check for any significant variation among tanks nested within a factor combination. During the course of the project several improvements were made to increase the power of experiments. Individual fish were identified with PIT tags. Fish were graded before the start of an experiment so that the coefficient of variation of the weight of fish in each tank was less than 15%.

Further detail on the methods used in the project can be found in the published papers and manuscripts appended.

RESULTS

Our first feeding trial showed that captive snapper fed a dry pellet diet can be grown to market size in less than two years at ambient water temperatures for the Sydney region (13 to 24°C). The fish were fed a dry sinking pellet with 42% protein. At the

start of the trial in April 1989, the snapper had a mean fork length of 121 ± 11 mm SD, an estimated mean weight of 50 g, and an estimated age of eight months. After 12.5 months their mean FL was 249 ± 15 mm SD and their mean weight was 403 ± 70 g SD. These results have been published (see Appendix 1). We have continued to hold and feed the group of snapper from our first feeding trial. At an estimated age of 2.8 years their mean fork length and weight was 341 mm and 949 g while at 3.7 years it was 400 mm and 1530 g. In contrast, wild snapper take between 3 and 5 years to reach 250 mm and a weight of about 360 g. This confirms that the growth rate of snapper continues to be increased significantly in captivity. Assuming isometric growth, the condition factor of our captive snapper was significantly greater than that of wild snapper (Fig. 1).

We collaborated with Dr Geoff Allan (BWFCRS) to formulate a dry pellet diet for snapper with fish meal (64%) as the protein source. This served as a reference diet. We also formulated a second diet by replacing 90% of the fish meal in the reference diet with poultry by-product meal and soybean meal. In our second feeding trial, the two diets were compared with two diets available commercially for other species. The juvenile snapper fed the reference diet had significantly higher growth rates and lower FCRs. There was no significant difference among the other three diets. Further details on the trial are in a publication (Appendix 2) and in a manuscript (Appendix 3) to be submitted for publication.

Replacing 90% of the fish meal in the reference diet with poultry by-product meal and soybean meal depressed growth and increased FCR. In our next feeding trial we compared four diets for which the amount of fish meal ranged from 64% (reference diet) to 10%. The ingredients and approximate nutrient composition of the diets are given in Table 1. The aim was to find approximately how much fish meal could be replaced before growth and FCR were affected. Under the conditions of our experiment, the growth and FCR of juvenile snapper fed the diet with 30% fish meal were not significantly different to that of those fed the reference diet (Table 2). Further reduction of the fish meal content of the diet to 20% gave a significantly lower growth rate compared to the reference diet (Table 2). The diet with 10% fish meal gave a significantly lower growth rate and a significantly higher FCR compared to the reference diet (Table 2).

In our final trial, the two diets (64 and 30% fish meal) were compared at two water temperatures; ambient (13 to 19°C) and ambient + 3 to 5°C. Over the four month trial (May to September 1992) we found that: (1) As before, there was no significant difference in the growth of juvenile snapper fed either diet, (2) increasing the seawater temperature by 3 to 5°C doubled the growth rate of juvenile snapper, and (3) there was no significant interaction of diet and temperature (Table 3). The lack of interaction between diet and temperature is important since commercial aquaculture sites will most probably be established where the ambient seawater temperatures are higher than in the Sydney region.

A paper describing the results of our last two trials and of a digestibility trial is in preparation and will be submitted for publication in the journal "Aquaculture". A reprint of the paper will be sent upon receipt.

Formal taste tests on wild and pellet-fed snapper were done in collaboration with Dr J Prescott (CSIRO). The overall acceptability of snapper reared on pellets was not significantly different to that of wild snapper (Appendix 4). We have done further formal taste tests comparing snapper fed the reference diet (64% fish meal) with snapper fed the diet with 30% fish meal. The results will be submitted for publication. A reprint of the paper will be sent upon receipt.

We tried several different methods for marking juvenile snapper. Visible Implant tags were not suitable because of the trauma caused implanting them. Microwire tags were difficult to read and the fish had to be killed to recover them. Therefore it was not possible to get data from individual fish without terminating the experiment. The formation of nodes on the dorsal fin rays was unreliable. After a series of experiments, we chose PIT tags for the following reasons: (1) they can be used on relatively small fish, (2) they can be implanted with little trauma to the fish, (3) they do not affect the growth of juvenile snapper (Appendix 5), (4) they can be read quickly and non-invasively thus minimising handling of the fish and (5) they are read electronically so the tag number can be directly entered into a computer.

A paper validating the use of otoliths to age young snapper has been published in the Australian Journal of Marine and Freshwater Research (Appendix 6).

The most serious disease that occurred during the project was caused by the infection of juvenile snapper with the protozoan *Crytocaryon irritans*. The organism was seen easily in wet preparations both of skin scrapings and gill filaments from moribund fish. When diagnosed early, the infection was treated effectively with a formalin (40% formaldehyde solution) bath for 1 hour at a concentration of 150 ppm v/v (approx. 160 mg/l) and which was repeated 48 and 96 hours later.

Collaborative work with Dr. Gary Reddacliff (EMAI) on the viral disease Lymphocystis, has been reported in The Australian Veterinary Journal (Appendix 7).

The results of collaborative work with Dr. Frank Roubal (Dept. of Parasitology, University of Queensland) on the ectoparasite *Anoplodiscus cirruspiralis*, has been reported in 2 papers (Appendices 8 and 9).

A paper on the collaborative work with Dr. Paul Canfield (Dept. of Veterinary Pathology, University of Sydney) on the haematology and haemochemistry of captive snapper has been accepted for publication in The Journal of Fish Biology. The manuscript is attached as Appendix 10.

DISCUSSION

OBJECTIVE 1. To determine whether the growth rate of snapper can be increased by varying water temperature and feeding regimes.

We have shown that captive snapper fed a dry pelletised diet can reach market size at about 21 months of age (Appendix 1). In contrast, wild fish take three to five years to reach market size. It is possible that market size could have been attained earlier if a better diet had been fed and the snapper had been captive during their first summer. Our work suggests that a lack of food limits the growth rate of wild juvenile snapper during their first winter.

Growth rate of juvenile snapper can be increased by increasing the water temperature. During winter, we doubled the specific growth rate of juvenile snapper by heating the water to between 3 and 5 °C above the ambient seawater temperature for the Sydney region (Table 3).

In our first feeding trial we fed a commercially available pellet formulated for another species of carnivorous marine fish. All of the pellet diets we formulated specifically for snapper were more cost effective than the commercial diet. Although we did not feed our snapper diets for as long as we fed the commercial diet, we see no reason why the significant improvement in growth rate seen with two of the snapper diets (64% and 30% fish meal), would not be maintained in the longer term.

The reference diet we formulated will be used as a benchmark for diets developed in the future.

OBJECTIVE 2. To assess the suitability of the Visible Implant tag for marking juvenile snapper.

We found the Visible Implant tag unsuitable for marking juvenile snapper. PIT tags however, were suitable for marking juvenile snapper as small as 130 mm FL and 60 g. The PIT tags were 11 mm long and 2 mm in diameter and implanted intraperitoneally with a twelve gauge hypodermic needle. Hence, we were reluctant to use them on smaller fish. With the much smaller PIT tags now available it should be possible to mark smaller fish.

OBJECTIVE 3. To validate estimates of the age of juvenile snapper based on growth rings deposited in otoliths.

The opaque marks in the otoliths of young snapper in this study formed annually and appeared in winter. The results were reliable because an opaque mark was formed in each of two successive years in one group of snapper, and during the first (and only) year in the second group of fish. Our work has established the capacity of otoliths to accurately record age under varying growth conditions.

IMPLICATIONS AND RECOMMENDATIONS

Snapper do have potential for mariculture in Australian temperate waters. However, the establishment of an industry depends on a number of factors. Firstly, hatcheries have to be established

to supply juveniles for grow-out. The supply of juveniles from the wild is unreliable. Secondly, suitable sites for the grow-out of snapper in net cages must be found. An important consideration in selecting a site will be the ambient water temperature. The use of waste hot water from power stations etc. should be examined. Thirdly, markets for farmed snapper have to be established.

The establishment of an snapper farming industry would be aided by research on: nutrition, management of captive broodstock, methods for rearing larvae extensively, transport of live fish and disease control.

Research on snapper nutrition should aim to find cost effective diets that besides producing faster growth rates also produce fish with the characteristics (e.g. conformation, muscle to fat ratio, skin colour etc.) demanded by the market. The search for low cost sources of protein to replace fish meal should continue.

The routine collection of eggs from captive brood stock is done in Japan. In Europe it is done for a related species the gilthead seabream, *Sparus auratus*. The techniques developed overseas could be adapted for use locally.

Extensive rearing of larvae would significantly lower the cost of producing juveniles for grow-out.

The development of a market for live snapper requires techniques

for transporting fish from the farm to the point of sale. Techniques are also required for transporting juveniles from hatcheries to grow-out farms.

Hatchery reared juvenile fish are particularly prone to disease when they are first stocked in net cages. We anticipate that as the industry develops, diseases of economic importance will occur. Research on controlling those diseases will be needed.

INTELLECTUAL PROPERTY

The results of the project and the methods developed cannot be regarded as intellectual property.

TECHNICAL SUMMARY OF INFORMATION DEVELOPED

The approximate nutrient composition and ingredients of the diets we formulated for snapper are given in Table 1. Specific growth rates (SGR) for juvenile snapper fed our diets are given in Tables 2 and 3.

During winter the SGR was approximately doubled by growing juvenile snapper in seawater heated to between 3 and 5°C above the ambient seawater temperature (Table 3).

PIT tags were used to mark juvenile snapper. They did not affect the growth rate of juvenile snapper.

Opaque marks in the otoliths of young snapper can be used to determine the age of the fish in years.

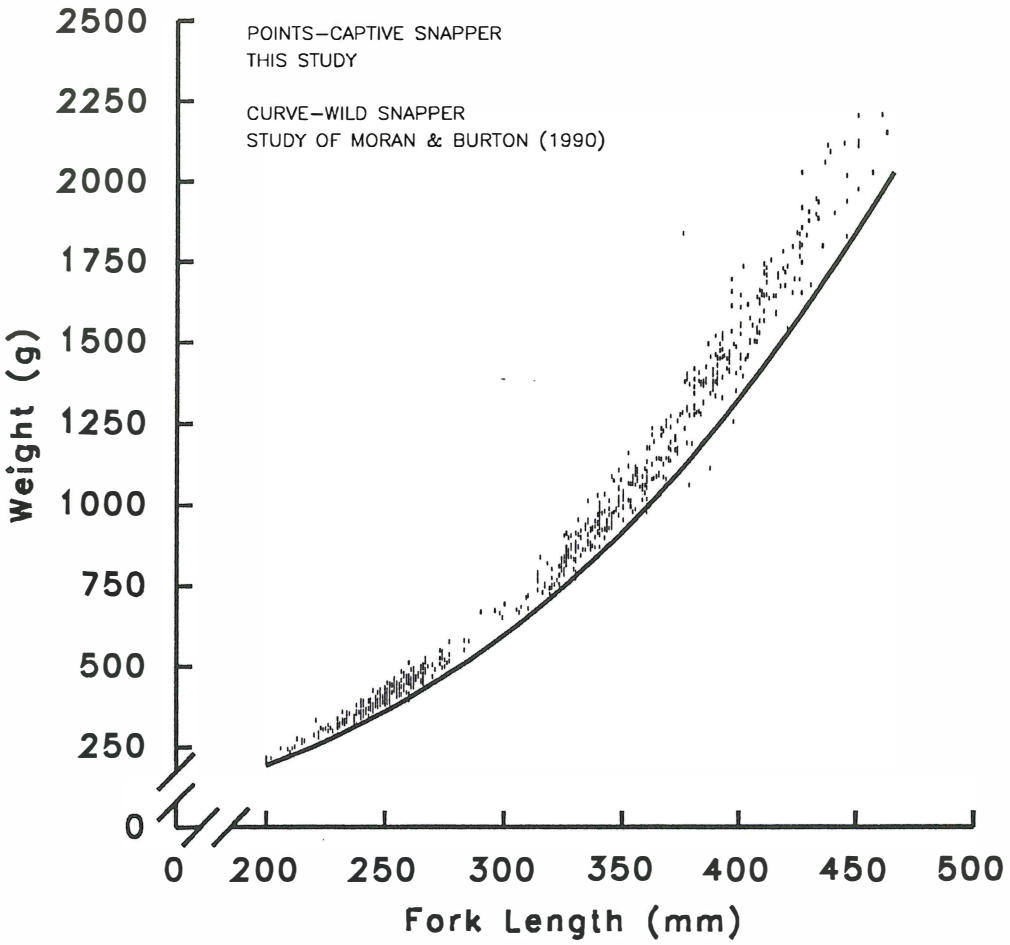

Figure 1. Length weight data for captive snapper. The fish were held at the Fisheries Research Institute and measured three times at intervals of approximately one year. The curve shown was fitted by Moran and Burton to the length weight data they obtained from wild snapper (see Moran and Burton, Fisheries Research Report No. 89 1990, Fisheries Department of Western Australia).

Table 1 Ingredients and Approximate Nutrient Composition of Snapper Diets

Diet	1	2	3	4
Ingredient(%)				
Fish meal	64.0	30.0	20.0	10.0
Lupins	7.0	7.0	7.0	7.0
Fish oil	3.1	5.6	6.3	7.1
Poultry meal	4.0	20.5	25.1	30.0
Sorghum	10.1	5.3	4.6	2.9
Soybean	0	20.0	25.0	30.0
Wheat	10.8	10.0	10.0	10.0
Vit & min premix	1.0	1.0	1.0	1.0
L-methionine	0	0.34	0.5	0.5
L-lysine	0	0.24	0.5	0.5
Approx. nutrient composition				
Protein (%)	49.5	50.0	49.8	49.9
Fat (%)	10.9	11.2	11.3	11.4
Linolenic series (%)	2	2	2	2
Fibre (%)	5.4	10.0	11.3	12.7
DE (MJ/kg)	14.4	14.4	14.4	14.4
Total lysine (%)	3.5	3.3	3.4	3.3
Total methionine (%)	1.3	1.3	1.3	1.3
Total methionine + cystine (%)	2.0	2.3	2.4	2.4

Table 2 Effects of the substitution of fishmeal with soybean meal and poutry by-product meal in prepared diets on the growth of snapper[1]

Fishmeal (%)	64	30	20	10
Weight gain (g)	$40.6^{a} \pm 1.4$	$36.0^{ab} \pm 2.1$	$31.1^{b} \pm 2.0$	$22.0^{c} \pm 2.4$
Relative weight gain[2]	$0.56^{a} \pm 0.03$	$0.49^{ab} \pm 0.03$	$0.42^{b} \pm 0.03$	$0.29^{c} \pm 0.03$
SGR[3] (%/day)	$0.36^{a} \pm 0.02$	$0.32^{ab} \pm 0.02$	$0.29^{b} \pm 0.02$	$0.20^{c} \pm 0.02$
Survival (%)	96 ± 2	93 ± 4	87 ± 4	93 ± 4
FCR[4]	$2.1^{b} \pm 0.1$	$2.3^{b} \pm 0.1$	$2.6^{b} \pm 0.3$	$3.9^{a} \pm 0.6$

[1] Values are means ± SE of three tanks. Means within a row having a common superscript were not significant ($P > 0.05$). Initial mean weight of snapper was 75 g.

[2] Relative weight gain = weight gain/initial weight.

[3] SGR = $(100/T) \log_e (W_T/W_i)$ where W_T is the weight of fish at time T, W_i is the initial weight of fish and T is the culture period in days.

[4] FCR = food conversion ratio:
weight of dry diet fed/wet weight gain of fish.

Table 3 Specific growth rates[1] (SGR) for juvenile snapper[2] grown on different diets with ambient and heated seawater.

Fishmeal (%)[3]	64	30
Seawater temperature		
ambient	0.34±0.02[a]	0.35±0.02[a]
heated[4]	0.74±0.02[a]	0.70±0.02[a]

[1] Defined in Table 2. Values are means ± SE. Means within a row having a common superscript were not significant ($P > 0.05$).

[2] Initial mean weight 38 g. Final mean weight of fish in heated water fed the 64% fishmeal diet was 93 g.

[3] Fishmeal content of the two diets used.

[4] Seawater was heated by between 3 and 5°C.

Appendix 1

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Growth of snapper, *Pagrus auratus*, from south-eastern Australia in captivity

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Abstract This study aimed to determine whether the relatively slow growth of wild *Pagrus auratus* in south-eastern Australia can be increased in captivity to a rate acceptable for aquaculture. Juvenile snapper from Port Hacking, New South Wales (34°47'S) were reared at ambient water temperatures. The fish were fed on a dry, sinking pellet with 42% protein. At the start of the trial in April 1989, the snapper had a mean fork length (FL) of 121 ± 11 mm SD, an average weight of 50 g, and an estimated age of 8 months. After 12.5 months their mean FL was 249 ± 15 mm and their mean weight was 403 ± 70 g. Our data indicate that, like the reproductively isolated population in Japan, growth of *Pagrus auratus* from Australasia can be rapid enough for aquaculture, and that there is potential to increase growth rate further.

Keywords snapper; *Pagrus auratus;* Sparidae; growth; aquaculture

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INTRODUCTION

The snapper, *Pagrus auratus*, forms the basis of important commercial and sport fisheries in Australasia (Paul 1986; MacDonald & Hall 1987). Like the reproductively isolated population of *Pagrus auratus* in Japan (see Paulin 1990) and the closely related species *Sparus auratus* in Europe (Foscarini 1988; Barnabé 1989), the Australasian population of *Pagrus auratus* has many attributes suitable for aquaculture. For example, it reproduces spontaneously in captivity (Smith 1986) and commands a high market price. A potential limiting factor to the culture of *Pagrus auratus* in Australasia is its apparent slow rate of growth to market size: wild fish take 3–5 years to reach a fork length (FL) of 250 mm (Crossland 1977; Horn 1986; GWH unpubl. data).

Here we report an initial trial on the growth of *Pagrus auratus* in south-eastern Australia at ambient water temperatures. Our aim was to determine whether growth of captive snapper could be increased to a rate acceptable for aquaculture, i.e., market size within 2 years. We achieved growth from a mean fork length of 121 mm, and average weight of 50 g, to a mean size of 249 mm FL and 403 g in 12.5 months. We also present evidence indicating that there is potential for further increase in growth rate of captive fish.

MATERIALS AND METHODS

In central (34°S) New South Wales (NSW), snapper spawn around October-November and juveniles recruit to marine bays and estuaries at 20–30 mm FL. Individuals of this size have an estimated age of 2 months (Kingsford 1986). The juveniles are vulnerable to commercial fishing gear by December at c. 50 mm FL (Fig. 1; Bell 1980). By the following March-April, the young-of-the-year have grown to a modal size of between 110 and 130 mm FL (Fig. 1). At an age of c. 18 months, and a mean FL of c. 180 mm, snapper in central NSW waters disperse to habitats offshore (Bell 1980).

