Final Report

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COMMERCIAL IN CONFIDENCE

Longer term holding of southern bluefin tuna
Thunnus maccoyii

David Ellis, Steven Clarke, Brian Jeffriess, Robert van Barneveld, Barbara Nowak, Phillip Thomas, Milena Fernandes, Maylene Loo and David Padula

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Australian Government

Fisheries Research and Development Corporation

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1. NON TECHNICAL SUMMARY

This project provided an understanding of the impact of longer term holding (LTH) on southern bluefin tuna (SBT) nutrition, product quality, health and the environment.

The nutrition component of this project has clearly defined significant production efficiencies through the delivery of optimised bait fish formulations using 'Formu-bait' software. Furthermore, it has identified that younger ranched SBT perform better and also some feeding behaviour characteristics that need to be managed carefully in LTH.

Product quality research has improved the understanding of flesh quality and residue status of SBT held for a longer period. This aspect of the project provides operators with confidence that there will be no adverse flesh quality effects with LTH SBT.

SBT health research identified that there is a need to further investigate health issues, especially in the first year of a LTH project. Whilst there were parasites in and on LTH SBT, the levels were lower in the second year compared with the first year. This provides confidence to operators that a 6-8 week mortality period appears to be related to SBT farmed in the first year.

Environmental research demonstrated the use of techniques developed during the Aquafin CRC to assess the effects of LTH on the environment and highlighted the potential issues for the future. The results have been adopted, with the Australian Southern Bluefin Tuna Industry Association applying for an extension to the aquaculture zone in the expectation that farm production will increase over the next few years through a possible increase in quota allocation of the wild fishery and longer term holding of SBT.

From a husbandry and production point of view, the benefits of this project highlighted processes that need to be considered for LTH. These include those associated with the management of lease sites and the operation of ranching equipment, with a strong emphasis on attention to minimising predators and poaching. It also defined regulatory issues that need to be considered when moving to LTH and compliance based environmental reporting.

The project demonstrated the economics of LTH of SBT, the use of infrastructure, crews and resources, and the associated financial risks. Ultimately, it demonstrated that marketing advantages such as a downturn in supply of global high-grade sashimi quality tuna can be met by holding SBT for longer periods.

This project has provided the nucleus for the development of LTH of SBT. One of the key findings to make LTH financially viable within current economic constraints and future economic forecasts is that operators will need to target smaller SBT (approximately 10kg). This will require industry and regulators to assess the current practice of rejecting SBT <10kg as part of AFMA's 40 fish weight sample process. Furthermore, if more SBT <10kg were removed from the wild fishery it may possibly have an impact on the wild fishery assessment.

This factor must be considered very carefully in context with sustainability.

However, the industry is confident that over the next few years the wild fishery stock assessment will improve largely due to the introduced regulations to minimise over catching in the fishery. It is therefore important that research continues in the area of LTH so that when the opportunity arises, the SBT ranching industry has available options of how best to utilise its quota allocation.

LTH research must focus on optimising nutrient inputs, feeding frequency and behaviour, product quality (flesh quality and residues), health and the environment.

This project was successful as it achieved its objectives and planned outcomes, and has positioned the SBT ranching industry with options to maximise quota allocation for the future.

Furthermore, it was an industry led research project that involved 10 Aquafin CRC partners which fulfilled the SBT ranching industry's commitment to the Aquafin CRC after the initial focus of *'SBT propagation'* was substituted with *'longer term holding of SBT'*.

2. ACKNOWLEDGEMENTS

Many of the research reports in this document appear in other Aquafin CRC final reports. These reports are as follows:

2001/249 (revised) Aquafin CRC - SBT Aquaculture Subprogram: development and commercial evaluation of manufactured diets.

2004/206 Aquafin CRC - SBT Aquaculture Subprogram: management of food safety hazards in farmed southern bluefin tuna to exploit market opportunities.

2004/209 Aquafin CRC - SBT Aquaculture Subprogram: dietary supplements for reducing oxidative stress and improving flesh quality attributes in SBT

2003/225 Aquafin CRC - SBT Aquaculture Subprogram: investigations of the relationship between farming practices and SBT health

3. BACKGROUND

The aquaculture of SBT has been a major success story in the expansion of the Australian aquaculture industry. SBT ranching, which started as research and development (R&D) in 1990, expanded to produce about 10,000 tonnes of gilled and gutted tuna, worth about $267 million in 2002/03 (SARDI, 2004). The industry has significant multiplier effects because of labour intensiveness, infrastructure requirements and the impetus it has created for the development of other industries and aquaculture sectors (Econsearch 1999). In economic terms, the industry is a high value seafood industry in Australia (behind Western Australian rock lobster), with almost all of its value coming from ranching/farming.

More recently, the value of the SBT ranching sector has declined substantially, $139 million in 2006/07 (Econsearch). The decline is due to the unfavourable financial exchange rate between Australia and Japan, and because product sale price has declined by greater than 30% due to increased volume of competitive species at the Japanese market. However in recent times, market prices are starting to improve due to implications related to 'over catch' in the fishery, and reduced supply by overseas producers. To minimise the constantly changing aspects of the market, the SBT ranching industry has and is continuing to refocus its research priorities on:

- Increased market competitiveness (ongoing projects on product quality, residue levels and a marketing strategy);
- Decreased operating costs (ongoing nutrition research but now primarily focused on optimising nutrition and feeding strategies using bait fish and existing environmental research focused on the development of novel and more efficient monitoring techniques);
- Increased production (continuing and new projects on SBT health management to further reduce the already low level of mortalities and LTH of SBT to increase the weight gain achieved from the quota managed stock that are ranched).

In 2002, a key focus for the Aquafin CRC was changed from "Tuna Propagation" to LTH. Establishing a specific and comprehensive research project to advance this concept proved challenging, primarily due to the limited knowledge about many aspects of SBT. The near completion/recent completion of a number of key Aquafin CRC projects in the areas of SBT biology, nutrition, product quality, food safety and health has now changed this position, providing new assessment tools and clarifying the key direction research should take.

A favorable economic assessment of LTH (Jeffriess 2004 – Aquafin CRC commercial in confidence document only available to the Board) and increasing concerns about the sustainability of the SBT fishery due to 'over fishing' have also focused the industry's interest in LTH.

A commercial company, DI Fishing Pty Ltd, agreed to collaborate with Aquafin CRC researchers and the ASBTIA formerly known as the Tuna Boat Owners Association of South Australia (TBOASA) on LTH, and held over sufficient SBT for a research trial that ran from April 2005 through to August. The company had SBT being ranched according to the normal production cycle (3 – 8 months) and comparisons were made with these SBT and those from LTH (about 18 months).

This project on LTH comprised a series of subprojects to assess the feasibility of holding SBT for a second ranching season. The subprojects included those associated with key knowledge deficiencies that may influence resource allocation, ranch husbandry and marketing:

- Production (i.e. growth, condition index and food conversion ratio);
- Product residue status (i.e. levels of dioxin, PCBs and heavy metals);
- Product quality (i.e. sensory evaluation, developed indices of quality);
- Environmental quality (i.e. status of the seafloor and benthic communities); and
- SBT Health.

4. CONSULTATION

Consultation has occurred on this topic between researchers and industry since 2003, when ASBTIA highlighted to the Aquafin CRC that their research priorities were now in this direction rather than in propagation. The subject has been discussed by the SBT Aquaculture Subprogram Steering Committee and Scientific Group at a number of 6 monthly meetings, as well as by the Aquafin CRC and FRDC Boards.

This project has been developed through extensive consultation between relevant researchers and industry representatives. The lead author, David Ellis, ASBTIA Research Manager and Steven Clarke, SBT Aquaculture Subprogram Leader, have driven this process.

The South Australian Fisheries Research Advisory Board has been kept informed of the development of this project.

5. NEED

This project is essential for the SBT aquaculture industry to address a number of its present threats, in particular:
- Continuing profitability related to fluctuations in currency exchange and market prices;
- Countering any possible decline in stock availability through a decrease in wild fisheries quota limits.

The project involves undertaking research as well as providing services to support these activities. The project:
- Was optimally matched with SBT industry sector outcomes, coordinated and the desired outcomes delivered;
- Optimised the use of all the available research and industry resources;
- Delivered on clearly distinguished activities and costs associated with the broad research needs of LTH;

Communication between researchers and ranchers will occur through SBT Aquaculture Subprogram meetings and presentations to enhance technology transfer.

DI Fishing Co Pty Ltd's investment in the project confirmed the industry's interest in the outcome of this research, further highlighting the potential value of LTH to the tuna ranching sector (Jeffriess, 2004).

6. OBJECTIVES

The key objectives of the project were as follows:

1. Assess the potential effects of LTH on benthic nutrient cycling and assimilative capacity.

2. Assess the environmental effects of LTH on macrobenthic infaunal communities using PCR assays and manual enumeration.

3. Communicate the economic analysis and benefits and costs, to the aquaculture industry sector, Commonwealth, State and Local Government regulators and the community.

4. Disseminate, where relevant and agreed by the SBT Aquaculture Subprogram Steering Committee, the results of all research investigation on LTH to relevant industry sector, government and community representatives.

5. Synthesise the relevant biological data into a revised economic assessment of LTH, to facilitate investment decisions by the SBT aquaculture industry sector.

6. Summarise other less quantitative data in terms relevant to the industry sector so that the benefits and risks associated with LTH are fully appreciated.

7. Evaluate changes in the redox potential and in the concentrations (and fluxes) of N and P in sediments.

8. Refine "Formu-Bait", a software package, to optimise bait fish feeding strategies during the spring and summer period, as well as during the second ranching season (LTH).

9. Define the residue (Hg, dioxins, PCBs etc) changes (concentration and body burden) that occur in SBT muscle as a result of LTH and use this along with sampling and testing for residues of bait fish used for the

SBT feed, to extend the single grow-out season predictive model to also address grow-out over a second season (i.e. LTH).

10   Describe SBT growth and condition during the spring and summer period (when SBT have previously not been ranched) as well as during the second ranching season (LTH).

11   Characterise the food conversion ratio (FCR) of SBT ranched during the spring and summer period, as well as during the second ranching season (LTH).

12   Extend the database for two Aquafin CRC postgraduate students working on related projects, which are studying the mercury content of SBT muscle and internal SBT organs, and dioxin and PCB content.

13   Using the data collected on mercury and dioxins etc, determine the effect of LTH against regulatory Minimum Residue Levels (MRLs) and tolerable intakes, and use this data for optimal positioning of the industry sector in relation to SBT product marketing.

14   Determine and characterise the interactions between SBT health (parasites etc) and residue levels.

15   Compare LTH fish to the competing product that is available on the Japanese market at the same time by: a) comparing LTH SBT product with the Northern Bluefin Tuna (NBT) product from Mexico (chemical and consumer acceptability/preference) in March 2006; and b) comparing the LTH SBT product with the SBT product produced here within the normal one season ranching cycle (chemical/instrumental only) in July 2006.

7. METHODS

SBT used for the LTH trial was captured in the Great Australian Bight in an area around 33°27'S, 132°04'E between 19 February and 3 March 2005 as part of a commercial catch by the purse-seine vessel FV Boston Bay and transferred into a towing pontoon. The SBT were then towed to Port Lincoln by the vessel FV Dampier, and on 27 March 2008 a 40 fish sample was taken from the tow cage to estimate mean weight of SBT >10 kg. Based on the average weight of 17.17kg, a total of 894 SBT for research were transferred into a 50m diameter commercial ranching pontoon together with an amount of SBT to be commercially ranched on 28 March 2005.

On 5 April 2005, 10 SBT from the 50m diameter commercial ranching pontoon were caught using a baited hook and hand-line for baseline samples for research focusing on nutrition, resides, health and product quality. In the period from 6 – 10 April 2005, a total of 879 SBT were caught using a baited hook and hand-line, tagged with conventional dart tags and transferred into 4 32m diameter research pontoons (approximately 220 SBT/pontoon), (Figure 1).

Once transferred into research pontoons the SBT were allowed to acclimatise, during which time they were fed a diet consisting mainly of local Australian sardines, *Sardinops neoSardineus*.

Eleven SBT from each research pontoon were inserted with archival tags but were not part of this study.

Figure 1. Catching and tagging SBT for the *'Formu-bait'* and *'Longer Term Holding'* Trial

On all occasions the length of the SBT was measured to the nearest centimetre, and when ocean conditions permitted, actual weights were recorded. It was possible to weigh and measure 108 SBT of the 879 tagged. These fish were used to establish a base level condition index (CI):

$$CI = \frac{Weight(kg)}{Length(m)^3}$$

A condition index of 18.21 was then used to calculate starting weights of unweighed SBT based on their measured length. Treatments (section 8.1) were randomly allocated to available pontoons. Tag data suggests that the 879 tagged SBT consisted of mainly 2-3 year old SBT, although different ratios of the cohorts were placed into research pontoons.

Treatments to research pontoons commenced on 18 April (section 8.1).
On 30 May 2005, the first harvest of 10 SBT were taken from each treatment. There was an intention to catch a further 20 SBT by hook and line from each treatment, record length and weight, and then return these to the relevant pontoons. However, the SBT were unable to be caught by baited hook and hand-line.

The two remaining harvests on July 11 and August 22, 2005 had 30 SBT harvested from each treatment, with 10 SBT from each treatment sampled for Aquafin CRC research projects.

Between 12 and 20 October, 2005, 519 SBT that were used for research in the 4 x 32m diameter research pontoons were transferred into a single research pontoon containing a cleaned and maintained net. The LTH research pontoon was then moved to another area on the lease site.

The LTH SBT were fed a diet similar to that described in section 8.1 (i.e. ~ 18.5% protein and 7.3% fat) and were harvested on 7 December 2005; 7, 14, 22 & 31 March 2006; and 14 August 2006.

For specific methods of sampling at research harvests refer to contents of sections 8.1-4.

8. RESULTS

The following sub-chapters describe research results for respective disciplines participating in the initial *'Formu-bait'* research and then the continuation of the LTH research. Some of these chapters can be found in their own final reports as identified in the acknowledgement section of this document.

Additional farm husbandry information not covered in any of the following sections is presented here.
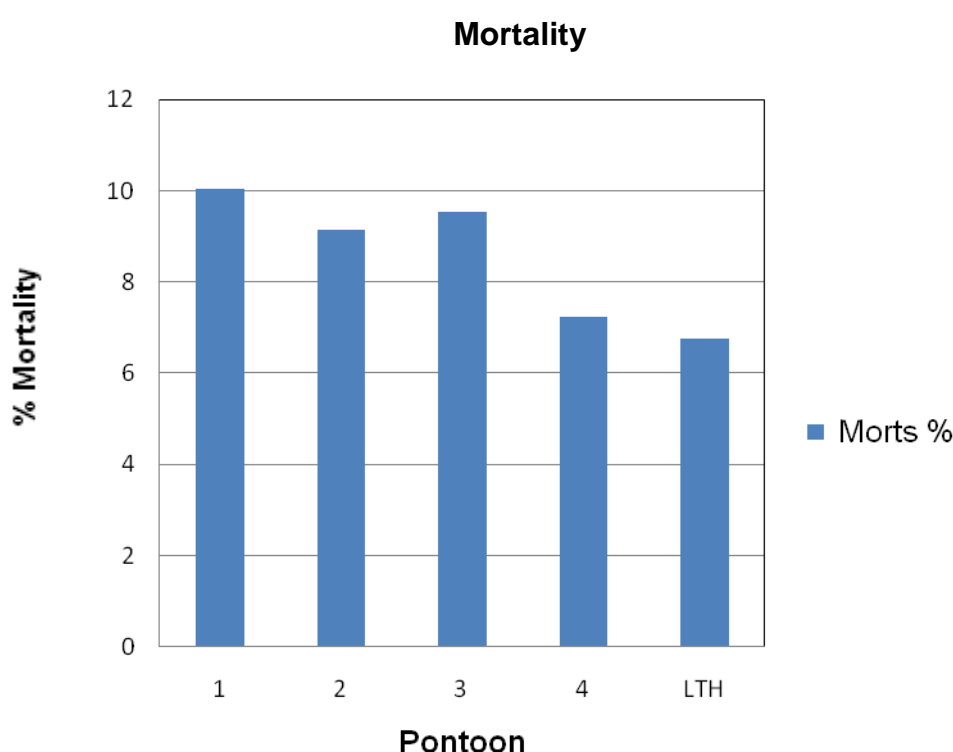
Mortality

Figure 2. Mortality expressed as percentage of stocked pontoon for research season 2005 (1-4) and LTH 2006.

Percentage mortality was higher across all pontoons (Figure 2) than the commercial industry average, which was 3.32% in 2005 and 3.58% in 2006. This can be partly explained by the extra handling involved with tagging the SBT for season 2005, but in realistic terms, it only requires a few mortalities in low stocked pontoons to overstate mortality figures. For example, if 10 SBT die in a commercial pontoon containing 1600 SBT as a result of tagging the mortality percentage would be 0.6% whereas, if the same amount dies in research pontoons that contain 220 SBT as a result of an identical process

the percentage mortality would be 4.5%. There were no significant mortalities during the 6-8 week mortality period as experienced by industry in general. The LTH pontoon experienced mortalities as a result of a seal attack and on one occasion bronze whaler sharks, *Carcharhinus brachyurus,* entered the pontoon.

Impact of tagging

Handling and tagging of fish is recognised as having an impact on their survival and growth (Crozier & Kennedy, 2002; Sumpton et al., 2008). As all SBT in this trial were tagged, it is therefore very important to interpret the following results as comparisons between treatments rather than absolute values, as the extent of the tagging impact was not measured.

Water temperature

The water temperature profile was consistent with previous monitoring performed by the ASBTIA. Water temperatures displayed a typical seasonal pattern with maxima of about 22°C in February - early March and a minima of about13.5°C in July (2006) - August (2005) (Figures 3 & 4).
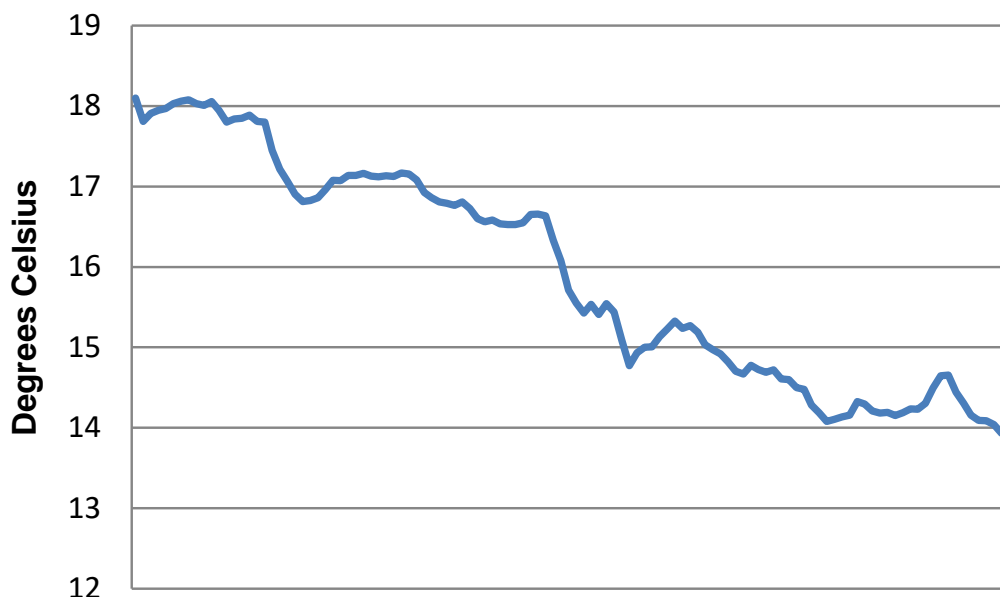
Figure 3. Water temperature for the *'Formu-bait'* trial (1st stage of LTH)

Figure 4. Water temperature for the *'LTH'* trial

References

Sumpton, W., Mayer, D., Brown, I., Sawynok, B., McLennan, M., Butcher, A. and Kirkwood, J., 2008. Investigation of movement and factors influencing post-release survival of line-caught coral reef fish using recreational tag-recapture data. *Fisheries Research,* 92: 189–195.

Crozier, W and Kennedy, G., 2002. Impact of tagging with coded wire tags on marine survival of wild Atlantic salmon (*Salmo salar* L.) migrating from the R. Bush, Northern Ireland. *Fisheries Research*, 59(2): 209-215.

8.1 Production – Nutrition & Growth

Influence of digestible energy intake on southern bluefin tuna (*Thunnus maccoyii*) growth rate, feed conversion efficiency and fat deposition using bait fish as a nutrient source – longer term holding Part 1

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Introduction

Capacity exists to improve the production efficiency of ranched southern bluefin tuna (SBT) through an improved focus on nutrient supply relative to nutritional requirements. Based on bait fish consumption over the course of a season, total weight gain and an assumed digestibility of bait fish, SBT grown over a 180 day period only retain 5-10% of the nitrogen (i.e. protein) they consume and deposit this as muscle (Fernandes et al., 2007. While a significant proportion of the ingested nitrogen may be used as an energy source (albeit an expensive one), it could be argued that this level of efficiency will quickly become unsustainable if the margin of over feed costs continues to decline.

Bait fish are still the predominant source of nutrients for farmed SBT. Evidence suggests that farmers have been blending bait fish supplies for some time in an attempt to improve the growth of their fish and the resulting product quality. Despite this, there is little information available on the most appropriate combinations of bait fish to feed over the course of a season. It is known that intake changes significantly as water temperatures drop and as

SBT gain condition, and hence opportunities exist to match nutrient supply from available bait fish to this intake pattern.

The aim of this research was to utilise *'Formu-bait'*, a computer program that formulates and optimises combinations of bait fish based on nutrient requirements of SBT and unit price. The research method varied protein and fat content of feed, in an attempt to exploit relative SBT growth and feed intake patterns over the course of a ranching season.

Methods

Selection of bait fish and diet formulation

Proximate analysis was performed on 22 shipments of various bait fish species held in freezers by DI Fishing Pty Ltd. This involved mincing a 20kg block randomly selected from a given shipment and taking a 700g subsample for analysis (Table 1). The proximate results were entered into *'Formu-bait'*, which was used to identify appropriate combinations of bait fish that could supply high protein (<19.5%) and low fat (<4%), medium protein (<18.5%) and medium fat (<7.5%), and low protein (<17.5%) and high fat (>10%) (Table 2).

Table 1. Proximate analysis of bait fish used in feeding experiments

Parameter	Protein	Moisture	Fat	Ash	Gross energy
Unit	%	%	%	%	MJ/kg
Sardinops sagax	16.35	63.80	14.87	2.97	8.63
Emmelichthys nitidus nitidus	19.80	72.30	2.50	5.70	4.29
Sardinops neoSardineus	20.35	73.80	2.00	4.00	4.96
Sardinops neoSardineus - PFP	20.40	70.80	4.30	3.60	5.21

Note: PFP refers to sardines sourced from the East Coast of Australia from Pelagic Fisheries Processors

Table 2. Formulated combinations of bait fish used in feeding experiments

Feed Specifications	Protein	Fat	*Sardinops sagax*	*Emmelichthys nitidus nitidus*	*Sardinops neoSardineus*
High protein/Low fat	19.50%	4.0%	15%	30%	55%
Med protein/Med fat	18.50%	7.3%	40%	35%	25%
Low protein/High fat	17.50%	10.5%	65%	35%	

Feeding strategies

Commercially, there is conjecture as to whether a low protein, high fat (LP/HF) diet at the beginning of the season or a high protein, low fat (HP/LF) diet is the most effective way to exploit the extremely high intakes of SBT at this time (up to 12% of body weight) (David Ellis Pers Comm). To address this, four feeding regimes were developed to examine SBT responses over the course of a growing season. These regimes included consistent supply of medium protein and medium fat (MP/MF) over the entire season, either low protein and high fat progressing to high protein and low fat or vice versa and supply of locally caught sardines only (Table 3). Locally caught sardines are typically low in fat, however at times lipid levels may reach 6%.

Table 3. Experimental feeding regimes over the course of the growing season

Treatment (Pontoon)	0-6wks (Period 1)	7-12wks (Period 2)	13-18wks (Period 3)
1 (10)	MP/MF	MP/MF	MP/MF
2 (11)	LP/HF	MP/MF	HP/LF
3 (12)	HP/LF	MP/MF	LP/HF
4 (13)	Local	Local	Local

Note – 'Local' refers to sardines caught by the South Australian sardine fishery

SBT management

An initial sample of 10 SBT was taken from a 50m pontoon on the 5th April 2005 for baseline compositional assessment for this research. In the period from the 5–10 April 2005, a total of 879 SBT were tagged with conventional dart tags and placed into 4 pontoons (approximately 220 SBT/pontoon).

On all occasions the length was measured and when ocean conditions permitted, actual weights were recorded. It was possible to weigh and measure a total of 108 SBT out of the 879 tagged. These fish were used to establish a base level condition index determined as follows:

$$CI = \frac{Weight(kg)}{Length(m)^3}$$

The condition index of 18.21 was then used to calculate starting weights of the remaining fish based on their measured length.

Treatments were randomly allocated to available pontoons.

Tag data suggests that the 879 tagged SBT consisted primarily of two cohorts (2-3 year old fish). Different ratios of the cohorts were placed in each of the research pontoons.

It was assumed that percentage length and weight increases were similar across year classes (cohorts).

Once transferred into the research pontoons, the SBT were allowed time to acclimatise and were fed a diet consisting mainly of local Australian sardines *Sardinops neoSardineus* during this period.

The experiment commenced on 18 April 2005 with 6 weekly harvest dates scheduled on the 30 May 2005, 11 July and 22 August 2005. SBT in all treatments were shovel fed the desired bait fish ratios for their particular treatment twice per day to satiation when weather conditions permitted.

On the first harvest 10 SBT were taken from each treatment. There was an intention to catch a further 20 SBT by hook and line from each treatment, record length and weight before returning them to the pontoon, but unfortunately, the SBT weren't willing participants in this exercise. The two remaining harvests (July and August) had 30 SBT harvested from each

treatment on the desired date.   Ten SBT from each treatment were destructively sampled for research projects within Aquafin-CRC.

All SBT had lengths and gilled & gutted (GG) weights were recorded at harvest. An industry standard of 13% weight loss from harvest was applied to all SBT to calculate whole weight of individual SBT (Brian Jeffriess pers comm).

One entire fillet from sampled SBT was minced into a homogenous state and sent for proximate analysis. In addition, a 2kg sample from every bait fish type used was randomly sampled at every feeding event and was stored. These samples were pooled on a weekly basis based on their type, fed through a commercial meat mincer until homogenous and then sent for proximate analysis.

Statistical Analyses

Comparison of proximate analyses (protein, moisture, fat, ash and energy) and condition index for each treatment over time, and between treatments within a time period were conducted by 1 way ANOVAs using Genstat, 10th edition, VSN International Ltd. All data were checked for homogeneity of variances and normality prior to analyses. Where differences were observed means were compared by Least Square Differences. As the experiment involved only one pontoon per treatment due to limitations on available resources, individual fish were used as replicates in statistical analysis, and as a consequence, care must be taken when interpreting these results.

Results

Close attention to bait fish combinations fed during the season resulted in provision of diets that closely matched the planned experimental feeding regime (Figures 1 and 2). While feed intake per se dropped over the course of the season, the percentage protein (Figure 1) and fat (Figure 2) was maintained for the respective treatments.
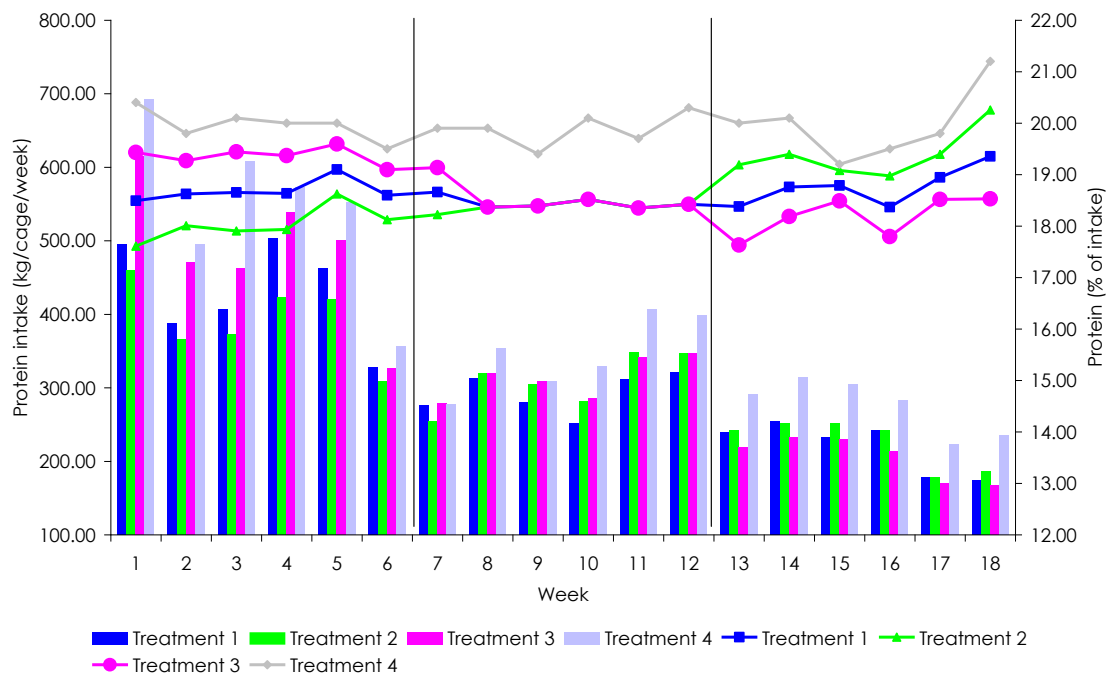
Figure 1. Protein intake and dietary protein content* during the experimental period, (Table 3 highlights the treatment conditions). Intake is represented on the right y-axis by lines and content on the left y-axis by bars.
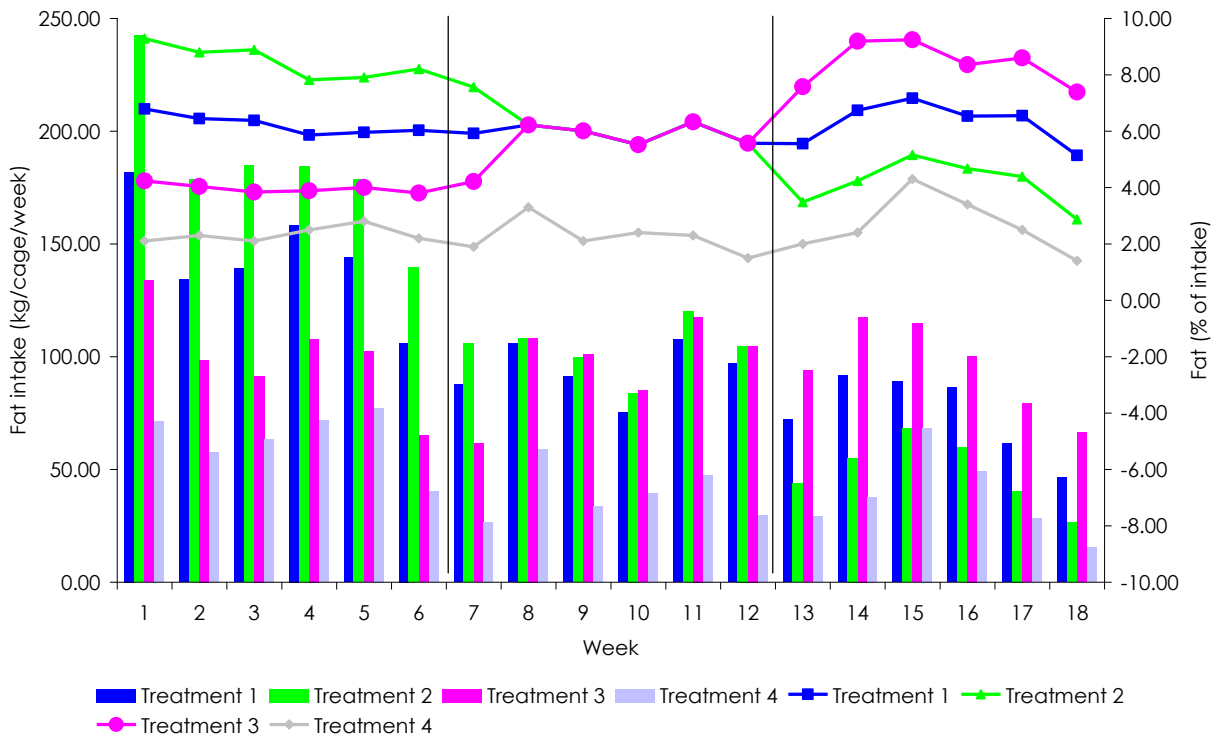
Figure 2. Fat intake and dietary fat content during the experimental period.

Again, intake is represented on the right y-axis by lines and content on the left y-axis by bars.
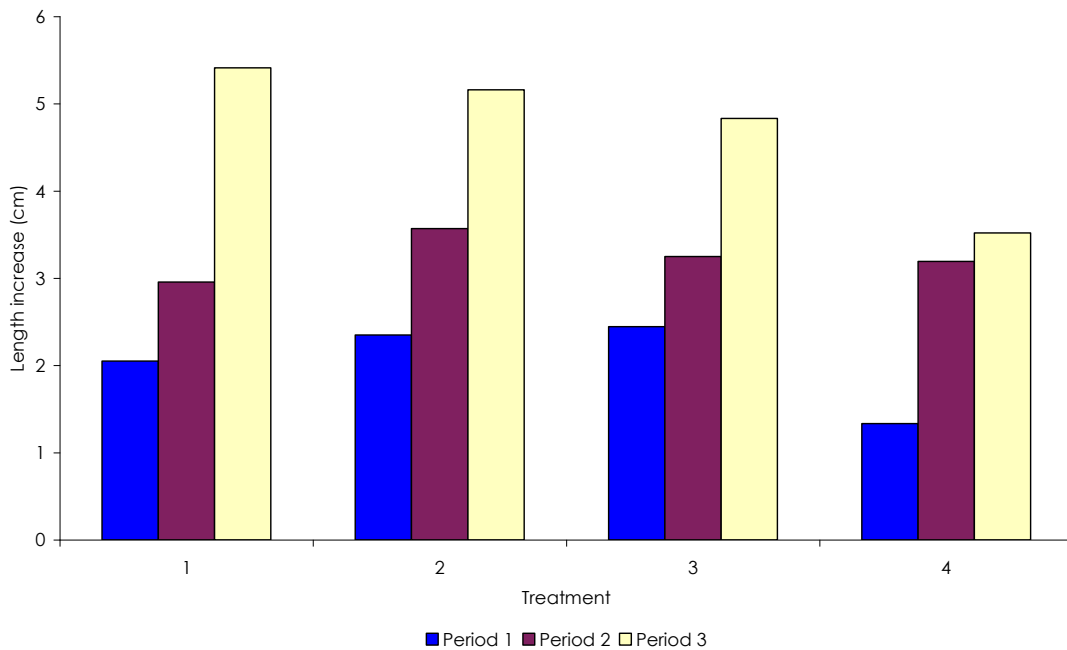
Figure 3. Calculated cumulative length increase of SBT fed varying fish combinations over the experimental periods.
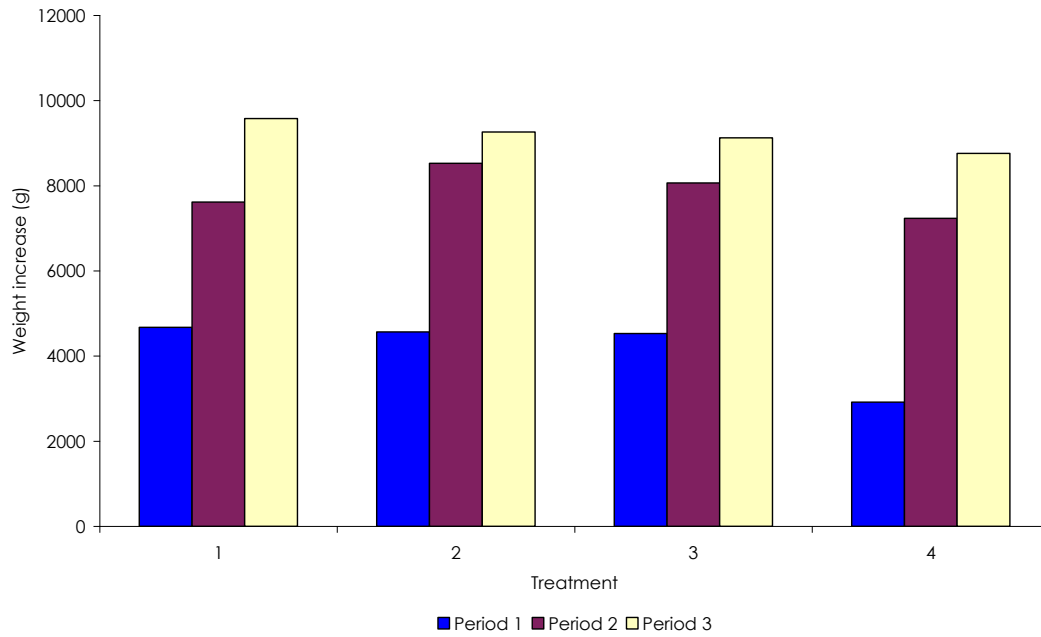
Figure 4. Calculated cumulative weight gain of SBT fed varying bait fish combinations over the experimental periods.
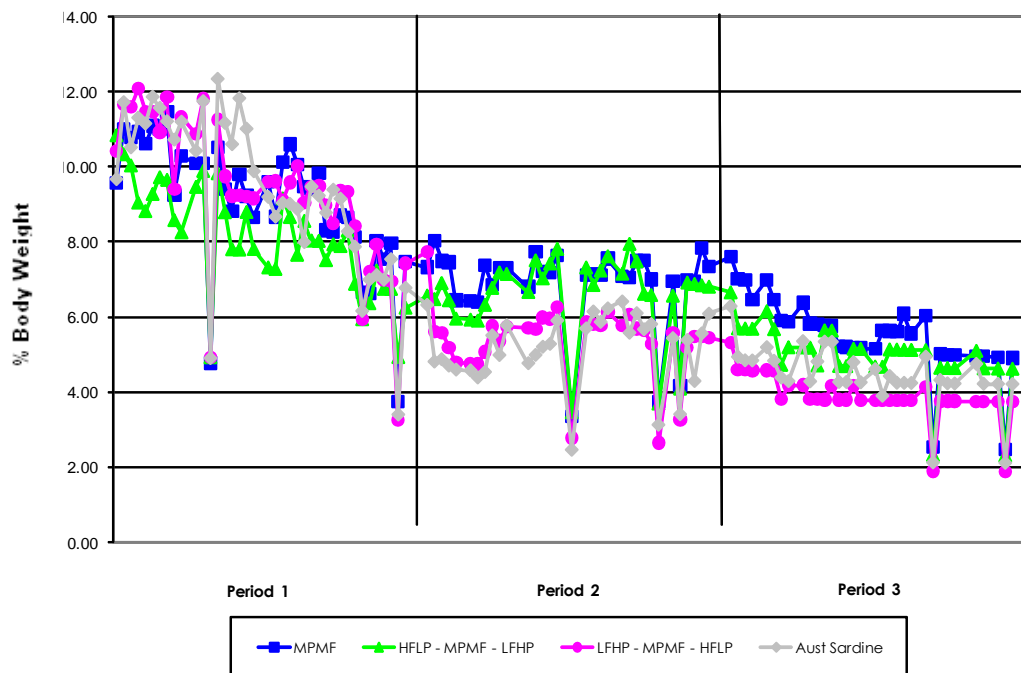
Figure 5. Feed intake as a proportion of body weight of SBT fed varying bait fish combinations over the experimental period.

8.1 Production – Nutrition & Growth
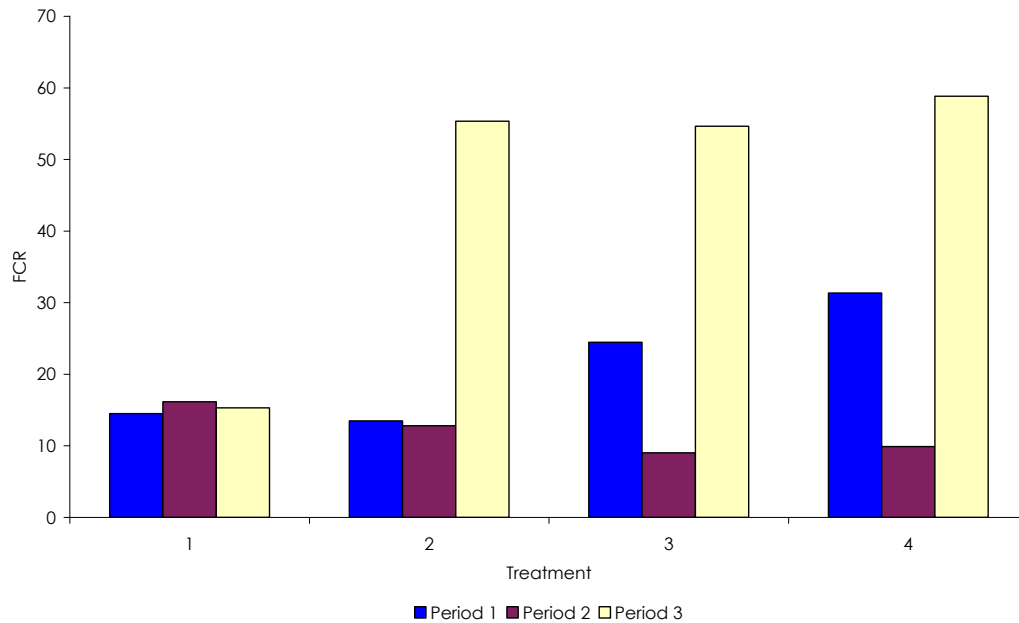
Figure 6. Feed conversion ratios of SBT fed varying bait fish combinations over the experimental period

Length, weight, feed intake and feed conversion data

Cumulative length increase and weight gains (Figures 3 and 4), intake as a percent of body weight (Figure 5) and feed conversion ratio (Figure 6) were calculated for each treatment.

