Aquaculture and Diet Development Subprogram: Post harvest Enhancement of Sea Urchin Roe for the Japanese Market

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SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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i) OBJECTIVES

1. Determining the feasibility of postharvest enhancement of sea urchin, *Heliocidaris erythrogramma*, roe through the use of commercially available feeds.

2. Determine the environmental conditions (time and temperature) under which roe enhancement can be manipulated.

3. Evaluate photoperiod manipulation of the gametogenic cycle as a method of increasing the availability of the highest quality roe.

4. Evaluate the best commercial growout options by assessing the technical and relative economic feasibility of both land based (eg. abalone) and in-water sub-tidal cage (eg. polyculture with Pacific Oysters).

5. Evaluate existing commercially available sea urchin diets (USA) and Australian abalone diets with a view to refinement for optimum post-harvest enhancement of *Heliocidaris erythrogramma* in Australia

6. Determine future research needs for the industry.

ii) OUTCOMES ACHIEVED

A diet suitable for sea urchin (*Heliocidaris erythrogramma*) roe enhancement has been identified and tested. The Australian Experimental Stockfeed Extrusion Centre is able to produce a water-stable semi-moist pellet that will produce commercial-level roe yields in a sea-cage environment. The diet is a locally-made version of a sea urchin feed produced by Wenger Manufacturing Inc.

Sea urchin husbandry techniques for aquaria and sea cages have been developed to the point where many of the major issues have been addressed or identified, *viz* the requirement for good water quality, the degree of aeration required, physical separation of waste products from sea urchin feeding areas, adequate feeding rates and cage design requirements.

A good working relationship with industry has been developed through the course of the project. Industry has been enthusiastically involved in many phases of the project, including the workshop, sea urchin collection, method standardisation and the field trial. The industry member with whom most of the collaboration has taken place is satisfied with progress towards a sea-cage roe enhancement diet.

An international network of sea urchin research and industry contacts has been developed, especially with the key people in nutrition research.

iii) Executive Summary

Five trials (Four laboratory-based and one sea-cage) trials were run in South Australia to determine the effect of environmental, physical and nutritional factors on roe enhancement in the purple sea urchin *Heliocidaris erythrogramma*. A sea urchin diet, made at the Australian Experimental Stockfeed Extrusion Centre (AESEC), was also sent to Wellington, New Zealand for comparison with locally produced and Norwegian roe enhancement diets. The trial, run by NIWA, assessed the effect of those diets on roe enhancement in the local sea urchin: kina, *Evechinus chloroticus.*

The most appropriate size for sea urchin roe enhancement was found to be about 72 mm test diameter (Mean size, 71.5 $\pm$ 0.40mm, 151g wet weight). Larger sea urchins (Mean size: 80.7$\pm$0.44mm) produced more and coarser roe than those of intermediate size and smaller animals (Mean size: 55.5 $\pm$ 0.47mm) produced a lot of fine-textured roe, but most of this was very light in colour, and, as a consequence, little could be classified as A grade.

It appears that *H. erythrogramma* is able to produce commercial-level roe yields over reasonable time frames in aquaria and in sea-cages using either available artificial diets or modifications of these in combination with naturally occurring macroalgae.

The results of trials using either an imported Wenger diet, or locally manufactured equivalent, suggest that between 70 and 90 days are necessary to produce an adequate roe yield; which, for lack of a better, commercially relevant, estimate for *H. erythrogramma*, is considered to be at least 10% of wet body weight. In laboratory trials using the Wenger diet, the final mean % roe yield, for medium-sized sea urchins, was 12.28% (initial: 4.01%) after 84 days with 74% of sea urchins producing yields of over 10%. In the field trial, 80 days were sufficient to achieve a mean yield of 11.2% (initial yield 2.63%) with 63% of sea urchins producing yields of over 10%. Both trials were run during spring/early summer with the result that a moderate percentage of sea urchins showed mature roe, prone to leakage, which in a commercial setting would reduce the value of the product. Thus it is recommended that sea-cage roe enhancement should be conducted during the cooler months while gonads are still premature; i.e. April to September, which may lengthen the time taken to achieve commercially useful roe yields but should optimise the roe quality.

Temperature and photoperiod had no effect on % roe yield or roe quality within the time period evaluated.

Higher dietary protein levels, ranging from 11% to 23% crude protein, did increase % roe yield using gelatin-bound diets, but did not with the Wenger-style diets, which varied in protein level from 21 to 27%, with a concomitant increase in metabolisable energy. It is difficult to directly compare the artificial diets given the differences in form and in the timing of the trials. However, it is clear that once semi-moist extruded pellets could be produced through the Wenger X-85 extruder at AESEC, % roe yield and roe quality improved markedly. Although diet form was not tested *per se*, it does appear that the gelatin-bound pellets used in Trials 1 and 2 did not provide adequate nutrition for roe enhancement.

At present the industry focus is on sea-cage culture rather than capital-intensive land-based systems. Given that the fishery is still very much in the development stage this is probably the option carrying the lowest risk. The sea-cage trial carried out in this study allowed a preliminary assessment of the potential of such a system and the early steps towards improvement. For example, rectangular cages were used with some success, however they proved hard to service (feed and clean) and some urchins were difficult to remove at harvest, having wedged themselves into the corners. It is suggested that midwater cylindrical cages be trialed in future and that feeding tubes be fitted to allow access from the surface. A modular, floating pontoon system is presented, to which cages could be moored in multiple rows.

Further research is necessary to determine the effect of initial % roe yield and gut index, harvest season and location, and diet composition on final % roe yield. Trials should also be run to refine the diet in terms of components, to move towards a nutritional optimum and to reduce cost. Additives such as cholesterol may not be necessary and, if the diet is to be used in sea cage culture, added carotenoids may also be superfluous.

A workshop which included local and interstate sea urchin industry members and researchers, fishery managers and abalone farmers, was held early in the project to provide a forum for discussions on research priorities and other issues affecting the fishery.

A study tour was undertaken to the UK, the USA and Canada to meet and talk with sea urchin researchers and sea urchin wild fishery and culture industry representatives. The trip was very successful with a great deal of useful information gained on nutritional, husbandry and wild fisheries issues (Appendix 4). A trip was also undertaken to Japan, funded by a private sea urchin processing company, to visit markets (Tsukiji Market, Tokyo), processing and culture facilities, again a very useful trip; the report is attached. (Appendix 5).

Acknowledgements

Many thanks to Terry Scott, of Coffin Bay, SA, for his enthusiasm and assistance during the project. Thanks also to Mr. Lance Schlipalius of Betatene Pty. Ltd., Cheltenham, Victoria, for the generous donation of the Algro required for diet making. Many thanks also to Dr John Lawrence of the University of South Florida, Tampa, Florida, USA, for his advice and encouragement and, with the assistance of Galen Rokey, of Wenger Manufacturing Inc., Sabetha, Kansas, USA, for donation of the sea urchin diet. Finally, thanks to all those volunteers from the Flinders University Workready program for invaluable assistance with the sea urchin trials.

Chapter 1 General Introduction

1.1 Background

In 1996 Japan imported over $US360 million worth of sea urchin roe from a world fishery in marked decline. These fisheries, principally in the US and Chile developed largely unregulated after the collapse of the Japanese urchin fishery and are now themselves in decline with little prospect for recovery (Keesing and Hall 1998).

Sea urchin stocks in Australia are extensive, but largely unexploited due to low rates of recovery (amount of roe per urchin), variation in roe quality (colour and texture) and variation in rate of recovery (in any batch of urchins there will be a wide range of recovery rates). All these factors together with the propensity of sea urchin fisheries to collapse through over harvesting, affect marketability and economic feasibility and therefore the viability of a traditional fishery. In 1996 Australia exported only 700 kg of urchin roe to Japan with a total value of $60,000 or an average of $85 per kg (includes both A and B grade roe). Over the same period roe exported from the USA received on average only $67 per kg. Peak prices paid for Tasmanian A grade *Heliocidaris erythrogramma* sea urchin roe in Japan in 1995 were $250 per kg (Sanderson et. al. 1996). South Australia, Victoria and Tasmania have divers licensed to collect urchins. Viability has been marginal due to low recovery rates, with profitability limited to a narrow window when high quality roe is available for collection. To develop this fishery the following is required:

1. Increase the recovery rate for A grade roe
2. Increase the period for harvesting sea urchins.

The resource is conservatively estimated at in excess of 5000 tonnes nationally. This means that for every tonne of sea uchins harvested, up to $10,000 in exports of roe could be achieved (based on 10% recovery @ $100/kg). Ten percent roe recovery/animal is considered to be an economically sustainable level for a sea urchin fishery.

Variation in roe quality and quantity in nature is doubtless due to temporal and spatial food availability, competition for food and variation in the reproductive cycle among individuals. The aim of this project was to enhance the rate of recovery and quality of urchin roe and reduce variability by holding and feeding sea urchins post-harvest. The advantage of this approach is that it will control quality and consistency of product and ensure sustainability by reducing the necessity to harvest large quantities of urchins simply to waste the majority that do not meet A grade standard.

The South Australian Government has recently awarded access to the State's sea urchin resources to five vertically integrated (diver to processor to exporter) operations in South Australia. As part of this, value adding through roe enhancement has been identified as a key opportunity for the developing industry.

1.2 Need

Development of sea urchin fisheries in Australia is currently limited by economic viability due to low recovery rates. This fishery has a large opportunity to expand given:

1. There is a large sea urchin resource
2. The high price urchin roe fetches
3. Low cost of fishing (relatively shallow water).

The opportunity to develop this fishery into a highly profitable one is dependent on the development of innovative solutions. This project examined the potential for enhancing recovery rates using supplementary feeding for short periods. If this can be done cost effectively it opens the opportunity to dramatically increase the profitability and size of this fishery. Without this technology the sea urchin fishery will not fully develop. With this technology the fishery has the potential to be worth millions of dollars per annum. This project has been initiated by support through both existing permit holders and aquaculturists.

1.3 Objectives

1. Determining the feasibility of postharvest enhancement of sea urchin, *Heliocidaris erythrogramma*, roe through the use of commercially available feeds.

2. Determine the environmental conditions (time and temperature) under which roe enhancement can be manipulated.

3. Evaluate photoperiod manipulation of the gametogenic cycle as a method of

increasing the availability of the highest quality roe.

4. Evaluate the best commercial growout options by assessing the technical and relative economic feasibility of both land based (eg. abalone) and in-water sub-tidal cage (eg. polyculture with Pacific Oysters).

5. Evaluate existing commercially available sea urchin diets (USA) and Australian abalone diets with a view to refinement for optimum post-harvest enhancement of *Heliocidaris erythrogramma* in Australia

6. Determine future research needs for the industry.

1.4 Variations to Project

The overall aim of this project was to evaluate the nutritional and environmental conditions necessary to assist the South Australian sea urchin industry in adding value to the resource, after harvest, in sea or land-based facilities.

During the course of the project it became clear that, in order for the results to be taken up by industry members, the objectives, as set out, would have to be modified to reflect the changing financial and infra-structural resources of industry. The resource base changed significantly over the period of the project, as did the numbers of active licence holders.

For example, at the beginning of the project a large Port Lincoln abalone farm had expressed an interest in running trials in tanks at its facility. This project was to be funded, in part, through AusIndustry, but as the grant application to that body was unsuccessful, the work did not take place. The industry focus is, at present, on sea-cage culture as the preferred method for the initial development of post harvest roe-enhancement techniques. A sea-cage trial run in Coffin Bay was successful, but comparison could not be made with land-based culture as intended (Objective 4).

The third objective necessitated differentiation between reproductive phases through which roe passes, then determination of which of these would provide the best roe yield upon feeding under conditions of varying photoperiod. It became apparent upon dissection of large numbers of sea urchins that any one sample would contain varying numbers of all three stages at any given time and that the only way to tell them apart was by dissection. An attempt was made to get around this problem using Nuclear

Magnetic Resonance Imaging (NMRI) at the Adelaide Hospital, that is, to assess the roe condition of the sea urchins without having to kill them, but this proved unsuccessful. A trial was run with varying photoperiod although manipulation of the gametogenic cycle was not attempted as it was considered impractical.

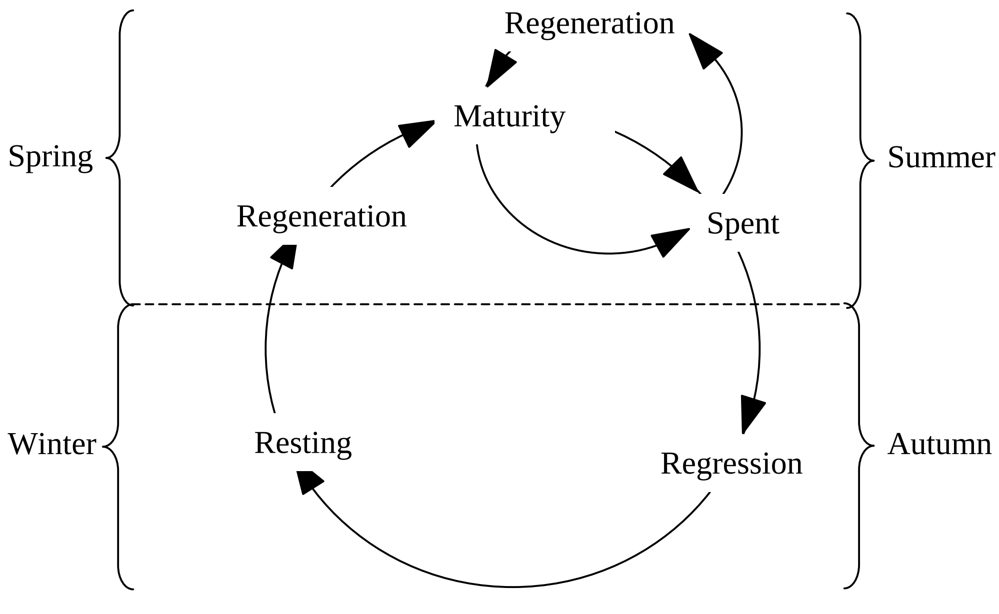
Finally, the fifth objective stated that trials with Australian abalone diets would be carried out alongside diets from the USA. As the sea urchins did not show any interest in the abalone diets the trials were carried out with diets from Canada and the USA instead.

FRDC was kept aware of these variations to the project plan through milestone reports.

1.5 Reproduction in *H. erythrogramma*

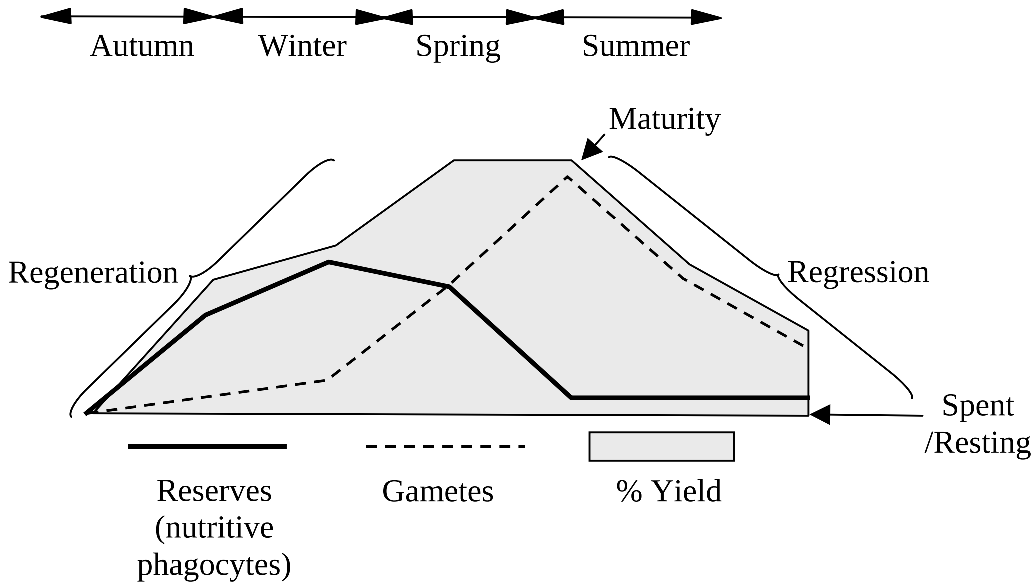
Williams and Anderson (1975), working on *H. erythrogramma* inhabiting rock platforms along the open sea coast near Sydney, NSW, found a five phase cycle for females, with one fewer for males (Fig 1.4.1). Roe regression and resting occurred from April to August; regeneration began in September, and between December and March, individual sea urchins went through mature, spent and regenerating phases two or three times.

Fig 1.4.1 Reproductive phases of the ovary of *H. erythrogramma*.
The male cycle is similar, lacking only Regression. From Williams and Anderson, 1975.



In Tasmania the timing of the phases may be slightly different. Dix (1977) reported that spent *H. erythrogramma* were predominant in late summer and early autumn (February-March), regeneration occurred from March to August with active storage cells (nutritive phagocytes, Fig 1.4.2) present and reserves were transferred from storage cells to gametes (active gametogenesis) during late winter-early spring with corresponding reduction in storage cell capacity. Mature gonads were most common during early summer (December-January). Dix was unable to confirm whether individual sea urchins spawned more than once as reported by Williams and Anderson (1975) although he did find that summer spawning was asynchronous, as reported in NSW, with individuals at all gametogenic stages present over this period.

Fig 1.4.2 Schematic view of the seasonal development of the gonad. Stage timing based on *Paracentrotus lividus*.
Modified from Blin, 1997.



At the time this study was carried out there was no published information on the periodicity of the reproductive cycle of *H. erythrogramma* in South Australian waters, other than a report that spawning occurs from November to April between $34^{o}40'$ and $35^{o}10'$ S (J Strzelecki, pers. comm), suggesting that the phasing is closer to that found in Tasmania than in NSW. Sea urchins were harvested from the wild, for Trial 1, in early April, theoretically in the NSW regression phase, however most showed gonadal growth. It could therefore be assumed that these individuals were actually either already in the regeneration phase, suggesting similarity with Tasmanian populations, or relatively high quality food and warmer temperatures had stimulated that regeneration. In 2001, Jeffrey did carry out a limited reproductive survey of a population of *H. erythrogramma* at Point Riley (137^{o} 33'E; 33^{o} 53'S) in South Australia between August 2000 and February 2001. He found that %GSI (Gonadosomatic index = Yield) peaked in August at 4.93% then decreased until October and remained low until the end of the study. Data are lacking for winter and autumn samples, limiting the conclusions that may be drawn from this work.

Hagen (1998) also reported out-of-season gonad production in a laboratory experiment using the green sea urchin *Strongylocentrotus droebachiensis*. He was able to produce commercial level % roe yield by providing abundant food in a protected environment, several months before the local harvesting season for wild populations.

Chapter 2 General Methods

2.1 Roe yield (%GSI), quality and gut index

Roe colours, textures and grades were standardised with reference to local industry members, to previously published work (Dix 1977, Sanderson, 1996) and using a Sea Urchin Roe Colour and Volume card issued by the Co-operative Extension Services Marine Program at the University of Maine, U.S.A and the Maine Department of Marine Resources Fisheries Technology Service. In addition, all observations were either carried out or checked by the author.

2.1.1 Yield (%GSI)

Excess water was drained from the each urchin for 2 minutes after removal from the holding tank. The animals were then weighed (to 0.01g) and test diameter (TD) measured (0.1mm) with vernier callipers then the test (i.e. shell) opened using purpose-designed urchin crackers (Fig 2.1.1). Haemocoelic fluid was then drained for 30secs on a mesh tray and the whole animal weighed again. The roe was then excised, excess moisture removed with a paper towel and weighed. Roe yield was calculated as gonad wet weight (g) / whole body weight (g) x 100 and varied from 1 to 28% depending on trial. The target %GSI in all trials was 10%, a figure usually equated with the commercially acceptable yield.

Fig 2.1.1 Sea urchin cracker.
Made in Japan.

2.1.2 Colour

Roe was rated for colour based on published work (Dix 1977, Sanderson 1996). The most common colours were orange, gold, brown and black with orange and gold showing a gradation from very light to dark. There was also a small amount of yellow roe, showing a similar gradation, and a similar amount of cream roe. Orange, gold and yellow roe were categorised as dark, medium and light or very light. Brown was dark only (Fig 2.1.2, Fig 2.1.3 and 2.1.4).

Fig 2.1.2. The most common sea urchin roe colours found during the study.

Fig 2.1.3 Fine dark gold and coarse light gold roe (left to right).

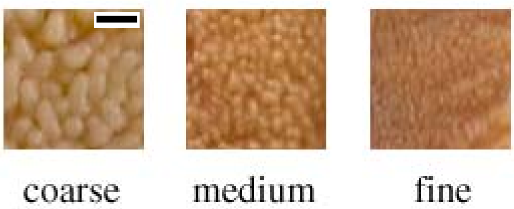
Fig 2.1.4 Orange coarse, and dark orange medium and fine roe (left to right)

Fig 2.1.5 Orange coarse, and dark orange fine roe, oral surface down (left to right)

2.1.3 Texture

Texture, also based on Sanderson (1996) ranged from fine (granulations to 0.5mm), through medium (0.5 – 1mm) to coarse (1-2mm) and very coarse (>2mm). Most roe fell within the first three categories (Fig 2.1.6).

Fig 2.1.6. Sea urchin roe textures found during the study.
Scale bar = 2mm



2.1.4 Grade

Colour and texture rating data were combined to form a roe index as both elements are considered together when assessing roe quality (**Table 2.1.1**). Grades were assigned after consultation with an industry representative. The grade assigned by the market may differ depending on the market and on the availability and quality of other product. For example, light brown to orange roe with fine texture may achieve an A grade and cream-fine may be assigned a B grade by a market relative to other product. C grade roe is generally not acceptable to the market.

Table 2.1.1 Colour and texture ratings for roe quality assessment based on Dix (1977).
Colour categories have been assigned numbers for ease of reference in the text.

Colour	Texture	Colour/ Texture Category	Grade
1. Black, brown, dark gold, orange or yellow	coarse	1.1	D
	medium	1.2	D
	fine	1.3	D
2. Gold, orange, yellow	coarse	2.1	C
	medium	2.2	B
	fine	2.3	A
3. Light gold, orange or yellow	coarse	3.1	C
	medium	3.2	B
	fine	3.3	B
4. Very light gold, yellow, orange or cream	coarse	4.1	D
	medium	4.2	C
	fine	4.3	C

2.1.4 Gut Index

The gut was also excised, carefully washed to remove faecal pellets then weighed after removal of excess moisture with a paper towel. The gut was used for calculation of a condition index (*sensu* Barker et al 1998) as gut wet weight (g)/whole sea urchin wet weight (g) x 100.

2.1.5 Data Analysis

For all trials the relationships between % roe yield (=% GSI, wet weight of roe/total body wet weight) and treatment were investigated, after testing for normality and arcsine transformation if necessary, using ANOVA on SPSS. Because of the abundance of zeros in the roe quality data, the relationships between texture and colour and treatment were analysed through analysis of deviance, calculated using Loglinear models on Genstat in consultation with Biometrics SA. Contingency tables were also used to compare treatment-specific counts of sea urchin roe. The relationships between gut indices and treatment were also investigated, after testing for normality and arcsine data transformation if necessary, using ANOVA on SPSS. Relationships between diet and leaching rate over time were analysed using ANCOVA on SPSS after transformation if necessary. If data could not be normalised through transformation, non-parametric methods (i.e. Mann Whitney U, Kruskal-Wallis) were used.

2.2. The effect of protein level and oil type on roe yield and quality

(Relevant Objectives: 1,6)

2.2.1 Introduction

Vadas (et al, 2000) reported that % roe yield in the green sea urchin, S*trongylocentrotus droebachiensis,* was positively correlated with the protein level of natural algal diets. Other work done on the same species using artificial diets, also suggests that the sea urchins use protein for gonadal growth (McBride et al, 1998). Artificial diets have obvious advantages over natural algal diets, including ease of handling, longer storage times and improved assimilation efficiencies (Robinson and Colbourne, 1997; Barker et al, 1998; McBride et al, 1998).

In keeping with Objective 1, it was therefore decided to run a trial to determine the effect of protein level on the % roe yield and roe quality in *Heliocidaris erythrogramma* using an artificial diet. The effect of oil source was also tested by inclusion of two oil types (linseed and fish) in the trial.

The base diet selected for this section was that formulated by Dr Shawn Robinson and Dr John Castell of the Canadian Department of Fisheries and Oceans Research Station in St Andrews, New Brunswick (refer Appendix 6: Study Tour Report). The initial contact was made with Dr Robinson, who subsequently extended an invitation to visit his laboratory. Both researchers have had considerable experience in the formulation of sea urchin diets and as the manufacture of the diet was relatively straightforward using existing equipment.

2.2.2 Methods

460 sea urchins were collected by hand using SCUBA in 2-3m of water off Pt Riley (137^{o} 33'E; 33^{o} 53'S) near Wallaroo in South Australia. They were transported back to the South Australian Aquatic Sciences Centre (SAASC) in 95L cool boxes full of fresh seawater. Continuous aeration was supplied to all boxes via air stones attached to compressed air cylinders. Mortality was less than 1% over 5 hours using this method. The urchins were then stored in four 2300L flow-through plastic tanks for three weeks to acclimate them to local conditions (**Fig 2.2.1)**

Fig 2.2.1 Flow-through storage tanks for sea urchins.

Tanks were at ambient temperature (15-17^{o}C) and were vigorously aerated. Urchins were fed on the 16% protein diet described below (2% body weight, twice a week) and, as the tanks were outside, the animals had access to filamentous algae growing on the sides of the tank.

After three weeks 144 urchins were randomly selected from the holding tanks and weighed (to 0.1g; mean ($\pm$ SE) wet weight = 148.8 $\pm$ 1.99g) and the test diameter (TD) measured (to 0.1mm; mean TD = 71.2 $\pm$ 0.03mm). They were then housed indoors in 28L bins individually supplied with flow-through water (0.5L/min) and constant aeration (**Figs 2.2.2 and 2.2.3**). 24 urchins were also taken as an initial sample for dissection and analysis of roe yield and quality.

Fig 2.2.2 Flow-through tank array for maintenance of sea urchins during roe enhancement trial

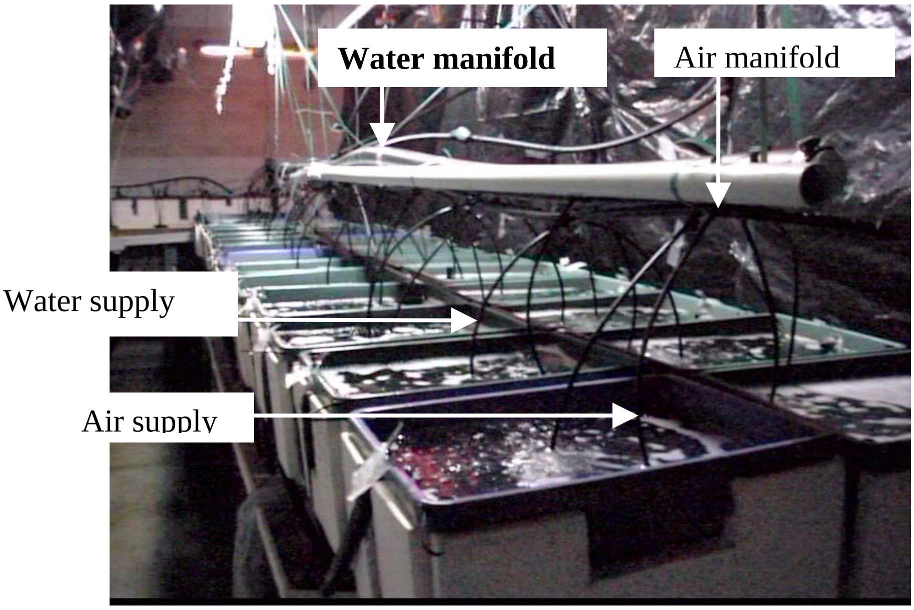
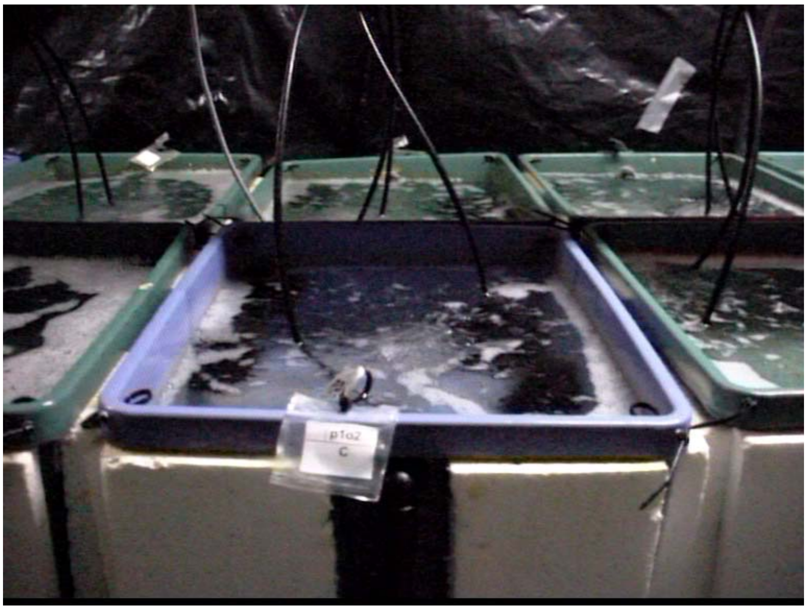


Fig 2.2.3 Individual tank within array



Within the tank array there were six treatments (6 diets), 4 tanks per treatment and 6 urchins per tank. Diets were fed at a rate of 2% body weight 3 times a week. Excess food was removed by siphoning and tanks cleaned before each feed. Temperature was constant at 19^{o}C, photoperiod was long (14:10LD) and the trial was run for 7 weeks.

The six diets used contained one of 3 protein levels (estimated crude protein levels of 13.6%, 18.4% and 25.7%) and each of 2 oil types, namely linseed and fish oil (mackerel). These diets were used following discussions with Dr Jeff Buchanan, a fish/invertebrate nutritionist at SARDI, Port Lincoln. The diet (**Table 2.2.1**) was based on a recipe designed by Dr John Castell and Dr Shawn Robinson of St Andrews

Biological Station (SABS), New Brunswick, Canada. The SABS diet has been successfully used as a baseline for the enhancement of the roe of the green sea urchin *Strongylocentrotus droebachiensis* in New Brunswick. The formulation used in the present work differs in that kelp powder (*Durvillea* spp) was added as an attractant. Its inclusion followed pre-experiment observations, which suggested that urchins would not eat the diet in its original form. The other modification was the inclusion of fish oil in one set of three diets varying in protein level (Table 2.2.1a and b) , the other set of three diets followed the SABS diet in using linseed oil as the lipid source. The three protein levels were achieved by manipulation of the soybean, wheat and canola meal content and by addition of the filler, diatomaceous earth. The differences between amino acid profiles of the diets were within acceptable limits (J. Buchanan, pers. comm.).

Table 2.2.1a Percent inclusion level of ingredients (dry matter basis) in the diets used to determine the effect of protein level and oil type on roe enhancement.
Based on a diet designed by Dr Shawn Robinson and Dr John Castell
of St Andrews Biological Station, St. Andrews, New Brunswick.

	Protein level		
Component	**Low**	**Medium**	**High**
Filler (diatomaceous earth)	25.0	5.5	-
[1]Kelp	14.00	14.00	14.00
Soybean Meal	12.00	12.50	20.22
Wheat meal	3.00	12.50	20.20
Canola Meal	3.00	12.50	20.3
Starch	17.72	17.72	-
Gelatin	5.00	5.0	5
Na Alginate	2	2	2
Linseed or Fish Oil	2	2	2
Lecithin	2	2	2
[2]Stay C (Vit C)	0.08	0.08	0.08
Ethoxyquin or BHT	0.20	0.20	0.20
[3]Algro+	10.00	10.00	10.00
Vitamin Mix	2	2	2
Mineral Mix	2	2	2
	100	**100**	**100**
% Crude Protein	**13.61**	**18.37**	**25.7**
Energy (MJ/kg)	8.68	13.21	13.37

[1] *Durvillaea potatorum*
[2]Ace Chemicals
[3]Equal mixture (2.92%) of soybean meal, wheat meal and canola meal plus 1.25% Algro (Betatene Pty. Ltd.), a 2% beta-carotene formulation obtained from spray-dried algae (*Dunaliella salina*).

Table 2.2.1b Vitamin and mineral mix used in the diet

Vitamins	Contribution g/tonne	Minerals	Contribution g/tonne
Available Zinc	1	Copper Sulphate	0.78
BHT ($CaCO_3$)	200	Ferrous Sulphate	30
Ethoxyquin 66%	80	Causmag Al 8	162.77
Ferrous sulphate (30%)	18	Manganous oxide	4
Pollard	4515.29	Rice fractions	900
Vitamin A500	10	Stock lime (Unical C300C)	256.77
Vitamin B1	12	Cobalt carbonate 21%	0.02
Vitamin B12 1% FG	18	Calcium Iodate 62%	0.18
Riboflavin B2 80% SD	9.98	White oil	20
Pyridoxine B6	4	Zinc Oxide	7
Biotin 2%	1200		
D-calpan	20		
Vitamin C EC	411.71		
Vitamin D3 500	0.21		
Folic Acid 80% SD	3.02		
Inositol	400		
Niacin pure	80		
White oil	60		

The diet was hand-mixed and extruded using a pasta machine with a 2cm die. The end result was a fettucine shape cut into approximately 2cm lengths (**Fig 2.2.4**). The diet was then oven-dried at 55°C for 24hours, allowed to come to room temperature then stored in sealed bags in a cold room (4°C) until used.

Fig 2.2.4 Diets used for protein and oil level trial. Left to right: 13.6%, 18.4% and 25.7% Crude Protein.
Oil type did not affect the diets' appearance.

At the end of the experiment all sea urchins were opened after weighing and test diameter (TD) measurement and organs removed for assessment as described above. Each colour and texture of roe was given a number to facilitate statistical analysis (**Table 2.2.2**). A further sample of sea urchins (n=18) was collected from Pt Riley at the end of the experiment to provide a comparison with the % yield achieved in the laboratory.

Percent gonad yield data were tested for normality (one sample K-S test) and homogeneity of variances (F_{max}) and arcsine-transformed if necessary. ANOVA was carried out on the data using the Statistics Package for the Social Sciences (SPSS). For ease of analysis and discussion, diets were coded depending on protein level and oil type. Crude protein levels 13.6%, 18.4% and 25.7% were assigned the codes 14, 18 and 26 respectively. Similarly linseed and fish oil were given the codes L and F respectively. Thus 14L referred to the diet containing 13.6% crude protein and linseed oil **(Table 2.2.2).**

Table 2.2.2 Diet identification codes used for analysis and in text elsewhere in this chapter

	Crude protein (%)	Oil type
14L	13.6	Linseed
14F	13.6	Fish
18L	18.4	Linseed
18F	18.4	Fish
26L	25.7	Linseed
26F	25.7	Fish

2.2.3 Results

Roe Yield

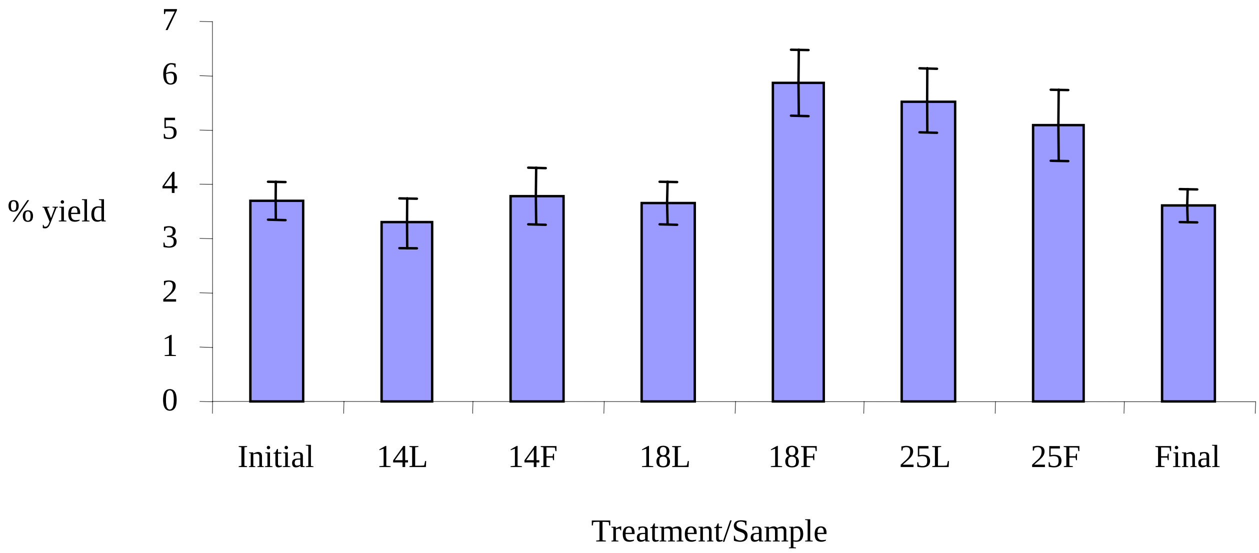
Roe yield varied between 1 and 13% and was affected by protein level in the diet (p=0.009), but not by oil (P=0.101, **Table 2.2.3**) (Fig 2.2.5), and there were no significant interaction effects (P=0.063). Data pooled over oil types, and analysed for the effect of protein alone, showed a significant increase in % yield with protein level (P=0.013, Tukeys HSD) between the 14% and the 18% protein treatments. There were no further significant effects when initial and final field samples were included in the analysis ($P \geq 0.07$).

Test diameter (TD) also had no effect on % roe yield (P=0.0.292). Percent change from initial roe yield was low, reaching a maximum of 1.9% for diet 26L (Fig 2.2.5). There was no significant change in mean sea urchin test diameter over the period of the trial (P>0.05). Data were pooled over tanks within treatments as there were no significant tank effects (P>0.05).

Table 2.2.3 ANOVA table: test of protein and oil effects on % roe yield

Source	Type III Sums of Squares	Df	Mean Square	F	Sig
Corrected Model	105.410	5	21.082	3.531	0.005
Intercept	2434.158	1	2434.158	407.637	<0.001
Protein	59.057	2	29.529	4.945	0.009
Oil	16.346	1	16.346	2.737	0.101
Protein x Oil	33.817	2	16.908	2.832	0.063
Error	680.737	114	5.971		
Total	3154.768	120			
Corrected Total	786.147	119			

Fig 2.2.5 Average % roe yield (±SE).
The codes 14, 18 and 25 refer to crude protein levels 13.6%, 18.4% and 25.7% respectively. Similarly the codes L and F refer to linseed and fish oil respectively. Thus 14L referred to the diet containing 13.6% crude protein and linseed oil



Roe Quality

Diet did not affect roe colour or texture (**Table 2.2.4**). Most roe fell into coarse or fine categories and colours 1 or 2 (**Table 2.2.5**)

Table 2.2.4 Kruskal-Wallis Test statistics for tests of the effect of dietary protein and oil on sea urchin roe colour and texture

Treatment	Roe Characteristic	χ^2	P
Protein	Colour	2.29	0.317
	Texture	0.754	0.686
Oil	Colour	1.70	0.193
	Texture	0.058	0.809

Table 2.2.5 Percentage of roe of each texture for each colour (n=134)
Data pooled over diets due to the non-significant treatment effects.

	Texture			
Colour	**Coarse**	**Medium**	**Fine**	**Roe of each colour as a percentage of total sample**
1. Black, brown, dark gold, orange or yellow	8.93	43.48	54.55	33.58
2. Gold, orange or yellow	75.00	30.43	29.09	48.51
3. Light gold, orange or yellow	16.07	26.09	9.09	14.93
4. Very light gold, orange yellow or cream	0.00	0.00	7.27	2.99
Roe of a each texture as percentage of total sample	41.79	17.16	41.04	100.00

Colour-texture categories grouped into approximate grades, as described in **Table 2.1.1,** show a predominance of C and D grade roe (**Table 2.2.6).** Diets 14F and 25L showed significant differences in the numbers of sea urchins in each grade (**Table 2.2.7**)

Table 2.2.6 Numbers and overall percentages of sea urchins found to have roe of each grade for each of the six diets used in the trial

	Grade				
Diet	**A**	**B**	**C**	**D**	**Totals**
14L	6	1	7	8	22
14F	2	2	7	12	23
18L	0	5	10	8	23
18F	1	6	6	5	18
25L	5	1	10	3	19
25F	1	5	10	7	23
Totals	**15**	**20**	**50**	**43**	**128**
Grade total as percent of total sample	**(12)**	**(16)**	**(39)**	**(34)**	**(100)**

Table 2.2.7 Significant Chi-Square tests on equality of numbers of sea urchins producing roe of each grade within each diet

Treatment	χ^2	**P**
14F	11.957	0.008
25L	9.421	0.024

Gut Indices

There were no significant differences in gut index between any of the treatments or between the treatments and the initial sample (P=0.124, ANOVA). The mean gut index (±SE) for the pooled data was 0.842 ± 0.031% (n=134).

2.2.4 Conclusions

Improvements in percent roe yield were limited although there was a significant increase in yield with protein and energy level. The increasing level of protein may have been responsible for this increase as it the primary contributor to gonad growth in other species (McBride et al, 1997). Oil type had no effect on % roe yield, roe colour or texture suggesting that either would be suitable for inclusion in this diet. Given the minimal improvement in % roe yield, further manipulation of this diet is clearly necessary if it is to be used as a commercial feed.

2.3. The effect of temperature and photoperiod on roe yield and quality.

(Relevant Objectives: 1, 2, 3, 6)

2.3.1 Introduction

The objective was to test the effect of temperature, photoperiod and diet type (artificial and natural) on roe yield and quality and the experiment was set up after consultation with Dr Robert van Barneveld.

