Southern Bluefin Tuna (*Thunnus maccoyii*) Aquaculture Subprogram Project 2: Development and optimisation of manufactured feeds for farmed Southern Bluefin Tuna.

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CRC for AQUACULTURE

UNIVERSITY OF TASMANIA

Project No: 97/362

97/362: Southern Bluefin Tuna (*Thunnus maccoyii)* Aquaculture Sub-Program Project 2: Development and optimisation of manufactured feeds for farmed Southern Bluefin Tuna.

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PIVOT Ltd (Trading as Gibsons Australia)

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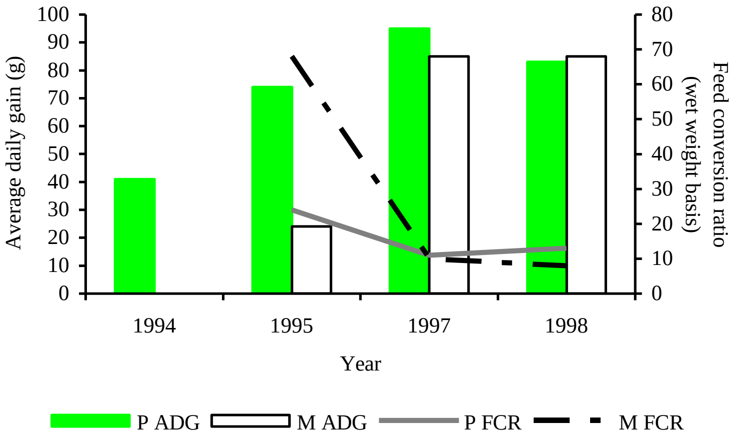
OBJECTIVES:

1. Develop a cost-effective, sustainable manufactured diet for farmed SBT that contains reduced levels of fish meal, trash fish and fish oils while maintaining growth performance and flesh characteristics.
2. Assess the response of farmed SBT to changes in diet moisture and protein content.
3. Assess the influence of artificial colour enhancers on the flesh characteristics of farmed SBT.
4. Develop near infrared spectrophotometry calibrations for the assessment of bait fish quality prior to inclusion in manufactured diets for SBT.
5. To determine the efficiency of digestion of farmed SBT fed manufactured diets.
6. Improve our understanding of the physiological responses by farmed SBT to manufactured diets.
7. Reduce nutrient excretion through improved knowledge of the nutritional value of diet ingredients for farmed SBT.

OUTCOMES ACHIEVED

Through this CRC project and projects preceding this one, we have developed a semi-moist manufactured diet that will produce growth rates and flesh characteristics equivalent to that achieved with pilchards. We are yet to produce a semi-moist diet on a commercial scale that produces growth and feed conversion responses equivalent to that achieved with bait fish, largely due to problems associated with achieving the same level of pellet binding with commercial scale production.

A review of research results from 1994 to 1998 demonstrates the significant progress that has been made towards the production of a commercially viable manufactured feed for SBT (summarised right; P ADG, pellet average daily gain; M ADG, manufactured diet average daily gain; P FCR, pellet feed conversion ratio; M FCR, manufactured diet feed conversion ratio). Growth rates of tuna fed manufactured feeds have more than doubled since 1994 and have plateaued at a level equivalent to that achieved with bait fish. Even more dramatic has been the improvement in feed conversion ratios when SBT are fed moist pellets, however, there is still scope for improvement in this area. We are at a stage where manufactured feeds could be used commercially for the production of SBT providing the moist pellet that has been tested to date can be manufactured commercially to the same specifications achieved on an experimental scale. With the above in mind, priorities for research into manufactured feeds has moved away from testing “the ideal diet” to examining some of the mechanisms that may be limiting SBT growth and feed conversion when they are offered any feed. To this end, we have improved our understanding of factors influencing the nutrition of SBT.



In addition to the development of manufactured diets, a major outcome from this project has been the development of near infrared spectrophotometry calibrations for the measurement of crude protein, moisture, crude fat and free fatty acids in processed whole (thawed or frozen), ground (thawed or frozen), or processed freeze-dried bait fish.

NON TECHNICAL SUMMARY:

Development and optimisation of manufactured diets for farmed Southern Bluefin Tuna (SBT) included the conduct of three core experiments (a fourth planned experiment was not undertaken for operational reasons) with SBT in experimental pontoons, plus peripheral research (Experiment 5) to develop near infrared spectrophotometer calibrations for bait fish quality.

Experiment 1: The influence of diet protein and fat content and diet ingredient combinations on the performance of caged SBT.

Experimental objectives:

1. Determine the performance of tuna fed the 1997 diet, but with some variation in the ingredients;
2. Establish the value of an artificial flesh colour enhancer;
3. Determine the influence of changes in the binding strength of the moist pellets on SBT performance;
4. Examine the response of SBT to changes in the pellet nutrient content.

Before embarking on a more detailed experimental program to define the specific requirements of a manufactured feed for SBT, an experiment was conducted to establish whether performance differences could be detected between SBT fed diets differing in crude protein and moisture content, respectively. Diets were formulated to contain significantly different levels of these components and fed twice daily to fish. This first growth experiment was also used to assess the value of adding colour enhancers to the diet. A single manufactured diet contained a colour enhancer (Carophyll pink) and the flesh colour of these fish were compared to those fed pilchards and other manufactured diets. To help understand the digestive efficiency of fish fed manufactured diets containing different levels of protein and moisture, a marker was included in the diets for seven days prior to harvest. Digesta from the distal intestine of two groups of five fish from each cage was pooled to determine the digestibility of amino acids, protein and energy.

Experiment 2: The influence of diet protein and fat content and diet ingredient combinations on the performance of caged SBT.

Experimental objectives:

1. To assess the influence of dietary fat content on the growth performance of SBT;
2. To reduce the level of fresh product and fish meal in manufactured feeds;
3. To assess the influence of restricted feeding on feed conversion ratio.

Experimental diets were formulated to be isonitrogenous and to contain 12, 15 or 18% fat. The feeding regimes varied between satiation and 80% of satiation intake based on crude protein intake on a dry matter basis. Intakes were adjusted to be self-regulating based on daily satiation intakes in the control pontoons. Daily feed intakes were adjusted based on past experience. A marker was included in the diets for seven days prior to harvest. Digesta from the distal intestine of two groups of five fish from each cage was pooled to determine the digestibility of amino acids, protein and energy.

Experiment 3: Changes in caged SBT nutritional requirements over a growing season.

Experimental objectives:

1. To determine whether the nutritional requirements of SBT and the mode of feeding change over the course of a growing season using diets differing in protein:energy ratio and bulk density.

Despite planning for this experiment, the SBT Aquaculture Steering Committee advised in January 2000 that no fish would be available in the experimental pontoons for research in that year. As a consequence, all resources from this project were redirected into the development of NIR calibrations for bait fish.

Experiment 4: Aspects of protein metabolism in SBT that may result in suboptimal physiological functioning.

Experimental objectives:

The focus of the third SBT experiment that was conducted was to measure indices of protein metabolism by focusing on the levels and changes in nutritional correlates (protein, RNA and DNA) and free amino acid pool concentrations. Muscle is most representative of overall patterns of growth and was selected as the focus for the study.

Experiment 5: Development of NIR calibrations for the measurement of protein fat and moisture in bait fish prior to inclusion in experimental diets.

Experimental objectives:

1. Develop NIR calibrations for the assessment of protein, fat and moisture in bait fish prior to incorporation into experimental diets.

In addition to SBT experiments, our ability to formulate manufactured diets was enhanced by the development of near infrared spectrophotometry (NIRS) calibrations for bait fish quality including crude protein, moisture, crude fat and free fatty acid concentration. NIRS calibrations permit very rapid analysis of samples with minimal preparation, so all samples can be analysed prior to inclusion in manufactured diets.

The conduct of the above experiments resulted in the following conclusions being drawn from the research:

- The composition of pilchards is highly variable over the course of an SBT growing season. This is likely to affect production efficiency. In contrast, manufactured diets can be produced with a high degree of consistency.

- The performance of SBT fed diets (moist pellets) containing reduced levels of pilchards does not equate to diets containing approximately 48% fresh product or fresh product alone. This may be due to a number of factors including reduced intake, or issues associated with pellet form and a more rapid breakdown of the pellet when less fresh product is included.

- There was no evidence of differences in growth rate between SBT fed diets with reduced levels of pilchards and significant differences in the content of dietary protein and fat. This suggests that we may be significantly overfeeding SBT in pontoons, resulting in feed wastage and an increase in the level of environmental waste.

- Higher fat diets may be more appropriate for farming SBT.

- Pellet quality and dietary protein content appear to have been two fundamental constraints to the conduct of the experiments in the current project.

- Numerical differences can be observed in the performance of SBT fed diets containing different levels of moisture, protein and/or crude fat.

- Dietary carotenoids do not influence the flesh characteristics of SBT fed manufactured diets.

- The crude protein, moisture, crude fat and free fatty acid content of bait fish can be adequately screened using near infrared spectrophotometry on samples of processed frozen, processed thawed and processed freeze-dried bait fish, the latter being the most accurate. Analysis can be undertaken using spectral ranges of 1100-2500 nm or 500-1050, the former being more accurate, but the latter acceptable.

- Manufactured diets with higher levels of crude fat have higher levels of digestible protein, dry matter and gross energy.

- The better the pellet binding or pellet integrity, the higher the dry matter, gross energy and crude protein digestibility. This is closely related to the anatomical structure of the tuna digestive tract and the comparatively short retention time of poorly bound pellets.

- Gut retention time appears to influence nutrient digestibility with improved digestibility observed with restricted feeding of manufactured diets.

- Higher gross energy, crude protein and dry matter digestibility is reflected by higher SBT growth rates.

- Positive correlations between tissue protein, RNA and DNA, IGF-I and liver status and nutritional status support their further use in nutrition experiments.

- Analysis free amino acid concentrations revealed extremely low levels of two essential amino acids which may contribute to poor feed efficiency of tuna fed formulated diets. However, the data need to be considered in relation to digestion and absorption of essential amino acids and concentrations in other tissues and plasma to further clarify the situation.

- Free histidine in muscle tissue did not relate to the nutritional status of the tuna and is not suitable as an indicator of such.

KEYWORDS: Southern Bluefin Tuna, Aquaculture, Nutrition.

Acknowledgements

This project represents a culmination of inputs from a range of sources. It was co-funded by the Fisheries Research and Development Corporation and the Aquaculture CRC Ltd with in-kind contributions from the South Australian Government, the Tuna Boat Owners Association of Australia (TBOAA), PIVOT Ltd and Barneveld Nutrition Pty Ltd. Additional scientific inputs were secured from Dr Chris Carter from the University of Tasmania who investigated temporal variation in the protein metabolism of tuna, and Dr Brett Glencross who was responsible for the daily management of the research experiments and the collation and interpretation of some data. Mr Steven Clarke (Subprogram Leader) was Principal Investigator on Southern Bluefin Tuna Aquaculture Subprogram Project 1 which provided the management of the tuna research pontoons. Dr Jeff Buchanan assisted with the collection of bait fish samples used in the NIR research. Some bait fish samples were also sourced via a TBOAA project being managed by Ms Kirsten Rough. The production of all experimental diets was completed under contract by Pivot Ltd using moist pellet production facilities established in Port Lincoln by this company. Technical support on the tuna research farm was provided by Mr R. Daw, Mr P. Musolino, Mr T. Musolino, Mr G. Bayly, Mr R. Harley and Mr E. Davis. Analytical services were provided by the SARDI Pig and Poultry Production Institute with contributions from Dr Y. Ru, Mr J. Kruk, Ms J. Hattam and Ms K. Swanson.

Background

The development of a suitable formulated feed is a high priority for the tuna farming industry. The desired outcome will enable the sashimi grade tuna product to be better matched with the colour and lipid (fat) requirements of the Japanese market, thereby increasing market price, as well as better suited to the nutritional requirements of the tuna which will enhance farm production and minimise environmental impacts. There is also an urgent requirement for a feed which minimises the importation and subsequent placement of overseas pilchards into Australian waters, an activity considered to present some risk for the importation and transfer of exotic diseases and pests (eg. Final draft of the National Task Force on the Importation of Fish and Fish Products). Through a number of completed and active research projects, scientists in close collaboration with industry have achieved the following towards the development of manufactured feeds for farmed Southern Bluefin Tuna:

1. A range of husbandry techniques that permit the distribution of fish to research pontoons with few or no mortalities in some years, routine tagging and weighing of the fish, and selective sampling of fish.

2. Weaning procedures that facilitate rapid consumption of manufactured semi-moist pellets.

3. A manufactured feed that is readily accepted by farmed Southern Bluefin Tuna and produces growth rates and flesh characteristics similar to that achieved with pilchards.

4. A detailed understanding of the digestive physiology of Southern Bluefin Tuna.

5. Some knowledge of the efficiency of digestion of manufactured diets and pilchards fed to farmed Southern Bluefin Tuna.

Despite these developments, much research is still required before a manufactured diet will be available for use in commercial systems. The nutrient density of the manufactured feeds should support growth rates that exceed that achieved with pilchards and there is room for improvement in the flesh characteristics of fish fed these diets. In addition, the existing manufactured diet is highly inflexible. Small changes in its composition can have significant effects on the ability of the diet to bind during processing and its acceptability to the fish.

To develop a cost-effective diet for farmed Southern Bluefin Tuna, we must first consider the objectives of the farming system. These include:

1. Maximise growth rates.
2. Increase carcase fat/lipid content.
3. Optimise feed conversion ratios.
4. Minimise feed wastage and pollution.

Unfortunately, the deposition of fat is a biologically inefficient process. In addition, fat deposition is usually maximised when a range of dietary nutrients, particularly protein, are in excess and are poorly balanced to the requirements of the fish. For this reason, the nutrient requirements of farmed Southern Bluefin Tuna may not be those that optimise growth and minimise pollution and it can be seen that a compromise must be reached in the development of manufactured feeds. Our immediate attention must be directed towards the form of the manufactured diets and the subsequent efficiency of digestion and utilisation of these diets.

The semi-moist pellet currently promoting growth of Southern Bluefin Tuna in experimental pontoons will serve as a useful research tool. It will allow us to assess the ability of the Southern Bluefin Tuna to respond to changes in dietary protein and moisture content, and the value of adding colour enhancers to the diet. We must then combine this knowledge with adequate processing technology, such as extrusion, to develop a manufactured pellet that is:

1. Easy to store.
2. Easy to handle.
3. Flexible in terms of ingredient composition.
4. Stable in water.
5. Highly acceptable by Southern Bluefin Tuna.
6. Able to promote acceptable growth rates.
7. Efficiently digested by Southern Bluefin Tuna.
8. Cost-effective.

Need

At present the tuna farming industry is almost entirely dependent on whole defrosted pilchards as a feed, with about 50% of the 15 - 20 thousand tonnes used in 1994/95 sourced overseas. The development of a suitable manufactured feed is a high priority with industry and government because:

a) International supplies of pilchards are variable in volume and quality (eg. Japanese supplies have declined markedly and the fat content of pilchards used in feeds varies from 1 - 22%).

b) Manufactured diets will provide the potential for improved product quality (in particular fat content, colour and texture) as they are more stable in storage than pilchards and can be altered to better meet the requirements of fish farming and the markets.

c) Manufactured diets will reduce industry feeding costs as their generally lesser moisture content and better feed conversion ratio will reduce the quantities required and therefore costs associated with feed storage and transport.

d) Manufactured diets and appropriate feeding strategies will greatly reduce environmental concerns associated with the present use of pilchards, including: reducing the overall requirement for pilchards, minimising risks of importing and dispersing undesirable diseases and pests, and reducing organic wastes in the farm environment which can harbour and promote diseases as well as detrimentally effect water quality.

The development of manufactured diets has been clearly recognised as a high priority by the Tuna Boat Owners Association of Australia (TBOAA) (numerous scientific workshops), the CRC for Aquaculture (Tuna Research Review Task Force) and the national Task Force on the Importation of Fish and Fish Products. Participating feed companies are also supportive as they will benefit from the desired outcome.

The economic benefits of the development of a suitable formulated feed has been estimated to be as high as $9.5 million/annum to the TBOAA and $5 million to successful feed manufacturers. Additional economic benefits would be expected to flow from ongoing research leading to further enhancements of feeds.

Objectives

The original objectives of this research project were to:

1. Develop a cost-effective, sustainable manufactured diet for farmed SBT that contains reduced levels of fish meal, trash fish and fish oils while maintaining growth performance and flesh characteristics.
2. Assess the response of farmed SBT to changes in diet moisture and protein content.
3. Assess the influence of artificial colour enhancers on the flesh characteristics of farmed SBT.
4. Identify extrusion techniques that will produce a cost-effective, acceptable manufactured feed for farmed SBT.
5. To determine the efficiency of digestion of farmed SBT fed manufactured diets.
6. Improve our understanding of the physiological responses by farmed SBT to manufactured diets.
7. Reduce nutrient excretion through improved knowledge of the nutritional value of diet ingredients for farmed SBT.

In order to meet objective 4, this project had a heavy reliance on the establishment of the Australasian Experimental Stockfeed Extrusion Centre (AESEC). Due to delays in the construction of this facility, objective 4 listed above was altered to the following in January, 2000:

4. Develop near infrared spectroscopy calibrations for the assessment of bait fish quality prior to inclusion in manufactured diets for SBT.

This change was ratified by the SBT Aquaculture Subprogram Steering Committee.

General Methodology

To meet the contracted objectives, three major experiments were designed to be undertaken using the experimental tuna research farm in Port Lincoln. Details of these experiments are as follows:

Experiment 1

Experiment title:	The influence of diet protein and fat content and diet ingredient combinations on the performance of caged SBT.
Related Objectives:	1,2,3,5 and 7
Animal Ethics Approval:	38/97
Experimental objectives:	

5. Determine the performance of tuna fed the 1997 diet, but with some variation in the ingredients.
6. Establish the value of an artificial flesh colour enhancer.
7. Determine the influence of changes in the binding strength of the moist pellets on SBT performance.
8. Examine the response of SBT to changes in the pellet nutrient content.

Material and methods

Diets

A total of four diets were used in Experiment 1 – pilchards as a control and three manufactured feeds (Table 1). The formulation basis for the manufactured feeds is as follows:

Pilchards: Industry standard used as a control in research experiments. When a manufactured diet is shown to exceed the performance currently achieved with pilchards, all experimental work will use this diet as a control and pilchards will be removed from the system.

CRC98A: This diet was formulated using the same ingredients as those used in CRC97. The ingredient additions were altered slightly to account for the use of local pilchards rather than imported pilchards. Local pilchards have a lower average oil content and a higher moisture content than imported pilchards. Extra oil was added to account for this. This approach maintained the binding properties of CRC98A while supplying the same nutrients as CRC97 as fed with the exception of crude fat content which was slightly depressed. This diet represents our base level manufactured diet and has been shown to produce growth rates and carcase characteristics equivalent to pilchards.

CRC98B: The basis of this formulation was to supply the same nutrients as CRC98A (and CRC97) using a different combination of ingredients. Specifically, this diet has a higher gluten content and lower fresh pilchard content. This formulation will allow us to assess the impact of fresh pilchard content on acceptance by SBT, and the ability to utilise other ingredients. The higher gluten content will result in different binding properties, however, our ability to assess these properties is limited and was not a major focus of this experiment. If fish performance is depressed when fed this diet, the program must try and identify the critical component of CRC98A that is influencing growth of SBT.

CRC98C: This diet was formulated to contain a higher crude protein content and lower crude fat content. The basis of the formulation was to determine our ability to assess differences in the performance of SBT fed significantly different levels of nutrients. If we cannot detect a significant difference in growth rate and flesh quality with this diet compared with CRC98A and CRC98B, the program must consider those factors having the greatest impact of performance. If no detectable improvement in SBT performance is evident, we will be able to conclude that either SBT derive a significant proportion of their dietary energy from oil, and they have in fact reached an energy limiting growth phase, or we have over-specified the protein and energy levels in the diet, and could achieve similar performance at a much reduced cost and nutrient levels.

Table 1. *Composition of experimental diets (g/kg, air-dry basis)*

Ingredients	Control	CRC98A	CRC98B	CRC98C
Additives				
Choline chloride	-	0.200	0.200	0.200
Lecithin for aquatic diets	-	1.000	1.000	1.000
Pre-mix vitamins and minerals*	-	0.300	0.300	0.300
Roche Stay-C vitamin C	-	0.048	0.048	0.048
Colour enhance (Carophyll pink)	-	0.001	0.001	0.001
Diluents and fillers				
Water	-	0.000	15.000	12.182
BO11C Pre-gelled starch	-	0.000	0.000	0.000
Energy				
Squid oil	-	9.000	9.339	3.395
Protein and amino acids				
Wheat gluten	-	10.000	15.000	15.000
Inual Antarctic krill meal	-	2.500	2.500	2.500
Fish meal Chilean 67% CP	-	27.387	28.888	35.737
Squid meal	-	2.500	2.500	2.500
Fresh pilchards	100.000	47.064	25.224	27.137

* See Table 3

When the diets are mixed, the mixing action on the gluten helps to bind the pellet. Moisture content of the pellets is critical to ensure optimal binding.

The diets were fed as formulated except for 7 days prior to each harvest. At this time, chromic oxide was included in all diets at a rate of 0.5% to facilitate digestibility measurements.

A synthetic astaxanthin (Carophyll Pink) was added to all manufactured diets. Feedback from the Centre for Food Technology suggests that a synthetic colour enhancer will have limited impact on flesh characteristics as the major influence on flesh colour is the myoglobin content of the flesh. The flesh colour of the fish fed the manufactured feeds was compared against the pilchard-fed fish. Full details of diet formulations are presented in Appendix III.

Table 2. *Nutrient contributions from the experimental diets (g/kg)*

Nutrient	CRC98A	CRC98B	CRC98C
Proximates			
Dry matter	622.7	623.8	633.0
Crude fat	147.1	147.3	97.0
Crude protein	389.2	389.2	439.2
Digestible nutrients			
Digestible crude protein	262.3	262.1	290.7
Amino acids			
Lysine	21.6	21.5	25.3
Threonine	12.2	12.9	14.8
Methionine	7.6	8.1	9.4
Isoleucine	14.8	15.6	17.8
Leucine	22.9	25.4	28.9
Tryptophan	2.6	2.84	3.4
Valine	15.7	17.0	19.4
Phenylalanine	13.8	15.3	17.1
Histidine	7.4	6.7	7.9
Arginine	21.5	22.3	25.9

Diet mixing

Diets were mixed and manufactured by Pivot Aquaculture Pty Ltd under the terms of a Memorandum of Understanding.

Fish and cages

Each diet was fed to two research cages of SBT. Fish were fed once daily to satiety (using visual inspection or video cameras to determine this point). The quantity of feed and pilchards provided to each pen was recorded daily.

Digestibility measurements

Samples of digesta were taken from fish at each harvest. These samples were used to determine the digestibility of protein, amino acids and energy in the manufactured feeds. The digestibility of nutrients in the pilchards was not determined.

Diets containing chromic oxide were fed to the SBT for a period of seven days prior to the collection period. At harvest, the digestive tract was removed from the fish and divided into four segments (stomach, pyloric caeca, proximal intestine, distal intestine) with suture or clamps. Tracts were kept on ice until the harvests were complete. The tracts were then emptied section by section into collection bottles. Digesta from up to five fish per cage were bulked until there is sufficient sample for the analysis of chromic oxide, protein and gross energy. The bulked samples comprised a single replicate, hence at least 10 fish had to be harvested from each cage to provide two replicates.

All samples were chilled and subsequently frozen following collection to prevent further digestion of digesta.

Table 3. *Composition of vitamin and mineral pre-mix used in 1998 experimental diets*

Ingredient	Units	unit/3kg	unit/kg
Vitamin A	MIU	3.000	1.000
Vitamin D3	MIU	0.500	0.167
Thiamine (B1)	g	15.000	5.000
Riboflavin (B2)	g	20.000	6.667
Pyridoxine (B6)	g	12.000	4.000
Vitamin B12	mg	30.000	10.000
Biotin	g	0.300	0.100
Vitamin K3	g	7.000	2.333
Pantothenic Acid	g	30.000	10.000
Niacin	g	65.000	21.667
Inositol	g	50.000	16.667
Vitamin E ADS	g	100.000	33.333
Folic acid S.D.	g	4.000	1.333
Ethoxyquin	g	150.000	50.000
Cobalt	g	1.000	0.333
Iodine	g	1.100	0.367
Copper	g	3.000	1.000
Magnesium	g	50.000	16.667
Manganese	g	20.000	6.667
Iron	g	20.000	6.667
Bioplex iron	g	20.000	6.667
Zinc	g	30.000	10.000
Aquastab C 42%	g	60.000	20.000

Statistics/Experimental Design

This experiment was based on a randomised block design and was analysed using a general linear model in SAS. Digestibility studies were based on at least four replications per diet (ie. two samples from each cage).

Experiment 2

Experiment title: The influence of diet protein and fat content and diet ingredient combinations on the performance of caged SBT.
Related Objectives: 1,2,5,6 and 7
Animal Ethics Approval: 38/97
Experimental objectives:

4. To assess the influence of dietary fat content on the growth performance of SBT.
5. To reduce the level of fresh product and fish meal in manufactured feeds.
6. To assess the influence of restricted feeding on feed conversion ratio.

To achieve the above objectives, three diets were planned for use in this experiment (99A, 99B and 99C) based on the compositions outlined below:

99A : 40% protein, 12% fat (38% water)
99B : 40% protein, 16% fat (34% water)
99C : 40% protein, 20% fat (29% water)

Due to difficulties with pellet integrity, the composition of the diets was altered midway through the experiment to contain the following:

99A : 40% protein, 12% fat (38% water)
99B : 40% protein, 15% fat (35% water)
99C : 40% protein, 18% fat (31% water)

Diets were manufactured by PIVOT Ltd to agreed specifications. From Week 15 until the end of the experiment the diets contained 0.5% chromic oxide (ie. 5 g/kg). Feed samples were tested fortnightly for crude protein, crude fat, gross energy and dry matter. Full details of diet formulations are presented in Appendix IV.

Feeding regimes

The objective of this experiment was to assess the influence of dietary fat content on SBT performance. With this in mind, the most meaningful results were obtained by equalising crude protein intake on a dry matter basis. This minimised the influence from other dietary variables while maintaining differences in fat intake. Equalising intake on a dry matter basis was undesirable as it favours lower fat diets due to the lower dry matter content of these diets – these fish would receive more protein and this feeding regime would reduce the differences between fat intakes of the fish on the various treatments. Equalising intake on a crude fat basis was undesirable as we wanted to detect differences between fish with different fat intakes.

A further innovation was suggested to ensure accurate results from this experiment. It is well known that daily feed intake decreases over the course of an experiment, possibly due to reductions in water temperature and day length. As a consequence, calculation of restricted feeding regimes is difficult and inaccuracies are likely. It was suggested that the 99A diet fed to satiation be used as a control for calculation of feeding levels of other diets fed restrictively. That is, the daily quantity of 99A consumed per fish was used to calculate the quantity of feed to be added to cages fed 99A, 99B and 99C fed restrictively. Full details of feed rate calculations are presented in Appendix V.

Based on the above, the following basic feeding regimes were applied:

- 99A to satiation twice daily (15 minute limit).
- 99A to 80% of actual daily satiation intake.
- 99B to 1.064 times the actual intake of 99A fed restrictively.
- 99C to 1.145 times the actual intake of 99A fed restrictively.

Digesta samples were collected from harvested fish for the calculation of dry matter, nitrogen and gross energy digestibility.

Experiment 3

Experiment title: Changes in caged SBT nutritional requirements over a growing season.
Related Objectives: 1,2,5,6 and 7
Animal Ethics Approval: 38/97
Experimental objectives:

2. To determine whether the nutritional requirements of SBT and the mode of feeding change over the course of a growing season using diets differing in protein:energy ratio and bulk density.

Hypothesis: At the time of planning this experiment, all caged SBT received a single source of nutrients over the course of a growing season (eg. in commercial farms bait fish were offered to SBT to satiety across the season, while in the experimental cages, a single moist pellet was employed). From research completed to date, it is clear that SBT fed manufactured feeds grow at a slower rate in the initial parts of the season, and while these fish reach the same final weight as the bait-fish fed SBT, the growth patterns over the course of the season favour the SBT fed bait-fish. Given that the intakes of the SBT change over the growing season, and as the composition of the fish also changes during this period, it is reasonable to suggest that there may be a need for a range of diets to meet differing intakes and nutritional requirements. It is possible that the present mode of feeding high energy density diets to SBT in the early part of the season may be compromising growth due to poor nutrient utilisation and may not in fact be taking advantage of the high intakes experienced during this period. In the latter part of the season, a higher energy density diet may be more appropriate. There is also evidence to suggest that SBT intake is related to dietary energy content rather than gut fill alone. In addition, research with any production animal supports the use of frequent, regular feeding of small proportions of daily nutrients in preference to single large daily meals. If the bulk of the diet can be increased to reduce energy density but increase gut fill, it may promote more regular feeding for a smaller acquisition of nutrients by the SBT, resulting in improved nutrient utilisation and subsequent growth.

Diets: At the time of planning this experiment, a total of two cages were available for new nutrition research in the Year 2000. If pilchard cages were utilised as control cages in the experiment and an additional two cages were utilised to examine the integrity of heated moist pellets produced by Pivot Ltd, then a nutrition experiment could be based on comparisons between 6 cages.

Heated moist pellets were based on a previously trialed diet formulation, and could be used as a positive control in this nutrition experiment.

To examine changing nutrient requirements over the course of a growing season, two diets were formulated (ie 2000B (1-7 weeks) and 2000B (8-14 weeks)). The following should be noted in relation to these diets:

- 2000B (1-7 weeks) was formulated to contain high levels of protein, low levels of fat and increased levels of bulk. As gut fill does not seem to influence satiety, it was hoped intake would be high, protein and energy levels would be adequate for muscle growth. Kaolin, which has a high water holding capacity, could influence digesta transit time and subsequently increase protein digestion. Protein excesses should be sufficient to promote fat deposition. Meat meal was added to ensure an adequate supply of calcium and phosphorus. Blood meal was used to boost dietary protein levels. Wheat gluten levels were increased to ensure adequate levels of binding. Distilled monoglyceride was added to assist incorporation of fat and water (using technology developed by the bread making industries). Diet 2000B (1-7 weeks) was to be offered for the first 7 weeks of the growing season accompanied by a mid-season harvest at the change-over point.

- 2000B (8-14 weeks) was formulated to contain low levels of protein and high levels of energy through dietary fat addition. This diet was designed to capitalise on reduced intakes experienced during the latter half of the growing season while maintaining adequate levels of fat deposition.

Full details of the experimental diets are presented in Appendix VI.

Diet manufacture: Experimental diets were to be manufactured in small quantities using Pivot Ltd equipment in Port Lincoln to first assess the pellet integrity. Formulations were to be altered until pellet integrity was in no way compromised by the pellet composition. Pellets were to be made fresh and stored for no longer than 24 hours prior to feeding.

Feeding: SBT were to be fed each diet to satiety twice a day, 6 days a week for half of the growing season, respectively. Feed offered was to be recorded and used in feed conversion calculations.

Experimental details: Fish were to be PIT-tagged for individual identification, weighed and measured prior to the commencement of the experiment during transfer to the experimental pontoons. A subsample of fish was to be harvested at the mid point of the growing season for weight measurements and digesta collections prior to changing diets in the pen. Seven days prior to the completion of the experiment (and prior to the mid-point harvest), an indigestible marker was to be added to the diets for use in digestibility calculations when the fish are harvested. At harvest, PIT-tag numbers, fish weights, fish lengths, flesh and blood samples and digesta samples were to be collected for analysis.

Important Note

Despite planning for this experiment, the SBT Aquaculture Steering Committee advised in January 2000 that no fish would be available in the experimental pontoons for research in that year. As a consequence, all resources from this project were redirected into the development of NIR calibrations for bait fish. A subsequent reprieve did provide some fish for use, however, changes had already been made to this project, and Experiment 3 did not proceed. A small experiment examining the performance of SBT fed heated moist pellets was conducted, however all results (with the exception of the digestibility data) from this research comprised part of Project 1 of this Subprogram and are reported elsewhere.

In addition to the above core experiments, a small experiment was conducted by Dr Chris Carter and aimed to examine aspects of protein metabolism in SBT in order to assess potential contributors to suboptimal SBT performance.

Experiment 4

Experiment title: Aspects of protein metabolism in SBT that may result in suboptimal physiological functioning.
Related Objectives: 1 and 6
Animal Ethics Approval: 38/97
Experimental objectives:

The focus of this experiment was to measure indices of protein metabolism by focusing on the levels and changes in nutritional correlates (protein, RNA and DNA) and free amino acid pool concentrations. Muscle is most representative of overall patterns of growth and was selected as the focus for the study. Amino acids in excess of requirements for protein synthesis tend to accumulate (within boundaries) in the muscle free pools due to the low specificity of catabolic pathways. It is therefore proposed that very low free pool concentrations of an essential amino acid relative to the other amino acids in the muscle indicate a low supply and a high rate of incorporation into proteins and suggest the potential for deficiency (Fuller and Garlick, 1994; Zello *et al*., 1995). In fish there is convincing evidence for this: when single essential amino acids were excluded from diets they (except for methionine) were present at their lowest concentration in the muscle free pool (Nose *et al*., 1978). Measurements of RNA concentration and its concentration relative to concentrations of protein and or DNA are indicative of levels of protein turnover and therefore reflect nutritional status (Houlihan *et al*., 1995).

Materials and Methods

SBT (13.9 ± 2.9 kg) held in a 32 m cage on the Tuna Research Farm, Port Lincoln, SA were used. Five fish were removed prior to feeding in the morning and further groups of five fish removed at 2, 4, 8, 12 and 24 hours after the morning feed. The fish were killed quickly by pithing, weight and fork length measured and tissue samples taken (Carter *et al*., 1998). Tissues samples were taken from the pylorus, liver and white muscle. At the same time the temperature of the white muscle and visceral cavity were measured. Blood was sampled from the bleed hole near the lateral line. The tissues were analysed for protein, DNA and RNA concentration (Carter *et al*., 1998) and muscle for free amino acid concentration (Carter *et al*., 1995). The gastrointestinal tract was removed intact, bagged and put on ice so that the organ weights and stomach contents could be weighed at a later time that evening (after the 12 hour sampling). The experiment ran between 10th and 11th February 1999.

Following revision of the project objectives in January, 2000, the following was undertaken to develop NIR calibrations for the composition of bait fish prior to inclusion in manufactured diets:

Experiment 5

Experiment title: Development of NIR calibrations for the measurement of protein fat and moisture in bait fish prior to inclusion in experimental diets.

Related Objectives: 1 and 4
Animal Ethics Approval: 38/97
Experimental objectives:

2. Develop NIR calibrations for the assessment of protein, fat and moisture in bait fish prior to incorporation into experimental diets.

Sample preparation

Bait fish samples (ie pilchards, mackerel) were obtained as whole frozen fish from commercial tuna farming operations in Port Lincoln, SA as well as from the Tuna Boat Owners Association of Australia as part of project being managed by Ms Kirsten Rough. In order to create a range of sample forms, the samples were homogenised in a food processor and freeze dried. As a result, the following sample forms were produced for each sample for use in calibration development:

1. Whole frozen fish (BF1)
2. Whole thawed fish (BF2)
3. Processed frozen fish (BF3)
4. Processed thawed fish (BF4)
5. Processed freeze-dried fish (BF5)

The samples initially formed two groups :

Group A – 79 samples intended for use as the calibration set
Group B – 28 samples intended for use as the validation set

Reference data

Chemical analyses: All pilchard samples were analysed in homogenised freeze-dried form. Chemical analyses were performed in duplicate except for the peroxide measurements. The moisture, crude protein and crude fat content were determined at the Pig and Poultry Production Institute Nutrition Research Laboratory, Roseworthy Campus, South Australia. The free fatty acid analysis and peroxide level measurements (Group A only) were conducted by Weston Food Laboratories, Enfield, New South Wales. The following methods were used to determine constituents under examination:

1. Moisture content – AOAC 7.003
2. Crude protein – Kjeldahl Method AOAC 24.027
3. Crude fat – AOAC 7.056
4. Free fatty acids – AOAC 28.029
5. Peroxide level – AOAC 28.023

Standard error of reference method: As part of the calibration validation, the Standard Error of the Laboratory (SEL) was calculated. The SEL is a standard error of variance between replicates analysed by the reference method and is defined as:

$$\text{SEL} = \sqrt{\frac{\sum_{i=1}^{N} (Y_1 - Y_i)^2}{N}}$$

The calculated SEL values for the constituents determined in freeze-dried form were as follows:

Moisture	= 0.10;
Crude protein	= 1.68;
Crude fat	= 0.66;
Free fatty acids	= 1.94.

The SEL for the analysis of peroxide was not calculated.

NIR scanning: NIR reflectance spectra of the bait fish samples were recorded using Foss NIRSystem Model 6500 Spectrophotometer (FossNIRSystem Inc., Silver Spring, MD, USA) and Intrasoft International (ISI) WINISI software (FossNIRSystem Inc., Silver Spring , MD, USA. Scanning was performed via transport module in reflectance mode over the wavelength range 1100-2500 nm at 2nm intervals.

Each sample was scanned in five different forms – BF1, BF2, BF3, BF4 and BF5. Collection of spectra of whole-frozen, whole-thawed, processed-frozen and processed-thawed samples was performed using polyethylene scanning bags placed in a rectangular quartz window cup. Processed freeze-dried samples were scanned using a small quartz window cup. Examination of final spectra was conducted in second derivative using SNV and Detrend scatter correction. Identical scanning procedures were applied for Group A and Group B.

Population structuring: The calibration sample set (Group A) was examined using the population structuring program CENTRE in order to identify spectral outliers. To identify patterns in the group of spectra that contribute the most to the variation among the spectra Principal Component Analysis (PCA) was used. An average Mahalanobis distance (Global H) was calculated and H values for individual samples were standardised by dividing by the average H value. Any sample with a spectrum more than 3.0 standardised units above the mean of the sample set was regarded as a spectral outlier. Identical population structuring procedures were applied to Group B samples. Spectral outliers were consequently excluded from both sample sets.

Calibration sets

The calibration modelling procedure was conducted for each constituent in each sample form. Two calibration sample sets were created:

1. Group A (79 samples)
2. Group A+Group B (total 107 samples) excluding randomly chosen samples for subsequent validations.

Modelling

The applied calibration technique involved SNV and Detrend scatter correction and modified partial least squares (MPLS) regression of derivatised spectra. The superlative math treatment was 2,8,8,1. The same calibration procedure was used for both calibration sample sets.

The calibration equations were produced for the following two segments of wavelengths:

1. Segment 1100-2500 nm
2. Segment 500 – 1050 nm

Calibration statistics

The Standard Error of Cross Validation (SECV) was used as the measure of accuracy of calibrations in each case. Final equations were chosen according to a combination of the lowest SECV and the highest 1-VR value (coefficient of determination – RSQ for cross validation).

Validation

The validation sample sets were used to test the performance of the calibration equations developed for predicting the four constituents in five different sample forms. The bait fish samples not included in the calibration sets were used for validation. In order to test the accuracy of calibrations, two validation sample sets were employed:

1. Group B samples (n = 28, excluding spectral outliers) – for validating calibrations based on Group A samples;
2. Group C - Randomly chosen samples (n = 12) to test the performance of the equations based on the Group A + Group B calibration set.

The MONITOR program was used to test the calibration equations. The NIR predicted constituent content for each sample form was compared with laboratory measured values.

The Standard Error of Prediction (SEP) was used as a calibration performance indicator. In addition, a ratio of SEP to Standard Deviation (SEP/StdDev) was used in the test. For superior calibrations this ratio should ideally be less than 0.3, although calibrations with the value below 0.6 are still regarded auspicious.

Detailed Experimental Outcomes Relative to Objectives

Objective 1: *Develop a cost-effective, sustainable manufactured diet for farmed SBT that contains reduced levels of fish meal, trash fish and fish oils while maintaining growth performance and flesh characteristics.*

Experiment 1

Diets

Analysis of pilchard samples (used as the control) and manufactured diets produced over the course of the experimental period clearly demonstrate the benefits of using manufactured diets in terms of consistency of nutrient supply. The dry matter, crude protein and crude fat content of pilchards (Figure 1) varied from 26-36 % (as received), 15-20% (as received) and 2-15% (as received), respectively. This must have an impact on the resulting performance of the tuna fed these pilchards over the course of the growing season. The peroxide activity in a number of the samples tested also demonstrates that a significant proportion of the pilchards contained fats that were in an active state of breakdown.

In contrast, the composition of the three manufactured diets was highly consistent over the course of the experimental period (Figures 2-4).

Growth performance and mortalities

As with previous experiments, there was significant variation between pontoons offered the same dietary treatment, including pilchards, at the 4 month harvest (Table 4). This variation was not as evident at the 3 month harvest. It is quite possible that disruptions caused during the preliminary harvest result in variable growth rates during the remaining month of the experiment.

The most significant outcome from this experiment was that the growth performance of SBT fed CRC 98A was equivalent to SBT fed pilchards. This is the first time SBT fed manufactured diets have demonstrated growth responses even remotely approaching that achieved with pilchards.

In experiments of this nature, there is insufficient replication to apply statistical analysis of any power. Based on the level of error associated with these experiments, significant differences were not evident between treatments. Examining the data available, the following points may warrant further investigation in a more robust experimental environment:

Table 4. *Growth performance of SBT fed manufactured diets or pilchards after 3 and 4 months of growth respectively.*

Treatment	3 month harvest Gain (kg)	SGR	ADG(g)	4 month harvest Gain (kg)	SGR	ADG (g)	Biomass gain (kg)	Mortalities (n/45)
CRC98A	8.93	0.43	102.6	10.93	0.41	94.2	434	2
CRC98A	6.64	0.38	81.0	9.02	0.41	76.4	353	0
Mean	**7.79**	**0.41**	**91.8**	**9.98**	**0.41**	**85.3**	**394**	
CRC98B	7.39	0.45	94.2	8.95	0.39	82.1	344	0
CRC98B	7.13	0.40	92.5	7.83	0.32	69.9	300	3
Mean	**7.26**	**0.43**	**93.4**	**8.39**	**0.36**	**76.0**	**322**	
CRC98C	6.25	0.52	84.8	6.93	0.44	69.5	256	6
CRC98C	6.97	0.37	90.5	8.58	0.32	76.6	357	2
Mean	**6.61**	**0.46**	**87.7**	**7.76**	**0.38**	**73.0**	**307**	
Pilchards	8.69	0.46	99.9	9.71	0.42	89.5	371	6
Pilchards	8.04	0.50	108.3	8.55	0.36	75.7	330	4
Mean	**8.37**	**0.48**	**104.1**	**9.13**	**0.39**	**82.6**	**351**	

Specific Growth Rate (SGR) = 100 x (ln(Final weight/Initial weight)/time).
Biomass gain (kg) refers to total biomass accrued between initial transfer date and harvest dates (fish harvested at 3 months inclusive). Does not account for fish lost due to mortalities, morbidities or poaching.

- Growth performance of the SBT fed pilchards was superior in the initial three months of experimentation. These differences were not evident after 4 months. A weaning phase onto manufactured diets may assist early uptake of these diets promoting improved growth in the initial phase of the season.

- The performance of SBT fed diets containing reduced levels of pilchards did not equate to diets containing approximately 48% fresh product or fresh product alone. This may be due to a number of factors including reduced intake, or issues associated with pellet form and a more rapid breakdown of the pellet when less fresh product is included.

- There was no evidence of a difference in growth rate between SBT fed on diets each of which contained a reduced level of pilchards, but in which the content and ratio of total protein and fat was varied. Numerically, CRC98C, which contained higher protein and lower fat resulted in the poorest SBT performance during this experiment. This suggests that we may be significantly overfeeding SBT in pontoons, resulting in feed wastage and an increase in the level of environmental waste. If the SBT were being fed to their requirements for growth, and the feeding strategy appropriate, then we would expect to detect a difference in the growth of fish fed these diets. The results also suggest that a higher fat diet is more appropriate for SBT.

Feed conversion efficiency

As expected, SBT fed manufactured diets had a superior feed conversion efficiency to SBT fed pilchards on a wet weight basis (Table 5). However, when converted to a dry matter basis, the feed conversion ratio of pilchards was superior to manufactured diets (Table 6).

Only small differences were observed between the FCR of SBT fed CRC98A and CRC98B. This suggests that intake alone may be contributing to differences in the performance of these fish rather than differences in nutrient availability. As a consequence, it appears that use of attractants to enhance the intake of manufactured diets with reduced levels of fresh bait fish would be desirable in the future. Differences between the FCR of CRC98A and CRC98C were more noticeable suggesting that the higher protein, lower fat diets are utilised less efficiently.

Table 5. *Feeding efficiency of SBT fed manufactured diets or pilchards after 3 and 4 months of growth respectively.*

Treatment	3 month harvest Gain (kg)	Feed (kg/fish)	FCR	4 month harvest Gain (kg)	Feed (kg/fish)	FCR	Biomass gain (kg)	Total fed (kg)	Gross FCR
CRC98A	8.93	75.87	8.49	10.93	89.68	8.20	434	3710	8.55
CRC98A	6.64	61.68	9.29	9.02	75.52	8.37	353	3187	9.03
Mean	**7.79**	**68.78**	**8.89**	**9.98**	**82.60**	**8.29**	**394**	**3449**	**8.79**
CRC98B	7.39	64.34	8.71	8.95	77.28	8.63	344	3204	9.31
CRC98B	7.13	61.67	8.65	7.83	73.74	9.42	300	3097	10.30
Mean	**7.26**	**63.00**	**8.68**	**8.39**	**75.51**	**9.02**	**322**	**3151**	**9.81**
CRC98C	6.25	63.33	10.10	6.93	77.78	11.22	256	3137	12.25
CRC98C	6.97	63.90	9.17	8.58	84.29	9.82	357	3510	9.82
Mean	**6.61**	**63.62**	**9.64**	**7.76**	**81.04**	**10.51**	**307**	**3324**	**11.04**
Pilchards	8.69	105.32	12.12	9.71	126.11	12.98	371	5019	13.53
Pilchards	8.04	88.99	11.07	8.55	115.57	13.51	330	4737	15.59
Mean	**8.37**	**97.16**	**11.60**	**9.13**	**120.84**	**13.25**	**351**	**4878**	**14.56**

Food Conversion Ration (FCR) = feed eaten(kg) / weight gain (kg).

Table 6. *Feeding efficiency (dry matter basis) of SBT fed manufactured diets or pilchards after 4 months of growth.*

Treatment	Dry Matter	Wet weight FCR	Dry weight FCR	Dry weight FCR (gross)
CRC98A	60.0	8.28	4.97	5.27
CRC98B	59.7	9.02	5.38	5.85
CRC98C	56.5	10.52	5.94	6.23
Pilchards	30.2	13.24	4.00	4.39

It is interesting to note that the FCR of pilchards was noticeably poorer after 4 months compared with the data derived from the 3 month harvest (Table 5). This may be due to overfeeding during the final growth phase. In fact, observation suggests that when SBT reach a certain level of condition, their inclination to eat is reduced. This may be related to their natural feeding behaviour, where as opportunistic feeders they eat intermittently. When body reserves are at a certain level, their need to eat may be reduced. In fact, the high FCR's observed for all treatments may be due to inappropriate feeding strategies and excessive feeding.