On 10 March 1989 we collected 405 young-of-the-year snapper with a seine net from Port Hacking,

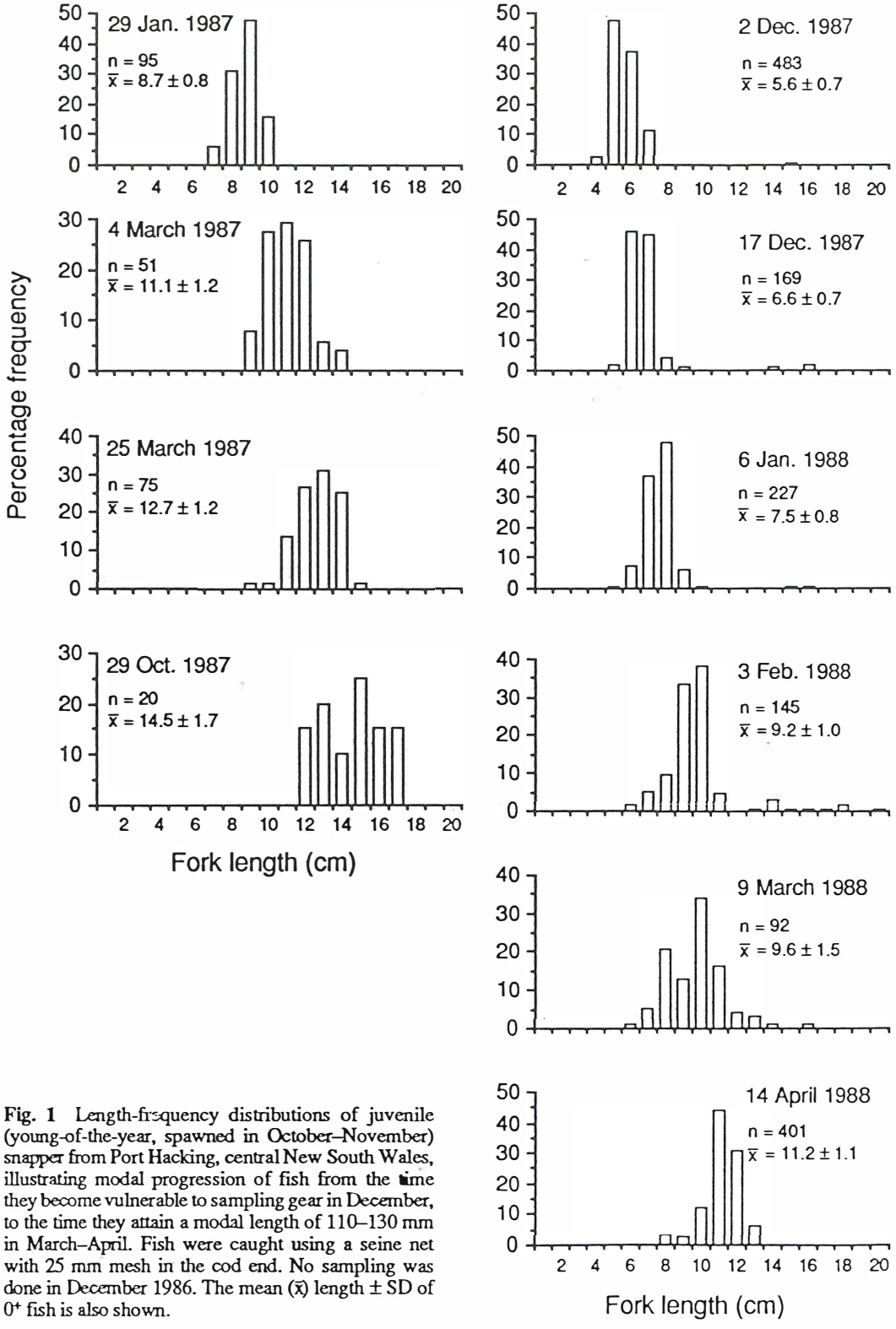

Fig. 1 Length-frequency distributions of juvenile (young-of-the-year, spawned in October–November) snapper from Port Hacking, central New South Wales, illustrating modal progression of fish from the time they become vulnerable to sampling gear in December, to the time they attain a modal length of 110–130 mm in March–April. Fish were caught using a seine net with 25 mm mesh in the cod end. No sampling was done in December 1986. The mean ($\bar{x}$) length ± SD of 0^+ fish is also shown.

NSW (34°47'S, 151°8'E). We placed the fish into a pen measuring 6.75 × 5 × 2.25 m within a large, flow-through, marine research pool (875 m^3) located 2 m from the shore, just inside the entrance to Port Hacking. Two sides of the pen were formed by a corner of the pool, the other two were made of 20 mm plastic mesh. The supply of sea water to the pool was drawn through a sand/gravel filter and spilled into this pen.

Fish in the pen started feeding within 24 h of capture and were maintained on a diet of chopped pilchards (*Sardinops neopilchardus*) until 12 April 1989, when they were netted with knotless mesh and measured. Their mean FL ± SD was 121 ± 11 mm and their average weight 50 g. Average weight was derived from an independent length-weight relationship, where $W = 0.000015\ L^{3.097}$, for wild 0^+ age snapper ($n = 659$). The fish used to calculate this relationship were caught in April 1990 from the same place as the 405 experimental fish. We did not weigh the 405 fish initially as stress from handling can inhibit growth of fish (Pickering 1981).

Between 12 and 21 April we weaned the fish onto a dry, sinking pellet by gradual substitution. The pellets were manufactured by Gibsons Stock Feeds (GPO Box 85a, Hobart, Tas 7001, Australia) as a diet for barramundi (*Lates calcarifer*). Their approximate composition was: protein 41.8% (mainly fish meal), carbohydrate 30%, fat 10.8%, lysine 3.2%, methionine 1.04%; their approximate metabolisable energy was 3550 kcal kg^{-1}. The maximum age of fish on 21 April was estimated to be 8 months.

From 22 April 1989, the fish were fed twice a day by hand, 6 days a week. During summer the feeding rate was 1–2% of estimated body weight per day. This was reduced to 0.5% in winter. We used pellets with a diameter of 2.5 mm at the start of the trial but increased this size to 4.5 mm after 6 months.

On 24 November 1989 we measured and weighed 381 fish. Some fish evaded capture and were not measured. Before returning the fish to the pen, 120 individuals were removed at random for use in another experiment. Fish returned to the pen resumed feeding within 12 h. On the same day we measured 95 snapper that had been caught from Port Hacking on 1 November 1989 and maintained elsewhere in the pool on a diet of pellets. These "wild" fish were the same age (1^+ yr) as our captive snapper, and representative of the length-frequency distribution of individuals of the same age present in estuaries in central NSW in October (Bell 1980).

On 2 May 1990 we measured and weighed all fish in the pen. It was not possible to measure a sample of wild fish of the same age because they disperse from inshore nursery areas between February and April (Bell 1980).

The flow rate of sea water through the pool varied during the trial. It was 100 $m^3\ day^{-1}$ between 10 March 1989 and 6 January 1990, and 600 $m^3\ day^{-1}$ from then on. Mean water temperature varied from a minimum of 13.6°C in July to a maximum of 23.9°C in February (Table 1). Salinity fluctuated non-seasonally from $30–35 \times 10^{-3}$ depending on rainfall in the catchment. Such variation was typical of nearby marine estuaries (Wolf & Collins 1979). Water quality was measured periodically. Dissolved oxygen never fell below 6 mg l^{-1}, and un-ionised ammonia and nitrites did not exceed 0.001 and 0.014 mg l^{-1}, respectively.

RESULTS AND DISCUSSION

On 24 November 1989 the mean FL of captive fish was 179 mm (Fig. 2). They were significantly ($t = -3.105$, $P < 0.001$, t-test) longer than fish assumed to be the same age taken recently from the wild (Fig. 2). On 2 May 1990 the mean FL of fish was 249 mm (Fig. 2), and their mean body weight ± SD was 403.3 ± 70.0 g. The density of fish in the pen at the end of the trial was 1.46 kg m^{-3}.

There were no outbreaks of disease during the trial, but 3% of fish had unilateral exophthalmia on 2 May.

Our data show that snapper can be grown to market size within 21 months. There are, however, at least three reasons to believe that faster rates of growth can be achieved. First, we did not start the trial until the end of summer, when fish were c. 8 months old.

Table 1 Mean (n = 10–27) monthly temperature ± SD of surface water in the research pool.

Year	Month	Temperature (°C)
1989	Apr	21.3 ± 0.7
	May	19.5 ± 1.0
	Jun	15.8 ± 0.5
	Jul	13.6 ± 0.9
	Aug	14.4 ± 0.6
	Sep	16.6 ± 0.8
	Oct	20.0 ± 1.0
	Nov	21.7 ± 0.6
	Dec	22.5 ± 0.5
1990	Jan	22.7 ± 0.8
	Feb	23.9 ± 1.3
	Mar	22.8 ± 0.4
	Apr	21.4 ± 0.8
	May	19.7 ± 1.3

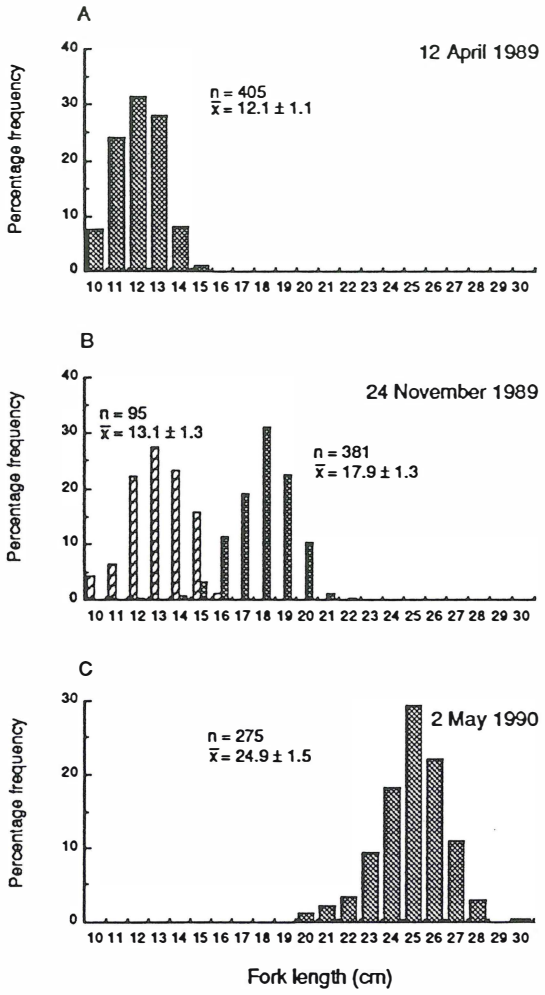

Fig. 2 Length-frequency distributions of **A**, juvenile fish at the start of the trial; **B**, captive and wild (hatched histogram) fish seven months later; and **C**, captive fish at the end of the trial. Mean ($\bar{x}$) length ± SD for each distribution is also shown.

Since growth of 0+ snapper is greater during summer than winter (Paul 1976; Bell 1980), it is reasonable to assume that snapper could be grown to 400 g more rapidly if they were fed during both their first and second summers. Second, we kept fish at ambient water temperatures, whereas growth of *Pagrus auratus* in Japan is enhanced under warm water conditions (Foscarini 1988). In south-eastern Australia there is such scope to increase growth by raising snapper towards the northern end of their distribution in southern Queensland, or by using warm water effluent from power stations in central NSW. Third, we used a pellet (42% protein) that had been formulated for another species. Growth of *Sparus auratus* in Europe and *Pagrus auratus* in Japan is optimal on a diet containing 46–50% protein (Alliot & Pastoureaud 1983; Foscarini 1988).

Our trial indicated that *Pagrus auratus* in south-eastern Australia have several attributes important for culture. Captive snapper showed greater growth than wild fish over the cooler half of the year. This suggests that slow winter growth of natural populations, reported by Lenanton (1974), Paul (1976), and Bell (1980), may result partly from a decline in abundance of food. Also, captive snapper resumed feeding within 12 h when captured and measured during November. These procedures were done carefully and quickly, without the use of anaesthetic, and indicate that sensible handling does not cause undue stress. Finally, the conversion ratio of pellets to total body weight was promising. We used 270 kg of pellets during the trial. After making allowance for the removal of 120 fish in November, the conversion ratio was 2:1. This approximates initial conversion ratios for culture of *Sparus auratus* in Europe (G. Barnabé, pers. comm.).

Clearly, growth rate of weaned fish need no longer be considered a major limiting factor for aquaculture of *Pagrus auratus* in Australasia. Experimental studies are now needed to confirm that optimal stocking densities and feeding rates, developed elsewhere for *Sparus auratus* and *Pagrus auratus*, also apply in Australasia. Since there is limited scope for local production of fish meal, we must also look for alternative ways of formulating cheap, high-protein feeds that are acceptable to snapper, and that produce satisfactory growth.

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Appendix 2

Fish meal substitution in a diet for Australian snapper, *Pagrus auratus*

Introduction

Snapper, *Pagrus auratus*, has been farmed successfully in Japan for about 20 years (Foscarini, 1988). In 1990 production by aquaculture was 40 000 t (Davy, 1990). Preliminary trials in New South Wales indicate that snapper have potential for farming in temperate Australia (Bell et al., 1991).

Fish meal is the highest-quality protein source for fish feeds (Lovell, 1989). However, increasing demand, reduced supply and increasing cost internationally (Barlow, 1989) have inspired a major research effort to replace fish meal with alternative animal and vegetable protein sources for a number of fish species (Mohsen and Lovell, 1990; Olvera-Navoa et al., 1990). In Australia, the production of large quantities of fish meal is not feasible because baitfish are scarce. Here we report an experiment where we evaluated two diets formulated for snapper: one is based on fish meal, and the other on soybean and poultry meal. Comparisons were also made with two commercially available fish diets.

Materials and methods

Juvenile snapper (mean initial weight 71.0 g, range 37.4 to 104.5 g) previously collected by seine net from Port Hacking, NSW (34° 5'S, 151° 8'E) were anaesthetised (50–70 mg L^{-1} Benzocaine) and stocked into 32 fibreglass tanks (200 L) at a density of eight fish per tank. Continuously flowing seawater (1.8 L min^{-1}) and aeration through an air-stone diffuser was provided for each tank. Natural lighting was augmented by fluorescent lights from 30 minutes after sunrise to 30 minutes before sunset.

Four dry-pellet diets were compared, using eight replicate tanks per diet. We formulated two diets for snapper following the recommended nutritional requirements for red sea bream, *Pagrus major* (Foscarini, 1988). In one diet, high-quality Chilean fish meal (68% protein) was used as the major source of protein and comprised approximately 60% of the diet, while in the other the fish meal content was reduced to approximately 10% and poultry meal and soybean meal were added (approximately 50 and 22% respectively). The two commercial diets used were a starter diet for Atlantic salmon, *Salmo salar* and a starter diet for barramundi (*Lates calcarifer*). Proximate analysis of the four diets gave the following ranges: protein (N x 6.25), 35–54%; fat, 12–19%; fibre, 6–17%; carbohydrate, 18–34% (Table 1).

The diameter of the pellets in all feeds was approximately 2 mm. The daily ration was set at 1.3% of the mean wet weight of fish per tank, and adjusted twice during the 12 week trial to compensate for weight increase. The fish were fed twice daily between 0900 and 1600.

Fish that died during the experiment were replaced with similar-sized fish with clipped pelvic fins. Replacement fish were easily identified at the end of the experiment and were not included in data analysis. The number of replacements never exceeded four per tank and was usually only one or two.

Temperature, salinity, dissolved oxygen (DO), ammonia and nitrite were measured as described by Allan et al. (1990). The weekly mean temperature increased from 15.9°C to 22.5°C during the experiment, while the DO and salinity were above 6.0 mg L^{-1} and 34‰ respectively. Ammonia and nitrite remained below 0.05 mg unionised ammonia-N L^{-1} and 0.03 mg NO_2-N L^{-1}, respectively.

At the end of the experiment the fish were anaesthetised and weighed, and the mean weight of surviving fish in each tank was calculated. Differences between treatments were assessed using ANOVA. Variances were homogeneous by Cochran's Test. When significant treatment effects occurred, means were compared by the Student-Newman-Keuls (SNK) procedure.

TABLE 1
Proximate analysis of experimental diets for snapper, *Pagrus auratus*[1]

Diet[2]	DM[3] (%)	Protein[4] (%)	ADF[5] (%)	Fat (%)	Carbohydrate[6]
SN1	87.94	51.19	6.23	11.99	28.76
SN2	88.45	53.75	6.54	14.03	23.40
GIB	92.08	35.13	16.56	18.99	34.25
AQU	94.17	51.06	6.58	12.61	17.59

[1] All results are expressed on a dry basis and are the mean of duplicate samples. The methods are those of Fairchney and White (1983)
[2] SN1 = snapper diet based on fish meal, SN2 = snapper diet based on soybean and poultry meal, GIB = salmon starter, AQU = barramundi diet
[3] DM = dry matter
[4] Protein = N x 6.25
[5] ADF = acid digestible fibre
[6] Carbohydrate = 100 — (ash + protein + fat)

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Results

Fish fed the fishmeal diet formulated for snapper were significantly heavier (mean final weight 118 g $fish^{-1}$; SE 6 g $fish^{-1}$; n = 8 replicate tanks) than fish fed the other three diets ($P<0.01$). There were no differences in the final weights of the fish fed the other three diets ($P>0.05$) (mean final weight 106 g $fish^{-1}$; SE 8 g $fish^{-1}$; n = 24 replicate tanks). The feed conversion ratio (FCR) (dry weight of pellets fed/increase in wet weight of fish) was lower for fish on the formulated fishmeal diet (mean = 1.6; SE = 0.01; n = 8 replicate tanks) than on the other three diets (mean = 2.2; SE = 1.0; n = 24 replicate tanks).

Discussion

We found that, in diets with similar protein, fat and fibre levels, replacement of fish meal by soybean and poultry meal reduced the growth rate and FCR of snapper. Other researchers with different fish have reached the same conclusion (Davis and Stickney, 1978; Fowler, 1980; Jackson et al., 1982; Viola et al., 1982). In contrast, Reinitz (1980) found no significant reduction in growth rate when soybean meal replaced 75% of the herring meal in a diet for rainbow trout (*Salmo gairdneri*).

Limiting essential amino acids may have reduced the growth of fish. Based on published values (NRC, 1983) the diet with 22% soybean and 50% poultry meal contained approximately 3.0% and 0.9% lysine and methionine respectively, while the diet with 60% fishmeal contained 3.5% and 1.3%, respectively. Yone (1976) recommended levels of 4.27% for lysine and 1.07% for methionine, although more recent recommendations for warmwater fish (carp, channel catfish) and Chinook salmon are lower (1.5–2.2% for lysine and 0.56–1.6% for methionine) (NRC, 1983).

As the digestibility of the soybean and poultry meal has not been assessed for snapper, the nutrients in these diets may have been less available than in the fish meal diet. Although formulated diets had similar levels of fibre and carbohydrate, the FCR of the soybean and poultry meal diet was higher than that for the fish meal diet. There is evidence that some species of fish find soybean meal unpalatable (Lovell, 1989). Finally, there was no evidence that any of the ingredients used in the diets were contaminated by fungi, and as the soybean meal was heat-treated during the oil extraction, most of the trypsin inhibitor in this meal would have been destroyed (Lovell, 1989).

Although the percentage of protein in the salmon starter diet was much less than in both the soybean and poultry meal snapper diet and the barramundi diet (Table 1), no significant difference in growth was found for the three diets. This indicates that there may be scope for reducing protein levels in snapper diets.

We conclude that, although commercially available diets for Atlantic salmon and barramundi are not ideal for snapper, similar growth rates to those recorded using these diets can be achieved using a diet containing poultry meal and soybean meal as the major sources of protein.

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Appendix 3

Substitution of fish meal in a diet for the carnivorous marine fish, *Pagrus auratus* (Bloch and Schneider) from south-eastern Australia

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ABSTRACT

The growth of Australian snapper (*Pagrus auratus*) (Bloch and Schneider) fed diets based on different sources of protein were compared. Two commercial diets for other species and two experimental diets for snapper were compared. Fish meal was the predominant source of protein for one experimental diet, while in the other 90% of the fish meal was replaced by soybean and poultry meal. Replacement of fish meal gave a lower growth rate and higher (poorer) food conversion ratio (FCR) compared to the fish meal diet. The growth rate and FCR obtained with the soybean and poultry meal diet was similar to that of the two commercial diets tested. Fatty acid analysis of the two experimental diets indicated that concentrations of essential fatty acids exceeded those reported to reduce growth of snapper. Concentrations of lysine and methionine in the diets associated with reduced growth were lower than those in the diet based on fish meal.

INTRODUCTION

Farming of carnivorous marine fish in temperate Australia is likely to be based on an indigenous species for which a suitable low cost diet can be produced. One of the species with promise for establishment of such an industry is the snapper, *Pagrus auratus* (Bloch and Schneider). Snapper has been farmed successfully in Japan for about 20 years (Foscarini, 1988; Fukusho, 1991), where it is known as red sea bream. In 1990, the Japanese production of snapper by aquaculture was 40,000 tonnes (Davy, 1990). Preliminary trials in New South Wales indicate that snapper should also be suitable for farming in temperate Australia (Bell, Quartararo and Henry, 1991).

Fish meal is the protein source of choice for fish feeds (Lovell, 1989) although increasing demand, reduced supply and increasing cost (Barlow, 1989) have contributed to a major research effort to replace fish meal with alternative sources of protein for a number of fish species (Olvera-Navoa, Campos, Salado and Martinez, 1990; Mohsen and Lovell, 1990). In Australia, the production of large quantities of fish meal is not feasible because baitfish are scarce. Therefore, a pre-requisite for large scale development of marine fish farming is the identification of a local supply of high quality protein to replace fish meal.

In this paper we describe an experiment that compares four diets for carnivorous fish. Two were formulated for snapper; one based on fish meal and the other with 90

% of the fish meal replaced with soybean meal and poultry meal. The other two were commercially available diets for other species.