Two experiments (T_1 and T_2) were carried out at the same time, simulating winter/spring and spring photoperiods respectively. These conditions were chosen as the industry was interested in appropriate timing for sea-cage culture, not manipulation of photoperiod for land-based intensive culture. There is more interest in the latter in northern hemisphere where out-of-season gonad production trials, for species such as *Strongylocentrotus droebachiensis*, have been successful in the laboratory (Hagen, 1998; Walker and Lesser, 1998).

2.3.2 Methods

Sea urchins (74.36$\pm$ 0.33 (SE) mm TD, n=300) were captured, transported, acclimated prior to the experiment and maintained during the trial, in the same way as reported for the previous trial. Three diets were used, *viz*: fresh *Ulva lactuca*, artificial diet (i.e. the 23% crude protein diet used in Trial 1) and a mixture (fed alternately with *Ulva* or artificial diet). Urchins were fed 3 times per week at 2% body weight for the artificial diet or 5% body weight for the *Ulva* per day. Uneaten food and faeces was removed by siphoning and tanks cleaned before each feed.

1. Winter/Spring Temperature, Winter Photoperiod (T_1)

Fifteen tanks were used for this experiment, each containing five sea urchins and fed on one of the three diets (Table 2.3.1a). Day length was held constant at the photoperiod on July 28th (10hrs 17 min L: 13hr 43min D), the beginning of the experiment, because of limitations in space and urchin numbers.

Table 2.3.1a Experimental design for Trial 2: Winter/Spring temperature, constant photoperiod (T_1)

Temperature	Photoperiod	Tanks	Diets/ Tanks		
(T_1) Winter/Spring	10hrs 17 min L: 13hr 43 min D	15	Ulva/5	Diet/5	Ulva + Diet/5

Initial temperature was set at 12^{o}C and increased at a rate of 0.45^{o}C/week to reach 15^{o}C by the end of week 6 (September 7th), the last week of the experiment. The rate of increase set for T_1 represents that expected in the area of collection (Nunes and Lennon, 1986) over the time that the experiment was run (Winter/Spring), based on ten years collected data.

2 Spring Photoperiod (T_2)

Thirty tanks were used for this experiment, each containing five sea urchins and fed on one of the three diets (Table 2.3.1b). Tank temperature was initially set at 15.3^{o}C and raised to 18^{o}C by increments of 0.45^{o}C/week. T_2 represents T_1 plus 6 weeks (September 8th to October 19th - Spring). The T_2 treatment was further subdivided into 2 photoperiod treatments, one constant at 12h 26min L: 11h 34min D (1st October - mid Spring), the other increasing in length by 11min 40secs each week to match the change in photoperiod appropriate to the 6 week period represented within the dates covered by T_2.

A further sample of sea urchins (n=12) was collected from Pt Riley at the end of the trials to provide a comparison with the % yield achieved in the laboratory. At the end of the experiment all sea urchins were treated as described in the previous trial.

Table 2.3.1b Experimental design for Trial 2: Spring photoperiod

Temperature	Photoperiod	Tanks	Diets/ Tanks		
Spring	Constant	15	Ulva/5	Diet/5	Ulva + Diet/5
Spring	Variable	15	Ulva/5	Diet/5	Ulva + Diet/5

2.3.3 Results

Roe Yield

Diet had a significant effect on % roe yield although temperature and photoperiod did not and there were no interaction effects (Table 2.3.2). Overall roe yield varied between 1 and 11.4% (**Fig 2.3.1**). When initial and final field samples were included in the analysis, (Table 2.3.3a and b), the % roe yield from initial and final field samples was observed to be lower than that produced by the artificial and the mixed diet treatments. The % roe yield produced by the *Ulva* treatment was no different from the field samples. There was also no significant change on mean sea urchin TD over the period of the trial (P>0.05).

Table 2.3.2 ANOVA table: test of the effects of photoperiod, temperature and diet on arcsine transformed % roe yield.
Initial and final field samples not included in analysis.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	2.476E-02	8	3.094E-03	1.820	.074
Intercept	10.253	1	10.253	6031.283	.000
Photoperiod	1.392E-03	1	1.392E-03	.819	.366
Temperature	2.115E-03	1	2.115E-03	1.244	.266
Diet	1.756E-02	2	8.782E-03	5.166	.006
Photoperiod*Diet	3.901E-03	2	1.951E-03	1.147	.319
Temperature*Diet	3.497E-03	2	1.748E-03	1.029	.359
Error	.376	221	1.700E-03		
Total	11.682	230			
Corrected Total	.400	229			

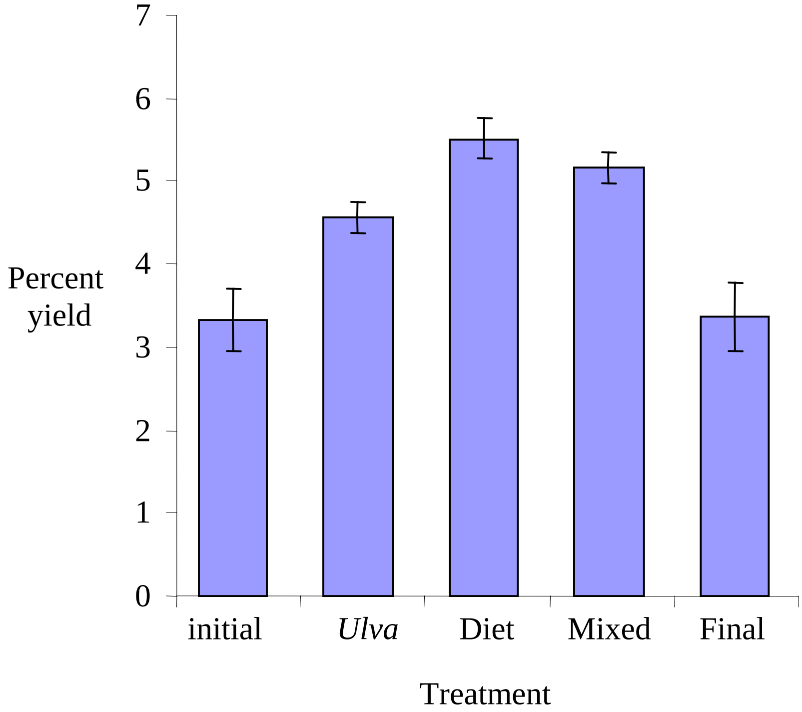
Table 2.3.3a ANOVA table: test of the effects of diet/sample origin on arcsine transformed % roe yield.
The analysis includes initial and final field samples

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	**0.020**	4	0.003	9.123	.000
Intercept	**0.282**	1	0.282	858.589	.000
Diet/sample origin	**0.020**	4	0.003	9.123	.000
Error	**0.084**	257	0.0003		
Total	**0.718**	262			
Corrected Total	**0.096**	261			

Table 2.3.3b Post Hoc Tests (Tukeys HSD) showing homogenous subsets.
Treatments listed in order of increasing mean % roe yield.

Treatment/Sample	Tukeys HSD	
Initial	|	
Final	|	
Ulva	|	|
Mixed		|
Diet		|

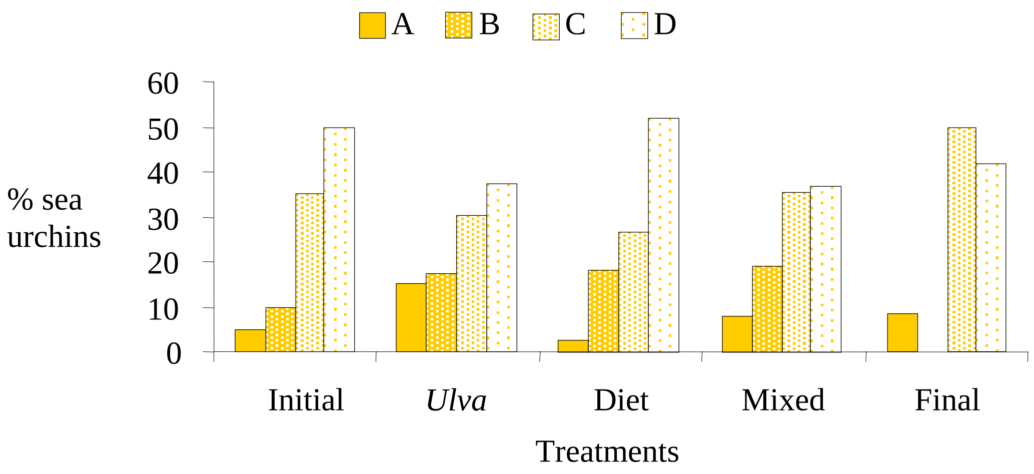
Fig 2.3.1 Average % roe yield (± SE) for three diets
Ulva, Artificial diet (23% estimated crude protein) and Mixture (fed alternately with *Ulva* or artificial diet) and initial and final samples from Point Riley



Roe Quality

Roe quality was not affected by photoperiod (P=0.394) or temperature (P=0.631). When roe quality data were grouped into grades as described in Table 2.1.1 there was a clear increase in A grade roe with *Ulva* (P=0.03, Fishers exact test, 2x2 Contingency Table) (**Fig 2.3.2**). Overall, most of the roe was C and D grade.

Fig 2.3.2 Percentage of sea urchins having roe within each of 4 grades



Gut Indices

These were not calculated as samples were damaged during processing.

2.3.4 Conclusions

Photoperiod and temperature had no effect on percent roe yield or roe quality; photoperiod probably, in part, because of the narrow range used. The lack of treatment effect may also have been exacerbated by the generally low % roe yield in all treatments although there was a significant diet effect with a trend to higher % yields with artificial feed, and an increase in A grade roe with *Ulva*. It is clear from this and the preceding trial that the Robinson and Castell diet does not produce the expected increases in % roe yield in this species; further work will explore other options.

2.4. The effect of a semi-moist extruded diet and sea urchin size on roe yield

(Relevant Objectives: 1,5,6)

2.4.1 Introduction

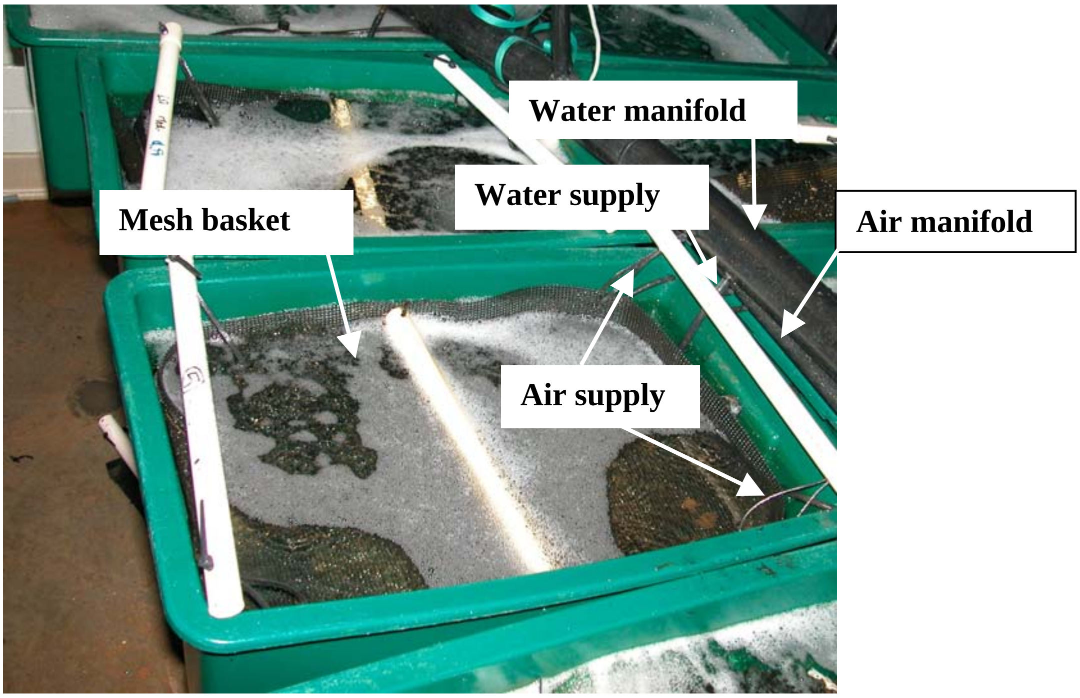
The Robinson and Castell formulated diet appeared to be inadequate for the enhancement of *H. erythrogramma* roe, as mentioned in the previous section. Soon after the previous trial commenced, the project was offered a quantity of a semi-moist extruded diet formulated for sea urchins by Dr John Lawrence of the University of South Florida, Tampa, Florida and Dr Addison Lawrence, of Texas A.& M. University, Port Aransas Texas, U.S.A.. Wenger International Inc. of Kansas City, Missouri, U.S.A. manufactured the diet and variations have been used widely in the U.S.A. (McBride et, 1997, 1998, 1999; Plank, et al 2002) and in New Zealand (Barker et al, 1998).

Urchin size was chosen as a variable of interest as discussions with industry members had suggested that roe of large urchins (circa 80 mm Test Diameter) was generally of coarser texture and therefore lower value than that of smaller animals. It was decided to test the hypothesis that roe quality is size-mediated, using representative sizes within the range found in the fishery.

2.4.2 Methods

Sea urchins (three size groups, 55.5 $\pm$ 0.47mm, 71.5 $\pm$ 0.40mm and 80.7$\pm$0.44mm TD) were collected, in September 2001, from shallow waters at Point Riley, bought back to SAASC and kept in outside tanks (temperature: 15^{o}C), without food, for 2 weeks to standardise gonad condition. Tanks were cleaned regularly to minimise algal growth. After the acclimation period, 42 sea urchins (approximately 14/size class) were killed and dissected, their gonads weighed and 1 gonad from each sea urchin preserved in Bouins fixative then 70% alcohol for later histological analysis. The remaining sea urchins were then measured and assigned to each of fifty-two 35L tanks (**Fig 2.4.1**) with individual air and flow-through water supplies (0.5L/min).

Fig 2.4.1 Tank array used in urchin size - roe enhancement comparison trial. Whole array not shown. All tanks were at waist level, arranged in two groups of 30, with walkways at edges and between groups.



Six sea urchins of each size group were randomly assigned to a given tank within the array. Sea urchins were suspended in a mesh basket within each tank (**Fig 2.4.2).** Water temperature was set at 17^{o}C. Tanks were cleaned and sea urchins fed 3 times a week (2% body weight/day) for twelve weeks.

Fig 2.4.2 Mesh basket used to support urchins within tanks

Water inlets and aerators (two 25mm air-stones per tank) were placed beneath the mesh basket to maximise water circulation and aeration.

Animals were fed with a sea urchin diet (**Table 2.4.1, Fig 2.4.3**) designed by the Drs Lawrence as mentioned. The diet was donated to the project by Wenger International Inc.

Table 2.4.1. Composition of sea urchin diet produced by Wenger Manufacturing Inc.
Moisture content approximately 17.74% (median, n=2)

Ingredients:	**Percent of dry Weight**
Corn meal	24
Wheat Middlings	24
Fish Meal	12
Norwegian kelp	14
Soy Flour	11
Lecithin	1
Cholesterol	0.20
Sodium Phosphate	1.30
Ethoxyquin	0.10
Vitamin/Mineral mixture	0.20
Potassium sorbate	0.30
B-carotene (Hy-Zea)	0.50
Fish oil (menhaden)	0.80
Glycerin	9
Phosphoric acid	1
Total	100

Fig 2.4.3 Wenger diet used to test the effect of sea urchin size on roe yield

At the end of the experiment all urchins were dissected and their organs weighed and treated as described earlier. Size-specific differences in roe yield (roe as a percentage of wet body weight), texture, and colour were investigated.

In this trial, a final sample was not obtainable from the field for comparison, due to inclement weather.

Histology

One of each of the five gonads from sea urchins within the initial and final samples (all size groups) was taken for histological analysis. Histological sections were prepared using standard methods and stained with haematoxylin and eosin, at the Medical School, University of Adelaide. Ova areas were measured using a Leitz Diaplan compound microscope connected to a PC running image analysis software (Sigmascan Version 4). Gonad stages were assigned after Dix (1977b) and Laegdsgaard et al (1991). The relationships between sea urchin size, sex and roe colour and texture, and between texture and ova size, were investigated.

2.4.3 Results

Roe Yield

As there were no differences in initial percentage yield between sizes (P=0.626, ANOVA), data were pooled, giving a mean of 4.01 % (± 0.35 SE, n=42). There was a significant increase in percentage yield (P<0.001, ANOVA, **Table 2.4.2**) over the period of the experiment with medium and small sea urchins producing similar yields, significantly higher than large sea urchins (P<0.003, Tukey HSD) (**Table 2.4.3).** Overall, 74% of sea urchins (large, 72%; medium, 77%; small, 74%) produced yields of 10% and above, the latter figure usually equated with the commercially acceptable yield, with the maximum yield being 29% of wet body weight. There was no significant change in mean sea urchin TD over the period of the trial (P>0.05).

Table 2.4.2 ANOVA table: test of the effect of size on % roe yield
There were no interaction effects.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	129.615	2	64.808	5.628	0.004
Intercept	40149.270	1	40149.270	3486.814	<0.001
Size	129.615	2	64.808	5.628	0.004
Error	3523.468	306	11.515		
Total	45364.933	309			
Corrected Total	3653.083	308			

Table 2.4.3: Final sea urchin roe yields (± SE) by size and post hoc tests (Tukeys HSD).
Treatments listed in order of increasing mean % roe yield within each table.

Sea Urchin Size	Mean Yield (%)	SE	% change from initial yield	Tukeys HSD
Large	10.786	0.325	6.78	|
Small	11.756	0.384	7.75	|
Medium	12.275	0.307	8.26	|

Roe Quality

Two-way interactions between final colour and texture, size and colour and size and texture were significant (**Table 2.4.4**, P<0.001).

Table 2.4.4. Loglinear regression statistics on size, colour, texture and interactions (Genstat).
na = not appropriate to test as higher order interactions were significant.

Fixed effect	Approx. Chi-square P-value
Size	Na
Colour	Na
Texture	Na
Colour. Texture	<.001
Size. Colour	<.001
Size. Texture	<.001
Size. Colour. Texture	0.439

Given the significance of all three 2-way interactions, it was not appropriate to consider any of the three factors (i.e. overall colour by texture or size by colour or size by texture) separately. Interactions were interpreted by referring to the table of observed counts (**Table 2.4.5**).

Table 2.4.5. Counts and % of urchins of each size having roe of each colour and texture.

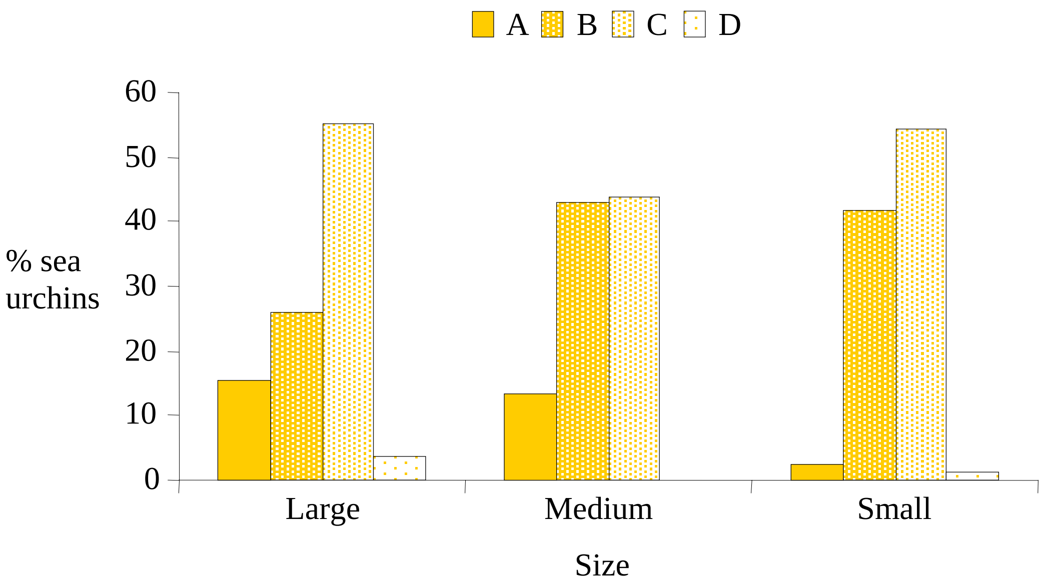
Size	Colour	Texture						Total	Total
		Coarse		Medium		Fine		n	%
		n	%	n	%	n	%		
Large	1	3	(5)	2	(7)	3	(13)	8	(7)
	2	53	(88)	20	(74)	17	(74)	90	(82)
	3	2	(3)	4	(15)	2	(9)	8	(7)
	4	2	(3)	1	(4)	1	(4)	4	(4)
Total n/(%)		60	(55)	27	(25)	23	(21)	110	(100)
		n	**%**	**n**	**%**	**n**	**%**		
Medium	1	2	(5)	1	(3)	2	(4)	5	(4)
	2	38	(95)	26	(87)	40	(71)	104	(83)
	3	0	(0)	2	(7)	10	(18)	12	(10)
	4	0	(0)	1	(3)	4	(7)	5	(4)
Total n/(%)		40	(32)	30	(24)	56	(44)	126	(100)
		n	**%**	**n**	**%**	**n**	**%**		
Small	1	0	(0)	0	(0)	0	(0)	0	(0)
	2	7	(88)	9	(90)	26	(43)	42	(53)
	3	0	(0)	1	(10)	21	(34)	22	(28)
	4	1	(13)	0	(0)	14	(23)	15	(19)
Total n/(%)		8	(10)	10	(13)	61	(77)	79	(100)

Table 2.4.5 suggests a number of trends across textures for each size and differences for each colour. For example, for large sea urchins, colour 2, most of the roe was coarse with numbers declining as the texture became finer. The counts for medium sea urchins, colour 2, were high for all textures, whereas for small sea urchins of colour 2 there was little coarse roe, the majority being of fine texture.

The size-colour interaction is due to the high number of observations in colours 2 and 3, for small sea urchins, and a large number of observations in colour 2 for large sea urchins. Large sea urchins had significantly more coarse roe than expected for colour 2 (Chi Square, $P<0.001$) whereas medium sea urchins showed no significant difference in texture for that colour (Chi Square, $P=0.191$). For small sea urchins, much of the roe was of colour 2 with most having fine texture. The size- texture interaction is likely due to the high number of large sea urchins with coarse roe compared to the large number of small sea urchins with fine roe.

When roe quality data were grouped into grades as described in Table 2.1.1 it was also apparent that large sea urchins contained relatively more low-grade roe (**Fig 2.4.4**) than medium sea urchins (P=0.042, Chi Square, 2x3 Contingency Table). Small urchins also contained very little A grade roe.

Fig 2.4.4 Percentage of sea urchins of each size having roe within each of 4 grades



Histology

Gonads were found at each of the 6 reproductive stages (**Figs 2.4.5, 2.4.6 and 2.4.7**). Most sea urchins, of both sexes, were found to be at Stage 2: regenerating. There were significantly more males than females in the resting (post-spawning) phase (X^2, P=0.028).

Fig 2.4.5 Percentages of males (n=57) and females (n=50) at each gonad stage. Stage 1 = resting, 2 = regenerating, 3 = late regenerating , 4 = mature, 5 = partly spawned, 6 = spent.

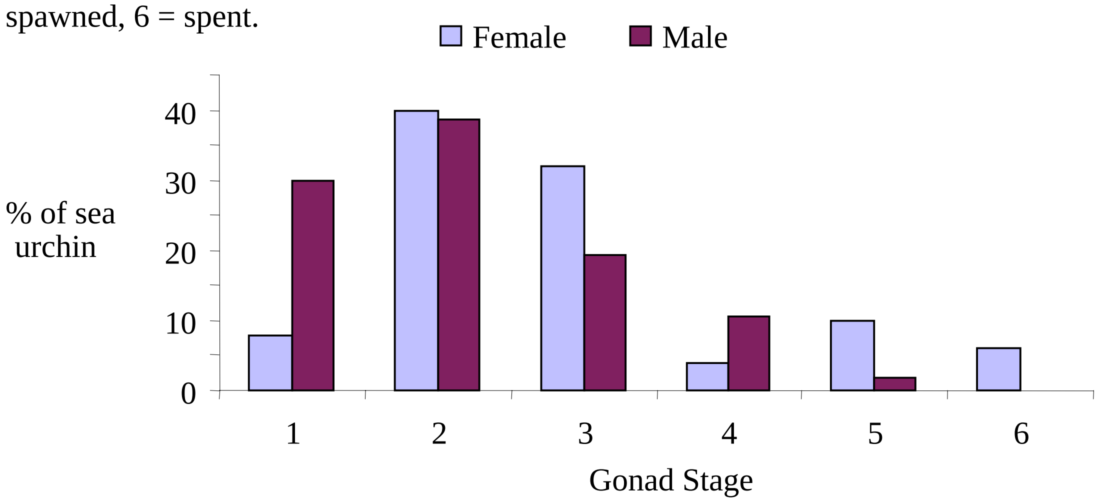
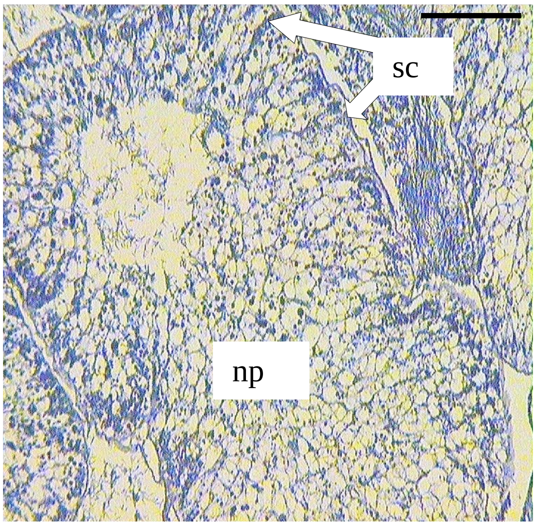
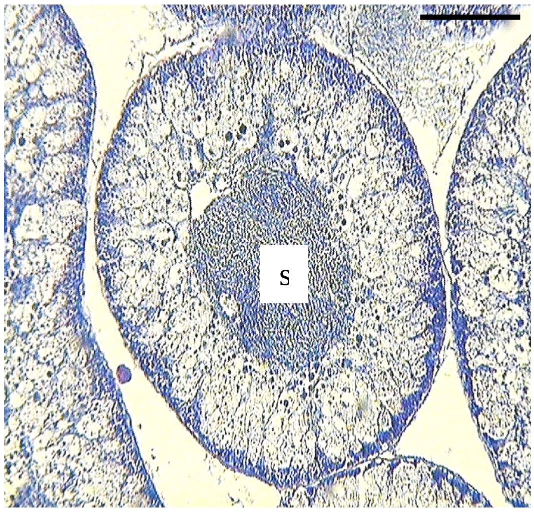


Fig 2.4.6 Testes Stages (after Laegdsgaard (1991)).
The photos show the progressive development of a tubule within the testes. No stage 6 testes were found. Spermatocytes (sc), spermatozoa (s), nutritive phagocytes (np). Scale bar = 100μm

a) Stage I: Recovering



b) Stage II: Growing



c) Stage III: Premature

d) Stage IV: Mature

e) Stage V: Partly spawned

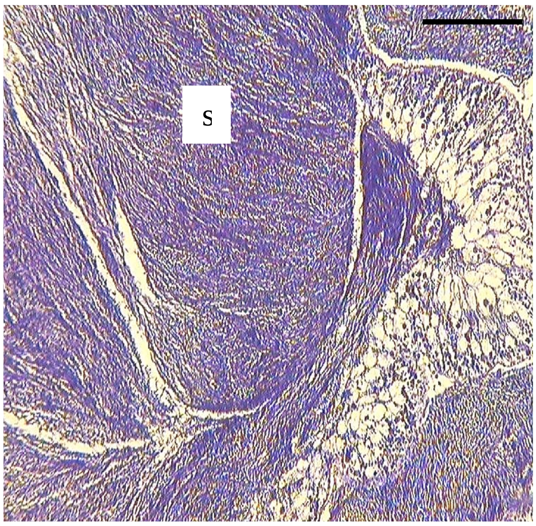
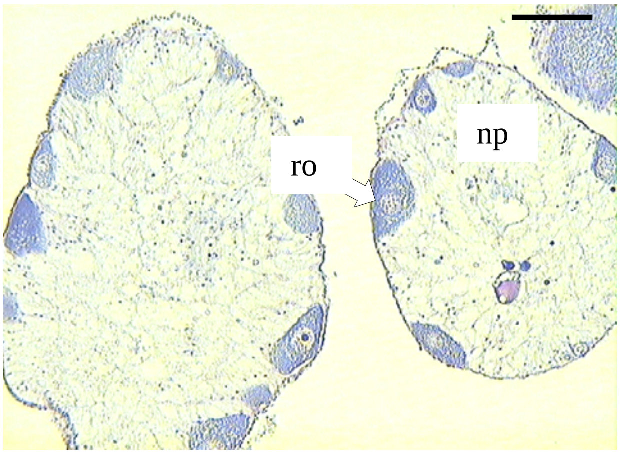
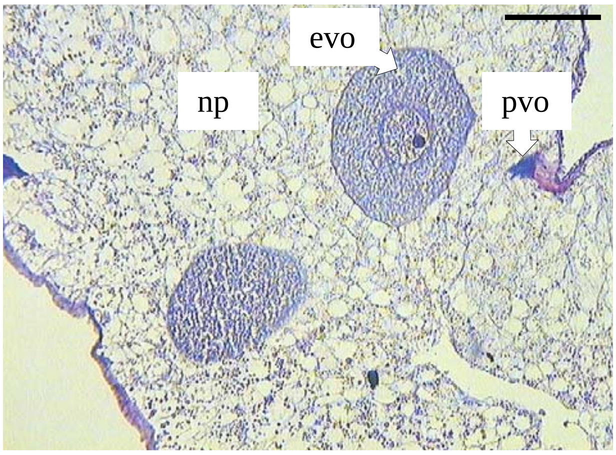


Fig 2.4.7. Ovarian Stages (after Laegdsgaard (1991)).
Stage 6 photos were damaged. Relict ovaries (ro), nutritive phagocytes (np), pre-vitellogenic oocytes (pvo), early vitellogenic oocytes (evo), late vitellogenic oocytes (lvo), oocytes (o), lumen (L). Scale bar = 100μm

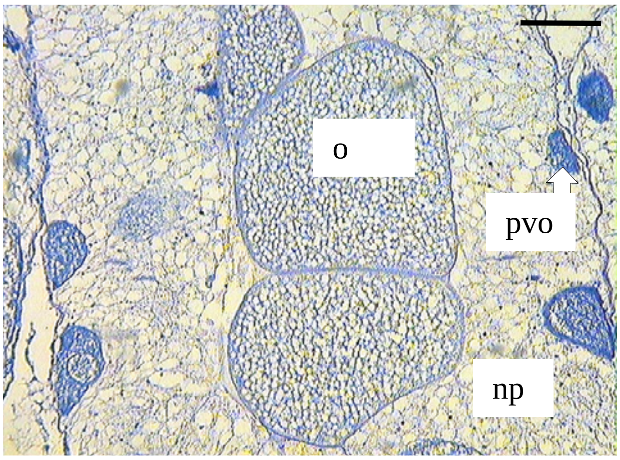
a) Stage I: Recovering



b) Stage II: Regenerating

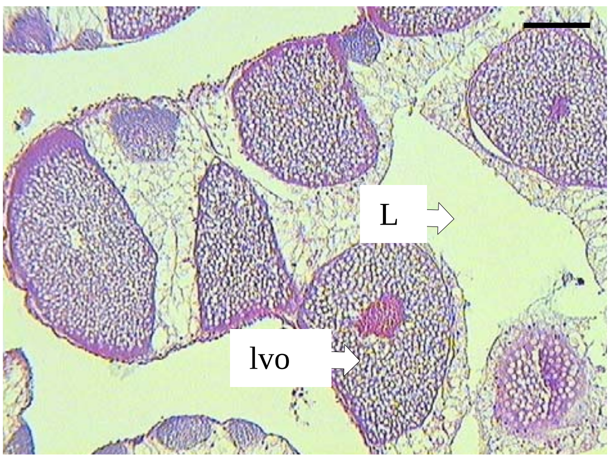


c) Stage III: Premature

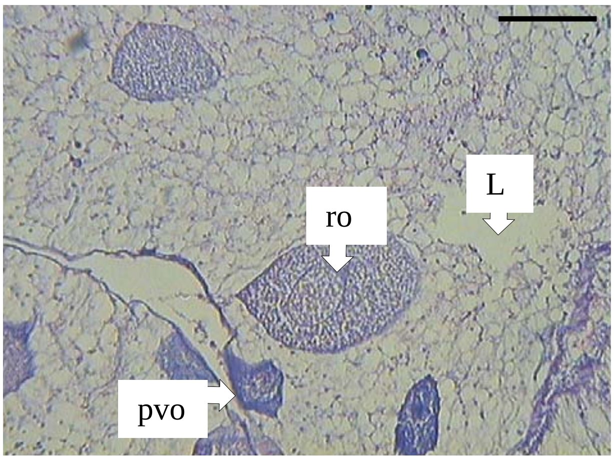


d) Stage IV: Mature

e) Stage V: Partly Spawned

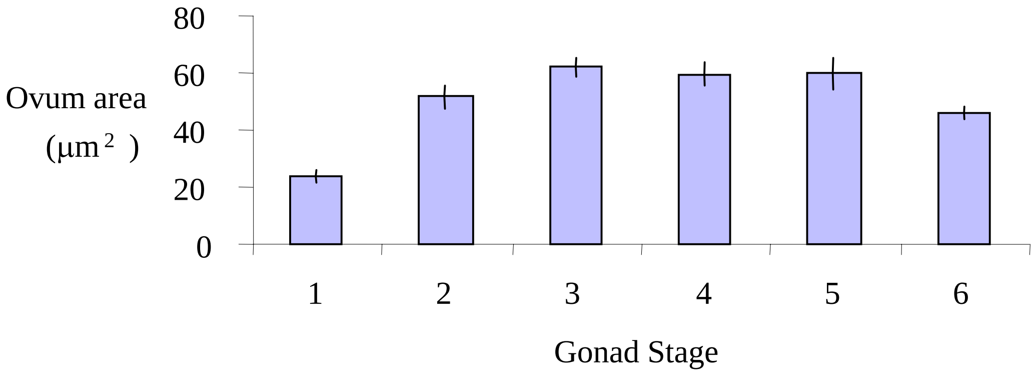


f) Stage VI: Spent



Gonad stage had a significant effect on the size of individual ova within the sampled ovaries (n=50, P=0.002, ANOVA). (**Fig 2.4.8**). Sea urchin size did not (P=0.244) have an effect and there were no significant interactions between gonad stage and sea urchin size (P=0.568).

Fig 2.4.8 Mean ovum area (μm ^{2} ±SE) - gonad stage comparison.
Stages after Dix (1977b) and Laegdsgaard et al (1991).



Ovum area was lowest in fine-textured roe (P<0.001, ANOVA) (**Fig 2.4.9**). The stage of ovaries did not affect their texture (Fig 2.4.10a) (Chi-Square, P>0.05). Finest-textured testes were found at the earliest gonad stages (Chi-Square = 19.182, P<0.001) (Fig 2.4.10b). .

Fig 2.4.9. Mean ovum area (μm^2) - roe texture comparison

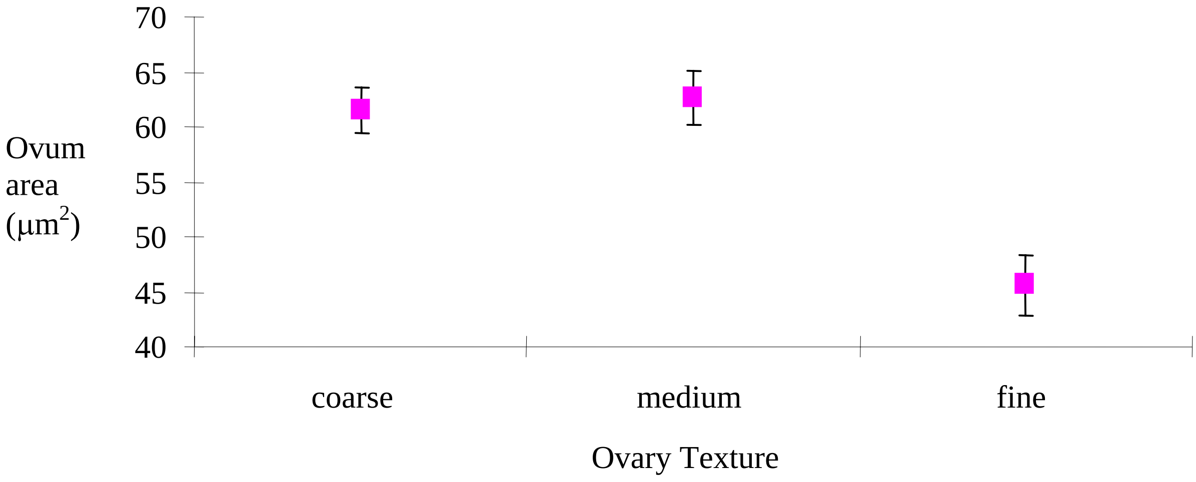
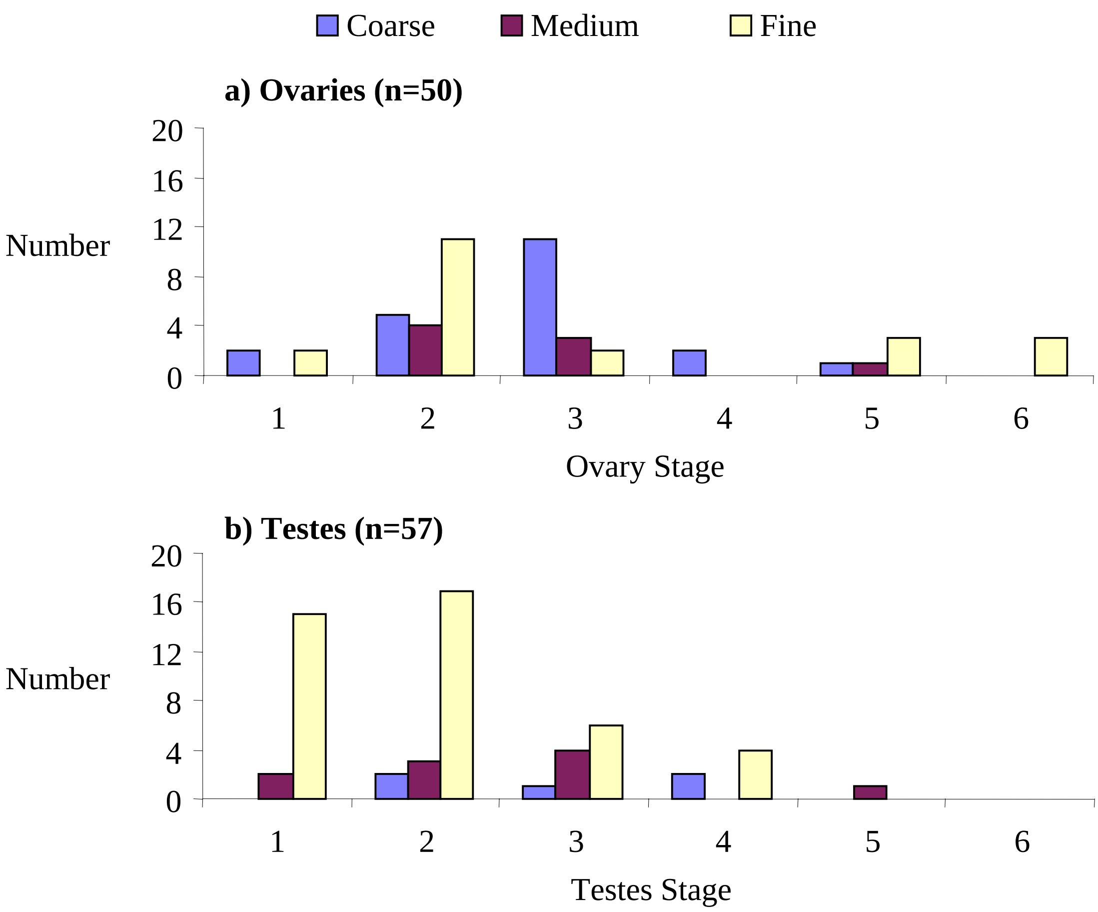


Fig 2.4.10 Number of coarse, medium or fine textured gonads at each gonadal stage



Gut Indices

There was no size effect on initial gut index (P=0.268, Mann-Whitney U) so the data were pooled and a mean calculated (1.108 + 0.12%, n=42). This was significantly lower (Mann-Whitney U, P<0.001) than those calculated for each size at the end of the trial. The final gut index also showed a significant decrease with sea urchin size (P<0.001, Mann-Whitney U) as follows: small, 3.53 ± 0.19% (n=79), medium, 2.86 ± 0.11% (n=121), large, 2.46 ± 0.08% (n=110).

2.4.4 Conclusions

The Wenger diet used in this trial produced a much higher average roe yield than that produced by the Robinson and Castell diet in the previous two trials. The condition of the sea urchins was also better, with the mean gut index in the present trial almost three times that achieved in Trial 1 (ref pg 35).

There were no differences in % roe yield produced by medium and small size groups. Large sea urchins produced more coarse roe and small sea urchins more fine roe than medium-sized animals.

Histology indicated that finer, and therefore higher quality, ovaries contained smaller ova than those in medium or coarse categories. The same was found for testes with finer roe also at a significantly earlier gonad stage.

2.5. *The effect of crude protein level and enhancement time on roe yield*

(Relevant Objectives: 2,5,6)

2.5.1 Introduction

As results from the previous trial suggested that medium-sized sea urchins were the most appropriate size for enhancement (see Results 2.4.3) these were used in subsequent trials. This trial investigated the effect of varying crude protein level and harvest time on yield and quality of sea urchin roe.