Based on the prevailing commercial conditions in 1998, to be cost-effective the manufactured feeds need to be consumed with an efficiency of better than 6.6:1 on a wet weight basis or the diet costs need to be reduced from $1,500/tonne to $1,066/tonne.

Micronutrient retention

Samples of bone and liver were sent for analysis of mineral components, specifically calcium, phosphorus, magnesium and iron (Table 7).

There were few differences in the bone content of these minerals (also accounting for differences in intake between fish fed pilchards and manufactured diets). Notable exceptions were the calcium content of bone in fish fed CRC98A, and the phosphorus content of fish fed CRC98C. This probably reflects the quantity of fish meal included in the manufactured diets. It is also interesting to note the comparatively low levels of iron in the liver of SBT fed manufactured diets relative to pilchards.

Given the higher level of these minerals in fishmeal included in manufactured diets relative to pilchards, the results suggest that the mineral content of manufactured diets could be significantly reduced without adverse affects, or alternatively, the bioavailability of minerals in the manufactured diets needs to be significantly improved.

Table 7. *Mineral composition of bone and liver from SBT fed manufactured diets or pilchards after a 4 month growth period.*

	CRC98A	CRC98B	CRC98C	Pilchards
Bone				
Ca (mg/100g)	8000	8400	8600	8500
Mg (mg/100g)	230	210	220	230
P (mg/100g)	7500	7400	8100	7900
Ash (mg/100g)	45.4	45.6	47.9	44.9
Liver				
Fe (mg/100g)	170	170	190	280

	Fishmeal	Pilchards
Feed		
Ascorbate (Vit C) mg/100g)	<1	1
Retinol (Vit A) (mg/100g)	200	150
DL-α-tocopherol / Vit E (mg/100g)	0.86	0.60
Ca (mg/100g)	3600	110
Fe (mg/100g)	25	2.9
Mg (mg/100g)	250	38
P (mg/100g)	2600	410
Zn (mg/100g)	6.4	1.2

Haematology

Routine analysis of blood samples collected as part of the 3 month harvest revealed very little difference between treatments (Table 8).

The most important parameter to note is haemoglobin as this has been shown to have the greatest influence on flesh colour. Clearly, there is no difference between the haemoglobin content of samples taken from SBT fed manufactured diets or pilchards. The general haematology analysis also suggests that there is little difference in the general health of the fish fed the respective dietary treatments.

Table 8. *Haematology results from blood samples collected from fish fed manufactured diets or pilchards after 3 months of growth.*

	CRC98A	CRC98B	CRC98C	Pilchards
Haematocrit	49.38	48.71	50.32	49.25
Leukocrit	1.36	1.32	1.27	1.23
Haemoglobin	15.38	15.49	15.60	15.27
Red cell count	2.78	2.85	2.88	2.83
Mean cell volume	179	172	175	180
Mean cell haemoglobin	55.64	54.70	54.28	55.83

Results are means (n=7) from April (3 months) harvests.

Market evaluation

At the conclusion of this experiment, Dr Brett Glencross arranged simultaneous distribution of fish to three Japanese markets (Table 9). While prices were highly variable, it can be seen that the Osaka market placed an equivalent value on SBT fed manufactured diets compared with SBT fed pilchards, while Tsukiji and Nagoya tended to favour the pilchard fed fish. In general, there was little difference between the value of fish fed the various manufactured diets although there was a trend for those fed CRC98C to receive lower prices. There are many reasons why fish fed different diets receive different prices and why the values differ between markets. These will be a combination of fish size, the preference for fat vs lean fish and the number of fish presented to the market in any one day to name a few. As a consequence, it is difficult to make a definitive assessment of the data collected.

Table 9. *Market data (Yen/kg) from experimental fish fed manufactured diets or pilchards sold after 4 months of growth to various Japanese outlets.*

Market	Date	CRC98A	CRC98B	CRC98C	Pilchards
Tsukiji	30th April	1133	1367	1100	1733
Nagoya	30th April	1250	1000	1200	1300
Osaka	30th April	1500	1667	1267	1600
Tsukiji	1st May	1000	1200	1167	1333
Nagoya	1st May	1600	2050	1400	1550
Osaka	1st May	1300	1650	1400	1800
Tsukiji	28th May	-	1467	-	1813
Nagoya	28th May	-	1633	-	1617
Osaka	28th May	-	2400	-	2000
Tsukiji	29th May	2023	-	-	-
Nagoya	29th May	2220	-	-	-
Osaka	29th May	1980	-	-	-
Tsukiji	30th May	-	-	1169	-
Nagoya	30th May	-	-	2067	-
Osaka	30th May	-	-	1567	-
Tsukiji	5th June	-	1767	1517	-
Nagoya	5th June	-	1957	1958	-
Osaka	5th June	-	1900	1520	-
Tsukiji	6th June	1283	-	-	2120
Nagoya	6th June	1940	-	-	1933
Osaka	6th June	2221	-	-	2650

Figures from 30th April and 1st May are mean $n \geq 2$ per treatment / market / day
Figures from 28th May to 6th June are means $n \geq 6$ per treatment / market / day

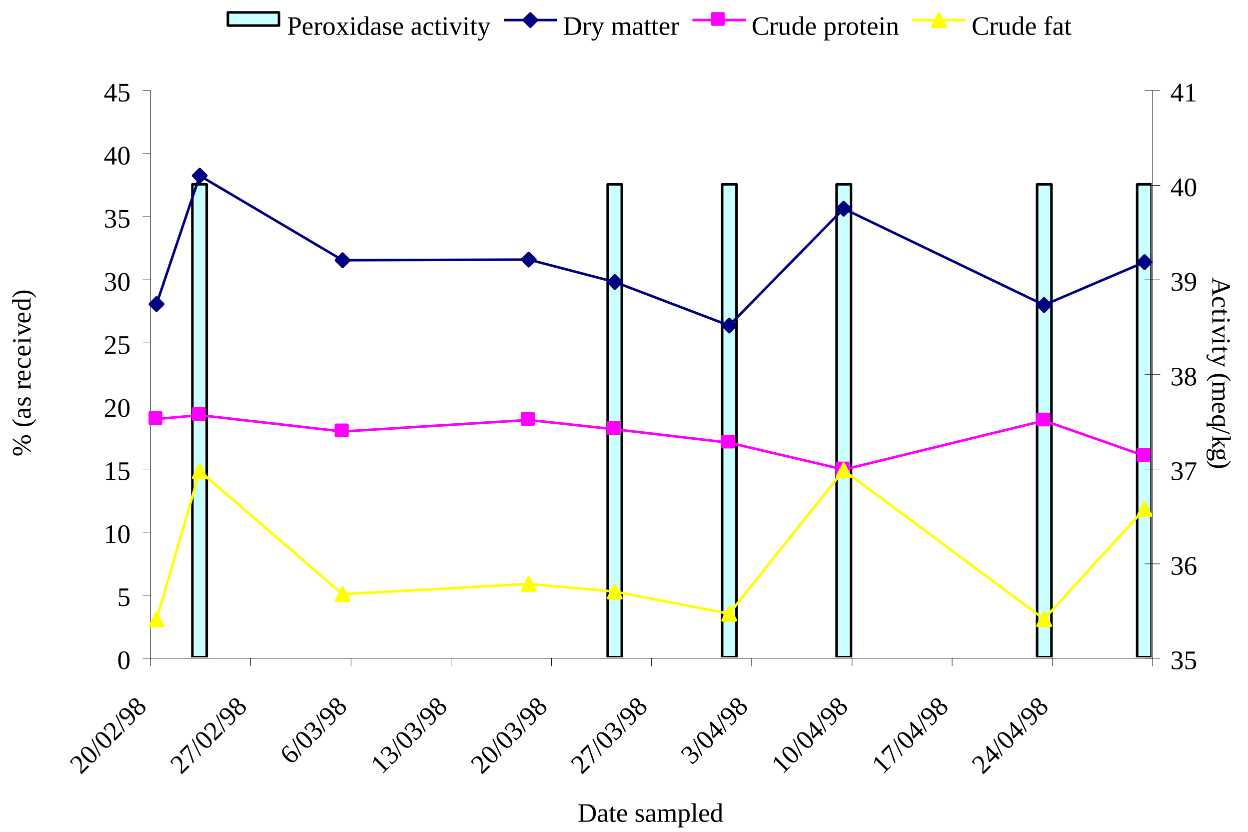


Figure 1. *Variation in the dry matter, crude protein, crude fat (%, as received) and peroxide activity (meq/kg) in pilchards fed to experimental pontoons in 1998.*

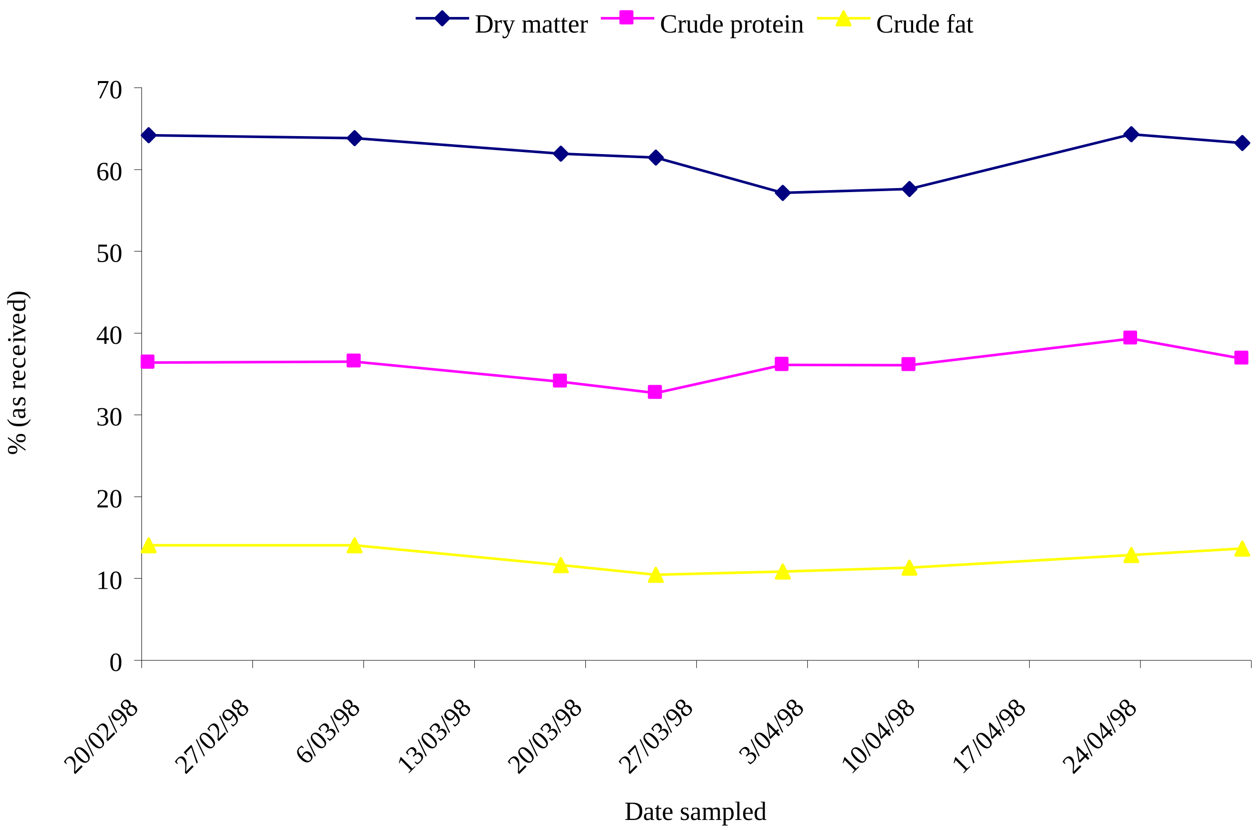


Figure 2. *Variation in the dry matter, crude protein and crude fat (%, as received) content of CRC98A fed to experimental pontoons in 1998.*

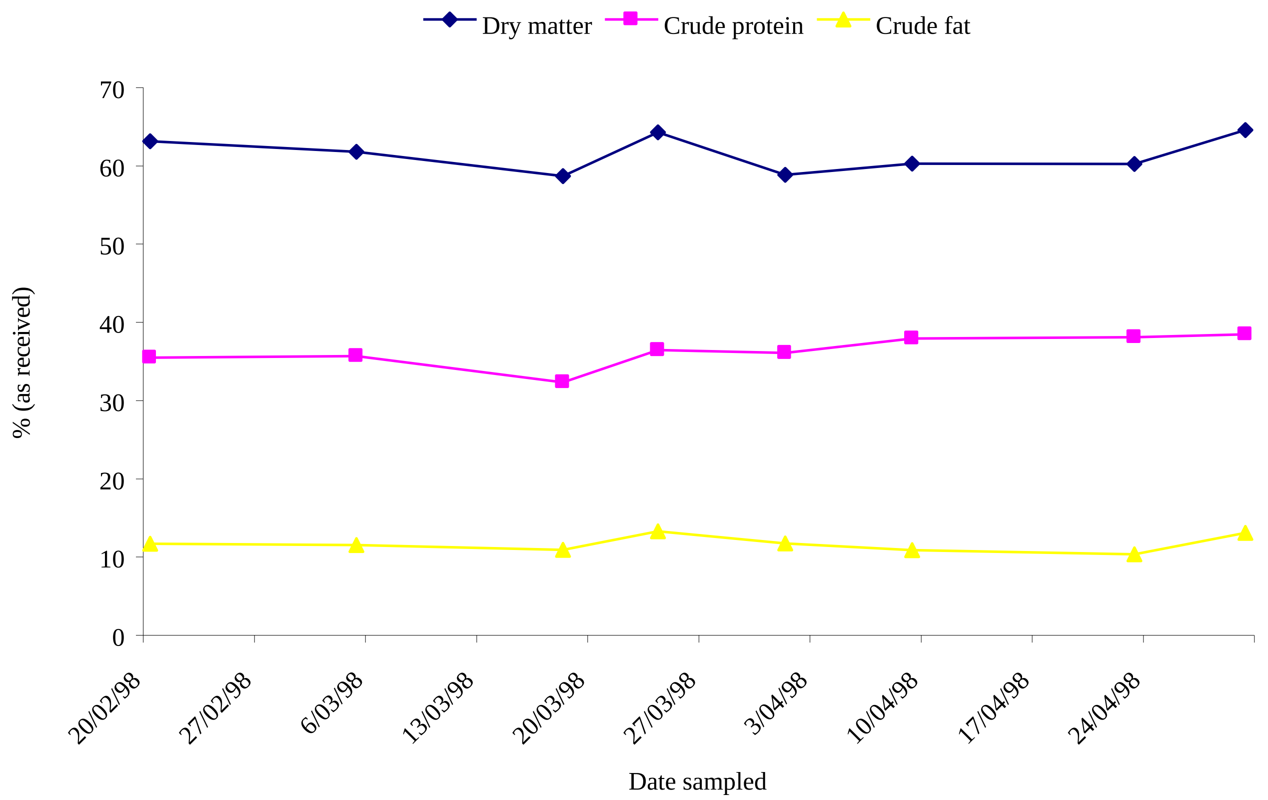


Figure 3. *Variation in the dry matter, crude protein and crude fat (%, as received) content of CRC98B fed to experimental pontoons in 1998.*

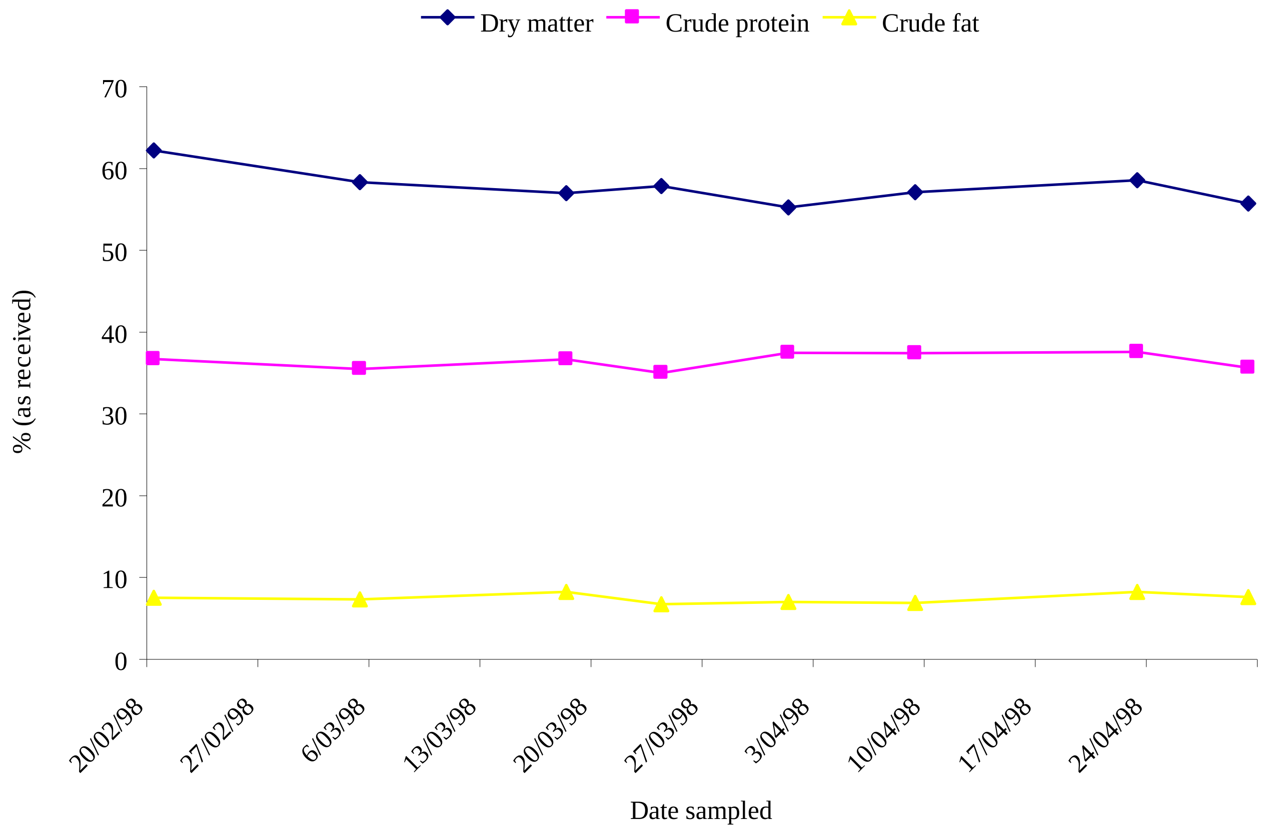


Figure 4. *Variation in the dry matter, crude protein and crude fat (%, as received) content of CRC98C fed to experimental pontoons in 1998.*

Experiment 2

Diets

As with Experiment 1, the composition of pilchards used in Experiment 2 was highly variable (Figure 5). Dry matter, crude protein, crude fat and gross energy content were equally variable. Unlike Experiment 1, the composition of the manufactured diets also varied considerably (Figures 6-8). This was largely due to problems with diet form prior to April 23, 1999, with manufactured diet composition stabilising after these issues were resolved. As the feeding rates of this experiment were closely monitored, this may have had an influence on the experimental outcomes, and serves as strong evidence for the need to ensure adequate diet manufacturing technology before embarking on this type of project.

Growth performance

The summary results by cage (Table 11) demonstrate noticeable differences between cages on the same treatment, particularly those fed diet C. This may be due to a lack of pellet integrity during the early stages of the experiment, but as with other experiments, this is a continual source of concern and places doubt over the value of experiments with such a small degree of replication.

Restricting feed intake did appear to improve FCR (Table 10), however, condition index gain and total weight gain were numerically compromised (although statistical analysis would reveal no significant difference). Optimising intake may be the best commercial strategy rather than optimising FCR, however, further research is required to confirm this.

Increasing dietary oil content (and hence the dietary energy supplied from oil) did not improve growth performance and appears to have reduced feed conversion efficiency. Experiment 1 demonstrated that tuna do not appear to place a heavy reliance on dietary protein as an energy source, hence the response is more likely to reflect poorer pellet quality with increasing oil levels.

FCR values obtained are extremely high compared with previous years. It appears that the delayed start to this experiment may have significantly influenced the results.

The results suggest that pellet quality and tuna protein intake are two fundamental constraints to tuna performance. It also appears that when feeding tuna pellets, early introduction to this feed is essential if growth performance is to be optimised.

Table 10. *Growth performance of SBT subjected to full or restricted feeding regimes and diets varying in protein and energy content.*

Parameter	99A Full	99A Restricted	99B Restricted	99C Restricted
Mean harvest weight (kg)	21.83	22.65	22.28	22.55
Mean harvest length (cm)	99.06	101.38	100.67	100.68
Average daily gain (g)	53.91	48.76	46.71	47.98
Condition index (final)	22.41	21.69	21.84	22.03
Specific Growth Rate	0.29	0.24	0.24	0.24
Weight gain (kg)	5.79	5.19	5.05	5.09
Length gain (cm)	4.83	4.42	4.32	4.64
CI gain	3.36	2.57	2.59	2.34
FCR*	18.72	17.01	18.37	20.27

* Based on (Mean feed intake/fish/day x days on)/weight gain (wet weight basis)

Table 11. *Growth performance by cage of SBT subjected to full or restricted feeding regimes and diets varying in protein and energy content.*

Parameter	A1	A3	A4	B1	B4	C1	C3	C4
Treatment	A Full	A Restricted	A Full	B Restricted	B Restricted	A Restricted	C Restricted	C Restricted
Mean harvest weight (kg)	22.49	23.43	21.16	21.71	22.84	21.87	23.96	21.14
Mean harvest length (cm)	100.37	101.66	97.74	99.81	101.52	101.10	102.40	98.95
Average daily gain (g)	49.70	54.01	58.11	43.17	50.25	43.51	56.83	39.13
Condition index (final)	22.24	22.24	22.58	21.82	21.85	21.13	22.29	21.76
Specific Growth Rate	0.25	0.26	0.33	0.22	0.25	0.22	0.27	0.20
Weight gain (kg)	5.42	5.67	6.16	4.71	5.38	4.70	5.91	4.27
Length gain (cm)	4.37	4.64	5.28	4.31	4.32	4.20	4.90	4.38
CI gain	2.97	2.84	3.74	2.33	2.85	2.29	2.82	1.85
Preliminary FCR*	19.71	15.18	17.73	19.44	17.30	18.84	16.54	24.00

* Based on (Mean feed intake/fish/day x days on)/weight gain (wet weight basis)

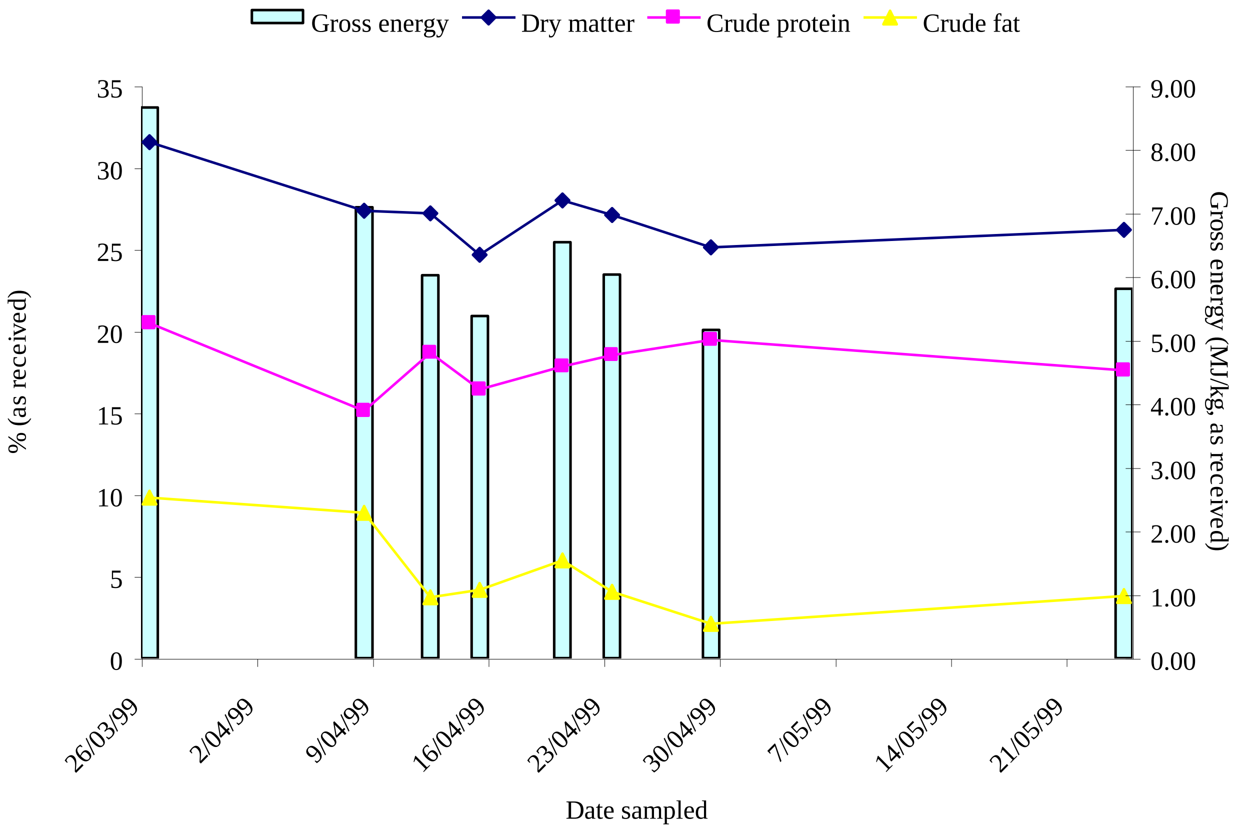


Figure 5. *Variation in the dry matter, crude protein, crude fat (%, as received) and gross energy (MJ/kg) content of pilchards fed to experimental pontoons in 1999.*

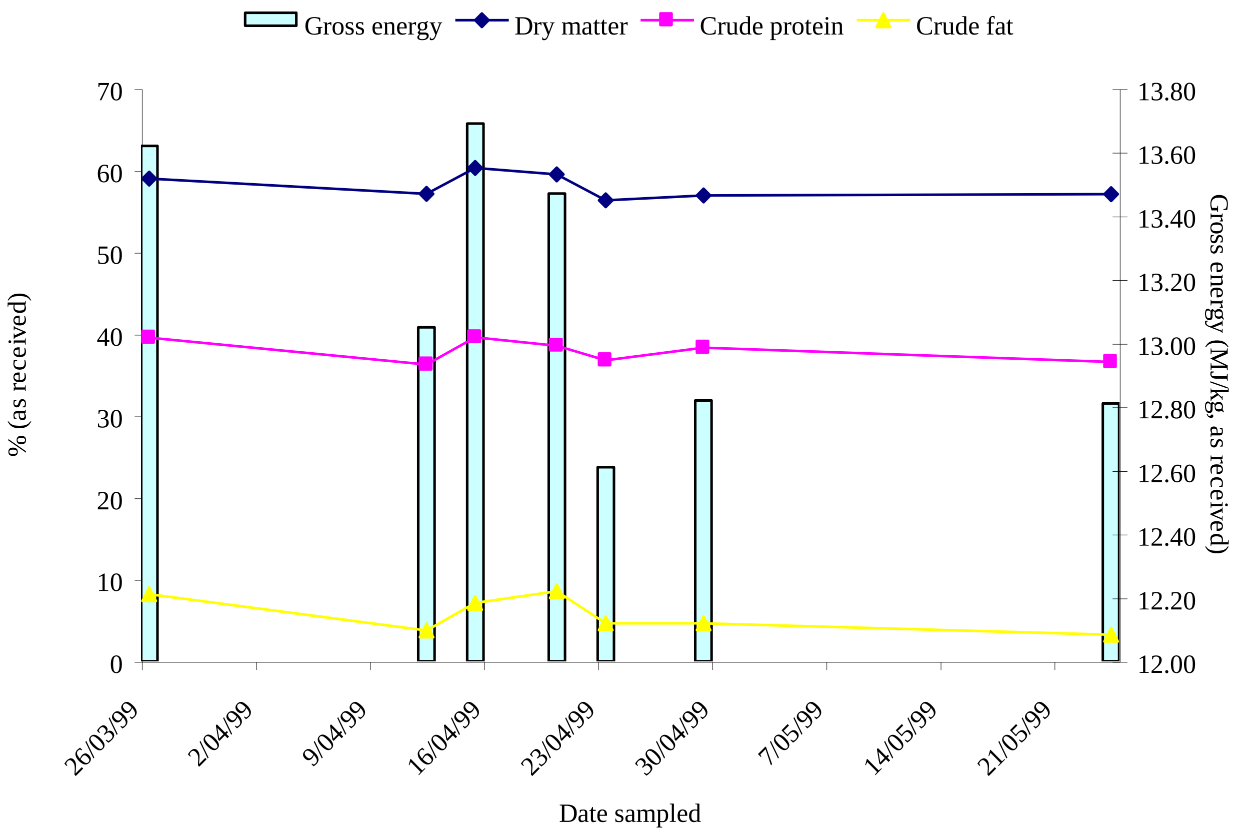


Figure 6. *Variation in the dry matter, crude protein, crude fat (%, as received) and gross energy (MJ/kg) content of diet 99A fed to experimental pontoons in 1999.*

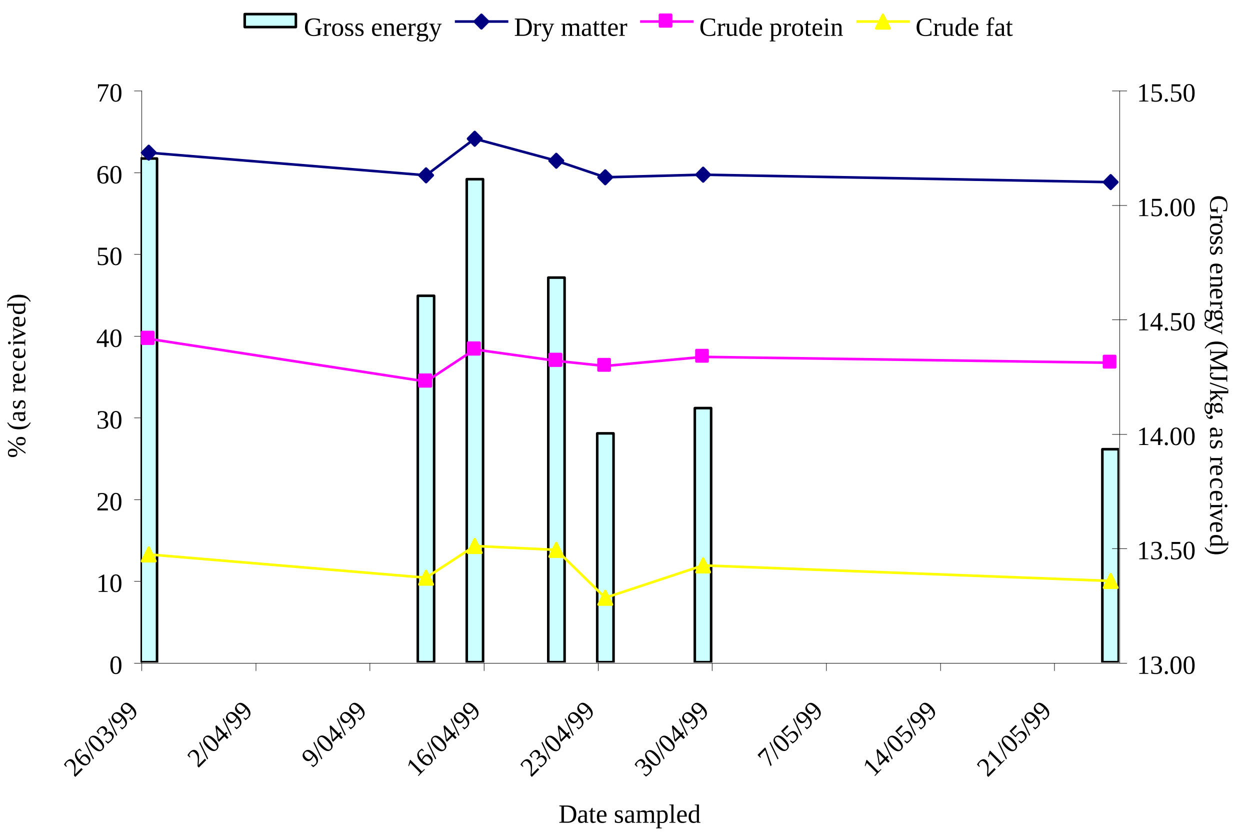


Figure 7. *Variation in the dry matter, crude protein, crude fat (%, as received) and gross energy (MJ/kg) content of diet 99B fed to experimental pontoons in 1999.*

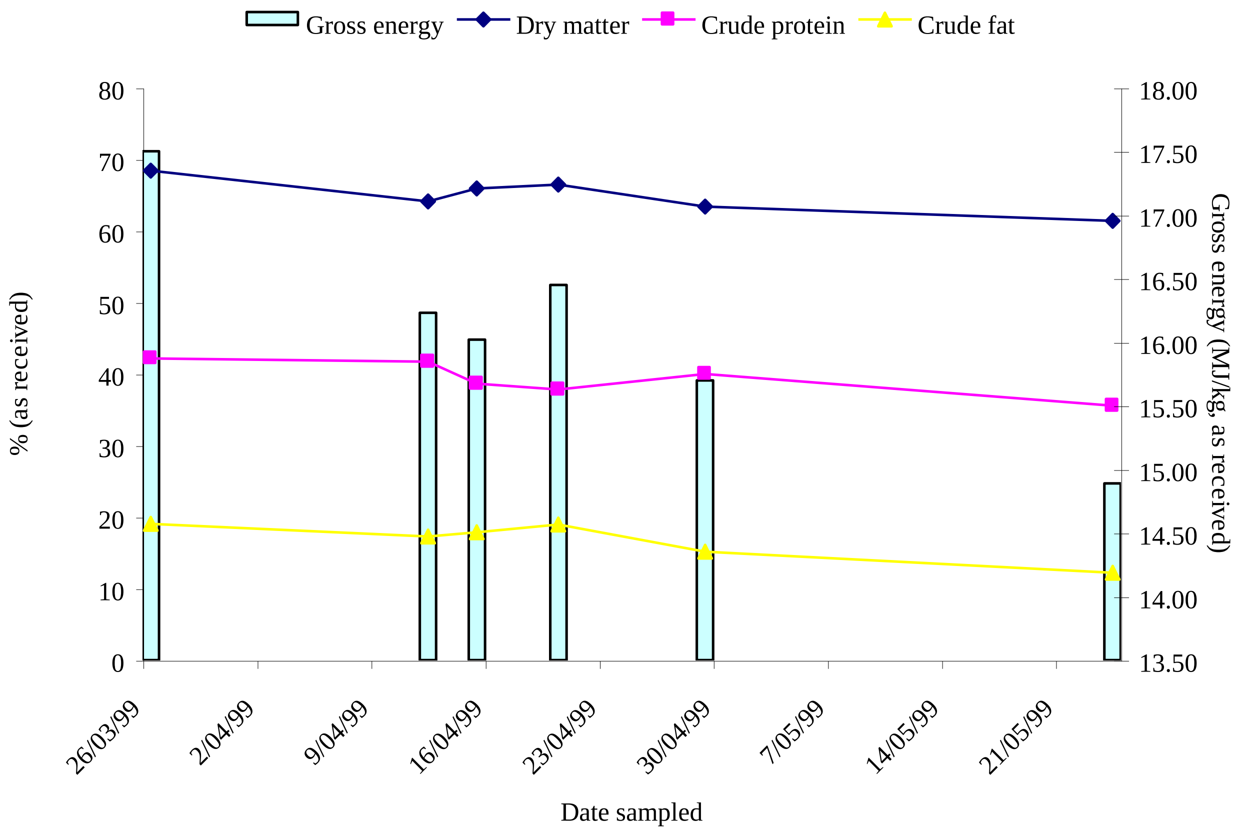


Figure 8. *Variation in the dry matter, crude protein, crude fat (%, as received) and gross energy (MJ/kg) content of diet 99C fed to experimental pontoons in 1999.*

Objective 2: *Assess the response of farmed SBT to changes in diet moisture and protein content.*

Experiment 1

An early objective of this project was to demonstrate that significant changes in diet composition could result in observable differences in growth response. Obviously, the degree of replication permitted by the available resources makes it difficult to demonstrate this statistically, so we must rely on numerical observations alone.

It can be seen from Experiment 1, described above, that differences can be detected between SBT fed diets differing in protein and moisture content (summary data presented in Figure 9). Based on results of Experiments 1 and 2, diet form, rather than protein source is critical, as is the supply of dietary energy in the form of fats or oils.

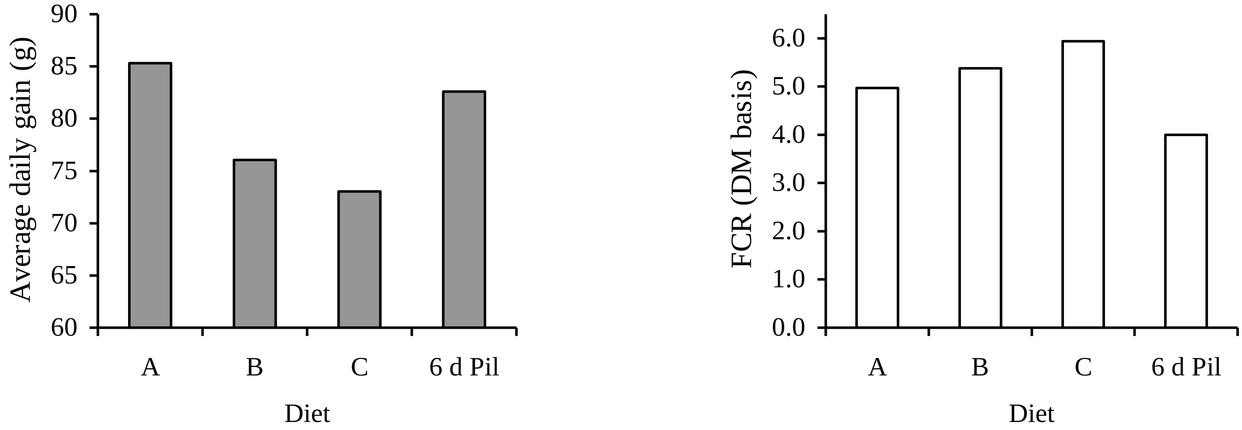


Figure 9. *Growth responses and feed conversion ratios of SBT fed diets differing in protein and moisture content.*

Objective 3: *Assess the influence of artificial colour enhancers on the flesh characteristics of farmed SBT.*

Experiment 1

As part of experiment 1, the influence of dietary carotenoids on flesh characteristics was assessed. A basal level of carotenoid was included in all manufactured diets at levels similar to those applied in other farmed finfish (higher levels were obviously possible, but would quickly become uneconomical if used routinely). The flesh characteristics of the manufactured feeds was compared with that observed for SBT fed pilchards.

Results presented in Tables 12 and 13 demonstrate that inclusion of carotenoids in manufactured diets for SBT had little influence on flesh characteristics, particularly in terms of colour (Table 12). Despite inclusion of carotenoids in the manufactured feeds, flesh levels of carotenoids was lower in SBT fed manufactured diets compared with pilchards (Table 13).

Table 12. *Carotenoid and iron content of SBT muscle from fish fed manufactured diets or pilchards.*

	CRC98A	CRC98B	CRC98C	Pilchards
Carotenoids 3mth (mg/kg)	0.3	0.3	0.4	0.5
Carotenoids 4mth (mg/kg)	0.4			
Fe (mg/kg)	8.1	17	24	15

Table 13. *Flesh characteristics of SBT fed manufactured diets containing carotenoids relative to SBT fed pilchards.*

Treatment	Colour	Fat level	Texture	Flavour
A	2.67	3.37	2.23	2.43
B	3.23	2.77	2.67	2.60
C	2.50	3.20	2.63	2.40
6d	2.57	3.23	2.66	1.93

Japanese (n=5) assessed flesh quality parameters

Colour:	Pale	0 1 2 3 4 5	Vivid	(Higher the better)
Fat:	Low	0 1 2 3 4 5	Excess	(Better in mid values)
Texture:	Mushy	0 1 2 3 4 5	Chewy	(Better in mid values)
Flavour:	Bad	0 1 2 3 4 5	Good	(Higher the better

Objective 4: *Develop NIR calibrations for the assessment of bait fish composition prior to inclusion in manufactured feeds*

Experiment 5

A range of NIR calibrations were developed as part of experiment 5 to assess the potential to predict the composition of bait fish prior to inclusion in experimental diets. Calibrations were developed across two wavelength ranges. The first, 1100-2500 nm, covered the most desirable spectral range on the scanning spectrophotometer being used, while the second, 500-1050 nm, was assessed to define the potential for calibrations to be developed on portable equipment using photodiode array detectors. Scanning and calibration development was completed by Mr Jurek Kruk from the South Australian Research and Development Institute – Pig and Poultry Production Institute.

Base data

A good range in values was obtained for the moisture, crude protein, crude fat and free fatty acid content of the bait fish samples analysed (Table 14) with moisture in bait fish (excluding squid) ranging from 62-77%, as received, crude protein ranging from 14.5-22.6%, as received, crude fat ranging from 0.5-11.3%, as received, and free fatty acids ranging from 0.80-14.6%, as received. Peroxide values were measured, however, it soon became obvious that development of an NIR calibration would be very difficult to achieve. The reason for this is that peroxide values tend to be either very low or very high depending on whether the fat in the sample is in an active state of breakdown or not. If a sample has become rancid, then the peroxide will revert to being low as the fats are not actively breaking down. This reduces the value of peroxide measurements in quality assurance schemes. The non-normal distribution of peroxide values forced its exclusion from the NIR calibration sets.

The range in moisture, crude protein, crude fat and free fatty acids in the validation set reflected the core data set (Table 15).