MATERIALS AND METHODS

Juvenile snapper were collected from Port Hacking, NSW (34^{o} 47'S, 151^{o} 8'E) by seine net in January 1990. The fork lengths and weights of these fish ranged from 70-90 mm and 5-20 g. The juvenile snapper were acclimated in 2000 l fibreglass tanks with flow-through (0.3 l sec^{-1}) aerated seawater at a stocking density of approximately 2 kg m^{-3}. Faeces and other particulate matter that settled on the bottom of the tank were removed daily by siphoning.

Proximate analysis of diets and fish were done using the methods outlined by Faichney and White (1983). Lipids were extracted from whole fish by the method of Folch, Lees and Stanley (1957). Neutral and polar lipid fractions were separated by chromatography and lipid classes were separated by thin layer chromatography as described by Anderson and Arthington (in press). Amino acid profiles were determined using Waters (Waters Chromatography Division, Millipore Pty Ltd, Lane Cove, NSW) Pico-Tag with a Waters HPLC. Samples were acid hydrolysed prior to amino acid analysis. As this technique degrades the sulphur amino acids, separate samples were oxidised using performic acid to determine methionine and cystine. Tryptophan was lost during acid hydrolysis and is not reported. Fish were freeze dried and homogenised prior to amino acid analysis.

Four dry pellet diets were compared; two were formulated by us and two were available commercially. The commercial diets were a starter diet for Atlantic Salmon (*Salmo salar*) and a starter diet for Barramundi (*Lates calcarifer*). One of the two experimental diets for snapper was based predominantly on fish meal as the source of protein and fulfilled the requirements for Japanese red sea bream given by Yone (1975). In the other diet, 90% of the fish meal was replaced by soybean and poultry meal. Proximate analysis of the four diets gave protein contents ranging from 35-54%, fat from 12-19%, fibre from 6-17% and carbohydrate from 18-34% (Table 1).

Pellet diameter was approximately 2 mm for all diets. The starting ration was 1.3% of the mean wet weight of fish per tank. The ration was adjusted twice during the trial to account for growth of fish.

Immediately prior to setting up the experiment, all fish were placed in a 2000 l tank. Groups of ten to twenty fish were caught at random and lightly anaesthetised with 50-70 mg l^{-1} Benzocaine (p-aminobenzoate). Individual fish were then taken at random, weighed, measured and distributed among 32 tanks by systematic interspersion. This process continued until there were eight fish per tank. Each tank held 200 l of aerated seawater at ambient temperature, giving an initial stocking density of approximately 2 kg m^{-3}.

The tanks were distributed on opposite sides of the room, each side had two rows of tanks at different heights (8 tanks per row, 16 tanks per side). The four diets were

randomly allocated to 2 tanks in each row so we could test for any effect of side and/or height on growth.

At the start of the experiment, there was no significant difference in the mean weight and fork length (FL) of fish per tank between the four diet groups ($F_{3,28}$ = 1.18, $P < 0.33$; $F_{3,28} = 0.82$, $P < 0.49$ resp.). The coefficient of variation (CV) for weight in each tank averaged 19% and ranged from 10 to 31%.

Fish that died during the experiment were replaced immediately by one of a similar size whose pelvic fins were clipped back to the body. Replacements were easily identified at the end of the experiment. The experiment commenced on 17 September and finished 12 weeks later on 11 December, 1990.

Water quality was maintained by a continuous supply of filtered seawater at 0.03 l sec^{-1}, and by siphoning the bottom of the tanks every second day. Water quality was assessed weekly by measuring dissolved oxygen using the modified Winkler's Method, calculating the unionised ammonia (NH_3) from the total ammonia concentration, estimating nitrite concentrations and measuring salinity (Major, Dal Pont, Klye and Newell, 1972; Dal Pont, Hogan and Newell, 1974; APHA, 1985). The dissolved oxygen always exceeded 6 mg l^{-1} and the salinity was between 34 and 35 ppt. Ammonia and nitrite never exceeded 0.05 mg l^{-1} and 0.03 mg l^{-1}, respectively.

Water temperatures were measured in each tank on at least four days during each week of the experiment. The weekly mean of the daily modal temperatures

increased from 15.9 to 22.5°C during the experiment (Fig. 1).

The natural photo-period was augmented by fluorescent lights which were switched on automatically in sequence half an hour after sunrise and switched off half an hour before sunset.

We used the mean weight and FL of the eight fish in each tank to test the null hypothesis that there was no effect of diet on growth. In tanks where some of the fish had died, the mean was based on the remaining original fish. The number of such fish in all tanks was always ≥4. Both the final mean weight and FL, and the difference in the mean starting and final weights and FLs, were used in a 3-way orthogonal ANOVA to test the effects of diet, side of the room and height of the tank on fish growth. All three factors were treated as fixed effects and the total number of degrees of freedom was 31. All variables had homogeneous variances by Cochran's Test. Significant differences among means were identified using the Student-Newman-Keuls (SNK) procedure.

RESULTS

Neither the side of the room on which the tank was located nor the height of the tank above the ground had any significant effect on any of the variables that we used to measure growth. After pooling non-significant terms (see Underwood, 1981), there was a significant effect of diet on the growth of snapper. This was true for

final mean weight, and final FL, and for the difference between the mean starting and final weights and FLs (Table 2). In all cases, the growth of fish fed the experimental diet with the most fish meal was significantly greater than fish fed the other three diets (Fig. 2, SNK tests). There were no significant differences among the fish on the latter three diets for any of the four variables (Fig. 2, SNK tests).

The food conversion ratio (weight of pellet fed/ increase in wet weight of fish)(FCR) was lower for fish fed the experimental fish meal diet (mean 1.6; SE 0.1; n = 8 replicate tanks) than for the other three diets (mean 2.2; SE 0.2; n = 24 replicate tanks).

The estimated protein and fat contents for fish sampled at the termination of the experiment reflected the contents of the diets. The highest fat content was measured in fish fed salmon starter diet which had the highest fat content of the four diets (Table 1). The protein content of fish before the experiment was 55.6 %, similar to that of three of the diets (1, 2 and 4), and the content of the fish after the experiment (Table 1). For fish fed the diet with the lowest protein content (35 %) the protein content was 46 % at the end of the experiment.

The fatty acid profiles of the experimental diets reflected the fatty acid profiles of the ingredients. The diet based on fish meal had higher concentrations of the linolenic series fatty acids and highly unsaturated fatty acids (HUFA's) than the diet based on soybean and poultry meal (Table 3). Compared with the fatty acid profile of fish fed the diet based on soybean and poultry meal, the fatty acid profile of fish fed the diet based on fish meal was closer to that of fish sampled before the

experiment commenced (Table 3).

Concentrations of all amino acids were higher in the fish sampled before the experiment commenced, and declined during the experiment (Table 4). The final concentrations of individual amino acids were lower in fish which had been fed diets with the lowest (Table 4).

DISCUSSION

The replacement of fish meal by soybean and poultry meal reduced the growth rate of snapper compared to the experimental diet based on fish meal. A number of other studies have found that the partial substitution of dietary fish meal with less expensive protein sources such as plant meal reduces growth rate (Davis & Stickney, 1978; Fowler, 1980; Jackson, Capper and Matty, 1982; Viola, Mokady, Rapport and Areili, 1982). In contrast, Reinitz (1980) found no significant reduction in growth rate when soybean meal replaced 75% of the herring meal in a diet for rainbow trout (*Oncorhynchus mykiss*).

Possible explanations for the reduction in growth rate include: 1) limiting levels of the essential fatty or amino acids, 2) the lower digestibility of soybean and/or poultry meal protein compared with fish meal, and 3) presence of toxins and/or substances reducing the availability of dietary protein.

Marine fish have a definite requirement for the n-3 series essential fatty acids, and specific requirements for n-3 highly saturated fatty acids have also been demonstrated (NRC, 1983; Halver, 1989). Snapper have been shown to require 2 % n-3 HUFA (Fujii and Yone, 1976). The diet based on soybean and poultry meal had lower levels of n-3 series and n-3 HUFA's than the diet based on fish meal; however, because 3 % fish oil was added to both diets the concentration of n-3 HUFA's was ≥2 % for both diets. Therefore the difference in growth recorded for fish fed the two formulated diets is unlikely to be related to a deficiency of essential fatty acids. Fatty acid profiles were not measured for the two commercial diets.

Concentrations of leucine, isoleucine, lysine and methionine were lower in the soybean and poultry meal diet and the two commercial diets compared with the experimental fish meal diet (Table 3). If reduced concentrations were below minimum requirements they may have contributed to reduced growth. As even the lowest concentrations of leucine or isoleucine were similar to, or above, the minimum requirements for a number of species (NRC, 1983; Halver, 1989) and the differences in concentrations between all the diets were minor, the levels of these amino acids are unlikely to have caused reduced growth. However, as both lysine and methionine concentrations were considerably reduced in the soybean and poultry meal diet, and the two commercial diets, compared with the fish meal diet, these amino acids may have been implicated in reduced fish growth. The lower concentrations of lysine and methionine were close to or below the minimum requirements (NRC, 1983; Halver, 1989). The concentration of tryptophan was not determined although this can also be a limiting amino acid (Halver, 1989).

Digestibility of the soybean and poultry meal has not been assessed for snapper. However, for other species, e.g. channel catfish, *Ictalurus punctatus*, digestibility of total protein and lysine and methionine are generally lower for plant protein sources than for fish meal (Robinson, 1989).

Although the fibre contents were similar in both experimental diets, the FCR of the soybean and poultry meal diet was higher than that for the fish meal diet. There is evidence that some species of fish find soybean meal unpalatable (Lovell, 1989). Finally, as the soybeans were heat treated when oil was extracted, it is unlikely that presence of trypsin inhibitor could have depressed growth.

Although the percentage of protein in the Gibsons salmon starter diet was significantly less than in both the low cost snapper diet and the Aquafeed barramundi diet (Table 1), no significant difference in growth was found for the three diets. This indicates that there may be scope for reducing protein contents in snapper diets.

We conclude that: 1) commercially available diets for Atlantic salmon and barramundi are not ideal for snapper; and 2) the growth rate of snapper on these commercial diets can be achieved at considerably lower cost by substituting a proportion of the fish meal with soybean and poultry meal.

In a future trial, we plan to examine a range of diets in which the substitution of fish meal falls between the extremes examined here.

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TABLE 1

Proximate composition (%) of experimental diets and of juvenile snapper (*Pagrus auratus*) sampled before the experiment commenced and when the experiment was terminated after 12 weeks[1]

Sample	Dry matter	Organic matter	Crude protein[2]	Fat	Fibre
Diets					
1[3]	87.94	91.94	51.19	11.99	6.23
2[4]	88.45	91.18	53.75	14.03	6.54
3[5]	92.08	88.37	35.13	18.99	16.56
4[6]	94.17	81.26	51.06	12.61	6.58
Fish					
Initial	97.97	82.74	55.56	26.94	
Fed diet 1[3]	97.14	82.93	58.00	24.54	
Fed diet 2[4]	98.02	84.13	53.69	30.56	
Fed diet 3[5]	97.50	87.44	46.25	40.76	
Fed diet 4[6]	95.75	80.20	59.13	18.64	

1 All results are expressed on a dry basis and are the mean of duplicate samples. Two fish (from separate replicates for those taken at the termination of the experiment) were freeze dried and combined. Methods used follow those of Faichney and White (1983)

2 Protein = Nx6.25

3 Diet formulated for snapper based on fish meal (SN1)

4 Diet formulated for snapper based on soybean meal and poultry meal (SN2)

5 Salmon starter diet (SSD)

6 Barramundi starter diet (BSD)

TABLE 2

F statistics obtained from ANOVA for the variables used to assess the effects of diet, side of the room and tank height on the growth of snapper (*Pagrus auratus*).

	Variable			
Source	Final FL	FL diff	Final Wt	Wt diff
diet (d)	7.15[1]	7.76[1]	5.50[1]	5.45[1]
side (s)	0.86	4.00[1]	0.89	3.26[1]
height(h)	1.04	0.20	3.51[1]	0.70
d*h	0.67	0.44	0.66	0.59
d*s	0.74	0.38	0.20	0.13
s*h	1.42[1]	2.37[1]	1.24	1.84[1]
d*s*h	0.28	0.21	0.17	0.66

[1] F statistic calculated after non-significant terms with P > 0.25 were pooled with the residual.

TABLE 3

Fatty acid composition (% of total fatty acids found) of two diets formulated for snapper (*Pagrus auratus*) and of juvenile snapper sampled before the experiment commenced, and when the experiment was terminated after 12 weeks.

Fatty acid[1]	Diet		Fish		
	1[2]	2[3]	Initial	Fed diet 1	Fed diet 2
14:0	6.53	3.17	4.38	4.16	3.41
14:1	0.19	0.47	0.51	0.70	0.54
16:0	20.93	20.42	15.33	16.97	17.70
16:1 (n-7)	9.09	7.42	8.69	8.23	7.87
16:2 (n-6)	0.27	0.67	0.71	1.22	0.89
18:0	2.55	5.44	5.09	5.13	5.18
18:1 (n-9)	16.28	30.28	20.60	20.46	26.63
18:2 (n-6)	7.41	10.71	8.75	8.98	10.22
20:0	0	0	0.01	0.01	0.14
18:3 (n-3) + 20:1 (n-9)	1.88	1.96	4.15	5.43	4.51
20:2 (n-6)	2.01	0.61	2.24	1.67	1.19
20:3 (n-6)	0	0.11	0.02	0.06	0.90
20:4 (n-6)	5.21	1.10	3.28	3.98	2.70
20:4 (n-3)	0.09	0	1.08	0.81	0.74
20:5 (n-3)	9.96	3.16	7.68	6.46	5.21
22:4 (n-6)	2.97	3.16	1.21	1.25	0.82
22:4 (n-3)	0.89	0.04	1.06	0.98	0.67
22:5 (n-3)	1.18	0.76	2.54	1.75	1.71
22:6 (n-3)	12.96	10.11	12.68	11.75	9.00

1 Two fish were combined and anlysed for initial fish. For fish fed diets 1 and 2, values are means for 3 and 4 fish respectively from different replicate tanks

2 Diet formulated for snapper based on fish meal

3 Diet formulated for snapper with 90 % of the fish meal replaced with soybean meal and poultry meal

TABLE 4

Amino acid composition (g/kg) of diets and juvenile snapper (*Pagrus auratus*) sampled before the experiment commenced, and when the experiment was terminated after 12 weeks[1].

Amino acid	Diet[2]				Fish				
	1	2	3	4	Initial	Fed diet1	Fed diet2	Fed diet3	Fed diet4
Asparagine	36.3	36.6	23.4	33.9	47.7	48.7	44.5	35.6	47.1
Glutamine	60.1	58.1	41.6	59.2	72.1	72.3	66.7	53.6	70.6
Serine	20.3	27.9	13.1	19.0	24.8	24.9	23.2	18.9	25.4
Glycine	24.8	32.9	21.0	41.3	45.2	49.4	44.3	43.9	53.8
Histidine	9.1	9.7	9.2	11.0	12.9	13.2	12.7	10.1	13.1
Arginine	19.9	23.2	15.1	23.3	29.1	29.5	29.7	23.9	33.6
Threonine	16.9	16.7	10.4	14.5	21.8	22.1	20.8	16.3	22.7
Alanine	26.7	24.1	18.5	30.1	35.8	37.4	34.1	30.3	38.6
Proline	21.7	32.6	18.1	31.6	30.3	32.5	30.0	28.0	35.2
Tyrosine	12.2	12.1	8.3	11.7	16.1	14.0	15.3	12.2	15.7
Valine	21.2	21.4	14.0	20.4	25.5	25.7	23.8	19.1	24.4
Methionine	11.7	6.5	6.5	6.6	15.0	13.3	11.6	9.6	14.0
Cystine	4.7	11.6	2.8	3.3	6.9	5.3	4.7	3.2	6.1
Isoleucine	18.8	17.2	11.8	16.8	23.6	23.6	21.3	16.7	21.7
Leucine	32.4	31.7	20.8	29.8	37.4	37.3	34.2	26.8	35.8
Phenyl -alanine	17.0	17.7	11.9	17.0	21.2	20.9	19.2	15.5	20.9
Lysine	30.7	22.1	20.1	29.2	44.5	44.1	41.4	32.3	43.0

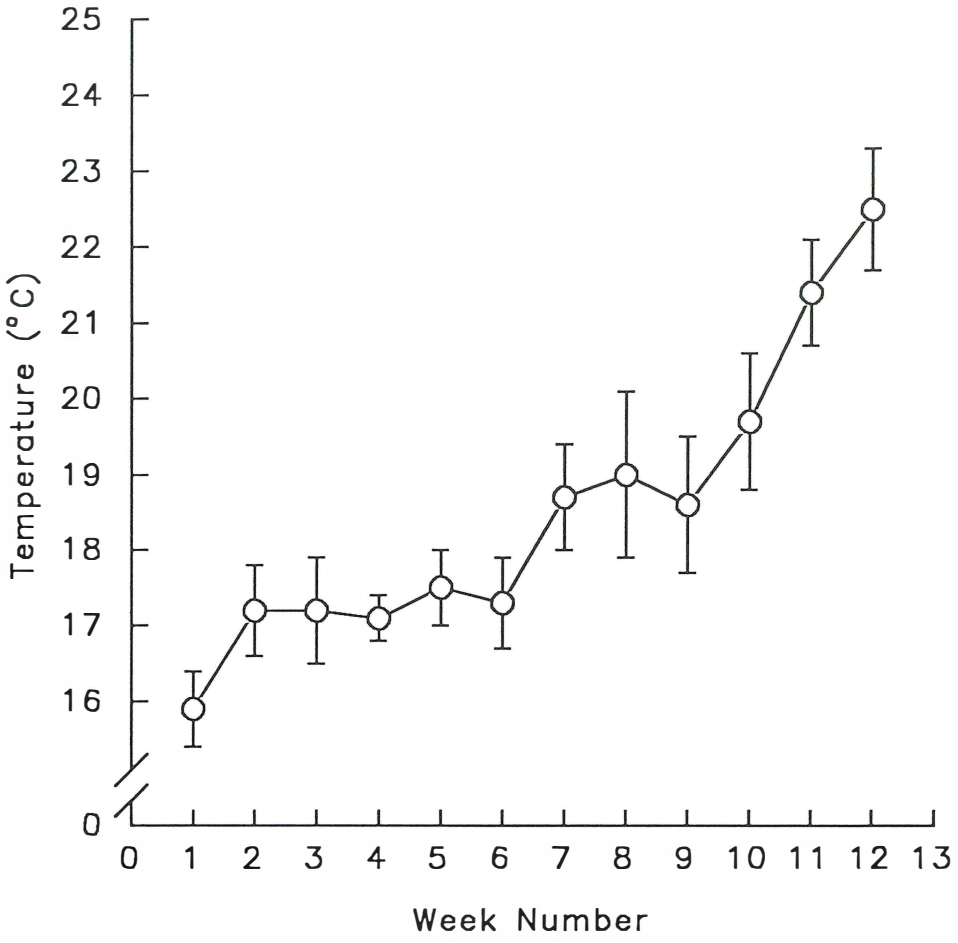
1 Results are expressed on an as is basis (moisture contents are given in Table 1) and are the mean of duplicate samples. Two fish (from separate replicates for those taken at the termination of the experiment) were freeze dried and combined

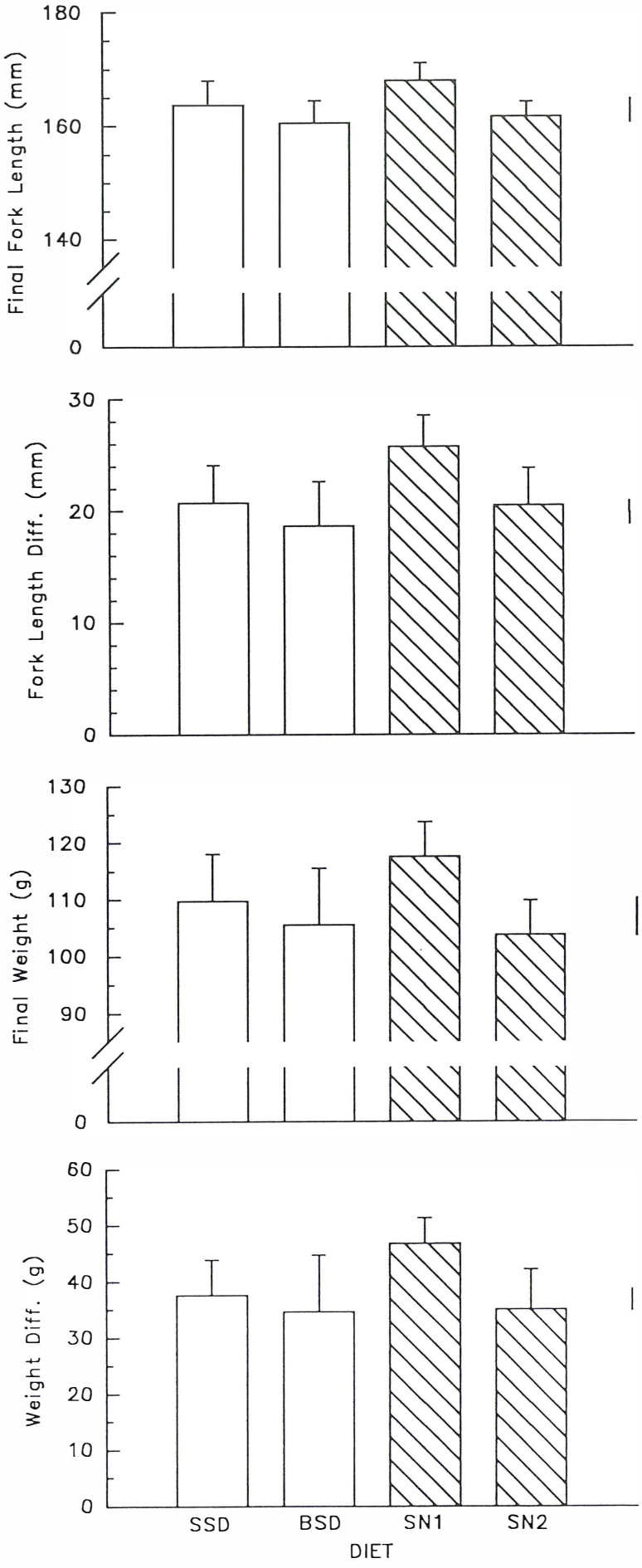
2 See Table 1 for a description of diets

FIGURE CAPTIONS

Figure 1 Weekly mean of daily modal temperatures during the twelve weeks of the experiment. Error bars give SD.