Comparison of proximate composition and condition index of fish

1. Within periods between treatment effects

No significant differences were found in the fat or moisture content of SBT fillets between treatments within any of the three time periods (Figures 7 and 8; $p > 0.05$). Similarly, no significant differences were found between treatments for fish condition index in period 1, energy content in period 1 and 3, and protein content in periods 2 and 3 (Figures 9, 10 and 12; $p > 0.05$). However, significant differences were observed between treatments in condition index in periods 2 and 3. In period 2, fish fed diet 4 had a significantly lower condition index than fish fed all other dietary treatments, with no differences being observed amongst fish fed diets 1, 2 and 3. Whilst in

period 3, fish fed diet 4 had a significantly lower condition index than fish fed all other dietary treatments except 3. In addition, fish fed dietary treatment 1 had a significantly higher condition index than fish fed all other treatments except diet 2 in period 3. With respect to protein content of the fish, significant differences were observed in period 1, with diet 3 fed fish having significantly lower protein than fish fed the other 3 dietary treatments. The ash content of the fish differed significantly between treatments in all 3 time periods (Figure 11; $p < 0.05$), with fish fed diet 2 having significantly lower ash content than fish fed all other diets except diet 3 in periods 1 and 2.

2. Within treatments over time effects

For dietary treatment 1 significant difference were observed in fish condition index and all proximates except protein over time (Figures 7, 8, 9, 10, 11 and 12; $p < 0.05$). Ash content was significantly higher in period 1 compared with 3, but not 2. Fat and condition index were significantly lower, whilst moisture was significantly higher in period 1 compared to both periods 2 and 3. Energy was significantly lower in period 1 than in period 3, but not than in period 2.

With dietary treatment 2 significant differences were found over time in fat, moisture, energy and condition index of the fish but not ash or protein content (Figures 7, 8, 9, 10, 11 and 12; $p < 0.05$). Fat and condition index were significantly lower, whilst moisture was significantly higher in period 1 than all other periods. Energy content of the fish was significantly lower in period 1 than period 3, but not period 2.

As for treatment 2, significant differences were found in fat, moisture, energy and condition index over time but not ash or protein content for treatment 3 (Figures 7, 8, 9, 10, 11 and 12; $p < 0.05$). Moisture content was significantly higher whilst energy and condition index were significantly lower in period 1 compared to periods 2 and 3. Fat was significantly lower in period 1 than in period 3 but not period 2.

A significant difference was observed over time for all parameters measured in fish fed dietary treatment 4 (Figures 7, 8, 9, 10, 11 and 12; $p < 0.05$). Ash and moisture were significantly higher in period 1 than periods 2 and 3 whilst fat, energy and condition index were all significantly lower in period 1 than in periods 2 and 3. Protein was significantly lower in period 3 than periods 1 and 2.

■ Treatment 1 ● Treatment 2 ▲ Treatment 3 ◆ Treatment 4
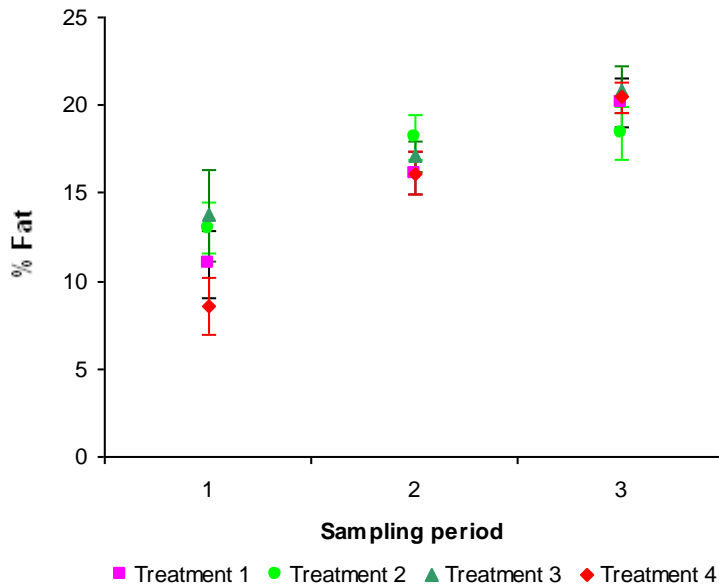
Figure 7. Change in % fat content of SBT fillets over time for each dietary treatment. Values are means ± SE.

■ Trreatment 1 ● Treatment 2 ▲ Treatment 3 ◆ Treatment 4
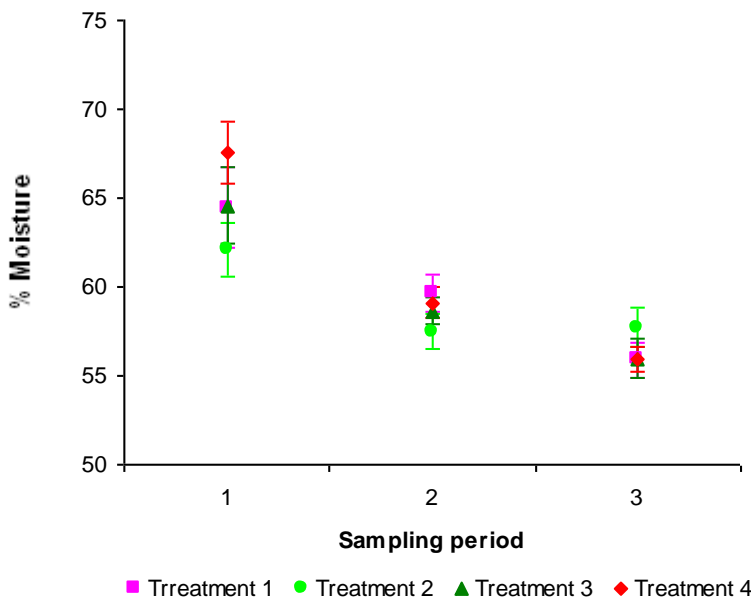
Figure 8. Change in moisture content of SBT fillet over time for each dietary treatment. Values are means ± SE.
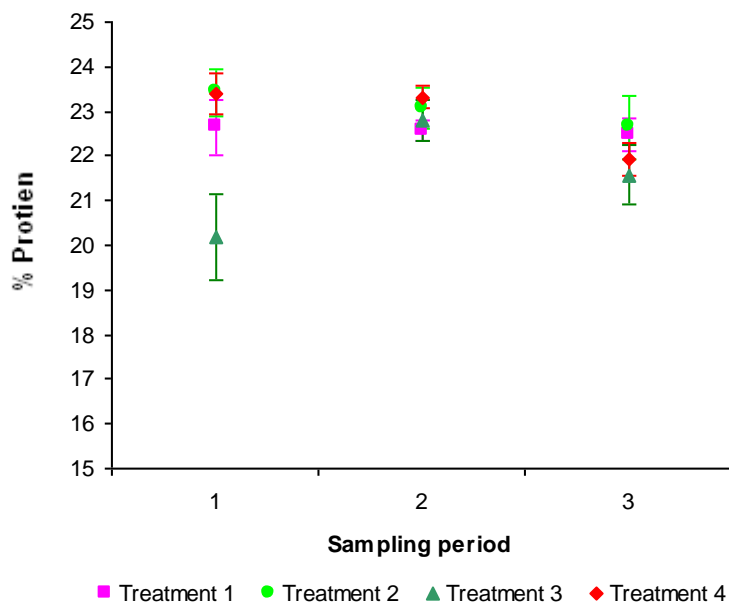
Figure 9. Change in protein content of SBT fillet over time for each dietary treatment. Values are means ± SE.
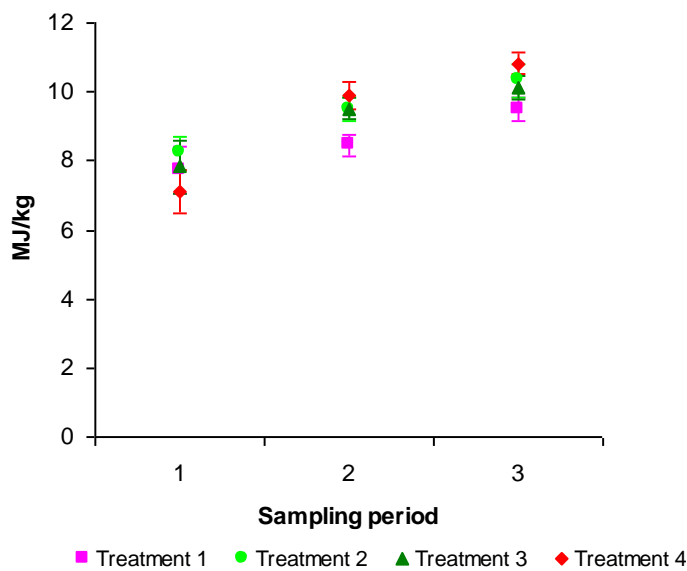
Figure 10. Change in energy content of SBT fillet over time for each dietary treatment. Values are means ± SE.
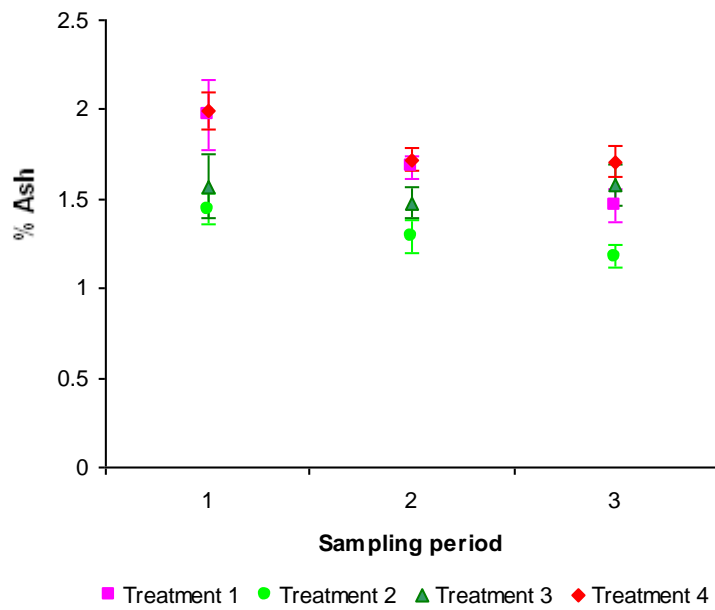
Figure 11. Change in ash content of SBT fillet over time for each dietary treatment. Values are means ± SE.
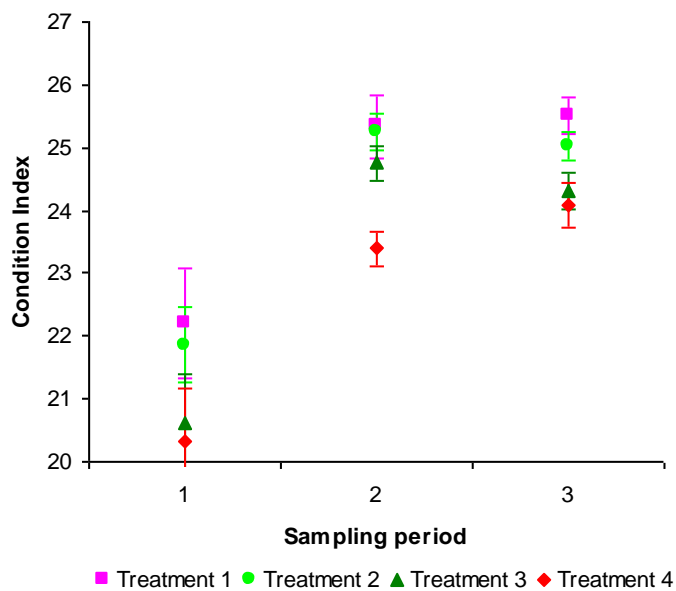
Figure 12. Change in condition index of SBT over time for each dietary treatment. Values are means ± SE.

The results suggest that varying bait fish combinations and subsequent nutrient supply can affect the relative growth efficiency of SBT over the course of the growing season. Maintaining a constant and balanced supply of protein and fat appears to be the most desirable feeding strategy for SBT. SBT fed a diet containing medium levels of protein and medium levels of fat for the entire experimental period had the greatest length increase, the greatest weight gain and the lowest feed conversion ratios. In particular, feed conversion ratios were maintained during the final trimester of growth compared to all other treatments. In addition, fish fed this treatment (treatment 1) had a significantly higher condition index than fish fed all other dietary treatments, except treatment 2 in the final harvest period of the experiment, which was slightly less although significantly similar.

There does not appear to be any advantage to supplying either high protein/low fat or low protein/high fat diets at the beginning of the season to exploit higher intakes and potential growth potential of the SBT.

It is also important to note that the poorest performance for the trial was achieved by SBT fed entirely local sardines. This treatment resulted in SBT with a significantly lower condition index than all other dietary treatments, except 3 in the final period of the experiment. However, the price of local sardines may still make them the most cost effective feed source, when compared with imported bait fish. This experiment highlights the price sensitivity of the relative treatments. Based on the differences observed, only a small shift in the cost of local sardines is required to make this treatment cost ineffective.

An interesting observation that can be made from these results is that when the diets are high in fat, relative intake by the SBT drops (Figure 5). This suggests that dietary energy content may have a regulatory role in intake. This could be particularly relevant for manufactured feeds which are energy dense. High fat levels (12%) may result in reduced intakes of manufactured feeds by SBT.

In summary, this experiment demonstrates that SBT performance can be manipulated by varying nutrient supply through different bait fish

combinations. In addition, the data suggests that feeding a combination of protein and fat over the growing season is more beneficial in terms of intake, particularly in the latter stages of the season. Finally, if intake is to be maximized, then dietary fat (or energy) levels need to be monitored and controlled, as high fat diets appear to limit intake.

Cost Benefit Analysis

This experiment demonstrated that maintaining a constant and evenly balanced supply of protein and fat in diets appears to be the most desirable feeding strategy for SBT, particularly in the final grow-out period. It can be seen from Table 4 that large increases in food conversion ratio's (FCRs) occurred for all feeding regimes except treatment 1. Furthermore, fish fed treatment 1 achieved the greatest weight gain during the final period (Table 4).

Table 4. Comparison of FCRs for the different treatments in the final period of the experiment.

Treatment	FCR in final period	Average weight gain per fish in final period (kg)
1 (MP/MF)	15.3	1.96
2 (LP/HF)	55.3	0.74
3 (HP/LF)	54.6	1.06
4 (Local sardines)	58.8	1.53

The FCR values in each treatment for the final period are possibly linked to the energy content of feed and the SBT physiology response. It would appear that higher fat feeds suppress intake whilst lower fat feeds increases intake (Refer Figure 5).

The costs of the growth recorded for each treatment for the final period based on their cost per kg and FCRs are outlined in Table 5.

Table 5. Calculation of the cost to feed 1 cage of 1900 SBT based on the figures derived from each treatment of this experiment for the final period.

Diet	Average weight gain per fish in final period (kg)	FCR in final period	Diet cost	Feed required for observed weight gain based on FCR (Kg)	Feed cost per SBT	Feed cost per pen if 1900 SBT in a pen
HP/LF	1.06	54.6	$0.66	57.9	$38.21	$72,606
MP/MF	1.96	15.3	$0.74	29.9	$22.13	$42,047
LP/HF	0.74	55.3	$0.79	40.9	$32.31	$61,389
Sardines	1.53	58.8	$0.50	89.9	$44.95	$85,405

The figures illustrated in Table 5 demonstrate that by feeding a MP/MF diet in the final grow-out period a rancher could save approximately $19,342. This savings figure is based on one cage of 1900 SBT being fed the MP/MF diet over the final six week period, instead of the next most cost effective treatment, the LP/HF diet. The local sardine diet worked out as the least cost effective diet to feed in the final trimester, with the MP/MF diet working out to be $43,358 cheaper based on one cage of 1900 SBT.

Influence of digestible energy intake on southern bluefin tuna (*Thunnus maccoyii*) growth rate, feed conversion efficiency and fat deposition using bait fish as a nutrient source – longer term holding Part 2

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Introduction

In 2002, a key focus for the Aquafin CRC changed from tuna propagation to longer term holding (LTH), however, at the time establishing a specific and comprehensive research project on this topic was not feasible, primarily due to the limited knowledge about many aspects of southern bluefin tuna (SBT).

The near completion/completion of a number of key Aquafin CRC projects in the areas of SBT biology, nutrition, product quality, food safety and health changed this position, providing new assessment tools and clarifying the key direction such research should take. A favourable economic assessment of LTH (Jeffriess 2004 – Aquafin CRC commercial in confidence document only available to the Board) and increasing concerns about the sustainability of the SBT fishery (due to recent CCSBT deliberations) also focused the industry's interest on LTH. A commercial company, DI Fishing Pty Ltd, collaborated with Aquafin CRC researchers and the TBOASA to perform research that included the LTH research trial. The company also had other commercial SBT being kept according to the normal ranching cycle, allowing comparisons to be made between SBT grown to current industry standards and those from LTH.

The project on LTH was comprised of a series of subprojects to assess the feasibility of holding SBT tuna for a second ranching season (from 3 to 8 months up to about 18 months).

The subprojects included those associated with key knowledge deficiencies that may influence resource allocation, ranch husbandry and marketing. One of the subprojects included production, specifically growth and feed conversion ratio. As such an experiment was devised to characterise SBT growth and condition during the spring and summer period (when SBT have previously not been farmed) as well as during the second farming season.

At the completion of the '*Formu-bait'* trial a total of 520 remaining SBT held in 4 x 32m pontoons were transferred into one 32m pontoon. The new pontoon had a clean net and was then relocated to another area of the lease site. The pontoon was fed a mixed diet, ideally representing the medium protein/medium fat diet (MP/MF), 18.5% and 7.3% respectively, that was used in Experiment 2.1. The diet was based on the availability of bait fish at the end of the commercial farming season and was fed for a period of 68 weeks.

Methods

Growth data

SBT were measured for fork length on six separate occasions during the LTH project. This included three measurements taken during the *Formu-bait* experiment - May 30th 2005, July 12th 2005 and August 22nd 2005. A further three measurements taken once the SBT were transferred into one pontoon on September 2005 - December 7th 2005, March 30th 2006 and August 15th 2006.

Change in proximate composition

Representative samples of the muscle composition of 5 harvested SBT and of the feed given to the SBT were taken and sent to Weston Food Laboratories for testing of fat, protein, moisture and energy content four times during LTH. A list of the proximate composition sampling dates is provided in Table 1.

Table 1. Dates of the four sampling periods during long term holding of SBT when muscle and feed samples were collected for proximate analyses.

Sampling Period	Date
1	August 2005
2	December 2005
3	March 2006
4	August 2006

Statistical analysis

1) Growth data

Analysis of the data was based on actual tag returns from tags placed into the SBT during initial stocking of pontoons for the *'Formu-bait'* in April 2005. At each harvest period, calculations on growth for both length and weight were based on initial starting points and measurements taken at harvest.

Food conversion ratio (FCR) was determined by tallying the average amount of feed received per pontoon per day. From this figure, average consumption per SBT per day was calculated, through knowledge of the number of SBT in each pontoon. Final FCR figures were derived by dividing the average consumption of an SBT by the total average growth achieved by SBT (determined by tag return data from harvested SBT for the period).

Average feed intake (consumption) was applied even though the pontoon consisted of 2 and 3 yr old SBT, as feed intake between cohorts could not be determined.

Condition index (CI) was calculated as:

$$CI = \frac{Weight(kg)}{Length(m)^3}$$

Total growth and FCR for sampling periods was based on the difference achieved between the mean results of SBT sampled for the periods.

2) Proximate composition data

To determine if SBT composition changed over time, proximate data as well as condition indices were analysed by 1 way ANOVA. Latin Square Design

was used to compare means where a significant difference was detected. All data were tested for normality by Shapiro-Wilk tests and homogeneity of variances by Bartlett's test prior to analysis. The author recognises that a preferred experimental design would involve having pontoons as replicates, and not individual fish within one pontoon. However, owing to the significant value of SBT and cost of conducting experiments with them, the experimental design had to be compromised.

Results are presented in Figures 1-5. It can be seen from Figures 2 and 3 that the cumulative weight gains and lengths and condition indices of the 2 and 3 year old cohorts followed a similar pattern. In addition, Figures 2 and 3 demonstrate that the weight gains and lengths of both cohorts at the end of the LTH were greater than the CSIRO SBT growth rate estimates (Polacheck et al. 2004). However, it can be seen from the combined growth rate data in Figure 4 that the average daily gain (g/day) of the tuna declined around week 12 (July 2005) onwards and did not start increasing until around week 50-51 (March 2006) when the condition index dropped to 21.5. Similarly FCRs increased gradually from week 8 and did not start declining again until weeks 50-51 (Figure 5).
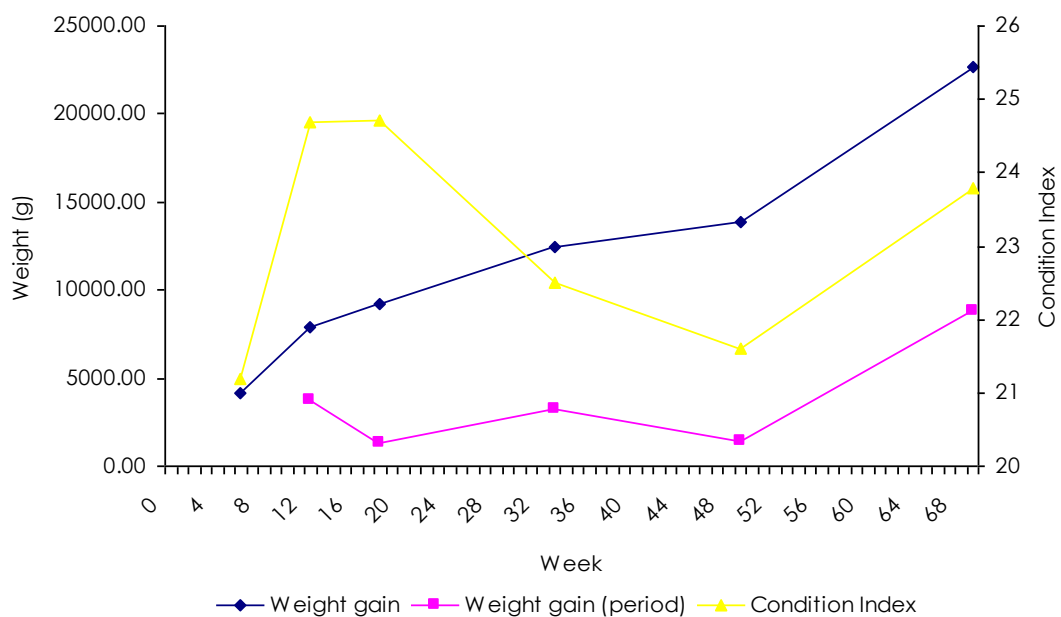
Figure 1. Cumulative weight gain (g), period weight gain (g) and condition index of all LTH SBT.
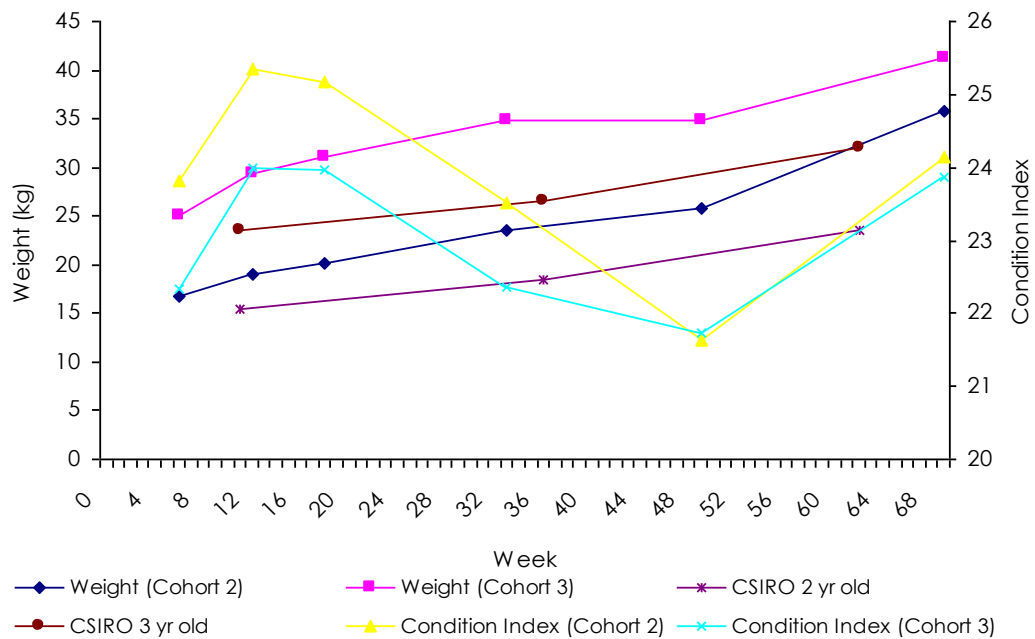
Figure 2. Cumulative weight gain (kg) of 2 year old and 3 year old cohorts of LTH SBT compared with projected weight gains for 2 and 3 year old wild SBT calculated by CSIRO.
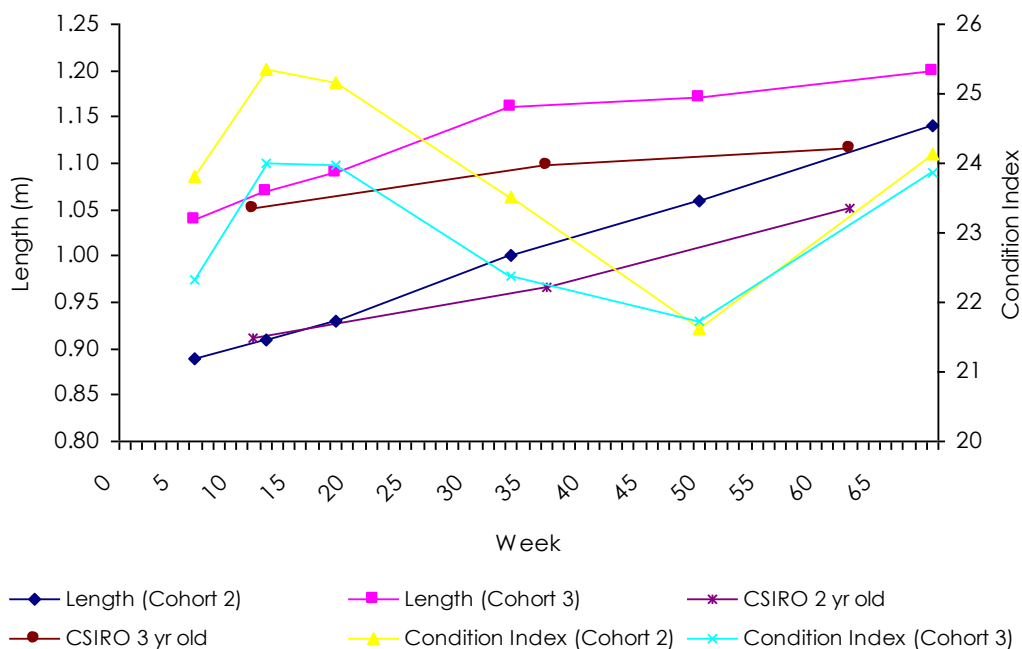
Figure 3. Cumulative lengths (m) of 2 year old and 3 year old cohorts of LTH SBT compared with projected lengths for 2 year old and 3 year old SBT calculated by CSIRO (Polacheck et al. 2004).

8.1 Production – Nutrition & Growth
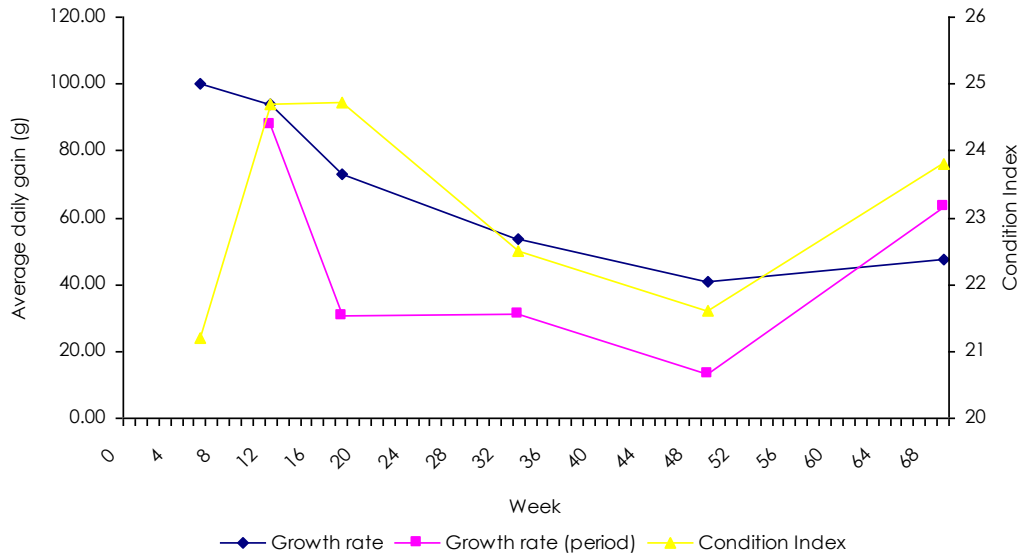
Figure 4. Growth rate (g/d) and growth rate per period (g) of all LTH SBT.
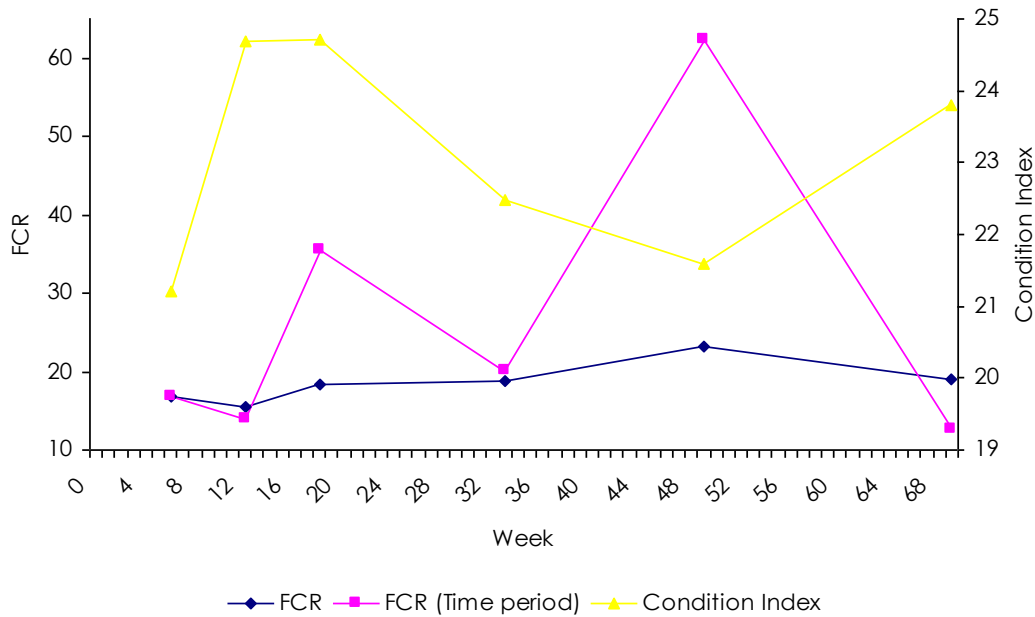
Figure 5. Cumulative feed conversion ratio (FCR) and FCR per period for LTH SBT.

A significant difference among sampling times was detected for all proximates measured and for condition index ($p < 0.05$). The fat and energy content of SBT decreased and reached their lowest values in the third sampling period in March 2006 (Figures 6 and 7).

Both the fat and energy content of SBT during this time were significantly lower than for any other sampling time. The levels of both fat and energy increased by the fourth sampling period (August 06) with energy returning to a level that was not significantly different from the first sampling time and fat returning to a level that was not significantly different from the second sampling time (December 05). The ash content of SBT also decreased significantly after the first sampling period and then remained constant, with no difference in ash content of SBT found between sampling periods 2, 3 and 4 (Figure 8). In contrast to the fat and energy content of SBT that decreased and then increased again by the fourth sampling period, protein and moisture increased and then decreased by the fourth sampling period (Figures 9 and 10). The protein content of SBT was significantly higher in sampling periods 2, 3 and 4 than in the first sampling period. The moisture content of SBT was significantly higher in sampling periods 2 and 3 than in sampling period 1, but by the fourth sampling period moisture levels had returned to a level not significantly different from the first sampling period.
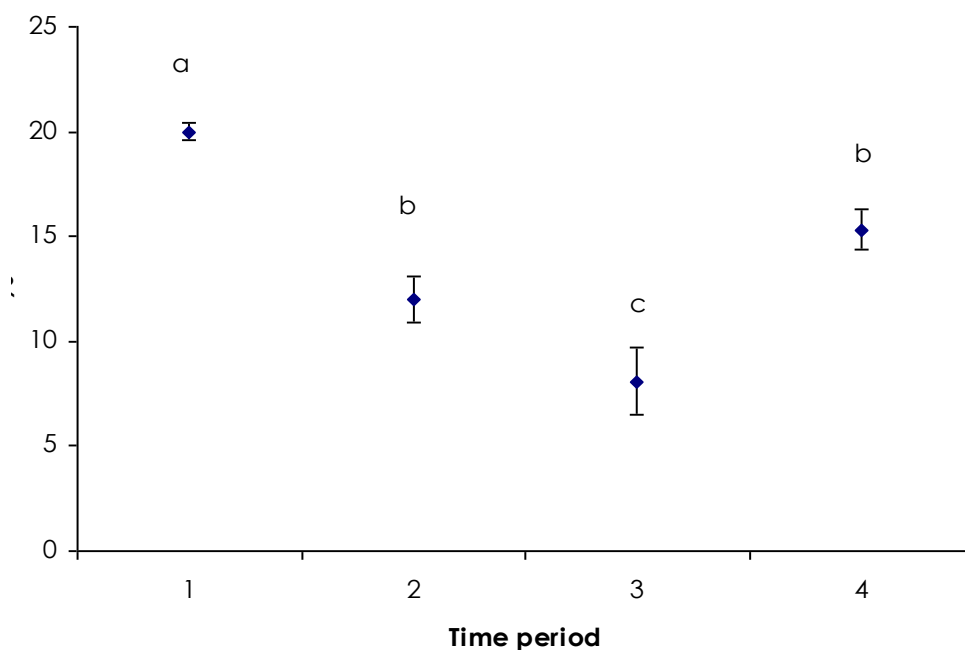
Figure 6. **Fat content of SBT muscle sampled at different times during LTH. Sampling times with different letters indicate significantly different muscle fat content.**
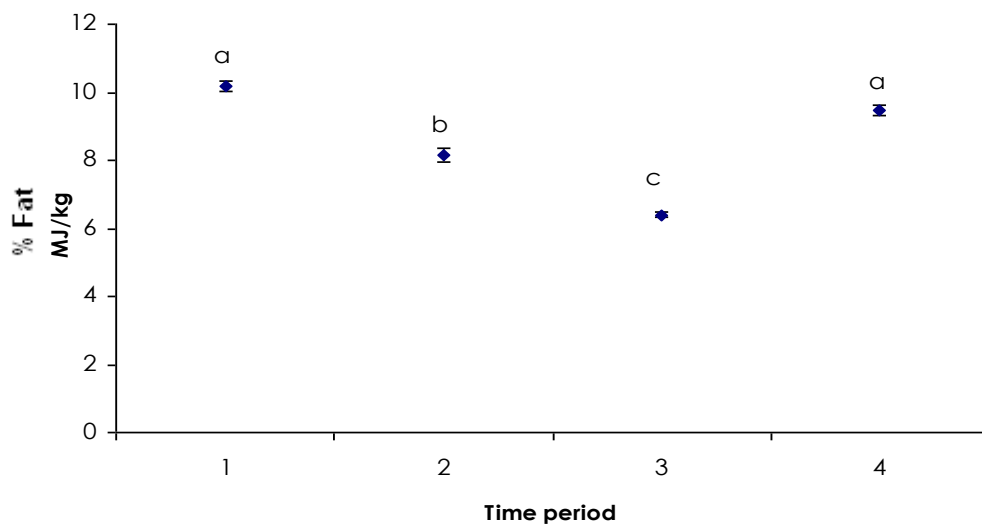
Figure 7. Energy content of SBT muscle sampled different times during LTH. Sampling times with different letters indicate significantly different muscle energy content.
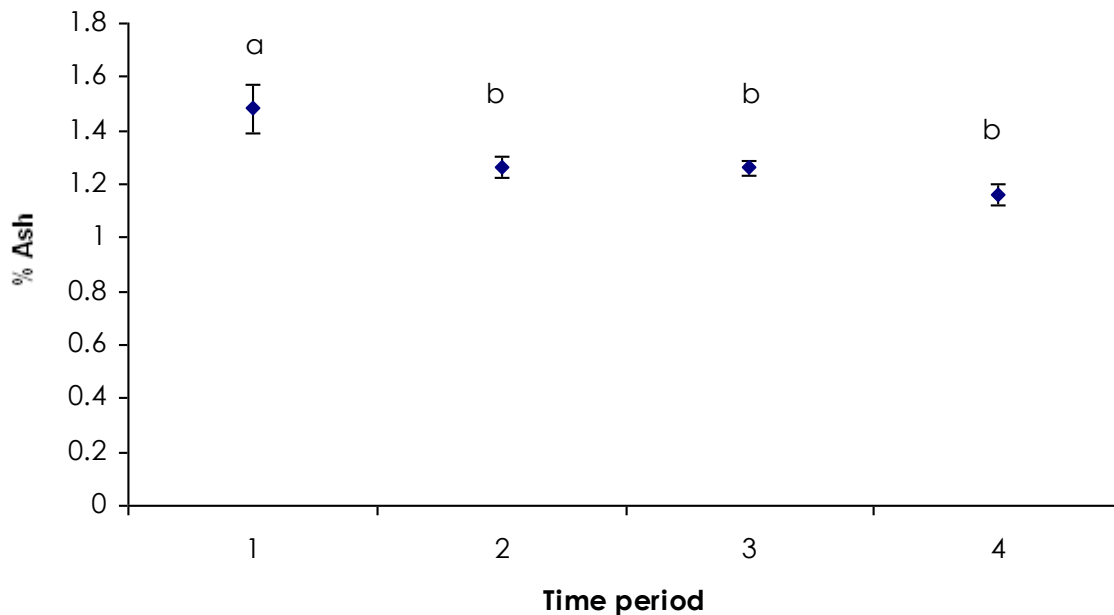
Figure 8. Ash content of SBT muscle sampled different times during long term holding. Sampling times with different letters indicate significantly different muscle ash content.

8.1 Production – Nutrition & Growth
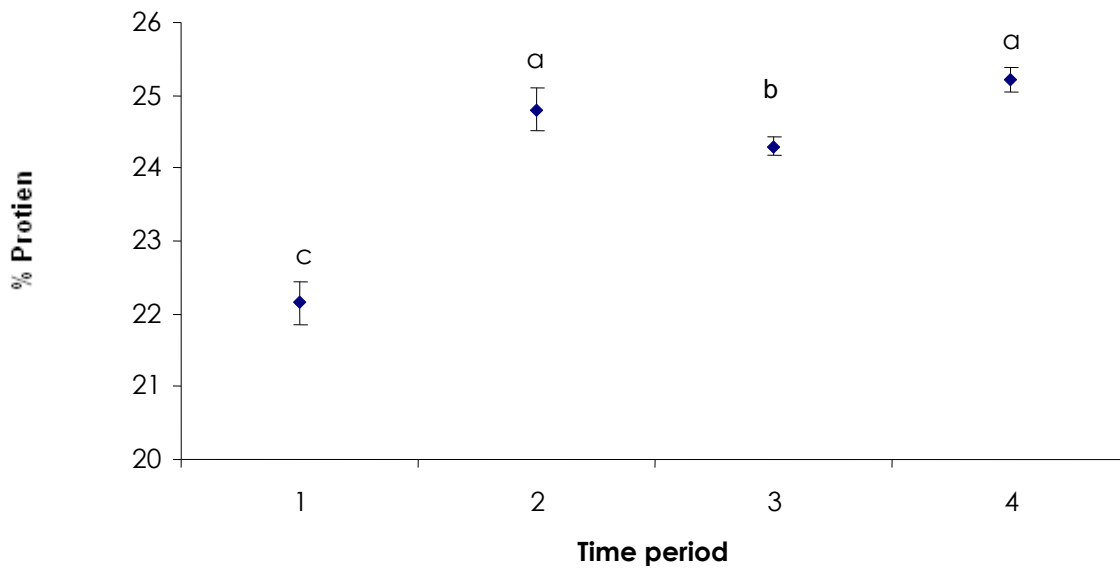
Figure 9. Protein content of SBT muscle sampled at different times during long term holding. Sampling times with different letters indicate significantly different muscle protein content.
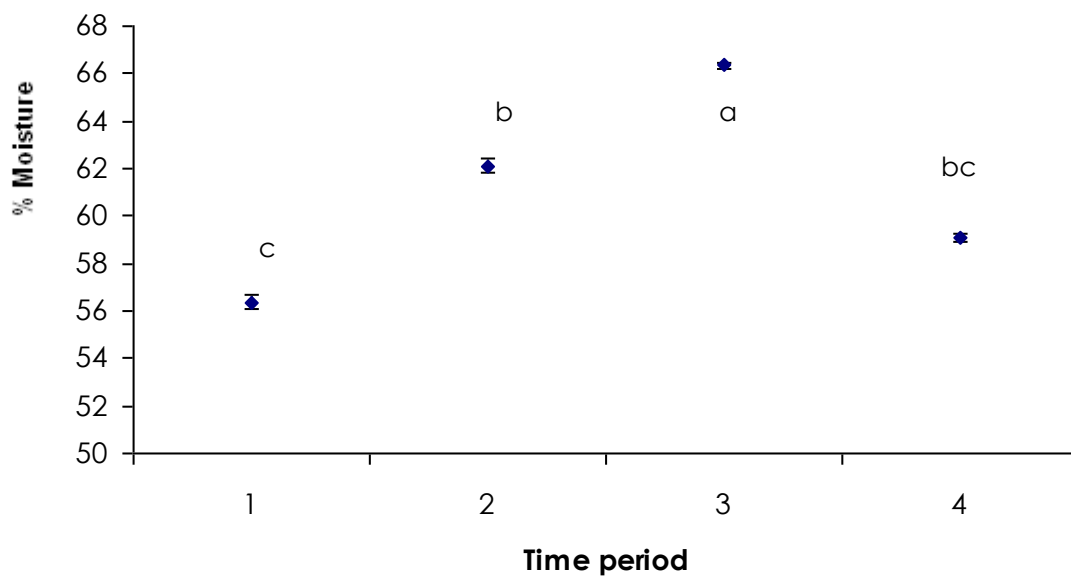
Figure 9. Moisture content of SBT muscle sampled different times during long term holding. Sampling times with different letters indicate significantly different muscle moisture content.