Table 14. *Reference data for the Group A NIR calibration set (AR, as received; DM, dry matter)*

No.	Sample ID	Lab No.	Moisture (%)	Crude Protein (%) (AR)	Crude Fat (%) (AR)	Free Fatty Acids (%) (AR)	Crude Protein (%) (DM)	Crude Fat (%) (DM)	Free Fatty Acids (%) (DM)	Peroxide Value (meg/kg) (DM)
1	Bight 28/4 Southern cals	A00639	72.11	17.69	1.71	8.79	63.43	6.12	31.53	69.0
2	Southern Cal Pilchards Bluefin	A00640	76.34	17.81	0.81	9.99	75.27	3.43	42.23	<0.1
3	19/4 Aust Pilchards Bluefin	A00641	72.24	18.02	2.51	3.82	64.91	9.04	13.75	77.0
4	Bight Southern Cal C	A00642	74.90	18.58	0.72	8.15	74.03	2.88	32.48	<0.1
5	Sardinops Sagax, Sth Cal, Mex	A00643	74.85	17.10	0.93	6.98	68.02	3.71	27.77	<0.1
6	Supu 1003596 Bight A 2/5	A00644	76.24	17.41	0.56	8.63	73.29	2.37	36.34	91.0
7	2/5 Bight C	A00645	74.35	17.72	1.46	7.62	69.08	5.69	29.70	88.0
8	CRXU 6099 Bight B 2/5	A00646	74.44	18.87	0.88	13.01	73.82	3.45	50.89	<0.1
9	Pilchards for RVB 10/4	A00647	72.90	19.70	0.97	10.93	72.72	3.57	40.33	<0.1
10	Bight 28/4 Southern Cal A	A00648	67.63	18.83	2.61	4.32	58.16	8.07	13.35	56.0
11	May B RVB	A00649	71.37	17.98	3.46	4.10	62.79	12.10	14.31	63.0
12	May C RVB	A00650	72.77	19.38	1.40	5.36	71.17	5.13	19.66	68.0
13	May E	A00651	75.62	18.13	0.70	10.97	74.35	2.86	45.00	73.0
14	A4 TBOA	A00652	75.40	17.91	0.46	7.15	72.78	1.87	29.06	91.0
15	A6 TBOA	A00653	71.64	18.95	2.30	7.82	66.81	8.11	27.57	101.0
16	A3TBOA	A00654	70.51	19.51	3.34	4.59	66.17	11.32	15.57	46.0
17	A8TBOA	A00655	73.60	18.56	1.33	6.81	70.28	5.04	25.81	50.0
18	A5TBOA	A00656	69.21	17.35	11.25	3.01	56.35	36.53	9.76	23.0
19	A9TBOA	A00657	72.14	16.85	3.08	4.75	60.49	11.06	17.06	47.0
20	A7TBOA	A00658	72.25	17.69	3.45	0.80	63.73	12.44	2.88	25.0
21	May D RVB	A00659	76.27	17.74	0.97	10.43	74.78	4.07	43.96	121.0
22	For RVB 27/4	A00660	69.47	18.57	2.11	4.79	60.84	6.92	15.68	55.0
23	May F	A00661	77.05	16.95	0.59	12.25	73.85	2.56	53.37	69.0
24	A1TBOA	A00662	69.31	18.76	2.99	4.88	61.13	9.75	15.92	71.0
25	A2TBOA	A00663	71.36	18.37	2.62	5.47	64.14	9.14	19.09	34.0
26	26/4 MFE Sth Cal Pilchard	A00664	76.26	16.93	0.60	6.96	71.32	2.54	29.34	80.0
27	26/4 MFE Dutch Herring	A00665	69.82	15.17	11.02	n/a	50.26	36.51	n/a	81.0
28	Sth'ear Semifrozen	A00666	74.17	17.34	1.39	7.98	67.11	5.38	30.87	72.0
29	May G	A00667	67.95	18.51	10.33	2.27	57.74	32.24	7.09	132.0

No.	Sample ID	Lab No.	Moisture (%)	Crude Protein (%) (AR)	Crude Fat (%) (AR)	Free Fatty Acids (%) (AR)	Crude Protein (%) (DM)	Crude Fat (%) (DM)	Free Fatty Acids (%) (DM)	Peroxide Value (meg/kg) (DM)
30	DI Heads & Guts 4/5	A00668	73.69	14.47	1.89	5.43	55.01	7.17	20.63	63.0
31	Bight C 4/5 CRUX 5155209	A00669	74.43	17.70	1.09	9.50	69.24	4.24	37.17	60.0
32	TRLU 105 968 O Bight B 4151	A00670	72.17	18.57	1.87	5.54	66.72	6.72	19.91	50.0
33	DI 45 Anchovies	A00671	63.54	22.62	6.79	7.42	62.02	18.63	20.35	84.0
34	DI Pilchards A Sth Cal 4/5	A00672	73.42	17.73	1.43	8.55	66.70	5.37	32.19	81.0
35	DI Pilchards B 4/5	A00673	76.51	16.62	0.83	9.66	70.74	3.52	41.11	65.0
36	Bight A 4/5 KNLU 2786800	A00674	76.45	16.51	1.04	8.84	70.12	4.42	37.55	73.0
37	AFE 1/6 2nd Anchovies Cal	A00675	71.62	18.97	2.69	7.48	66.85	9.49	26.35	95.0
38	Bight Pilchards 2/6 A	A00676	72.81	19.36	0.96	8.39	71.20	3.52	30.85	85.0
39	Bight Pilchards 2/6 B	A00677	75.24	16.90	1.10	n/a	68.26	4.46	n/a	90.0
40	Bight Pilchards 25/5 C	A00678	74.65	18.80	0.75	10.17	74.16	2.98	40.13	119.0
41	Bight Pilchards 25/5 D	A00679	75.83	17.87	0.55	11.83	73.91	2.28	48.95	166.0
42	Bight Squid 2/6	A00680	77.96	16.88	0.91	5.76	76.57	4.14	26.14	57.0
43	Blue Fin Anchovies 2/6	A00681	70.42	18.29	2.60	4.06	61.82	8.80	13.73	113.0
44	AFE Anchovies Cal 1/6	A00682	72.40	19.52	1.93	14.56	70.72	7.01	52.75	84.0
45	POLV 28/5 281057L Bight A 28/5	A00683	76.57	17.48	0.69	9.50	74.59	2.93	40.54	136.0
46	Blue Fin Japanese Sardine 2/6	A00684	68.19	18.43	7.20	2.16	57.95	22.65	6.79	211
47	AFE Argentina Squid 1/6	A00685	76.14	17.95	2.64	1.19	75.25	11.07	4.99	16.0
48	Marnikol Pilchards 2/6	A00686	74.40	17.87	1.30	7.26	69.80	5.08	28.36	598.0
49	SVDU 301299/8 Bight 28/5 (B)	A00687	62.31	18.93	4.33	3.51	50.23	11.49	9.32	77.0
50	AFE Pilchards Cal 1/6/00	A00688	73.94	18.79	0.89	9.42	72.08	3.40	36.14	119.0
51	Sth Cal Pilch Tony's Tuna1/6/00	A00689	74.75	19.04	0.77	7.56	75.42	3.06	29.96	225.0
52	Bight 5/6/00 (A) TOLU 5677375	A00690	72.41	16.68	2.88	3.63	60.45	10.42	13.14	56.0
53	Bight 5/6/00 (B) LRXU 5157500	A00691	74.95	17.09	1.11	5.60	68.21	4.44	22.36	137.0
54	Marnikol Anchovies 2/6 (A)	A00692	71.76	14.83	9.07	n/a	52.53	32.13	n/a	120.0
55	Marnikol Anchovies 2/6 (B)	A00693	76.09	15.04	4.67	3.88	62.92	19.55	16.22	105.0
56	Bight 7/6 5C24 8259577	A00694	72.51	19.91	1.06	6.94	72.41	3.84	25.24	25.0
57	Blasl0v WA Pilchards 6/6	A00695	75.33	18.50	0.67	9.45	74.96	2.72	38.31	<0.1
58	Australian Pilchards Bluefin 19/4	A00696	71.01	18.62	2.77	3.00	64.22	9.55	10.35	105.0
59	Blaslov 6/6 Squid	A00697	79.38	16.24	1.32	8.31	78.76	6.39	40.31	<0.1
60	Blaslov's 6/6 Cal Pilchard March	A00698	74.52	18.64	1.53	5.83	73.17	6.02	22.87	53.0
61	Tony's Southern Cal. 19/4	A00775	74.51	17.41	1.23	5.81	68.31	4.82	22.80	110.0

No.	Sample ID	Lab No.	Moisture (%)	Crude Protein (%) (AR)	Crude Fat (%) (AR)	Free Fatty Acids (%) (AR)	Crude Protein (%) (DM)	Crude Fat (%) (DM)	Free Fatty Acids (%) (DM)	Peroxide Value (meg/kg) (DM)
62	Blaslov 6/6 Cal. Pilchards Feb	A00776	67.13	17.86	3.13	4.69	54.32	9.51	14.26	127.0
63	Lukin (B) 7/6	A00777	73.06	19.45	1.25	6.54	72.22	4.64	24.27	106.0
64	Kinkawooka 7/6/00 103D	A00778	72.99	17.12	2.12	4.70	63.38	7.84	17.39	62.0
65	Bight 7/6 (B) LPIU 5603056	A00779	74.50	18.19	1.11	7.58	71.35	4.35	29.75	61.0
66	Kinkawooks 7/6/00 51C 60	A00780	71.10	18.63	1.39	4.05	64.45	4.82	14.01	91.0
67	Lukin (A) 7/6	A00781	75.02	17.16	1.00	8.41	68.69	4.00	33.67	90.0
68	Squid (?)	A00782	77.07	17.47	1.26	6.01	76.19	5.49	26.23	20.0
69	Blaslov Pacific Mackarel 6/6	A00783	73.22	17.49	3.87	5.66	65.30	14.46	21.14	128.0
70	Bight 9/6 Squid	A00784	75.74	17.71	1.14	6.49	72.99	4.69	26.77	29.0
71	Kalis 6/4 (B) Black squid Sth trewane	A00785	76.90	17.58	2.78	2.83	76.08	12.02	12.27	22.0
72	Kalis 9/6 Red Squid	A00786	69.80	12.64	15.78	0.25	41.86	52.27	0.83	4.0
73	Ajka Pilchards 9/6 (B) Batch 55 27/4/00	A00787	72.73	18.70	2.08	5.07	68.57	7.62	18.58	129.0
74	Bight 9/6 (A)	A00788	65.50	17.46	3.91	3.13	50.60	11.33	9.08	50.0
75	Ajka 9/6 Pilchards (A) Batch 122	A00789	72.66	19.20	1.86	3.24	70.22	6.82	11.84	258.0
76	Bight 9/6 (B) SCX48259577	A00790	71.12	20.41	1.16	5.87	70.69	4.01	20.34	59.0
77	Kalis US Pilchards 9/6	A00791	74.87	18.77	0.74	8.07	74.69	2.93	32.10	83.0
78	Kalis 9/6 Mex. Pilchards FESU 2967014	A00792	70.67	17.59	1.15	5.53	59.96	3.91	18.86	51.0
79	Bight 9/6 (B)	A00793	61.30	19.11	5.66	3.71	49.38	14.62	9.59	290.0
		Mean	72.88	17.94	2.51	6.51	66.90	8.75	24.96	91.15
		Min	61.30	12.64	0.46	0.25	41.86	1.87	24.96	4.00
		Max	79.38	22.62	15.78	14.56	78.76	52.27	24.96	290.00
		StdDev	3.66	1.56	3.10	3.06	8.12	9.98	24.96	81.89

Table 15. *Reference data for the Group B NIR validation set (AR, as received; DM, dry matter)*

No.	Sample ID	Lab No.	Moisture (%)	Crude Protein (%) (AR)	Crude Fat (%) (AR)	Free Fatty Acids (%) (AR)	Crude Protein (%) (DM)	Crude Fat (%) (DM)	Free Fatty Acids (%) (DM)
1	22/8/00 Bight (C) Pilchards	A00916	70.82	20.24	2.37	6.54	69.36	8.11	22.42
2	22/8/00 Bight (A) Pilchards	A00917	72.26	18.31	2.18	5.86	66.00	7.85	21.14
3	22/8/00 Bight (B) Pilchards	A00918	72.10	16.99	3.33	7.79	60.87	11.94	27.90
4	22/8/00 DI Norway Pilchards	A00919	58.00	15.29	23.39	n/a	36.39	55.67	n/a
5	10/6/00 DI Mixed Pilchards and Anchovies	A00920	74.63	17.64	1.59	7.27	69.53	6.26	28.67
6	17/8/00 Bight Pilchards	A00921	75.06	18.28	1.83	10.86	73.29	7.35	43.55
7	22/8/00 Bluefin Pilchards	A00922	72.86	17.83	2.14	6.87	65.71	7.89	25.32
8	17/8/00 Bight (A) Pilchards	A00923	71.53	19.08	2.31	5.75	67.02	8.12	20.19
9	17/8/00 Bight Squid	A00924	74.88	19.24	1.48	25.70	76.61	5.88	102.32
10	27/4/00 KNLU 2777433 Pilchards	A00925	71.96	19.34	1.35	17.28	68.98	4.82	61.64
11	17/8/00 Lukin A Pilchards	A00926	67.82	19.73	4.32	5.08	61.32	13.42	15.80
12	10/8/00 Blaslov Pilchards ("low fat")	A00927	75.44	17.39	1.07	11.95	70.80	4.35	48.67
13	10/8/00 Blaslov Pilchards ("high fat")	A00928	73.20	18.43	1.31	7.70	68.77	4.87	28.71
14	23/8/00 Calif Pilchards POCW 2829564	A00929	70.37	18.70	1.90	7.19	63.12	6.41	24.28
15	2/2/00 Sandinella Pilchards 020200-2	A00930	63.17	17.86	14.43	n/a	48.48	39.18	n/a
16	2/2/00 Nth. Californian Pilchards	A00931	65.83	17.52	8.32	1.02	51.26	24.34	3.00
17	14/3/00 Gibson's batch 120 300-1 Pilchards	A00932	68.97	18.25	3.43	9.07	58.79	11.05	29.24
18	23/8/00 Lukin A Pilchards	A00933	66.46	15.17	6.50	2.71	45.24	19.38	8.09
19	5/4/00 EASV 570 244 Pilchards	A00934	74.53	19.64	0.78	10.64	77.11	3.08	41.80
20	29/5/00 Pilchards	A00935	72.90	19.24	1.16	25.73	70.99	4.28	94.97
21	DI Sculy Pilchards	A00936	67.63	20.73	6.06	0.76	64.04	18.73	2.35
22	14/4/00 Pilchards	A00937	73.37	18.05	1.19	12.30	67.80	4.46	46.18
23	23/3/00 Calif Pilchards POCU 282 9567	A00938	71.99	19.32	1.27	10.76	68.97	4.55	38.40
24	23/2/00 DI Mixed Bait (C)	A00939	68.84	16.43	8.13	2.15	52.74	26.11	6.89
25	8/9/00 Bluefin Pilchards U.S.A.	A00940	71.44	16.38	3.31	4.73	57.35	11.58	16.56
26	8/9/00 Lukin U.S.A.	A00941	61.93	16.55	16.27	n/a	43.48	42.73	n/a
27	Lukin 8/9/00	A00942	70.72	18.02	4.42	3.48	61.53	15.11	11.87
28	8/9/00 Morroco (1)	A00943	72.03	17.88	1.27	7.52	63.93	4.56	26.87

No.	Sample ID	Lab No.	Moisture (%)	Crude Protein (%) (AR)	Crude Fat (%) (AR)	Free Fatty Acids (%) (AR)	Crude Protein (%) (DM)	Crude Fat (%) (DM)	Free Fatty Acids (%) (DM)
		Mean	70.38	18.13	4.54	8.67	62.48	13.65	31.87
		Min	58.00	15.17	0.78	0.76	36.39	3.08	2.35
		Max	75.44	20.73	23.39	25.73	77.11	55.67	102.32
		StdDev	4.68	1.49	6.15	7.02	11.05	14.70	27.67

Tables 16-19 display the finalised calibration statistics for moisture, crude protein, crude fat and free fatty acids in the group A samples of bait fish scanned as whole frozen (BF1), whole thawed (BF2), processed frozen (BF3), processed thawed (BF4) and processed freeze-dried (BF5) over the 1100-2500 nm scanning range.

Table 16. *Group A - Calibration statistics for moisture (1100-2500 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	66	73.05	0.55	1.80	0.44
BF2	72	72.96	0.92	1.62	0.76
BF3	71	72.86	0.98	0.69	0.96
BF4	72	73.13	0.98	0.64	0.96
BF5	65	4.26	0.93	0.40	0.88

Table 17. *Group A - Calibration statistics for crude protein (1100-2500 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	69	18.15	0.66	1.04	0.19
BF2	74	18.08	0.39	1.06	0.22
BF3	71	18.04	0.81	0.61	0.69
BF4	75	17.99	0.85	0.58	0.73
BF5	67	64.31	0.97	1.31	0.96

Table 18. *Group A - Calibration statistics for crude fat (1100-2500 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	64	1.75	0.48	0.88	0.36
BF2	69	1.85	0.62	0.89	0.55
BF3	69	2.06	0.96	0.57	0.91
BF4	73	2.07	0.96	0.68	0.88
BF5	67	7.11	0.97	1.46	0.94

Table 19. *Group A - Calibration statistics for free fatty acids (1100-2500 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	66	6.55	0.49	2.02	0.41
BF2	72	6.38	0.49	2.11	0.42
BF3	65	6.66	0.61	1.91	0.51
BF4	71	6.61	0.84	1.49	0.69
BF5	66	24.32	0.88	5.13	0.80

Tables 20-23 display the finalised calibration statistics for moisture, crude protein, crude fat and free fatty acids in the group A samples of bait fish scanned as whole frozen (BF1), whole thawed (BF2), processed frozen (BF3), processed thawed (BF4) and processed freeze-dried (BF5) over the 500-1050 nm scanning range.

Table 20. *Group A - Calibration statistics for moisture (500-1050 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	65	73.02	0.19	2.31	0.10
BF2	72	73.24	0.17	2.65	0.02
BF3	70	72.92	0.92	1.29	0.85
BF4	73	73.14	0.92	1.13	0.88
BF5	67	4.22	0.84	0.65	0.70

Table 21. *Group A - Calibration statistics for crude protein (500-1050 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	65	18.17	0.07	0.95	-0.12
BF2	70	18.14	0.16	0.90	-0.03
BF3	71	18.04	0.50	0.93	0.29
BF4	74	18.01	0.53	0.91	0.30
BF5	66	64.70	0.91	2.39	0.85

Table 22. *Group A - Calibration statistics for crude fat (500-1050 nm.*

	N	Mean	RSQ	SECV	1-VR
BF1	63	1.86	0.18	1.29	-0.04
BF2	70	1.84	0.47	1.11	0.25
BF3	66	1.86	0.84	0.84	0.59
BF4	72	1.85	0.68	0.92	0.50
BF5	64	5.83	0.76	1.73	0.69

Table 23. *Group A - Calibration statistics for free fatty acids (500-1050 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	66	6.64	0.41	2.60	0.14
BF2	72	6.38	0.21	2.72	0.04
BF3	68	6.71	0.62	2.22	0.40
BF4	70	6.50	0.81	1.63	0.63
BF5	66	24.32	0.87	5.63	0.76

Tables 24-27 display the finalised calibration statistics for moisture, crude protein, crude fat and free fatty acids in the group A + group B samples of bait fish scanned as whole frozen (BF1), whole thawed (BF2), processed frozen (BF3), processed thawed (BF4) and processed freeze-dried (BF5) over the 1100-2500 nm scanning range.

Table 24. *Groups A+B - Calibration statistics for moisture (1100-2500 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	79	72.09	0.65	2.13	0.59
BF2	79	72.17	0.81	1.69	0.73
BF3	78	72.23	0.98	0.73	0.95
BF4	81	72.22	0.97	0.72	0.95
BF5	74	4.77	0.95	0.41	0.91

Table 25. *Groups A+B - Calibration statistics for crude protein (1100-2500 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	83	18.10	0.53	0.84	0.35
BF2	94	18.12	0.39	1.11	0.24
BF3	73	18.04	0.81	0.56	0.75
BF4	83	18.12	0.78	0.62	0.67
BF5	79	63.00	0.98	1.38	0.96

Table 26. *Group s A+B - Calibration statistics for crude fat (1100-2500 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	76	2.36	0.54	1.60	0.38
BF2	77	2.29	0.45	1.52	0.37
BF3	76	2.30	0.95	0.78	0.86
BF4	77	2.05	0.78	0.73	0.76
BF5	75	7.17	0.98	1.54	0.93

Table 27. *Groups A+B - Calibration statistics for free fatty acids (1100-2500 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	74	6.60	0.50	2.31	0.42
BF2	70	6.76	0.42	2.05	0.37
BF3	75	6.64	0.83	1.59	0.69
BF4	78	6.41	0.82	1.57	0.66
BF5	74	23.80	0.90	4.78	0.82

Tables 28-31 display the finalised calibration statistics for moisture, crude protein, crude fat and free fatty acids in the group A + group B samples of bait fish scanned as whole frozen (BF1), whole thawed (BF2), processed frozen (BF3), processed thawed (BF4) and processed freeze-dried (BF5) over the 500-1050 nm scanning range.

Table 28. *Groups A+B - Calibration statistics for moisture (50-1050 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	77	72.02	0.38	2.72	0.30
BF2	80	72.29	0.25	2.84	0.10
BF3	78	72.28	0.94	1.15	0.88
BF4	81	72.16	0.94	1.35	0.84
BF5	76	4.72	0.87	0.62	0.80

Table 29. *Groups A+B - Calibration statistics for crude protein (50-1050 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	79	18.17	0.09	0.94	0.00
BF2	87	18.16	0.18	0.92	0.13
BF3	72	18.06	0.58	0.96	0.31
BF4	82	18.06	0.63	0.83	0.41
BF5	77	63.19	0.94	2.27	0.89

Table 30. *Groups A+B - Calibration statistics for crude fat (50-1050 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	76	2.43	0.25	1.96	0.09
BF2	78	2.22	0.12	1.69	0.06
BF3	77	2.14	0.85	0.83	0.76
BF4	79	2.05	0.61	1.03	0.52
BF5	74	6.79	0.83	2.76	0.69

Table 31. *Groups A+B - Calibration statistics for free fatty acids (50-1050 nm).*

	N	Mean	RSQ	SECV	1-VR
BF1	75	6.55	0.41	2.94	0.07
BF2	77	6.61	0.19	2.87	0.02
BF3	74	6.69	0.73	1.80	0.60
BF4	77	6.34	0.72	1.72	0.57
BF5	75	24.02	0.78	6.17	0.70

Tables 32-35 display the validation statistics for moisture, crude protein, crude fat and free fatty acids in the group A samples of bait fish compared against the group B samples scanned as whole frozen (BF1), whole thawed (BF2), processed frozen (BF3), processed thawed (BF4) and processed freeze-dried (BF5) over the 1100-2500 nm scanning range.

Table 32. *Group A vs Group B - Validation statistics for moisture (1100-2500 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	3.43	4.34	0.49	0.79
BF2	3.31	4.22	0.44	0.78
BF3	1.13	3.52	0.91	0.92
BF4	0.83	2.99	0.92	0.28
BF5	0.85	0.54	0.13	1.57

Table 33. *Group A vs Group B - Validation statistics for crude protein (1100-2500 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	1.31	1.39	0.10	0.94
BF2	1.22	1.37	0.19	0.89
BF3	1.09	1.28	0.45	0.85
BF4	0.89	1.01	0.66	0.88
BF5	2.15	5.52	0.85	0.39

Table 34. *Group A vs Group B - Validation statistics for crude fat (1100-2500 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	5.59	5.55	0.33	1.01
BF2	5.19	5.35	0.33	0.97
BF3	2.53	3.94	0.80	0.64
BF4	2.19	3.14	0.87	0.70
BF5	2.49	5.03	0.77	0.50

Table 35. *Group A vs Group B - Validation statistics for free fatty acids (1100-2500 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	6.00	6.62	0.37	0.91
BF2	6.30	6.38	0.13	0.99
BF3	6.20	6.38	0.31	0.97
BF4	6.06	6.53	0.21	0.69
BF5	6.32	13.84	0.84	0.46

Tables 36-39 display the validation statistics for moisture, crude protein, crude fat and free fatty acids in the group A samples of bait fish compared against the group B samples scanned as whole frozen (BF1), whole thawed (BF2), processed frozen (BF3), processed thawed (BF4) and processed freeze-dried (BF5) over the 500-1050 nm scanning range.

Table 36. *Group A vs Group B - Validation statistics for moisture (500-1050 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	4.67	4.34	0.13	1.08
BF2	4.93	4.22	0.22	1.17
BF3	1.80	3.52	0.74	0.51
BF4	1.89	2.99	0.71	0.63
BF5	2.15	0.54	0.10	3.98

Table 37. *Group A vs Group B - Validation statistics for crude protein (500-1050 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	1.28	1.38	0.18	0.93
BF2	1.35	1.37	0.01	0.99
BF3	1.38	1.28	0.17	1.08
BF4	1.40	1.10	0.06	1.27
BF5	3.99	5.52	0.61	0.72

Table 38. *Group A vs Group B - Validation statistics for crude fat (500-1050 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	5.82	5.55	0.13	1.05
BF2	5.60	5.35	0.26	1.05
BF3	2.59	3.94	0.85	0.66
BF4	2.66	3.14	0.59	0.85
BF5	3.52	5.03	0.71	0.70

Table 39. *Group A vs Group B - Validation statistics for free fatty acids (500-1050 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	14.66	12.81	0.11	1.14
BF2	14.00	12.43	0.15	1.13
BF3	13.93	11.72	0.28	1.19
BF4	12.16	10.31	0.01	1.18
BF5	8.71	13.84	0.61	0.63

Tables 40-43 display the validation statistics for moisture, crude protein, crude fat and free fatty acids in the combined group A and group B samples of bait fish compared against the group C samples scanned as whole frozen (BF1), whole thawed (BF2), processed frozen (BF3), processed thawed (BF4) and processed freeze-dried (BF5) over the 1100-2500 nm scanning range.

Table 40. *Group AB vs Group C - Validation statistics for moisture (1100-2500 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	2.89	3.81	0.37	0.76
BF2	3.58	3.78	0.27	0.95
BF3	1.53	2.99	0.83	0.51
BF4	0.82	3.75	0.95	0.22
BF5	0.95	1.84	0.72	0.52

Table 41. *Group AB vs Group C - Validation statistics for crude protein (1100-2500 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	1.13	1.61	0.60	0.70
BF2	1.17	1.61	0.52	0.73
BF3	1.01	1.41	0.52	0.72
BF4	1.02	1.61	0.63	0.63
BF5	1.92	8.05	0.97	0.24

Table 42. *Group AB vs Group C - Validation statistics for crude fat (1100-2500 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	3.10	3.85	0.55	0.81
BF2	3.91	4.51	0.33	0.87
BF3	2.47	4.60	0.81	0.54
BF4	3.44	4.94	0.93	0.70
BF5	2.06	5.53	0.89	0.37

Table 43. *Group AB vs Group C - Validation statistics for free fatty acids (1100-2500 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	1.96	2.84	0.60	0.69
BF2	2.64	3.60	0.53	0.73
BF3	2.47	3.76	0.53	0.66
BF4	1.61	3.66	0.91	0.44
BF5	6.45	13.56	0.78	0.48

Tables 44-47 display the validation statistics for moisture, crude protein, crude fat and free fatty acids in the combined group A and group B samples of bait fish compared against the group C samples scanned as whole frozen (BF1), whole thawed (BF2), processed frozen (BF3), processed thawed (BF4) and processed freeze-dried (BF5) over the 500-1050 nm scanning range.

Table 44. *Group AB vs Group C - Validation statistics for moisture (500-1050 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	2.61	3.81	0.64	0.69
BF2	2.57	3.78	0.51	0.68
BF3	2.84	2.99	0.76	0.95
BF4	1.18	1.84	0.60	0.64
BF5	3.46	3.75	0.72	0.92

Table 45. *Group AB vs Group C - Validation statistics for crude protein (500-1050 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	1.52	1.61	0.10	0.94
BF2	1.58	1.61	0.00	0.98
BF3	1.38	1.41	0.04	0.98
BF4	1.23	1.61	0.54	0.76
BF5	5.60	5.61	0.38	1.00

Table 46. *Group AB vs Group C - Validation statistics for crude fat (500-1050 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	3.15	3.85	0.37	0.82
BF2	4.20	4.51	0.39	0.93
BF3	2.30	4.60	0.75	0.50
BF4	3.20	4.94	0.85	0.65
BF5	3.06	5.53	0.69	0.55

Table 47. *Group AB vs Group C - Validation statistics for free fatty acids (500-1050 nm).*

	SEP	StdDev	RSQ	SEP/StdDev
BF1	2.68	2.83	0.20	0.95
BF2	3.15	3.60	0.27	0.88
BF3	3.41	3.76	0.12	0.91
BF4	3.35	3.66	0.58	0.92
BF5	7.87	13.56	0.64	0.58

As expected, we observed higher accuracy of calibrations with the increased homogeneity of fish samples. The unprocessed samples in both frozen and thawed forms performed very poorly. The calibration results are not encouraging. The above was also confirmed by validation performance. The likely reason for these poor results could be the physical presentation of the sample during scanning combined with uneven distribution of measured constituents in the sample.

The calibrations for samples presented in freeze dried form were the best performing ones. This was confirmed by validation results showing the lowest errors and highest correlation coefficients as compared with samples in other forms. It is recognised that NIR has a potential to accurately test samples of fish, although the necessity to prepare samples for NIR measurement reduces the speed of the test. This seems to question the application of the test in field conditions. However, the positive outcome appears to be the elimination of the actual chemical analysis in the laboratory after freeze-drying.

Although slightly less reliable than for freeze-dried samples, the results for processed pilchards, in both frozen and thawed forms, present potential. The calibrations' statistics show good performance. Validation outcome is also positive except for the relatively high error (SEP) for crude fat measurements. However, it should be noted that the calibration model is constructed on a basis of laboratory wet chemistry results, which themselves showed relatively high errors. The validation results for free fatty acids were better for processed wet fish than for freeze-dried fish. Overall, the NIR calibration results for processed pilchards demonstrate potential for future application.

In order to assess the potential application of portable NIR equipment for these tests, the wavelength range was shortened accordingly to 500-1050 nm.

As demonstrated previously, the more homogenous samples, the more precise the NIR measurements. The results obtained when testing unprocessed (frozen and thawed) fish samples, show very high calibration and validation errors and are poorly correlated with wet chemistry data. Based on the outcome when testing various combinations of samples, it appears that the NIR analysis of whole fish samples proved rather unreliable in this exercise.

Consistent with the previous experiment with an extended NIR wavelength range, the 500-1050 nm range produced the best results for freeze-dried fish samples. This applies to both calibration and validation sets. However, this outcome seems irrelevant, since the expected benefit of using portable NIR instrument lies with less unprocessed fish material. Therefore, this assessment should not take into account freeze-dried samples.

Although, the results are less reliable than for full NIR wavelength range, there seems to be some potential using reduced wavelengths. The best performing measurement are for moisture and crude protein. The measurements for crude fat present a relatively high error when validating the calibration but, again, it must be seen in conjunction with standard error of reference data used. As observed with the full NIR wavelength range, prediction of free fatty acids is the weakest measurement.

We have sufficient confidence in the results of this research to offer commercial analysis of bait fish samples using NIR as a screen for chemical composition for use in routine QA programs.

Objective 5: *To determine the efficiency of digestion of farmed SBT fed manufactured diets.*

Experiments 1 and 2

As a routine component of experimentation, chromic oxide was added as an indigestible marker to all manufactured diets 7 days prior to harvest. This was to permit digesta collection for assessment of nutrient digestibility.

Growth results from 1998 were extremely encouraging promoting SBT performance to levels similar to those achieved with pilchards. The digestibility data associated with these diets reflects the performance levels (Table 48). Digestibility coefficients for dry matter, N and gross energy were all in the vicinity of 0.85-0.90, suggesting that there was minimal waste and environmental loading. In addition, statistical analysis was possible on these samples revealing that gross energy digestion in diets containing reduced levels of dietary fat was significantly lower.

Table 48. *Digestibility of dry matter, N and gross energy in manufactured diets fed to SBT in experimental pontoons in 1998*

	Diet			Statistics		
	CRC98A	CRC98B	CRC98C	Pr>F	Diet	SEM
Dry matter digestibility	0.88	0.85	0.84	0.101	NS	0.016
N digestibility	0.88	0.84	0.81	0.061	NS	0.020
Gross energy digestibility	0.90^{a}	0.85^{a}	0.81^{b}	0.015	*	0.019

NS, not significant: *, P<0.05: SEM, standard error of the mean: ^{a,b} Values in a row with different superscripts differ significantly

The poorer growth responses of SBT in the 1999 experiments was reflected by a poorer digestibility of nutrients in the manufactured diets, particularly in diets fed more frequently (Table 49, 99A). Feeding the same diet restrictively improved nutrient digestion by approximately 10%. Interestingly, nutrient digestion was superior in diets containing elevated levels of fat despite a poorer pellet quality and integrity.

Table 49. *Digestibility of dry matter, N and gross energy in manufactured diets fed to SBT in experimental pontoons in 1999*

	Diet			
	99A (frequent)	99A (restricted)	99B (restricted)	99C (restricted)
Dry matter digestibility	0.53	0.57	0.69	0.72
N digestibility	0.71	0.80	0.85	0.85
Gross energy digestibility	0.69	0.79	0.86	0.85

As a service to project 1 of the SBT Aquaculture Subprogram, the digestibility of diets assessed in 2000 was determined. It can be seen that the heat treatment of the moist pellets had little impact on the digestibility of nutrients in these pellets (Table 50).

Table 50. *Digestibility of dry matter, N and gross energy in manufactured diets fed to SBT in experimental pontoons in 2000*

	Diet		Statistics	
	Standard	Heated	Diet	SEM
Dry matter digestibility	0.65	0.63	NS	0.032
N digestibility	0.83	0.85	NS	0.020
Gross energy digestibility	0.84	0.86	NS	0.018

NS, not significant, P>0.05: SEM, standard error of the mean.

Objective 6: *Improve our understanding of the physiological responses by farmed SBT to manufactured diets.*

Experiment 4

Feeding: Five hundred kilograms of feed was fed to the cage of tuna. If it is assumed that this contained 1000 tuna with an average weight of 13.9 kg it was equivalent to an average ration of 500 g per fish or 3.6 % body weight (BW). The stomach contents of the 2 h group were equivalent to 0.75 % BW and taken to be the closest approximation of the daily intake of the fish. All fish at this time had stomach contents although there appeared to be differences between individuals. Pellets were present in the stomachs of fish from the 2 to 12 h sample times but there were no pellets in the stomachs of fish taken 24 h after feeding. Two of the stomachs from fish taken at between 2 and 12 h had no contents, both fish were taken at 4 h but it is difficult to use these data to conclude that 90% of fish were feeding.

There were significant differences in the digestive tract (DT) temperature at different times and these were significantly higher at 8 h (Figure 10). Muscle temperature fluctuated over the day although there were no significant differences between times there was a trend ($P < 0.07$) for maximum temperature at 8 h after feeding and for a minimum immediately after feeding. The mean muscle temperature was 26.0 ± 1.2 °C. DT temperature was about 1°C lower than the muscle temperature and both showed similar temporal changes (Figure 1). These data are suggestive of an increase in metabolic activity within the SBT that is highly likely to be due to the Heat Increment of Feeding (HIF) as well as changes in activity. The increase in temperature in the DT partly reflects the heat released during digestion and partly the metabolic activity associated with the synthesis of digestive enzymes and the absorption of nutrients through the intestine. In the muscle tissues the increase in heat will to a larger extent reflect the heat produced by synthesis of protein as part of the HIF (Houlihan *et al*., 1995). It is of interest that in a previous experiment (Carter *et al*., 1998) muscle temperature correlated with growth rate and with the RNA: protein ratio that is indicative of protein synthesis.

Digestive Tract and Liver Nutritional Correlates

The relative size (% body weight) of the empty stomach (1.17 ± 0.05 %BW), pylorus (1.57 ± 0.07 %BW) and liver (1.18 ± 0.03 %BW) were not affected by the time of sampling and used further in the assessments of nutritional status of the tuna (see below).

Nutritional correlates were measured in the liver and protein, RNA and DNA content showed little change following feeding (Table 51). Overall mean (± SEM) values for protein, RNA and DNA were 212 ± 22 mg/g, 8.2 ± 0.2 mg/g and 3.0 ± 0.2 mg/g, respectively, and markedly higher than observed in tuna sampled in feed trials in 1995 (Carter *et al*., 1998). For 34 kg tuna with a specific growth rate of 0.2 %/d when fed pilchards values were 151 mg/g, 2.2 mg/g and 1.1 mg/g, respectively (Carter *et al*., 1998).

The RNA: protein ratio did not change significantly with time but there was a suggestion of higher values over the last 12 hours ($P < 0.1$). The liver is associated with high levels of metabolism and very high rates of protein synthesis following feeding and partly due to catabolism of dietary amino acids as well as the export of amino acids, peptides and proteins to other tissues. This is reflected by high RNA:protein ratios observed in tuna liver compared with muscle but they are also within the range found in other feeding and growing fish. In fact, the concentrations and pattern of increase towards the end of the 24 h period are very similar to those observed in Atlantic cod (Lyndon *et al*., 1993).

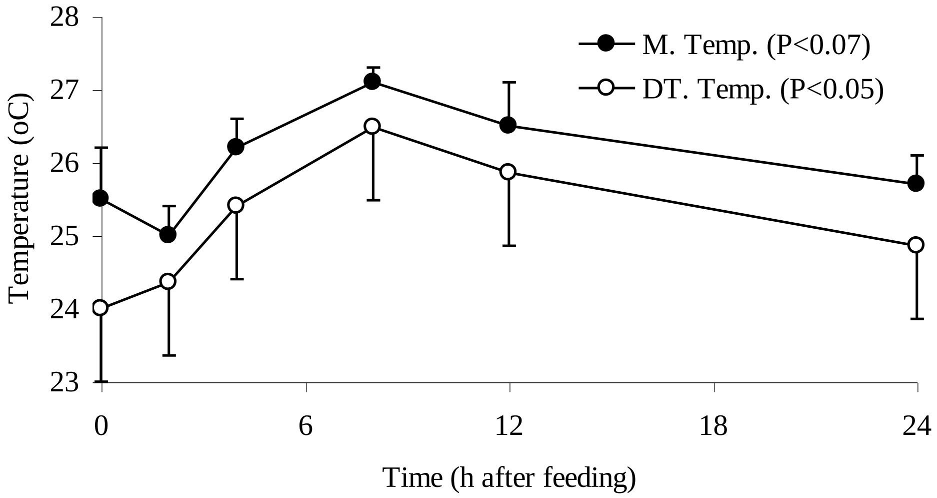


Figure 10. *The temperature of the digestive tract (DT) and muscle (M) of tuna following feeding*

Table 51. *Liver nutritional correlates following feeding*

Liver	0h	2h	4h	8h	12h	24h	P
Protein (mg/g)	231 ± 54	212 ± 63	271 ± 68	216 ± 22	177 ± 57	167 ± 67	NS
RNA (mg/g)	8.7 ± 0.6	8.5 ± 0.3	7.2 ± 0.6	8.2 ± 0.5	8.4 ± 0.4	8.1 ± 0.7	NS
DNA (mg/g)	3.2 ± 0.4	2.8 ± 0.5	3.2 ± 0.4	2.9 ± 0.2	2.5 ± 0.3	3.4 ± 0.5	NS
RNA: protein	43.1±6.5	49.1±7.4	34.2±8.4	39.0±3.1	61.0±12.7	71.9±14.8	<0.09
RNA: DNA	2.8 ± 0.2	3.3 ± 0.5	2.5 ± 0.5	3.0 ± 0.4	3.5 ± 0.4	2.8 ± 0.7	NS

Muscle Nutrition Correlates

Nutritional correlates in the muscle did not show any significant changes following feeding (Table 52). Overall mean (± SEM) values for protein, RNA and DNA were 147 ± 3 mg/g, 0.6 ± 0.0 mg/g and 0.5 ± 0.0 mg/g, respectively. In comparison to the tuna from 1995, discussed above, the muscle protein and RNA content was lower but the DNA higher (Carter *et al*., 1998). This resulted in the RNA: protein (4.4 ± 0.2) being about half and the RNA:DNA (2.1 ± 0.3) being about a third of the value reported in 1995. This is of interest since generally the higher ratios would suggest higher potential for growth. However, very high ratios may indicate poor nutrition and attempts by an animal to compensate for this by increasing rates of protein metabolism (Millward *et al*., 1973).

The immediate regulation of protein synthesis is via both the amount and the activity of RNA (rRNA). The amount of RNA is reflected in the RNA: protein ratio. The activity of RNA cannot be assessed without measurement of protein synthesis and this was not possible in the present experiment. In fish on a stable feeding regime the amount of RNA is expected to remain relatively constant over 24 h and to indicate a general level of metabolism. It is for this reason that RNA: protein and DNA: RNA ratios often correlate with growth rate of juvenile fish (Houlihan *et al*., 1995). However, rates of protein synthesis will change within a 24 hour period following feeding (Lyndon *et al*., 1993) and this regulation will be in terms of RNA activity. Following feeding there were no significant changes in muscle concentrations of RNA, DNA or protein or in the RNA: DNA and RNA: protein ratios and broadly expected from research on other fish species. Despite the lack of statistical difference there was a suggestion of temporal variation in RNA concentration and ratios (Figure 11) that was similar to that of liver and may suggest a period of higher metabolism over the 12-24 h period.

Table 52. *Muscle nutritional correlates following feeding*.

Muscle	0h	2h	4h	8h	12h	24h	P
Protein (mg/g)	145 ± 14	141 ± 11	137 ± 19	153 ± 12	154 ± 11	150 ± 21	NS
RNA (mg/g)	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	NS
DNA (mg/g)	0.5 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	NS
RNA: protein	4.9 ± 0.6	3.9 ± 0.2	3.6 ± 0.3	4.4 ± 0.7	4.7 ± 0.6	4.7 ± 0.6	NS
RNA: DNA	2.2 ± 0.7	2.1 ± 0.5	1.2 ± 0.3	2.2 ± 1.0	2.8 ± 0.9	2.4 ± 0.7	NS

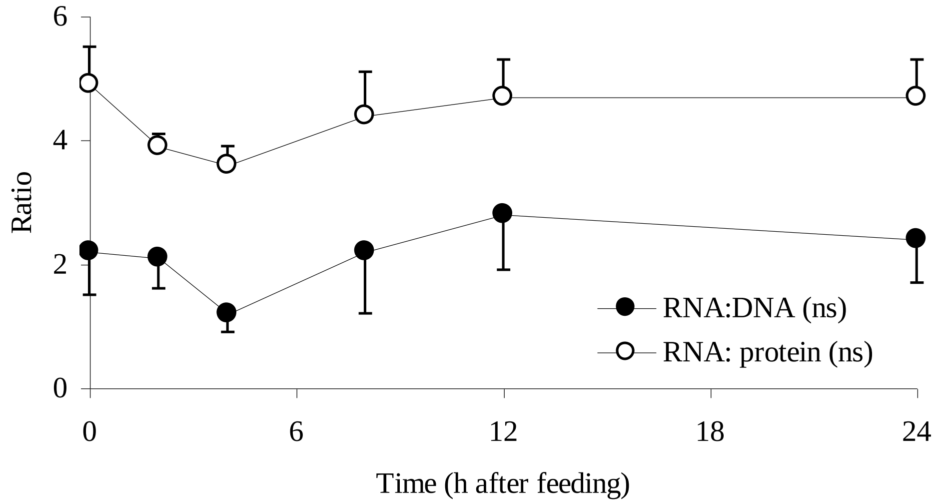


Figure 11. *Muscle RNA: protein and RNA: DNA ratios of tuna following feeding*

The mean value of 4.4 ± 0.2 mg RNA / g protein for the RNA: protein ratio was used to make an assessment of the nutritional status of these tuna. The relationship between RNA:protein ratio and rates of protein synthesis has been described by PS = 2.3 RNA:protein – 6.4 (Carter *et al*., 1993). The tuna RNA: protein ratio was used to predict an expected minimum rate of protein synthesis of 3.7 %/d. This is relatively similar to those found in other teleosts. Furthermore, it is only 1.3 times higher than typical values even when the very different weight and temperature of the tuna are accounted for by using established scaling relationships. Consequently, these data tend to suggest that SBT are similar to other fish in the basic mechanisms involved in muscle protein turnover (Table 52). However, it should be noted that this analysis does not take into account the maximum rates of synthesis that might be attained at the measured RNA concentrations.

Muscle Free Amino Acid Pools

After feeding the free pool concentrations of selected essential amino acids showed some variation. However, changes were relatively small and this low response has been observed previously in teleosts fed large (Carter *et al*., 1995) and small (Carter *et al*., unpublished) meals (Figure 12).

Table 53. *Comparison of nutritional status in tuna (SBT) and Atlantic salmon fed similar diets*.

	SBT	Atlantic salmon	SBT scaled*
Weight (kg)	13.85 (2.94)	0.51 (0.08)	0.5
Feed intake (% bodyweight)	0.75 (0.34)	1	
Muscle RNA	0.63 (0.18)	0.85 (0.06)	1.2
Muscle RNA:DNA	2.11 (1.62)	5.92 (0.59)	3.0
Muscle RNA:Protein	4.37 (1.19)	4.35 (0.14)	7.4
Plasma IGF-I (ng/ml)	38.8 (16.1)	25.2 (3.0)	

*Tuna data scaled to account for differences in size and temperature.

The most interesting data concerned the concentrations of individual essential amino acids. Both lysine and threonine were at very low concentrations compared to those expected from other fish (Atlantic salmon and rainbow trout) or from the composition of the feed. Concentrations were less than 10% of those expected and threonine appeared to be at the lowest relative concentration (Figure 13). This may reflect a species difference but given the similarity in concentrations of other essential amino acids it may also indicate low availability of these essential amino acids. This analysis needs further investigation in relation to digestibility as well as concentrations in other tissues and the plasma. It would be useful to discount the hypothesis that threonine or lysine were limiting protein metabolism and growth.

The muscle free pool histidine concentration was extremely high in the tuna and expected from other studies (Abe, 1995) that have found ranges between 20 and 120 µmol/g muscle in a range of scombrid species. The principle explanation relates to the function of histidine-related dipeptides in swimming function, particularly during anaerobic metabolism. It is of further interest that histidine has also been proposed as a direct energy source during non-feeding and this suggests a potential use as an indicator of nutritional status. The only systematic study showed a decrease from 80-90 µmol/g in feeding skipjack tuna to less than 5 in one fish starved for 12 d (Abe, 1995). The tuna in the present study showed considerable individual variation (Figure 14) and muscle histidine was investigated further as an indicator of nutritional status (see below).

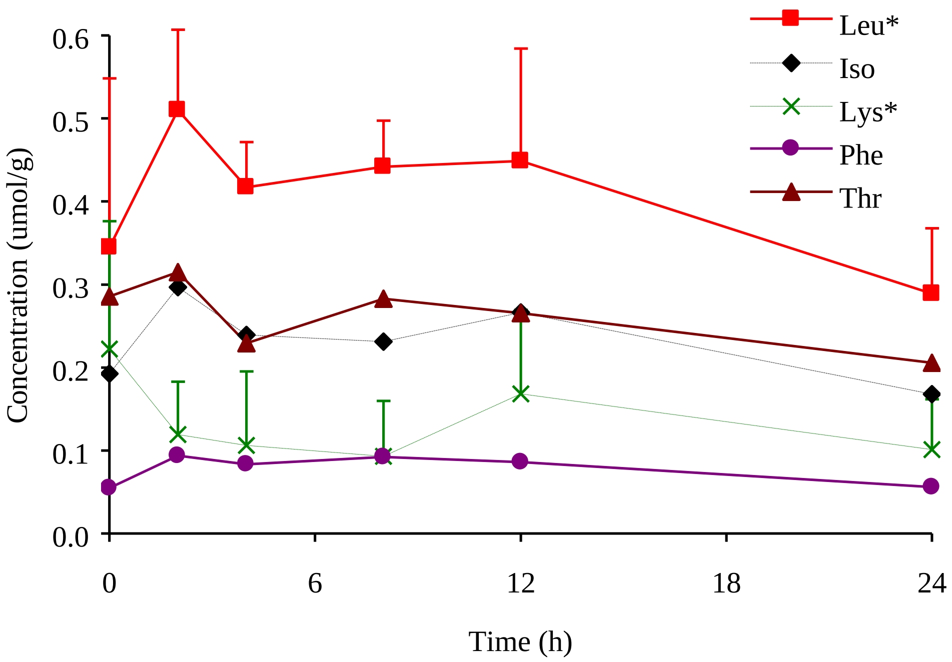


Figure 12. *Muscle free pool concentrations of essential amino acids of tuna following feeding*

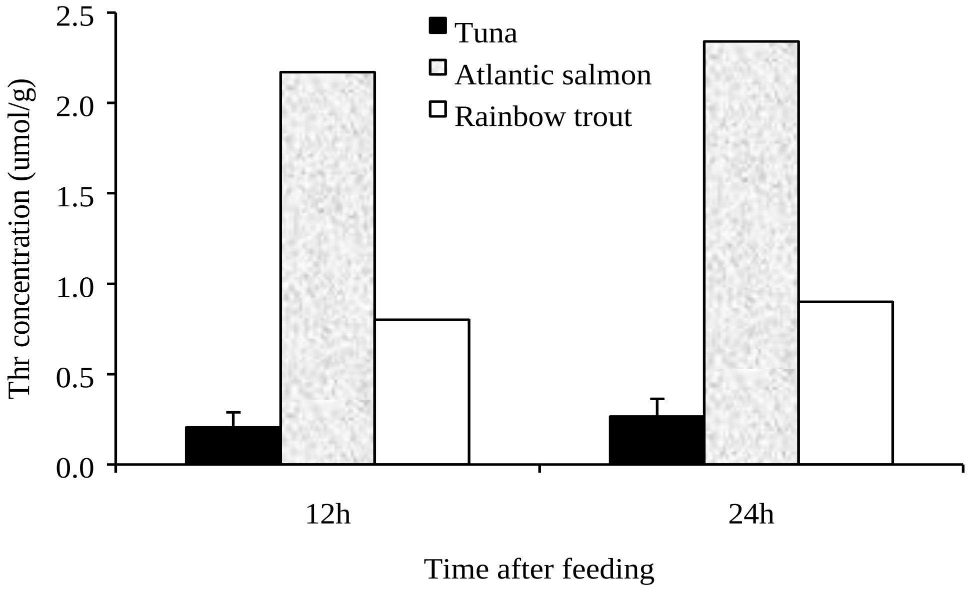


Figure 13. *Comparison of free threonine concentration in muscle tissues of feeding tuna, Atlantic salmon and rainbow trout at 12 and 24 h after feeding.*

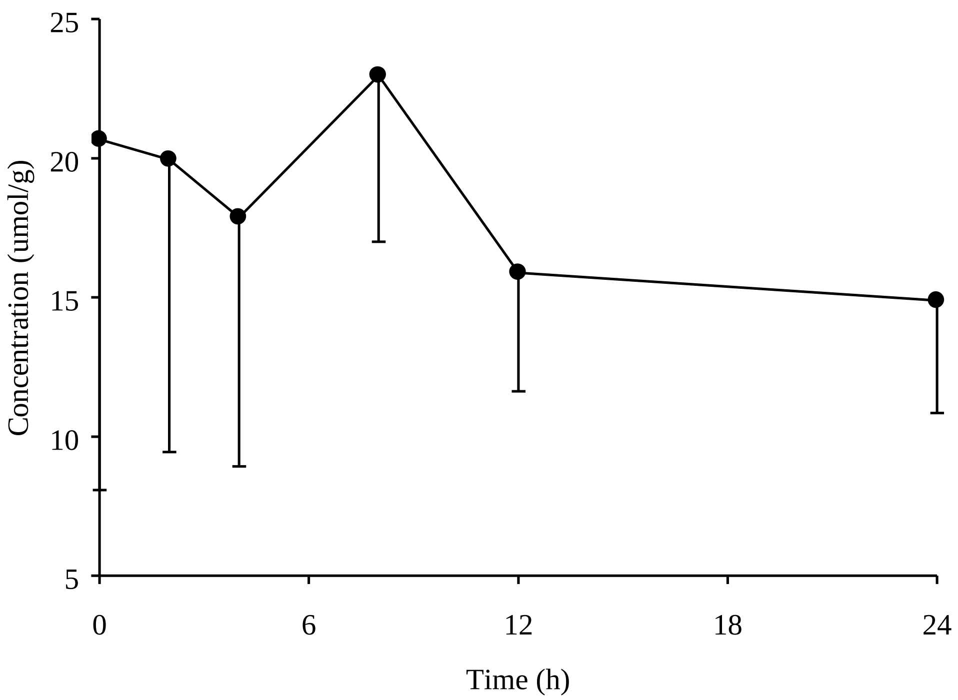


Figure 14. *Muscle free pool histidine concentration of tuna following feeding*

Overview of Nutritional Status

Growth was not measured in the present study and nutritional status was examined by investigating the correlations between the various indices measured. In addition to the measurements presented above collaboration (P.M.Thomas and J.F.Carragher, Flinders University) enabled IGF-I as a measure of growth potential and cortisol as a measure of stress level to be included in the analyses.