2.5.2 Methods

Sea urchins (69.43 $\pm$ 0.34 mm TD) were collected from shallow waters at Point Riley, bought back to SAASC and kept in outside tanks (temperature: 15^{o}C), without food, to standardise gonad condition. The acclimation period was to have been 2 weeks as before but had to be extended to 6 weeks because of delays in diet production. During this period some food was available as the outside tanks supported growth of filamentous algae. After the acclimation period, 36 sea urchins were killed and dissected. The remaining sea urchins were randomly assigned to each of three diets (15 tanks) and three harvest treatments within an array of forty-five 35L tanks described in the previous trial (i.e. 5-7 sea urchins/tank, **Figs 2.4.1 and 2.4.2**). Water temperature was set at 17^{o}C. Tanks were cleaned and sea urchins fed 3 times a week (2% body weight/day). Five tanks from each diet treatment were harvested at 46, 70 and 92 days and dissected as detailed in previous trials.

Sea urchins were fed one of three semi-moist diets (**Fig 2.5.1**) formulated by Dr Rob van Barneveld (Barneveld Nutrition Pty. Ltd.) (further details in Appendix 3).

Fig 2.5.1 Semi-moist diets used to test the effect of harvest time and protein level on % roe yield

Diets used are the lighter forms, top left to right: 21%, 24% and 27% crude protein. The Wenger diet (bottom), used in the previous trial, is included for comparison.

The formulations (Appendix 3) were based on the Wenger diet used in the previous trial (Diet 1 being analogous to the latter) and contained increasing concentrations of protein, the level of which was manipulated by manipulating the soybean meal and fish-meal concentrations. Protein and energy concentrations were estimated prior to formulation then measured, once the pellets were made, using Kjeldahl and bomb calorimetry techniques respectively (Table 2.5.1). Duplicate samples were run in each case. Calculated protein levels were close to those measured (Table 2.5.1).

Table 2.5.1 % Crude protein (CP) and gross energy (MJ/Kg)) for the diets used in testing the effect of nutrient density on roe yield.

Diet	Estimated Crude Protein (%)	Measured Crude Protein (%)	Measured Gross Energy (MJ/Kg)	CP(g/Kg):E(MJ/Kg)
1	21.0	22.03	17.51	12.59
2	24.0	24.20	16.98	14.25
3	27.0	26.76	17.03	15.72

2.5.2 Results

Roe Yield

The initial mean percent roe yield was low at 1.63 ± 0.184% (n=36), reaching a mean of 7.32 ± 0.323% (n=72) after 92 days (**Fig 2.5.2a**), a difference of 5.69%. Twenty-one percent of sea urchins had yields of over 10% after 92 days (**Fig 2.5.2b**). Harvest day significantly affected % roe yield (P<0.001) although diet did not (P=0.355) (**Table 2.5.2**). There was no significant change in mean sea urchin TD over the period of the trial (P>0.05).

Fig 2.5.2a Mean % yield (+SE) for each diet at each harvest day.
* and ** indicate significant differences (P<0.05 and P<0.001 respectively) between successive harvest days using pooled data (Tukeys HSD Test).

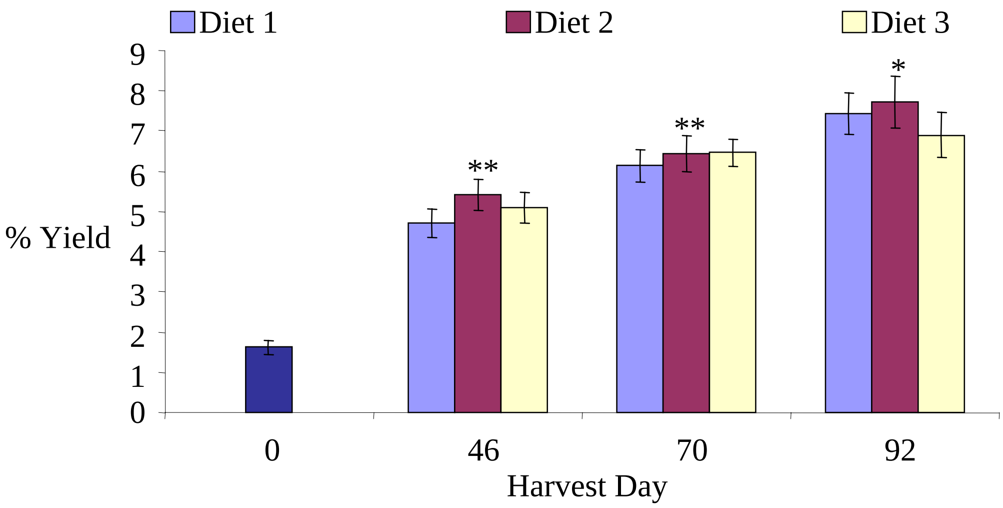


Fig 2.5.2b Percent sea urchins with 10% roe yield or above

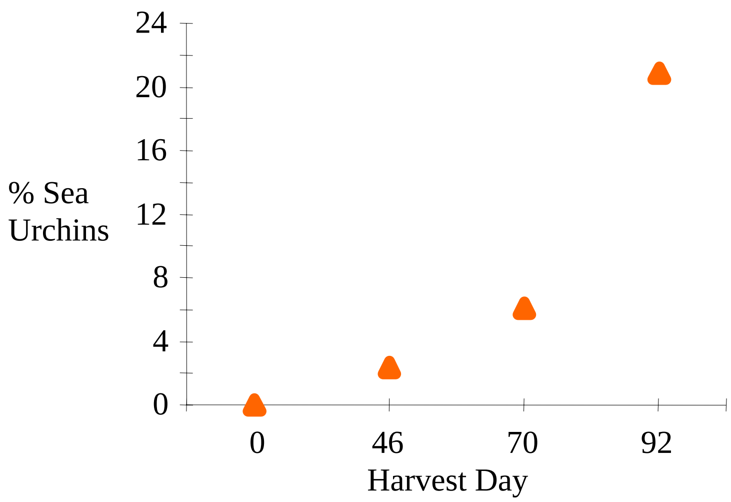


Table 2.5.2 ANOVA table: test of the effect of harvest day and diet on % roe yield

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	858.517	9	95.391	19.948	<0.001
Intercept	7244.776	1	724.776	1515.015	<0.001
Harvest day	217.167	2	108.584	22.707	<0.001
Diet	9.954	2	4.977	1.041	0.355
Diet*Harvest day	7.923	4	1.981	0.414	0.798
Error	1272.007	266	4.782		
Total	10738.126	276			
Corrected Total	2130.524	275			

Roe Quality

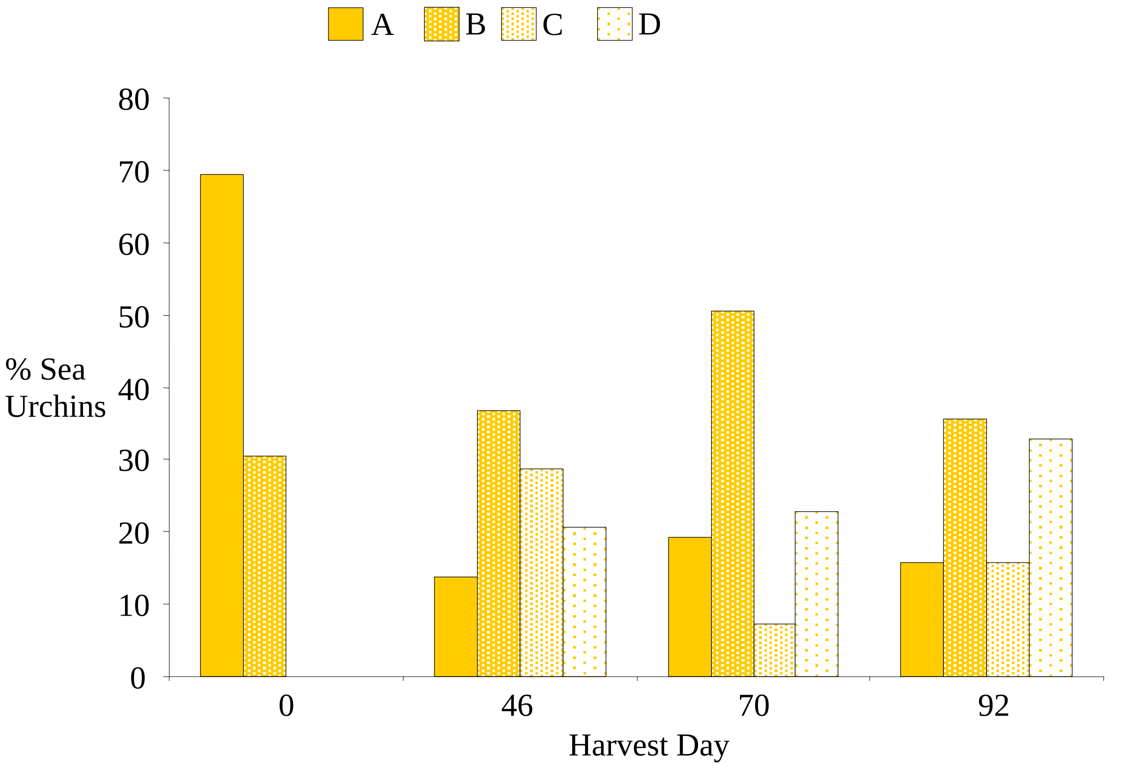
Diet also had no effect on roe colour and texture (P<0.964, Analysis of Deviance) although harvest day did (P<0.001), largely as a result of the narrow range of quality for the initial sample **(Table 2.5.3a).** Colour and texture varied significantly (P<0.001) and there were several significant two-way interactions (**Table 2.5.4**. Interaction between harvest day and colour (P=0.004) occurred as a result of variation in colours one and two between harvests. Texture/harvest interactions (P=0.007) occurred as a result of decreases in coarse and increases in medium texture with harvest day, and colour/texture interactions (P=0.002) occurred as a result of low counts in colours 1 and 4 and in colour 3 for coarse texture.

Table 2.5.3 Accumulated analysis of deviance for harvest day, diet and roe colour and texture plus significant interaction effects

Parameter change			**mean deviance (approximate)**		
	d.f.	**deviance**	**deviance ratio**	**Chi**	**P**
+ harvest day	2	0.7612	0.3806	0.38	0.683
+ diet	2	0.0735	0.0367	0.04	0.964
+ colour	3	73.5663	24.5221	24.52	<0.001
+ texture	2	65.8449	32.9225	32.92	<0.001
+ harvest day .colour	6	18.8903	3.1484	3.15	0.004
+ harvest day.texture	4	14.0743	3.5186	3.52	0.007
+ colour.texture	6	21.3926	3.5654	3.57	0.002
Residual	24	18.2221	0.7593		
Total	107	266.2441	2.4883		

When roe quality data were grouped into grades as described in Table 2.1.1 and subjected to contingency table analysis it was apparent that there was little change in quality over the period of the experiment (Fig 2.5.3). The apparent change between harvest days 0 and 46 is not biologically interesting as very small roe yields are usually fine irrespective of conditions (pers obs.). There was a significant change in the quantity of B and C grade roe between days 46 and 70 (P<0.001, Fishers exact test).

Fig 2.5.3 Percentage sea urchins having roe within each grade at each harvest day



Gut Indices

There were no significant differences between diets (P = 0.276) or harvest days (P = 0.348) with respect to gut index (ANCOVA). The data were therefore pooled and a mean (2.82 $\pm$ 0.046%, n=240) calculated.

Mature Roe

Mature roe is prone to leakage of fluid as observed during the histology work reported in Section 2.4.2. Leakage was an issue in this trial, mainly due to the late start to the experiment, caused by delays in the delivery of the experimental diets. Roe from 50 sea urchins, or 20% of the total (246), was mature and leaked on removal. Of those, 4 were of A, 24 were of B, 13 were of C and 9 were of D grade colour and texture. Sea urchins with mature roe were evenly spread over diets and harvest days so the analysis was not affected by their inclusion, although clearly the grade allocated would be, if this were a commercial trial.

Diets

All diets should have contained 5 ppm, β-carotene as Algro, as specified in Appendix 3. However, this was left out of the formulation of Diet 1 by the diet manufacturer, as discovered during an independent assay carried out by Betatene Pty Ltd, who supplied the Algro. Diet 1 contained less than 1 ppm β-carotene, whereas Diets 2 and 3 contained the expected 5ppm.

2.5.4 Conclusions

Percent final roe yield and percent change in roe yield were lower than expected, due in part, to the low initial % yield. However, in terms of net yield (final yield – mean initial yield) the figures were actually no different from those for the same size sea urchins in the previous trial (Mann-Whitney U, $P = 0.078$). Elucidation of diet-specific consumption and assimilation, and subsequent diet refinement, is clearly necessary, however it appears that at least 3 months are required for significant roe enhancement.

It was also significant that there was no discernible difference in roe colour between diets, given that Diet 1 contained no β-carotene. Apart from Diet 1, analysis of diets, after extrusion, produced values matching the quantity expected from the recipe. In addition, there were no noticeable differences in roe colour between urchins fed artificial diets and those from initial/final field samples in other trials. Although further work is necessary to properly assess this, it may be that additional β-carotene is not necessary to achieve an acceptable roe colour given the presence of kelp in the diet.

2.6. *Field comparison of artificial and natural diets*

(Relevant Objectives: 4,6)

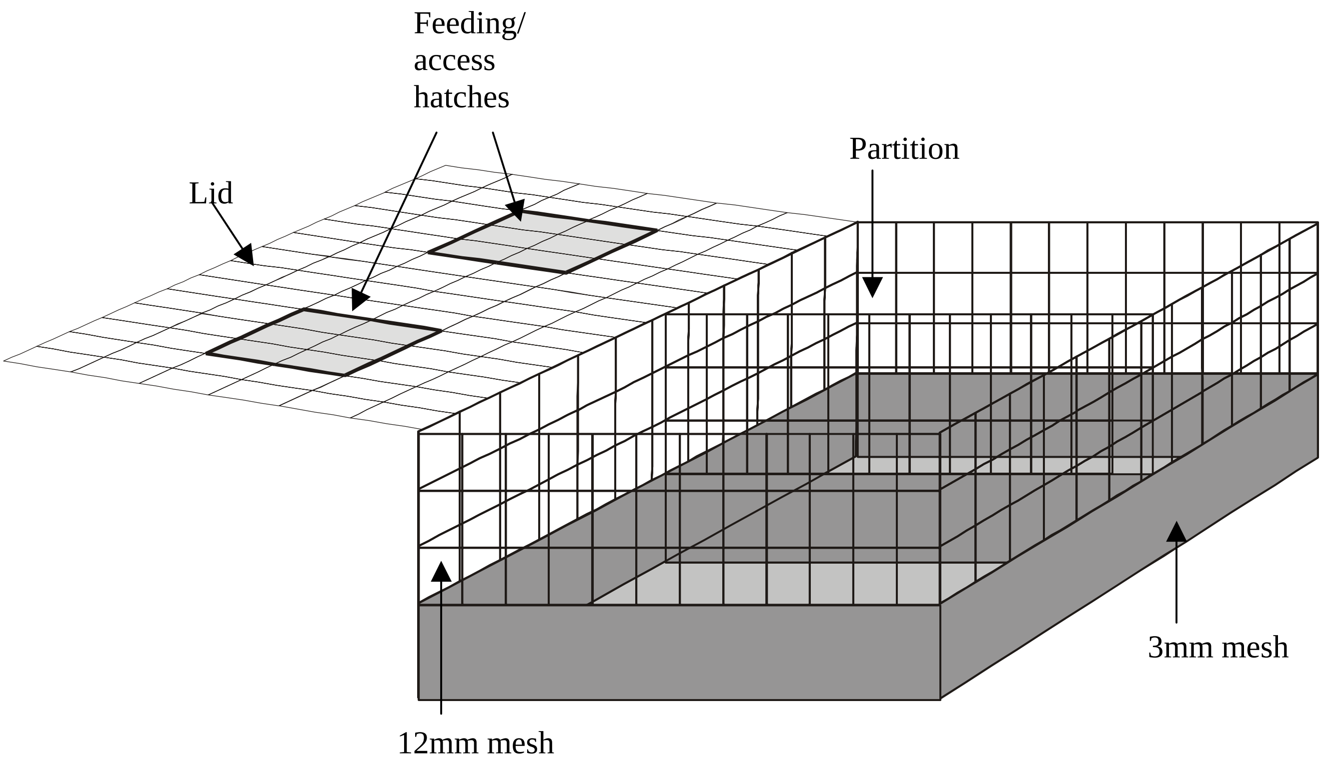
2.6.1 Introduction

A field experiment was run to provide an industry-relevant comparison with the laboratory trials. The work was carried out in collaboration with Terry Scott, a sea urchin fisherman, from Coffin Bay who built the holding cages and fed the sea urchins during the trial.

2.6.2 Methods

Four cages were set up on the shoreward side of an oyster lease offshore from the township at Coffin Bay (34^o 37'S 135^o 28'E). The cages were set up in a row, attached by cable ties to unused oyster racks in 1.5-2m of water, each cage being about 0.3m below the water surface at low tide. The cages **(Fig 2.6.1**) were rectangular, 0.9m long, 0.7m wide and 0.35m high, divided in half vertically by a partition giving a surface area of 1.19 m^2 per section. Two gauges of black plastic mesh were used in construction; the upper two thirds of the cage wall and the lid consisting of 12mm mesh, the lower third, and the floor, of 3mm mesh. Each section had a lockable feeding/access hatch in the top to allow feeding and cleaning of cages.

Fig 2.6.1 Cage used in field trial



Sea urchins (mean TD 66.34±1.38 mm) were harvested from a small embayment on the northern side of the entrance to Kellidie Bay, north-west of the Coffin Bay township on the 27th of September 2002. An initial sample (20 sea urchins) was placed on ice for air transport back to SAASC in Adelaide for dissection and roe evaluation, as described in earlier trials. The remainder (300 sea urchins) were subdivided into 8 groups (4 of 25 and 4 of 50) for assignment to two density and two feed treatments (Fig **2.6.2**) with duplication of densities within diets. The densities selected were 21 and 42 animals/m^2 (25 and 50 per cage section respectively) and the two feed types were the third Wenger diet described in the previous trial (27% CP) and a natural diet consisting of freshly-harvested *Entermorpha* sp and *Ulva lactuca,* both algae forming part of the natural diet of sea urchins in the area from which the experimental animals were harvested. Sea urchins were fed, and cages cleaned, 3 times a week at 4% body weight per day. Data loggers (ibutton, Temperature Technologies Inc.) were placed in the cages to record hourly sea temperature and the trial run for 11 weeks. As the logistics of the experiment prevented initial weight and TD measurement of all animals going into cages, data collected during dissection of the initial sample was used instead.

Fig 2.6.2 Experimental layout for Coffin Bay field trial showing numbers of sea urchins per cage section for each diet

Diet

Artificial	**Natural**	**Artificial**	**Natural**
25	25	50	50
50	50	25	25

After 11 weeks all urchins were removed from the cages, placed on ice and airfreighted back to SAASC in Adelaide for dissection and roe evaluation as described earlier.

2.6.3 Results

Sea urchin test diameter changed significantly in the natural diet treatment during the experiment (ANOVA on log-transformed data, **Table 2.6.1**), although wet weight did not.

Table 2.6.1. Mean test diameter (TD) (±SE) and mean weights (±SE) for each sample/diet in field trial.
Differences tested using ANOVA, and Post Hoc comparisons tested with Tukey HSD

Sample/Diet	**TD(mm)**	**SE**	**N**	**Comparison**	**P**
1.Initial	66.34	1.380	20	1 vs 2	0.009
2. Final Natural	71.22	0.966	108	2 vs 3	<0.000
3. Final Artificial	67.36	0.577	86	1 vs 3	0.773
Total	69.21	0.571	214		
Sample/Diet	**Weight (g)**	**SE**	**N**	**Comparison**	**P**
1. Initial	111.94	8.515	20	1 vs 2	0.273
2. Final Natural	124.95	3.414	106	2 vs 3	0.271
3. Final Artificial	117.16	3.605	85	1 vs 3	0.817
Total	120.58	2.393	211		

Roe Yield

The initial percent roe yield was low at 2.63 ± 0.673 (n=20), reaching a maximum of 19.9% at 11 weeks with the artificial diet showing the highest mean percentage yield (11.16±0.388) (**Table 2.6.2a and b),** an 8.56% change from initial % yield.

It is possible that these yield figures are slightly overestimated because of dehydration of sea urchins during transport on ice from from Port Lincoln. Laboratory trials 3 and 4 had mean TDs of 68.67 ± 0.593mm and 69.43 ±0.34 mm and mean wet weights of 145.5±3.03g and 151.82±2.03g respectively. Animals from laboratory trials were taken directly out of the tanks, allowed to drain for 2 minutes then weighed and initial samples for laboratory trials were brought back alive in seawater then frozen. The present trial had a very similar mean TD (69.43+0.607) but a lower mean wet weight (120.58±2.393, pooled data); possibly making a difference of 1 to 2% in the percentage yield.

Table 2.6.2a Mean initial and final treatment % roe yields ($\pm$ SE) by diet

Diets	Mean % yield	SE	% change from initial yield
Initial	2.63	0.673	
Natural	7.41	0.304	4.85
Artificial	11.16	0.388	8.56

Table 2.6.2b ANOVA table: test of the effect of diet on % roe yield

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	656.700	1	**656.700**	59.624	<0.001
Intercept	16081.457	1	16081.457	1460.099	<0.001
Diet	656.700	1	656.700	59.624	<0.001
Error	2059.608	187	11.014		
Total	**18275.805**	189			
Corrected Total	2716.308	188			

Sixty-three percent of sea urchins fed the artificial diet produced yields of over 10% after 11 weeks (**Table 2.6.3**) but the mortality rate for this treatment was substantially higher than for the natural diet, largely due to water flow problems within the first month. Lifting the cages further away from the seafloor rectified this problem.

Table 2.6.3 Sea urchins with yield above 10% wet weight and % mortality for each sample/treatment

Sample/Diet	% of remaining sea urchins within treatment with yield above 10%	% mortality from beginning of experiment
Initial (n=20)	5	-
Natural (n=108)	20	28
Artificial (n=86)	63	43

Roe Quality

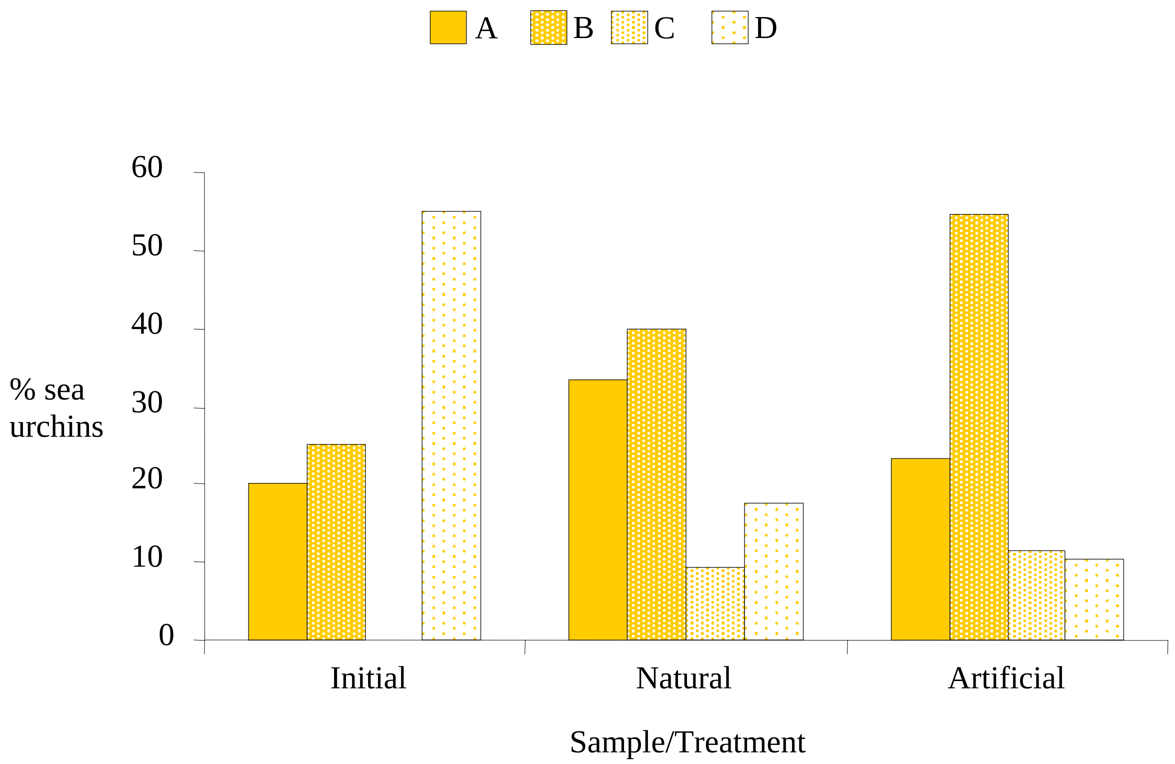
Roe colour differed significantly between treatments (P<0.001) as did texture (P<0.001) (**Table 2.6.4**). There were also significant diet x colour (P< 0.001) and colour x texture (P<0.001) interactions.

Table 2.6.4 Counts of roe of each colour and texture in sea urchins fed on natural and artificial diets

Treatment	**Colour**		**Texture**		
		coarse	**medium**	**fine**	**Totals**
Natural Diet	**1**	0	2	17	**19**
	2	8	29	36	**73**
	3	2	7	7	**16**
	Totals	**10**	**38**	**60**	**108**
Artificial Diet	**1**	1	2	6	**9**
	2	5	16	20	**41**
	3	5	20	11	**36**
	Totals	**11**	**38**	**37**	**86**

As in previous trials the roe quality count data were grouped into grades as described in Table 2.1.1 and subjected to contingency table analysis. There were significant differences in quality between urchins fed the natural and those fed the artificial diet, with the former producing significantly more A-grade roe (P=0.037, Fishers Exact Test) (**Fig 2.6.3**).

Fig 2.6.3 Percentage of sea urchins having roe of each grade within each sample/treatment



Gut Indices

The initial gut index was 0.86 $\pm$ 0.280% (n=20); the final value for the sea urchins on the artificial diet was 1.36 $\pm$ 0.069% (n=84) and on the natural diet it was 1.73 $\pm$ 0.097% (n=106). Final gut index increased significantly (Mann-Whitney U) in the order initial>artificial>natural ($P \leq 0.005$).

The highest mean gut index (i.e. natural diet) was significantly lower than the pooled mean value from the previous trial (i.e. 2.82 $\pm$ 0.046%) ($P<0.001$, ANOVA).

Mature Roe

Mature roe, prone to leakage, was an issue in this trial, mainly due to the late start to the experiment, caused by delays in the delivery of the experimental diets. Forty-nine sea urchins or 23% showed mature roe, out of the 214 animals dissected. Of those, 12 were of A, 23 were of B, 5 were of C and 9 were of D grade colour and texture. Roe from five (i.e. 25%) sea urchins out of the initial sample was found to be mature and, at the final dissection, 16 (14%) of those fed the natural diet and 28 (32%) of those on the artificial diet were also at this stage.

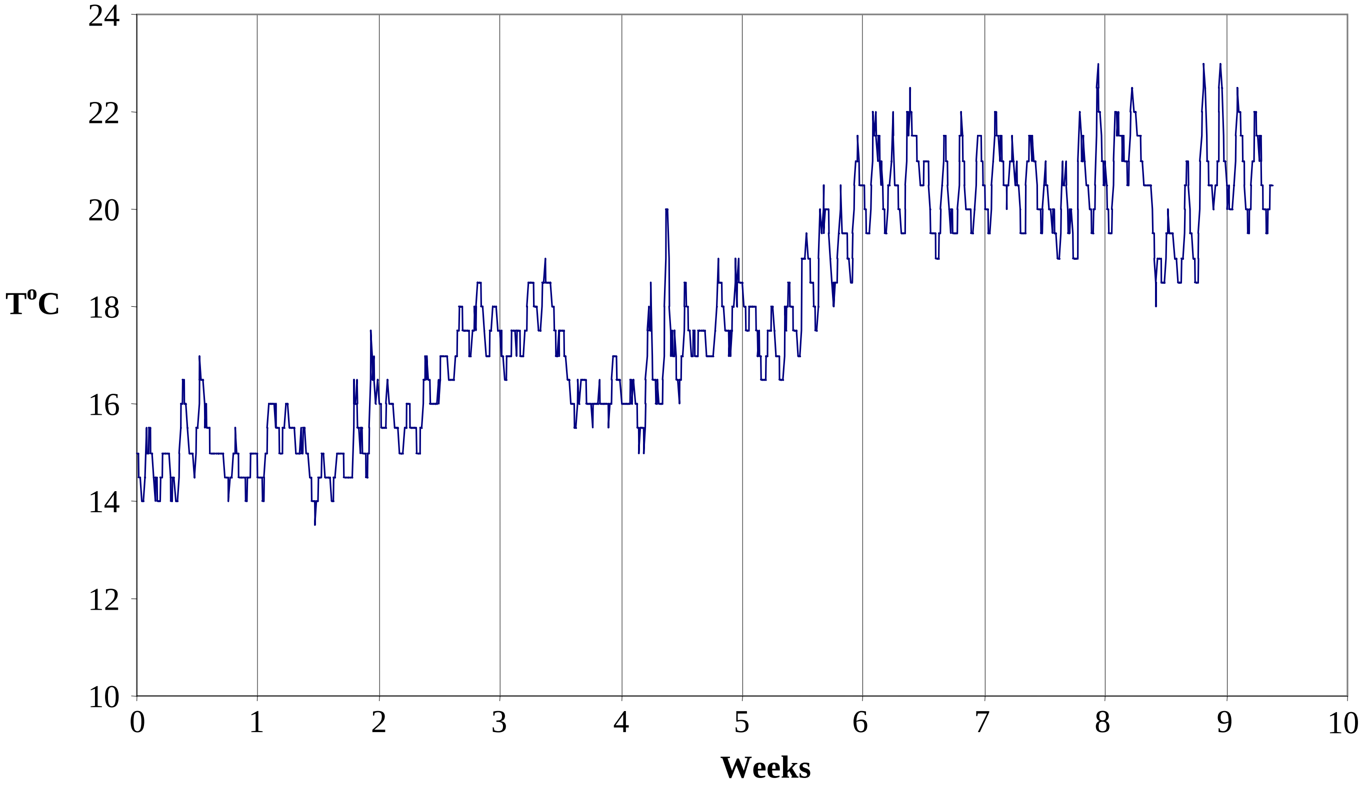
Taste

An industry representative tasted several samples of “A” grade roe from both artificial and natural diet treatments. He was unable to distinguish between the two treatments, and pronounced the roe from the trial to be of high quality.

Field Temperature

Temperature **(Fig 2.6.4)** rose steadily from 15.17°C (± 0.148, n=48) at the beginning of the experiment to 19.92°C (± 0.130, n=48) after 6 weeks, and then changed relatively little, apart from a slight decline at 8 weeks, to reach a mean of 20.70°C (± 0.462, n=48) in the 9th week. The loggers were stolen in the second half of the 9th week so further data were not available.

Fig 2.6.4 Sea water temperature (°C) in Coffin Bay 28/09/02 to 2/12/02.
Mean water depth 2m



2.6.4 Conclusions

The artificial diet produced the highest percent roe yield although the natural diet produced more A grade roe. The sea urchins in the artificial diet treatment did have access to *Ulva* and *Enteromorpha* growing on the cages, however supplementary feeding with locally available aquatic macrophytes may be necessary to boost roe quality to the level achievable with a natural diet. This would allow the use of the current iteration of the diet without further refinements such as increasing carotenoid levels, which would increase diet cost unnecessarily.

The condition of sea urchins was lower than expected, given the previous trial, although it was still higher than that achieved with the Robinson and Castell diet used in the first trial. This was perhaps due to the more energetically-expensive environment of the sea-cages, that is, the need to maintain position and obtain food against swell and tidal influences.

2.7 Comparison between Diet 1, a natural food (*Macrocystis pyrifera*) and diets produced in New Zealand and Norway.

(Relevant Objective(s):1,5)

2.7.1 Introduction

A quantity of Diet 1 (21% CP) was sent over to National Institute of Water and Atmospheric Research (NIWA) in Wellington, New Zealand, for comparison with a sea urchin diet made by NIWA, a Norwegian diet and a local alga (*Macrocystis pyrifera*) over a period of 10 weeks. The sea urchins used (*Evechinus chloroticus*), were housed in plastic-coated wire mesh baskets suspended in tanks supplied with recirculated seawater. There is an expanding aquaculture industry for these urchins, locally known as kina. As the report is produced from the trials is confidential, the methods cannot be detailed here. A results summary is included below. Further details of methodology and results may be available from Dr Phillip James of NIWA in Wellington, N.Z..

2.7.2 Results

Roe Yield

There was no significant difference in mean % roe yield between *E. chloroticus* fed the NIWA diet (10.86%) and those on Diet 1 (11.33%) over a 10 week feeding period. The sea urchins fed both these diets had significantly greater increases in % yield than those fed the Norway diet (P=0.0014) or the algal diet, the latter producing the lowest % yield (mean = 9.48%).

Roe Quality

The algal diet produced roe of the most acceptable colour although the NIWA diet consistently produced the highest percentage of roe of acceptable colour, with Diet 1 producing the least, 10 to 20% less than the NIWA diet. Colours were analysed using a Minolta spectrophotometer, a “Roche” egg yolk colour card and the “Maine” sea urchin roe colour card.

2.7.3 Conclusion

The diet produced by NIWA produced the highest yield of the most acceptable colour roe for *E. chloroticus*. Diet 1 produced equally good yield but colour problems were evident, probably due to the fact that β-carotene had been left out of the formulation by the manufacturer. These differences were not evident during trials (Chapter 2.5) in South Australia possibly due to species-specific differences in carotenoid requirements and to lower resolution in discrimination between roe colours. It is likely that Diet 1 would produce as good a roe colour as the NIWA diet if the β-carotene had been included.

2.8 *Stability of artificial diets used in Trials 3 to 6.*

2.8.1 Introduction

Laboratory trials were run to test the stability of the Wenger diet and the three AESEC diets. Physical (weight loss) and biochemical change (protein and energy) were monitored over 48 hours and sinking rates were tested, the latter on the three AESEC diets only.

2.8.2 Methods

Leaching and biochemical change

The Wenger diet used in Trial 3 and the extruded Wenger-style diets made at AESEC and used in Trials 4 to 6 were tested for loss of weight, energy and protein during leaching, based on a method developed by Dr Dave Smith, QDPI, Queensland.

In each case duplicate 15-20g samples of each diet were weighed into tared, plastic, 70ml containers with 300μm mesh ends and the containers placed in the tanks, previously used in Trials 3 and 4, for periods up to 48 hours. For each 48 hour trial, 6 pairs of duplicate samples of each diet were prepared with a pair removed from the water at 1, 2, 3, 17, 24 and 48 hours. After removal from the tanks, containers (with samples) were rinsed in distilled water to remove excess salt and dried in an oven (55^{o}C) for 16 hours. The containers (with samples) were allowed to come to room temperature in press-seal bags with desiccant then removed from the bags and weighed to 0.1mg. Duplicate initial samples (i.e. Time 0) were also prepared as controls (i.e. no leaching) and dried along with the experimental samples. The difference in container (with sample) weights before and after the trial minus the weight loss measured after drying of the controls was taken as the weight loss due to leaching.

Dry samples of leached diet and controls were then finely ground with a mortar and pestle then analysed in duplicate for crude protein (%) and energy (MJ/Kg) by staff at the SARDI Pig and Poultry Production Institute. Protein was determined by the Soil NC method using a LecoCN2000, "SoilNC" combustion at 1350^{o}C. This method determines the nitrogen content in the sample which is then multiplied by 6.25 to estimate the protein content. Gross Energy was determined using a Parr 1281 Bomb Calorimeter.

Sinking Rates

Standard sinking rate tests were carried out on the three SARDI AESEC diets. Twenty-five pellets of each diet were dropped one at a time into a 3m cylinder of salt water from a distance of ten cm above the water surface. The time taken to reach the bottom was recorded.

2.8.2 Results

Leaching and biochemical change

Significant % dry weight loss occurred over the 48hr period (Fig 2.8.1, Table 2.8.1, ANCOVA), with up to 20% loss recorded for Diet 3 (26.7% CP) and the Wenger Diet. The only overall significant difference arose between Diet 2 and the Wenger diet (P=0.003, Tukey HSD).

Fig 2.8.1 Weight loss in four diets as a percentage of initial weight after leaching for 48 hours.
Duplicates at each time point are shown.

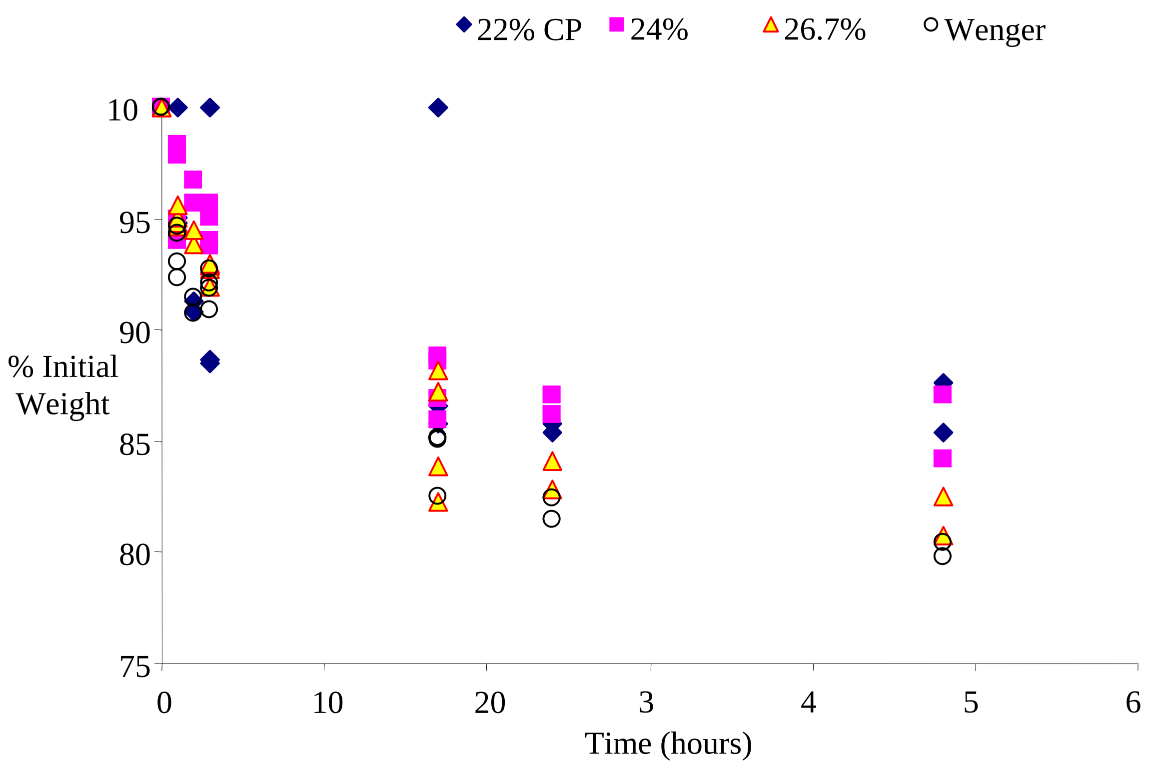


Table 2.8.1 Weight loss in four diets after leaching for 48 hours.
ANCOVA table for comparison between diets with hours as the covariate

Source	Type III Sums of Square	df	Mean Square	F	P
Corrected model	1595.708	4	398.927	35.521	<0.001
Intercept	1426.345	1	1426.345	127.003	<0.001
Hours	1488.338	1	1488.338	132.523	<0.001
Diet	105.978	3	35.326	3.145	0.031
Error	774.925	69	11.231		
Total	8742.619	74			
Corrected Total	2370.633	73			

All diets showed a significant loss in protein and energy content over the 48 hour leaching period (ANCOVA, Tables 2.8.2a and 2.8.2b). All three AESEC diets showed significantly more energy (MJ/Kg) and % protein change than the Wenger diet (both P<0.001, Tukey HSD) (**Table 2.8.2a and b, Fig 2.8.2a and b)**. The marked increase in % protein at 48 hours , especially in the 26.7% diet, was presumably due to loss of other components.

Table 2.8.2a. Percent protein loss in four diets after leaching for 48 hours.
ANCOVA table for comparison between diets with time as the covariate.

Source	Type III Sums of Squares	df	Mean Square	F	P
Corrected model	14.495	4	3.624	24.976	<0.001
Intercept	5945.903	1	5945.903	40981.995	<0.001
Time	5.727	1	5.727	39.470	<0.001
Diet	10.886	3	3.629	25.011	<0.001
Error	4.498	31	0.145		
Total	10048.347	36			
Corrected Total	18.992	35			

Table 2.8.2b Energy loss (MJ/Kg) in four diets after leaching for 48 hours. ANCOVA table for comparison between diets with time as the covariate. Data were arcsine transformed.

Source	Type III Sums of Squares	df	Mean Sq	F	P
Corrected model	0.0149	4	0.0037	24.994	<0.001
Intercept	0.601	1	0.601	40247.032	<0.001
Time	0.0059	1	0.0059	39.454	<0.001
Diet	0.0012	3	0.0037	25.042	<0.001
Error	0.0046	31	0.00015		
Total	1.014	36			
Corrected Total	0.0195	35			

Fig 2.8.2a Change in energy content (MJ/Kg) in four diets after leaching for 48 hours. Duplicates shown at each time point.