The LTH results have proved extremely useful. While SBT continue to grow over the entire period (as can be seen from the cumulative growth data), daily weight gains and FCR are significantly compromised until the condition index of the SBT falls below 21.5. This condition index level appears critical if we separate out the weight gains and lengths of individual cohorts of fish in the pool – regardless of upper condition index achieved; feeding intensity was not renewed until condition index reached 21.5.

Based on this data, it is clear that the cost-effectiveness of LTH will be strongly dependent on the feeding regime adopted until the condition index returns to 21.5. It is also clear that further enhancement of feeding efficiency in SBT may be driven by an understanding of the factors that influence feed intake and growth when SBT have significant body reserves of fat or are lean relative to their length.

Adiposity is thought to exert a negative feedback control on feed intake in SBT. Such an inhibitory effect of elevated body fat on feed intake in fish has been reported in studies by Metcalfe and Thorpe (1992), Jobling and Miglavs (1993), Shearer et al. (1997), Jobling and Johansen (1999), Silverstein et al. (1999), Silverstein and Plisetskaya (2000), Johansen et al. (2002, 2003).

Studies on Atlantic salmon have investigated whether feed intake is under lipostatic regulation by producing high fat and low fat fish by feeding diets with different fat concentrations and thereafter observing the intake of the two groups when offered high and low fat feeds (Johansen et al., 2002, 2003). Johansen et al. (2002) found that salmon had a general preference for leaner feeds irrespective of adiposity level, but leaner fish consumed more feed, grew faster and deposited more body fat than their fatter counterparts. Over time, body compositions were found to converge among treatments, and differences in feed intake ablated, indicating lipostatic regulation of feed intake.

In a similar subsequent study by Johansen et al. (2003) with larger salmon (740g vs 165g), fat fish consumed 30% less feed than lean fish, which resulted in corresponding differences in growth. However, the differences in adiposity seen at the end of the preconditioning phase were still evident at the

trial end. Groups of fish, which were preconditioned with the same feed during the build-up phase, had similar feed consumption and growth to each other during the experimental phase, indicating that body fat was playing an important role in the regulation of feed intake.

Given that the feeding intensity of SBT in this study was not renewed until their condition index dropped to 21.5 it appears that, as for salmon, feed intake in tuna is under lipostatic regulation. This theory is also in agreement with the results obtained in Experiment 2.1b which investigated relative SBT growth rates and feed intake patterns over the course of a ranching season when fed bait fish diets containing varying combinations of fat and protein. In this experiment relative intake by SBT dropped when the diets were high in fat. According to the model of lipostatic regulation of feed intake, feed consumption of an animal will be negatively correlated to its amount of adipose tissue (Kennedy, 1953; Loftus, 1999; Woods and Seeley, 2000).

If this is the case then there is little opportunity to encourage SBT to increase feed intake when they have significant body reserves of fat or are lean (relative to their length). In the study by Johansen et al. (2003) feed intake of the fat fish was suppressed to such an extent, irrespective of whether they were fed high or low fat diets, that growth became impaired. Other studies have also reported growth impairment in fish with increased adiposity (Regost et al., 2001; Jobling et al., 2002; Johansen et al., 2002). This may explain the significantly compromised growth rates of SBT with a condition index above 21.5 in this study.

Similar condition indices to those recorded in our study have been reported for captured and conditioned Atlantic bluefin tuna, *Thunnus thynnus*. *T. thynnus* that had been held for 5 months and fattened on bait fish had a mean condition index of 19.9 and maximum of 27.5 (Aguado-Giménez and García García, 2005). Tičina et al. (2007) reported slightly higher mean and maximum condition indices of 23.3 and 33.1, respectively, for *T. thynnus* that had been held in cages and fattened for 511 days on bait fish. However, the fish were much smaller than in the study by Aguado-Giménez and García García (2005) with an average initial weight of 6.4kg and final weights ranging from 20 to 43kg as compared to an average final weight of 167kg in the study

by Aguado-Giménez and García García (2005). The SBT in our study, which were held and fed bait fish for 478 days were closer in size to those in the study by Tičina et al. (2007) with a mean initial weight of 19kg and mean final weights ranging from 27-47kg. At the end of the 478 days they had an average condition index of 23.8 and a maximum of 26.4. When comparing between studies, not only should genetic differences between species be taken into account, but also that SBT in our study were handled during tagging, causing stress. Consequently, growth rates and condition indices may not be as high as what they would have been had the fish not been handled, as was the case in the studies by Aguado-Giménez and García García (2005) and Tičina et al. (2007). This is evident in the study by Tičina et al. (2007) who looked at the specific and relative growth rates of a group of tagged fish. They recorded negative specific growth rates and thermal-unit growth coefficients for 19 out of 20 *T. thynnus* that were harvested 44-53 days after tagging and concluded tagging related stress may have significantly adverse effects on bluefin tuna condition and growth performance.

The specific growth rate recorded in our study for 2-3 yr old SBT held for 478 days (0.24%/day) is the same as what was reported by Katavic et al. (2003). In their study small *T. thynnus* with an average weight of 12kg reached approximately 45kg after a 540 day ranching period, corresponding to a specific growth rate of 0.245%. Tičina et al. (2007) reported a slightly lower specific growth rate for 2 year old *T. thynnus* that were tagged and held for between 572 and 597 days (0.20%/day), although they recorded a slightly higher specific growth rate for 1 year old *T. thynnus* that were tagged and held for between 507 and 526 days (0.28%/day). Tičina et al. (2007) also reported that small *T. thynnus* (average initial weight of 5kg), not disturbed by tagging achieved even higher specific growth rates (0.298–0.332%/day) after 511 days of farming (final weights of 25-30kg). Thus it appears that both tagging and tuna size affects specific growth rates. In addition, conditioning period has also been observed to have an effect. Based on tag recapture data Tičina et al. (2007) found that 2 year old *T. thynnus* held for 171-190 days achieved a specific growth rate of 0.31%/day as compared to 0.20%/day recorded for 2 year old fish held for between 572-597 days. It should be taken into account

that first specific growth rates related mostly to growth during the summer months whilst the second lower specific growth rate related to combined winter and summer growth. According to Cort (2003) summer growth of 1-3 year old SBT in the wild is on average five to six times higher than their winter growth. In comparison to ranched tuna, the maximum condition index reported for wild *T. thynnus* by Aguado-Giménez and García García (2005) was 21.5 with a mean of 16.5. Similarly, Tičina et al. (2007) reported a maximum of 22.5 and mean of 19.5 for wild tuna of the same species. Aguado-Giménez and García García, (2005) found that the difference in condition index between wild and ranched bluefin tuna was more pronounced when fork length was greater than 180cm. The younger ranched specimens showed similar or slightly higher condition indices to wild bluefin tuna. Below 180cm, ranched bluefin tuna were not as overweight as the larger specimens. The high growth and metabolic rate of smaller tuna mean that they may devote most of the energy input to maintaining standard requirements (Brill, 1987).

Katavic et al. (2002) compared the length–weight relationship of small ranched bluefin tuna, *T. thynnus* (60–160cm fork length), with data of wild bluefin tuna and found that differences appeared only above 110cm fork length. This information suggests that the compromised growth rates reported with LTH of SBT in this study might not have occurred if smaller fish were used owing to their higher growth and metabolic rates which would prevent them from depositing fat as easily as larger fish. However, the high condition indices reported for small fish in the study by Tičina et al. (2007) that were only 20 to 43kg in weight at the end of the 511 days and had an average condition index of 23.3 seems to dispel this theory and suggests that high condition indices, at least much higher than found in wild stock of the same size, can be obtained with small fish.

The implication of this study for LTH is that if feed intake is under lipostatic regulation then feeding rates of SBT will be governed by their condition index and there is really no opportunity to try and make them eat more to improve growth rates when their condition index is above 21.5. Thus the best management tool that can be suggested is to try and improve FCRs by

reducing the amount of feed offered, providing ranched SBT with only what will be consumed, as a result feed wastage will also diminish.

The amount they should be fed to ensure optimum FCRs will depend on how high their condition index is – the fatter the SBT, the greater the down regulation of their feed intake and thus the less feed that should be provided to prevent feed wastage. Increasing the amount of feed provided when the FCR is high will not encourage SBT to eat more regardless of whether the feed is reduced in fat content. This has been demonstrated in the study by Johansen et al. (2003), which found that Atlantic salmon reduced their feed intake to such an extent that growth was impaired. Feed intake of the fat salmon did not differ, regardless of whether they were offered low fat or high fat food. There was also no difference in specific growth rates or final body weights between fat salmon fed the high fat food and fat salmon fed the low fat food for 7 weeks, although the fat salmon fed the low fat food had a significantly better FCR (Johansen et al., 2003).

There is potential to exploit feed intake in SBT through genetic manipulation and selective breeding programs, should captive breeding of SBT eventuate, or through manipulation of hormones that control feed intake as is being experimented with in humans. Hormones that have been found to be involved in regulating feed intake in mammals include orexins, ghrelin and leptin (Arora and Anubhuti, 2006). Ghrelin is produced in cells lining the human stomach that stimulate appetite. Levels of ghrelin increased before a meal and decreased after it. It is considered the counterpart of the hormone leptin, produced by adipose tissue, which induces satiation when present at high levels. Orexins, also called hypocretins, are a pair of excitatory neuropeptide hormones produced by a small population of cells in the lateral and posterior hypothalamus. The orexin system has been shown to be involved in the stimulation of food intake based on the finding that central administration of orexin A/hypcretin-1 increases food intake (Sakurai, 2006). Hypocretin producing cells have been shown to be inhibited by leptin (by leptin receptors) but are activated by ghrelin.

Recently, intracerebroventricular (ICV) administration of ghrelin, orexin and neuropeptide Y (NPY) has been shown to stimulate food intake in goldfish

suggesting that similar mechanisms involving central and peripheral ghrelin signals acting through central orexin and NPY may be present as in rats (Miura et al., 2007).

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8.2 Production – Residue

Effect of longer term holding on residue and contaminant levels in cultured southern bluefin tuna (*Thunnus maccoyii*)

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Abstract

Ranched southern bluefin tuna (SBT) were cultured across two grow-out seasons, a total of 16 months over 2005 and 2006. A total of 50 tuna were sampled from seven harvests and analysed for residues and contaminants. Residues and contaminants were selected to meet both regulatory and trade access requirements and included metals, metalloids, dioxins, PCBs and pesticides.

The extended culture period did not lead to violative levels of residues or contaminants. Overall, there were measurable levels found of the pesticide DDT which showed a seasonal accumulation trend reflecting the fat content of the fish sampled. A similar pattern of accumulation was demonstrated for dioxins and PCBs. An inverse relationship was observed between fat content and mercury concentration.

Introduction

The southern bluefin tuna (SBT) industry has been appraising the possibility of developing long term holding (LTH) strategies for cultured fish in Port Lincoln. This would give the industry flexibility to supply overseas markets at different times of the year and take advantage of supply shortfalls from elsewhere in the global market. As part of this work the industry needed to better understand the impact of this strategy on accumulation of residues and contaminants in harvested product.

Nutritional properties of bait fish (e.g. fat, amino acid profiles) have been characterised for nearly two dozen species of bait fish (Jeff Buchanan/David Ellis pers. comm.) and the information has been compiled as part of the development of the *'Formu-bait'* feeding decision support tool. However, the characterisation of residue and contaminants in wild-caught bait fish is less well advanced.

The only major work on the levels of residues in bait fish used in Port Lincoln was completed in 2004 (Padula et al., 2004a). A range of bait fish species from different geographical locations including sardines (Australia, South Africa, Oregon, California and Morocco), herring, mackerel, redbait and squid were analysed during 2002 and 2003. The types of residues for which the bait fish were tested included; metals, metalloids, dioxins (PCDD/F), polychlorinated biphenyls (PCBs) and pesticides (OC/OP).

Previous research (Padula et al., 2004a) identified substantial variation in the presence and levels of residues in bait fish of the same species and from varying geographical locations. For example, the DDT metabolite, p,p DDE, was only regularly found in bait fish sourced from the west coast of North America. Analysis for dioxins and PCBs was only performed on eight samples, with Californian bait fish having the greatest concentrations. The report noted the need for longitudinal surveys of residue levels in bait fish to determine trends, taking into account geographical variations.

A qualitative assessment for the potential bioaccumulation of residues in ranched SBT from bait fish was made by Padula et al. (2004b). Bioaccumulation was shown to occur for a number of residues including total DDT (in the form of p,p DDE), arsenic, dioxin and PCBs. Mercury levels were not statistically different between the wild and ranched SBT. It was noted that the relationship between the residue levels in bait fish and the final harvested SBT was not clear. SBT harvested from individual pontoons with known feed histories would be needed in order to develop predictive models for residue levels. Additionally, the need for on-going testing of feeds and harvested SBT would be necessary to fulfil national and international requirements for traceability of market product.

A follow up survey of residues in ranched SBT across the whole-of-industry in 2004 (Padula et al., 2005) highlighted large differences in levels between SBT companies, especially for fat-soluble residues such as PCBs and dioxin. This study highlighted the importance of feeding practices on the residue levels in ranched SBT. The National Dioxins program noted that tuna ranchers should consider bait fish sources in order to minimise the amount of dioxin and PCBs entering the food chain.

A shortcoming of these earlier studies was the lack of information on the effect of culture, time and diet in the bioaccumulation of residues. Additionally, there was no information on transfer dates, weights at transfer, diet histories (species and quantity fed) or the representativeness of the ranched SBT provided.

To overcome these limitations an interdisciplinary project was initiated to investigate the effect of these factors on the bioaccumulation of residues in farmed SBT: Aquafin CRC - Southern Bluefin Tuna Aquaculture Subprogram: Longer term holding of southern bluefin tuna (*Thunnus maccoyii*).

Residue subproject objectives of LTH study
1. Investigate the residue (Hg, dioxin, PCBs etc) changes (concentration and body burden) that occur in SBT muscle because of LTH.

2. Sample and test bait fish to establish residue levels in the feed fed to the SBT used in the trial.

3. Extend the single grow-out season predictive model (presently under development by Aquafin CRC in Project 2004/306 using the 2005 research data) to address ranching including a second season (i.e. LTH).

4. Extend the database for two Aquafin CRC postgraduate students working on related projects, that is studying the mercury content of SBT muscle and internal organs (Sita Balshaw), and dioxin and PCB content (Samuel Phua).

5. Using the data collected on mercury and dioxins etc, evaluate the effect of LTH against regulatory MRLs and tolerable intakes and use this data for optimal positioning of the industry sector in relation to SBT product marketing.

6. Determine and characterise the interactions associated with SBT health (parasites etc) and residue levels.

Overall project objectives in relation to residues in long term holding study

1. Define the residue (Hg, dioxins, PCBs etc) changes (concentration and body burden) that occur in SBT muscle as a result of LTH and use this along with sampling and testing for residues of bait fish used for the SBT feed, to extend the single grow-out season predictive model to also address grow-out over a second season (i.e. LTH).

2. Extend the database for two Aquafin CRC postgraduate students working on related projects, that is studying the mercury content of SBT muscle and internal SBT organs, and dioxin and PCB content.

3. Using the data collected on mercury and dioxins etc, determine the effect of LTH against regulatory MRLs and tolerable intakes, and use this data for optimal positioning of the industry sector in relation to SBT product marketing.

4. Determine and characterise the interactions between SBT health (parasites etc) and residue levels.

Methodology

Feeding Design

The experimental design incorporated the feeding of a mix of Australian and imported wild caught bait fish species. The nutritional profile of these bait fish is summarised in Table 1.

Table 1. Nutritional profiles of bait fish species diet fed during long term holding.

Common name	Species	Code [2]	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Gross Energy (MJ/kg)
Californian Sardine	*Sardinops sagax*	CS	16.35	63.80	14.87	2.97	8.63
Redbait	*Emmilichthys nitidus nitidus*	RB	19.80	72.30	2.50	5.70	4.29
Australian Sardine	*Sardinops neophilchardis*	AS	20.35	73.80	2.00	4.00	4.96
Australian Sardine[1]	*Sardinops neophilchardis*	AS	20.40	70.80	4.30	3.60	5.21
Indonesian Herring	*Sardinella lemuru*	IH	18.5	69	7.4	4.9	5.74
Blue Mackerel	*Scomber australasicus*	AM	19	67.6	10.2	2.5	5.86

[1]Frozen product from the east coast of Australia;

[2]The two letter code used for figures in the results section

Prior to the LTH study, all fish were part of a nutrition study (refer section 7.1). Four sea-cages were stocked with SBT and fed on diets with variable nutrition content. These diets are summarised in Tables 2 and 3.

Table 2. Experimental treatments and ratios of bait fish used during the initial 18 week trial

Treatment	Protein (%)	Fat (%)	Percentage of feed offered		
			Sardinops sagax	*Emmelichthys nitidus nitidus*	*Sardinops neoSardineus*
High Protein/Low Fat	19.5	4.0	15%	30%	55%
Medium Protein/Medium Fat	18.5	7.3	40%	35%	25%
Low Protein/High Fat	17.5	10.5	65%	35%	0%
Australian Sardine	0%	0%	0%	0%	100%

Table 3. Feeding regime for the initial 18 week feeding trial

Sea-cage	Weeks 1-6	Weeks 7-12	Weeks 13-18
1	Medium protein/Medium fat	Medium protein/Medium fat	Medium protein/Medium fat
2	Low protein/High fat	Medium protein/Medium fat	High fat/Low protein
3	High fat/Low protein	Medium protein/Medium fat	Low protein/High fat
4	Australian Sardine	Australian Sardine	Australian Sardine

At the completion of the initial 18 week study all remaining SBT were pooled into one pontoon.

Harvest of southern bluefin tuna from the long term holding study

SBT were harvested at weeks 0, 6, 12, 18, 36, 52, 76 and were provided either frozen or fresh chilled in commercial tuna cartons as per export bound product.

Sampling methodology

SBT were processed using a modified version of the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) approach. In addition, some SBT were sampled following the approach of the Japanese Ministry of Health, Labour and Welfare (MHLW) using individual filleted cuts for testing as has been implemented under the new Japanese Positive List System of MRLs (refer picture 1.)
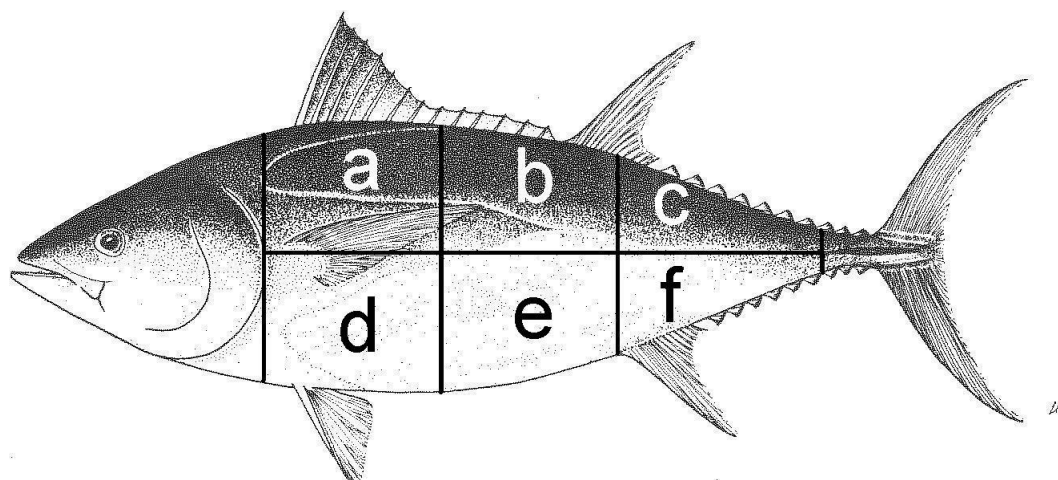
Picture 1. Diagrammatic representation of the Japanese MAFF sampling method for Bluefin Tunas.

Dioxin (PCDD/F) and PCB analysis – HRGC/HRMS

HRGC/HRMS analysis was used for the measurement of dioxin (PCDD/F) and PCBs in all samples. Internationally recognised published methods were used for the extraction, clean up and analysed analysis including USEPA 1613B for PCDD/F and USEPA 1688A for PCBs.

Testing for PCBs included the non-ortho PCBs: 77, 81, 126, 169 and mono-ortho PCBs: 105, 114, 118, 123, 156, 157, 167, 189. In addition, a group of indicator PCBs were measured: 1, 3, 4, 15, 19, 28, 37, 44, 49, 52, 54, 70, 74, 99, 101, 104, 110, 138, 153, 155, 170, 180, 183, 187, 188, 194, 196, 199, 202, 205, 206, 208, 209. Total PCB concentration was determined by the summation of the individual concentrations of all these PCB congeners with

non-detects treated as being equal to the LOD i.e. upper-bound values reported as per the European Commission requirements (EC No 2375/2001).

Sample Preparation

The sample was blended with sodium sulphate and was then loaded into a Soxhlet extractor, fortified with a labelled internal standard and extracted with organic solvent (methylene chloride:hexane 1:1) for 16 hours. A clean-up and recovery standard was then added and the extract was evaporated to constant weight and the lipid weight determined gravimetrically.

Clean-up was affected by solid phase clean-up techniques using acid and base modified silica gel. The extract was then purified with column chromatography techniques using activated alumina and carbon.

The final extract was concentrated and fortified with recovery standards and analysed by HRGC-HRMS.

PCB and Dioxin Analysis

Analysis was carried out on a Micromass Autospec Ultima High Resolution Mass Spectrometer (HR-MS) instrumentation interfaced to an Agilent 6890 chromatograph operating in the splitless mode and equipped with Zebron ZB-5 capillary columns for PCB and PCDD/PCDF analysis. Confirmatory analysis of the 2,3,7,8-TCDF and 2,3,7,8-TCDD congeners was performed on Supelco SP-2331 capillary columns.

HRMS analysis was carried out in the electron impact mode. Native and labelled compounds were acquired by Selected Ion Monitoring (SIM) with the mass resolution being maintained at 10,000 (10% valley) throughout the analysis.

Chromatographic data was processed using a Waters QUANLYNX™ (V4.0) software package. Levels of target analytes were determined via the isotope dilution technique. All data was corrected for recoveries.

Mammalian WHO TEF values were used for the calculation of TEQ's reported in this study (van den Berg et al 1998). Non-detects were treated as equal to the Limit of Detection (LOD) following EU requirements for reporting of upperbound results (EC No 2375/2001).

Metals analysis

Determination of trace elements was performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

A subsample (~1g) of the original sample provided (approximately 150g) was digested with re-distilled nitric acid. After making up to appropriate volume the digest was analysed for trace elements using ICP-MS and or ICP-AES.

The analysis was performed on an ICPMS: Elan 6100DRC (Perkin Elmer), ICPAES: Vista Pro (Varian) and High Resolution ICPMS: Element (Finnigan). This followed published methods of USEPA 6010, 6020, AOAC, 16th Edition, Method 986.15, 974.14.

Pesticide analysis by GC-MS

The analysis of organochlorines, organophosphates, fungicides, herbicides, synthetic pyrethroids and carbamates in the samples was performed via serial extraction with acetone/hexane then concentrated. Extracts were cleaned up with Gel Permeation Chromatography (GPC).

Analysis on the extracts was performed using an HP-5973 Gas Chromatograph Mass Spectrometer (GC-MS) with Programmed Temperature Vaporizing (PTV) injector.

The extraction followed method USEPA3510 with the analysis of the extract following USFDA, Pesticide Analytical Manual Volume 1 (PAM) 3rd Edition, 1996.

Lipid Analysis

Total lipid was determined by soxhlet extraction. An appropriate amount of sample, between 1.5g and 5g, dependent on the expected fat content and homogeneity of the matrix, was weighed to 0.1mg, into a pre-dried soxhlet fat determination thimble.

The thimble was placed into a soxhlet apparatus and extracted for at least 16 hours using diethyl ether on a water bath at approximately 90°C.

The extracted material was collected into a flask pre-weighed to 0.1mg. After collection for 16 hours the flask was dried in a convection oven at 102°C for two hours. Once removed from the oven, the flask was allowed to cool in a desiccator to room temperature. The flask was then re-weighed and returned to the oven where it underwent the re-weighing process until no further weight loss occurred between successive dryings.

The fat content of the sample was calculated by subtracting the final flask weight from the original flask weight and dividing this figure by the original sample mass (multiply by 100 to obtain the percentage value or g/100g).

Table 5. Accredited laboratories that performed testing for this study

Analysis performed	Laboratory
Metals and metalloids	AgriQuality, Auckland, New Zealand
Pesticides	AgriQuality, Wellington, New Zealand
Moisture content	AgriQuality, Wellington, New Zealand
Total fat content	AgriQuality, Wellington, New Zealand
Dioxins & PCBs	AgriQuality, Wellington, New Zealand

Tables 6-8 summarise residue testing performed for bait fish and SBT including dioxins, PCBs, pesticides, metals and metalloids.

Table 6. Summary of metals and metalloid analyses with Limit of Reporting (LOR) values.

Metals & Metalloids	Limit of Reporting (mg/kg)
Antimony	0.01
Arsenic	0.1
Cadmium	0.001
Chromium	0.1
Copper	0.1
Lead	0.01
Mercury	0.01
Selenium	0.1
Tin	0.03
Zinc	0.1

Table 7. Summary of dioxins and furan analyses by congener.

Chlorinated dibenzo-p-dioxins	Chlorinated dibenzofurans
2,3,7,8-TetaCDD	2,3,7,8-TetraCDF
1,2,3,7,8-PentaCDD	1,2,3,7,8-PentaCDF
1,2,3,4,7,8-HexaCDD	2,3,4,7,8-PentaCDF
1,2,3,6,7,8-HexaCDD	1,2,3,4,7,8-HexaCDF
1,2,3,7,8,9-HexaCDD	1,2,3,6,7,8-HexaCDF
1,2,3,4,6,7,8-HeptaCDD	1,2,3,7,8,9-HexaCDF
OctaCDD	2,3,4,6,7,8-HexaCDF
	1,2,3,4,6,7,8-HeptaCDF
	1,2,3,4,7,8,9-HeptaCDF
	OctaCDF

PCB analyses

Targeted PCB congeners included the regulatory significant non-ortho PCBs: 77, 81, 126, 169 and the mono-ortho PCBs 105, 114, 118, 123, 156, 157, 167, 189. In addition a group of indicator PCBs: 1, 3, 4, 15, 19, 28, 37, 44, 49, 52, 54, 70, 74, 99, 101, 104, 110, 138, 153, 155, 170, 180, 183, 187, 188, 194, 196, 199, 202, 205, 206, 208, 209.

Table 8. Summary of pesticide analyses with Limit of Reporting (LOR) values.

Compound	LOR	Compound	LOR	Compound	LOR	Compound	LOR
Acephate	0.05	DDT (*p,p*)	0.01	Flutriafol	0.01	Phosmet	0.01
Acetochlor	0.01	Deltamethrin	0.01	Fluvalinate	0.02	Phosphamidon	0.01
Alachlor	0.01	Demeton-s-methyl	0.01	Folpet	0.02	Piperonyl butoxide	0.01
Aldrin	0.01	Diazinon	0.01	Furalaxyl	0.01	Pirimicarb	0.01
Alpha-cypermethrin	0.01	Dichlobenil	0.01	Furathiocarb	0.01	Pirimiphos-methyl	0.01
Atrazine	0.01	Dichlofenthion	0.01	Haloxyfop-etotyl	0.01	Prochloraz	0.05
Azaconazole	0.01	Dichlofluanid	0.01	Haloxyfop-methyl	0.01	Procymidone	0.01
Azinphos methyl	0.01	Dichlorvos	0.01	Heptachlor	0.01	Prometryn	0.01
Azoxystrobin	0.01	Dicloran	0.01	Heptachlor-epoxide	0.01	Propachlor	0.01
Benalaxyl	0.01	Dicofol	0.01	Hexachlorobenzene	0.01	Propargite	0.01
Bendiocarb	0.01	Dicrotophos	0.01	Heptenophos	0.01	Propazine	0.01
Benodanil	0.01	Dieldrin	0.01	Hexaconazole	0.01	Propetamphos	0.01
BHC (alpha)	0.01	Difenoconazole	0.01	Hexazinone	0.01	Propham	0.01
BHC (beta)	0.01	Diflufenican	0.01	Indoxacarb	0.01	Propiconazole	0.01
Bifenthrin	0.01	Dimethanamid	0.01	Iodofenphos	0.01	Propoxur	0.01
Binapacryl	0.01	Dimethoate	0.01	Iprodione	0.01	Propyzamide	0.01
Bioresmethrin	0.02	Dimethomorph	0.01	Isofenphos	0.01	Prothiofos	0.01
Bitertanol	0.01	Diphenamid	0.01	Karbitulate	0.05	Pyrazophos	0.01

Compound	LOR	Compound	LOR	Compound	LOR	Compound	LOR
Bromacil	0.01	Diphenylamine	0.01	Kresoxim-methyl	0.01	Pyrimethanil	0.01
Bromophos ethyl	0.01	Disulfoton	0.01	Lindane	0.01	Pyriproxyfen	0.01
Bromophos methyl	0.01	Endosulfan I	0.01	Linuron	0.01	Quintozene	0.01
Bromopropylate	0.01	Endosulfan II	0.01	Malathion	0.01	Quizaolofop-ethyl	0.01
Bupirimate	0.01	Endosulfan sulphate	0.01	Metalaxyl	0.01	Sethoxydim	0.05
Buprofezin	0.01	Endrin	0.01	Methacriphos	0.01	Simazine	0.01
Captan	0.01	EPN	0.01	Methidathion	0.01	Tau-fluvalinate	0.01
Carbaryl	0.01	Epoxyconazole	0.01	Methiocarb	0.01	Tebuconazole	0.01
Carbofuran	0.01	EPTC	0.01	Metolachlor	0.01	Tebufenpyrad	0.01
Carboxin	0.01	Esfenvalerate	0.01	Metribuzin	0.01	Terbacil	0.01
Chlordane-cis	0.01	Ethiofencarb	0.01	Mevinphos	0.01	Terbufos	0.01
Chlordane-trans	0.01	Ethion	0.01	Monocrotophos	0.01	Terbufos sulfone	0.1
Chlorfenvinphos	0.01	Ethoxyquin	0.01	Myclobutanil	0.01	Terbumeton	0.01
Chlorfluazuron	0.1	Etridiazole	0.01	Naled	0.05	Terbuthylazine	0.01
Chlornitrofen	0.01	Etrimfos	0.01	Napropamide	0.01	Terbutyrn	0.01
Chlorobenzilate	0.01	Famphur	0.01	Nitrofen	0.01	Tetrachlorvinphos	0.01
Chlorothalonil	0.01	Fenamiphos	0.01	Nitrothal isopropyl	0.01	Tetradifon	0.01
Chlorpropham	0.01	Fenarimol	0.01	Norflurazon	0.01	Thiometon	0.01
Chlorpyrifos	0.0	Fenchlorvos	0.0	Omethoate	0.1	Tolclofos-	0.0

Compound	LOR	Compound	LOR	Compound	LOR	Compound	LOR
	1		1			methyl	1
Chlorpyrifos methyl	0.01	Fenitrothion	0.01	Oryzalin	0.1	Tolyfluanid	0.01
Chlorthal-dimethyl	0.01	Fenoxaprop-ethyl	0.01	Oxadiazon	0.01	Tralkoxydim	0.01
Chlozolinate	0.01	Fenoxycarb	0.02	Oxadixyl	0.01	Triadimeton	0.01
Clomazone	0.01	Fenpiclonil	0.01	Oxamyl	0.1	Triadimenol	0.01
Coumaphos	0.01	Fenpropathrin	0.01	Oxyfluorfen	0.01	Triallate	0.01
Cyanazine	0.01	Fenpropimorph	0.01	Paclobutrazol	0.01	Triazophos	0.01
Cyfluthrin	0.02	Fensulfothion	0.01	Parathion	0.01	Trichlorfon	
Cyhalothrin	0.01	Fenthion	0.01	Parathion-methyl	0.01	as~Dichlorvos	0.05
Cypermethrin	0.03	Fenvalerate	0.01	Penconazole	0.01	Trifloxystrobin	0.01
Cyproconazole	0.01	Fipronil	0.01	Pencycuron	0.02	Trifluralin	0.01
Cyprodinil	0.01	Flamprop-methyl	0.01	Pendimethalin	0.01	Vinclozolin	0.01
DDD (*o,p*)	0.01	Fluazifop-p-butyl	0.01	Permethrin	0.01		
DDD (*p,p*)	0.01	Fluazinam	0.1	Phorate	0.01		
DDE (*o,p*)	0.01	Fludioxonil	0.01	Phorate sulphone	0.05		
DDE (*p,p*)	0.01	Fluometuron	0.01	Phorate sulphoxide	0.05		
DDT (*o,p*)	0.01	Flusilazole	0.01	Phosalone	0.01		

Results and Discussion

Baseline levels of residues and contaminants in wild-caught SBT The commentary for each of these residues and contaminants is discussed in the following pages and graphs. There are few standards set for these residues and contaminants in Japan. Of interest is the seasonality effect of time of sampling which followed the fat content changes. The data overall confirmed that LTH fish sampled at any time throughout the duration of the study fully met Australian and Japanese regulatory standards.

Table 9. Summary of metals, pesticides, dioxins and PCBs concentrations in wild-caught SBT at transfer

Residue	Mean (mg/kg)	Min (mg/kg)	Max (mg/kg)	Standard (mg/kg)
Metals				
Antimony	**<0.01**	**<0.01**	**<0.01**	**Not set**
Arsenic (Total)	**0.79**	**0.67**	**0.92**	**Not set**
Cadmium	**0.0086**	**0.009**	**0.011**	**Not set**
Copper	**0.306**	**0.27**	**0.33**	**2**
Chromium	**<0.1**	**<0.1**	**<0.1**	**Not set**
Lead	**<0.01**	**<0.01**	**<0.01**	**0.5**
Mercury	**0.514**	**0.41**	**0.59**	**1**
Selenium	**0.98**	**0.85**	**1.20**	**2**
Tin	**<0.03**	**<0.03**	**<0.03**	**Not set**
Zinc	**4.36**	**4.1**	**4.8**	**15**
Pesticides				
Aldrin and Dieldrin	**N.D.**[1]	N.D.	N.D.	**0.1**
BHC	N.D.	N.D.	N.D.	**0.01**
Chlordane	N.D.	N.D.	N.D.	**0.05**
DDT	N.D.	N.D.	N.D.	**1**
Hexachlorobenzene	N.D.	N.D.	N.D.	**0.1**
Heptachlor	N.D.	N.D.	N.D.	**0.05**
Lindane	N.D.	N.D.	N.D.	**1**
Polychlorinated biphenyls				
PCBs (upper bound basis)	0.001132	0.000502	0.00171	0.5
Dioxins	Mean	Min	Max	Standard

	(pg-TEQ/g)	(pg-TEQ/g)	(pg-TEQ/g)	(pg-TEQ/g)
Dioxins (upper bound basis)	0.055	0.039	0.074	4
Dioxins and dioxin-like PCBs (upper bound basis)	0.166	0.112	0.227	8

[1] N.D. not detected

Metal and metalloid residues

Arsenic

The arsenic concentration increased over the initial 18 week study period, however decreased during the LTH part of the study. This probably reflects the lower arsenic content in the bait fish species fed during long term holding. It is suggested that the form of arsenic present in SBT is lipophilic (as arsenolipids), suggesting that this element is not present in the toxic inorganic form.

There is no ML/MRL set for arsenic (total) in fish. There is an Australian ML set for the inorganic form of arsenic. This inorganic form of arsenic makes up no more than 5% of the total arsenic content. There is no Japanese MRL for arsenic in fish.

Cadmium

Cadmium does not appear to bio-accumulate with extended culture period in SBT.

Cadmium declined during extended culture period suggesting that growth dilution is the major factor influencing the concentration. There was an apparent increase in March 2006; this may reflect a loss in condition at this harvest point and there is no Australian or Japanese ML/MRL set for cadmium in fish. Cadmium levels in wild caught bait fish were higher in imported species than Australian caught product.

Copper

There appeared to be no bio-accumulation of copper in this study.

Levels of copper remained relatively stable throughout the long term holding study suggesting that the concentration of this element is regulated by the tuna. Chemically, copper is found in the first row of the Transition metals and it is likely that it has an important biological activity.

There is no Australian or Japanese ML/MRL set for copper in fish.

There appears to be no difference between imported or Australian caught bait fish products in terms of copper content.

Chromium

Chromium is an important micronutrient involved in prevention of diabetes in humans. Chromium was first detected in the December 2005 harvest and then again in March 2006. Surprisingly, the concentrations dropped below the detection limit by August 2006. Chemically Chromium is found in the first row of the Transition metals and may therefore have an important biological activity in SBT.

Only some species of bait fish, such as Australian redbait, had measurable levels of chromium present.

There is no Australian or Japanese ML/MRL set for chromium in fish.

Lead

Lead was generally undetected in SBT from the study with the highest concentration being 0.013mg/kg. This is just above the Limit of Detection (LOD) of 0.01mg/kg. The Australian ML for lead in fish is 0.5mg/kg. There is no Japanese MRL set for lead in fish.

In terms of bait fish, Indonesian herring had high levels of lead present, while all other bait fish had very low or undetected levels of lead present. This may reflect the inclusion of skin and bones in the testing matrix.

Mercury

In Japan, all *Thunnus spp.* are exempt from the Food Sanitation Law standard for mercury in fish. In Australia SBT must comply with a total mercury standard of 1mg/kg and bait fish with a total mercury standard of 0.5mg/kg.

Mercury levels declined through time (Figure 1) across the long term holding study reflecting an inverse relationship with fat. Growth dilution is clearly an important factor influencing the concentration. The age class of the SBT is also important to consider when examining mercury data of cultured SBT.
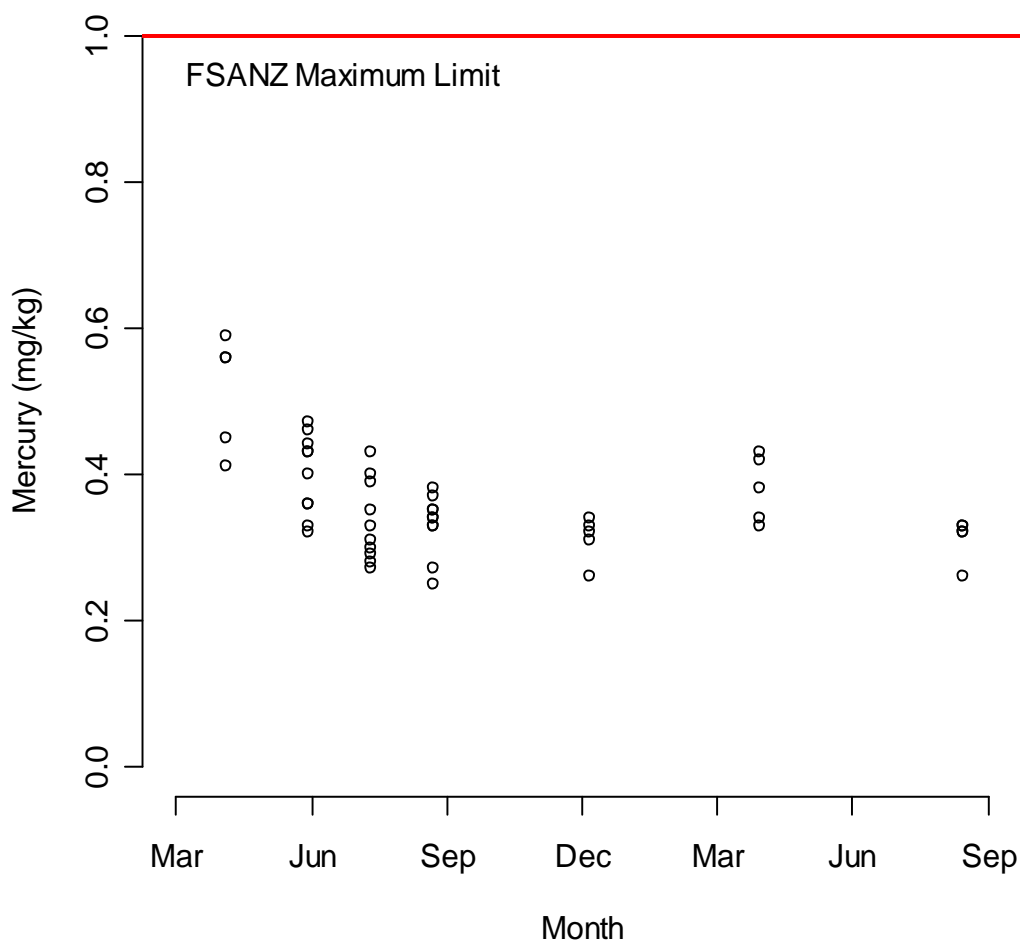
Figure 1. Total mercury concentration of SBT throughout the LTH study. Of interest is the apparent inverse relationship between mercury with fat content of the SBT.