Figure 2 Means for each diet for each four variables used to measure growth of snapper (*Pagrus auratus*). From top to bottom: final FL, difference in FL, final weight and difference in weight. Diets are defined in Table 1. Vertical lines adjacent to histograms link means not significantly different at $\alpha = 0.05$ by the SNK test. Error bars give the SD.


25
24
23
22
21
20
19
18
17
16
0
Temperature (°C)
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Week Number


Final Fork Length (mm)
180
160
140
0
Fork Length Diff. (mm)
30
20
10
0
Final Weight (g)
130
120
110
100
90
0
Weight Diff. (g)
60
50
40
30
20
10
0
SSD
BSD
SN1
SN2
DIET

Appendix 4

Sensory Evaluation of Australian Snapper (*Pagrus auratus*) Raised in Captivity

J. Prescott and J. D. Bell

Interest in the aquaculture of marine fish in Australia is gaining momentum. One species with considerable potential for farming is snapper (*Pagrus auratus*). The same species is cultured widely in Japan, where it is called the red sea bream. In Japan, the industry is based on an abundant supply of small, cheap fish (mainly pilchards, sardines and anchovies) fed to red sea bream held in sea cages (Foscarini, 1988).

Although snapper held in captivity can be grown rapidly to market size in Australia (Bell, Quartararo and Henry, 1991), there is little scope for farming snapper in Australia using cheap fish for food. The reason is that populations of small, cheap fish around the Australian coastline are very low by world standards. For example, an average of only 5564 tonnes of baitfishes (mainly pilchards, scaly mackerel and anchovies) were landed throughout Australia in 1980-85 (information supplied by Bureau of Rural Resources, Canberra, 1991).

Aquaculture of snapper in Australia will have to be based on formulated diets, as done for closely related species in Europe (Barnabe, 1990). Before development of such an industry can proceed, investors need to know whether snapper raised on pellet diets based on fish meal are as acceptable as the wild fish, or as those held in captivity and fed on cheap fish. The second part of the question is important because it may affect entry of Australian cultured snapper to the Japanese market.

In this paper, we report a sensory evaluation of three groups of snapper: wild fish, snapper fed on a pelleted diet based on fish meal, and those raised on fish flesh.

The two groups of snapper reared on fish flesh and pellets were caught from the wild at a mean fork length (FL) of 12 cm, and a mean weight of 50 g, in March 1989. They were held in adjacent 60 m^3 pens within a large (860 m^3) "flow-through" pool of sea water at the NSW Fisheries Research Institute on the shore of Port Hacking, Sydney. The fish reared on fish flesh were fed mainly pilchards, at ~ 3% of their body weight day^{-1}, although prawns and squid were used occasionally. The pelleted diet used to feed the other group was based on fish meal and contained 42% protein (see Bell, *et al* (1991) for more details of the diet and conditions under which fish were reared). The pellets were fed at the rate of 1-2% of body weight day^{-1}.

Between 9 and 10 am on 29 October 1990, 13 fish were caught by line from each of the two groups of captive fish. On the same day, 13 snapper were caught from the wild offshore from Port Hacking between 6 and 8.30 am. Th mean length and weight of fish from all three groups ranged from 244-276 mm and 384-538 g (Table 1), i.e. the fish were plate size.

Table 1 Mean lengths (measured to the fork in the tail) and weights (g) of wild snapper, and snapper fed on pellets and fish flesh used in the sensory evaluation. Sample sizes and Standard Deviations are also shown.

	Fork Length (mm)			Weight	
Group	n	Mean	SD	Mean	SD
Wild	13	244	43	384	260
Pellet	13	276	20	538	100
Flesh	13	262	19	454	100

Immediately after capture, fish from all three groups were killed by severing the spinal cord and were placed between crushed ice. All fish were filleted and prepared for storing between 11 am and 1 pm on 29 October, 1990. This involved removing the skin and bones from the fillets, blotting the flesh with paper towels and storing the fillets from each group of fish in separate plastic bags on ice. The fillets were kept like this for 48 h before sensory evaluation, to simulate normal shelf life.

Just prior to sensory evaluation, fillets from each group were cut into pieces of approximately 25 g. The pieces were then placed individually into covered dishes and cooked in a microwave oven (output: 700 W) for 20 sec on the highest setting. They were served hot to a taste panel.

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The taste panel consisted of 30 members of CSIRO staff (17 women, 13 men), all of whom had experience in sensory evaluation of food.

Each panellist received a single sample of each type of fish separately and sequentially for evaluation. The pieces of fish were coded for identification, and the order of presentation was counterbalanced across the subjects to avoid bias. Graphic rating scales were used for the evaluation (see e.g., Lawless and Malone, 1986). The panellists were required to place a mark on a 150 mm line which had descriptors at each end. The characteristics evaluated, and their end point descriptors, were:

(a) Colour of flesh (not at all acceptable to very acceptable),
(b) Texture (extremely firm to extremely soft),
(c) Oiliness (extremely dry to extremely oily),
(d) Flavour (extremely poor to extremely good),
(e) Off flavour (none to extremely strong),
(f) Fresh taste (extremely fresh to extremely stale),
(g) Overall acceptability (extremely poor to extremely good).

Following evaluation, data were digitised and converted to scores within the range 0 to 100. Note that a high score indicates increased acceptability for Flavour, Colour of flesh, Fresh taste and Overall acceptability, whereas a low score indicates decreased Off flavour. For Texture and Oiliness a score of 50 indicates a point which is neither firm nor soft, or neither dry nor oily, respectively (i.e., a neutral point).

Data within a fixed range (e.g. percentages) often have heterogeneous variances, and require transformation (e.g. arcsine) (Underwood, 1981). Inspection of residual plots for each ANOVA revealed that the model fitted the data well for each variable with the exception of Off flavours, which showed an increase in variance with fitted values. For Off flavours, homogeneity was achieved after $\log_e$ transformation.

For each variable, we used a randomised block design ANOVA to test the null hypothesis of no difference among the three groups of fish. In this analysis, each panellist was treated as a block, which removed 29 degrees of freedom from the residual mean square, thereby accounting for variation among subjects. Where ANOVA revealed a significant difference at alpha = 0.05, we used the test for least significant differences (Snedecor and Cochran, 1989) to identify how the three groups of fish differed.

RESULTS AND DISCUSSION

The mean (± SE) ratings for Flavour, Colour of flesh, Fresh taste, Off flavours, and Overall acceptability are shown in Figure 1, while the ratings for texture and oiliness are shown in Figure 2 in relation to the neutral rating of 50.

Flavour, Colour of flesh, Fresh taste and Off Flavours

The mean ratings for Flavour, Colour of flesh and Fresh taste exceeded 50 for each type of fish, whereas the rating for presence of Off flavours was low for all three types of fish (Figure 1). This suggests that all types of fish were relatively acceptable with respect to these attributes. For each of these four variables, there were minor, but non-significant, differences between the three types of fish (Figure 1). The results of the ANOVA for each variable were: Flavour ($F_{2,58}$=2.94, p=0.061), Colour of flesh ($F_{2,58}$=2.89, p=0.064), Fresh taste ($F_{2,58}$=1.78, p=0.178) and Off flavours ($F_{2,58}$=2.45, p=0.095).

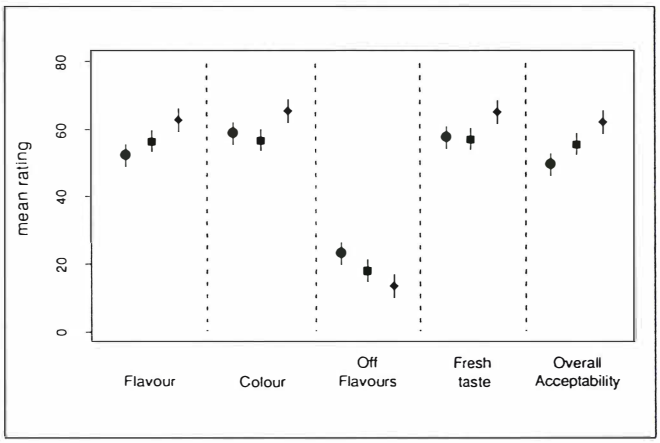

Figure 1 Mean (± SE) ratings of flavour, colour of flesh, freshness, off flavours, and overall acceptability for three groups of snapper.

● = fish flesh ■ = pellet ◆ = wild

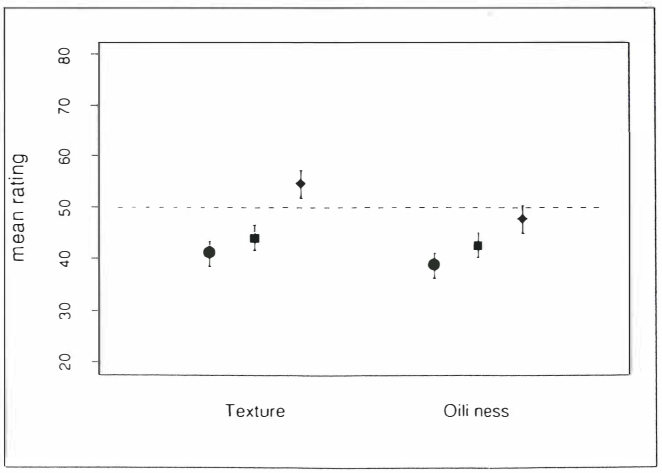

Figure 2 Mean (± SE) ratings of texture and oiliness for three groups of snapper. The dotted line indicates the neutral point.

● = fish flesh ■ = pellet ◆ = wild

Texture and Oiliness

The mean ratings for Oiliness of the flesh varied from 39 to 48 (Figure 2), but did not differ significantly among the three groups of fish ($F_{2,58}$=2.9, p=0.063). This was not true for the analysis of Texture. The texture of wild snapper was rated as significantly softer than either of the two groups of captive snapper ($F_{2,58}$=5.4, p=0.007, 5% LSD=8.42) (Figure 2).

Overall acceptability

There was a significant difference in the Overall acceptability of the three groups of fish ($F_{2,58}$=3.51, p=0.036) (Figure 1). Snapper fed on fish flesh were not as acceptable as wild fish, but there was no significant difference between the acceptability ratings of wild snapper and those fed on pellets, or between snapper fed on fish flesh and those fed on pellets (5% LSD=9.40).

Our results are encouraging for the development of aquaculture of snapper based on pelleted diets for at least two reasons. First, the lack of a significant difference in overall acceptability of wild snapper and those fed on pellets indicates that snapper reared on a formulated diet should have similar consumer appeal to the wild fish. A factor that may influence this general conclusion is the texture of the flesh. The firmer texture of cultured fish may enhance their appeal on the Australian market, as the snapper's relatively soft flesh can pose a problem for handling and storage (N. Ruello, pers. comm).

In addition to the attributes evaluated in the present study, appearance of the fish is also important because snapper are generally sold whole, and are usually served this way in restaurants. Cultured snapper have most of the markings and attributes that currently appeal to consumers of the wild product, but they have shorter tails and a slightly darker colour than wild fish. The acceptability of cultured snapper served whole remains to be determined.

Second, there were no significant differences between fish raised on flesh and pellets for any of the variables we measured. This suggests that snapper fed on pellets in Australia could be acceptable to Japanese consumers of cultured red sea bream. However, there may be differences in taste, and common methods of fish consumption, between Japan and Australia. For example, red sea bream are often eaten raw as sashimi in Japan. We have demonstrated that Australian consumers are unlikely to differentiate between the cooked flesh of snapper reared on fish flesh and pellets, but the acceptability of cooked and raw flesh from both these types of fish to the Japanese palate remains to be investigated.

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Appendix 5

Aquaculture and Fisheries Management 1992, **23**, 543–548

Effect of intraperitoneal passive implantable transponder (PIT) tags on the growth and survival of juvenile snapper, *Pagrus auratus* (Bloch and Schneider)

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Abstract. This study examined whether passive implantable transponder (PIT) tags could be used to mark individually juvenile snapper, *Pagrus auratus* (Bloch and Schneider), without affecting their growth. Fifty juvenile snapper (25 tagged and 25 untagged controls) were placed in each of four 2000-l tanks. At the start of the experiment the snapper had a mean weight of 59 ± 18 g (SD). After 70 days, the mean weight of all fish was 115 ± 31 g (SD) and there was no significant difference between the growth of tagged and untagged fish. Apparent tag loss ranged from 4 to 8%.

Introduction

The potential of snapper, *Pagrus auratus* (Bloch and Schneider), for aquaculture in south-eastern Australia is being evaluated partly through a series of growth trials (Bell, Quartararo & Henry 1991; Quartararo, Allan & Bell 1992). One way of carrying out powerful experiments to detect differences in growth due to different diets, water temperatures, etc., is to tag individual fish, nest aquaria within treatments and analyse the data using ANOVA (Underwood 1981). The criteria for a tag in such experiments are that it identifies individual fish permanently, can be read and recorded easily without killing the fish, and has a minimal effect on growth rate. The majority of methods developed for tagging fish individually do not meet all these criteria (Emery & Wydoski 1987).

Passive implantable transponder (PIT) tags have been used to identify individual fish (Harvey & Campbell 1989; Prentice, Flagg & McCutcheon 1990) and have potential for use in growth experiments. However, although PIT tags meet the first two of the above criteria, their effect on the growth of juvenile fish has only been described for salmonids (Prentice *et al.* 1990). Here we report a growth trial to determine if PIT tags affect survival and growth of juvenile snapper.

Materials and methods

Juvenile snapper were caught by beach seine in Port Hacking, New South Wales (340°47′S, 151°8′E) and acclimated for 6 months in 2000-l fibreglass tanks with a flow through of 0·3 l/s of aerated sea water. Stocking density was below 2 kg/m^3.

To reduce costs, we used 'dummy' tags donated by AVID (Norco, CA, USA). 'Dummy' tags consisted only of the sealed glass casing of functional PIT tags and were 14 mm long with

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a diameter of 2 mm. Prior to application, the tags were soaked overnight in 70% ethanol and then rinsed with sterile saline.

Tags were placed intraperitoneally using an applicator consisting of a modified 3-ml plastic hypodermic syringe fitted with a 12-gauge stainless steel needle. A snug fitting stillet, attached to the syringe plunger and passed through the needle, was used to eject tags.

Before tagging, fish were anaesthetized with Benzocaine (ethyl p-aminobenzoate, 50–75 mg/l in sea water) and then held ventrodorsally with their head away from the person doing the tagging. The needle containing the tag was inserted anteriorly through the midline of the ventral surface approximately midway between the vent and the attachment of the pelvic fins. During insertion, the angle between the needle and the ventral body surface was approximately 30°. After penetration of the peritoneal cavity the tag was ejected and the needle withdrawn.

Fish used in this trial were chosen randomly from the acclimation tanks, anaesthetized and weighed. Every second fish was tagged. The fish were systematically interspersed to four 2000-l tanks with a flow through of 0·3 l/s of aerated sea water at ambient temperature. At the start of the trial, each of the four tanks was stocked with 25 tagged and 25 untagged (control) fish. Five fish failed to recover from anaesthesia: two control and three tagged fish. Gross (i.e. with the naked eye) inspection of the abdominal cavity of the three tagged fish revealed no trauma to internal organs. The deaths were attributed to the effects of handling and anaesthesia. The five dead fish were replaced.

The mean weight and fork length of all fish at the start of the trial was 59 ± 18 g (SD) and 132 ± 13 mm (SD), respectively. There was no significant difference in the frequency distributions of weights between the two groups in each tank at the start of the trial (Kolmogorov-Smirnov (KS) test, Siegel 1956). The initial stocking density was approximately 1·5 kg/m^3 and the trial ran for 70 days beginning on the 21 October 1990. The water temperature varied from 17°C at the start of the trial, to 24°C at the end. Water quality was assessed weekly by measuring dissolved oxygen (DO, >6 mg/l), nitrite (<0·03 mg NO_2-N/l), ammonia (<0·05 mg unionized NH_3-N/l) and salinity (34 ppt).

At the end of the trial, the KS test was used to test whether there was a significant difference in weight frequency distributions between the control and tagged fish in each tank. The null hypothesis was that the PIT tag did not affect growth. Survival was also compared for the two groups of fish in each tank. At the end of the trial, tags were recovered by inspecting the peritoneal cavity and if a tag was not found, the organs in the abdominal cavity were carefully dissected out and examined for tags.

Results

The number of control and tagged fish in each tank at the start and end of the trial, the mortality of fish and the apparent tag loss during the trial are shown in Table 1. In some cases where a tag appeared to be lost it was possible that it may still have been in the body of the fish. Thus the apparent tag loss may be greater than the true tag loss. This problem would have been avoided if functional tags had been used; however, the cost of using functional tags could not be justified in preliminary trials.

Apparent tag loss ranged from 4 to 8% in the four tanks. Even if this was the true tag loss, the use of PIT tags to mark fish individually would not be precluded for experimental designs based on ANOVA where tanks are nested within treatments. It may mean, however, that more

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Table 1. Numbers of control and tagged fish in each of four tanks at the start of the experiment, and apparent numbers at the end. Mortality is also shown

Tank	Group	Start	End	Mortality	Apparent tag loss (%)
1	Control	25	26*	0	
	Tagged	25	24	0	4
2	Control	25	26*	1	
	Tagged	25	22	1	8
3	Control	25	25*	1	
	Tagged	25	24	0	4
4	Control	25	27*	0	
	Tagged	25	23	0	8

*Gives the number of fish in which a tag could not be found at the end of the trial (note that the actual number should have been 25).

individuals will need to be tagged to allow for some tag loss, thereby maintaining the desired number of degrees of freedom in the residual when using ANOVA.

Three fish died during the trial, two from tank 2 on days 11 and 68, and one from tank 3 on day 52. The fish that died in tank 2 on day 11 was tagged and showed grossly visible signs of peritonitis. This was most probably caused by tagging. The cause of death of the other two apparently untagged fish was not determined. In every case where a tag was recovered at the end of the trial, no gross signs of irritation of the peritoneal cavity were found.

The growth of juvenile snapper was not affected by intraperitoneal PIT tags during the 70-day experiment when the fish increased their weight by 95% (Fig. 1). We found no significant difference ($P>0{\cdot}1$) in weight frequency distributions between the control and tagged groups in each tank at the end of the trial (KS test).