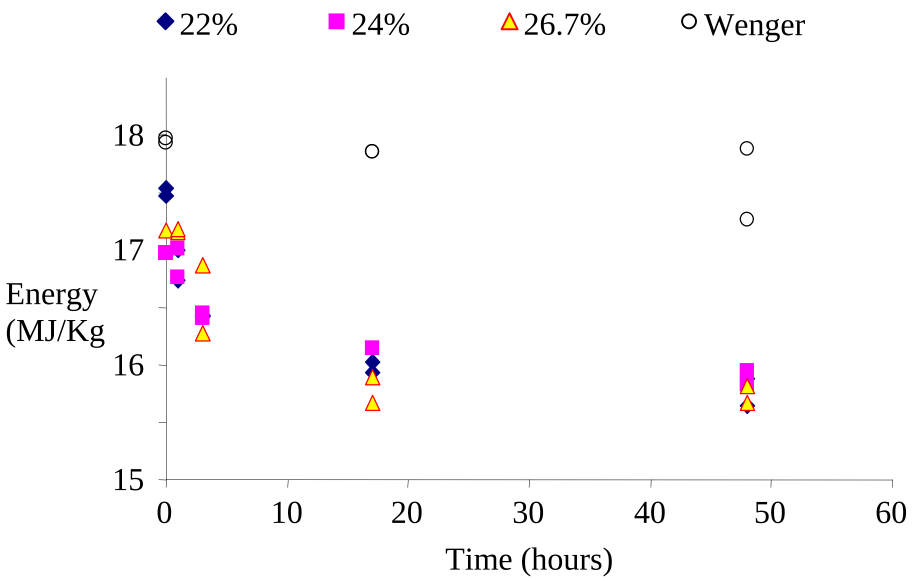
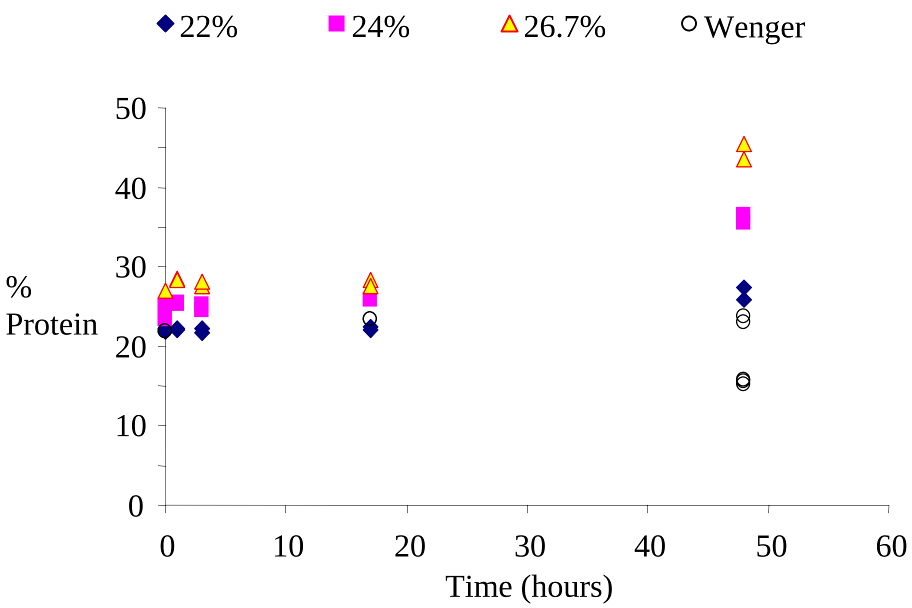


Fig 2.8.2b Change in % protein in four diets after leaching for 48 hours.



Sinking rates

Sinking times varied from 30.3 (±1.482) seconds for Diet 1 (21%CP) to 18.6 (± 0.375) seconds for Diet 3 (27% CP) (Table 2.8.3). The difference in sinking time was significant (Table 2.8.4a) with the more nutrient-dense diets sinking faster (Table 2.8.4b).

Table 2.8.3 Mean sinking times (seconds) (±SE) for each of three AESEC diets
CP 21%, 24% and 27%. The test for Diet 1 was repeated as a control.

	Diets			
	21%	**21%**	**24%**	**27%**
Mean	30.3	29.3	22.0	18.6
SE	1.48	1.17	0.34	0.38

Table 2.8.4a ANCOVA table: comparison between sinking times of three AESEC diets.
CP 21%, 24% and 27%. Analysis carried out on log-transformed data. Two pellets from Diet 1 floated and are not included in the analysis.

Source	**Type III Sums of Squares**	**Df**	**Mean Square**	**F**	**P**
Corrected model	0.698	3	0.233	48.947	<0.001
Intercept	187.681	1	187.681	39501.271	<0.001
Diet	0.698	3	0.233	48.947	<0.001
Error	0.447	94	0.0048		
Total	188.595	98			
Corrected Total	1.144	97			

Table 2.8.4b Pair-wise comparisons for the above analysis

Diet	**21% repeated**	**24%**	**27%**
21%	0.585	<0.001	<0.001
24%			<0.001

Conclusions

The three AESEC diets showed significantly more energy loss than the Wenger diet over the 48hour trial period. The % protein level in the AESEC diets actually increased, at least in part due to the loss in energy. Diet stability is an important issue and improvements are clearly necessary in his area.

Sinking rate is an issue in sea-cage culture with swell and tide acting on the cage environment. Further work is necessary to optimise nutrient density in relation to diet cost.

Chapter 3 General Discussion

3.1 Photoperiod, temperature and season

Photoperiod and temperature had no effect on % roe yield within the timeframe allocated. This experiment looked at a constant natural average Spring photoperiod (12h 26min L: 11h 34min D) and compared it with ambient Spring photoperiod for urchins which had been harvested in the middle of Winter (early July). Devin and Walker (2001) also ran laboratory trials looking at the effect of photoperiod, in this case sourcing animals from a Maine (USA) population of the green sea urchin, *Strongylocentrotus droebachiensis*. They also compared fixed (12h light; 12 h dark) with ambient, in this case, winter, photoperiod and achieved a significant result with the fixed photoperiod producing the highest % roe yield (24.6 $\pm$ 4.4%) over 3 months from an initial yield of 8.6 $\pm$ 3.5%. Their ambient treatment produced a yield of 20.8 $\pm$ 3.4%. Both Devin and Walker's treatments were fed the same artificial diet, a Wenger diet, similar that used in Trial 3 of this study. They ran the experiment again (12L:12D, and summer photoperiods) in summer; however in this case there were no significant photoperiod effects, perhaps not surprising given that S *droebachiensis* roe undergoes the reserve accumulation and gonads mature during autumn and winter (Walker and Lesser, 1998). In the present study, the diet was certainly sub-optimal but the result for photoperiod was highly non-significant (P=0.315), suggesting a much longer period would be necessary to achieve a significant result. Alternatively, the difference in photoperiod between treatments may have been too subtle to be effective.

Trial 3 suggests that a higher yield is possible with a mean initial roe yield of 4% increasing to mean of 12.2% over twelve weeks for medium sized sea urchins in the laboratory and, in Trial 5 in the field, from 2.6% to 11.2% over a 11 week period. Manipulation of photoperiod and temperature have become of less importance since this project's inception, as the industry has become more interested in rapid, cost effective, sea-cage-based enhancement of roe yield. The latter notwithstanding, temperature does have some significance from the point of view of the appropriate season for enhancement. The appropriate season is critical, if roe is left too long, becomes mature and leakage occurs on removal from the animal, the product is of limited value. The field trial was initially planned for the Autumn-Winter period as spawning begins during November-December in Coffin Bay, but this was prevented because of delays in diet production by the AESEC. Given that some of the roe from sea urchins in the trial showed evidence of maturity, this suggests, as above, that enhancement of roe be carried out during the cooler months, April to September.

3.2 Sea urchin size and roe yield

It was suggested (Section 2.4) that medium-sized sea urchins (circa 70mm TD, 150g wet weight) were the most appropriate for further work, and by extension, enhancement in general. Larger sea urchins produced more coarse roe than those of intermediate size and smaller animals produced a lot of fine-textured roe, but most of this was very light in colour, and, as a consequence, little could be classified as A grade. This choice is echoed by industry in that roe from large sea urchins is generally considered too coarse to be economically useful and such animals would usually be the first harvested from a virgin stock to allow smaller animals access to food and space (T. Scott pers comm). Further to this, **Table 3.2.1** illustrates the effect that differences in yield may have on the final production of the different grades for each size within an enhancement facility, based on the results of Trial 3, and assuming that the relative yields per grade found in this trial would translate to the field. This example suggests that medium sea urchins may produce 75% more high-grade (A or B) roe per tonne than large sea urchins and 18% more than small sea urchins. Also, in order to achieve the same production levels shown by 1 tonne of medium-sized sea urchins, a facility would require 1.3 tonnes and 2.5 tonnes of large animals to produce the same amount of A and B grade roe respectively. Similarly 3.9 tonnes and 1.4 tonnes of the small sea urchins would be required to equal the production achieved by 1 tonne of medium-sized sea urchins.

Table 3.2.1. Estimates of size, diet and grade-specific roe production levels for culture based on the results of Trial 3 (laboratory)
*[1] Based on mean yield (refer Table 2.4.3) *[2]Tonnage of sea urchins required for Trial 3 is that for large and small sea urchins to get to medium sea urchin production levels for grades A and B.

	Kg roe in 1 tonne of sea urchins	**Percentage of roe of each grade from trial**		**Estimated roe (kg) of each grade per tonne**			**Tonnage of sea urchins required per grade*[2]**	
Size		**A**	**B**	**A**	**B**	**A+B**	**A**	**B**
Large	107.86	8.3	25.9	9.0	28.0	37.0	1.3	2.5
Medium	122.75	9.8	42.6	12.1	52.3	64.4		
Small	117.56	2.6	42.9	3.1	50.4	53.4	4.0	1.4

Furthermore, in 1998, Hagen suggested that there is a 'physiologically determined optimum size' at which gonad yield is maximised. This optimum size for *S. droebachiensis* was in the intermediate range, as found in the present study. Above this level, in his case 55-60mm TD, roe yield declined, suggesting a form of senescence in larger sea urchins. Data from Dix 1977) suggests a similar phenomenon for a Tasmanian population of *H. erythrogramma*. Samples taken at 20 sites (n=3215) around the Tasmanian coast showed a mean yield peaking at 10.4% between 80 and 85mm TD, declining to 4.9% for sea urchins between 85 and 90mm TD. The large sea urchins in the present study (mean 80.7mm TD, range 75.1-88.3 mm TD), as the largest found in the population from which they were harvested, may also have been undergoing senescence although the difference (Medium sea urchins (70.5mm) 12.3%, large urchins 10.8%) was not as marked. This issue clearly requires further investigation. Such senescence has also been reported for *S. purpuratus* (Gonor, 1972) and *Evechinus chloroticus* (McShane and Anderson, 1997). McShane and Anderson reported a reduction in size of the gonads of very large individuals (>150mm TD), suggesting that the maintenance of a large body mass may limit the energy available for reproduction.

The small sea urchins (55.5$\pm$0.47 mm TD, range 44.1-59.9mm) although bracketing the size of maturity (SOM) reported by Dix (1977 – 55mm TD) may have been too young to produce well-developed roe. In the sample taken for histology, all small sea urchins showed signs of reproductive activity. Some proportion of non-functional gonads might have been expected at such a size, given the proximity to the SOM, however as Barker et al (1998) reported for *H. erythrogramma*'s co-famililial *Evechinus chloroticus*, in a study using artificial diets (Barker et al, 1998), precocious gonad formation does occur, with sexual maturity determined by the availability of nutrients, not sea urchin size. This cannot be confirmed for *H. erythrogramma,* as initial histological samples were not taken.

It appears that *H. erythrogramma* is able to produce commercial-level roe yields over reasonable time frames in aquaria and in sea-cages using either available artificial diets or modifications of these in combination with naturally occurring aquatic macrophytes. The latter is suggested by the increase in the proportion of higher quality roe in the artificial diet treatment in the sea-cage trial in comparison with that using the same diet in the laboratory. Although not quantified, it was clear from gut contents that sea urchins in the artificial diet treatment were also feeding on algae (*Ulva* and *Enteromorpha*) growing on the cages. The faeces were deep green in colour and closer examination, with a dissecting microscope, showed algal fragments.

As discussed above, Devin and Walker (2001), using the green sea urchin (30-45mm TD), *Strongylocentrotus droebachiensis,* achieved a % roe yield of 24.6 $\pm$ 4.4% over 3 months from an initial yield of 8.6 $\pm$ 3.5%, a mean of 1.3%/week. Similarly, Robinson et al (2002) achieved a mean roe yield of 17% from 3.2% over 65 days, a rate of 1.6%/week using the same species (50mm TD) fed artificial diets differing in β–carotene source. Using the size:weight relationship published for *S. franciscanus,* a commercial species found off the coast of California, USA, (Mc Bride et al 1998) and assuming its applicability to *S. droebachiensis,* this is equivalent to about 10g and about 7g roe/urchin respectively for the two studies (i.e. Devin and Walker, 2001 and Robinson et al 2002), or between 5 and 7% roe yield for *H. erythrogramma* of 70mm used in the present study (**Table 3.2.2**).

The accumulation rate, in the present study was lower, at a maximum of 0.68%/week for laboratory trial 3 and 0.72%/week for the field trial. A range of sizes (40-45mm, 60-65mm and 70-75mm TD) of *Loxechinus albus* produced 28%, 17% and 15.8% yield respectively, from initial yields of 3%, 8.5% and 9.5% (Olave *et al* 2001) using a similar diet and the same timeframe (12 weeks) used in the present study. The respective accumulation rates declined with size *viz*: 2.08%, 0.71%, 0.46%/week. There appears to be a general decline in gonad production rate with size, possibly mediated by the increasing costs of maintenance, as urchins become larger and/or a form of senescence as discussed earlier. A similar decline was evident in the present study, at least for the larger size classes (medium 71.5mm $\pm$ 0.40mm and large 80.7mm $\pm$

0.44mm TD) with rates of 0.71% and 0.53%/week respectively, although this is not always the case as the small size class (55.5 ± 0.47mm) used in Trial 3 accumulated roe at only 0.62%/week. *Evechinus chloroticus* (Barker et al, 1998) showed slightly increasing rates for 50-60mmTD and 70-80mmTD sea urchins at 1.13 and 1.25%/week over a twelve week period. In the latter case, larger sea urchins were not tested; it is not known whether the pattern that appears in the present study would also occur for *E. chloroticus.*

Table 3.2.2. Conversion from % roe yield to roe wet weight (g) for *H. erythrogramma* at a range of sizes.
Whole Weight = $0.0012*TD^{2.764}$. The commercial cut off point is usually considered to be 10% roe yield (dashed line).

Sea urchin wet weight (g) (TD)	59.58 50	98.63 60	151.02 70	218.43 80	302.49 90
% Yield	**Roe wet weight (g)**				
1					
2			3		6
3		3	5	7	9
4		4	6	9	12
5	3	5	8	11	15
6	4	6	9	13	18
7	4	7	11	15	21
8	5	8	12	17	
9	5	9	14	20	
10	6	10	15	22	
11	7	11	17		
12	7	12	18		
13	8	13	20		
14	8	14	21		
15	9	15			
16	10	16			
17	10	17			
18	11	18			
19	11	19			
20	12	20			
21	13	21			
22	13	22			

The above suggests that, in terms of yield, *H. erythrogramma* is able to produce quantities of roe that compare well with other species for which there has been interest in roe enhancement. Further to this, the figure of 10% yield, which is often quoted as necessary for commercial-viable operations, may be a little misleading. This figure appears to have originated with small northern hemisphere species such as *S. droebachiensis* (Motnikar et al, 1997). Smaller species such as *Paracentrotus lividus,* which reaches market size at 40mm TD (cf *S. droebachiensis* 50-60mm TD), require a yield of 20% of wet body weight to make the harvesting and processing of roe viable (Moylan, 1997). Most of the processing cost is in the labour involved in individually opening sea urchins to extract roe; a reduction in yield may have a significant effect on cost and viability. For example, processing 50-55mm *S. droebachiensis* with 12-14%

yield has a labour cost component of 20% of that of urchins with 7% yield (pers comm. Carl Parsons, Green Seafoods, Newfoundland. (Refer Appendix 6 for further details). Given that a processing operation would be based on the absolute yield (i.e. kg roe produced per tonne of sea urchins) it is probable that the optimum % yield for a *H. erythrogramma* fishery would be less than 10%. The level required to sustain an additional roe enhancement operation, with additional husbandry costs (eg food, labour), would presumably be somewhat higher but is beyond the scope of this report.

3.3 Gut index

The sea urchin gut wall functions as a storage organ, particularly of lipids and carbohydrates (Lawrence, 1976; Lawrence and Lane 1982; Fernandez, 1997) and therefore may be used as an index of nutritional state (Lawrence, 1976). The gut index (GTI) generally shows seasonal patterns correlating with both somatic and gonadal growth (Lawrence and Lane, 1982; Klinger et al 1997).

In the present study, the GTI was low in Trial 1 (0.842%) (Table 3.3.1) with no effect of diet and no change observed during the experiment, by contrast in Trial 3 there was a significant increase in GTI during the trial and a significant decline in final GTI with increasing size from 3.53% for the smallest to 2.46% for the largest sea urchins. Fernandez and Pergent (1998) reported a similar size effect, feeding a variety of artificial diets to *Paracentrotus lividus.* The final pooled mean GTI, and therefore the condition, for sea urchins in Trial 1 was also less than the initial mean GTI for Trial 3 (0.842% cf 1.108%). This, in combination with the lower yield in the first trial, suggests that the Canadian diet is nutritionally less adequate than the Wenger diet for *H. erythrogramma*.

Table 3.3.1 Mean gut index (gut wet weight (g)/whole sea urchin wet weight (g) x 100) for each trial.
A mean based on pooled data is given where there were no significant differences between treatments within a trial.

Trial	Name	Treatment	Mean gut index (%)	SE
1	The effect of protein level and oil type on roe yield and quality	Pooled	0.842	0.031
3	The effect of a semi-moist extruded diet and sea urchin size on roe yield	Initial	1.108	0.12
		Small	3.53	0.194
		Medium	2.86	0.106
		Large	2.46	0.083
4	The effect of protein level and enhancement time on roe yield	Pooled	2.82	0.046
5	Field comparison of artificial and natural diets	Initial	0.86	0.280
		Artificial diet	1.36	0.069
		Natural diet	1.73	0.097

McBride et al (1998), feeding artificial diets varying in protein level to *Strongylocentrotus franciscanus*, and Fernandez and Pergent (1998), working with *Paracentrotus lividus*, reported that food type had no effect on GTI, as found in Trial 4 (Table 3.3.1). However, this was not the case in the field trial where the natural diet produced a significantly higher GTI but a lower % roe yield than the artificial diet. GTI does not appear to correlate with the % roe yield *per se*, perhaps being more indicative of storage of excess carbohydrate and/or lipid from the diet. Trial 3, using the Wenger diet produced a high % roe yield and high GTI, Trial 4, using a locally made diet based on the Wenger formulation produced a low roe % yield and a high GTI, almost identical to that produced by the same-sized sea urchins in the previous trial. The protein level in the Wenger diet in Trial 3 was the same as that used in Diet 1 in Trial 4. It was also of interest that the field trial, based on sea urchins harvested from Coffin Bay, produced lower initial and final GTI's than recorded in Trial 3 and 4, which were based on sea urchins sourced from Wallaroo. Further work is clearly necessary to determine the effect of initial condition (i.e. GTI), harvest season and area and diet composition on final GTI and roe yield.

3.4 Roe enhancement time

The results of the fourth laboratory trial (mean final roe yield 7.32%) suggested that between 70 and 92 days were necessary to ensure that at least 20% of sea urchins produced an adequate roe yield; which, for lack of a better, commercially relevant, estimate for *H. erythrogramma*, was considered to be at least 10% of wet body weight. Presumably the length of time necessary to achieve a similar level of enhancement in Trial 3 would have been reduced, as the final mean % yield, for similar-sized sea urchins, was higher (12.28%) after 84 days, as was the initial yield (4.01% compared with 1.63%). In this case, 74% of sea urchins produced yields of over 10%. In the field trial, 80 days were sufficient to achieve a mean yield of 11.2% (initial yield 2.63%), a similar % increase to that found in Trial 3 (Trial 3:8.27%, Trial 5: 8.57%). In this case, 63% of sea urchins produced yields of over 10%.

It is suggested that 2.5 to 3 months are sufficient to achieve a good average yield of reasonable quality roe at the time of year/temperature the trials were run. Other workers have used similar time periods. For example, high % roe yields were achieved by Robinson *et al* (2002) who ran a trial over two months, in New Brunswick, Canada, from mid Summer to early Spring using *S. droebachiensis* and by Lawrence *et al* (1997) who ran a series of trials, using *Loxechinus albus*, in tanks and sea-cages over 3 months from mid-Winter to mid-Spring and over Summer in Chile. In 2001, Olave *et al* used the same period for *Loxechinus albus* in sea-cages and also achieved reasonable yields (Table 3.4.1).

Table 3.4.1 Mean percent roe yield achieved in published studies over two to three months

Author	Site	Diet	Time (months)	Initial roe yield (%)	Final roe yield (%)
Robinson et al 2002	Laboratory	Artificial	2	7	20
Lawrence et al 1997	Sea-cages	Artificial	3	5.5	22
	Laboratory	Artificial	3	5.3	21
Olave et al 2001	Seacages	Artificial	3	8.5	17

Season and temperature may have some impact on enhancement time although this is not necessarily the case, as indicated in the present study and as reported by McBride *et al* (1997) when investigating the effect of temperature (12.9 and 16.1^{o}C) on gonad production in *S. franciscanus*. McBride *et al* found that sea urchins increased feeding rate at the higher temperature but they reported no difference in final % roe yield between the two treatments. In the present study, late delivery of diets by the manufacturer precluded the carrying out of winter laboratory and field trials with Wenger-style diet formulations.

3.5 Roe quality

Artificial diets produced higher gonad yields than natural (i.e. macroalgae) equivalents for this and other species of sea urchin (Robinson and Colbourne, 1997; Lawrence et al 1997; Barker *et al*, 1998; Cook et al, 1998; Olave et al 2001). This not surprising given the greater nutrient concentration of the former and the higher nutritive variability of the latter.

Achieving good quality roe is not as straightforward. Quality is a combination of colour, texture and taste. High quality roe (i.e. that which sells on the Japanese market) is generally fine and bright orange, yellow or gold in colour and when placed on the tongue, melts without leaving a residue or a bitter taste. We were unable to carry out extensive taste-testing in this project, however the industry representative with whom we were working was very satisfied with the quality, including taste, of the roe from both artificial and natural diet treatments in the field trial carried out at Coffin Bay.

Gonad colour in sea urchins is due to a variety of carotenoids, which function to buffer eggs and sperm against oxidation (Tsushima et al 1995). For *H. erythrogramma* the most important of these is β-echinenone (Tsushima et al 1995), derived from β-carotene in the diet (Tsushima et al 1993). Other pigments found in this species include α-isocryptoxanthin, from which β-echinenone may be derived as it is in *Pseudocetrotus depressus* (Tsushima et al 1993) but neither the ovaries or the testes of *H. erythrogramma* contain astaxanthin, commonly used in the fish culture industry to colour the flesh of salmonids (Robinson et al 2002). The latter pigment has been shown to be ineffective in adding colour to sea urchin gonads (Motnikar et al, 1997; Harvardsson et al, 1999)

Barker *et al* (1998) found that *E. chloroticus* produced very light white to cream-coloured roe when fed on artificial diets. This included one diet containing 14% kelp meal, suggesting that either the presence of kelp does not necessarily ensure satisfactory roe colour or that chemical changes occurring during air-drying of the kelp, in preparation for inclusion in the diet, affected its nutritional value (De Jong Westman *et al*, 1995). Barker *et al* (1998) reported that sea urchins fed on an algal diet (a mixture of *Macrocystis pyrifera* and *Ulva lactuca*), produced roe that was more yellow to orange. In the present study, the quantity of A-grade roe increased with the quantity of fresh algae in the diet. Thus 10% of roe, from medium–sized sea urchins, was of this grade in Trial 3, using three artificial diets in the laboratory; increasing to 23% (22% without mature roe) in the artificial diet treatment in the field with algae consumed as well, and to 33% (32% without mature roe) with natural diet in the field. However, in this case, this relative reduction in roe quality produced by the artificial diet in the field trial was balanced by the higher yield (**Table 3.5.1**). In addition, it is estimated that more than twice the quantity of B-grade roe would be produced by the artificial diet than by its natural counterpart.

Table 3.5.1. Percent roe yield (without the sea urchins showing mature roe) from sea urchins producing roe of each grade within the sea-cage trial and estimated relative roe production from 1 tonne of sea urchins using artificial and natural diets in sea-cages in Coffin Bay.
*Estimated weight of sea urchins based on percentage of urchins producing roe of each grade within trial

	% Yield/Diet		***Estimated sea urchins (kg)**		**Estimated roe (kg)**	
Grade	**Natural**	**Artificial**	**Natural**	**Artificial**	**Natural**	**Artificial**
A	8.06	12.03	326	228	26.3	27.4
B	7.14	11.5	404	561	28.9	64.6
C	10.08	13.35	90	123	9.1	16.4
D	5.35	9.89	180	88	9.6	8.7

With *H. erythrogramma* roe texture does appear to relate to ova size, with finer roe containing smaller ova, but this species is unusual in that it has a large lipid-rich egg (450 μm diameter) that develops into an abbreviated non-feeding larva in about 3.5 days (direct development; Williams and Anderson, 1975). Such a strategy occurs in only 5% of sea urchins for which there are data (Edward and Minor, 2001). 65% of sea urchins (Edward and Miner, 2001) develop indirectly, including most, if not all, other species of interest to aquaculture, such as *Evechinus chloroticus*, *Loxechinus albus*, *Lytechinus* *variegatus*, *Paracentrotus lividus* and *S. droebachiensis* (Lawrence, 2001). The remainder (30%) are termed brooders as they retain offspring among the spines (Edward and Minor, 2001). Indirect development is characterised by very small eggs, a feeding pluteus larva with a planktonic phase of weeks to months and small juveniles (Lawrence, 2001). For example, *Lytechinus variegatus* has a small (70-250μm) egg that develops via a typical plankotrophic larva over 5-100 days, producing a juvenile of 250-700μm, compared with the 500-3000μm juvenile produced by *H. erythrogramma* (Lawrence, 2001). The fact that most sea urchin research is carried out on indirect developers may explain, in part, why texture is not perceived as an issue, small ova/eggs may naturally produce fine roe, thus the focus has been on colour and taste instead. Roe texture is clearly a concern in *H. erythrogramma* and the use of diet components such as cholesterol, which appear to enhance egg size (De-Jong Westman et al 1995) in other species (i.e. *S. droebachiensis*), and also increase the cost of the diet, should be revisited.

There is also the potential for selection by fishers/culturists in response to market demand, for urchins containing premature roe, that is, gonads that are not completely full of gametes and beginning to show signs of gamete release (ie. having mature roe) as was the case in Trials 4 and 5 in this study. As shown above (**Figs 1.4.1 and 1.4.2),** sea urchin gonads go through an annual cycle of reserve accumulation and spawning with optimum yield achieved just prior to spawning.

3.6 Diets

It is difficult to directly compare the various artificial diets used in this study given the differences in form and in the timing of the trials. However, it is clear that once semi-moist extruded pellets could be produced through the extruder at AESEC, % yield and quality improved markedly. Although diet form was not tested *per se*, it does appear that the gelatin-bound pellets of the formulation used in Trials 1 and 2 did not provide adequate nutrition to be considered for roe enhancement. Extruded diets, in general were denser and more water-stable than bound equivalents and have been used successfully for roe enhancement in other sea urchin species (i.e. *Loxechinus albus*, Lawrence et al, 1997, Olave et al 2001; *Strongylocentrotus franciscanus* McBride et al, 1998; *Strongylocentrotus droebachiensis* Walker and Lesser, 1998, Robinson et al, 2002). There are exceptions, as the NIWA's gelatin-bound diet produced a very similar mean % roe yield to that achieved with the first AESEC formulation. The roe colour was also more acceptable with the NIWA diet, but this was probably because β-carotene had been inadvertently left out of the AESEC formulation, however the latter did not appear to affect roe quality in Trial 4.

The Wenger (USA) diet used in Trial 3 produced the best roe of any of the laboratory trials. However, its iterations, varying in nutrient density, did not produce roe of comparable size or quality, except in the field trial. There is still some way to go to produce an optimum diet for roe enhancement, but despite current limitations, the local-produced version does produce reasonable yields of quality roe in the environment in which culture is likely to occur. It is clear that further trials will have to be run to refine the diet in terms of stability and formulation, to move towards a nutritional optimum and to reduce cost. It may be that cholesterol is not necessary and, if the diet is to be used in sea cage culture, added carotenoids may also be superfluous. Dr Bob Hooper (Memorial University of Newfoundland) has achieved very good roe colour in *Strongylocentrotus droebachiensis* by adding kelp alone to a diet rather than relatively expensive carotenoids (pers comm).

4. Benefits

This research has provided a basis for post harvest roe enhancement by the sea urchin industry as intended in the original application. Industry has been enthusiastically involved in many phases of the project, including the workshop, sea urchin collection, method standardisation and the field trial. The industry member with whom most of the collaboration has taken place is very satisfied with progress towards a sea-cage roe enhancement diet and, in 2004, intends to use the results in a commercialisation-level sea-cage-based project at Coffin Bay. It is likely that funding will be sought from AusIndustry for this work.

5. Further Development

Diet remaining from trials 4 and 5 will be used to further develop culture techniques with industry as soon as possible. The current project and the forthcoming trials will form the basis for an application for Ausindustry funding to undertake a commercialisation-level sea-cage-based project at Coffin Bay.

6. Planned Outcomes

A baseline diet for sea urchin roe enhancement has been developed. The Australian Experimental Stockfeed Extrusion Centre is able to produce a water-stable semi-moist pellet that will produce commercial-level roe yields in a sea-cage environment. The diet is an iteration of a sea urchin feed produced by Wenger Manufacturing Inc.

Sea urchin husbandry techniques for sea cages and aquaria have been developed to the point where many of the major issues have been addressed or identified, *viz* the requirement for good water quality, the degree of aeration required, physical separation of waste products from sea urchin feeding areas, adequate feeding rates and cage design requirements.

A very good working relationship with industry has been developed through the course of the project.

An international network of sea urchin research and industry contacts has been developed, especially with the key people in nutrition research.

7. Conclusion

There were six objectives to this project:

1 Determine the feasibility of postharvest enhancement of sea urchin, *Heliocidaris erythrogramma*, roe through the use of commercially available feeds.

2 Determine the environmental conditions (time and temperature) under which roe enhancement can be manipulated.

3 Evaluate photoperiod manipulation of the gametogenic cycle as a method of increasing the availability of the highest quality roe.

4 Evaluate the best commercial growout options by assessing the technical and relative economic feasibility of both land based (eg. abalone) and in-water sub-tidal cage (eg. polyculture with Pacific oysters).

5 Evaluate existing commercially available sea urchin diets (USA) and Australian abalone diets with a view to refinement for optimum post-harvest enhancement of *Heliocidaris erythrogramma* in Australia.

6 Determine future research needs for the industry

Objectives 1 and 5 were met. Although it was clear that the urchins would not consume the abalone diet; it does appear that post-harvest enhancement of purple sea urchin roe is possible using iterations of purpose-made commercially available feeds.

Objective 2 was met. It is suggested that between 70 and 90 days are necessary to ensure that a substantial proportion of sea urchins have commercial-level roe yields. However, it remains to be seen if this may change with diet development and time of year.

As the industry is interested in rapid (6-12 week), cost effective, sea-cage based, enhancement of roe, manipulation of photoperiod and temperature (Objectives 2 and 3), are, at this stage, more of academic interest although the latter has some significance from the point of view of the appropriate season for enhancement. The appropriate season is critical, if roe is left too long, it becomes mature and leakage occurs and the product is of limited value. As some of the sea urchins in the field trial showed mature roe at the end of September, it is suggested that roe enhancement be carried out during the cooler months, April to September.

Objective 4 was partially completed. At the beginning of the project a large Port Lincoln abalone farm had expressed an interest in running sea urchin trials in tanks at its facility for comparison with sea-cage trials (Objective 4). This project was to be funded, in part, through AusIndustry, but as the grant application to that body was unsuccessful, the work did not take place. The industry focus is, at present, on sea-cage culture as the preferred method for the initial development of post harvest roe-enhancement techniques. The sea-cage trial run in Coffin Bay was successful, but comparison could not be made with land-based culture as intended.

Objective 6 was met in full. Future research needs for industry should include:

- Diet refinement for sea-cage culture with respect to stability and formulation, to move towards a nutritional optimum, maximising yield and quality and reducing cost.
- Once stability is improved, estimation of food conversion ratios should be carried out as an aid to diet refinement.
- Investigation of the effect of initial condition (i.e. gut index), harvest season and location on final gut index and roe yield.

- Investigation of the optimum cage and facility design and sea urchin density/cage. Suggestions for practical sea urchin culture are attached (Appendix 5).

Appendix 1: Intellectual Property

There are no intellectual property issues arising from this work.

Appendix 2: Staff

Dr Richard Musgrove

Mr Geoff Wyatt

Dr Robert van Barneveld

Appendix 3: Diets

The following are the formulations and extruder settings for the diets used in Trials 4-6

Production of Sea Urchin Feeds

Prepared for Dr Richard Musgrove by Dr Robert van Barneveld,
Consultant Research Scientist (Nutrition)
Manager, Australasian Experimental Stockfeed Extrusion Centre
c/- Barneveld Nutrition Pty Ltd, 19-27 Coonan Rd, South Maclean, Qld, 4280. Ph: 07 5547 8611. Fax: 07 5547 8624.
E-mail: rob@barneveld.com.au
April, 2002

A3.1. Composition

The formulated diets are based on an existing formulation that has been assessed in Australia experimentally resulting in a significant increase in the yield of roe.

A total of three diets have been prepared (Table 1). The protein:energy ratio has been maintained in all diets while increasing the crude protein content from 21% to 27%. This should allow us to assess whether sea urchins have further capacity to increase roe yield with more nutrient dense diets, without confounding the combined response to changing levels of protein and energy. The base level of 21% is consistent with the crude protein content of the diet that has been examined previously.

A number of unknowns exist around the composition of these diets. These include the nutrient contributions from kelp meal, the need for purified forms of cholesterol, and the most appropriate source of phosphorus.

Potassium sorbate has been added as an antimicrobial agent, Banox is an antioxidant, phosphoric acid is a preservative and glycerol is designed to increase the degree of slip in the barrel so that the level of starch cook is minimised thus allowing the pellet to sink.

Vitamin and mineral premixes have been formulated for finfish and crustaceans (generic) and it is recommended that the crustacean mix be used in these diets in the absence of any experimental data outlining specific vitamin and mineral requirements. The composition of the vitamin and mineral premix is presented in Table 2.

Table A3.1.1. Composition of experimental sea urchin diets

Diet 1

Formula basic data

Code : SU1000	Name : SEA URCHIN BASE DIET	
Sell price: 0.0	Batch [Kg]: 1000.0	Group code:
Cost : 3335.584	Created : 15/04/02	Version :
Margin : -3335.584	Updated : 15/04/02	FM origin : SU1000
Tonnes : 0.0	User name : Administra	VM key : SU1000

External reference:
Script file name :

Raw material	%	[Kg]	Tonnes	Cost	Diet cost
10740 MAIZE 10.0	16.291581	162.916	0.0	220.0	35.841
15970 WHEAT POLLARD 15.0	34.712957	347.13	0.0	200.0	69.426
17150 KELP MEAL	12.5	125.0	0.0	1000.0	125.0
34640 SOYBEAN MEAL 48.0	11.698987	116.99	0.0	600.0	70.194
41200 FISH MEAL 60.0	11.286474	112.865	0.0	1000.0	112.865
45200 FISH OIL	0.5	5.0	0.0	1000.0	5.0
45740 LECITHIN	1.0	10.0	0.0	2000.0	20.0
48210 DEFLOUR ROCK PHOSPHATE	1.3	13.0	0.0	700.0	9.1
52200 VITAMIN C	0.075	0.75	0.0	10500.0	7.875
54150 CAROTENE 11-12	0.02	0.2	0.0	24000.0	4.8
60520 BANOX	0.015	0.15	0.0	3000.0	0.45
90000 CRUSTACEAN AQUACULTURE V+M PREMIX	1.0	10.0	0.0	6000.0	60.0
92000 CHOLESTEROL	0.3	3.0	0.0	900000.0	2700.0
93000 GLYCERINE	8.0	80.0	0.0	1000.0	80.0
94000 PHOSPHORIC ACID	1.0	10.0	0.0	600.0	6.0
94500 POTASSIUM SORBATE	0.3	3.0	0.0	550.0	1.65
	100.0	1000.0	0.0		3308.201

Analysis

[VOLUME]	% :	100.0	ISOLEUCINE %	:	0.852206	SODIUM %	:	0.128483
DRYMATTER	% :	82.989356	LYSINE %	:	1.265696	CHLORIDE %	:	0.199156
MOISTURE	% :	16.990644	METHION %	:	0.4	FAT/EE %	:	4.028831
PROTEIN	% :	21.0	THREONINE %	:	0.805318	PIGMENT MG/KG	:	0.0
C_FIBRE	% :	8.232515	TRYPTOPHAN %	:	0.246088	CAROTENOID MG/KG	:	0.0
AME_C_MJ	MJ/KG :	8.155988	M+C %	:	0.703287	XANTHOPHYL MG/KG	:	0.0
DE_PIG_MJ	MJ/KG :	10.280206	ASH %	:	6.37272	ASTAXAN MG/KG	:	0.0
ARGININE	% :	1.291057	CALCIUM %	:	1.225868	CANTAXAN MG/KG	:	0.0
LEUCINE	% :	1.4	PHOSPHORUS %	:	0.973428	#CP/AME	:	2.574796

Diet 2

Formula basic data

Code : SU3000	Name : SEA URCHIN 24%CP	
Sell price: 0.0	Batch [Kg]: 1000.0	Group code:
Cost : 3378.048	Created : 15/04/02	Version :
Margin : -3378.048	Updated : 15/04/02	FM origin : SU1000
Tonnes : 0.0	User name : Administra	VM key : SU1000

External reference:
Script file name :

Raw material	%	[Kg]	Tonnes	Cost	Diet cost
10740 MAIZE 10.0	16.118767	161.119	0.0	220.0	35.461
15970 WHEAT POLLARD 15.0	27.889258	278.893	0.0	200.0	55.779
17150 KELP MEAL	12.813322	128.133	0.0	1000.0	128.133
34640 SOYBEAN MEAL 48.0	15.067427	150.674	0.0	600.0	90.405
41200 FISH MEAL 60.0	15.248119	152.481	0.0	1000.0	152.481
45200 FISH OIL	2.853107	28.531	0.0	1000.0	28.531
45740 LECITHIN	1.0	10.0	0.0	2000.0	20.0
48210 DEFLOUR ROCK PHOSPHATE	1.3	13.0	0.0	700.0	9.1
52200 VITAMIN C	0.075	0.75	0.0	10500.0	7.875
54150 CAROTENE 11-12	0.02	0.2	0.0	24000.0	4.8
60520 BANOX	0.015	0.15	0.0	3000.0	0.45
90000 CRUSTACEAN AQUACULTURE V+M PREMIX	1.0	10.0	0.0	6000.0	60.0
92000 CHOLESTEROL	0.3	3.0	0.0	900000.0	2700.0
93000 GLYCERINE	5.0	50.0	0.0	1000.0	50.0
94000 PHOSPHORIC ACID	1.0	10.0	0.0	600.0	6.0
94500 POTASSIUM SORBATE	0.3	3.0	0.0	550.0	1.65
	100.0	1000.0	0.0		3350.665

Analysis

[VOLUME]	% :	100.0	ISOLEUCINE %	:	1.00499	SODIUM %	:	0.163623
DRYMATTER	% :	85.624092	LYSINE %	:	1.514589	CHLORIDE %	:	0.246167
MOISTURE	% :	14.355908	METHION %	:	0.474747	FAT/EE %	:	6.49836
PROTEIN	% :	24.0	THREONINE %	:	0.938868	PIGMENT MG/KG	:	0.0
C_FIBRE	% :	8.0	TRYPTOPHAN %	:	0.283079	CAROTENOID MG/KG	:	0.0
AME_C_MJ	MJ/KG :	9.230769	M+C %	:	0.8	XANTHOPHYL MG/KG	:	0.0
DE_PIG_MJ	MJ/KG :	11.169406	ASH %	:	6.97954	ASTAXAN MG/KG	:	0.0
ARGININE	% :	1.485116	CALCIUM %	:	1.429255	CANTAXAN MG/KG	:	0.0
LEUCINE	% :	1.6	PHOSPHORUS %	:	1.064539	#CP/AME	:	2.6

Diet 3

Formula basic data

Code : SU4000	Name : SEA URCHIN 27%CP		
Sell price: 0.0	Batch [Kg]: 1000.0	Group code:	
Cost : 3409.741	Created : 15/04/02	Version :	
Margin : -3409.741	Updated : 15/04/02	FM origin : SU1000	
Tonnes : 0.0	User name : Administra	VM key : SU1000	

External reference:
Script file name :

Raw material	%	[Kg]	Tonnes	Cost	Diet cost
10740 MAIZE 10.0	10.771593	107.716	0.0	220.0	23.698
15970 WHEAT POLLARD 15.0	26.042261	260.423	0.0	200.0	52.085
17150 KELP MEAL	12.612501	126.125	0.0	1000.0	126.125
34640 SOYBEAN MEAL 48.0	21.917309	219.173	0.0	600.0	131.504
41200 FISH MEAL 60.0	16.171363	161.714	0.0	1000.0	161.714
45200 FISH OIL	6.344602	63.446	0.0	1000.0	63.446
45740 LECITHIN	1.0	10.0	0.0	2000.0	20.0
48210 DEFLOUR ROCK PHOSPHATE	0.430371	4.304	0.0	700.0	3.013
52200 VITAMIN C	0.075	0.75	0.0	10500.0	7.875
54150 CAROTENE 11-12	0.02	0.2	0.0	24000.0	4.8
60520 BANOX	0.015	0.15	0.0	3000.0	0.45
90000 CRUSTACEAN AQUACULTURE V+M PREMIX	1.0	10.0	0.0	6000.0	60.0
92000 CHOLESTEROL	0.3	3.0	0.0	900000.0	2700.0
93000 GLYCERINE	2.0	20.0	0.0	1000.0	20.0
94000 PHOSPHORIC ACID	1.0	10.0	0.0	600.0	6.0
94500 POTASSIUM SORBATE	0.3	3.0	0.0	550.0	1.65
	100.0	1000.0	0.0		3382.36

Analysis

[VOLUME]	% :	100.0	ISOLEUCINE %	: 1.162262	SODIUM	%	:	0.172908
DRYMATTER	% :	88.166937	LYSINE %	: 1.736778	CHLORIDE	%	:	0.255971
MOISTURE	% :	11.813063	METHION %	: 0.52	FAT/EE	%	:	9.879294
PROTEIN	% :	27.0	THREONINE %	: 1.063425	PIGMENT	MG/KG	:	0.0
C_FIBRE	% :	8.0	TRYPTOPHAN %	: 0.326204	CAROTENOID	MG/KG	:	0.0
AME_C_MJ	MJ/KG :	10.384615	M+C %	: 0.881819	XANTHOPHYL	MG/KG	:	0.0
DE_PIG_MJ	MJ/KG :	12.415223	ASH %	: 7.331336	ASTAXAN	MG/KG	:	0.0
ARGININE	% :	1.716129	CALCIUM %	: 1.2	CANTAXAN	MG/KG	:	0.0
LEUCINE	% :	1.8	PHOSPHORUS %	: 0.949164	#CP/AME		:	2.6

A3.2. Mixing

All ingredients need to be mixed prior to extrusion with the exception of the fish oil, the glycerine and the phosphoric acid. Mix sheets will need to be prepared to for the appropriate batch size and accounting for the exclusion of the above three components. Mixed ingredients will need to be hammer-milled through a 2.3mm screen.