There was a correspondence between the muscle nutritional correlates and plasma IGF-I concentrations so that significant and positive correlations were observed between IGF-I and muscle RNA ($r=0.55$; $P<0.01$), RNA:DNA ($r=0.49$; $P<0.01$), protein ($r=0.44$; $P<0.05$) and RNA:protein ($r=0.43$; $P<0.05$) as well as more weakly with temperature ($r=0.38$; $p<0.05$). These data are suggestive of the relationships between whole animal growth and mechanisms of protein accretion found previously (Millward *et al*., 1973).

Indices such as condition factor (K: 100 x (W/L^3) and liver size (HSI: % BW) have often been used as indices of nutritional status. K did not correlate with the muscle indices of nutritional status, with liver indices or with IGF-I concentration. In contrast, HSI was positively correlated with some of the other indices of nutritional status: IGF-I ($r=0.50$; $P<0.01$), muscle temperature ($r=48$; $P<0.01$), muscle RNA ($r=0.36$, $P<0.05$) as well as with liver protein ($r=0.55$, $P<0.001$).

Muscle free histidine concentration was not correlated with tuna weight, K or other indices of nutritional status and did not appear to be sensitive to the nutritional status of the tuna. Cortisol was not correlated with other parameters measured in the study. Thus, there seemed no evidence for smaller fish having greater stress levels than larger fish (due to a hierarchy effect) or there being any relationships between stress level and the indices of nutritional status.

Objective 7: *Reduce nutrient excretion through improved knowledge of the nutritional value of diet ingredients for farmed SBT.*

Based on the results described above, nutrient excretion can be effectively reduced by focussing on diet form and by ensuring a significant proportion of dietary energy is derived from fat. The presence of dietary crude fat levels exceeding 14% appears preferable. Diet form may be significantly enhanced by developing a steam extruded pellet for SBT to replace the existing moist pellets. Further research is also required to address feeding strategies and feeding frequency to ensure caged SBT are not being overfed.

Benefits

The primary benefit arising from the conduct of this experiment has been the further enhancement of our knowledge of those factors that influence the nutrition of farmed SBT. In particular, results arising from this project will allow:

1. The production of semi-moist manufactured diets that produce growth responses equivalent to that achieved with pilchards, but with less efficient feed conversion;

2. Reductions in the levels of bait fish in manufactured diets without a significant compromise to SBT performance;

3. Knowledge that SBT performance can be manipulated by manipulating the nutrient composition of the diet;

4. Knowledge that diet form and early weaning of SBT onto manufactured pellets is essential if manufactured diets are to be used in commercial SBT production systems.

5. Reduced levels of nutrient excretion into the surrounding environment by optimising the level of dietary fat in the form of oil.

While feed conversion efficiency was improved during this project, there is still significant room for improvement. There is also scope to reduce feed costs through improved pellet integrity and more efficient modes of pellet manufacture.

Further development

Based on the outcomes of this project, a number of new initiatives are suggested for subsequent research in the field of tuna nutrition.

A $1.35 million Australasian Experimental Stockfeed Extrusion Centre is being established as a direct result of a need from tuna research projects for improved diet manufacturing technology (however, there will obviously be additional uses for the facility once it is fully operational). This will facilitate the production of extruded feeds with high levels of added fat and attractants and a high level of pellet integrity.

It is suggested that subsequent research programs address the following core research areas:

Nutrition research: Identify nutrition research surrogates for SBT that can be used to define ingredient specifications and nutritional requirements more cost-effectively and accurately than currently possible using SBT on the tuna research farm in Pt Lincoln.
Pellet technology: Develop production techniques for extruded tuna feeds that will facilitate the use of the widest possible range of feed ingredients while maintaining acceptability of the product by SBT.
Pellet technology: Identify those characteristics of extruded and semi-moist feeds that have the greatest influence on SBT performance, including the digestibility and acceptability of the pellet using small-scale, intensive experiments and growth experiments on the tuna research farm in Pt Lincoln.
Feed evaluation: Using surrogates, develop a feed evaluation system defining the availability of key nutrients for SBT.
Nutrient requirements: Utilise surrogates, growth correlates and nutrient partitioning to define the requirements for key nutrients such as protein and energy and the fate of ingested nutrients in SBT.
Feeding strategies: Examine the influence of feeding strategy on the efficiency of nutrient use using surrogates and SBT.
Communication: Extend project results to commercial tuna farmers using workshops, publications and individual meetings.

Conclusions

The following conclusions can be drawn from the conduct of this research:

- The composition of pilchards is highly variable over the course of an SBT growing season. This is likely to affect production efficiency. In contrast, manufactured diets can be produced with a high degree of consistency.

- The performance of SBT fed diets (moist pellets) containing reduced levels of pilchards does not equate to diets containing approximately 48% fresh product or fresh product alone. This may be due to a number of factors including reduced intake, or issues associated with pellet form and a more rapid breakdown of the pellet when less fresh product is included.

- There was no evidence of a difference in growth rate between SBT fed on diets each of which contained a reduced level of pilchards, but in which the content and ratio of total protein and fat was varied. This suggests that we may be significantly overfeeding SBT in pontoons, resulting in feed wastage and an increase in the level of environmental waste.

- Higher fat diets may be more appropriate for farming SBT.

- The physical quality of pellets and dietary protein content appear to have been two fundamental constraints to the conduct of the experiments in the current project.

- Numerical differences can be observed in the performance of SBT fed diets containing different levels of moisture, protein and/or crude fat.

- Dietary carotenoids do not influence the flesh characteristics of SBT fed manufactured diets.

- The crude protein, moisture, crude fat and free fatty acid content of bait fish can be adequately screened using near infrared spectrophotometry on samples of processed frozen, processed thawed and processed freeze-dried bait fish, the latter being the most accurate. Analysis can be undertaken using spectral ranges of 1100-2500 nm or 500-1050 nm, the former being more accurate, but the latter acceptable.

- Manufactured diets with higher levels of crude fat have higher levels of digestible protein, dry matter and gross energy.

- Improved binding and pellet integrity is closely related to nutrient digestibility.

- Gut retention time appears to influence nutrient digestibility with improved digestibility observed with restricted feeding of manufactured diets.

- Higher gross energy, crude protein and dry matter digestibility is reflected by higher SBT growth rates and improved feed conversion efficiency.

- Positive correlations between tissue protein, RNA and DNA, IGF-I and liver status and nutritional status support their further use in nutrition experiments.

- Analysis free amino acid concentrations revealed extremely low levels of two essential amino acids which may contribute to poor feed efficiency of tuna fed formulated diets. However, the data need to be considered in relation to digestion and absorption of essential amino acids and concentrations in other tissues and plasma to further clarify the situation.

- Free histidine in muscle tissue did not relate to the nutritional status of the tuna and is not suitable as an indicator of such.

Appendix I – Intellectual Property

It is difficult to identify those outcomes from this research that may represent intellectual property, in particular, whether the diet formulations *per se* have any value outside the scope of this research project. Further discussion between the stakeholders is required to clarify this. In the interim, the author nominates the following as potential sources of IP.

- A potential key piece of intellectual property relates to the production of semi-moist pellets to specifications reflective of those produced for use in experiments in 1998 (ie CRC98A, CRC98B, and CRC98C). These pellets were produced on small scale experimental equipment and resulted in a high level of binding and water stability. It appears that the same level of binding could not be produced on a commercial scale. As stated in the "Outcomes Achieved" section of this report, manufactured diets can be produced to promote growth responses in SBT equivalent to that achieved with pilchards providing they can be produced with the same level of integrity as those used in the experiments. As a consequence, the exact procedure used to produce the experimental diets CRC98A, CRC98B and CRC98C and measurements of these pellets should be documented and protected.

- A further piece of intellectual property that has resulted from this project is in the form of the NIR calibrations for the measurement of bait fish quality. This technology is comparatively easy to protect due to the fact that the calibrations cannot easily be transferred between machines. Commercialisation of this technology could be in the form of commercial services for the measurement of bait fish quality (already in place) and the transfer of the calibrations to portable equipment. These commercialisation processes extend beyond the scope of this project.

Appendix II - Staff

The following staff participated in the conduct of the project:

Name and affiliation	Position	FTE on project (%)
Barneveld Nutrition Pty Ltd		
Dr Robert van Barneveld	Consultant Research Scientist	20
South Australian Research and Development Institute		
Dr Brett Glencross	Research Scientist	30
Mr Steven Clarke	Aquaculture Program Leader	5
Mr Jurek Kruk	Technical Officer	50
Mr Rowan Daw	Research Assistant	20
Dr Yingjun Ru	Research Scientist	5
Ms Janet Hattam	Technical Officer	5
Mr Kylee Swanson	Technical Officer	5
University of Tasmania		
Dr Chris Carter	Senior Lecturer	15
Mr Matthew Bransden	PhD Student	20
Mr Rhys Hauler	Technical Officer	20
Tuna Boat Owners Association of Australia		
Mr Paul Mussolino	Farm hand	5
Mr Tor Mussolino	Farm Manager	5
Mr Edward Davis	Farm Hand	5

Appendix III:
Experiment 1 Diet Formulations - 1998

Ingredients

Amino acid	Content (g/kg)						
	Pilchards	Chilean	FM70	Krill meal	Squid	Blood	Gluten
Lysine	7.600	52.600	55.710	28.000	52.600	74.040	15.900
Threonine	3.540	26.800	29.320	16.000	26.800	40.890	21.600
Methionine	1.300	18.500	20.200	14.000	18.500	10.490	10.800
Isoleucine	4.990	30.000	31.950	17.000	30.000	8.280	30.100
Leucine	4.000	50.000	51.740	28.000	50.000	108.180	53.300
Tryptophan	0.000	8.100	8.860	0.000	8.100	12.690	2.000
Valine	3.750	34.030	38.160	19.000	34.030	76.290	32.400
Phenylalanine	3.840	26.200	28.340	18.000	26.200	54.630	37.400
Histidine	4.480	16.310	17.520	8.700	16.310	54.480	1.560
Arginine	6.430	50.000	40.380	23.000	50.000	36.140	29.600

Vitamins and Minerals

Ingredient	Units	Gibsons 98	
		unit/3kg	unit/kg
Vitamin A	MIU	3.000	1.000
Vitamin D3	MIU	0.500	0.167
Thiamine (B1)	g	15.000	5.000
Riboflavin (B2)	g	20.000	6.667
Pyridoxine (B6)	g	12.000	4.000
Vitamin B12	mg	30.000	10.000
Biotin	g	0.300	0.100
Vitamin K3	g	7.000	2.333
Pantothenic Acid	g	30.000	10.000
Niacin	g	65.000	21.667
Inositol	g	50.000	16.667
Vitamin E ADS	g	100.000	33.333
Folic acid S.D.	g	4.000	1.333
Ethoxyquin	g	150.000	50.000
Cobalt	g	1.000	0.333
Iodine	g	1.100	0.367
Copper	g	3.000	1.000
Magnesium	g	50.000	16.667
Manganese	g	20.000	6.667
Iron	g	20.000	6.667
Bioplex iron	g	20.000	6.667
Zinc	g	30.000	10.000
Aquastab C 42%	g	60.000	20.000

Notes:

Without knowing the activities and forms of the vitamins and minerals in this premix, it is difficult to make a comparison with the RP mix used in CRC 1/97
Costs should not be an issue in experimental diets.

Formulated by Craig Foster (Gibsons) 15/01/02

Diet Name: **CRC98A** **Formulation date:** **28/01/98**

Ingredient Inclusion

Ingredient	Inclusion (%)	g/5kg	kg/100kg	kg/200kg	Price $/kg	$/kg
Additives						
Choline chloride	0.200	10.000	0.200	0.400	1.900	0.004
Lethicin for aquatic diets	1.000	50.000	1.000	2.000		0.000
Pre-mix minerals*	0.200	10.000	0.200	0.400	8.780	0.018
Pre-mix vitamins*	0.100	5.000	0.100	0.200	43.840	0.044
Roche Stay-C vitamin C	0.048	2.400	0.048	0.096	27.800	0.013
Colour enhancer	0.001	0.050	0.001	0.002	100.000	0.001
Diluents and Fillers						
Water	0.000	0.000	0.000	0.000	0.000	0.000
BO11C Pre-gelled starch	0.000	0.000	0.000	0.000	1.450	0.000
Energy						
Squid oil	9.000	450.000	9.000	18.000	0.800	0.072
Proteins and amino acids						
Wheat gluten	10.000	500.000	10.000	20.000	2.444	0.244
Inual Antartic krill meal	2.500	125.000	2.500	5.000	2.000	0.050
Fish meal Chilean 67% CP	27.387	1369.345	27.387	54.774	0.908	0.249
Squid meal	2.500	125.000	2.500	5.000	1.500	0.038
Fresh pilchards	47.064	2353.205	47.064	94.128	0.900	0.424
TOTAL	**100.000**	**5000.000**	**100.000**	**200.000**		**1.156**

* Gibsons tuna premix added at 3 kg/tonne (vitamins and minerals combined)

Chemical Composition

Ingredient	Composition (g/kg)				
	Dry matter	Fat	CP	DCP coeff	DCP
Choline chloride	900	0	0	0	0
Lethicin for aquatic diets	900	0	0	0	0
Pre-mix minerals	900	0	0	0	0
Pre-mix vitamins	900	0	0	0	0
Roche Stay-C vitamin C	900	0	0	0	0
Water	0	0	0	0	0
BO11C Pre-gelled starch	900	0	0	0	0
Squid oil	995	995	0	0	0
Wheat gluten	900	31	738	0.90	664.20
Inual Antartic krill meal	900	180	580	0.42	243.60
Fish meal Chilean 67% CP	920	120	670	0.55	368.50
Squid meal	920	120	650	0.30	195.00
Fresh pilchards	311	74	177	0.83	146.91

Nutrient Contributions

	Contributed (g/kg)			
Ingredient	Dry matter	Fat	CP	Digest CP
Choline chloride	1.800	0.000	0.000	0.000
Lethicin for aquatic diets	9.000	0.000	0.000	0.000
Pre-mix minerals	1.800	0.000	0.000	0.000
Pre-mix vitamins	0.900	0.000	0.000	0.000
Roche Stay-C vitamin C	0.432	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000
BO11C Pre-gelled starch	0.000	0.000	0.000	0.000
Squid oil	89.550	89.550	0.000	0.000
Wheat gluten	90.000	3.100	73.800	66.420
Inual Antartic krill meal	22.500	4.500	14.500	6.090
Fish meal Chilean 67% CP	251.959	32.864	183.492	100.921
Squid meal	23.000	3.000	16.250	4.875
Fresh pilchards	146.369	34.827	83.303	69.142
TOTAL	**637.311**	**167.842**	**371.346**	**247.448**

Dietary Amino Acid Contributions

Amino acid	Content (g/kg)	Ratio:Lys	Req Ratio	Req Amt	Def/Ex	Add
Lysine	21.587	100.00	100	14.854	6.734	-8.633
Threonine	12.236	56.68	56	6.852	5.384	-5.494
Methionine	7.571	35.07	30	2.271	5.300	-5.408
Isoleucine	14.750	68.32	57	8.407	6.342	-6.472
Leucine	22.856	105.88	90	20.570	2.286	-2.332
Tryptophan	2.621	12.14	10	0.262	2.359	-2.407
Valine	15.650	72.50	62	9.703	5.947	-6.069
Phenylalanine	13.828	64.05	47	6.499	7.329	-7.478
Histidine	7.357	34.08	30	2.207	5.150	-5.255
Arginine	21.505	99.62	73	15.698	5.806	-5.925

	Actual	Req ratio	Req Amt
Lys:CP	0.058	0.040	14.854
Lys:DCP	0.087		

Notes:

Formulated by Dr Robert van Barneveld 15/01/02

Diet Name: **CRC98B** **Formulation date:** **28/01/98**

Ingredient Inclusion

Ingredient	Inclusion (%)	g/5kg	kg/100kg	kg/200kg	Price $/kg	$/kg
Additives						
Choline chloride	0.200	10.000	0.200	0.400	1.900	0.004
Lethicin for aquatic diets	1.000	50.000	1.000	2.000		0.000
Pre-mix minerals*	0.200	10.000	0.200	0.400	8.780	0.018
Pre-mix vitamins*	0.100	5.000	0.100	0.200	43.840	0.044
Roche Stay-C vitamin C	0.048	2.400	0.048	0.096	27.800	0.013
Colour enhancer	0.001	0.050	0.001	0.002	100.000	0.001
Diluents and Fillers						
Water	15.000	750.000	15.000	30.000	0.000	0.000
BO11C Pre-gelled starch	0.000	0.000	0.000	0.000	1.450	0.000
Energy						
Squid oil	9.339	466.950	9.339	18.678	0.800	0.075
Proteins and amino acids						
Wheat gluten	15.000	750.000	15.000	30.000	2.444	0.367
Inual Antartic krill meal	2.500	125.000	2.500	5.000	2.000	0.050
Fish meal Chilean 67% CP	28.888	1444.387	28.888	57.775	0.908	0.262
Squid meal	2.500	125.000	2.500	5.000	1.500	0.038
Fresh pilchards	25.224	1261.213	25.224	50.449	0.900	0.227
TOTAL	**100.000**	**5000.000**	**100.000**	**200.000**		**1.098**

* Gibsons tuna premix added at 3 kg/tonne (vitamins and minerals combined)

Chemical Composition

Ingredient	Composition (g/kg)				
	Dry matter	Fat	CP	DCP coeff	DCP
Choline chloride	900	0	0	0	0
Lethicin for aquatic diets	900	0	0	0	0
Pre-mix minerals	900	0	0	0	0
Pre-mix vitamins	900	0	0	0	0
Roche Stay-C vitamin C	900	0	0	0	0
Water	0	0	0	0	0
BO11C Pre-gelled starch	900	0	0	0	0
Squid oil	995	995	0	0	0
Wheat gluten	900	31	738	0.90	664.20
Inual Antartic krill meal	900	180	580	0.42	243.60
Fish meal Chilean 67% CP	920	120	670	0.55	368.50
Squid meal	920	120	650	0.30	195.00
Fresh pilchards	311	74	177	0.83	146.91

Formulated by Dr Robert van Barneveld 15/01/02

Nutrient Contributions

Ingredient	Contributed (g/kg) Dry matter	Fat	CP	Digest CP
Choline chloride	1.800	0.000	0.000	0.000
Lethicin for aquatic diets	9.000	0.000	0.000	0.000
Pre-mix minerals	1.800	0.000	0.000	0.000
Pre-mix vitamins	0.900	0.000	0.000	0.000
Roche Stay-C vitamin C	0.432	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000
BO11C Pre-gelled starch	0.000	0.000	0.000	0.000
Squid oil	92.923	92.923	0.000	0.000
Wheat gluten	135.000	4.650	110.700	99.630
Inual Antartic krill meal	22.500	4.500	14.500	6.090
Fish meal Chilean 67% CP	265.767	34.665	193.548	106.451
Squid meal	23.000	3.000	16.250	4.875
Fresh pilchards	78.447	18.666	44.647	37.057
TOTAL	**631.570**	**158.404**	**379.645**	**254.103**

Dietary Amino Acid Contributions

Amino acid	Content (g/kg)	Ratio:Lys	Req Ratio	Req Amt	Def/Ex	Add
Lysine	21.512	100.00	100	15.186	6.326	-8.111
Threonine	12.945	60.18	56	7.249	5.696	-5.812
Methionine	8.105	37.68	30	2.431	5.673	-5.789
Isoleucine	15.615	72.59	57	8.901	6.714	-6.851
Leucine	25.398	118.06	90	22.858	2.540	-2.592
Tryptophan	2.842	13.21	10	0.284	2.558	-2.610
Valine	16.962	78.85	62	10.517	6.446	-6.577
Phenylalanine	15.252	70.90	47	7.169	8.084	-8.249
Histidine	6.701	31.15	30	2.010	4.691	-4.786
Arginine	22.331	103.81	73	16.301	6.029	-6.152

	Actual	Req ratio	Req Amt
Lys:CP	0.057	0.040	15.186
Lys:DCP	0.085		

Notes:

Formulated by Dr Robert van Barneveld 15/01/02

Diet Name: **CRC98C** **Formulation date:** **8/02/98**

Ingredient Inclusion

Ingredient	Inclusion (%)	g/5kg	kg/100kg	kg/200kg	Price $/kg	$/kg
Additives						
Choline chloride	0.200	10.000	0.200	0.400	1.900	0.004
Lethicin for aquatic diets	1.000	50.000	1.000	2.000		0.000
Pre-mix minerals*	0.200	10.000	0.200	0.400	8.780	0.018
Pre-mix vitamins*	0.100	5.000	0.100	0.200	43.840	0.044
Roche Stay-C vitamin C	0.048	2.400	0.048	0.096	27.800	0.013
Colour enhancer	0.001	0.050	0.001	0.002	100.000	0.001
Diluents and Fillers						
Water	12.182	609.111	12.182	24.364	0.000	0.000
BO11C Pre-gelled starch	0.000	0.000	0.000	0.000	1.450	0.000
Energy						
Squid oil	3.395	169.769	3.395	6.791	0.800	0.027
Proteins and amino acids						
Wheat gluten	15.000	750.000	15.000	30.000	2.444	0.367
Inual Antartic krill meal	2.500	125.000	2.500	5.000	2.000	0.050
Fish meal Chilean 67% CP	35.737	1786.837	35.737	71.473	0.908	0.324
Squid meal	2.500	125.000	2.500	5.000	1.500	0.038
Bloodmeal (Ring-dried 85% CP)	0.000	0.000	0.000	0.000	1.000	0.000
Fresh pilchards	27.137	1356.834	27.137	54.273	0.900	0.244
TOTAL	**100.000**	**5000.000**	**100.000**	**200.000**		**1.130**

* Gibsons tuna premix added at 3 kg/tonne (vitamins and minerals combined)

Chemical Composition

Ingredient	Composition (g/kg)				
	Dry matter	Fat	CP	DCP coeff	DCP
Choline chloride	900	0	0	0	0
Lethicin for aquatic diets	900	0	0	0	0
Pre-mix minerals	900	0	0	0	0
Pre-mix vitamins	900	0	0	0	0
Roche Stay-C vitamin C	900	0	0	0	0
Water	0	0	0	0	0
BO11C Pre-gelled starch	900	0	0	0	0
Squid oil	995	995	0	0	0
Wheat gluten	900	31	738	0.90	664.20
Inual Antartic krill meal	900	180	580	0.42	243.60
Fish meal Chilean 67% CP	920	120	670	0.55	368.50
Squid meal	920	120	650	0.30	195.00
Bloodmeal	900	8.4	850	0.80	680.00
Fresh pilchards	311	74	177	0.83	146.91

Nutrient Contributions

Ingredient	Contributed (g/kg)			
	Dry matter	Fat	CP	Digest CP
Choline chloride	1.800	0.000	0.000	0.000
Lethicin for aquatic diets	9.000	0.000	0.000	0.000
Pre-mix minerals	1.800	0.000	0.000	0.000
Pre-mix vitamins	0.900	0.000	0.000	0.000
Roche Stay-C vitamin C	0.432	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000
BO11C Pre-gelled starch	0.000	0.000	0.000	0.000
Squid oil	33.784	33.784	0.000	0.000
Wheat gluten	135.000	4.650	110.700	99.630
Inual Antartic krill meal	22.500	4.500	14.500	6.090
Fish meal Chilean 67% CP	328.778	42.884	239.436	131.690
Squid meal	23.000	3.000	16.250	4.875
Blood meal	0.000	0.000	0.000	0.000
Fresh pilchards	84.395	20.081	48.032	39.866
TOTAL	**641.389**	**108.899**	**428.918**	**282.151**

Dietary Amino Acid Contributions

Amino acid	Content (g/kg)	Ratio:Lys	Req Ratio	Req Amt	Def/Ex	Add
Lysine	25.260	100.00	100	17.157	8.103	-10.389
Threonine	14.848	58.78	56	8.315	6.533	-6.666
Methionine	9.397	37.20	30	2.819	6.578	-6.712
Isoleucine	17.765	70.33	57	10.126	7.639	-7.795
Leucine	28.899	114.41	90	26.009	2.890	-2.949
Tryptophan	3.397	13.45	10	0.340	3.057	-3.120
Valine	19.365	76.66	62	12.006	7.359	-7.509
Phenylalanine	17.120	67.78	47	8.046	9.074	-9.259
Histidine	7.904	31.29	30	2.371	5.533	-5.645
Arginine	25.878	102.45	73	18.891	6.987	-7.130

	Actual	Req ratio	Req Amt
Lys:CP	0.059	0.040	17.157
Lys:DCP	0.090		

Notes:

Formulated by Dr Robert van Barneveld 15/01/02

Appendix IV:
Experiment 2 Diet Formulations - 1999

Ingredients

Amino acid	Content (g/kg)						
	Pilchards	Chilean	Meat meal	Krill	Casein	Blood	Gluten
Lysine	7.600	52.600	25.630	28.000	72.800	74.040	15.900
Threonine	3.540	26.800	16.020	16.000	40.300	40.890	21.600
Methionine	1.300	18.500	7.010	14.000	27.100	10.490	10.800
Isoleucine	4.990	30.000	13.620	17.000	50.800	8.280	30.100
Leucine	4.000	50.000	29.880	28.000	83.300	108.180	53.300
Tryptophan	0.000	8.100	2.900	0.000	14.900	12.690	2.000
Valine	3.750	34.030	22.570	19.000	63.100	76.290	32.400
Phenylalanine	3.840	26.200	16.780	18.000	46.400	54.630	37.400
Histidine	4.480	16.310	8.340	8.700	27.100	54.480	1.560
Arginine	6.430	50.000	34.010	23.000	35.300	36.140	29.600

Amino acid	Content (g/kg)	
	Canola	Soybean
Lysine	20.260	29.630
Threonine	15.740	18.350
Methionine	7.400	6.590
Isoleucine	13.740	23.020
Leucine	24.920	36.960
Tryptophan	4.280	6.960
Valine	17.850	23.570
Phenylalanine	14.200	23.700
Histidine	9.670	12.240
Arginine	21.820	35.770

Vitamins and Minerals

Ingredient	Units	Gibsons 98	
		unit/3kg	unit/kg
Vitamin A	MIU	3.000	1.000
Vitamin D3	MIU	0.500	0.167
Thiamine (B1)	g	15.000	5.000
Riboflavin (B2)	g	20.000	6.667
Pyridoxine (B6)	g	12.000	4.000
Vitamin B12	mg	30.000	10.000
Biotin	g	0.300	0.100
Vitamin K3	g	7.000	2.333
Pantothenic Acid	g	30.000	10.000
Niacin	g	65.000	21.667
Inositol	g	50.000	16.667
Vitamin E ADS	g	100.000	33.333
Folic acid S.D.	g	4.000	1.333
Ethoxyquin	g	150.000	50.000
Cobalt	g	1.000	0.333
Iodine	g	1.100	0.367
Copper	g	3.000	1.000
Magnesium	g	50.000	16.667
Manganese	g	20.000	6.667
Iron	g	20.000	6.667
Bioplex iron	g	20.000	6.667
Zinc	g	30.000	10.000
Aquastab C 42%	g	60.000	20.000

Notes:

Vitamin and mineral levels to be adjusted for 1999 experiments

Diet Name: **99A** **Formulation date:** **21/04/99**

Ingredient Inclusion

Ingredient		Incl. (%)	g/5kg	kg/100kg	kg/200kg	Price $/kg	$/kg
Additives							
Choline chloride	0.200	0.195	9.739	0.195	0.390	1.900	0.004
Lethicin for aquatic diets	1.000	0.974	48.693	0.974	1.948	1.000	0.010
Pre-mix minerals	0.200	0.195	9.739	0.195	0.390	8.780	0.018
Pre-mix vitamins	0.100	0.097	4.869	0.097	0.195	43.840	0.044
Roche Stay-C vitamin C	0.048	0.047	2.337	0.047	0.093	27.800	0.013
Diluents and Fillers							
Water	4.000	3.895	194.772	3.895	7.791	0.000	0.000
Energy							
Fish oil	3.177	3.094	154.687	3.094	6.187	1.100	0.035
Proteins and amino acids							
Wheat gluten	10.000	9.739	486.930	9.739	19.477	3.000	0.300
Blood	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Meat meal	0.000	0.000	0.000	0.000	0.000	0.550	0.000
Soybean meal	0.000	0.000	0.000	0.000	0.000	0.480	0.000
Canola meal	0.000	0.000	0.000	0.000	0.000	0.380	0.000
Casein	0.000	0.000	0.000	0.000	0.000	1.500	0.000
Inual Antartic krill meal	2.500	2.435	121.733	2.435	4.869	2.500	0.063
Fish meal Chilean 67% CP	32.907	32.047	1602.341	32.047	64.094	1.400	0.461
Fresh pilchards	48.552	47.283	2364.160	47.283	94.566	0.900	0.437
TOTAL	**102.684**	**100.000**	**5000.000**	**100.000**	**200.000**		**1.384**

Chemical Composition

	Composition g/kg)				
Ingredient	Dry matter	Fat	CP	DCP coeff	DCP
Choline chloride	900	0.000	0	0	0
Lethicin for aquatic diets	900	0.000	0	0	0
Pre-mix minerals	900	0.000	0	0	0
Pre-mix vitamins	900	0.000	0	0	0
Roche Stay-C vitamin C	900	0.000	0	0	0
Water	0	0.000	0	0	0
Canola meal (solvent extracted)	900	25.000	350	0.6	210
Fish oil	995	995.000	0	0	0
Wheat gluten	900	31.000	738	0.90	664.20
Inual Antartic krill meal	900	180.000	580	0.42	243.60
Fish meal Chilean 67% CP	920	120.000	670	0.55	368.50
Casein	920	12.000	877	0.90	789.30
Fresh pilchards	340	92.000	210	0.83	174.30
Soybean meal (solvent)	900	25.000	480	0.6	288.00
Meat meal (50% CP)	930	100.000	500	0.8	400.00
Blood meal (85% Ring-dried)	900	8.400	850	0.85	722.50

Nutrient Contributions

Ingredient	Composition g/kg)			
	Dry matter	Fat	CP	Digest CP
Choline chloride	1.800	0.000	0.000	0.000
Lethicin for aquatic diets	9.000	0.000	0.000	0.000
Pre-mix minerals	1.800	0.000	0.000	0.000
Pre-mix vitamins	0.900	0.000	0.000	0.000
Roche Stay-C vitamin C	0.432	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000
Canola meal (solvent extracted)	0.000	0.000	0.000	0.000
Fish oil	31.609	31.609	0.000	0.000
Wheat gluten	90.000	3.100	73.800	66.420
Inual Antartic krill meal	22.500	4.500	14.500	6.090
Fish meal Chilean 67% CP	302.744	39.488	220.477	121.262
Casein	0.000	0.000	0.000	0.000
Fresh pilchards	165.078	44.668	101.960	84.627
Soybean meal (solvent extracted)	0.000	0.000	0.000	0.000
Meat meal (50% CP)	0.000	0.000	0.000	0.000
Blood meal (85% Ring-dried)	0.000	0.000	0.000	0.000
TOTAL	**609.503**	**120.141**	**400.000**	**271.122**

Dietary Amino Acid Contributions

Amino acid	Content (g/kg)	Ratio:Lys	Req Ratio	Req Amt	Def/Ex	Add
Lysine	23.289	100.000	100	16.000	7.289	-9.345
Threonine	13.098	56.240	56	7.335	5.763	-5.881
Methionine	8.149	34.991	30	2.445	5.704	-5.821
Isoleucine	15.730	67.542	57	8.966	6.764	-6.902
Leucine	24.426	104.880	90	21.983	2.443	-2.492
Tryptophan	2.865	12.304	10	0.287	2.579	-2.632
Valine	16.734	71.853	62	10.375	6.359	-6.489
Phenylalanine	14.676	63.017	47	6.898	7.778	-7.937
Histidine	7.916	33.989	30	2.375	5.541	-5.654
Arginine	23.110	99.233	73	16.871	6.240	-6.367

	Actual	Req ratio	Req Amt
Lys:CP	0.058	0.040	16.000
Lys:DCP	0.086		

Notes:
Utilises 99A mash.

Formulated by Dr Robert van Barneveld 15/01/02

Diet Name: **99B** **Formulation date:** **21/04/99**

Ingredient Inclusion

Ingredient		Incl. (%)	g/5kg	kg/100kg	kg/200kg	Price $/kg	$/kg
Additives							
Choline chloride	0.200	0.192	9.620	0.192	0.385	1.900	0.004
Lethicin for aquatic diets	1.000	0.962	48.102	0.962	1.924	1.000	0.010
Pre-mix minerals	0.200	0.192	9.620	0.192	0.385	8.780	0.018
Pre-mix vitamins	0.100	0.096	4.810	0.096	0.192	43.840	0.044
Roche Stay-C vitamin C	0.048	0.046	2.309	0.046	0.092	27.800	0.013
Diluents and Fillers							
Water	4.000	3.848	192.406	3.848	7.696	0.000	0.000
Energy							
Fish oil	6.574	6.324	316.197	6.324	12.648	1.100	0.072
Proteins and amino acids							
Wheat gluten	10.000	9.620	481.015	9.620	19.241	3.000	0.300
Blood	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Meat meal	0.000	0.000	0.000	0.000	0.000	0.550	0.000
Soybean meal	0.000	0.000	0.000	0.000	0.000	0.480	0.000
Canola meal	0.000	0.000	0.000	0.000	0.000	0.380	0.000
Casein	0.000	0.000	0.000	0.000	0.000	1.500	0.000
Inual Antartic krill meal	2.500	2.405	120.254	2.405	4.810	2.500	0.063
Fish meal Chilean 67% CP	34.979	33.651	1682.542	33.651	67.302	1.400	0.490
Fresh pilchards	44.346	42.662	2133.124	42.662	85.325	0.900	0.399
TOTAL	**103.947**	**100.000**	**5000.000**	**100.000**	**200.000**		**1.412**

Chemical Composition

	Composition g/kg)				
Ingredient	Dry matter	Fat	CP	DCP coeff	DCP
Choline chloride	900	0.000	0	0	0
Lethicin for aquatic diets	900	0.000	0	0	0
Pre-mix minerals	900	0.000	0	0	0
Pre-mix vitamins	900	0.000	0	0	0
Roche Stay-C vitamin C	900	0.000	0	0	0
Water	0	0.000	0	0	0
Canola meal (solvent extracted)	900	25.000	350	0.6	210
Fish oil	995	995.000	0	0	0
Wheat gluten	900	31.000	738	0.90	664.20
Inual Antartic krill meal	900	180.000	580	0.42	243.60
Fish meal Chilean 67% CP	920	120.000	670	0.55	368.50
Casein	920	12.000	877	0.90	789.30
Fresh pilchards	340	92.000	210	0.83	174.30
Soybean meal (solvent)	900	25.000	480	0.6	288.00
Meat meal (50% CP)	930	100.000	500	0.8	400.00
Blood meal (85% Ring-dried)	900	8.400	850	0.85	722.50

Nutrient Contributions

Ingredient	Composition g/kg)			
	Dry matter	Fat	CP	Digest CP
Choline chloride	1.800	0.000	0.000	0.000
Lethicin for aquatic diets	9.000	0.000	0.000	0.000
Pre-mix minerals	1.800	0.000	0.000	0.000
Pre-mix vitamins	0.900	0.000	0.000	0.000
Roche Stay-C vitamin C	0.432	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000
Canola meal (solvent extracted)	0.000	0.000	0.000	0.000
Fish oil	65.407	65.407	0.000	0.000
Wheat gluten	90.000	3.100	73.800	66.420
Inual Antartic krill meal	22.500	4.500	14.500	6.090
Fish meal Chilean 67% CP	321.807	41.975	234.359	128.898
Casein	0.000	0.000	0.000	0.000
Fresh pilchards	150.777	40.799	93.127	77.296
Soybean meal (solvent extracted)	0.000	0.000	0.000	0.000
Meat meal (50% CP)	0.000	0.000	0.000	0.000
Blood meal (85% Ring-dried)	0.000	0.000	0.000	0.000
TOTAL	**639.195**	**149.865**	**399.999**	**268.121**

Dietary Amino Acid Contributions

Amino acid	Content (g/kg)	Ratio:Lys	Req Ratio	Req Amt	Def/Ex	Add
Lysine	24.059	100.000	100	16.000	8.059	-10.332
Threonine	13.504	56.129	56	7.562	5.942	-6.063
Methionine	8.478	35.236	30	2.543	5.934	-6.055
Isoleucine	16.142	67.091	57	9.201	6.941	-7.083
Leucine	25.293	105.129	90	22.764	2.529	-2.581
Tryptophan	3.033	12.608	10	0.303	2.730	-2.786
Valine	17.281	71.828	62	10.714	6.567	-6.701
Phenylalanine	15.057	62.585	47	7.077	7.980	-8.143
Histidine	8.065	33.523	30	2.420	5.646	-5.761
Arginine	23.876	99.238	73	17.429	6.447	-6.578

	Actual	Req ratio	Req Amt
Lys:CP	0.060	0.040	16.000
Lys:DCP	0.090		

Notes:
Utilises existing 99B mash.

Formulated by Dr Robert van Barneveld 15/01/02

Diet Name: **99C** **Formulation date:** **21/04/99**

Ingredient Inclusion

Ingredient		Incl. (%)	g/5kg	kg/100kg	kg/200kg	Price $/kg	$/kg
Additives							
Choline chloride	0.200	0.193	9.670	0.193	0.387	1.900	0.004
Lethicin for aquatic diets	1.000	0.967	48.349	0.967	1.934	1.000	0.010
Pre-mix minerals	0.200	0.193	9.670	0.193	0.387	8.780	0.018
Pre-mix vitamins	0.100	0.097	4.835	0.097	0.193	43.840	0.044
Roche Stay-C vitamin C	0.048	0.046	2.321	0.046	0.093	27.800	0.013
Diluents and Fillers							
Water	5.000	4.835	241.744	4.835	9.670	0.000	0.000
Energy							
Fish oil	10.063	9.730	486.523	9.730	19.461	1.100	0.111
Proteins and amino acids							
Wheat gluten	10.000	9.670	483.487	9.670	19.339	3.000	0.300
Blood	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Meat meal	0.000	0.000	0.000	0.000	0.000	0.550	0.000
Soybean meal	0.000	0.000	0.000	0.000	0.000	0.480	0.000
Canola meal	0.000	0.000	0.000	0.000	0.000	0.380	0.000
Casein	0.000	0.000	0.000	0.000	0.000	1.500	0.000
Inual Antartic krill meal	2.500	2.417	120.872	2.417	4.835	2.500	0.063
Fish meal Chilean 67% CP	36.809	35.593	1779.667	35.593	71.187	1.400	0.515
Fresh pilchards	37.496	36.257	1812.864	36.257	72.515	0.900	0.337
TOTAL	**103.415**	**100.000**	**5000.000**	**100.000**	**200.000**		**1.415**

Chemical Composition

	Composition g/kg)				
Ingredient	Dry matter	Fat	CP	DCP coeff	DCP
Choline chloride	900	0.000	0	0	0
Lethicin for aquatic diets	900	0.000	0	0	0
Pre-mix minerals	900	0.000	0	0	0
Pre-mix vitamins	900	0.000	0	0	0
Roche Stay-C vitamin C	900	0.000	0	0	0
Water	0	0.000	0	0	0
Canola meal (solvent extracted)	900	25.000	350	0.6	210
Fish oil	995	995.000	0	0	0
Wheat gluten	900	31.000	738	0.90	664.20
Inual Antartic krill meal	900	180.000	580	0.42	243.60
Fish meal Chilean 67% CP	920	120.000	670	0.55	368.50
Casein	920	12.000	877	0.90	789.30
Fresh pilchards	340	92.000	210	0.83	174.30
Soybean meal (solvent)	900	25.000	480	0.6	288.00
Meat meal (50% CP)	930	100.000	500	0.8	400.00
Blood meal (85% Ring-dried)	900	8.400	850	0.85	722.50

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Nutrient Contributions

Ingredient	Dry matter	Fat	CP	Digest CP
	Composition g/kg)			
Choline chloride	1.800	0.000	0.000	0.000
Lethicin for aquatic diets	9.000	0.000	0.000	0.000
Pre-mix minerals	1.800	0.000	0.000	0.000
Pre-mix vitamins	0.900	0.000	0.000	0.000
Roche Stay-C vitamin C	0.432	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000
Canola meal (solvent extracted)	0.000	0.000	0.000	0.000
Fish oil	100.125	100.125	0.000	0.000
Wheat gluten	90.000	3.100	73.800	66.420
Inual Antartic krill meal	22.500	4.500	14.500	6.090
Fish meal Chilean 67% CP	338.643	44.171	246.620	135.641
Casein	0.000	0.000	0.000	0.000
Fresh pilchards	127.485	34.496	78.741	65.355
Soybean meal (solvent extracted)	0.000	0.000	0.000	0.000
Meat meal (50% CP)	0.000	0.000	0.000	0.000
Blood meal (85% Ring-dried)	0.000	0.000	0.000	0.000
TOTAL	**669.808**	**180.236**	**400.000**	**264.473**

Dietary Amino Acid Contributions

Amino acid	Content (g/kg)	Ratio:Lys	Req Ratio	Req Amt	Def/Ex	Add
Lysine	24.501	100.000	100	16.000	8.501	-10.899
Threonine	13.752	56.129	56	7.701	6.051	-6.174
Methionine	8.727	35.619	30	2.618	6.109	-6.234
Isoleucine	16.349	66.726	57	9.319	7.030	-7.173
Leucine	25.934	105.849	90	23.341	2.593	-2.646
Tryptophan	3.182	12.985	10	0.318	2.863	-2.922
Valine	17.647	72.026	62	10.941	6.706	-6.843
Phenylalanine	15.274	62.339	47	7.179	8.095	-8.260
Histidine	8.057	32.883	30	2.417	5.640	-5.755
Arginine	24.350	99.385	73	17.776	6.575	-6.709

	Actual	Req ratio	Req Amt
Lys:CP	0.061	0.040	16.000
Lys:DCP	0.093		

Notes:
Utilises existing 99C mash.

Formulated by Dr Robert van Barneveld 15/01/02

Appendix V:
Experiment 2 - Feeding Rate Calculations

Calculation Instructions

1. Feed fish on diet 99A to satiation (maximum 15 minutes)
2. Record the weight of feed offered
3. Use the "Daily Feed Intake Calculator" to determine the feed offered per fish for that day

Example: If 25 kg of feed was offered and there are 28 fish in the pen then they were offered 893 g feed per fish

4. Repeat for the replicate cage on diet 99A fed to satiation
5. Mean the g feed/fish/day for the two cages
6. Round the g feed/fish/day UP TO the nearest 10 g

Example: If 893 g feed per fish was offered this will round up to 900 g feed per fish

7. Use the 99A 80% Restriction Feed Quantity Calculator to determine the kg of feed that needs to be added to the cages fed 99A restrictively.

Example: If 900 g feed per fish was offered to cages on 99A to satiation and there are 27 fish in the cage on 99A restricted, then 19.4 kg of feed is to be offered to this cage

8. Repeat for the second cage on 99A restricted

9. Use the 99B Restriction Feed Quantity Calculator to determine the kg of feed that needs to be added to the cages fed 99B restrictively.

Example: If 900 g feed per fish was offered to cages on 99A to satiation and there are 27 fish in the cage on 99B restricted, then 20.7 kg of feed is to be offered to this cage

10. Repeat for the second cage on 99B restricted

11. Use the 99C Restriction Feed Quantity Calculator to determine the kg of feed that needs to be added to the cages fed 99C restrictively.