Discussion

Studies on radio and acoustic tags implanted surgically in the peritoneal space of fish have found that the tags elicited a chronic inflammatory response: tags were encapsulated by fibrous tissue and there were adhesions between the parietal and visceral peritoneum (Summerflet & Mosier 1984; Marty & Summerfelt 1986; Lucas 1989). This was not evident in the present study. The relative lack of tissue response could possibly be explained by the different methods of tag application. In most cases, surgical implantation causes more tissue damage and bleeding than does the insertion of a hypodermic needle: it is known that blood clots aid in the formation of adhesions. It is possible that the heat-sealed glass used for PIT tags causes less tissue reaction than the paraffin-coated polystyrene or polypropylene used in radio and acoustic tags. The two classes of tag differ markedly in size, which may also help explain the difference in tissue reaction. Lucas (1989) used dummy transmitter tags measuring 12·5 × 48 mm (the smallest in the literature surveyed) in rainbow trout, *Oncorhynchus mykiss* (Walbaum), with a mean weight of 349 g. The PIT tags used in the present study measured 2 × 14 mm and were inserted in snapper with a mean weight of 59 g. Thus, the ratio of the volume of the tag to that of the fish is appreciably greater for dummy transmitter tags.

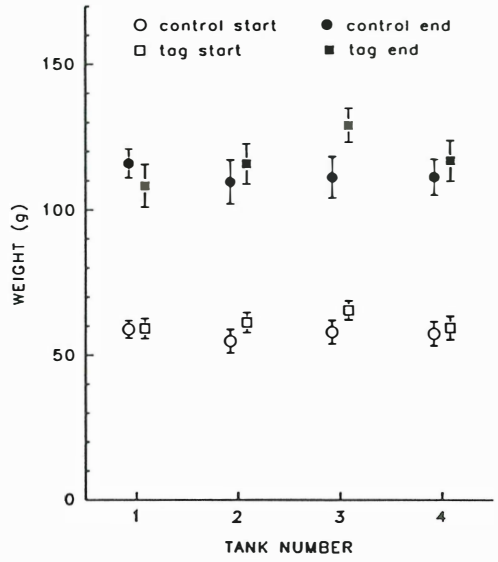

Figure 1. Mean weights of control and tagged fish in each tank at the start and end of the trial. Error bars give the SE.

Our growth trial had two weaknesses. Firstly, the data were not independent because control and tagged fish were mixed together and there may have been scope for the tagged fish to affect the growth of the control fish. However, the growth of control fish (0·9% wt/day) was comparable to that of previous trials in which control fish were kept on their own (0·8% wt/day, from Ferrell, Henry, Bell & Quartararo 1992). Secondly, our experimental design was not of the form we advocate for growth trials. The reason for this was that there was no way of individually marking control fish, and consequently nesting tanks within the 'control' treatment.

Despite these minor weaknesses, we conclude that the lack of difference in growth rate between tagged and control fish in all four tanks shows that PIT tags do not affect the growth of juvenile fish and therefore have application for aquaculture and fisheries management beyond the marking of broodstock (Harvey & Campbell 1989; Jenkins & Smith 1990). PIT tags meet the criteria for examining growth of individual snapper as small as 130 mm FL and 60 g.

Acknowledgments

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Appendix 6

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Validation of Annual Marks in the Otoliths of Young Snapper, *Pagrus auratus* (Sparidae)

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Abstract

In April 1989, young-of-the-year snapper (*Pagrus auratus*) were captured from the wild and injected with tetracycline. The fish were reared on natural food in a large pool with flowing sea water under ambient marine conditions. Two fish were removed every two months for two years, and the growth of their otoliths, relative to the tetracycline mark, was measured. Growth of the otoliths was greatest in spring and summer and least during winter. Opaque marks appeared in the otoliths of fish during both winters of the study. Our results confirm that the opaque marks in otoliths of young snapper can be used to determine the age of the fish in years.

Introduction

Pagrus auratus is widely distributed in the western Pacific (Paulin 1990), where it supports extensive fisheries in both the Northern and Southern Hemispheres (Crossland 1980; Foscarini 1988). Age estimates of wild *P. auratus* based on scales and otoliths have been used to describe growth differences among stocks around New Zealand (Paul and Tarring 1980). The accuracy of age estimates is critical where such information influences management of commercial fisheries (Beamish and McFarlane 1983).

Until recently, the only validations of annual formation of marks in bony tissues of *P. auratus* have relied on seasonal progression of growth increments at the margins of scales (Cassie 1956; Paul 1976) or on the association of counts of marks on scales or otoliths with year-class cohorts (Lenanton 1975; Paul 1976). However, Francis *et al.* (1992) have used fish marked with tetracycline to validate the formation of annual marks in otoliths.

Our study was designed to validate the periodicity and timing of the formation of annual marks in otoliths of *P. auratus* in the first two years of life by examining the growth of the otolith subsequent to a tetracycline mark. Our study complements that of Francis *et al.* (1992) in two ways: we use fish of a size and age not used in their validation, and regular sampling of fish allows us to plot the position of annual marks against the growth of the otolith throughout the study.

Materials and Methods

Two groups of *P. auratus* specimens were used in this study. The first group comprised 50 young-of-the-year collected from Port Hacking (34°47′S,151°8′E) on 10 March 1989 by Bell *et al.* (1991) to investigate growth in captivity. These fish had a mean fork length (FL) of $107{\cdot}2 \pm 4{\cdot}5$ s.d. mm. They were given an intraperitoneal injection of tetracycline hydrochloride at a dosage of 50 mg kg^{-1} (McFarlane and Beamish 1987). The fish were then placed in an 8-m^3 floating cage within an 875-m^3 pool that was supplied with flowing sea water at ambient temperatures and salinities (see Bell *et al.* 1991 for more details). The fish were fed chopped pilchards and prawns and otherwise maintained as described by Bell *et al.* (1991). Two fish were sampled from the cage every two months between May 1989 and February 1991. Otoliths were removed from these fish, then cleaned and stored dry in envelopes.

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The second group of fish was used to test that our procedure for injecting *P. auratus* with tetracycline did not affect growth. Three 4000-L tanks were each stocked with 55 *P. auratus* juveniles (mean FL for each tank ranged from 92 to 94 mm) that had been injected with tetracycline (as above) on 1 February 1990. After 155 days, the mean ($n=3$ tanks) increase in FL of injected fish was compared with the mean ($n=3$) from tanks stocked with 55 control fish of the same size. Mean growth of injected fish ($41\cdot7\pm2\cdot1$ s.d. mm) and control fish ($40\cdot0\pm1\cdot0$) was not significant different (two-tailed $t=1\cdot25$, $P=0\cdot28$). Mortality of injected fish ($6\%\pm3\cdot6$ s.d.) was not significantly different from that of control fish ($9\cdot7\%\pm6\cdot0$ s.d.) (two-tailed $t=0\cdot80$, $P=0\cdot44$, arcsin-transformed data). At the end of this experiment, the fish marked with tetracycline were placed in an 8-m^3 cage and reared under the same conditions as the first group of 50 fish described above. Otoliths were removed from 10 individuals of this second group of fish on 20 February 1991 (i.e. 12 months later, towards the end of their second summer).

Otoliths were prepared for reading by methods based on those of Beamish (1979). Thin sections were made by mounting otoliths in blocks of resin and using a diamond saw to make a cut, in the transverse plane, through the core. The cut surface was then polished by using a series of emery papers and abrasive films, the finest being 3 μm. The polished surface was attached to a microscope slide with resin. The procedure was repeated from the other end of the block, leaving a thin section of between 50 and 100 μm on the glass slide. The finished sections were viewed by using reflected light with a compound microscope at magnifications of $\times25$ and $\times100$. Identification and measurement of the position of fluorescent marks was made separately with a compound microscope equipped with an ultraviolet-light source.

The distance from the outer edge of the otolith to opaque marks (light bands when viewed by reflected light), or to a fluorescent mark, was measured along the axis between the focus of the otolith and the ventral surface of the otolith just ventral to the sulcus (Fig. 1). Identification numbers were masked and recoded prior to making any measurements or counts in order to coneal the identity of the specimen.

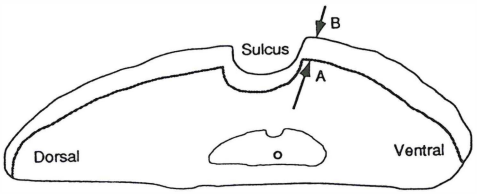

Fig. 1. Schematic diagram of transverse section of a snapper otolith. Opaque marks and tetracycline marks were measured from the mark (Point A, and represented by shaded line) to the edge of the otolith at Point B.

Results

The fish injected with tetracycline had otoliths with fluorescent marks, except for two individuals, one sampled in May 1990, the other in July 1990. The transverse sections revealed that more material was laid down on both the dorsal and the ventral edges of the otolith than on the interior, sulcal surface. Except at the dorsal and ventral tips of the otolith, there was no fluorescence on the antisulcal surface of any otolith.

Opaque marks were observed in otoliths from the first group of fish in two successive years between July and October. The otoliths grew in the three months following tetracycline treatment in March 1989, but this growth did not include an opaque margin (Fig. 2). However, otoliths collected from fish in early October 1989 had opaque margins. In all successive samples, the distance between the opaque mark and the tetracycline mark remained similar, indicating that the opaque mark had formed at a similar time in 1989 in all of the experimental fish. Otoliths from the single fish collected in August 1990 had an opaque margin, and all fish sampled after that date had two opaque marks (Fig. 2).

The distance from the edge of the otolith to the tetracycline mark did not increase regularly between successive samples, and growth of the otolith was greatest in the period between December and April (Fig. 2). The most rapid growth of the otoliths was in the months following incorporation of the fluorescent mark in March 1989. The slower growth of the otoliths after May 1989 was associated with the appearance of the opaque mark.

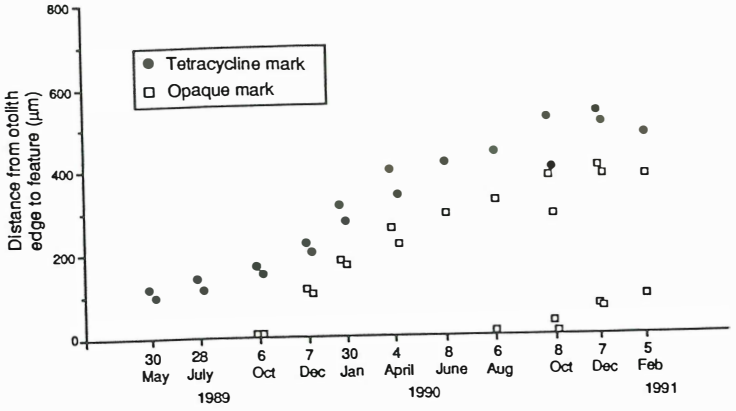

Fig. 2. The growth of otoliths, depicted by the distance from the edge of the otolith to tetracycline marks and to opaque marks. Measurements from individual fish are aligned vertically; points from fish collected the same day are offset for clarity.

This pattern appeared to be repeated in the winter of 1990, but there was also an increase in variation between individuals and absence of replicates from some samples in the same period.

The location of the fluorescent mark on the otoliths of the ten 1+ fish from the second group sampled on 20 February 1991 was similar to that of the fish sampled in January 1990 from the first group of fish; it averaged 0·36 mm (s.d. = 0·07 mm) from the ventral margin near the sulcus. Each fish had a single opaque mark that was found an average of 0·19 mm (s.d. = 0·02 mm) from the ventral margin.

Discussion

The opaque marks in the otoliths of young snapper in this study were formed annually and appeared in winter (i.e. when other studies of marks in snapper scales have suggested otoliths should form an annual mark) (Lenanton 1975; Paul 1976). The results are reliable because an opaque mark was formed in each of two successive years in the first group of fish, and during the first (and only) year in the second group of fish.

The appearance of an opaque mark at the margin of the otolith in late winter or spring has been found previously in *P. auratus* (Paul 1976; Horn 1986) and many other species (Bagenal and Tesch 1978). It was unfortunate that the spacing of collections every two months, and the small sample of fish each time, did not allow us to quantify variation in the timing of formation of the opaque marks. It appears, however, that the opaque margin formed earlier in the year in the second winter of the study. Opaque margins were not visible in otoliths taken in July 1989, but the single fish sampled in July 1990 did have an opaque edge to the otolith. The source of this variation is not known but could be due to interannual differences in wter temperature. The mean temperatures for July (the coldest part of the year) in the pool were more than 1°C higher in 1990 than in 1989 (14·6°C ± 1·0 s.d. versus 13·6°C ± 0·9; Bell *et al.*, 1991 and unpublished data).

Francis *et al.* (1992) suggest that some proportion of age assignments will be wrong because of incorrect interpretation of marks, particularly on the edges of otoliths. There were no obvious misinterpretations in our study; however, our winter collections of snapper had opaque otolith margins in different months, suggesting that interannual or individual

variation in the timing of formation of the opaque mark is possible. Such variation would add to the uncertainty in assigning age classes accurately at those times of the year when opaque marks are difficult to detect.

One reason for caution in applying our validation is that the fish were reared in conditions where they grew up to twice the rate of wild fish (Bell *et al* 1991). For example, the mean length of the fish sampled on 7 December 1991 was 274 mm FL, more than 70 mm greater than for fish of a similar age in New Zealand (Paul 1976; Horn 1986). Although holding captive fish is an acceptable way of attaining the validation of ageing procedures (McFarlane and Beamish 1987; Murphy and Taylor 1990), there can be problems associated with this general method. For example, Campana (1984) found daily increments in starry flounder larvae reared in the laboratory to be less clear than increments in wild larvae.

Nevertheless, we believe that our results provide a validation of the general timing of opaque-mark formation. Opaque marks were formed in otoliths in the numbers and at the times expected. Our work, done under conditions of rapid growth, supports that of Francis *et al.* (1992), who show that the formation of opaque marks is not affected in *P. auratus* when growth varied by more than a factor of two among years. We conclude that both our work and that of Francis *et al.* (1992), demonstrates the value of otoliths for age determination in *P. auratus* by establishing the capacity of otoliths to accurately record age under varying growth conditions.

Acknowledgments

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Appendix 7

Lymphocystis in cultured snapper (*Pagrus auratus*) and wild kingfish (*Seriola lalandi*) in Australia

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Lymphocystis affects a wide variety of the more highly evolved freshwater, brackish and saltwater fish. It is caused by an iridovirus, first isolated by Wolf *et al* (1966), and has been reported overseas from 125 species representing 42 families (Smail and Munro 1989). In Australia it was first reported from imported paradise fish (*Macropodus opercularis*) held in quarantine (Ashburner 1975), and gross lesions consistent with lymphocystis have been described affecting leatherjackets (family Balistidae) and flounder) (family Pleuronectidae) in Sydney Harbour (Reddacliff 1985). Pearce *et al* (1990) described the disease in captive barramundi (*Lates calcarifer*) in the Northern Territory, while Anderson (personal communication) regularly observes lymphocystis in a variable percentage of juvenile barramundi reared in marine cages off the Queensland coast. We report here further evidence of lymphocystis in Australian native fish.

Three hundred and seventy juvenile snapper (*Pagrus auratus*), body length approximately 80 mm, weight approximately 20 g, were captured in Port Hacking (34°47'S, 151°8'E) during March/April 1990, and held in a 2000 litre, 2.5 m diameter fibreglass tank, using water from the adjacent estuary in a continuous flow-through system. Water temperature ranged from 22°C in March to 14°C in July. Fish were fed a commercial salmon starter diet.

Lesions on fins were first noted in July 1990, when about 5% of the fish were grossly affected. During the next month the prevalence increased until it stabilised at about 50%. The infection persisted until November 1990, when an interruption to the water supply resulted in the death of the affected group of fish. Several fish from the original group, which were held in other similar tanks as part of a tagging trial, still had gross lesions in December 1990. No mortality attributable directly to lymphocystis occurred, and the behaviour and feeding of the fish was considered normal. Subsequently, juvenile snapper captured in April 1991 and kept under similar conditions developed identical lesions.

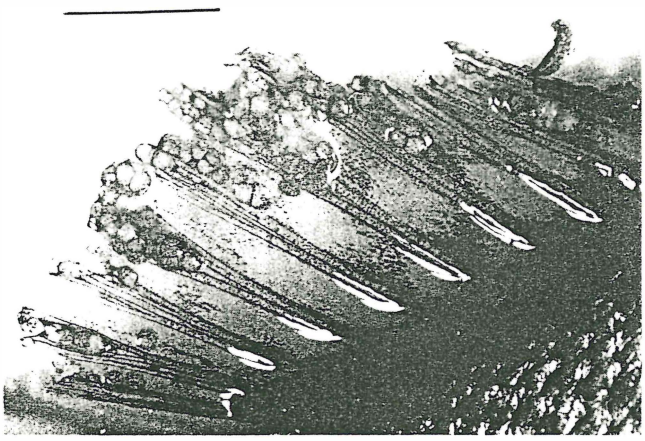

Figure 1. Lymphocystis lesions on the dorsal fin of a snapper (*Pagrus auratus*). Bar represents 5 mm.

In July 1990 3 live fish were submitted to the laboratory for pathological investigation. Grossly the lesions were pale and multi-nodular, ranging from 1 to 10 mm in size and were confined to the fins (Figure 1). Affected fins were fixed in 10% buffered neutral formalin, and processed routinely for histopathology. The microscopic appearance was typical of lymphocystis, with characteristic massively hypertrophied cells in the sub-epithelial connective tissue, each surrounded by a thick, PAS positive, hyaline capsule (Figure 2). Further fish were submitted in August 1990, and unstained wet preparations from typical lesions were examined under routine bright-field microscopy. The characteristic hyaline capsule and intracytoplasmic inclusions were readily seen (Figure 3), allowing a rapid presumptive diagnosis to be made. Histopathologic findings were as above, and the presence of icosahedral viral particles in the cytoplasm of hypertrophied cells was confirmed by transmission electron microscopy.

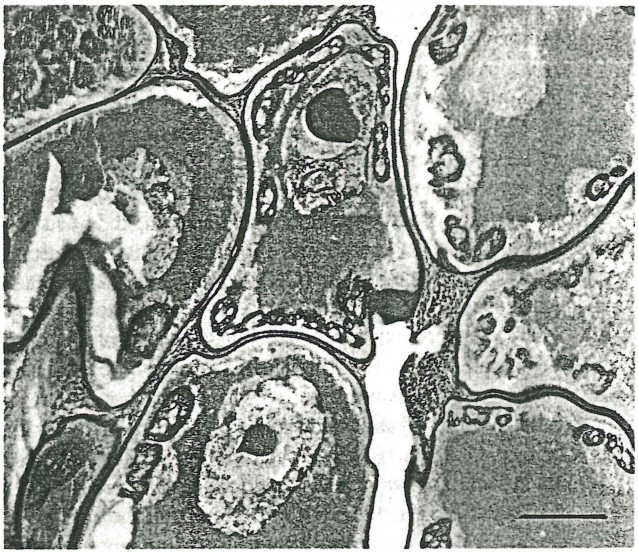

Figure 2. Histological appearance of hypertrophic cells from a lymphocystis lesion from the fin of a snapper. Note the thick hyaline capsule, the peripherally arranged, vesicular inclusions and the hypertrophied nucleus and nucleolus. Haematoxylin and eosin; bar represents 100 µm.

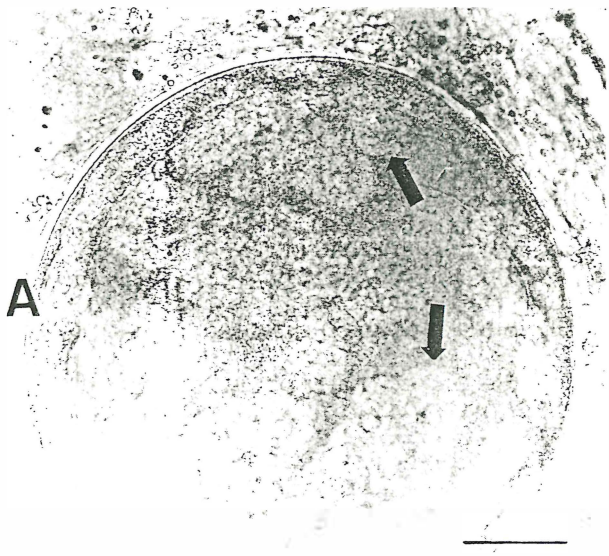

Figure 3. Wet squash preparation of a lymphocystis lesion from the fin of a snapper. The thick hyaline capsule (A) and abundant intracytoplasmic inclusions (arrows) in a single, round hypertrophic cell are readily visible. Bar represents 100 µm.