Levels of mercury found in the bait fish species were very low or unmeasurable (Figure 2). Higher levels were found in the Australian redbait (mean 0.036mg/kg) than either of the sardine species fed during the initial 18 week study. It is interesting to note that the Australian redbait was also higher

in selenium. Selenium is suggested to ameliorate the harmful effects of mercury.
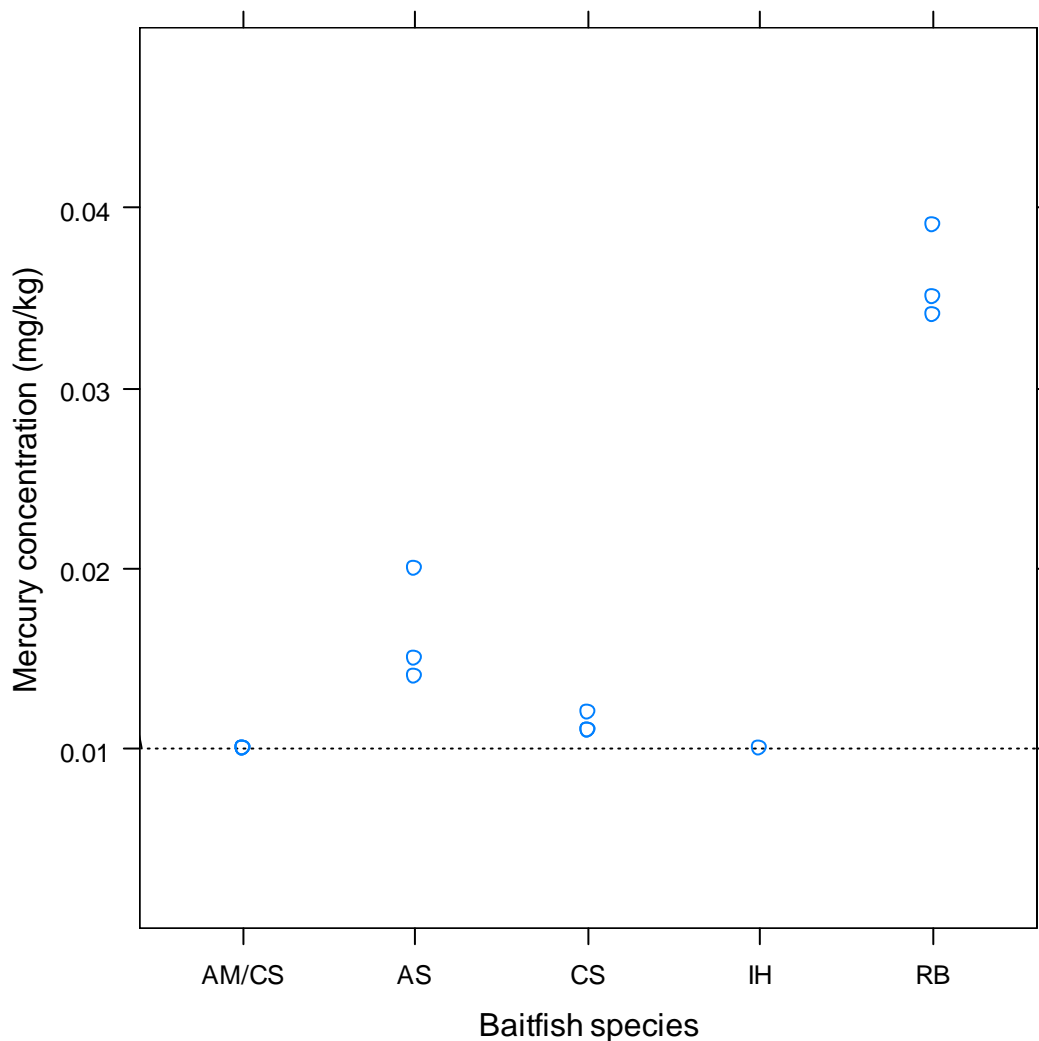
Figure 2. Total mercury content of bait fish fed during the LTH study. The limit of detection (0.01mg/kg) is shown as a dashed horizontal line.

Selenium

Selenium is an important micronutrient involved in the immune response. There is an apparent decline in selenium concentration (Figure 3) in SBT with extended culture period Selenium levels appeared to show an inverse relationship (similar to mercury) with fat content. SBT concentrations appear to be related to the dietary levels in bait fish (Figure 3).
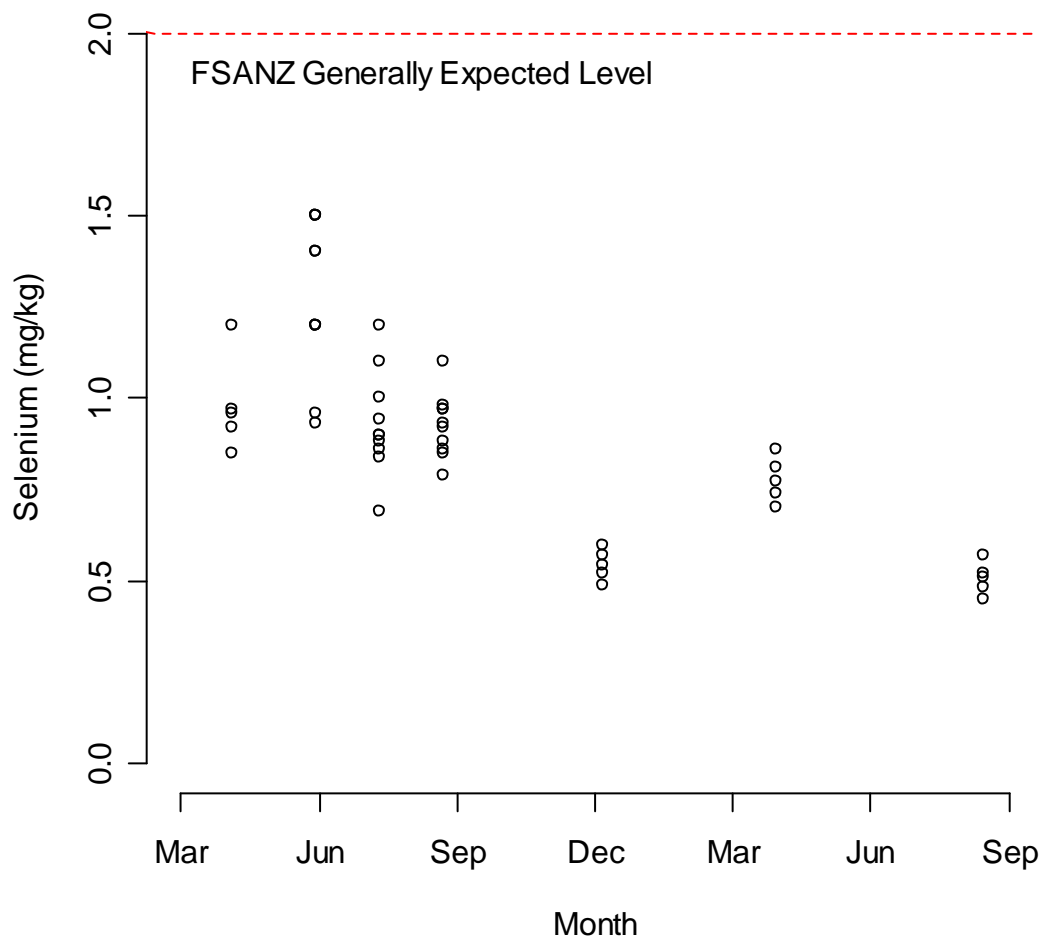
Figure 3. Selenium content of SBT throughout the LTH study.

As with mercury, an apparent inverse relationship is observed with fat content. Selenium may also counteract the harmful effects of mercury. The Australian redbait had the highest levels of selenium present. The apparent trend observed with the selenium content of redbait (Figure 4) and its corresponding mercury content is particularly interesting. The higher levels of these elements in redbait may reflect the lower fat content of these baits.
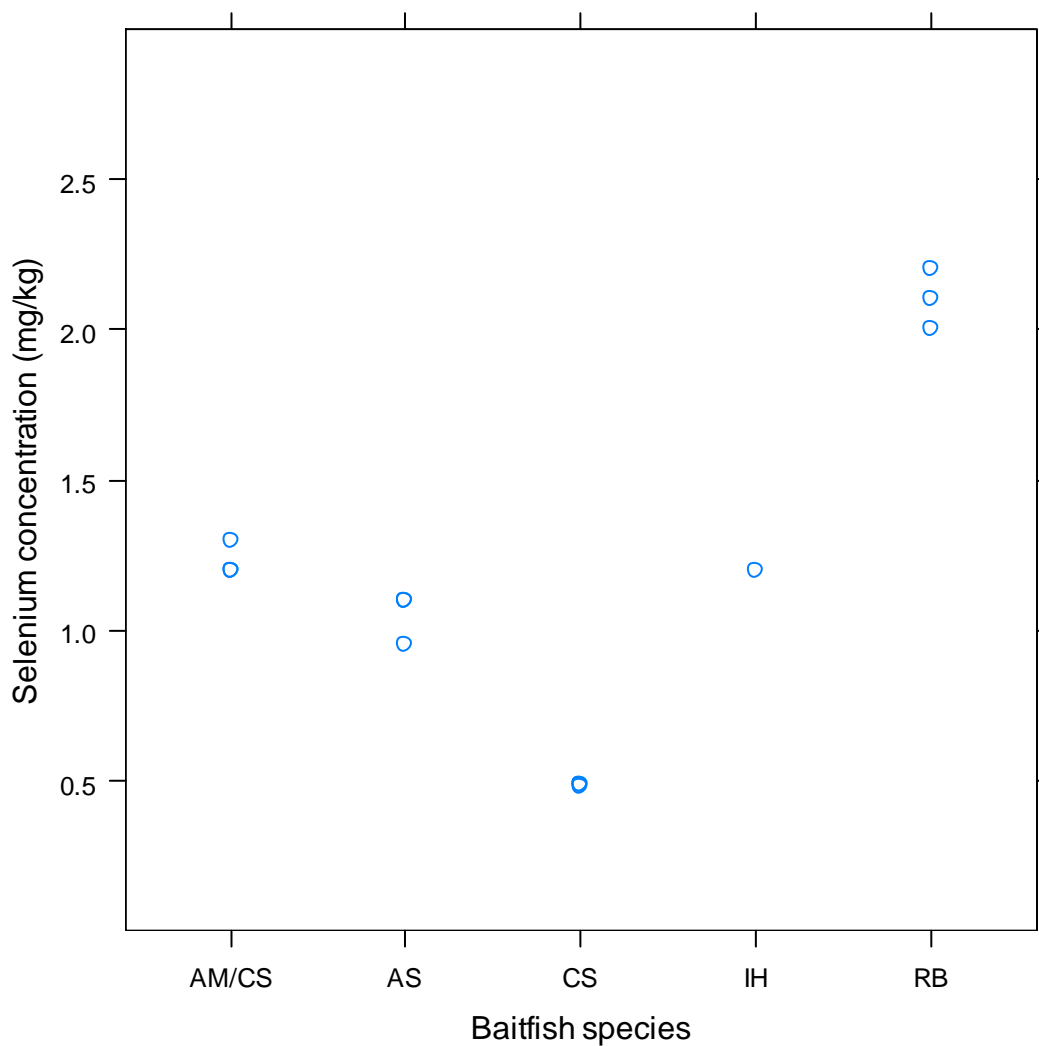
Figure 4. Selenium content of bait fish species fed during the LTH study.

Tin

Tin was not detected in any SBT during the LTH study. Low levels of tin were found in all bait fish during the first 18 weeks of the study. This may reflect the inclusion of the skin and bones in the testing.

Zinc

Zinc, like copper, is a biologically active metal and will be actively regulated. All levels found were well below the FSANZ 90th percentile GEL (Figure 5).
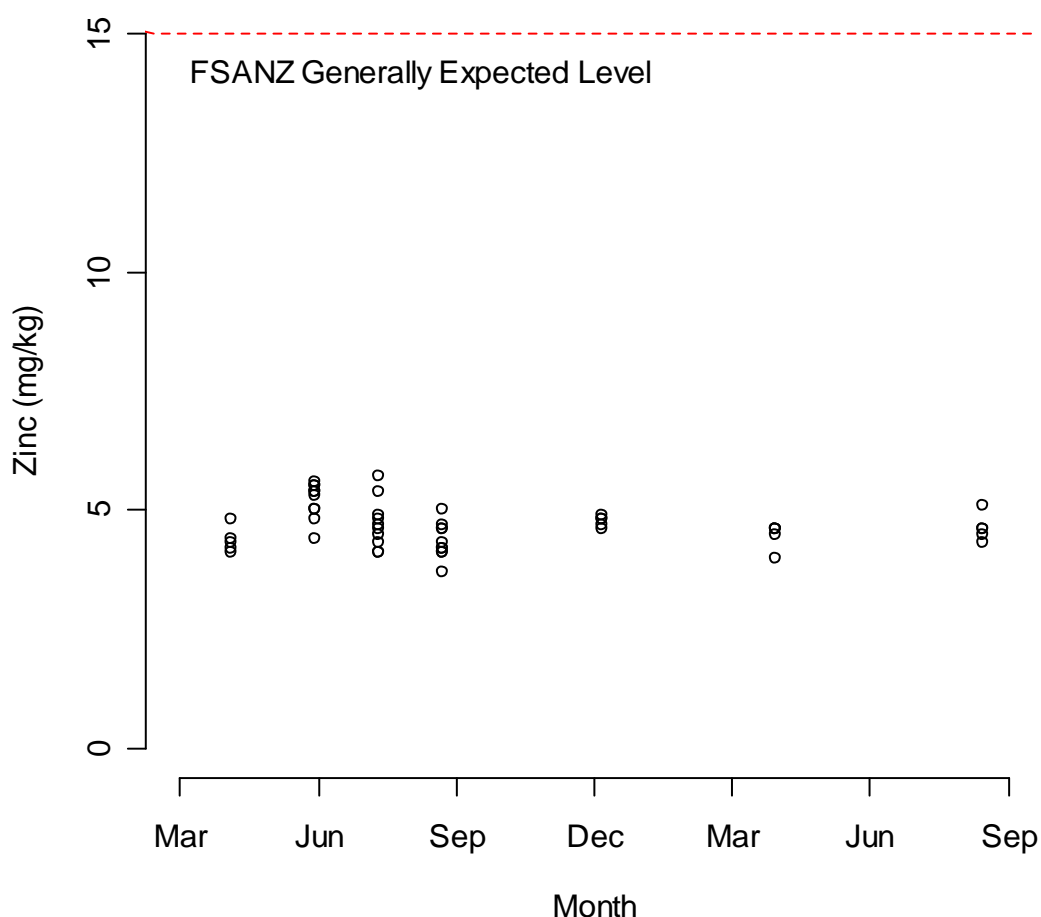
Figure 5. Zinc content of SBT throughout the LTH study.

It would appear that zinc is actively regulated within SBT regardless of diet source. Dietary sources of zinc including Australian and imported bait fish species had very similar levels of zinc regardless of source (Figure 6).

The higher levels found in these bait fish may reflect the fact that whole bait fish were tested compared to skinless, boneless, edible portions of SBT. The skin is known to contain high levels of metals such as zinc which may act as a natural antifouling layer.
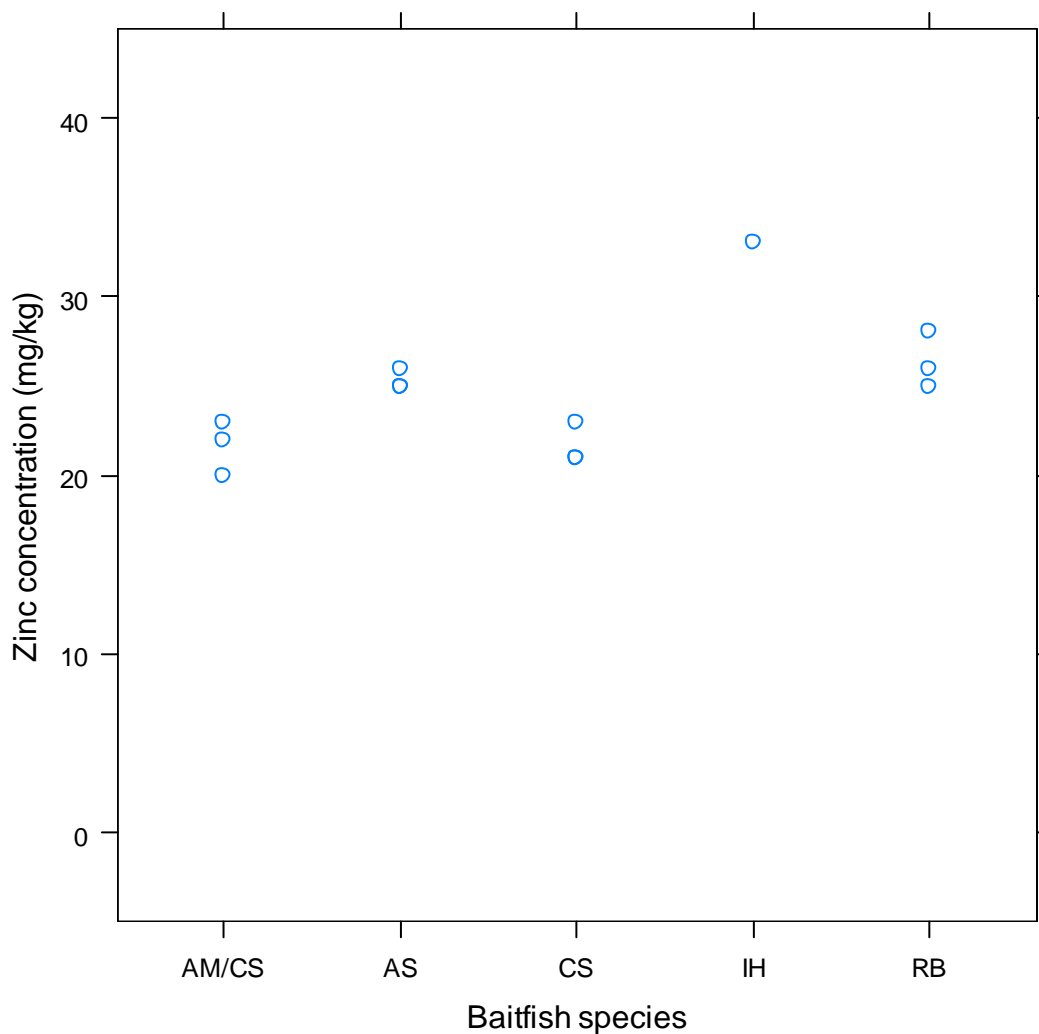
Figure 6. Zinc content of bait fish species fed during the LTH study.

Chemical properties of metals in biological systems

Copper and zinc are neighbours in the first row of the Transition metals block of the periodic table of the elements. Both metals are found in enzymes important for biological processes with the body. Iron is another example of a first row transition metal that is important biologically, as it is critical for the oxygen transportation capacity of both haemoglobin and myoglobin.

By contrast, metals in the second and third row of the periodic table (e.g. mercury) generally do not have biological functions.

Pesticides

Pesticides may be present in cultured fish from several sources e.g. dietary or from terrestrial land run-off after application.

DDT

The pesticide DDT was found as its metabolite p,p DDE in the LTH SBT. Figure 7 shows one of the metabolites of the pesticide DDT is present (DDE) in SBT from the long term holding study. DDT is a fat-soluble pesticide and is extremely persistent in the marine environment due to its lipophilic nature and resistance to degradation. It has been banned in Australia since the late 1980's and in many parts of the world. Its presence indicates historical terrestrial usage as a crop protection agent. It is still used in some parts of the world for malaria control purposes. Bait fish species from the United States are the main source of DDE in farmed SBT.
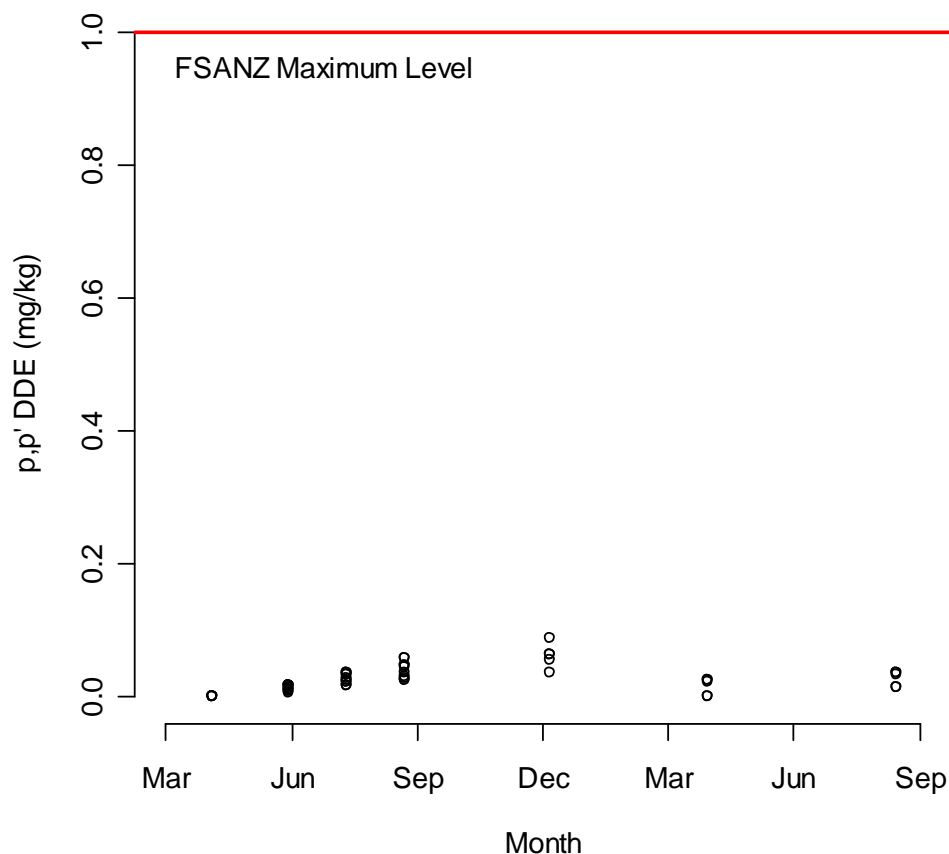
Figure 7. The pesticide DDT was found as its metabolite DDE throughout LTH study.

Polychlorinated biphenyls (PCBs)

The Australian and Japanese Maximum level based on Aroclors and Kaneclors, respectively is set at 0.5 mg/kg (red horizontal line, Figure 8).
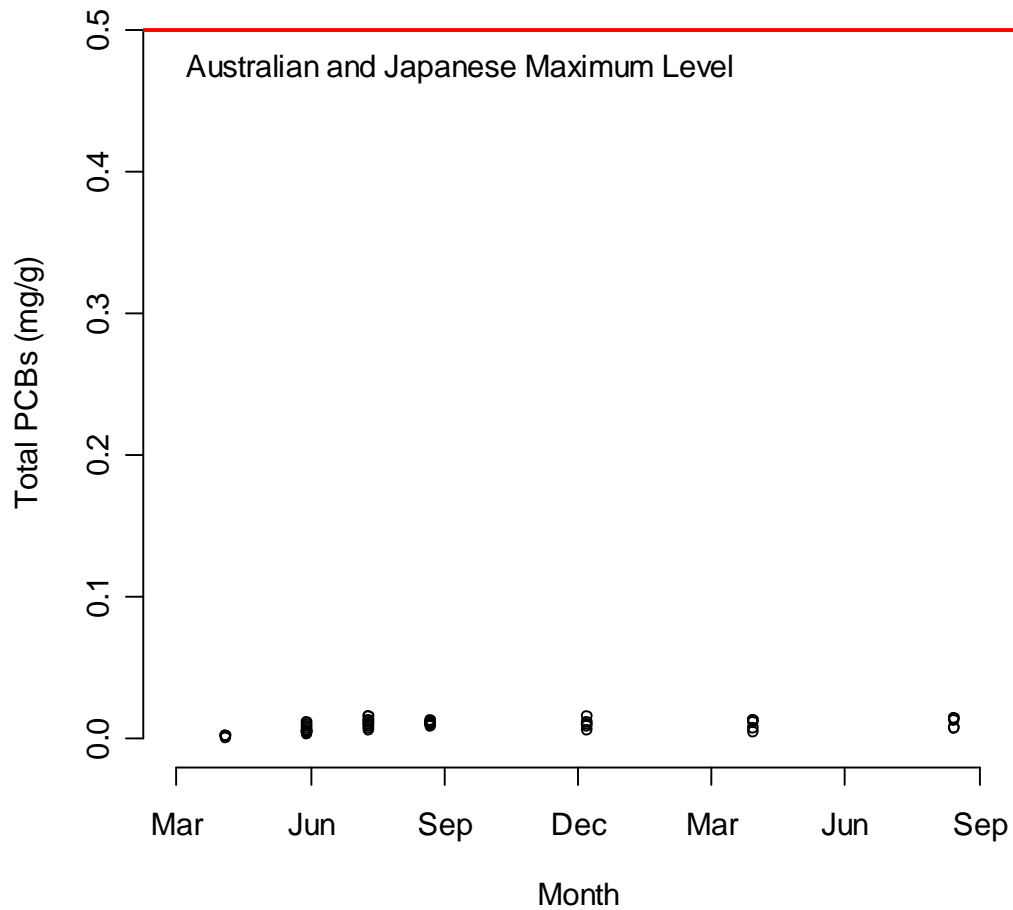
Figure 8. Total PCBs content of SBT throughout the LTH study.

Dioxins and dioxin-like PCBs

Dioxins (pg-TEQ/g)

The red line (Figure 9) is the European Commission Maximum Level for dioxins and furans of 4 pg-TEQ/g.
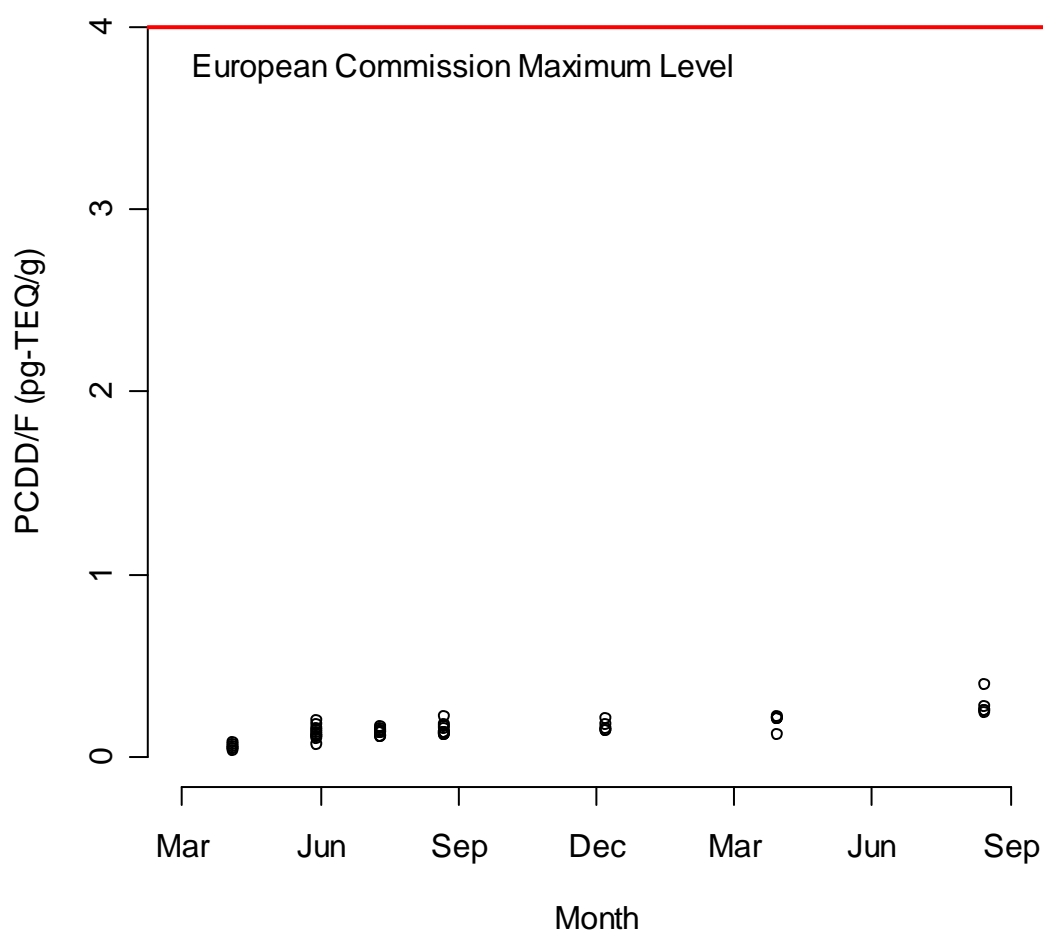
Figure 9. Upperbound Dioxin and Furan Toxic Equivalent concentration (pg-TEQ/g).

Dioxins and PCBs (sum) (pg-TEQ/g)

The levels of the regulatory significant dioxins and the dioxin-like PCBs can be seen in Figure 10. These compounds are extremely persistent in fatty foods of animal origin including fish. Their presence has arisen from a variety of sources including high temperature incineration, pesticide manufacture, paper bleaching and from natural terrestrial sources such as bushfires.
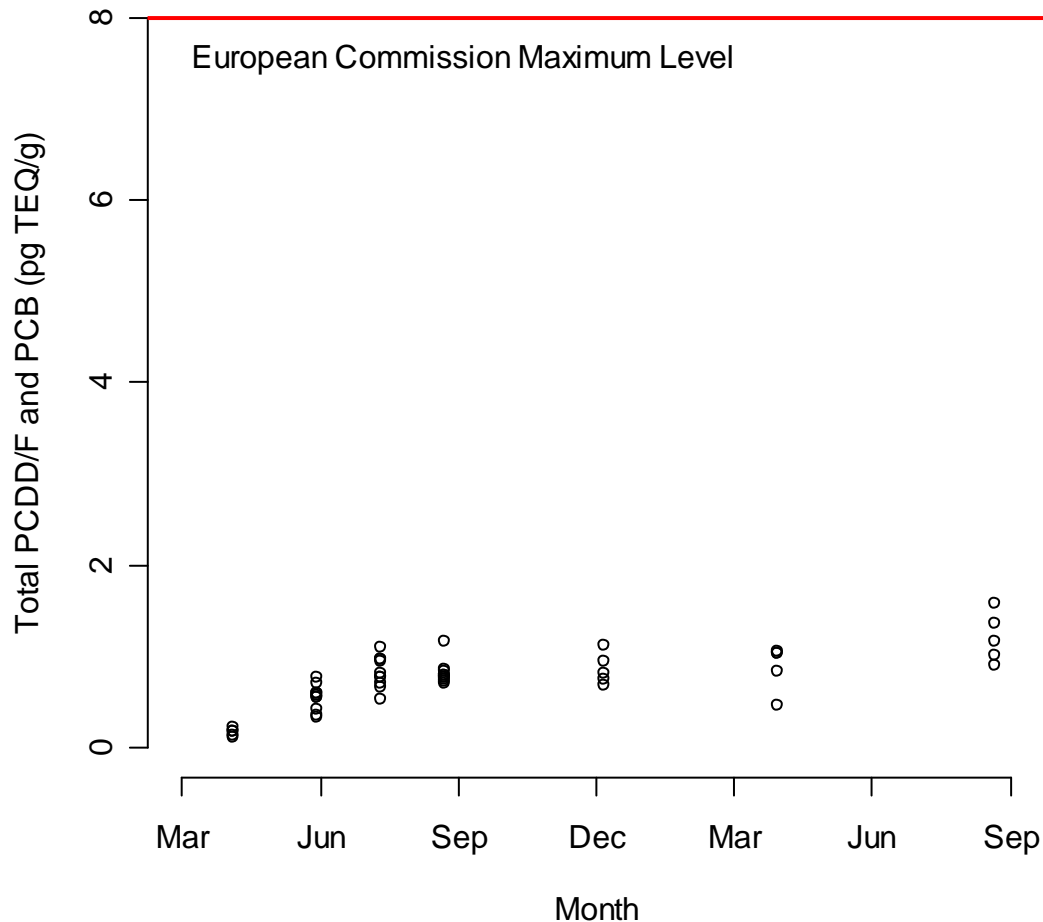
Figure 10. Effect of culture period and levels of the environmental contaminants dioxins and the dioxin-like PCBs in SBT.

PCB contaminants reflect the lipid content of SBT as they are lipid soluble contaminants (Figure 10). The lipid content of SBT during the LTH study changed as a result of dietary intake (Refer Section 8.1). The levels found are extremely low by international comparison with competitors products entering the Japanese market typically having levels 10 fold higher. Californian sardine had the highest levels of dioxins and the dioxin-like PCBs present (Figure 11). As the origins of these compounds have come from different sources the types of dioxins and dioxin-like PCBs also differs. There are 209 different types of PCBs, each called a congener. The analysis of these compounds is specialised, requiring the use of laboratory instruments that can detect levels down to one-trillionth of a gram. The introductions of new regulatory standards in the European Union in November 2006 highlight the growing ongoing trade importance of these compounds for seafood in international trade.
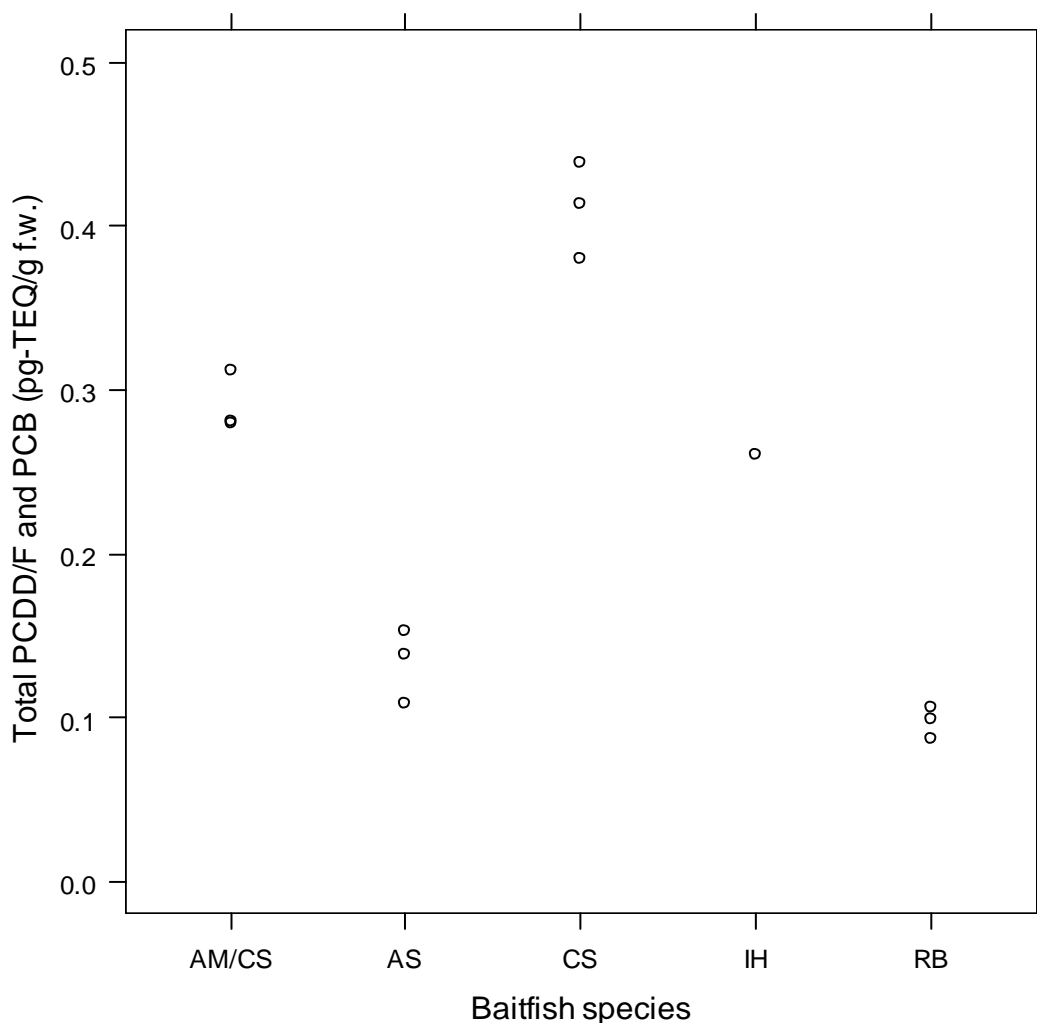
Figure 11. Dioxin and dioxin-like PCB levels in bait fish by species fed during the LTH study

The levels of fat-soluble residues such as dioxins and PCBs reflected the lower fat content of the fish.

A number of regulatory changes have occurred with respect to product in international trade. The Japanese Government has implemented a Positive List System of Maximum Residue Limits (MRLs) that prescribes regulatory standards for seafood; this is in addition to the introduction of a Uniform Limit. The Uniform Limit gives permission for trace amounts of residues and contaminants (with no adverse public health effects) to be present in seafood sold in Japan. In the European Union (EU) amendments have been made to standards for dioxins and the dioxin-like Polychlorinated Biphenyls (PCBs) that will come into effect in November 2006.

The effect of these new regulatory standards will be most strongly felt by competitors with products produced in other parts of the world.

Industry outcomes from the residue assessment of LTH study

1. Assurance of safety of product sold in Japanese market.
2. LTH did not lead to any adverse residue levels being found.
3. Full compliance with the Japanese Positive List System of Maximum Residue Limits (MRLs) which limits the need for Japanese Positive List insurance for product in trade.
4. Levels of dioxins and PCBs were approximately one-tenth of levels in Bluefin Tuna cultured in the Mediterranean and one-fifth of the levels of Bluefin Tuna cultured in Mexico.
5. Ability to meet new European Union (EU) standards for the dioxins and the related dioxin-like PCBs that came into effect in November 2006.

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8.3 Production – Flesh Quality

Quality characteristic comparisons of southern bluefin tuna (*Thunnus maccoyii*) held over from the 2005 ranching season for longer term holding

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Introduction

The quality of farmed or ranched southern bluefin tuna (SBT) is assessed using a combination of empirical and quantified measures of carcass attributes, that over time, have been recognised by wholesale buyers as important to the price they will receive when on-selling the product (Thomas, 2007). A comprehensive study of the quality attributes of farmed SBT has been carried out over several years (D'Antignana, 2007; Thomas, 2007) and in these studies, not only have important quality attributes been recognised but repeatable methods of assessing them have been developed.   The first major quality attributes used for assessing SBT carcases are:

Size – usually assessed as weight.

Shape – assessed as Condition Index (CI) which is expressed as the relationship between the length and weight of individual SBT (see methods below).

External damage – i.e. marks, blemishes, and breaks and holes in the skin of the fish.

The size and shape of the SBT, in combination with the amount of food fed within the ranching period, are attributes that are also used by ranchers and researchers as a measure of production efficiency. This explains why the measurement of fish production in SBT ranching is often used to describe fish "quality".

With this point acknowledged, it may be useful for the reader to refer to sections 7.1 and 7.2, in 2004/209 Aquafin CRC - SBT Aquaculture Subprogram: dietary supplements for reducing oxidative stress and improving flesh quality attributes in SBT, in order to gain understanding of the importance of the quality attributes of size and shape of SBT and the use of these descriptors in general ranching terms. The external damage on fish is used as an indicator of the harvest and post harvest treatment of the fish, with quality downgrades of SBT occurring as the external damage becomes more obvious (pers. observations of the lead author). However, generally this quality attribute is not measured in ranched SBT; as the ranching process gives a greater level of control over harvesting and pre and post- mortem fish handling. This control results in little to no assessable external damage occurring to fish prior to delivery to the market.

Other quality characteristics of SBT include attributes of the flesh. Most important of the flesh attributes are the fat content and the shelf life. As the fat content increases the oxidative pressure on the flesh increases (Buchanan and Thomas, in press Aug 2008; Thompson et al., 2006) this can result in a cascade of oxidative processes that is most obviously recognised by the loss of the bright red colour of the flesh. This red colour is due primarily to the presence of high muscle concentrations of myoglobin. The oxidation of myoglobin results in a colour change of the flesh from red to brown; a process that is accelerated if low storage temperatures are not maintained (Chen et al., 2004). Within the flesh of SBT (and all animals) there are natural protection mechanisms which are enzymic and non-enzymic (Gorelik and Kanner, 2001). The endogenous vitamins E and C are important non-enzymic antioxidants within food animals (Decker et al., 2000) including SBT. Vitamin E is a natural free radical scavenger and breaks the chain of lipid oxidation. Vitamin C is also able the scavenge free radicals and prevent lipid oxidation, but in addition has a role in replenishing vitamin E within the fish muscle. Together these actions result in the inhibition of the changes to the myoglobin in SBT muscle that ultimately delay the post mortem browning process or colour shelf life of the product.