Example: If 900 g feed per fish was offered to cages on 99A to satiation and there are 27 fish in the cage on 99C restricted, then 22.3 kg of feed is to be offered to this cage

12. Repeat for the second cage on 99C restricted

13. Complete the above procedure at each feeding

Daily Feed Intake Calculator (g feed/fish/day)

	Number of fish per cage										
Kg Fed	20	21	22	23	24	25	26	27	28	29	30
10.0	500	476	455	435	417	400	385	370	357	345	333
10.5	525	500	477	457	438	420	404	389	375	362	350
11.0	550	524	500	478	458	440	423	407	393	379	367
11.5	575	548	523	500	479	460	442	426	411	397	383
12.0	600	571	545	522	500	480	462	444	429	414	400
12.5	625	595	568	543	521	500	481	463	446	431	417
13.0	650	619	591	565	542	520	500	481	464	448	433
13.5	675	643	614	587	563	540	519	500	482	466	450
14.0	700	667	636	609	583	560	538	519	500	483	467
14.5	725	690	659	630	604	580	558	537	518	500	483
15.0	750	714	682	652	625	600	577	556	536	517	500
15.5	775	738	705	674	646	620	596	574	554	534	517
16.0	800	762	727	696	667	640	615	593	571	552	533
16.5	825	786	750	717	688	660	635	611	589	569	550
17.0	850	810	773	739	708	680	654	630	607	586	567
17.5	875	833	795	761	729	700	673	648	625	603	583
18.0	900	857	818	783	750	720	692	667	643	621	600
18.5	925	881	841	804	771	740	712	685	661	638	617
19.0	950	905	864	826	792	760	731	704	679	655	633
19.5	975	929	886	848	813	780	750	722	696	672	650
20.0	1000	952	909	870	833	800	769	741	714	690	667
20.5	1025	976	932	891	854	820	788	759	732	707	683
21.0	1050	1000	955	913	875	840	808	778	750	724	700
21.5	1075	1024	977	935	896	860	827	796	768	741	717
22.0	1100	1048	1000	957	917	880	846	815	786	759	733
22.5	1125	1071	1023	978	938	900	865	833	804	776	750
23.0	1150	1095	1045	1000	958	920	885	852	821	793	767
23.5	1175	1119	1068	1022	979	940	904	870	839	810	783
24.0	1200	1143	1091	1043	1000	960	923	889	857	828	800
24.5	1225	1167	1114	1065	1021	980	942	907	875	845	817
25.0	1250	1190	1136	1087	1042	1000	962	926	893	862	833
25.5	1275	1214	1159	1109	1063	1020	981	944	911	879	850
26.0	1300	1238	1182	1130	1083	1040	1000	963	929	897	867
26.5	1325	1262	1205	1152	1104	1060	1019	981	946	914	883
27.0	1350	1286	1227	1174	1125	1080	1038	1000	964	931	900
27.5	1375	1310	1250	1196	1146	1100	1058	1019	982	948	917
28.0	1400	1333	1273	1217	1167	1120	1077	1037	1000	966	933
28.5	1425	1357	1295	1239	1188	1140	1096	1056	1018	983	950
29.0	1450	1381	1318	1261	1208	1160	1115	1074	1036	1000	967
29.5	1475	1405	1341	1283	1229	1180	1135	1093	1054	1017	983
30.0	1500	1429	1364	1304	1250	1200	1154	1111	1071	1034	1000

* Round the calculated feed intake/fish/day up to the nearest 10 (ie 1154 becomes 1160)

** Kg Fed is based on the mean quantity of feed offered to the two experimental cages fed diet 99A to satiation

99A 80% Restriction Feed Quantity Calculator (kg feed/cage)

	Number of fish per cage										
99A/fish*	20	21	22	23	24	25	26	27	28	29	30
300	4.8	5.0	5.3	5.5	5.8	6.0	6.2	6.5	6.7	7.0	7.2
310	5.0	5.2	5.5	5.7	6.0	6.2	6.4	6.7	6.9	7.2	7.4
320	5.1	5.4	5.6	5.9	6.1	6.4	6.7	6.9	7.2	7.4	7.7
330	5.3	5.5	5.8	6.1	6.3	6.6	6.9	7.1	7.4	7.7	7.9
340	5.4	5.7	6.0	6.3	6.5	6.8	7.1	7.3	7.6	7.9	8.2
350	5.6	5.9	6.2	6.4	6.7	7.0	7.3	7.6	7.8	8.1	8.4
360	5.8	6.0	6.3	6.6	6.9	7.2	7.5	7.8	8.1	8.4	8.6
370	5.9	6.2	6.5	6.8	7.1	7.4	7.7	8.0	8.3	8.6	8.9
380	6.1	6.4	6.7	7.0	7.3	7.6	7.9	8.2	8.5	8.8	9.1
390	6.2	6.6	6.9	7.2	7.5	7.8	8.1	8.4	8.7	9.0	9.4
400	6.4	6.7	7.0	7.4	7.7	8.0	8.3	8.6	9.0	9.3	9.6
410	6.6	6.9	7.2	7.5	7.9	8.2	8.5	8.9	9.2	9.5	9.8
420	6.7	7.1	7.4	7.7	8.1	8.4	8.7	9.1	9.4	9.7	10.1
430	6.9	7.2	7.6	7.9	8.3	8.6	8.9	9.3	9.6	10.0	10.3
440	7.0	7.4	7.7	8.1	8.4	8.8	9.2	9.5	9.9	10.2	10.6
450	7.2	7.6	7.9	8.3	8.6	9.0	9.4	9.7	10.1	10.4	10.8
460	7.4	7.7	8.1	8.5	8.8	9.2	9.6	9.9	10.3	10.7	11.0
470	7.5	7.9	8.3	8.6	9.0	9.4	9.8	10.2	10.5	10.9	11.3
480	7.7	8.1	8.4	8.8	9.2	9.6	10.0	10.4	10.8	11.1	11.5
490	7.8	8.2	8.6	9.0	9.4	9.8	10.2	10.6	11.0	11.4	11.8
500	8.0	8.4	8.8	9.2	9.6	10.0	10.4	10.8	11.2	11.6	12.0
510	8.2	8.6	9.0	9.4	9.8	10.2	10.6	11.0	11.4	11.8	12.2
520	8.3	8.7	9.2	9.6	10.0	10.4	10.8	11.2	11.6	12.1	12.5
530	8.5	8.9	9.3	9.8	10.2	10.6	11.0	11.4	11.9	12.3	12.7
540	8.6	9.1	9.5	9.9	10.4	10.8	11.2	11.7	12.1	12.5	13.0
550	8.8	9.2	9.7	10.1	10.6	11.0	11.4	11.9	12.3	12.8	13.2
560	9.0	9.4	9.9	10.3	10.8	11.2	11.6	12.1	12.5	13.0	13.4
570	9.1	9.6	10.0	10.5	10.9	11.4	11.9	12.3	12.8	13.2	13.7
580	9.3	9.7	10.2	10.7	11.1	11.6	12.1	12.5	13.0	13.5	13.9
590	9.4	9.9	10.4	10.9	11.3	11.8	12.3	12.7	13.2	13.7	14.2
600	9.6	10.1	10.6	11.0	11.5	12.0	12.5	13.0	13.4	13.9	14.4
610	9.8	10.2	10.7	11.2	11.7	12.2	12.7	13.2	13.7	14.2	14.6
620	9.9	10.4	10.9	11.4	11.9	12.4	12.9	13.4	13.9	14.4	14.9
630	10.1	10.6	11.1	11.6	12.1	12.6	13.1	13.6	14.1	14.6	15.1
640	10.2	10.8	11.3	11.8	12.3	12.8	13.3	13.8	14.3	14.8	15.4
650	10.4	10.9	11.4	12.0	12.5	13.0	13.5	14.0	14.6	15.1	15.6
660	10.6	11.1	11.6	12.1	12.7	13.2	13.7	14.3	14.8	15.3	15.8
670	10.7	11.3	11.8	12.3	12.9	13.4	13.9	14.5	15.0	15.5	16.1
680	10.9	11.4	12.0	12.5	13.1	13.6	14.1	14.7	15.2	15.8	16.3
690	11.0	11.6	12.1	12.7	13.2	13.8	14.4	14.9	15.5	16.0	16.6
700	11.2	11.8	12.3	12.9	13.4	14.0	14.6	15.1	15.7	16.2	16.8
710	11.4	11.9	12.5	13.1	13.6	14.2	14.8	15.3	15.9	16.5	17.0
720	11.5	12.1	12.7	13.2	13.8	14.4	15.0	15.6	16.1	16.7	17.3
730	11.7	12.3	12.8	13.4	14.0	14.6	15.2	15.8	16.4	16.9	17.5
740	11.8	12.4	13.0	13.6	14.2	14.8	15.4	16.0	16.6	17.2	17.8
750	12.0	12.6	13.2	13.8	14.4	15.0	15.6	16.2	16.8	17.4	18.0
760	12.2	12.8	13.4	14.0	14.6	15.2	15.8	16.4	17.0	17.6	18.2
770	12.3	12.9	13.6	14.2	14.8	15.4	16.0	16.6	17.2	17.9	18.5
780	12.5	13.1	13.7	14.4	15.0	15.6	16.2	16.8	17.5	18.1	18.7
790	12.6	13.3	13.9	14.5	15.2	15.8	16.4	17.1	17.7	18.3	19.0
800	12.8	13.4	14.1	14.7	15.4	16.0	16.6	17.3	17.9	18.6	19.2
810	13.0	13.6	14.3	14.9	15.6	16.2	16.8	17.5	18.1	18.8	19.4
820	13.1	13.8	14.4	15.1	15.7	16.4	17.1	17.7	18.4	19.0	19.7
830	13.3	13.9	14.6	15.3	15.9	16.6	17.3	17.9	18.6	19.3	19.9
840	13.4	14.1	14.8	15.5	16.1	16.8	17.5	18.1	18.8	19.5	20.2
850	13.6	14.3	15.0	15.6	16.3	17.0	17.7	18.4	19.0	19.7	20.4
860	13.8	14.4	15.1	15.8	16.5	17.2	17.9	18.6	19.3	20.0	20.6
870	13.9	14.6	15.3	16.0	16.7	17.4	18.1	18.8	19.5	20.2	20.9
880	14.1	14.8	15.5	16.2	16.9	17.6	18.3	19.0	19.7	20.4	21.1
890	14.2	15.0	15.7	16.4	17.1	17.8	18.5	19.2	19.9	20.6	21.4
900	14.4	15.1	15.8	16.6	17.3	18.0	18.7	19.4	20.2	20.9	21.6
910	14.6	15.3	16.0	16.7	17.5	18.2	18.9	19.7	20.4	21.1	21.8
920	14.7	15.5	16.2	16.9	17.7	18.4	19.1	19.9	20.6	21.3	22.1

99A 80% Restriction Feed Quantity Calculator (kg feed/cage)

Number of fish per cage

99A/fish*	20	21	22	23	24	25	26	27	28	29	30
930	14.9	15.6	16.4	17.1	17.9	18.6	19.3	20.1	20.8	21.6	22.3
940	15.0	15.8	16.5	17.3	18.0	18.8	19.6	20.3	21.1	21.8	22.6
950	15.2	16.0	16.7	17.5	18.2	19.0	19.8	20.5	21.3	22.0	22.8
960	15.4	16.1	16.9	17.7	18.4	19.2	20.0	20.7	21.5	22.3	23.0
970	15.5	16.3	17.1	17.8	18.6	19.4	20.2	21.0	21.7	22.5	23.3
980	15.7	16.5	17.2	18.0	18.8	19.6	20.4	21.2	22.0	22.7	23.5
990	15.8	16.6	17.4	18.2	19.0	19.8	20.6	21.4	22.2	23.0	23.8
1000	16.0	16.8	17.6	18.4	19.2	20.0	20.8	21.6	22.4	23.2	24.0
1010	16.2	17.0	17.8	18.6	19.4	20.2	21.0	21.8	22.6	23.4	24.2
1020	16.3	17.1	18.0	18.8	19.6	20.4	21.2	22.0	22.8	23.7	24.5
1030	16.5	17.3	18.1	19.0	19.8	20.6	21.4	22.2	23.1	23.9	24.7
1040	16.6	17.5	18.3	19.1	20.0	20.8	21.6	22.5	23.3	24.1	25.0
1050	16.8	17.6	18.5	19.3	20.2	21.0	21.8	22.7	23.5	24.4	25.2
1060	17.0	17.8	18.7	19.5	20.4	21.2	22.0	22.9	23.7	24.6	25.4
1070	17.1	18.0	18.8	19.7	20.5	21.4	22.3	23.1	24.0	24.8	25.7
1080	17.3	18.1	19.0	19.9	20.7	21.6	22.5	23.3	24.2	25.1	25.9
1090	17.4	18.3	19.2	20.1	20.9	21.8	22.7	23.5	24.4	25.3	26.2
1100	17.6	18.5	19.4	20.2	21.1	22.0	22.9	23.8	24.6	25.5	26.4
1110	17.8	18.6	19.5	20.4	21.3	22.2	23.1	24.0	24.9	25.8	26.6
1120	17.9	18.8	19.7	20.6	21.5	22.4	23.3	24.2	25.1	26.0	26.9
1130	18.1	19.0	19.9	20.8	21.7	22.6	23.5	24.4	25.3	26.2	27.1
1140	18.2	19.2	20.1	21.0	21.9	22.8	23.7	24.6	25.5	26.4	27.4
1150	18.4	19.3	20.2	21.2	22.1	23.0	23.9	24.8	25.8	26.7	27.6
1160	18.6	19.5	20.4	21.3	22.3	23.2	24.1	25.1	26.0	26.9	27.8
1170	18.7	19.7	20.6	21.5	22.5	23.4	24.3	25.3	26.2	27.1	28.1
1180	18.9	19.8	20.8	21.7	22.7	23.6	24.5	25.5	26.4	27.4	28.3
1190	19.0	20.0	20.9	21.9	22.8	23.8	24.8	25.7	26.7	27.6	28.6
1200	19.2	20.2	21.1	22.1	23.0	24.0	25.0	25.9	26.9	27.8	28.8
1210	19.4	20.3	21.3	22.3	23.2	24.2	25.2	26.1	27.1	28.1	29.0
1220	19.5	20.5	21.5	22.4	23.4	24.4	25.4	26.4	27.3	28.3	29.3
1230	19.7	20.7	21.6	22.6	23.6	24.6	25.6	26.6	27.6	28.5	29.5
1240	19.8	20.8	21.8	22.8	23.8	24.8	25.8	26.8	27.8	28.8	29.8
1250	20.0	21.0	22.0	23.0	24.0	25.0	26.0	27.0	28.0	29.0	30.0
1260	20.2	21.2	22.2	23.2	24.2	25.2	26.2	27.2	28.2	29.2	30.2
1270	20.3	21.3	22.4	23.4	24.4	25.4	26.4	27.4	28.4	29.5	30.5
1280	20.5	21.5	22.5	23.6	24.6	25.6	26.6	27.6	28.7	29.7	30.7
1290	20.6	21.7	22.7	23.7	24.8	25.8	26.8	27.9	28.9	29.9	31.0
1300	20.8	21.8	22.9	23.9	25.0	26.0	27.0	28.1	29.1	30.2	31.2
1310	21.0	22.0	23.1	24.1	25.2	26.2	27.2	28.3	29.3	30.4	31.4
1320	21.1	22.2	23.2	24.3	25.3	26.4	27.5	28.5	29.6	30.6	31.7
1330	21.3	22.3	23.4	24.5	25.5	26.6	27.7	28.7	29.8	30.9	31.9
1340	21.4	22.5	23.6	24.7	25.7	26.8	27.9	28.9	30.0	31.1	32.2
1350	21.6	22.7	23.8	24.8	25.9	27.0	28.1	29.2	30.2	31.3	32.4
1360	21.8	22.8	23.9	25.0	26.1	27.2	28.3	29.4	30.5	31.6	32.6
1370	21.9	23.0	24.1	25.2	26.3	27.4	28.5	29.6	30.7	31.8	32.9
1380	22.1	23.2	24.3	25.4	26.5	27.6	28.7	29.8	30.9	32.0	33.1
1390	22.2	23.4	24.5	25.6	26.7	27.8	28.9	30.0	31.1	32.2	33.4
1400	22.4	23.5	24.6	25.8	26.9	28.0	29.1	30.2	31.4	32.5	33.6
1410	22.6	23.7	24.8	25.9	27.1	28.2	29.3	30.5	31.6	32.7	33.8
1420	22.7	23.9	25.0	26.1	27.3	28.4	29.5	30.7	31.8	32.9	34.1
1430	22.9	24.0	25.2	26.3	27.5	28.6	29.7	30.9	32.0	33.2	34.3
1440	23.0	24.2	25.3	26.5	27.6	28.8	30.0	31.1	32.3	33.4	34.6
1450	23.2	24.4	25.5	26.7	27.8	29.0	30.2	31.3	32.5	33.6	34.8
1460	23.4	24.5	25.7	26.9	28.0	29.2	30.4	31.5	32.7	33.9	35.0
1470	23.5	24.7	25.9	27.0	28.2	29.4	30.6	31.8	32.9	34.1	35.3
1480	23.7	24.9	26.0	27.2	28.4	29.6	30.8	32.0	33.2	34.3	35.5
1490	23.8	25.0	26.2	27.4	28.6	29.8	31.0	32.2	33.4	34.6	35.8
1500	24.0	25.2	26.4	27.6	28.8	30.0	31.2	32.4	33.6	34.8	36.0

* Quantity of 99A fed to fish on the satiation feeding regime (calculated previously)

99B Restriction Feed Quantity Calculator (kg feed/cage)

Number of fish per cage

99A/fish*	20	21	22	23	24	25	26	27	28	29	30
300	5.1	5.4	5.6	5.9	6.1	6.4	6.6	6.9	7.2	7.4	7.7
310	5.3	5.5	5.8	6.1	6.3	6.6	6.9	7.1	7.4	7.7	7.9
320	5.4	5.7	6.0	6.3	6.5	6.8	7.1	7.4	7.6	7.9	8.2
330	5.6	5.9	6.2	6.5	6.7	7.0	7.3	7.6	7.9	8.1	8.4
340	5.8	6.1	6.4	6.7	6.9	7.2	7.5	7.8	8.1	8.4	8.7
350	6.0	6.3	6.6	6.9	7.2	7.4	7.7	8.0	8.3	8.6	8.9
360	6.1	6.4	6.7	7.0	7.4	7.7	8.0	8.3	8.6	8.9	9.2
370	6.3	6.6	6.9	7.2	7.6	7.9	8.2	8.5	8.8	9.1	9.4
380	6.5	6.8	7.1	7.4	7.8	8.1	8.4	8.7	9.1	9.4	9.7
390	6.6	7.0	7.3	7.6	8.0	8.3	8.6	9.0	9.3	9.6	10.0
400	6.8	7.2	7.5	7.8	8.2	8.5	8.9	9.2	9.5	9.9	10.2
410	7.0	7.3	7.7	8.0	8.4	8.7	9.1	9.4	9.8	10.1	10.5
420	7.2	7.5	7.9	8.2	8.6	8.9	9.3	9.7	10.0	10.4	10.7
430	7.3	7.7	8.1	8.4	8.8	9.2	9.5	9.9	10.2	10.6	11.0
440	7.5	7.9	8.2	8.6	9.0	9.4	9.7	10.1	10.5	10.9	11.2
450	7.7	8.0	8.4	8.8	9.2	9.6	10.0	10.3	10.7	11.1	11.5
460	7.8	8.2	8.6	9.0	9.4	9.8	10.2	10.6	11.0	11.4	11.7
470	8.0	8.4	8.8	9.2	9.6	10.0	10.4	10.8	11.2	11.6	12.0
480	8.2	8.6	9.0	9.4	9.8	10.2	10.6	11.0	11.4	11.8	12.3
490	8.3	8.8	9.2	9.6	10.0	10.4	10.8	11.3	11.7	12.1	12.5
500	8.5	8.9	9.4	9.8	10.2	10.6	11.1	11.5	11.9	12.3	12.8
510	8.7	9.1	9.6	10.0	10.4	10.9	11.3	11.7	12.2	12.6	13.0
520	8.9	9.3	9.7	10.2	10.6	11.1	11.5	12.0	12.4	12.8	13.3
530	9.0	9.5	9.9	10.4	10.8	11.3	11.7	12.2	12.6	13.1	13.5
540	9.2	9.7	10.1	10.6	11.0	11.5	12.0	12.4	12.9	13.3	13.8
550	9.4	9.8	10.3	10.8	11.2	11.7	12.2	12.6	13.1	13.6	14.0
560	9.5	10.0	10.5	11.0	11.4	11.9	12.4	12.9	13.3	13.8	14.3
570	9.7	10.2	10.7	11.2	11.6	12.1	12.6	13.1	13.6	14.1	14.6
580	9.9	10.4	10.9	11.4	11.8	12.3	12.8	13.3	13.8	14.3	14.8
590	10.0	10.5	11.0	11.6	12.1	12.6	13.1	13.6	14.1	14.6	15.1
600	10.2	10.7	11.2	11.7	12.3	12.8	13.3	13.8	14.3	14.8	15.3
610	10.4	10.9	11.4	11.9	12.5	13.0	13.5	14.0	14.5	15.1	15.6
620	10.6	11.1	11.6	12.1	12.7	13.2	13.7	14.2	14.8	15.3	15.8
630	10.7	11.3	11.8	12.3	12.9	13.4	13.9	14.5	15.0	15.6	16.1
640	10.9	11.4	12.0	12.5	13.1	13.6	14.2	14.7	15.3	15.8	16.3
650	11.1	11.6	12.2	12.7	13.3	13.8	14.4	14.9	15.5	16.0	16.6
660	11.2	11.8	12.4	12.9	13.5	14.0	14.6	15.2	15.7	16.3	16.9
670	11.4	12.0	12.5	13.1	13.7	14.3	14.8	15.4	16.0	16.5	17.1
680	11.6	12.2	12.7	13.3	13.9	14.5	15.0	15.6	16.2	16.8	17.4
690	11.7	12.3	12.9	13.5	14.1	14.7	15.3	15.9	16.4	17.0	17.6
700	11.9	12.5	13.1	13.7	14.3	14.9	15.5	16.1	16.7	17.3	17.9
710	12.1	12.7	13.3	13.9	14.5	15.1	15.7	16.3	16.9	17.5	18.1
720	12.3	12.9	13.5	14.1	14.7	15.3	15.9	16.5	17.2	17.8	18.4
730	12.4	13.0	13.7	14.3	14.9	15.5	16.2	16.8	17.4	18.0	18.6
740	12.6	13.2	13.9	14.5	15.1	15.7	16.4	17.0	17.6	18.3	18.9
750	12.8	13.4	14.0	14.7	15.3	16.0	16.6	17.2	17.9	18.5	19.2
760	12.9	13.6	14.2	14.9	15.5	16.2	16.8	17.5	18.1	18.8	19.4
770	13.1	13.8	14.4	15.1	15.7	16.4	17.0	17.7	18.4	19.0	19.7
780	13.3	13.9	14.6	15.3	15.9	16.6	17.3	17.9	18.6	19.3	19.9
790	13.4	14.1	14.8	15.5	16.1	16.8	17.5	18.2	18.8	19.5	20.2
800	13.6	14.3	15.0	15.7	16.3	17.0	17.7	18.4	19.1	19.7	20.4
810	13.8	14.5	15.2	15.9	16.5	17.2	17.9	18.6	19.3	20.0	20.7
820	14.0	14.7	15.4	16.1	16.8	17.4	18.1	18.8	19.5	20.2	20.9
830	14.1	14.8	15.5	16.2	17.0	17.7	18.4	19.1	19.8	20.5	21.2
840	14.3	15.0	15.7	16.4	17.2	17.9	18.6	19.3	20.0	20.7	21.5
850	14.5	15.2	15.9	16.6	17.4	18.1	18.8	19.5	20.3	21.0	21.7
860	14.6	15.4	16.1	16.8	17.6	18.3	19.0	19.8	20.5	21.2	22.0
870	14.8	15.6	16.3	17.0	17.8	18.5	19.3	20.0	20.7	21.5	22.2
880	15.0	15.7	16.5	17.2	18.0	18.7	19.5	20.2	21.0	21.7	22.5
890	15.2	15.9	16.7	17.4	18.2	18.9	19.7	20.5	21.2	22.0	22.7
900	15.3	16.1	16.9	17.6	18.4	19.2	19.9	20.7	21.5	22.2	23.0
910	15.5	16.3	17.0	17.8	18.6	19.4	20.1	20.9	21.7	22.5	23.2
920	15.7	16.4	17.2	18.0	18.8	19.6	20.4	21.1	21.9	22.7	23.5

99B Restriction Feed Quantity Calculator (kg feed/cage)

Number of fish per cage

99A/fish*	20	21	22	23	24	25	26	27	28	29	30
930	15.8	16.6	17.4	18.2	19.0	19.8	20.6	21.4	22.2	23.0	23.7
940	16.0	16.8	17.6	18.4	19.2	20.0	20.8	21.6	22.4	23.2	24.0
950	16.2	17.0	17.8	18.6	19.4	20.2	21.0	21.8	22.6	23.5	24.3
960	16.3	17.2	18.0	18.8	19.6	20.4	21.2	22.1	22.9	23.7	24.5
970	16.5	17.3	18.2	19.0	19.8	20.6	21.5	22.3	23.1	23.9	24.8
980	16.7	17.5	18.4	19.2	20.0	20.9	21.7	22.5	23.4	24.2	25.0
990	16.9	17.7	18.5	19.4	20.2	21.1	21.9	22.8	23.6	24.4	25.3
1000	17.0	17.9	18.7	19.6	20.4	21.3	22.1	23.0	23.8	24.7	25.5
1010	17.2	18.1	18.9	19.8	20.6	21.5	22.4	23.2	24.1	24.9	25.8
1020	17.4	18.2	19.1	20.0	20.8	21.7	22.6	23.4	24.3	25.2	26.0
1030	17.5	18.4	19.3	20.2	21.0	21.9	22.8	23.7	24.5	25.4	26.3
1040	17.7	18.6	19.5	20.4	21.2	22.1	23.0	23.9	24.8	25.7	26.6
1050	17.9	18.8	19.7	20.6	21.5	22.3	23.2	24.1	25.0	25.9	26.8
1060	18.0	18.9	19.8	20.8	21.7	22.6	23.5	24.4	25.3	26.2	27.1
1070	18.2	19.1	20.0	20.9	21.9	22.8	23.7	24.6	25.5	26.4	27.3
1080	18.4	19.3	20.2	21.1	22.1	23.0	23.9	24.8	25.7	26.7	27.6
1090	18.6	19.5	20.4	21.3	22.3	23.2	24.1	25.1	26.0	26.9	27.8
1100	18.7	19.7	20.6	21.5	22.5	23.4	24.3	25.3	26.2	27.2	28.1
1110	18.9	19.8	20.8	21.7	22.7	23.6	24.6	25.5	26.5	27.4	28.3
1120	19.1	20.0	21.0	21.9	22.9	23.8	24.8	25.7	26.7	27.6	28.6
1130	19.2	20.2	21.2	22.1	23.1	24.0	25.0	26.0	26.9	27.9	28.9
1140	19.4	20.4	21.3	22.3	23.3	24.3	25.2	26.2	27.2	28.1	29.1
1150	19.6	20.6	21.5	22.5	23.5	24.5	25.5	26.4	27.4	28.4	29.4
1160	19.7	20.7	21.7	22.7	23.7	24.7	25.7	26.7	27.6	28.6	29.6
1170	19.9	20.9	21.9	22.9	23.9	24.9	25.9	26.9	27.9	28.9	29.9
1180	20.1	21.1	22.1	23.1	24.1	25.1	26.1	27.1	28.1	29.1	30.1
1190	20.3	21.3	22.3	23.3	24.3	25.3	26.3	27.3	28.4	29.4	30.4
1200	20.4	21.5	22.5	23.5	24.5	25.5	26.6	27.6	28.6	29.6	30.6
1210	20.6	21.6	22.7	23.7	24.7	25.7	26.8	27.8	28.8	29.9	30.9
1220	20.8	21.8	22.8	23.9	24.9	26.0	27.0	28.0	29.1	30.1	31.2
1230	20.9	22.0	23.0	24.1	25.1	26.2	27.2	28.3	29.3	30.4	31.4
1240	21.1	22.2	23.2	24.3	25.3	26.4	27.4	28.5	29.6	30.6	31.7
1250	21.3	22.3	23.4	24.5	25.5	26.6	27.7	28.7	29.8	30.9	31.9
1260	21.5	22.5	23.6	24.7	25.7	26.8	27.9	29.0	30.0	31.1	32.2
1270	21.6	22.7	23.8	24.9	25.9	27.0	28.1	29.2	30.3	31.3	32.4
1280	21.8	22.9	24.0	25.1	26.1	27.2	28.3	29.4	30.5	31.6	32.7
1290	22.0	23.1	24.2	25.3	26.4	27.5	28.5	29.6	30.7	31.8	32.9
1300	22.1	23.2	24.3	25.5	26.6	27.7	28.8	29.9	31.0	32.1	33.2
1310	22.3	23.4	24.5	25.6	26.8	27.9	29.0	30.1	31.2	32.3	33.5
1320	22.5	23.6	24.7	25.8	27.0	28.1	29.2	30.3	31.5	32.6	33.7
1330	22.6	23.8	24.9	26.0	27.2	28.3	29.4	30.6	31.7	32.8	34.0
1340	22.8	24.0	25.1	26.2	27.4	28.5	29.7	30.8	31.9	33.1	34.2
1350	23.0	24.1	25.3	26.4	27.6	28.7	29.9	31.0	32.2	33.3	34.5
1360	23.2	24.3	25.5	26.6	27.8	28.9	30.1	31.3	32.4	33.6	34.7
1370	23.3	24.5	25.7	26.8	28.0	29.2	30.3	31.5	32.7	33.8	35.0
1380	23.5	24.7	25.8	27.0	28.2	29.4	30.5	31.7	32.9	34.1	35.2
1390	23.7	24.8	26.0	27.2	28.4	29.6	30.8	31.9	33.1	34.3	35.5
1400	23.8	25.0	26.2	27.4	28.6	29.8	31.0	32.2	33.4	34.6	35.8
1410	24.0	25.2	26.4	27.6	28.8	30.0	31.2	32.4	33.6	34.8	36.0
1420	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.1	36.3
1430	24.3	25.6	26.8	28.0	29.2	30.4	31.6	32.9	34.1	35.3	36.5
1440	24.5	25.7	27.0	28.2	29.4	30.6	31.9	33.1	34.3	35.5	36.8
1450	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.0
1460	24.9	26.1	27.3	28.6	29.8	31.1	32.3	33.6	34.8	36.0	37.3
1470	25.0	26.3	27.5	28.8	30.0	31.3	32.5	33.8	35.0	36.3	37.5
1480	25.2	26.5	27.7	29.0	30.2	31.5	32.8	34.0	35.3	36.5	37.8
1490	25.4	26.6	27.9	29.2	30.4	31.7	33.0	34.2	35.5	36.8	38.0
1500	25.5	26.8	28.1	29.4	30.6	31.9	33.2	34.5	35.8	37.0	38.3

* Quantity of 99A fed to fish on the satiation feeding regime (calculated previously)

99C Restriction Feed Quantity Calculator (kg feed/cage)

	Number of fish per cage										
99A/fish*	20	21	22	23	24	25	26	27	28	29	30
300	5.5	5.8	6.0	6.3	6.6	6.9	7.1	7.4	7.7	8.0	8.2
310	5.7	6.0	6.2	6.5	6.8	7.1	7.4	7.7	8.0	8.2	8.5
320	5.9	6.2	6.4	6.7	7.0	7.3	7.6	7.9	8.2	8.5	8.8
330	6.0	6.3	6.7	7.0	7.3	7.6	7.9	8.2	8.5	8.8	9.1
340	6.2	6.5	6.9	7.2	7.5	7.8	8.1	8.4	8.7	9.0	9.3
350	6.4	6.7	7.1	7.4	7.7	8.0	8.3	8.7	9.0	9.3	9.6
360	6.6	6.9	7.3	7.6	7.9	8.2	8.6	8.9	9.2	9.6	9.9
370	6.8	7.1	7.5	7.8	8.1	8.5	8.8	9.2	9.5	9.8	10.2
380	7.0	7.3	7.7	8.0	8.4	8.7	9.1	9.4	9.7	10.1	10.4
390	7.1	7.5	7.9	8.2	8.6	8.9	9.3	9.6	10.0	10.4	10.7
400	7.3	7.7	8.1	8.4	8.8	9.2	9.5	9.9	10.3	10.6	11.0
410	7.5	7.9	8.3	8.6	9.0	9.4	9.8	10.1	10.5	10.9	11.3
420	7.7	8.1	8.5	8.8	9.2	9.6	10.0	10.4	10.8	11.2	11.5
430	7.9	8.3	8.7	9.1	9.5	9.8	10.2	10.6	11.0	11.4	11.8
440	8.1	8.5	8.9	9.3	9.7	10.1	10.5	10.9	11.3	11.7	12.1
450	8.2	8.7	9.1	9.5	9.9	10.3	10.7	11.1	11.5	12.0	12.4
460	8.4	8.8	9.3	9.7	10.1	10.5	11.0	11.4	11.8	12.2	12.6
470	8.6	9.0	9.5	9.9	10.3	10.8	11.2	11.6	12.1	12.5	12.9
480	8.8	9.2	9.7	10.1	10.6	11.0	11.4	11.9	12.3	12.8	13.2
490	9.0	9.4	9.9	10.3	10.8	11.2	11.7	12.1	12.6	13.0	13.5
500	9.2	9.6	10.1	10.5	11.0	11.5	11.9	12.4	12.8	13.3	13.7
510	9.3	9.8	10.3	10.7	11.2	11.7	12.1	12.6	13.1	13.5	14.0
520	9.5	10.0	10.5	11.0	11.4	11.9	12.4	12.9	13.3	13.8	14.3
530	9.7	10.2	10.7	11.2	11.7	12.1	12.6	13.1	13.6	14.1	14.6
540	9.9	10.4	10.9	11.4	11.9	12.4	12.9	13.4	13.8	14.3	14.8
550	10.1	10.6	11.1	11.6	12.1	12.6	13.1	13.6	14.1	14.6	15.1
560	10.3	10.8	11.3	11.8	12.3	12.8	13.3	13.8	14.4	14.9	15.4
570	10.4	11.0	11.5	12.0	12.5	13.1	13.6	14.1	14.6	15.1	15.7
580	10.6	11.2	11.7	12.2	12.8	13.3	13.8	14.3	14.9	15.4	15.9
590	10.8	11.3	11.9	12.4	13.0	13.5	14.1	14.6	15.1	15.7	16.2
600	11.0	11.5	12.1	12.6	13.2	13.7	14.3	14.8	15.4	15.9	16.5
610	11.2	11.7	12.3	12.9	13.4	14.0	14.5	15.1	15.6	16.2	16.8
620	11.4	11.9	12.5	13.1	13.6	14.2	14.8	15.3	15.9	16.5	17.0
630	11.5	12.1	12.7	13.3	13.8	14.4	15.0	15.6	16.2	16.7	17.3
640	11.7	12.3	12.9	13.5	14.1	14.7	15.2	15.8	16.4	17.0	17.6
650	11.9	12.5	13.1	13.7	14.3	14.9	15.5	16.1	16.7	17.3	17.9
660	12.1	12.7	13.3	13.9	14.5	15.1	15.7	16.3	16.9	17.5	18.1
670	12.3	12.9	13.5	14.1	14.7	15.3	16.0	16.6	17.2	17.8	18.4
680	12.5	13.1	13.7	14.3	14.9	15.6	16.2	16.8	17.4	18.1	18.7
690	12.6	13.3	13.9	14.5	15.2	15.8	16.4	17.1	17.7	18.3	19.0
700	12.8	13.5	14.1	14.7	15.4	16.0	16.7	17.3	18.0	18.6	19.2
710	13.0	13.7	14.3	15.0	15.6	16.3	16.9	17.6	18.2	18.9	19.5
720	13.2	13.8	14.5	15.2	15.8	16.5	17.1	17.8	18.5	19.1	19.8
730	13.4	14.0	14.7	15.4	16.0	16.7	17.4	18.1	18.7	19.4	20.1
740	13.6	14.2	14.9	15.6	16.3	16.9	17.6	18.3	19.0	19.7	20.3
750	13.7	14.4	15.1	15.8	16.5	17.2	17.9	18.5	19.2	19.9	20.6
760	13.9	14.6	15.3	16.0	16.7	17.4	18.1	18.8	19.5	20.2	20.9
770	14.1	14.8	15.5	16.2	16.9	17.6	18.3	19.0	19.7	20.5	21.2
780	14.3	15.0	15.7	16.4	17.1	17.9	18.6	19.3	20.0	20.7	21.4
790	14.5	15.2	15.9	16.6	17.4	18.1	18.8	19.5	20.3	21.0	21.7
800	14.7	15.4	16.1	16.9	17.6	18.3	19.1	19.8	20.5	21.3	22.0
810	14.8	15.6	16.3	17.1	17.8	18.5	19.3	20.0	20.8	21.5	22.3
820	15.0	15.8	16.5	17.3	18.0	18.8	19.5	20.3	21.0	21.8	22.5
830	15.2	16.0	16.7	17.5	18.2	19.0	19.8	20.5	21.3	22.0	22.8
840	15.4	16.2	16.9	17.7	18.5	19.2	20.0	20.8	21.5	22.3	23.1
850	15.6	16.4	17.1	17.9	18.7	19.5	20.2	21.0	21.8	22.6	23.4
860	15.8	16.5	17.3	18.1	18.9	19.7	20.5	21.3	22.1	22.8	23.6
870	15.9	16.7	17.5	18.3	19.1	19.9	20.7	21.5	22.3	23.1	23.9
880	16.1	16.9	17.7	18.5	19.3	20.2	21.0	21.8	22.6	23.4	24.2
890	16.3	17.1	17.9	18.8	19.6	20.4	21.2	22.0	22.8	23.6	24.5
900	16.5	17.3	18.1	19.0	19.8	20.6	21.4	22.3	23.1	23.9	24.7
910	16.7	17.5	18.3	19.2	20.0	20.8	21.7	22.5	23.3	24.2	25.0
920	16.9	17.7	18.5	19.4	20.2	21.1	21.9	22.8	23.6	24.4	25.3

99C Restriction Feed Quantity Calculator (kg feed/cage)

99A/fish*	Number of fish per cage									
	20	21	22	23	24	25	26	27	28	29	30
930	17.0	17.9	18.7	19.6	20.4	21.3	22.1	23.0	23.9	24.7	25.6
940	17.2	18.1	18.9	19.8	20.7	21.5	22.4	23.2	24.1	25.0	25.8
950	17.4	18.3	19.1	20.0	20.9	21.8	22.6	23.5	24.4	25.2	26.1
960	17.6	18.5	19.3	20.2	21.1	22.0	22.9	23.7	24.6	25.5	26.4
970	17.8	18.7	19.5	20.4	21.3	22.2	23.1	24.0	24.9	25.8	26.7
980	18.0	18.9	19.7	20.6	21.5	22.4	23.3	24.2	25.1	26.0	26.9
990	18.1	19.0	20.0	20.9	21.8	22.7	23.6	24.5	25.4	26.3	27.2
1000	18.3	19.2	20.2	21.1	22.0	22.9	23.8	24.7	25.6	26.6	27.5
1010	18.5	19.4	20.4	21.3	22.2	23.1	24.1	25.0	25.9	26.8	27.8
1020	18.7	19.6	20.6	21.5	22.4	23.4	24.3	25.2	26.2	27.1	28.0
1030	18.9	19.8	20.8	21.7	22.6	23.6	24.5	25.5	26.4	27.4	28.3
1040	19.1	20.0	21.0	21.9	22.9	23.8	24.8	25.7	26.7	27.6	28.6
1050	19.2	20.2	21.2	22.1	23.1	24.0	25.0	26.0	26.9	27.9	28.9
1060	19.4	20.4	21.4	22.3	23.3	24.3	25.2	26.2	27.2	28.2	29.1
1070	19.6	20.6	21.6	22.5	23.5	24.5	25.5	26.5	27.4	28.4	29.4
1080	19.8	20.8	21.8	22.8	23.7	24.7	25.7	26.7	27.7	28.7	29.7
1090	20.0	21.0	22.0	23.0	24.0	25.0	26.0	27.0	28.0	29.0	30.0
1100	20.2	21.2	22.2	23.2	24.2	25.2	26.2	27.2	28.2	29.2	30.2
1110	20.3	21.4	22.4	23.4	24.4	25.4	26.4	27.5	28.5	29.5	30.5
1120	20.5	21.5	22.6	23.6	24.6	25.6	26.7	27.7	28.7	29.8	30.8
1130	20.7	21.7	22.8	23.8	24.8	25.9	26.9	27.9	29.0	30.0	31.1
1140	20.9	21.9	23.0	24.0	25.1	26.1	27.2	28.2	29.2	30.3	31.3
1150	21.1	22.1	23.2	24.2	25.3	26.3	27.4	28.4	29.5	30.5	31.6
1160	21.3	22.3	23.4	24.4	25.5	26.6	27.6	28.7	29.8	30.8	31.9
1170	21.4	22.5	23.6	24.6	25.7	26.8	27.9	28.9	30.0	31.1	32.2
1180	21.6	22.7	23.8	24.9	25.9	27.0	28.1	29.2	30.3	31.3	32.4
1190	21.8	22.9	24.0	25.1	26.2	27.3	28.3	29.4	30.5	31.6	32.7
1200	22.0	23.1	24.2	25.3	26.4	27.5	28.6	29.7	30.8	31.9	33.0
1210	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0	32.1	33.3
1220	22.4	23.5	24.6	25.7	26.8	27.9	29.1	30.2	31.3	32.4	33.5
1230	22.5	23.7	24.8	25.9	27.0	28.2	29.3	30.4	31.5	32.7	33.8
1240	22.7	23.9	25.0	26.1	27.3	28.4	29.5	30.7	31.8	32.9	34.1
1250	22.9	24.0	25.2	26.3	27.5	28.6	29.8	30.9	32.1	33.2	34.4
1260	23.1	24.2	25.4	26.5	27.7	28.9	30.0	31.2	32.3	33.5	34.6
1270	23.3	24.4	25.6	26.8	27.9	29.1	30.2	31.4	32.6	33.7	34.9
1280	23.4	24.6	25.8	27.0	28.1	29.3	30.5	31.7	32.8	34.0	35.2
1290	23.6	24.8	26.0	27.2	28.4	29.5	30.7	31.9	33.1	34.3	35.4
1300	23.8	25.0	26.2	27.4	28.6	29.8	31.0	32.2	33.3	34.5	35.7
1310	24.0	25.2	26.4	27.6	28.8	30.0	31.2	32.4	33.6	34.8	36.0
1320	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.9	35.1	36.3
1330	24.4	25.6	26.8	28.0	29.2	30.5	31.7	32.9	34.1	35.3	36.5
1340	24.5	25.8	27.0	28.2	29.5	30.7	31.9	33.1	34.4	35.6	36.8
1350	24.7	26.0	27.2	28.4	29.7	30.9	32.2	33.4	34.6	35.9	37.1
1360	24.9	26.2	27.4	28.7	29.9	31.1	32.4	33.6	34.9	36.1	37.4
1370	25.1	26.4	27.6	28.9	30.1	31.4	32.6	33.9	35.1	36.4	37.6
1380	25.3	26.5	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	37.9
1390	25.5	26.7	28.0	29.3	30.6	31.8	33.1	34.4	35.7	36.9	38.2
1400	25.6	26.9	28.2	29.5	30.8	32.1	33.3	34.6	35.9	37.2	38.5
1410	25.8	27.1	28.4	29.7	31.0	32.3	33.6	34.9	36.2	37.5	38.7
1420	26.0	27.3	28.6	29.9	31.2	32.5	33.8	35.1	36.4	37.7	39.0
1430	26.2	27.5	28.8	30.1	31.4	32.7	34.1	35.4	36.7	38.0	39.3
1440	26.4	27.7	29.0	30.3	31.7	33.0	34.3	35.6	36.9	38.3	39.6
1450	26.6	27.9	29.2	30.5	31.9	33.2	34.5	35.9	37.2	38.5	39.8
1460	26.7	28.1	29.4	30.8	32.1	33.4	34.8	36.1	37.4	38.8	40.1
1470	26.9	28.3	29.6	31.0	32.3	33.7	35.0	36.4	37.7	39.0	40.4
1480	27.1	28.5	29.8	31.2	32.5	33.9	35.2	36.6	38.0	39.3	40.7
1490	27.3	28.7	30.0	31.4	32.8	34.1	35.5	36.9	38.2	39.6	40.9
1500	27.5	28.9	30.2	31.6	33.0	34.4	35.7	37.1	38.5	39.8	41.2

* Quantity of 99A fed to fish on the satiation feeding regime (calculated previously)

Appendix VI:
Experiment 3 Diet formulations - 2000

Ingredients

Amino acid	Content (g/kg)						
	Pilchards	Chilean	Meat meal	Krill	Casein	Blood	Gluten
Lysine	7.600	52.600	25.630	28.000	72.800	74.040	15.900
Threonine	3.540	26.800	16.020	16.000	40.300	40.890	21.600
Methionine	1.300	18.500	7.010	14.000	27.100	10.490	10.800
Isoleucine	4.990	30.000	13.620	17.000	50.800	8.280	30.100
Leucine	4.000	50.000	29.880	28.000	83.300	108.180	53.300
Tryptophan	0.000	8.100	2.900	0.000	14.900	12.690	2.000
Valine	3.750	34.030	22.570	19.000	63.100	76.290	32.400
Phenylalanine	3.840	26.200	16.780	18.000	46.400	54.630	37.400
Histidine	4.480	16.310	8.340	8.700	27.100	54.480	1.560
Arginine	6.430	50.000	34.010	23.000	35.300	36.140	29.600

Amino acid	Content (g/kg)	
	Canola	Soybean
Lysine	20.260	29.630
Threonine	15.740	18.350
Methionine	7.400	6.590
Isoleucine	13.740	23.020
Leucine	24.920	36.960
Tryptophan	4.280	6.960
Valine	17.850	23.570
Phenylalanine	14.200	23.700
Histidine	9.670	12.240
Arginine	21.820	35.770

Vitamins and Minerals

Ingredient	Units	Gibsons 98 unit/3kg	Gibsons 98 unit/kg
Vitamin A	MIU	3.000	1.000
Vitamin D3	MIU	0.500	0.167
Thiamine (B1)	g	15.000	5.000
Riboflavin (B2)	g	20.000	6.667
Pyridoxine (B6)	g	12.000	4.000
Vitamin B12	mg	30.000	10.000
Biotin	g	0.300	0.100
Vitamin K3	g	7.000	2.333
Pantothenic Acid	g	30.000	10.000
Niacin	g	65.000	21.667
Inositol	g	50.000	16.667
Vitamin E ADS	g	100.000	33.333
Folic acid S.D.	g	4.000	1.333
Ethoxyquin	g	150.000	50.000
Cobalt	g	1.000	0.333
Iodine	g	1.100	0.367
Copper	g	3.000	1.000
Magnesium	g	50.000	16.667
Manganese	g	20.000	6.667
Iron	g	20.000	6.667
Bioplex iron	g	20.000	6.667
Zinc	g	30.000	10.000
Aquastab C 42%	g	60.000	20.000

Notes:

Vitamin and mineral levels to be adjusted for 1999 experiments

Diet Name: **2000B (1-7 weeks)** **Formulation date:** **10/12/99**

Ingredient Inclusion

Ingredient		Incl. (%)	g/5kg	kg/100kg	kg/200kg	Price $/kg	$/kg
Additives							
Choline chloride	0.200	0.200	10.000	0.200	0.400	1.900	0.004
Lethicin for aquatic diets	1.000	1.000	50.000	1.000	2.000	1.000	0.010
Pre-mix minerals	0.200	0.200	10.000	0.200	0.400	8.780	0.018
Pre-mix vitamins	0.100	0.100	5.000	0.100	0.200	43.840	0.044
Roche Stay-C vitamin C	0.048	0.048	2.400	0.048	0.096	27.800	0.013
Kaolin	2.000	2.000	100.000	2.000	4.000	27.800	0.556
Diluents and Fillers							
Water	5.000	5.000	250.000	5.000	10.000	0.000	0.000
Energy							
Distilled monoglyceride	0.500	0.500	25.000	0.500	1.000	1.100	0.006
Fish oil	0.343	0.343	17.172	0.343	0.687	1.100	0.004
Proteins and amino acids							
Wheat gluten	15.000	15.000	750.000	15.000	30.000	3.000	0.450
Blood	2.500	2.500	125.000	2.500	5.000	1.000	0.025
Meat meal	5.000	5.000	250.000	5.000	10.000	0.550	0.028
Soybean meal	0.000	0.000	0.000	0.000	0.000	0.480	0.000
Canola meal	0.000	0.000	0.000	0.000	0.000	0.380	0.000
Casein	0.000	0.000	0.000	0.000	0.000	1.500	0.000
Inual Antartic krill meal	0.000	0.000	0.000	0.000	0.000	2.500	0.000
Fish meal Chilean 67% CP	32.613	32.613	1630.674	32.613	65.227	1.400	0.457
Fresh pilchards	35.495	35.495	1774.754	35.495	70.990	0.900	0.319
TOTAL	**100.000**	**100.000**	**5000.000**	**100.000**	**200.000**		**1.932**

Chemical Composition

	Composition g/kg)				
Ingredient	Dry matter	Fat	CP	DCP coeff	DCP
Choline chloride	900	0.0	0.0	0.0	0.0
Lethicin for aquatic diets	900	0.0	0.0	0.0	0.0
Pre-mix minerals	900	0.0	0.0	0.0	0.0
Pre-mix vitamins	900	0.0	0.0	0.0	0.0
Roche Stay-C vitamin C	900	0.0	0.0	0.0	0.0
Kaolin	900	0.0	0.0	0.0	0.0
Water	0	0.0	0.0	0.0	0.0
Canola meal (solvent extracted)	900	25.0	350.0	0.6	210.0
Distilled monoglyceride	995	995.0	0.0	0.0	0.0
Fish oil	995	995.0	0.0	0.0	0.0
Wheat gluten	900	31.0	738.0	0.9	664.2
Inual Antartic krill meal	900	180.0	580.0	0.4	243.6
Fish meal Chilean 67% CP	920	120.0	670.0	0.6	368.5
Casein	920	12.0	877.0	0.9	789.3
Fresh pilchards	340	92.0	210.0	0.8	174.3
Soybean meal (solvent)	900	25.0	480.0	0.6	288.0