Lymphocystis was also diagnosed histopathologically from formalin fixed tissues of 2 wild kingfish (*Seriola lalandi*) caught off Tuggerah Reef (33° 20'S, 151°40'E) in January 1991. Gross lesions were remarkable for the amount of melanin pigment present. They appeared as irregular, roughened, black patches on both body and fins. Histologic appearance was as above, except that melanocytes were particularly prominent within the dermal inflammatory response. The hypertrophic cells were themselves unpigmented.

This report extends the confirmed occurrence of lymphocystis in Australian native fish. The occurrence in wild kingfish is undoubtedly a natural infection, whilst infection in the snapper was most likely to have been introduced to the facility directly from the wild, either unnoticed on the fish at time of capture, or via the open circulation water system. The high morbidity observed in this outbreak was possibly associated with the husbandry system. Minor traumatic fin lesions are common in young snapper kept in small tanks, allowing a ready route of entry for the virus. Paperna *et al* (1982) recorded similar high morbidity in sea-cage-reared *Sparus aurata*, a closely related species, in the Red Sea, and observed the densest clustering of hypertrophic cells where abrasions occurred on the skin.

Whilst lymphocystis can be a serious disease in some fish culture systems (Tonguthai and Chinabut 1987; Cheong *et al* 1983), and may be of economic importance in fishing operations by reducing the aesthetic appeal of infected fish, it is usually regarded as a self-limiting condition, and often, light infestations may be overlooked. One author (GR) has observed 2 separate lymphocystis outbreaks in aquarium species in the Sydney region, where the aquarists concerned regarded the infection as just another "white spot" condition that was refractory to the usual treatments. Both were presumptively diagnosed with wet squash preparations as described above and subsequently confirmed with histopathology. One case involved a native Barrier Reef species, the percula clown fish (*Amphiprion ocellatus)*, obtained through commercial aquarium suppliers but collected from the wild. The other case involved a common exotic aquarium species, the golden gourami (*Trichogaster* sp) obtained from a local retail outlet. Langdon (1988) has also observed the disease in fish of this genus in quarantine facilities in Western Australia. The wet squash is a simple and fast technique, available to every veterinary practice at minimal cost. Greater awareness and the use of this technique should allow the prevalence, distribution and host-range of lymphocystis in Australia to be better defined.

We thank K Walker and R Whittington for the initial pathological investigation, S Wilson for electron microscopy and J Mathews for the gross photography.

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Appendix 8

Journal of Fish Diseases 1992, **15**, 409–415

Infection of captive *Pagrus auratus* (Bloch & Schneider) by the monogenean, *Anoplodiscus cirrusspiralis* Roubal, Armitage & Rohde (Anoplodiscidae) in Australia

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Abstract. The monogenean *Anoplodiscus cirrusspiralis* infects the fins and nares of snapper, *Pagrus auratus* (Bloch & Schneider). The epidermis beneath the haptor in both microhabitats is eroded and the parasite attaches to the basement membrane by an adhesive secretion. Captive snapper suffered fin damage through high levels of infection by this parasite. Laboratory experiments showed *A. cirrusspiralis* to be adversely affected by reduced salinity and killed within 1 h by diluted sea water of <5‰ salinity.

Introduction

Sparid fish are important aquaculture species in several parts of the world; for example, red sea bream, *Pagrus major* (Temminck & Schlegel), and black sea bream, *Acanthopagrus schlegeli* (Bleeker), in Japan, and gilthead bream, *Sparus aurata* L., in the Middle East and the Mediterranean. In Australia, there is an increasing interest in farming native marine fish, and the snapper, *Pagrus* (*Chrysophrys*) *auratus* (Bloch & Schneider), is currently under investigation.

Parasitic diseases are a problem in the culture of sparids, as well as other fish species. The copepod ectoparasite *Alella macrotrachelus* (Brian) is a major parasitic disease of *Acanthopagrus schlegeli* in Japan (Ueki & Sugiyama 1979; Kawatow, Muroga, Izawa & Kasahara 1980) and *Pagrus major* is afflicted with pathogenic parasites that include the ciliate *Cryptocaryon*, the protophytan *Oodinium* and the monogenean *Bivagina tai* (Foscarini 1988; Ogawa 1990).

Individuals of snapper, held in large concrete ponds at the Fisheries Research Institute, Cronulla, New South Wales, Australia, presented with hyperaemic and ragged fins. These fish were found to be heavily infected with the monogenean *Anoplodiscus cirrusspiralis* Roubal, Armitage & Rohde, 1983. We report here on the levels of infection and the histopathology of infection by *Anoplodiscus cirrusspiralis* on captive snapper. Studies were done *in vitro* to determine the tolerance of *A.cirrusspiralis* to diluted sea water and to fresh water as a possible control measure.

Materials and methods

Seventeen wild *P. auratus* were collected by handline off Sydney in March 1991. Young-of-the-year *P. auratus* were collected from Port Hacking, Sydney, in November 1989 by seine net and placed in a $8\,m^3$ net cage in large ponds containing older *P. auratus* at the Fisheries Research Institute, Cronulla. Seventeen *P. auratus* were sampled from the cage on 15 June 1990. The experiment was repeated in 1990/1991 and 15 *P. auratus* sampled on 7 May 1991.

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Fish were killed by spinal severance, the gut cut open and the fish dropped into 10% formalin (4% formaldehyde). The fish were soaked for 24 h in fresh water (three changes) and all sediment was examined for detached parasites. The fish were measured and the external body surface, including fins, and nares and operculum were examined for parasites. Pieces of infected tissue were removed, embedded in wax, sectioned at 5 μm and stained with haematoxylin and eosin (H&E).

Juvenile worms were identified by their small size and lack of vitellaria. Abundance, prevalence and mean intensity are defined by Margolis, Esch, Holmes, Kuris & Schad (1982). Correlation analysis was done with SAS (Ver. 6.03, SAS Institute Inc., Box 8000, Cary, North Carolina, USA).

To test the survival of *A. cirrusspiralis* in water of different salinities (at 20°C), individuals of caged snapper were killed by spinal severance and small pieces of caudal or pectoral fin with attached adult parasites were placed in 50 ml of either fresh water or sea water diluted to various salinities; nine parasites were used in each treatment. Sea water (34‰) served as the control. The time for the parasites to die was measured by their lack of response to gentle prodding with a blunt probe and their subsequent inability to revive when returned to undiluted sea water. The water at each dilution was replaced after 8 h and the experiment run for 25 h.

Results

Infection levels

A summary of infection levels by *Anoplodiscus cirrusspiralis* on wild and captive *Pagrus auratus* is given in Table 1. It is clear that the captive fish were more heavily infected, and

Table 1. Number of infected fish, abundance and range of infection by *Anoplodiscus cirrusspiralis* on caged and wild *Pagrus auratus*

	Caged fish		
	1990 sample	1991 sample	Wild fish
Sample size	17	15	17
Average length*	18·1	18·2	13·3
(S.D., range)	1·17, 15·3–20·3	1·21, 16·5–20·3	2·4, 10·4–18·2
Fins†			
Adult parasites	10, 6·7, 0–27	14, 18·2, 0–63	1, 0·07, 0–1
Juvenile parasites	11, 2·8, 0–12	13, 7·0, 0–20	0
Skin			
Adult parasites	0	0	0
Juvenile parasites	0	6, 1·4, 0–7	0
Nares			
Adult parasites	13, 2·8, 0–12	3, 0·2, 0–2	3, 0·3, 0–2
Juvenile parasites	1, 0·06, 0–1	0	0
Total‡			
Adult parasites	8·41	28·47	0·35
Juvenile parasites	3·3	9·07	0

* Length-to-caudal fork (cm); S.D., standard deviation.
† Number of fish infected, abundance, range of infection.
‡ Overall abundance (includes attached and unattached parasites).

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the level of infection in caged fish was greater in 1991 than in 1990. Only four of the 17 wild *P. auratus* were infected, and neither juvenile parasites nor parasites detached during preservation of fish in formalin were found in this sample.

All 17 fish examined in the 1990 sample of caged *P. auratus* were infected with a total of 163 (115 adult, 48 juvenile) attached parasites and 36 (28 adult, eight juvenile) parasites in the sediment. Fourteen of 15 fish were infected in the 1991 sample of caged *P. auratus* with 402 (276 adult, 126 juvenile) attached parasites and 161 (151 adult, 10 juvenile) unattached parasites.

No adult parasites were found on the flanks of the fish, but 21 juvenile parasites were found on the sides of six fish in the 1991 sample of caged fish; these infections were associated with high levels of infection on the fins by both adult and juvenile parasites.

The nares were infected by adult parasites in all samples, but only one juvenile was found in this microhabitat (caged fish, 1990 sample). Although there was a positive correlation ($r = 0{\cdot}64$, $P < 0{\cdot}01$) between parasite abundance on the fins and nares in the 1990 sample of caged fish, there were few parasites in the nares of the wild fish and in the 1991 sample of caged fish.

The pectoral fins of more fish in the 1990 sample ($n = 13$, $\bar{x} = 4{\cdot}69$, range 1–21) were infected, but less heavily, than the caudal fin ($n = 8$, $\bar{x} = 6{\cdot}62$, range 1–14). The pectoral fins were more heavily infected ($n = 13$, $\bar{x} = 17{\cdot}53$, range 4–51) than the caudal fin ($n = 14$, $\bar{x} = 10{\cdot}7$, range 1–38) in the 1991 sample of caged fish. Twenty-five per cent and 29% of parasites on the caudal and pectoral fins, respectively, in the 1991 sample were juvenile (separate data were not kept for the 1990 sample). In neither sample was parasite abundance correlated with size of fish.

Histopathology

A wide area of inflammation was associated with heavy infections of the parasites on the caudal and pectoral fins (Figs 1 & 2). The inflammation extended along the fin between the fin rays. There was no evidence of inflammation of the lamellae in the nares where the parasite attached (Fig. 3).

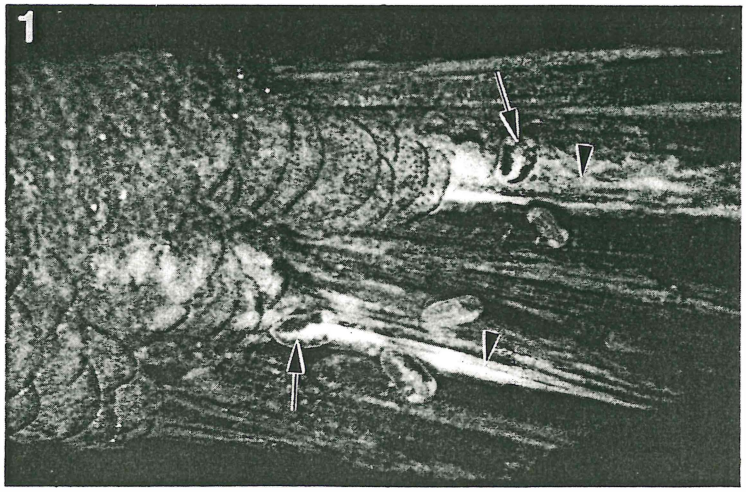


Figure 1. Several *Anoplodiscus cirrusspiralis* (arrows) near base of caudal fin of a snapper. Inflammation in vicinity of parasite (arrowheads) (×7).

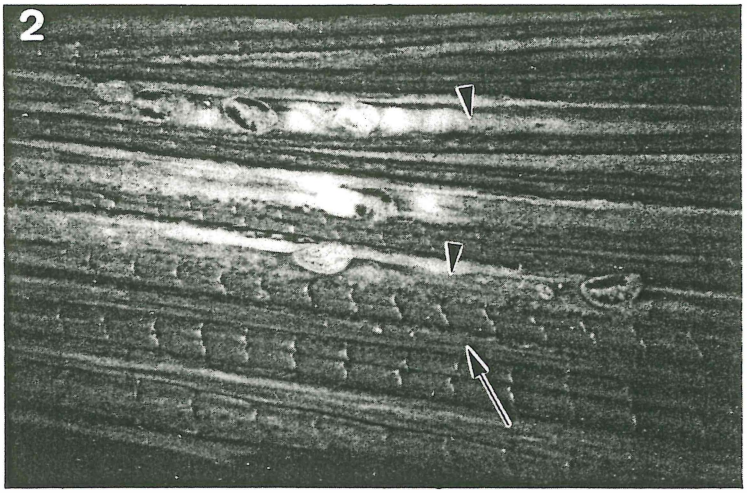


Figure 2. *Anoplodiscus cirrusspiralis* on pectoral fin of snapper with widespread inflammation (arrowheads) in vicinity of parasite, especially in inter-ray tissue. Nearby tissue appears unaffected (arrow) (×7).

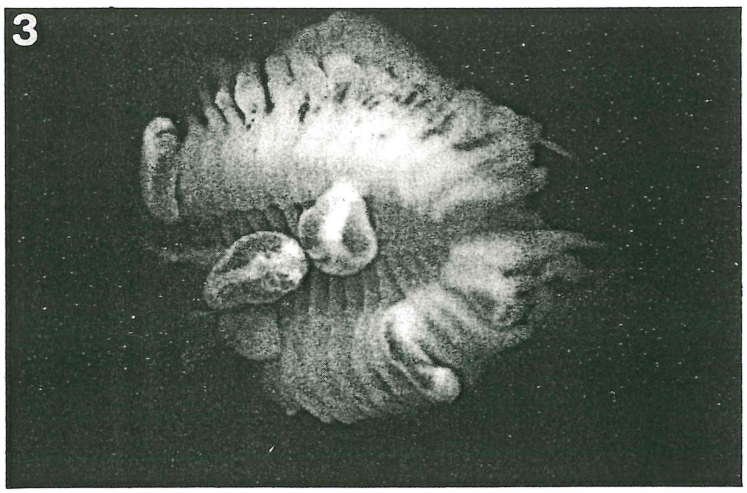
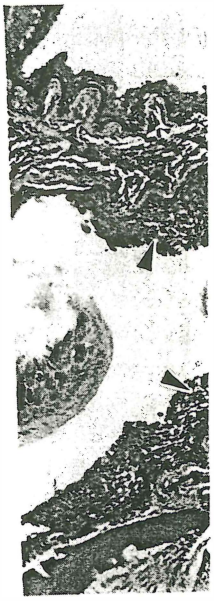
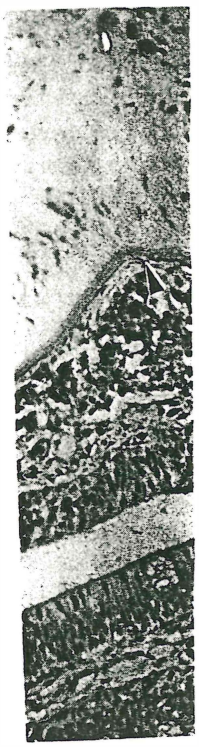


Figure 3. Several individuals of *Anoplodiscus cirrusspiralis* attached to the nares of snapper. No pathology is evident (×10).

The epidermis beneath the haptor of *A. cirrusspiralis* on the fins was eroded, and proliferated epidermis was evident on either side of the attached worm but not on the opposite side of the fin; there was little cellular infiltration (Fig. 4). Adult and juvenile parasites, including very small juveniles, were attached to the basement membrane by an eosinophilic layer beneath the haptor.

Parasites attached to the nasal lamellae also eroded the epidermis beneath the haptor, and the epidermis adjacent to the parasite was deformed (Fig. 5). There was no tissue reaction evident in the same or adjacent lamellae (Fig. 6). An eosinophilic layer was again present between the haptor and the basement membrane (Fig. 7).





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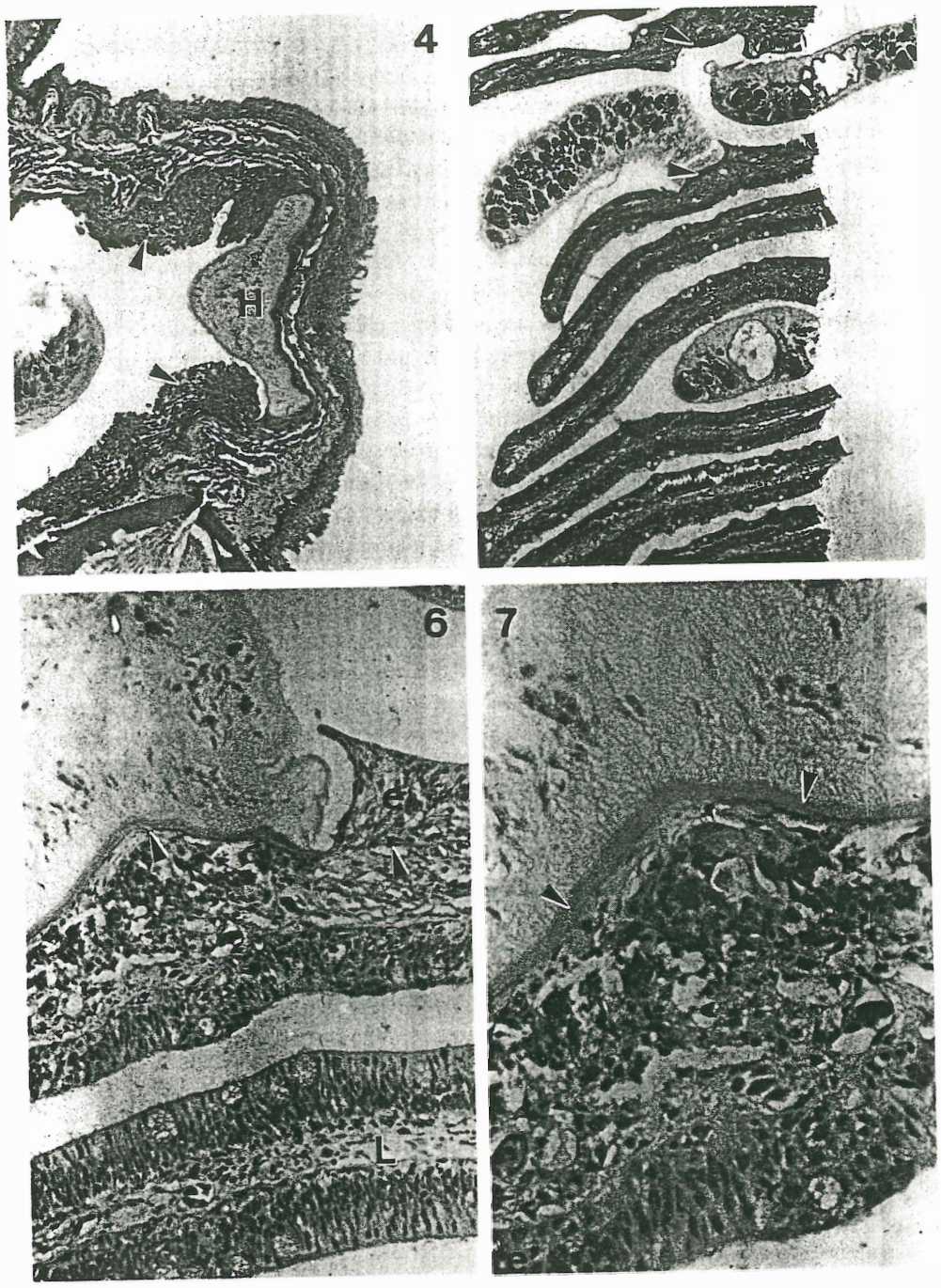

Figure 4. *Anoplodiscus cirrusspiralis* attached to caudal fin of snapper showing eroded epidermis beneath haptor (H) and thickened neighbouring epidermis (arrowheads) (×130).

Figure 5. Several *Anoplodiscus cirrusspiralis* attached to nares (arrowheads). Tissue damage restricted to vicinity of parasite (×50).

Figure 6. Attachment site on nasal lamella. Epidermis (e) eroded and parasite attached to basement membrane (arrowheads). Adjacent lamella (L) appears normal (×260).

Figure 7. Enlargement of attachment site to show eroded epidermis and parasite secretion (arrowheads) beneath haptor. Normal epidermis (e) on other side of lamella (×520).

Salinity tolerance

All *A. cirrusspiralis* survived in undiluted sea water for 24 h, but one had died after 25 h (Fig. 8). A reduced salinity resulted in lower survival such that in fresh water all were dead within 15 min. An increase in salinity improved survival. All parasites were dead within 1 h at 5‰, but at 10‰, all survived at least 8 h.