Selenium is important to fish because it participates in a range of biological systems and is an integral part of a large range of selenoproteins (i.e. enzymic systems). These selenoproteins are important in the management of oxidative stress and therefore play a part in the maintenance of post-mortem colour retention in SBT.

The aim of this research was to compare the quality characteristics of SBT held over from the 2005 ranching season for Longer Term Holding (LTH) to the competing product that is available to the Japanese market at the same time.

To achieve this we intended to compare the LTH product with the product produced within the normal ranching season at Port Lincoln and harvested in July 2006

Reported here is the sampling of SBT in July 2006, comparing LTH SBT with One Season (OS) SBT that were farmed by SEKOL Farm Tuna Pty Ltd and harvested at the same time as the LTH fish which were farmed by DI Fishing Pty Ltd.

Methods

The LTH fish compared in this research were SBT carried over from the 2005 season which were fed a standard commercial diet that was similar to the diet described in the 2005 research as medium fat and medium protein (i.e. ~ 18.5% protein and 7.3% fat). These LTH fish were harvested on the 7th December 2005, 31st of March, 2006, and then on the 15th August 2006 (i.e. @ 360 and then 498 days of culture). The OS SBT were also harvested at this time in 2006. The OS SBT were fed on a standard commercial diet (i.e. a medium fat and medium protein) as for the LTH SBT and were held in culture for 50 and 151 days for Harvest 1 and Harvest 2, respectively.

Analysis of the samples of 10 fish from each harvest included:

Biochemical measures

- Crude fat
- Measure of oxidation (TBARS)
- Vitamin E (α-tocopherol)
- Vitamin C (ascorbic acid)
- Selenium

Physical measures:

- Colour (visual and instrumental)

Fish husbandry

Longer Term Holding SBT

The cohort of SBT that were subjects of the '*Formu-bait*' trial and subsequently the current LTH study, were transferred from a towing pontoon to 50 Ø m ranching pontoons on the 30th March, 2005.   Ten fish from this group were harvested on 5th April 2005 to obtain muscle core and blood samples. The farm husbandry practices of the 2005 on- growing season is documented in section 8.1 of this volume and will not be elaborated in this section.

At the end of the '*Formu-bait*' trial in August 2005, 519 SBT remaining from an earlier feeding trial were transferred to a 32 m Ø ranching pontoon on the DI Fishing Co. Pty. Ltd. lease site. These SBT, referred to as the Long Term Holding group (LTH), were on-grown for a second ranching season, which ended on the 15th August 2006. During the extended on-growing period, the LTH group were fed a bait fish diet that was similar to the medium fat / medium protein (i.e. ~ 18.5% protein and 7.3% fat) diet described in the 2005 '*Formu-bait*' trial (refer Section 8.1). Other relevant details of the husbandry practices employed during the 2006 on-growing period can be found in this volume (refer Section 7).

One Season SBT

During the 2006 ranching season SBT that were on-grown, on the Sekol Ranched Tuna Pty Ltd (Sekol) lease or DI Fishing Co Pty Ltd (DI) lease, and harvested at the same time that the LTH fish, were referred to as One Season 1 (OS1) and One Season 2 (OS2) respectively. For Sekol, OS1 tuna were transferred from the wild to a 50 m Ø ranching pontoon on the 13th February 2006. From then they were fed a bait fish diet that was classified as medium to high in fat which was fed at rate 2kg bait fish/SBT/day, for 46 days (pers. comm. David Warland, Farm Manager, Sekol).

In the case of the OS2 group, SBT with a CI of 20.0 (pers. comm. Verne Lindsay DI) were transferred from the wild to a DI Fishing Pontoon 5a on 17th of March 2006, and fed a diet similar to that of the LTH fish group (i.e. medium to high in fat) for 151 days.

Harvest One

On 31st March 2006, following 359 days of culture, 10 SBT were harvested from the LTH pontoon. At this time, due to commercial constraints, there was no opportunity to harvest SBT ranched for only one season from the DI Fishing farm lease and OS1 SBT were sampled from a harvest that was carried out by Sekol on their lease on the same day as the LTH pontoon harvest. During this harvest time, muscle and blood were sampled from 10 LTH SBT and, at an onshore tuna processing facility, muscle was sampled from 10 OS1 fish that had been cultured for 46 days. For the LTH SBT, immediately following net harvest SBT were quickly killed following standard industry practice; briefly described as: *A sharp, 13mm ∅, stainless steel rod was inserted into the brain (ike-jime). Following ike-jime, a wire cable was briefly inserted down the spinal cord. Fish were then bled by cutting the cutaneous lateral artery, located under the pectoral fin. At this time blood was collected from the wound using a 10ml syringe that had been treated with heparin. Following weighing and length measurement, a muscle sample was excised from the fish using a 17mm ∅ stainless steel coring tool inserted into*

the bleed wound. The resulting muscle was stored on ice for post mortem colour assessment.

The weight and length of the harvested SBT was recorded and Condition Index (CI) was calculated on whole fish wet weight as:

$$CI = \frac{Weight(kg)}{Length(m)^3}$$

The OS1 fish had been held in ice slurry between harvest and processing. For these fish the harvest and muscle sample collection was carried out as described above. However no blood sample was collected for the OS1 tuna.

Harvest 2

On 15th August 2006, following 497 days of culture, 10 SBT were harvested from the LTH pontoon on the DI Fishing lease. Approximately 1 hour later 10 fish were harvested from Pontoon 5b from the same farm, i.e OS2 fish that had been cultured a total of 151 days. Muscle and blood samples were collected from all fish as per Harvest 1.

Sample preparation

The muscle core samples, anterior chutoro and akami combined, were homogenised to a fine mince. Several grams of sub sample were retained at 4°C for colour shelf life determination; the remainder was stored at –80°C for biochemical analysis. Plasma was taken from centrifuged blood then stored at –80°C for biochemical analysis.

Analysis

Muscle collected from each fish was minced and a sub-sample was analysed for levels of vitamin E, C and selenium.   The vitamin E (α-tocopheryl) concentration was determined by a HPLC method based on the method of Huo et al. (1999).

Vitamin C was determined by a technique based on the HPLC fluorescence detection method of Brown and Miller (1992) at the laboratory at the Lincoln Marine Science Centre. For the measurement of TBARS approximately 1g of tissue was homogenized in 5ml of 0.6M perchloric acid. Five ml of the homogenate was added to 5ml of thiobarbituric acid in stoppered test tubes. The test tubes were placed in a water bath of 100°C for 35 minutes. The supernatant was read against a blank in a spectrophotometer at 540nm. The concentration of TBARS was determined as $mg.kg^{-1}$ of tissue (w/w) by comparison to a standard curve. Selenium level was determined using a method based on the fluorimetric technique of (Watkinson, 1966) modified as per (Paynter et al., 1993). The Crude Fat Content (CFC) of the muscle was determined gravimetrically by a method based on the Norwegian Standard method (NS 9402 E). Approximately 10g of chopped tissue, 40g of anhydrous sodium sulphate and 80ml of ethyl acetate was agitated in a stomacher mixer (IUL Instruments) for three minutes, and the resulting homogenate was filtered (Whatmans GF/C filter papers). The filtrate was decanted into plastic beakers and evaporated in a fume hood until no solvent was evident. The beakers were then placed in an oven at 60°C for approximately 12 hours and then weighed to determine the fat weight (g), which was expressed as a percentage of the muscle wet weight (g) using the following formula:

$$CFC = \frac{(Evaporated\ Homogenate\ wt\ /\ Solvent\ Dilution)}{(Extracted\ Muscle\ wwt\ g)} \times 100$$

Colour shelf life was measured the day after harvest and then every 24hr for eight days. Minced muscle samples were placed in small plastic containers and randomly placed on numbered white tiles, covered with clear plastic cling wrap and kept in refrigerated storage at 4°C. At each sample time, a panel of 5 people gave a subjective 'blind' colour rating to each muscle sample using a predetermined grading system ranging from red to brown, using scores between 0 and 6 (Table 1).

Table 1. Colour criteria for visual assessment of SBT muscle sample browning over time.

Score	Criteria Description
0	Red meat (not bloomed)
1	Bright red meat (bloomed)
2	Change in red colour i.e. a dulling of the bloomed meat
3	Either a significant darkening of the meat or margins beginning to brown
4	Either a significant darkening of the meat i.e. heading towards red/black or brownness spreading from the margins/general change of blocks
5	Either a blackening of the meat or an overall browning of the meat but, for either, still with a pink/red undertone
6	Either a black sample or a brown/green sample; no red/pink undertone visible

At the same time an objective colour measurement was taken from the surface of the centre of each muscle core using a Minolta colour meter. The colour of each sample was measured, and from CIE a* and b* values the angle of hue and chroma were calculated using the following equations:

$$Hue = \arctan(b*/a*)$$
$$a* > 0 \quad b > 0*$$

and

$$Chroma = \sqrt{(a*^2 + b*^2)}$$

The hue angle is a descriptor of what is generally understood to be the true colour, and the chroma is the intensity or degree of saturation of the colour.

Statistical analysis

Results were reported as LTH and OS group means + SE (n=10) and tested for differences with t-test using statistical software Sigma Stat 3.1. Data were log transformed where necessary to satisfy assumptions of normality and homogeneity of variance. Differences were significant where P<0.05.

Results

Fish Condition

The CI of the LTH fish increased with culture time (Table 2). The crude fat content of the sampled muscle increased from 3.2% at transfer (March 31st 2006) to 11.2%, following 497 days of culture (i.e. on August 15th). Correspondingly during this culture period the whole weight of the sampled fish increased from 28.8kg to 39.0kg. At harvest 1 (March 31st) the LTH1 group crude fat was significantly lower than in OS1 tuna at 3.2% and 5.2% respectively. LTH1 CI was also significantly lower than in OS1 fish at 20.9 and 24.0 respectively. However, whole weight was similar for both groups, at ~30kg. At the final grow-out harvest on 15th August 2006, there were no significant difference in the CI of LTH2 and OS2 fish groups and crude fat of the two groups was similar at ~11.5%. Whole weight and CI of the two groups was also similar at ~35kg and ~23.5kg, respectively.

Table 2. Mean crude fat, whole weight and CI in SBT transferred from the wild to culture pontoons in 2005 and harvested following 359 (LTH1) and 497 days of culture (LTH2) and SBT held for One Season, following 46 (OS1) and 151 (OS2) days of culture.

Holding group and sampling date			Crude fat (% wet wt)	Whole weight (kg)	Condition Index (CI)
LTH	Transfer**		no sample	13.4 ± 1.3	17.7 ± 0.4
OS1	46	days	5.2 ± 0.5	31.4 ± 1.4	24.0 ± 0.5
LTH1	359	days	3.2 ± 0.4*	28.8 ± 2.0	20.9 ± 0.4*
OS2	151	days	11.6 ± 1.0	34.7 ± 2.1	24.0 ± 0.3
LTH2	497	days	11.2 ± 0.6	39.0 ± 1.5	23.1 ± 0.4

* indicates values ± SE (n=10) that are significantly different (P<0.05) to fish group harvested at the same time.

**Transfer values not included in the comparisons.

Muscle Antioxidant Levels

At the first harvest, the muscle vitamin E level for the LTH 1 group was significantly lower than the OS1 group; however at the second harvest the OS2 and LTH2 group levels were similar (Table 3). The vitamin E level of the LTH Transfer group was within the range of the levels measured for the other harvest groups during the culture period. At the first harvest the ascorbic acid level of the flesh of the OS1 group was significantly lower than the LTH1 group. At the time of the second harvest the ascorbic acid level of the flesh of the OS2 and LTH2 groups were similar to one another. Generally the fish groups that were harvested closest to the time of transfer had much lower muscle ascorbic acid levels than harvest groups that had been cultured for a longer period of time. At first harvest, the muscle selenium level of the LTH1 group was significantly lower than the OS1 harvest group. At the time of the second harvest the muscle selenium concentration of the OS2 group and the LTH2 group were similar. With the exception of the selenium tissue level of the OS1 group there was a trend indicating a drop in muscle selenium concentration with time in culture.

Table 3. Muscle tissue antioxidant levels in SBT transferred from the wild to culture pontoons in 2005 and harvested following 359 (LTH1) and 497 days of culture (LTH2) and SBT held for One Season, following 46 (OS1) and 151 (OS2) days of culture

Holding group and sampling date	α-tocopherol (mg.kg^{-1}wet wt)	Ascorbic acid (mg.kg^{-1}wet wt)	Selenium (mg.kg^{-1}wet wt)
LTH Transfer** **	5.12 ± 0.21	1.87 ± 0.18	0.82 ± 0.05
OS1　46　days	6.13 ± 0.31	1.48 ± 0.70	0.92 ± 0.03
LTH1　359　days	4.60 ± 0.24*	8.03 ± 0.57*	0.77 ± 0.03*
OS2　151　days	6.51 ± 0.22	12.64 ± 0.99	0.68 ± 0.02
LTH2　497　days	6.02 ± 0.38	11.38 ± 0.78	0.70 ± 0.02

* indicates values ± SE (n=10) that are significantly different (P<0.05) to fish group harvested at the same time.

**Transfer values not included in the comparisons.

Muscle Post Mortem Change - biochemical

At transfer the TBARS concentration in LTH tuna was 0.18mg (Table 4). At the time of the first harvest, the LTH1 group TBARS (0.11mg.kg^{-1}) was significantly higher than in the OS1 group at 0.07mg.kg^{-1}. Following 5 days post mortem storage (i.e. colour change end point) TBARS was similar in both groups but had increased to ~0.90mg.kg^{-1}. At the time of the second harvest the LTH2 and OS2 groups had similar TBARS levels at ~0.17mg.kg^{-1}. Following 4 days post mortem storage (i.e. colour change end point) TBARS were still similar in both groups but had increased to ~ 1.10mg.kg^{-1}.

Table 4. Thiobarbituric Acid Reactive Substances (TBARS) in SBT transferred from the wild to culture pontoons in 2005 and harvested following 359 (LTH1) and 497 days of culture (LTH2) and SBT held for One Season, following 46 (OS1) and 151 (OS2) days of culture

Holding group	Sampling date	TBARS – at harvest (malonaldehyde mg.kg^{-1}wet wt)	TBARS – post mortem (mg.kg^{-1}wet wt)
LTH Transfer**	5/4/05	0.18 ± 0.02	no extended
OS1 46 days	31/3/06	0.07 ± 0.00	0.82 ± 0.10 (Day 5)
LTH1 359 days	31/3/06	0.11 ± 0.02*	0.98 ± 0.07 (Day 5)
OS2 151 days	15/8/06	0.16 ± 0.01	1.01 ± 0.07 (Day 4)
LTH2 497 days	15/8/06	0.17 ± 0.01	1.20 ± 0.07 (Day 4)

* indicates values ± SE (n=10) that are significantly different (P<0.05) to fish group harvested at the same time.

**Transfer values not included in the comparisons.

TBARS defined as malonaldehyde concentration in muscle.

Muscle Post Mortem Change – colour

During the 5 days of storage at 4°C following the first grow-out harvest, there were no significant differences in muscle hue values between OS1 and LTH1 fish groups. Hue values were stable in the OS1 group (at ~0.4) for 3 days post mortem and then increased rapidly until reaching the colour end point at day 5 (no remaining red) of ~0.9 (Figure1). LTH1 hue showed a similar pattern, but a sharp increase in hue occurred 2 days post mortem with a trend for slightly higher values than in the OS1 group evident during storage. Muscle chroma in both groups decrease similarly and in a linear fashion for both fish groups, during post mortem storage. Although the pattern of decrease in chroma was similar for both groups LTH1 values were significantly lower than the OS1 group from day 3 onwards (Figure 2). For SBT from harvest 2, the muscle hue in both groups increased steadily, during cold storage, until reaching the colour end point on day 4. The LTH2 fish group had significantly higher hue than in OS2 fish group on day 2 with values of 0.60 and 0.53, respectively (Figure 3).

The hue of the meat of both groups increased during cold storage and the difference between the groups became greater until on day 4 of storage, reaching 1.05 for the LTH2 group and 0.84 for the OS2 group. Muscle chroma decreased with storage time in both fish groups but was only significantly different on day 0 at 16.71 and 14.56 for LTH and OS fish, respectively (Figure 4).

Figure 1. Muscle hue (mean + SE, n=10) in samples stored at 4°C for 5 days post mortem from SBT held for One Season (OS1, 46 days) and for a longer term (LTH1, 359 days).
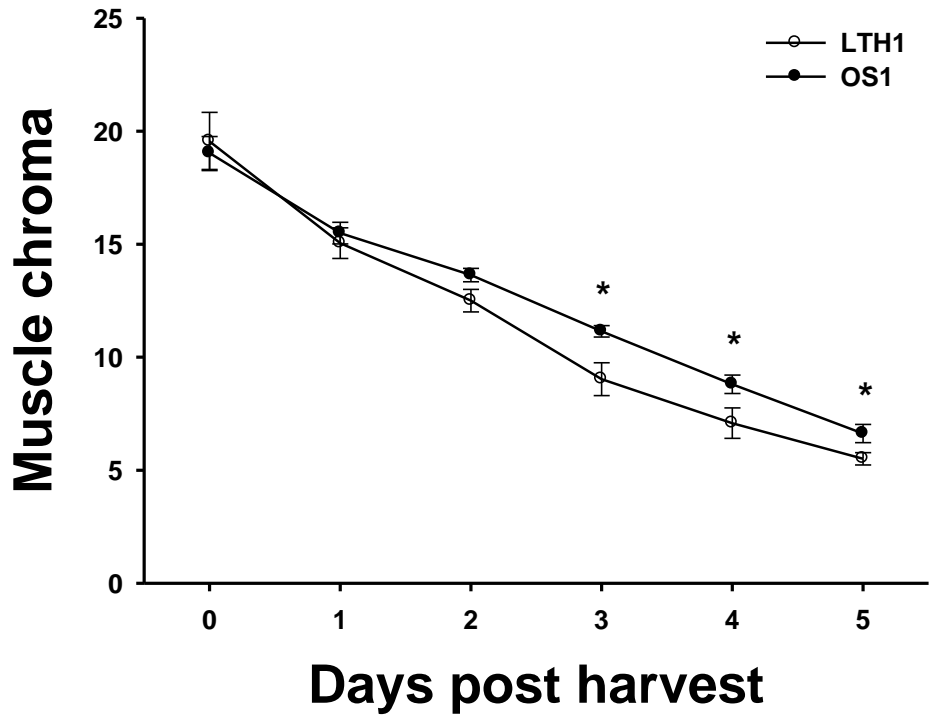
Figure 2. Muscle chroma (mean + SE, n=10) in samples stored at 4°C for 5 days post mortem from SBT held for One Season (OS1, 46 days) and for a longer term (LTH1, 359 days).
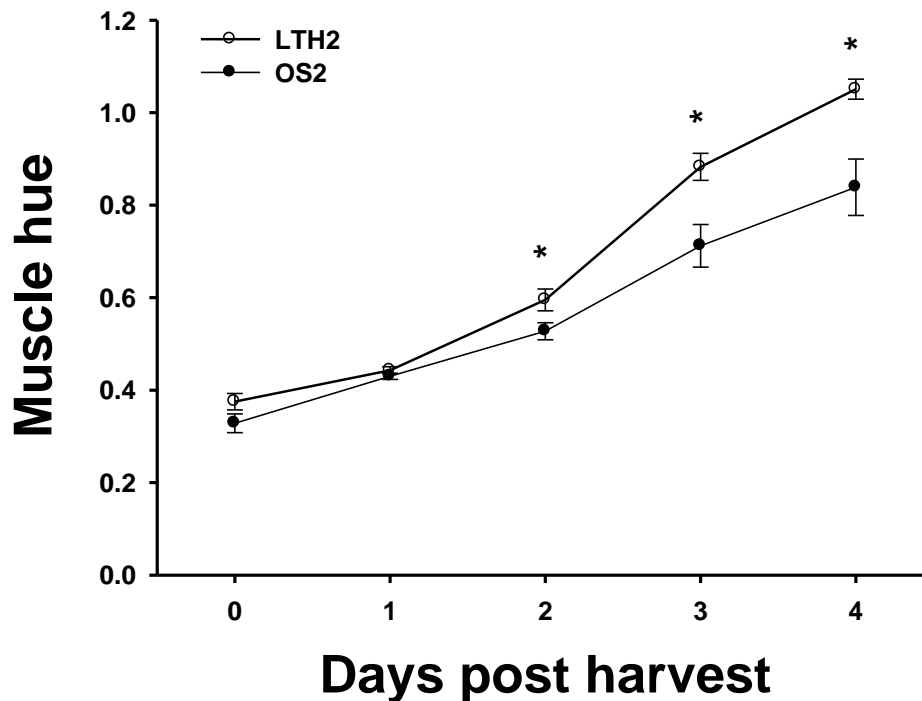
Figure 3. Muscle hue (mean + SE, n=10) in samples stored at 4°C for 5 days post mortem from SBT held for One Season (OS2, 151 days) and for a longer term (LTH1, 497 days).
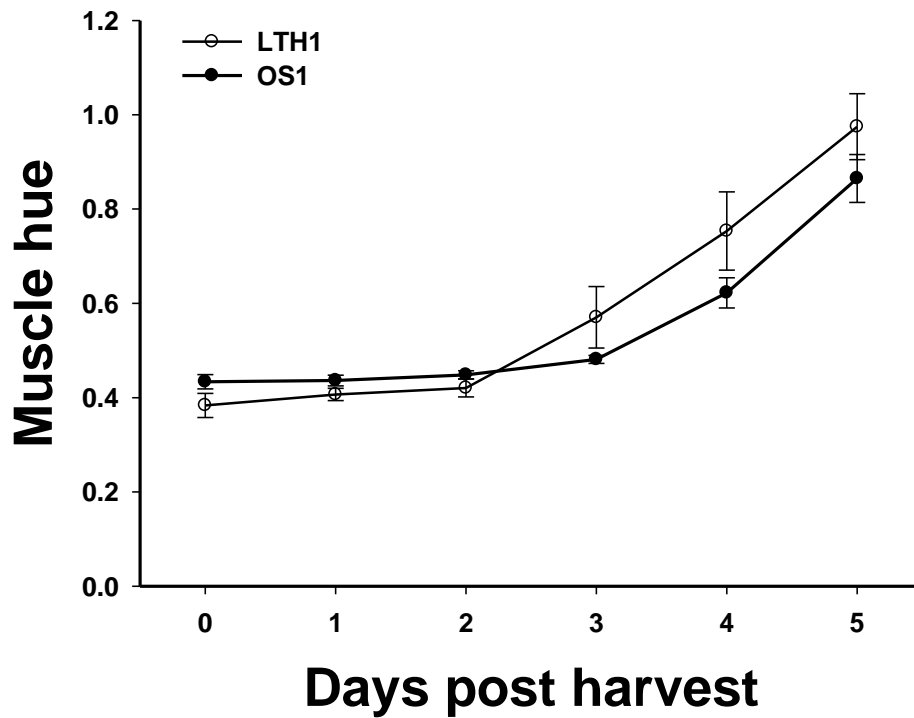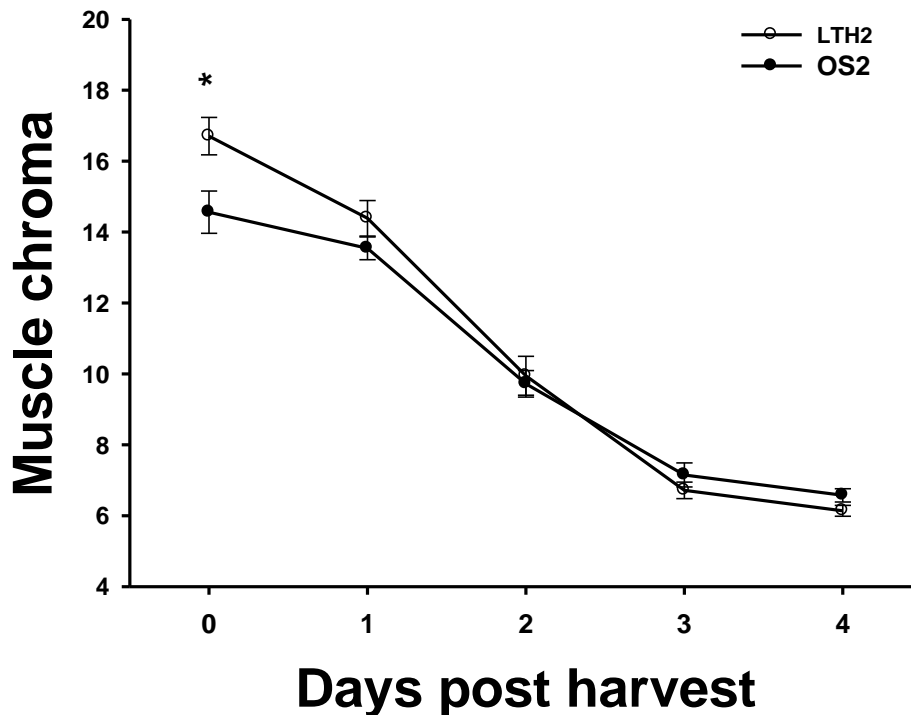
Figure 4. Muscle hue (mean + SE, n=10) in samples stored at 4°C for 5 days post mortem from SBT held for One Season (OS2, 151 days) and for a longer term (LTH1, 497 days).

Results of the subjective colour scores (where 0 was fresh red and 6 was brown with no visible red) indicated that the LTH2 fish group browned faster than the OS2 group. From day 2 up to and including 5 days of storage at 40°C there was a significantly lower frequency of low colour scores given to LTH2 muscle than OS2 muscle (Figure 5). Under the same storage conditions after the second grow-out harvest both groups browned at a similar rate over 4 days of cold storage with no significant differences in frequency of scores between LTH and OS fish on any day (Figure 6).

Figure 5. Colour score frequency distribution of SBT muscle stored at 4°C for 5 days post mortem from fish held for One Season (OS1) (46 days) and longer term (LTH1) (359 days).

Figure 6. Colour score frequency distribution of SBT muscle stored at 4°C for 5 days post mortem from fish held for One Season (OS2) (151 days) and longer term (LTH2) (497 days).

Discussion
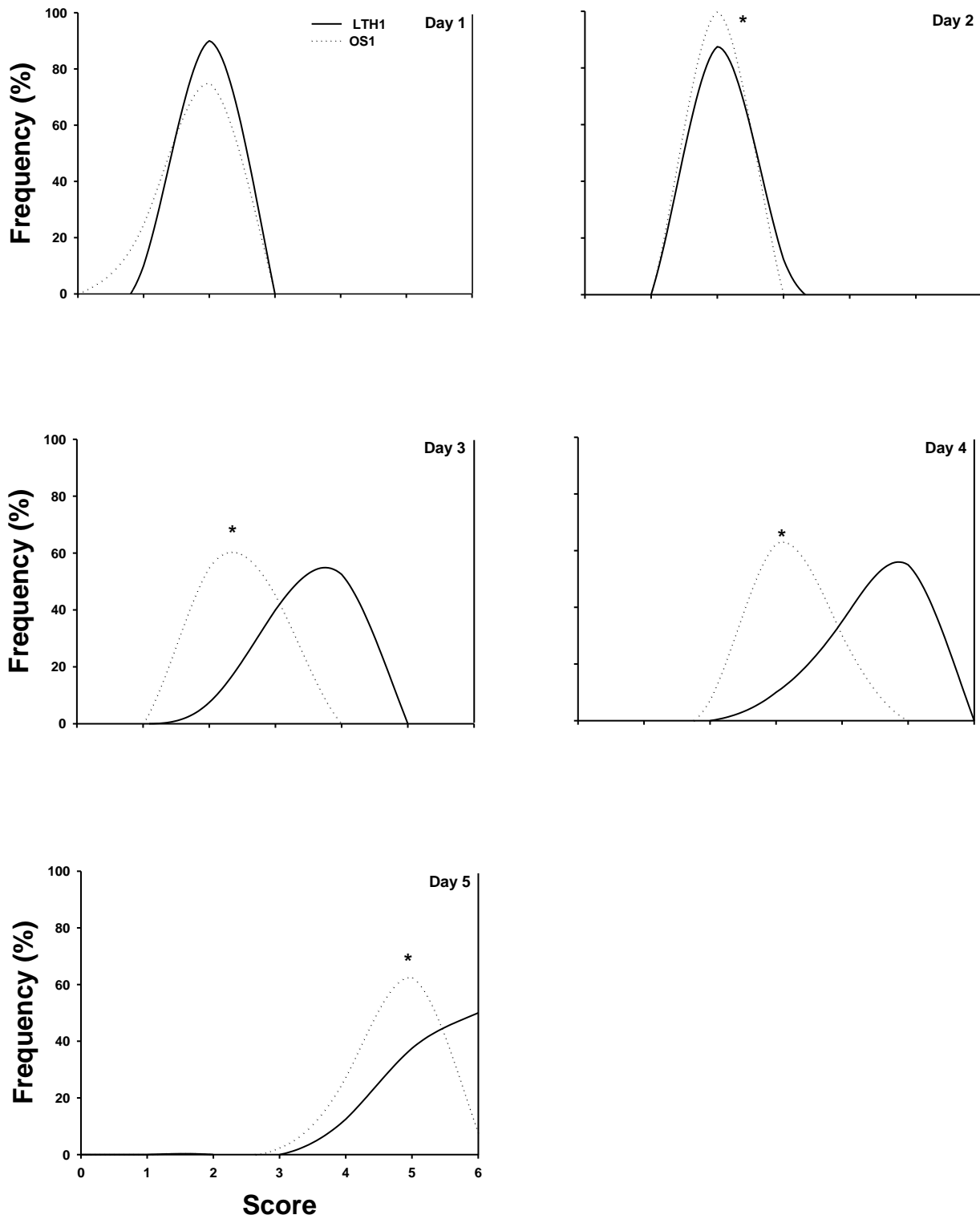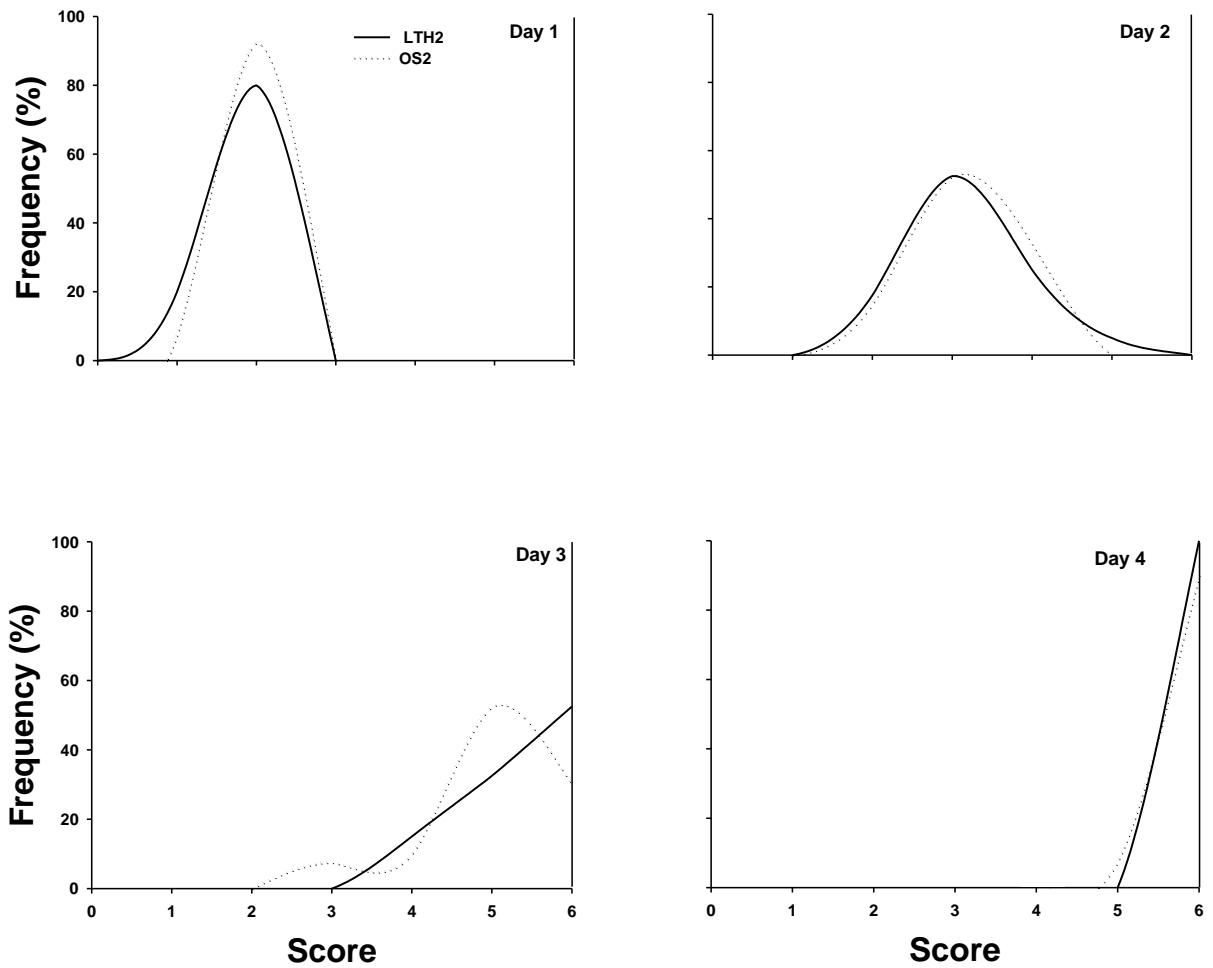
The muscle vitamin E concentration of the OS1 fish was higher than the LTH1 fish, but this may have been due to the higher fat level or CI of the OS1 fish. It should also be noted that the OS1 fish muscle vitamin E concentration at transfer time in 2006 is unknown but may have been high relative to transferred fish in 2005.

Muscle vitamin C concentration was also higher in the OS1 than the LTH1 fish. The muscle vitamin C concentration of the OS1 at transfer is unknown and so there is no way of knowing what influence the start of season level may have had on the muscle vitamin C level at harvest.

Consistent with the antioxidant vitamins E and C, muscle selenium was lower in the LTH1 fish than the OS1 fish. This is consistent with the observation that muscle selenium level in farmed SBT drops during the season (Thomas, 2007). The range of SBT carcass selenium levels is quite large compared to levels in samples reported previously (Thomas, 2007;Thomas and Buchanan, 2006) (core samples containing akami and chutoro). The levels measured in the flesh of SBT in the current experiment are in the lower and higher ends of the range reported previously (D'Antignana, 2007; Thomas, 2007; Thomas and Buchanan, 2006) (i.e. LTH1 and OS1 fish respectively). From the current results it is possible that the longer ranching period of the LTH fish resulted in gradual depletion of muscle selenium over time.

The fat level of the LTH1 group was ~ 2% lower than the OS1 group. This is probably a reflection of a lower CI of the LTH1 fish sampled at the time of harvest. Despite the lower fat level of the LTH1 fish, the muscle TBARS concentration was higher in this group. This is surprising as the oxidative products measured in the TBARS assay are derived from lipid, and it might be expected to increase in fish tissue with a higher fat concentration. However, the actual difference in flesh fat concentration was not large and may not have had any measurable effect on oxidative pressure. It should also be noted that the overall differences in TBARS, overtime and between groups within each sample time, was small and therefore the relevance of the changes should not be over interpreted.

The visual colour of the LTH1 fish muscle was not as stable as that of the OS1 fish. This means that the OS1 fish muscle retained a red colour longer during storage than the muscle of the LTH1 fish. High fat level is thought to provide an initiator of post mortem browning so it is surprising that muscle with lower fat would brown faster than more fatty muscle. Nevertheless the difference in fat concentration is not large and may not have had a large influence on post mortem muscle browning and other factors not measured here may have had an overriding influence on colour retention. The visual colour change seen in the muscle samples is supported by the instrumental colour measurements of hue and chroma. This indicates that the difference between the browning rates was large enough to be detected by the colour meter. As the colour meter is known to be less sensitive than the visual assessment, this detected difference strengthens the observation that the shelf life of the LTH1 fish muscle was shorter than the OS1 fish muscle.

Overall the differences in the concentration of Vitamin E, C and selenium that were apparent between the LTH fish and the OS fish at first harvest, were not found at the later time of Harvest 2. This lack of difference between the LTH and OS fish at Harvest 2 was likely because the levels of vitamin E and C in the flesh of the OS fish was maintained, but for the LTH fish the levels were higher and closer to those found in the OS muscle. The selenium level between the groups was similar at Harvest 2 but the trend of dropping muscle selenium during culture (Thomas and Buchanan, 2006) may have been evident here. At all times post mortem the TBARS levels in the flesh of fish of Harvest 2 were generally slightly higher than those of Harvest 1. However no difference between the groups, at either post-mortem sample time, indicated that the post-mortem oxidation process was occurring at a similar rate in the muscle of both groups.

That there was a significantly higher hue value for the LTH2 group on day 2, 3 and 4 is difficult to interpret. This difference is indicative of a faster browning of the LTH group; however this conclusion is not supported by the chroma values or the visual colour scores of the samples collected from Harvest 2.

For the chroma values, it is unusual to encounter colour differences on the day of harvest but this result may have been an artefact of the different delay between harvest and sample processing of the two groups. Particularly because, for all the sampling points following the initial one there was no difference in chroma between the two groups detected.

Summary

LTH SBT

Changes in the parameter measured in the LTH SBT over the sample period indicated that in these SBT:

From the transfer level muscle vitamin E concentration declined during 2005 and then appeared to recover by March 2006, remaining at that level until the last harvest (August 2006). Muscle vitamin C was low at transfer (as has been observed in other SBT groups at this time (D'Antignana, 2007; Thomas, 2007) but the level increased significantly by March and then again in August. Muscle selenium deceased from transfer and then remained at approximately the lower level up to the last sample period in August. The CI and muscle fat level showed a gradual increase over the ranching period from approximately 17 at transfer to 23 at the final harvest (August, 2006).

Production (OS), Long Term Holding (LTH) Comparison

The commercial constraints of this study, collection of samples and groups of SBT sampled from were less than desirable as a basis for a robust comparison. When viewing the conclusions, reference should be made to the methods and results. That said, conclusions should be considered with caution and the study used primarily as an indication of aspects of the LTH of SBT that might justify further investigation within the product quality area.

For Harvest 1, muscle vitamin E, C and selenium in addition to muscle fat concentration and fish CI were lower in the LTH fish group that the OS group. However, these parameters were not different for the two groups harvested at the time of the second harvest (i.e. August). Although it is difficult to compare the OS groups to one another, it is apparent that the LTH SBT vitamin levels rose during culture and the fat and condition increased. Although there was some evidence indicating that TBARS was slightly higher in the LTH group than the OS at both harvest times, the differences were small. However, from the results of this study, it is possible that post-mortem TBARS accumulation occurs more quickly in SBT that have been ranched for longer. More evidence would be required, than is presented here, in order to confirm this observation.

The instrumental colour and the visual colour scores indicated that for the SBT harvested in March (Harvest 1) the LTH group had faster colour deterioration than the OS fish. There was little evidence to indicate that there was any difference in the rate of colour deterioration between production fish and LTH fish later in the season (i.e. August – Harvest2).

Overall, in March (Harvest 1) lower muscle antioxidants and some evidence of higher oxidative products in LTH SBT correlated with reduced colour retention in this group compared to the one season production fish (OS). However, there was little difference in the muscle antioxidant or shelf life indicators between the production (OS) and LTH SBT later in the season (i.e. August).

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Acknowledgments

8.4 SBT Health

Investigation of the health of southern bluefin tuna (*Thunnus maccoyii*) farmed longer term (over 12 months)

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Introduction

The aquaculture of southern bluefin tuna (SBT) has been a major contributor to the expansion of the Australian aquaculture industry. The industry started in 1991 and increased with 5,185 tonnes of SBT being stocked into pontoons in 2002.

Despite the fact that most of Australia's available SBT quota is already entering the aquaculture sector, continued industry growth is expected due to further technical development. This includes: improved survival of SBT, LTH of SBT; improvements in product quality and value adding leading to better market prices; advances in feeds, feeding strategies, and ranching technologies leading to reduced operating costs.

Under the current production system, the SBT captured from the wild for grow-out are generally three years old, with assumed natural mortality levels in the wild of 20-30% per annum (CCSBT 2002). Therefore, the SBT captured for ranching have survived three years or more of health challenges. While the SBT industry has not been affected by disease outbreaks, further intensification of the SBT industry may increase the risk of health problems. Diseases can be prevented only when the risks are recognised and managed before the diseases occur. The identification of risk factors (hazard

identification or a risk register) is crucial for developing preventative measures (Thrushfield, 1995). Revision of SBT health risks (Nowak et al, 2003, FRDC 2001/253) discovered that very little information exists.

While a range of parasites has been identified in wild species of tuna (Langdon 1990, Oldewage 1993, Murugesh and Madhavi 1995, Petter and Cabaret 1995, Moravec et al 1999, Kohn et al 2001), few caused health problems in a culture situation (Munday et al 1997, Cribb et al 2000, Colquitt et al 2001). However, it is well known that an intensification of other aquaculture industries can result in the emergence of new health problems. Additionally, it has been suggested that some environmental factors, for example blooms of raphidophyte flagellate *Chattonella marina* may contribute to SBT mortalities during ranching (Munday and Hallegraeff 1998).

Aquafin CRC Project 3.1 (FRDC project 2001/253) identified the main risks in SBT health and recommended the design of a health surveillance and monitoring program. Parasites, in particular the blood fluke, *Cardicola forsteri*, ranked of highest concern. FRDC Project (2003/225) provided the link between the above mentioned desktop study and novel fish health research. At the time when this project started there was a general lack of knowledge about SBT health. This research provided a basic understanding of the effect of ranching practices on SBT health and epidemiology of the blood fluke. It also identified parasites and forms a basis for future SBT health research (including LTH), as well as developed and established sampling methodology, a health database and industry operational procedures and training for an innovative SBT health surveillance program.