The fish oil, glycerine and phosphoric acid need to be combined (in the same proportion as they exist in the diet – a separate mix sheet will need to be prepared for this), and added to the second entry port of the DDC using a liquid pump. Addition rate needs to coincide with the combined dietary inclusion of the three ingredients in proportion to the feed rate into the DDC (ie if the feed rate is 270 kg/hr and the dietary inclusion of the three ingredients is 12%, then the liquid addition rate to the DDC needs to be 32.4 kg/hr. It would be wise to prepare an addition matrix for feed rates of 200-600 kg/hr prior to extrusion).

A3.3. Extruder stackup

The extruder stackup for production of these feeds is similar to that used for a semi-expanded salmon feed. Heads to be used (in order starting from the DDC downspout) are 68781-001, 687782-001, 68783-001, 68783-001, 68783-001, 68785-001 (vented), 68784-001. Vented head should be open.

The rotating elements in order from the DDC downspout are 68792-001, 68793-001, 68805-013, 68794-003, 68794-003,68806-001(F), 68806-001(F), 68806-001(F), 68795-001, 68806-003(F), 68806-003(F), 68806-003(R), 68806-003(R), 68806-003(R), 68806-003(R), 68805-023, 68794-001, 68805-003, 68796-001 (These numbers represent screws, shear locks and lobe locks in order).

A3.4. Extruder operating conditions

The following operating conditions are recommended as a starting point for extrusion of this product:

Feed Rate:	270 kg/hr
Steam to DDC:	34 kg/hr
Water to DDC:	38 kg/hr
Slurry to DDC:	Dietary inclusion (%) of feed rate.
Extruder speed:	600 rpm
Steam to extruder:	20 kg/hr
Water to extruder:	10 kg/hr

Temperature at the die face should be minimised. Dies should 7 mm with 4 openings in the die plate.

Drying and cooling

Extruded pellets should be dried and cooled at temperatures not exceeding 70° applied over periods of 20 minutes on the first dryer belt, 25 minutes on the second dryer belt and 20 minutes in the cooler.

A3.5. Vacuum infusion

Not required.

A3.6. Vitamins and Minerals

Table A3.6.1. Vitamin and mineral premix composition.

Vitamin	
Fat-soluble vitamins	
Vitamin A, IU/kg	5,000,000
Vitamin D, IU/kg	3,000,000
Vitamin E, IU/kg	100
Vitamin K, mg/kg	5
Water-soluble vitamin, mg/kg	
Riboflavin	25
Pantothenic acid	75
Niacin	40
Vitamin B12	0.2
Biotin	1
Folate	10
Thiamin	60
Vitamin B6	50
Vitamin C	200
myo-Inositol	400
Minerals, mg/kg	
Magnesium (as magnesium sulphate)	3,000
Iron (as iron sulphate)	
Zinc (as zinc sulphate)	100
Manganese (as manganese sulphate)	
Copper (as copper sulphate)	40
Iodine (as potassium iodide)	
Selenium (as sodium selenite)	0.4
Cobalt (as cobalt sulphate)	

Appendix 4: Suggested Guidelines for System Design and Management for Captive Enhancement of Sea Urchins in Sea-based Systems

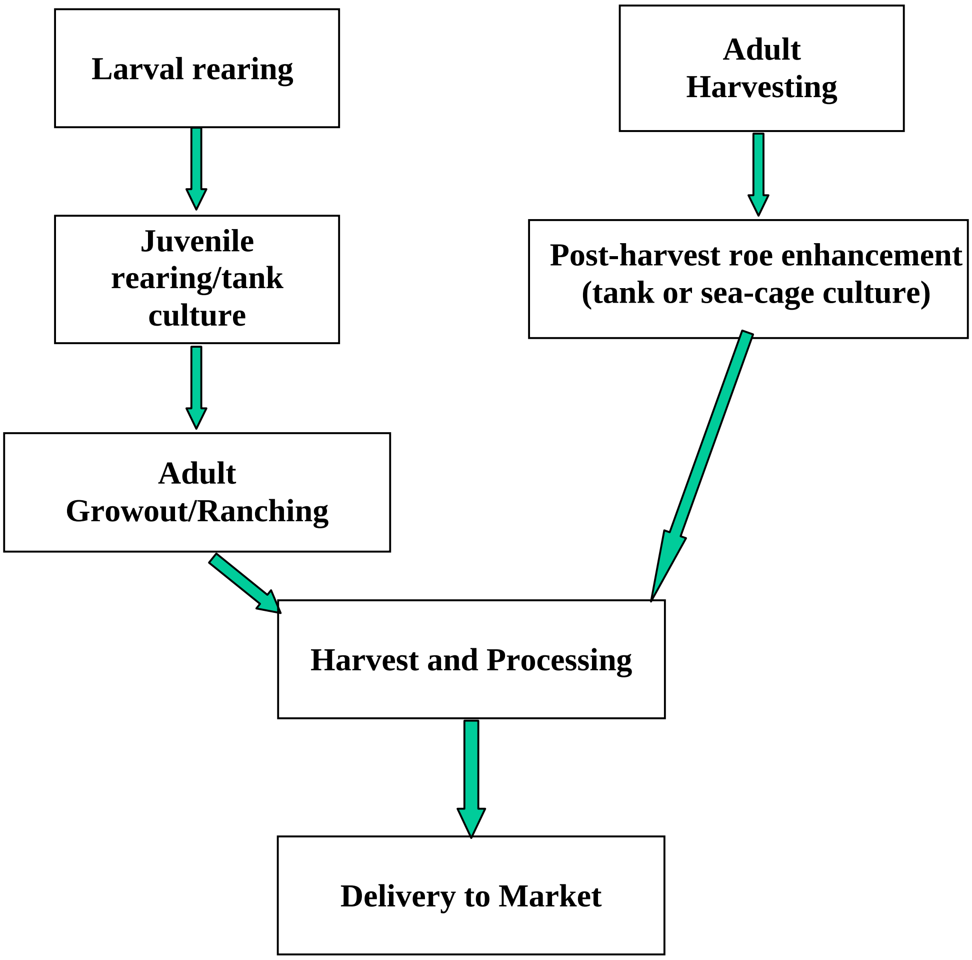
DISCLAIMER

Although the research organisation involved in the sea urchin roe enhancement project has taken all reasonable care in preparing the information contained within this document, neither it nor its officers accept any liability resulting from the interpretation or use of this information. Please also recognise that the information contained within this document is preliminary in nature and subject to change without further notice.

A4.1 Introduction

There are two avenues that sea urchin culture generally follows (Fig A4.1.1), although they are not mutually exclusive. The first, stock enhancement, involves spawning adults and raising the progeny through to the juvenile stage in tanks. In Japan, these are then transplanted out to sea where they grow into adults and are harvested 2 to 3 years later.

Fig A4.1.1 Sea Urchin Culture



This report deals with the second approach; captive enhancement of sea urchins on land or in sea cages.

Clearly, in order to ensure survival of sea urchins in tanks, procedures have to be followed to ensure that the animals are in good condition upon arrival at the enhancement facility. This means that the post harvest handling procedures must aim to minimise stress on sea urchins at each phase of the process; harvest, boat transfer/ land transport to enhancement tanks/cages and treatment in enhancement facility.

A4.2. Harvest and treatment on boats

The spawning season for sea urchins in South Australia, at a latitude of 35S, is between November and April (pers comm J. Strzelecki, Adelaide University) although it is likely that urchins will go through at least two spawning-regeneration cycles during that period (Williams and Anderson, 1975). There is also likely to be some spatial variation in the timing of spawning as sea urchin populations are found from Point Lowly, near Whyalla (a latitude of 33S) to Port Macdonald (a latitude of 38S) and as such are exposed to a wide range of sea temperatures as well as density and food availability effects. Seasonal effects and the reproductive cycle are important to the harvesting and enhancement schedules as the market requires pre-mature roe (i.e. eggs not fully developed) with fine texture.

A4.3. Collection

In South Australia, sea urchins are usually collected with a short-handled, three-pronged garden rake or similar device. In New Brunswick in Canada, a rake has been designed to fit over the glove of the diver (Fig A4.3.1).

Fig A4.3.1 Purpose-designed harvesting rake for sea urchins (New Brunswick, Canada)

This rake works well, especially for small sea urchins, and may be less likely to damage the tests (shells) of the harvested animals.

Care should also be taken, after harvesting, not to hit the side of the boat with the catch bag (Fig A4.3.2). In California they use a derrick for this task. Once on board individual urchins should be handled as little as possible. Apart from being an inefficient way of moving the catch, each handling episode increases stress on the animals and the likelihood that they will die.

Fig A4.3.2 Landing the catch in Japan

In Newfoundland, trials with catch cages have proven successful. Urchins are collected by the divers and loaded directly into cages constructed of green plastic coated wire mesh (Fig 4.3.3).

Fig A4.3.3 Cages for holding urchins

Cages are about 700 by 500 mm and the mesh about 38 mm. They are floated off buoys until all are filled at which point the diver loads the cages into bins on the boat then brings the urchins back to the facility in the cages to be placed in the live tanks. This reduces handling, damage and stress on the urchins although the diver would still have to assess the roe yield before putting the urchins in the cages.

A4.4 Boat transport to enhancement facility

Although the purple urchin is hardier than the other commercial Australian species (the black urchin, C*entrostephanes rodgersii*) it will dry out rapidly once out of water, particularly in the summer. The solution to this is to keep them in water, either in the well of the boat or in a large insulated tub (i.e. "Xactics" bin). The well's pump or the deck hose should be used to keep a continual supply of fresh seawater running through the catch and out the scuppers. Simply using wet hessian sacks to cover sea urchins housed in Nally bins, as has been done with catch destined for the processors, is not adequate, particularly if there is a long journey to be undertaken to the enhancement facility.

A4.5 An enhancement facility

There are two common options: sea-cages or land-based tanks/raceways. Both are in the developmental stage at present.

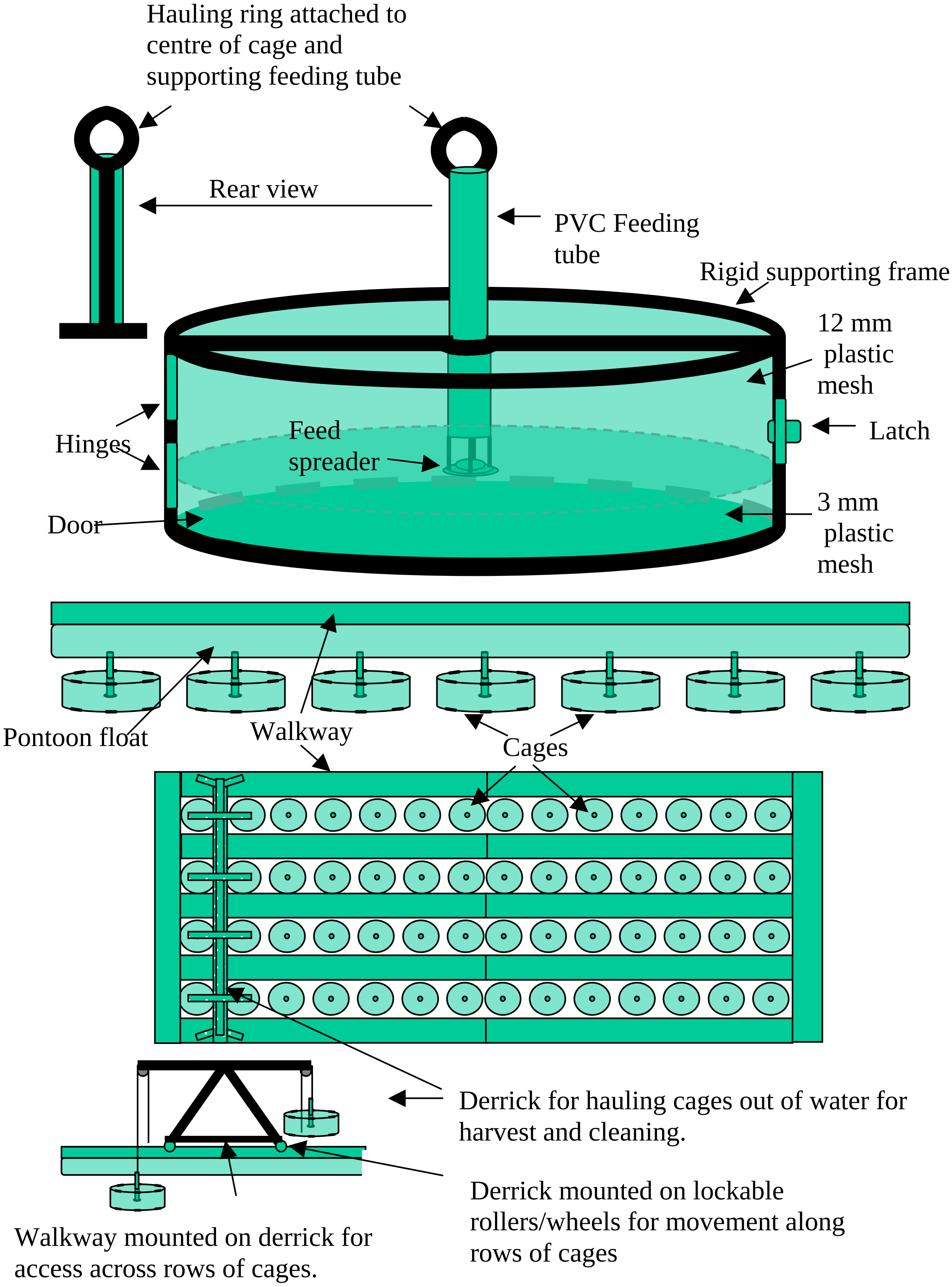
A4.5.1 Sea-cages

This may be structured as either a monoculture (i.e. sea urchins only) or a polyculture (e.g. with oysters). Monoculture involves feeding the animals with either aquatic macrophytes grown for the purpose or an artificial diet, within moored cages or nets, in sheltered waters with a good tidal flow to reduce potential impacts of waste feed on local flora and fauna. The cages should be towable to allow flexibility with site selection, particularly at times of high water temperature. Aquatic macrophytes could be grown for food, as in Japan, on droplines tied to moored and buoyed surface lines. Artificial feeds could also be used as shown in the present study, either in combination with kelp or as a sole source of nutrition. Sea-cages will also naturally attract fouling organisms, such as *Ulva* and *Enteromorpha*, which would then be consumed by the sea urchins. They also have the advantage of lower infrastructure costs than land-based facilities.

The optimum cage design is a matter of opinion. However, during the present study, which used rectangular cages moored to an oyster lease, the necessity for modifications became apparent. The cages did moderately well, however feeding was labour intensive and urchins were hard to remove from corners of cages at harvest. The following design attempts to rectify these problems.

The cage (Fig A4.5.1) is cylindrical in shape with a door in the side that opens outward, allowing for easier access. The cage is made of black plastic mesh supported on a rigid frame; the upper three quarters of the cage wall and the top consists of 12mm mesh, the lower quarter and the floor of 3mm mesh. This allows for retention of the artificial diet while maximising water circulation through the cage. A feeding tube (PVC pipe) is inserted into the centre of the cage to allow feeding from the surface. The tube is supported by a strut that terminates in a hauling ring, used for removal of the cage from the water and by a piece of the frame that bisects the top of the cage. The latter also support the hauling strut. The lower end of the feeding tube has a cone attached which acts to spread feed over the bottom of the cage, preventing accumulation of feed in the centre. Cages are hung from a pontoon and positioned just below the water line. They are spaced far enough apart so that water circulation through a given cage is not blocked. If large numbers of cages are used, a derrick system may be built to facilitate removal of cages from the water. It should be noted that this is a only a concept at this stage, no trials have been run in the field with the system. It is however clear that rectangular cages accessed by divers on hookah, as used in the present study, are inefficient and expensive to maintain. A move to a cylindrical cage design with a feeding tube to the surface is the next logical step. Such feeding tubes have been used with success in experimental lobster live-holding systems in the field.

Fig A4.5.1. Suggested floating sea-cage roe enhancement facility.
Each cage is approximately 1m across.



In support of the idea, the Japanese common sea urchin has been successfully cultured in China using rafts of rearing cages previously used for scallop and abalone. In this case, the sea urchins were raised from the juvenile stage, however enhancement of adults could be carried out using the same infrastructure.

Trials in Scotland (Kelly *et al* 1998) have shown that the sea-cage system provides significantly greater roe development (up to about 15% yield) in the local sea urchin (*Psammechinus miliaris*) than found in wild populations (about 3%) at the same time of year. In this case the sea urchins were fed on *Laminaria* spp, including *Laminaria saccharina* and housed in unused Atlantic salmon cages.

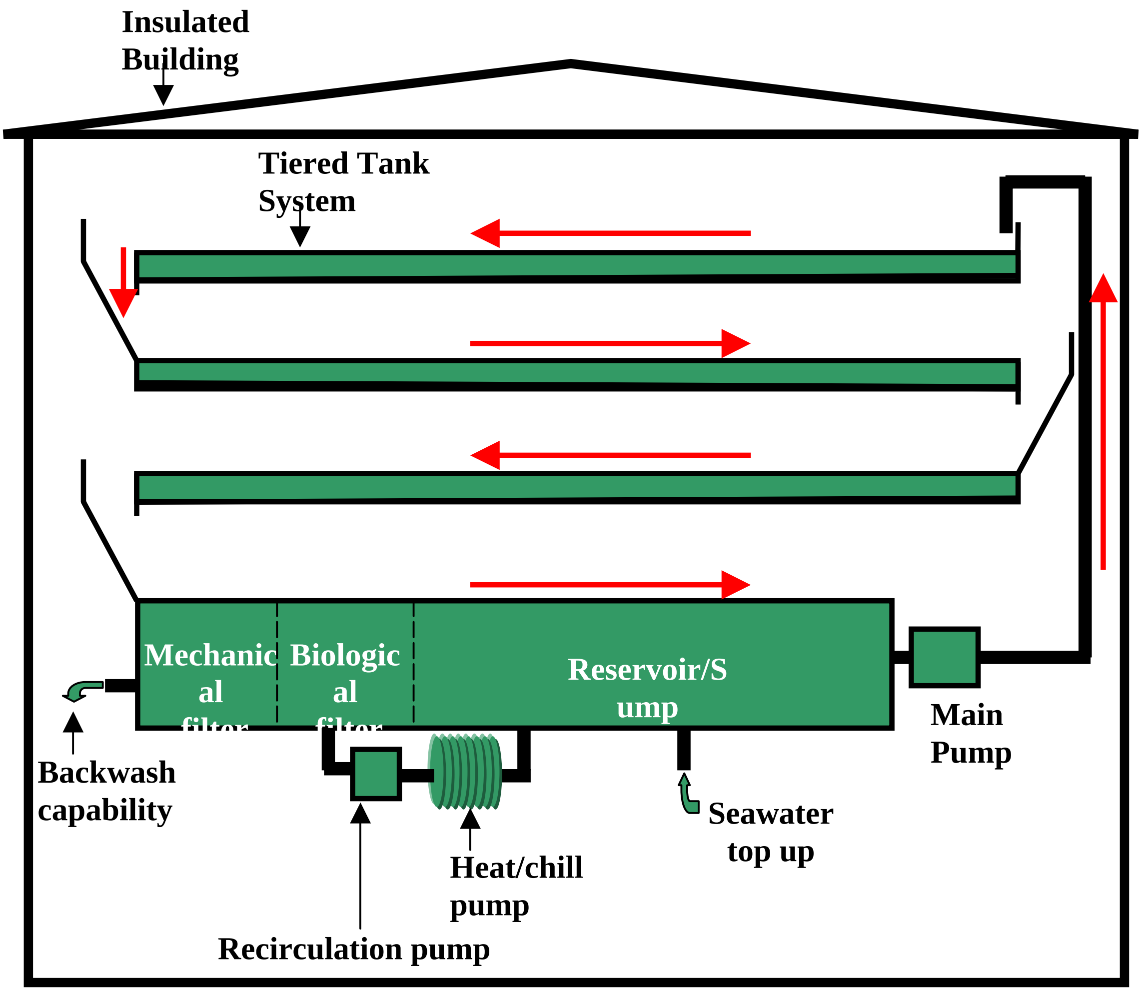
Even greater roe enhancement (up to 22%) was achieved when urchins were put into cages with salmon (ie. polyculture). In this case, although kelp was fed to the sea urchins, they preferred the waste salmon food and salmon faeces. There is, however, an issue here of roe taste, as roe from urchins fed on fish products has been reported (pers. comm. S. Robinson, New Brunswick, Canada) to have a bitter taste however it may be possible to overcome this problem by switching feed to kelp in the last few weeks of the enhancement process (pers. comm. M. Kelly, Oban, Scotland). In these trials it was also found that the sea urchins significantly reduced fouling (primarily *Enteromorpha* and *Cladophora*) on the nets without significantly compromising net strength.

A4.5.2. Tanks/Raceways

Tank or raceway enhancement of sea urchin roe is very much in the experimental phase worldwide. The key points to remember with such systems are the maintenance of good water quality (including appropriate temperatures and minimum ammonia levels) and the provision of high quality, cost effective feed.

Most of the culture work to date has focused on the production of juveniles in hatcheries and rearing to commercial size (ie. in France: Blin, 1996) in tanks/raceways or out-planting the juveniles into depleted areas (ie. in California: Rogers-Bennett et al, 1994). There are also fully commercial juvenile-production operations, which sell stock to sea urchin fishing co-operatives for restocking depleted areas in Japan. Clearly the same juvenile-rearing infrastructure may be used to fatten adults. An example of a fattening system, based on a French juvenile rearing facility, is illustrated (Fig A4.5.2).

Fig A4.5.2 Suggested roe enhancement system (tiered tank set-up based on Blin (1996)).
Red arrows indicate flow direction.



The raceways, which, in South Australia, should be housed in an insulated building, or at least covered to reduce evaporation, are built above filter and seawater storage tanks. Each raceway is inclined slightly so the water brought up by the main pump descends by gravity back down to the mechanical filter and through the system. Water replacement is determined by the ammonia build-up in the system and also by the need to replace backwashing (typically 5-10% loss/backwash) and evaporative losses. A heat/chill pump on the continuous recirculation loop between the reservoir and the biological filter controls temperature. In each raceway, there is just enough seawater to cover the sea urchins, allowing for maximal gas exchange between air and water. Further exchange occurs as the water falls between each raceway. Each raceway should have a false mesh floor to separate animals from their faeces as described in the present study. This maximises water quality around the sea urchins, facilitates removal of faeces and waste products and, if mesh baskets are used to contain the animals rather than a one-piece mesh floor, allows for more efficient harvesting of animals.

A Newfoundland sea urchin processing company, broadening its operations to include aquaculture, has reported that raceways were superior to tanks in roe enhancement trials (Parsons, 2000). Raceways gave a slightly higher roe yield, provided a better water flow/gas exchange and were much easier to clean than tanks. In addition, both removal of urchins and the monitoring of mortalities were easier.

A4.5.3. Diseases

There is concern over potential disease problems in such a polyculture system, particularly as the urchins are feeding on salmon faeces as well as uneaten salmon food. Diseases such as infectious pancreatic necrosis (IPN), viral haemorhagic septicemia (VHS) and infectious salmonid anaemia (ISA) are found in salmon sea-cage culture and could potentially be passed on to the urchins.

Other diseases recorded from sea urchins, and potentially a problem in the high density monoculture, include those caused by bacterial infection, amoebae and nematodes (Tajima and Lawrence, 2001). Their occurrence has not yet been investigated in *H. erythrogramma*.

A4.5.4. Seasonality

The market requirement for pre-mature (fine) roe delimits the season for enhancement in outdoor facilities such as sea-cages where the water temperature is not controlled. Recent work on the purple urchin suggests that spawning temperature is around 19PP^{o}C (J Strzelecki, Adelaide University, pers. comm.), in Gulf St Vincent, SA, is from November/December to about April. Thus the outdoor enhancement season at this latitude should probably take place between May and October, but favouring the earlier months in this range.

A4.5.5. Diets

The most successful diet for gonad enhancement has been that produced by Wenger Manufacturing Inc (Kansas, USA). The diet, produced in collaboration with scientists from Texas A&M University and the University of South Florida is a semi-moist extruded preparation. It has been used successfully for gonad enhancement for sea urchin species in Europe, Israel, New Zealand, Chile, USA (Lawrence et al. 2001) and South Australia, as described in the present study. Further refinements are required to optimise the diet for *H. erythrogramma* (eg. substitution/removal of expensive components, improvements in stability) but, in its present form, it is capable of enhancing the roe of this species over a reasonable time period.

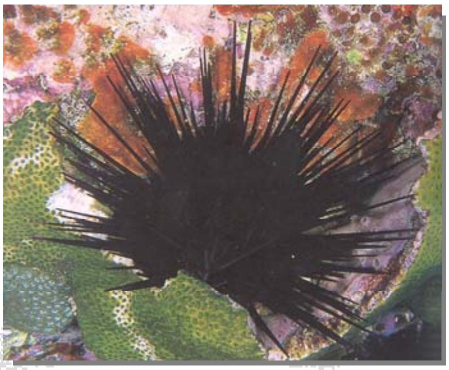
APPENDIX 5: SEA URCHIN WORKSHOP PROCEEDINGS

This workshop was supported by FRDC and had the following aims:

1. To raise industry's awareness of trends in sea urchin culture and research in Australia and overseas
2. To provide a forum for informal discussion of matters of importance to industry.
3. To facilitate the creation of a Sea Urchin Working Group.

THE PROCEEDINGS OF THE FIRST SEA URCHIN WORKSHOP

Editors:
Richard J. Musgrove and Marion B. Claremont



South Australian Aquatic Sciences Centre
16TH JUNE 2000

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

PIRSA
Primary Industries and Resources South Australia

INTRODUCTION

WELCOME

Tania Kiley, PIRSA Fisheries welcomed all attendees on behalf of Primary Industries and Resources South Australia (PIRSA) Fisheries and Aquaculture.

Dr Richard Musgrove, South Australian Research and Development Institute Aquatic Sciences, also welcomed everybody, especially those who had travelled all the way from NSW and Victoria. He then advised that the proceedings would be recorded on tape and a workshop document prepared using the tape as a basis.

AIMS

This aims of the workshop were:

1. To raise industry's awareness of trends happening in sea urchin culture and research in Australia and overseas. One of the speakers, Lance Schlipalius, who has visited the Canadian team working on sea urchin diets in New Brunswick, will present some of their work today. We are using the Canadian diets in trials at present.

2. To provide informal discussion of matters which are of importance to industry given that it is industry people who are driving the conference, and to facilitate the creation of the Sea Urchin Working Group which would then decide where industry wants to head.

THE WORKSHOP

The Impact of Harvesting on Sea Urchin Stocks – Dr Scoresby Shepherd (SARDI Aquatic Sciences):

Dr Shepherd is a scientist who has worked for many years on abalone population dynamics but has an ongoing interest in sea urchin population dynamics as well.

Sea urchins and abalone have lots of things in common, so principles that apply to abalone populations, by and large, apply to sea urchins as well. I will go through some of the features in common. I am going to talk about world fisheries and their status, and then about some of the biological aspects of sea urchins which make them very much like abalone. *Heliocidaris* has a larval stage of about five days, which is about the same as abalone. The larvae are lecithotrophic, which means that they have an egg yolk and feed on it without depending on plankton. Hence, the larval life is short and the larvae don't move very far from their natal site, that is, where they are born. Sea urchins are sedentary, (ie. they don't move much in the adult stage). This is important because it means that the spatial structure, that is, the way sea urchins are dispersed on the bottom, is critically important. Basically they live where they settle and their settlement site is determined by the coastal topography and local water currents. So the larvae are concentrated in certain areas, and establish local populations. Another local population may be fifty metres away, but the larvae are passively transported over distances of hundreds of metres to kilometres so that many local populations are linked by larval dispersal and form meta-populations which are the genetic units.

The world sea urchin fisheries look reasonably stable, but this picture is deceptive because many fisheries are declining as others develop. The Japanese fishery is declining. Current global production is about 116,000 tonnes. In Chile the coast is divided into regions and the fishers are gradually moving down the coast to reach southern waters, over-exploiting as they go. Russia, Korea and the USA have declining fisheries, especially in California where serious depletion is occurring. However in the northeast Pacific (Alaska), the fishery is controlled by rolling closures and is stable. Oregon and Washington fisheries were reasonably stable but then native fishing claims upset their management regime and they are now again trying to recover some stability.

BIOLOGY

Sea urchins spawn eggs and larvae into the water where fertilisation occurs. The larval life of a sea urchin is about five days, they then settle on the bottom and grow as they graze micro- and later macro-algae.

Because sea urchins have a metapopulation structure and different populations may have a unique set of population characteristics, their fisheries need to be managed at a fine scale of 10-50 km along the coast. If one population is overfished it cannot be replenished from a neighbouring population, hence the need for fine-scale management.

Maintaining minimum densities of spawning stock in a population is critically important for the survival of that population. Studies on the fertilisation success of sea urchins show that if individuals are more than 50 cm apart the likelihood that eggs are fertilised falls to very low levels of a few percent. Hence it is necessary to maintain densities of 0.2-0.5 urchins per square metre if the population is not to crash.

Sea urchins should be considered as a clump of living gonad surrounded by protective shell and spines, and little else. The primary function of the sea urchin is to reproduce enough young to maintain the population.

QUESTIONS

Q: John Vairy (Sea Urchin Processor, NSW): What is the mechanism that makes sea urchins spawn? The purple urchin is getting ready to spawn now.

A: In Japan the physical wave crash on the coast during severe storms such as typhoons induces epidemic spawning. Conversely, in southern Australia, *Heliocidaris* spawns on really calm days, so there it may be the opposite mechanism, the complete absence of water movement. We also think there is a lunar signal, but that has not been proven. The Mediterranean sea urchin has a lunar spawning cycle and they spawn either before or after the full moon.

Q: Is the survival of the young coming on improved by the presence of adults?

A: There are no data to support or preclude this theory.

A Diver's Perspective on the Industry - Terry Scott (Sea Urchin Licensee, Coffin Bay, SA):

Firstly I would like to talk about harvesting. I started diving in 1990 after a friend contacted me about a diving job in 1987. I had grand ideas of driving a Mercedes Benz in no time, however I had a lot to learn. We went through pretty frustrating times because we were starting off anew without knowing anything about the industry.

We began to do small and awkward amounts of sea urchin harvesting, just trying to make some money out of it, which was very difficult as the economy was down. I think that the hardest thing was that I had to learn entirely by myself, I didn't have any information at that stage. I met Dr John Keesing (previously SARDI) and he helped me out tremendously with books etc.

I harvest sea urchins using a three-pronged garden rake and 15 to 18 kilogram bags. I did find sea urchins occasionally two to three high, which was exciting. My aim was to develop some harvesting methods. I left one third of the sea urchins behind for regeneration and found after the second and third year it worked quite well, even though I noticed the sea urchin size had dropped considerably. Although I found a lot of big sea urchins, which I harvested, I was told that the roe was not good and I had to get better quality sea urchins. This was frustrating but I also realised, from the little I had read, that I had to get rid of the larger sea urchins to let the young ones come into peak condition. Thus, although the size dropped after harvesting a spot for two to three years the quality and recovery weight improved quite dramatically.

I discussed the possibility of using old sea urchin shells as fertiliser with contacts in Port Lincoln, because I felt there could be a by-product spin-off which would be an incentive to harvest them. After a while I started to find that I could look from the boat to the seabed and, especially if there was a fair bit of seagrass, pick out the sea urchins that would be satisfactory, ie. the roe quality would be good. I still get it wrong now and again, but I have developed a certain pattern.

I keep a dive diary and find my experiments very interesting, especially with spawning. Up until today I have completed approximately 140 dives, some would be about 0.5 hour at the most, but I have been doing a lot by looking fairly hard, although there is no money to be earned.

My licence covers the Eyre Region zone which is a fairly big area that goes right around into the gulf to between Arno Bay and Port Nedham. However, although I haven't done a lot of diving in the gulf. I had a few spot dives at Wallaroo and did not find anything really promising there, although it had reasonable quality sea urchins. Venus Bay is out of my licence area.

The South Australian Industry - Steve Wright (Sea Urchin Licensee, Victor Harbour, SA)

In terms of roe the return could be as low of $50/kg for our "A" grade due to poor processing. There were transport problems and other difficulties which meant the roe only realised up to $40-$50/kg. I think John Vairy was getting $80/kg for "A" grade and about $50/kg for B+. This was the landed price, and at the bottom end of the market; it did not pay to do it. The aim should be for the upper end of the market where the price range is anything from $50/kg to $500/kg. The Chileans went through a period where their roe was regarded as an inferior product because of where it was coming from and how it was getting there. Competition with the Japanese fisheries market operations impacted on the Chilean price.

The danger is that if we work in a piecemeal manner our industry could get a bad reputation. South Australian products can be top grade. We have clean, clear waters which have the potential to yield a really good product. We need to work together in the open market place, not compete against each other. A rogue processor just sending roe off to make a quick dollar (ie. a poor product), would give all South Australian sea urchin products a bad reputation. It is very hard to put out a product and expect to get top price, the best line of promotion would be to send a small shipment to give potential buyers a sample to taste and make a judgement on the product.

Q: Mark Thring, Louth Bay Abalone: With the work that you have done, have you gone through the financials of it? Are you convinced that it is a viable industry?

A: I think, with the proviso that we work in a co-ordinated manner and with researchers like SARDI, it will be. However, working individually, I have reservations because of the quantities needed, 2-4 tonnes of live weight of product have to go into a processing plant per week to maintain its viability, including keeping people who are skilled. Being able to process, crack open and then do a professional job of selection, grading and packaging, you need a semi-skilled workforce which is hard to maintain due to the seasonal vagaries of whether or not you can get into the water. All of the problems with diving conditions that you have in the abalone industry may be multiplied by ten because these conditions vary so much from the South-East, to the West Coast and in the Gulf St Vincent. Spawning time and quality also vary. I think we can certainly reduce the variability in quality by roe enhancement in an aquaculture situation, however you need regular supplies from people who are trained urchin divers.

It would be destructive to decimate one area if we want a long-term sustainable industry so that our grand-children can be part of it in the future. We then need to be able to maintain those stocks and ensure their sustainability while at the same time we have to make some money to keep people employed and keep the industry viable. Therefore we have to be able to ensure that we have enough live product coming in, being looked after and sent live to a centralised processing plant. Given the differences in spawning periodicity, we will need one processing plant as close as possible to the airport in Adelaide. Harvested urchins from Port Lincoln or from the south coast could be sent to that one processing plant. Some of the top labels from California for example, have five to seven people employed in the processing plant. It is possible to operate with five, where you have packers, shellers, graders and a manager.

We should aim at getting one plant up and operating in South Australia. A viable, sustainable plant operating with a permanent workforce, avoiding casual labour. The continual need to train new people to the appropriate level can have a negative effect on the quality of the roe sent to market. Quality assurance is needed, with top Australian Quarantine and Inspection Service (AQIS) standards being met, a very good marketing and export program and a business plan. It may cost $300,000 for the plant to be set up with the appropriate refrigeration, shelling equipment and building lease. These aspects should be looked at first and then arrangements made so that the divers are treated fairly. I would also like to see the divers, processors, exporters and marketers work together as a cooperative, there are good examples where this has worked elsewhere. Although it is difficult and takes a bit longer, it will maintain the viability of the industry.

Making representation to the State and Federal governments where there are grants available on a dollar for dollar basis for research and development requires a good business plan and a model of working together. Separate applications will dissipate the available resources for this particular industry considering we have to compete with all the other industries across the state and across the country.

We have been through the process of creation of a business plan and come up with about 10 different versions. It depends on the assumptions that you make in the beginning. At the end of the day, if you try to do it alone, you could fail because of the possibility of others coming in, cutting corners inappropriately, getting a bad reputation, which could influence the market's response to your product too.

Q: Mark Thring, Louth Bay Abalone: So you need roughly 4-4.5 tonnes per day?

A: No, about 2-4 tonnes per day. Realistically about 2 tonnes per day, per dock. You have to look at every individual component including all diving and processing costs. Processing costs include setting up costs, labour, ice, water, having everything ready, clean-up costs and sterilising of all equipment. Then you get into the major one which is the break-even point. It cost me about $110 to send 3 kilograms of sea urchins to Japan. It is necessary to have volume to pack your freight properly. 1000 kilograms is the absolute minimum, if you can process 2 tonnes a day it cuts your overhead costs down.

Q: Craig Blount (NSW Fisheries Research Institute): In terms of grading, it seems we have sea urchins with both yellow and orange coloured roe. Can they both make "A" grade, and what size of roe are you looking for as "A" grade? Secondly in terms of building a $300,000.00 plant in SA to centralise the product, you were saying that most of the product that is out there is "B" grade yet you are talking about the niche market in Japan for "A" grade. Aren't we behind the 8 ball to start with, with a "B" grade product?

A: John Vairy (Sea Urchin Processor, NSW): Yes, but we must achieve a high quality of product beforehand.

Steve Wright (Sea Urchin Licensee, Victor Harbour): Exactly, that's why we have backed off. I don't think that we have got the industry unless what Richard is doing and what industry is doing can really improve a B+ grade to an "A" grade, which is yet to be determined.

Terry Scott has done some work, but it is early days for the industry. Part of the reason it hasn't taken off in the past, and people have been looking at it for the last 10 years, is because there are so many variables and difficulties. With market demands, there is about 2.7 tonne shortfall of "A" grade product each week. Our products are patchy in terms of "A" grade.

Q: Craig Blount (NSW Fisheries Research Institute): Yellow and orange roe?

A: Yes, the roe is sort of a browny/yellow colour with some of it bright yellow. The "A" grade is really bright yellow/orange about the same size as the indigenous sea urchin. We do get some of the brighter yellow and orange colour, more from Coffin Bay and we get patches of it in other areas down the south coast.

Markets and Marketing: a Chef's Perspective - Jean Pierre Rival (Chef Consultant, Adelaide):

I have been a chef for about 30 years and have always specialised in fish and seafood. I arrived in Australia about 13 years ago and went to Port Lincoln to start a fish and seafood restaurant. When I tried to put sea urchin on my menu people were surprised, they knew in France we eat snails, but sea urchins – that was really crazy!

The point I would like to make is regarding the national market because we can't export sea urchin all around the world. The French probably pay about $8-9 dollars each for a nice sea urchin, with orange roe. So far I haven't seen anyone in Australia making money exporting sea urchin roe, therefore I would like to talk about the market and also the product. I have been using sea urchin for 20 years and I am really happy that there are people who know what they are talking about. Unfortunately the average person is unaware of sea urchin roe as cuisine.

Twelve years ago no one in Australia knew what sea urchins were, even chefs did not know about sea urchin roe. This made it impossible to promote if the chefs didn't know about the product. By trying to sell sea urchin and cook sea urchin I was always getting into trouble because there was no distribution, I could not buy sea urchin anywhere. Also, if I did put sea urchin on the menu, no one would order it because they did not know what it was.

To exacerbate the problem, no one could give me an accurate price per kilogram. I could not put it on my menu without knowing the cost factor.

It is essential we calculate a method of pricing, then we could begin to put our sea urchin on the menu; at which time, if people are prepared to try it, we will have to create a market and create a distribution. Possibly Angelakis could sell it.

Many people like the roe but have no idea of how to keep it refrigerated. To get the product from Coffin Bay to Adelaide and keep it really fresh is extremely difficult in my view. I would like to see sea urchin roe come from the sea to the restaurant using the most efficient method of keeping it in optimum condition.

The question of how long can you can keep sea urchin roe needs to be answered before putting it onto the market.

There are people in other countries who are conversant with how to handle sea urchin roe, and we need to tap into this knowledge to enable us to deliver the sea urchin roe at its best. Once we master this and achieve recognition our industry will eventually get a good name, however, it is going to be a long road.

South Australia has the reputation as being "The State of Food", which is really good, but we need to ensure we have appropriate quality control and a knowledgable workforce.

How do we position the product? Our product would be positioned well, notwithstanding the cost of processing and packaging. This product could reach top price as a delicacy, for example sea urchin pate is a product well known overseas.

Q: Richard Musgrove (SARDI Aquatic Sciences): It sounds to me like the local market would be a safer bet in the early stages of the industry, would you agree with that?