Meat meal (50% CP)	930	100.0	500.0	0.8	400.0
Blood meal (85% Ring-dried)	900	8.4	850.0	0.9	722.5

Nutrient Contributions

	Composition g/kg)			
Ingredient	Dry matter	Fat	CP	Digest CP
Choline chloride	1.800	0.000	0.000	0.000
Lethicin for aquatic diets	9.000	0.000	0.000	0.000
Pre-mix minerals	1.800	0.000	0.000	0.000
Pre-mix vitamins	0.900	0.000	0.000	0.000
Roche Stay-C vitamin C	0.432	0.000	0.000	0.000
Kaolin	18.000	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000
Canola meal (solvent extracted)	0.000	0.000	0.000	0.000
Distilled monoglyceride	4.975	4.975	0.000	0.000
Fish oil	3.417	3.417	0.000	0.000
Wheat gluten	135.000	4.650	110.700	99.630
Inual Antartic krill meal	0.000	0.000	0.000	0.000
Fish meal Chilean 67% CP	300.044	39.136	218.510	120.181
Casein	0.000	0.000	0.000	0.000
Fresh pilchards	120.683	32.655	74.540	61.868
Soybean meal (solvent extracted)	0.000	0.000	0.000	0.000
Meat meal (50% CP)	46.500	5.000	25.000	20.000
Blood meal (85% Ring-dried)	22.500	0.210	21.250	18.063
TOTAL	**665.052**	**90.044**	**450.000**	**319.741**

Dietary Amino Acid Contributions

Amino acid	Content (g/kg)	Ratio:Lys	Req Ratio	Req Amt	Def/Ex	Add
Lysine	25.370	100.000	100	18.000	7.370	-9.448
Threonine	15.060	59.363	56	8.434	6.626	-6.762
Methionine	8.728	34.402	30	2.618	6.109	-6.234
Isoleucine	16.958	66.844	57	9.666	7.292	-7.441
Leucine	29.920	117.936	90	26.928	2.992	-3.053
Tryptophan	3.404	13.417	10	0.340	3.064	-3.126
Valine	20.325	80.116	62	12.602	7.724	-7.881
Phenylalanine	17.722	69.857	47	8.330	9.393	-9.585
Histidine	8.922	35.170	30	2.677	6.246	-6.373
Arginine	25.633	101.038	73	18.712	6.921	-7.062

	Actual	Req ratio	Req Amt
Lys:CP	0.056	0.040	18.000
Lys:DCP	0.079		

Diet Name: **2000B (8-14 weeks)** **Formulation date:** **10/12/99**

Ingredient Inclusion

Ingredient		Incl. (%)	g/5kg	kg/100kg	kg/200kg	Price $/kg	$/kg
Additives							
Choline chloride	0.200	0.200	10.000	0.200	0.400	1.900	0.004
Lethicin for aquatic diets	1.000	1.000	50.000	1.000	2.000	1.000	0.010
Pre-mix minerals	0.200	0.200	10.000	0.200	0.400	8.780	0.018
Pre-mix vitamins	0.100	0.100	5.000	0.100	0.200	43.840	0.044
Roche Stay-C vitamin C	0.048	0.048	2.400	0.048	0.096	27.800	0.013
Kaolin	0.000	0.000	0.000	0.000	0.000	27.800	0.000
Diluents and Fillers							
Water	2.000	2.000	100.000	2.000	4.000	0.000	0.000
Energy							
Distilled monoglyceride	0.500	0.500	25.000	0.500	1.000	1.100	0.006
Fish oil	7.880	7.880	394.005	7.880	15.760	1.100	0.087
Proteins and amino acids							
Wheat gluten	15.000	15.000	750.000	15.000	30.000	3.000	0.450
Blood	0.000	0.000	0.000	0.000	0.000	1.000	0.000
Meat meal	5.000	5.000	250.000	5.000	10.000	0.550	0.028
Soybean meal	0.000	0.000	0.000	0.000	0.000	0.480	0.000
Canola meal	0.000	0.000	0.000	0.000	0.000	0.380	0.000
Casein	0.000	0.000	0.000	0.000	0.000	1.500	0.000
Inual Antartic krill meal	0.000	0.000	0.000	0.000	0.000	2.500	0.000
Fish meal Chilean 67% CP	15.511	15.511	775.533	15.511	31.021	1.400	0.217
Fresh pilchards	52.561	52.561	2628.063	52.561	105.123	0.900	0.473
TOTAL	**100.000**	**100.000**	**5000.000**	**100.000**	**200.000**		**1.348**

Chemical Composition

	Composition g/kg)				
Ingredient	Dry matter	Fat	CP	DCP coeff	DCP
Choline chloride	900	0.0	0.0	0.0	0.0
Lethicin for aquatic diets	900	0.0	0.0	0.0	0.0
Pre-mix minerals	900	0.0	0.0	0.0	0.0
Pre-mix vitamins	900	0.0	0.0	0.0	0.0
Roche Stay-C vitamin C	900	0.0	0.0	0.0	0.0
Kaolin	900	0.0	0.0	0.0	0.0
Water	0	0.0	0.0	0.0	0.0
Canola meal (solvent extracted)	900	25.0	350.0	0.6	210.0
Distilled monoglyceride	995	995.0	0.0	0.0	0.0
Fish oil	995	995.0	0.0	0.0	0.0
Wheat gluten	900	31.0	738.0	0.9	664.2
Inual Antartic krill meal	900	180.0	580.0	0.4	243.6
Fish meal Chilean 67% CP	920	120.0	670.0	0.6	368.5
Casein	920	12.0	877.0	0.9	789.3
Fresh pilchards	340	92.0	210.0	0.8	174.3
Soybean meal (solvent)	900	25.0	480.0	0.6	288.0

Meat meal (50% CP)	930	100.0	500.0	0.8	400.0
Blood meal (85% Ring-dried)	900	8.4	850.0	0.9	722.5

Nutrient Contributions

	Composition g/kg)			
Ingredient	Dry matter	Fat	CP	Digest CP
Choline chloride	1.800	0.000	0.000	0.000
Lethicin for aquatic diets	9.000	0.000	0.000	0.000
Pre-mix minerals	1.800	0.000	0.000	0.000
Pre-mix vitamins	0.900	0.000	0.000	0.000
Roche Stay-C vitamin C	0.432	0.000	0.000	0.000
Kaolin	0.000	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000
Canola meal (solvent extracted)	0.000	0.000	0.000	0.000
Distilled monoglyceride	4.975	4.975	0.000	0.000
Fish oil	78.407	78.407	0.000	0.000
Wheat gluten	135.000	4.650	110.700	99.630
Inual Antartic krill meal	0.000	0.000	0.000	0.000
Fish meal Chilean 67% CP	142.698	18.613	103.921	57.157
Casein	0.000	0.000	0.000	0.000
Fresh pilchards	178.708	48.356	110.379	91.614
Soybean meal (solvent extracted)	0.000	0.000	0.000	0.000
Meat meal (50% CP)	46.500	5.000	25.000	20.000
Blood meal (85% Ring-dried)	0.000	0.000	0.000	0.000
TOTAL	**600.220**	**160.001**	**350.000**	**268.401**

Dietary Amino Acid Contributions

Amino acid	Content (g/kg)	Ratio:Lys	Req Ratio	Req Amt	Def/Ex	Add
Lysine	15.820	100.000	100	14.000	1.820	-2.333
Threonine	10.059	63.582	56	5.633	4.426	-4.516
Methionine	5.523	34.914	30	1.657	3.866	-3.945
Isoleucine	12.472	78.838	57	7.109	5.363	-5.472
Leucine	19.347	122.295	90	17.412	1.935	-1.974
Tryptophan	1.701	10.755	10	0.170	1.531	-1.562
Valine	13.238	83.679	62	8.207	5.030	-5.133
Phenylalanine	12.531	79.212	47	5.890	6.642	-6.777
Histidine	5.536	34.991	30	1.661	3.875	-3.954
Arginine	17.276	109.202	73	12.611	4.664	-4.760

	Actual	Req ratio	Req Amt
Lys:CP	0.045	0.040	14.000
Lys:DCP	0.059		

Appendix VII:
Experiment 2 Detailed data collection

99 Tuna Harvest Growth Results - Cage A1

Selection Number	Allocation Date	Pit Tag Number	Treatment	Cage	Ing Cage ID	Starting Weight	Starting Length (cm)	Start CI
528	2/03/99	0169D2B3	A Full	A1	1	16.08	94.0	19.36
529	2/03/99	01C82F22	A Full	A1	1	19.01	100.0	19.01
530	2/03/99	01C81F92	A Full	A1	1	17.00	97.0	18.63
531	2/03/99	0169DDC4	A Full	A1	1	17.84	96.0	20.16
532	2/03/99	01CD263B	A Full	A1	1	15.46	93.0	19.22
533	2/03/99	01CB2B18	A Full	A1	1	19.00	99.5	19.29
534	2/03/99	01CB272D	A Full	A1	1	18.62	101.5	17.81
535	2/03/99	01CD3275	A Full	A1	1	16.54	94.0	19.91
536	2/03/99	0216C229	A Full	A1	1	17.74	96.0	20.05
537	2/03/99	01C824E7	A Full	A1	1	16.40	99.0	16.90
538	2/03/99	01CD1DF7	A Full	A1	1	16.45	97.0	18.02
539	2/03/99	01C81F89	A Full	A1	1	15.54	94.5	18.41
540	2/03/99	0169C837	A Full	A1	1	16.87	97.5	18.20
541	2/03/99	01EFDC14	A Full	A1	1	19.26	96.5	21.43
542	2/03/99	01F09005	A Full	A1	1	14.77	94.0	17.78
543	2/03/99	01E28835	A Full	A1	1	19.14	99.0	19.73
544	2/03/99	01E53FF5	A Full	A1	1	19.36	97.5	20.89
545	2/03/99	01F05A41	A Full	A1	1	15.10	90.0	20.71
546	2/03/99	01F07F10	A Full	A1	1	18.28	97.0	20.03
547	2/03/99	01C831F1	A Full	A1	1	16.16	94.0	19.46
548	2/03/99	01C717FC	A Full	A1	1	16.34	94.0	19.67
549	2/03/99	01C7252E	A Full	A1	1	17.42	98.5	18.23
550	2/03/99	01C7FF6F	A Full	A1	1	18.06	97.0	19.79
551	2/03/99	01F16625	A Full	A1	1	16.68	97.0	18.28
552	2/03/99	01C72CA6	A Full	A1	1	17.42	96.5	19.39
553	2/03/99	01C7F5A7	A Full	A1	1	20.48	105.0	17.69
554	2/03/99	01C7273D	A Full	A1	1	17.78	96.5	19.79
555	2/03/99	01C712C3	A Full	A1	1	17.22	96.5	19.16
556	2/03/99	01C83208	A Full	A1	1	14.25	91.0	18.91
557	2/03/99	01C7212F	A Full	A1	1	17.32	96.5	19.27
		MEANS						19.17

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99 Tuna Harvest Growth Results - Cage A1

Selection Number	Allocation Date	Pit Tag Number	Harvest Date	Harvest Number	Tail Tag Number	Bag Tag Number	Harvest Weight
528	2/03/99	0169D2B3	18/06/99				
529	2/03/99	01C82F22	18/06/99	13	B13	A112	23.50
530	2/03/99	01C81F92	18/06/99	12	B12	A111	23.38
531	2/03/99	0169DDC4	18/06/99	5	B5	A14	24.20
532	2/03/99	01CD263B	18/06/99	21	B21	-	21.84
533	2/03/99	01CB2B18	18/06/99	3	B3	A12	23.08
534	2/03/99	01CB272D	18/06/99				
535	2/03/99	01CD3275	18/06/99	4	B4	A13	21.30
536	2/03/99	0216C229	18/06/99	10	B10	A19	22.06
537	2/03/99	01C824E7	18/06/99	15	B15	A114	20.68
538	2/03/99	01CD1DF7	18/06/99	18	B18	-	22.02
539	2/03/99	01C81F89	18/06/99	23	B23	-	19.44
540	2/03/99	0169C837	18/06/99				
541	2/03/99	01EFDC14	18/06/99	14	B14	A113	24.54
542	2/03/99	01F09005	18/06/99	20	B20	-	19.66
543	2/03/99	01E28835	18/06/99				
544	2/03/99	01E53FF5	18/06/99	6	B6	A15	24.86
545	2/03/99	01F05A41	18/06/99	2	B2	-	20.38
546	2/03/99	01F07F10	18/06/99	9	B9	A18	24.72
547	2/03/99	01C831F1	18/06/99	22	B22	-	23.32
548	2/03/99	01C717FC	18/06/99				
549	2/03/99	01C7252E	18/06/99	7	B7	A16	22.44
550	2/03/99	01C7FF6F	18/06/99	11	B11	A110	23.10
551	2/03/99	01F16625	18/06/99	19	B19	-	22.36
552	2/03/99	01C72CA6	18/06/99				
553	2/03/99	01C7F5A7	18/06/99				
554	2/03/99	01C7273D	18/06/99	8	B8	A17	23.04
555	2/03/99	01C712C3	18/06/99	16	B16	A115	23.70
556	2/03/99	01C83208	18/06/99	17	B17	-	20.00
557	2/03/99	01C7212F	18/06/99	1	B1	A11	23.62
		MEANS					22.49

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99 Tuna Harvest Growth Results - Cage A1

Selection Number	Allocation Date	Pit Tag Number	Harvest Length	Days on	Weight Gain (kg)	Length Gain (cm)	Final CI	CI Gain	ADG (g)
528	2/03/99	0169D2B3		109					
529	2/03/99	01C82F22	103.0	109	4.49	3.00	21.51	2.50	41.19
530	2/03/99	01C81F92	101.0	109	6.38	4.00	22.69	4.07	58.53
531	2/03/99	0169DDC4	100.0	109	6.36	4.00	24.20	4.04	58.35
532	2/03/99	01CD263B	98.0	109	6.38	5.00	23.20	3.98	58.53
533	2/03/99	01CB2B18	104.0	109	4.08	4.50	20.52	1.23	37.43
534	2/03/99	01CB272D		109					
535	2/03/99	01CD3275	98.0	109	4.76	4.00	22.63	2.72	43.67
536	2/03/99	0216C229	101.0	109	4.32	5.00	21.41	1.36	39.63
537	2/03/99	01C824E7	101.0	109	4.28	2.00	20.07	3.17	39.27
538	2/03/99	01CD1DF7	101.0	109	5.57	4.00	21.37	3.35	51.10
539	2/03/99	01C81F89	97.0	109	3.90	2.50	21.30	2.89	35.78
540	2/03/99	0169C837		109					
541	2/03/99	01EFDC14	102.0	109	5.28	5.50	23.12	1.69	48.44
542	2/03/99	01F09005	96.0	109	4.89	2.00	22.22	4.44	44.86
543	2/03/99	01E28835		109					
544	2/03/99	01E53FF5	100.0	109	5.50	2.50	24.86	3.97	50.46
545	2/03/99	01F05A41	95.0	109	5.28	5.00	23.77	3.06	48.44
546	2/03/99	01F07F10	102.0	109	6.44	5.00	23.29	3.27	59.08
547	2/03/99	01C831F1	100.0	109	7.16	6.00	23.32	3.86	65.69
548	2/03/99	01C717FC		109					
549	2/03/99	01C7252E	105.0	109	5.02	6.50	19.38	1.16	46.06
550	2/03/99	01C7FF6F	101.5	109	5.04	4.50	22.09	2.30	46.24
551	2/03/99	01F16625	101.0	109	5.68	4.00	21.70	3.43	52.11
552	2/03/99	01C72CA6		109					
553	2/03/99	01C7F5A7		109					
554	2/03/99	01C7273D	101.0	109	5.26	4.50	22.36	2.58	48.26
555	2/03/99	01C712C3	101.0	109	6.48	4.50	23.00	3.84	59.45
556	2/03/99	01C83208	99.0	109	5.75	8.00	20.61	1.70	52.75
557	2/03/99	01C7212F	101.0	109	6.30	4.50	22.93	3.65	57.80
		MEANS	100.37		5.42	4.37	22.24	2.97	49.70

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99 Tuna Harvest Growth Results - Cage A1

Selection Number	Allocation Date	Pit Tag Number	Specific Growth Rate
528	2/03/99	0169D2B3	
529	2/03/99	01C82F22	0.19
530	2/03/99	01C81F92	0.29
531	2/03/99	0169DDC4	0.28
532	2/03/99	01CD263B	0.32
533	2/03/99	01CB2B18	0.18
534	2/03/99	01CB272D	
535	2/03/99	01CD3275	0.23
536	2/03/99	0216C229	0.20
537	2/03/99	01C824E7	0.21
538	2/03/99	01CD1DF7	0.27
539	2/03/99	01C81F89	0.21
540	2/03/99	0169C837	
541	2/03/99	01EFDC14	0.22
542	2/03/99	01F09005	0.26
543	2/03/99	01E28835	
544	2/03/99	01E53FF5	0.23
545	2/03/99	01F05A41	0.28
546	2/03/99	01F07F10	0.28
547	2/03/99	01C831F1	0.34
548	2/03/99	01C717FC	
549	2/03/99	01C7252E	0.23
550	2/03/99	01C7FF6F	0.23
551	2/03/99	01F16625	0.27
552	2/03/99	01C72CA6	
553	2/03/99	01C7F5A7	
554	2/03/99	01C7273D	0.24
555	2/03/99	01C712C3	0.29
556	2/03/99	01C83208	0.31
557	2/03/99	01C7212F	0.28
		MEANS	0.25

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99 Tuna Harvest Growth Results - Cage A3

Selection Number	Allocation Date	Pit Tag Number	Treatment	Cage	Ing Cage ID	Starting Weight	Starting Length (cm)	Start CI
438	3/03/99	01C831F6	A Restricted	A3	3	17.60	97.0	19.28
439	3/03/99	01C70AA1	A Restricted	A3	3	17.98	96.5	20.01
440	3/03/99	01C7FF95	A Restricted	A3	3	16.98	98.5	17.77
441	3/03/99	01C71526	A Restricted	A3	3	20.70	100.0	20.70
442	3/03/99	01C7043B	A Restricted	A3	3	20.76	100.0	20.76
443	3/03/99	01C70630	A Restricted	A3	3	17.72	96.0	20.03
444	3/03/99	01C72A66	A Restricted	A3	3	17.22	94.0	20.73
445	3/03/99	01C724E3	A Restricted	A3	3	16.46	96.0	18.60
446	3/03/99	01F08340	A Restricted	A3	3	18.64	97.0	20.42
447	3/03/99	01C718F4	A Restricted	A3	3	18.70	98.0	19.87
448	3/03/99	01C72C6D	A Restricted	A3	3	17.60	98.5	18.42
449	3/03/99	01C70984	A Restricted	A3	3	15.88	93.5	19.43
450	3/03/99	01C7223B	A Restricted	A3	3	19.50	100.5	19.21
451	3/03/99	01C71A61	A Restricted	A3	3	18.50	98.0	19.66
452	3/03/99	01C73030	A Restricted	A3	3	17.66	99.0	18.20
453	3/03/99	01C7170B	A Restricted	A3	3	19.40	98.0	20.61
454	3/03/99	01C70A4B	A Restricted	A3	3	18.08	97.5	19.51
455	3/03/99	01C7FEF4	A Restricted	A3	3	18.22	100.5	17.95
456	3/03/99	01C7C336	A Restricted	A3	3	16.14	95.0	18.82
457	3/03/99	01C80184	A Restricted	A3	3	16.10	95.5	18.48
458	3/03/99	01C71286	A Restricted	A3	3	17.16	96.0	19.40
459	3/03/99	01C7F388	A Restricted	A3	3	18.52	98.0	19.68
460	3/03/99	01F05BE6	A Restricted	A3	3	19.24	101.0	18.67
461	3/03/99	01C70E09	A Restricted	A3	3	16.84	94.0	20.27
462	3/03/99	01C707C9	A Restricted	A3	3	12.64	88.0	18.55
463	3/03/99	01CF2F9E	A Restricted	A3	3	20.84	100.0	20.84
464	3/03/99	01C71A16	A Restricted	A3	3	17.92	95.0	20.90
465	3/03/99	01F0B9CC	A Restricted	A3	3	19.54	101.0	18.97
466	3/03/99	01C70E2E	A Restricted	A3	3	13.42	91.0	17.81
467	3/03/99	01C7FA03	A Restricted	A3	3	16.22	94.0	19.53
		MEANS						19.44

NOTE: Five fish were missing pit tags (weighing 25.50, 28.92, 24.70, 23.98, 27.68 kg, respectively)

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99 Tuna Harvest Growth Results - Cage A3

Selection Number	Allocation Date	Pit Tag Number	Harvest Date	Harvest Number	Tail Tag Number	Bag Tag Number	Harvest Weight
438	3/03/99	01C831F6	15/06/99				
439	3/03/99	01C70AA1	15/06/99	18	W18	A315	23.66
440	3/03/99	01C7FF95	15/06/99	7	W7	A37	21.58
441	3/03/99	01C71526	15/06/99	16	W16	A313	27.08
442	3/03/99	01C7043B	15/06/99	1	W1	A31	26.94
443	3/03/99	01C70630	15/06/99	12	W12	A310	23.66
444	3/03/99	01C72A66	15/06/99	15	W15	A312	22.04
445	3/03/99	01C724E3	15/06/99	11	W11	A39	23.40
446	3/03/99	01F08340	15/06/99	25	W25	-	24.40
447	3/03/99	01C718F4	15/06/99				
448	3/03/99	01C72C6D	15/06/99	17	W17	A314	23.14
449	3/03/99	01C70984	15/06/99	5	W5	A35	20.30
450	3/03/99	01C7223B	15/06/99	2	W2	A32	26.88
451	3/03/99	01C71A61	15/06/99				
452	3/03/99	01C73030	15/06/99	26	W26	-	23.06
453	3/03/99	01C7170B	15/06/99	20	W20	-	25.82
454	3/03/99	01C70A4B	15/06/99				
455	3/03/99	01C7FEF4	15/06/99	22	W22	-	23.54
456	3/03/99	01C7C336	15/06/99	3	W3	A33	21.80
457	3/03/99	01C80184	15/06/99	24	W24	-	19.04
458	3/03/99	01C71286	15/06/99				
459	3/03/99	01C7F388	15/06/99	9	W9	A38	24.70
460	3/03/99	01F05BE6	15/06/99	14	W14	A311	26.20
461	3/03/99	01C70E09	15/06/99	19	W19	-	22.26
462	3/03/99	01C707C9	15/06/99				
463	3/03/99	01CF2F9E	15/06/99				
464	3/03/99	01C71A16	15/06/99	4	W4	A34	23.66
465	3/03/99	01F0B9CC	15/06/99				
466	3/03/99	01C70E2E	15/06/99	6	W6	A36	18.84
467	3/03/99	01C7FA03	15/06/99				
		MEANS					23.43

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99 Tuna Harvest Growth Results - Cage A3

Selection Number	Allocation Date	Pit Tag Number	Harvest Length	Days on	Weight Gain (kg)	Length Gain (cm)	Final CI	CI Gain	ADG (g)
438	3/03/99	01C831F6		105					
439	3/03/99	01C70AA1	102.0	105	5.68	5.50	22.30	2.29	54.10
440	3/03/99	01C7FF95	101.0	105	4.60	2.50	20.95	3.18	43.81
441	3/03/99	01C71526	106.0	105	6.38	6.00	22.74	2.04	60.76
442	3/03/99	01C7043B	106.0	105	6.18	6.00	22.62	1.86	58.86
443	3/03/99	01C70630	102.0	105	5.94	6.00	22.30	2.27	56.57
444	3/03/99	01C72A66	96.5	105	4.82	2.50	24.53	3.79	45.90
445	3/03/99	01C724E3	99.7	105	6.94	3.70	23.61	5.01	66.10
446	3/03/99	01F08340	102.0	105	5.76	5.00	22.99	2.57	54.86
447	3/03/99	01C718F4		105					
448	3/03/99	01C72C6D	102.8	105	5.54	4.30	21.30	2.88	52.76
449	3/03/99	01C70984	98.3	105	4.42	4.80	21.37	1.94	42.10
450	3/03/99	01C7223B	105.5	105	7.38	5.00	22.89	3.68	70.29
451	3/03/99	01C71A61		105					
452	3/03/99	01C73030	102.7	105	5.40	3.70	21.29	3.09	51.43
453	3/03/99	01C7170B	103.0	105	6.42	5.00	23.63	3.02	61.14
454	3/03/99	01C70A4B		105					
455	3/03/99	01C7FEF4	105.3	105	5.32	4.80	20.16	2.21	50.67
456	3/03/99	01C7C336	98.5	105	5.66	3.50	22.81	3.99	53.90
457	3/03/99	01C80184	98.3	105	2.94	2.80	20.05	1.56	28.00
458	3/03/99	01C71286		105					
459	3/03/99	01C7F388	102.0	105	6.18	4.00	23.28	3.60	58.86
460	3/03/99	01F05BE6	104.6	105	6.96	3.60	22.89	4.22	66.29
461	3/03/99	01C70E09	99.4	105	5.42	5.40	22.67	2.39	51.62
462	3/03/99	01C707C9		105					
463	3/03/99	01CF2F9E		105					
464	3/03/99	01C71A16	101.2	105	5.74	6.20	22.83	1.93	54.67
465	3/03/99	01F0B9CC		105					
466	3/03/99	01C70E2E	98.1	105	5.42	7.10	19.96	2.15	51.62
467	3/03/99	01C7FA03							
		MEANS	101.66		5.67	4.64	22.24	2.84	54.01

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99 Tuna Harvest Growth Results - Cage A3

Selection Number	Allocation Date	Pit Tag Number	Specific Growth Rate
438	3/03/99	01C831F6	
439	3/03/99	01C70AA1	0.26
440	3/03/99	01C7FF95	0.23
441	3/03/99	01C71526	0.26
442	3/03/99	01C7043B	0.25
443	3/03/99	01C70630	0.28
444	3/03/99	01C72A66	0.24
445	3/03/99	01C724E3	0.34
446	3/03/99	01F08340	0.26
447	3/03/99	01C718F4	
448	3/03/99	01C72C6D	0.26
449	3/03/99	01C70984	0.23
450	3/03/99	01C7223B	0.31
451	3/03/99	01C71A61	
452	3/03/99	01C73030	0.25
453	3/03/99	01C7170B	0.27
454	3/03/99	01C70A4B	
455	3/03/99	01C7FEF4	0.24
456	3/03/99	01C7C336	0.29
457	3/03/99	01C80184	0.16
458	3/03/99	01C71286	
459	3/03/99	01C7F388	0.27
460	3/03/99	01F05BE6	0.29
461	3/03/99	01C70E09	0.27
462	3/03/99	01C707C9	
463	3/03/99	01CF2F9E	
464	3/03/99	01C71A16	0.26
465	3/03/99	01F0B9CC	
466	3/03/99	01C70E2E	0.32
467	3/03/99	01C7FA03	
		MEANS	0.26

Prepared by Dr Robert van Barneveld 15/01/02

99 Tuna Harvest Growth Results - Cage A4

Selection Number	Allocation Date	Pit Tag Number	Treatment	Cage	Ing Cage ID	Starting Weight	Starting Length (cm)	Start CI
2	1/03/99	01CD2E6CT	A Full	A4	20	8.58	75.50	19.94
3	1/03/99	01C71511T	A Full	A4	20	15.26	93.50	18.67
4	1/03/99	01C7F660T	A Full	A4	20	13.74	91.50	17.94
5	1/03/99	01C7116BT	A Full	A4	20	14.96	94.00	18.01
6	1/03/99	01C72B4FT	A Full	A4	20	11.18	82.00	20.28
8	1/03/99	01C7118CT	A Full	A4	20	10.42	85.00	16.97
355	1/03/99	01CB01BET	A Full	A4	20	15.16	95.00	17.68
356	1/03/99	01F013CCT	A Full	A4	20	17.26	96.00	19.51
357	1/03/99	01C70E49T	A Full	A4	20	11.6	84.00	19.57
358	1/03/99	01C7301ET	A Full	A4	20	16.22	99.00	16.72
359	1/03/99	01C72AD6T	A Full	A4	20	15.78	91.00	20.94
360	1/03/99	01F1333CT	A Full	A4	20	20.02	101.00	19.43
361	1/03/99	01C7FDDBT	A Full	A4	20	15.64	96.00	17.68
362	1/03/99	01C7ED62T	A Full	A4	20	23.98	108.00	19.04
363	1/03/99	01C73216T	A Full	A4	20	15.36	94.00	18.49
364	1/03/99	01C7F27ET	A Full	A4	20	17.1	95.00	19.94
365	1/03/99	01F160C0T	A Full	A4	20	16.86	95.00	19.66
366	1/03/99	01F158C6T	A Full	A4	20	17.06	98.00	18.13
367	1/03/99	01C712B1T	A Full	A4	20	16.64	97.00	18.23
368	1/03/99	01C71A8ET	A Full	A4	20	10.27	84.50	17.02
369	1/03/99	01C71B8AT	A Full	A4	20	15.3	95.00	17.85
370	1/03/99	01C73032T	A Full	A4	20	15.92	95.00	18.57
371	1/03/99	01C72172T	A Full	A4	20	16.18	95.00	18.87
372	1/03/99	01C820B0T	A Full	A4	20	18.9	103.00	17.30
373	1/03/99	01C82ES1T	A Full	A4	20	13.88	91.00	18.42
374	1/03/99	01C82B52T	A Full	A4	20	15.64	92.50	19.76
375	1/03/99	0169CCD1T	A Full	A4	20	16.6	92.00	21.32
376	1/03/99	0169D6B0T	A Full	A4	20	13.9	94.00	16.74
377	1/03/99	0216BDB3T	A Full	A4	20	18.96	97.50	20.46
		MEANS						18.73

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99 Tuna Harvest Growth Results - Cage A4

Selection Number	Allocation Date	Pit Tag Number	Harvest Date	Harvest Number	Tail Tag Number	Bag Tag Number	Harvest Weight
2	1/03/99	01CD2E6CT	14/06/99	23	B23	-	14.38
3	1/03/99	01C71511T	14/06/99	17	B17	-	22.44
4	1/03/99	01C7F660T	14/06/99	20	B20	-	18.48
5	1/03/99	01C7116BT	14/06/99	16	B16	A415	21.68
6	1/03/99	01C72B4FT	14/06/99	12	B12	-	15.76
8	1/03/99	01C7118CT	14/06/99	3	B3	A43	16.84
355	1/03/99	01CB01BET	14/06/99	14	B14	A413	22.80
356	1/03/99	01F013CCT	14/06/99	19	B19	-	22.38
357	1/03/99	01C70E49T	14/06/99	15	B15	A414	17.72
358	1/03/99	01C7301ET	14/06/99	1	B1	A41	22.34
359	1/03/99	01C72AD6T	14/06/99	21	B21	-	24.18
360	1/03/99	01F1333CT	14/06/99				
361	1/03/99	01C7FDDBT	14/06/99	24	B24	-	18.60
362	1/03/99	01C7ED62T	14/06/99				
363	1/03/99	01C73216T	14/06/99	4	B4	A44	23.06
364	1/03/99	01C7F27ET	14/06/99	6	B6	A46	22.22
365	1/03/99	01F160C0T	14/06/99	2	B2	A42	24.12
366	1/03/99	01F158C6T	14/06/99				
367	1/03/99	01C712B1T	14/06/99				
368	1/03/99	01C71A8ET	14/06/99				
369	1/03/99	01C71B8AT	14/06/99				
370	1/03/99	01C73032T	14/06/99	10	B10	A410	21.10
371	1/03/99	01C72172T	14/06/99	13	B13	A412	22.64
372	1/03/99	01C820B0T	14/06/99	22	B22	-	25.88
373	1/03/99	01C82ES1T	14/06/99	8	B8	A48	20.68
374	1/03/99	01C82B52T	14/06/99	11	B11	A411	21.18
375	1/03/99	0169CCD1T	14/06/99	18	B18	-	22.62
376	1/03/99	0169D6B0T	14/06/99	9	B9	A49	20.32
377	1/03/99	0216BDB3T	14/06/99	5	B5	A45	25.36
		MEANS					21.16

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99 Tuna Harvest Growth Results - Cage A4

Selection Number	Allocation Date	Pit Tag Number	Harvest Length	Days on	Weight Gain (kg)	Length Gain (cm)	Final CI	CI Gain	ADG (g)
2	1/03/99	01CD2E6CT	85.0	106	5.80	9.50	23.42	3.48	54.72
3	1/03/99	01C71511T	98.1	106	7.18	4.60	23.77	5.10	67.74
4	1/03/99	01C7F660T	94.7	106	4.74	3.20	21.76	3.82	44.72
5	1/03/99	01C7116BT	100.0	106	6.72	6.00	21.68	3.67	63.40
6	1/03/99	01C72B4FT	87.0	106	4.58	5.00	23.93	3.66	43.21
8	1/03/99	01C7118CT	96.0	106	6.42	11.00	19.03	2.07	60.57
355	1/03/99	01CB01BET	101.0	106	7.64	6.00	22.13	4.45	72.08
356	1/03/99	01F013CCT	99.0	106	5.12	3.00	23.07	3.56	48.30
357	1/03/99	01C70E49T	91.3	106	6.12	7.30	23.28	3.71	57.74
358	1/03/99	01C7301ET	103.5	106	6.12	4.50	20.15	3.43	57.74
359	1/03/99	01C72AD6T	98.3	106	8.40	7.30	25.46	4.52	79.25
360	1/03/99	01F1333CT							
361	1/03/99	01C7FDDBT	99.3	106	2.96	3.30	19.00	1.32	27.92
362	1/03/99	01C7ED62T		106					
363	1/03/99	01C73216T	97.5	106	7.70	3.50	24.88	6.39	72.64
364	1/03/99	01C7F27ET	101.5	106	5.12	6.50	21.25	1.30	48.30
365	1/03/99	01F160C0T	100.0	106	7.26	5.00	24.12	4.46	68.49
366	1/03/99	01F158C6T		106					
367	1/03/99	01C712B1T		106					
368	1/03/99	01C71A8ET		106					
369	1/03/99	01C71B8AT		106					
370	1/03/99	01C73032T	97.0	106	5.18	2.00	23.12	4.55	48.87
371	1/03/99	01C72172T	99.0	106	6.46	4.00	23.33	4.46	60.94
372	1/03/99	01C820B0T	107.0	106	6.98	4.00	21.13	3.83	65.85
373	1/03/99	01C82ES1T	96.0	106	6.80	5.00	23.37	4.96	64.15
374	1/03/99	01C82B52T	96.5	106	5.54	4.00	23.57	3.81	52.26
375	1/03/99	0169CCD1T	98.8	106	6.02	6.80	23.45	2.14	56.79
376	1/03/99	0169D6B0T	97.0	106	6.42	3.00	22.26	5.53	60.57
377	1/03/99	0216BDB3T	104.5	106	6.40	7.00	22.22	1.77	60.38
		MEANS	97.74		6.16	5.28	22.58	3.74	58.11

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99 Tuna Harvest Growth Results - Cage A4

Selection Number	Allocation Date	Pit Tag Number	Specific Growth Rate
2	1/03/99	01CD2E6CT	0.49
3	1/03/99	01C71511T	0.36
4	1/03/99	01C7F660T	0.28
5	1/03/99	01C7116BT	0.35
6	1/03/99	01C72B4FT	0.32
8	1/03/99	01C7118CT	0.45
355	1/03/99	01CB01BET	0.39
356	1/03/99	01F013CCT	0.25
357	1/03/99	01C70E49T	0.40
358	1/03/99	01C7301ET	0.30
359	1/03/99	01C72AD6T	0.40
360	1/03/99	01F1333CT	
361	1/03/99	01C7FDDBT	0.16
362	1/03/99	01C7ED62T	
363	1/03/99	01C73216T	0.38
364	1/03/99	01C7F27ET	0.25
365	1/03/99	01F160C0T	0.34
366	1/03/99	01F158C6T	
367	1/03/99	01C712B1T	
368	1/03/99	01C71A8ET	
369	1/03/99	01C71B8AT	
370	1/03/99	01C73032T	0.27
371	1/03/99	01C72172T	0.32
372	1/03/99	01C820B0T	0.30
373	1/03/99	01C82ES1T	0.38
374	1/03/99	01C82B52T	0.29
375	1/03/99	0169CCD1T	0.29
376	1/03/99	0169D6B0T	0.36
377	1/03/99	0216BDB3T	0.27
		MEANS	0.33

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Selection Number	Allocation Date	Pit Tag Number	Treatment	Cage	Ing Cage ID	Starting Weight	Starting Length (cm)	Start CI
558	2/03/99	01CD255E	B Restricted	B1	26	15.68	94.0	18.88
559	2/03/99	01CD31A2	B Restricted	B1	26	15.46	94.0	18.61
560	2/03/99	01CD2498	B Restricted	B1	26	15.90	95.0	18.54
561	2/03/99	01F0FC67	B Restricted	B1	26	20.12	101.0	19.53
562	2/03/99	0169D0AB	B Restricted	B1	26	18.10	95.0	21.11
563	2/03/99	01C7245F	B Restricted	B1	26	17.57	96.0	19.86
564	2/03/99	01C7175D	B Restricted	B1	26	18.26	97.0	20.01
565	2/03/99	01C71944	B Restricted	B1	26	16.66	97.5	17.97
566	2/03/99	01C7F81E	B Restricted	B1	26	17.90	98.0	19.02
567	2/03/99	01C722FD	B Restricted	B1	26	18.34	97.0	20.09
568	2/03/99	01C70469	B Restricted	B1	26	19.01	100.0	19.01
569	2/03/99	01C71A3F	B Restricted	B1	26	16.53	98.0	17.56
570	2/03/99	01C72B2C	B Restricted	B1	26	17.34	98.0	18.42
571	2/03/99	01C80023	B Restricted	B1	26	19.60	103.5	17.68
572	2/03/99	01C7093F	B Restricted	B1	26	18.40	98.0	19.55
573	2/03/99	01CD1DE6	B Restricted	B1	26	17.21	99.0	17.74
574	2/03/99	01C72F2A	B Restricted	B1	26	16.78	95.5	19.27
575	2/03/99	01C7F60F	B Restricted	B1	26	16.60	98.0	17.64
576	2/03/99	01C71650	B Restricted	B1	26	18.08	95.0	21.09
577	2/03/99	01F15619	B Restricted	B1	26	16.38	96.0	18.51
578	2/03/99	01C71BFE	B Restricted	B1	26	12.82	87.0	19.47
579	2/03/99	01C7FB2E	B Restricted	B1	26	17.22	92.0	22.11
580	2/03/99	01C705AA	B Restricted	B1	26	16.93	97.0	18.55
581	2/03/99	01C7173D	B Restricted	B1	26	17.40	97.0	19.06
582	2/03/99	01C709BF	B Restricted	B1	26	15.46	91.5	20.18
583	2/03/99	01C70D26	B Restricted	B1	26	17.52	98.0	18.61
584	2/03/99	01C710F3	B Restricted	B1	26	14.84	97.0	16.26
585	2/03/99	01C7FAD6	B Restricted	B1	26	18.58	98.0	19.74
586	2/03/99	01F09021	B Restricted	B1	26	17.76	96.5	19.76
587	2/03/99	01C82D31	B Restricted	B1	26	13.44	88.5	19.39
		MEANS						19.11

Selection Number	Allocation Date	Pit Tag Number	Harvest Date	Harvest Number	Tail Tag Number	Bag Tag Number	Harvest Weight
558	2/03/99	01CD255E	18/06/99				
559	2/03/99	01CD31A2	18/06/99	17	W17	-	20.28
560	2/03/99	01CD2498	18/06/99				
561	2/03/99	01F0FC67	18/06/99	9	W9	B18	28.60
562	2/03/99	0169D0AB	18/06/99	16	W16	B115	20.68
563	2/03/99	01C7245F	18/06/99	3	W3	B13	22.88
564	2/03/99	01C7175D	18/06/99				
565	2/03/99	01C71944	18/06/99				
566	2/03/99	01C7F81E	18/06/99	18	W18	-	23.28
567	2/03/99	01C722FD	18/06/99				
568	2/03/99	01C70469	18/06/99	13	W13	B112	21.30
569	2/03/99	01C71A3F	18/06/99				
570	2/03/99	01C72B2C	18/06/99	15	W15	B114	22.36
571	2/03/99	01C80023	18/06/99				
572	2/03/99	01C7093F	18/06/99				
573	2/03/99	01CD1DE6	18/06/99	8	W8	B17	21.20
574	2/03/99	01C72F2A	18/06/99	1	W1	B11	20.62
575	2/03/99	01C7F60F	18/06/99				
576	2/03/99	01C71650	18/06/99	14	W14	B113	23.80
577	2/03/99	01F15619	18/06/99				
578	2/03/99	01C71BFE	18/06/99	11	W11	B110	18.08
579	2/03/99	01C7FB2E	18/06/99	12	W12	B111	23.04
580	2/03/99	01C705AA	18/06/99	5	W5	B15	21.84
581	2/03/99	01C7173D	18/06/99	2	W2	B12	20.10
582	2/03/99	01C709BF	18/06/99	6	W6	B16	19.28
583	2/03/99	01C70D26	18/06/99	10	W10	B19	23.10
584	2/03/99	01C710F3	18/06/99				
585	2/03/99	01C7FAD6	18/06/99				
586	2/03/99	01F09021	18/06/99	4	W4	B14	22.36
587	2/03/99	01C82D31	18/06/99	7	W7	-	18.02
		MEANS					21.71

Selection Number	Allocation Date	Pit Tag Number	Harvest Length	Days on	Weight Gain (kg)	Length Gain (cm)	Final CI	CI Gain	ADG (g)
558	2/03/99	01CD255E		109					
559	2/03/99	01CD31A2	99.0	109	4.82	5.00	20.90	2.29	44.22
560	2/03/99	01CD2498		109					
561	2/03/99	01F0FC67	105.0	109	8.48	4.00	24.71	5.18	77.80
562	2/03/99	0169D0AB	99.0	109	2.58	4.00	21.31	0.20	23.67
563	2/03/99	01C7245F	101.0	109	5.31	5.00	22.21	2.35	48.72
564	2/03/99	01C7175D		109					
565	2/03/99	01C71944		109					
566	2/03/99	01C7F81E	104.0	109	5.38	6.00	20.70	1.68	49.36
567	2/03/99	01C722FD		109					
568	2/03/99	01C70469	102.0	109	2.29	2.00	20.07	1.06	21.01
569	2/03/99	01C71A3F		109					
570	2/03/99	01C72B2C	102.0	109	5.02	4.00	21.07	2.65	46.06
571	2/03/99	01C80023		109					
572	2/03/99	01C7093F		109					
573	2/03/99	01CD1DE6	103.5	109	3.99	4.50	19.12	1.38	36.61
574	2/03/99	01C72F2A	100.5	109	3.84	5.00	20.31	1.05	35.23
575	2/03/99	01C7F60F		109					
576	2/03/99	01C71650	100.0	109	5.72	5.00	23.80	2.71	52.48
577	2/03/99	01F15619		109					
578	2/03/99	01C71BFE	92.0	109	5.26	5.00	23.22	3.75	48.26
579	2/03/99	01C7FB2E	96.0	109	5.82	4.00	26.04	3.93	53.39
580	2/03/99	01C705AA	102.0	109	4.91	5.00	20.58	2.03	45.05
581	2/03/99	01C7173D	99.0	109	2.70	2.00	20.72	1.65	24.77
582	2/03/99	01C709BF	96.0	109	3.82	4.50	21.79	1.61	35.05
583	2/03/99	01C70D26	102.0	109	5.58	4.00	21.77	3.15	51.19
584	2/03/99	01C710F3		109					
585	2/03/99	01C7FAD6		109					
586	2/03/99	01F09021	100.0	109	4.60	3.50	22.36	2.60	42.20
587	2/03/99	01C82D31	93.5	109	4.58	5.00	22.05	2.66	42.02
		MEANS	99.81		4.71	4.31	21.82	2.33	43.17

99 Tuna Harvest Growth Results - Cage B1

Selection Number	Allocation Date	Pit Tag Number	Specific Growth Rate
558	2/03/99	01CD255E	
559	2/03/99	01CD31A2	0.25
560	2/03/99	01CD2498	
561	2/03/99	01F0FC67	0.32
562	2/03/99	0169D0AB	0.12
563	2/03/99	01C7245F	0.24
564	2/03/99	01C7175D	
565	2/03/99	01C71944	
566	2/03/99	01C7F81E	0.24
567	2/03/99	01C722FD	
568	2/03/99	01C70469	0.10
569	2/03/99	01C71A3F	
570	2/03/99	01C72B2C	0.23
571	2/03/99	01C80023	
572	2/03/99	01C7093F	
573	2/03/99	01CD1DE6	0.19
574	2/03/99	01C72F2A	0.19
575	2/03/99	01C7F60F	
576	2/03/99	01C71650	0.25
577	2/03/99	01F15619	
578	2/03/99	01C71BFE	0.32
579	2/03/99	01C7FB2E	0.27
580	2/03/99	01C705AA	0.23
581	2/03/99	01C7173D	0.13
582	2/03/99	01C709BF	0.20
583	2/03/99	01C70D26	0.25
584	2/03/99	01C710F3	
585	2/03/99	01C7FAD6	
586	2/03/99	01F09021	0.21
587	2/03/99	01C82D31	0.27
		MEANS	0.22

Selection Number	Allocation Date	Pit Tag Number	Treatment	Cage	Ing Cage ID	Starting Weight	Starting Length (cm)	Start CI
378	1/03/99	01C82BFE	B Restricted	B4	21	14.32	90.0	19.64
379	1/03/99	0169D4F7	B Restricted	B4	21	15.92	97.0	17.44
380	1/03/99	0169D432	B Restricted	B4	21	21.16	105.0	18.28
381	1/03/99	01CB2A4B	B Restricted	B4	21	16.06	94.0	19.34
382	1/03/99	01F15081	B Restricted	B4	21	16.42	98.0	17.45
383	1/03/99	0169D9A0	B Restricted	B4	21	18.38	101.0	17.84
384	1/03/99	0169D78A	B Restricted	B4	21	17.78	97.0	19.48
385	1/03/99	01E501E7	B Restricted	B4	21	16.00	95.0	18.66
386	1/03/99	01C82437	B Restricted	B4	21	19.14	99.0	19.73
387	1/03/99	0169D59C	B Restricted	B4	21	14.82	94.0	17.84
388	1/03/99	01C81C74	B Restricted	B4	21	15.08	94.0	18.16
389	1/03/99	0169D304	B Restricted	B4	21	17.48	94.0	21.05
390	1/03/99	0169DAAB	B Restricted	B4	21	15.52	98.5	16.24
391	1/03/99	01F08DC5	B Restricted	B4	21	19.48	98.0	20.70
392	1/03/99	01C8273B	B Restricted	B4	21	16.86	93.0	20.96
393	1/03/99	01C80071	B Restricted	B4	21	18.14	98.0	19.27
394	1/03/99	01CB27D3	B Restricted	B4	21	17.16	100.0	17.16
395	1/03/99	01C717B4	B Restricted	B4	21	17.56	98.0	18.66
396	1/03/99	01C71176	B Restricted	B4	21	15.66	93.5	19.16
397	1/03/99	01C715B9	B Restricted	B4	21	15.88	92.0	20.39
398	1/03/99	01C70F06	B Restricted	B4	21	19.56	104.0	17.39
399	1/03/99	01C7188A	B Restricted	B4	21	17.24	94.0	20.76
400	1/03/99	01C7301D	B Restricted	B4	21	17.84	96.0	20.16
401	1/03/99	01C7287B	B Restricted	B4	21	15.04	93.0	18.70
402	1/03/99	01C74C42	B Restricted	B4	21	15.98	94.0	19.24
403	1/03/99	01C7C34B	B Restricted	B4	21	17.74	97.0	19.44
404	1/03/99	01C70415	B Restricted	B4	21	13.80	90.0	18.93
405	1/03/99	01C8263F	B Restricted	B4	21	14.24	92.0	18.29
406	1/03/99	01C7FEFE	B Restricted	B4	21	18.04	97.0	19.77
407	1/03/99	01C722AA	B Restricted	B4	21	18.12	98.0	19.25
		MEANS						18.98
		Excluding 01C81C74						19.01