This investigation will provide an indication health in an environment where SBT are held for a longer culture period.

Methods

Two and three-year-old wild southern bluefin tuna (SBT) (*Thunnus maccoyii Castelnau*) were purse-seined in the Great Australian Bight (map reference 33°27'S, 132°04'E) on February 19, 2005, transferred into a towing pontoon and towed to Port Lincoln. This cohort of SBT was then transferred into four pontoons each of 32m diameter by catching each individual SBT using a baited, barbless hook and line, measuring the length and weight and inserting a conventional dart tag before release into the destination pontoon. SBT were transferred on the following dates: April 5th (Pontoon 1), 6th (Pontoon 2), 8th (Pontoon 3) and 10th (Pontoon 4). SBT in each pontoon were fed on a different diet (Pontoon 1 - medium protein/medium fat, Pontoon 2 - low protein/high fat changed to medium protein/medium fat for week 7-12 post transfer and then to high protein/low fat for week 13-18 post transfer, Pontoon 3 - high protein/low fat, changed to medium protein/medium fat for week 7-12 post transfer and then to low protein/high fat for week 13-18 post transfer, Pontoon 4 - control diet/local Australian sardines) as part of a collaborative research project, and maintained at similar stocking densities (Table 4). Before stocking the pontoons, ten SBT were initially examined on April 5th, 2005.

Further samples of SBT (based on 10 tuna per pontoon) were examined for gill parasites at approximately six-weekly intervals, on May 31st, July 11th, and August 22nd, over a total period of just over 4.5 months. After this date, on 12th of October 2005, all tuna remaining in these four pontoons were transferred into a single pontoon, where they were maintained until the end of the experiment. All SBT were from the same cohort and diets fed to SBT were frozen bait fish, composed from different species to achieve different fat/protein ratios (see Table 1).

Table 1. Diets and stocking densities of SBT in pontoons in which ectoparasite burdens on gills were monitored.

Cage no.	Initial diet	Initial no. tuna	Stocking density (kgm^{-3}) a) initial	b) final
i) April-October 2005				
1	medium protein	219	0.456	0.466
2	high fat, low protein	219	0.487	0.499
3	low fat, high protein	220	0.529	0.529
4	local sardines	221	0.584	0.601
ii) October 2005-August 2006				
5	medium fat, medium protein			0.331

Thereafter, SBT were sampled at approximately three-monthly intervals: 30 on December 6th 2005; 30 in March 2006 (10 each on March 7th , 14th and 31st); and 30 on August 15th 2006 (Table 2).

In the second year of the trial, for comparison with parasite burdens on the LTH SBT, samples of *T. maccoyii* from cohorts farmed commercially within 2006 were also examined: 30 in March (20 SBT on March 24th and 10 SBT on March 28th; originally transferred to pontoon on February 15th, 2006); and 20 on August 18th, 2006 (originally transferred to the pontoon on March 17th, 2006).

Table 2. Sample data for a cohort of SBT examined over a 17 month trial ranching period (April in 2005 to August 2006), and two samples of control tuna farmed and harvested within 2006 (March and August 2006).

Sample Date	Water Temp (°C)	Weight kg ± SD	Length cm ± SD	Condition Index ± SD
A. Samples from LTH trial				
5 April 2005	19.3	13.4 ± 4.0	90.4 ± 7.3	17.7 ± 1.2
31 May 2005	17.7	23.0 ± 5.3	102.3 ± 8.7	21.3 ± 2.5
11 July 2005	16.5	20.6 ± 5.0	93.0 ± 7.6	25.1 ± 2.5
22 August 2005	14.6	25.4 ± 6.4	99.9 ± 9.0	25.0 ± 1.5
12 December 2005	19.1	30.6 ± 6.5	109.2±8.6	23.3±1.1
7, 14, 31 March 2006	20.5	30.8 ± 5.6	112.6±7.5	21.2±1.8
15 August 2006	13.6	39.8 ± 7.1	118.9±7.2	23.5±1.1
B. 2006 control cohort samples (from commercial farms)				
24, 28 March 2006	20.5	30.5 ± 6.8	107.0±8.5	24.6±1.7
18 August 2006	13.6	36.5 ± 8.0	114.5±9.2	24.0±1.3

At the time of experimental sampling, SBT were caught and processed according to standard commercial harvesting techniques. SBT were sampled using a small scale purse seine net within pontoons to isolate a portion of the school; each SBT was then captured individually by hand by divers on snorkel, and transferred onto the harvest vessel. SBT were then immediately pithed (spiked) through the head, bled from pectoral bleed cuts, cored using a *'Taniguchi tool'*, wired to destroy the upper spinal nerves, and the gills and

viscera were then excised. Fish were tagged and lengths and weights were recorded later, at the time of packaging on shore. CI was calculated using the South Australian tuna industry formula:

$$CI = \frac{Weight(kg)}{Length(m)^3}$$

Blood samples were collected for total blood haemoglobin concentration analysis and for the determination of specific anti- *Cardicola forsteri* antibody levels in blood serum (Aiken et al., 2008). Blood and blood serum samples were kept frozen until analysis. On board the harvest vessel, SBT were examined for presence of *Caligus spp.* on the skin and eyes. SBT gills were examined for parasites with the naked eye as soon as possible after each SBT was caught and processed (usually within 30min of death). Parasites were identified after referring to Cressey and Cressey (1980) and Hayward et al., (2007). *Cardicola forsteri* counts were determined as described in following section. Parasite infections were characterised, for each sampling date (and for the three pooled dates within March 2006), by prevalence (the number of host infections as a proportion of the population at risk) and mean intensity (the average number of parasites in each infected host) (Bush et al., 1997). As many zeroes were present in some intensity data in some sample dates (resulting in bootstrap confidence intervals for mean intensities being zero), mean abundance (the average number of parasites in all hosts - Bush et al., 1997) was also calculated for statistical comparison of parasite counts on different sample dates.

Sterne's exact 95% confidence intervals were calculated for prevalences, and 95% Bootstrap confidence intervals (with 2000 replications) were calculated for mean intensities and abundances, using the software 'Quantitative Parasitology 3.0' (Reiczigel and Rózsa, 2005). The data for each sample date were compared with other sample dates in a pairwise fashion using Fischer's Exact Test for prevalence, and Bootstrap t-Test for mean abundances. Given the high total number of pairwise comparisons (n=216), an alpha level of 0.01 was regarded as significant for these statistics.

A standard indirect ELISA was used to detect and quantify specific serum antibodies in SBT against *C. forsteri* (Aiken et al., in prep). Assay conditions were optimized empirically and reagent concentrations determined by chequerboard titrations (Crowther, 1995). Colour was allowed to develop for 30min and the optical density was measured at 405nm using a Spectra Rainbow Thermo microplate reader (TECAN Trading AG, Switzerland). Positive and negative standard sera, previously chosen from the preliminary ELISA and Western blot analysis, were titrated in duplicate on each plate

Caligus spp. infections

In 2005, an epizootic of *C. chiastos* was characterised by a significant increase in prevalence in the first six weeks after transfer to farms from the wild (from 0% to 55%); this was followed by a significant decline in this parameter over the next 12 weeks (to 0%, Figure 1); a single specimen of a second species (*Caligus* sp.) was also detected within this 4.5 month period (Hayward et al 2008b, 2009). In 2006, we recorded a third species of sea louse for the first time on SBT: *C. amblygenitalis* Tripathi, 1961. In March 2006, a second epizootic peak occurred (affecting both 2005 and 2006 cohorts), this time with mixed infections of *C. chiastos* and *C. amblygenitalis*, with a combined prevalence of 100% in 2005 SBT cohort. The peak was also present in 2006 SBT cohort, however the prevalence and mean abundance were significantly lower than in 2005 SBT cohort (Figure 1). The prevalence of both sea lice species then declined significantly over the winter period (Hayward et al 2009).

Mean intensity of infection also peaked in autumn (including second autumn in culture) and declined in winter (Figure 2). On all but one date that sea lice were detected, sea lice counts were significantly associated with the severity of gross eye damage. Because both peaks in infection occurred in the warmest months, we conclude that any risk that sea lice pose to the ranching of *T. maccoyii* within Spencer Gulf is increased under certain summer conditions (Hayward et al 2009).
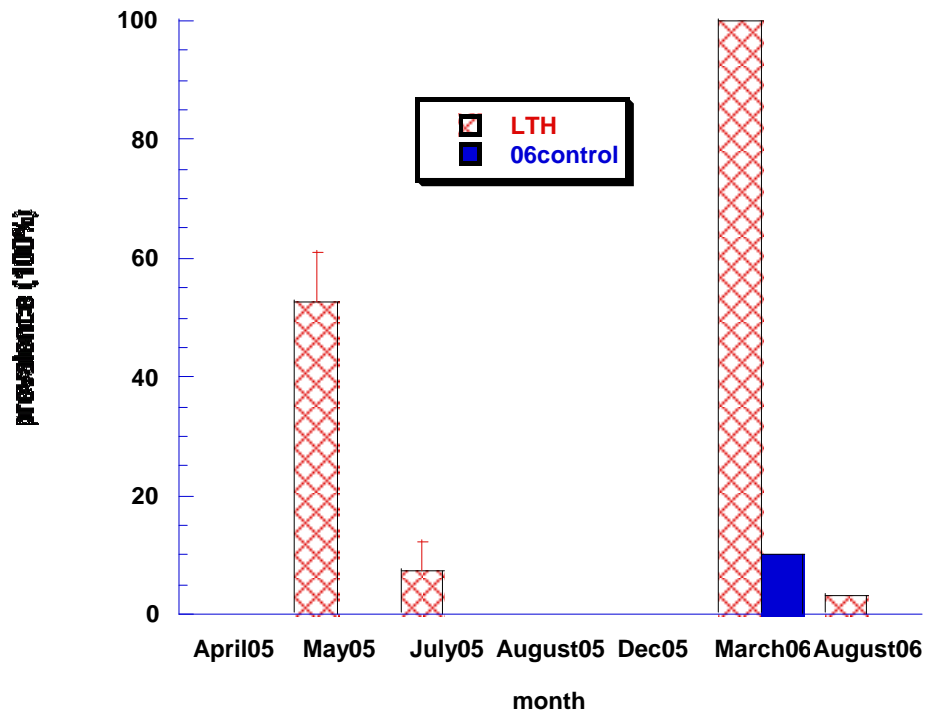
Figure 1. Prevalence of infection with skin copepod *Caligus spp.* in 2005 SBT cohort during LTH; prevalence in 2006 SBT cohort shown for comparison.
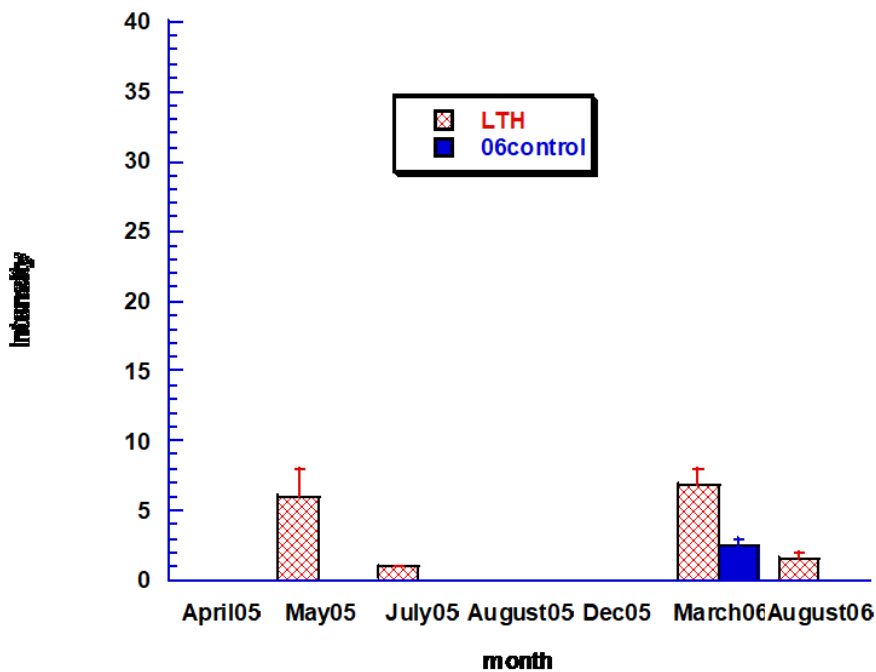

Figure 2. Mean intensity of infection with skin copepod *Caligus spp.* in 2005 SBT cohort during LTH; mean intensity for 2006 SBT cohort shown for comparison.

Gill parasites loads

Four species of metazoans were detected on the gills of *T. maccoyii*: two copepods, *Pseudocycnus appendiculatus* (Pseudocycnidae) and *Euryphorus brachypterus* (Euryphoridae); a polyopisthocotylean (monogenean) flatworm, *Hexostoma thynni* (Hexostomatidae), and an unidentified Cymothoidae gen sp. isopod. Among the long-term cohort of *T. maccoyii*, the overall prevalence for one of these species was over half of all the SBT (57.3% for *P. appendiculatus*, compared with 5.9% for *E. brachypterus*, 32.7% for *H. thynni* and 0.91% for the isopod), and this species was also generally more abundant in these fish (grand mean intensity was 6.87 for *P. appendiculatus*, whereas it was 1.38 for *E. brachypterus*, and 2.61 for *H. thynni* and 1.00 for the isopod). The maximum intensities on any single SBT reached for each species was 58 individuals of *P. appendiculatus*, 3 *E. brachypterus*, 23 *H. thynni*, and 1 isopod (Hayward et al 2008a).

A clear trend was evident for the gill copepod *Pseudocycnus appendiculatus*: initial prevalence rose significantly from an intermediate (40.0%) to peak in July 2005 (85.0%), then declined significantly over summer months to an intermediate level (43.3%), and declined even further in the second winter to the lowest level in this study (13.3%). In contrast, infection parameters of *Euryphorus brachypterus* and *Hexostoma thynni* remained at lower levels over the course of the LTH trial. For *E. brachypterus*, there were no significant differences in prevalences over time (despite the absence of this species from August 2005 onwards); for *H. thynni*, although the prevalence differed significantly between two pairs of months (being significantly higher in July 2005 (at 55.0%) than in both December 2005 and August 2006 (both 20.0%)), there were no significant differences among the remaining 21 combinations of monthly data, and thus no clear trend was evident over time (Hayward et al 2008a). Isopods were recorded on only two of 30 SBT in the August 2006 sample.

For each parasite species, very similar patterns to those observed in prevalence occurred in mean intensities and abundances. For *P. appendiculatus*, a clear trend was again evident, with mean intensity peaking

in the first winter (increasing from 1.75 per infected fish at the start of the study in April 2005, reaching 10.35 in July 2005), then gradually but significantly declining after this time, reaching a low of 2.75 in August 2006. For both *E. brachypterus* and *H. thynni*, numbers per fish remained much lower than those of *P. appendiculatus* throughout the study, with no significant differences between any months (Hayward et al 2008a). Both SBT infected with isopods in the August 2006 sample were infected with a single isopod each.
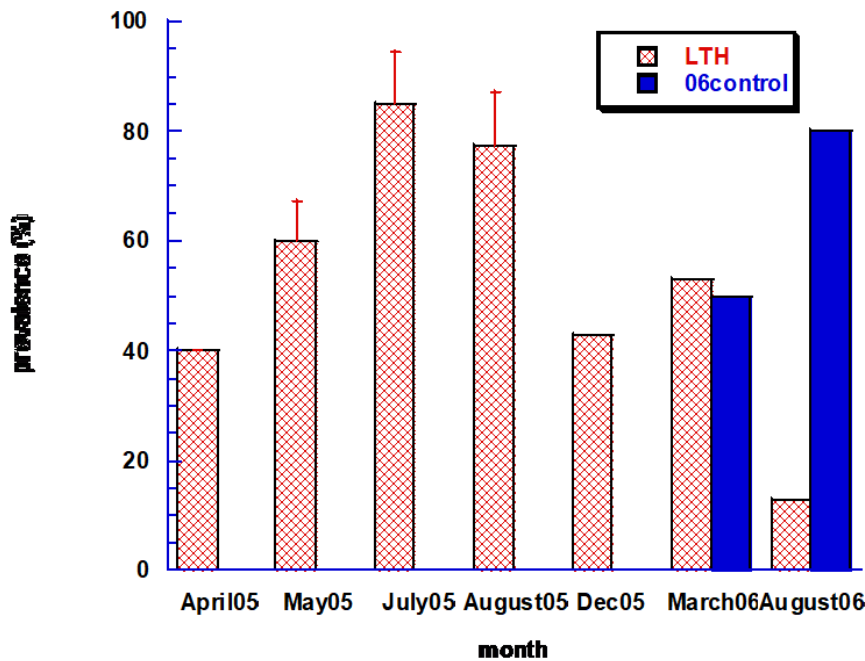

Figure 3. **Mean prevalence of infection with gill copepod *Pseudocycnus appendiculatus*. in 2005 SBT cohort during LTH; prevalence in 2006 SBT cohort shown for comparison.**
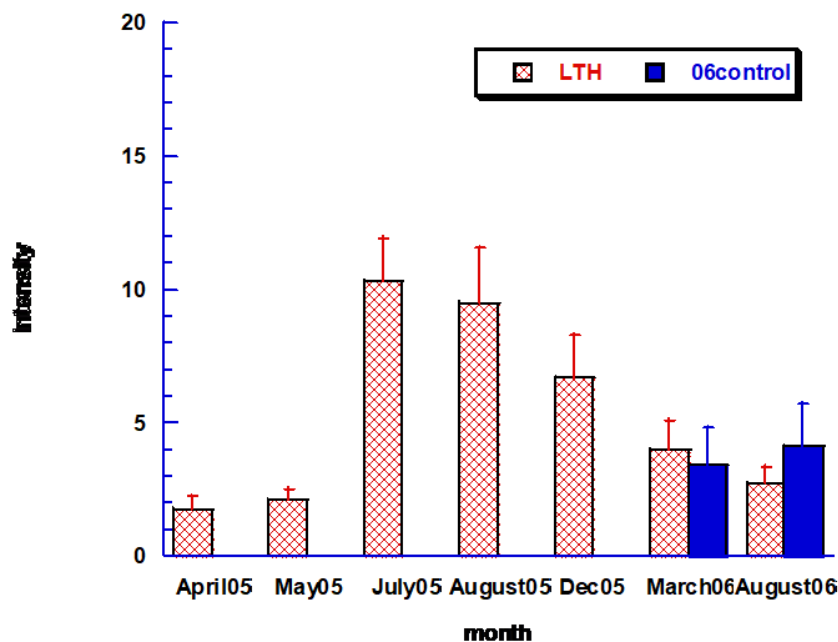
Figure 4. Mean intensity of infection with gill copepod *Pseudocycnus appendiculatus*. in 2005 SBT cohort during LTH, prevalence in 2006 SBT cohort shown for comparison.

In the control cohort of *T. maccoyii* in the second year of the study (that is, *T. maccoyii* farmed and harvested within 2006), prevalence and mean intensities of two species of parasites (*E. brachypterus* and *H. thynni*) did not differ significantly from those in the long-term trial fish, sampled after almost a year longer in captivity (Hayward et al 2008a). For *P. appendiculatus*, there was no significant difference in prevalence in trial and control fish in March 2006 (Figure 3); however, in August 2006, prevalence was significantly higher in 2006 cohort fish (80.0%) than in the 2005 cohort of fish (13.3%), but very similar to the level in 2005 cohort during their first winter on the farm (77.5%). For mean intensities of *P. appendiculatus*, in contrast, there were no significant differences among control and trial fish in either March or August 2006 samples (Figure 4). This suggests that the high prevalence observed in *P. appendiculatus* infections occurs only during the first winter on the farm (Figure 3).

Cardicola forsteri infections and specific antibody levels

No flukes were detected in the hearts of transfer SBT. Fluke prevalence peaked in May (2005) at 97.5% and then declined to reach a plateau from December (2005) (12.5%) to August (2006) (10%). Fluke intensity and abundance also increased after transfer to reach a peak intensity of 10.92 (S.E. = 1.72) fluke per infected SBT in May and 10.65 (S.E. = 1.7) mean abundance in May. The maximum number of flukes observed during the study was 42 from a fish harvested in May (2005). Blood fluke intensity and abundance also did not differ between cages for any month (Intensity: $ANOVA_{May}$, P=0.56, $ANOVA_{July}$, P =0.71, $ANOVA_{Aug}$, P=0.82, Abundance: $ANOVA_{May}$, P=0.71, $ANOVA_{July}$, P =0.78, $ANOVA_{Aug}$ P=0.27) and data from cages were pooled. Fluke intensity decreased after the peak in May (2005) to one fluke per infected SBT in December (2005) and abundance to 0.17 (S.E. = 0.07) mean fluke per fish examined in December (2005). Intensity in May (2005) was greater than all other sampling points (ANOVA, Fisher LSD test, P<0.05) and abundance in May and July (2005) was significantly greater than all other sampling points (ANOVA, Fisher LSD test, P<0.05). Infection abundance was significantly greater in 2006 cohort than 2005 cohort in March (2006) (ANOVA, P=0.011), and in August (2006) (ANOVA, P=0.007). However, significant differences were not observed between fluke intensities of the two cohorts in March 2006 and in August 2006. Fluke abundance of 2006 cohort was significantly greater in March (2006) than in August (2006) (ANOVA, P=0.009). Pearson Chi-square analysis showed that fluke prevalence was significantly greater in 2006 cohort than 2005 cohort in March (2006) (χ^2_{March}=22.86, P<0.001) and in August (2006) (χ^2_{August}=22.86, P=0.001).

Mean anti-*Cardicola forsteri* antibody level increased until December 05, then decreased in March and increased again in August 06, however there were no statistically significant differences detected (Figure 5, ANOVA, $P > 0.05$, Aiken et al 2008).
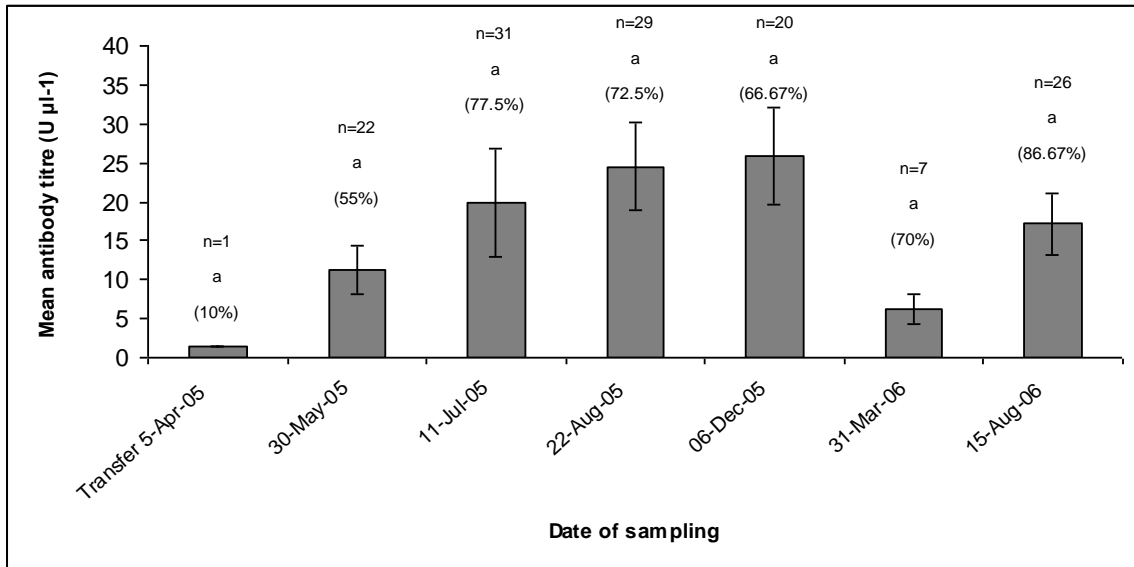
Figure 5. Mean anti- *Cardicola forsteri* antibody titre in SBT in 2005 SBT cohort during LTH.

However, there was a statistically significant difference between cohort 2005 and cohort 2006 in August 06 (Figure 6). Antibody titre for Cohort 2006 was approximately 4 times greater than for cohort 2005 at the same time post-transfer (August 05). This could be due to variation in immune response between the two cohorts or due to differences in the pattern of blood fluke infection in 2005 and 2006 (Aiken et al 2008).
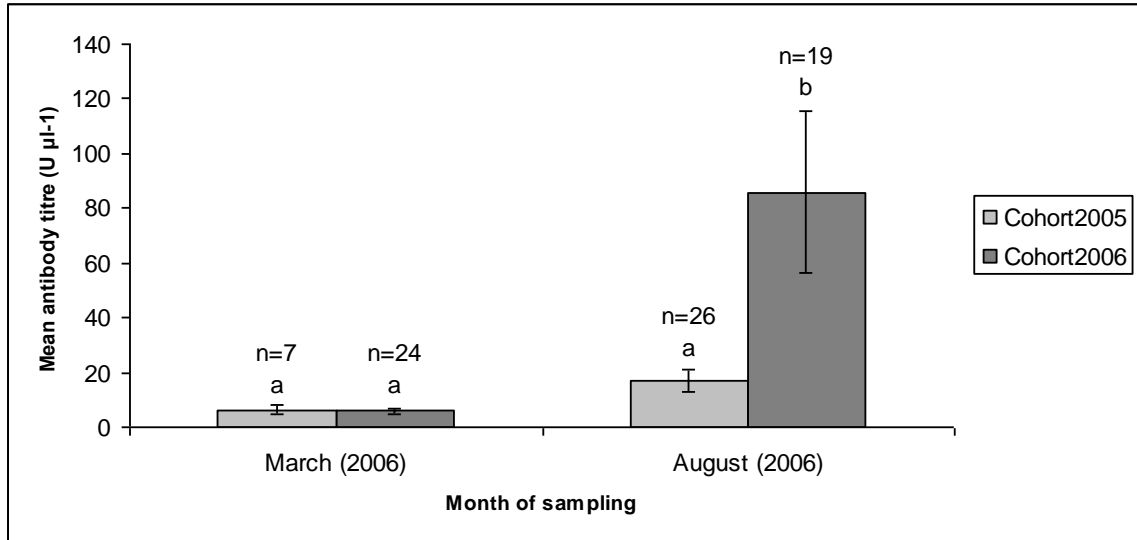
Figure 6. Mean anti- *Cardicola forsteri* **antibody titre in SBT in 2005 SBT cohort and 2006 cohort in March and August 2006.**

Haemoglobin

LTH had a significant effect on haemoglobin level in SBT blood. From day 139 in culture, blood haemoglobin concentration was significantly increased, peaking in December 05 (day 245 in culture). After day 139 blood haemoglobin concentration was greater than reported in wild SBT (Rough et al., 2005).
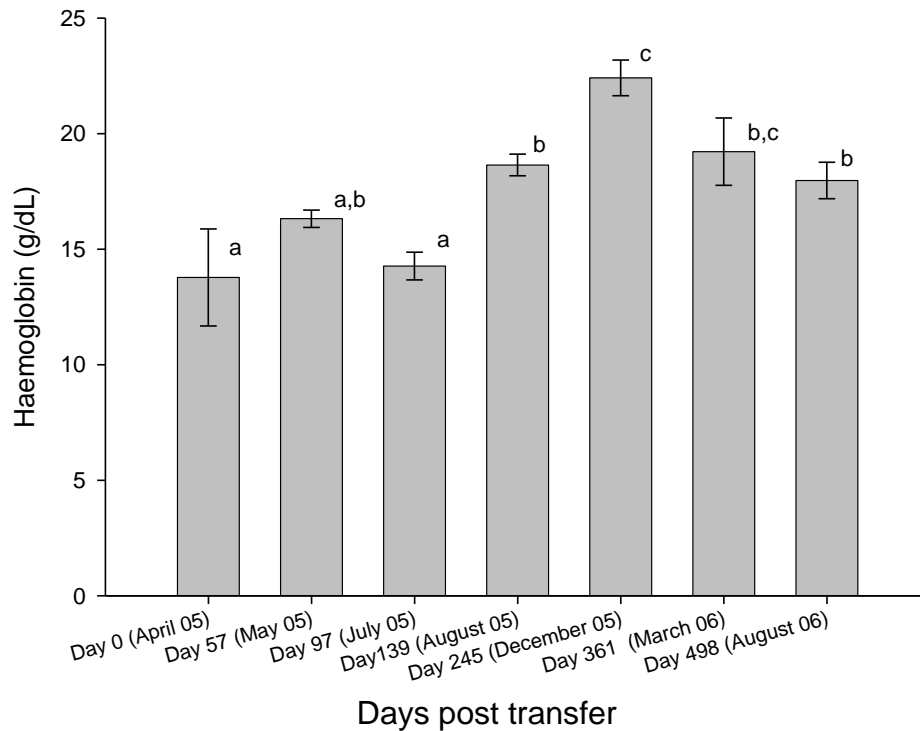
Figure 7. Time in culture had a significant effect on blood haemoglobin level in SBT (ANOVA). Different letters show means which were significantly different (P<0.05).

Overall, LTH did not have any adverse effects on SBT health. In particular, the prevalence and intensity of infection with gill copepod *Pseudocycnus appendiculatus* and blood fluke *Cardicola forsteri* were lower in the second season of farming. The only parasite which prevalence and intensity of infection increased in the second year and were greater than the 2006 cohort was skin copepod *Caligus* spp. There was a relationship between the numbers of this parasite and eye damage. It should be also noted that the final stocking density in the trial cage was very low. Furthermore, there was no cage replication over summer and in the second season.

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8.5 Environment

Effects of long term holding of southern bluefin tuna on infaunal assemblages

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Introduction

Holding southern bluefin tuna (SBT) for 12 to 15 months (i.e. longer term holding – LTH), longer than the current 3 to 8 month stocking period, will have implications for the ability of the sediments to assimilate any farm/ranch wastes reaching the seafloor. In particular, if the SBT are held over summer, the period when ranches were previously fallowed, warmer water temperatures may increase microbial activity, resulting in conditions that are different from those previously studied (late summer to autumn).

Macrobenthic infauna are the most widely known and accepted biological indicators of environmental degradation and restoration in marine sediments (e.g. Clarke and Green 1988; Austen et al., 1989; Warwick et al., 1990; Weston, 1990; Warwick and Clarke, 1991; Agard et al., 1993; Ferraro et al., 1994), because they encompass a diverse range of species covering a multiplicity of sizes, reproductive strategies, feeding behaviours and life histories. Collectively these communities change in response to a series of parameters including water quality, physico-chemical status of the benthos, and nutrient and organic carbon loading (Bilyard, 1987).

Macrobenthic infauna is expected to continue to be used for determining if the industry is meeting its regulatory requirements in terms of environmental impacts. From the now completed "Aquafin CRC/FRDC Project 2001/103: Aquafin CRC - Southern Bluefin Tuna Aquaculture Subprogram: Tuna environment subproject - Development of novel methodologies for cost effective assessment of the environmental impact of aquaculture", a complete

system has been developed to rapidly assess the "environmental health" of the seabed in the vicinity of SBT pontoons (Loo et al., 2006).

The full system, from sample collection through to data analysis and interpretation, has been implemented since 2002 by regulatory authorities in South Australia for routine environmental compliance monitoring of SBT aquaculture. With this move to using DNA-based assays for compliance monitoring, there is a need to better understand the response of this new technique to changes in infaunal assemblages. Therefore the work in this project involved assessing the effects of LTH of SBT on infaunal assemblages using both the traditional technique of sorting, identifying and enumerating infauna, and DNA-based assays of target infauna. Comparing both techniques for detecting any changes in infaunal assemblages resulting from LTH of SBT allows the DNA-based technique to be further validated, and thereby increases the sensitivity for setting trigger points for management purposes.

Materials and Methods

Field sampling and laboratory processing

Field sampling was carried out in October 2005 (before stocking with SBT already ranched for one season) to assess the natural variability of the site and again in February and April 2006 when the pontoon was stocked.

In October 2005, sediment samples were collected at various distances along a transect running north from the proposed location of the pontoon (0, 10, 20, 40 and 80m), as well as at a control site 1km away from any stocked pontoon. In February 2006, samples were collected from the 0m and 1km sites, and it was realised that due to an error in translation of the coordinates for the proposed pontoon site, the pontoon was located 160m from where it was originally expected to be. Therefore Sites PA1 to PA5 were considered to be pre-stocked sites located within the lease boundaries, and could have been in an area where ranching had previously occurred. In April 2006, samples were collected from 0m, as well as 60 and 150m north of the pontoon. The sampling design for April 2006 with three sites at increasing distance from the

stocked pontoon was again to investigate the potential gradient effect of ranching. The site codes and coordinates of all the sampling sites are given in Table 2.

Sixteen replicate sediment samples were collected using a HAPS Bottom Corer fitted with a 67mm diameter sampling tube. Eight of the samples were extruded from the sampling tube into 2-litre pre-labelled plastic jars, and then preserved in 10% Bennett's solution (1:1 propylene glycol and formalin). The other eight samples were extruded from the sampling tube into pre-labelled aluminium foil trays, sealed and frozen.

In the laboratory, the Bennett's solution in the eight preserved samples was decanted before the samples were processed. The samples were gently washed and screened using 1.0mm sieves. After the fine (<1mm) sediment had been washed from the sample, the retained material was washed into a large Petri dish. Animals in the retained sediment were picked out with the aid of a stereomicroscope and identified. The animals were then enumerated and preserved in 70% ethanol for storage. The eight frozen samples were dried before being processed using the SARDI DNA extraction system. Total DNA extracted was then quantified for the targeted infauna using the real-time PCR assays developed in the Aquafin CRC PCR project (Loo et al., 2006).

Table 2. Coordinates of the pontoon and control sites sampled in October 2005, February and April 2006 in decimal degrees (WGS84) and Northing and Easting (GDA94, Zone 53).

Sites		Latitude	Longitude	Northing	Easting
October 2005					
Control	CA	-34.69840	136.00225	6159945	591793
Pre-stocked pontoon					
0m	PA1	-34.71178	135.98490	6158477	590189
10m	PA2	-34.71172	135.98497	6158484	590196
20m	PA3	-34.71167	135.98497	6158489	590196
40m	PA4	-34.71207	135.98485	6158445	590184
80m	PA5	-34.71143	135.98557	6158515	590251
February 2006					
Control	CB	-34.69800	136.00148	6159990	591723
0m (stocked)	PB	-34.71022	135.98408	6158651	590116
April 2006					
Control	CC	-34.69820	136.00140	6159968	591715
Stocked pontoon					
0m	PC1	-34.71012	135.98398	6158662	590107
60m	PC2	-34.71058	135.98392	6158611	590101
150m	PC3	-34.71102	135.98327	6158563	590041

Data analysis

Multivariate techniques were used to analyse the two sets of data. Similarity matrices were computed using the Bray-Curtis similarity coefficient to eliminate the effects of joint absences of taxa. Prior to analysis, the data for manual enumeration were square root transformed to decrease the influence of dominant species, while the data for DNA assays were range standardised to reduce the effects of variable animal sizes on amount of extracted DNA.

Non-metric multidimensional scaling analyses (nMDS) were carried out on the Bray-Curtis similarity matrices, with goodness-of-fit in the ordination plots measured as stress with Kruskal's stress formula I (Kruskal and Wish, 1978). Each nMDS plot was examined to look for differences between control and pontoon sites and also across sampling times.

The Bray-Curtis similarity matrices were then compared to determine if there was any relation between the manual enumeration technique and the DNA assays. However, before comparison, the data for manual enumeration were also range standardised to ensure consistency in the treatment of the different data sets. Range standardisation has been used because it puts differently scaled variables on the same footing, thereby eliminating any signal other than relative amounts (McCune and Mefford, 1999). The multivariate analyses were carried out using routines in the software package PRIMER (Plymouth Routines in Multivariate Ecological Research) (Clarke, 1993).

Results and Discussion

Enumerated Infauna Data

Multivariate analyses of the data from manual enumeration of infauna indicated differences across sites and through time. The nMDS plot of abundance of infaunal assemblages for only the pontoon sites (PA1 to PA5, PB and PC1 to PC3) showed distinct separation of sites through time (Figure 10a). Sites sampled in October 2005 (PA1 to PA5) grouped to the left of the ordination plot and sites sampled in April 2006 (PC1 to PC3) grouped to the right while the site sampled in February 2006 (PB) was in the middle of the plot (Figure 10a). The horizontal axis of the ordination plot could represent either sampling time (seasonal variation) or increasing impact (left to right).

The control site for the three sampling times was positioned as close to the first sampling (CA) as possible based on GPS readings taken in October 2005, although subsequent locations were up to 50m distant.

When data from the control site were included in the analysis, the ordination plot showed the control sites sampled in February 2006 (CB) and April 2006 (CC) were in the middle of the plot between the PA and PC sites, while the control site sampled in October 2005 (CA) was well separated from the rest of the sites (Figure 10b). If the trajectory of the control sites indicated change in infaunal community structure due to seasonal variation, then none of the pontoon sites (both pre- and post-stocking) followed this trajectory. The infaunal community structure at the pontoon sites remained grouped according to pre-stocked sites (PA1 to PA5) and stocked sites sampled in February 2006 (PB) and in April 2006 (PC1 to PC3). This result indicates that, in addition to any seasonal variations, the pontoon sites may be responding to a temporal gradient of increasing organic enrichment. Sites PC1 and PC2, sampled latest (April 2006) in this study and in closer proximity to the pontoon (located at 0m and 60m from the pontoon), are presumably more organically enriched and are therefore separated to the right of the ordination plot (Figure 10a and b). Sites PA1 to PA5, located within the ranch lease boundary, sampled in October 2005 before stocking, were not grouped with the control site CA sampled at the same time, suggesting that there could be some impact of ranching on these sites carried over from the previous season.

As for the control site, harvesting for the 2004/05 ranching season would have been completed in October, and the new ranching season (2005/06) did not begin until late December to January. As such, no leases in the area had any stocked pontoons, explaining the separation of Site CA from all other sites (Figure 10b). Barring seasonal variation, Sites CB and CC might be expected to be generally more similar to CA and would group together in the ordination plot rather with the pontoon sites. However, Sites CB and CC grouped with the pre-stocked pontoon sites, in particular with Site PA2, indicating that these sites had infaunal assemblages more similar to Site PA2 than to Site CA. Furthermore Site CC is also close to PC3, sampled at the same time and located 150m from the pontoon.

This was substantiated by the Bray-Curtis similarity between these sites where CB and PA2 had a similarity of 67% while CB and CA had a similarity of 55%. This was also the case for Site CC where the Bray-Curtis similarity between Site CC and PA2 was 68%, with PC3 was 68% and with CA was 51%.

These results suggest that there is a trajectory of increasing organic enrichment moving from the control site (CA) sampled at a time when no stocked pontoons were in the area, to sites that may have been previously ranched (PA1 to PA5), to stocked pontoon sites PB sampled in February 2006 and PC3 located 150m from the pontoon sampled in April 2006, to potentially most impacted sites PC1 and PC2 (located at 0m and 60m respectively from the pontoon). There appears to be a lag in the move of the control site CB and CC towards the pontoon sites. This lag suggests that there might be a wider impact of ranching from surrounding leases, since most leases would have stocked pontoons by February 2006, even though the site is located at least 1km from any lease boundary.
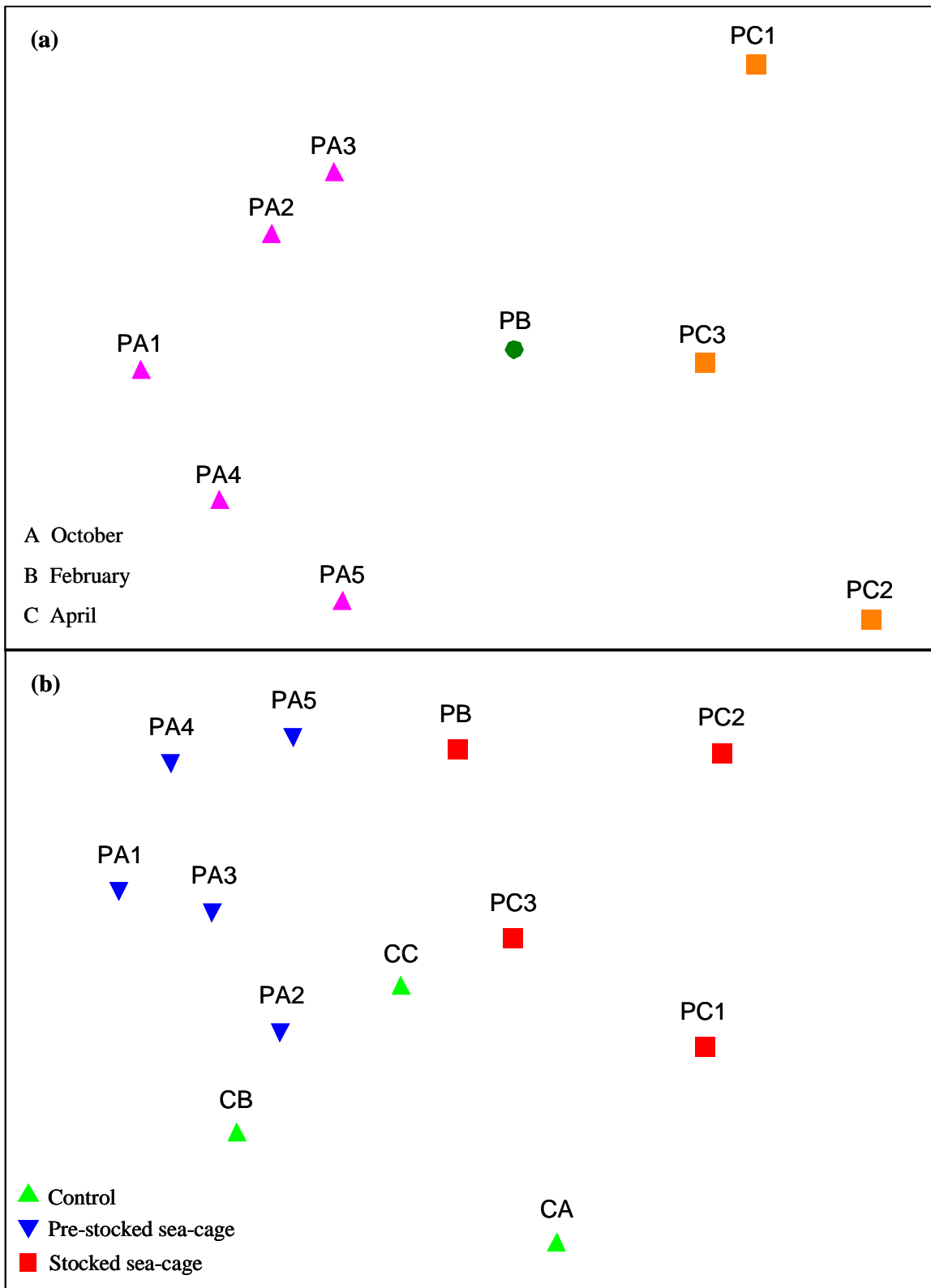
Figure 10. Two-dimensional nMDS ordination plots of square root transformed abundance data of infauna from manual enumeration of (a) pontoon sites only (stress = 0.09) and (b) all pontoon and control sites (stress = 0.13).