Discussion

Infection levels of *A. cirrusspiralis* on wild populations of *P. auratus* are usually low (prevalence: 23%, abundance: 0·35, this study). The prevalence and abundance of infection by the closely related *Anoplodiscus australis* Johnston on wild populations of sparids, *Acanthopagrus* spp., are also low: *Acanthopagrus australis* (Günther) (prevalence <1%, abundance <0·01, Roubal 1990; 7·5–13·6%, 0·08–0·32, Byrnes 1986); *A. butcheri* (none infected, Byrnes 1986); *A. berda* (Forskal) (2·5–55%, 0·03–2·8, Byrnes 1986) and *A. latus* (Houttuyn) (20–45%, 0·33–4·1, Byrnes 1986). It is not known to what extent factors such as salinity, locality, host size and season affect these data. The large populations of *A. cirruspiralis* seen in caged *P. auratus* in the present study presumably arise by cycling of the parasite within the experimental ponds.

The majority of juvenile *A. cirrusspiralis* occur on the pectoral and caudal fins, and at high levels of infection juveniles attach to the flanks of the fish. Infection of the nares is inconsistent. Those that do attach to the nasal lamellae fail to produce the deposits of melanin-like pigment scattered throughout the body of parasites on the external surfaces (Roubal & Quartararo 1992). Site selection on the host occurs early in the infection phase and post-settlement movement may be limited. The oncomiracidium of *Anoplodiscus* has eight pairs of larval hooklets, but these are soon absorbed into the parasite tissue (Ogawa & Egusa 1981). Juvenile worms erode the epidermis and in all microhabitats the worms attach permanently to the basement membrane by an adhesive secretion (Roubal & Whittington 1990).

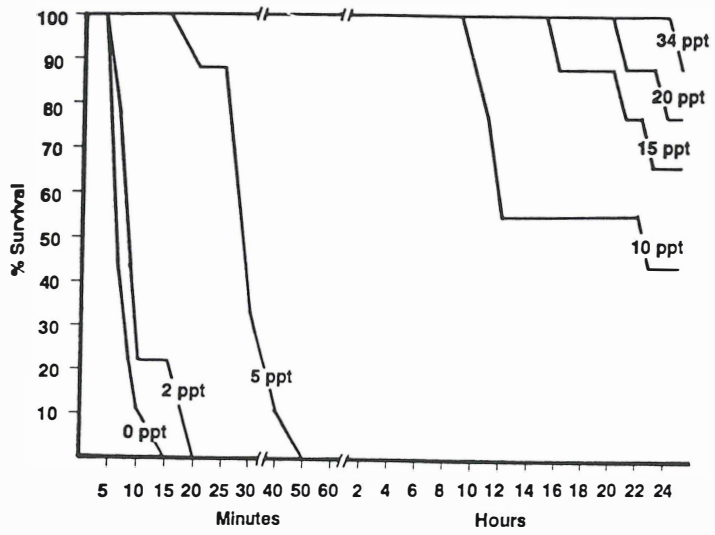

Figure 8. Percentage survival of *Anoplodiscus cirrusspiralis* over 25 h at different salinities.

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The epithelium adjacent to the parasite proliferates, but there is little host response in the dermis. This indicates that once the epidermis is eroded, the parasite does not irritate significantly the underlying tissue. None of the damage associated with feeding by *Benedenia monticelli* (Parona & Perugia) on the skin of mullets (Paperna, Diamant & Overstreet 1984) and by *Capsala martinierei* (Bosc) in the epidermis of *Mola mola* (L.) (see Logan & Odense 1974) is evident for *Anoplodiscus cirrusspiralis*.

Treatment of infection appears to be done easily by bathing the fish in fresh water. In the laboratory, all *A. cirrusspiralis* were killed by fresh water within 15 min, and within 60 min at 5‰ salinity. However, at 10‰, all survived for at least 8 h. This implies that the parasite may be euryhaline to some extent as an adaptation to the estuarine environment.

Acknowledgments

This study was supported by the Australian Research Council and the Fisheries Research Institute, Cronulla. Mr D. Graham and Ms M. Horton assisted with fish dissection.

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Appendix 9

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OBSERVATIONS ON THE PIGMENTATION OF THE MONOGENEANS, *ANOPLODISCUS* SPP. (FAMILY ANOPLODISCIDAE) IN DIFFERENT MICROHABITATS ON THEIR SPARID TELEOST HOSTS

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†Fisheries Research Institute, Cronulla, New South Wales 2230, Australia

(*Received* 18 *November* 1991; *accepted* 23 *January* 1992)

Abstract—ROUBAL F. R. and QUARTARARO N. 1992. Observations on the pigmentation of the monogeneans, *Anoplodiscus* spp. (family Anoplodiscidae) in different microhabitats on their sparid teleost hosts. *International Journal for Parasitology* **22**: 459–464. The monogeneans *Anoplodiscus australis* and *A. cirrusspiralis* infect the fins of their respective hosts, the yellowfin bream, *Acanthopagrus australis*, and the snapper, *Pagrus auratus*. In these habitats the adult parasite contains melanin-like pigment deposits within the body. Very young parasites lack the pigment but deposits of pigment appear and coalesce into dense deposits within the parasite as it grows. The evidence suggests that the pigment is synthesized within the body of the parasite rather than acquired through feeding on the host epidermis. However, adults of *Anoplodiscus cirrusspiralis* from the nares of snapper lack this pigment, which suggests that environmental cues may promote the production of pigment as a possible aid to camouflage.

INDEX KEY WORDS: Monogenea; *Anoplodiscus*; pigmentation; microhabitat.

INTRODUCTION

Anoplodiscus australis has been described from the fins of the yellowfin bream, *Acanthopagrus australis* (Pisces: Sparidae) (see Roubal, 1981) and *Anoplodiscus cirrusspiralis* from the fins of snapper, *Pagrus auratus* (Pisces: Sparidae) (see Roubal, Armitage & Rohde, 1983). These descriptions noted the presence of pigment within the body of the adult parasite. However, in a recent investigation of the parasite fauna of captive snapper at the Fisheries Research Institute, Cronulla, Sydney, it was found that *Anoplodiscus cirrusspiralis* also infects the nares of its host, unlike *Anoplodiscus australis*. Furthermore, these nasal-dwelling adult parasites lacked the pigment.

Pigment, apparently derived from the skin of the host, has been found in the gut of the monocotylid monogenean *Dendromonocotyle kuhlii* (see Kearn, 1979) and the capsalid monogenean *Capsala martinieri* (see Logan & Odense, 1974). However, Kearn (1979) pointed out that pigment could also be manufactured within the body of the parasite.

We made observations on post-settlement, developing *Anoplodiscus* to determine if (i) the pigment is associated with the gut of the parasite which would indicate acquisition through the diet, or (ii) the pigment is produced locally within the parasite body irrespective of the distribution of gut caeca and diverticula.

MATERIALS AND METHODS

Individuals of yellowfin bream, *Acanthopagrus australis*, were collected by handline or net in southern Queensland and northern New South Wales, killed by spinal severance, the gut cut open and the whole fish dropped into 10% formalin (4% formaldehyde); live fish were anaesthetized in benzocaine and the skin and fins examined. Snapper were collected by handline off Sydney or from captive populations at the Fisheries Research Institute, Cronulla, Sydney and preserved in formalin or anaesthetized and examined. The fins and body surface of anaesthetized and preserved fish, as well as the buccal and opercular cavities and nares of preserved fish, were examined for *Anoplodiscus*. Whole mounts of formalin-fixed parasites were unstained or stained with Grenacher's alum carmine or Semichon's acetocarmine. Formalin-fixed parasites were also washed, stored in 70% ethanol and mounted temporarily in lactic acid for examination. Individuals of *Anoplodiscus*, either detached or attached to pieces of tissue, were embedded in wax, sectioned and stained with haematoxylin and eosin or left unstained. Formalin-fixed parasites and pieces of caudal fin with melanophores were stained with Nile blue for melanin (see Humason, 1972) or washed in distilled water and placed in 10% hydrogen peroxide for 48 h.

TABLE 1—PIGMENTATION OF ADULT *Anoplodiscus* SPP. FROM DIFFERENT SPECIES OF BREAM (*Acanthopagrus* SPP.) AND FROM SNAPPER *Pagrus auratus*

Locality	Parasite (Host) Microhabitat	Host size*	Pigmented?	Reference
Anoplodiscus australis (*Acanthopagrus australis*) (nares uninfected)				
Coffs Harbour (NSW)	Fins	2.4–8.8	Yes	Roubal, 1981
Moreton Bay (QLD)	Fins	2.2–9.8	Yes	This study
Anoplodiscus spari (*Acanthopagrus schlegeli*)				
Japan (sea-cage)	Fins	<5	No	Ogawa & Egusa, 1981 Roubal, personal observation
Anoplodiscus cirrusspiralis (*Pagrus auratus*)				
Sydney (NSW) (caged)	Fins	4.5–6.5	Yes	This study
	Nares		No	This study
Sydney (wild)	Fins	3.5–6.0	Yes	This study
	Nares		No	This study
Western Australia	Fins	9.5–18	No	This study
	Nares		No	This study
S, E Australia, N. Z.	Fins	4–10	Yes	Roubal *et al.*, 1983
	Nares		NA	

*Head length (cm) range. S, E, southern, eastern; N. Z., New Zealand; NA, not available.

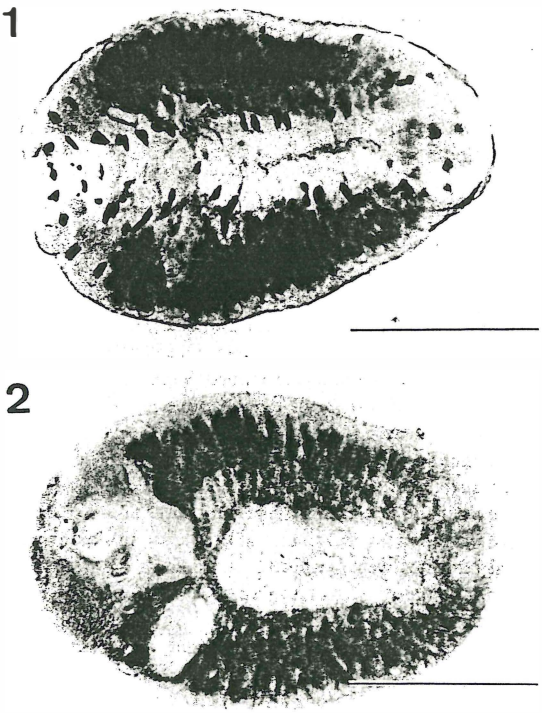

FIG. 1. Unstained wholemount of *Anoplodiscus cirrusspiralis* from fins of snapper. Pigment scattered throughout body. Scale bar, 500 μm.

FIG. 2. Unstained wholemount of *Anoplodiscus cirrusspiralis* from nares of snapper. Note the absence of pigment deposits within body. Lactic acid preparation. Scale bar, 500 μm.

RESULTS

Adult individuals of *A. cirrusspiralis* from the fins of some samples of snapper have deposits of a dark pigment scattered throughout the body (Fig. 1), as do adult *Anoplodiscus australis* from the fins (pectoral and caudal) of bream (Table 1). However, those adult *A. cirrusspiralis* infecting the nares of snapper lack this pigment (Fig. 2) as do worms from the inner side of the pectoral fin of large snapper from Western Australia (none of the latter had parasites on the outer surface of the pectoral fin or on the caudal fin) (Table 1). Furthermore, the smallest juvenile worms of both species from either the nares (snapper only) or fins (snapper and bream) lack this pigment.

Pigment deposits are not associated with gut caeca; some are arranged across gut diverticula (Fig. 3) and occur in the anterior end of the body where no gut processes extend (Fig. 4).

Histological sections show the pigment deposits to consist of fine, homogeneous particles but without a definite border (Fig. 5). Melanophores in the fins of bream and snapper are restricted to the outer region of the dermis and none were seen in the epidermis (Fig. 6). Both the melanin in pieces of fin and the pigment in whole worms were stained by Nile blue and both were faded to the same extent by the hydrogen peroxide.

Observations on juvenile individuals of *A. australis* and *A. cirrusspiralis* of increasing size indicate that pigment deposition begins before vitellogenesis and gonadal maturation. Pigment formation begins in

FIG. 3. Margin of body of pigmented *Anoplodiscus* showing pigment passing across gut diverticula (arrowheads). Lactic acid preparation. Scale bar, 100 μm.

FIG. 4. Anterolateral margin of worm showing pigment deposits anterior to (at left) and away from any gut diverticulum (arrowhead). Lactic acid preparation. P, Pharynx. Scale bar, 100 μm.

diffuse streaks along the length of the body and is not restricted to the vicinity of the intestinal caecum and lateral branches. Initially the pigment consists of few scattered particles but as the parasite matures, the pigment consolidates into more discrete deposits with denser particles (Figs. 7–10).

The vitellaria and remainder of the parasite body are translucent in live *Anoplodiscus*. Fixation in formaldehyde precipitates the proteins and makes the body opaque. The distribution of the pigment in live worms resembles that of the melanophores in the underlying fin, and the sluggish movement of the live parasite makes the worm difficult to see on the surface of the fin where it is attached. The vitellaria in gill-dwelling monogenea on snapper and bream tend to be more opaque than that in *Anoplodiscus*. Furthermore, individuals of the skin-dwelling copepod *Caligus epidemicus* are largely translucent whereas individuals of *Caligus epinepheli* that live in the buccal cavity of bream are more opaque.

DISCUSSION

As pointed out by Kearn (1979) the pigment in the body of skin-dwelling parasites may be produced locally or it may be ingested from the skin of the host and incorporated into the body of the parasite. Kearn (1979) found evidence that the gut pigment of *Dendromonocotyle kuhlii* was derived from pigment cells in the epidermis of the ray, *Amphotistius kuhlii*, and Logan & Odense (1974) found melanin granules in the digestive tract of *Capsala martinieri* resulting from the browsing of that parasite on the epidermis and stratum spongiosum in the skin of *Mola mola*. The available evidence, however, suggests that the pigment in individuals of *Anoplodiscus* spp. is produced within the parasite body. As the parasite grows the pigment appears throughout the body and not only in the vicinity of the gut. The latter would be expected if the parasite was ingesting the melanin from the fin.

It seemed reasonable initially to assume that as the parasite grew it could penetrate further into the host tissue during feeding. Since there are no melanophores in the nasal lamellae, parasites in this microhabitat could not obtain the dark pigment. However, to obtain melanin from the fin, the parasite would have to breach the epidermis and enter the dermis to reach the melanophores. There is no evidence that such is the case.

The presence or absence of pigmentation in the adults of *Anoplodiscus* spp. is far from uniform.

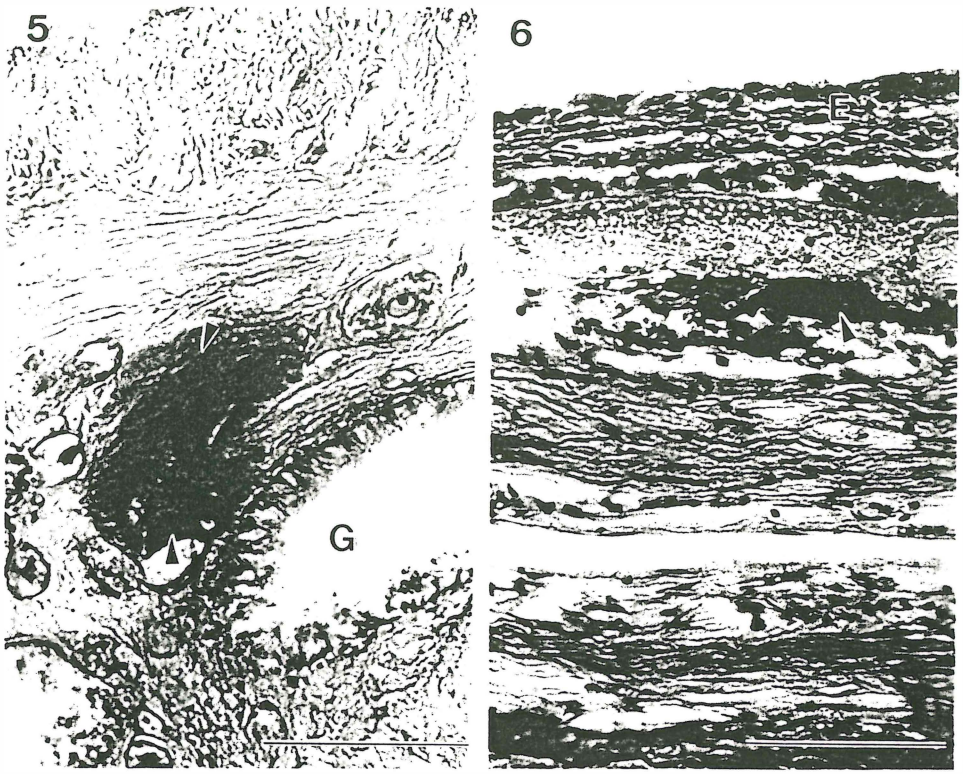

FIG. 5. Section through worm showing pigment deposit (arrowheads) next to gut caecum (G). Scale bar, 50 μm.

FIG. 6. Section through caudal fin of snapper. Melanophores (arrowhead) in dermis. Epidermis (E) lacks pigment. Scale bar, 50 μm.

Individuals of *Anoplodiscus australis* from the fins of bream, *Acanthopagrus australis*, were pigmented as were those reported by Byrnes (1986) from *Acanthopagrus australis*, *A. berda* and *A. latus*. However, *Anoplodiscus spari* from the fins of sea bream, *Anoplodiscus schlegeli*, cultured in Japan lacked pigmentation (Ogawa & Egusa, 1981; Roubal, personal observation). Adult *Anoplodiscus cirrus-spiralis* from the fins of wild and cultured snapper in Sydney had the deposits of pigment, but those in the nares did not. Also, *A. cirrusspiralis* from the fins of wild snapper in New South Wales, South Australia and New Zealand were pigmented (Roubal *et al.*, 1983); nares from these fish were not examined. In contrast, *A. cirrusspiralis* from the fins and nares of large wild snapper in Western Australia lacked pigment.

The function of the pigment in *Anoplodiscus* is unclear, as are the cues initiating its production in one microhabitat and not another. One explanation is that the pigment plus translucent body of fin-inhabiting *Anoplodiscus* make them difficult to see, and may act as camouflage against possible cleaning organisms; the same could be said for the translucent skin-dwelling *Caligus epidemicus*. It is also significant that worms and copepods on the gills or in the buccal cavity of these sparid hosts tend to be more opaque. Kearn (1978) found evidence that in an aquarium the monogenean *Pseudoleptobothrium aptychotremae* on the skin of the shovel-nosed ray, *Aptychotrema banski*, was preyed upon by small leatherjackets, *Paramonacanthus oblongus*. However, experiments need to be done to investigate the survival of *Anoplodiscus* in aquaria with possible cleaning organisms.