A: Yes. Chefs are very creative, so give them some new product and they will produce a variety of very creative dishes.

A: John Vairy (Sea Urchin Processor, NSW): There is still a large potential, but the product has got to be there. You asked about storage, it has to be kept almost spoilage proof. If we do a tray it's preferable to keep it at 1^0C. How long depends on the processor, processing is quite intricate, you have to control bacteria. In Japan they use a mixture of water and alcohol to spray over the roe to kill the bacteria. Ten days to two weeks is a reasonable maximum length of storage time.

We also have to look at the food regulations. Putting roe in a drink fridge is not acceptable. If the fridge the roe is stored in is opened and shut continually, the shelf life will be very poor.

When we pack for export, quite often we will keep all the trays packed in boxes and kept in the freezer and actually put ice packs in to keep the temperature as low as possible. Even when you are processing, chilled water is necessary to keep the temperature down. Under 5^0C the bacteria do not multiply and spread rapidly. Chilled brine can also be used.

Sea urchin nutrition research in Canada - Lance Schlipalius (Director, Carotenoids, Betatene Pty Ltd) :

I am probably the person in this room that knows least about sea urchins. I have just arrived from the United States this morning so I'll have to keep this fairly lively, otherwise I am going to be the first person to fall asleep.

As Richard has said, we have been working with the people in Canada at the St Andrews Biological Station which is over the border from Maine in the United States. The next thing I should do is tell you of how we fit into all this, because it is a little bit obtuse. We were approached by the people at St Andrews Biological Station about 6 months ago because we produce a material called Algro Natural, some of you in the abalone area may even know about it. The product comes from Whyalla and we have been producing it for some 15 years, with most of it going into places like Japan for aquaculture. The Canadians were interested in trying this material because it is of algal origin and they wanted to get an enhancement program going for their sea urchin industry in the Bay of Fundy. They have a small industry, $US2-4 million per annum, but seasonal. It fluctuates, but overall it is declining. It was much larger, apparently reaching the $US10m mark annually and they were getting in the order of $CAN30-50 per kilogram for roe when I was there in December last year.

There are two men working in this enhancement area for the Department of Oceans and Fisheries. St Andrews is a small town of about 1700 people and biological studies are one of the main features of the town. Dr Shawn Robinson is basically a marine ecologist looking at wild catches and other ecological factors, and Dr John Castell is a nutritionist, so together they both form a pretty good team.

The species which they study is *Strongylocentrotus droebachiensis*, that is the green sea urchin. This is seemingly the only endemic sea urchin in that area, and they believe that the catch has declined due to the fact that the animals are about 10 years old, and some of the larger animals are up to thirty years or more. It is proposed that in that area at least some of the animals are indeed living in an almost subsistence situation and it may even be that that's the case more generally, because once you give them good feed the roe enhancement is very rapid as I think Terry pointed out earlier, from experiments that he has done. Originally they had in the order of 3-4% roe from the animals in the wild but there is quite a seasonal influence. In this particular species I gather the main influence with the spawning is the day length. Because they are so far north they have a pretty substantial day/night variance throughout the year. I was there in the middle of December and it was light for about 4 or 5 hours per day. That seems to be the main influence in the spawning feature, but they can also induce the sea urchin to spawn artificially by using potassium chloride. Their interest at the moment is to do some enhancement by developing some holding systems and then to utilise enrichment diets, optimising those for roe enhancement. Stage two is to go into aquaculture in total. They ran an experiment on our Algro Natural material compared with the rates of other particular diets which they had accumulated from various sources.

Their tanks are round, about a metre in diameter and about 30 centimetres deep. They contain small baskets which hold about ten urchins at a time. The feed they originally developed is a sort of porridge type mash with a mainly cereal base and some additives in terms of vitamins and minerals. They have now progressed to moist extruded pellets which are made with a pasta type machine which produces feed with a long thick noodle type appearance (7-8 mm in diameter). The feed that they use for our product is based on soybean, wheat and canola meal with some added starch gel and alginate, linseed oil provided with free fatty acids, lecithin, vitamins and minerals, ascorbic acid for some antioxidant activity as well. Those of you involved in the animal feed area will understand that those things are pretty standard. The test period for this was two months, sea urchins were taken from the wild and put into an enhancement system then the animal compared with the roe prior to being enhanced and then with similar animals after enhancement.

These are the results that they obtained:

Originally they had 3.4% roe in these animals which were taken from the wild and fed on the various diets for two months (Table 1).

Table 1 Roe yield for sea urchins fed various diets in a laboratory system over a two month period. Initial mean yield = 3.4% The Control is the basic diet without additives. The units for the Algro additive are mg/kg

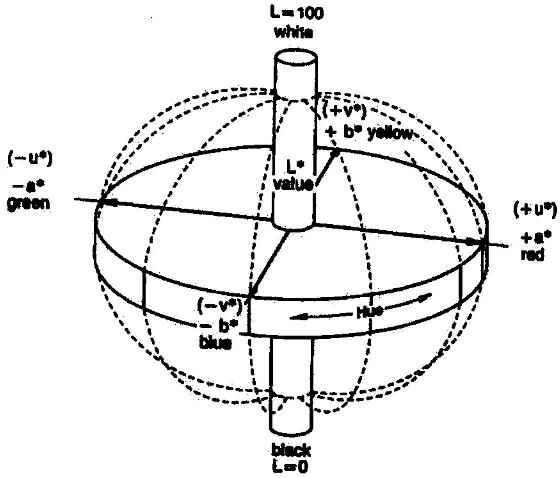
Diet	**Roe Yield (%)**
Control	17.3
" + Carrot	15.6
" + Beet	16.8
" + Algro 50 mg/kg	16.6
" + Algro 100	15.4
" + Algro 250	16.2
" + Algro 500	16.6
Shurgain	14.6

The control, is the basic diet described above, with none of the additives so it just provides them with some basic material, some protein sources and grain meals. They added carrot as a source of carotenoid to try to develop the colour. An explanation about the colour might be helpful here, the colour which is mainly being reported in the roe of sea urchins in the literature is a material called echinone, that's a carotenoid. There's a small spectrum of carotenoids in the roe, but the main one there is echinone and that is just a few steps up the biochemical pathway from betacarotene, the main carotenoid found in most living things. Betacarotene is the main carotenoid component in our Algro Natural.

The Shurgain diet has been developed by a feed producer in Canada and contains another betacarotene product – a synthetic betacarotene. There are three different levels of use of our particular material (Algro Natural) in the feed going up to a concentration of 5% of the total feed. So you can see that regardless of what you use you get a substantial increase in the roe yield in the end.

They also interpret the colour because they are looking at quality as well. They measured this with a colour meter that had an electronic device with which you measure brightness and colour (as used in tuna aquaculture product quality research: ed). The parameters are measured using a spectrophotometer attached to a computer. The computer software reads the colour and its brightness as a function of its position within a 3-dimensional space (Fig 1). The "a" axis grades from red (+ve) to green (-ve), the "b" axis from yellow (+ve) to blue (-ve) and the brightness (L) axis from black to white.

Figure 1 A 3-dimensional representation of colour. Any colour can be plotted on the a-b axis and the brightness can be plotted on the L axis. Figure from Robinson, S., Castell, J, Kennedy, E. and Peters, L (2000), A summary of sea urchin culture at the St. Andrews Biological Station. In: Workshop on the Co-ordination of Green Sea Urchin Research in Atlantic Canada, St Andrews Biological Station, New Brunswick, Canada.



The dominant feature of the colour is the brightness, which has gone up substantially and is one of the main determining features for quality in the roe. In terms of the flavour these were also judged by comparison with those taken from the wild. There was an improvement in some of the test diets, in particular they gave a less bitter characteristic, the taste was less sharp and more rounded – probably more acceptable. I had the opportunity of looking at a few of these and certainly there did appear to be a benefit with the Algro product compared to some of the others. The Shurgain product actually had quite a bitter flavour, which is apparently less acceptable to the market and downgraded the score. In other words, there is quite a range of criteria which are important to the end product. There have been more tests and since then, we have looked to see what was happening in Australia with sea urchins and met with Richard Musgrove.

Basically that is what is happening in Canada and there is this joint effort world-wide to try to get something together in terms of a diet which looks as if it may be acceptable for improving gonad condition and to allow the rating of the various species.

Sea Urchin Research In NSW and Victoria - Craig Blount (NSW Fisheries Research Institute):

I am going to give a general overview of what is happening in research and development in NSW and eastern Victoria. Duncan Worthington and myself worked on a FRDC-funded project in Victoria. The project goes for three years and we are only at the beginning of our first year. It came about because of interest in the last couple of years in sea urchins in NSW and eastern Victoria. There was also a need for the project because the fishery is experiencing rapid development and there is very little information to base management decisions upon. It appears we are at the same stage as everywhere else in terms of interest.

I will be mainly discussing stock assessment and roe enhancement which are the two major objectives of our FRDC research project.

Stock Assessment

It is essential to get a good stock assessment process going for our resources. Stock assessment was identified as an important objective to be met due to the vulnerability of sea urchin fisheries to over-exploitation. Roe enhancement is also of high priority due to variability in the quality of roe that occurs in sea urchins in the wild.

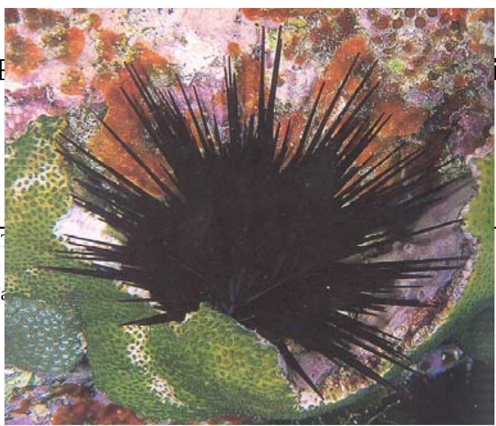
Roe Enhancement

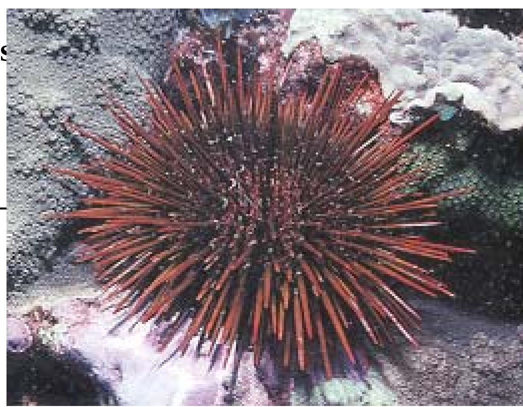
There is a considerable variability in roe quality in wild sea urchins, and it would appear that you have similar problems down here. John Vairy from NSW will be giving the industry perspective later.

Live Fisheries

There have been a few pushes to get it going over the last couple of decades but they have all failed, probably because of the market. However over the last year or so it seems to be going well thanks to a lot of R&D by industry.

There have been several attempts to develop the fishery, and the latest appears to be the most organised and most likely to succeed. There are 37 permit holders in NSW and 22 in eastern Victoria. It is a multi-species fishery, harvesting the black (Fig 1), red and purple sea urchin species.





Present management is based upon area closures only. There are no size limit and no quota restrictions.

The black sea urchin is a large animal and is abundant throughout NSW. The red urchin is larger but has a more restricted distribution. It can occur in high abundance in certain spots, particularly close to the shore although it is not as dispersed as the black urchin. The purple urchin occurs more in eastern Victoria. There are a lot of purple urchins in NSW, however they are quite small.

In terms of management, at the moment we are working on area closures only, for two reasons. One because the industry has only been going for the last couple of years and we want the industry to develop unrestricted, we do not want to introduce size limits and quotas just yet. In NSW we have approximately 85 fishing zones and only about thirty of those are for sea urchin fishing.

I mentioned that stock assessment was one of our main objectives. What we are trying to do is estimate the size and productivity of the fishery and the effects of harvesting in order to determine how to manage the resource properly.

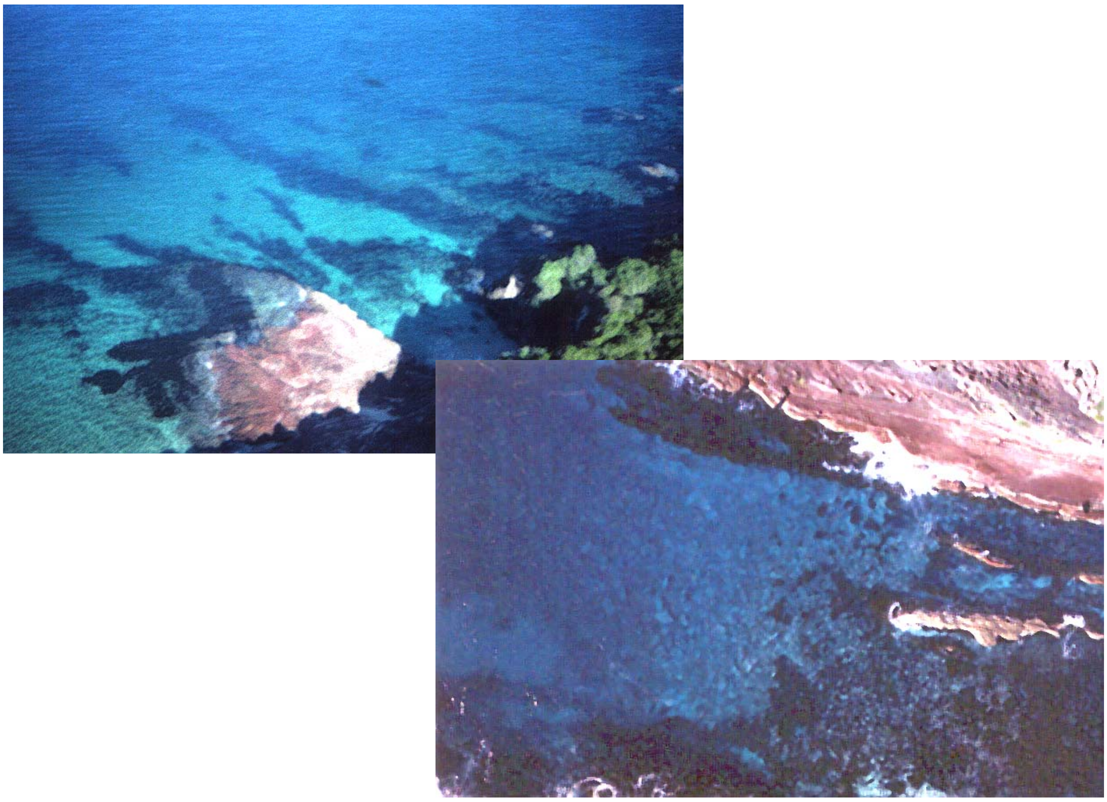
In estimating the size of the resource we are compiling a database that combines information about the area of sea urchin habitat in NSW and eastern Victoria with estimates of the density of sea urchins within these habitats in specific regions. Estimates of habitat area are obtained from aerial photos.

We have taken a number of aerial photos to demonstrate our work. Nearshore reef (Fig. 2) in NSW is generally composed of areas of large brown algae on the shallow tops of the reef and areas of Barrens (white rock with sea urchins) in deeper areas and gutters.

Fig 2 Nearshore Reef

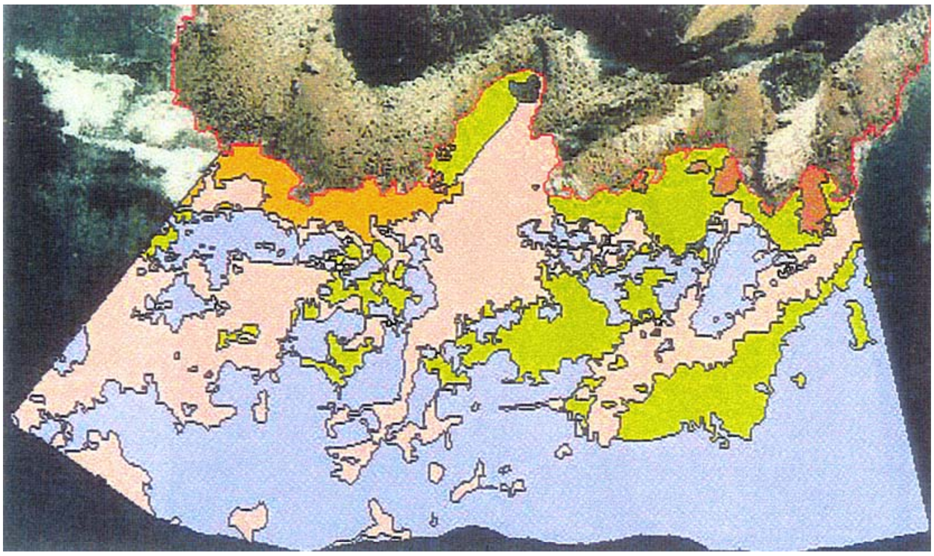
These distinctly different areas are recognisable from aerial photos (Fig. 3).

Fig 3 Nearshore reef from the air



Aerial photos can be converted to a habitat map in a GIS format (Fig. 4). We have mapped habitat at 60 sites in NSW.

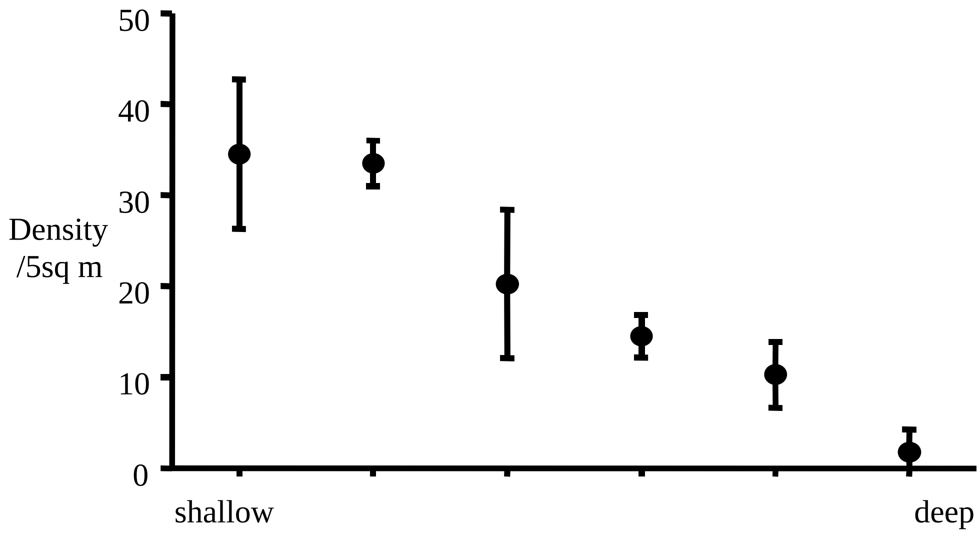
Fig 4 GIS habitat map of 60 sites in NSW



From time series of aerial photos of the same site we know that the boundaries of these habitats can shift over time.

We have surveyed the density of sea urchins at approximately 70 sites in NSW and eastern Victoria. In our surveys we swim along two transects that run offshore at each site. At points along these transects we count urchins within specific size classes to get estimates of density.
We keep a photographic record and underwater map of each site so we can return to the exact area at some time in the future. The data we obtain are estimates of the density in each of three size classes of sea urchin, at specific distances from the shore (Fig 5).

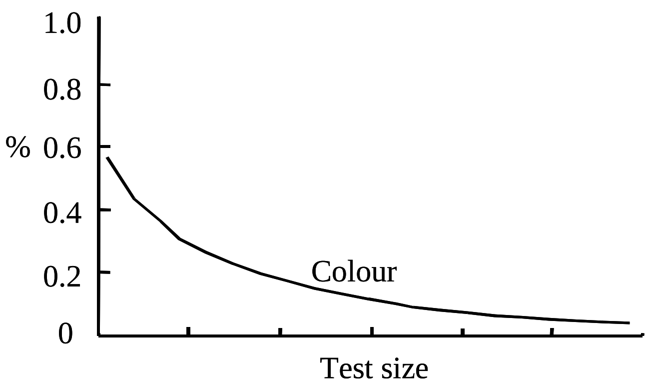
Fig 5. Density (/5sq m) by depth: red urchins 50-100 mm



In addition to our estimates of resource size we are measuring commercial catch rates and sizes. We are also comparing how populations change in fished vs non-fished areas.
Although fishing is currently in shallow algal habitats the majority of the resource is in Barrens habitat, representing an enormous, untouched resource. However, there are problems with recovery and colour of the roe from urchins in Barrens areas.

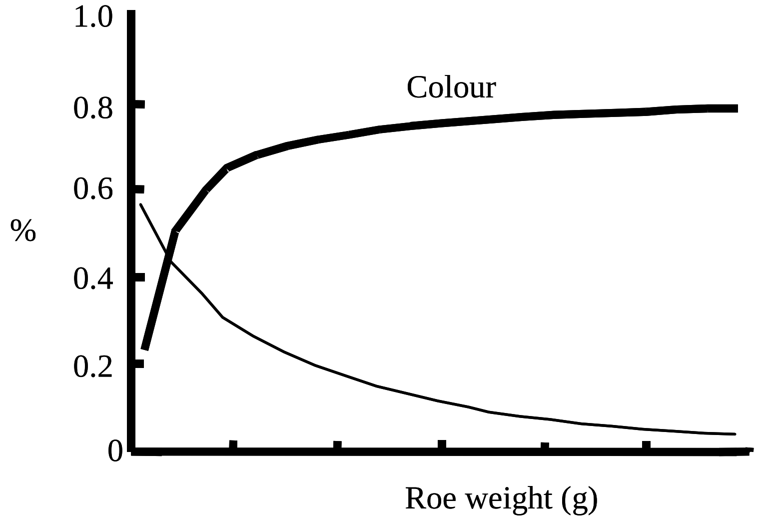
The ultimate goal of our enhancement work is sizeable roe of a consistently good colour. There are many factors affecting the colour of roe. For example, the colour of roe is related to the size of the urchin (Fig 6).

Fig 6. The effect of test size on roe colour



Colour of roe is also related to roe weight. It is the smaller urchins with heavier roe weights that have the best colour (Fig 7)

Fig 7. Roe weight and colour



We are in the process of designing a series of field experiments to investigate ways of enhancing the roe of sea urchins. One of these is a thinning experiment in which the density of sea urchins is reduced. The density of sea urchins in the Barrens were reduced to a proportion of the original density and the roe of these sea urchins compared to the roe from sea urchins in which the density was not manipulated.

Where sea urchins were thinned out the roe was improved significantly (Fig 8) relative to the controls (unthinned). The proportion of sea urchins with roe of a good colour was significantly increased, as was the average recovery. This was probably due to an increase in the availability of food to sea urchins at the thinned sites.

Fig 8 The effect of density reduction on the size of roe recovered from remaining sea urchin

Recovery was significantly improved in sea urchins from the thinned treatments relative to controls, but not to the level found in Fringe habitats. Roe enhancement experiments will also involve transplanting younger animals to more productive areas and estimating the time-scale, and cost-benefit of enhancement. Industry is helping with the application of roe enhancement techniques by thinning on a large scale and keeping a logbook of the effort needed to make it work.

Processing Sea Urchin Roe - John Vairy (NSW Industry Representative):

I have been in the abalone industry for about 30 years and been toying with sea urchins for about 15 years. I also have about 10 years experience with exporting. I have seen a lot of sea urchin projects fail.

The new AQIS regulations determining processing of this product include specifications which are very rigid. They include a fully enclosed working space, filtered air, air locks on the doors and cool room. Whenever a person wishes to enter, they are obliged to walk through a foot bath prior to entering the work area.

Room temperature ideally should be about 5^{o}C, but as that is a little difficult for people to work in, 15^{o}C is probably a suitable temperature. The processing temperature impacts heavily on the quality of the roe.

Another factor is the quality of the sea water. Natural sea water is not suitable as it contains substantial quantities of bacteria, it is preferable to use town water with 3% salt added. Water should be tested regularly for chlorine levels.

Workers should be obliged to wear hair nets (this is critical), rubber gloves and aprons. Care should be taken to prevent fibres falling out of clothing.

Plastic boxes with chilled salt water should be used. The whole object is to clean the roe and get rid of as much bacteria as possible, as it can reduce the shelf life considerably.

Basket design and composition is important, if you are going to do it purchase the proper baskets from Japan or America. The baskets should stack inside each other. The fit must be tight enough so as not to crush the roe. Processing speed and quality control are major issues.

People most often fail because they have not adhered to the principles mentioned above.

Q: Mark Thring (Louth Bay Abalone): Is processing at sea an option?

A: Because animals are under stress the minute they come out of the water, processing at sea is an excellent option. The earlier the processing starts the better.

For storage in salt water, a shallow elongated container is preferable to a deep container. This limits the number of boxes which can be stacked on top of each other, however it reduces the potential for damage through crushing and air bubble movement. Trapped air bubbles may damage the roe as they move between stacks of boxes.

The cost to set up a “State of the Art” processing plant would be in excess of $100,000. I have a room which is about 3.6m^2, however this is overcrowded.

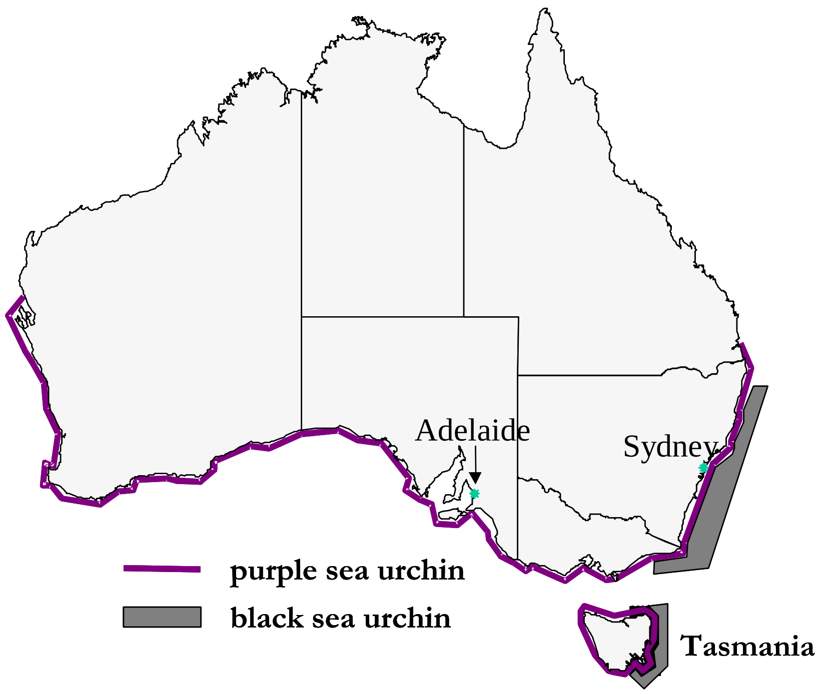
Sea urchin Nutrition Research in South Australia - Richard Musgrove, (SARDI Aquatic Sciences)

Background

There are about 20 species of sea urchin in Australia, 18 of which are unique to the region. Two of these are being exploited commercially, that is, the black sea urchin (*Centrostephanes rodgersii*) and the purple sea urchin (*Heliocidaris erythrogramma).* Their distributions within Australia are as follows (Fig 1).

Having just heard of the good work of Craig Blount and Duncan Worthington, it is apparent that one of the major problems we face in South Australia is the dearth of knowledge about the local species: population size, age structure, age at sexual maturity and diets. Until information is gathered covering these issues we are fishing in the dark. We have no idea of the impact of fishing pressure and what level is sustainable.

Fig 1. Australian distribution of the purple sea urchin (*Heliocidaris erythrogramma)* and the black sea urchin (*Centrostephanes rodgersii*)



Having said that we are fortunate that this is a new fishery in South Australia, the opportunity exists for sustainable management from the outset.

There is clearly a market for these animals. The domestic market in Japan is worth about $AU760 million with imports from the rest of the world adding another $AU240 million. Australia is a very small part of that; in 1999 about 13.2 tonnes of “green” (whole, chilled) sea urchins went through the Sydney fish market fetching an average of $3/kilogram. The same year 360 kilograms of processed roe went through, selling for $27 to $46/ kilogram. Recently processed A grade purple sea urchin roe from Coffin Bay in SA has been quoted at

$130-160/kilogram in Japan and Tasmanian purple sea urchin A grade roe fetches up to $250/kilogram.

Current project : Refer to main body of Final Report

Rules and Regulations - Ben Loiterton (PIRSA Fisheries and Aquaculture)

A number of people have access to the sea urchin fishery in South Australia through either miscellaneous fisheries licences or five developmental miscellaneous exemptions. Those exemptions have been running since July 1999 and will be re-assessed at the end of June 2001.

As far as access to the fishery goes, that is about it for the time being, there is no new access in the interim period whilst those permits are current. I have been looking after the sea urchin fishery, however Samara Miller, another fisheries manager who was unable to make it today, will be taking over from me. Still, feel free to contact me and I will either pass the enquiry on to her or answer it myself. Feel free to ask any questions you might have.

At the end of the two year experimental period, the five permits will be reviewed and any future access will be established at that time but there is no guarantee of ongoing access on the basis of those developmental permits.

Q: Steve Wright (Sea Urchin Licensee, Victor Harbour) What is the information you have gathered so far as to the prognosis of the licence fees?

A: Some zones are doing better than others however that information is confidential.

The permits were put in place because there was interest in sea urchins, and it is relatively unknown in South Australia what the potential is. The data collected will give PIRSA an opportunity to have a look right across the state to see what is out there and what potential exists. More formal arrangements will be considered at the end of the two year period, in terms of how much people are catching etc.

GENERAL DISCUSSION

RICHARD MUSGROVE (SARDI Aquatic Sciences)

The thought is that we should form some sort of working group, industry body, whatever you would like to call it, in order to foster a co-operative approach to harvesting, marketing and research. Instead of individuals working autonomously, everyone working together can achieve much more.

With that in mind I have asked Steve Wright to chair the discussion period.

STEVE WRIGHT (Sea Urchin Licensee, Victor Harbour)

What I would like to do just before we kick off with that is to perhaps ask John Vairy and our friends from interstate who have a handle on this discussion as to where things are at and where they are not at. Given your experience in Tasmania, Victoria and New South Wales you might be able to give us some dot points before we get into a general discussion in what we need to do with the work committee. If you had your time over again, given your experience and given the situation here, (ie. the state of the industry), what do you suggest we should do? We would like to pick your brains a little whilst we have you here.

Conversely we would like you to feel free to keep contact with Richard Musgrove or in fact come to the meetings so that we can try to develop something in terms of a southern ocean perspective and share information in terms of marketing and exporting an Australian product. We are open to working with our interstate colleagues as much as possible.

Major Points:

1. John Vairy: You need representatives from different areas; from the divers' point of view as well as processors, marketing experts, researchers (SARDI) and representatives from professional and recreational fishers.

2. Steve Wright: Marketing and management people might include The Fish Factory, Michael Angelakis, Simon Koh, Samara Miller etc. The ideal number would be a group of seven people working on the basis of seeking ways of achieving support from the South Australian government, especially with support from PIRSA and SARDI. The seven representatives might call on anything up to twelve people from their particular area of expertise. The emphasis should be on progressing things in unison, bringing in synergy and energy to achieve an outcome.

3. Brett Williams: The meetings can be rotated around the state, with each person hosting in turn.

4. Steve Wright: Thank you to our interstate colleagues in terms of sharing. Things could possibly be done federally so that we are doing things as an industry, particularly the focus on the Japanese market. Looking from an "Australian perspective" might be of benefit as we are up against the Americans, the Russians and the Chileans.

5. Jean-Pierre Rival: We should look at other possible markets eg. Europe.

6. Steve Wright: It is unfortunate that “fly by night” processors are spoiling the market for everyone. We need to look at ideas on how we can control this? Without legislation at this point there is no way of dealing with this issue except quality control. Who has the carriage of this, SARDI or PIRSA? Samara Miller does a good job, however Richard Musgrove will be considerably involved from a R&D point of view. An agenda issue to consider is the possibility of self regulation. An industry body seems to be the most appropriate to make representations through SARDI and PIRSA to the Minister voicing our fears that we may have “the rug pulled from underneath us”, and we are interested in growing the industry in a positive way.

7. John Vairy: There are concerns that the wrong person could get their hands on the funding, it reflects back on industry and hurts everyone.

8. Richard Musgrove: FRDC require substantial industry support before funding is given. Also a development plan is necessary for the sea urchin fishery.

9. Ben Loiterton: Eventually we will have to have a management plan put into place.

10. John Vairy: The critical things are money and manpower to run it.

11. Steve Wright: Can we confront FRDC with an information committee? I think the first thing we must do is to approach FRDC nationally.

PARTICIPANTS

NAME	Title/association
Nicholas Pluker	Sea Urchin Licensee
Terry Scott	Sea Urchin Licensee
Mr and Mrs Williams	Sea Urchin Licensee
Mark Westcott	Sea Urchin Licensee
Barry Lienert	Sea Urchin Licensee
Graeme Hartwich	Sea Urchin Licensee
Jim and Rosalie Pope	Sea Urchin Licensee
Steven Wright	Sea Urchin Licensee
John Vairy	Sea Urchin Fisher/Processor, NSW
Dr Richard Musgrove	Sea Urchin Project Leader, SARDI Aquatic Sciences
Dr Scoresby Shepherd	SARDI Senior Research Scientist
Tania Kiley	PIRSA Client Manager, Shellfish
Marcus Strauss	NSW Sea Urchin Fisher
Craig Blount	NSW Fisheries Research Institute
Dr Duncan Worthington	NSW Fisheries Research Institute
Jean-Pierre Rival	Chef Consultant
Steve Edwards	Manager, Granite Island Adventure Park
Luke Horlin -Smith	Biologist, Granite Island Adventure Park
Ben Loiterton	PIRSA Fisheries Manager
Graham Ween	Louth Bay Abalone
Mark Thring	Louth Bay Abalone
Don Morrison	Louth Bay Abalone
Gabor Karl	Flinders University
Jon Lark	Flinders University

APPENDIX 6: STUDY TOUR REPORT

This study tour was part of the original proposal and was intended to allow direct observation of state-of-the-art technology and practice within established sea urchin industries. The information obtained and the contacts made facilitated technology transfer to South Australian industry (fishers, processors and aquaculturists). The trip also enabled the exploration of opportunities for the establishment of collaborative research programs.

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Scope

Visits were made to sea urchin researchers in Scotland, in New Hampshire, Florida and California in the USA and in New Brunswick and Newfoundland in Canada. A visit was also made to the Lobster Institute in Maine, USA.

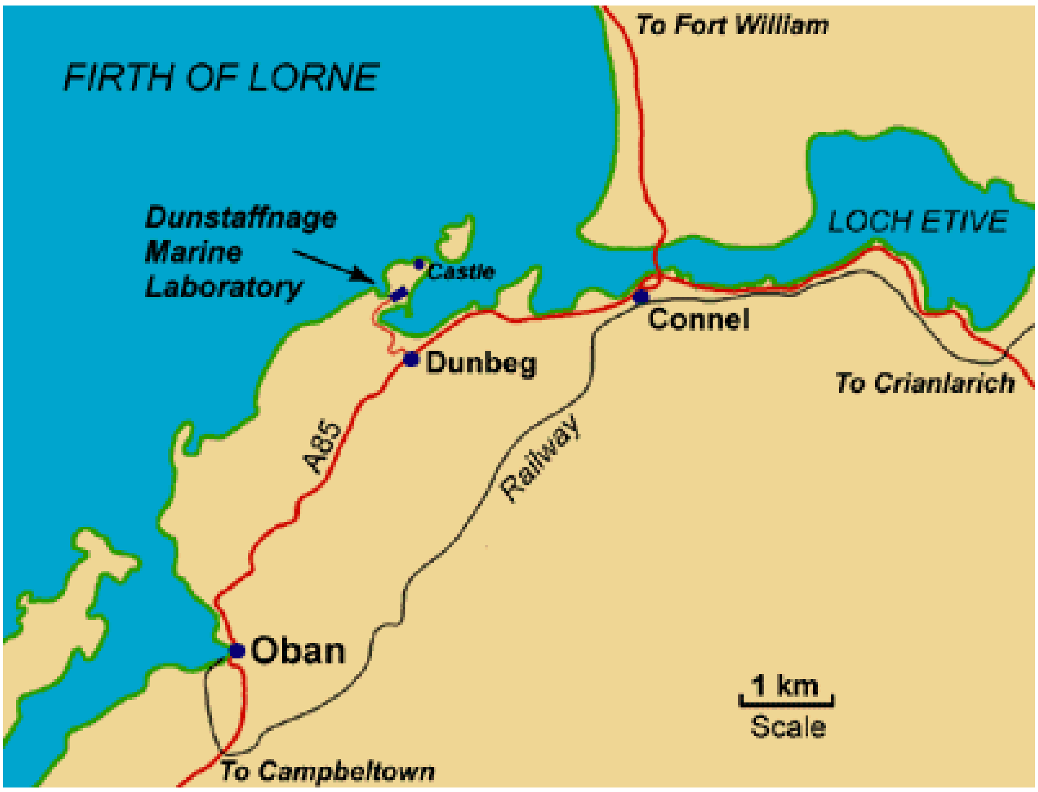
Aim

This trip allowed the author to directly observe state-of-the-art technology and practice within established sea urchin industries. The information obtained and the contacts made will facilitate technology transfer to South Australian industry (fishers, processors and aquaculturists). The trip also enabled the exploration of opportunities for the establishment of collaborative research programs.

Scotland, UK

The initial visit was to the Dunstaffnage Marine Laboratory (DML) (Map 1), on the Firth of Lorn near Oban, about three hours west of Glasgow (Map 2) by bus.

Map 1 Dunstaffnage Marine Laboratory



Map 2 The UK, showing Glasgow in Scotland (red arrow)

DML is a research station within the Scottish Association for Marine Science (SAMS). The laboratory has many strengths, for example: Geochemistry, Deep-Sea Benthic Dynamics, Deep Water Fish Biology, Biotechnology, Invertebrate Biology and Mariculture, Microbial Ecology and Zooplankton Dynamics, to name a few.

The Invertebrate Biology and Mariculture group works mainly on sea-urchin and abalone cultivation under the guidance of its leader, Dr Maeve Kelly, a Post Doctoral Research Fellow at SAMS. She showed me around the laboratory, in particular its aquarium facilities with research in progress and I was fortunate to met many of DML's other staff and students. Extensive discussions were held on sea urchin nutrition, (particularly relevant to the FRDC project I am working on) especially with regard to their current work.

Dr. Kelly's focus at present is on "marine macroalgivores of commercial importance". Specifically, she is studying the potential for commercial farming of the sea urchin *Psammechinus miliaris*. Dr Kelly's group is leading a consortium of nine academic and industrial partners within the EC, and is addressing scientific and technical issues faced by this new culture industry. The industrial partners include Scottish, Irish, French, Portuguese and Norwegian companies.

P. miliaris differs from *Heliocidarias erythrogramma* in several significant areas (**Table 1**), for example, there are both intertidal and subtidal populations with the latter yielding the biggest roe and highest growth rates, probably due to the relative greater abundance of invertebrate food in that zone.

Table 1 Comparison between *Heliocidaris erythrogramma* and *Psammechinus miliaris*

	Heliocidaris erythrogramma	*Psammechinus miliaris*
Maturity	50-60mm	5mm
Av Size	70-80mm	30mm
Price	$AU130-160	$AU27-45
Habitat	Subtidal	Intertidal and subtidal
Food	Omnivorous	Omnivorous
Main Market	Japan	France

Culture

There is a particular interest in polyculture with salmon. Trials of the urchins have been successful thus far, both in terms of roe enhancement and using the urchins as a means to keep the cages free of fouling. The following is an abstract from a recent paper on the subject.

Sea urchins in polyculture: the way to enhanced gonad growth?

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Corresponding author.

[2] Joseph Johnson and Sons, Lower Badcall, Scourie, UK

In Echinoderms: San Francisco, Mooi and Telford (eds), 1998, Balkema, Rotterdam.

Abstract

In polyculture wild *P. miliaris* were used to stock Atlantic salmon (*Salmo salar*) sea cages. Urchins in cages with salmon showed a significantly enhanced gonad growth compared to urchins in similar cages with no salmon (monoculture). The survival rates were good in cages that did not use an air-lift to evacuate fish mortalities. Both urchins in poly- and monoculture had significantly enhanced gonad growth compared to local wild populations. In tank-based trials grazing *P. miliaris* made a significant reduction in the amount fouling on nets lining the tanks. The data suggests urchin polyculture may have advantages for growing urchins to marketable size with a minimal outlay for feeds and cages.

More interesting information from this paper

- The paper was divided into two sections, the first a polyculture trial in sea cages, the second a net cleaning trial in tanks. During the former the urchins were loose in the cages which were part of a commercial salmon farm. Urchin density was 2 m^{-2} of cage netting using 20-30mm diameter animals. During the latter, the there were 300 urchins per tank , equivalent to $150m^{-2}$ of net surface. The urchins were the same size as those used in the polyculture trial.

Polyculture trial

- All the urchins in the polyculture trial were fed with *Laminaria* spp, including *L. saccharina*. The urchins in the treatment without salmon showed significantly reduced gonad growth compared with those in cages with salmon. Also, the urchins in the polyculture did not feed on the *Laminaria*, but on the excess salmon food and faecal material.

Net Trial

1. At the completion of the trial there was a significantly greater amount of fouling (primarily *Enteromorpha* and *Cladophora*) on the ungrazed net compared to the grazed nets.

2. During the trial some of the tanks were fed *L. saccharina* and some were left unfed. The latter had significantly smaller gonads than those which were fed

suggesting that the fouling alone was not enough to sustain the urchins at the high stocking density used.

3. After the trial, breaking strain tests showed that there was no significant reduction in net strength due to urchin grazing although closer examination showed the grazed nets had a slightly fluffy appearance.