DNA Assay Infauna Data

Similar multivariate analyses carried out on data from the DNA assays also showed differences across sites and through time. The nMDS plot of DNA assay data for only the pontoon sites (PA1 to PA5, PB and PC1 to PC3) showed separation of sites through sampling times (Figure 11a). Pre-stocked pontoon sites PA1 to PA5 sampled in October 2005, and stocked pontoon sites PC1 to PC3 sampled in April 2006, were separated to the left and right of the ordination plot respectively. Site PB sampled in February 2006 was between these two groups (Figure 11a). This ordination was similar to that obtained for the enumerated infaunal data (Figure 10a) and again suggested that the horizontal axis of the plot could represent either seasonal variation or increasing impact (left to right).

Including the data from the control site in the multivariate analysis resulted in the pontoon sites maintaining a left to right ordination (PA1 to PA5, PB and PC1 to PC3). The control site sampled in October 2005 (CA) was again separated from all the other sites at the bottom of the plot while Site CB (sampled in February 2006) and Site CC (sampled in April 2006) grouped in the middle of the plot (Figure 11b).

Again, if the trajectory of the control sites followed seasonal variation, the pontoon sites (both pre-stocked and stocked) did not follow this trajectory. The pontoon sites remained grouped according to pre-stocked conditions (PA1 to PA5) and stocked conditions (PB and PC1 to PC3), indicating that the pontoon sites might be responding to a temporal gradient of increasing organic enrichment. The trajectory of change at the control site appeared to be towards the pontoon sites (Figure 11b). Site CB sampled in February 2006 was closer to pre-stocked pontoon sites (PA1 to PA5) than to Site CA. Site CC sampled in April 2006 was also closer to pontoon sites (PC1 to PC3) than to Site CA (Figure 11b). This was further substantiated by the Bray-Curtis similarity between CB and PA1 to PA5 (66%) being higher than CB and CA (44%).

Similarly, the similarity between CC and PC1 to PC3 (63%) was higher than CC and CA (35%). This trajectory of change in targeted infauna from DNA assays for the control site suggested that the differences might not be due to seasonal variation and instead might be indicative of increasing organic enrichment at the control site.
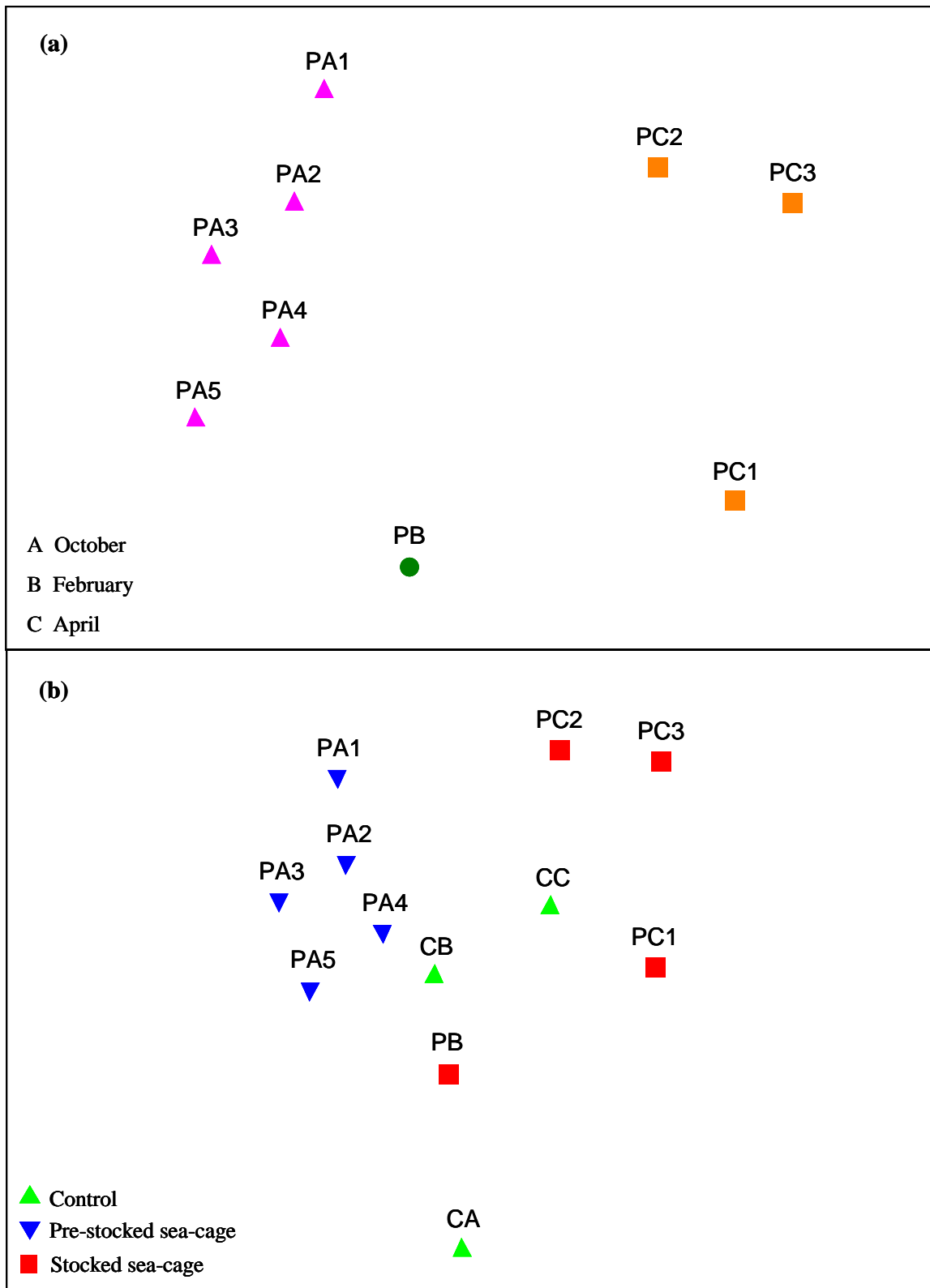
Figure 11. Two-dimensional nMDS ordination plots of standardised DNA assay data of (a) pontoon sites only (stress = 0.08) and (b) all pontoon and control sites (stress = 0.09).

Comparison of Enumerated and DNA Assay Infauna Data

Visual comparison of the nMDS ordination plots for enumerated infauna data and for DNA assay infauna data showed that the two plots were similar. For both plots, the pontoon sites (PA1 to PA5) grouped together on the left of the plot while stocked pontoon sites (PC1 to PC3) from April sampling grouped to the right and Site PB was in between (Figure 3 a and b). The control sites were also plotted similarly for both ordinations with Site CA separated from the other sites and Site CB and CC close to the pontoon sites (**Error! Reference source not found.**a and b).

Using the RELATE routine in PRIMER, comparisons can be made of how closely related two sets of multivariate data are for a matching set of sites. Comparisons of Bray-Curtis similarity matrices from both the enumerated infaunal data and DNA assay infaunal data gave a ρ statistic of 0.513, with a significance level of 0.6% for pontoon sites only and a ρ statistic of 0.249, with a significance level of 2.5% for all sites. These results meant that the ρ statistics were significantly different than zero ($\rho = 0$ for the null hypothesis of no relation between the two similarity matrices), indicating that the similarity matrices of the DNA assays and the manual enumeration were in agreement.
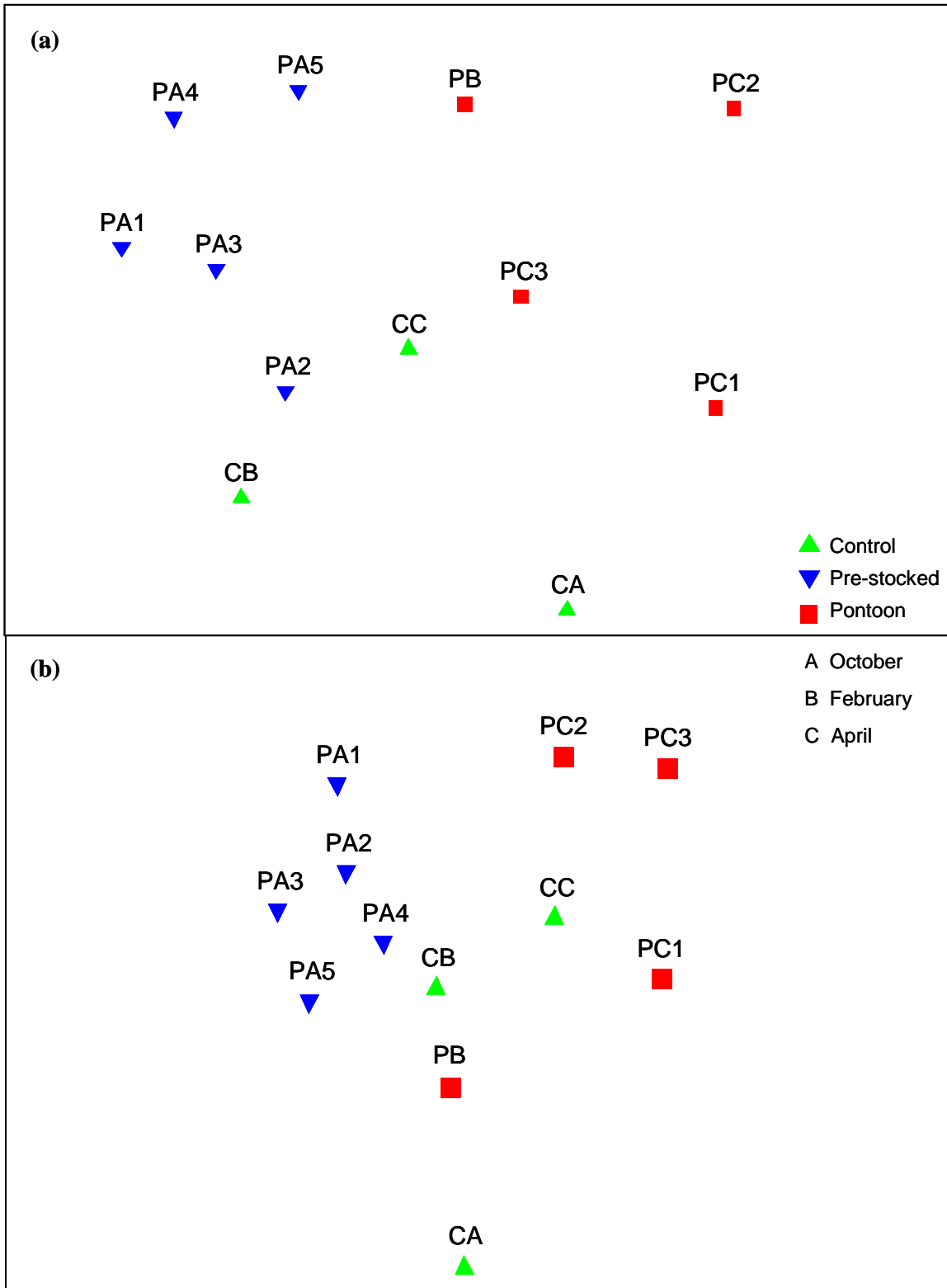
Figure 12. Two-dimensional nMDS ordination plots of (a) square root transformed abundance data of infauna from manual enumeration (stress = 0.13) and (b) standardised DNA assay data of infauna (stress = 0.10).

Conclusions

Both sample processing techniques, manual enumeration and DNA assays of benthic infauna, gave similar results and could differentiate control (CA, CB and CC) and pontoon sites (PB, PC1 to PC2) and even intermediate pre-stocked sites (PA1 to PA5). These results suggested that the changes in benthic infaunal assemblages through time were probably due to a temporal gradient, most likely increasing organic enrichment at the pontoon sites with LTH of SBT in the pontoon. Furthermore, the change in benthic infaunal assemblages at the control site towards one more similar to the pontoon sites indicated that these changes might be due to a wider impact of ranching in the region. However, this is not conclusive as there was only one control site and the purpose of this study was to investigate the effects of LTH on infaunal assemblages and not the effects of ranching in the aquaculture zone. Moreover, the work was carried out on a research pontoon where the stocking density is less than that of a commercial farm; therefore the extent of impact cannot be fully predicted for a commercial farm holding SBT throughout the year.

The comparable results obtained from the DNA-based technique also further validated the sensitivity of this technique and that it may be used confidently for environmental compliance monitoring of SBT aquaculture in Port Lincoln.

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Acknowledgements

Effects of long term holding of southern bluefin tuna on benthic assimilative capacity

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Introduction

The release of organic wastes from fish aquaculture into the marine environment increases nutrient availability, and reduces oxygen contents, in both the water column and sediments (Wu, 1995; Strain & Hargrave, 2005). The ranching of southern bluefin tuna in South Australia occurs in offshore waters where flushing is intense, resulting in the rapid dispersal of aquaculture wastes whilst reducing adverse effects to the pelagic and benthic ecosystems (Bierman, 2005). The unusually high metabolic rates of these endothermic fish account for a higher nitrogen load to the environment when compared to other commercial species such as salmon (Fernandes et al., 2007a). Although high, nitrogen loads are primarily delivered as dissolved wastes and the effects to the benthos are small. Benthic nutrient fluxes and oxygen uptake rates increase rapidly underneath the pontoons as solid wastes reaching the seafloor are remineralized and released back into the overlying water column (Lauer, 2005; Fernandes et al., 2007b). As a consequence, accumulation of nutrients in the sediments is small and only noticeable towards the end of the stocking period.

Despite minimal known effects at current stocking densities and holding periods, tuna operators are required to fallow farmed sites for 12 months. This waste mitigation strategy aims at reducing localized effects through remineralization and assimilation of excess organic matter over time. Current practices also mean that sites are stocked towards the end of summer and harvested towards the end of winter, with a 4 to 7 month grow-out period. As a

consequence, the entire offshore zone is free of ranching roughly between September and January, giving the system at least 4 months for recovery.

Holding southern bluefin tuna for 12 to 15 months could have implications for the ability of sediments to turnover nutrient inputs, particularly if fish are held over summer. Warmer water temperatures in summer could increase microbial activity and lead to conditions that are detrimental to ranching (e.g. low oxygen, degassing from sediments, high ammonia levels). In this study, the effects of these seasonal changes on the capacity of sediments to recycle nutrients were investigated. For this purpose, nutrient benthic standing stocks and fluxes, oxygen uptake rates, and sediment redox profiles, at both a research pontoon and a control site were monitored in the spring of 2005, and summer and autumn of 2006. This sampling strategy was used to determine if the carrying capacity of the region is limited by processes occurring over the summer period, when fish have traditionally not been held. To assess interannual and seasonal variability, results from the control site monitored in this work were combined with data from a control site monitored in the same area in 2004 using the same methods (Lauer, 2005; Fernandes et al., 2007b). To assess the impact of holding fish over summer, data from the research pontoon in this work were compared with a commercial pontoon stocked at the end of summer in the same area and monitored in 2004 using the same methods (Lauer, 2005; Fernandes et al., 2007b).

Materials and Methods

Sampling

Samples were collected in October 2005 (before stocking) and in February and April 2006, at the edge of the research pontoon and at a control site more than 1 km from any stocked pontoon.

Sediments were collected with 67 mm (i.d.) stainless steel tubes using a HAPS Corer (KC Denmark). Upon retrieval, the overlying water in the tube

was carefully discarded to minimise surface disturbance and the sediment extruded onto a clean stainless steel table. Four cores were collected for the analyses of organic carbon, total nitrogen and total phosphorus. The top layer (0-1 cm) of each core was sliced, transferred into a pre-combusted glass jar and stored on ice in the field before long-term freezer storage at -30 °C. Two cores were collected for the analysis of nutrients in porewaters. The top layer (0-2 cm) of each core was sliced, transferred into a centrifuge tube and stored on ice for up to 3 h before transfer to the laboratory.

Four to six intact cores were collected for determination of redox profiles and nutrient fluxes at the sediment-water interface. The tubes used for collection of these cores were made of PVC and impervious to light, with an internal diameter of 105 mm. The cores had a visibly undisturbed sediment surface, a minimum of 800 mL of clear overlying *in situ* bottom seawater and at least 10 cm depth of sediment.

Analytical procedures

Organic carbon, total nitrogen and total phosphorus

Sediment samples were freeze-dried, sieved to 500 □m to remove large shell fragments, and homogenized with a mortar and pestle. Aliquots for organic carbon analysis were pre-treated with 1 N hydrochloric acid to remove carbonates, rinsed with MilliQ water to remove hygroscopic salts and oven-dried at 40 °C using a method modified from Hedges & Stern (1984). Organic carbon contents were determined in a LECO TruSpec CNS Elemental Analyser and corrected for carbonate content, measured with a pressure transducer (RS Components, part 348-8065, Iso-Tech voltameter IDM 91) after acidification of aliquots with 5.5 N hydrochloric acid.

Total nitrogen contents were determined by Continuous-Flow stable Isotope Ratio Mass Spectrometry (CF IRMS) using a Europa Scientific ANCA-SL elemental analyser coupled to a Geo 20-20 mass spectrometer.

For determination of total phosphorus, samples collected in October and February were digested with nitric acid, hydrogen peroxide and hydrochloric acid (US Environmental Protection Agency, 1991), whereas samples collected in April were digested with aqua regia (1:3 HNO_3/HCl mixture) (Standards Australia, 1997). The extracts were analysed in a Varian Vista Axial ICP-AES.

Porewaters

Samples were centrifuged at 3,000 rpm (1,400 g) for 10 min, filtered (0.45 µm) and stored frozen (-30 °C). Ammonium (APHA-AWWA-WPCF, 1998a) and phosphate (APHA-AWWA-WPCF, 1998b) were determined by flow injection analysis with a QuickChem 8000 Automated Ion Analyser.

Benthic nutrient fluxes and oxygen uptake rates

Benthic fluxes were measured on board immediately after sampling with a manually operated incubation system (Lauer, 2005), consisting of a temperature sensor connected to a data logger (DT 50, Datataker) and a temperature-controlled water bath. The water bath consisted of a 200 L PVC outer container filled with ice and a 60 L polystyrene inner container filled with freshwater. The PVC barrels sealed at both ends were placed in the inner container, which had a pump to circulate the water and an aquarium heater to maintain water temperature. Each core was stirred with a single blade (7 mm wide, 4 mm long) magnetic stirrer to prevent stratification. The temperature was set at ambient bottom seawater temperature measured at the time of collection. The system maintained temperatures to ± 0.8 °C of the set value. Incubations were designed to last for 2 to 4 hours to limit the likelihood of non-linear changes. Dissolved oxygen concentrations were determined with a HQ-10 (Hach) DO-meter and samples of the overlying seawater were taken in duplicate from each core, filtered (0.45 µm) and stored frozen (-30 °C), at both the start and end of the incubation. Ammonium (APHA-AWWA-WPCF, 1998a), nitrate/nitrite (NOx) (APHA-AWWA-WPCF, 1998c) and phosphate

(APHA-AWWA-WPCF, 1998b) were determined by flow injection analysis with a QuickChem 8000 Automated Ion Analyser.

Redox potential

Oxidation-reduction potential (redox) profiles were measured at the end of the incubations using a platinum electrode with a calomel reference (ORP, Phoenix). Surface redox was obtained by inserting the 10 mm electrode tip into the top of each core, whereas depth profiles were measured 20 mm apart (centre to centre) to a depth of 8 cm. These profiles were obtained by inserting the electrode into 12 mm diameter holes pre-drilled along the side of the PVC barrels, after removal of the rubber grommets used to seal the holes during collection and incubation. The redox probe tip was rinsed in seawater before each insertion and measurements were allowed to stabilize for 20 s. Voltage output (mV) measurements were adjusted relative to the normal hydrogen electrode. Redox measurements were not made in April 2006 due to equipment failure.

Statistical analysis

Results were analysed with the software package STATISTICA (StatSoft, Tulsa, OK). Analysis of variance (ANOVA) was used to identify statistical differences ($\square$ = 0.05) with treatment (pontoon *versus* control) and/or season as fixed effects. When required, data were inverse-transformed prior to analysis to improve normality and heterogeneity of variances. In the case of benthic nutrient fluxes, data were $\log_{10}$ transformed after a constant was added to each score so that the smallest score was 1. Oxygen uptake rates were also $\log_{10}$ transformed. Although data were transformed for statistical analysis, results were plotted in the figures as the original untransformed data.

Results and Discussion

A 2-way ANOVA with season and treatment as fixed effects indicated that the concentration of organic carbon ($F_{(2, 18)}=0.19$, $p=0.825$) and nitrogen ($F_{(2, 18)}=0.26$, $p=0.773$) in sediments did not change over time, while the concentration of phosphorus significantly increased under the pontoon after stocking ($F_{(2, 18)}=9.29$, $p=0.002$) (Figure 1). The higher phosphorus contents observed in autumn for both the research pontoon and its control site might reflect a different extraction procedure used for these samples. The increase in phosphorus concentrations after stocking was only 15% above background levels, whereas concentrations measured under a commercial pontoon in the same area in 2004 were up to 3 times higher than natural values (Figure 2). Commercial pontoons are stocked in summer, and although there is no significant build up of organic carbon in sediments throughout the season, higher levels of nitrogen and phosphorus are observed towards the end of the stocking period in winter, which persist until late spring (Figure 2). Although the commercial pontoon used for comparison purposes was monitored two years earlier in 2004, levels recorded at control sites in the same time period suggest negligible interannual and seasonal variability when compared to the impact of commercial pontoons (Figure 3).
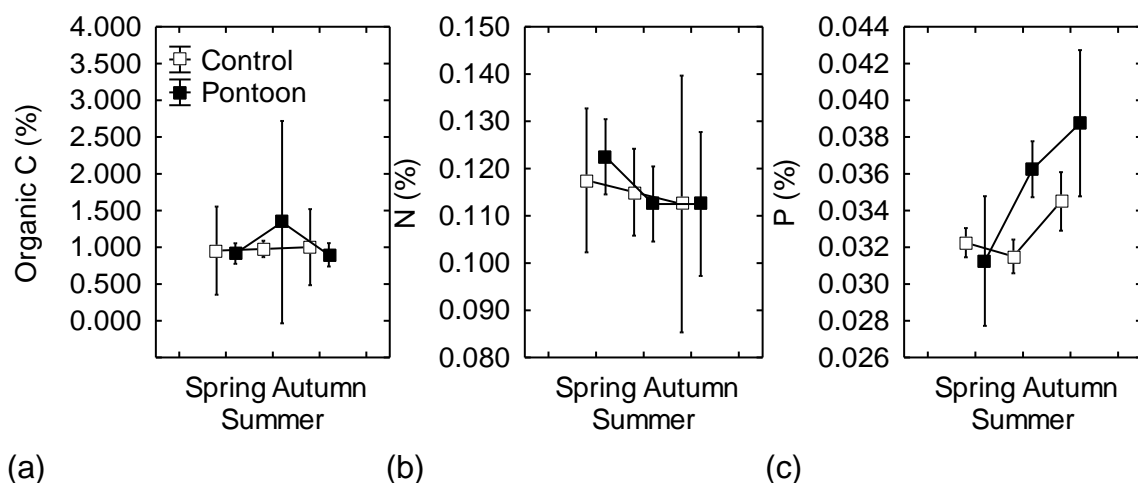
Figure 1. Organic carbon (a), nitrogen (b) and phosphorus (c) contents of sediments under the research pontoon *versus* its control site in the spring of 2005 (before stocking) and

summer and autumn of 2006. Values are reported as the mean and error bars denote 95% confidence intervals (± 1.96 SE, n = 4).

(a)

(b)

(c)
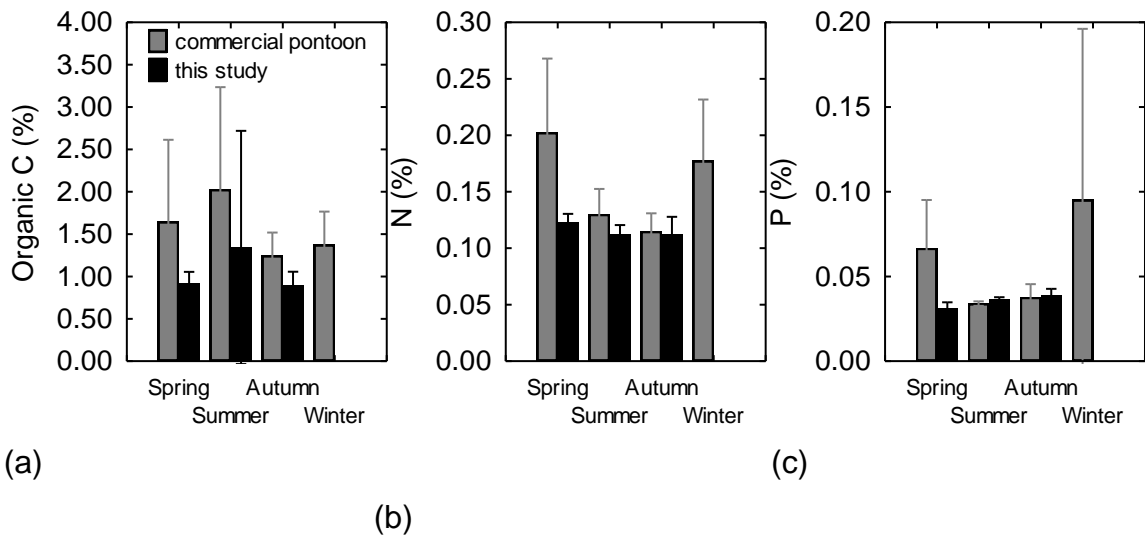
Figure 2. Organic carbon (a), nitrogen (b) and phosphorus (c) contents of sediments under the research pontoon monitored in this study in 2005/2006 against a commercial pontoon monitored in the same area in 2004 (Lauer, 2005; Fernandes et al., 2007b). Values are reported as the mean and error bars denote 95% confidence intervals (± 1.96 SE, n = 4).

(a)

(b)

(c)
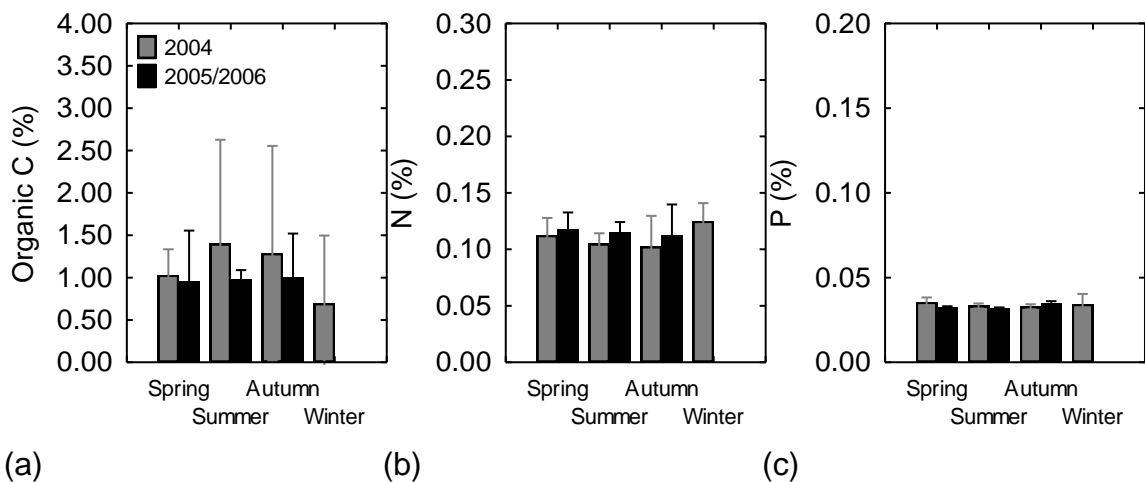
Figure 3. Organic carbon (a), nitrogen (b) and phosphorus (c) contents of sediments in the control site monitored in this study in 2005/2006 against a control site monitored in the same area in 2004 (Lauer, 2005; Fernandes et al., 2007b). Values are reported as the mean and error bars denote 95% confidence intervals (± 1.96 SE, n = 4).

The same general trend was observed for the standing stocks of nitrogen and phosphorus in porewaters and associated benthic fluxes (Figures 4 and 5). While a 2-way ANOVA with season and treatment as fixed effects showed no change of ammonium concentrations in porewaters ($F_{(2, 6)}=0.57$, $p=0.591$) and inorganic nitrogen fluxes (ammonium + nitrate/nitrite) ($F_{(2, 20)}=0.87$, $p=0.433$) above background levels, phosphate concentrations ($F_{(2, 6)}=6.73$, $p=0.029$) and fluxes ($F_{(2, 18)}=11.82$, $p=0.0005$) were significantly higher under the pontoon after stocking. Inorganic nitrogen fluxes were typically dominated by ammonium, with highest relative input in summer and under the pontoon in autumn, although this trend was not significant (Figure 6). Phosphate concentrations in porewaters were up to 5 times background values in summer, and 8 times in autumn; to put these data in perspective, values recorded under a commercial pontoon in the same area in 2004 reached as much as 20 times background values. The impact of the research pontoon on phosphate fluxes was even more marked, with values reaching up to 14 times background values in summer and 22 times in autumn. However, this increase is still small when compared to a commercial pontoon, where phosphate fluxes from sediments can reach more than 220 times background values. Data from control sites monitored in this work and in 2004 indicate no significant interannual variability of background conditions. A 1-way ANOVA with season as fixed factor revealed slightly higher concentrations of ammonium in porewaters ($F_{(2, 9)}=8.74$, $p=0.008$) and benthic fluxes ($F_{(2, 19)}=5.00$, $p=0.018$) in summer than spring, but no clear seasonal pattern for phosphate concentrations ($F_{(2, 9)}=2.89$, $p=0.107$) and fluxes ($F_{(2, 18)}=2.87$, $p=0.083$).

Figure 4. Temporal variability in ammonium (a) and phosphate (b) concentrations in sediment porewaters under the research pontoon *versus* its control site in the spring of 2005 (before stocking) and summer and autumn of 2006. Values are reported as the mean and error bars denote 95% confidence intervals (± 1.96 SE, n = 2).
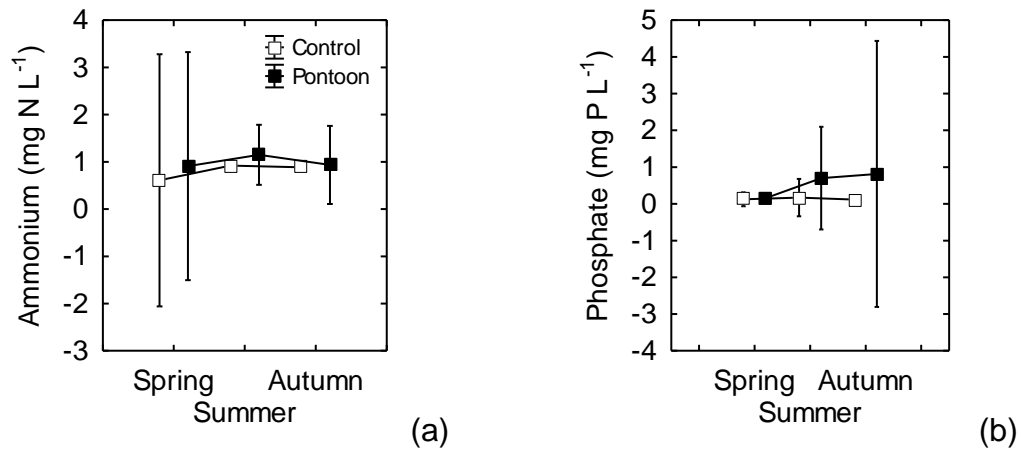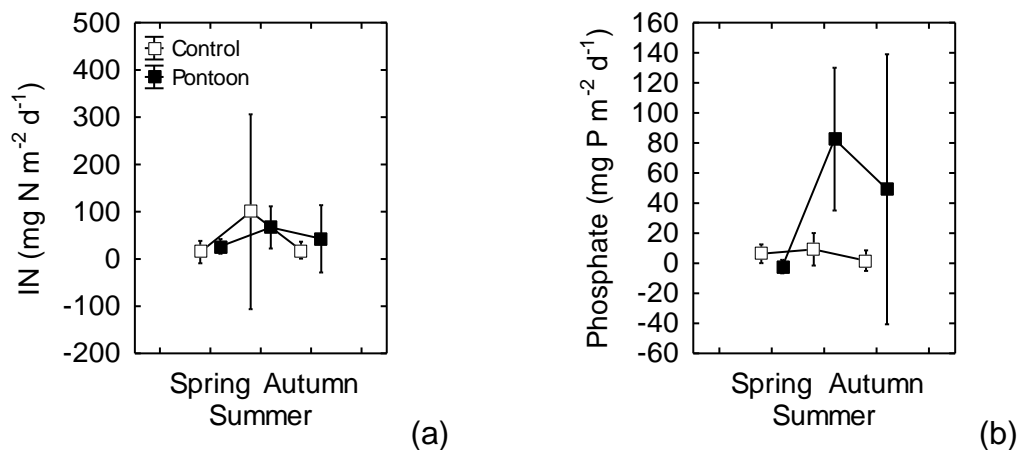

Figure 5. Temporal variability in inorganic nitrogen (ammonium + nitrate/nitrite) (a) and phosphate (b) benthic fluxes under the research pontoon *versus* its control site in the spring of 2005 (before stocking) and summer and autumn of 2006. Values are reported as the mean and error bars denote 95% confidence intervals (± 1.96 SE, n = 4 or 6).
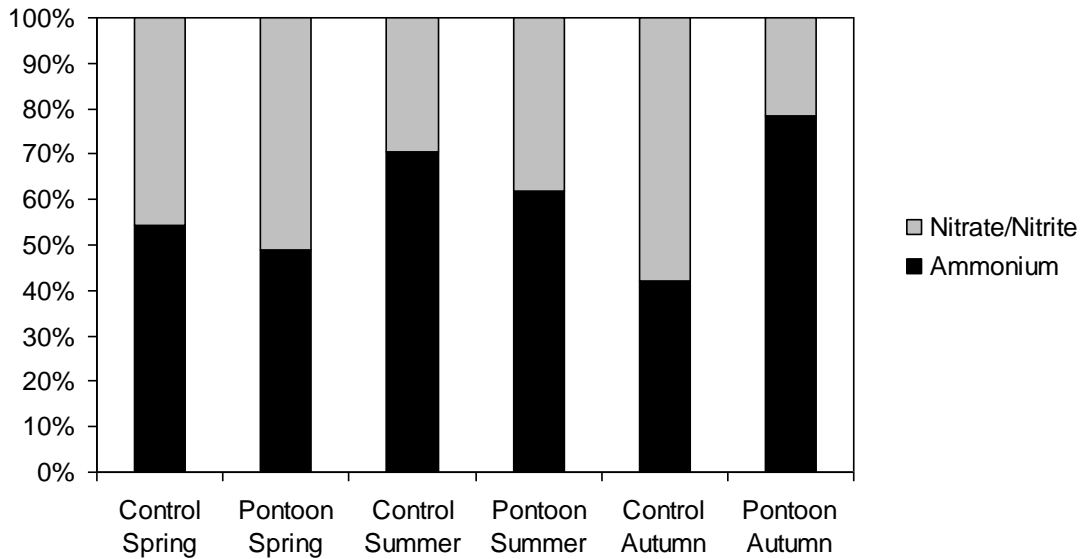
Figure 6. Mean contribution of ammonium and nitrate/nitrite to inorganic nitrogen benthic fluxes.

Although higher oxygen uptake rates were recorded after the research pontoon was stocked (Figure 7), this increase was not significant due to the variability of the dataset, suggesting that any effect of ranching was not homogeneous across the seafloor. Although somewhat higher oxygen uptake rates were recorded in summer at the control sites monitored in 2004 and 2006, this increase was also not significant. Based on these data, there is no marked interannual or seasonal variability in benthic oxygen uptake rates.

There was also no significant change in sediment redox profiles as a result of ranching over summer, although a 2-way ANOVA with season and depth as fixed effects showed a decrease in oxygen penetration in summer ($F_{(4, 80)}=5.73$, $p=0.0004$) leading to negative redox values below 2 cm depth for both control and pontoon sites (Figure 8).

Figure 7. Temporal variability in benthic oxygen uptake rates (OUR) under the research pontoon *versus* its control site in the spring of 2005 (before stocking) and summer and autumn of 2006. Values are reported as the mean and error bars denote 95% confidence intervals (± 1.96 SE, n = 4 or 6).
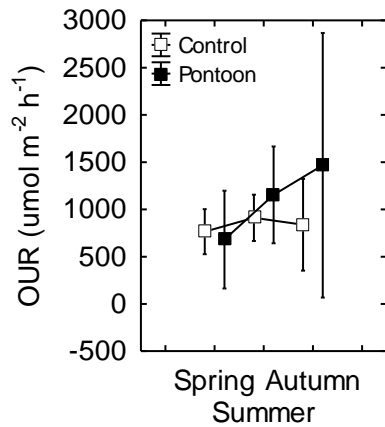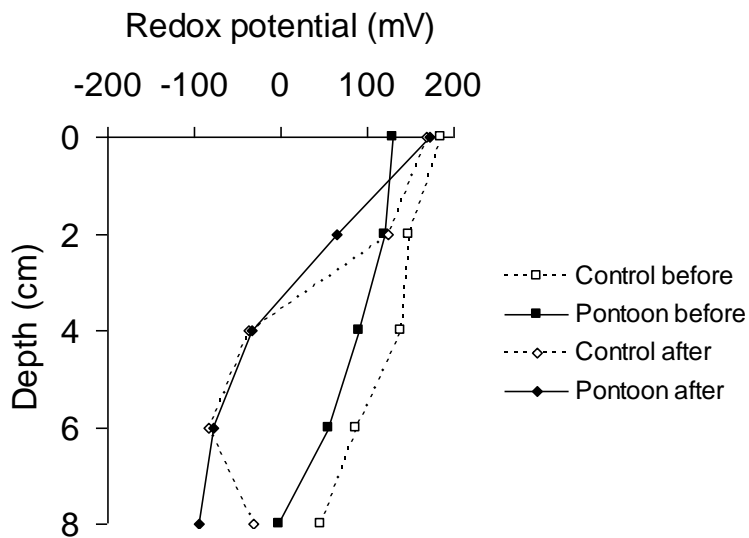

Figure 8. Redox potential profiles at the research pontoon *versus* its control site before stocking in October 2005 and after stocking in February 2006. Values are reported as the mean (n = 4 to 6).

Conclusions

This very limited dataset suggests no marked seasonal trends in benthic assimilative capacity. Although the slightly higher concentrations of ammonium in porewaters, higher ammonium benthic fluxes, and lower oxygen penetration during summer indicate an increase in benthic activity at this time of the year, these changes were small.

Phosphorus mineralization increased under the research pontoon after stocking, but values were significantly lower than found under a commercial pontoon. While the impact of the research pontoon was comparatively small, this pontoon was stocked to only a fraction of commercial capacity. It is therefore difficult to predict the impact of a commercial operation stocking tuna throughout the year based on these data. If long-term holding of southern bluefin tuna was to become a significant practice within the industry, research should focus on determining the effects of pontoons stocked to commercial capacity over an entire stocking cycle.

Acknowledgements

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8.6 Economic Analysis

Economic analysis of longer term holding of southern bluefin tuna

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Introduction

In 2003, the southern bluefin tuna (SBT) industry sought to change one program in the Aquafin CRC from propagation to longer term holding (LTH). After considerable debate, the CRC agreed to this change and had it ratified by the Federal Government CRC Secretariat.

It became clear to the SBT farming/ranching industry in 2003 that LTH was a major priority for the industry. This was due to the following factors – largely economic and market-driven:

1. SBT ranching is a very young industry and is rapidly developing. It was only in 2003-2004 that the longer-term outlook became clearer.

2. The Australian SBT ranching industry is a global producer, with tuna ranching sites in Europe and North America, the other two major ranching regions. From 2002 we gained considerable experience in LTH sites in Croatia. This indicated that many of our previous concerns about LTH (e.g. flesh quality, and possibly growth rates) were not justified.

3. The major barrier to LTH of SBT has always been the marginal cost. This marginal cost is largely feed. In 2002 and 2003, it became clearer that the Australian feed supply was sustainable at a high level.

Therefore, the feed cost appears to have been structurally and substantially reduced to a level to make longer term grow-out economic, given certain assumptions.

4. It became clearer that there is a structural change in Japan, the dominant market for sashimi tuna in the world. This reflects much slower economic growth - resulting in a stalling of total demand for high quality tunas. The only way for us to address this is to add value to our current product.