There are no data concerning the cues that initiate pigment production in *Anoplodiscus*. These cues may include rate of water flow, substrate topography, composition of mucus, etc. as well as genetic predetermination, i.e. those parasites destined to be unpigmented select certain microhabitats. Experimental transfer of adult and juvenile parasites from the nares to the fins and from the fins of large fish to small fish would indicate at what stage in ontogeny pigment production is determined and to what extent microhabitat influences this. Because adult *Anoplodiscus* does not reattach to the host once removed, we are unable to transfer the parasite from

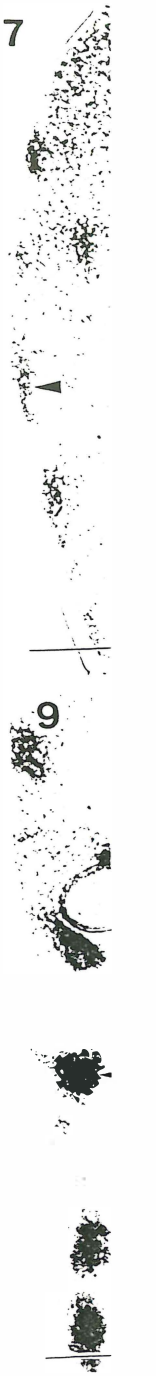

FIG. 7. Juvenile *An*

FIG. 8. Larger juv

FIG. 9. Large

FIG. 10. Subadult

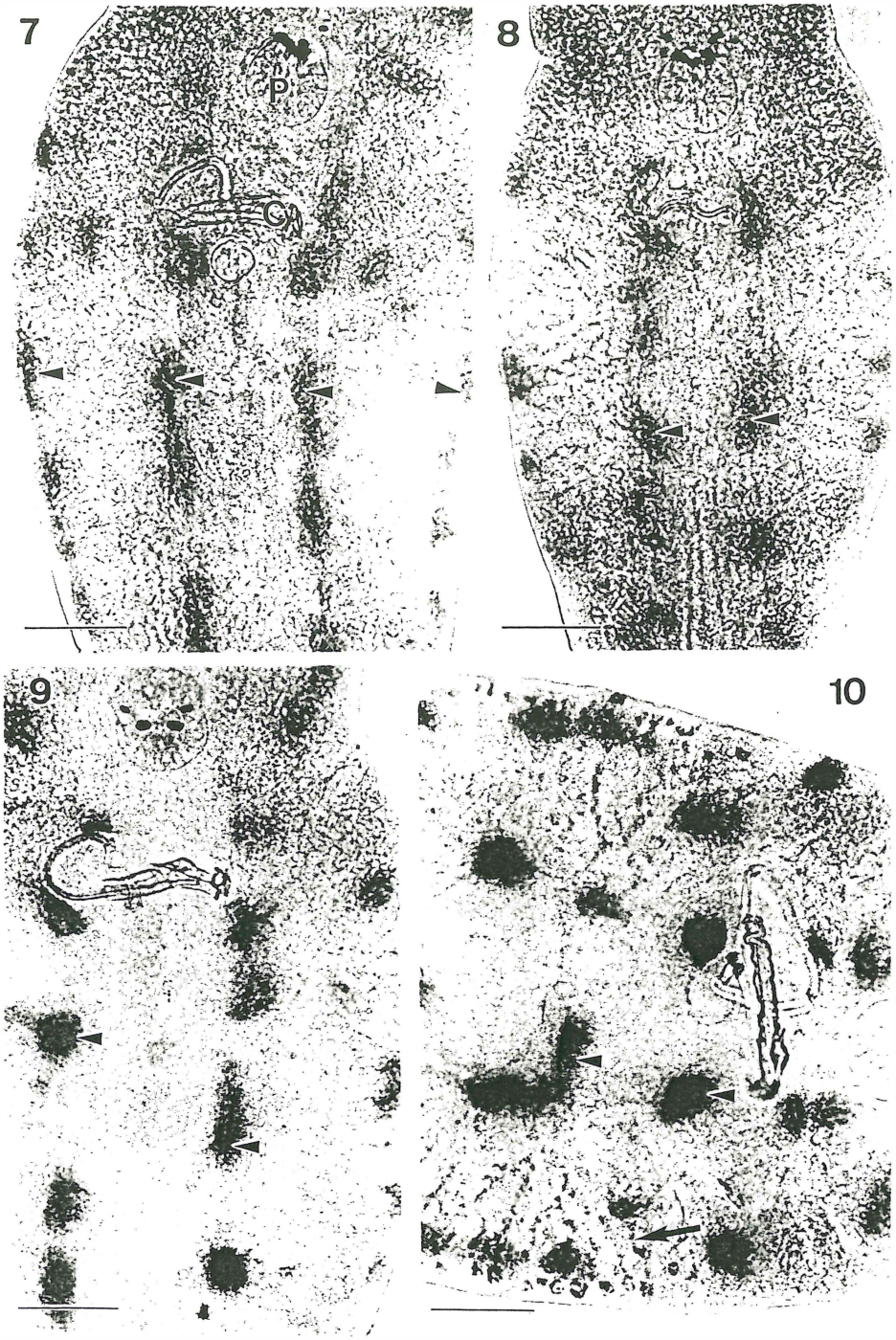

FIG. 7. Juvenile *Anoplodiscus cirrusspiralis* with pigment as streaks along length of body (arrowheads). C, penis; P, pharynx. Scale bar, 100 μm.

FIG. 8. Larger juvenile than Fig. 7 with pigment starting to coalesce into discrete deposits (arrowheads). Scale bar, 100 μm.

FIG. 9. Larger juvenile than Fig. 8 with denser pigment now in discrete deposits (arrowheads). Scale bar, 100 μm.

FIG. 10. Subadult worm with well-developed cirrus, distinct, dark pigment deposits (arrowheads) and vitellogenesis evident (arrow). Scale bar, 100 μm.

one microhabitat to another. However, current studies on the biology of *Anoplodiscus cirrusspiralis* will provide us with the opportunity to attempt the transfer of juvenile parasites from the nares to the fins and vice versa.

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Appendix 10

RUNNING HEAD: BLOOD REFERENCE VALUES FOR CAPTIVE SNAPPER

Haematological and biochemical reference values for captive Australian snapper, *Pagrus auratus*, Bloch & Schneider

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ABSTRACT

Reference values for common haematological and biochemical tests were established for 64 captive Australian snapper, *Pagrus auratus* (Bloch & Schneider). Fish were bled from caudal vertebral sinuses after benzocaine anaesthesia. Haematological results were extremely variable for total leucocytes and types of leucocytes. There was no correlation between leucocyte counts and leucocrit values (R^2 = 0.37). Thrombocytes were not determined by direct cell counting due to problem of aggregation. Polychromasia was common but did not correlate with reticulocyte counts (R^2 = 0.08). Biochemical results were extremely variable for creatine kinase and aspartate transaminase but this was considered a direct result of muscle damage during collection.

Key words: Snapper, *Pagrus auratus*, normal haematology and biochemistry.

I. INTRODUCTION

The necessity to establish reference haematological values in fish with a view to assessment of health status and the subsequent diagnosis of disease is widely indicated (Blaxhall, 1972; Alexander *et al.*, 1980; Campbell, 1988; Cameron, 1989; Campbell & Murra, 1990); and, indeed, have proved useful in the investigations of certain fish diseases (Barham *et al.*, 1980; Bollard *et al.*, 1989; Cameron & Foster, 1990). The usefulness of biochemical investigations in fish disease has been less well documented but Hille (1982) has mentioned the use of certain tests in the investigation of diseases in rainbow trout, *Oncorhynchus mykiss*, while Shieh (1978) has commented on the use

of blood enzymes for investigating furunculosis in brook trout, *Salvelinus fontinalis*.

The economic viability of farming Australian snapper, *Pagrus auratus* (Bloch & Schneider) in NSW is being assessed by growth trials in tanks and seacages. This study was done to establish reference ranges for haematological and biochemical analytes for healthy captive snapper that may be useful in the investigation of future disease problems.

II. MATERIALS AND METHODS

FISH

Approximately 300 juvenile snapper (estimated age 1+ yr) were caught by beach seine net in Port Hacking NSW (34°5'S) in November 1989, and 200 selected for growth trials and kept in a circular, flow-through fibreglass tank with a volume of 4000 l. The flow rate of aerated filtered seawater into the tank was 18 l min^{-1}. The bottom of the tank was siphoned daily to remove faeces and uneaten food. The fish were transferred to a seacage (2 x 2 x 2 m) in a seawater pool with a volume of 875 m^3 and a flow through rate of 600 m^3/day on 23 January 1990. The cage was rotated weekly for defouling by air drying. The water in the pool was mixed and aerated by air lift pumps.

The snapper were fed twice daily, 6 days of each week, with a dry, sinking pellet with 42% protein (Gibsons Stock Feeds, Hobart, Tasmania). The daily ration was 1% of the estimated live weight of fish. The snapper were kept at ambient seawater temperatures which ranged from 24°C in February to 14°C in July. Seawater quality was assessed in July from the concentrations of unionised ammonia (NH_3) and nitrite ions (NO_2^-) in one surface and one bottom sample taken from 6 sites in the pool.

ANAESTHESIA AND SAMPLING

Blood was taken on four occasions between August and October when water temperature varied from 14.2°C to 17.6°C. About 1 hr before blood was collected, up to 20 snapper from the sea cage were selected at random with a dip net and placed in a circular fibre glass tank containing 2000 l of aerated seawater.

Fifteen minutes before blood collection, the fish were sedated with 15-20 mg/l benzocaine (ethyl p-aminobenzoate). Groups of up to 3 snapper were taken and anaesthetised (to Stage III) in 30 l of aerated seawater with 50-75 mg/l of benzocaine. Fish were bled immediately from the caudal vertebral sinus using a 21 gauge needle attached to a 5 ml plastic syringe and then measured for length and weight. Blood collection was attempted from 70 fish and successful from 64. Two to three ml of blood was collected in plain and/or lithium heparin (for biochemistry) and dipotassium EDTA-lined (for haematology) tubes. Not all tests were performed on fish producing less than the required volume of blood. Collection of blood from each batch of fish took less than 1 hr.

HAEMATOGICAL TECHNIQUES

Tests were performed on blood within 3 hr of collection. Haematocrit (Hct) and total plasma protein were determined by the microhaematocrit method and refractometry respectively. Leucocrit values (Lct) were determined by the microhaematocrit method (Mcleay and Gordon, 1977). Erythrocyte numbers (RBCC) were derived by determining total nucleated cells using the Coulter DN (Coulter Electronics Ltd, Luton, England) and subtracting total leucocyte counts (including thrombocytes). Total leucocyte counts (LCC) were performed using the method of Natt and Herrick (1952). Haemoglobin (Hb)

was determined on both pre- and post-centrifuged blood (3000g for 10 min), utilising the cyanomethaemoglobin method and the Coulter Haemoglobinometer (Coulter, England). Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration (MCHC) were determined utilising only post-centrifugation haemoglobin values. Both pre- and post-centrifuged haemoglobin values are presented as other articles on fish haematology differ on what values are presented. Reticulocyte counts were performed on films stained with brilliant cresyl blue. Blood films for differential leucocyte counts were made directly from withdrawn blood, air-dried, fixed in methanol, and stained with a commercial Romanowsky-type stain (Diff Quik®, Lab-Aids, New South Wales). All values for leucocyte types are presented as absolute figures (percentage value multiplied by total leukocyte count).

BIOCHEMICAL TECHNIQUES

Serum was separated from the cellular components of blood by centrifugation. Biochemical analysis was done using a computerised spectrophotometer (Cobas Mira, Hoffman - La Roche, Basle, Switzerland) except for sodium, potassium (K) and chloride which were analysed by ion specific electrodes (Nova 4, NovaBiomedical, Massachusetts).

Uric acid, aspartate aminotransferase (AST), alanine aminotransferase, alkaline phosphatase, creatine kinase (CK), glucose, cholesterol, calcium, inorganic phosphate and magnesium were analysed utilising Trace reagent kits (Trace Scientific, New South Wales) whilst triglycerides were analysed utilising a Roche reagent kit (Hoffman - La Roche, Basle, Switzerland). All enzyme assays were performed at 37°C.

STATISTICAL METHODS

Data was analysed with SAS (SAS Institute Inc., North Carolina). To assess normality, the UNIVARIATE procedure was used to produce a normal probability plot and calculate either the Shapiro-Wilk or Kolmogorov D statistic depending on the sample size. Reference values (95% of the distribution) are given as the mean ± 2SD to account for effect of sample size. Two-sided nonparametric tolerance limits are given for data that deviated significantly ($P < 0.2$) from normality.

III. RESULTS

Prior to blood sampling, both NH_3 and NO_2^- were less than 20 µg/l in the seawater of the holding pool. The concentration of dissolved oxygen (DO) and salinity were checked at intervals of 2 weeks. DO was always greater than 5 mg/l and salinity was between 33 and 35 ppt. For the fish sampled, fork length varied from 18.4 to 23.8 cm (mean ± SD = 20.8 ± 1.25, n = 64) and weight varied from 155 to 324 g (224 ± 39, n = 64).

HAEMATOLOGY

Summary results for haematology are presented in Table 1. There was no correlation between total leucocyte counts and leucocrit values ($R^2 = 0.37$). Thrombocytes were not determined by direct cell counting due to the problem of aggregation, and as it was impossible to distinguish all of them from small lymphocytes. Even on differential counts in blood films, it was difficult to distinguish all small mononuclear cells. Consequently, where some doubt existed as to the origin of these cells they were identified as thrombocytes as they were considered to be altered

thrombocytes. These cells occurred in addition to obvious large and small lymphocytes, and oval thrombocytes.

Monocytes were similar in appearance to those in other reported fish species. Granulocytes were primarily heterophils. Eosinophils and basophils were detected in only two films. Polychromasia occurred in 44 of the 52 blood films examined, and varied from 1-14 in 100 erythrocytes. There was no correlation with reticulocyte counts ($R^2 = 0.08$). Cells were designated as reticulocytes only if they contained dense granules or more than five fine granules (one to five fine granules were commonly seen in erythrocytes). Most brilliant cresyl blue positive granules were present perinuclearly.

BIOCHEMISTRY

Summary results for biochemistry are presented in Table 2. Levels of CK reflected the extent of muscle damage associated with blood collection. Variable levels of AST were shown to correlate with levels of CK ($R^2 = 0.83$), but levels of K did not show significant correlation with CK or AST ($R^2 = 0.0004$ and 0.0001 respectively).

IV. DISCUSSION

To limit variability, blood collection was restricted to immature fish of a set size during late winter and early spring. Seasonal variations, especially water temperature and photoperiod, are known to influence values for Hct and Hb in Baltic salmon, *Salmo salar*, due to fluctuations in erythropoeitic activity (Hardig and Hoglund, 1984). These authors also investigated the effect of age on blood variables and came to the conclusion that in immature fish very little variation occurred with ageing.

The selection of anaesthetic, the site of blood sampling, and the anticoagulant can have marked effects on the values obtained for various haematological and biochemical values in fish (Blaxhall, 1972; Cameron, 1989; Klontz, 1972; Wedemeyer, 1970). For example, Wedemeyer (1970) reported that unbuffered tricaine caused marked decreases in blood glucose, while Hille (1982) mentioned that cardiac puncture has less effect on the levels of certain blood enzymes compared to blood taken from caudal sinuses. The type of anticoagulant to use is contentious. For haematology, some workers prefer to use EDTA (Blaxhall, 1972) but others prefer heparin as they suggest EDTA can alter several haematological tests (Barham, Smit & Schoonbee, 1979). More recent workers suggest that both anticoagulants can be used (Campbell & Murru, 1990).

It is accepted that blood sampled from the caudal vertebral sinuses may show elevated CK compared to blood obtained from cardiac puncture (Gaudet, Racicot & Leray, 1975). In our study, marked elevations of CK appeared to relate to difficulties in obtaining the blood sample and suggests that the increases occurred following damage to skeletal muscle by repeated repositioning of the needle, and contamination from muscle fluid. This negates the use of this method of collection if a suspected myopathy is being investigated as CK is the main diagnostic enzyme for muscle damage in fish (Cameron, 1989). AST in domestic animal species is also known to rise in muscle damage (Duncan & Prasse, 1986) and in our study CK was correlated with AST. Salte, Asgard & Liestol (1988) found CK elevations were not followed by AST elevations in 'Hitra disease' in farmed Atlantic salmon.

The problem of determining whether small, round mononuclear cells are small lymphocytes or round thrombocytes is not unusual in fish. Roubal (1986) noted that *Acanthopagrus australis* (Günther), thrombocytes resembled small lymphocytes and,

furthermore, large lymphocytes were often difficult to separate from monocytes. However, the problem may be species dependent in fish, as Breazile *et al.* (1982) found no difficulty in distinguishing thrombocytes from small lymphocytes in channel catfish, *Ictalurus punctatus*. However, Ellsaesser *et al.* (1985) suggested that small lymphocytes and thrombocytes became difficult to distinguish in channel catfish when there was a delay of more than two hours in making blood films: then thrombocytes became round, lost their cytoplamic extensions and resembled small lymphocytes. In our study, blood films were made at the time of collection and yet thrombocytes appeared to occur in both oval and round forms. The absolute values derived for thrombocytes and small lymphocytes from our study could be innacurate as special methods, such as cytofluorography (Ellsaesser *et al.*, 1985), were not employed to distinguish the two populations.

Haemoglobin values in fish have been determined by the oxyhaemoglobin method (Summerfelt, 1967), the Sahli acid haematin method (Hesser, 1960) and the cyanomethaemoglobin method (Murachi, 1959). In comparing the three methods with direct measurement of the iron content of haemoglobin, Larsen and Sniezko (1961) found that correction factors were necessary for all three methods in fish but that the cyanomethaemoglobin method was most appropriate. We considered the analysis of the post-centrifugation samples to reflect the more accurate values for haemoglobin as suspended erythrocyte nuclei interfered with optical density. Centrifugation of samples for haemoglobin determination appears not to be universally utilized but Sniezko (1960) used it for the acid haematin method while Alexander *et al.* (1980) employed centrifugation for the cyanomethaemoglobin method. Campbell & Murru (1990) recommended centrifugation of blood samples analysed by the cyanomethaemoglobin

method.

The LCC cannot be determined using electronic counters because of the presence of free erythrocyte nuclei after lysis of erythrocytes (Campbell, 1988). Manual counting of leucocytes is commonly employed but errors for this method have been well documented (Dacie & Lewis, 1975). Consequently, other methods have been employed to estimate leucocytes. Estimation of leucocytes from a blood film, utilising total cell determinations, has been suggested by Cameron (1989), while McLeay & Gordon (1977) applied the Lct method to fish. In our study, there was no correlation between the LCC and the Lct but this has been acknowledged by other workers (Houston & DeWilde, 1968; Wedemeyer, Gould & Yasutake, 1983). Both methods have inherent difficulties but for ease of measurement the Lct is best.

Acknowledgments

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TABLE I. Haematological reference values for captive snapper *(Pagrus auratus)*

Observation	Reference Values	Mean	Mini-mum	Maxi-mum	Disparity[a]	Tolerance[b]
Haematocrit (%)	26-54	40	18	58	3/56	--
Haemoglobin[c] g/l	98-200	147	72	196	2/56	--
Haemoglobin[d] g/l	48-115	82	45	126	3/55	--
Total cell count $x10^{12}/l$	2.3-4.8	3.6	1.8	5.0	3/55	--
White blood cell count $x10^{9}/l$	4.5-55	19	4.5	55	0/48	0.90
Reticulocyte count (%)	0-22	4	0	22	0/40	0.90
Leucocrit (% x 10^{2})	0.2-2.4	0.9	0.2	2.4	0/35	0.85
Erythrocytes $x10^{12}/l$	2.5-4.5	3.5	2.5	4.5	0/38	--
Mean corpuscular volume $x10^{15}/l$	85-141	113	84	145	1/38	--
Mean corpuscular haemoglobin (pg)	19-27	22	19	28	0/37	--
Mean corpuscular haemoglobin concentration (g/l)	160-290	208	160	290	0/37	0.90
Granulocytes $x10^{9}/l$	0-11.3	2.2	0	11.3	0/48	0.90
Lymphocytes $x10^{9}/l$	0.1-7.7	2.1	0.1	7.7	0/48	0.90
Monocytes $x10^{9}/l$	0-2.8	0.5	0	2.8	0/48	0.90
Thrombocytes $x10^{9}/l$	2.4-42.7	13.4	2.4	42.7	0/48	0.90

TABLE I Footnotes:

[a] The number of fish falling outside the reference values

[b] The fraction of the population within the range of the sample at the 95% confidence level

[c] Haemoglobin concentration before centrifugation

[d] Haemoglobin concentration after centrifugation

TABLE II. Biochemical reference values for captive snapper *(Pagrus auratus)*

Constituent	Reference Values	Mean	Mini-mum	Maxi-mum	Disparity[a]	Tolerance[b]
Total protein g/l	36-69	53	32	75	2/56	--
Alkaline phosphatase U/l	0-98	44	0	100	1/22	--
Alanine transaminase U/l	8-427	97	8	427	0/43	0.85
Aspartate transaminase U/l	9-199	33	9	199	0/43	0.85
Creatine kinase U/l	104-8087	1291	104	8087	0/43	0.85
Cholesterol mmol/l	3.3-7.0	5.1	2.6	6.8	1/41	--
Triglycerides mmol/l	0.9-5.2	2.0	0.9	5.2	0/41	0.85
Creatinine µmol/l	19-119	56	19	119	0/41	0.85
Glucose mmol/l	3.5-24.6	9.3	3.5	24.6	0/43	0.85
Uric acid nmol/l	6-100	30	6	100	0/38	0.85
Calcium mmol/l	2.5-3.7	3.1	2.4	3.6	1/41	--
Magnesium mmol/l	1.1-2.3	1.7	1.0	2.3	1/41	--
Inorganic phosphate mmol/l	2.3-4.2	3.2	2.3	4.2	0/39	--
Sodium mmol/l	187-221	204	185	219	1/24	--
Potassium mmol/l	0-12.2	6.0	1.5	14.3	1/23	--
Chloride mmol/l	163-194	178	166	194	0/24	--

[a] The number of fish falling outside the reference values

[b] The fraction of the population within the range of the sample at the 95% confidence level