Related work

In other work (in SAMS Annual Report 1998-1999) using sea urchin cages designed for suspended culture, *P. miliaris* at a density of 0.5-1.2 individuals m^{-2} kept the cages free of fouling organisms while the controls (unstocked cages) were covered with encrusting organisms. It was suggested that this species may be used to control biofouling. Other density trials showed that stocking at 450 individuals m^{-2} did not inhibit somatic growth but gonadal growth was significantly reduced compared with urchins stocked at 150 m^{-2}.

There is concern over potential disease problems, particularly as the urchins are feeding on salmon faeces as well as uneaten salmon food. Diseases such as infectious pancreatic necrosis (IPN), viral haemorhagic septicemia (VHS) and infectious salmonid anaemia (ISA) are found in salmon seacage culture and could potentially be passed on to the urchins.

Other current work

Work of particular interest to the FRDC sea urchin roe enhancement project involves the use of diatoms (*Phaeodactylum tricorunutum*) in diets for the urchins. When included in artificial diets these have been found to significantly improve roe yield and roe colour.

Other work in preparation for publication includes

- The effect of artificial diets on growth, lipid utilisation and gonad biochemistry in *P. miliaris*.
- Work on the biosynthesis of eicospentaenoic acid (a fatty acid) in the urchin.

- Fatty acid compositions of gonadal material and diets for the urchin with regard to trophic and nutritional implications.

Abstracts for the above manuscripts (reproduced with permission):

Effect of artificial diets containing carotenoid-rich Microalgae on gonad growth and colour in the sea urchin *Psammechinus miliams (gmelin)*

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Corresponding author.

Abstract

Gonad growth and colour was examined in the echinoid *Psammechinus miliaris* when fed diets containing either micro- or macroalgal supplements. Urchins receiving diets containing the microalgae *Phaeodactylum tricornutum* and Tahitian *Isochrysis* sp. showed significantly greater gonad growth at the end of the 12 week experimental period in comparison to urchins fed the artificial diet with no added algae. An improvement in gonad colour, compared to the control, was observed for both treatments receiving microalgae, whereas those fed the macroalgae *Laminaria saccharina* diet showed no significant improvement in colour. The *P.tricornutum* diet improved gonad colour more rapidly than the other algal diets. These results show that cultured microalgae, incorporated into an artificia1 diet, have a positive effect on the gonad colour of *P. miliaris,* with promising implications for commercial echinoculture.

Biosynthesis of eicosapentaenoic acid in the sea urchin *Psammechinus miliaris*

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Abstract

The sea urchin *Psammechinus miliaris* (Gmelin) (Echinodermata: Echinoidea) was shown by using a deuterated tracer and quantitation by negative chemical ionisation gas chromatography mass spectrometry (GC-MS) to convert 18:3n-3 to 20:5n-3. The rate of conversion was very slow, corresponding to 0.09 pg.g tissue.mg 18:3n-3 eaten over 14 days. The great majority of the tracer was catabolized. Intermediate fatty acids 18:4n-3 and 20:4n-3 were found together with the elongation products 20:3n-3 and 22:3n-3. No deuterated 22:6n-3 was found.

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Fatty acid compositions of gonadal material and diets of the sea urchin, *Psammechinus miliaris*: Trophic and Nutritional Implications

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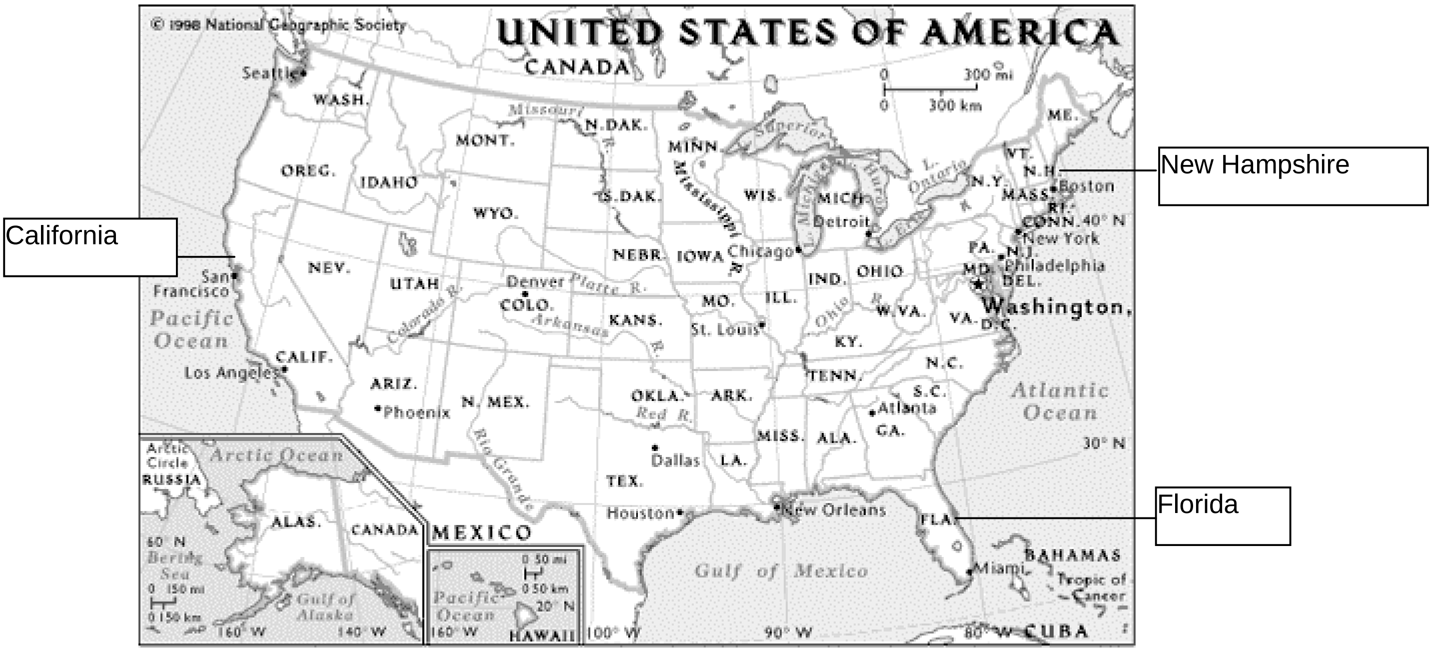
Abstract

The fatty acid compositions of gonadal material was examined for the sea urchin *Psamechinus miliaris* (Gmelin) held in aquaria and fed either salmon feed pellets or the macroalga, *Laminaria saccharina* for 18 months. Gonadal material was also examined from *P. miliaris* collected from four field sites, including commercial scallop lines encrusted with the mussel, *Mytilus edulis*, sea cages stocked with Atlantic salmon *Salmo salar* and two intertidal sea-loch sites, characterised by either a fine mud or a macroalgal substratum. The fatty acid compositions of known and potentia1 dietary material was examined. The proportions of certain fatty acids in the gonads of *P. miliaris* were significantly affected by diet type and location. Docosahexaenoic acid (DHA) 22:6 *n - 3* was significantly higher in the gonads of the sea urchins fed salmon feed in aquaria and collected from the salmon cages and scallop lines than in the gonads of the sea urchins fed *L. saccharina* in aquaria and collected from the intertidal sea loch sites. The salmon feed and the mussel tissue also contained a high proportion of this fatty acid. Stearidonic acid 18:4 *n* - 3 and arachidonic acid 20:4 *n* - 6, however, were found in significantly higher proportions than DHA in the gonads of the sea urchins fed *L. saccharina* and collected from the two intertidal sea-loch sites. *L. saccharina* was also found to contain high proportions of stearidonic and arachidonic acid. The gonads of the sea urchins collected from the intertidal site, characterised by a mud substratum, and from the scallop lines were found to contain a lower 18:1 *n - 9/* 18:1 *n - 7* ratio and a higher proportion of branched and odd-chained fatty acids, signifying a high dietary bacterial input, than the sea urchins held in the aquaria and collected from the salmon cage. 20:2 and 22:2 non-methylene-interrupted-dienoic fatty acids (NMIDs)
were found in *P. miliaris* fed diets lacking these fatty acids suggesting *de novo* biosynthesis. These results, therefore, suggest that the proportions/ ratios of certain fatty acids in the gonads of *P. miliaris* could be used to give an indication of the predominant diet type of this species in the wild.

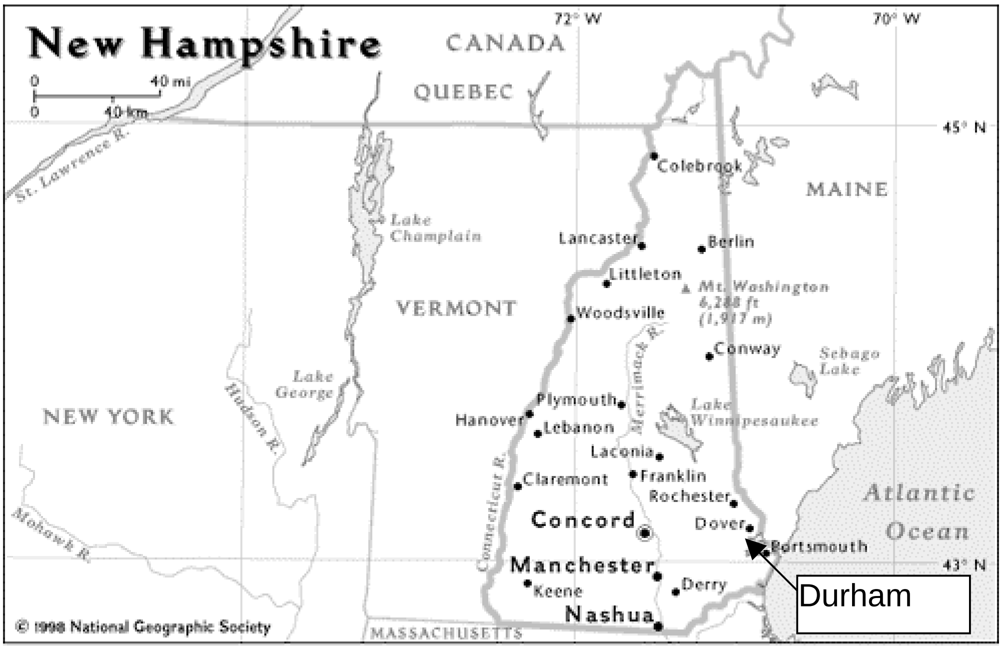
USA

Visits were made to sea urchin researchers and industry facilities in New Hampshire, Florida and California.



New Hampshire

From Scotland the next visit was for discussions with Dr Chuck Walker, Dr Mike Lesser and Dr Larry Harris of the Biology Department, University of New Hampshire (UNH) at Durham, south-west of Dover (see map below) New Hampshire, USA. All three are involved in an effort to re-establish the green sea urchin (*Strongylocentrotus droebachiensis*) fishery in the Gulf of Maine. Most of their work in this area revolve around hatchery development and manipulation of the gametogenic cycle using photoperiod.



Although the current FRDC project revolves around short-term enhancement of roe rather than closing the reproductive cycle and developing a hatchery, it must be acknowledged that these aspects will be important for ensuring the sustainability of a local fishery. As has been the case overseas, the wild resource may not sustain the heavy fishing pressure that could follow an increase in interest due to a rise in market price for roe. Trial shipments of purple sea urchin roe from South Australia have been quoted at $130-$160/ kg, above the current US average price ($US30/lb – equivalent to $AU125/kg).

The following is an article by Christine Fagan (UNH Sea Grant) who puts the New Hampshire efforts into perspective.

UNH Researchers Working to Revitalize Gulf of Maine Urchin Industry

By Christine Fagan
UNH Sea Grant
October 3, 2000

DURHAM, N.H. -- During the past 10 years, the Gulf of Maine green sea urchin (*Strongylocentrotus droebachiensis*) (Fig NH1) industry has gone from boom to bust. University of New Hampshire researchers are working to bring it back.

Fig NH1 The Green Sea Urchin

After being ignored by New England commercial fisherman for hundreds of years, the prickly urchins became popular in the early 1980s. They migrated en masse through Northeastern waters, gorging themselves on kelp beds and leaving the bottom of the ocean barren. The effects were disastrous for lobsters.

Larry Harris, UNH professor of zoology, has been studying marine communities for 30 years. He explains that after the urchins took over, commercial fishermen began to tap into the large Japanese market for sea urchin gonads. Known as uni, the gonads are considered a delicacy. During the late '80s this new fishery was a gold rush.

By 1993 the resource had peaked and urchin populations began to decline. Harris, who had started monitoring young urchins in 1983, found last year's population reached an all-time low.

Sea Grant and the UNH Agricultural Experiment Station are now funding Harris to find the most efficient way to grow large numbers of sea urchins in hatcheries. This type of production may be the only way the Gulf of Maine will have any long-term role in the sea urchin market.

Harris is experimenting with different types of growing containers, including rafts and panels. Urchins are cultured until they reach a suitable size, then outplanted or released into the ocean because storing them is expensive and labor intensive. Cultured urchins can be used to replenish wild stocks or aquaculture efforts on leased sections of the ocean bottom.
During the past academic year, Harris served as adviser for a Tech 797 project in which students developed an urchin growth system. Tech 797 is a year-long course

that allows interdisciplinary teams of undergraduates to collaboratively address marine issues. The students Laura Marshall, Seung Suk and Brian Sullivan, members of the class of 2000, won the David Drew Memorial Award for their efforts. The award is given annually to the year's best project.

Harris says the growth system shows great promise. Another project team is developing it further this semester. He hopes UNH will have an active sea urchin aquaculture hatchery in operation by early 2002. Currently there is only one hatchery in operation in the region. Located in Lubec, Maine, and operated by Peacock Canning Co., it is a side effort in a salmon hatchery.

Natural settlement of urchins occurs in June and July in the Gulf of Maine, and this is also when outplanting might be expected to take place. The problem, Harris says, is that this is also the time when the urchins' predators -- crabs and certain fish -- are most active. The alternate plan is to outplant urchins in the winter when urchin predators are inactive. Initial results suggest that winter outplanting is effective and further trials are planned.

By outplanting early, however, the sea urchins' reproductive cycles are out of sync. This is a problem that researchers Charles Walker, UNH professor of zoology, and Michael Lesser, UNH associate professor of zoology, are addressing with funding from Sea Grant, the UNH Agricultural Experiment Station and the U.S. Department of Commerce.

Light plays an important role in the sea urchin reproductive cycle, and by manipulating photoperiod (daily exposure to light), Walker and Lesser have been able to induce reproduction at different times during the year. Thus, the urchins can be outplanted earlier while the water is still very cold, sharply reducing the activity of predators and allowing the urchins time to become established.

Walker has been using his knowledge of the urchin reproductive system to develop land-based techniques to be used in the industry for producing optimal uni. While Japan is the largest market for uni, there are smaller markets in France, Belgium, Greece, Italy and Turkey. The ideal gonad is classified by its large size, firmness, color, texture and taste. Gonads are only marketable during a specific phase of the reproductive cycle, so by manipulating photoperiod it is possible to have ideal gonads available continuously.

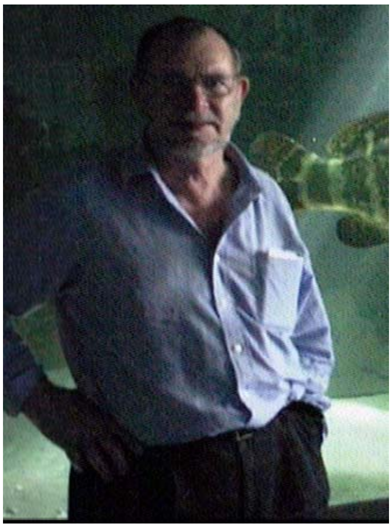
Michael Devon is an aquaculturist in Darling, Maine, who sells sea urchins to the Japanese market and has implemented some of Walker's findings. Walker hopes to help others like Devon, and is currently building a web site to make his results more accessible to the public. The site is still under construction, but can be visited at http://zoology.unh.edu/faculty/walker/urchin/gametogenesis.html.

New Hampshire Sea Grant is a component of the National Sea Grant College Program, a network of university-based research, education and extension efforts that promotes the wise use, conservation and development of our marine resources.

Florida

The next stop was The University of South Florida, in Tampa (red arrow, map below) where I visited Dr John Lawrence (Fig F1) for two days.

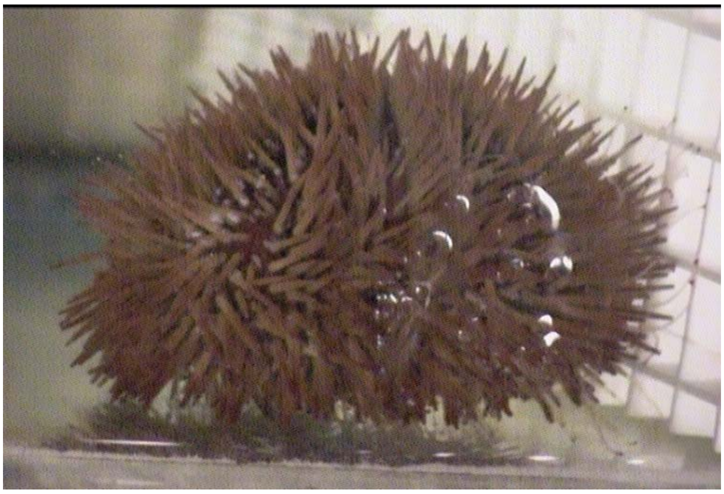
Fig F1 Dr John Lawrence



Dr Lawrence has done a tremendous amount of work on sea urchin nutrition and, with his brother, Addison, has developed a diet which is manufactured commercially by the aquaculture diet development arm of Wenger International. The diet has been used extensively in growth trials in many parts of the world (i.e. NZ, Canada, Chile) The Drs Lawrence are collaborating with workers in Chile in the development of a diet for the culture of their edible sea urchin (*Loxechinus albus*).

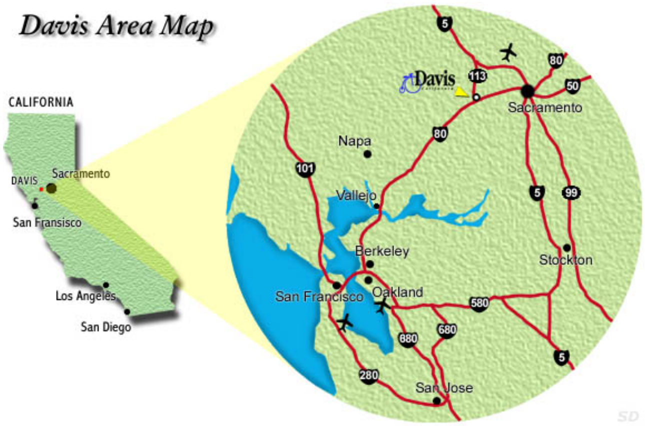
Dr Lawrence is written into the FRDC Sea Urchin project as an adviser and we had some interesting and useful discussions about his work on *Lytechinus variegatus* (Fig F2) and mine on *H. erythrogramma*. Although there is no sea urchin fishery in Florida, the visit was still productive with regard to meeting and talking with Dr Lawrence. Since then he has also sent me 150kg of urchin food (at no charge) for trialing with *H. erythrogramma* within the FRDC roe enhancement project.

Fig F2 *Lytechinus variegatus*



California

I met with Dr Susan McBride in Davis, California, 89km north-east of San Francisco (map below). Dr McBride works at Eureka, in Northern California, for the US Sea Grant Program.



She informed me of the status of the urchin industry in California and of her research into *Strongylocentrotus franciscianus* (the red sea urchin) nutrition. She has done some very good work on this topic over a number of years. Most recently she produced papers on the effect of temperature and protein concentration, in prepared and natural feeds, on growth and assimilation efficiency (abstracts below *).

Up until recently there was an urchin roe enhancement business running off the Californian coast. Unfortunately it was destroyed in a hurricane and was not re-established. I was given several reports on the status of the fishery for *Strongylocentrotus franciscianus* (the red sea urchin) and *S. purpuratus* (the purple sea urchin) which are available on request.

The fishery, which concentrates on the red urchin (99%), is in decline. It began in the early 1970's as a pest control measure in the face of an expanding sea urchin population and associated decline in kelp beds in Southern California. The kelp acts as a breakwater, protecting the beaches from erosion. The status of the urchin changed as the yen began to rise against the dollar, and roe exports to Japan became more and more profitable. After peaking at 23,577 tonnes in 1988, the state-wide catch had declined to 10,086 tonnes by 1995. Its value in 1996 was about

$US80million ($AU151million/year), most of which was exported to Japan. In terms of the fishery the state is divided into two areas – Northern and Southern California. Central California is not fished due to the lack of marketable sea urchins, largely the result of predation by sea otters. Most of the decline in the fishery has been in the north, where the catch declined from a 1988 peak of 13,605 tonnes to 2,148 tonnes in 1996. There are two excellent reviews (S. Kato and S. Schroeter (1985) Biology of the red sea urchin *S. franciscanus* and its fishery in California, Marine Fisheries Review 47 (3) and P. Kalvass and J. Hendrix (1997) The California red sea urchin, *S. franciscanus*, fishery, catch, effort and management trends, Marine Fisheries Review 59 (2) which are available on request.

***Abstracts mentioned above**

The effect of protein concentration in prepared feeds on growth, feeding rate, total organic absorption, and gross assimilation efficiency of the sea urchin *Strongylocentrotus franciscanus*. Journal of Shellfish Research, Vol. 17, No. 5, 1563 – 1570, 1998.

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[4]Department of Fisheries, Humboldt State University, Arcata, California 95521 USA

Abstract Small *Strongylocentrotus franciscanus* (mean + SD = 18.3 + 13.1 g wet body weight) were placed in individual containers on 27 March 1997 and fed prepared feeds with 30, 40, and 50% protein until 30 January 1998 (n = eight/treatment). Growth, increase in wet body weight (including coelomic fluid) did not differ significantly with treatment [analysis of variance (ANOVA) p = .461]. The gross assimilation efficiency (increase in g dry body weight/g dry feed consumed) was calculated for each individual. The assimilation efficiencies for the 40 and 50% treatments did not differ significantly, but the efficiencies for the 30 and 40% treatments and the 30 and 50% treatments did differ significantly (p = .009). Total organic absorption was significantly different between the 50% and the other two diets treatments (p = .02) in July and January. Pooled male and female gonad protein and lipid concentration as a percentage of dry matter were significantly different, t-test, p = .002, p = .008, respectively. This suggests physiological regulation of feeding, with feeding rate decreasing with increased level of protein. The gonads of small *S. franciscanus* were in the premature stage of development at the end of the study.

The effect of temperature on production of gonads by the sea urchin *Strongylocentrotus franciscanus* fed natural and prepared diets. Journal Of The World Aquaculture Society Vol. 28, No. 4, 1997

Susan C. McBride[1], William D. Pinnix[2] , John M. Lawrence [3] , Addison L. Lawrence[4] and [2]Timothy M. Mulligan
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Abstract
Strongylocentrotus franciscanus (248 ± 76 g wet body weight, mean and standard deviation) were maintained in the laboratory at 12.9 and 16.1°C and fed either *Nereocystis luetkeana* or a prepared feed for 4 mo (July through October). The size and histological condition of the gonads of an initial sample, the experimental animals, and a sample from the commercial fishery were evaluated. The gonad index of experimental *S. franciscanus* increased from an initial 3.4% ± 1.4 to 19.2% ± 3.3 (mean and standard deviation) for the four treatments. The feeding rate was greater for individuals fed *N. luetkeana* and for both foods at the higher temperature. Gonad production was not significantly different among the experimental treatments, indicating the prepared feed was nutritionally superior to the algal diet and an increase in catabolism at the higher temperature balanced the increase in food intake. Histological preparations determined the urchin gonads developed from an initial spent condition to the premature stage for both experimental and fishery samples, indicating neither food or temperature affected gametogenesis.

Canada

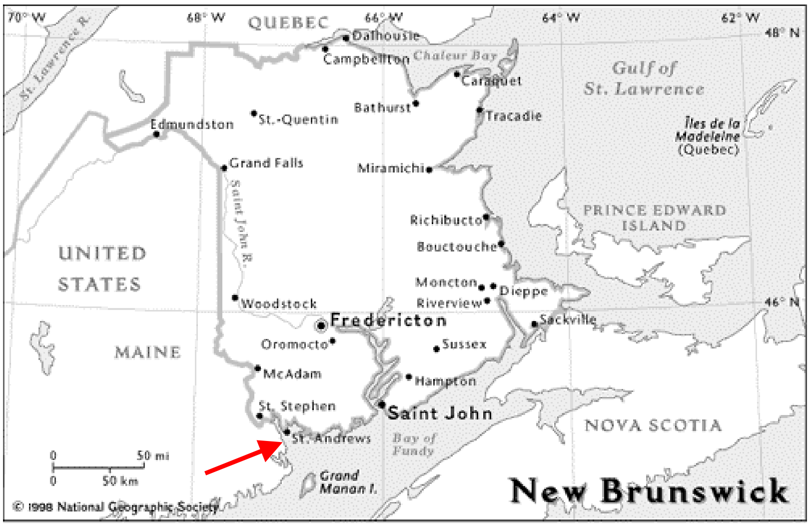
Visits were then made to sea urchin researchers and industry facilities in New Brunswick and Newfoundland on the south-eastern coast of Canada

New Brunswick

The first Canadian destination was the St. Andrews Biological Station (SABS) (Fig NB1), a federal research laboratory of the Department of Fisheries and Oceans (DFO) Canada.

Fig NB1 St. Andrews Biological Station

It is in the town of St Andrews-By-the-Sea (red arrow on map below) in south-eastern New Brunswick, half an hour from the US border.



At the station I met with Dr Shawn Robinson and colleagues. A workshop was originally planned but, when I arrived, I found it had been called off as Dr John Castell, who is working with Dr Robinson on sea urchin nutrition, roe enhancement and grow-out, had to attend a workshop in Japan. Nevertheless the visit was worthwhile as I spent some time with Dr Robinson and went out on an urchin fishing boat for a day to observe the operation and talk with industry. Once again the focus was the green sea urchin, *Strongylocentrotus droebachiensis.*

Nutrition and gonad enhancement work at SABS

The following is the latest iteration of the diet used at SABS as a baseline for their nutritional trials.

SABS - Sea Urchin Reference Diet - 2000

(designed by J.D. Castell and S.M.C. Robinson)

Basic Composition

Ingredient	%
Soybean meal	17.00
Wheat Meal	17.00
Canola Meal	17.00
Starch	10.72
Gelatin (dissolve in hot water)	5.00
Sodium Alginate	2.00
Linseed Oil	2.00
Lecithin	2.00
Vitamin Mix [a)]	2.00
Mineral Mix [b)]	15.00
Stay C	0.08
Ethoxyquin	0.20
Algro Natural beta-carotene & filler [c)]	10.00
Total	100

Mix water and dry mix together (~35:65)

a) The vitamin mix is a SABS in-house preparation of:

Ingredient	%
Vitamin A (500,000 I.U./g)	**0.133**
Vitamin D3 (400,00 I.U./g)	**0.075**
Vitamin E (500 I.U./g)	**3.333**
Vitamin K (menadione Na bisulphite)	**0.300**
Thiamin . HCl	**0.500**
Riboflavin	**0.400**
d-Calcium pantothenate	**1.200**
Biotin (1%)	**0.800**
Folic Acid	**0.167**
Vitamin B12 (0.1%)	**0.667**
Niacin	**1.333**
Pyridoxine . HCl	**0.400**
Ascorbic Acid (Stay C)	**6.667**
Inositol	**1.333**
Ethoxyquin (75%)	**0.889**
Wheat middlings	**81.803**

b) The mineral mix is a Modified Barnhart Tomarelli Salt mix that we buy commercially

c) We use beta-carotene from the Betatene Pty. Ltd. from Australia. (Fax: 61 3 9584 8348). It is a 2% formulation obtained from spray-dried algae (*Dunaliella salina*) so you also get some other components from the algae as well. We found 250 mg/Kg of dry feed worked well. Therefore, for 1 Kg of dry feed we used 12.5g Algro and 87.5g soybean:wheat:canola to make up the 10% weight.

Notes:

1. We have intentionally avoided fishmeal in the preparation. Firstly, because of its relative expense compared to plant-based protein and secondly, because of the possibility of imparting a bad taste to the sea urchin roe through fish oils. Thus the use of vegetable oil.
2. The diet is presently in a moist extruded formulation (although it can be air-dried with some loss of pigment due to oxidisation). This extruded form was found to have the best stability in some of our previous trials.

Prepared by: Shawn Robinson, St. Andrews Biological Station
06/10/00

I was also given a summary paper on the nature of the fishery as well as past and present sea urchin culture work at SABS. The following is an abstract, the full version is available on request.

Shawn M.C. Robinson, John D. Castell, Eddy J. Kennedy and Lisa Peters

Biological Station, Dept. Fisheries and Oceans, St Andrews, N.B., E5B2L9,

Canada

Resume de l'elevage des oursins de mer a la station biologique de St-Andrews/ A summary of sea urchin culture at the St Andrews Biological Station

Work on the green sea urchin (*Strongylocentrotus droebachiensis*) at the St. Andrews Biological Station began in 1989. Early research indicated that most of the animals within the local stocks were growing relatively slowly and that most of the animals of legal size were 12 years old on average, suggesting that food limitation was occurring and that a potential existed for culture. In 1995, early feeding trials were initiated using a Wenger sea urchin feed as well as an experimental mixture that utilised waste vegetable material from commercial sources. Performance of the diets in growth rate of the roe were superior to the natural kelp controls, however the formulation using vegetable material was not practical due to storage problems associated with fermentation of the product. In 1997, experiments were initiated to investigate the somatic growth potential of juvenile sea urchins to determine whether full cycle growout would be feasible for this species. Results from this experiment indicated that growth of the test could be accelerated by six-fold as animals grew from 10mm to 50mm in 18 months.

The results of the growth study led to further work being done into the essential ingredients (proteins and lipids) required in a juvenile sea urchin diet in order to maximise growth of the test. Results from the ongoing study are indicating that high-level protein (over 25%) is not necessarily required in juveniles and that they can grow successfully on plant proteins rather than animal proteins. Performance of the animals also differs depending on the type and concentration of fatty acids they receive in their diet.

Recent work on diets has led to the development of a standardised open diet for sea urchins that is available to all interested parties so that better comparisons can be made between studies. Improvements in the quality of the roe in colour were made by incorporating beta-carotene produced by the algae, *Dunaliella salina*, into the diet.

Catches in the local green sea urchin fishery have risen from 0 to over 1700 tons per year from 1988 to 1999 and, in some areas, there are signs that harvesting capacity has been reached. Therefore, in 1999, research into the production of sea urchin juveniles from a hatchery was initiated.

Ageing Sea Urchins

Dr Robinson has recently published a paper on ageing in the green sea urchin[1] , based on the rings to be found in parts of the Aristotle's Lantern, the jaw. The theory is the same as that used for ageing work in fish using otoliths. The method is still very much in the experimental stage but still potentially very useful in South Australia. We have no information on the age of urchins in our fishery. It is vital that such information is collected in order that the fishery is managed sustainably. Dr Robinson's method provides a place to start with ageing work which should be a priority in future research on *Heliocidaris erythrogramma* in this state. Dr Robinson demonstrated the method to me as follows:

The urchin opened (Fig NB 2.1 (next page)) and the rotules are dissected out (Fig NB2.2). These structures (Fig. NB2.3) are situated on the proximal end of each of the five parts of the jaw. The separated rotules are then charred over a flame (Fig NB2.4) until brown then put aside to cool. A microscope slide is heated briefly over the same flame and a small amount of thermoplastic glue (Crystalbond 509 or equivalent) applied (Fig NB 2.5). This melts onto the slide and stays fluid for several minutes to allow embedding of the rotules which are laid on their sides in the glue. The slide is then cooled in water and the preparation sanded with 400 wet-dry sandpaper (or equivalent) (Fig NB 2.6) to about the level of the rotules' notches (Fig NB2.3). A drop of histoclear is then applied to clear the preparation and the slide is ready for viewing (Fig NB2.7). The urchin shown in this example appeared to be in its fourth year. This fitted in well with the expected age, the urchin was put into Dr Robinson's aquarium system 2.5 years earlier and its age at that time was estimated (from size) at 1.5 years. Research is currently focussed on the transfer of this method to the field.

[1] Robinson, S.M.C. and MacIntyre, A.M. (1997) Ageing and growth of the green sea urchin. Bull. Aqua. Assoc. Can. 97, 56-60

Fig NB2 Ageing green sea urchins

Fig NB 2.1

Fig NB 2.2

Fig NB 2.3

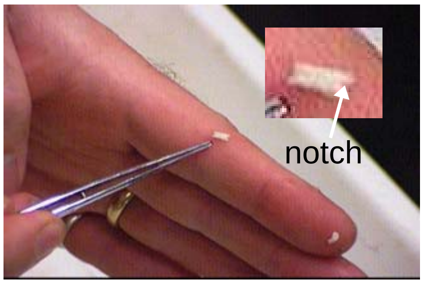


Fig NB 2.4

Fig NB 2.5

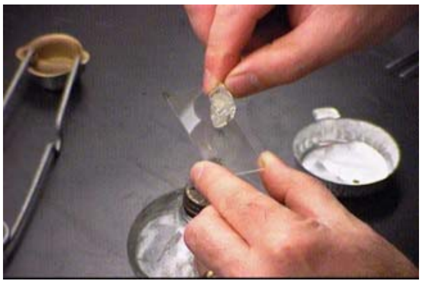
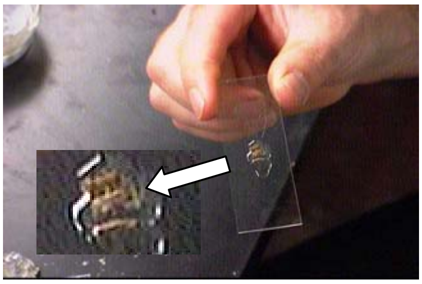


Fig NB 2.6

Fig NB 2.7



The Fishery

As mentioned above, I was taken out on a commercial sea urchin fishing vessel and was able to observe the operation and talk to the fishers. The vessel (Miss Back Bay) (Fig NB 3) is fairly typical for the Bay of Fundy sea urchin fishery, about 38ft (11.5m) long, wooden, with urchin sorting facilities aboard.

Fig NB 3 Miss Back Bay

She has two tenders (Fig NB 4), sturdy wooden boats about 15 ft (4.5m) long, which are towed to the fishing area and used there for diver transport to and from the mother vessel.

Fig NB 4 Tenders

Divers are dry-suited and on SCUBA. Collection of urchins from kelp beds is done using a hook (Fig NB5). Divers work two to a tender, the tender driver moving between them to pick up the catch, exchanging full catch bags (20-23 kg) (Fig NB6) for empty ones.

Fig NB5 The hook used to collect urchins

Fig NB6 Catch bags. Dr Shawn Robinson is on the right of each photo

Divers will then either swim to the surface and exchange there or attach their bag to a buoy which will be hauled up to be exchanged by the driver. The work is done at low tide because the Bay of Fundy, has a 7.6m tidal range, at high tide they would be diving in 13-14 m of water, making the exercise much more difficult and time consuming. As it is they spend 4-5 hours a day in the water which reaches a maximum of 13^0C during the season, 1 October to 31 March, the coldest months in that part of the world.

Urchins are taken back to the mother ship in tubs where they are sorted (Fig NB7) at a specially constructed bench – those which make it to the end of the bench are kept, those which can fit between the rails of the bench are returned to the sea.

Fig NB7 Sorting bench

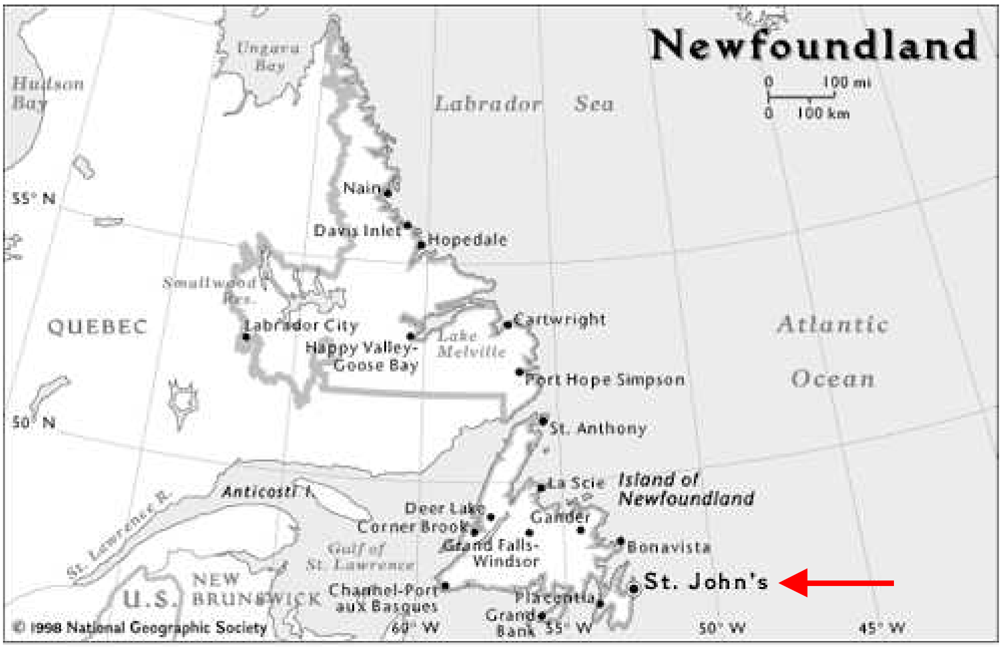
A two inch (50mm) gauge is used both to measure the urchins and as a sorting tool (Fig NB8).

Fig NB8 Urchin gauge

Urchins are periodically cracked to assess the yield, divers get $CN1.30 - $CN2.30/lb for whole urchins containing 10% roe. On top of that, they get 10 cents more per lb for every percentage point above 10% ($AU0.42/kg).

Newfoundland

The next stop was St John's, Newfoundland (red arrow, map below), where I met up with Dr Bob Hooper and colleagues at the Canadian Centre for Fisheries Innovation (CCFI). I was also taken to two processing facilities by Marc Kielley, who does the Industry Liaison work at CCFI. Finally, I gave a talk to a third year aquaculture class on work underway at SAASC and on my research with sea urchins and lobsters.



The Fishery

Once again the focus was the green sea urchin, *Strongylocentrotus droebachiensis.* The fishery was commercialised in 1994. Once again the minimum test diameter is 2" (50mm). Urchins are fished mostly north of St John's in Notre Dame Bay, Bonavista Bay and Trinity Bay. Some 36$^+$ sea urchin permits have been issued (4 divers per licence).

Most of the harvest is from October to Christmas though the season runs from October 1 to March 31. Fishing is carried out from an open boat 25-30ft (8-9.7m) long which carries 2-6 divers and has a quota of 160,000lb (72.6 tonnes). The divers will spend 5-6 hours in the water per day, some on hookah, most on SCUBA, those with the latter have a recreational diving licence only. Three divers can collect 2000lb (908kg) per day and can make $Can8,000 ($AU10,000) per week each and up to $Can100,000 ($125,000) a season. I didn't see these divers in action although I was told that the methods are very similar to those in New Brunswick.

Processing

The yield on the wharf may be 12% which is reduced to 9% at pack-out, a loss of 3% during the process. Roe may fetch $20 to $30/lb ($55 to $82/kg) - there is a concentration on volume rather than quality, which keeps the average price down. About 80% of the roe that is processed is "A" grade, the remainder "B" with the darker grades and deep orange discarded. "A" grade roe is fine grained and bright orange, "B" grade is coarser (eggs >1mm) with poorer colour. Processing urchins with 12-14% yield has a labour cost component of 20% of that of urchins with 7% yield, thus it is much more economical to process urchins with a greater yield.

The processing plants I visited, New Found Foods Inc and Green Seafoods Ltd, were both of the standard fish processing type, very clean, with stainless steel benches, chilled seawater and the staff in hairnets and appropriate clothing. The facilities are up to Canadian standard and are regularly inspected by the local authorities and by representatives of the Japanese buyers. I was allowed to take photos of individual parts of the processing operation but not of the staff at work or panoramic shots of the inside of either factory.

Urchins are opened using a pair of urchin pliers and the roe carefully scooped out using a small stainless steel spoon (Fig NF1 and NF2).

Fig NF1 Tools for urchin processing: pliers and scoop

Fig NF2 Scoop in action

The roe is then placed in stackable plastic mesh trays and soaked in salt water to wash off other viscera and gut contents (Fig NF3). Artificial seawater is used for hygiene.

Fig NF3 Soaking roe

The roe trays are then soaked in another artificial seawater bath containing no more than 1.5% alum to firm up the roe. Alum is actually a dehydrating agent and is used on all the roe processed in Newfoundland. I was also told that the Japanese use a refractometer to measure the salinity of the fluid in the test (haemocoelic fluid) and they adjust the alum concentration accordingly. The roe is then graded and placed in larger mesh trays (Fig NF4) for packing into either small, solid bottomed, 100g plastic trays, or if AA grade, into handmade wooden trays.

Fig NF4 Larger trays with roe. A grade (left), B grade(right)

Seventy-five plastic trays or 50 wooden trays may be packed into a styrofoam box (esky) (Fig NF4) and 20-25 eskys stacked on a “skid” (pallet), the export unit. The plastic trays are sourced from Pacific Packaging, an American firm.

Fig NF4 a) Wooden Trays. b) Plastic trays. c) Esky with cardboard inserts used for packing

a)

b)

c)

Roe has a 6-7 day shelf life at 4^{o}C, once processed, so timing of harvest and export is critical. The processor also has only an approximate idea of the price that a given shipment will receive once it reaches the market, as so much depends on the demand (what else is at the market at the time of sale). Most is sold at auction at the Tsukiji Market in Tokyo.

This uncertainty can have quite an impact, given the two day journey to Japan from Newfoundland, especially if a shipment attracts a low price. One of the processors told me of making a $CAN15,000 profit one week, only to loose it the following week because of problems at the market. Their roe usually attracts a price of $AU50 - $AU70 a kilo, with breakeven at just over $AU60. Occasionally they achieve $AU100 to $AU120/kg.