5. For value adding, it was also clear that the market is maintaining a large advantage for SBT over 40kg (compared with under 40kg). With our current production system, we are averaging harvest weights of 27-33kg. Longer-term grow-out would allow fish over 40kg.

6. A major competitive advantage for Australian SBT ranching is very low mortality rates. Neither of our major competitors - the Mediterranean and Mexico - have these advantages. Long term grow-out would maximise Australia's advantage.

7. It was also more evident that SBT has a significant yield disadvantage compared with our major competitor, Northern Bluefin Tuna (NBT) in the Mediterranean and Mexico. The yield disadvantage decreases as we move along the SBT growth curve.

LTH of SBT will also provide marketing flexibility. Ranchers will have the ability to hold stock over until markets improve. The industry was financially damaged in 2003 and 2004 when a low price offered by Japanese buyers led to early selling or costly storage because the option of LTH of live SBT had not been thoroughly investigated.

Economic Assumptions and LTH Assessment

The following economic assumptions have been generalised and do not accurately reflect individual SBT ranching company structures. SBT ranching company's assessing this information will be required to use their own business structure information when evaluating the economic potential of LTH.

Table 1. The general business structure of current SBT ranching of 3-6 month grow-out

Item	Assumption
Average catch weight[1]	18kg
Cost of catching/towing to farm[3]	$3.50/kg
Cost of feed - 2002[3]	$0.90/kg
Cost of feed - 2004[3]	$0.51/kg
Feed conversion ratio[3]	12:1
Whole weight added in farms (3-6 mths)[1]	93%
Feed cost of adding kg - 2002 (feed cost)[3]	$10.80/kg
Feed cost of adding kg - 2004 (feed cost)[3]	$6/kg
Av. whole weight of individual SBT harvested[1]	34.7kg
Av. whole weight of individual SBT added in farm[1]	16.7kg
Processed/whole weight ratio[3]	0.87
Av. processed (market) weight of individual SBT[3]	30.19kg
Ex-farm price (at $=Yen 70)[2]	$20/kg

Notes to the table:
[1]Australian Fisheries Management Authority. Average weight 15kg.
[2]Japanese Ministry of Finance, assuming $A = Y70
[3]Australian Southern Bluefin Tuna Industry Association Ltd

This initial cost can increase in some cases by:

1. The cost of any leased-in quota. However, arms length leased quota is only around 6% of total tonnage into farms (B Jeffriess pers. com. - based on AFMA data).

2. Any interest on borrowings to buy quota. The quota purchased in the last three years is only around 7% of tonnage into farms (B Jeffriess pers.com. - based on AFMA data). Also, borrowings are largely in Yen, at under 2.5% interest rate (including line fees).

Changes in the Japanese Market

The following table shows general price trends since 2002. Points to highlight in this table are that the market for ranched Australian SBT was very buoyant until it felt the effect of global competition in 2004 (Mexico and the Mediterranean). Since 2004 the market has shown a growing interest in NBT compared with SBT which can be significantly attributed to the bigger size of NBT.

Table 2. Import value of Australian ranched SBT

Year	Yen/kg[1]	$A/kg	Currency conversion[2]
2002	2250	33.58	67
2003	2181	27.26	80
2004	1544	20.32	76
2005	1572	18.71	84
2006	1824	21.46[3]	85
2007	2100	20.19	104

Notes to the table:
[1]Japanese Ministry of Finance. Landed Japan.
[2]JPY to AUD on 30th June every year – www.x-rates.com
[3] Prices received for SBT during the LTH trial were consistent regardless of size at harvests in March '06' and August '06'.

Marketing Flexibility from Longer-Term Grow-out

Australia's SBT ranch harvest is marketed in two categories, super low temperature frozen (SLT) and fresh fish by air freight. Approximately 80-90% of produce is marketed as SLT, with the remainder going fresh by air.

There is limited flexibility in varying the frozen/fresh mix because increasing the fresh SBT supply reduces the auction price (i.e. Australia is a price maker on the fresh auction market).

The frozen price of SBT each year is negotiated en bloc by the Japanese buyers. The harvest occurs between June and August. In 2003, the buyers declined to offer a realistic price. Some farmers sold for a low price, while others tried to store the frozen SBT in Japan. The result was large financial losses because Japanese buyers knew the stock in Japan was losing money. Inflexibility of the harvest period (to achieve the 3-6 month grow-out) contributed to this market situation. Holding SBT longer will give the industry marketing and volume flexibility, preventing the buyers from declining realistic prices in the future.

LTH could provide SBT ranchers with flexible options to take advantage of value added opportunities or fluctuating volumes of tuna on the fresh market.

Table 3. Fixed costs for LTH trial assessment.

Costs	*Item*	*Amount $AU*
Fixed Costs - Capture	Total capture cost	192000
	Fuel	68474
	Labour (Skippers & Deck-hands)	67821
	Diving	45000
	Tow Cage Equipment (depreciation)	25000
	Feed	20000
	Transfer Costs	9864
	Consumables	12000
	TOTAL	**440159**
Fixed Cost - Ranching	Admin., Licences & Management[1]	325000
	Repairs and Maintenance[1]	62000
	Equipment & Tools[1]	11000
	Depreciation of Ranching Structures	100000
	Consumables	19000
	Insurance[1]	50000
	Freezers and Operation Costs[1]	65000
	Trucks & Forklifts & Operation costs[1]	18000
	TOTAL	**650000**

Notes to table

Lease quota omitted from table

[1] General costs associated with ranching operations linked to medium operator

Table 4. Ranch information based on LTH trial results

Farm Info	0 weeks	6 weeks	12 weeks	18 weeks	36 weeks	52 weeks	70 weeks
Initial Weight Tagged Fish (kg)[1]	17.17	16.73	14.96	14.79	18.25	16.85	15.15
Weight kg (whole)		21.4	23.48	24.88	30.64	30.53	37.84
Weight kg (gilled and gutted)		18.62	20.43	21.65	26.65	26.56	32.92
Growth Rate %		27.91	56.95	68.22	67.89	81.19	149.77
Avg Feed/SBT (kg)		65.43	49.05	44.88	66.67	127.5	109.6
Feed Used During Period (kg)		452717	322413	277303	378983	652292	493430
Cumulative Feed Use (kg)[4]		452717	775131	1052434	1431417	2083709	2577139
FCR		14.01	13.44	15.79	18.24	25.84	20.41
Cumulative Mortality %[3]		1	5	6	8	10	12
Stock at Hand[2]	6989	6919	6640	6570	6430	6290	6150
Total Weight kg (whole)	120001	148069	155897	163453	197012	192037	232728
Total Weight kg (gilled and gutted)	104401	128834	135646	142233	171356	167065	202469

Notes to the table
[1] Initial tag weight i.e. calculations based on recaptured tagged SBT at harvest and their initial average weight at tagging
[2] Economic assessment based on 1 stocked 40m tow pontoon – 120t SBT
[3] Mortality based on industry average and LTH trial (Refer Figure 2, Chapter 7.1)
[4] Feed – Medium Protein/Medium Fat Profile

Table 5. Economic assessment of LTH trial based on research information

Farm Info $	6 weeks	12 weeks	18 weeks	36 weeks	52 weeks	70 weeks
Fixed Costs (quota lease)[1]	960000	960000	960000	960000	960000	960000
Fixed Costs (capture)	440159	440159	440159	440159	440159	440159
Fixed Costs (ranching)	650000	650000	650000	650000	650000	650000
Proportion of Ranching Fixed Costs (for following year)						216666
Cumulative Feed Costs[2]	335011	573597	778801	1059249	1541945	1907083
Cumulative Vessel Costs[3]	37382	74765	112147	224294	323981	436128
Cumulative Diving Costs[4]	48000	96000	144000	288000	416000	560000
TOTAL	2470552	2794521	3085107	3621702	4332085	5170036
Stock Value (gilled and gutted)[5]	2764774	2910963	3052323	3677306	3585216	4344975
BALANCE	294222	116442	-32784	55605	-746869	-825061

Notes to the table
[1] SBT lease quota based on $8/kg
[2] Based on feed cost of $0.74/kg
[3] Vessel costs are for a standard industry vessel and crew operating 6 days per week.
[4] Diving costs are contract rates
[5] Stock values are based on $21.46 kg – From Table 2. They do not reflect changing prices from individual harvest or year to year prices.

The figures presented in Tables 5, 6 and 7 are based on the tow pontoon being transferred into 4 ranching pontoons.

Table 6. Economic assessment of LTH trial based on research information and optimising feed inputs

Farm Info $	6 weeks	12 weeks	18 weeks	36 weeks	52 weeks	70 weeks
Fixed Costs (quota lease)[1]	960000	960000	960000	960000	960000	960000
Fixed Costs (capture)	440159	440159	440159	440159	440159	440159
Fixed Costs (ranching)	650000	650000	650000	650000	650000	650000
Proportion of Ranching Fixed Costs (for following year)						216666
Cumulative Feed Costs[2]	262576	449576	610412	830222	1208551	1494741
Vessel Costs[3]	37382	74765	112147	224294	323981	436128
Diving Costs[4]	48000	96000	144000	288000	416000	560000
TOTAL	2398117	2670500	2916718	3392675	3998691	4757694
Stock Value (gilled and guttted)[5]	2764774	2910963	3052323.16	3677306	3585216	4344975
BALANCE	366657	240463	135605	284631	-413475	-412719

Notes to the table
[1] SBT lease quota based on $8/kg
[2] Based on feed cost of $0.58/kg
[3] Vessel costs are for a standard industry vessel and crew operating 6 days per week.
[4] Diving costs are contract rates
[5] Stock values are based on $21.46 kg – From Table 2. They do not reflect changing prices from individual harvest or year to year prices.

Table 7. Economic assessment of LTH trial based on assumptions of optimising farm husbandry

Farm Info $	6 weeks	12 weeks	18 weeks	36 weeks	52 weeks	70 weeks
Fixed Costs (quota lease)[1]	960000	960000	960000	960000	960000	960000
Fixed Costs (capture)	440159	440159	440159	440159	440159	440159
Fixed Costs (ranching)	650000	650000	650000	650000	650000	650000
Proportion of Ranching Fixed Costs (for following year)						216666
Cumulative Feed Costs[2]	262576	449576	610412	682748	701558	1098535
Vessel Costs[3]	37382	74765	112147	224294	323981	436128
Diving Costs[4]	48000	96000	144000	288000	416000	560000
TOTAL	2398117	2670500	2916718	3245201	3491698	4361488
Stock Value (gilled and gutted)[5]	2764774	2972247	3117266	3757248	3664888	4443724
BALANCE	366657	301747	200548	512047	173190	82236

Notes to the table

[1] SBT lease quota based on $8/kg

[2] Based on feed cost of $0.58/kg

[3] Vessel costs are for a standard industry vessel and crew operating 6 days per week.

[4] Diving costs are contract rates

[5] Stock values are based on $21.46 kg – From Table 2. They do not reflect changing prices from individual harvest or year to year prices.

In addition to the notes to the table, information that underpins the economic assessment assumes mortality is 4% each year/season and FCR does not exceed 18:1 following 6 months in culture.

Discussion

Farm Gate Market Price

If reference is made to the 6 week harvest in Tables 5, 6 and 7 it appears that based on economics the SBT should have been sold after 6 weeks ranching to return the maximum amount. Unfortunately the value of the SBT at this point would more than likely be below the industry average as the SBT would not normally be market ready. This is due to the SBT having not reached a preferable market size and condition. As the tables don't reflect this issue, the figure should be dismissed in the economic analysis. Similarly, there was a bias in sampling at 36 weeks in culture which results in overstating the value of SBT at this point in time.

The SBT that were sold during March 2006 achieved one of the objectives of the LTH project in that it allowed the operator involved to meet a market shortfall on the fresh market in Japan. This was reflected in the market price whereby the price received for LTH SBT was $1.78/kg higher than what was received in the August 2006 harvest.

The SBT were sold on the 'Fresh' Japanese market and were competing against single season ranched SBT that were graded from a frozen harvest. There were no differences between the results received for the LTH and single season SBT and industry average prices. One aspect that should be emphasised is that there appeared to be no market perceptions or differences between SBT grown over 1 season or 2, which could lead to the conclusion that LTH does not lead to quality deterioration – e.g. colour.

In 2005, the average industry farm gate price for SBT was $18.71 and $21.46 in 2006 (Table 2). This difference between industry average prices clearly shows the potential for LTH and offers operators flexibility in maximising returns from year to year.

Feed Management

The medium protein - medium fat feeding profile used appears to deliver the best ratio of protein to fat for growing SBT (Section 8.1). Using the computer program '*Formu-bait*' to optimise nutrients can have a dramatic response in changing the economics of the project. Reducing feed costs from $0.74 to $0.58/kg (Tables 5 and 6) could represent a significant saving to the operator and will more than likely not compromise nutrient input or SBT performance.

The FCR experienced during the first part of the trial and consequent FCR during the trial may have been compromised by the initial extra handling of all SBT (Section 7.1). The high cumulative FCR after the first year in culture was not expected and as mentioned in Section 7.1 of the report, is probably linked to feed intake during this period. There appears to be a physiological process that inhibits feed intake during the warmer water temperatures in the second season. Future LTH trials should focus on formulating diets or bait mixes that reflect the physiological requirements of the SBT during the different stages of growth to maximise economic returns. For example, it may be better to feed the SBT a diet that enhances length growth and maintains condition index at 21 until it is time to prepare them for harvest, at which the feeding profile would change to maximise SBT condition to suit market requirements. Ultimately, the high cumulative FCR at the end of the trial (Table 4) is an area that needs to be addressed to make LTH attractive.

SBT Growth

One of the assumptions of the LTH trial was that the final weight of SBT would exceed 40kg, which would allow the SBT industry to take advantage of a recognised marketing opportunity (i.e. a higher price for SBT larger than 40kg). However, the growth of the SBT was not able to meet this expectation (Table 4). It was proposed that SBT in the second season would repeat the high late summer feed intake and consequent fast growth as experienced in the first season, but according to information presented in Section 8.1 this was not the case.

The project did highlight the potential of using 2 year old SBT (8-12kg) for LTH (Section 8.1). Indications suggest that this cohort of SBT is ideally suited for LTH due to their FCR, robustness for ranching and good growth rate.

Policy Issues

However, to develop LTH further there is also a critical policy issue that needs to be addressed. This relates to the Australian Fisheries Management Authority (AFMA) rules associated with the 40 fish weight sample in the existing SBT fishery management plan. The plan stipulates that all SBT less than 10kg are not part of the 40 fish sample that is used to obtain the average weight of the wild caught SBT. This has the potential to disadvantage any industry member that wishes to undertake LTH by over estimating the average weight of SBT at transfer.

Mortality

The mortality experienced in the first part of the trial (Table 4) was unacceptable by industry standards and is possibly linked to the tagging and handling as demonstrated in previous SBT research. Mortalities experienced in the LTH component of the trial (after August 22nd, 2005) could be attributed to the influence of seals and on one occasion the interaction of bronze whaler sharks. Table 7 produces figures using 4% mortality for each season and further improvements that can be made in this area will underpin the success of LTH. Possibly the use of 2 year old SBT and commercial ranching conditions would minimise mortality.

Diving

The assumptions made for the farm diving component are commercial rates for providing a diving service per pontoon (Refer Table 3). A Remote Operated Vehicle can deliver significant savings if used for inspections on a frequent basis in SBT ranching operations.

Assessment of Outcomes

It is always difficult to interpret the economic implications of research trials of this type. The reasons include the difficulty of separating mortalities due to tagging from the mortalities due to holding fish longer (e.g. any weakening in immune systems, concentrating predators on the trial pontoons); the problem of separating market price changes due to supply and quality; and the question whether a different feeding regime would have produced more growth. These questions can only be resolved by more research trials and/or by commercial uptake.

The results of this trial indicate that LTH is presently a higher risk approach to SBT ranching investment. The results from this LTH trial indicate that there is no economic benefit within the scope of the trial. However, there is a potential economic benefit if the results are applied to commercial ranching operations and steps are undertaken to address issues as highlighted in this report.

However, the fundamentals of SBT ranching in Australia continue to suggest that LTH must be part of the future SBT ranching structure. These fundamentals are:

(1) The positive outcomes of this trial included no deterioration in flesh quality with LTH.

(2) The trial also showed that LTH did provide the flexibility to market quality fish in a period of high market demand that cannot presently be supplied.

(3)   The major shortfall in the trial was the lack of fish weight increase in the early summer period (i.e. November/January), and the resulting high FCR's. As mentioned, this might be addressed by a change in diet, regularity of feeding, and/or focus on length growth rather than weight gain.

(4)   As mentioned, in a quota-based fishery the challenge for the industry is to optimise the performance of each fish as the amount of fish that can be ranched is limited in tonnage each year. This is in addition to the normal need for continuous productivity improvement. The industry has a number of research projects to improve the productivity of each fish (e.g. fouling management, nutrition, health and product quality). However, the biggest potential gain is in LTH – the question may be the LTH period.

(5)   Point (4) is reinforced by the emerging shortage of premium tuna supplies in Japan as the Mediterranean supply decreases. This gap will be partly filled by LTH of NBT in Japan (i.e. grow-out from 200g). There is a major market opportunity for Australia in the next 10 years.

(6)   There is an emerging trend of bluefin propagation which will possibly have an effect on the premium sashimi market. LTH has the potential to appeal to a different market demand of larger SBT.

(7)   LTH is also attractive because it will make the most of the high fixed costs of tuna ranching, by extending the short period industry resources are currently used each year. This opportunity will be even greater in the future with the capital costs of operating further offshore.

(8)   It remains the case that LTH only has to cover the direct (incremental) costs to justify the investment. As mentioned, the major direct cost is feed. The Australian feed sources are now stable at a high supply level, and affordable prices. This is required to underpin LTH.

(9)   The Australian industry relies heavily on currency hedging to justify the investment in the current single season grow-out system. This hedging involves purchasing currency at a given exchange rate rather

than accepting the spot rate. Currency hedging will underpin the investment in LTH, and eliminate possibly the biggest risk.

(10) The trial confirmed that LTH needs to utilise smaller fish in order to maximise its profitability (akin to the Croatian NBT experience). In Australia, the government policy of excluding fish less than 10kg from weight samples used to debit catch against quota is a major barrier to LTH. It is expected that this policy will be changed as soon as emerging average weight verification technology is implemented. This could be in 2011.

Summary

The results from this project have demonstrated the economic potential of LTH whilst highlighting issues that need further research to enhance the economic viability. Since this project began, the fundamentals in the SBT fishery, industry and market have shifted even further in favour of LTH. These shifts appear likely to last at least another decade.

The project was an important step along the difficult road to characterise the technical and financial challenges of LTH. It clearly showed that the next step to develop LTH is to research diets and feeding patterns which address the growth capacity of SBT over the summer period, particularly the 2-3 year olds. If this research is successful, then the only barriers to commercial adoption of LTH are reducing mortality below that experienced during the research and alteration of AFMA's average weight collection method. Industry expects AFMA to address the 10kg exclusion in their average weight policy by 2011.

9. BENEFITS & ADOPTION

This study greatly expands our knowledge of holding SBT for a greater period than the existing ranching cycle.

SBT ranch operators now have a much better understanding of the impact of LTH on SBT health, the environment, and production issues associated with growth, condition index, feeding behaviour, and product quality in respect to flesh characteristics and residue levels.

The nutrition component of this project has clearly defined significant production efficiencies through the delivery of optimised bait fish formulations using '*Formu-bait'* software. It has emphasised that to make LTH viable, 2 year old SBT must be the focus of wild capture activities and on-grown, which will result in better growth rates and reduced food conversion ratios. Furthermore, some feeding behaviour characteristics in LTH of SBT need to be managed carefully.

Product quality research has improved the understanding of flesh quality and residue status of SBT held for a longer period. This aspect of the project provides operators with confidence that there will be no adverse product safety or flesh quality issues associated with LTH of SBT.

SBT health research identified that there is a need to further investigate health issues, especially in the first year of LTH. The research project demonstrated that the level of parasites in the second year LTH of SBT was significantly less than in the first year.

Environmental research demonstrated the use of techniques developed in the Aquafin CRC to assess environmental impact and highlight potential issues for the future. The ASBTIA has already sought to address the results by applying to the SA Government for a greater ranching zone in the expectation that ranching production will increase over the next few years through a possible increase in quota allocation of the wild fishery and LTH of SBT.

From a husbandry and production point of view the benefits of this project highlighted processes that need to be considered and include management of lease sites and improved ranching equipment with a strong emphasis on reducing predation and poaching. The project also defined regulatory issues that need to be considered when moving to LTH of SBT with respect to compliance based environment reporting.

LTH demonstrated the economics of holding SBT and the associated financial risks. Ultimately, it showed that marketing advantages such as a downturn in supply of global high-grade sashimi quality tuna can be met by holding by LTH of SBT.

There are many aspects of this research that have been adopted by industry which include formulating bait fish mixes based on known proximate requirements, feeding of bait fish to meet residue regulatory requirements and the benefit of fresh local bait fish for delivery of some vitamins. Although not reported in this paper, there have been advances in new husbandry techniques such as handling and tagging of SBT and operational logistics of having SBT in a pontoon in the offseason with respect to managing crews and infrastructure. Furthermore, it has been part of industry discussions on how to proceed with another LTH project in the future and how research can be incorporated into this to investigate the use of pellet diets, improved weaning on to these diets, market opportunities and value adding options.

10. FURTHER DEVELOPMENT

This project has provided the nucleus for the development of long term holding of southern bluefin tuna. One of the key findings is that to make LTH financially viable within current trade constraints and future economic forecasts it will require SBT ranches to target younger SBT (approximately 10kg) for grow-out. This will require the industry and regulators to assess the rejection of SBT <10kg as part of the 40 fish weight sample which is currently used to determine the total weight of wild caught SBT that can be attributed to an operators quota allocation. Furthermore, if more SBT were removed from the wild fishery it may possibly have an impact on the wild fishery. This factor must be considered very carefully in context with sustainability.

However, the industry is confident that over the next few years the wild fishery stock assessment will improve due to the most recently introduced regulations to minimise over catching in the fishery. It is therefore important that research continues in the area of LTH so that when the opportunity arises, the Australian SBT ranching industry is presented with options of how to best optimise quota allocation.

In the meantime, for further improvement in LTH, industry needs to adopt and commercialise existing research results and together with researchers continue research efforts in the following areas:

1. Nutrition

- There is a need to further refine feed formulation to obtain a better food conversion ratio (FCR). The production of well conditioned SBT is very expensive with respect to the provision of feeds (nutrients). The ideal optimised diet for LTH should meet the metabolic and growth requirements of SBT until they are ready to be conditioned for the market.

- Investigate the application of using pelleted diets to better match nutritional requirements of SBT and benefits such as handling and delivery of diets for LTH.

- Feed formulation and condition index of SBT appear to be inter-related and impact on the feeding behaviour of SBT in LTH grow-out. This relationship needs to be understood further.

- Need to understand the optimal frequency of feeding as there are significant overheads in the delivery of feed to commercially ranched SBT.

- Sourcing and securing quality feed to accommodate an increase in production.

2. Product Quality (Residues)

- Refine feed formulation and thereby manage residue content to ensure ranched SBT have a high product safety status with respect to residue levels.

3. Product Quality (Flesh)

- Refine feed formulation to ensure ranched SBT are of high quality and meet market requirements in respect to fat content, colour, drip loss and shelf life.

- Investigate options to improve colour, minimise drip loss and enhance shelf life.

4. Marketing

- The production and availability of market ready SBT should be all year round. There will be a requirement to develop product lines and promote the flexibility of the product, whether it be on overseas or local markets.

5. Health

- Whilst the research suggested that LTH of SBT resulted in lower parasite loads than occurs on SBT held for one season this should be repeated to increase confidence in these results. It is also very

important to understand the epidemeology of the parasites and the factors that cause their rise and fall in infection level.

6. Environment

- There is a need to investigate feed formulation to minimise environmental impacts.
- Continued research on fouling management strategies to improve water quality conditions to LTH of SBT.
- Continued research in the area of physiology and metabolism and environmental impacts needs to be enhanced and understood.

11. PLANNED OUTCOMES

The key planned outcome of this project was to assess longer term holding (LTH) of southern bluefin tuna (SBT) and this was achieved.

The series of subprojects (Section 8) reported and achieved outcomes in the following areas:

1. Characterise the SBT growth and condition during the spring and summer period (when SBT have previously not been farmed) as well as during the second ranching season (LTH).

2. Characterise the food conversion ratio (FCR) of SBT farmed during the spring and summer period, as well as during the second ranching season (LTH).

3. Define the residue (Hg, dioxins, PCBs etc) changes (concentration and body burden) that occur in SBT muscle as a result of LTH and use this along with sampling and testing for residues of baitfish used for the SBT feed, to extend the single growout season predictive model to also address growout over a second season (ie. LTH).Extend the database for two Aquafin CRC postgraduate students, one studying the mercury content of SBT flesh and internal SBT organs, and the other dioxin and PCB content of SBT flesh.

4. Using the data collected on mercury and dioxins, etc to determine the effect of LTH against regulatory MRLs and tolerable intakes, and use this data for optimal positioning of the industry sector in relation to SBT product marketing.

5. Residues of bait fish used for the SBT feed, to extend the single grow-out season predictive model to also address grow-out over a second season (i.e. LTH).

6. Compare LTH of SBT to the competing product that is available on the Japanese market by comparing the LTH SBT product with the current SBT product (one season ranching cycle). This

research would expand on the study of chemical/instrumental parameters conducted in July 2006.

7. Determine and characterize the interactions between SBT health (parasites, etc) and residue levels.

8. Discover the potential effects of LTH on benthic nutrient cycling and assimilative capacity.

9. Investigate the environmental effects of LTH on macrobenthic infaunal communities using PCR assays and manual enumeration.

10. Evaluate changes in the redox potential and in the concentrations (and fluxes) of N and P in sediments.

11. An economic analysis and cost benefit analysis for the SBT ranching industry.

12. Summarise other less quantitative data in terms relevant to the industry sector so that the benefits and risks associated with LTH are fully appreciated.

12. CONCLUSION

This project was successful as it achieved most of its objectives and planned outcomes, and has positioned the SBT ranching industry with options to maximise quota allocation for the future.

The project was also important as it was industry led and involved strong collaboration between many of the science discipline research teams established as a result of the Aquafin CRC. Those involved included:

1. The Fisheries Research and Development Corporation

2. The Australian Southern Bluefin Tuna Industry Association (ASBTIA)

3. Two actively involved ASBTIA members in DI Fishing Pty Ltd and Sekol Farmed Tuna Pty Ltd

4. The South Australian Research & Development Institute - Aquatic Sciences

5. The South Australian Research & Development Institute - Food Safety

6. University of Tasmania

7. Flinders University

8. University of Adelaide

9. Barneveld Nutrition Pty Ltd

This project also fulfilled the SBT ranching industry's commitment to the Aquafin CRC after the initial focus of *'SBT propagation'* was replaced with *'long term holding of SBT'*.

13. APPENDIX ITEMS

APPENDIX 1: Intellectual Property

There is no specific intellectual property issues associated with this project. The ASBTIA Ltd request that the results of this project remain 'Commercial-in-Confidence' until results can be validated further in additional research trials.

This project involved the application of a range of research tools developed as a result of earlier Aquafin CRC projects; some of which include intellectual property.

APPENDIX 2: Summary of 2004 projects requiring R&D Support

Aquafin CRC - Southern Bluefin Tuna Aquaculture Subprogram: Provision of Research Platforms for Projects requiring Port Lincoln based R&D Support

Project Objectives

1. Provide and maintain a managed technical service as required by other project PIs undertaking tuna research activities using the commercial (industry) pontoon research platform in Port Lincoln.

2. Ensure, to the level of resources available, that the commercial (industry) pontoon research platform operations are world best practice

3. Coordinate and therefore optimize the use of the limited resources available for research requiring live SBT as part of the commercial (industry) pontoon research platform, through the development of an agreed project Annual Operating Plan

4. Ensure completion, in consultation with other project PIs, of the planned activities designated in the project Annual Operating Plan on the commercial (industry) pontoon research platform.

5. Support improved communication between project PIs and industry partners.

Projects Supported

The following projects were supported by organising or co-ordinating resources to meet project objectives:

2000/221 Aquafin CRC – SBT Aquaculture Subprogram: Quality and nutritional evaluation of bait fish used for tuna ranching.

2001/102 Aquafin CRC - SBT Aquaculture Subprogram: Tuna environment subproject 1: Development of novel methodologies for cost effective assessment of the environmental impact of aquaculture.

2001/103 Aquafin CRC - SBT Aquaculture Subprogram: Tuna environment subproject 2 - Evaluation of waste composition and waste mitigation strategies.

2001/104 Aquafin CRC - SBT Subprogram: Tuna environment subproject 3 - Development of regional environmental sustainability assessments for tuna sea-cage aquaculture.

2001/200 Aquafin CRC: SBT Aquaculture Subprogram: Tuna cell line development and their application to tuna aquaculture health surveillance.

2003/225: Aquafin CRC - SBT Aquaculture Subprogram: Investigation of the relationship between ranching practices and southern bluefin tuna health.

2003/227: Aquafin CRC - SBT Aquaculture Subprogram: Development and validation of bait fish sampling methods to address international residue standards for southern bluefin tuna (*Thunnus maccoyii*).

Rotten Bay Infrastructure

Infrastructure and coordination of resources was provided to the following project based in the sheltered waters of Rotten Bay.

2003/228 Aquafin CRC - SBT Aquaculture Subprogram: Activity metabolism in live-held southern bluefin tuna (*Thunnus maccoyii*).

DI Fishing Research Platform

The delivery of research platforms to the following projects was performed on a commercial tuna ranching lease site owned by DI Fishing Pty Ltd. In 2004, SBT research was performed on 3 x 32m diameter research pontoons and access was provided to a commercial pontoon for research comparisons. The methods for each project using this platform can also be found in the following project final reports.

2001/201 Aquafin CRC - SBT Aquaculture Subprogram: Commercialisation trials for a manufactured tuna feed.

2001/248 Aquafin CRC - SBT Aquaculture Subprogram: Maximising the control of quality in farmed SBT.

2001/249 Aquafin CRC - SBT Aquaculture Subprogram: Development and commercial evaluation of manufactured diets.
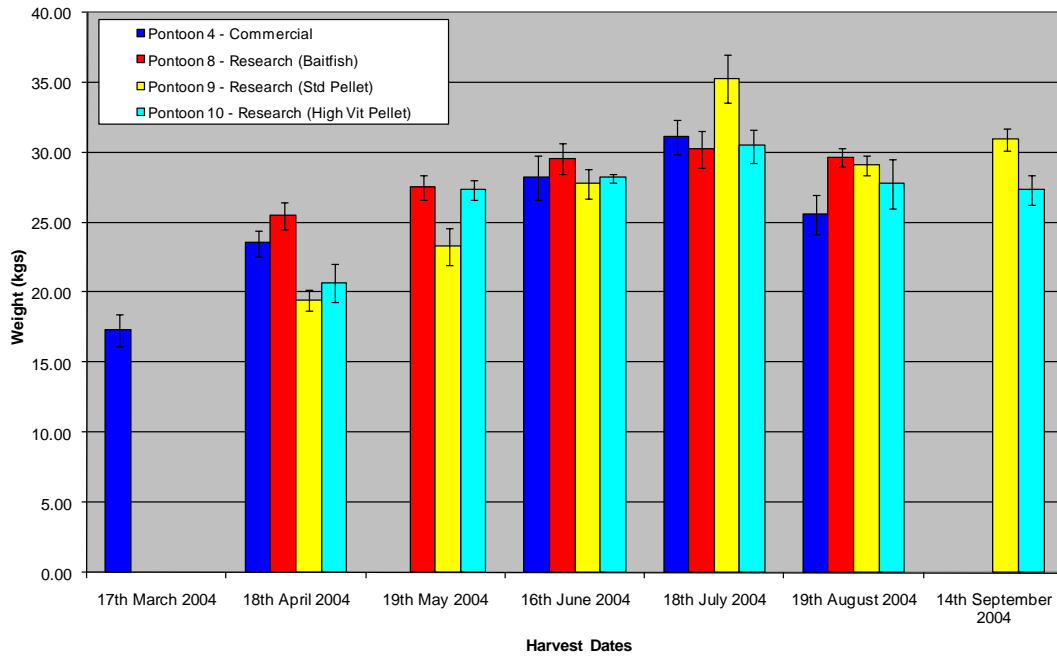
2003/225: Aquafin CRC - SBT Aquaculture Subprogram: Investigation of the relationship between ranching practices and southern bluefin tuna health.

The following Figures provide a summary of research ranching information from season 2004.
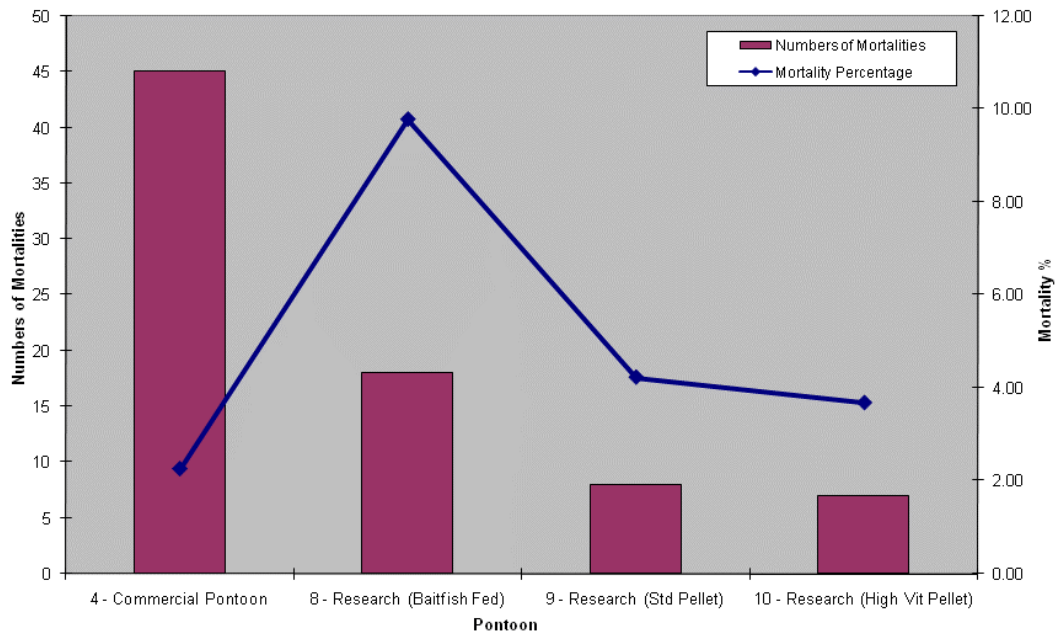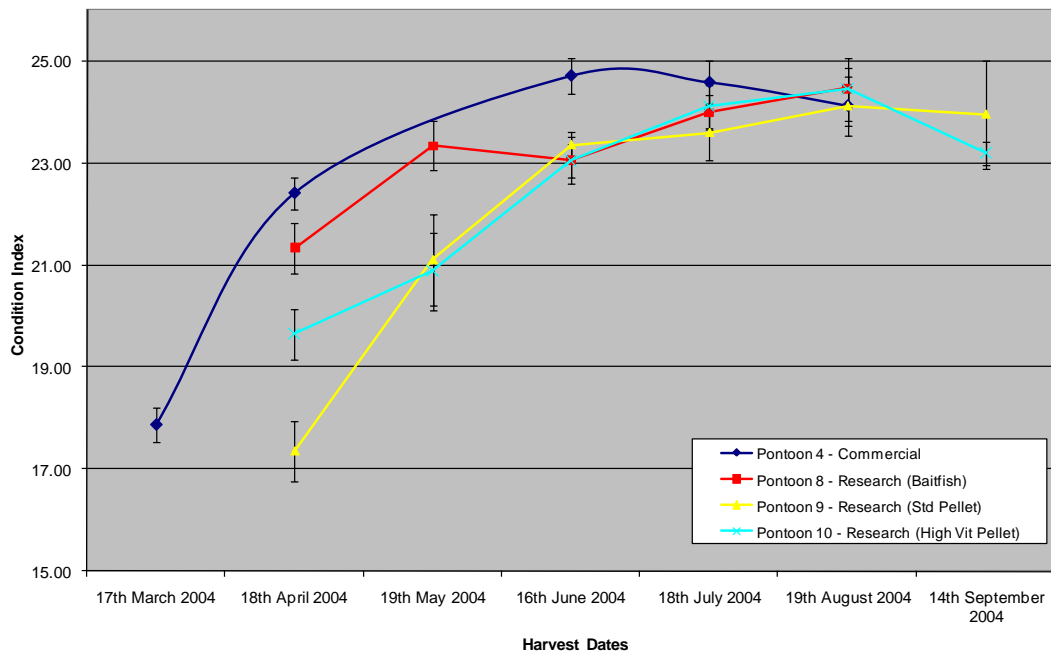

Figure 1. SBT mortality expressed as actual numbers and % of initial pontoon stocking numbers.

Figure 2. Average SBT harvest weight from every treatment

Note: Pontoons 9 and 10 were part of a digestibility trial and were harvested on the 14th September

Figure 3. Condition index of harvested research SBT

APPENDIX 3: Australian and Japanese Positive List System for Maximum Residue Limits (MRLs)

In May 2006 the Japanese Government through the Japanese Ministry of Health, Labour and Welfare, amended the existing *Food Sanitation Law*. The *Food Sanitation Law* was established in the late 1960's following the events of mercury contamination of seafood associated with Minamata and the PCB contamination of rice oil in Yusho, Japan. Japan has moved towards a *Positive List System* of regulating agricultural chemical residues in foods, that is permission is given for the presence of these compounds within prescribed limits in foods. There are 119 new *provisional* Maximum Residue Limits (MRLs) established under the *Positive List System* that apply specifically to Bluefin Tuna. Bluefin Tuna has been grouped under its Latin fish order of *Perciformes* in the new *Positive List System*.

Australian residue standards for fish

Table 1: Australian Maximum Levels (MLs), Extraneous Residue Limits (ERL) and Generally Expected levels (GELs) for residues and contaminants in fish

Compound	Type of Standard	Standard (mg/kg)
Metals		
Arsenic (inorganic)	**ML**	**2**
Copper	**GEL (90th percentile)**	**2**
Lead	**ML**	**0.5**
Mercury	**ML**	**1**
Selenium	**GEL (90th percentile)**	**2**
Tin (canned fish)	**ML**	**250**
Zinc	**GEL (90th percentile)**	**15**
Pesticides		
Aldrin & Dieldrin	**ERL**	**0.1**
BHC	**ERL**	**0.01**
Chlordane	**ERL**	**0.05**
DDT	**ERL**	**1**
Hexachlorobenzene	**ERL**	**0.1**
Heptachlor	**ERL**	**0.05**
Lindane	**ERL**	**1**
Polychlorinated Biphenyls (PCBs)		
PCBs	**ML**	**0.5**

An ML/MRL or ERL is only set by FSANZ where it serves an effective risk management function to protect public health.

Changes to toxicity weighting factors for dioxins and dioxin-like PCBs

The World Health Organisation (WHO) has undertaken a major re-evaluation of the toxicity of dioxins and PCBs. For a number of years there has been an internationally agreed weighting factor called a Toxic Equivalence Factor (TEF) system used so data can be compared from around the world. This scheme has recently been updated with new TEF values. Overall, the effect of

APPENDIX 3: Australian and Japanese Positive List System for Maximum Residue Limits (MRLs)

this is that the contribution of the dioxin-like PCBs to the total Toxic Equivalence (TEQ) value is lessened. The dioxin-like PCBs typically contribute more than three-quarters of the total TEQ value in fish.

Congener	WHO 1998 TEF	WHO 2005 TEF
Chlorinated dibenzo-*p*-dioxins		
2,3,7,8-TetraCDD	1	1
1,2,3,7,8-PentaCDD	1	1
1,2,3,4,7,8-HexaCDD	0.1	0.1
1,2,3,6,7,8-HexaCDD	0.1	0.1
1,2,3,7,8,9-HexaCDD	0.1	0.1
1,2,3,4,6,7,8-HeptaCDD	0.01	0.01
OctaCDD	0.0001	**0.0003**
Chlorinated dibenzofurans		
2,3,7,8-TetraCDF	0.1	0.1
1,2,3,7,8-PentaCDF	0.05	**0.03**
2,3,4,7,8-PentaCDF	0.5	**0.3**
1,2,3,4,7,8-HexaCDF	0.1	0.1
1,2,3,6,7,8-HexaCDF	0.1	0.1
1,2,3,7,8,9-HexaCDF	0.1	0.1
2,3,4,6,7,8-HexaCDF	0.1	0.1
1,2,3,4,6,7,8-HeptaCDF	0.01	0.01
1,2,3,4,7,8,9-HeptaCDF	0.01	0.01
OctaCDF	0.0001	**0.0003**
Non-*ortho*-substitututed PCBs		
PCB 77	0.0001	0.0001
PCB 81	0.0001	**0.0003**
PCB 126	0.1	0.1
PCB 169	0.01	**0.03**
Mono-*ortho*-substitututed PCBs		
PCB 105	0.0001	**0.00003**
PCB 114	0.0005	**0.00003**
PCB 118	0.0001	**0.00003**
PCB 123	0.0001	**0.00003**
PCB 156	0.0005	**0.00003**
PCB 157	0.0005	**0.00003**
PCB 167	0.00001	**0.00003**
PCB 169	0.0001	**0.00003**

Table 2. Summary of 1998 and 2005 Toxic Equivalence Factor (TEF) values for dioxins and the dioxin-like PCBs.

Adapted from van den Berg *et al.* (2006). Values in bold indicate where TEF values have changed.

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