Selection Number	Allocation Date	Pit Tag Number	Harvest Date	Harvest Number	Tail Tag Number	Bag Tag Number	Harvest Weight
378	1/03/99	01C82BFE	15/06/99				
379	1/03/99	0169D4F7	15/06/99	7	B7	B45	20.60
380	1/03/99	0169D432	15/06/99	8	B8	B46	26.58
381	1/03/99	01CB2A4B	15/06/99				
382	1/03/99	01F15081	15/06/99	19	B19	-	19.42
383	1/03/99	0169D9A0	15/06/99	20	B20	-	25.46
384	1/03/99	0169D78A	15/06/99	4	B4	-	24.02
385	1/03/99	01E501E7	15/06/99	10	B10	B48	22.24
386	1/03/99	01C82437	15/06/99	15	B15	B413	24.56
387	1/03/99	0169D59C	15/06/99				
388	1/03/99	01C81C74	15/06/99	12	B12	B410	15.00
389	1/03/99	0169D304	15/06/99				
390	1/03/99	0169DAAB	15/06/99				
391	1/03/99	01F08DC5	15/06/99	2	B2	B42	23.84
392	1/03/99	01C8273B	15/06/99	13	B13	B411	21.40
393	1/03/99	01C80071	15/06/99	18	B18	-	22.44
394	1/03/99	01CB27D3	15/06/99	6	B6	B44	19.96
395	1/03/99	01C717B4	15/06/99	5	B5	B43	24.20
396	1/03/99	01C71176	15/06/99				
397	1/03/99	01C715B9	15/06/99				
398	1/03/99	01C70F06	15/06/99	1	B1	B41	26.78
399	1/03/99	01C7188A	15/06/99	9	B9	B47	25.68
400	1/03/99	01C7301D	15/06/99	14	B14	B412	20.94
401	1/03/99	01C7287B	15/06/99	17	B17	B415	20.64
402	1/03/99	01C74C42	15/06/99	16	B16	B414	20.90
403	1/03/99	01C7C34B	15/06/99	3	B3	-	25.36
404	1/03/99	01C70415	15/06/99	11	B11	B49	19.24
405	1/03/99	01C8263F	15/06/99				
406	1/03/99	01C7FEFE	15/06/99	21	B21	-	22.52
407	1/03/99	01C722AA	15/06/99				
		MEANS					22.47
		Excluding 01C81C74					22.84

Selection Number	Allocation Date	Pit Tag Number	Harvest Length	Days on	Weight Gain (kg)	Length Gain (cm)	Final CI	CI Gain	ADG (g)
378	1/03/99	01C82BFE		107					
379	1/03/99	0169D4F7	98.3	107	4.68	1.30	21.69	4.24	43.74
380	1/03/99	0169D432	108.6	107	5.42	3.60	20.75	2.47	50.65
381	1/03/99	01CB2A4B		107					
382	1/03/99	01F15081	99.6	107	3.00	1.60	19.65	2.21	28.04
383	1/03/99	0169D9A0	106.8	107	7.08	5.80	20.90	3.06	66.17
384	1/03/99	0169D78A	99.1	107	6.24	2.10	24.68	5.20	58.32
385	1/03/99	01E501E7	99.9	107	6.24	4.90	22.31	3.65	58.32
386	1/03/99	01C82437	103.4	107	5.42	4.40	22.22	2.49	50.65
387	1/03/99	0169D59C		107					
388	1/03/99	01C81C74	94.4	107	-0.08	0.40	17.83	-0.32	-0.75
389	1/03/99	0169D304		107					
390	1/03/99	0169DAAB		107					
391	1/03/99	01F08DC5	103.0	107	4.36	5.00	21.82	1.12	40.75
392	1/03/99	01C8273B	96.3	107	4.54	3.30	23.96	3.00	42.43
393	1/03/99	01C80071	109.0	107	4.30	11.00	17.33	-1.95	40.19
394	1/03/99	01CB27D3	103.5	107	2.80	3.50	18.00	0.84	26.17
395	1/03/99	01C717B4	102.0	107	6.64	4.00	22.80	4.15	62.06
396	1/03/99	01C71176		107					
397	1/03/99	01C715B9		107					
398	1/03/99	01C70F06	107.7	107	7.22	3.70	21.44	4.05	67.48
399	1/03/99	01C7188A	102.0	107	8.44	8.00	24.20	3.44	78.88
400	1/03/99	01C7301D	99.2	107	3.10	3.20	21.45	1.29	28.97
401	1/03/99	01C7287B	96.2	107	5.60	3.20	23.18	4.49	52.34
402	1/03/99	01C74C42	96.5	107	4.92	2.50	23.26	4.02	45.98
403	1/03/99	01C7C34B	102.8	107	7.62	5.80	23.34	3.91	71.21
404	1/03/99	01C70415	95.4	107	5.44	5.40	22.16	3.23	50.84
405	1/03/99	01C8263F		107					
406	1/03/99	01C7FEFE	101.0	107	4.48	4.00	21.86	2.09	41.87
407	1/03/99	01C722AA		107					
		MEANS	101.18		5.12	4.13	21.66	2.70	47.82
		Excluding 01C81C74	101.52		5.38	4.32	21.85	2.85	50.25

Selection Number	Allocation Date	Pit Tag Number	Specific Growth Rate
378	1/03/99	01C82BFE	
379	1/03/99	0169D4F7	0.24
380	1/03/99	0169D432	0.21
381	1/03/99	01CB2A4B	
382	1/03/99	01F15081	0.16
383	1/03/99	0169D9A0	0.30
384	1/03/99	0169D78A	0.28
385	1/03/99	01E501E7	0.31
386	1/03/99	01C82437	0.23
387	1/03/99	0169D59C	
388	1/03/99	01C81C74	0.00
389	1/03/99	0169D304	
390	1/03/99	0169DAAB	
391	1/03/99	01F08DC5	0.19
392	1/03/99	01C8273B	0.22
393	1/03/99	01C80071	0.20
394	1/03/99	01CB27D3	0.14
395	1/03/99	01C717B4	0.30
396	1/03/99	01C71176	
397	1/03/99	01C715B9	
398	1/03/99	01C70F06	0.29
399	1/03/99	01C7188A	0.37
400	1/03/99	01C7301D	0.15
401	1/03/99	01C7287B	0.30
402	1/03/99	01C74C42	0.25
403	1/03/99	01C7C34B	0.33
404	1/03/99	01C70415	0.31
405	1/03/99	01C8263F	
406	1/03/99	01C7FEFE	0.21
407	1/03/99	01C722AA	
		MEANS	0.24
		Excluding 01C81C74	0.25

Selection Number	Allocation Date	Pit Tag Number	Treatment	Cage	Ing Cage ID	Starting Weight	Starting Length (cm)	Start CI
588	2/03/99	0169C9A1	A Restricted	C1	25	18.62	97.5	20.09
589	2/03/99	0216C736	A Restricted	C1	25	15.94	92.0	20.47
590	2/03/99	0169D335	A Restricted	C1	25	19.18	102.0	18.07
591	2/03/99	0169C8E2	A Restricted	C1	25	16.52	96.0	18.67
592	2/03/99	01F062B3	A Restricted	C1	25	17.30	94.5	20.50
593	2/03/99	01F10526	A Restricted	C1	25	12.34	86.0	19.40
594	2/03/99	0169DCC8	A Restricted	C1	25	15.58	96.0	17.61
595	2/03/99	01F13FE9	A Restricted	C1	25	17.40	97.0	19.06
596	2/03/99	01F09131	A Restricted	C1	25	14.00	94.5	16.59
597	2/03/99	01C82776	A Restricted	C1	25	18.52	99.5	18.80
598	2/03/99	01CD2669	A Restricted	C1	25	16.70	93.0	20.76
599	2/03/99	01CD1E53	A Restricted	C1	25	17.38	98.5	18.19
600	2/03/99	01C73208	A Restricted	C1	25	20.30	99.0	20.92
601	2/03/99	01C8324F	A Restricted	C1	25	19.10	99.5	19.39
602	2/03/99	01CD323E	A Restricted	C1	25	17.36	96.0	19.62
603	2/03/99	01E518EC	A Restricted	C1	25	18.46	100.5	18.19
604	2/03/99	01F08889	A Restricted	C1	25	19.66	101.0	19.08
605	2/03/99	01F0948A	A Restricted	C1	25	17.14	99.0	17.66
606	2/03/99	01F0928F	A Restricted	C1	25	15.86	96.0	17.93
607	2/03/99	01C7FEB9	A Restricted	C1	25	18.77	101.0	18.22
608	2/03/99	01C82F10	A Restricted	C1	25	15.43	97.0	16.91
609	2/03/99	01F08C12	A Restricted	C1	25	15.03	96.5	16.73
610	2/03/99	0169D005	A Restricted	C1	25	16.20	96.5	18.03
611	2/03/99	01F10345	A Restricted	C1	25	15.94	95.0	18.59
612	2/03/99	0169CB5B	A Restricted	C1	25	17.05	98.0	18.12
613	2/03/99	01F08BE1	A Restricted	C1	25	15.80	97.0	17.31
614	2/03/99	0169D7E9	A Restricted	C1	25	17.42	98.0	18.51
615	2/03/99	01C82882	A Restricted	C1	25	18.36	99.0	18.92
616	2/03/99	0169D34D	A Restricted	C1	25	17.56	97.0	19.24
617	2/03/99	010824E9	A Restricted	C1	25	17.32	97.0	18.98
		MEANS						18.69

99 Tuna Harvest Growth Results - Cage C1

Selection Number	Allocation Date	Pit Tag Number	Harvest Date	Harvest Number	Tail Tag Number	Bag Tag Number	Harvest Weight
588	2/03/99	0169C9A1	17/06/99	11	B11	C111	24.06
589	2/03/99	0216C736	17/06/99	16	B16	-	21.86
590	2/03/99	0169D335	17/06/99	19	B19	-	24.60
591	2/03/99	0169C8E2	17/06/99	25	B25	-	19.60
592	2/03/99	01F062B3	17/06/99	18	B18	-	22.62
593	2/03/99	01F10526	17/06/99	20	B20	-	16.00
594	2/03/99	0169DCC8	17/06/99				
595	2/03/99	01F13FE9	17/06/99	4	B4	C14	21.16
596	2/03/99	01F09131	17/06/99	22	B22	-	18.36
597	2/03/99	01C82776	17/06/99	14	B14	C114	22.80
598	2/03/99	01CD2669	17/06/99	10	B10	C110	22.00
599	2/03/99	01CD1E53	17/06/99	15	B15	C115	21.08
600	2/03/99	01C73208	17/06/99	1	B1	C11	22.84
601	2/03/99	01C8324F	17/06/99	2	B2	C12	19.20
602	2/03/99	01CD323E	17/06/99	7	B7	C17	23.26
603	2/03/99	01E518EC	17/06/99	17	B17	-	19.14
604	2/03/99	01F08889	17/06/99	9	B9	C19	25.86
605	2/03/99	01F0948A	17/06/99	23	B23	-	21.74
606	2/03/99	01F0928F	17/06/99	6	B6	C16	24.88
607	2/03/99	01C7FEB9	17/06/99				
608	2/03/99	01C82F10	17/06/99	8	B8	C18	20.78
609	2/03/99	01F08C12	17/06/99				
610	2/03/99	0169D005	17/06/99				
611	2/03/99	01F10345	17/06/99	12	B12	C112	21.88
612	2/03/99	0169CB5B	17/06/99	24	B24	-	24.76
613	2/03/99	01F08BE1	17/06/99	21	B21	-	17.86
614	2/03/99	0169D7E9	17/06/99	13	B13	C113	22.82
615	2/03/99	01C82882	17/06/99	3	B3	C13	23.82
616	2/03/99	0169D34D	17/06/99	5	B5	C15	23.84
617	2/03/99	010824E9	17/06/99				
		MEANS					21.87

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99 Tuna Harvest Growth Results - Cage C1

Selection Number	Allocation Date	Pit Tag Number	Harvest Length	Days on	Weight Gain (kg)	Length Gain (cm)	Final CI	CI Gain	ADG (g)
588	2/03/99	0169C9A1	103.0	108	5.44	5.50	22.02	1.93	50.37
589	2/03/99	0216C736	99.0	108	5.92	7.00	22.53	2.06	54.81
590	2/03/99	0169D335	105.0	108	5.42	3.00	21.25	3.18	50.19
591	2/03/99	0169C8E2	98.0	108	3.08	2.00	20.82	2.15	28.52
592	2/03/99	01F062B3	105.0	108	5.32	10.50	19.54	-0.96	49.26
593	2/03/99	01F10526	90.0	108	3.66	4.00	21.95	2.55	33.89
594	2/03/99	0169DCC8		108					
595	2/03/99	01F13FE9	104.0	108	3.76	7.00	18.81	-0.25	34.81
596	2/03/99	01F09131	95.5	108	4.36	1.00	21.08	4.49	40.37
597	2/03/99	01C82776	101.5	108	4.28	2.00	21.80	3.00	39.63
598	2/03/99	01CD2669	98.0	108	5.30	5.00	23.37	2.61	49.07
599	2/03/99	01CD1E53	103.5	108	3.70	5.00	19.01	0.83	34.26
600	2/03/99	01C73208	102.0	108	2.54	3.00	21.52	0.60	23.52
601	2/03/99	01C8324F	100.0	108	0.10	0.50	19.20	-0.19	0.93
602	2/03/99	01CD323E	101.0	108	5.90	5.00	22.58	2.95	54.63
603	2/03/99	01E518EC	102.0	108	0.68	1.50	18.04	-0.15	6.30
604	2/03/99	01F08889	108.0	108	6.20	7.00	20.53	1.45	57.41
605	2/03/99	01F0948A	103.5	108	4.60	4.50	19.61	1.94	42.59
606	2/03/99	01F0928F	102.0	108	9.02	6.00	23.44	5.52	83.52
607	2/03/99	01C7FEB9		108					
608	2/03/99	01C82F10	100.0	108	5.35	3.00	20.78	3.87	49.54
609	2/03/99	01F08C12		108					
610	2/03/99	0169D005		108					
611	2/03/99	01F10345	100.0	108	5.94	5.00	21.88	3.29	55.00
612	2/03/99	0169CB5B	102.5	108	7.71	4.50	22.99	4.88	71.39
613	2/03/99	01F08BE1	98.0	108	2.06	1.00	18.98	1.66	19.07
614	2/03/99	0169D7E9	101.0	108	5.40	3.00	22.15	3.64	50.00
615	2/03/99	01C82882	102.0	108	5.46	3.00	22.45	3.52	50.56
616	2/03/99	0169D34D	103.0	108	6.28	6.00	21.82	2.58	58.15
617	2/03/99	010824E9		108					
		MEANS	101.10		4.70	4.20	21.13	2.29	43.51

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99 Tuna Harvest Growth Results - Cage C1

Selection Number	Allocation Date	Pit Tag Number	Specific Growth Rate
588	2/03/99	0169C9A1	0.24
589	2/03/99	0216C736	0.29
590	2/03/99	0169D335	0.23
591	2/03/99	0169C8E2	0.16
592	2/03/99	01F062B3	0.25
593	2/03/99	01F10526	0.24
594	2/03/99	0169DCC8	
595	2/03/99	01F13FE9	0.18
596	2/03/99	01F09131	0.25
597	2/03/99	01C82776	0.19
598	2/03/99	01CD2669	0.26
599	2/03/99	01CD1E53	0.18
600	2/03/99	01C73208	0.11
601	2/03/99	01C8324F	0.00
602	2/03/99	01CD323E	0.27
603	2/03/99	01E518EC	0.03
604	2/03/99	01F08889	0.25
605	2/03/99	01F0948A	0.22
606	2/03/99	01F0928F	0.42
607	2/03/99	01C7FEB9	
608	2/03/99	01C82F10	0.28
609	2/03/99	01F08C12	
610	2/03/99	0169D005	
611	2/03/99	01F10345	0.29
612	2/03/99	0169CB5B	0.35
613	2/03/99	01F08BE1	0.11
614	2/03/99	0169D7E9	0.25
615	2/03/99	01C82882	0.24
616	2/03/99	0169D34D	0.28
617	2/03/99	010824E9	
		MEANS	0.22

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99 Tuna Harvest Growth Results - Cage C3

Selection Number	Allocation Date	Pit Tag Number	Treatment	Cage	Ing Cage ID	Starting Weight	Starting Length (cm)	Start CI
498	3/03/99	01F08FC5	C Restricted	C3	27	20.32	101.0	19.72
499	3/03/99	01F08EB7	C Restricted	C3	27	18.42	98.5	19.27
500	3/03/99	01C82A2C	C Restricted	C3	27	18.40	99.0	18.96
501	3/03/99	01C802F1	C Restricted	C3	27	17.08	97.0	18.71
502	3/03/99	01C82693	C Restricted	C3	27	18.80	100.5	18.52
503	3/03/99	01C824D3	C Restricted	C3	27	19.88	103.0	18.19
504	3/03/99	0169CFEB	C Restricted	C3	27	18.16	97.0	19.90
505	3/03/99	01C8257B	C Restricted	C3	27	18.74	99.0	19.31
506	3/03/99	01C820D0	C Restricted	C3	27	16.82	92.0	21.60
507	3/03/99	01C9CA44	C Restricted	C3	27	20.60	101.0	19.99
508	3/03/99	01C82FA9	C Restricted	C3	27	16.36	94.5	19.39
509	3/03/99	01C829A3	C Restricted	C3	27	18.12	95.5	20.80
510	3/03/99	01C82DBA	C Restricted	C3	27	14.38	89.0	20.40
511	3/03/99	01C831EA	C Restricted	C3	27	18.02	97.0	19.74
512	3/03/99	01CD2461	C Restricted	C3	27	18.94	100.0	18.94
513	3/03/99	0169DBEE	C Restricted	C3	27	19.90	100.0	19.90
514	3/03/99	01C82A36	C Restricted	C3	27	18.60	96.0	21.02
515	3/03/99	0169DA66	C Restricted	C3	27	18.20	99.0	18.76
516	3/03/99	01E51700	C Restricted	C3	27	18.68	98.0	19.85
517	3/03/99	01CD1DAA	C Restricted	C3	27	18.14	96.0	20.50
518	3/03/99	0169DB69	C Restricted	C3	27	17.80	97.0	19.50
519	3/03/99	01F08BF8	C Restricted	C3	27	20.98	102.5	19.48
520	3/03/99	01C82B5F	C Restricted	C3	27	18.22	97.0	19.96
521	3/03/99	01F0856A	C Restricted	C3	27	18.44	99.5	18.72
522	3/03/99	016900F8	C Restricted	C3	27	17.42	97.0	19.09
523	3/03/99	01C8210A	C Restricted	C3	27	16.76	98.5	17.54
524	3/03/99	01C825BC	C Restricted	C3	27	15.66	95.5	17.98
525	3/03/99	0169D166	C Restricted	C3	27	16.32	95.0	19.03
526	3/03/99	01E28B94	C Restricted	C3	27	18.76	97.5	20.24
527	3/03/99	01F0FC2E	C Restricted	C3	27	17.36	99.0	17.89
		MEANS						19.43

NOTE: Two fish recovered without pit tags (weighing 26.64 and 24.28)

Prepared by Dr Robert van Barneveld 15/01/02

99 Tuna Harvest Growth Results - Cage C3

Selection Number	Allocation Date	Pit Tag Number	Harvest Date	Harvest Number	Tail Tag Number	Bag Tag Number	Harvest Weight
498	3/03/99	01F08FC5	14/06/99	4	W4	-	27.60
499	3/03/99	01F08EB7	14/06/99	8	W8	C37	25.38
500	3/03/99	01C82A2C	14/06/99	12	W12	C311	24.22
501	3/03/99	01C802F1	14/06/99	22	W22	-	22.74
502	3/03/99	01C82693	14/06/99	23	W23	-	22.96
503	3/03/99	01C824D3	14/06/99	3	W3	C33	27.26
504	3/03/99	0169CFEB	14/06/99	6	W6	C35	25.14
505	3/03/99	01C8257B	14/06/99	11	W11	C310	24.00
506	3/03/99	01C820D0	14/06/99	25	W25	-	23.84
507	3/03/99	01C9CA44	14/06/99	10	W10	C39	27.82
508	3/03/99	01C82FA9	14/06/99				
509	3/03/99	01C829A3	14/06/99	26	W26	-	23.10
510	3/03/99	01C82DBA	14/06/99	21	W21	-	18.94
511	3/03/99	01C831EA	14/06/99	1	W1	C31	24.70
512	3/03/99	01CD2461	14/06/99				
513	3/03/99	0169DBEE	14/06/99	9	W9	C38	26.60
514	3/03/99	01C82A36	14/06/99	7	W7	C36	26.28
515	3/03/99	0169DA66	14/06/99				
516	3/03/99	01E51700	14/06/99	19	W19	-	23.76
517	3/03/99	01CD1DAA	14/06/99				
518	3/03/99	0169DB69	14/06/99	5	W5	C34	24.36
519	3/03/99	01F08BF8	14/06/99				
520	3/03/99	01C82B5F	14/06/99	13	W13	C312	23.64
521	3/03/99	01F0856A	14/06/99				
522	3/03/99	016900F8	14/06/99	15	W15	C314	21.84
523	3/03/99	01C8210A	14/06/99	18	W18	C315	21.66
524	3/03/99	01C825BC	14/06/99	2	W2	C32	21.32
525	3/03/99	0169D166	14/06/99	14	W14	C313	22.80
526	3/03/99	01E28B94	14/06/99	24	W24	-	24.26
527	3/03/99	01F0FC2E	14/06/99	20	W20	-	20.86
		MEANS					23.96

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99 Tuna Harvest Growth Results - Cage C3

Selection Number	Allocation Date	Pit Tag Number	Harvest Length	Days on	Weight Gain (kg)	Length Gain (cm)	Final CI	CI Gain	ADG (g)
498	3/03/99	01F08FC5	104.7	104	7.28	3.70	24.05	4.33	70.00
499	3/03/99	01F08EB7	104.2	104	6.96	5.70	22.43	3.16	66.92
500	3/03/99	01C82A2C	104.0	104	5.82	5.00	21.53	2.57	55.96
501	3/03/99	01C802F1	102.0	104	5.66	5.00	21.43	2.71	54.42
502	3/03/99	01C82693	104.0	104	4.16	3.50	20.41	1.89	40.00
503	3/03/99	01C824D3	106.7	104	7.38	3.70	22.44	4.25	70.96
504	3/03/99	0169CFEB	102.5	104	6.98	5.50	23.34	3.45	67.12
505	3/03/99	01C8257B	104.0	104	5.26	5.00	21.34	2.02	50.58
506	3/03/99	01C820D0	99.6	104	7.02	7.60	24.13	2.53	67.50
507	3/03/99	01C9CA44	105.1	104	7.22	4.10	23.96	3.97	69.42
508	3/03/99	01C82FA9		104					
509	3/03/99	01C829A3	101.1	104	4.98	5.60	22.35	1.55	47.88
510	3/03/99	01C82DBA	93.8	104	4.56	4.80	22.95	2.55	43.85
511	3/03/99	01C831EA	101.0	104	6.68	4.00	23.97	4.23	64.23
512	3/03/99	01CD2461		104					
513	3/03/99	0169DBEE	105.6	104	6.70	5.60	22.59	2.69	64.42
514	3/03/99	01C82A36	101.6	104	7.68	5.60	25.06	4.03	73.85
515	3/03/99	0169DA66		104					
516	3/03/99	01E51700	103.1	104	5.08	5.10	21.68	1.83	48.85
517	3/03/99	01CD1DAA		104					
518	3/03/99	0169DB69	104.0	104	6.56	7.00	21.66	2.15	63.08
519	3/03/99	01F08BF8		104					
520	3/03/99	01C82B5F	103.0	104	5.42	6.00	21.63	1.67	52.12
521	3/03/99	01F0856A		104					
522	3/03/99	016900F8	101.0	104	4.42	4.00	21.20	2.11	42.50
523	3/03/99	01C8210A	102.0	104	4.90	3.50	20.41	2.87	47.12
524	3/03/99	01C825BC	98.1	104	5.66	2.60	22.58	4.60	54.42
525	3/03/99	0169D166	101.0	104	6.48	6.00	22.13	3.09	62.31
526	3/03/99	01E28B94	103.4	104	5.50	5.90	21.94	1.70	52.88
527	3/03/99	01F0FC2E	102.0	104	3.50	3.00	19.66	1.77	33.65
		MEANS	102.40		5.91	4.90	22.29	2.82	56.83

99 Tuna Harvest Growth Results - Cage C3

Selection Number	Allocation Date	Pit Tag Number	Specific Growth Rate
498	3/03/99	01F08FC5	0.29
499	3/03/99	01F08EB7	0.31
500	3/03/99	01C82A2C	0.26
501	3/03/99	01C802F1	0.28
502	3/03/99	01C82693	0.19
503	3/03/99	01C824D3	0.30
504	3/03/99	0169CFEB	0.31
505	3/03/99	01C8257B	0.24
506	3/03/99	01C820D0	0.34
507	3/03/99	01C9CA44	0.29
508	3/03/99	01C82FA9	
509	3/03/99	01C829A3	0.23
510	3/03/99	01C82DBA	0.26
511	3/03/99	01C831EA	0.30
512	3/03/99	01CD2461	
513	3/03/99	0169DBEE	0.28
514	3/03/99	01C82A36	0.33
515	3/03/99	0169DA66	
516	3/03/99	01E51700	0.23
517	3/03/99	01CD1DAA	
518	3/03/99	0169DB69	0.30
519	3/03/99	01F08BF8	
520	3/03/99	01C82B5F	0.25
521	3/03/99	01F0856A	
522	3/03/99	016900F8	0.22
523	3/03/99	01C8210A	0.25
524	3/03/99	01C825BC	0.30
525	3/03/99	0169D166	0.32
526	3/03/99	01E28B94	0.25
527	3/03/99	01F0FC2E	0.18
		MEANS	0.27

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99 Tuna Harvest Growth Results - Cage C4

Selection Number	Allocation Date	Pit Tag Number	Treatment	Cage	Ing Cage ID	Starting Weight	Starting Length (cm)	Start CI
408	1/03/99	01E54440	C Restricted	C4	22	17.42	99.0	17.95
409	1/03/99	01F0848D	C Restricted	C4	22	16.68	94.0	20.08
410	1/03/99	01F04FAD	C Restricted	C4	22	18.04	99.0	18.59
411	1/03/99	01F0F70A	C Restricted	C4	22	10.96	83.5	18.83
412	1/03/99	01CB2AAC	C Restricted	C4	22	16.50	100.0	16.50
413	1/03/99	01CD2A66	C Restricted	C4	22	17.84	97.0	19.55
414	1/03/99	01F05CF6	C Restricted	C4	22	15.50	93.5	18.96
415	1/03/99	01C82E25	C Restricted	C4	22	15.02	91.5	19.61
416	1/03/99	0169D8A9	C Restricted	C4	22	16.46	94.5	19.50
417	1/03/99	0169D933	C Restricted	C4	22	19.92	96.0	22.52
418	1/03/99	0169D529	C Restricted	C4	22	18.16	98.0	19.29
419	1/03/99	0169DB2B	C Restricted	C4	22	14.32	86.0	22.51
420	1/03/99	01C81D38	C Restricted	C4	22	18.32	94.0	22.06
421	1/03/99	01C82073	C Restricted	C4	22	18.32	100.0	18.32
422	1/03/99	01CD2A07	C Restricted	C4	22	18.72	98.0	19.89
423	1/03/99	01C81DED	C Restricted	C4	22	18.50	96.0	20.91
424	1/03/99	0169D231	C Restricted	C4	22	16.08	91.0	21.34
425	1/03/99	0169DB38	C Restricted	C4	22	16.36	96.5	18.21
426	1/03/99	01CB26C9	C Restricted	C4	22	17.84	95.5	20.48
427	1/03/99	0169D651	C Restricted	C4	22	19.20	99.0	19.79
428	1/03/99	01E2941E	C Restricted	C4	22	18.06	96.0	20.41
429	1/03/99	01F089AA	C Restricted	C4	22	16.38	92.0	21.04
430	1/03/99	0169D365	C Restricted	C4	22	15.32	92.0	19.67
431	1/03/99	016A1198	C Restricted	C4	22	17.26	96.5	19.21
432	1/03/99	01C82D15	C Restricted	C4	22	20.52	100.0	20.52
433	1/03/99	021354D3	C Restricted	C4	22	17.06	94.0	20.54
434	1/03/99	01CD254D	C Restricted	C4	22	16.66	94.0	20.06
435	1/03/99	01E27348	C Restricted	C4	22	19.94	99.0	20.55
436	1/03/99	01C7124E	C Restricted	C4	22	19.04	100.0	19.04
437	1/03/99	0169CF86	C Restricted	C4	22	17.14	93.0	21.31
		MEANS						19.91
		Excluding 0169D231						20.59

NOTE: Three fish recovered without pit tags (weighing 25.68, 23.62 and 20.60)

Prepared by Dr Robert van Barneveld 15/01/02

99 Tuna Harvest Growth Results - Cage C4

Selection Number	Allocation Date	Pit Tag Number	Harvest Date	Harvest Number	Tail Tag Number	Bag Tag Number	Harvest Weight
408	1/03/99	01E54440	17/06/99	4	W4	C43	23.22
409	1/03/99	01F0848D	17/06/99	18	W19	C415	16.80
410	1/03/99	01F04FAD	17/06/99	20	W18	-	24.20
411	1/03/99	01F0F70A	17/06/99	17	W17	C414	17.12
412	1/03/99	01CB2AAC	17/06/99				
413	1/03/99	01CD2A66	17/06/99	16	W16	C413	19.08
414	1/03/99	01F05CF6	17/06/99	14	W14	C411	20.72
415	1/03/99	01C82E25	17/06/99	22	W22	-	20.86
416	1/03/99	0169D8A9	17/06/99	24	W24	-	15.66
417	1/03/99	0169D933	17/06/99				
418	1/03/99	0169D529	17/06/99	8	W8	C45	23.62
419	1/03/99	0169DB2B	17/06/99	10	W10	C47	19.64
420	1/03/99	01C81D38	17/06/99	3	W3	C43	23.82
421	1/03/99	01C82073	17/06/99	9	W9	C46	23.68
422	1/03/99	01CD2A07	17/06/99	12	W12	C49	23.32
423	1/03/99	01C81DED	17/06/99	15	W15	C412	23.78
424	1/03/99	0169D231	17/06/99	1	W1	C41	14.22
425	1/03/99	0169DB38	17/06/99				
426	1/03/99	01CB26C9	17/06/99	13	W13	C410	22.32
427	1/03/99	0169D651	17/06/99	23	W23	-	21.00
428	1/03/99	01E2941E	17/06/99				
429	1/03/99	01F089AA	17/06/99	19	W20	-	23.86
430	1/03/99	0169D365	17/06/99	25	W25	-	16.02
431	1/03/99	016A1198	17/06/99	7	W7	C44	23.04
432	1/03/99	01C82D15	17/06/99				
433	1/03/99	021354D3	17/06/99	11	W11	C48	21.66
434	1/03/99	01CD254D	17/06/99				
435	1/03/99	01E27348	17/06/99				
436	1/03/99	01C7124E	17/06/99				
437	1/03/99	0169CF86	17/06/99	21	W21	-	20.62
		MEANS					20.83
		Excluding 0169D231					21.14

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99 Tuna Harvest Growth Results - Cage C4

Selection Number	Allocation Date	Pit Tag Number	Harvest Length	Days on	Weight Gain (kg)	Length Gain (cm)	Final CI	CI Gain	ADG (g)
408	1/03/99	01E54440	102.0	109	5.80	3.00	21.88	3.93	53.21
409	1/03/99	01F0848D	94.0	109	0.12	0.00	20.23	0.14	1.10
410	1/03/99	01F04FAD	100.5	109	6.16	1.50	23.84	5.25	56.51
411	1/03/99	01F0F70A	92.0	109	6.16	8.50	21.99	3.16	56.51
412	1/03/99	01CB2AAC		109					
413	1/03/99	01CD2A66	101.0	109	1.24	4.00	18.52	-1.03	11.38
414	1/03/99	01F05CF6	98.0	109	5.22	4.50	22.01	3.05	47.89
415	1/03/99	01C82E25	96.0	109	5.84	4.50	23.58	3.97	53.58
416	1/03/99	0169D8A9	96.0	109	-0.80	1.50	17.70	-1.80	-7.34
417	1/03/99	0169D933		109					
418	1/03/99	0169D529	103.5	109	5.46	5.50	21.30	2.01	50.09
419	1/03/99	0169DB2B	93.5	109	5.32	7.50	24.03	1.51	48.81
420	1/03/99	01C81D38	100.0	109	5.50	6.00	23.82	1.76	50.46
421	1/03/99	01C82073	104.5	109	5.36	4.50	20.75	2.43	49.17
422	1/03/99	01CD2A07	102.0	109	4.60	4.00	21.97	2.09	42.20
423	1/03/99	01C81DED	104.0	109	5.28	8.00	21.14	0.23	48.44
424	1/03/99	0169D231	92.0	109	-1.86	1.00	18.26	-3.08	-17.06
425	1/03/99	0169DB38		109					
426	1/03/99	01CB26C9	99.0	109	4.48	3.50	23.00	2.52	41.10
427	1/03/99	0169D651	102.0	109	1.80	3.00	19.79	0.00	16.51
428	1/03/99	01E2941E		109					
429	1/03/99	01F089AA	97.0	109	7.48	5.00	26.14	5.11	68.62
430	1/03/99	0169D365	95.5	109	0.70	3.50	18.39	-1.28	6.42
431	1/03/99	016A1198	101.5	109	5.78	5.00	22.03	2.83	53.03
432	1/03/99	01C82D15		109					
433	1/03/99	021354D3	98.0	109	4.60	4.00	23.01	2.47	42.20
434	1/03/99	01CD254D		109					
435	1/03/99	01E27348		109					
436	1/03/99	01C7124E		109					
437	1/03/99	0169CF86	98.0	109	3.48	5.00	21.91	0.60	31.93
		MEANS	98.64		3.99	4.23	21.60	1.63	36.58
		Excluding 0169D231	98.95		4.27	4.38	21.76	1.85	39.13

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99 Tuna Harvest Growth Results - Cage C4

Selection Number	Allocation Date	Pit Tag Number	Specific Growth Rate
408	1/03/99	01E54440	0.26
409	1/03/99	01F0848D	0.01
410	1/03/99	01F04FAD	0.27
411	1/03/99	01F0F70A	0.41
412	1/03/99	01CB2AAC	
413	1/03/99	01CD2A66	0.06
414	1/03/99	01F05CF6	0.27
415	1/03/99	01C82E25	0.30
416	1/03/99	0169D8A9	-0.05
417	1/03/99	0169D933	
418	1/03/99	0169D529	0.24
419	1/03/99	0169DB2B	0.29
420	1/03/99	01C81D38	0.24
421	1/03/99	01C82073	0.24
422	1/03/99	01CD2A07	0.20
423	1/03/99	01C81DED	0.23
424	1/03/99	0169D231	-0.11
425	1/03/99	0169DB38	
426	1/03/99	01CB26C9	0.21
427	1/03/99	0169D651	0.08
428	1/03/99	01E2941E	
429	1/03/99	01F089AA	0.35
430	1/03/99	0169D365	0.04
431	1/03/99	016A1198	0.26
432	1/03/99	01C82D15	
433	1/03/99	021354D3	0.22
434	1/03/99	01CD254D	
435	1/03/99	01E27348	
436	1/03/99	01C7124E	
437	1/03/99	0169CF86	0.17
		MEANS	0.19
		Excluding 0169D231	0.20

Prepared by Dr Robert van Barneveld 15/01/02

Appendix VIII:
Animal Ethics and Welfare Submission and Approval

PIRSA ANIMAL ETHICS COMMITTEE
APPROVAL FORM

APPLICANT: **Dr Robert van Barneveld**

DEPARTMENT: **SARDI Pig and Poultry Production Institute**

TITLE OF PROJECT: **SOUTHERN BLUEFIN TUNA (*THUNNUS MACCOYII*) AQUACULTURE SUB-PROGRAM 2. DEVELOPMENT AND OPTIMISATION OF MANUFACTURED FEEDS FOR CAGED SOUTHERN BLUEFIN TUNA.**

ANIMAL ETHICS COMMITTEE APPROVAL: **YES**

APPLICATION NUMBER: **38/97**

FOR TERM OF THE PROTOCOL DESCRIBED: **3 Years**

CONDITIONS: **Nil.**

SIGNED:......Andrew M Barton...... Date:....4/12/97.........
Chairperson

Ms Patricia Carter
Executive Officer
PIRSA Animal Ethics Committee
Pig & Poultry Production Institute
Roseworthy Campus
ROSEWORTHY S Aust 5371
Phone: (08) 8303 7683
Fax: (08) 8303 7977
Email: carter.patricia@pi.sa.gov.au

AE7085

APPLICATION NO. 38/97

Date Received..........................

SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE
ANIMAL ETHICS COMMITTEE

APPLICATION FOR ETHICS APPROVAL OF PROJECT INVOLVING ANIMALS

Refer to attached "Guidelines for Seeking Ethics Approval" for details of requirements before filling out this application form. 2. Submit this form typewritten plus 7 duplicate copies. N.B. Applications submitted otherwise than in accordance with the guidelines will inevitably suffer delays. 3. If space is insufficient for full information use additional sheets and attach to your application.

APPLICANT: Dr R.J. van Barneveld	QUALIFICATIONS: B.Agr.Sc. (Hon) PhD	Telephone: (08) 8303 7787
BRANCH/REGION: Pig and Poultry Production Institute		After hours: (085) 244 375 Ext No: -
OTHERS INVOLVED: Mr A. Smart Mr S. Clarke Mr B. Glencross Mr Jason Nichols	QUALIFICATIONS: B. Sc B.Sc, M.Sc B.Sc.~~Agr.~~ (HONS) M.Sc. B.Sc.M.Sc.	Telephone: (08) 86 83 3631 (08) 200 2443 (08) 86 83 3631 (08) 86 83 3631
1. TITLE OF PROJECT: Southern Bluefin Tuna (*Thunnus maccoyii*) Aquaculture Sub-Program 2. Development and optimisation of manufactured feeds for caged Southern Bluefin Tuna.		
STARTING DATE: November, 1997	SOURCE OF FUNDING: FRDC/CRC	
ESTIMATED DURATION: 3 years		
IS THIS AN EXTENSION OF WORK PREVIOUSLY APPROVED BY THE AEC?	YES	
IF SO, GIVE PREVIOUS APPROVAL NUMBER(S):	-21/96 (UofA: W/042/96)	
HAVE REPORTS ON THESE PROJECTS BEEN LODGED?	YES	

DECLARATION

To the best of my knowledge this proposal conforms with the "Code of practice for the Care and Use of Animals for Scientific Purposes" (NH&MRC/CSIRO/AAC 1990) and the Prevention of Cruelty to Animals Act 1985. I have read Section 3 of the Code of Practice which sets down the responsibilities of investigators. I accept those responsibilities.

SIGNED: .... DATE 26/11/97
Applicant

I am satisfied that the applicant has appropriate qualifications and experience to carry out the work with minimum distress to the animals. I believe the project conforms with the requirements of the "Code of Practice for the Care and Use of Animals for Scientific Purposes" (NH&MRC/CSIRO/AAC 1990) and the Prevention of Cruelty to Animals Act, 1985.

SIGNED: Andrew M Pointon .... DATE 28/11/97
Principal Research Officer/Chief

ETHICS COMMITTEE APPROVAL - APPLICATION NO.
APPROVED BY THE ANIMAL ETHICS COMMITTEE at its meeting held on
FOR THE PERIOD UNTIL:
SUBJECT TO:

SIGNED: .... DATE: ....
Chairman, The South Australian Department of Agriculture Animal Ethics Committee

2. ANIMALS REQUIRED: (Common and Scientific Names)					
SPECIES	STRAIN	SEX	AGE/SIZE	NUMBER /MONTH	TOTAL NUMBER
Thannus maccoyii	Southern Blue Fin	M/F	20-30 kg	450	1350

WHERE WILL EXPERIMENTAL PROCEDURES TAKE PLACE? Boston Bay, Pt Lincoln

SOURCE OF ANIMALS: Tuna Boat Owners Association of Australia

Note: Ethical approval of a project does not guarantee that the animals, or space for holding them, will be available; it is for the experimenter to ensure this availability. It is strongly recommended that you discuss these matters with the people concerned prior to applying for Ethics Approval.

PLACE WHERE ANIMALS WILL BE HELD: Boston Bay, Pt Lincoln

MAXIMUM NUMBER OF ANIMALS TO BE HOUSED AT ANY ONE TIME: 450

IS ANY SPECIAL FEEDING, HANDLING OR ISOLATION OF THE ANIMALS REQUIRED:

Animals have been caught in the wild and are held in sea cages anchored in Boston Bay, Pt Lincoln. Cages vary in sizes but are capable of holding in excess of 200 fish. Only 10 fish per cage will be required for these experiments.

3. IS THE ACQUISITION, RETENTION OR USE OF THE ANIMALS SUBJECT TO ANY PERMIT, LAW OR REGULATION OF THE STATE OR COMMONWEALTH (eg: protected native or imported species?) No

IF SO, DO YOU HOLD A SPECIALIST LICENCE RELEVANT TO THIS EXPERIMENT? -

4. DESCRIBE IN DETAIL WHAT WILL ACTUALLY BE DONE TO THE ANIMALS:
(A full protocol for the project should be attached. In addition two copies of any grant application with which this project is linked should be attached to the original.)

Three major growth experiments will be conducted with 8-10 cages (45 fish per cage) in Boston Bay, Port Lincoln from January to April in 1998, 1999 and 2000. Growth experiments deviate little from standard management practices. Following harvest of these fish, as many samples as possible are collected to make maximum use of this limited resource and to optimise the amount of information collected from each experiment.

Fish will be kept in sea cages and managed using industry standard practices. They will be weighed and tagged prior to allocation to the cages and weighed at least once, but no more than twice over the growing period (up to 6 months). They will be fed either pilchards (industry standard) or moist pellets manufactured from fish meals, oils, and alternative protein and energy sources. At the completion of their growing period, a subsample of fish will be harvested using standard industry practices (hooked, pithed and bled). Following harvest, the digestive tract will be removed and digesta collected.

The procedures involve minimum handling of the fish. Experimental requirements deviate little from standard management practices.

A full project proposal is attached.

5. LIST ALL DRUGS (GENERIC NAMES, WITH DOSES) TO BE ADMINISTERED

NIL

6. EXPERIMENTAL CATEGORY (tick where appropriate)

A Minor procedures with or without anaesthesia, eg: immunisation, injections, blood sampling, diet experiments, breeding. ✓

B Non-survival, eg: anaesthetised and/or humanely killed, ie: for organ removal, etc. ✓

C Survival following anaesthesia with minor procedures, eg: biopsies, castration, cannulation.

D Survival following anaesthesia with major procedures, eg: major surgical grafts.

E Induction of some form of illness, eg: toxicity experiments, burn studies, tumour, implant or radiation studies.

F Experiments on unanaesthetised animals (curarised or equivalent).

G Other, eg: behavioural studies.

7. PAIN CLASSIFICATION (tick where appropriate)

A No pain or distress or mild pain or distress ✓

B Moderate pain or distress

C Severe or chronic pain or distress

8. (a) IDENTIFY AND JUSTIFY ALL PROCEDURES WITH POTENTIAL TO CAUSE PAIN OR DISTRESS. WHAT STEPS WILL BE TAKEN TO AVOID OR MINIMISE SUCH PAIN OR DISTRESS. (Include full details of anaesthesia and analgesia including doses, route of administration and the person responsible)

NIL

(b) IF NEURO-MUSCULAR BLOCKADE IS TO BE USED IN THE PROJECT, WHAT MONITORING PROCEDURES WILL BE USED TO AVOID PAIN OR DISTRESS?

N/A

(c) WHAT POST-OPERATIVE CARE WILL THE ANIMALS REQUIRE? GIVE DETAILS OF ANALGESIA AND THE NAME OF THE PERSON PROVIDING THE CARE?

N/A

(d) HAVE ANY OF THE ANIMALS TO BE USED BEEN THE SUBJECT OF A PREVIOUS EXPERIMENT? IF SO EXPLAIN WHY THEY ARE TO BE USED AGAIN.

NO

(e) WHAT IS THE PLANNED END-POINT OF THE EXPERIMENT AND THE REASON FOR ITS CHOICE? WHAT WILL HAPPEN TO THE ANIMAL FOLLOWING TERMINATION OF THE EXPERIMENTAL PROCEDURE? IF IT IS TO BE KILLED, WHAT METHOD IS TO BE USED?

Experiments will end after a 14 week growth period. This durationis necessary to detect a growth response. In addition, water temperatures start to drop and limit the capacity to conduct feeding experiments. At the end of the experiments, the animals will be harvested using standard industry harvesting practices and this fish sold on the Japanese sashimi market.

(f) DO YOU BELIEVE THE PROJECT INVOLVES SPECIAL ETHICAL CONSIDERATIONS? IF SO PLEASE IDENTIFY THESE AND ADD ANY REMARKS YOU THINK MAY BE OF ASSISTANCE TO THE COMMITTEE.

NO

EXPERIMENTAL PROCEDURES ONLY

9. Irrespective of whether or not the experiments are likely to cause distress to the animals, the Committee is obliged by both the Prevention of Cruelty to Animals Act and the Code of Practice to assess whether or not the use of animals will allow worthwhile scientific objectives to be met.

(a) WHAT NEW INFORMATION AND/OR UNDERSTANDING IS SOUGHT FROM THIS EXPERIMENT (In answering this question highlight the potential value of the information for the understanding of humans or animals, to the maintenance and improvement of human or animal health and welfare, to the improvement of animal management or production or to the achievement of educational objectives).

At present, caged Southern Blue Fin Tuna are fed a diet of pilchards and mackerel. If this industry is to be sustainable, then alternative protein sources must be found and manufactured diets must be developed. To achieve both of these objectives, a central aim must be the assessment of amino acid and energy digestibility in a range of alternative proteins (vegetable proteins, animal by-products), and evaluation of manufactured diets with a view to feeding a semi-moist pellet. This research is designed to achieve this aim.