Past Experimental work and work in progress.

There have been several studies on Newfoundland populations of the green sea urchin. These began in 1993 with a study on urchin maturation timing, age, growth rates, feed, habitat and roe value by the province's Department of Fisheries and Aquaculture. In 1994 work began on the first phase of a series of feeding and ranching experiments in collaboration with the CCFI. Phase II began in 1995.
The details of these experiments may be accessed in reports by McKeever et al (1996) and Cuthbert et al (1995) (available on request).

Abstracts for the above reports

Sea Urchin Aquaculture - Phase I
Sea Urchin Feeding and Ranching Experiments

CCFI Project: AUI-503 by Fiona M. Cuthbert, Robert G. Hooper Biology Department and Bonne Bay Biology Station Memorial University of Newfoundland, St. John's, Nfld. A1B 3X9

and Tom McKeever, Aquaculture Unit, Marine Institute, March 1995.

Abstract
Sea urchins with low roe yield and quality were experimentally reared on specific seaweed diets. A diet of *Laminaria digitata* or *L. longicruris* led to major (more than

20% increases in roe yield and quality in both aquarium and cage trials. *Ascophyllum nodosum* produced mediocre results while *Alaria esculenta, Fucus vesiculosus and Agarum cribosum* produced poor yield and quality. Growth rates were better in warm water than in cold. Consumption of *Laminaria* was as much as 5% of animal weight per day in urchins held in warm water and lower in those held in colder temperatures. Consumption of *Ascophyllum* was initially 3 to 4% but decreased after the first three weeks. Consumption rates of the other species were lower. Mortality was minimal in urchins fed on *Laminaria* but higher in urchins fed on *Ascophyllum* and *Fucus.* Mortality was observed to be higher in cages than in aquaria.

Harvest of *Laminaria* spp. was most effective using scuba diving techniques. Harvested kelps could be kept in good condition, practically indefinitely, when held in cold flowing seawater tanks. Field cage results were comparable to results obtained in experimental tanks although urchins were observed to feed additiona11y on filamentous algae growing on the cages and on diatoms in the substrate below. Some of the feed in the cages was stolen by snails and invading small sea urchins.

Sea urchin aquaculture - Phase II:
Ration size, seasonal growth rates, mixed kelp diets, fish diet, old urchin growth, and baiting confinement experiments.
by
Robert G. Hooper, Fiona M. Cuthbert Bonne Bay Biology Station, Biology Department, Memorial University of Newfoundland
Thomas McKeever, Aquaculture Unit, Marine Institute, Memorial University of Newfoundland

Sponsored by: Canadian Centre for Fisheries Innovation, Government of Newfoundland and Labrador, and Memorial University of Newfoundland
March 1996

Abstract

Various experimental diets were fed to sea urchins between the spring of 1995 and the winter of 1996 at the Bonne Bay Marine Research Laboratory. Ration size experiments have shown that diets considerable below satiation rates can be satisfactory in producing good gonad yield and quality. Rations as low as 1.25% urchin weight per day give moderate results though 2% urchin weight/day was better. A mixed diet of *Laminaria digitata* and *L. longicruris* produced a greater increase in gonad yield and better quality roe than *L longicruris* alone. Seasonal trials to compare growth rates during different seasons were initiated at intervals since June and are still continuing through the winter. Growth rates between trials started from June to September were uniformly good. Growth rates and feeding rates in the winter were significantly reduced. Fresh fish diets were investigated and found to produce good roe yield but with undesirable flavour and texture. Large, low-quality sea urchins were shown to respond to feeding in the same manner as smaller, young sea urchins but at a slower rate. No evidence of major senescence was found.

Experiments were carried out to determine whether the presence of food was adequate to keep sea urchins in an area. Dispersal of fed sea urchins was much lower than that of unfed sea urchins but was sufficient to prove that some form of fencing is necessary for sea urchin ranching. Exclusion of alien sea urchins is another reason for requiring fencing.

The effort spent on harvesting seaweed was recorded and the condition of the harvested sites was superficially monitored. Large quantities of weed were harvestable with minima1 effort by divers and efficiency could be greatly improved for larger scale harvest. Slow regeneration was noted in harvested plots, largely by growth of young/small plants previously shaded in the understorey of the large kelps. More detailed evaluation of harvestable kelp and wild sea urchin resources are required.

Current Research

At present Dr Bob Hooper, in conjunction with Green Seafoods, is running a series of trials with various extruded diets, the base formulation of which is as follows:

Formulations for Sea Urchin Feed

Ingredient Cost Summary

Final Weight	Formulations 1, 2 and 3	Formulatio Cost							Cost/lb	
Ground Kelp	35.0%	35.000%							0	
Corn Meal	12.5%	12.500%	$ 2.50	kg	$	1.14	lb	$	0.14	lb
Soya Meal	12.5%	12.500%	$ 2.50	kg	$	1.14	lb	$	0.14	lb
Wheat Mid	12.5%	12.500%	$ 2.50	kg	$	1.14	lb	$	0.14	lb
Ground Barley	12.5%	12.500%	$ 2.50	kg	$	1.14	lb	$	0.14	lb
Gelatin	7.0%	7.000%	$10.00	kg	$	4.55	lb	$	0.32	lb
Lecithin	5.0%	5.000%	$ 9.50	kg	$	4.32	lb	$	0.22	lb
Water	3.0%							$	-	
Propylene Glycol		2.998%	20	kg	$	9.09	lb	$	0.27	lb
Sodium Benzoate		0.002%	1000	kg	$	454.55	lb	$	0.01	lb
Totals	100%	100.000%						$	1.38	lb

Diets are made using the CCFI's extruder (Fig NF5) and fed to urchins in tanks at Green Seafoods facility at Winterton.

Fig NF5 The diet extruder

In addition to diets they are testing a collection cage system at present. Urchins are collected by the divers and loaded directly into cages constructed of green plastic coated wire mesh (Fig NF6).

Fig NF6 Cages for holding urchins

Cages are about 700 by 500 by 500 mm and the mesh about 38 by 38 mm. They are floated off buoys until all are filled at which point the diver brings the urchins back to the facility in the cages to be placed in the tanks. This reduces handling, damage and stress on the urchins although the diver would still have to assess the roe yield before putting the urchins in the cages. Staff at Green Seafoods are feeding the urchins, still in the cages and will assess yield throughout the experimental period. Although the mesh size is large, urchins cover the walls of each cage, presumably little food is wasted. The work is at the pilot stage, using cylindrical flow through tanks of about 500L capacity (Fig NF7a to e, following page). They are also linked to each other in a series, that is, water flows out the bottom of one down to an adjacent tank below the level of the first.

Fig NF7 Experimental tank system

Fig NF7a Tank showing the standpipe used to control the water level and the frame used to support the cages

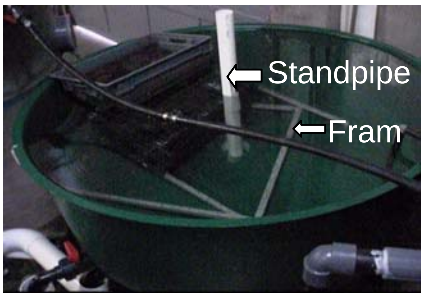


Fig NF9b Tanks linked in series

Fig NF7c Close-up of tanks linked in series

Fig NF7d Cages in tanks. Marc Keilley (left) and Carl Parsons

Fig NF7e Close-up of cages in tanks showing the supporting frame

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APPENDIX 7: JAPAN TOUR REPORT

A short trip was made to Japan by the author and Ms Samara Miller of PIRSA Fisheries, Port Lincoln at the invitation of Miyako International, a seafood exporting company. Miyako International had expressed interest in developing the South Australian sea urchin resource and met all trip costs. During the trip we visited sea urchin aquaculture and processing facilities and subsequently presented a final report to PIRSA fisheries and Miyako International. The section in the original report covering Miyako's potential involvement in the SA sea urchin fishery has been removed from the enclosed document.

JAPAN TOUR REPORT
JAPANESE SEA URCHIN MARKETS, AQUACULTURE CENTRE AND PROCESSING FACILITIES

6 June 2001 to 13 June 2001

Dr Richard Musgrove, SARDI Aquatic Sciences Centre, West Beach
and
Ms Samara Miller, PIRSA Fisheries, Port Lincoln

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DISCLAIMER

The figures given in this report are based on the information provided by various people during the study tour. Neither Ms Samara Miller nor Dr Richard Musgrove make any warranty of any kind expressed or implied concerning the figures and technical information mentioned in this report.

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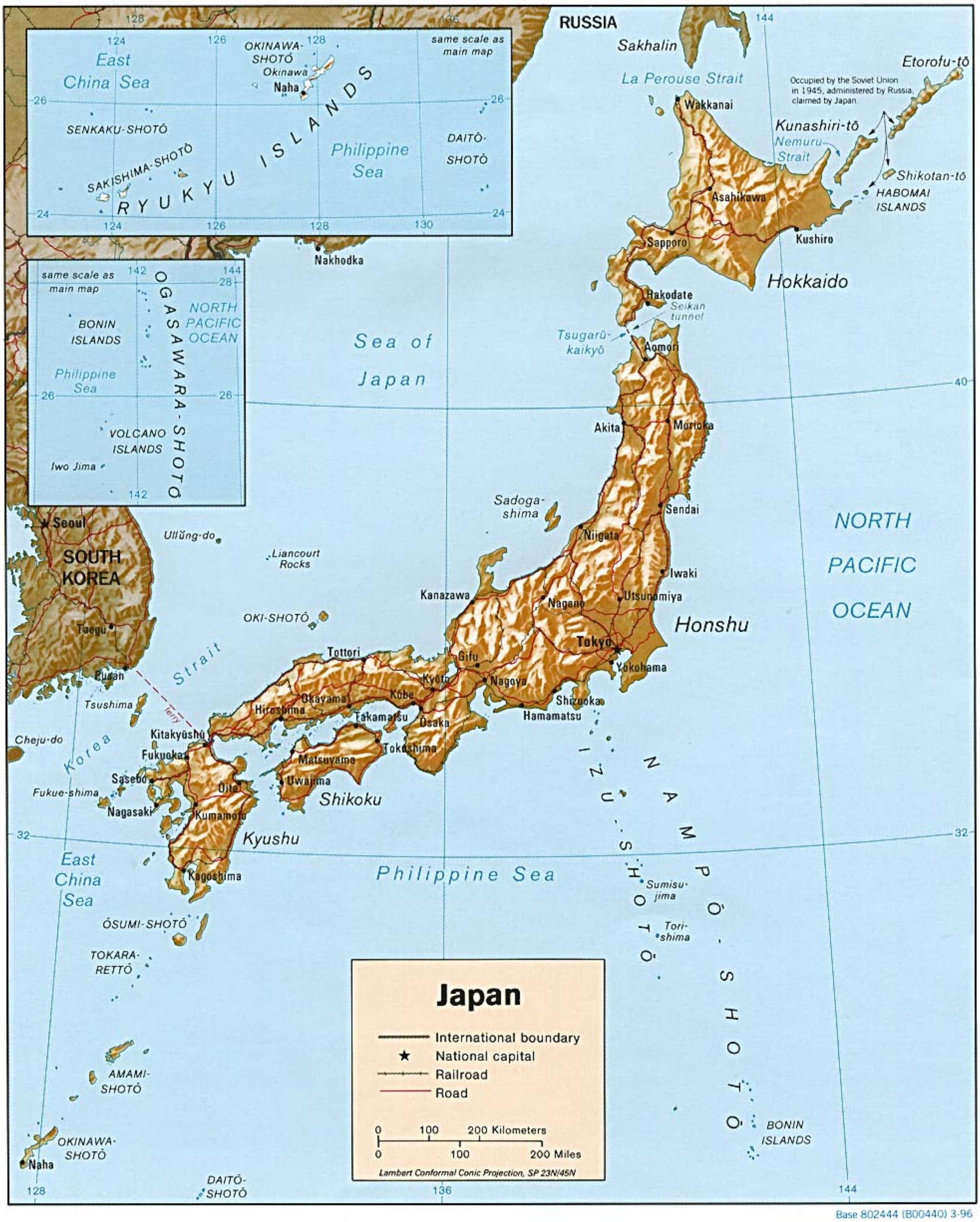
ACKNOWLEDGMENTS

Many thanks to our hosts, Miyako International, especially Mr Takeshi Takagi, Special Adviser, Mr Mark Spence, Managing Director and Mr Hiroyuki Takagi, Director.

Map of Japan showing the cities visited during the trip



Itinerary

6 June - **Sydney**
Met with Dr Duncan Worthington and Mr Craig Blount of NSW Fisheries, Mr Takeshi Takagi, Mr Hiroyuki Takagi, Mr Mark Spence and Mr Simon Elliott, Directors of Miyako International Seafood Pty Ltd, and Mr Rutland Cheung of Blessington Judd Solicitors and Notaries.

7 June - **Tokyo**
Met with an associate of Miyako International Seafood Pty Ltd, Mr Takashi Sudo, Chief Executive Officer of Sudo Construction Inc.

8 June - Tour of Tokyo sea urchin processing facility with Mr Osaka, Osaka Inc., Kasai.
Met with Mr Kenzo Kubota, President, Tokyo Kanda Rotary Club, Akasaka.
Met with Senator Tadashi Igarashi, Vice President, Tokyo Metropolitan Assembly, Shinjuku.

9 June - Tour of Tsukiji fish and vegetable markets with Mr Mochizuki, General Manager.
Met with Mr Chotaro Orihara, Director, General Manager, Sales Administration Department, Daito Gyorui Co. Ltd, Tsukiji Market.
Met with Mr Mitsuru Kawasaki, Senior General Manager, Sushi-related Fish and Shellfish Department, Daito Gyorui Co. Ltd, Tsukiji Market.
Met with Mr Shuji Tsukamoto, Staff Manager, Restaurant Fish Department, Chuo Gyorui Co. Ltd.
Met with Mr Shimoda, Sushi-related Fish and Shellfish Department, Daito Gyorui Co. Ltd.

10 June - **Hakodate, Hokkaido.**
Tour of Hakodate sea urchin processing facility with Mr Masashi Murakami, President, Murakami Shouten Co. Ltd.

11 June - Tour of Esan Aquaculture Centre, Hokkaido with Mr Yuzawa, Manager of Centre and Mr Hiragima.

12 June - Met with Mr Mitsuru Kawasaki, Senior General Manager, Sushi-related Fish and Shellfish Department, Daito Gyorui Co. Ltd, Tsukiji Market.
Met with Mr Robert Gumley, Chief Representative, and Mr Andrew Buckley, Project Officer, Trade and Investment, Australian Business Centre, South Australian Government, Tokyo.

13 June - Return to Australia.

1 The Japanese Sea Urchin Fishery

The Japanese sea urchin fishery includes about 16 species, 6 of which are of importance, *viz*: *Stronglylocentrotus intermedius* (bafun) and *S. nudus* (purple) in the north; *Anthocidaris crassipina* and *S. pulcherrimus* in the central region and *Tripnuestes gratilla* (white), and *Pseudocentrotus depressus* (red) in the south. The facilities we visited during our stay were involved with the production or processing of the bafun, and the purple sea urchins which, together, account for more than 80% of the Japanese production. Bafun is the smaller of the two species, (Fig 2), about 50mm or so in diameter, similar in appearance to the green sea urchin (*Strongylocentrotus droebachiensis*) found in waters off south-eastern Canada/ north-eastern USA. It is most common on rocky substrate at 1-2 metres depth, but can be found down to 6 metres. The purple sea urchin is a deeper water species (down to 20 meters) and is similar in appearance to the black sea urchin (*Centrostephanes rodgersii*) found in New South Wales and on the east coast of Tasmania.

2. Sea Urchin Aquaculture Centre

A visit was made to the Esan Aquaculture Centre (Fig 1), a government-subsidised facility in the village of Esan, an hour east of Hakodate, on the northern island of Hokkaido.

Fig 1. Esan Aquaculture Centre

Esan is situated on a small bay which flows directly into the Pacific Ocean. The Centre is one of 50 scattered around the island of Hokkaido and was built in 1992. It is considered to be one of the smaller centres on the island.

The facility cost A$1.8m to build and has an annual operating budget of about A$200,000. The average sea urchin aquaculture centre costs about A$5 million to build and A$500,000 to run. By way of comparison, an average abalone aquaculture centre, in Japan, would cost more than A$70 million to build.

2.1 Function

The centre is focused on the production of juvenile sea urchins for reseeding the fishery. Reseeding is an ongoing process and each centre is linked to the fishery through town Fishing Associations (FA) to which they sell juveniles for reseeding at a cost of about A30 cents for each 5mm (6 month old) sea urchin. Each year a given FA will decide how many juveniles to buy from an aquaculture centre based on catch in the previous season. The operation is really more akin to sea ranching than a wild fishery.

At Esan it was acknowledged that it would actually be better to grow urchins up to 3 cm but the cost would be prohibitive.

Both the purple and the bafun sea urchin (Fig 2) are cultured at the Esan facility. Bafun have a considerably slower growth rate than the purple sea urchin but are considered to produce a better-tasting roe.

Fig 2. Bafun (foreground) and purple sea urchins

2.2 Capacity

The Centre occupies an area of 1,525.93 m^2 and has three main buildings, *viz*, a Breeding Tank Building (287.55m^2), a Pump Room (12.63m^2) and a Food Cultivation Room (296.80m^2).

Water comes into the centre via a an inlet situated 100 metres offshore and 40 metres below the surface. The pump room contains the following:

- Two 3.7kw inlet pumps,
- Two 5.5kw inlet pumps
- Two 7.5kw pumps for pushing water through the filters
- One 11kw back-washing pump
- Two 5.5kw air blowers
- One cooling unit

The water is pumped into a seawater tank (56,000l) then through two large pressurised filters (85,000l and 60,000l/ hour) containing layers of small stones, sand and charcoal. It is then pumped into a storage tank (54,000l) and distributed as needed into the following:

- Eight larval breeding tanks (@ 1,000l)
- Five larval settlement tanks (@ 6,000l)
- Thirty growout tanks (@ 7,500l) costing approximately A$7,000-A$10,000 each.
- Six food (algae) cultivation tanks (@ 7,500l)
- Two water interchange tanks (@ 1,000l)

Ultraviolet filters are not used for water sterilisation at Esan as they are very expensive. They may be used at other, larger, centres.

Before water passes into the larval tanks it is pumped through 10 micron and 1 micron bacterial filters. Water is turned over in the growout tanks 3 times per month and the tanks are chlorinated after each grow-out cycle. Water is pumped back into the ocean after use.

Filters are back-flushed 3 times per day and cleaned once every 5 years. Their replacement cost is about A$50,000. Inlet pipes are replaced once every 5 years.

2.3 Culture of juvenile sea urchins

The process of ranching begins with the collection of broodstock. Bafun broodstock are collected twice a year (spring and autumn) and purple sea urchin broodstock once a year (autumn). The broodstock are fed kelp (brown algae) which is grown by the Centre. We were unable to ascertain the species but the blades are thin and approximately 40cm in width. Thin-bladed kelp is preferred by the broodstock as it can be ingested easily[1]. The Centre grows kelp using 120m buoyed ropes with vertical weighted ropes at intervals. Typically, four 120m ropes are used to provide all the food for the broodstock over the spawning season.

[1] There was also a suggestion that artificial diets are used at some centres.

Broodstock urchins are kept in tanks inside the facility. Spawning is initiated artificially, using Potassium Chloride (KCL) and once larvae are produced, they are transferred to rearing tanks kept at 17-20°C. Larvae begin to feed approximately 2 days after hatching depending on their condition, that of the female parent and water temperature. In culture they are fed on the microalga, *Chaetoceros gracilis*. Settlement onto batteries of polycarbonate corrugated sheets[2] occurs after 22 days. Sheets are conditioned in tanks prior to larval settlement in order to provide food for the larvae (marine microalgae). The planktonic larval period is much longer than that of the South Australian purple urchin, *Heliocidaris erythrogramma*, which settles after 3 to 5 days. At approximately 25 days the batteries are placed in raceway growout tanks outside the main building (Fig 1 and Fig 3), where the juvenile sea urchins (Fig 4) are fed kelp.

Fig 3. Raceway growout tank

They also graze on the algae growing on the sheets. The juveniles are kept in the tanks for six months then sold one of the local Fishing Associations (see Section 3) for on-growing at sea.

Fig 4. Juvenile sea urchins with a battery of corrugated plastic sheets in the background

The most critical time for the sea urchins is the first few weeks, in particular the first 2 to 3 days after spawning. Water chillers and shade-cloth are used for the inside larval settlement

[2] The sheets cost $2.00 each and last for about 3 years. This centre has approximately 3,000 sheets.

tanks (Fig 5) to control water temperature during summer. This is a necessary as the south-facing building has large windows to allow algal growth.

Fig 5. Larval settlement tanks

2.4 Predation by Birds

Wire is strung above the tanks to deter birds which would otherwise prey on the juvenile urchins. The winds are too strong to use scarecrows and it would cost too much to use ribbons.

2.5 Production

Esan produces approximately 3,000,000 5mm sea urchins per year, with two batches produced in Spring and two in Autumn. Production figures for the last few years have been as follows (Table 1).

Table 1. Production figures for the Esan Aquaculture Facility. (Source: brochure produced by the Esan facility)

Esan Town	Juveniles (kg)	Price Yen	Yen per kg	AUD$ per kg
89	32,000	453,000,000	14,156	$208.18
90	17,000	298,000,000	17,529	$257.79
91	13,000	215,000,000	16,538	$243.21
92	18,000	337,000,000	18,722	$275.33
93	16,000	278,000,000	17,375	$255.51
94	15,000	238,000,000	15,867	$233.33
95	7,000	104,000,000	14,857	$218.49
96	10,000	132,000,000	13,200	$194.12

Centre Sales	Price Yen	Pieces	Yen per kg	$ per kg
Hiura FA	4,738,000	200,000	24	$0.35
Shikeuti FA	10,800,000	600,000	18	$0.26
Kotakei FA	3,348,000	250,000	13	$0.20
Esan FA	15,600,000	1,200,000	13	$0.19
Total	34,486,000	2,250,000	17	$0.25

* FA = Fisherman's Association

2.6 Disease and Temperature

Extremes of water temperatures may cause mortality in culture tanks and in the shallow subtidal juvenile ranching areas.

Aside from such direct effects, a rise in temperature can increase the incidence of diseases such as "black spot" which causes the spines to fall off, leaving the cultured juvenile sea urchins bald.

3. Fishing Associations

As mentioned above, aquaculture centres are usually associated with fishing associations, with a given coastal town often having more than one such organisation depending on its size. Each year an association will buy a quantity of juvenile sea urchins from a local aquaculture centre. The quantity bought will depend on recent catch history and the aquaculture centre's production. The urchins will be ranched at sea for 2 to 3 years then harvested by divers who are licensed by their local association and pay annual fees for the privilege. The licences remain the property of the association.

An association will release the 5mm sea urchins into subtidal areas in shallow bays where they reside for about 12 months, growing to approximately 3cm (Fig 6). After this period they are recaptured and moved to deeper water (down to 20m) to grow for an additional 2 to 3 years.

Fig 6. A juvenile ranching area. Collecting is taking place to move urchins to adult grow-out areas in deeper water

They reach maturity at 3 to 4 years of age and commercial size at 4.5 cm, with size and growth rate depending on food availability and temperature. There is a degree of natural spawning which adds to the resource. It is anticipated that up to 90% of juveniles may be lost through predation, movement from tides and currents, interaction with humans and natural mortality. The remaining 10% is considered sufficient to sustain the industry.

Urchins are not given artificial feed during the ranching process, but will be placed in food-rich areas to optimise growth/gonad production. One of the problems identified within these programs is the development of urchin "barrens" caused by high-density bottom feeding by sea urchins.

An association will decide where sea urchins will be ranched and will also permit access to these areas by the public. Although sea urchins are a lucrative resource, the theft of juveniles is low given the lack of markets for this size, the complexity involved in developing markets in the first place and the high cost of establishing an aquaculture centre to further enhance their growth.

In addition, in a coastal village/town, a large proportion of the population is employed either directly or indirectly in the sea urchin industry and thus have a stake in the industry's success. There is also a benefit in the location of urchins becoming common knowledge as information is required to enable an association to monitor and balance the urchin densities in the wild. If there are too few, they will not be able to spawn and if there are too many, they will compete for food.

4 Sea Urchin Processing Facilities

4.1 Tokyo

The processing factory at Kensai, in Tokyo, employs about 20 people with 16 workers and three packers. The factory pays a fixed price for sea urchin roe (written in English as "uni", pronounced "ewni") with little regard for quality. This is a common practice and allows processors to cover themselves for fluctuations in product quality (i.e. money lost in the troughs is made up in the peaks). Poor quality or damaged uni may be sold as paste. We were told that damage to roe during transport was common when sellers first enter the marketplace. At the processor we visited, quality is dependent on colour, texture, shape and dryness, with less emphasis placed upon taste. However, B grade may attract a higher price than A grade uni based on taste. Gold-orange coloured uni is preferred over a bright orange or yellow product (Fig 7).

Fig 7. Gold-orange roe is preferred.

All the uni we saw was very fine in texture, to the extent that the "grain" was barely visible. Upon tasting, the uni melted in the mouth without leaving a residue.

This processor obtains approximately eight hundred kilograms of uni per day from Korea, Canada, Chile and Unites States and he indicated that he could accept a minimum of 100 kilograms of uni from Australia every two days. Miyako suggested that the South Australian product could be combined with that supplied by New South Wales to make up the quantities required.

The two major species of urchin in the Japanese fishery (Bafun and purple) contain uni which must be processed differently because of differing textures and requirements for alum (see below).

Uni comes into the processors either in chilled eskys or in "wet packs". The latter are plastic bags filled with uni and a little water. The bag is sealed under pressure, removing all air and ensuring that the product is not damaged in transit. Upon arrival at the facility the uni is placed in plastic trays and washed in seawater then placed in a chiller tank held between 5 and 8^{o}C and the dehydrating agent, alum (aluminium potassium sulphate) is added at a concentration of about 1%. The length of time the uni is left in the alum depends upon its initial water content which is gauged by eye and feel. The aim is to produce a firm uni.

Once the dehydration process is completed, the uni is taken to the packing room (Fig 8)

Fig 8. The packing room at the processing factory in Kensai in Tokyo

where it is graded and placed in either plastic (B grade) or wooden trays (A or an A grade/B grade mix). In the case of the mixed A/B, the A grade is placed on the top of the tray and the remainder of the box filled with B grade. Great care is taken in handling the uni as it is easily damaged during the process. Once packed the trays are placed in foam eskys and stored, prior to dispatch to market, in a chiller at 0^{o}C.

4.2 Hakodate

The processor we visited in Hakodate handles approximately 300 to 400 kilograms of uni per day, most of which is obtained from North and South Korea and Chile. Domestic product is bought from fishing associations. The volume of domestic product peaks in the rainy season (June - July).

It was interesting to note that the facility manages its own food safety practices without government regulation. Pressure for high standards of cleanliness comes from buyers who will inspect a processing plant prior to entering into a supply agreement. The success of this self regulation ethos may also be grounded in the Japanese familiarity with the handling of sushimi (raw seafood).

The processing and packaging operation takes place in one large room (Fig 9). This contrasts with the move, in Australia, to separate rooms, considered safer and more hygienic. It is the experience of one of us (RM) that the Japanese model is more common in other countries (Canada).

Fig 9. The uni processing factory at Hakodate

5. Markets

There are 88 central wholesale markets in 56 cities in Japan of which 54 markets are for the buying and selling of fish. Nineteen markets exist for flowers and 10 for meat. The Tsukiji Market is the largest fish market in Japan.

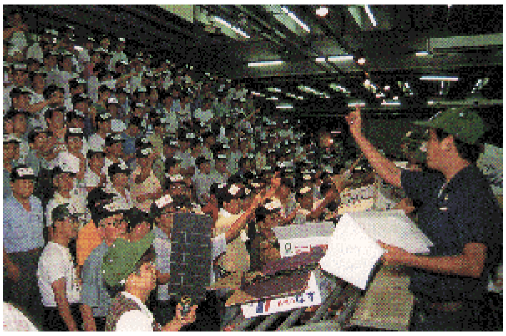
700 million tonnes of fish and fish products are consumed domestically in Japan each year. Of that 200 to 300 million tonnes are imported from China. A$800 million worth of fish product comes from Australia, with tuna and tuna products making up approximately A$350 million. 50% of tuna harvested worldwide, or about 1 million animals, becomes sushimi on the Japanese market. The emphasis in Japan is on quality rather than quantity of produce.

5.1 Tsukiji Market

The Tsukiji Market covers a large area of approximately 230,000 m^2 with produce arriving by boat, train, ship and truck. About 52,000 people pass through the Tsukiji market on any given day. Because of lack of space for further expansion at its present site the Market will be moved to new premises on the other side of the Sumida River over the next 10 to 15 years.

We observed three different auctions during our tour of the Market, *viz* uni, tuna and vegetables (Fig 10). Preliminary inspection and grading by potential buyers occurs while waiting for the auction to commence. When the auction begins the quantity of produce and the price is called out by the auctioneer and the transaction occurs almost instantaneously. The same style appeared to be adopted for each of the auctions observed.

Fig 10. Uni (left) and vegetable auctions at Tsukiji Market



5.2 Sea Urchin Roe at Tsukiji

There are US$240 million worth of uni imported to Japan annually from countries such as North Korea, China, Russia, Chile and the USA (Boston and San Francisco). Added to this is domestic production worth about $700 million. The total value of the uni market in Japan is therefore approximately $1 billion per year. An incredible 30 tonnes (approx.) of sea urchin uni is sold each day in Japan, 5 to 6 days per week.

Fig 11. The uni hall at Tsukiji Market

Tsukiji market has 10% of the Japanese market which represents 3 tonnes or about 10,000 trays of uni per day (Table 2). The remainder is sold through the other 53 fish markets in the country. Uni is packaged predominantly as "300 gram trays". However 80 and 100 gram trays are also sold in the market. There is an expectation that the trade in sea urchins will increase in the future.

Table 2. Uni prices at the Tsukiji Market, Tokyo, for the 16th of July 2001 (converted to Australian dollars using $A1=60 Japanese Yen). Red sea urchin is *Pseudocentrotus depressus* and white sea urchin is *Tripneustes gratilla*.

Country of Origin	Common Name	Tray Size	Grams of uni per tray	Number of trays sold	Minimum $A/kg	Maximum $A/kg	Average $A/kg
China and Korea	-	Large	280	4811	48	179	107
		Medium	150	1170	56	61	58
Japan	Red	Large	300	1944	144	500	267
		Medium	150	902	89	333	167
	White	Large	300	1437	100	667	194
		Medium	150	400	111	167	122
Others	-		80	2349	58	83	69
South America	-	Large	300	250	83	100	89
US West Coast	-	Large	300	144	50	100	72
			Total Trays	13407			

5.3 Imports

Three to four years ago, uni imports from China peaked. There has since been a decline in Chinese uni, however that from Russia has increased. This increase has been of such magnitude that there is now a glut which has depressed prices paid for Japanese uni. Recently the Russian Government introduced regulations in response to over-fishing and it is now thought that uni imports will decline from that country.

Most Russian uni comes from around Sakhalin Island and the Kuril Islands (between the northern tip of the Japanese island of Hokkaido and the Kamchatka Peninsula). Fishing takes place year round but there is a seasonal northward movement to avoid areas in which urchins are spawning. The latter occurs along the coast north of North Korea between June and September and off the Kuril Islands between October and June. At present 100 tonnes of live sea urchin comes from the Kuril Islands per day, 5 days per week. The recovery rate is highly variable.

Sea urchin roe has two functions for the animal; storage of nutrients and production of eggs. The nutrients are stored in special cells called nutritive phagocytes and used both as an energy source by the adult in times of food scarcity and, after transfer to the eggs immediately prior to spawning, by the developing embryos and larvae. The Japanese market targets uni which is at the pre-transfer stage, that is, the preferred product is really the nutritive phagocytes not the eggs. At this stage the uni is dull gold in colour and has a very fine texture, to the extent that individual eggs are not visible.

Mr Murakami (Hakodate sea urchin processing facility) believes that sea urchins can never be fished out from the many small islands off Hokkaido and pointed out that there were large areas of untouched stocks in the 1000 kilometres of coastline off the Sea of Japan. Although this theme of an inexhaustible ocean recurred several times during the trip, some individuals did regard sustainability of stocks as a high priority. In this case, the sea urchin industry is supported by 50 aquaculture facilities which are continually reseeding the urchin beds. Their presence alone suggests that the Japanese government is concerned about the longevity of the fishery.

5.4 Australian Uni

The black sea urchin (*Centrostephanes rodgersii*) found in New South Wales is unable at this stage to command a high price in Tsukiji market compared to its competitors. It was conveyed that in general the South Australian product has a better reputation than that from New South Wales. However, we were advised that the Boston (USA)-based Explorer Company sent approximately 500 kg of live sea urchins to Japan from South Australia. The colour of this product was considered to be poor and Mr Shimoda suggested that exporting South Australian uni without consideration to requirements of the market would tarnish its image.

5.5 Australian Business Centre, Tokyo

The Australian Business Centre (ABS) promotes South Australian seafood in Japan, organises Japanese delegations to South Australia and works to attract investment. The Centre works with the Japanese Government to promote various products through the Embassy and mediates between buyers in Japan and the Australian seafood industry. They also raise the level of awareness in Japan that South Australia is able to provide good quality product. The office is part of the Department of Industry, Tourism and Trade and operates on a “fee for service” basis. Japanese companies can contract the ABS to source seafood product and they pass relevant information to Food Adelaide, a separate organisation, which then makes contact with South Australian producers through their membership. The ABS are also involved with FoodEx which is a trade show held, in Tokyo, in February each year. Last year thirteen South Australian companies exhibited.

With regard to the seafood industry, it was conveyed by the ABS, and subsequently by several processors, that consistency of supply is paramount. Currently there is concern from Japanese buyers that product quality and quantity would decline if there was an off-season in the fishery.

6. Implications for South Australia

The information gleaned from the study tour has assisted PIRSA Fisheries in considering the future of a fishery for sea urchins in South Australia.

Throughout the study tour it became increasingly obvious that markets for uni in Japan are “delicate”, in that many factors are considered in determining what price will be paid for the final product. In addition to the texture, taste, colour, dullness and shape of the sea urchin uni, consistency of product and established business arrangements/networks are integral to the final price paid. Miyako International Seafood Pty Ltd, a seafood exporting company, have suggested that to overcome problems in providing consistent quantities, wet packs of uni from South Australia and New South Wales could be sent in the first few shipments. Once experience had been gained and subsequent acceptance of the product, the uni could then be packaged in plastic trays.

Miyako have suggested that they would like to work towards a harvest of approximately 150kg of uni per day from South Australia. Assuming a yield of between 7 and 10% of uni per urchin this equates to a harvest of between 1.5 and 2 tonnes of urchins per day. At this stage the sustainability of such a harvest is unknown. If the price paid at Tsukiji Market was $100/kg, the gross turnover would be $15,000 per day or $75,000 per week.

In assessing the quantity of product necessary for a commercial operation it has become evident that aquaculture should be the primary focus of the sea urchin industry in South Australia, given the risk of overfishing the resource. Support should be given to licence applicants who incorporate aquaculture as part of their long-term business plans as this indicates their intention to operate in an ecologically sustainable manner.

It may be that providing access to a specified area for fishers, or a single company, would encourage the level of responsibility for the fishery demonstrated in Japan. This is also seen in the South Australian River Fishery where one operator has exclusive commercial access to a pre-determined area. If this policy is adopted, consideration must be given to the size and number of areas as each must be an economically, and ecologically, sustainable unit. At the same time, the policy must also take into account the existing fishery licence holders who currently have access to sea urchins on a state-wide basis.

The possibility of developing a sea urchin fishery in South Australia was discussed with NSW Fisheries to draw on their past experiences. The point was made that little is known of the biological characteristics of sea urchins in SA and therefore any further authorities issued for sea urchin harvesting should be provided with the ability to reduce effort significantly, even to the extent of revoking fishing licences if indicators suggested that the resource was diminishing. This ability will also encourage stronger liaison between Government regulators and industry. It was also clear from these discussions that a program should be put in place for the biological data collection and monitoring of the sea urchin stocks.

New South Wales currently has a sea urchin fishery in which Miyako International Seafood participates. The fishing licences issued in New South Wales are joint abalone/sea urchin licences. As higher prices are paid for abalone, less fishing effort is directed at sea urchins by divers. In South Australia it is suggested that separate fishing licences be issued for sea urchins.

The buying of fishing licences and subsequent non-activity or speculation by licence holders is an issue which is often raised in regard to the development of new fisheries in South Australia and in other States. The requesting of business plans, which include financial projections, if fishing licences are to be tendered in the future, may assist Government in the identification of companies or individuals who will utilise the licence. It is suggested that this apply to future sea urchin licences if tendered. The non-transferability of any licence issued may also discourage a degree of speculation.

APPENDIX 8: Honours theses co-supervised by the author during the project

Evaluation of Techniques for Gonad Enhancement in the Purple Sea Urchin *Heliocidaris erythrogramma*

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2001

A thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Technology; Aquaculture (Honours)

ABSTRACT

A decrease in production of sea urchin fisheries has lead to diversification in strategies relating to the continued exploitation of this resource. The management of these fisheries has been met with variable levels of success, prompting new techniques that gain more control over the life cycle of sea urchins to be developed. A widely used enhancement technique involves providing supplemental feed and optimal environmental conditions to improve the quality and the quantity of roe recovered. This study investigates enhancement techniques in the Purple Sea Urchin *Heliocidaris erythrogramma.* Gonad yield (GSI) of *Heliocidaris erythrogramma* located at Point Riley, South Australia was greatest in August (4.93%), decreasing in October to maintain at around 3.6% until February. During the period of this study urchins developed through all reproductive stages, from recovering spent in August to spent in February. Reproductive development *of H. erythrogramma* was accelerated for females, which were ripe in October, compared with males that were still in the regenerating phase. The low gonad yield of *H. erythrogramma* from Point Riley suggests this population would never form a major part of a sustainable sea urchin fishery.

Urchins fed a prepared diet and exposed to different photoperiods showed no increase in GSI compared with an initial and fmal wild sample of the same population. Gonad yield in all photoperiod treatments decreased in comparison with the wild samples. There was no clearly different pattern in the reproductive stage of urchins in any photoperiod treatment. Most urchins spawned or resorbed gonad material in captivity and remained in a spent reproductive phase. Samples taken of wild sea urchin had a high percentage of gonads with a colour that would command a superior market price. The only experimental photoperiod that produced a colour distribution similar to the wild samples was the dark treatment. *H.erythrogramma* was fed a prepared or macroalgal diet and exposed to different photoperiods. There was no significant interaction between photoperiod and diet on GSI, however GSI was significantly influenced by diet. Urchins fed a formulated diet maintained GSI in comparison with a wild sample of the same population taken at the beginning of the experiment. In contrast, urchins fed a macroalgal diet *(Ecklonia radiata)* had a significantly smaller GSI than animals maintained on formulated feeds. Unlike the previous experiment, urchins exposed to a 'light photoperiod' of 14 hours dark / 10 hours light had a greater percentage of gonads of the preferred market colour (bright to pale orange/yellow).

Assessing Techniques for the Direct Aging and Tagging of the Sea Urchin, *Heliocidaris erythrogramma*

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A thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours)

ABSTRACT

The determination of population dynamics is essential when determining the appropriate management strategy for a species. In South Australia, the understanding of population biology of the sea urchin *Heliocidaris erythrogramma* is virtually non-existent. Aging provides us with important population dynamics information such as growth rates and is performed through the interpretation of annual increments that are formed in the animal's calcium carbonate structures. Sea urchins have numerous hard structures that can be used in aging. The structure that seems to offer a great deal of success is the rotule, which is found in Aristotle's lantern. One method for acquiring information on population dynamics for sea urchins is through tagging, and the appropriate tagging technique will be species-specific due to the variety in size and test structure. This study was concerned with the development of appropriate methodological approaches for aging studies and the development of an appropriate tagging technique for the sea urchin, *H. erythrogramma.*

Various volume and concentration amounts of the antibiotic tetracycline were injected into sea urchins to determine the optimal amount to use for further aging studies. This was achieved through the ease of interpretation of tetracycline bands on rotules and monitoring growth and survivorship. Sea urchins were injected with 0.5, 1 or 3% concentrations of tetracycline, and volumes of 0.01, 0.1, 0.15ml per 10g wet weight of sea urchin using a universal concentration of 1% tetracycline. The different concentrations and volumes were compared against a control that was injected with 0. lml/10g wet weight of seawater. A subset of sea urchins was sampled after approximately 4 months. As dosage of tetracycline increased so did the amount present in the rotules. Differences in growth between the various treatments were not significant for the volume and concentration experiments respectively. There was no significant difference in average mortality between the various treatments for the volume and concentration experiments respectively. However, overall mortality was 42.5%. Monthly samples of 30 sea urchins were collected from Pt. Riley for marginal increment analysis work. Marginal increment analysis showed that dark (translucent) increments were formed during the cooler months of the year. These were easily determined in smaller sea urchins, but as sea urchins increased in size there were some incomplete translucent bands that made it difficult to count accurately.

Various tag types were trialed to try and establish an appropriate tagging technique for *H.*

erythrogramma. Further work was conducted using dart tags, which proved to be the most successful tagging methodology. There was 100% tag retention in the third tagging experiment. In contrast, the majority of non-invasive tag types used were lost in the first 10 days of the experiment.

Future aging validation work on *H. erythrogramma* should be conducted using a 1% tetracycline concentration with a 0.1 ml/10g wet weight injection. Field tagging work should be done using dart tags with the application of the surgical glue, Histoacryl around the base of the tag and drill hole. The indication from this study is that a count of translucent and opaque increments in the transverse section of rotules can be used to estimate the age of sea urchins.

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