(b) WHY ARE ANIMALS NECESSARY FOR THE PROJECT AND WHY HAVE TECHNIQUES NOT INVOLVING THE USE OF ANIMALS BEEN REJECTED?

SBFT have a highly specific mode of feeding and digestive process and hence the digestibility of alternative proteins must be assessed in the target species. Other techniques have not been rejected but are not available. Some work is now been conducted using salmon as a model for tuna to limit the number of tuna experiments required.

(c) WHAT PUBLISHED ARTICLE DESCRIBES WORK CLOSEST TO THIS PROJECT?

van Barneveld *et al.* (1997). Nutritional Management of Sea-Caged Southern Bluefin Tuna (see attached).

(d) HOW DOES THIS PROPOSAL DIFFER, OR FOLLOW FROM PREVIOUS OR CONCURRENT WORK? EXPLAIN WHY ANY REPETITION OR PREVIOUSLY PERFORMED WORK IS NECESSARY?

This work is a continuation of previous research. Extensive progress has been made towards the development of manufactured feeds to date, yet there is still considerable work required with alternative feeding strategies and diet formulations before a manufactured feed can be used commercially.

(e) IF IT IS NOT PROPOSED TO PUBLISH THE RESULTS, PLEASE EXPLAIN WHY?

It is anticipated that the completed work will be submitted for publication in scientific journals

10. HOW DO YOU JUSTIFY THE <u>NUMBER</u> AND SPECIES OF ANIMALS NEEDED?

There are only 12 cages available to complete caged tuna research. In addition, results are extremely variable between cages. The number of fish in each cage is to allow a growth and digestibility response to be assessed with a moderate degree of confidence in the results.

11. DOES THIS PROJECT INVOLVE EXPERIMENTATION OF HUMAN SUBJECTS, WORK WITH RECOMBINANT DNA OR THE USE OF CARCINOGENS OR TERATOGENS?
If so, it is necessary to make separate application to the relevant Committee (see Guidelines) for approval.

NO

12. DOES THIS PROJECT POSE ANY HEALTH RISKS TO OTHER ANIMALS OR STAFF?
If so, explain the nature of the risk and precautions to be taken.

NO

Appendix IX:
Publications

Publication List

Carter, C.G., Sceto, G.S., Smart, A., Clarke, S. and van Barneveld, R.J. (1999). Correlates of growth and condition in southern bluefin tuna, *Thunnus maccoyii* (Casteluau). *Fish Physiology and Biochemistry* (Accepted)

Glencross, Brett, D., van Barneveld, Robert, J., Carter, Chris, G. and Clarke, Steven, M. (1999). Factors influencing feed intake and feed conversion in farmed Southern Bluefin Tuna (*Thunnus maccoyii*). *Proceedings of the World Aquaculture Society.* 287.

Smart, A., Clarke, S., Carter C.G., and van Barneveld, R.J. (1998). The development of methodology for the evaluation of growth of farmed southern bluefin tuna, *Thunnus maccoyii* (Castelnau). *Journal of Experimental Biology*. (submitted).

van Barneveld, R.J., Smart, A.R., Clarke, S.M., Carter, C.G., Davis, B.J., Tivey, D.R. and Brooker, J.D. (1997). Nutritional management of sea-caged Southern Bluefin Tuna (*Thunnus maccoyii*). In *Recent Advances in Animal Nutrition in Australia, 1997* [J.L. Corbett, M.Choct, J.V. Nolan and J.B.Rowe, editors] pp.88-97 Department of Animal Science: University of New England. *

van Barneveld, Robert, Glencross, Brett, Carter, Chris, Clarke, Steven, Foster, Craig, and Bayly, Geoff. (1998). Manipulation of the ingredient and nutrient content of manufactured feeds fed to caged Southern Bluefin Tuna (*Thunnus maccoyii*) and subsequent production responses. *Proceedings of the Australian Marine Science Association.*

van Barneveld, R.J., Glencross, B.D., Carter, C.G., Clarke, S.M. and Smart, A.R. (1999). Diet development for pelagic finfish: The Southern Bluefin Tuna (*Thunnus maccoyii*) Model. *Proceedings of the World Aquaculture Society*. 787

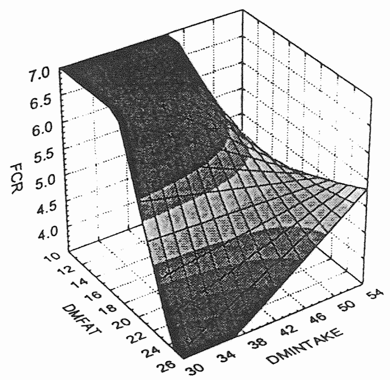
FACTORS INFLUENCING FEED INTAKE AND FEED CONVERSION IN FARMED SOUTHERN BLUEFIN TUNA (*Thunnus maccoyii*)

Brett D. Glencross*, Robert J. van Barneveld, Chris G. Carter, Steven M. Clarke

South Australian Research and Devlopment Institute,
PO Box 120, Henley Beach, SA 5020, Australia.

The southern bluefin tuna (SBT) aquaculture industry in South Australia is reliant on the feeding of bait-fish for the on-growing of the SBT. The risks associated with the feeding of bait-fish are considerable and varied, foremost being the reliance on a single feed source. Consequently, the focus of current research is on the development of a manufactured feed. Three moist-pellet feeds were formulated to examine effects of alternative feed ingredients and different energy levels. A four month growth trial was conducted, with all feeds fed twice daily to satiety, six days a week. By chance, one replicate of one of the moist-pellet feeds appeared to not have been fed to satiation, allowing examination of restriction feeding effects.

There were no significant differences in growth between any of the moist-pellet or bait-fish fed SBT. Feed intake by SBT fed the moist-pellet was significantly ($P < 0.05$) lower than feed intake by the bait-fish fed SBT and there were also significant ($P < 0.05$) differences in feed intake between the moist-pellet feeds. Daily feed intake varied between about 0.8 and 2.2 kg per SBT. Feed intake was higher at the beginning of the trial and declined as it progressed. This correlated strongly with a decline in water temperature, but the rate at which feed intake in each of the treatments and even replicates within treatments declined, suggests that there are also other factors influencing feed intake.



Three dimensional modelling of dry matter intake (kg/fish : DMINTAKE) and diet dry matter fat content (% : DMFAT) and food conversion ratio (kg eaten/kg gain : FCR) for farmed SBT.

Dry matter (DM) feed conversion ratio (FCR) was lowest for bait-fish fed SBT. Moist-pellet fed SBT had higher FCR than those fed the bait-fish and differences were evident between each of the three moist-pellet types. Moist-pellet FCR was correlated with fat content of the feed, with lower FCR correlating with high dietary fat content. Further examination of FCR as a function of fat content in all the feeds (bait-fish included) identified this factor as being strongly correlated to FCR. However, FCR was also correlated with DM intake. Modelling of this data demonstrates that the combination of both these factors is likely to improve the FCR achievable from prospective feeds (Figure).

The results of this study support that future feed development for SBT should target increasing the fat content of the moist-pellets. That FCR can also be improved by minor feed restriction suggests that there is also capacity to reduce feed usage to some degree, maintain growth, decrease the cost of feeding and reduce environmental impact of SBT farming.

Nutritional management of sea-caged Southern Bluefin Tuna *(Thunnus maccoyii)*

R.J. van Barneveld[1,6], A.R. Smart[2,6], S.M. Clarke[5,6] C.G. Carter[3,6] B.J. Davis[1], D.R. Tivey[4] and J.D. Brooker[4]

[1]SARDI-Pig and Poultry Production Institute, Nutrition Research Laboratory, The University of Adelaide, Roseworthy Campus, Roseworthy, SA 5371
[2]SARDI-Tuna Research, Lincoln Marine Science Centre, PO Box 2023 Port Lincoln, SA 5606
[3]University of Tasmania, Department of Aquaculture, Launceston Campus, PO Box 1214 Launceston, TAS 7250
[4]Department of Animal Science, University of Adelaide, Waite Agricultural Research Institute, Glen Osmond, SA 5061
[5]SARDI Aquatic Sciences Centre, PO Box 120 Henley Beach, SA 5022
[6]Co-operative Research Centre for Aquaculture, University of Technology, Sydney, PO Box 123 Broadway, NSW 2007

Summary

The farming of Southern Bluefin Tuna (SBT) in sea–cages in Boston Bay, South Australia, is one of Australia's fastest growing and most valuable aquaculture industries. The fish are fattened for sale on the Japanese sashimi market with the industry having the potential to produce 3 000 tonnes of tuna in 1996/97 worth about $A90 million. At present, the industry relies heavily on the use of local and imported 'trash fish' such as pilchards, mackerel and herring for the nutrition of the sea-caged SBT. If the industry is to be sustainable, however, there is a need to reduce the nutritional reliance on trash fish. The development of a manufactured feeds for SBT is the highest industry priority. Research to date has quantified the potential for manufactured feeds to replace trash fish and significant advances have been made in the development of research methods necessary to assess the performance of SBT fed these feeds. Despite this, a manufactured feed that can promote SBT growth and flesh quality equivalent to pilchards still eludes the industry, and many questions pertaining to the nutritional management of sea-caged SBT are still to be answered.

Introduction

Pressure on wild stocks of fish, global population growth, and research suggesting a need to increase the proportion of seafood in western diets has resulted in the rapid development of many aquaculture systems. Csavas (1994) has suggested that seafood consumption will reach 84 million tonnes by the year 2000. This is a 17% increase since 1990. In contrast, the FAO (1994) estimate that global fisheries landings peaked in 1989 at 87 million tonnes and have since fluctuated near this level. This suggests that fisheries stocks are being harvested at close to their maximum sustainable yield. As a consequence, market demand for aquaculture products is expected to increase from 19.3 million tonnes, worth $US32.5 billion, in 1992 to about 22-24 million tonnes by the end of this century (Chamberlain and Rosenthal, 1995). In addition, the health benefits of consuming seafood in terms of reduced cardio–vascular disease are becoming widely documented and will result in an increase in the proportion of seafood in western diets. This will place further pressure on global seafood supplies and demands on aquaculture systems.

The farming of SBT has been a major success story in the expansion of the Australian aquaculture industry, although it has not been without its problems. The industry, started in 1990, produced approximately 2 000 tonnes of tuna with a market value of $60 million in 1994/95 and has the potential to produce about 3 000 tonnes worth about $90 million in 1996/97. The industry also has a significant economic multiplier effect because of its labour intensiveness and infrastructure requirements. It is significant because of the employment it has created in a regional area facing economic decline, and because of the impetus it has provided in South Australia for the development of associated service industries (infrastructure construction and maintenance, research and technical services, etc.) and of industry sectors (mussel farming and longer term holding of rock lobster).

Tuna farming has developed in the last few years as Australia's fastest growing aquaculture industry. Its initial success was due to the adoption of technologies from Japan. However, some of these technologies are not compatible with ecologically sustainable development. In particular the development of feeds that do not rely on imported frozen pilchards and that reduce the nutrient input into the water are seen as urgent priorities both by industry and government regulatory agencies.

Compared to more traditional animal production systems, there is great scope to improve our knowledge of the nutrition of many aquaculture species including SBT. Fish nutrition, however, presents many new challenges to the researcher due to the nature of the industry, and the difficulty associated with conducting nutrition experiments under water.

The aims of this paper are to:

- Describe the development of SBT fanning in South Australia;
- Outline the current commercial nutritional management of SBT;
- Discuss the development of manufactured feeds and nutrition management strategies for SBT;
- Suggest future research directions for SBT nutrition research.

Background to Southern Bluefin Tuna farming in South Australia

Description of Southern Bluefin Tuna

Southern Bluefin Tuna (*Thunnus maccoyii*) are one of 13 species of tuna in the Scombridae family. Close relations are butterfly mackerel and billfishes such as swordfish, marlins and spearfish. Its closest relative is the Northern Bluefin Tuna (*Thunnus thunnus*). *The* SBT is a large fish that can reach weights of up to 200 kg and lengths of up to two metres. They are pelagic living near the surface of the ocean. The lifespan of a SBT is in excess of 20 years, and they reach maturity at approximately 8 years.

Tuna are adapted to maximise feeding success in an environment where food is sparse and patchy, by being able to locate, capture and process food rapidly. Thus, tuna have very high energy demands associated with continuous swimming, gill ventilation and anaerobic swimming during feeding and an aerobic capacity that exceeds that of most other fishes (Korsmeger et *al.* 1996).

Breeding occurs in the warm Indian Ocean, south of Indonesia. As the tuna grow, they move southwards towards major feeding grounds in the Southern Ocean. The tuna migrate from the spawning ground, around the Western Australian coast to the Great Australian Bight.

Development of the sea-caged Southern Bluefin Tuna industry

The sea-caged SBT industry began in response to the decrease in the quotas for ocean caught tuna. It became increasingly more difficult for the Port Lincoln fishing community to make a profit from canned ocean caught tuna sold on the domestic market. By enhancing product quality through supplementary feeding in sea cages, the Japanese sashimi market became a very profitable target while still allowing the tuna industry to work within the reduced quotas.

The tuna farming process begins with a catch of wild fish between December and February each year. Commercial tuna fishing fleets track schools of tuna west of the Eyre Peninsula in South Australia. Once a suitable catch is located 'purse seines' are used for capture of the tuna destined for the sea-cages. Purse seines are nets that are towed around the school of tuna. The bottom of the net is then closed off, like a purse string, and in general, the tuna are then swum from the purse seine net through an opening into an attached 'Bridgestone' type towing cage. The tuna are towed slowly (1 to 2 knots) back to Boston Bay, Port Lincoln, with towing sometimes required for several hundred kilometres. Once in the bay, the tuna are swum from the towing cage into moored sea-cages being counted during the process using underwater video. Fish swim and feed normally through the towing process and enter the moored cages in a relaxed and healthy state. The non-handling of the tuna during the capture process contributes to the minimisation of stress and injury which occurs if the tuna are poled during capture.

The sea-cages used in the farming process are made of high density black polyethylene plastic (HDPE) usually with plastic-moulded stanchions. The diameter of commercial cages averages 40 metres. Two mesh nets are suspended from the floating pontoon. The inner net contains the fish, and the mesh size ranges from 60 – 90 mm. The outer net is a net to prevent access by predators, with a mesh size of 150 to 200 mm. The inner net drops approximately 10 metres, and the predator net falls to the sea floor, weighted by a chain (Evans, 1992). It is important that the nets have some fouling by marine growths, so that the SBT can clearly distinguish them and avoid entranglement.

It initially was planned that the fattening of tuna would occur over a period of six months. It soon became apparent, however, that marketable fish could be produced in three months, dependent on their initial stocking size. This, and earlier initial stocking dates, has led to the possibility of two farming cycles per year.

Current nutritional management of sea-caged Southern Bluefin Tuna

The current commercial nutritional management of sea-caged SBT is very basic. It essentially involves providing approximately $20 million (15–20 000 tonnes) worth of 'trash fish' to about one hundred thousand sea-caged fish distributed across the commercial farms.

The 'trash fish' is predominantly pilchards but also includes jack mackerel, blue mackerel and herring. Approximately 50% of the 15-20 000 tonnes of trash fish used annually is from overseas. The smaller trash fish such as pilchards are fed whole while larger species are chopped prior to feeding. In some cases, the trash fish have been coated with vitamin and mineral premixes in an attempt to maximise the performance of the SBT. The benefits of this practice are hard to quantify, however, and as most of the vitamins and minerals are washed from the pilchards prior to consumption by the tuna, it is unlikely that this supplementation strategy is cost-effective.

In some instances, frozen trash fish are thawed prior to being manually shovelled into the pens. Other delivery methods have been tried with the most recent and common practice being the provision of frozen blocks of pilchards into mesh floating containers that deliver feed as they defrost.

The industry recognises a number of issues associated with their current feeding methods that could affect their sustainability. These include:

- The natural stocks of the trash fish are limited in Australia and shipments of such fish from overseas imposes a quarantine risk;
- International supplies of pilchards are variable in volume and quality (Japanese supplies have, for example, declined markedly and the fat content of pilchards used in feeds varies from 1–22%);
- As the industry develops and operating costs need to be reduced, it will become increasingly important to reduce feed costs through mechanisation.
- A trash fish diet may not allow the tuna to grow to their full potential.
- Poor utilisation of the trash fish diet by SBT may be resulting in significant amounts of waste nutrients being released into Boston Bay.

In addition, the current feeding practices are costly, labour intensive and inefficient in terms of feed conversion and wastage. As a consequence the development of a manufactured feed and alternative feeding strategies has been deemed the highest industry priority. It is perceived that:

- Manufactured feed can be better matched to the nutritional requirements of farmed tuna thereby enhancing growth and fish health, which translates into increased farm production levels;
- Manufactured feed will provide the potential for improved product quality (in particular fat content, colour and texture) as they are more stable in storage than trash fish and can be altered to better meet the requirements of fish farming and the markets;
- Manufactured feed will reduce industry feeding costs as its generally lower moisture content and promotion of better feed conversion ratio will reduce the quantities required and also, therefore, costs associated with feed storage and transport;
- Manufactured feed will greatly reduce environmental concerns associated with the present use of trash fish, including reducing the overall requirement for pilchards, minimising risks of importing and dispersing undesirable diseases and pests, and reducing organic wastes in the farm environment which can harbour and promote diseases as well as detrimentally affect water quality.
- Development of a manufactured feed will allow the selection and incorporation of more cost-effective feed ingredients and a reduction in the quantity of fresh fish, fish meal and fish oils in the feed, supplies of which are rapidly diminishing.

The economic benefits of the development of a suitable manufactured feed has been estimated to be as high as $9.5 million/annum to the Tuna Boat Owners of Australia and $5 million/annum to successful feed manufacturers. Additional economic benefits would be expected to flow from ongoing research leading to further enhancement of these feeds.

Development of manufactured feeds and nutrition management strategies for sea-caged Southern Bluefin Tuna

The process of developing a suitable manufactured feedand alternative feeding strategies for SBT has been difficult. One of the main reasons for this is that all research conducted with these fish is pioneering and every research technique must be developed before valuable results can be obtained. In addition, we have a poor knowledge of the behaviour of the SBT in a caged environment and it takes time to understand their physical needs.

Special considerations for nutrition research with sea-caged Southern Bluefin Tuna

Compared to traditional nutrition research with terrestrial animal species, there are many physical impediments to conducting nutrition research with SBT. These include:

- The ability to maintain an experimental diet underwater with minimal nutrient loss;
- Difficulties associated with measuring growth or nutritional parameters (e.g. little potential for routine faeces or digesta collection).
- The high value of the experimental fish;
- The inability to maintain a constant experimental environment;
- High accommodation costs resulting in a limited ability to replicate experimental treatments;
- Difficulties associated with getting SBT to accept experimental diets.

A nutritional parameter that requires special consideration in aquaculture systems is feed conversion ratio (FCR). Difficulties associated with measuring growth rate, collection of wasted feed and associated leaching will all contribute to inaccurate estimates of FCR. When interpreting results from aquaculture systems, one must ask whether FCR is an appropriate measure of fish performance during nutritional studies.

The high value of experimental animals can limit the number used in experiments, and the ability to conduct flesh analysis as part of nutritional studies. Farmed SBT can attract more than $A54 per kilogram on the Japanese sashimi market.

The effects of environment on the nutrient requirements of terrestrial production animals is well established. In addition, when conducting nutrition experiments with terrestrial species, the environment can often be closely controlled. This is far more difficult when conducting nutrition experiments with SBT in an ocean environment.

Digestive physiology of Southern Bluefin Tuna

When developing a manufactured feedand feeding strategy, a knowledge of the digestive physiology and natural feeding habits of the target species is a logical starting point.

Digestive anatomy

A detailed investigation into the comparative anatomy and systemics of the tunas (genus *Thunnus)* was completed by Gibbs and Collette (1966). The oesophagus merges indistinguishably into the stomach which forms a blind sac posteriorly (Figure 1). The intestine rises from the anterior end of the stomach, and a very large caecal mass is attached to its origin by several ducts that are not externally apparent. The intestine proceeds caudad for half or more the length of the body cavity (straight intestine), forms a loop, runs craniad (ascending portion) almost to the pylorus, then forms another loop and continues in a nearly straight line (descending portion) to the anus. The spleen is located between the straight and ascending portions of the intestine. The gall bladder is a long, tubular sac rising from the right lobe of the liver, attached to the dorsal wall of the left side of the straight intestine (Figure 1).

The specific anatomy of the SBT has a number of nutritional implications. The blind stomach has a massive capacity. Individuals are easily able to consume 10% of their body weight in pilchards in one feeding period. This may indicate an ability to feed infrequently while still maintaining a constant flow of digesta, and hence nutrient absorption, through the intestine. The lower intestine of the SBT is very short, with little difference in the morphology of the intestinal segments defined above. The functions of the whole SBT intestine are likely to resemble the small intestine of terrestrial animals. Anatomically, there are few digestive sites that would be suited to the fermentation of high fibre diets.

Digestive enzymes

Homogenates of pyloric caeca have been tested at the University of Adelaide for pancreatic proteolytic enzyme profiles (D.Tivey *et al.* unpublished data). The presence of major proteolytic enzymes, similar to those found in terrestrial animals, has been observed. In particular, a high activity of a dipeptidyl peptidase IV-like enzyme has been demonstrated. It appears that diet type (pilchards vs manufactured feed) has little effect on the resident proteolytic enzymes in the pyloric caeca.

The results of Tivey et *al.* (unpublished data) indicate that the expression of enzymes in the digestive tract is not rate limiting to protein digestion *in vivo.* Factors which may influence the digestion of protein include protein type, differences in the mechanical processing of the diet prior to feeding, the residence time of the food in the pyloric caeca, the rate of pancreatic secretion in response to food in the digestive tract and the effect of microenvironment (mucin type and quantity) of the pyloric caeca on luminal enzyme activities and absorption of digestion end products.

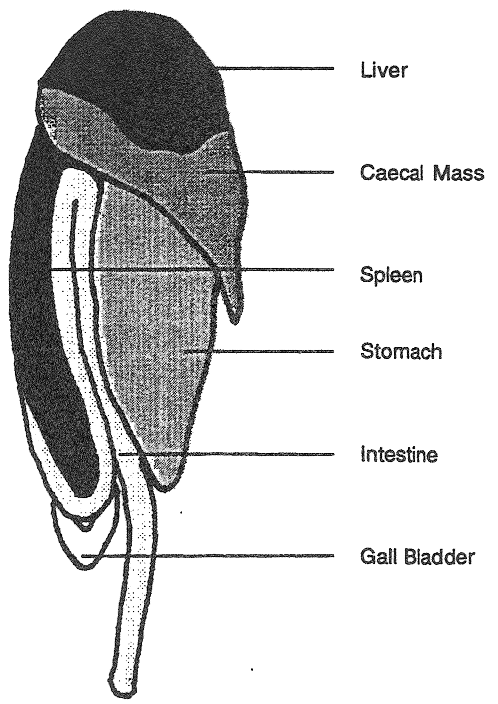


Figure 1 Schematic representation of the digestive tract of a Southern Bluefin Tuna (*Thunnus maccoyii*)

Digestive microbiology

The bacterial population of the digestive tract of SBT has been isolated and characterised at the University of Adelaide by J. Brooker et *al.* (unpublished data). Up to 10^8 bacteria/ml were detected in samples collected from the pyloric caeca and intestine. There was a diverse range of microorganisms present and these appeared to be a resident population in the digestive tract rather than derived from the feed. The populations differed depending on whether pilchards or manufactured diets were fed, but were highest in the pyloric caeca.

Brooker *et al.* (unpublished data) found that bacteria isolated from the pyloric caeca were mainly proteolytic with a small number being amylolytic as well. In contrast, many bacterial isolates from the intestine were either lipolytic or proteolytic and lipolytic. This suggests that there is a separation of functions in the digestive tract with bacteria in the intestine contributing to the digestion of lipid in the diet. Lipase expressing bacteria were more prominent when SBT were fed pilchards compared with those fed a manufactured feed.

Scanning electron microscopy has revealed different modes of action of the microbial populations in the digestive tract of SBT (Brooker et *al.* unpublished data). The pyloric caeca contains populations that are mainly attached to small particles of feed in the lumen. In contrast, the intestine contains microbial populations that appear to be attached to the intestinal wall. Bacterial attachment to feed in the pyloric caeca may have a role in the ability of SBT to digest fibrous feed ingredients.

What are the nutritional requirements of Southern Bluefin Tuna?

The initial strategy for the development of a manufactured feed for SBT was to match the diet specifications to the requirements of the SBT with the aim of reducing feed costs, minimising environmental pollution, ensuring even growth rates, maximising carcass quality and exploiting desirable carcase traits whilst allowing the selection of the most cost–effective ingredients. Unfortunately, a number of these objectives are conflicting.

Unlike most terrestrial and aquaculture production systems, the optimum farmed SBT car-case contains a significantly higher level of intramuscular fat compared to wild caught fish. Other systems specifically target a reduction in carcase fat. Deposition of fat is an inefficient process. It takes approximately five times as much energy to deposit a gram of fat compared to a gram of lean meat (van Barneveld et *al.* 1997). In addition, carcase fat deposition is increased when the diet specifications do not match the requirements of the fish for optimum growth. Excesses of protein, amino acids and energy or an imbalance of these respective nutrients can result in increased fat production. Hence, to maximise carcase quality traits, it is unlikely that feeding efficiency and growth rates will be maximised and environmental pollution may be higher than if the diets were tailored for maximum lean growth. For this reason, it is difficult to define a basis for diet formulation for SBT.

As there is no existing information on the nutritional requirements of SBT, best estimates have been made to facilitate diet formulations based on the requirements of other pelagic fmfish. These estimates have been used to ensure that there is no deficiency of any one nutrient, rather than to accurately match diet specifications to nutrient requirements. We must also consider that diet form appears to have a major impact on the nutritional value of manufactured feeds, and we have no knowledge of the sensitivity of caged SBT to changes in the protein and energy content of their diet. For this reason, highly tuned diet specifications may not result in improved performance of the fish. In addition, due to the difficulties associated with conducting nutrition research with SBT, experiments to specifically define requirements for certain nutrients are unlikely in the near future.

An 'ideal' dietary amino acid profile has been defmed from the amino acid balance of red and white muscle in wild tuna (van Barneveld, 1996; Table 1). Comparison of this profile with the balance used in diet formulations for SBT prior to 1997 indicates that there are excesses of methionine, valine, leucine, phenylalanine, and arginine while there is a notable deficiency of histidine. If diets formulated for SBT contain high levels of protein, the amino acid balance is likely to have little relevance due to the large amount of amino acids that will be wasted and deaminated. As the protein content of these diets is reduced, however, and the quantity of fresh fish and fish meal is replaced through the use of alternative protein sources, the ideal amino acid profile will have greater relevance.

Progress in the development of a manufactured diet for sea-caged Southern Bluefin Tuna.

Four large-scale feeding experiments have been conducted with sea-caged SBT since 1994 through the Cooperative Research Centre for Aquaculture. Due to the time of harvest and the duration of experiments, only one large scale feeding experiment is possible per year. Smaller, more intensive experiments such as digestibility and transit time studies are conducted following the large scale growth experiments using the remaining fish.

Feeding and growth experiment 1(1994)

Southern Bluefin Tuna are extremely selective about the form of a manufactured feed they will accept. They appear sensitive to shape, colour, texture, odour, moisture content, oil content and fish or fish meal content. As a consequence, the number of potential diet presentation options and diet ingredients is limited.

The acceptance of semi-moist 'sausage-type' pellets and extruded pellets was compared with pilchards using SBT held in three cages anchored in Boston Bay, SA (van Barneveld et *al.* 1995). This experiment was also used to refine experimental methodology for the handling, tagging and performance monitoring of SBT in research cages.

The tuna could be handled, measured and tagged with only 10% mortality. Tuna would not consume extruded pellets (>75% DM) but slowly accepted semi–moist 'sausage-type' pellets (52–70% DM). An extensive weaning period of 42 days was required before the SBT would readily consume the pellets resulting in substantial weight loss. Feed conversion ratios (FCR) of 7:1 were recorded for SBT consuming pellets compared to 13: 1 by those fed pilchards. The growth and meat quality of SBT fed pellets appeared comparable to those fed pilchards. Capture, weighing, tagging and transfer of fish was shown to have a negative impact on the performance of SBT, and hence care must be taken when interpreting these results for use in a commercial environment.

The results suggested that there was potential for manufactured feeds to be offered as a semi-moist 'sausage-type' pellet to replace pilchards as the diet for caged SBT. In addition, the long time taken for the fish in this experiment to take the pellets as their sole diet may have been due to initial low water temperatures and the fact that the SBT had previously been fed pilchards for prolonged periods.

Feeding and growth experiment 2 (1995)

The second feeding and growth experiment evaluated the time to wean recently caught SBT held in warmer waters onto manufactured feeds and compared the growth performance of SBT fed pilchards or manufactured diets (van Barneveld et *al.* 1995). A diet of known composition was formulated to contain a variety of animal proteins, fish proteins and cereals and to meet the estimated requirements of SBT. During manufacture, however, this diet was significantly extended with water and flour to facilitate processing into sausage skins. The experiment used six cages containing SBT allocated following tagging, and measurements of weight and length. The SBT were fed to satiety and the weight of feed added to each cage was recorded daily. After 105 d, the SBT were harvested, identified and re-weighed. Tuna mortality was high initially in cages with unfouled nets and through handling when the fish were in poor condition. In addition, poaching resulted in significant losses of fish fiom some cages and made the interpretation of the results difficult (determination of FCR etc.).

The SBT were successfully weaned onto artificial diets within 14 days. SBT fed the manufactured feed had lower weight gains and higher feed conversion ratios than SBT fed pilchards (Table 2). The variability associated with the performance of SBT fed the manufactured feed was also higher than those fed pilchards. There was no consistent relationship between

Table 1 Essential amino acid content of red and white muscle in wild SBT (g/kg, dry matter), dietary amino acid balance (used prior to 1997) and proposed ideal amino acid balance (from van Bameveld, 1996).

Amino acid	Red muscle	White muscle	Dietary balance[1]	Ideal balance[1]
Methionine	11.0	10.9	49	35
Threonine	18.2	17.2	56	56
Valine	20.4	19.2	74	63
Isoleucine	18.7	17.1	61	57
Leucine	29.3	27.6	110	90
Phenylalanine	15.1	14.9	63	47
Lysine	32.5	30.7	100	100
Histidine	30.5	30.3	37	96
Arginine	23.9	22.3	93	73

[1]Balance on a DM basis.

Table 2 Growth performance of SBT fed pilchards and a manufactured feed (from van Bameveld *et al.* 1995).

	Pilchards		Manufactured feed	
Cage	**1**	**2**	**1**	**2**
Number of SBT	15	5	16	15
Average final weight (kg)	28.4	37.9	22.0	30.0
Average length (cm)	106.4	117.1	102.0	111.1
Average weight gain (kg)	6.8	8.7	0.9	4.0
Average daily gain (g)	65	83	9	38
Feed conversion ratio	26	23	110	27

stocking density and the growth performance of the SBT. The growth response from one cage of SBT fed the manufactured feed suggests that there is a high potential for a manufactured feed to replace pilchards in this aquaculture system. The dilution of nutrients in this diet during pelleting may be one reason for the lower than anticipated performance.

Results from the above experiments had significant outcomes in terms of improving experimental methods and identifying gaps in our knowledge. In particular, the effects of nutritional history on the performance of SBT on manufactured feeds, the role of attractants and flavour enhancers in manufactured feeds, the nutritive value of alternative protein sources for SBT, and the specific nutrient requirements of SBT are all areas where our knowledge is limited.

Feeding and growth experiment 3 (1996)

The use of sausage skins was a slow and inefficient way of making manufactured feeds for SBT. The aim of the third large-scale feeding and growth experiment was to assess the value of a spray-coated pellet formulated to meet the nutritional requirements of SBT and to accommodate water and binder addition at the time of manufacture. This experiment was also designed to achieve a higher level of replication than previous experiments using three cages per experimental treatment.

The formulated feed contained a combination of Chilean fish meal, blood meal, Antarctic krill meal, DL-methionine, squid oil, Jack Mackerel oil, wheat gluten, pre-gelled starch, water, vitamins, minerals and squid flavouring. The pellet was well accepted after a short period of introduction, but growth performance of the SBT on this diet was not determined due to sudden SBT deaths that occurred across the whole industry in April, 1996 following a storm. A report of this incident (Clarke, 1996) concluded that the storm stirred sediment on the bay floor resulting in gill irritation, subsequent mucous production and asphyxiation of the SBT. The research farm lost 75% of its fish while the commercial farms lost in the vicinity of $50 million worth of fish. The majority of commercial farms have now been moved to deeper waters outside the bay.

Feeding and growth experiment 4 (1997)

The previous growth experiments showed some potential for manufactured feeds, but growth performance of the SBT was always substantially lower than pilchard-fed fish. Results from morphology studies and digestibility experiments in conjunction with the growth experiments suggested that ingredient particle size and diet form may play an important role in the utilisation ofnutrients (Davis, 1997). It appears that when diets are offered in sausage skins or spray-coated pellets they rapidly break down in the stomach. Due to the small particle size of the ingredients, they are quickly passed from the stomach into the pyloric caeca and intestine. Rapid transit time of the small particles through the short intestine results in poor utilisation. In contrast, pilchards and well bound diets are slowly broken down in the stomach, and the constant flow of small quantities of nutrients through the intestine results in better absorption and subsequent utilisation. Further evidence of this phenomenon is demonstrated when SBT are fed whole pilchards or pilchards chopped and included in sausage skins. Despite an identical nutritional value, SBT fed pilchards in skins had poorer growth performance than fish fed whole pilchards (Davis, 1997).

The objectives of this experiment were to compare the growth performance of SBT fed one of the following feed forms. The feed forms were:

- Pilchards;
- Cooperative Research Centre (CRC) for Aquaculture Mash: 'Mash' feeds are defined as feeds that contain a proportion of fresh trash fish in combination with a concentrate. The advantage of this feed is that acceptance is still high, while the overall requirement for trash fish is reduced;
- Northern Bluefin Tuna Mash: This diet was based on fresh trash fish and a concentrate of unknown composition that has been imported from Japan where it has been used successfully with Northern Bluefin Tuna;
- Extruded pellet: This is a high quality, low moisture feed form that has been proven for use in other aquaculture systems. It is highly flexible, hygienic and extruders can produce large quantities of feed quickly and efficiently. It could only be produced in limited quantities due to the remote location of the equipment and there was insufficient to assess growth performance;
- Two commercial diet preparations: Composition of these diets was unknown.

All feeds were well accepted after a short introduction period. To enhance acceptance, SBT were fed an alginate bound pellet from the time of capture and during towing into Boston Bay.

The growth response with a manufactured feed was the best so far achieved (Table 3). The CRC Aquaculture Mash promoted mean growth rates that were only 45% of those achieved with pilchards, however, the variation surrounding the growth of the fish fed manufactured feeds was significantly less than that observed on pilchards. It appears that exceptional growth of one fish fed pilchards has greatly increased the mean growth rate of this treatment. The condition of fish fed the CRC Aquaculture Mash was similar to those fed pilchards. The growth rate and condition of fish fed the Northern Bluefin Tuna Mash and proprietary manufactured feeds was poor in comparison with pilchards and the CRC Aquaculture Mash, and in some instances, the fish lost weight and condition (Table 3).

The CRC Aquaculture Mash was based on pilchards, Chilean fish meal, krill meal, free amino acids, squid oil, vitamins, minerals, anti-oxidants and water. On an 'as-fed basis, the CRC Aquaculture Mash had a higher nutrient density than pilchards (Table 4), but despite a slightly reduced feed intake, was unable to support growth rates similar to those observed with pilchards.

Evaluating feed ingredients for use in manufactured diets

If a suitable trash fish replacement is to be found for use in SBT manufactured feeds, a number of other protein sources (animal proteins, grain legumes, cereals) need to be evaluated. A knowledge of the nutritional value of feed ingredients other than trash fish and fish meals will allow us to cost-effectively replace these protein sources while maintaining the required balance of amino acids and lipids.

Experiments have been made to assess the *in vivo* digestibility and transit time of feed ingredients, manufactured feeds and pilchards fed to SBT (Davis, 1997; van Barneveld et *al.* unpublished data). The digestibility of dry matter, nitrogen and energy along the digestive tract has been determined for Peruvian fish meal fed to SBT in 'sausage skins' (Table 5). Negative digestibilities for nitrogen were calculated in the stomach and pyloric caeca. This is likely to be due to large contributions of endogenous N in these regions. Endogenous N contributions are also likely to have resulted in an underestimate of the nitrogen digestibility in the distal intestine.

Table 3 Mean growth performance (and standard deviation) of Southern Bluefin Tuna fed pilchards and manufactured feed with varvina forms.

	Pilchards		CRC Mash		NBT Mash			
	Cage 1	Cage 2	Cage 1	Cage 2	Cage 1	Cage 2	Man A	Man B
Fish sampled	9	8	9	6	10	10	10	10
Weight gain (kg)	12.95	7.46	5.62	3.68	1.33	1.59	–0.85	0.44
	(10.18)	(1.89)	(1.60)	(1.29)	(1.00)	(0.53)	(1.09)	(1.07)
Length gain (cm)	5.39	5.69	4.39	3.92	2.05	1.85	1.20	1.30
	(2.07)	(1.87)	(2.41)	(1.16)	(1.91)	(1.45)	(0.82)	(1.46)
Condition index*	24.42	23.50	22.55	21.35	18.84	19.87	17.40	18.89
	(0.93)	(0.84)	(1.26)	(0.70)	(1.46)	(1.06)	(1.43)	(1.09)
Condition index gain	11.83	4.77	3.21	2.04	0.57	0.85	–1.85	1.29
	(12.40)	(1.44)	(1.52)	(1.81)	(1.66)	(1.01)	(1.61)	(1.62)

* Condition index is a subjective measurement based on the weight and length of the fish.
Man A, Commercially manufactured diet A; Man B, Commercially manufactured diet B; NBT, Northern Bluefin Tuna; CRC, Cooperative Research Centre.

Table 4 Composition of CRC Aquaculture Mash and pilchards (g/kg, as-fed) fed to Southern Bluefin Tuna during the 1997 large-scale feeding and growth experiment in Port Lincoln, SA.

Nutrient	CRC Aquaculture Mash	Pilchards
Crude protein	363.00	210.00
Dry matter	615.00	340.00
Fat	152.00	92.00
Lysine	27.32	7.60
Threonine	15.61	3.54
Methionine	10.68	1.30
Isoleucine	31.68	34.99
Leucine	28.73	4.00
Valine	19.61	3.75
Phenylalanine	17.72	3.84
Histidine	10.49	4.48
Arginine	26.05	6.43

A range of problems was encountered with experiments conducted to determine the digestibility of nutrients in the distal intestine of SBT fed manufactured feeds and pilchards. Poor mixing and a wide range in ingredient particle size is thought to have resulted in negative amino acid digestibility estimates being obtained in this experiment. Digesta transit time was assessed in SBT fed manufactured feeds and pilchards using a marker dilution technique (Davis, 1997). The manufactured diet was estimated to have a transit time of 5.3 hours. Sampling of the fish fed pilchards was not long enough to dilute the marker to 80% of the equilibrium concentration and hence an exact transit time could not be assessed, yet it was longer than that observed with the manufactured feed. The significant difference in transit times for manufactured feeds compared to pilchards may help explain the poorer utilisation of the former.

Future nutrition research directions

Having identified a manufactured feed that is accepted by the SBT as well as promoting about 70% of the growth and condition achieved by using a trash fish diet, the opportunity exists to use this feed as a research tool and enhance our knowledge of SBT nutrition. The following research program is proposed for the SBT research farm in Boston Bay, SA from July 1997.

- Utilise the CRC Aquaculture Mash feed to assess the ability of caged SBT to respond to changes in the protein and moisture content of their diet;
- Assess the potential of natural colour enhancers, such as astaxanthans, in manufactured feeds;
- Assess the influence of frequency of feeding on the growth performance of SBT. In the short term, the ability to feed less frequently while maintaining performance represents massive cost savings to the tuna farms.

Table 5 Dry matter, nitrogen and energy digestibility in the stomach, pyloric caeca, proximal intestine and distal intestine, in a Peruvian fish meal based diet fed to caged Southern Bluefin Tuna, determined 2.5 and 5.0 hours after feeding.

	Collection time (hours)		Statistics		
	2.5	5.0	SEM (2.5h)	SEM (5.0h)	Time
Dry matter digestibility					
Stomach	0.00	0.06	0.021	0.021	NS
Pyloric caeca	-0.08	-0.24	0.024	0.029	**
Proximal intestine	0.17	0.06	0.065	0.088	NS
Distal intestine	0.42	0.35	0.048	0.048	NS
Region*Time	NS				
Region	***				
SEM	0.044				
Nitrogen digestibility					
Stomach	-0.13	-0.07	0.020	0.019	NS
Pyloric caeca	0.02	-0.05	0.076	0.082	NS
Proximal intestine	0.11	-0.01	0.062	0.077	NS
Distal intestine	0.38	0.33	0.062	0.059	NS
Region*Time	NS				
Region	***				
SEM	0.047				
Energy digestibility					
Stomach	0.12	0.20	0.023	0.022	*
Pyloric caeca	0.21	0.19	0.068	0.073	NS
Proximal intestine	0.41	0.29	0.049	0.060	NS
Distal intestine	0.62	0.59	0.041	0.039	NS
Region*Time	NS				
Region	***				
SEM	0.039				

NS, not significant; * P<0.05; *** P<0.001; SEM, standard error of the mean (after Davis, 1997).

- Develop suitable extruded feed forms and trial these forms on a large scale;
- Assess the potential to reduce the quantity of fresh fish, fish meal and fish oils in manufactured feeds.

These experiments will be run in conjunction with experiments aimed at modelling the energetic expenditure of SBT in sea-cages, improving the flesh quality of SBT by pre- and post-harvest handling and storage techniques and the identification of growth correlates to improve the efficiency of research experiments.

Conclusions

A manufactured feed that can promote growth and flesh quality characteristics in SBT similar to that currently achieved with trash fish is likely to be developed soon. Research conducted over the past 4 years has revealed some potential for manufactured feeds in this aquaculture system but progress has been slow due to wide ranging difficulties associated with researching a large, valuable, pelagic species. Enhanced research techniques and diet manufacturing technology (e.g. extrusion) combined with a collaborative research program will greatly assist development of a manufactured feed in the future. Together with specific feeding strategies, advanced nutritional management of SBT will reduce the dependence on trash fish, ensuring the viability of this industry in the long term.

Acknowledgments

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Manipulation of the ingredient and nutrient content of manufactured feeds fed to caged Southern Bluefin Tuna (*Thunnus maccoyii*) and subsequent production responses.

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Previous research has shown Southern Bluefin Tuna (SBT) will accept a semi-moist pellet (~600 g/kg dry matter) containing at least 500 g/kg fresh pilchards, or equivalent, and more than 380 g/kg crude protein (CP). The objectives of this experiment were to assess the production responses of SBT fed semi-moist pellets containing reduced levels of fresh product and the performance of SBT fed diets containing varying levels of nutrients. Three diets were formulated for this experiment and were compared against a pilchard control. Diet A contained 500 g/kg pilchards and supplied 390 g/kg CP and 160 g/kg crude fat (CF). Diet B supplied the same level of nutrients as Diet A, but contained only 250 g/kg of fresh pilchards. Diet C supplied 50 g/kg more CP and 60 g/kg less CF than Diets A and B. Preliminary data suggests there is no difference in the specific growth rate or average daily gain of SBT fed the three semi-moist pellet diets and the pilchard diet and that growth rates can be sustained on diets with reduced levels of fresh pilchards. On a dry weight basis, the feed conversion ratio (FCR) of SBT fed pilchards was superior, with no differences in the FCR of fish fed semi-moist pellets. Market data suggests the flesh characteristics of fish fed Diet C were inferior. It can be concluded from these results that protein is not limiting in SBT diets, but rather the protein:energy ratio, and the source of dietary energy may be influencing SBT performance and flesh characteristics.

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DIET DEVELOPMENT FOR PELAGIC FISH: THE SOUTHERN BLUEFIN TUNA (*Thunnus maccoyii*) MODEL

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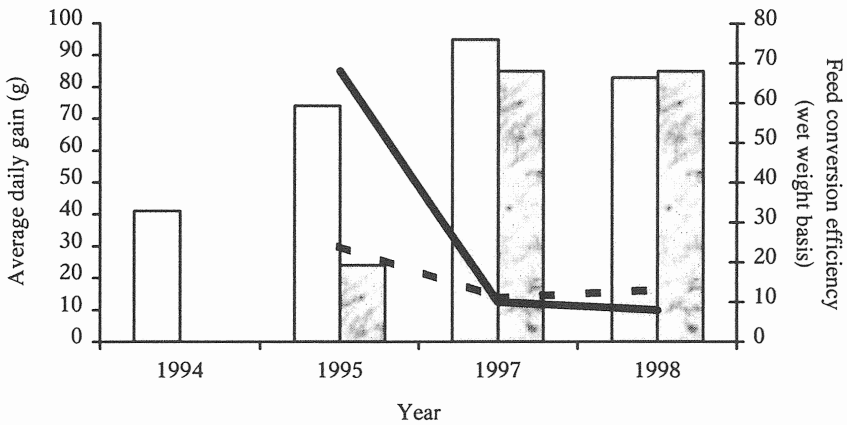
Development of manufactured feeds for farmed Southern Bluefin Tuna (SBT) is recognised as a key factor in the sustainability of this industry. The development of diets to replace bait fish as the primary nutrient source in this aquaculture system has presented many challenges and could serve as a model for the development of feeds for other pelagic aquaculture species. The objective of this paper is to outline the processes undertaken to develop manufactured feeds for SBT and the resulting improvements in feeding efficiency over a four year period.

Diet development for SBT and other pelagic fish is plagued by many physical impediments including the ability to maintain an experimental diet underwater, difficulties associated with measuring growth and other nutritional correlates, the high value of the experimental fish, an inability to maintain a constant experimental environment and high experimental costs. For these reasons, little progress was made with diet development for SBT until manufacturing procedures were established for the production of moist pellets that were readily accepted by the fish. Significant advances were also required in fish husbandry, particularly in relation to the measurement of growth and feed intake, and the weaning of fish onto manufactured feeds following capture. Introduction of moist pellets and improved weaning procedures in the third year of the research program resulted in significant improvements in the growth rates and feed conversion efficiency of SBT to levels comparable with bait fish. These diets also formed a base on which future nutrition research could build.



Improvements in average daily gain (g) of SBT fed bait fish (□) or manufactured diets (□) and the feed conversion efficiency of SBT fed bait fish (▬) or manufacturd diets (▪▪▪▪) over 4 years of diet development

Moist pellet development was based on diet transit time studies, basic fish morphology and digestive anatomy, endogenous enzyme profiles and digestive microbiology. This information was used to establish suitable binding properties and the most appropriate base ingredients. Having established an acceptable diet form, subsequent research focussed on 1) establishing the response of the SBT to changes in dietary nutrient content, 2) reducing the levels of bait fish, fish meals and fish oils in the moist pellet, 3) establishing the most appropriate dietary energy source and 4) *in vitro* and *in vivo* digestibility studies to assess diets and component ingredients. Future research will examine the suitability of extruded pellets with a reduced moisture content and a wider ingredient base while maintaining acceptable product quality. Similar approaches may be useful for other pelagic species.